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IMPACT FACTOR

“THINK BEYOND TO CREATE MIRACLES”
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The name 'Enthralling' to this edition was suggested by Lieutenant Jyotishk Tiwari. We thank him whole heartedly. The word enthralls stands for 'hold spellbound' i.e. capturing interest as if by a spell. In simple English language, it is one of the synonyms of the word delight.
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Editorial Note

Analysis and Research in a high-integrity organization- an environment of absolute integrity where knowledge is kept intact. Departures from set codes of knowledge are made in respect of “Innovations”. Performance can be evaluated quickly.

Researcher seeks to see the problem on their feet and pick up things quickly- a researcher has an interest into abysmal digging until roots of the problem, understand it to the maximum and commit.


What prompts one to bear an interest into these areas are the associated dynamics with which societies are attributed with. Study of society, and consequently the economy, is an imperative but an ardent exercise since the laboratory for experiments is the society itself.

A Researcher has an endeavor to construct valid sets of explanations to events that ratify, repose and redress the concerns of the society. He vouches to study principles laid down as a technical question, irrespective of the purpose, the enterprise, the personnel composing it, or any constitutional, political, or social theory underlying its creation.

The researcher may face constraints and limitations that inhibit the fusion or synthesis of researches, but, takes them as an opportunity. I believe that thinkers, with different backgrounds, different perceptions and different expectations would tread a common path, with a common approach and with a common purpose.

One also understands and incorporates the limitations, at integrating the several streams of thoughts and of impact of culture. To my understanding, no organization can function beyond the pale of cultural constraints. For, culture of society exerts immense influence on organizations and on those who work in them. Therefore, researchers must take into account the cultural context. May be an issue of making incidental references to the places other than writers’ own place of origin, affects the universal validity of the theory.

Values have been changing through the passage of time. Gone are the days when researcher was concerned with only such values as economy and efficiency, and gone are the days when it was concerned with only psychological and emotional factors involved in group behavior. Today societies of the world are in a flux and social transformation is taking place at a faster rate, research is expected to meet and handle these challenges.

One may opine that the classical, behavioral, approaches in the researches are, but, segments of a unified and integrated field of scientific research, the bewilderment about scientific approaches is a consequence of a myopic view of science and its attributes of definiteness and universal validity. These attributes lose significance when applied in isolation. They are to be meaningful and useful.

One may feel that perplexity about values shall deepen when someone considers the efforts of theory building a sterile exercise in the manipulation of abstract and empty concepts. But the objective of researches is not to provide readymade solutions to the problems of social order or disorder, or the discovery of Laws. One may endeavor to elaborate conceptual tools that might help empirical investigation by suggesting useful ways of looking at realities. Such tools do not provide, prefabricated answers to questions or problems, they simply prepare the ground for their adequate handling. It is also to be emphasized that no conceptualization will ever be final, definite and settled. It is and must be, subject to continual alterations, elaborations, and refinements- all this being necessary in order to keep theory always at the service of empirical research. Thought process beyond the contours of defined knowledge lies at the base of innovation. As life grows, one is required to make a significant contribution to the society.

Mr. Apoorav Srivastava
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SYNERGISTIC HEPATOCARDIOPROTECTIVE AND ANTIOXIDANT EFFECTS OF MYRRH AND ASCORBIC ACID AGAINST DIAZINON-INDUCED TOXICITY ON RABBITS

Mohamed M. Abdel-Daim & Safaa M. Halawa

1Pharmacology Department, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt, 2Department of Plant Protection, Faculty of Agriculture, Benha university, Benha, Egypt

Abstract:
Diazinon (DZN) is a widely used organophosphorus synthetic and acaricide widely used for agricultural and veterinary purposes. However, its human and animal exposure leads to hepatocardiotoxicity. Our experimental objective was to evaluate protective effects of Myrrh; Commiphora molmol oil and ascorbic acid; vitamin C against DZN-induced hepatocardiotoxicity in healthy male white New Zealand rabbits (Oryctolagus cuniculus). DZN-treated animals revealed significant alterations in serum biochemical parameters related to hepatic and cardiac injuries. There was a significant increase in hepatic and cardiac lipid peroxidation and significant inhibition in tissue antioxidant biomarkers due to DZN intoxication. Both myrrh and vitamin C protect against DZN-induced serum as well as hepatic and cardiac tissue biochemical parameters when used alone or in combination along with DZN-intoxication. Furthermore, both myrrh and vitamin C produced synergetic hepatocardioprotective and antioxidant effects. Therefore, it could be concluded that myrrh and/or vitamin C administration able to minimize the toxic effects of DZN by its free radical-scavenging and potent antioxidant activity.

Introduction
Organophosphorous pesticides have fully replaced the chlorinated pesticides in the 4 decades ago. The main advantage of the organophosphorous compounds was their low cumulative effect and short-term persistence in the ecosystem (Zavon, 1971). Although the organophosphates have been replaced by pyrethroids within the last 10–15 years, there is still a very intensive use of organophosphorus insecticides (Salem and Olajos, 1988). They are used throughout the world for control of agricultural, veterinary and domestic insect pests. Diazinon [phosphoric acid, O, O-diethyl O (2-isopropyl-6-methyl-4-pyridinyl)] phosphorothioate is an organophosphorus insecticide widely used in agricultural, veterinary and domestic practice throughout the world to control ticks, flies, lice, fleas and other insect pests of ornamental plants, food crops and domestic animals (Larkin and Tjeerdema, 2000). Toxic effects of Diazinon on target and non-target organisms are due to the inhibition of acetyl cholinesterase (AChE). Diazinon exerts its toxicity through binding its oxygen analog to the neuronal enzyme AChE, resulting in the accumulation of the endogenous neurotransmitter; acetylcholine in neurons and effectors organ (Larkin and Tjeerdema, 2000). Furthermore, it affects mitochondrial membrane transport in rat liver and disrupts cytochrome P450 system in human liver (Sams et al., 2004). The toxic effects of diazinon on mammalian cells were studied by some researchers (Al-Attar and Abu Zeid, 2013; ElMazoudy and Attia, 2012; Elmazoudy et al., 2011; Larkin and Tjeerdema, 2000; Razavi et al., 2013). Diazinon toxicity induced oxidative stress resulting in hematological changes, neurotoxicity, hepatotoxicity, nephrotoxicity, cardiotoxicity and both male and female reproductive toxicity (Al-Attar and Abu Zeid, 2013; ElMazoudy and Attia, 2012; Elmazoudy et al., 2011; Razavi et al., 2013).

The cells combat oxidative stress by either removing the damaged nucleotides and lipid peroxidation products or directly scavenging oxygen radicals via endogenous enzymatic and non-enzymatic antioxidants (Abdel-Daim et al., 2013; Azab et al., 2013; Madkour and Abdel-Daim, 2013). Myrrh is a yellow aromatic oleo-gum resin obtained from the stems of a number of small, thorny tree species of the genus Commiphora, particularly C. molmol and C. myrrha (Burseraceae family). It consists of approximately 30–60% water-soluble gum, 20–40% alcohol soluble resin and 3–8% volatile oil (al-Harbi et al., 1997). It also contains eugenol, cuminic aldehyde, terpenes and sesquiterpenes (al-Harbi et al., 1997). It has been demonstrated that myrrh has many pharmacological activities, including: local anesthetic, analgesic, anti-inflammatory and anti-ulcer, immunomodulatory, antimicrobial and antiparasitic (al-Harbi et al., 1997; Ashry et al., 2010; Dolara et al., 2000; Shen et al., 2012). It has a potent anticancer activity against Ehrlich ascites carcinoma (Qureshi et al., 1993). Myrrh protected liver against diethylnitrosamine-induced injury and hepatocarcinogenesis (El-Shahat et al., 2012). It reduced Pb-induced oxidative damage in hepatic mouse tissue by reducing lipid peroxidation and enhancing GST activity (Ashry et al., 2010; El-Ashmawy et al., 2006). Myrrh active constituent; guggulsterone induced cardioprotective effects against doxorubicin cardiomyocyte injury (Wang et al., 2012). Its antioxidant properties might be through its free radical-scavenging activities (Ashry et al., 2010; El-Ashmawy et al., 2006). The free radical-scavenging effect of myrrh essential oils also provide a protection against lipid peroxidation induced by lipophilic pharmaceuticals and cosmetic preparations (Auffray, 2007).

Ascorbic acid; vitamin C is probably the most commonly used vitamin and perhaps the most important antioxidant in extracellular fluids. It is an essential component in the diet of some mammals, including human and rabbits. It is highly water soluble and acts as an effective reductant. It is the most effective antioxidant in inhibiting lipid peroxidation initiated by peroxyl radicals, and considered as an effective radical scavenger of ROS (El-Demerdash et
It may also regenerate other antioxidants such as vitamin E (Carr and Frei, 1999). To our knowledge, the role of myrrh and vitamin C against DZN-induced alterations in serum biochemical parameters as well as lipid peroxidation and antioxidant status in rabbits has not been studied yet. Therefore, in the present study, was designed to investigate the alterations in serum biochemical parameters related to liver and heart injuries as well as hepatic and cardiac lipid peroxidation and oxidative stress induced by DZN in rabbits. Moreover, the role of myrrh or/and vitamin C supplementation in alleviating these DZN-induced hazard effects could be evaluated.

Material and Methods

**Chemicals:**
Diazinon® and ascorbic acid were purchased from Adwia Pharmaceuticals, Cairo, Egypt. Diazinon® 60 was applied as a commercial emulsifiable formulation containing 60% active ingredient. It was diluted in deionized water for the final required concentration. Myrrh oleo-resin emulsion from was prepared by Pharco Pharmaceuticals, Alexandria, Egypt. Briefly, known weight of myrrh powder was extracted with ethanol by percolation at room temperature. The extracts were evaporated under vacuum at 40°C to give a yellowish-brown semisolid residue. The yield of myrrh ethanol extract was stored in a refrigerator. The used emulsion was prepared according to (Massoud and Labib, 2000). The concentration used in the experiment was based on the dry weight of the extract. All kits used for evaluation of serum biochemical parameters, tissue lipid peroxidation and antioxidant biomarkers were purchased from Randox Laboratories Ltd, U.K. and CK was purchased from Stanbio™ CK-NAC (UV-Rate) kit (Texas, USA). Other chemicals used in this study were analytical grade.

**Animals and experimental design**
The design of the experiments conforms to the guidelines of the National Institutes of Health (NIH) and was approved by a local committee at Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt and Faculty of Agriculture, University, Moshtohor, Egypt. The present work was carried out at Rabbit Farm, Animal Production Department, Faculty of Agriculture, University, Moshtohor, Egypt. Total forty healthy male white New Zealand rabbits (Oryctolagus cuniculus) (weighing 1500± 200 g) were used in the current study. All animals were housed in metal batteries and supplemented with the basal diet and water ad libitum through the experiment. After one weeks of acclimatization, all rabbits were randomly divided into five experimental groups of nine rabbits each. Groups I (control) received saline, and II (DZN) orally received the emulsifying agent at a dose of 5.28 mg/kg body weight (Tsitsimpikou et al., 2013) and served as positive controls, respectively. Groups III (DZN-CME) and IV (DZN-AA) were administered with CME in a dose rate of 50 mg/kg body weight(Ashry et al., 2010), and AA at a dose of 20 mg/kg body weight (Ozdem et al., 2011) respectively, while rabbits at group V (DZN-CME-AA) were administered both CME and AA at the same dose and regimen used for the group III and IV. DZN, CME and AA were orally administered using intra-gastric intubation every 48 hours for successive 30 days (Table 1). At the end of the experiment, blood samples were collected at room temperature, left to clot and centrifuged at 3000 rpm for 15 minutes to obtain clear sera, and then all animals were slaughtered and sacrificed, and livers and hearts were excised for further preparation for estimation of lipid peroxidation and antioxidant biomarkers.

**Serum biochemical analysis**
Freshly separated sera were used for estimation of serum hepatic and cardiac injury biomarkers according to manufacturer protocol. The appropriate kits were used for the determination of the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) according to (Reitman and Frankel, 1957). Alkaline phosphatase (ALP) was determined according to (Tietz et al., 1983). The enzyme activity was expressed as units/liter computed directly from the absorbance values. The level of total proteins were evaluated according to (Lowry et al., 1951). While total bilirubin was determined according to (Schattmann, 1952). Nevertheless, cholesterol was measured according to (Allain et al., 1974; Richmond, 1973). Serum LDH activity was determined enzymatically the according to the manufacturer's protocol using kits from Randox Laboratories Ltd, U.K. (BuHl and Jackson, 1978). CK activity was estimated according to the method developed by (Szasz et al., 1979) using Stanbio™ CK-NAC (UV-Rate) kit (Texas, USA).

**Evaluation of lipid peroxidation and antioxidant biomarkers**
Hepatic and cardiac tissues of the collected from each rabbit group were rapidly removed, cleaned from any extraneous materials and immediately perfused with cold saline. The tissues were homogenised in cold phosphate buffer saline (0.1 M pH 7.4) then the homogenate was filtered and centrifuged at 1500 rpm for 20 min .The supernatant was stored in -80°C until use for biochemical analysis of lipid peroxidation and antioxidant biomarkers.
Lipid peroxidation was evaluated using measurement of MDA content in the tissues according to (Mihara and Uchiyama, 1978). Oxidative stress was assessed by evaluation of the enzymatic antioxidant biomarker; superoxide dismutase (SOD) according to (Nishikimi et al., 1972), and the non-enzymatic antioxidant marker; reduced glutathione (GSH) according to (Beutler et al., 1963).

Statistical analysis
All data were expressed as means ± S.E.M. and statistically analyzed using SPSS 17.0 for Windows (SPSS Inc, Chicago, IL). Statistical significance of differences among different study groups were evaluated using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test for post-hoc analysis. Statistical significance was acceptable to a level of P≤0.05.

Result and Discussion
Reactive oxygen species (ROS) are continuously generated inside the body as a result of exposure to many exogenous drugs and xenobiotics in our environment and/or a plenty of endogenous metabolic events involving redox enzymes and electron transport mechanism (Abdel-Daim et al., 2013; Azab et al., 2013; Madkour and Abdel-Daim, 2013). Under normal conditions, there is equilibrium between the ROS generated and the antioxidants present as the ROS generated are neutralized by the endogenous antioxidants (Abdel-Daim et al., 2013; Sun, 1990). Deleterious effects caused by ROS occur as a consequence of an imbalance between the formation and inactivation of these species leading to irregularities in cellular physiology and different pathological conditions (Abdel-Daim et al., 2013; Sun, 1990). Free radicals have been implicated in the aetiology of many degenerative diseases such as cancer, cataract, coronary heart disease, stroke, rheumatoid arthritis, diabetes, Alzheimer's disease and ageing process (Abdel-Daim et al., 2010a, b; Funasaka et al., 2012; Wilcox et al., 2004).

Diazinon; an organophosphorus insecticide has been widely used in industrial agriculture and veterinary practice worldwide that would be potentially an exposure risk to human and animals, including rabbits (Larkin and Tjeerdema, 2000). Although several reports about the toxicity of DZN have been published, little study has been performed about the use of natural products for prevention of such toxicity and mechanism of their ameliorative action (Al-Attar and Abu Zeid, 2013; ElMazoudy and Attia, 2012; Elmazoudy et al., 2011; Larkin and Tjeerdema, 2000; Razavi et al., 2013).

In the present study, hepatic and cardiac injuries caused by DZN may be attributed to the oxidative stress resulted from free radical production. DZN intoxication significantly (P≤0.05) increased serum liver and heart injury biomarkers; AST, ALT, ALP, cholesterol and total bilirubin, LDH and CK. Moreover, it significantly (P≤0.05) reduced serum total protein level (Table 2). DZN treatment significantly (P≤0.05) increased lipid peroxidation through elevated hepatic and cardiac MDA level, significantly (P≤0.05) decreased hepatic and cardiac enzymatic; SOD and CAT as well as non-enzymatic; GSH antioxidant level (Tables 3 &4).

All these effects are involved in the cascade of events leading to DZN-mediated hepatocardiac oxidative stress and toxicity. This indicates that hepatocardiaco injuries induced by DZN is the result from oxidative stress that arises as a result of excessive generation of ROS, which have been reported to attack various biological molecules, including lipids and causing lipid peroxidation. The activities of antioxidant enzymes, including the enzymes involved in glutathione metabolism were also perturbed in DZN treated group (Tables 3 and 4) indicating the involvement of oxidative stress in DZN-mediated hepatocardiaco injuries. These results are consistent with the literature (Al-Attar and Abu Zeid, 2013; Razavi et al., 2013) and point towards the role of ROS in DZN-mediated injury and toxicity (Al-Attar and Abu Zeid, 2013; Larkin and Tjeerdema, 2000; Razavi et al., 2013; Salem and Olajos, 1988).

The organophosphorus insecticide; DZN caused an increase in AST, ALT, ALP, LDH, CK, CK-MB enzymes activity, cholesterol total protein, albumin and total bilirubin as well as hepatic and cardiac MDA levels and reduced GSH, CAT, SOD, glutathione peroxidase (GPx) and glutathione S-transferase (GST) in mice and rats (Al-Attar and Abu Zeid, 2013; El-Demerdash and Nasr, 2013; Razavi et al., 2013; Tsitsimpikou et al., 2013). These alterations might differ dependent on exposure dose and duration.

In the current study, the pre-administration of CME (50 mg/kg) as well as AA (20 mg/kg) reduced the serum hepatic and cardiac injury biomarkers. Moreover, they reduced the lipid peroxidation in hepatic and cardiac tissues. In addition, there were elevations of hepatocardiaco antioxidant enzymes and glutathione levels due to both preventive agents administration. Both myrrh and vitamin C induced synergistic protective effects against DZN-induced serum and tissues biochemical alterations (Tables 2, 3 &4).

The antioxidant and protective effects of myrrh are owed to their content of antioxidant active constituents such as eugenol, cuminic aldehyde and sesquerpenes (al-Harbi et al., 1997; Shen et al., 2012). Many previous literatures showed the hepatoprotective, cardioprotective and antoxygen effects of myrrh and its active constituents against drugs, chemicals and xenobiotics (Ashry et al., 2010; Affray, 2007; El-Ashmawy et al., 2006; El-Shahat et al., 2012; Shen et al., 2012; Wang et al., 2012).
Pre-treatment with vitamin C might play a role in reducing the toxic effect of DZN, and its antioxidant properties seem to mediate such a protective effect, indicated by the reduction of MDA as well as the elevation of GSH and SOD, CAT levels in hepatic and tissue (Devrim et al., 2008; El-Demerdash et al., 2005; Kojo, 2004). Rabbit is used for many researches as an important livestock for production of meat in most Mediterranean countries, offering more than 30% of world production. Besides, rabbits reproductive capacity, a feature that is attractive for its use as lab animal too. (Lazzaroni et al., 2009). The protective effect of myrrh and/or vitamin C against DZN-induced oxidative stress in our rabbit model could be either direct by inhibiting lipid peroxidation and scavenging free radicals or indirect through the enhancement of the activity superoxide dismutase and CAT; the enzymatic free radicals' scavengers in the cells. Therefore, myrrh and vitamin C could be used in combination to prevent and treat hepatic and cardiac diseases, especially those induced by oxidative damage.

References


Table 1: Summary of different rabbit groups and their treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>DZN</th>
<th>CME</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DZN</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DZN-CME</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>DZN-AA</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>DZN-CME-AA</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Diazinon 5.28 mg/kg body weight, every 48 hours for 4 weeks (DZN), Commiphora molmol; myrrh emulsion at 50 mg/kg body weight, every 48 hours for 4 weeks, 1 hour before DZN dose (CME), Ascorbic acid; vitamin C 20 mg/Kg body weight every 48 hours, 1 hour before DZN administration (AA)

Table 2: Serum enzymes activity and biochemical parameters in control and different treated groups

<table>
<thead>
<tr>
<th></th>
<th>AST U/L</th>
<th>ALT U/L</th>
<th>ALP U/L</th>
<th>Cholesterol</th>
<th>T. protein</th>
<th>T. bilirubin</th>
<th>LDH U/L</th>
<th>CK U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>49.72±2.06</td>
<td>54.42±3.08</td>
<td>15.31±0.49</td>
<td>60.80±1.38</td>
<td>8.23±0.14</td>
<td>1.44±0.02</td>
<td>43.90±1.89</td>
<td>119.48±3.41</td>
</tr>
<tr>
<td>DZN</td>
<td>123.4±7.95</td>
<td>151.6±8.18</td>
<td>29.9±2.61</td>
<td>87.3±2.06</td>
<td>6.28±0.11</td>
<td>1.91±0.02</td>
<td>72.19±2.75</td>
<td>337.03±13.21</td>
</tr>
<tr>
<td>DZN-CME</td>
<td>76.05±3.66</td>
<td>76.28±4.09</td>
<td>20.15±1.02</td>
<td>70.51±1.54</td>
<td>7.26±0.11</td>
<td>1.57±0.06</td>
<td>54.67±2.43</td>
<td>179.23±10.09</td>
</tr>
<tr>
<td>DZN-AA</td>
<td>60.26±1.46</td>
<td>69.26±8.88</td>
<td>18.91±1.28</td>
<td>65.5±1.42</td>
<td>7.58±0.12</td>
<td>1.42±0.03</td>
<td>50.62±2.25</td>
<td>145.4±7.28</td>
</tr>
<tr>
<td>DZN-CME-AA</td>
<td>48.32±2.65</td>
<td>52.7±2.27</td>
<td>16.16±0.45</td>
<td>61.35±1.84</td>
<td>7.89±0.17</td>
<td>1.40±0.03</td>
<td>45.4±1.56</td>
<td>124.23±5.03</td>
</tr>
</tbody>
</table>
Data are expressed as means ± SE (n=8).

Values having different superscripts within the same row are significantly different (P≤0.05).

Diazinon 5.28 mg/kg body weight, every 48 hours for 4 weeks (DZN), Commiphora molmol; myrrh emulsion at 50 mg/kg body weight, every 48 hours for 4 weeks, 1 hour before DZN dose (CME), Ascorbic acid; vitamin C 20mg/Kg body weight every 48 hours, 1 hour before DZN administration (AA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (T. protein), total billirubin (T. billirubin), lactatic dehydrogenase (LDH), creatine kinase (CK).

Table 3: Hepatic oxidative stress markers and antioxidant parameters in control and different treated groups.

<table>
<thead>
<tr>
<th></th>
<th>MDA nmol/gm</th>
<th>GSH mg/g</th>
<th>SOD u/g</th>
<th>CAT u/g</th>
<th>TAC mmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.78±2.39</td>
<td>54.52±1.98</td>
<td>36.06±1.91</td>
<td>2.75±0.13</td>
<td>48.01±1.35</td>
</tr>
<tr>
<td>DZN</td>
<td>76.06±2.57</td>
<td>34.68±1.61</td>
<td>13.34±0.90</td>
<td>0.84±0.04</td>
<td>33.15±0.72</td>
</tr>
<tr>
<td>DZN-CME</td>
<td>52.54±1.92</td>
<td>43.44±1.49</td>
<td>24.14±1.78</td>
<td>1.49±0.09</td>
<td>42.36±1.29</td>
</tr>
<tr>
<td>DZN-AA</td>
<td>41.03±5.08</td>
<td>45.64±4.23</td>
<td>31.16±3.64</td>
<td>1.84±0.23</td>
<td>44.40±1.75</td>
</tr>
<tr>
<td>DZN-CME-AA</td>
<td>32.30±2.34</td>
<td>50.82±1.66</td>
<td>35.96±1.52</td>
<td>2.11±0.10</td>
<td>47.71±0.84</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE (n=8).
Values having different superscripts within the same row are significantly different (P≤0.05).

Diazinon 5.28 mg/kg body weight, every 48 hours for 4 weeks (DZN), Commiphora molmol; myrrh emulsion at 50 mg/kg body weight, every 48 hours for 4 weeks, 1 hour before DZN dose (CME), Ascorbic acid; vitamin C 20mg/Kg body weight every 48 hours, 1 hour before DZN administration (AA), malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (TAC).

Table 4: Cardiac oxidative stress marker and antioxidant parameters in control and different treated groups.

<table>
<thead>
<tr>
<th></th>
<th>MDA nmol/gm</th>
<th>GSH mg/g</th>
<th>SOD u/g</th>
<th>CAT u/g</th>
<th>TAC mmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>43.17±3.59</td>
<td>28.69±1.04</td>
<td>11.27±0.60</td>
<td>1.37±0.06</td>
<td>816.2±22.87</td>
</tr>
<tr>
<td>DZN</td>
<td>120.3±4.81</td>
<td>15.96±0.82</td>
<td>3.94±0.35</td>
<td>0.44±0.01</td>
<td>529.7±23.20</td>
</tr>
<tr>
<td>DZN-CME</td>
<td>68.79±4.30</td>
<td>22.86±0.79</td>
<td>7.55±0.56</td>
<td>0.79±0.05</td>
<td>720.1±21.98</td>
</tr>
<tr>
<td>DZN-AA</td>
<td>52.28±3.76</td>
<td>23.65±2.65</td>
<td>9.74±1.14</td>
<td>0.92±0.12</td>
<td>754.8±29.82</td>
</tr>
<tr>
<td>DZN-CME-AA</td>
<td>39.92±2.99</td>
<td>29.06±1.85</td>
<td>11.37±0.47</td>
<td>1.07±0.05</td>
<td>803.0±14.25</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE (n=8).
Values having different superscripts within the same row are significantly different (P≤0.05).

Diazinon 5.28 mg/kg body weight, every 48 hours for 4 weeks (DZN), Commiphora molmol; myrrh emulsion at 50 mg/kg body weight, every 48 hours for 4 weeks, 1 hour before DZN dose (CME), Ascorbic acid; vitamin C 20mg/Kg body weight every 48 hours, 1 hour before DZN administration (AA), malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (TAC).
Objective: Various compounds of medicinal plants have been widely investigated since ancient times for their possible immunomodulatory properties in the body’s immune system. In the present study, the immunostimulatory effect of Leptadenia pyrotechnica crude extract was evaluated in vivo. Results: Our research revealed that at doses (150 mg/kg), Leptadenia pyrotechnica crude extract increased the phagocytic activity in a dose dependant manner when compared with the control and thus the clearance rate of carbon was faster after the administration of the plant extract P=0.001.

Introduction

The immune system is the most complex biological systems in the body. At the time of infection immune system go under the attack of a large number of viruses, bacteria and fungi. There are two branches of immunity response: humoral immunity and cellular immunity.

In order to perform phagocytic function, cells of reticuloendothelial system must be transformed to the active state. This specific ability is significantly suppressed by the action of physiological and pathological factors in nature. However, it is possible to influence this ability using certain immunomodulating agents. Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions. In the innate immune the nature killer cell plays an important role to the defiance against virus – infected and malignant cell to destroy the abnormal cell. Medicinal plants which are used as immunomodulatory effect to provide alternative potential to conventional chemotherapy for a variety of diseases, especially in relation to host defense mechanism. The use of plant product like polysaccharides, lectins, peptides, flavonoids and tannins has been the immune response or immune system in various in vitro models.

Leptadenia pyrotechnica (Forssk.) Decne (Synonym-L.Spartinum Wight) locally known as Khimp or Khip (Rajasthan), Khimparlo, Thahawar, Ranser (Gujarat), Broom bush (English) is an erect, ascending, shrub up to 1.5 m high with green stem and pale green alternating bushy branches with watery sap. Leaf is rarely found and are deciduous when present are 2.5-6.5x 0.2-0.3 cm, sessile, narrowly linear to linear lanceolate, caduceus. Flowers are in cluster lateral umbellate cymes, greenish yellow. Corolla – lobes valvate, outer corona is of 5 scales, stamens corona of raised undulate fleshy ring. Each flower is bisexual pentamerous actinomorphic, sepals joined as base only, corolla sympetalous. Follicles 7.0-14.0x0.5-0.8 cm, terete, lanceolate, tapering to selender beak, glabrous. Seeds are 5-7 mm long, ovate lanceolate, glabrous, comose (hairy) with tufted hairs 2.5-3.5 cm long. Flowering and fruiting occurs from August to January.

It is common throughout the state of Rajasthan and found in dry habitats particularly in desert zones. In India it is commonly found in Banswara, Palod, Dungarpur and Kota. Whole plant seeds and flowers are used for different purpose. Its fiber is used as antihistaminic and expectorant. Fresh juice of the plant is used for abortion. Plant sap is applied to eczema and other skin disease and is also given in diabetes. Whole plant is used in treating wound in Yemen folks and proved to have antibacterial activity against Staphylococcus aureus & Bacillus subtilis. The latex or the leaf paste is applied over the thorn injury for thorn removal. Whole plant infusion is mixed with buttermilk and given for uterine prolapse and stomach disorders in sariska region of Rajasthan. It is used to cure constipation and is considered good for health in Bikaner region of Rajasthan. In the sudanodeccanian region of central Sahara it is traditionally used in fever, cough, kidney disorders, stones, urinary disease. The present investigation was undertaken to evaluate the immunostimulatory effect of the crude extract obtained from methanolic extracts of the whole parts of L. pyrotechnica using phagocytic responses by carbon clearance test in vivo experimental model.

Materials and methods

Plant material

The plant of Leptadenia pyrotechnica was collected from “Kagore” village of Jaipur, Rajasthan, India in the month of January. It was authenticated by Dr. D. C. Saini, Sr. Scientist, Birbal Sahni Institute of Palaeobotany, Lucknow, Uttar Pradesh, India. [Voucher registration number 15531]
Preparation of extract
The successive extraction of powdered material was carried out in several batches using different solvents in increasing order of polarity in a soxhlet apparatus by hot percolation technique. The solvents used were petroleum ether, chloroform, acetone, methanol and distilled water. The powdered material of *Leptadenia pyrotechnica* was evenly packed in a soxhlet extractor for about 36 hours with different solvents. The temperature was maintained (25°C - 100°C) on an electric heating mantle with thermostat control. The extracts were then concentrated by evaporating the solvent under reduced pressure. Preliminary phytochemical studies were carried out on methanolic extract to assess the presence of various phytoconstituents and Immunomodulatory activity of the methanol extract of whole plant of *Leptadenia pyrotechnica*.

Experimental animals
Wistar albino rats of either sex, weighing 150 to 200 gm, were housed in groups of four per cage under controlled light (12:12 light: dark cycle) and temperature (25 ± 2°C). Environmental and behavioral assessment was conducted during the light cycle. Food (Golden feed, New Delhi, India) and water *ad libitum* was provided. The animals were acclimatized to laboratory conditions for seven days before commencement of experiments. All the procedure described, were reviewed and approved by Institutional Animal Ethical Committee.

Toxicity studies
Acute toxicity study was performed for Methanolic extracts of *Leptadenia pyrotechnica* according to the acute toxic classic method as per OECD guidelines. (Ecobichon, 1997). Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the various extracts were administered orally at the dose of 150 mg/kg and observed for 14 days. If mortality was observed in two animals out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such 50,100,150,200, 400,500 & 2000mg/kg body weight. The animals were observed for toxic symptoms for 72 h.

Immunomodulatory Activity

Carbon clearance test (Phagocytic activity)
Phagocytic index was determined by a reported method (Gonda et al., 1990). Swiss albino mice of either sex weighing between 25-30g were used for the screening. They were divided into 4 groups of 6 animals each, and then the animals were marked for their identity. Animal groups I serves as control received distilled water, group II serves as standard received Levimasole (50mg/kg) and group III - IV received methanolic extracts of *Leptadenia pyrotechnica* dissolved in distilled water at doses 50 mg/kg and 150mg/kg body weight by oral administration for 5 consecutive days. 48 hours after the last dose tail vein injection of carbon ink suspension (10μl/gm) was injected. At the interval of 0 minutes and 15 minutes the blood sample was withdrawn through the retro-orbital puncture from each mice. 25 μl of each blood sample was mixed with 2ml of 0.1% w/v Na₂CO₃. The carbon clearance i.e. the rate of elimination of carbon from the blood was determined by using UV spectrophotometer at 650nm.

Biostatistical Interpretation
The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett’s Test. The values were expressed as mean ± SEM and *P*<0.05 was considered significant.

Result & Discussion

Immunomodulatory activity
Carbon clearance test
Immunomodulatory activity of the methanolic extracts of the whole parts of *L. pyrotechnica* was evaluated using the Carbon clearance test in mice using Indian ink. The whole plant methanolic extract at a dose of 50, 150 mg/kg body weight and Levimasole as standard at a dose of 50 mg/kg was used and the results were shown in Table no 1.00, Fig no. 1.00

<p>| Table no. 1.00: Effect of <em>L. pyrotechnica</em> on the Carbon clearance test (MEL – methanolic extract of <em>L. pyrotechnica</em>) |</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>No. of animals</th>
<th>Phagocytic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Saline</td>
<td>6</td>
<td>0.043±0.016</td>
</tr>
<tr>
<td>II</td>
<td>Levimasole</td>
<td>6</td>
<td>0.083±0.0016</td>
</tr>
<tr>
<td>III</td>
<td>MEL 50</td>
<td>6</td>
<td>0.046±0.005</td>
</tr>
<tr>
<td>III</td>
<td>MEL 150</td>
<td>6</td>
<td>0.069±0.002</td>
</tr>
</tbody>
</table>

Immunomodulatory effect of methanolic extract of *L. pyrotechnica* by carbon clearance. All the values are shown as mean ± Sem \( n = 6 \), *\( p < 0.05 \), **\( p < 0.01 \), ***\( p < 0.001 \) vs control.

The Phagocytic index of carbon clearance test by the injection of Indian ink was found to be low in control and in the MEPL (methanolic extract of *L. pyrotechnica* at a dose of 50 mg/kg dose) and a significant difference in the mean Phagocytic index were observed in standard (levimasole 50 mg/kg) and in MEPL (methanolic extract of *L. pyrotechnica*) 150 mg/kg dose in a dose dependant manner.

Indian ink which was injected through the tail vein acts as granuloma foreign body. The Indian ink was phagocytized and cleared by mononuclear macrophage after it went to the circulation. Ninety percent of it was phagocytized by liver kuffer cell while the rest was phagocytized by spleen macrophage. The clearance rate of granular foreign body in circulation reflected the phagocytic function of mononuclear macrophage.

**Discussion**

Stimulation of reticuloendothelial system and activity of macrophage is evident from carbon clearance assay. In this, in vivo assay macrophages are known to secrete a number of cytokines, which in turn stimulates other immunocytes. This may enhance the defense ability to counter the infectious stress. Levimasole showed higher phagocytic phagocytic index than methanolic whole plant extract of *L. pyrotechnica* which reflect a marked increase in the rate of carbon clearance a significant enhancement in the phagocytic function of the macrophages and thus, non-specific immunity. Phagocytosis by macrophages is important against the smaller parasites and its effectiveness is markedly improved with the administration of levimasole.
enhanced by opsonisation of the parasites with the antibody and complement C3b, leading to more rapid clearance of parasites from the blood.

Conclusion

In vivo investigations showed that the crude extract of *L. pyrotechnia* at concentrations of 150mg/kg increased the phagocytic index, corrected α and decreased the rate of carbon clearance this immunomodulatory effect of *L. pyrotechnia* could be attributed to its interesting chemical composition. It is essentially characterized by the presence of unsaturated fatty acids, antioxidant compounds (Vitamin E family), phenolic compounds, triterpenoids, sterols and saponins.

References

22. R.H. Gokani, S.K. Lahiri, D.D santani and M.B. shah. "evaluation of immunomodulatory activity of Clerodendrum phlomidi phgocytic index, corrected α and decreased the rate of carbon clearance this immunomodulatory effect of *L. pyrotechnia* could be attributed to its interesting chemical composition. It is essentially characterized by the presence of unsaturated fatty acids, antioxidant compounds (Vitamin E family), phenolic compounds, triterpenoids, sterols and saponins.

THE END


**Techniques for dissolution enhancement**

**Particle size reduction**

Dissolution is the process in which the solid substance is dissolved in the given solvent and dissolution rate is the amount of solid dissolve in the solvent per unit time. Solubility has an important role in controlling the dissolution rate of dosage forms. According to Noyes Whitney equation by reducing the particle size there is an increase in effective surface area consequently the contact time with the dissolution media and increase in the dissolution rate.

Dissolution rate is given by Noyes Whitney equation:

\[ \frac{dc}{dt} = k(Cs-Cb) \]

Where \( \frac{dc}{dt} \) is the dissolution rate of the drug

\( k \) = dissolution rate constant

\( Cs \) =concentration of drug in stagnant layer

\( Cb \) =concentration of the drug in the bulk of the solution at time \( t \) (Kumar et al 2011).

Most frequently method to increase the water solubility of active ingredients is to reduce particle size so that surface area is increase and increase interaction with the solvent. Although, this method is cost effective and easy to carry out but not always produce desired result .Another easy way to reduce the particle size of the substance is to micronize the substance by using different class of ball mills but the drawback of this method is that morphological and physical properties of the particles produced cannot be controlled and it can also change the crystalline nature of the formulation. Spray drying is effective method to resolve these limitation .it produce particle with reduced/control size and is the way to control particle morphology and properties.(Serajuddin et al 1999).

**Supercritical technology**

Supercritical fluids are substances above their critical temperature and pressure. Examples of the techniques that utilize supercritical fluids for preparation of pharmaceuticals are; Rapid Expansion of Supercritical Solutions (RESS ) and Supercritical Anti-Solvent (SAS) (Sharma et al ,2012 and Kumar et al ,2011).

**Nano suspension**

This technique involves poor water soluble drug without matrix dispersed in suspension. It is effective and an alternative method to resolve the limitation of poorly water soluble. This technology applied to the drugs which are insoluble in both water and oils. There are various method for the preparation of nanosuspension include Precipitation Method, High Pressure Homogenization, High Pressure, Homogenization in non-aqueous media, combination of precipitation and High Pressure Homogenization and Media Milling (Patel et al.,2011).

**Cryogenic technology**

Cryogenic techniques have been developed to enhance the dissolution rate of poorly water soluble drugs by creating nanostructured amorphous drug particles with high degree of porosity at very low-temperature conditions. Cryogenic inventions can be defined by the type of injection device (capillary, rotary, pneumatic, and ultrasonic nozzle), location of nozzle (above or under the liquid level), and the composition of cryogenic liquid (hydrofluoralkanes, nitrogen ,argon oxygen and organic solvents). After cryogenic processing, dry powder can be
obtained by various drying processes like spray freeze drying, atmospheric freeze drying, vacuum freeze drying and lyophilisation (Savjani et al., 2012).

**Solid dispersion**
The concept of solid dispersion was originated by Sekiguchi and Obi in 1960, who investigated the generation and dissolution performance of eutectic, melts of a sulphonamide drug and a water soluble carrier. Solid dispersion refers to the incorporation of poorly water soluble drug in inert water soluble carrier. It is a powerful pharmaceutical technique to change crystallinity of the drug in an attempt to enhance solubility, dissolution rate, wettability, in vivo absorption (Kumar A et al, 2011).

Spray drying and spray drying with excipients
Spray drying is widely used in pharmaceutical industry because of its distinct features like rapid drying and an alternative to the milling method to reduce particle size. Dissolution by using spray drying enhanced by adding small amount excipients like surfactant which improve wetting properties. Spray drying of ibuprofen is improved by using sodium lauryl sulphate and gelatine which improve initial dissolution rate of the drug in simulated gastric fluid. (Elkordy et al., 2010). Excipients which are soluble are used to increase the solubility of poorly water soluble drug.

**Microencapsulation by spray drying**
Because of rapid evaporation rate, spray drying is used for drying of pharmaceutical heat sensitive materials and food products. It may also consider as dehydration process and is used in the encapsulation process to entrap the active material within the matrix which should be protective and chemically inert to the material being entrapped. It can be used as an alternative technique to conventional method of microencapsulation which include solvent evaporation or phase evaporation. Microsphere of progesterone was prepared by solvent evaporation method and one of the problem which occur during this was the spontaneous crystallisation of progesterone in the aqueous phase. This problem does not occur during the spray drying of progesterone (Taylor & Francis 1998). The study carried out by Gharsallaoui (2007) showed the art and way of use of spray drying in food industry. This process has been successfully used in food industry for a long time ago. The study described the practical and theoretical information about this process. Spray drying is considered to be cheap and most commonly used technique in the microencapsulation of food products and 30 -50 times cheaper than freeze drying. Microencapsulation by spray drying involves three steps (Dziezak 1988).preparation of emulsion/dispersion, homogenization, atomization of the mass into drying chamber. According to the Shahidi and Han (1993) there are four stages in the microencapsulation by spray drying preparation of dispersion/emulsion, homogenization, atomization of emulsion and dehydration of atomized particles.

Microencapsulation by spray drying is used to settle down the volatile compounds. Several binders were used for this purpose which include gelatine, gum arabic, polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), carboxymethylcellulose (CMC), methylcellulose (CMC), methylcellulose and tragacanth (Kawashima et al., 1972). Spray drying is also used in the microencapsulation of oil soluble vitamins such as A and D and in the aromatic oils which are used in the flavouring of pharmaceuticals. Spray drying is also used in the taste masking, in the preparation of the polymer coated microcapsules (Deasy, 1984). Spray drying technique is also used to prepare...
the microsphere which contain paracetamol and Eudragit, spherical particles having the size 5-20 μm and used to produce tablets with slow release properties (Becirevic et al., 1989).

Microsphere of Chlordiazepoxide
According to Birish Sarkar (2011) the solubility and dissolution rate of chlordiazepoxide was improved by preparing microsphere of chlordiazepoxide by using different ratios of drug and polymer by spray drying technique. Fourier transform infrared spectroscopy was used to study the of resultant microsphere. Differential scanning calorimetry spectroscopy DSC and XRD were used to investigate the properties of prepared microsphere. The prepared microsphere showed decrease in crystalline properties and increase in dissolution and solubility compared to pure sample of chlordiazepoxide. Hence spray drying was found to effective technique to increases the solubility and dissolution of poorly water soluble drug chlordiazepoxide (Sarkar et al. 2011).

Granulation
Spray dried is a useful alternative method to wet granulation for tablet formulations that cannot be directly compressed. Up to 60% of slurries can be dried by spray drying. Spray dried granulation has better distribution of drug, improved flow, colour uniformity, improved stability, improved hardness and require less lubricant than wet mass products. It gives rise to strong tablet and maximum use of binder (Patel et al. 2009). Spray drying is also used in the production of slow release granulation and less amount of binder is needed in granulation by spray drying as compared to granulation by other conventional methods (Kornblum 1969).

Examples of spray dried Excipients Base process
With the spray drying one can co-precipitate an API with a polymer in a stable amorphous solid dispersion, thereby greatly improve the dissolution rate of many poorly water soluble drugs including, indomethacin, ibuprofen, tolbutamide and excipients (carbohydrates, disintegrant, glidant, surfactant (Gonnissen, et al. 2007). Spray drying was also used to bind the aluminium silicate and magnesium carbonate with different binders to improve the flow and other flow properties related to tableting and also used to prepare slow release magnesium carbonate (Takenaka et al., 1971). It is also used to prepare prolonged release sulfaphenylthiadiazole (SETD) granulations (Askar and Becker 1966).

Spray drying in the formulation of orally disintegrating tablets
Orally disintegrating tablets (ODT) are becoming more popular nowadays than classical methods of tablets production due to their ease in administration and they are suitable for the patients suffering from dysphagia and also no water is required for swallowing tablets and hence suitable for geriatric, paediatric and travelling patients. Orally disintegrating tablets (ODT) dosage form is a better alternative for oral medication to improve quality of life and patient compliance. ODTs can be prepared by different methods like direct compression, freeze drying spray drying, sublimation and wet granulation method. Two model drugs, one with good aqueous solubility (Metoclopramide HCL) and another with poor aqueous solubility (Valdecoxib) were used. Metoclopramide (an antiemetic) is used in the motion sickness and is beneficial to the travelling patients who may have no access to water at the time of taking medication. Valdecoxib (COX-2 selective) is used in rheumatoid arthritis and osteoarthritis associated with elderly patients. Spray dried excipient base was used for the formulation of ODTs tablets to achieve faster disintegration in the oral cavity without water. Dissolution rate was enhanced along with faster disintegration by using superdisintegrants like Ac-Di-Sol, sodium starch glycolate (SSG) and Kollidon CL in the formulation of tablets. Other excipients include diluent (mannitol) along with sweetening agent (aspartame) were used in the formulation of ODTs tablets. The tablets were evaluated for hardness, friability, water absorption, disintegration time (DT) and in vitro drug release. Maximum drug release and minimum DT were observed with Kollidon CL excipients base as compared to tablets prepared by direct compression, revealed the advantage of spray dried excipients base technique over direct compression technique. (Mishra et al., 2006)

Spray drying excipients in pulmonary drug delivery system
The use of sodium carboxymethylcellulose (NaCMC) as a spray dried excipients in the preparation of inhalable formulation offer an alternative method for the preparation of stable formulations for pulmonary delivery. Spray drying which is one step process for the preparation of dry powder with respirable range has been used to prepare dry powder for inhalation use. (Li et al., 2010)

Current application of spray drying in pharmaceutical technology
In pharmaceutical field, spray drying is commonly used as a dehydrating tool and to obtain the dry extracts of the products with specific properties. (Cal et al., 2010)

Some examples of spray dried process are given below:
Spray dried excipients and co spray dried composites
The use of lactose in combination with alginate is comprehend to be very effective in the formulation of spray-dried products (Takeuchi et al., 2005). Lactose is one of the most widely and extensively used excipients in pharmaceutical technology due to its stable physicochemical properties like unhygroscopicity. In recent years there has been extensively research on the effect of spray drying on the mixture of drug substance sand powder properties and various types of excipients with respect of direct tableting ability. (Takeuchi, et al 1998 ; Makai et al.,2006)

Water solubility of active ingredients in drug formulation
The main goal of spray drying with excipients is to enhance the aqueous solubility of the drug The issue to enhance the water solubility is main issue as about 40% of the new active substances have low water solubility (Gursoy et al 2001). By improving the aqueous solubility of the drug it is possible to develop new formulation methods.

Spray drying to enhance water solubility of poor water soluble drugs
One example of this artemisinin (antimalarial )which is poorly water soluble drug and has been spray dried with different concentration of maltodextrin (excipient/food additive) under different conditions of process circumstances. The water solubility of spray dried material was related to the to the process conditions like inlet temperature, flow rate and concentration in the feed which have the effect on the properties of powders, responsible for the solubility (Sahoo , et al 2009). One other important example is of griseofulvin which belong to BCS class II and belong to antifungal is poor water soluble drug . Dissolution and solubility of griseofulvin was improved by spray drying using 0.05% of poloxamer 407. The resultant powdered product was formulated in gelatine capsules and administered to the rats. The study showed that spray dried griseofulvin has better dissolution and solubility than pure sample of griseofulvin (Wong et al 2006, Chaubal et al. 2008).

Nano-Spray drying in protein therapy
Protein Nano -therapies are widely used in diseases such as cancer, diabetes and asthma. Therapeutics proteins are preferred to be administered parentally because of their low availability, enzyme degradation, poor membrane permeability and short half-life in GIT and intestinal tract when they administered orally. (Lee et al., 2007; Shaji and Patole, 2008).
To achieve better patient compliance alternative novel drug delivery dosage forms are developed like controlled peroral delivery, pulmonary delivery, nasal delivery and transdermal delivery (Cleland et al., 2001). Some of these require powder formulation and spray drying is very well known to produce powder from liquid phase. It is used in preparation of proteins intended for pulmonary nasal and control oral delivery as it has advantage of drying and formation of particles in one step via particle engineering and particles properties like morphology, size and flow can be precisely controlled (Lee et al. 2006). So, it is a versatile technique for formulation of proteins and peptide.

Spray drying in encapsulation of vitamin C
The study carried out by Esposito (2001) showed the production of methacrylate microparticles for the delivery (administration) of ascorbic acid via the oral route. Spray drying was used in the preparation of vitamin C/ Eudragit microsphere. Vitamin C which is an anti-oxidant and use to reduce the risk of colon rectal cancer by neutralising the oxygen reactive species and free radicals that can harm to DNA (Jacobs et al., 2001). Microparticle which were produced by spray drying, characterized by size and morphology by scanning electron microscopy and in vitro kinetic release study was performed these microparticles showed a good morphology and size distribution that permit to propose them as candidate for the delivery of vitamin C as associated therapy in the treatment of colorectal cancer by oral route. (Esposito et al. 2001)

Improvement of dissolution of tolbutamide by spray drying
Tolbutamide which is first generation potassium channel blocker, sulfonylurea and used as oral hypoglycaemic agent and is a poorly water soluble drug . Dissolution of tolbutamide was improved by spray drying solvent deposition and disintegrates ( utilizing diluted ammonia solution of the drug containing either hydroxypropylcellulose(L-HPLC) or partly pregelatinized corn starch (PCS) as disintegrates. After performing the whole experiment and study it was obvious that dissolution rate of the drug from spray dried particle was more rapid than that of powdered drug alone or with the disintegrant (Takeuchi et al 1987).

Improvement of dissolution and solubility of Celecoxib by spray drying
Celecoxib which is a selective COX-2 inhibitor and mainly used in the treatment of rheumatoid arthritis and belongs to class II compound according to biopharmaceutical classification system, having low solubility, high permeability. It has poor aqueous solubility, dissolution and flow properties. So, its oral bioavailability depends on dissolution rate in the GIT tract. According to the research carried out by Dixit et al (2011) microsphere of celecoxib was prepared by
spray drying technique by using Pluronic F 127 (powder, bio reagent suitable for cell culture. The prepared microspheres was observed by differential scanning calorimeter (DSC), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and scanning electron microscopy (SEM). Dissolution rate and solubility profile of prepared microsphere of celecoxib was compared to pure sample of celecoxib. Spray dried microsphere showed decrease crystallinity. The dissolution and solubility of microsphere which contained different amount of drug and polymer, was improved as compare with physical mixture and pure sample of drug (celecoxib).

![Fig 2: SEM of celecoxib, pluronic F127, celecoxib and pluronic F127 physical mixtures and different ratios of microsphere (adapted from Mudit Dixit et al 2011)](image)

**Dissolution enhancement of Ibuprofen**

Ibuprofen which is an NSAID and belongs to BCS class II is poorly water soluble drug. Dissolution rate of this hydrophobic drug was improved by preparing microsphere by utilising spray drying and spray chilling technique. Surfactant excipients which was used in this study was Poloxamer 127 which is hydrophilic polymer and enhance the wettability of particles and dissolution rate. The prepared microsphere of drug particles was observed and characterized by using differential scanning calorimetry (DSC), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR). Dissolution studies were performed which showed that dissolution rate of spray dried microsphere of ibuprofen was improved when compared to control ibuprofen and spray is more effective technique to improve dissolution rate, flow properties and wettability of poorly water soluble drug than spray chilled technology. (Elkordy et al., 2010)

**Dissolution enhancement of Tinidazole by spray drying**

Tinidazole belongs to BCS class II and an anti-parasitic used in protozoan infection is a poor water soluble drug. According to the study carried out by Puckiraj Chhaprel et al. (2012), the performance of spray drying in the enhancement of dissolution of tinidazole was observed using solid dispersion technique. Various aqueous soluble carriers like polyethylene glycol (PEG 4000), hydroxypropylmethylcellulose (HPMC 5cps) and cyclodextrin were employed for this experiment. After performing the phase solubility it was notify that by increasing the concentration of water soluble carrier, there is improvement in the dissolution and solubility of poor water soluble drug utilising spray dry. Differentiation of solid dispersion was performed using SEM, XRD, FT-IR, and DSC showed enhancement of dissolution rate. Even the insignificant amount of change in physical or chemical in active ingredients in pharmaceutical dosage form effect the dissolution and bioavailability. The study carried out by Martins, R. M et al. 2012 revealed the effect of spray drying on physical properties and in vitro dissolution rate on four different drugs, carabmzepine, piroxicam, indomethacin, nifedipine. Each of the drug was suspended in ethanol: water (70:30) and then spray dried. Spray dried drugs were differentiate by using DSC, SEM, XRPD. Dissolution rate of spray dried drug showed an improvement as compared pure sample of drug. So, spray drying is an effective technique to alter physical properties and improvement in sanative effect. (Martins et al., 2012)
Spray drying to enhance the solubility and dissolution of biopharmaceutical drug classification system class II molecules

Many known active pharmaceutical ingredients and new chemical entity are hydrophobic having poor aqueous solubility and therefore exhibit poor dissolution and bioavailability if they designed or formulated in unreformed forms. Among other conventional techniques to improve solubility and dissolution like self-emulsification, solubilisation and enhancement in solubility and dissolution by making complex utilising cyclodextrin, another novel technique is spray drying using copolymer which is developed especially for improvement of dissolution and solubility of poorly water soluble APIs. This was also tested on human trials and successfully used at commercial marketing. Physicochemical and excipients compatibilities studies are extensively performed on API and screened for their effect on solubility and permeability. In this process, API is first dissolved in water miscible organic solvent, commonly used ethanol then mixture of an amphiphatic and a hydrophilic polymer are formulated as mixed aqueous solvent solution. The mixture of aqueous polymer and organic API are mixed under controlled temperature and shake well to from cloudy mixture which is then subjected to spray drying process. Related to this technique, amphiphilic polymers are those which have dual nature and soluble in both organic and aqueous solvent. Examples include polyethylene oxides (PEO or also known as polyethylene glycol PEG), PEO derivatives, PEO copolymers like PEO/polypropylene glycol (PPG) copolymers, PEG modified starches, poloxamers, poloxamines, and hydroxypropyl cellulose and also include esters like vinyl acetate. Hydrophilic polymers are those which are soluble in aqueous solvent or combination of organic and aqueous solvent but are insoluble in organic solvent. Example include polyvinyl alcohol, sodium alginate, carboxymethylcellulose, hydroxyethylcellulose and chitosan (David et al. 2012).

Another study carried out by Htendra S. Mahaja and others in 2012 showed the usage of spray drying with excipients to improve the dissolution rate and enhance the solubility rate of gliclazide belongs to BCS class II which is an oral hypoglycaemic drug. In this experiment solid dispersion of gliclazide was prepared using different carrier of drug and carrier. DSC, SEM, XRD and FT-IR studies were performed which revealed that spray dried solid dispersion result in the improvement of dissolution rate and bioavailability. Hence spray drying is very useful in enhancing the solubility and dissolution rate of poorly water soluble drug.

Spray drying of hydrophobic drugs by using hydrophilic excipients

As mentioned above. Spray drying is a traditional method which is carried out in single step to produce dry powder from suspension or liquid. Spray drying is widely used in the formulation of powder dosage form but its application is restricted to the spray drying of water soluble drug in aqueous media together with hydrophilic excipients. In case of poorly water soluble drug spray drying cannot be progressed in aqueous media and carried out in organic or non-aqueous solvent. Now, a days spray drying of hydrophobic drugs is carried with the aid of hydrophilic component most commonly used is hydrophilic pharmaceutical excipients which result in the formation of mixture of dry powder having both properties i.e. hydrophilic and hydrophobic. This method is used in pharmaceutical formulation composition in which hydrophilic portion is drug which is use in the range of 0.01 to 95% of the powder and hydrophilic portion is pharmaceutical excipients which is use in the range of 99.99% of the powder. The modern and develop method of spray drying is consistent with at least most of the pharmaceutical excipients which are hydrophilic in nature such as povidone and polymers, citric acid, mannitol, pectin and water soluble carbohydrate and hydrophilic drugs, especially with those excipients which are used in the formulation of pulmonary and inhalation dosage forms. Mostly include mannitol, povidone, pectin, lactose sodium citrate and sodium chloride. Use of each excipient is specific according to formulation and use, for example lactose, sodium citrate and sodium chloride are commonly used in the formulation of pulmonary dosage forms as they are known as generally recognized safe (GRAS) for such formulation. This method is effective for wide range of hydrophobic drugs, nutrients, steroids and salt. Examples are beclamethasone, betamethasone, predisone, methylprednisone, peptides Cyclosporine, Vitamins A, D, K and other water insoluble derivatives. Hydrophobic drugs shows poor solubility or sparingly or insoluble in water as they have solubility below 5mg/ml mostly below 1mg/ml. In context with hydrophilic excipients they are water soluble and have ability to forms gels which are reversible and have solubility greater than 5mg/ml or 50 mg/ml and even more. In spray dried formulation for pulmonary use, excipients should free of toxic signs and should fine for patient compliance. Examples are carbohydrates and compounds from, povidone, pectin, mannitol, citric acid, lactose, sodium citrate, sodium chloride. The latter four are very common and have advantage in pulmonary dosage forms. Pharmaceutical excipients are selected in spray drying process according to their stability, dispensability, consistency and binding properties to powder particles to make sure uniform composition and delivery. The above method is depend on the choice selection media for dissolving hydrophobic drugs or other component and hydrophilic excipients or other component and also on method of connecting the components to the spray dryer and introduced through nozzle of spray drying apparatus, the nozzle should be of specific size. This is conventional method for dissolving hydrophobic drugs and hydrophilic excipients by suitable selection of solvent media, water is
selected for hydrophilic excipients and organic solvent is generally selected for hydrophobic drugs. One other advantage of this process is that it minimizes the use of solvent. (Gordon et al., 2000).

Lactose is commonly used as excipients and binder in pharmaceutical industry and spray dried lactose was first used in the 1960s to the pharmaceutical industry as excipients that ease the formulation and manufacturing processes. It is prepared from the spray drying of lactose concentrate, which had been a commonly used method for lactose preparation. The main boon of spray dried lactose over commonly prepared lactose is that it can be directly compress and well suited to direct compress application in tableting (Broadhead et al., 1992. Bolhuis 2004). By modifying the spray dry method the properties of powders like particle size, density, porosity and water content can also be changed and controlled (Master 1985,Newton 1966). One other excipients which is commonly used in spray dried excipients is mannitol because of its hardness and inert nature. Study was carried out to check whether the type of excipients has any effect on the hardness or dissolution of the tablet or not. The result showed that the spray dried mannitol (SDM) is suitable for CD formulation of naproxen sodium (Nuguru et al., 2008).

References
COMPARATIVE STUDY OF PHARMACEUTICAL PARAMETERS AND ANTIMICROBIAL PROPERTIES OF CEFTRIAXONE, CIPROFLOXACIN AND CEFIXIME IN INJECTION & TABLET DOSAGE FORM

Mamta Arora* & Amrik Singh†
Department of Biotechnology, A.S.B.A.S.I.S.M.College, Bela, Roopnagar, Punjab

Abstract
In the present work different types of cephalosporins i.e. Ciprofloxacin, Cefixime, Ceftriaxone were evaluated with respect to pharmaceutical parameters and Minimum Inhibitory Concentration. Cefixime and Ciprofloxacin were in tablet form and Ceftriaxone in injection form. Physical parameters were within the range when compared with IP data. Friability test, Hardness test, Pfizer test guaranteed the strength and durability of the drugs. Weight variation test confirmed uniformity of drugs in the tablets. Both tablets samples were in agreement with the printed data according to weight variation calculations. Minimum inhibitory concentration were calculated against three different microorganisms E. coli (MTCC 1652), P. fluorescens (MTCC 2421), S. aureus (MTCC 3160). Among the three dosage forms Ceftriaxone and ciprofloxacin were equally effective against S. aureus, in broad of minimum inhibition concentration range of 2 to more than 16µg/ml. Cefixime was least effective. In tablets Ciprofloxacin was more effective against S. aureus. Ceftriaxone available in injection form was more effective in the less concentration range than Ciprofloxacin and Cefixime. It was also confirmed by the spectroscopic absorbance obtained for the drugs at their respective λmax values.

Introduction
World Health Organisation survey reported 7% of the world’s marketed drugs are counterfeit. Out of that antibiotics contribute to 45%. Therapeutic failure and emergence of drug resistant strains may be serious problems due to suboptimal level of the drug. Therefore the quality in the manufacture of medicine is utmost important with particular importance to antibiotics. Out of these antibiotics Cephalosporin particularly Ceftriaxone, Ciprofloxicin and Cefixime are especially important. These are semisynthetic and broad range antibiotics. Therefore in the present study basic parameters and comparative study of three dosage form of cephalosporin have been done. Similarly MIC have been evaluated. MIC i.e. Minimum Inhibitory Concentration is the lowest concentration to inhibit the growth of microorganisms.

The minimum inhibitory concentration (MIC) represents the concentration of antimicrobial at which there is complete inhibition of growth of organism. Different methods are available for the determination of MIC. Dilution methods are used to determine the minimum inhibitory concentrations (MICs) of antimicrobial agents and are the reference methods for antimicrobial susceptibility testing against which other methods, such as disk dilution, are calibrated. MIC methods are widely used in the comparative testing of new agents. In clinical laboratories they are used to establish the susceptibility of organisms that give equivocal results in disk tests, for tests on organisms where disk tests may be unreliable, and when a more accurate result is required for clinical management. The present study was conducted among the various antibiotics as well as the comparative minimum inhibitory concentration study of three brands of 2nd and 3rd generation cephalosporins. All the methods are extremely sensitive to variations in performance. Many factors influence the result as following:

- Size of inoculum
- Contents and acidity (pH) of the broth or agar
- Reading procedures (Farzana et al., 2011)

Antimicrobial susceptibility test of the isolated organisms were done by disc diffusion method. On antibiotic susceptibility test, MRSA strains showed 100% resistant against penicillin, oxacillin, cloxacillin and amoxycillin. (Islam et al., 2008)

Material and Methods

Three Different cephalosporins were compared by different biotechnological and pharmaceutical methods. Different tests were performed for the comparative study of three different drug samples.

Evaluation Parameters: Following parameters were measured according to IP

1) Shape and Size:
2) Organoleptic Properties:
3) Hardness:
4) Friability:
5) Weight Variation

Minimum Inhibitory Concentration (MIC)

MIC was determined by agar plate dilution method

Serial two fold dilution concentrations of 3 antibiotics of cephalosporin group of antibiotics belonging to 2nd and 3rd generation for test were prepared against S. aureus, E. coli, P. fluorescens procured from MTCC Chandigarh...
Stock solutions of all antimicrobials were prepared. Four to five well isolated colonies S. aureus, E. coli, P. fluorescens isolates from agar plates were inoculated in tube containing 5 ml of Nutrient broth and incubated at 35°C until it was achieved or the turbidity of 0.5 standards. Then this diluted 1 ml inoculum was transferred on Nutrient agar plates containing various concentrations of antibiotics with the help of micropipette. Agar plates containing antibiotic was prepared and agar was cooled to 50°C after autoclaving. Dilution series of antimicrobial agents were added depending upon antimicrobial agent. The containers were mixed thoroughly and agar was poured into already labeled sterile petri plates on a leveled surface. MIC was evaluated (Farzana et al., 2011).

A stock solution of concentration 0.05 mg/ml was prepared by first dissolving 0.02 g of crystalline ciprofloxacin with 10 ml of distilled water in a 100 ml beaker. The resulting solution was then transferred quantitatively into a 100 ml volumetric flask, swirled for complete dissolution and topped to the mark with distilled water. A 25 ml of this solution was taken into another 100 ml volumetric flask, topped to the mark with distilled water, corked and labeled stock A. The procedure was repeated to obtain stock B. (Ali A.E., 2011)

Standard solutions of concentrations ranging from 0.002 mg/ml to 0.012 mg/ml at intervals of 0.002 mg/ml were prepared. From each of the stock, 2 ml, 4 ml, 6 ml, 8 ml, 10 ml and 12 ml were taken into separate 50 ml volumetric flasks using a 10 ml graduated pipette and topped to the mark with distilled water. These were corked and labeled to obtain two sets of standards from the respective stock.

**Preparation of the Sample**

Four brands of ciprofloxacin sodium chloride pharmaceutical infusions were obtained from various Retail stores. Sample solutions of concentration 0.004 mg/ml were prepared. A 20 ml aliquot of the drug sample was transferred into a 100 ml volumetric flask and topped to the mark with distilled water. A 1 ml aliquot of the resulting solution was taken into another 100 ml volumetric flask and distilled water added to the mark.

**Measurement of Absorbance**

The SHIMADZU 1800 ultraviolet spectrophotometer was used for the measurement of absorbance. The equipment was warmed for 5 minutes, after which a wavelength scan was done to obtain the wavelength of maximum absorption. The wavelength of maximum absorption obtained was 272 nm, 598nm and 340 nm for Ciprofloxacin, Cefixime and Ceftriaxone respectively. The spectrophotometer was then set to a single wavelength mode and absorbance for each sample was determined. The spectrophotometer was zeroed using distilled water. The cuvette was rinsed and filled with standard and the absorbance was recorded. This was repeated for the remaining standards. The cuvette was rinsed with methanol followed by distilled water in between measurements. The cuvette was then rinsed and filled with the first sample prepared from the ciprofloxacin pharmaceutical infusion and absorbance taken. Same was repeated with cefixime and ceftriaxone. The absorbance of all the samples including the sample blank was measured. (Brown M. et al., 2013)

**Table 1. Length determination**

<table>
<thead>
<tr>
<th>Length</th>
<th>Ciprofloxacin</th>
<th>Cefixime</th>
<th>Ceftriaxone</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>16.801 mm</td>
<td>12.221 mm</td>
<td>NA</td>
</tr>
<tr>
<td>L2</td>
<td>16.814 mm</td>
<td>12.244 mm</td>
<td>NA</td>
</tr>
<tr>
<td>L3</td>
<td>16.816 mm</td>
<td>12.229 mm</td>
<td>NA</td>
</tr>
<tr>
<td>Average Length</td>
<td>16.810 mm</td>
<td>12.231 mm</td>
<td></td>
</tr>
</tbody>
</table>

**Thickness**: Thickness was also determined with Digital Micrometer taking three tablets from each type of drug and their average thickness was hence calculated.
### Table 2. Thickness determination

<table>
<thead>
<tr>
<th>Thickness</th>
<th>Ciprofloxacin</th>
<th>Cefixime</th>
<th>Ceftriaxone</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>4.647 mm</td>
<td>4.476 mm</td>
<td>NA</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>4.725 mm</td>
<td>4.469 mm</td>
<td>NA</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>4.735 mm</td>
<td>4.369 mm</td>
<td>NA</td>
</tr>
<tr>
<td>Average Thickness</td>
<td>4.702 mm</td>
<td>4.438 mm</td>
<td></td>
</tr>
</tbody>
</table>

**Hardness:** Hardness was Determined By Using Pfizer detector. Three tablets were taken to check their hardness and their average hardness was determined.

### Table 3. Hardness determination

<table>
<thead>
<tr>
<th>Hardness</th>
<th>Ciprofloxacin</th>
<th>Cefixime</th>
<th>Ceftriaxone</th>
</tr>
</thead>
<tbody>
<tr>
<td>H&lt;sub&gt;1&lt;/sub&gt;</td>
<td>6.2 kg/cm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>6.1 kg/cm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>NA</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;</td>
<td>6.3 kg/cm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>6.3 kg/cm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>NA</td>
</tr>
<tr>
<td>H&lt;sub&gt;3&lt;/sub&gt;</td>
<td>6.8 kg/cm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>6.2 kg/cm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>NA</td>
</tr>
<tr>
<td>Average Hardness</td>
<td>6.4 kg/cm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>6.2 kg/cm&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

**Friability:** It was Determined with Friabilator. Eight tablets were taken in case of Ciprofloxacin and eighteen tablets of Cefixime were used to determine the friability using the below stated formula.

### Table 4. Friability calculation

<table>
<thead>
<tr>
<th></th>
<th>Ciprofloxacin</th>
<th>Cefixime</th>
<th>Ceftriaxone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Weight</td>
<td>6.29 gm</td>
<td>6.25 gm</td>
<td>NA</td>
</tr>
<tr>
<td>Final Weight</td>
<td>6.25 gm</td>
<td>6.22 gm</td>
<td>NA</td>
</tr>
<tr>
<td>Friability</td>
<td>6.29-6.25 × 100&lt;br&gt;6.29</td>
<td>6.25-6.22 × 100&lt;br&gt;6.25</td>
<td></td>
</tr>
<tr>
<td>Friability</td>
<td>0.6 %</td>
<td>0.48 %</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Weight Variation:** Determined for 10 tablets of each type and their weight variation range was calculated.
### Table 5. Weight variation

<table>
<thead>
<tr>
<th>Weight</th>
<th>Ciprofloxacin</th>
<th>Cefixime</th>
<th>Ceftriaxone</th>
</tr>
</thead>
<tbody>
<tr>
<td>W&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.67 gm</td>
<td>0.35 gm</td>
<td></td>
</tr>
<tr>
<td>W&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.71 gm</td>
<td>0.31 gm</td>
<td></td>
</tr>
<tr>
<td>W&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.70 gm</td>
<td>0.34 gm</td>
<td></td>
</tr>
<tr>
<td>W&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.70 gm</td>
<td>0.33 gm</td>
<td></td>
</tr>
<tr>
<td>W&lt;sub&gt;5&lt;/sub&gt;</td>
<td>0.72 gm</td>
<td>0.32 gm</td>
<td>NA</td>
</tr>
<tr>
<td>W&lt;sub&gt;6&lt;/sub&gt;</td>
<td>0.72 gm</td>
<td>0.31 gm</td>
<td></td>
</tr>
<tr>
<td>W&lt;sub&gt;7&lt;/sub&gt;</td>
<td>0.68 gm</td>
<td>0.29 gm</td>
<td></td>
</tr>
<tr>
<td>W&lt;sub&gt;8&lt;/sub&gt;</td>
<td>0.73 gm</td>
<td>0.31 gm</td>
<td></td>
</tr>
<tr>
<td>W&lt;sub&gt;9&lt;/sub&gt;</td>
<td>0.69 gm</td>
<td>0.30 gm</td>
<td></td>
</tr>
<tr>
<td>W&lt;sub&gt;10&lt;/sub&gt;</td>
<td>0.70 gm</td>
<td>0.32 gm</td>
<td></td>
</tr>
<tr>
<td><strong>Total Weight</strong></td>
<td><strong>7.02 gm</strong></td>
<td><strong>3.18 gm</strong></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Average</th>
<th>Ciprofloxacin</th>
<th>Cefixime</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 gm = 0.70</td>
<td>10 gm = 0.31</td>
<td></td>
</tr>
</tbody>
</table>

**Weight variation Range**: 0.63-0.77 gm for Ciprofloxacin, 0.28-0.34 gm for Cefixime.

Weight variation range for Ciprofloxacin was determined between 0.63 gm and 0.77 gm. It was checked for the 10 tablets whose weight was calculated and all the tablets were within the range of the weight variation limit. For Cefixime, weight variation limit was between 0.28 gm to 0.34 gm and all the 10 tablets were within the range of weight variation.

**Disintegration Time**: Determined by **Disintegration Test Apparatus** for 6 tablets of each sample. Ciprofloxacin is dispersible tablet and hence its disintegration time was 1 minute. Cefixime has disintegration time of 5 minutes. Ceftriaxone injection was disintegrated in less than one minute in the water. Results were confirmed from the IP.
The Minimum inhibitory concentration

The Minimum inhibitory concentration of antibiotics (Ciproflaxocin, cefixime and ceftriaxome) were examined on microorganisms *E. coli*, *P. fluorescens*, *S. aureus* in different concentrations. MICs were determined with an inoculum of approximately $5 \times 10^6$ CFU/ml by the microdilution method and with controls. Ciproflaxocin was inhibitory at concentrations of ≤1 µg/ml to 90% of *E. coli*, *P. fluorescens*, *S. aureus*. The MICs of Ciproflaxocin, cefixime and ceftriaxone are listed in the table below.

Table 7. Minimum Inhibitory Concentration (µg/ml)

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>TCC No.</th>
<th>Range</th>
<th>MIC$_{50}$</th>
<th>MIC$_{90}$</th>
<th>Range</th>
<th>MIC$_{50}$</th>
<th>MIC$_{90}$</th>
<th>Range</th>
<th>MIC$_{50}$</th>
<th>MIC$_{90}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ciproflaxocin</td>
<td>Cefixime</td>
<td>Ceftriaxone</td>
<td>Ciproflaxocin</td>
<td>Cefixime</td>
<td>Ceftriaxone</td>
<td>Ciproflaxocin</td>
<td>Cefixime</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>1652</td>
<td>≤0.07-16</td>
<td>0.26</td>
<td>0.6</td>
<td>≤0.07-16</td>
<td>0.12</td>
<td>0.5</td>
<td>≤0.07-16</td>
<td>0.07</td>
<td>07</td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td>2421</td>
<td>0.5-&gt;15</td>
<td>&gt;15</td>
<td>&gt;15</td>
<td>0.25-15</td>
<td>&gt;15</td>
<td>&gt;15</td>
<td>≤0.06-15</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>3160</td>
<td>2-16</td>
<td>4</td>
<td>&gt;16</td>
<td>4-16</td>
<td>16</td>
<td>&gt;16</td>
<td>2-16</td>
<td>4</td>
<td>16</td>
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</tbody>
</table>

Fig 1 Graphical representation of Minimum Inhibitory Concentration for three drugs
### Table 8. Standard curve of sample

<table>
<thead>
<tr>
<th>Volume (ml)</th>
<th>Standard A 1</th>
<th>Standard A 2</th>
<th>Standard A 3</th>
<th>Standard B 1</th>
<th>Standard B 2</th>
<th>Standard B 3</th>
<th>Absorbance ± 0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>2</td>
<td>0.173</td>
<td>0.181</td>
<td>0.174</td>
<td>0.184</td>
<td>0.179</td>
<td>0.175</td>
<td>0.177 ± 0.007</td>
</tr>
<tr>
<td>4</td>
<td>0.324</td>
<td>0.320</td>
<td>0.317</td>
<td>0.334</td>
<td>0.336</td>
<td>0.320</td>
<td>0.325 ± 0.001</td>
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<tr>
<td>6</td>
<td>0.472</td>
<td>0.478</td>
<td>0.480</td>
<td>0.482</td>
<td>0.471</td>
<td>0.473</td>
<td>0.476 ± 0.000</td>
</tr>
<tr>
<td>8</td>
<td>0.634</td>
<td>0.635</td>
<td>0.622</td>
<td>0.635</td>
<td>0.629</td>
<td>0.625</td>
<td>0.63 ± 0.000</td>
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<tr>
<td>10</td>
<td>0.740</td>
<td>0.747</td>
<td>0.724</td>
<td>0.747</td>
<td>0.784</td>
<td>0.916</td>
<td>0.776 ± 0.003</td>
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</tbody>
</table>

### Table 9. Absorbance of sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance ± 0.001</th>
<th>Average absorbance</th>
<th>Standard deviation</th>
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</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>0.345</td>
<td>0.343</td>
<td>0.343</td>
</tr>
<tr>
<td>Sample B</td>
<td>0.377</td>
<td>0.379</td>
<td>0.376</td>
</tr>
<tr>
<td>Sample C</td>
<td>0.422</td>
<td>0.423</td>
<td>0.425</td>
</tr>
<tr>
<td>Blank</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
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</tbody>
</table>

Corrected Absorbance

<table>
<thead>
<tr>
<th>Sample</th>
<th>Corrected Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>0.343±0.0006</td>
</tr>
<tr>
<td>Sample B</td>
<td>0.377±0.002</td>
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<tr>
<td>Sample C</td>
<td>0.424±0.005</td>
</tr>
</tbody>
</table>

### Table 10. Manufacturer’s Specified Concentration and Experimental Concentration Data

<table>
<thead>
<tr>
<th>Sample</th>
<th>Manufacturing date</th>
<th>Expiry date</th>
<th>Manufacturer’s Concentration (mg/100 ml)</th>
<th>Experimental Concentration (mg/100 ml)</th>
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<tbody>
<tr>
<td>A</td>
<td>JAN.,2013</td>
<td>DEC.,2015</td>
<td>200</td>
<td>215.32</td>
</tr>
<tr>
<td>B</td>
<td>JULY 2013</td>
<td>MARCH 2015</td>
<td>200</td>
<td>237.94</td>
</tr>
<tr>
<td>C</td>
<td>APRIL 2012</td>
<td>MAY 2014</td>
<td>200</td>
<td>258.34</td>
</tr>
</tbody>
</table>
Discussion and Conclusion

In the present work different types of cephalosporins i.e. Ciprofloxacin, Cefixime, Ceftriaxone were taken. Cefixime and Ciprofloxacin were in tablet form and Ceftriaxone in injection form. Different parameters are analyzed for these antibiotics. First of all physical parameters were taken and these are within the range when compared with IP data. Friability test and Hardness test along with Pfizer test assured the strength and durability of the drugs. Weight variation test confirmed uniformity of drug in the tablets. Both tablets samples were in agreement with the printed data according to weight variation calculations.

Minimum inhibitory concentration was checked for individual antibiotic to observe effectiveness against three different microorganisms *E. coli* (MTCC 1652), *P. fluorescens* (MTCC 2421), *S. aureus* (MTCC 3160). Cefixime, Ceftriaxone and Ciprofloxacin were used to determine the Minimum Inhibitory Concentration against the above stated microbial culture. In all the given cultures microbial growth is inhibited. Results are confirming the efficiency of each drug sample.

Among the three dosage forms Ceftriaxone and ciprofloxacin were equally effective against *S. aureus*, in broad of minimum inhibition concentration range of 2 to more than 16µg/ml. Cefixime was least effective. In tablets Ciprofloxacin was more effective against *S. aureus*. Comparatively ceftriaxone injection was more effective against microbial growth and was inhibiting taken cultures microbial growth.

Hence it was concluded that Ceftriaxone available in injection form was more effective in the less concentration range than Ciprofloxacin and Cefixime, which are available in tablet form. Same conclusion was also confirmed by the spectroscopic absorbance obtained for the drugs at their respective $\lambda_{\text{max}}$ values.

References


Abstract:

The purpose of the study is to formulate rectal suppositories of atenolol, an antihypertensive agent, using various hydrophilic and hydrophobic polymers such as gelatin, Polymethylene glycol-400 and hydrogenated vegetable oil along with or propylene glycol as plasticizer and beeswax as hardening agent by fusion or pour molding method. The in-vitro release data was statistically evaluated and was found that the drug release mechanism from all the prepared suppositories formulation was diffusion controlled (r=0.9427 to 0.9894). All the suppository formulations has obeyed zero order release kinetics except those which were prepared by utilizing 10% beeswax w/w and gelatin suppositories without PEG-400. The formulation containing 30% of PEG-400 w/w of gelatin has displayed zero order release kinetics (r=0.9936) and released 99.10 % of atenolol within 2.5 hr. The DSC and FT-IR studies reveal that there is mild to no interaction between polymer and the drug. The stability studies data suggested that there was no significant change in the drug content after a period of 6 months (p<0.005). The overall results conclude that atenolol rectal suppositories can be conveniently formulated by fusion method utilizing hydrogenated vegetable oil and gelatin-PEG 400 bases.

The absorption of antibiotic and polypeptides is more efficient from the small intestine than from the rectum and hence can be formulated utilizing various absorption promoting polymers. The human rectum represents a body cavity in which drugs can be easily inserted and retained well. Rectal route is also good for drug that irritates gastrointestinal mucosa, other reasons for preferring the rectal route over the oral route is when the drug is extensively metabolized or deactivated by liver enzymes. The superior rectal vein perusing the upper part of the rectum drains into the portal vein and subsequently into the liver, on the other hand, the middle and inferior rectal vein drains the lower part of the rectum and venous blood is returned to inferior venacava. Hence the drug absorbed in the latter system will be delivered preferentially to the systemic circulation by passing first pass metabolism. The rectal route is much preferred in pediactric and geriatric patients with difficulties in swallowing solid oral dosage forms. These are also the dosage forms of choice for unconscious and semiconscious patients. Major factors affecting the absorption of drugs from suppositories are anorectal physiology, suppository vehicle and physicochemical properties of drug. Considering the above facts suppositories can be stated as having greatest potential as convenient dosage form in the treatment of chronic health disorders such as rheumatism and cardiovascular diseases especially among the elderly patients.

Atenolol a beta adrenergic-blocking agents decrease the oxygen demand of the myocardium by lowering both the rate and force of contractin of the heart. They suppress the activation of the heart by blocking β1 receptors, and they reduce the work of the heart by decreasing heart rate, contractility, cardiac output and blood pressure. With β-blockers, the demand for oxygen by the myocardium is reduced both during exertion and rest. All the β blockers are non selective at high doses and can inhibit β2 receptors. The β-blockers reduce the frequency and the severity of angina attacks, these agents are particularly useful in the treatment of patients with myocardial infarction and have shown prolong survival. About 50% of a dose is absorbed following oral administration. peak plasma concentrations are reached in 2 to 4 hours. Atenolol has low lipid solubility. It crosses the placenta and distributed into breast milk where concentrations higher then in maternal plasma have been achieved. Only small amounts a reported to cross the blood brain-barrier, and plasma protein binding is minimal. The plasma half life is about 6 to 7 hours. Atenolol undergoes little or no hepatic metabolism and is excreted mainly in urine. It is removed by hemodialysis. In the present study the suppositories of Atenolol were prepared by using different hydrophilic and hydrophobic polymeric bases like gelatin, PEG-400 and hydrogenated vegetable oil using propylene glycol as plasticizer and beeswax as...
Materials and Methods
Atenolol was a generous gift from Glenmark Pharmaceuticals, Mumbai, Maharashtra, India. Propylene glycol, polyethylene glycol 400 (PEG 400) was purchased from Sd Fine Chem Ltd, Mumbai. Gelatin, propyl paraben were purchased from Qualigens Fine Chemicals, Loba Chemie Pvt Ltd and Merck Specialities Pvt Ltd, Mumbai respectively. All other chemicals were of analytical reagent grade.

Mould Calibration:
To prevent the variation in the mould capacity the molds were calibrated before preparing the suppositories and were standardized. The melted base was poured into the mould and freeze dried, after freezing the suppositories were removed from the mould and weighed individually and the mean weight was taken as true capacity of the mould. The procedure was repeated for different bases. The calibrated mould capacities ranged from 1.02 to 1.21 g for gelatin-PEG 400 base and 1.02 to 1.12 g for vegetable oil beeswax suppositories.

Suppository Preparation:
The displacement value for fatty base was calculated and was found that approximately 1.232 g Atenolol displaces 1 g of hydrogenated vegetable oil. Suppositories were prepared using fusion (pour moulding) method. a) Fatty base (hydrogenated vegetable oil) was taken in beaker and was melted by heating, drug was thoroughly mixed with base with continuous stirring until the drug is completely dispersed in the base. Melted base along with the dispersed drug was transferred in to calibrated suppository mould (holing of suppositories is possible with immediate cooling hence should be avoided). b) Gelatin was taken in a beaker and small amount of water is added to it and heated on a magnetic stirrer after complete melting of the gelatin, accurately weighed amount of propylene glycol(PG), polyethylene glycol-400 (PEG), drug along with the preservative (PG) was incorporated in the gelatin solution and was continuously stirred until a uniform mixture is obtained, the beaker is removed from the magnetic stirrer and initially cooled for few seconds to remove the air entrapment and then the solution was filled in to the calibrated moulds and immediately cooled because rapid cooling will result in good suppository if not cooled immediately the defective suppositories will be obtained with pitting and fissures. The drug loaded in each suppository is 50 mg of atenolol. The formulated suppositories were packed in aluminium foil and store under refrigeration composition of suppository formulation are shown in table 1.

Evaluation of Suppositories:
The formulated suppositories were analysed for physical appearance by visual examination, weight variation, content uniformity, liquefaction time/softening temperature and micromelting range test, stability studies, DSC was performed to study the thermal properties of the drug. Formulated rectal suppositories were evaluated for physical appearance by visually inspecting the selected formulated suppositories by cutting along the longitudinal section and the surfaces were observed for uniform dispersion of drug within the base and for any pits or fissures by observing with the naked eye.

Weight uniformity:
The individual and the average weight of 20 formulated suppositories were calculated. As per I.P the prepared passes the weight variation test if no suppository deviates by 5% of average weight and not more than two suppositories should deviate by more than 7.5% of average weight.

Determination of Drug Content:
Ten suppositories were cut into small pieces. Small pieces equivalent to 100 mg drug weighed accurately and transferred into 100ml volumetric flask and containing 80ml pH 7.4 buffer by vigorous shaking for thirty minutes. The volume was adjusted up to mark with buffer. The absorbance was measured at 224.5nm for atenolol against the solvent blank. The drug content was obtained from the calibration curve. Average of three determinations was considered as average drug content of the formulated suppositories and shown in table.

Liquefaction Time/ Temperature:
A pipette with narrow opening at the bottom and wide opening at the top was utilized for this test. The pipette was immersed in beaker containing hot water maintained at 37ºC± 1ºC in such a way that the bottom of the pipette touches the surface of hot water. The formulated suppository was inserted into the pipette from broad opening by carefully pushing the suppository downwards until it reaches the narrow part of the pipette fig-1. A plunger of glass was placed over the suppository from the broad end of the pipette. The time at which the glass rod reaches narrow
end of pipette after complete melting of suppository is termed as liquefaction time and the temperature at which the glass rod just move downwards (initially) is recorded as liquefaction temperature table

**Micro melting range test:**
This test is performed by using capillary tube of 10 cms long. The formulated suppository were inserted into the capillary tube upto 1cm and inserted in to beaker containing water which was heated by means of a heating mantle. While the temperature is raised the solid sample will liquefy, the temperature at which the solid liquefy is recorded as micro melting range of suppository table

**In vitro Dissolution Studies:**
The dissolution studies atenolol from the prepared suppositories was conducted using USP-XXIII dissolution test apparatus I (Electrolab) employing a basket stirrer. Phosphate Buffer pH7.4 at 37° ± 0.5°C was used as the medium of dissolution and the basket was rotated at 50 rpm. Single suppository was utilized in each analysis. 5ml of the sample were taken out by means of a syringe fitted with a prefilter at appropriate time interval and immediately replaced with same amount of medium. The absorbance of the sample was measured 224.5nm using spectrophotometer after suitable dilutions. The studies were carried out in triplicate. All the studies were run in triplicate (n=3).

**Stability Studies:**
This was performed at a temperature of 25° ±3°C/ 60± 5% Relative humidity for a period of 6 months on the promising suppository formulations (ATPG3 and AVS4). Fifteen suppositories were individually wrapped in aluminum foil and packed in card-board boxes and kept in a humidity chamber for 180 days. Sample is analyzed for drug content uniformity at monthly intervals and at the end of six month period dissolution test was performed to determine the drug release profile. The data is presented in table

**Results and Discussion**
Suppositories of atenolol, were prepared by fusion method employing hydrogenated vegetable oil (ALFS₀ to ALFS₃), gelatin-PEG 400 (ALGS₀ to ALGS₃) as base, beeswax for increasing the melting point of hydrogenated vegetable oil and propylene glycol as plasticizer. The prepared formulations were analyzed for physical appearance, weight variation, content uniformity, liquefaction time and temperature, micro-melting range, *in vitro* dissolution and stability.

The moulds (1 g capacity) were calibrated for different bases used. The calibrated mould capacities ranged from 1.02 to 1.21 g for gelatin-PEG 400 base and 1.02 to 1.12 g for vegetable oil beeswax suppositories. All the suppositories were free from pits, fissures and cracks. The longitudinal section of the suppositories was opaque and uniform in appearance, indicating complete and even distribution of drug in the base.

The formulated suppositories were evaluated for uniformity of weight and the results are given in table-2. The percent deviation from the mean weights of all batches was found to be within the prescribed limits as. The drug content was found to be in the range of 97.3% to 99.5%. The low standard deviation values indicate uniform drug content formulated suppositories. Melting/softening time is the time at which the suppositories withstand body temperature of 37° C, which is helpful in convenient handling and release of the drug after administration in the rectum.

The suppositories prepared with hydrogenated vegetable oils alone were soft with low melting below 37° C, to increase the hardness and melting beeswax was added in the formulation. In case of hydrogenated vegetable oil-beeswax suppositories the liquefaction time and temperature were found to be in the range of 03 to 06.51 min at 37±1°, while with gelatin-PEG 400 suppositories the liquefaction time was found to be in the range of 03.3 to 5.11 min at 37±1° C. The temperature for micro melting (Fig-1) of hydrogenated vegetable oil suppositories was between 37° to 38° C and for gelatin-PEG 400 suppositories it was found to be in between 37.5° to 38° C in all the batches.

*In vitro* dissolution studies on the formulated hydrogenated vegetable oil-beeswax suppositories (ALFS₀ to ALFS₃) and the gelatin-PEG 400 base suppositories (ALGS₀ to ALGS₃) were carried out in pH 7.4 phosphate buffer and the various dissolution parameters, viz., percent drug dissolved, t₉₀% and t₁₀₀% are shown in table-3 and dissolution profile are depicted in Fig-2 and 3. This data shows that hydrogenated vegetable oil-beeswax suppositories have released upto 99.20% (ALFS₂ containing 7.5% bees wax) of the drug in 150 min while gelatin-PEG 400 suppositories has released upto 99.10% (ALGS₃ containing 30% of PEG-400) of the drug in 150 min. In case of the formulations ALFS₀ to ALFS₃ consist of hydrogenated vegetable oil-beeswax suppository base the
release rate decrease as the concentration of the beeswax increases this might be due to the increase in melting time.

**Drug Release Kinetics:**
The *in vitro* drug release data was subjected to goodness of fit test by linear regression analysis, according to first-order kinetic equations, zero order, Higuchi and Peppas models to determine the mechanism of drug release. The regression coefficient ‘r’ values for Higuchi’s equation range from 0.9427 to 0.9894 indicating that the drug release is by diffusion mechanism, and those of ‘n’ values of Peppas equation range from 0.5536 to 1.51. As per the ‘n’ values of Peppa’s equation the prepared suppositories have shown non-Fickian (ALFS1, ALFS2, ALFS3, ALGS0, ALGS1, ALGS2 and ALGS3 n>0.45), and super case-II transport (ALFS0 n>1.0) release mechanism. The super case-II transport release mechanism may attributed to burst effect displayed by these formulations, The formulation ALGS3 containing 30% PEG-400 has displayed zero order release rate (r=0.9936) hence this formulation can be considered as promising formulation and it has released 99.10% AT within 150 min. All the formulations except ALGS0 and ALFS3 have shown zero order drug release kinetics.

**Stability Studies:**
Stability studies of the above formulation indicated that there are no significant changes in drug content during a period of 6 months (p=0.005) Table-4.

**FT-IR:**
FT-IR was done to evaluate interactions between the drug and polymer. IR spectra for pure drug, gelatin and formulation of drug with gelatin are shown in Fig 4-6 were analyzed. The IR spectra of Atenolol had shown characteristic peaks at 889 cm⁻¹, (C=CH₂ stretch), 1612 cm⁻¹ (Conjugated C=C (aromatic) stretch), 1637 cm⁻¹ (O=C-NH₂ stretch), 1705 cm⁻¹ (C=O stretch) and 2868 cm⁻¹ (C-H stretch) 2923 cm⁻¹ (CH₂ stretch), 2965 cm⁻¹ (ester C-CH₃ stretch) respectively. Whereas the prominent peak of secondary amine was appeared at 3240-3277 cm⁻¹ and of hydroxyl group appeared at 3355 cm⁻¹. The IR spectra of Atenolol formulation ATPGS had exhibited similar characteristic peaks of pure drug which confirmed that there was mild to no interaction between the drug and the polymer.

**Differential Scanning Colorimetry:**
The DSC thermogram of atenolol has exhibited a sharp endothermic peak at 153.29°C with an onset of 152.49°C corresponding to its melting point with ΔH =161.86 J/g. The thermogram of ATPGS3 formulation has shown endothermic peak at 124.69°C with less intense peaks of drug indicating decrease in crystallinity due to mixing or may be due to change in the heat capacity of atenolol: polymer, Finally the DSC result suggest that there is a lowering in the atenolol endothermic peak corresponding to the melting point of the atenolol indicating a possible mild interaction at molecular level between the drug and the polymer without affecting the integrity the drug Fig 7 and 8.

**Conclusion:**
The overall evaluation results suggest that atenolol suppositories can be conveniently formulated by fusion method utilizing hydrogenated vegetable oil and gelatin-PEG 400 bases.

**References**


Table-1: Composition of 10 suppositories

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<tr>
<th>Sl.No</th>
<th>Ingredients</th>
<th>ALFS₀</th>
<th>ALFS₁</th>
<th>ALFS₂</th>
<th>ALFS₃</th>
<th>ALGS₀</th>
<th>ALGS₁</th>
<th>ALGS₂</th>
<th>ALGS₃</th>
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<tbody>
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<td>1</td>
<td>Hydrogenated vegetable oil (g)</td>
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<td>---</td>
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<tr>
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<td>Gelatin</td>
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<tr>
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<td>1.00</td>
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<td>Propylene glycol (g)</td>
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<td>Propyl paraben (g)</td>
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<td>Distilled Water (ml)</td>
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<td>Drug (gm)</td>
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</table>

Table-2: Evaluation of suppositories

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<tr>
<th>Formulation code</th>
<th>Weight variation* (mean±SD)</th>
<th>Drug content* (mean±SD)</th>
<th>Liquefaction time/ temperature</th>
<th>Micro melting range (ºC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Time (min)</td>
<td>Temp (ºC)</td>
</tr>
<tr>
<td>ALFS₀</td>
<td>1.12±0.008</td>
<td>99.23±0.00318</td>
<td>3.0±1.16</td>
<td>36.73±2.00</td>
</tr>
<tr>
<td>ALFS₁</td>
<td>1.015±0.00707</td>
<td>99.10±0.002516</td>
<td>5.45±2.645</td>
<td>37.66±0.58</td>
</tr>
<tr>
<td>ALFS₂</td>
<td>1.02±0.0107</td>
<td>97.91±0.001527</td>
<td>5.83±1.153</td>
<td>36.66±0.58</td>
</tr>
<tr>
<td>ALFS₃</td>
<td>1.03±0.0164</td>
<td>97.3±0.002516</td>
<td>6.51±1.00</td>
<td>37.0±0.00</td>
</tr>
<tr>
<td>ALGS₀</td>
<td>1.212±0.0139</td>
<td>99.5±0.00353</td>
<td>3.3±0.0131</td>
<td>36.6±0.577</td>
</tr>
<tr>
<td>ALGS₁</td>
<td>1.21±0.0116</td>
<td>99.07±0.0002</td>
<td>4.9±0.5773</td>
<td>38.0±0.00</td>
</tr>
<tr>
<td>ALGS₂</td>
<td>1.021±0.00737</td>
<td>99.25±0.00305</td>
<td>5.11±1.527</td>
<td>36.33±0.58</td>
</tr>
<tr>
<td>ALGS₃</td>
<td>1.04±0.0117</td>
<td>98.20±0.00316</td>
<td>4.00±0.023</td>
<td>37.00±0.58</td>
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</tbody>
</table>
Table 3: In-vitro drug release data

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Formulation Code</th>
<th>( t_{50}) (min)</th>
<th>( t_{70}) (min)</th>
<th>Cumulative drug release*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ALFS₁</td>
<td>99.0</td>
<td>118.5</td>
<td>99.04±0.557</td>
</tr>
<tr>
<td>2</td>
<td>ALFS₁</td>
<td>76.5</td>
<td>100.5</td>
<td>98.19±0.981</td>
</tr>
<tr>
<td>3</td>
<td>ALFS₂</td>
<td>77.0</td>
<td>106.5</td>
<td>99.20±0.997</td>
</tr>
<tr>
<td>4</td>
<td>ALFS₃</td>
<td>27</td>
<td>39</td>
<td>98.34±0.577</td>
</tr>
<tr>
<td>5</td>
<td>ALGS₀</td>
<td>94.5</td>
<td>114.0</td>
<td>97.4±1.009</td>
</tr>
<tr>
<td>6</td>
<td>ALGS₁</td>
<td>109.5</td>
<td>135.0</td>
<td>97.00±1.230</td>
</tr>
<tr>
<td>7</td>
<td>ALGS₂</td>
<td>94.5</td>
<td>108.0</td>
<td>98.99±0.987</td>
</tr>
<tr>
<td>8</td>
<td>ALGS₃</td>
<td>63.0</td>
<td>94.5</td>
<td>99.10±0.124</td>
</tr>
</tbody>
</table>

Table 4: Stability data (Drug content)

<table>
<thead>
<tr>
<th>Trial</th>
<th>A (1st Day)</th>
<th>B (180th Day)</th>
<th>A-B</th>
<th>Mean ± SD</th>
<th>p=0.005</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>98.20</td>
<td>98.0</td>
<td>0.20</td>
<td>98.616 ± 0.5014</td>
<td>0.296</td>
</tr>
<tr>
<td>02</td>
<td>99.11</td>
<td>98.91</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>03</td>
<td>98.21</td>
<td>98.00</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>04</td>
<td>98.35</td>
<td>97.91</td>
<td>0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05</td>
<td>99.21</td>
<td>98.78</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>98.616</td>
<td></td>
<td></td>
<td>0.296</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.5014</td>
<td></td>
<td></td>
<td>0.1270</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1: Micro melting Test Apparatus

Figure 2: Cumulative percent drug release versus time plots of formulations ALGS0, ALGS1, ALGS2 and ALGS3
Figure-3: Cumulative percent drug remained versus time plot of formulations of ALFS0, ALFS1, ALFS2, ALFS3 and ALFS4

Figure-4: IR Spectra of Atenolol

Fig 5: IR Spectra of Gelatin
Fig 6: IR Spectra of ATPGS3

Figure 7: DSC thermogram of Atenolol

Figure 8: DSC thermogram of Formulation ATPGS3

THE END
DEVELOPMENT AND VALIDATION OF NEW SPECTROPHOTOMETRIC METHODS FOR THE QUANTITATIVE ESTIMATION OF SILODOSIN IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

Abstract:
Three simple, sensitive, accurate and rapid spectrophotometric methods A, B and C have been developed for the quantitative estimation of Silodosin in bulk drug and also pharmaceutical formulations. Method A is a UV-spectrophotometric method in which Silodosin was dissolved in alcohol showing $\lambda_{max}$ 269nm. The method is linear in the concentration range of 10-50$\mu$g/ml. Methods B and C are based on oxidation followed by complex formation reaction. In method B and C Silodosin reacts with 1,10-phenanthroline and 2,2'-bipyridyl to form orange red colored chromogen which shows maximum absorbance at 507nm and 518nm for methods B and C respectively. Linearity range for method B was between 2-10$\mu$g/ml and that for method C was between 4-20$\mu$g/ml. Results of the analysis were validated statistically. All the validation parameters were within the acceptable range and prove the precision, sensitivity and applicability of the methods for the routine quantitative determination of Silodosin in its dosage form.

Introduction
Silodosin is a highly selective alpha 1A-adrenoreceptor antagonist approved for the treatment of the signs and symptoms of benign prostatic hyperplasia (BPH). Its clinical pharmacology profile offers a number of advantages including uroselectivity, once daily (QD) dosing, a standard dose of 8 mg QD that does not need to be adjusted according to age and the feasibility of concomitant treatment with phosphodiesterase type 5 (PDE 5) inhibitors and antihypertensive agents. Relative to Tamsulosin, Silodosin has less cardiovascular side effects. Silodosin, a selective antagonist of alpha-1 adrenoreceptors, has chemical name:

$1\text{-}(3\text{-Hydroxypropyl})\text{-}5\text{-}[2\text{R}]-2\text{-}[2\text{-(2,2,2\text{-Trifluoroethoxy})phenoxyl\text{-}ethyl\text{-}amino}]\text{propyl}\text{-}2\text{,3\text{-dihydro}\text{-}1H\text{-indole\text{-}7\text{-carboxamide}}}$

The structural formula of Silodosin is:

![Silodosin Structural Formula](image)

Literature survey reveals that number of analytical methods are available for estimation of Doxazosin, Tamsulosin, Gabapentine and other BPH drugs but only one UV spectrophotometric method and one HPLC method has been developed for the quantitative estimation of Silodosin in formulation and one LC-MS/MS method for the determination of Silodosin in human plasma. Silodosin is a key drug for the treatment of BPH with a number of advantages including uroselectivity, once daily dosing, standard dose of 8 mg QD that does not need to be adjusted according to age and the feasibility of concomitant treatment with phosphodiesterase type 5 (PDE 5) inhibitors and antihypertensive agents. Lack of analytical methods for the quantitative estimation drives us for the development of spectrophotometric methods for the routine analysis of Silodosin.

Experimental Work:
EQUIPMENT: Electronic balance, UV-Visible Spectrophotometer (Systronic 2203) with matched quartz cells.
REAGENTS: Reagents required such as alcohol, 1,10-phenanthroline, ferric chloride, 2,2'-bipyridyl are of analytical grade, purchased from different sources, capsule dosage form of Silodosin (Silodal 4mg and 8mg) was purchased from local market.

Karazgi Kishwar Jahan* & Malipatil SM
*JIT University, Vidyanagri Churu-Bisau Rd., Jhunjhunu, Rajasthan, ¹HKE’S College of Pharmacy, Sadam Road, Gulbarga
PREPARATION OF STANDARD STOCK SOLUTION: Standard stock solution was prepared by dissolving 100mg of Silodosin in about 40ml alcohol in a 100ml volumetric flask and volume was made upto the mark with alcohol (1000µg/ml).

PREPARATION OF WORKING STOCK SOLUTION: 10ml of standard stock solution was taken in a 100ml volumetric flask and volume was made upto the mark with alcohol (100µg/ml).

Method A: 10, 20 and 30µg/ml solutions were prepared by diluting working stock solution with alcohol and scanned between 200 to 400 nm and 269nm was selected as λmax.

Five different aliquots were prepared by taking 1, 2, 3, 4 and 5ml from working stock solution in different 10ml volumetric flask and final volume was made upto 10ml with alcohol. Calibration curve was plotted using absorbance values against concentration.

Method B: In this method five aliquots from 2 to 10 µg/ml were prepared by taking 0.2 to 1ml solution from working stock solution in different 10 ml volumetric flasks, to each of the flask 0.5ml of 0.5% ferric chloride was added followed by 1ml of 0.2% w/v of 1,10-phenanthroline. Aliquots were heated at 60°C for about 10 minutes to complete the reaction, allow to cool at room temperature and then volume was made upto the mark with alcohol. Absorbance of aliquots was measured at 507nm against reagent blank and calibration curve was prepared.

Method C: Aliquots of drug were prepared by pipetting 0.4, 0.8, 1.2, 1.6 and 2ml of working stock solution in different 10ml volumetric flasks. To each of the flask 0.5ml of 0.5% w/v ferric chloride and 1ml 0.02M 2,2'-bipyridyl were added respectively. The solutions were heated on water bath at 60°C for 10 minutes for complete development of color. Cool at room temperature and volume was made upto the mark with alcohol. Absorbance was measured against reagent blank prepared simultaneously selecting 518nm as λmax and calibration curve was prepared.
### Method – C

![Chemical structure](image)

**Table No 1: Optical Characteristic and Regression Analysis Data.**

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Optical Characteristic</th>
<th>Method A</th>
<th>Method B</th>
<th>Method C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>( \lambda_{\text{max.}} )</td>
<td>269</td>
<td>507</td>
<td>518</td>
</tr>
<tr>
<td>2.</td>
<td>Linearity range</td>
<td>10-50</td>
<td>2-10</td>
<td>4-20</td>
</tr>
<tr>
<td>3.</td>
<td>Sandell’s sensitivity ( \mu g/cm^2 )</td>
<td>0.03</td>
<td>0.009</td>
<td>0.012</td>
</tr>
<tr>
<td>4.</td>
<td>Molar absorptivity</td>
<td>( 4.2218 \times 10^4 )</td>
<td>( 3.1119 \times 10^4 )</td>
<td>( 2.3785 \times 10^4 )</td>
</tr>
<tr>
<td>5.</td>
<td>Correlation coefficients(r)</td>
<td>0.99991</td>
<td>0.9996</td>
<td>0.9994</td>
</tr>
<tr>
<td>6.</td>
<td>Slope (b)</td>
<td>0.01585</td>
<td>0.06215</td>
<td>0.046925</td>
</tr>
<tr>
<td>7.</td>
<td>Intercept (a)</td>
<td>0.0133</td>
<td>0.0047</td>
<td>0.0327</td>
</tr>
<tr>
<td>8.</td>
<td>RSD of Precision</td>
<td>0.76438</td>
<td>1.146</td>
<td>0.464</td>
</tr>
<tr>
<td>9.</td>
<td>Average recovery</td>
<td>100.28±0.26</td>
<td>99.9±0.75</td>
<td>100.02±0.478</td>
</tr>
<tr>
<td>10.</td>
<td>Color Stability period</td>
<td>---------------</td>
<td>80min</td>
<td>75min</td>
</tr>
<tr>
<td>11.</td>
<td>LOD</td>
<td>0.779</td>
<td>0.237</td>
<td>0.0153</td>
</tr>
<tr>
<td>12.</td>
<td>LOQ</td>
<td>2.36066</td>
<td>0.71868</td>
<td>0.614</td>
</tr>
<tr>
<td>13.</td>
<td>Percentage assay of formulation(Mean±SD)</td>
<td>100.16±0.268</td>
<td>99.66±0.353</td>
<td>99.83±0.749</td>
</tr>
<tr>
<td>14.</td>
<td>Range of error</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05 confidence limit</td>
<td>( 1.64687 \times 10^{-3} )</td>
<td>( 3.9837 \times 10^{-3} )</td>
<td>( 2.6246 \times 10^{-3} )</td>
</tr>
<tr>
<td></td>
<td>0.01 confidence limit</td>
<td>( 2.18687 \times 10^{-3} )</td>
<td>( 5.28999 \times 10^{-3} )</td>
<td>( 3.4853 \times 10^{-3} )</td>
</tr>
<tr>
<td>15.</td>
<td>Standard error of method</td>
<td>( 6.25 \times 10^{-3} )</td>
<td>( 1.51185 \times 10^{-3} )</td>
<td>( 9.9608 \times 10^{-3} )</td>
</tr>
</tbody>
</table>
### Table No 2: Analysis of Silodosin Capsule Formulation With Statistical Evaluation (N=6)*

**(METHOD A, B, C)**

<table>
<thead>
<tr>
<th>Method</th>
<th>Label Claim</th>
<th>Reference Method Mean</th>
<th>%Drug estimated Mean± SD</th>
<th>%RSD</th>
<th>SEM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4mg</td>
<td>98.2</td>
<td>99.97±0.20</td>
<td>0.201</td>
<td>0.082</td>
</tr>
<tr>
<td></td>
<td>8mg</td>
<td>98.7</td>
<td>100.35±0.67</td>
<td>0.667</td>
<td>0.247</td>
</tr>
<tr>
<td>B</td>
<td>4mg</td>
<td>98.2</td>
<td>99.91±1.195</td>
<td>1.196</td>
<td>0.488</td>
</tr>
<tr>
<td></td>
<td>8mg</td>
<td>98.7</td>
<td>99.41±0.98</td>
<td>0.986</td>
<td>0.400</td>
</tr>
<tr>
<td>C</td>
<td>4mg</td>
<td>98.2</td>
<td>99.30±0.95</td>
<td>0.962</td>
<td>0.387</td>
</tr>
<tr>
<td></td>
<td>8mg</td>
<td>98.7</td>
<td>100.36±0.48</td>
<td>0.479</td>
<td>0.196</td>
</tr>
</tbody>
</table>

*Mean of 6 determinations

### Table No 3: Recovery Studies Using the Proposed Method With Statistical Evaluation (N=6)*

**(METHOD A, B, C)**

<table>
<thead>
<tr>
<th>Method</th>
<th>Concentration of formulation</th>
<th>Label claim</th>
<th>Pure drug spiked</th>
<th>Statistical results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean± SD %RSD SEM*</td>
</tr>
<tr>
<td>A</td>
<td>50%</td>
<td>8mg</td>
<td>4mg</td>
<td>100.04 0.136 0.136 0.055</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100%</td>
<td>8mg</td>
<td>100.56 0.55 0.547 0.224</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150%</td>
<td>8mg</td>
<td>100.26 0.568 0.567 0.231</td>
</tr>
<tr>
<td>B</td>
<td>50%</td>
<td>8mg</td>
<td>4mg</td>
<td>99.1 1.338 1.350 0.546</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100%</td>
<td>8mg</td>
<td>100.16 1.401 1.399 0.571</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150%</td>
<td>8mg</td>
<td>100.56 0.685 0.681 0.279</td>
</tr>
<tr>
<td>C</td>
<td>50%</td>
<td>8mg</td>
<td>4mg</td>
<td>100.15 0.88 0.878 0.359</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100%</td>
<td>8mg</td>
<td>99.49 0.811 0.812 0.331</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150%</td>
<td>8mg</td>
<td>100.42 0.682 0.679 0.278</td>
</tr>
</tbody>
</table>

*Mean of 6 determinations
Table No 4: Color Stability Studies (METHOD B, C).

<table>
<thead>
<tr>
<th>Method</th>
<th>Concentration</th>
<th>6µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time(min)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Absorbance</td>
<td></td>
</tr>
<tr>
<td>Method B</td>
<td>0.375</td>
<td>0.376</td>
</tr>
<tr>
<td>Method C</td>
<td>0.609</td>
<td>0.608</td>
</tr>
</tbody>
</table>

Results and Discussion

1. Optical parameters
In order to ascertain the optimum wavelength of maximum absorption (λ max) of UV spectrophotometric method and of the colored species formed in each of the two visible spectrophotometric methods, specified amount of Silodosin was taken and the colors were developed following the above mentioned procedures individually. The absorption spectra were scanned on spectrophotometer in the wavelength region of 200-380nm for UV spectrophotometric method and 380-800 nm for colorimetric methods against corresponding reagent blanks. Appropriate λ max for the three methods was selected.

2. Optical Characteristics
The absorbance at appropriate wave lengths of a set of solutions containing different amounts of Silodosin and specified amount of reagents (as described in the recommended procedure) were noted against corresponding reagent blank.
The Beer’s law plot of the system illustrated graphically. Least square regression analysis was carried out for the slope, intercept and correlation coefficient. Beer’s law limits, molar absorptivity, Sandell’s sensitivity for Silodosin with each of mentioned reagents was calculated table-1.

3. Parameters fixation
In developing these methods, a systematic study of the effects of various relevant parameters in the methods concerned were undertaken by verifying one variable at a time (OVAT) and controlling all other parameter to get the maximum color development, reproducibility and reasonable period of stability of final colored species formed.

4. Linearity range
The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyzed sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated within a suitable level of precision, accuracy and linearity. Linearity ranges of the three proposed methods were given in table no 1.

5. Method
The results obtained in colometric methods were based on oxidation followed by complex formation reaction of Silodosin with 1,10-phenanthroline and 2,2’-bipyridyl using ferric chloride to form an orange red colored chromogen that exhibited maximum absorption at 507nm and 518 nm respectively against the corresponding reagent blanks. The effect of various parameters such as concentration, volume of reagents, order of addition of reagents and solvent for final dilution were studied by means of control experiments varying one parameter at a time.

6. Precision
Precision of each one among the three proposed spectrophotometric methods were ascertained separately from the absorbance values obtained by actual determination of a fixed amount of Silodosin in final solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods and presented in table no 1.
7. Analysis of formulations
Commercial formulations of Silodosin were successfully analyzed by the proposed methods. The result obtained by developed methods was compared with reported HPLC method (reference method) and found in good agreement with the labeled amount in formulation. This shows applicability of the proposed methods for the routine analysis of the drug. Results are shown in table-2.

8. Accuracy
Recovery studies were carried out at three different levels i.e., 50%, 100% and 150%, of label claim following standard addition method. Results were statistically calculated and found between the range of 100.04-100.56, 99.1-100.56 and 99.49-100.42 for methods A, B and C respectively. This shows high accuracy of the proposed methods (table 3).

9. Color Stability:
To study the stability of the developed color for proposed methods middle concentration of linearity range was selected. Color was developed by adding 0.5ml of 0.5% w/v FeCl₃ solution and 1ml of 0.2%w/v solution of 1,10-phenanthroline to drug. The resulting solution was heated at 60°C for 10 min, allow to cool at room temperature and volume was made up to 10 ml with alcohol. Color stability was measured against time.
For method C, to the drug solution, 0.5ml of 0.5% w/v solution of FeCl₃ and 1ml of 0.02M 2,2'-Bipyridyl was added. The resulting solution was heated at 60°C for 10 min, cooled at room temperature and volume was made up to 10 ml with alcohol. Colors for the two methods were found to be stable for sufficient period of time. Results for the color stability studies were provided in table no 4.

Fig-1: Absorbtion Spectrum of Silodosin

Fig-2: Calibration curve of Silodosin

Conclusion
The proposed methods can be used for determination of Silodosin in bulk drug as well as in formulations. These methods are rapid, simple and have great sensitivity and accuracy. Developed methods make use of simple reagents, which an ordinary analytical laboratory can afford. Methods are sufficiently sensitive to permit determination even down to 10µg/ml. Hence we can conclude that the proposed methods are suitable for routine determination of Silodosin in its formulation.

References
**IN VITRO ANTIFUNGAL EFFECTS OF DIFFERENT INDIGENOUS PLANTS FROM ARACHI (PAKISTAN) ON PATHOGENIC FUNGUS ASPERGILLUS NIGER.**

Wafa Arshad* & Dr. Huma Ikram

1Department of Microbiology, 2Assistant Professor, Department of Biochemistry, University of Karachi, Karachi, Pakistan.

**Abstract:**

The objective was to design an experimental approach towards evaluation of antifungal activity of seven plants found indigenously in nature. Plant extracts were prepared dissolving 20 grams in 70% methanol. The fungus to be tested was cultivated in a PDA medium. Using a 5-day old colony of *Aspergillus niger* incubated in the heat chamber at 28 °C and keep in the dark for 10 days. Well inoculation method was performed by inoculating two wells on a single plate for a control (ethanol) and test (extracts) with fungi with centre filled well for all seven plant leaves extract. Zone of inhibition were measured and statistical evaluations were reported. These extracts showed maximum activity even at low concentrations. We conclude from the above experimental approach that all the seven leaves extracts exhibit amazing fungicidal properties that support their traditional use as antiseptics for tropical fungal skin infections, in ointments.

**Introduction**

Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. In fruit and vegetables, there is a wide variety of fungal genera causing quality problems related to aspect, nutritional value, organoleptic characteristics, and limited shelf life (Agrios, 2004). In addition, in some cases fungi are indirectly responsible for allergic or toxic disorders among consumers because of the production of mycotoxins or allergens. Since antiquity, the plant kingdom has provided a variety of compounds of known therapeutic properties, like analgesics, anti-inflammatory, medicines for asthma, and others. In recent years, antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world (Cowan, 1999). For example, a large proportion of the South American population use plant extracts obtained from traditional medicinal plants as medicine for many infectious diseases. Several works have demonstrated in laboratory trials that different plant tissues, such as roots, leaves, seeds and flowers possess inhibitory properties against bacteria, fungi and insects (Davicioni et al., 2007).

Currently, there is little evidence on the antifungal aspect/properties of the medicinal plants under investigation against pathogenic fungi. Fungi are ubiquitous in the environment, and infection due to fungal pathogens has become more common. *Aspergillus niger* is usually found in common mesophilic environments such as soil, plants, and enclosed air environments. *A. niger* is not only a xerophilic fungi (mold that doesn’t require free water for growth, can grow in humid environments), but is also a thermotolerant organism (capable of growing at high temperatures). Because of this property, the filamentous fungi exhibits a high tolerance to freezing temperatures.

*Aspergillus niger* is relatively harmless compared to other filamentous fungi. Despite this fact, there have been some medical cases that have been accounted for, such as lung infections or ear infections in patients that have an weakened immune system, or an immune system that has been impaired by a disease or medical treatment. In the case of ear infections, *A. niger* invades the outer ear canal which can cause damage to the skin it came in contact with.

*Aspergillus niger* is the most abundant species of Aspergillus in nature as it can grow on a large variety of substances. Aspergillus niger can even grow in environments with very little nutrients available.

In houses it is often found growing on damp walls. Of the Aspergillus species, *Aspergillus niger* infects humans the third most often. A fungal ball in the lungs is eventually created by Aspergillus niger after it infects a person's lungs and begins to grow. The health effects of Aspergillus niger include hearing problems and even hearing loss.

Indigenous plants (sometime also called native plants) are plants that have evolved over thousands of years in a particular region. They have adapted to the geography, hydrology, and climate of that region. These plants occur in communities, that is, they have evolved together with other plants. As a result, a community of indigenous plants provides habitat for a variety of native wildlife species such as songbirds and butterflies.
Extracts of leaves were prepared from seven different indigenous plants:
1. Liriodendron
2. Aster thomsonii
3. Jasminium Auriculatum
4. Chrysanthemum
5. Cosmos sulphurous
6. Gazania gaerta and
7. Vinca rosea

**Liriodendron:**
Liriodendron (common name: tulip tree) is a genus of two species of characteristically large deciduous trees in the magnolia family (Magnoliaceae). These trees are widely known by their large flowers superficially resembling tulips, though they are not closely related to the true tulips. Native Americans were the first to use the tulip tree for medicine, making decoctions of the tree's roots for use as a tonic and heart stimulant. These remedies remained in use long after the Colonial period. Liriodendron was included in the 1892 edition of the "U.S. Pharmacopoeia," which recommended the powdered root bark for relief of dyspepsia and dysentery. The powdered substance could also be combined with whiskey as a cough treatment. In his 1903 book "Specific Medications and Specific Medicines," Dr. John M. Scudder of Cincinnati recommended using a tincture of the root bark and alcohol as a tonic to remedy nervous complaints.

Poultices and ointments made from tulip tree leaves and buds were used by Native Americans, including the Cherokee, for wounds, burns and inflammation. Some Native Americans also used the leaves in decoctions to treat arthritis. The bark, which contains an alkaloid called tulipferine, was thought by some Native Americans to have an aphrodisiac effect when chewed. Teas made from it were used to treat fever and digestive ailments. Early colonists adopted the custom of using bark teas to treat fever. A bark extract can also be used as a cinchona (quinine) substitute.

The Chinese tulip (Liriodendron chinense), has been used for centuries in traditional Chinese medicine. As with its American counterpart, the bark has been made into medicine to treat fevers. Closely related magnolias have also been a part of traditional Chinese medicine.

The extracts prepared from Liriodendron tulipifera Linn., L. chinense (Hemsl.) Sarg., and their hybrid L. chinense x L. tulipifera, were investigated for their cytotoxic abilities in vitro against five human cancer cell lines: MDA-MB-231 and MCF-7 breast cancer cells, SGC-7901 gastric cancer cells, HuH-7 hepatocarcinoma cells, and HCT-15 colon carcinoma cells, and then measured their phenols and alkaloids contents. Of these plant extracts, some of them, especially the lower polar extracts from barks, exhibited potent cytotoxic effects on five tested tumor cell lines.

**Aster thomsonii**
Aster thomsonii (Thomson's Aster), is growing naturally on various places of northern areas of Pakistan including Shugran. Flower-heads are purplish with a yellow center, usually arising singly at ends of branches. They are with many light purple ray florets, 2 cm long. Bracts are linear-lanceshaped long-pointed, hairy, leaf-like. Leaves are ovate to elliptic, pointed or long-pointed, coarsely toothed, narrowed below and half stem-clasping. Stems are shaggy-haired, erect, branched, 1-3 ft tall.

It has been used for a long time in curing of diseases. This plant has been used traditionally as expectorant diuretic, antitumor, antibacterial, antiviral and antiulcer natural source for a long time. Its aqueous and ethanolic extracts of stalks, leaves, and roots have low toxicity. Phytochemical analysis showed the presence of saponins. Saponins have been found to be potentially useful secondary metabolites for the treatment of hypercholesterolemia which suggests that saponins might be acting by interfering with intestinal absorption of cholesterol20. Analysis also showed presence of Tannins which had been widely used as an application to sprains, bruises and superficial wounds. Also the presence of cardiac glycosides showed that plant species can be use in the treatment of hypertension.

**Jasminium Auriculatum**
Jasminium auriculatum is a species of jasmine, in the family Oleaceae. A spreading woody shrub grows up to 2 meters in height. Leaves trifoliate with lower small two, often deciduous, and a large, rounded, acuminate, central one. Flowers white fragrant, in paniculate cyme. Fruits globose black berries. Used in the treatment of a number of ailments, including burning sensation, diuretic, hyperdesia, ulcers, odontalgia, stomatopathy, ophthalmopathy,
cardiopathy, urolithiasis, nephrolithiasis, strangury and dermatopathy. The roots are useful in skin diseases especially for ring-worm.

The flowers are fragrant, bitter, acrid, astringent, cardiotonic, diuretic, depurative, sweet and refrigerant. They are useful in burning sensation, hyperdipsia, odontalgic, thermogenic, aphrodisiac, antiseptic, emollient, anthelmintic, ulcers, stomatopathy, ophthalmopathy, urolithiasis, nephrolithiasis, strangury, deobstruant, suppurative, leprosy, skin diseases, wounds, corns and aromatherapy. The juice of the leaves in the form of jelly, on local application to a linear-uniform excised wound in the rats was found to promote wound healing, as assessed by histological, biochemical and contraction rate studies. The fresh juice of the leaves was found to increase and early gain of the tensile strength in the treated linear wounds in rats. Leaves extracts injected intramuscularly showed wound healing promoting activity on musculo-peritoneal wounds on the abdomen of rats.

**Chrysanthemum**

Chrysanthemums, sometimes called mums or chrysanths, are flowering plants of the genus Chrysanthemum in the family Asteraceae. They are native to Asia and northeastern Europe. Most species originate from East Asia and the center of diversity is in China. There are about 40 valid species.

It is a rich source of vitamin C and is therefore very effective in treating viral and bacterial infections. One of the medicinal uses of chrysanthemum tea is that it helps relieve the symptoms of sinusitis and cold and eases head heaviness. One of the other chrysanthemum health benefits is that it is a natural coolant and helps lower body temperature while suffering from fevers or heat strokes. Chrysanthemum is also a well-known natural remedy for acne and pimples. You can either drink it in tea form or use it as an herbal poultice for the topical treatment of acne and pimples. Experts also recommend using chrysanthemum for skin problems like boils, skin sores, irritation, and allergic skin reactions. Its powerful anti-inflammatory and antiseptic properties help in treating these problems effectively. Chrysanthemum tea helps regulate high blood pressure and is therefore very beneficial to hypertension patients. It is also a good remedy for calming the nerves and easing anxiety, stress, and mental fatigue. One of the health benefits of chrysanthemum is that it increases blood flow to the heart, thereby making it effective for maintaining good cardiac health. Chrysanthemum tea consumed with meals helps in boosting digestion, especially in the case of oily and greasy foods. Being low in calories, chrysanthemum tea is also good for obese as well as weight conscious people.

Research shows that chrysanthemum tea may also be good for people suffering from neurodegenerative diseases like Parkinson’s disease. Chrysanthemum contains neuro-protective properties that help prevent neuron injury and degeneration. Chrysanthemum is also a good treatment for other problems like eye irritation, conjunctivitis, ulcerative colitis, and migraines.

**Cosmos sulphureus**

Cosmos sulphureus is also known as Sulfur Cosmos and Yellow Cosmos. Its native habitat is Central America. It is a annual herb growing to 60 cm tall. Leaves are opposite, pinnately cut; with narrow lanceolate lobes. Flower stalk is up to 20 cm long.

An ethnobotanical study showed Cosmos sulphureus to have activity against Plasmodium. Butein has been isolated from Cosmos sulphureus. Butein has been reported to be a powerful antioxidant against lipid and LDL peroxidation and has also exhibited anti-inflammatory activities, aromatase inhibition, cyclooxygenase inhibition and angiotensin converting enzyme inhibition, etc.

**Gazania gaerta**

Gazania is a genus of flowering plants in the family Asteraceae, native to Southern Africa. They produce large, daisy-like composite flowers in brilliant shades of yellow and orange, over a long period in summer. They are often planted as drought-tolerant groundcover. Not much research is present on Gazania gaerta as a possible antifungal so our research interestingly provide insight on its antifungal properties.

**Vinca rosea**

Catharanthus roseus, , is a species of Catharanthus native and endemic to Madagascar. Other English names occasionally used include Cape periwinkle, rosy periwinkle, and “old-maid”. It is an evergreen subshrub or herbaceous plant growing to 1 m tall. The leaves are oval to oblong, long and broad, glossy green, hairless, with a pale midrib and a short petiole; they are arranged in opposite pairs. The flowers are white to dark
pink with a darker red centre, with a basal tube and a corolla diameter with five petal-like lobes. The fruit is a pair of follicles 2–4 cm long and 3 mm broad.

Extracts from it have been used against numerous diseases, including diabetes, malaria, and Hodgkin's lymphoma. The substances vinblastine and vincristine extracted from the plant are used in the treatment of leukemia and Hodgkin's lymphoma. Daily supplements made with the active ingredients found in Vinca rosea help to improve blood supply to the brain and increase the level of oxygen and glucose that the brain can effectively utilize. These supplements are also highly effective in preventing the abnormal coagulation of blood and in raising the levels of serotonin, the blood neurotransmitter, in the brain. Serotonins are bunches of neurons in the central nervous system that play a critical role in memory, sleep, appetite, heart function and muscle control. Deficiencies of serotonin are likely to cause schizophrenia, phobias, migraine and bulimia.

The main alkaloid in Vinca rosea is known as vincamine. Vincamine has blood thinning and memory-enhancing properties and is effective in the treatment of vascular dementia. The condition is caused when the arteries that supply blood to the brain develop plaques. Vascular dementia is the most common dementia after Alzheimer disease. Vascular dementia is not one disease but a number of syndromes related to numerous vascular mechanisms. This form of dementia is a preventable condition and can be corrected with early detection and diagnosis.

Materials and Methods
Sample Collection
Different plant leaves were collected from the plants within the University of Karachi campus and were identified by experts from the Department of Botany, University of Karachi. The plants used in the study were:

1. Liriodendron
2. Aster thomsonii
3. Jasmium Auriculatum
4. Chrysanthemum
5. Cosmos sulphurous
6. Gazania gaerta and
7. Vinca rosea

Preparation of plant extracts
Plant leaves were used as explants for in vitro studies. These plants were thoroughly washed under tap water and were fine powdered using pestle and mortar. The plants were then rinsed with 70% methanol, and were surface sterilized for 4 min under UV source.

Medium composition
For the routine cultivation and identification of fungi, we used Potato Dextrose Agar.

Ingredients:
- Potato dextrose agar (Oxoid CM139) 39 g
- Distilled water 1000 ml

Method:
1. Soak potato dextrose agar in small amount of the water in a stainless steel jug.
2. Boil remaining water, add to soaking ingredients, bring to the boil, stirring constantly.
3. Dispense for slopes as required.
4. Autoclave at 121°C for 15 minutes. Remove and slope or pour for plates as required.

Test organism:
Aspergillus niger was isolated, using a 5-day old colony of Aspergillus niger incubated in the heat chamber at 28 °C and kept in the dark for 10 days.

Antifungal Assay:
For the Antifungal assay, Well inoculation method was performed. As the main focus of the experiment was to check the inhibition of the strain of Aspergillus niger by these seven leaves extracts.

Aspergillus niger was grown on PDA plate, and confirmed by microscopy and morphological characteristics.

Colonies on potato dextrose agar at 25°C are initially white, quickly becoming black with conidial production. Reverse is pale yellow and growth may produce radial fissures in the agar. Microscopically, hyphae are septate and
Hyaline. Conidial heads are radiate initially, splitting into columns at maturity. The species is biseriate (vesicles produce sterile cells known as metulae that support the conidiogenous phialides). Conidiophores are long (400-3000 µm), smooth, and hyaline, becoming darker at the apex and terminating in a globose vesicle. Metulae and phialides cover the entire vesicle. Conidia are brown to black, very rough, globose, and measure 4-5 µm in diameter.

There was a single PDA plate used for single leave extract. One well for ‘control’ and other for ‘test’. The plates were spot inoculated with fungal strain and kept for 7-8 days for fungal growth and meanwhile wells were made by borer technique in which we inoculated a fixed amount of our extracts and ‘ethanol’ being used as a control. The inhibition was observed after two days of the fungal strain. The zones of clearance were measured in millimetres. Experiment was performed in triplicate in order to confirm the results accuracy.

1) Apergillus niger growth on PDA plate
2) Microscopic view of Asp. Niger

Results:

Following table shows zones of inhibitions (in millimetres):

<table>
<thead>
<tr>
<th>Leave extracts</th>
<th>I.</th>
<th>II.</th>
<th>III.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Extract</td>
<td>Control</td>
</tr>
<tr>
<td>1. Liriodendron</td>
<td>45mm</td>
<td>20mm</td>
<td>45mm</td>
</tr>
<tr>
<td>2. Aster thomsonii</td>
<td>45mm</td>
<td>22mm</td>
<td>45mm</td>
</tr>
<tr>
<td>3. Jasium auriculatum</td>
<td>40mm</td>
<td>0.5mm</td>
<td>40mm</td>
</tr>
<tr>
<td>4. Crysanthemum</td>
<td>40mm</td>
<td>10mm</td>
<td>40mm</td>
</tr>
<tr>
<td>5. Cosmos sulphureus</td>
<td>45mm</td>
<td>15mm</td>
<td>45mm</td>
</tr>
<tr>
<td>6. Gazania gaerta</td>
<td>40mm</td>
<td>0.5mm</td>
<td>45mm</td>
</tr>
<tr>
<td>7. Vinca rosea</td>
<td>45mm</td>
<td>15mm</td>
<td>45mm</td>
</tr>
</tbody>
</table>

The following table shows the invitro effect of the leaves extract on the growth of Aspergillus niger. Experiment was performed in triplicate in order to confirm the accuracy of the results which are interpretable.

Usually, for all seven extracts used, ‘control’ zones were measured of about 40 mm or 45 mm in triplicate and the respective ‘extract’ zones were half times inhibiting the growth of Aspergillus which depicts that all these seven extracts have some antifungal potential as they clearly show susceptibility of the grown fungus on the plate and clear zones were obtained.
Minor fluctuations were observed in triplicate while measuring zone diameters in all of the seven extracts used which could be personal hand error but the changes are minor which shows no great effect on the obtained results of susceptibility.

Of all the seven extracts, *Aster thomsonii* gave best inhibitory results, showing bigger zones of growth inhibition in accordance with the control. Secondly, *Liriodendron* had good inhibitory effects.

*Gazania gaerta* extracts are still under research trials of antimicrobial activities and specifically to our knowledge there is no such publications on antifungal studies, thus our research confirmed the specie of Gazania as having antifungal potential inhibiting the growth of *Aspergillus niger*.

All seven plant leaves extracts were from indigenous sources in nature and thus their potential of fungal growth inhibition under right conditions provided, can be applied in many areas of biotechnology and industrial use.
No growth on plant extract well

Inhibition of growth around extract well
Discussion:
The present study tested the antifungal activity of crude extracts and their respective dilutions from medicinal plants belonging to seven plant families. The results obtained from this work showed that plant extracts of Pakistani medicinal plants screened exhibit antifungal effects. The experiment carried out in triplicate assured the inhibition results in which all seven extracts according to their antifungal potential inhibited the Aspergillus strain, few fluctuations in the results did not affect the susceptibility results. The mean was plotted of all the three readings in a graph showing highest activities of Aster thomsonii and Liriodendron plants. Jasmiu and Gazania leaves extract gave minimum antifungal inhibition but maybe due to low or insufficient quantity of the leaves extract isolated but their zones of inhibition were measured inhibiting Aspergillus niger.

There are several different antifungal preparations that are used to treat various fungal infections. They come as cream, ointments, shampoo, pessaries, medicine to take by mouth and injections. The length of treatment depends on what type of fungal infection you have, how severe it is and if you have any other health problems - for example, problems with your immune system. Some courses of treatment can be as short as a few days (for example, for vaginal thrush), other courses can be a long as ten weeks (for example, for ringworm infection of the scalp).
Various types of fungus can cause internal infections. For example:
- Aspergillosis most commonly affects the lungs, but sometimes infects other organs.
- Cryptococcosis is uncommon, but can cause meningitis.
- Histoplasmosis is rare, but can cause serious infections of the lung and other organs.

Thus these studies could be further evaluated and studies that what are the other bioactive compounds involved that inhibit the pathogenic strain of *Aspergillus niger*.

Therefore these extracts from native grown plants could be used in pharmaceutical preparations, in treatment of topical fungal infection or infection associated with *Aspergillus niger*.

The higher antifungal activity may be due to the presence of phytochemicals like phenolic compounds, flavonoids, coumarin, quinines, formulations or topical formulations and also to support their traditional use as antiseptics for tropical fungal skin infections, in ointments so as to prevent and reduce prevalence of fungal infections which require more long treatment durations and also become life threatening so it is therefore important to acknowledge the usage from these provided natural indigenous sources.

Thus it can be concluded that by knowing antifungal potential of used plants extracts, we can apply this knowledge for the treatment of infections specially being caused by pathogenic strain of *Aspergillus niger*, many antifungal preparations can use these leaves extracts, or in natural sprays ,in fungicides as well.

### References

Abstract:

Clinical Data Management (CDM) was once perceived as the set of processes which resulted in a reviewed database. But this very perspective is in a state of dynamic flux today. Various CDM activities broadly include finalization of Case Report Forms (CRF), Annotation of CRF, Design of Database in Oracle Clinical, Edit Checks programming, Double Data Entry, Validation of Data (Query Management), Data Coding, relevant Quality Control (QC) activities and Database lock etc. With respect to the scenario in India, though the significance of Clinical Data Management in the field of Clinical Research is well entrenched and the expected CDM outcomes are outlined in the guidelines but there is no structured operational framework for its implementation and measures to monitor progress of the implementation. This may result in ambiguity. The variable application of the CDM process definition and lack of operational guidelines makes comparison challenging among different trials types. Further, with the advent of recombinant products, biosimilars, vaccines etc. there is a need to develop and harmonize the way clinical data is processed and managed in the context of specific trial. This article shares the authors experience and elaborates the activities carried out to manage clinical trial data of in-house case study conducted for Mylive™ vaccine, Panacea Biotec Ltd.

Introduction

Clinical Data Management (CDM) encompasses activities carried out to manage clinical trial data in order to have an accurate and unbiased output for the study. As a product of the clinical development process, clinical data is recognized to be a key corporate asset and provide critical documented evidence in support of medicine’s efficacy and safety, and of its potential economic value. Thus, poorly managed data may jeopardize the whole objective of the clinical trial. The output obtained should meet applicable regulatory requirements, ethical principles and must be fool proof in terms of data quality & corresponding standard operating procedures so that any rechallenge should not give transformed data values and analysis result(s). In India the conduct of clinical trials is directed by Schedule Y of Drug and Cosmetic Rules. General guidelines for carrying out clinical trials and expected framework for managing CDM related activities are defined by CDSCO (Central Drugs Standard Control Organization).
### Table 1: Study Protocol in Brief

<table>
<thead>
<tr>
<th>Study Title</th>
<th>A Randomized, Multicenter, Open Label, Comparative Study to Evaluate the Immunogenicity and Reactogenicity of a Fully Liquid Pentavalent DTwP-HepB-Hib Vaccine (Myfive™, Panacea Biotec Ltd.) with Pentavalent DTwP-rHepB-Hib vaccine (Pentavac SD/MD, Serum Institute of India Ltd.) in Healthy Infants.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase of Development</td>
<td>Phase II/III</td>
</tr>
<tr>
<td>Study Design</td>
<td>Open Label, multi-centric, randomized, parallel group, comparative study (active control)</td>
</tr>
<tr>
<td>Study Centre</td>
<td>7 centers</td>
</tr>
<tr>
<td>Dosage</td>
<td>3 doses of test or comparator vaccine to be administered 4 weeks apart</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>600 (300 in each arm). Initially, the study was conducted in 48 healthy infants.</td>
</tr>
<tr>
<td>Study population</td>
<td>Healthy infants, 6-10 weeks of age</td>
</tr>
<tr>
<td>Dose administration</td>
<td>0.5ml per dose by deep intramuscular injection</td>
</tr>
<tr>
<td>Site of administration</td>
<td>Antero-lateral aspect of thigh</td>
</tr>
<tr>
<td>Duration of protocol therapy</td>
<td>3 months</td>
</tr>
</tbody>
</table>

### Material and Methods

A questionnaire was formulated after reviewing existing literature and Indian GCP guidelines document; expected to augment data quality as compared with using none. The questionnaire was debated with CDM team. The following parameters were considered with focusing vaccine trial, though most of the parameters considered may be applied to other therapeutic segments too.

- Compliance to regulatory standards
- Achieving data quality for very small quantity of data
- Achieving data quality for very large quantity of data
- Remote Data Captured
- Randomization of data
- Maintenance of Blinding
- Adapted Clinical Trials
- Support to multi-arms study
- Subject Confidentiality maintained
- Double Data Entry
- Data access by authorized person
- Data security system maintained
- Recorded audit trial
- Hardware & software - validation
- No deletion of data once it has been entered
- Adequate backup
- Credibility of the data based on the study design
- Data contained in the final report must match with the original observations
- Easy identification of the individual subjects, without compromising confidentiality & allocation of unique subject identification code
- Accuracy, completeness, legibility, and timeliness of the data reported should be there
- Data discrepancies handling
- Easy work flow
- Language restrictions
- Less Training Time
- Less Cost of Software Purchase/Validation/Maintenance
- Easy installation & implementation of procedures
The members had to select either of the two options: ‘Possible or Not Possible’ against the above parameters for the two options ‘Myfive™ CDM Methodology (denoted by \( p_{cdmsm} \))’ and ‘without any specific methodology and CDMS (denoted by \( p_{nccdmsm} \))’. The response was analyzed as below.

The null hypothesis was established as follows:

\[
H_0: p_{cdmsm} = p_{nccdmsm}
\]

\[
H_a: p_{cdmsm} \neq p_{nccdmsm}
\]

Chi square statistics was calculated using SAS 9.3 on windows platform to see if using ‘Myfive™ CDM Methodology’ does aid in handling the above listed parameters. A chi-square value obtained was 15.1667. The probability of obtaining a chi-square value of 15.1667 or greater by chance alone is < 0.0001. So the null hypothesis was rejected. A significant difference in data management was presumed by the use of ‘Myfive™ CDM Methodology’ as compared to using none from the observed chi square statistics. However, there is a need for bigger survey/parameters related to data issues.

**Operation Methodology Adopted for CDM Activities**

The primary objective of CDM processes is to provide high-quality data by keeping the number of errors and missing data as low as possible and gather maximum reliable and accurate data for statistical analysis\(^2\). To achieve the outlined goals and objectives, following steps were performed and each step is depicted by a flow chart.

**Finalization of Case Report Forms (CRF)**

After the finalization of the study protocol, comments on draft CRF were provided by CDM unit. Comments/suggestions were given and incorporated in order to align the CRF with the study-specific protocol to ensure that all the required information was captured, to avoid redundant and duplicate data points. Also, CRF was finalized in a manner so as to facilitate the database programmer to have consistent database entry screens by the use of standard questions from the global library. Pages which were repeated in the subsequent visits, with same data points, were structured consistently. This further helped the programmer by enabling to copy the pages from previous visits in database. Refer to the flow chart number 01, for the quick snapshot of the procedures involved.

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**Flowchart 01: Finalization of CRF**

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Flowchart 01: Finalization of CRF

1. Final protocol and draft CRF provided to CDM unit
2. Draft CRF review to align with protocol and to facilitate database design
3. Use of standard fields and pages from global library
4. Suggestion accepted for finalization of CRF
   - YES: Final CRF
   - NO: Draft CRF re-created by clinical operation team

End
```
**Annotation of CRF**

Once the CRF was finalized, each field of the same was assigned with the names consistent with the Clinical Data Acquisition Standards Harmonization (CDASH) and Clinical Data Interchange Standards Consortium (CDISC) conventions, as applicable, known as CRF annotation. Annotations are done to assist in database designing, validation programming, data extraction and analysis and for subsequent regulatory submissions. Consistent use of annotations has the added advantage of quick rollout of extension/sister studies and easy meta-analysis. Final annotated CRF was assigned Version 1, with its effective date. Any further changes to the document were updated and the document was assigned new version number incrementally. Refer to the flowchart number 02, for snapshot view of the procedures involved.

**Data Management Plan (DMP)**

DMP being a live document, with version control, was prepared to give insight of the anticipated life cycle of the project, right from the conception of CDM activities till the archiving of the project related documents. It highlighted phase-wise planning according to various time points defined for the study, list of team members involved in the study, task ownership matrix, SOPs/guidelines to be followed, list of self-evident corrections, data entry guidelines, applicable lab ranges, handling of non CRF data etc. Refer to the flowchart number 03, for quick snapshot of the procedures involved.
**Design of Database**

Clinical Database Management System (CDMS) used for the study was Oracle Clinical Applications Version 4.5.3. Based on the final approved annotated CRF, database designing was initiated. Designing of each CRF panel was done so as to have data entry screens exact replicas of the CRF. As pertinent, tasks listed below, including but not limited to, the following were accomplished, as and wherever applicable:

- Use of Correct Default prompt and SAS Labels
- Correct use of Discrete Value Group (DVG)
- Marking mandatory fields
- Applying upper and lower bounding (ranges) to the questions
- Hard Coding the required fields
- Use of qualifying questions
- Use of repeat questions groups for the tables
- Use of indicator questions
- Navigations through conditional branching
- Cursor movement
- Use of subsets in Data Collection Module (DCM)
- Character layout generation

Database once designed was released for User Acceptance Test (UAT) i.e. test data entry, before releasing the database in production. UAT was done for both the first and second pass data entry. A well designed database avoids snags during validation programming, production data entry and discrepancy management. In a way this imparts endurance to the CDM project and prevents it from getting crumpled. Refer to the flow chart number 04, for the quick understanding of the procedures involved.

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**Flowchart 04: Flow Chart for Study Database Designing in CDMS**

1. Final annotated CRF
2. Use of standard fields &/or pages from global library or from previous studies
3. Creation of study specific fields &/or pages
4. Screen Design
5. UAT by tester
6. Comments accepted
7. Database ready for validation programming
8. End
**Edit Checks programming**

Edit checks were programmed based on the approved data validation guidelines so that the data was in conformance and qualified the protocol constraints. Usually for several but not all, repeat or standard pages, validation procedures are picked from previous similar studies or from the global library. However, all study specific validations are programmed after consultation with medical monitor and clinical operations team. A History of version control is properly maintained and the document is signed off once finalized. Each modification to the edit checks was captured in the subsequent versions and respectively programmed. All programming was checked in test data for accuracy before releasing the procedures for live data authentication. Refer to the flowchart number 05, for easy understanding of the procedures involved.

**Flowchart05: Flow Chart for Edit Checks programming**

**Double Data Entry**

For the study, double data entry was executed. This was done to minimize errors during data entry and possible bias, resulting into overall quality data. The first pass data entry operator entered the data from paper CRF in the study database. The process was repeated by the second pass data entry operator, who re-entered the same data. The entries made by the first pass operator were not visible to the second pass operator. In case of errors or mismatch during second pass entry, the system generated signals for mismatched fields. Same was corrected by the second pass operator after verification from the CRF. Both the entries were done by different operators. Until first pass was not complete, second pass data entry was not initiated. Reconciliation(s) and rectification(s), as applicable, between first and second pass data entry were done by a third CDM personnel. Refer to the flowchart number 06, to simplify procedural details.
Validation of Data

Validation checks were run on the live data as soon as it was entered into the study database. This process was carried out by the system or through manual intervention as and when desired. System sensitized batch validations were run during a fixed schedule of the day, incrementally to the live data, resulting in the generation of all the multivariate discrepancies as per the programmed edit checks. Refer to flow chart number 07, for the quick snapshot of the procedures involved.
**Query Management**

Query/Discrepancy management was done to provide accurate, clean, complete, consistent, reliable and statistically sound data. This process included generation of queries for the data which was erroneous, illegible and/or incomplete. Queries were generated by OC through pre-written as well as manually applied programs. Queries were generated time to time in the Conduct phase of the study and sent to the investigator at respective sites seeking resolution to the queries through Data Clarification Forms (DCF). Some of the queries which did not require investigator's intervention were resolved in-house in consultation with the Clinical Operations Team. Subsequently, based on the resolution of the queries, the same were updated in the OC database by CDM personnel and all the queries were assigned closed/resolved status. Query tracking log was maintained in order to facilitate and trace the DCF flow to maintain turn-around-time (TAT), as per the applicable Standard Operating Procedures (SOPs). Refer to flowchart number 08, where procedural details have been simplified for easy understanding.

**Data View**

It is the tool, which after necessary programming, has enabled to create different views of the data stored in Oracle Clinical in SAS formats. These SAS datasets were directly exported to the biostatistician for analysis, without any manual intervention. However, for the purpose of Quality Control (QC), data was extracted in MS Excel sheet by connecting with Open Database Connectivity (ODBC). Refer to the flowchart number 09, for the quick understanding of the procedures involved.
Data Coding
Data coding of ‘Serious Adverse Events (SAE) and Adverse Events (AEs)’ was done with the help of Medical Dictionary for Regulatory Activities (MedDRA) to categorize and standardize the verbatim terms in the ‘SAE/AE panel’ of the CRF, so that they can be harmonized to facilitate easy review and successive analysis. Coded terms were documented as per the ‘MedDRA Coding guidelines’ for the study. Refer to flowchart number 10, for the quick snapshot of the procedures involved.

Serious Adverse Event (SAE) Reconciliation
This process was completed to reconcile number of SAEs in CDMS and Pharmacovigilance (PVG) safety database. Apart from number of SAEs, all the relevant information in both the databases, related with the SAEs, had to be alike, e.g. Subject ID, verbatim term, concomitant medication etc. In case of any discrepant data, DCFs were raised and resolved by the investigator and updated in the OC database to complete the SAE reconciliation process. Refer to the flowchart number 11, to simplify understanding of procedural details.

---

**Flowchart 10: Flow Chart for Data Coding**

1. Data set ready for coding
   - Manual coding
   - Auto-coding
   - Coding Reviewed & Comments provided
     - Yes, required
     - Corrections done as per comments & recoded
   - Not required
   - Coding Documented
   - End

**Flowchart 11: Flow Chart for SAE Reconciliation**

1. Manual SAE reconciliation
2. Auto-SAE reconciliation
   - Miss match found in CDM dataset, comment provided
     - YES
     - Comments reviewed
     - Comments accepted
     - Documentation done
     - End
     - NO
     - DCF generated, sent for resolution
     - DCF generated, sent for resolution
   - Refer Flow Chart for Query Management, till all queries are resolved
   - End
**Quality Control (QC) activities**

To have consistent, accurate and reliable data for the purpose of report generation for a clinical trial, one of the important processes is having a robust quality control system in place. QC includes the operational techniques and activities undertaken within the system of Quality Assurance (QA) to verify that the requirements for quality of the trial related activities have been fulfilled. QC activities concern everybody involved in planning, conducting, monitoring, evaluating, data handling and reporting.

“The objective of QC is to avoid exposure of study subjects to unnecessary risks and maintain reliability of data to draw appropriate conclusions from the study”.

In CDM, clinical trial data is entered into the clinical database and data cleaning is done by applying study-specific validation procedures. However, the aim is always to have a nearly 100% error free data pool for statistical analysis. In order to meet this requirement various QC measures including, but not limited to the following were applied:

- QC Report of CRF annotation
- QC Report of Database Designing and Entry Screen Layout
- QC report of 1st and 2nd Pass Test Data Entry
- QC Report of Validation Procedures
- QC Report of View Definitions, View templates and Data Extraction
- QC Report of Subject Enrollment
- QC Report of MedDRA Coding
- QC Report of Discrepancy Status in OC
- 100% QC of Critical Data Points
- 100% QC of Random sample size using √n+1
- QC Report of DCFs

QC findings were resolved / updated as required and all the signed QC reports were documented in the Master Data Management File (MDMF).

**Database lock**

Study database lock was carried out to ensure the integrity of the clinical trial data so that it is ready for export to the biostatistician for statistical analysis and submission to the regulatory authorities. It was done to ensure that there are no unauthorized manipulations to the database and its security is maintained. Before the database lock, all the team members were informed about the same and it was checked that all CDM activities were compiled and completed as per applicable SOPs. Some of the activities that were completed to ensure that the database is now ready for lock are listed below including but not limited to the following:

- All Study Data Entered
- MedDRA coding done
- SAE reconciliation accomplished
- All discrepancies reviewed, resolved and closed with the appropriate resolutions
- Final data QC completed
- QA activities completed
- Approved and signed documents placed in specific folders in MDMF

Based on the above procedures, study database was locked and study access was revoked for CDM team members. Refer to the flowchart number 12, for snapshot view of the procedures involved.
Challenges
There are still fundamental problems in clinical data management practices. There exists a great diversity in the procedure adopted for data processing and handling across industry. Some of the challenges faced while adopting this procedure were:

- The overall process attracts initial higher cost
- It involves comparatively more training time
- Risks of Data Security
- More time needed for data cleaning and discrepancy management
- Need to evolve current Indian regulatory guidance
- It is required to have in-house SOPs in conformance with regulatory standards
- Customized codes are required to overcome the regional language barriers
- Lack of technical experts to monitor the implementation

Authors believe that more work needs to be done to overcome these barriers, as the outcomes of this study will be implemented on more trials.

Results
The activities carried out to handle data from the Myfive™ vaccine clinical trial were as per in-house SOPs which complied with regulatory guidelines. An audit was conducted at the end of the study by QA Department of Panacea Biotec and there was no major adverse finding. To respond to the audit report, Corrective and Preventive Action (CAPA) was sent after taking appropriate preventive/corrective measures, as required.

Data was thus ready for analysis and reporting.

Discussion
Vaccine studies are different from other studies, as they are done on healthy subjects. Unlike other studies where the focus is on the outcome of the treatment, in vaccine trials antibody titer are measured to check the immunogenicity. Thus, the CDM activities of Myfive™ study were based on the steps including but not limited to the following: inputs for CRF design given in ‘CRF and Protocol Alignment Document’ were exclusively based on a typical vaccine trial design. For example, immunization history was collected along with medical history; AE pages included solicited (systemic/local reactions) and unsolicited reactions, diary data was essential, many follow up visits etc. These changes in the data acquisition methodology resulted in necessary alterations in overall operational activities, like database designing had to be modified, timelines for different activities were different, type of edit checks applied in the study varied drastically, data extract views were adjusted accordingly, because of the unavoidable time consuming lab analysis for antibody titres database lock was delayed etc.

Conclusion
There are no specific guidelines in India which elaborates step by step methodology of management of data of vaccine trials once the CRF has been received from the site. With the operational aspects of CDM system in place, the errors could be minimized and the data processing could be completed in a time and cost effective manner. We have been able to replicate and reproduce the operational model developed in another vaccine trial (NUCOVAC™-Pneumococcal Polysaccharide Conjugate Vaccine, Phase I), which is the subject of another publication.

CDM of vaccine development is an extended, multi-step procedure where critical design steps must be considered prior to the start of study and to avoid multitude of errors the corrective actions taken in synergy. CDM activities undertaken for managing data for this vaccine study were partially similar to that of any other trial (e.g. drug trial), but the difference was in the way the tools were used to carry out the tasks. These tasks and processes undertaken met the quality standard and satisfied QA personnel. As a result, analysis ready data was obtained. It seemed logical that the success from this model could be applied seamlessly into the CDM activities of other vaccine trials in future, leaving scope for revisiting, revamping, revising and revitalizing the activities as per the individual project requirements.

References

THE END
Components that constitute the composition of seeds include 20.85% protein, 38.20% fats (cholesterol, campestrol, stigmastrol, betasitosterol), 4.64% moisture, 4.37% ash, 7.94% crude fiber, 31.94% carbohydrate. Mineral elements include potassium, phosphorous, sodium and iron (Riaz et al 1996; Salma et al 2007).

Unfortunately in Pakistan, its production is restricted to small scale. It is cultivated in the NWFP. In Pakistan its annual consumption is 200-250 tons (Ahmad and Ghafoor 2004). It is insufficient to fulfill the requirements. For this purpose it is imported from Bangladesh and India. Cultivation, propagation and production of kalonji (Nigella sativa) are highly suitable with the environment in Pakistan (Iqbal et al 2010; Rabbani et al 2011). Inspite of its potential of higher yield, our country has low yield as compared to other countries. Weed competition is one of the major factors that are responsible for its low yield. About 60-85% yield of the crop is reduced by weed interference (Ahmad and Ghafoor 2004).

Biotic and abiotic stresses are responsible for reduced crop production. During last two decades beneficial effects on crop plants by inoculating seeds with Azospirillum strains have reported by several studies. (Okon 1985; Sarig et al 1988; Das et al 2007; Verma 2011; Pereyra et al 2012). Remarkable positive changes have been observed by inoculation of plants by Azospirillum (Kolb and Martin 1985; Sarig et al 1988). Best inoculation results were observed in semi-arid lands which have non optimal conditions for plant growth (Bashan and Holguin 1997). Organic substances that are able to promote or inhibit plant growth, active in very low concentrations are referred as plant growth regulators (Bhatia and Parashar 1996). Plant growth is affected by their application. There are several researches which explain their positive effect on crop yield (Hernandez 1997; Ashraf et al 1987, 1989). During germination, rate of metabolism is increased by these substances which are responsible for increased growth (Verma and Tandon 1998). Their application is valuable in obtaining important commercial products (Davies 1982). Auxins are naturally occurring plant growth compounds that are found throughout the kingdom Plantae. Auxins control several processes of plant life i.e. elongation of stem, biosynthesis of ethylene and differentiation of vascular bundles (Nordstrom et al 1991). Chemically auxins are similar to Indole-3- acetic Acid (IAA). It is the major auxin in many plants.

Gibberellins are tetracyclic diterpene growth factors. Gibberellins are a class of endogenous plant growth substances that exert pleiotropic effects on plant developmental processes (Davies 1995). These are necessary for stem elongation and other developmental processes (Hooley 1994; Swain and Olszewski 1996). A naturally occurring plant hormone, gibberellic acid known as GA₃ naturally exists in plants, is responsible for growth of plants. Increased length of plants is the result of its application which is due to cell division and cell elongation. According to Kende and Zeevaart (1997) cell division and cell elongation is significantly influenced by this hormone. This hormone is responsible for cell expansion, cell elongation and elongation of internodes (Huttley and Phillips 1995). In internodes, it improves osmoregulation as a result increase in plant length takes place (Azuma et al 1997). GA₃ is also involved in increased production of ethylene leading to the seedless oranges (Abeles and Rubenstein 1964). However Scort and Leopold (1967) found antagonistic effect to ethylene in lettuce seedlings.
Materials and Methods

A pot experiments was carried out to study the response of black seed to foliar applications of IAA and GA3. The experiment was conducted at the Department of Botany, University of Gujrat, Gujrat, Pakistan. It was conducted during the session of 2012-2013. The experiment was laid down in field area allotted by Botany Department in the university. The experiment was laid down in a completely randomized design (CRD) under natural day/night conditions: average 12 hour photoperiod. Homogenous seeds of *Nigella sativa* were procured from the seed shop of Gujranwala, Pakistan for this research.

To improve germination seeds were soaked in tap water for about 12 hours. The seeds were then sown in pots (25cm in diameter) filled with sandy loam soil. Each pot was filled with about 4-5Kg of soil. Each treatment had three replicates. After sowing, each pot was provide with 100 cm³ of full strength Hoagland’s solution to accompany better germination and growth of seedlings. 10 seeds were sown in each pot. After germination only 5 uniform plants were left in each pot. Treatments were applied 15 days after germination. Two levels of each IAA (30ppm, 60ppm) and GA3 (30ppm, 60ppm) were applied as foliar spray after 15 days of germination. Parameters of first harvest (seedling stage) were studied 21 days after treatment (DAT) while second harvest (vegetative stage) was measured after 42 days.

Levels of Hormones:
Following levels of hormones were used as foliar spray. Solutions were sprayed at the rate of 50 ml per plant. Hormones were sprayed onto the plants using a hand atomizer. Detergent was used as surfactant. Surfactant is responsible for the penetration of exogenous hormones within leaf tissues.

Treatments:

To = Control (Tap water)
T1 = 30ppm of IAA
T2 = 60ppm of IAA
T3 = 30ppm of GA3
T4 = 60ppm of GA3

Study of parameters:
Effects of growth regulators on growth at various concentrations were studied. After the foliar application of hormones following parameters were studied.

Morphological parameters:

a) Plant fresh weight (g):
For measuring plant fresh weight, plant of each treatment of each replicate was uprooted carefully, washed with water and then separated into roots and shoots and then their weight was recorded in grams (g) using electrical balance.

b) Plant dry weight (g):
Plant's roots and shoots were dried in oven at 65⁰C for 5 days and then dry weight was recorded in grams (g).

c) Root length (cm):
The root length was measured from base to the tip of the longest root. The measurement was done by centimeter scale.

d) Shoot length (cm):
The shoot length was measured from base to the tip of the youngest leaf. The measurement was done by centimeter scale.

e) Relative growth rate (RGR) (g g⁻¹ day⁻¹):
Relative growth rate was calculated by using the formula given below:

\[ RGR = \frac{1}{W} \times \frac{\Delta W}{\Delta T} \]

Where as

\( W \) = Shoot dry weight at first harvest.

\( \Delta W \) = Shoot dry weight at second harvest - shoot dry weight at first harvest.

\( \Delta T \) = Number of days b/w first and second harvest.

Results

Root fresh weight (g):
The figure 1 shows the effect of two levels of IAA (30 ppm and 60 ppm) and \( \text{GA}_3 \) (30 ppm and 60 ppm) on root fresh weight of black seed. Results showed that these hormones effected root growth. Both harvests showed maximum growth at \( \text{T}_2 \) (IAA 60 ppm). On the other hand \( \text{GA}_3 \) had negative effect on root growth leading to reduced fresh weight. The ANOVA table 1a. showed the same trend. Due to the positive effect of IAA the results were more significant as compared to the \( \text{GA}_3 \). On the other hand table 1b showed all the significant results of both levels of both hormones.

Shoot fresh weight (g):
The figure 2. Illustrates the effect of two levels of IAA (30 ppm and 60 ppm) and \( \text{GA}_3 \) (30 ppm and 60 ppm) on shoot fresh weight of black seed. Results showed that these hormones significantly affected the development of shoot and it was maximum at \( \text{T}_2 \) (IAA 60 ppm). On the other hand \( \text{GA}_3 \) showed negative effect on shoot growth leading to reduced fresh weight due to thinning of shoot (in the first harvest). In second harvest \( \text{GA}_3 \) showed pronounced effect on growth increment which was more than \( \text{T}_0 \) (control) but less than both levels of IAA (\( \text{T}_1 \), \( \text{T}_2 \)). The ANOVA tables 2a and 2b showed significant effect of hormones.

Root dry weight (g):
The figure 3.illustrates the significant effect of two levels of IAA (30 ppm and 60 ppm) and \( \text{GA}_3 \) (30 ppm and 60 ppm) on root dry weight of black seed. Root dry weight significantly (table 3a) increased under the influence of IAA which was maximum at \( \text{T}_2 \) in both harvests as IAA increased the growth of roots. \( \text{T}_2 \) (60 ppm IAA) is more efficient than \( \text{T}_1 \) (30 ppm IAA).Whereas \( \text{GA}_3 \) application showed significant (table 3b) reduction in dry weight of roots in first harvest. But in second harvest the growth regulating activity of \( \text{GA}_3 \) enhanced the growth of roots and root weight was greater than control. While the ANOVA results of second harvest (vegetative stage) are non-significant except for \( \text{GA}_3 \).

Shoot dry weight (g):
Analysis of variance (ANOVA) of data given in table 4a and 4b shows that hormones had significant effect on the shoot dry weight in both harvests(seedling as well as vegetative stage). Shoot weight was increased by subsequent applying two levels of factor IAA (30 ppm and 60 ppm). Due to thinning of shoot minimum growth was shown by \( \text{T}_3 \) plants shown Figure 4.

Root length (cm):
The figure 5 illustrates the effect of two levels of IAA (30 ppm and 60 ppm ) and \( \text{GA}_3 \) (30 ppm and 60 ppm ) on root length of black seed.\( \text{GA}_3 \) had positive and significant (table 5a and b) effect on root length increment as compare to IAA. IAA showed decrease in root length due to extensive branching of main root causing an increase in root weight and thus inhibiting root length. 60 ppm level of \( \text{GA}_3 \) was more efficient than 30 ppm in increasing this growth attribute.

Shoot length (cm):
Analysis of variance (ANOVA) of data presented in table 6a and 6b shows that hormones affected this characteristic. The results of shoot length are in harmony to the results of root length. Both levels of \( \text{GA}_3 \) influenced the shoot growth of black seed and caused an increase in the shoot length of this plant. 60 ppm level of \( \text{GA}_3 \) is more efficient than 30 ppm. On the other hand IAA had annulled effect on the length of shoot. Minimum shoot length was observed in \( \text{T}_1 \) (30 ppm IAA). The results of \( \text{GA}_3 \) are significant as well as non-significant at seedling and vegetative stage respectively. While IAA gave significant results in both harvests (seedling stage and vegetative stage) Figure 6.
Relative growth rate (g g\(^{-1}\) day\(^{-1}\)):

The figure 7 shows that treatments of GA\(_3\) had constructive effect on this variable. Foliar application of hormones affected the relative growth rate of plant. ANOVA given in table 7 describes the significant effect of hormones on Nigella plant. Minimum relative growth rate was observed in T1 plants that are under the effect of 30 ppm IAA.

Discussion

From the results, indicated the wide-ranging reaction of black seed towards hormonal application. The present investigation monitored changes caused GA\(_3\) and IAA treatments in growth and morphological parameters of black seed. Results are in harmony with the findings of following workers. Uses of some plant hormones improve the growth attributes containing fresh weight, dry weight root and shoot length. Parallel consequences were identified by Abraham and Ataga 1981; Onyebunchi 1981; Chippa and Lal 1988 through worked on several crop plants. IAA showed most operative effect in enhancing the morphological parameters i.e. root and shoot weights (fresh as well as dry: Fig. 4.1, Fig. 4.2, Fig. 4.3 and Fig. 4.4). The explanations can be validated that IAA causes cell expansion and cell elongation resulting into increased biomass. (Arif et al., 2001). The same results were reported by Vamili et al. (2011) on Bambusa arundinacea. According to Naeem et al (2004) IAA triggered the expansion of leaves leading to increased photosynthesis resulting into enhanced plant growth. A similar increase in growth characteristics was reported by Jamro et al (1990) in soybean and Kakkar and Rai (1993) in Phaseolus vulgaris.

Gillaspy et al (1993) reported significant role of IAA on increasing fruit size through its action on cell expansion on chilli pear. Agusti et al (1994) also found the same results on Clausellina. From the results distinct effect of IAA ad GA\(_3\) on underground (root) and upper ground parts (shoot) of plant respectively was observed. Hartman et al (1990) and Davies (1995) reported the same findings. Weaver (1972) described early rooting in IAA treated pots. This may be because auxins stimulate root initials (Gurumurthi et al., 1984 and Klass et al 1987). Ahmed et al (2012) reported increased growth in IAA treated orange plants. The reason of this result was enlarged leaf areas. In this reverence, Leopold and Kriedemann (1975) reported the capability of IAA on cell division and cell extension. Leben and Barton (1957); Castro and Bergann (1973) on bean crop and Agarwal et al (1994) on Trifolium reported increase in dry matter production after the foliar application of growth regulators. In present study GA\(_3\) showed reduction in plant biomass and the results are friendly with the findings of with the findings of Saleem et al (2007) who worked on Citrus sinensis. Stewart et al (1951) also reported the same results. The present results are not in harmony with the findings of Ouzounidou et al (2011) who reported increment in plant biomass while working on onion and garlic.

GA\(_3\) caused increase in plant dry matter and the results are not in accordance to the work of Tanimoto et al (1990); Maske et al (1997) who worked on soybean. Sharma and Kumar (2011) reported low and high quantities showed boosted effect of IAA and GA on the growth attributes of Chlorophyllum tuberosum and Pergularia daemia respectively. Shah (2007) stated that supplementation of plants with GA\(_3\) markedly improved the productivity (Fig. 4.1 and Fig. 4.2,) and (dry weight) of black seed. Present results (Fig. 4.3 and Fig. 4.4) are not loyal with this statement. Phytohormones i.e. IAA, GA\(_3\) showed significant effect on Coleus barbatus tubers (Gupta et al., 2010). Mukhtar (2004) and Chauhan et al (2009) agree with these findings. It might be due to increased absorption of soil nutrients that is triggered by plant hormones. Biswas and Chaudhari (1997) and Malik et al (1986) performed trials on Arachis hypogea and revealed the same findings. Qadeer (1996) reported broad, plush green and slender, nimble green leaves treated with IAA and GA\(_3\) in maize respectively.

Fig. 4.5 and Fig. 4.6 displayed that there is increase in shoot as well as in root length under the influence of GA\(_3\). On the other hand IAA caused decrease in these parameters. The reason may be expansion in diameter and the results are in agreement with the findings reported by Pilot and Saugy (1985). Lee et al (2000) working on Zinnia cultures also reported similar results. GA\(_3\) caused cell division and cell elongation and the results are similar with the findings of Daykin et al (1997) and Hisamatsu et al (1998). These results are committed with the findings of Ahmed et al. (2012) worked on Valencia orange trees. IAA is responsible for the induction of roots with increased number of root hairs and root laterals subsequently there is meaningful increase in the shoot and root weight Shahab et al (2009). GA\(_3\) showed more pronounced effect on plant height (shoot length and root length) and this statement is supported by the work of Liu and Loy (1976). This may be due to effect of GA\(_3\) on wall extensibility (Huttly and Phillips 1995) and expansion, elongation of internodes (Moore 1989) due to influence of GA\(_3\) on improved absorption during cell division. Increase in plant length exhibited narrowing of shoot.

Naeem et al (2004) reported marked increase in the shoot of lentil after GA\(_3\) application. Analogous findings were reported by Chaudhry (1997). The increase in length was accompanied by inhibition in the diameter. Reason of length interpreted asGA\(_3\) decreased the availability of available sugars causing thinning of shoot diameter. Moreover, Chaudhry and Zahir (1992) working on Abelmoschus esculentus L. and Chudharyand Khan (2000) working on Cicer arietnum. reported same findings. Hernandez, 1997; Bagatharia and Chanda 1998 also reported the upsurge of internodes in a number of crops. Gibberellins created elongation of stem nodes by enhancing cell division and cell...
wall extensibility (Taiz and Zeiger 1998). Unamba et al (2009) documented the effect of gibberellins on increased germination and plant length while working on dwarf okra. Brumbaugh (2009) received same results on pea. It was concluded that the plant hormones have substantial influence on the morphological nature of the Nigella plant. Observations presented in this study showed a broad covenant with previous studies described in this research.

References
FIG. 1: EFFECT OF TWO LEVELS OF IAA AND GA$_3$ ON ROOT FRESH WEIGHT OF BLACK SEED

To = Control (Tap water), T1 = 30ppm of IAA, T2 = 60ppm of IAA, T3 = 30ppm of GA$_3$, T4 = 60ppm of GA$_3$.

FIG. 2: EFFECT OF TWO LEVELS OF IAA AND GA$_3$ ON SHOOT FRESH WEIGHT OF BLACK SEED

To = Control (Tap water), T1 = 30ppm of IAA, T2 = 60ppm of IAA, T3 = 30ppm of GA$_3$, T4 = 60ppm of GA$_3$. 
To = Control (Tap water), T1 = 30ppm of IAA, T2 = 60ppm of IAA, T3 = 30ppm of GA3, T4 = 60ppm of GA3.
To = Control (Tap water), T1 = 30 ppm of IAA, T2 = 60 ppm of IAA, T3 = 30 ppm of GA₃, T4 = 60 ppm of GA₃.

FIG. 5: EFFECT OF TWO LEVELS OF IAA AND GA₃ ON ROOT LENGTH OF BLACK SEED

FIG. 6: EFFECT OF TWO LEVELS OF IAA AND GA₃ ON SHOOT LENGTH OF BLACK SEED

To = Control (Tap water), T1 = 30 ppm of IAA, T2 = 60 ppm of IAA, T3 = 30 ppm of GA₃, T4 = 60 ppm of GA₃.
To= Control (Tap water), T1= 30ppm of IAA, T2= 60ppm of IAA, T3= 30ppm of GA3, T4= 60ppm of GA3.

**Table: 1a ANALYSIS OF VARIANCE (ANOVA) FOR ROOT FRESH WEIGHT OF BLACK SEED TO FOLIAR APPLICATION OF IAA AND GA3 AT SEEDLING STAGE**

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA</td>
<td>0.011</td>
<td>2</td>
<td>0.006</td>
<td>11.804</td>
<td>0.003 **</td>
</tr>
<tr>
<td>GA3</td>
<td>3.645</td>
<td>2</td>
<td>1.823</td>
<td>0.377</td>
<td>0.035 *</td>
</tr>
<tr>
<td>IAA x GA3</td>
<td>2.19</td>
<td>4</td>
<td>0.547</td>
<td>0.113</td>
<td>0.975 ns</td>
</tr>
<tr>
<td>Error</td>
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<td>9</td>
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<td>Total</td>
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<td>17</td>
<td></td>
<td></td>
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</table>

**Table: 1b ANALYSIS OF VARIANCE (ANOVA) FOR ROOT FRESH WEIGHT OF BLACK SEED TO FOLIAR APPLICATION OF IAA AND GA3 AT VEGETATIVE STAGE**

<table>
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<tr>
<td>IAA</td>
<td>0.016</td>
<td>2</td>
<td>0.008</td>
<td>7.598</td>
<td>0.011 *</td>
</tr>
<tr>
<td>GA3</td>
<td>0.024</td>
<td>2</td>
<td>0.012</td>
<td>11.371</td>
<td>0.003 **</td>
</tr>
<tr>
<td>IAA x GA3</td>
<td>0.027</td>
<td>4</td>
<td>0.007</td>
<td>6.587</td>
<td>0.009 **</td>
</tr>
<tr>
<td>Error</td>
<td>0.009</td>
<td>9</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.076</td>
<td>17</td>
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</table>
### Table: 2a ANALYSIS OF VARIANCE (ANOVA) FOR SHOOT FRESH WEIGHT OF BLACK SEED TO FOLIAR APPLICATION OF IAA AND GA\textsubscript{3} AT SEEDLING STAGE

<table>
<thead>
<tr>
<th>Source</th>
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<th>MS</th>
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<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA</td>
<td>0.234</td>
<td>2</td>
<td>0.117</td>
<td>93.089</td>
<td>0.000***</td>
</tr>
<tr>
<td>GA\textsubscript{3}</td>
<td>0.066</td>
<td>2</td>
<td>0.033</td>
<td>26.167</td>
<td>0.000***</td>
</tr>
<tr>
<td>IAA x GA\textsubscript{3}</td>
<td>0.053</td>
<td>4</td>
<td>0.013</td>
<td>10.523</td>
<td>0.001**</td>
</tr>
<tr>
<td>Error</td>
<td>0.011</td>
<td>9</td>
<td>0.001</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td>0.364</td>
<td>17</td>
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### Table: 2b ANALYSIS OF VARIANCE (ANOVA) FOR SHOOT FRESH WEIGHT OF BLACK SEED TO FOLIAR APPLICATION OF IAA AND GA\textsubscript{3} AT VEGETATIVE STAGE

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>IAA</td>
<td>0.137</td>
<td>2</td>
<td>0.069</td>
<td>3.433</td>
<td>0.048*</td>
</tr>
<tr>
<td>GA\textsubscript{3}</td>
<td>0.007</td>
<td>2</td>
<td>0.003</td>
<td>0.171</td>
<td>0.846 ns</td>
</tr>
<tr>
<td>IAA x GA\textsubscript{3}</td>
<td>0.528</td>
<td>4</td>
<td>0.132</td>
<td>6.619</td>
<td>0.009**</td>
</tr>
<tr>
<td>Error</td>
<td>0.179</td>
<td>9</td>
<td>0.019</td>
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<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
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### Table: 3a ANALYSIS OF VARIANCE (ANOVA) FOR ROOT DRY WEIGHT OF BLACK SEED TO FOLIAR APPLICATION OF IAA AND GA\textsubscript{3} AT SEEDLING STAGE

<table>
<thead>
<tr>
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<tr>
<td>IAA</td>
<td>5.41</td>
<td>2</td>
<td>2.705</td>
<td>0.412</td>
<td>0.067**</td>
</tr>
<tr>
<td>GA\textsubscript{3}</td>
<td>5.336</td>
<td>2</td>
<td>2.668</td>
<td>0.406</td>
<td>0.068**</td>
</tr>
<tr>
<td>IAA x GA\textsubscript{3}</td>
<td>6.521</td>
<td>4</td>
<td>1.630</td>
<td>0.248</td>
<td>0.904 ns</td>
</tr>
<tr>
<td>Error</td>
<td>5.909</td>
<td>9</td>
<td>6.566</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>7.636</td>
<td>17</td>
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</table>
### Table 3b: Analysis of Variance (ANOVA) for Root Dry Weight of Black Seed to Foliar Application of IAA and GA3 at Vegetative Stage

<table>
<thead>
<tr>
<th>Source</th>
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</tr>
</thead>
<tbody>
<tr>
<td>IAA</td>
<td>0.009</td>
<td>2</td>
<td>0.004</td>
<td>1.229</td>
<td>0.337 ns</td>
</tr>
<tr>
<td>GA3</td>
<td>3.690</td>
<td>2</td>
<td>1.845</td>
<td>0.052</td>
<td>0.095 *</td>
</tr>
<tr>
<td>IAA x GA3</td>
<td>0.002</td>
<td>4</td>
<td>5.868</td>
<td>0.164</td>
<td>0.951 ns</td>
</tr>
<tr>
<td>Error</td>
<td>0.032</td>
<td>9</td>
<td>0.004</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.044</td>
<td>17</td>
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### Table 4a: Analysis of Variance (ANOVA) for Shoot Dry Weight of Black Seed to Foliar Application of IAA and GA3 at Seedling Stage

<table>
<thead>
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<tr>
<td>IAA</td>
<td>0.003</td>
<td>2</td>
<td>0.002</td>
<td>42.111</td>
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<tr>
<td>GA3</td>
<td>3.556</td>
<td>2</td>
<td>1.7778</td>
<td>4.607</td>
<td>0.042 *</td>
</tr>
<tr>
<td>IAA x GA3</td>
<td>3.248</td>
<td>4</td>
<td>8.119</td>
<td>2.104</td>
<td>0.163 ns</td>
</tr>
<tr>
<td>Error</td>
<td>3.473</td>
<td>9</td>
<td>3.859</td>
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</tr>
<tr>
<td>Total</td>
<td>0.004</td>
<td>17</td>
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### Table 4b: Analysis of Variance (ANOVA) for Shoot Dry Weight of Black Seed to Foliar Application of IAA and GA3 at Vegetative Stage

<table>
<thead>
<tr>
<th>Source</th>
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</tr>
</thead>
<tbody>
<tr>
<td>IAA</td>
<td>0.089</td>
<td>2</td>
<td>0.044</td>
<td>0.869</td>
<td>0.045 *</td>
</tr>
<tr>
<td>GA3</td>
<td>0.068</td>
<td>2</td>
<td>0.034</td>
<td>0.659</td>
<td>0.540 ns</td>
</tr>
<tr>
<td>IAA x GA3</td>
<td>0.161</td>
<td>4</td>
<td>0.040</td>
<td>0.782</td>
<td>0.564 ns</td>
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<tr>
<td>Error</td>
<td>0.464</td>
<td>9</td>
<td>0.052</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
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### Table 5a: Analysis of Variance (ANOVA) for Root Length of Black Seed to Foliar Application of IAA and GA₃ at Seedling Stage

<table>
<thead>
<tr>
<th>Source</th>
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<tr>
<td>IAA</td>
<td>6.574</td>
<td>2</td>
<td>3.287</td>
<td>2.648</td>
<td>0.125 ns</td>
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<tr>
<td>GA₃</td>
<td>4.909</td>
<td>2</td>
<td>2.454</td>
<td>1.977</td>
<td>0.013 *</td>
</tr>
<tr>
<td>IAA x GA₃</td>
<td>5.068</td>
<td>4</td>
<td>1.267</td>
<td>1.021</td>
<td>0.447 ns</td>
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<tr>
<td>Error</td>
<td>11.173</td>
<td>9</td>
<td>1.241</td>
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<tr>
<td>Total</td>
<td>27.724</td>
<td>17</td>
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</table>

### Table 5b: Analysis of Variance (ANOVA) for Root Length of Black Seed to Foliar Application of IAA and GA₃ at Vegetative Stage

<table>
<thead>
<tr>
<th>Source</th>
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<tr>
<td>IAA</td>
<td>1 2.434</td>
<td>2</td>
<td>6.217</td>
<td>4.027</td>
<td>0.050 *</td>
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<tr>
<td>GA₃</td>
<td>39.605</td>
<td>2</td>
<td>19.803</td>
<td>12.828</td>
<td>0.002 **</td>
</tr>
<tr>
<td>IAA x GA₃</td>
<td>19.843</td>
<td>4</td>
<td>4.961</td>
<td>3.214</td>
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<tr>
<td>Error</td>
<td>13.893</td>
<td>9</td>
<td>1.544</td>
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### Table 6a: Analysis of Variance (ANOVA) for Shoot Length of Black Seed to Foliar Application of IAA and GA₃ at Seedling Stage

<table>
<thead>
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<tr>
<td>IAA</td>
<td>45.868</td>
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<td>22.934</td>
<td>7.394</td>
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<tr>
<td>GA₃</td>
<td>34.445</td>
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<td>17.223</td>
<td>5.553</td>
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<tr>
<td>IAA x GA₃</td>
<td>19.93</td>
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<td>4.983</td>
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<td>Error</td>
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<td>3.101</td>
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<tr>
<td>Total</td>
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<td>--------</td>
<td>----</td>
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<tr>
<td>IAA</td>
<td>846.79</td>
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<td>423.395</td>
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<tr>
<td>GA₃</td>
<td>3261.627</td>
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<td>1630.814</td>
<td>1.206</td>
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<tr>
<td>IAA x GA₃</td>
<td>771.988</td>
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<td>12213.62</td>
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<td>357.069</td>
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Total 17094.025 17

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<tr>
<td>IAA</td>
<td>2.079</td>
<td>2</td>
<td>1.039</td>
<td>71.729</td>
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</tr>
<tr>
<td>GA₃</td>
<td>1.6812</td>
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<td>0.841</td>
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<td>0.000 ***</td>
</tr>
<tr>
<td>IAA x GA₃</td>
<td>2.085</td>
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<td>0.521</td>
<td>35.865</td>
<td>0.000 ***</td>
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<tr>
<td>Error</td>
<td>0.130</td>
<td>9</td>
<td>0.014</td>
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</table>

Total 5.976 17
The principal aim of the investigation was to explore the anti-diabetic potential of ethanolic extracts of aerial parts of Flemingia chappar Graham (Fabaceae) (FCE) against streptozotocin induced diabetes in rats. The shade dried leaves were pulverized and extracted by successive extraction in a soxhltor. Phytochemical studies showed that FCE contain significant quantities of flavanoids, saponins, carbohydrates and phytosterols. Results of acute toxicity studies suggest that extracts can be considered as category 4 as per OECD guidelines 420. In oral glucose tolerance test, FCE at the dose of 400mg/kg, p.o showed significant decrease in elevated blood glucose levels within 30 min compared to 150 min in normal animals. Anti-diabetic potential of test drug FCE at three different doses (100, 200, and 400 mg/kg b.w p.o) was studied on STZ induced diabetic rats for 21 days. Normal control, diabetic control and Standard (Glibencamide) groups were also conducted in similar fashion. Biochemical parameters such as blood glucose, TG, TC, HDLC, SGOT, SGPT, ALP, glycosylated Hb and weight of animals were noted at 0,7,14 and 21 days of anti-diabetic studies in STZ induced rats. Glycogen content in the liver and skeletal muscle was estimated after 21 days of treatment. The test drug showed progressive (P<0.001) reduction in blood glucose, TG, TC, HDLC, SGOT, SGPT, ALP, glycosylated Hb levels and elevation in HDLC, Liver & muscle glycogen levels and in a dose related manner during the period of study. The results suggested that the FCE at 400 mg/kg dose (96.13 %) after 21 days showed equipotent effect with that of standard drug glibenclamide (97.8 %).

Materials and Methods

Plant material
The plant was authenticated by Dr. B.Prathibha Devi, Professor & Head, Department of Pharmacy, Osmania University, Hyderabad. A.P. India and the specimens have been preserved in our research lab (GPWKDP/2009/YP/01).

Extraction
Shade dried crude drug was pulverized, sieved (10/44) and stored in air-tight containers. About 5000 grams of crude drug was extracted using AR grade solvents Petroleum ether (60 – 80°C), Benzene, Chloroform, Acetone, Ethyl acetate and Ethanol (95%) by successive soxhltion method until the phytoconstituents were completely exhausted. All the extracts were concentrated by using rota–vacuum evaporator (Buchi type, Mumbai, India) until a semisolid extract is obtained, dried at less than 50°C, comminuted in a ball mill and preserved in air tight containers kept in dessicators prior to its studies and labeled as FCE.

Materials
Streptozotocin was purchased from Sigma-Aldrich, India and the solution was prepared by freshly dissolving in citrate buffer (0.01 M, pH 4.5). Glibenclamide was procured from Cipla Ltd. Diagnostic kits used in this study were procured from Span Diagnostics Ltd., India. All the other chemicals used were of analytical grade.

**Phytochemical investigation**

Phytochemical tests for the extract was carried out (Kokate, 1994) to find out the presence of phytoconstituents viz flavonoids, phytosterols, phenolic compounds, carbohydrates, tannins, triterpenoids etc. and the results were given in Table 1.

**Pharmacological Studies**

**Acute toxicity studies: OECD Guidelines No. 420**

Female wistar rats (nulliparous and non-pregnant) of 8 to 10 weeks old weighing 200 – 250gms supplied by National Institute of Nutrition, Hyderabad, India, were individually housed in polypropylene cages lined with husk renewed every 24 h in well-ventilated rooms at 22±3°C and RH between 50 to 60, under artificial lighting12:12 h light and dark cycle in hygienic condition for at least five days prior to the study. The rats were fed with standard laboratory pellet diet (Hindustan lever) and water ad libitum. The studies were performed according to OECD Guidelines 420 and the protocol was approved by the Institutional Animal Ethics Committee (Reg. No. 1472/PO/a/CPCSEA).

**Sighting study**

Animals were fasted over-night prior to dosing and weighed. The test substance was administered to single animals in a sequential manner following the flow charts in Annex 2 of OECD 420. The starting dose for the sighting study was selected from the fixed dose levels of 300 mg/kg (as there is no evidence from in vivo and in vitro data). The next dose used for this study was 2000 mg/kg. The Test substances were administered in a constant volume of 2 mL/100g body weight in the form of suspension. After the substance has been administered, food was withheld for a further 3-4 h. A period of at least 24 hours was allowed between the dosing of each animal. All animals were observed for at least 14 days.

**Main study**

A total of five female wistar rats were used for each dose level investigated and the animals were made up of one animal from the sighting study dosed at the selected dose level together with an additional four animals. The time interval between dosing at each level was 3 or 4 days.

**Observations**

Animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 h with special attention given during the first 4 h and daily thereafter, for a total of 14 days. All observations were systematically recorded individually for each animal. Observations include changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous systems, somatomotor activity and behaviour pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Individual weights of animals should be determined shortly before the test substance was administered and at least weekly thereafter. Weight changes were calculated and recorded. At the end of the test surviving animals were weighed and then humanely killed. All animals were subjected to gross necropsy and pathological changes were recorded. Microscopic examination of organs was also done for evidence of gross pathology in animals surviving 24 or more hours after the initial dosing.

**Acute toxicity studies: OECD Guidelines No. 425**

Animals were divided into two groups of 3 animals each. Group I was treated with vehicle (distilled water) and was kept as a control. Group II was treated with 5000 mg/kg dose according to their body weight. Blood and tissue were collected on 14th day. Hematological and biochemical parameters were measured in treated group as well as in control group. The organs were quickly blotted and weighed in a digital balance. Gross necropsy of heart, liver and kidney were observed.

**SUB ACUTE TOXICITY STUDIES: OECD GUIDELINES NO. 407**

The plant extract at the dose of 250, 500 and 1000 mg/kg body weight were administered orally to 4 groups of six rats respectively to every 24 h for 28 days and control received vehicle at the same volume. The toxic manifestation such as body weight, mortality, and food and water intake was monitored. After 28 days all surviving animals were fasted overnight and anaesthetized with ether. The heparinised blood samples were collected for determining
haematological parameters and the serum from non-heparinised blood was carefully collected for determining clinical blood chemistry. Animals were sacrificed after blood collection and the internal organs were removed and weighed to determine the relative organ weights and observed for gross lesions. The internal organs were preserved in 10% buffered formaldehyde solution for histological examination.

**Oral Glucose Tolerance Test for FCE**

Wistar rats of either sex weighing 200 – 250gms obtained from National Institute of Nutrition, Hyderabad, India, were individually housed in polypropylene cages lined with husk renewed every 24 h in well-ventilated rooms at 22±3°C and RH between 50 to 60, under artificial lighting12:12 h light and dark cycle in hygienic condition for at least five days prior to the study. The rats were fed with standard laboratory pellet diet (Hindustan lever) and water *ad libitum*. The protocol was approved by the Institutional Animal Ethics Committee.

The animals were divided into five groups of six rats in each group.
Group-I: Administered glucose (2 g/kg p.o. b.w.)
Group-II: Administered glibenclamide (0.5 mg/kg) and glucose (2 g/kg p.o. b.w.).
Group-III: Administered FCE (100 mg/kg) and glucose (2 g/kg p.o. b.w.).
Group-IV: Administered FCE (200 mg/kg) and glucose (2 g/kg p.o. b.w.).
Group-V: Administered FCE (400 mg/kg) and glucose (2 g/kg p.o. b.w.).

The animals were fasted overnight and treated with above dosage schedule orally. The FCE and glibenclamide were administered half an hour before administration of glucose solution. Blood glucose levels were determined at 0 (before glucose challenge) 30, 90, 150th min after glucose administration. Serum was separated and glucose levels were measured immediately. The results were given in Table 2.

**Antidiabetic Activity on Streptozotocin Induced Diabetic Rats**

Wistar rats of either sex weighing 200 – 250gms obtained from National Institute of Nutrition, Hyderabad, India, were individually housed in polypropylene cages lined with husk renewed every 24 h in well-ventilated rooms at 22±3°C and RH between 50 to 60, under artificial lighting12:12 h light and dark cycle in hygienic condition for at least five days prior to the study. The rats were fed with standard laboratory pellet diet (Hindustan lever) and water *ad libitum*. The protocol was approved by the Institutional Animal Ethics Committee.

**Experimental Protocol**

About 40 animals as described above were selected for the experiment; six animals were kept separately as normal control group (Group I). Remaining 34 animals were made diabetic by a single intraperitoneal injection of Streptozotocin (50 mg/kg of body weight) dissolved in citrate buffer (0.01 M, pH 4.5). The blood glucose level was checked before and 72 h after STZ injection to confirm the development of diabetes. The diabetic animals were stabilized for five days and the next day (day 0) experiment was started. Only those animals which showed blood glucose levels >250 mg/dL were separated and used for the study (Kumar. S. et al. 2012) The rats were provided with 5% glucose solution bottles in their cages for the next 24 h to prevent hypoglycaemia (Stanley et al., 1998).

The rats were divided into nine groups each consisting of six rats.
Group-1: Normal control animals - Administered 1%w/v sodium CMC p.o.
Group-2: Diabetic control animals - Administered STZ (50 mg/kg, i.p.).
Group-3: STZ (50 mg/kg, i.p.) induced diabetic animals administered glibenclamide 0.5 mg/kg p.o. once daily for 21 days
Group-4: STZ (50 mg/kg, i.p.) induced diabetic animals administered FCE 100 mg/kg in 1%w/v sodium CMC p.o. once daily for 21 days.
Group-5: STZ (50 mg/kg, i.p.) induced diabetic animals administered FCE 200 mg/kg in 1%w/v sodium CMC p.o. once daily for 21 days.
Group-6: STZ (50 mg/kg, i.p.) induced diabetic animals administered FCE 400 mg/kg in 1%w/v sodium CMC p.o. once daily for 21 days.

**Biochemical estimations**

Blood samples were collected from the animals prior to the treatment with above schedule and after 30 min of Glibenclamide/test drug administration on 7th, 14th and 21st day. Blood obtained from the retro orbital venous plexus of rats under ether anesthesia using a glass capillary tube, centrifuged (2,500 rpm/10 min) to separate serum. The
serum was used for biochemical estimation of blood glucose, triglycerides, Total cholesterol, HDL-cholesterol, Glycosylated haemoglobin, SGOT, SGPT and ALP.

Collection of organs
After 21 days of daily feeding of the test and the standard drugs orally, the animals were euthanized by overdose of ether anaesthesia. Liver and skeletal muscle tissue samples were collected for the assessment of glycogen content.

Analysis

**Estimation of Plasma glucose, serum total cholesterol (TC), HDL-cholesterol, serum triglycerides (TG), SGOT, SGPT, ALP, Glycogen content**

Glucose levels were estimated by commercially available glucose kits (Span Diagnostics Ltd, Surat, India) based on glucose oxidase method. The total cholesterol was estimated by the one step method (Wybenga et al., 1970). HDL-cholesterol level was determined by the commercially available reagent kit (Erba Mannheim, Transansa biomed &Daman, India) based on phosphotungustate method (Herbert, 1984). The serum triglyceride level is estimated by the method Enzymatic GPO (Werner et al., 1981). Serum transaminase activity was measured according to the method of Reitman and Frankel(1957). Serum alkaline phosphatase was estimated by following the method of Kind and King’s (1976). Blood glycosylated hemoglobin was measured using spectrophotometric method. (Tinder P. 1969). Glycogen content in the liver/skeletal muscle tissue was estimated by the method of Plummer et al., 1978 and the glycogen content was expressed as g/g of tissue. The results were given in Tables 3,4,5&6 and represented graphically in Figures 1,2, 3 & 4.

**Weight of animals**
The results were given in Table 6 and represented graphically in Fig 5.

Statistical analysis

Data were expressed as mean ± SEM, (n=6). Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison. Values were considered statistically significant when at p<0.05.

Results and Discussion

Phytochemical studies

Table 1 represents the results of phytochemical analysis of the ethanolic extracts of *Flemingia chappar* Linn (Fabaceae) (FCE). The results showed the presence of flavonoids, glycosides, phenolic compounds, proteins, saponins, and phytosterols in varied quantities in appreciable amounts. As many as 150 plants having flavanoids, steroids and tannins as active principles were found to possess antidiabetic potential (Pusparaj et al., 2000; Meiselman et al., 1976; Choi et al., 1991; Ernmenisogiu et al., 1995). Hence the extracts were selected for antidiabetic studies.

Acute oral toxicity studies

In acute and subacute oral toxicity study, the FCE did not show any sign and symptoms of toxicity or mortality up to 2000 mg/kg. Hence, these could be considered as category 4 as per OECD guidelines.

Effect on oral glucose tolerance

Table 2 represents the results of oral glucose tolerance test. The blood glucose levels in the control group (G-I) increased to peak level at 30 min after glucose load and decreased to near normal at 150 min. Group-II (glibenclamide 0.5 mg/kg, p.o) and Group V (FCE 400mg/kg, p.o) showed significant decrease in elevated blood glucose levels at 30 min. Group III and IV showed significant decrease in blood glucose levels at 90 min. Results also suggest that the FCE have not decreased the blood glucose levels below normal levels.

Antidiabetic Activity of FCE

Effect on serum glucose levels

Table 5 and Fig 1 represent the results of the study of antidiabetic effect of FCE on STZ induced diabetic rats. The diabetic control group (G-II) showed a significant increase in the serum glucose levels on the 7th, 14th and 21st day in comparison to the normal control (G-I) indicating STZ induced persistent diabetes mellitus. Significant decrease in
blood glucose levels on the 7th day in Standard group (G-III) administered glibenclamide, 0.5 mg/kg, p.o, once daily. The serum glucose levels reached to near normal on day 14 and 21. Similarly in groups G-IV to G-VI (FCE 100, 200 and 400 mg/kg, p.o, once daily), a significant and dose dependent decrease in the serum glucose levels on 7th, 14th and 21st day was observed. G-VI and G-VI showed equipotent activity with G-III. The plausible mechanism behind the antidiabetic potential of FCE may be due to the presence of flavanoids, steroids and tannins which may have increased the activity of enzymes responsible for utilization of glucose by insulin-dependent pathway or regenerate β-cells in pancreatic islets. (Lenzen and panten, 1988; Ahmed et al., 1991; Jorns et al., 1997; Sakurai et al., 2001)

**Effect on biochemical parameters**

Serum levels of TC, TG, SGOT, SGPT, ALP, Glycosylated haemoglobin were decreased progressively after 21 days of treatment in a dose-related manner. It is also observed that HDL-Cholesterol levels were increased progressively during the period of study. In this study, the FCE at 400 mg/kg p.o significantly decreased TC (88.79%), TG (89.29%), SGOT (89.3%), SGPT (95.1%), ALP (97.4%), Glycosylated haemoglobin (87.2%) and significantly increased HDL-cholesterol level (56.32%). Liver & muscle glycogen contents were increased significantly after 21 days of treatment of test extracts in a dose-related manner. Similarly, the weight of the animals also gained to normal in the treated animals. The increase in HDL-cholesterol levels is very beneficial because of its protective effects in prevention of cardiovascular diseases. This could be due to the presence of other hypolipidemic agents such as β-sitosterol in the leaf extract (Rajalingam et al., 1993; Pathak et al., 1981; Kaleem et al., 2006).

Since STZ permanently destroys the pancreatic β cells, lowering of blood glucose level in Streptozotocinised rats after administration of the extracts indicates that the extract possesses extra pancreatic effects (Bhupesh et al., 2008). On the basis the antidiabetic activity of FCE may be due to presence of flavanoids, steroids and tannins (Iwu, 1983; lwu, 1980).

**Conclusion**

The ethanolic extract of *Flemingia chappar* at the dose of 400 mg/kg body weight showed a significant antidiabetic activity and its efficacy is on par with the standard drug, Glibenclamide 0.5 mg/kg, p.o. This may be due to the presence appreciable quantities of phytoconstituents such as flavanoids, glycosides, phenolic compounds, proteins, saponins, Proteins, Triterpenoids and steroids. One possible mechanism of action postulated is stimulation of insulin secretion there by improvement of glycogenesis process. Further studies are in progress to isolate the active principle(s) in the extracts as well as to elucidate their exact mechanism(s) of action. As the extracts are found to be considered as potentially safe i.e. category 4 as per the OECD guidelines, and showed equipotent antidiabetic efficacy, these extracts may be used as alternative for patients in the management of type 2 diabetes mellitus.

**Table 1: Phytochemical analysis of FCE**

<table>
<thead>
<tr>
<th>Chemical Constituents</th>
<th>FCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+++</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+++</td>
</tr>
<tr>
<td>Phenolics</td>
<td>++</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+++</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
</tr>
</tbody>
</table>

+ Positive, - Negative, FCE= ethanolic extract of *Flemingia chappar*. 
### Table 2: Effect of FCE on Oral glucose tolerance test

| Group | Blood glucose levels (mg/dL) |
|---|---|---|---|---|
|    | Initial | 30 min | 90 min | 150 min |
| I  | 83.67 ±0.33 | 139.2 ±1.10 | 127.9 ±1.66 | 89.50 ±1.05 |
| II | 79.83 ±0.60 | 119.5 ±0.76 | 86.0 ±1.50*** | 75.33 ±1.3*** |
| III| 79.33 ±0.61 | 131.0 ±0.41 | 122.8 ±0.98** | 84.33 ±0.95* |
| IV | 80.50 ±0.71 | 126.7 ±1.74 | 100.7 ±1.02** | 79.83 ±1.16* |
| V  | 79.67 ±0.55 | 123.3 ±3.30 | 94.33 ±0.66*** | 76.0 ±1.23** |

Each value represents the mean ± SEM. n = 6 number of animals in each group. Values**P<0.001, *P<0.01, #P<0.05.compared to positive control

### Table 3: Effect of FCE on serum levels of Total Cholesterol, HDL-Cholesterol and Triglycerides

<table>
<thead>
<tr>
<th></th>
<th>TC</th>
<th>HDL-Cholesterol</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
<td>Day 14</td>
</tr>
<tr>
<td>Group 1</td>
<td>103±4.5</td>
<td>102±3.3</td>
<td>103±3.6</td>
</tr>
<tr>
<td>Group 2</td>
<td>206±6.5</td>
<td>204±5.6</td>
<td>205±6.4</td>
</tr>
<tr>
<td>Group 3</td>
<td>204±6.1</td>
<td>171±4.3</td>
<td>139±7.5</td>
</tr>
<tr>
<td>Group 4</td>
<td>205±6.5</td>
<td>197±6.5</td>
<td>173±4.6</td>
</tr>
<tr>
<td>Group 5</td>
<td>205±6.5</td>
<td>181±5.6</td>
<td>153±4.6</td>
</tr>
<tr>
<td>Group 6</td>
<td>204±6.3</td>
<td>175±4.7</td>
<td>142±6.8</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM. n = 6 number of animals in each group. Values**P<0.001, *P<0.01, P<0.05.compared to positive control

### Table 4: Effect of FCE on SGOT, SGPT and ALP

<table>
<thead>
<tr>
<th></th>
<th>SGOT</th>
<th>SGPT</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
<td>Day 14</td>
</tr>
<tr>
<td>Group 1</td>
<td>33±2.4</td>
<td>34±2.5</td>
<td>35±2.4</td>
</tr>
<tr>
<td>Group 2</td>
<td>73±2.2</td>
<td>74±2.1</td>
<td>74±2.0</td>
</tr>
<tr>
<td>Group 3</td>
<td>73±2.3</td>
<td>61±2.4</td>
<td>43±2.3</td>
</tr>
<tr>
<td>Group 4</td>
<td>73±4.3</td>
<td>69±2.4</td>
<td>51±2.4</td>
</tr>
<tr>
<td>Group 5</td>
<td>74±2.1</td>
<td>67±2.1</td>
<td>47±1.6</td>
</tr>
<tr>
<td>Group 6</td>
<td>72±6.8</td>
<td>63±2.3</td>
<td>45±0.9</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM. n = 6 number of animals in each group. Values**P<0.001, *P<0.01, #P<0.05.compared to positive control
Table 5: Effect of FCE on serum levels of Glycosylated haemoglobin and Glucose

<table>
<thead>
<tr>
<th>Group</th>
<th>Glycosylated Hb (%)</th>
<th>Glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
</tr>
<tr>
<td>Group 1</td>
<td>8.1±0.9</td>
<td>9.1±0.9</td>
</tr>
<tr>
<td>Group 2</td>
<td>13.5±1.2</td>
<td>12.7±1.5</td>
</tr>
<tr>
<td>Group 3</td>
<td>13.9±1.5</td>
<td>11.5±1.3</td>
</tr>
<tr>
<td>Group 4</td>
<td>13.7±0.9</td>
<td>12.9±1.4</td>
</tr>
<tr>
<td>Group 5</td>
<td>13.6±0.4</td>
<td>12.5±1.5</td>
</tr>
<tr>
<td>Group 6</td>
<td>13.8±1.1</td>
<td>11.9±1.4</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM. n = 6 number of animals in each group. Values**P<0.001, *P<0.01, #P<0.05 compared to positive control

Table 6: Effect of FCE on Liver glycogen, Muscle glycogen and Weight of the animals

<table>
<thead>
<tr>
<th>Group</th>
<th>Glycosylated Hb</th>
<th>Glycogen (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
</tr>
<tr>
<td>Group 1</td>
<td>8.1±0.9</td>
<td>9.1±0.9</td>
</tr>
<tr>
<td>Group 2</td>
<td>13.5±1.2</td>
<td>12.7±1.5</td>
</tr>
<tr>
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<td>13.9±1.5</td>
<td>11.5±1.3</td>
</tr>
<tr>
<td>Group 4</td>
<td>13.7±0.9</td>
<td>12.9±1.4</td>
</tr>
<tr>
<td>Group 5</td>
<td>13.6±0.4</td>
<td>12.5±1.5</td>
</tr>
<tr>
<td>Group 6</td>
<td>13.8±1.1</td>
<td>11.9±1.4</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM. n = 6 number of animals in each group. Values**P<0.001, *P<0.01, #P<0.05 compared to positive control

Figure 1: Effect of FCE on serum levels of Glucose

Each value represents the mean ± SEM. n = 6 number of animals in each group.
Figure 2: Effect of FCE on serum levels of Total Cholesterol, HDL-Cholesterol and Triglycerides

Each value represents the mean ± SEM. n = 6 number of animals in each group.

Figure 3: Effect of FCE on SGOT, SGPT and ALP

Each value represents the mean ± SEM. n = 6 number of animals in each group.

Figure 4: Effect of FCE on serum levels of Glycosylated haemoglobin and Liver and Muscle Glycogen

Each value represents the mean ± SEM. n = 6 number of animals in each group.
Figure 5: Effect of FCE on Weight of animals

Each value represents the mean ± SEM. n = 6 number of animals in each group.

References
SPRAY DRYING: A REVIEW

Ayesha Sethi¹, Hira Ijaz¹, Farzana Chaudry²
¹College of Pharmacy, Govt. College University Faisalabad, ²Director
University of Veterinary and Animal Sciences, Lahore, Pakistan

Abstract:
Development of hydrophobic drugs in oral dosage form is a great challenging because of their poor wetting properties, bioavailability and dissolution. Oral dosage form containing hydrophobic drugs have problems like poor aqueous solubility, poor wetting, adhesion, poor powder flow and poor drug content uniformity. There are many different approaches which may be adopted to attempt to solve these problems. These include solid dispersion, particle size reduction, supercritical fluid technology and etc. Spray drying has a broad range of different application in pharmaceutical industry and it looks like increasingly the enhancement of the solubility and dissolution rate of poorly water soluble drug will be one of most important.

Introduction
In pharmaceutical technology, spray drying appears as unique drying technique. It is a unit operation that alters the fluid into desiccated product in single step. It is a conversion of feed from a liquid form into a dehydrated particles form by spraying the feed into a hot drying medium. One of important purpose of drying by this method in pharmaceutical technology is to obtain dry powder particles with specific characteristics. This method removes moisture and humidity instantly and converts pump-able liquid into dry powder.

Because of relative simplicity of this method, there are several inherent advantages which make this process ideal for handling a wide variety of products (Cal et al., 2010, Parikh 1997). In the pharmaceutical field it is used for simple drying operations to particle engineering of bulk pharmaceutical ingredients (API) and excipients, encapsulation and pulmonary infection (Vehring et al., 2007). It is also used for processing biopharmaceutical products such as peptides and proteins and vitamins. Spray drying process is very rapid solvent evaporation (Paudel et al., 2012).

Background of The Spray Drying Process
The idea of spray drying, comprising of converting of the feed from fluid state into a transformed dried particulate form by spraying the feed into a gaseous drying medium. The pioneer application of this method originate from the year 1860 and the first patent concerning spray drying process was registered in 18721. The true great prosperity or rapid development in this technology was given by World War II, during which the need for transport of huge amounts of food raised, which cause to develop and formulate new methods to reduce food's weight and volume, as well as explore for new and more useful methods. During all these research and struggling, spray drying emerged as the best drying process to accomplish all requisition and specifications. Many years researches into spray drying processes, cause it to be most commonly and frequently used technique in drying process and pharmaceutical technology and is the area of interest for scientist concerning drug discovery and development (Cal et al., 2010). Spray drying has been employed for decades and found and was the first method to use in dairy industry.

Spray Drying and Overview of General Principle of Spray Drying Process
Spray drying process changes the solution, suspension or emulsion into a solid powder in one step. It is a well-known process in many manufacturing sectors such as pharmaceuticals, food, chemical or material science. The process of spray drying encompasses three important stages. The first step is to produce atomization of the fluid stream by suitable tool. Further, fine droplets of the feed are forced to contact with a drying gas at sufficient elevated which is higher than feed temperature. In the meantime of drying period, the solvent within the dispersion droplets is evaporated which result in the accumulation of the solid product particles. At the last, the particles which are dried, must be detached from the drying gas by appropriated tool (cyclone device) and must be collected by a receptacle tank. Each of the phase which are described and condition under which these phases are carried out, showed impact on the productiveness of drying process and quality of final product or dosage form. (Cal et al., 2010).
Spray Drying and Poorly Water Soluble Drugs

The principal aim of spray drying is to produce amorphous materials and it is also used to obtain particle size reduction, drying method and to generate nanoparticles of hydrophobic drugs (Heng et al., 2011, Peltonen et al., 2012, Vehring et al., 2008).

To carry out the spray drying of poorly aqueous soluble drugs, it should dissolve in volatile organic solvent or mixture of solvent but the final state of the product depend on the chemical nature of drug (Corrigan, et al., 1995). The spray drying of API like indomethacin and itraconazole produce particles which have glassy shape with substantial solubility while the drug product like naproxen shows no amorphization on spray drying (Mahlin et al., 2011).

Dissolution and solubility of hydrophobic drugs

The oral route is very much a convenient and feasible route of drug administration. It gives rise to better patient compliance and administration is easy. For a drug to be administered in the oral dosage form it has to be dissolved in gastric fluid. The formulation of hydrophobic drugs which are poorly water soluble is therefore difficult and requires special consideration and techniques. For hydrophobic drugs dissolution is the rate limiting step (Elkordy et al., 2010). Accordingly there is a need to improve the dissolution properties of hydrophobic drugs to ensure good bioavailability.

This poor solubility and poor dissolution of hydrophobic drugs is a major league for Scientists concerning research and development. Poor aqueous solubility is the major barrier in the formulation and development of new drugs to beat poorly water soluble drugs in terms of their in vivo performance due to their insufficient property to be dissolved and wetted by body fluid. Bearing this in mind, drugs may be classified according to their solubility and permeability (Table 1) and also drug may be described according to their solubility (Table 2). It has been estimated that about 40% of pharmaceutical compounds may be considered as poorly water soluble. Some illustrative examples are shown in Table 3.

### Table 1. The Biopharmaceutical Classification System

<table>
<thead>
<tr>
<th>CLASS</th>
<th>SOLUBILITY</th>
<th>PERMEABILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>HIGH</td>
<td>HIGH</td>
</tr>
<tr>
<td>II</td>
<td>LOW</td>
<td>HIGH</td>
</tr>
<tr>
<td>III</td>
<td>HIGH</td>
<td>LOW</td>
</tr>
<tr>
<td>IV</td>
<td>LOW</td>
<td>LOW</td>
</tr>
</tbody>
</table>
The dissolution and solubility of hydrophobic drugs is improved by spray drying. It mainly aimed at generating amorphous materials. It is possible when they are soluble in a volatile organic solvent or a mixture of solvents.

**Importance of Solubility and Dissolution**

The poor solubility and low dissolution of hydrophobic drugs in gastrointestinal fluid cause imperfect bioavailability particularly for class II substances according to BCS (as mentioned above in Table 1), the bioavailability can be enhanced by increasing the dissolution and solubility in GIT fluid. (Savjani, et al., 2012)

Breakdown of the pharma new chemical entity pipeline through Biopharmaceutical Classification System is shown in Fig 1

The negative effect of compounds with low solubility include poor absorption and bioavailability, insufficient solubility for IV dosing, development challenges leading to increasing the development cost and time, burden shifted to patient (frequent high-dose administration. (Savjani et al., 2012).

The compounds which have aqueous solubility less than 10 mg / mL may exhibit an incomplete, erratic and/or slow absorption rate (Lloyd V et al., 2005). Like cyclosporine which is an immunosuppressant and sparing water soluble and is the cause of unwanted effects such as unpredictable oral absorption profile, poor oral bioavailability and cause confusion in formulation processes (Boukhris et al., 2012).

<table>
<thead>
<tr>
<th>Definition</th>
<th>Part of solvent required for one part of solute</th>
</tr>
</thead>
<tbody>
<tr>
<td>very soluble</td>
<td>&lt;1</td>
</tr>
<tr>
<td>freely soluble</td>
<td>1-10</td>
</tr>
<tr>
<td>Soluble</td>
<td>10-30</td>
</tr>
<tr>
<td>sparingly soluble</td>
<td>30-100</td>
</tr>
<tr>
<td>slightly soluble</td>
<td>100-1000</td>
</tr>
<tr>
<td>very slightly soluble</td>
<td>1000-10000</td>
</tr>
<tr>
<td>Insoluble</td>
<td>&gt;10000</td>
</tr>
</tbody>
</table>
Lead compound having poor "drug like "properties led to incomplete absorption at the administration site which contribute to the high clinical failure because of poor pharmacokinetics. (Viral et al., 2010)

The complications are more extreme for drugs such as itraconazole and carbamazepine (belonging to class III as classified by Washington 1996), they are poorly soluble in both aqueous and water media and for a drug having log p value 2 .Such drugs often have an incomplete and slow absorption profile and bioavailability is uncertain because their performance is dissolution-rate limited and is affected by the fed/fasted state of the patient (Patravale et al., 2003).

Table 3: List of water insoluble drug , category and solubility profile .(adapted from Kaur et al.,2012)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Category</th>
<th>Solubility profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ibuprofen</td>
<td>Anti-inflammatory</td>
<td>Ibuprofen is only slightly soluble in water (&lt;1 mg/mL)</td>
</tr>
<tr>
<td>2 furosemide</td>
<td>diuretics</td>
<td>Much more soluble in alcohol/water mixture</td>
</tr>
<tr>
<td>3 gliclazide</td>
<td>Anti-diabetic</td>
<td>Soluble in acetone ,sparsingly soluble</td>
</tr>
<tr>
<td>4 glipizide</td>
<td>Anti-diabetic</td>
<td>Ethanol (95%), slightly soluble in ether sparingly soluble in dichloromethane, slightly soluble in ethanol 95%. Soluble in ether, sparingly soluble ethanol (95%), slightly soluble in acetone</td>
</tr>
<tr>
<td>5 aceclofenac</td>
<td>Anti-inflammatory. Analgesic</td>
<td>Practically insoluble in water, free soluble in acetone, soluble in ethanol in 95%</td>
</tr>
<tr>
<td>6 indomethacin</td>
<td>Anti-inflammatory, analgesic</td>
<td>Soluble in chloroform sparingly soluble in ethanol 95%.</td>
</tr>
<tr>
<td>7 ketoprofen</td>
<td>Anti-inflammatory, analgesic</td>
<td>Freely soluble in ethanol 95 %, chloroform and ether.</td>
</tr>
<tr>
<td>8 diclofenac</td>
<td>Anti-inflammatory</td>
<td>Freely soluble in methanol, Soluble in ethanol(95%), sparingly soluble in water and glacial acetic acid</td>
</tr>
<tr>
<td>9 felodipine</td>
<td>Calcium Channel blocker</td>
<td>Sparingly soluble in dichloromethane, slightly soluble in ethanol 95%.</td>
</tr>
<tr>
<td>10 loperamide</td>
<td>Anti diarrheal</td>
<td></td>
</tr>
<tr>
<td>11 morphine</td>
<td>NSAID</td>
<td>Soluble in water, Freely soluble in hot Water, more soluble in ethanol.</td>
</tr>
<tr>
<td>12 naproxen</td>
<td>Anti-inflammatory</td>
<td>Soluble in water, freely soluble in hot Water, more soluble in ethanol.</td>
</tr>
<tr>
<td>13 numodipine</td>
<td>Calcium Chanel Blocker</td>
<td>Poor water soluble drug.</td>
</tr>
<tr>
<td>14 ofloxacin</td>
<td>Antibiotic</td>
<td>Soluble in ethanol and chloroform, insoluble in ether.</td>
</tr>
<tr>
<td>15 griseofulvin</td>
<td>Antifungal</td>
<td>Practically insoluble in water</td>
</tr>
<tr>
<td>16 carbamazepine</td>
<td>Antiepilectic</td>
<td>Practically insoluble in water</td>
</tr>
<tr>
<td>17 itraconazole</td>
<td>Antifungal</td>
<td>Poor soluble in both aqueous and organic media</td>
</tr>
<tr>
<td>18 simvastatin</td>
<td>Cholesterol lowering agent</td>
<td>Poor soluble in both aqueous and organic media</td>
</tr>
</tbody>
</table>

Uses of Spray Drying Process
Spray drying has wide range of application within the chemical industry, food industry, and Biochemical and Pharmaceutical industry. It is also used in the processing of milk, eggs, ceramics, and several chemicals. (Broadhead et al 1992)

The process of spray drying can be applied for drying to any substances in solution or suspension.it is most useful for thermolabile materials .Examples of both soluble and insoluble substances that are spray dried are citric acid, calcium phosphate, sodium phosphate,., gelatine ,starch and barium sulphate. The process is also used in the formulation of dried antibiotic where spray dried powder is packed and distributed and reconstituted as syrup at the time of dispensing.
Application of Spray Drying to Pharmaceuticals

Spray drying is not a new technology as far as pharmaceutical industry is concerned. It has been used successfully since early 1960’s. It is one of the useful method to process pharmaceutical products since it offers a way to obtain powders with predetermined properties like particle size and shape. In addition to this, a variety of formulation process like encapsulation, complex formation and even polymerization can be accomplished in one step in a spray dryer. It is also convenient method for drying heat sensitive drugs like proteins with minimal loss of activity. (Broadhead et al, 1992)

Spray drying has been used in the pharmaceutical industry since the 1940 for the production of drug substances and various excipients. It is used to modify the biopharmaceutical properties of the drug substances such as to improve or increase the dissolution rate of hydrophobic drugs (Parikh, 1997).

Some of the applications of spray drying and spray dried excipients technology to the pharmaceutical industry is given below:

Advantages of Spray Drying

Spray drying is a continuous process. As long as liquid can continue to be supplied to the drying system the powder product will continue to be produced. In some case, this process has been operated and continued for months without interruption. In actual, this process is very rapid, with the main portion of the evaporation taking place in less than a few second. This cause minimal exposure time with evaporative cooling, creates a very lenient process relative to the thermal effect. So, this process is well suited for heat sensitive products. The great advantage can be gained by the selection of equipment choices and manipulation process variables gives the operator a degree of control over the physical properties of powder produced (Parikh, 1997).

It is simple and cost effective and it is 30-50 times less expensive than freeze drying. It produce particle having dust free and fine. Able to used in pharmaceutical aseptic processing to ceramic operation. It has fully automated control system that allowed continuously monitoring procedure. It can apply to both heat resistant and heat sensitive products. It has high precision over particle size control. There is wide range of spray dryer to meet product specification. (Kaur et al., 2012)

Disadvantages of Spray Drying

The main disadvantage of spray drying is its cost in terms of operation and equipment. Spray dryers have poor thermal efficiency because of hot air circulate in the chamber without contacting with particles unless higher drying temperature is used but this is impossible as majority of pharmaceuticals products degrade at extreme temperature. Spray drying process needs laboratory and pilot scale testing (Broadhead et al., 1992).

Conclusion and Future Prospects

Dissolution is the rate limiting step for oral absorption of drugs which are poor water soluble and this can affect the in vivo absorption of the drug. Solubility is the main and basic requirement to develop the formulation and different dosage forms. Spray drying technology applied to particles manufacturing to produce the products ranging from pharmaceutical direct compression excipients to microencapsulated flavours (Tajus et al., 2012).

Spray drying technique is beneficial to pharmaceutical technique as it is a continuous process to obtain uniform composition of powders for direct tabletting compression and precise physical properties and also reduce the steps from traditional manufacturing process and decrease considerable amount of operator handling (Parikh, 1997).

As this review indicates, spray drying has wide range of application in pharmaceutical industry. In future, there are possibilities to extend the use of spray drying and to introduce the newest technology into production. Data from the literature showed that the most important area of are protein therapy, anticancer drugs, and enhancement in the aqueous solubility of the drugs and other fields that are important for modern pharmaceutical technology. Spray drying technique include nanosuspension, microemulsion and spray drying self-emulsification are awaiting for utilization in pharmaceutical industry. According to Master 1991 multistage processes, modern spray drying technique and temperature gradient technique hold promise for future application in pharmaceutical industry.

References


THE END
ANTIOXIDANT ACTIVITIES OF SELECTED SAMPLES OF GLYCINE MAX L. N.

Abstract:
Two seed samples Glycine max Linn. (S1, S2) were purchased from two retail stores of local market and were evaluated. Sprouted and non-sprouted seed powder of samples were extracted separately with methanol (100%, 50%) by cold maceration to obtain methanolic and hydroalcoholic extract of Glycine max Sample 1 was designated as MES1 and HES1 respectively and sample 2 as MES2 and HES2. The total phenolic and flavonoid content of these samples were studied. The total phenolic content of samples MES1, HES1, MES2 and HES2 with respect to gallic acid was found to be 310µg/ml, 275.5µg/ml, 210µg/ml and 199.3µg/ml respectively. The flavonoid content of samples MES1, HES1, MES2 and HES2 with respect to quercetin was found to be 52.9µg/ml, 41.1µg/ml, 72.5µg/ml and 47.2µg/ml respectively. This potential was compared to sprouted seeds. In vitro antioxidant activity of methanolic and hydroalcoholic extracts of two seed samples of Glycine max. were assessed using three different methods (Reducing power, Total antioxidant activity and Ferric reducing antioxidant power activity). The effect of sprouting on in-vitro antioxidant activity on these two samples were evaluated. There was significant increase in antioxidant activity with increase in concentration of the sample. Maximum activity was reported in (Total antioxidant activity i.e. 0.635) at concentration 100µg/ml which was comparable to the standard. In total anti-oxidation activity, there was visible increase in total antioxidant power of methanolic extract of sprouted seeds with increase in concentration which was evident by increase in percentage activity from 0.493 to 0.599 at concentration of 60 µg/ml in MES1.

Introduction
Nutraceuticals are products that provide health and medicinal benefits including the prevention and treatment of disease. Such products may range from herbal products to genetically engineered foods, dietary supplements and processed foods. The term Nutraceutical was defined as a product isolated from foods and sold in medicinal forms. Phytochemicals and antioxidants are two specific types of nutraceuticals. Research has proved that foods with Phytochemicals may help to provide protection from many diseases such as heart disease, diabetes, cancer and hypertension. Antioxidants may be helpful in avoiding chronic diseases. Soybean (Glycine max Linn.) is rich source of phytochemicals constituents. Soybean reduces the risk of range of hazardous diseases like osteoporosis, atherosclerosis, uterus cancer, breast cancer and prostrate cancer. People in India are becoming aware about the benefits of consuming soy. The health promoting activity associated with soy consumption is due to the presence of isoflavones and soybean known as a functional food due to the presence of beneficial secondary metabolites such as isoflavones, isoflavones found in soybean exist in four chemical forms: aglycone, glucoside, malonylglucoside, and acetylglyceride. Many phenolic components found in plant are potential antioxidants: tannins, flavonoids, and lignin. Therefore it is stipulated now days to explore the potential of these plants.

Materials and Methods
Collection of material:
Two seed samples Glycine max Linn. (S1, S2) purchased from two retail stores of local market in the month of January 2013. The seeds were washed well using tap water, then it was dried. The dried samples were grinded properly using a grinder, to obtain the powderd form. The powder of the seeds were stored in air tight containers.

Seed germination and preparation of extracts:
Soybean seeds were selected and washed with water. A cotton bed was prepared to spread and germinate seeds. 100 gram of seeds was spread on wet-cotton bed at room temperature. Seeds were covered with similar type of cotton covering. Water was sprinkled as and when required to keep bedding wet. Sprouts with 1.5-3.0 cm germination length were picked up for analysis. It took about 72 hours for seeds to germinate up to this length. Fine powder of raw clean seeds and their sprouts were prepared for extraction. Sprouting are better techniques to improve the phenolic content and antioxidant activity.

Cold Maceration:
Sprouted and non-sprouted seed powder of samples was extracted separately with methanol (100%, 50%) by cold maceration to obtain methanolic and hydroalcoholic extract of Glycine max Sample 1 (MES1 and HES1 respectively) and for sample S2 (MES2 and HES2). Seed powders (25g) of each was extracted trice with 250 ml of methanol for 3 hour in an electrical shaker at 40°C. The extracts were filtered through Whatman No.2 filter paper and evaporated under vacumm using rotary evaporator. Yield of the extracts is weighed on the weighing balance. Each extract were transferred to glass vials and kept at 4°C.

Estimation of Total Phenolic Content and Flavanoid Content of seed extracts:
The total phenolic and flavonoid content of methanolic and hydroalcoholic extracts of these samples were studied according to the method described by FolinCiocalteu. The FCR reagent oxidizes phenols and changes in the dark blue colour (k = 765 nm) are monitored by UV-visible. AlCl₃ (0.1 gm/ml) and CH₃COONa (1 M) were used to check the flavnoid content and kept for 30 minutes after that absorbance was taken at 415 nm by using UV-visible.

Determination of in-vitro antioxidant activity of methanolic and hydroalcoholic extracts of Glycine max Linn. with the help of different models discussed below:

**Reducing power method:**
It was determined by using the procedure with slightly modifications. Take 1 ml of extract and added 2.5 ml phosphate buffer and 2.5 ml of pot. Ferricyanide and then mixture was incubated at 50°C for 20min. 2.5mL of trichloroacetic acid was added to the mixture, which was then centrifuged at 3000rpm for 10min. The upper layer of the solution was separated and mixed with 2.5 ml of distilled water and 0.5 ml FeCl₃ and take absorbance at 700 nm. BHT was used as positive control.

**Total antioxidant activity by Ammonium molybdate method:**
0.1 ml of the sample were mixed with 1 ml of the reagent solution. The tubes were covered with silver foil and incubated at 95ºC for 90 min. The tubes were cooled to room temperature and the absorbance was measured at 695 nm against a blank. Ascorbic acid was used as a standard.

**Ferric Reducing Antioxidant Powder activity:**
FRAP assay is based on the ability of antioxidants to reduce Fe³⁺ to Fe²⁺ in the presence of TPTZ, forming an intense blue Fe²⁺TPTZ complex. 0.2ml of the extract is added to 3.8 ml of FRAP reagent and the reaction mixture is incubated at 37 °C for 30 min. and absorbance is measured at 593 nm. FeSO₄ is used for calibration. BHT, BHA, ascorbic acid, quercetin can be used as a positive control.

**Results**
**Total phenolic content:** The total phenolic content of non-sprouted seed samples MES1, HES1, MES2 and HES2 with respect to gallic acid was found to be 310µg/ml, 275.5µg/ml, 210µg/ml and 199.3µg/ml respectively. The phenolic contents were found to be maximum in MES1 among all the samples. Phenolic content (113%) enhanced clearly in sprouted seeds.

**Total flavonoid content:** The flavonoid content of non-sprouted seed samples MES1, HES1, MES2 and HES2 with respect to quercetin was found to be 52.9µg/ml, 44.1µg/ml, 72.5µg/ml and 47.2µg/ml respectively. The highest among these were found to be in MES2. Clearly potentiation of flavonoid content of sprouted seeds. Flavonoid content (291%) enhanced.

**In-vitro antioxidant activity of non-sprouted and sprouted seed samples:**

**Reducing power assay:**

![Figure 1: Reducing power of methanolic and hydroalcoholic extracts of non-sprouted seed samples MES1, HES1, MES2 and HES2](image-url)
There was visible increase in reducing power of methanolic extract of sprouted seeds particularly at higher concentration which was evident by increase activity from 0.578 to 0.591 at concentration 100µg/ml in MES2.

**Total antioxidant activity**: 

There was visible increase in total antioxidant power of methanolic extract of sprouted seeds with increase in concentration which was evident by increase percentage activity from 0.493 to 0.599 at concentration 60 µg/ml in MES1.
Ferric reducing antioxidant power assay:

Figure 5: Ferric reducing antioxidant power of methanolic and hydroalcoholic extracts with non-sprouted samples MES1, HES1, MES2 and HES2

Figure 6: Ferric reducing antioxidant power of methanol extracts of sprouted seeds MES1, HES1, MES2 and HES2

In this study, there was concentration dependent increase of ferric reducing antioxidant power in both cases i.e. non-sprouted and sprouted seeds. But there was visible increase in ferric reducing antioxidant power of methanol extracts of sprouted seeds with increase in concentration which was evident by increase percentage activity from 0.499 to 0.534 at concentration 80 µg/ml in MES2.

Conclusion

In-vitro antioxidant activity in various methanol extracts of Glycine max Linn. seed was assessed using three different methods i.e Reducing power, total antioxidant activity and ferric reducing antioxidant power activity. The results revealed that the Glycine max methanolic extracts of MES2 exhibited highest antioxidant activity followed by MES1, HES1 and HES2 extracts whereas sprouting and soaking increased the antioxidant activity of seed samples. Therefore it is recommended to use this plant after soaking and sprouting.

Since Soybeans are “healthy food” due to the presence of various valuable constituents like phenolic and flavonoid content as evaluated in the present study. Biological compounds of this plant needs to be more precisely defined. The extracts can be used in different industries like food, pharmaceutical and as an additive in cosmetic products.

References

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**Abstract:**

Pharmacy, derived from the word *pharmakon* meaning *drug or medicine*. Pharmacy can be described as the science or practice of the preparation and dispensing of medicinal drugs. The discovery of drugs like sulphonamides and penicillin brought a major revolution in pharmaceutical field. Paracelsus (16th century) declared that the only difference between a medicine and a poison was in the dose. All medicines were toxic. It was cure or kill. Prescription drugs like Anti diabetic drugs, Anti convulsant drugs, Anti viral drugs etc and Non Prescription drugs like cough suppressants, topical antibiotics, anti-acne drugs, decongestants etc are used in day to day life.

The evolution of the Indian Pharmaceutical Industry can be divided into four principal epochs. First era from 1850 to 1945, second period from 1945 to late 1970’s, the third epoch for development is from the early 1980’s to the early 1990’s and the fourth memorable events spans from the early 1990’s to the present time. In India, before the advent of British rule the indigenous forms of medicine used are ayurvedic and unani. A major breakthrough was the therapeutic revolution, which marked the beginning of the period 1945 to mid 1970’s, resulted in a phenomenal growth of the global pharmaceutical industry located mainly in Germany, Switzerland etc.

Two major public sector units, the Hindustan Antibiotics Ltd (HAL) in 1954 and the Indian Drugs and Pharmaceuticals Limited (IDPL) in 1961, were established, and the production of drugs from its basic stage started in India. HAL was the first company in India to manufacture a number of antibiotic drugs like penicillin, streptomycin, sulfate, Ampicillin etc from the basic stage. The IDPL was established with the support and assistance of the Soviet Union to produce antibiotics, synthetic drugs and surgical instruments. In 1952 the total turn over for the pharmaceutical sector was around Rs 32 crore, which increased to Rs 75 crores approx for bulk drugs and Rs 370 crore for formulation products in 1970. Contraceptive drugs were developed, which reduced population growth rates in industrialized countries. The first truly synthetic pain reliever, antipyrine, was produced from quinoline derivatives. Digitalis from foxglove and strophanthint from an African dogbane were both botanicals purified for use against heart disease.

**Some of the evolutionary changes in the 18th century are**

The United States pharmacopoeia (USP) published in 1820, Gregor Johann Mendel (the father of modern genetics) research work led to the discovery of particulate inheritance, dominant and recessive traits, genotype and phenotype, and the concept of homozygous and heterozygous(3). The Alkaloid quinine(1820) was first extracted from the bark of cinchona trees by two French chemists, Diamorphine(1874), Aspirin(1899).

**Some of the epochs of the 19 th century are**

In 1900, Karl Landsteiner discovered the first human blood groups: O,A,B. In 1902, proteins were first shown to be polypeptides and the AB blood group was discovered. In 1909, Harvey cushing demonstrated the link of pituitary hormone to Gigantism. Salvarsan, the first magic bullet drug effective against syphilis(1910), Alexander Fleming, discovery of penicillin had changed the world of modern medicine by introducing the age of antibiotics, Ibuprofen synthesized in 1961, Introduction of Adverse Drug reaction yellow card scheme(1964) in response to the thalidomide tragedy of 1961.
**Landmark changes in the 20th century are**

Placebo-controlled, randomized, blinded clinical trials became a powerful tool for testing new medicines. X-rays became powerful diagnostic tool for wide spectrum of diseases from bone fractures to cancer. New psychiatric drugs were developed. These include antipsychotics for treating hallucinations and delusions, and antidepressants for treating depression. The invention and development of immunosuppressive drugs and tissue typing made organ and tissue transplantation a clinical reality. New methods for cancer treatment, including chemotherapy, radiation therapy, and immunotherapy, were developed. At the end of 20th century the world was undergoing its second major period of globalization the first, started in the 18th century. Norman Borlaug, Father of green revolution, is often credited with saving over a billion people worldwide from starvation.

**Some of the major innovations of the 21st century are**

Abiocor Artificial heart, Bio-artificial liver, camera pill, bio-ionic contact lens, iLimb bionic hand, Elekta Axsesse, eye writer etc. Though they are innovations which indirectly help in the field of pharmacy in usage of drugs.

**Growth of Private Sector in manufacturing of drugs in India**

The Serum Institute of India (1966) supplies low-cost, life saving vaccines and the world’s largest producer of measles and DPT vaccines, Novartis India launched calcium Sandoz as an OTC supplement, Piramal life sciences has initiated phase-1 trials of a new experiment drug for Diabetes metabolic syndrome in Canada, Biocon has 7 and wockhardt has 10 new chemical entities in their pipelines. Reliance life sciences has launched three biosimilars - Relipoietin, ReliGrast, and Reliferon in the domestic market.

**Conclusion**

Government policies like the New Drug Policy, Patent Acts, Foreign Exchange Regulation Act (FERA), and the Hathi committee (1974) played a pivotal role for the growth and development of the pharmaceutical sector. With a humble beginning Rs 32 crores (1952) turnover, today the pharma industry has achieved an annual turnover of US$10.04 (july2010) and still growing. This phenomenal growth/revolution took place with the guiding masters like late Sri Sarabhai and new entrepreneurs both in public and private sectors, besides Government’s thrust through legislation( for motivation/incentives) to earn more foreign exchange besides meeting local demands. Pharma Revolution made available medicines to under privileged also at a very reasonable price. Thus, there is a little bit of pharma in everybody’s life. Thanks for Pharma Revolution.

**References**

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Abstract:
In this study, evaluation of the antifungal potential of mushroom extracts of different solvent viz., petroleum, chloroform and methanol against nine plants pathogenic fungi viz., A. alternate, A. flavus, A. solani, A. tomentosa, C. capsici, D. dematium, L. lindenuthianum, F. oxysporum and F. solani, ten human pathogenic fungi viz., M. gypseum, T. equinum, T. kanei, C. albicans, C. indicum, C. krusei, C. merdanum, C. zonatum, E. floccosum and T. rubrum were investigated. The Clevaria rosea of mushroom was picked from the litter and decaying soil surface, with help of forceps and then they were cleaned and air dried in an hot air oven at 40 °C for 48 h. dried mushroom samples were powdered mechanically for further use. All extracts were concentrated by using rotary flash evaporator. The phytochemical screening was carried out to know the compounds responsible for these activities. The antifungal assays in this study were performed by the agar-well diffusion method. All species were incubated for 3 days at 28 °C in the dark so that they could be quantified by measuring the zones of growth inhibition diameters. The susceptibility of the bacteria to the crude extracts on the basis of zones of growth inhibition varied according to microorganism and extracting solvent.

Introduction
Man has been hunting for the wild mushrooms since antiquity. During the early days of civilization, mushrooms were consumed mainly for their palatability and unique flavours. Higher fungi have been used by mankind for millennia. Firstly they are used as part of regular diet for their nutritional value, completing population food intake. They contain minerals, vitamins and nutritive compounds such as proteins and polysaccharides and have low fat content. Secondly mushrooms fruiting bodies are also appreciated for delicacy. Indeed their palatability is exploited as taste and flavor enhancers when associated in food preparation and cooking. Thirdly, higher fungi are used for medicinal purpose. Their pharmacological action and therapeutic interest in promoting human health are known for thousands of years.

Asian traditional medicine practices and nowadays modern medicine in Eastern countries as China, Japan, Korea and several Asian countries still use mushrooms for treatment of major diseases. Ancient traditional uses are now clinically confirmed and constitute a strong base for intensive research and development of Basidiomycota biologically active metabolites (BAM) isolated or in combination. According to literature, more than 270 medicinal fungi are reported in traditional Chinese medicine for their preventive and/or curative effects. In Japan, the knowledge of biological activities from mushrooms is the same as in China. Four species are really very popular in medical care, i.e., Shiitake (Lentinula edodes (Berk.) Pegler), Reishi or Mannentake (Ganoderma lucidum (Curtis) P. Karst.), Maitake (Grifola frondosa (Dicks.) Gray) and Enokitake (Flammulina velutipes (Curtis) Singer). They are sold in streets to consumers as a source of good health and lifetime prolongation. Some Japanese people can even run around for kilometers in order to pick up wild fungi as Reishi growing on very old plum trees and considered to be effective against cancer and degenerative diseases.

Mushrooms also possess bioactive natural products that are antitumor, antiviral and antibacterial. They contain many different bioactive compounds with diverse biological activity depending on the way they prepared and consumed. In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists for searching new antimicrobial substances from various sources that are the good sources of novel antimicrobial chemotherapeutic agents. Bender et al.’s culture extracts of A. bisporus and other Agaricus sp. showed antibacterial activity against Staphylococcus saprophyticus. To the best of our knowledge, no research has been available on antifungal activity of a mushroom Clevaria rosea. Therefore, the aim of the present work is to evaluation of the antifungal potential of mushroom extracts against human and plant pathogenic fungi.

Materials and Methods:

a. Clevaria rosea:
The Clevaria rosea were collected from semi evergreen forest region (13°51’56.30"N, 75°03’12.50"E) which is located in Haniya, Hosanagar taluk, Shimoga district, Karnataka, India, during the month of June to August 2012. The Clevaria rosea of mushroom was picked from the litter and decaying soil surface, with help of forceps and then they were cleaned and air dried in an oven at 40 °C for 48 h. dried mushroom samples were powdered mechanically
for further use. Identification was done by comparing their morphological, anatomical and physiological characteristics with the help of standard literatures like. The voucher specimen (KUABARN-265) has been deposited at the herbarium of mycology laboratory, Department of Applied Botany, Kuvempu University, Jnana Sahyadri, Shimoga district, Karnataka, India.

b. **Chemicals and reagents:**
All chemicals and reagents used in the present study were purchased from reliable firms like HiMedia Laboratories Pvt. Ltd and were of analytical grade.

c. **Preparation of Clevaria rosea extracts:**
The dried and powdered by grinder (Bajaj Electrical Limited-Twister Mixer) mushroom material (200 g) was extracted successively with 2000 ml pet ether following chloroform and methanol with a Soxhlet extractor for 48 h at temperature not exceeding the boiling point of the solvent. The extract was filtered with Whatsman filter paper no.1 and the filter was concentrated in a vacuum at 40˚C using a rotary evaporator. For the entire analysis, compounds of extract were dissolved in dimethylsulfoxide (DMSO). The yield of extracts obtained from pet ether was 3.04gm, followed by chloroform 2.52 gm and methanol 32.98 gm (Table 1). Each extract was transferred to glass vials and kept at 4˚C before use.

d. **Phytochemical analysis of Clevaria rosea extracts:**
The phytoconstituents present in the organic extracts were determined qualitatively according.

e. **Microorganisms:**
Pure cultures of all experimental fungi were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. American Type Culture Collection (ATCC). The viability of the organisms was maintained by regular transfer into freshly prepared on Potato dextrose agar (PDA) at 28˚C and stored at 4˚C until used. For the present study pure fungal cultures were taken (Table 2).

f. **Preparation and its Sterilization:**
For the agar well-diffusion method of antimicrobial susceptibility were tested on solid (Agar-agar) media in petriplates. For the fungal assay, PDA (39 gm/L), were used for developing surface colony growth. The suspension culture, for fungal cell growth was done by preparing 2.4% (w/v) PDB (Potato dextrose broth) was taken for evaluation. All the media prepared were then sterilized by autoclaving the media at (121˚C) for 20 minutes.

g. **Antifungal testing of Clevaria rosea extracts:**
C. rosea extracts were tested for antifungal activity by agar – well diffusion technique with a little modification. The fungal spore suspension was prepared by the addition of a loopful of fungal spores in a 5 ml of sterile distilled water and 1 ml Tween 20. Then fungal spore suspension was spread evenly on the petriplate containing 20 ml of solidified potato dextrose agar. Four wells were punched at the corner by using sterile cork borer of 6 mm diameter. The different solvent extracts of C. rosea were loaded to the four wells by using 100μl micropipette in 4 different concentrations i.e., 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml respectively. Clotrimazole, Fluconazole, Mancozeb and Captan are used as a positive control and DMSO is used as a negative control. All the plates were incubated at 23±2˚C fungal growth was determined by measuring the diameter of zone of inhibition after 5 days of incubation. The test was done in triplicates to arrive concordant result.

**Results:**
The yield of the crude extract obtained in the mushroom samples shows that minimum yield in petroleum ether, chloroform and maximum yield in methanol (Table 1).

A. **Secondary metabolic constituents:**
The qualitative analysis of secondary metabolite screening of different solvent extracts from Clevaria rosea has revealed the presence of various secondary metabolites of therapeutic importance namely steroids, saponins, glycosides, flavonoids and phenols whereas absent of alkaloids, triterpenoids and tannins. The secondary phytoconstituents like phenols, glycosides and flavonoids is strongly present in all solvent extracts shown in (Table 3).
Phytoconstituents such as alkaloid, sesquiterpine, phenolic compounds and glycosides have been reported to inhibit bacterial growth and to be protective to plants against bacterial and fungal infections.

B. Antifungal test:
The antifungal activity of different solvent extracts (viz., Petroleum ether, Chloroform and Methanol) from C. rosea were evaluated against nine plant pathogens viz., A. alternate, A. flavus, A. solani, A. tomentosa, C. capsici, C. dematiuom, C. lindemuthianum, F. oxysporum and F. solani and ten human pathogens viz., M. gypseum, T. equinum, T. kanei, C. albicans, C. indicum, C. krusei, C. merdarium, E. floccosum and T. rubrum. The antifungal activity was determined using the agar well-diffusion method summarized in (Table 4 and 5). The antifungal activity of all the solvent extracts were calculated by measuring inhibition zone formed around the well in millimeter (mm).

In 100% concentration, the highest inhibition zone is obtained from A. tomentosa (10 mm), followed by C. dematiuom (9 mm), A. flavus and C. capsici (7 mm) and the moderate activity shown that is 2.6 mm to 6.6 mm in all lower concentration of petroleum ether extract. In case of chloroform extract more inhibition zone against F. oxysporum (11 mm) and C. lindemuthianum (9 mm) at 100% concentration, same time lower concentration decrease the value of antifungal activity. The highest activity shows against A. tomentosa (12 mm), followed by C. dematiuom (9 mm) and F. solani (7 mm) at 100% concentration in methanol extract of C. rosea (Table 4). The least activity (2.3mm to 3.3mm) has shown in lower concentration of both petroleum ether and methanol extracts of C. rosea against A. flavus (Figure 1).

The three organic solvent extracts, showed moderate activity against all the tested fungi. The maximum antifungal activity of petroleum ether and methanol extracts of C. rosea was found against C. merdarium (14 mm and 11 mm), followed by C. albicans (12 mm and 11.3 mm), T. equinum (12 mm and 11 mm), M. gypseum (11 mm), E. floccosum (12mm and 11 mm) and C. krusei (12 mm and 11 mm) at 100% concentration in all three solvent (Table 5). There is no inhibition of zone against T. rubrum in all the three different solvent of C. rosea extracts (Figure 2).

The extract shows increasing inhibitory activity with increase in concentration (12.5% -100%). The antifungal activity of different solvent extracts of mushroom is changeable and has a lower antifungal activity as to comparison of antibiotics viz., Clotrimazole, Fleuconazole, Mancozeb and Captan (Table 6).

Discussion:
The search for antimicrobials from natural sources has received much attention, and efforts have been put in to identify compounds that can act as suitable antimicrobial agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines for controlling the growth of microorganisms. These compounds have a significant therapeutic application against human pathogens, including bacteria, fungi, or virus. Numerous studies have been conducted with the extracts of various plants, screening of antimicrobial activity, as well as for the discovery of new antimicrobial compounds.

In the present investigation, different extracts of C. rosea were evaluated for exploration of their antifungal activities against multi-drug resistant, clinically isolated microorganisms. The use of mushrooms with potential therapeutic properties raises global interests from the scientific and clinical community based on two main reasons. First, mushrooms demonstrate their efficiency against numerous diseases and metabolic disturbances as serious as cancer or degenerative diseases. These therapeutic effects seem to lay on multiple and complex pharmacological actions on different cellular and molecular targets. Mushroom compounds would act in combination to influence cell surface receptors, and to trigger various downstream signaling events leading to high pharmacological efficiency and specificity.

Secondly, fungal bioactive metabolites can be obtained from many origins both wild and cultivated fruiting bodies or from mycelial biomass and supernatant of submerged cultured using bioreactors. Isolation and purification of natural or hemisynthetic active components (namely polyphenols, polysaccharides, protein-bound polysaccharides, sesquiterpenes, triterpenoids) require common analytical procedures. Consequently, higher fungi present major advantages as putative valuable drug candidates for various pathologies as reported. Different mushroom species possess different constituents and in different concentration, which account for the differential antimicrobial effect, as suggested. The broad spectrum of antimicrobial activity may be attributed to the presence of bioactive metabolites of various chemical types in mushrooms compounds.

Conclusion:
The data obtained from the present study suggest the presence of various phytochemicals in the different solvent extracts of Clevaria rosea. Total phenolic content was higher in all the four solvent extract. Flavonoids content was also found to be higher except petroleum ether extract of C. rosea. Antifungal activity of mushroom extract has been
shown that *Clevaria rosea* has a broad spectrum of activity which can be used as leads in developing the novel therapeutic bioactive agents.

References


Table: 1. Total yield of the fruiting body of mushrooms extracts obtained in different organic solvents (200 gm in 2000 ml)

<table>
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<tr>
<th>Mushroom species</th>
<th>Organic solvents</th>
<th>Yield of extract in gms</th>
<th>% of yield</th>
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<tr>
<td><em>Clevaria rosea</em></td>
<td>Petroleum ether</td>
<td>3.04</td>
<td>1.52</td>
</tr>
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<td></td>
<td>Chloroform</td>
<td>2.52</td>
<td>1.26</td>
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<td></td>
<td>Methanol</td>
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<td>16.49</td>
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### Table 2. Fungal cultures

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<th>ATCC</th>
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<tbody>
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<td>7202</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Aspergillus flavus</td>
<td>9170</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Alternaria solani</td>
<td>26934</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Alternaria tomentosa</td>
<td>16404</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Colletotrichum capsici</td>
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</tr>
<tr>
<td>6</td>
<td>Colletotrichum dematium</td>
<td>60192</td>
<td></td>
</tr>
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<td>7</td>
<td>Colletotrichum lindemuthianum</td>
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<td>8</td>
<td>Fusarium oxysporum</td>
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<td><strong>Plant pathogens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Candida albicans</td>
<td>10231</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Chrysosporium indicum</td>
<td>4266</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Candida krusei</td>
<td>6258</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Chrysosporium merdarium</td>
<td>900628</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Chrysosporium zonatum</td>
<td>845981</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Epidermophyton floccosum</td>
<td>613</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Trichophyton rubrum</td>
<td>1538</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Microsporum gypseum</td>
<td>2157</td>
<td></td>
</tr>
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<td>18</td>
<td>Trichophyton equinum</td>
<td>6275</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Trichophyton kanei</td>
<td>2091</td>
<td></td>
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<td></td>
<td><strong>Human pathogens</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. Secondary metabolite analysis of Clevaria rosea

<table>
<thead>
<tr>
<th>Tests</th>
<th>PE</th>
<th>CE</th>
<th>ME</th>
<th>AQ</th>
</tr>
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<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Triterpenoids</td>
<td>Flavonoids</td>
<td>Phenols</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>---------------</td>
<td>------------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>Petroleum ether extract</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Methanol extract</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Note: ‘+’ is Present, ‘-’ is Absent, PE-Petroleum ether extract, CE-Chloroform extract, ME-Methanol extract, AQ-Aqueous extract.

Table 4. Antifungal activities of Clevaria rosea extract against plant pathogenic fungi by agar well diffusion method.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Petroleum ether extract (Conc. mg/ml)</th>
<th>Chloroform extract (Conc. mg/ml)</th>
<th>Methanol extract (Conc. mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>A. alternate</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A. flavus</td>
<td>7</td>
<td>6</td>
<td>4.6</td>
</tr>
<tr>
<td>A. solani</td>
<td>-</td>
<td>6</td>
<td>4.3</td>
</tr>
<tr>
<td>A. tomentosa</td>
<td>10</td>
<td>6.6</td>
<td>-</td>
</tr>
<tr>
<td>C. capsici</td>
<td>7</td>
<td>5.3</td>
<td>4</td>
</tr>
<tr>
<td>C. dematium</td>
<td>9</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>C. lindemuthianum</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F. oxysporum</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F. solani</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Note: ‘-‘- No activity.
Table 5. Antifungal activities of Clevaria rosea extract against human pathogenic fungi by agar well diffusion method

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Petroleum ether extract (Conc. mg/ml)</th>
<th>Chloroform extract (Conc. mg/ml)</th>
<th>Methanol extract (Conc. mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100%  50%  25%  12.5%</td>
<td>100%  50%  25%  12.5%</td>
<td>100%  50%  25%  12.5%</td>
</tr>
<tr>
<td>M. gypseum</td>
<td>11  10  -  -</td>
<td>9  -  -  -</td>
<td>-  -  -  -</td>
</tr>
<tr>
<td>T. equinum</td>
<td>9  8  -  -</td>
<td>12  10  8  -</td>
<td>11  10  9  7</td>
</tr>
<tr>
<td>T. kanei</td>
<td>6  5  4.6  3.3</td>
<td>7.3  5.6  4  -</td>
<td>7  6  5  -</td>
</tr>
<tr>
<td>C. albicans</td>
<td>12  11  9.6  -</td>
<td>12  10  -  -</td>
<td>11.3  10.3  9  -</td>
</tr>
<tr>
<td>C. indicum</td>
<td>-  -  -  -</td>
<td>-  -  -  -</td>
<td>-  -  -  -</td>
</tr>
<tr>
<td>C. krusei</td>
<td>-  -  -  -</td>
<td>11  10  8  7</td>
<td>12  10  -  -</td>
</tr>
<tr>
<td>C. merdarium</td>
<td>11  10  8  14</td>
<td>14  1  -  -</td>
<td>-  -  -  -</td>
</tr>
<tr>
<td>C. zonatum</td>
<td>-  -  -  -</td>
<td>-  -  -  -</td>
<td>11  9  7  4</td>
</tr>
<tr>
<td>E. floccosum</td>
<td>11  9  8  6</td>
<td>-  -  -  -</td>
<td>12  11  9  5</td>
</tr>
<tr>
<td>T. rubrum</td>
<td>-  -  -  -</td>
<td>-  -  -  -</td>
<td>-  -  -  -</td>
</tr>
</tbody>
</table>

Note: ‘–’ No activity.
### Table 6. Antifungal activity of standard drug and control against plant and human pathogenic fungi

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Test organism</th>
<th>Standard</th>
<th>Control</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Clotrimazole</td>
<td>Fleuconazole</td>
</tr>
<tr>
<td>1</td>
<td>A. alternata</td>
<td>x</td>
<td>X</td>
</tr>
<tr>
<td>2</td>
<td>A. flavus</td>
<td>x</td>
<td>X</td>
</tr>
<tr>
<td>3</td>
<td>A. solani</td>
<td>x</td>
<td>X</td>
</tr>
<tr>
<td>4</td>
<td>A. tomentosa</td>
<td>x</td>
<td>X</td>
</tr>
<tr>
<td>5</td>
<td>C. capsici</td>
<td>x</td>
<td>X</td>
</tr>
<tr>
<td>6</td>
<td>C. dematium</td>
<td>x</td>
<td>X</td>
</tr>
<tr>
<td>7</td>
<td>C. lindemuthianum</td>
<td>x</td>
<td>X</td>
</tr>
<tr>
<td>8</td>
<td>F. oxysporum</td>
<td>x</td>
<td>X</td>
</tr>
<tr>
<td>9</td>
<td>F. solani</td>
<td>x</td>
<td>X</td>
</tr>
</tbody>
</table>

#### Plant pathogenic fungi

- A. alternata
- A. flavus
- A. solani
- A. tomentosa
- C. capsici
- C. dematium
- C. lindemuthianum
- F. oxysporum
- F. solani

#### Human pathogenic fungi

- M. gypseum
- T. equinum
- T. kanei
- C. albicans
- C. indicum
- C. krusei
- C. merdarium
- C. zonatum
- E. floccosum
- T. rubrum

*Note: 'x' - Not applicable, '-' - No activity.*
**Figure 1.** Mean zones of inhibition of different solvent extract of *C. rosea* against plant pathogenic fungi

**Figure 2.** Mean zones of inhibition of different solvent extract of *C. rosea* against human pathogenic fungi
Red blood cells are known as Erythrocytes, it have been potential carrier capabilities for the delivery of drugs. It life cycle in our body is 120 days. Erythrocytes are natural products of the body, biodegradable in nature, isolation of these is easy and large amount of drug can be loaded in small volume of cells, non immunogenic in action and can be targeted to disease tissue or organ, prolong the systemic activity of the drug about 120 days while residing for a longer time in the body, act as a carrier for number of drugs, target the drugs within the reticuloendothelial system (RES) as well known RES organs/sites. Various method like physical, osmosis based system and chemical method can be used to load the drug. Resealed erythrocytes have wide application including drug targeting to RES and NON RES organ, in enzyme therapy, antiviral agent delivery etc. Potential clinical indication for "RES targeting" include iron over storage disease, parasitic disease hepatic tumors, cancer and lysosomal storages diseases carriers.

Novel drug delivery systems are one of the widely used delivery systems. In the present scenario, amongst them, “Drug Loaded Erythrocytes” is one of the growing and potential systems for delivery of drugs and enzymes. Carrier erythrocytes have been evaluated in thousands of drug administration in humans proving safety and efficacy of the treatments. Carrier erythrocytes, resealed erythrocytes loaded by a drug or other therapeutic agents, have been exploited extensively in recent years for both temporally and spatially controlled delivery of a wide variety of drugs and other bioactive agents owing to their remarkable degree of biocompatibility, biodegradability and a series of other potential advantages.

Characteristics of blood
Erythrocytes have 120 days life cycles, abundant in human body, Blood contains about 55% of plasma and 45% of corpuscles or formed elements. Normal blood cells have extensile, elastic, biconcave and non nucleated configuration with a diameter ranging from 6-9 µ and the thickness is nearly 1-2 µ. Erythrocytes have a solid content of about 35% most of which is Hb and rest 65% being water. Lipid content of erythrocytes includes cholesterol, lecithin and cephalins. pH the normal pH range of blood is 7.35 to 7.45, which is slightly alkaline. Venous blood normally has a lower pH than does arterial blood because of the presence of more carbon dioxide. Viscosity—this means thickness or resistance to flow. Blood is about three to five times thicker than water. Viscosity is increased by the presence of blood cells and the plasma proteins, and this thickness contributes to normal blood pressure. The surface area of mature, biconcave RBCs is about 136 µm$^2$ but can swell to a sphere of approx 150 fl. It is noteworthy that RBCs can also cross undamaged capillaries of 2-3 µm in diameter. The RBC membrane is strictly connected with the membrane skeletal proteins which are organized in a uniform shell. The RBC shape can undergo a number of reversible transformations. An important determinant of RBC survival is its deformability. Key factors affecting deformability are internal viscosity (mainly contributed by RBC hemoglobin), the surface/volume of the cell and the intrinsic deformability of the membrane. The RBCs have other very interesting properties namely they behave as an osmometer since they shrink when placed into a hypertonic solution or swell when placed into a hypotonic solution. The RBCs can reach a critical hemolytic volume giving rise to holes on the membrane ranging from 10 nm up to 500 nm. These processes are usually reversible and following haemolysis the holes close and the cell resumes its biconcave shape. Red blood cells constitute potential biocompatible carriers for different bioactive substances, including protein drugs, since they feature some unique advantages, for examples, they are completely biodegradable without generation of toxic.

Erythrocytes as drug carriers
Many of the carriers has been used for drug targeting, among which cellular carrier is most advantages and related to non-immunogenicity, non-pathogenicity, bio degradability, and also a high drug loading efficiency. In cellular carrier erythrocytes have been great potential in novel drug delivery system. Resealed erythrocytes have been great
capability to circulate throughout the body because it has zero order release kinetics, reproducibility, ease of isolation. Most of resealed erythrocytes used as drug carriers are rapidly taken up from blood by macrophages of reticuloendothelial system, which is present in liver, lung, and spleen of the body. The newer RBC has the capacity to synthesize hemoglobin. The adult RBC however loses their capacity and serves only to carry hemoglobin. The use of RBC or cells as drug delivery system requires that the drug which are normally unable to permeate the membrane, should be made to traverse the membrane without causing any irreversible changes in membrane structure and permeability. Further the cells should be able to release the drug in controlled manner upon reaching the desired target.

Advantages of Resealed Erythrocytes

- It has biocompatibility,
- Biodegradable in nature,
- Against entrapped drug it not shows any desired immune response,
- Erythrocytes have longer life-span as a carrier as compare to other carrier system,
- In size and shape shows uniformity,
- Protection of the loaded compound from unwanted degradation within the host body inactivation by the endogenous factors;
- Possibility of targeted drug delivery to the reticuloendothelial system organs;
- As compare to conventional drug-administration methods remarkable decrease in concentration fluctuations at steady state
- Availability of the knowledge, techniques, and facilities for handing, transfusion, and work with erythrocytes;
- Release of drug follows ideal zero-order kinetics;
- Wide variety of compounds with the capability of being entrapped within the erythrocytes;
- Possibility of loading a relatively high amount of drug in a small volume of erythrocytes, which in turn assures the dose sufficiency in clinical as well as animal studies using a limited volume of erythrocyte samples;
- Modification of the pharmacokinetic and pharmacodynamics parameters of the drug
- Considerable increase in drug dosing intervals with drug concentration in the safe and effective level for a relatively long time;
- Possibility of decreasing the drug side effects;
- Due to their natural roles, they are ideal carriers for intravascular drug delivery;
- Possibility of using synthetic erythrocyte counterparts (artificial erythrocytes);
- Possibility of using special apparatus for loading (i.e. red cell loader) and thus completely controllable loading procedure;
- Possibility of loading without extracting the erythrocytes out the patient body (in-vivo loading of circulating erythrocytes).

Disadvantages

- Being biodegradable, they are removed in-vivo by the reticuloendothelial system.
- Rapid leakage of certain entrapped substances from the loaded erythrocytes.
- May alter the physiology of the erythrocyte.
- Being from biological origin, entrapped erythrocytes may present variability and lesser standardization in their preparation, compared with other systems.
- Inaccessibility of many important therapeutic targets like solid tumours, extravascular tissue components, and central nervous system.
- Safety and technical concerns related to the storage of the loaded erythrocytes.
- Liability to biological contamination due to the origin of the blood, the equipment and the environment, such as air.
- Rigorous controls are required accordingly for the collection and handling of the erythrocytes.
- The treatment of carrier erythrocytes with membrane stabilizing agents, such as glutaraldehyde can increase their osmotic resistance, thus resulting in a decrease in the drug release rate.
- Structural changes in erythrocytes may occur during the drug loading procedure, and this can also lead to the recognition of erythrocytes by macrophages.

Delivery Strategies
Two major strategies for drug delivery using erythrocytes as a carrier includes
- Targeted drug delivery
- Intravenous slow drug release strategy

**Intravenous slow drug release strategy**

Erythrocytes circulate in intravenous and used as slow – release carrier for the delivery of drug, enzyme, and peptide and cardiovascular drugs among others. Lot of proposed mechanism for drug release through erythrocytes including passive diffusion, specialized membrane-associated carriers, phagocytes of the carrier cells by the macrophages of RES and, then, depletion of the drug into circulation, accumulation of the drug in RES upon lysis of the carrier and slow release from this system into circulation. accumulation of the carrier erythrocytes in lymphatic nodes following subcutaneous injection of the cells and drug release upon haemolysis in this sites, and, finally, haemolysis in the injection sites (in cases of the injection routes other than IV).

**Targeted drug delivery**

RES or non-RES ‘targeting’ is another important strategy using erythrocytes as carriers.

a. **RES targeting**

At the time of aging physiological condition and metabolic pathway of erythrocytes loses its natural integrity, flexibility, and chemical composition due to this change destruction of cell and spleen tubecules. Peritoneal macrophages, hepatic Kupffer cells, and alveolar macrophages of the lung, peripheral blood monocytes, and vascular endothelial cells are other site for abnormal erythrocytes. Aging and other factor which causes the change in erythrocytes recognized by

The phagocytic macrophages via change in chemical composition of erythrocytic membrane. Changing in erythrocytes during loading was trapped by liver and spleen it was shows that some limit of cell damage during loading, the spleen was preferred site for storage of carrier erythrocyte.

b. **Non-RES targeting**

Recently, carrier erythrocytes have been used to target organs outside the RES. The various examples are:

- The magnetically guided erythrocytes have been tested successfully for targeting ibuprofen and diclofenac to inflamed tissues.
- Photosensitized erythrocytes have been studied as a photo triggered carrier and delivery system for methotrexate in cancer treatment.
- Many other example are available in which erythrocytes has been used to target organ outside the RES.

**Source and Isolation**

Different species of mammal and its erythrocytes used as sources of drug loading, sealing, of drug and enzyme delivery like mice, rabbit, monkey, goat, sheep, dog. Blood is collected into heparin zed tubes by venipuncture.

- Blood is withdrawn from cardiac/splenic puncture (in small animal) and through veins (in large animals) in a syringe containing a drop of anti-coagulant.
- The whole blood is centrifuged at different condition for 5 min. at 4 ±10 °C in a refrigerated centrifuge.
- After washing erythrocytes are diluted with PBS and stored at 40 C until used.
- Fresh blood encapsulation capacity was higher as compare to aged blood so used fresh of mammel. Total fresh blood was stored at cool (4C) temp and stored for less than two days.

**Various conditions for isolation and centrifugal force for isolation of erythrocytes:**

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Species</th>
<th>Washing buffer</th>
<th>Centrifugal force (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cow</td>
<td>10-15mmol KH2PO4/NaHPO4</td>
<td>1000</td>
</tr>
<tr>
<td>2</td>
<td>Sheep</td>
<td>10mmol KH2PO4/NaHPO4</td>
<td>500-1000</td>
</tr>
<tr>
<td>3</td>
<td>Human</td>
<td>154mmol NaCl</td>
<td>&lt;500</td>
</tr>
<tr>
<td>4</td>
<td>Horse</td>
<td>2mmol MgCl2,10mmol glucose</td>
<td>1000</td>
</tr>
<tr>
<td>5</td>
<td>Rabbit</td>
<td>10 mol KH2PO4/NaHPO4</td>
<td>500-1000</td>
</tr>
<tr>
<td>6</td>
<td>Mouse</td>
<td>10mmol KH2PO4/NaHPO4</td>
<td>100-500</td>
</tr>
<tr>
<td>7</td>
<td>Pig</td>
<td>10mmol KH2PO4/NaHPO4</td>
<td>500-1000</td>
</tr>
<tr>
<td>8</td>
<td>Dog</td>
<td>15mmol KH2PO4/NaHPO4</td>
<td>500-1000</td>
</tr>
</tbody>
</table>
Evaluation of hematological parameters

Once the encapsulation has been completed in the erythrocytes, determination is made in normal and resealed erythrocytes of such standard hematological parameters such as:

- Protein content,
- Creatine content,
- Reduced glutathione (GSH) content
- Glutathione disulphide (GSSG) content,

Other haematological parameters, such as mean corpuscular volume (MCV), mean corpuscular hemoglobin, mean hemoglobin corpuscular concentration (MCHC) and red cell distribution width (RDW) may be characterised using an automated hematology analyser. These parameters determine the influence of the encapsulation process on the hematological properties of the erythrocytes.

Method of drug loading

The following methods have been employed for drug loading in erythrocytes:

- Hypo-osmotic lysis method: This method is based on capability of erythrocytes to retain their shape and morphology when placed in isotonic saline after being challenged with altered tonicity environment
  - Dilution method,
  - Dialysis method,
  - Preswell method,
  - Isotonic osmotic lysis method.
- Electro-insertion/Electro encapsulation method
- Endocytosis method
- Membrane perturbation method
- Lipid fusion method

Dilution method

In hypotonic solution (0.4%) fresh erythrocytes are placed; ruptured erythrocytes membrane are ruptured and escape cellular content due to this swelling of cell up to 1.6 times of its original volumes, pore diameter increases 200-500Å, due to this allows equilibrium of the intracellular and extracellular concentration, One volume of the washed erythrocytes could be treated with 2-20 volume of material to be loaded in hypotonic buffer at 0 °C for 5 minutes, The solution tonicity is then restored by adding a hypertonic buffer,

- This method is rapid and simplest especially for low molecular weight drugs,
- But major drawback is low entrapment efficiency (1-8%),
- Hypotonic dilution is used for loading enzymes such as galactosidase, glucosidase, asparginase etc

Dialysis method

Erythrocytes suspension and drug solution mix and place into dialysis bag and both end are tied, the dialysis bag is inflated with an air bubble and sealed in such a way that erythrocytes suspension occupies not more than 75 % of internal volume. Bubbles are critical for lysis and resealing. A tube was placed on bag containing 100 ml swelling solution. The bottle are placed at 4C for desired lysis time and shake then dialysis tube was placed in 100 ml isotonic phosphate buffer saline (PBS), pH 7.4 at room temperature for resealing. Then loaded erythrocytes washed with cold PBS at 4C. finally the erythrocytes suspended in PBS.

This method has high entrapment efficiency in the order of 30–50%, cell recovery of 70–80%. It takes long processing time and the need for special equipment.

This method has been used for loading enzymes such a galactosidase, desferroxamine, glucoserebrosidase, inositolhexaphosphatase, human recombinant erythropoietin.

Press well method

Erythrocytes are placed in slightly hypotonic solution due to this swelling of erythrocytes without lysis, swollen cell are recovered by centrifugation at low RPM. Then small volume of aqueous drug solution are added to the point of lysis slow swelling of cells leads to good retention of the cytoplasmic constituent and hence good survival in vivo. This method is simpler and quicker.

Drugs encapsulated in erythrocytes using this method include Propranolol, asparginase, cyclophosphamide, metotrexate, insulin, metronidazole, levothyroxine, isoniazide etc.
Isotonic osmotic lysis method
Erythrocytes are incubated in the solution of a substance which have high membrane permeability. The solute will diffuse into the cells due to concentration gradient. This process is followed by an influx of water to maintain osmotic equilibrium. Chemicals used for isotonic hemolysis such as urea solution, polyethylene glycol, and ammonium chloride. Incubated erythrocyte was diluted with glycol free buffer medium for resealing. This method is used for small molecules.

Electro-insertion/Electro encapsulation method
This method is based on the observation that electrical shock brings about irreversible changes in an erythrocyte membrane. The use of transient electrolysis to generate desirable membrane permeability for drug loading into RBC. The erythrocyte membrane could open by a dielectric breakdown. Subsequently, the pores can be resealed by incubation at 37°C in an isotonic medium. Erythrocytes was suspending in an isotonic buffer solution in an electrical discharge chamber. Electric break down is evident when the membrane is polarized for microsecond using 2kV/cm for 20 micro second. Electrohemolysis and resealing is implemented in three step.
- Washed erythrocytes are suspended in pulsation medium and kept at 4°C
- 0.15 ml of erythrocytes suspension is transferred to a high voltage pulsation device equipped with a single electric pulse 2.2kV/cm for duration of 20 microsecond, macromolecules added to the erythrocytes suspension, which could subsequently treated with a 3.7kV/cm, for 20 microsec electric pulse in a medium composed of a 3:7 mixture of isotonic NaCl and iso-osmotic sucrose. The cell suspension is transferred to a pre cooled tube and kept at 4°C.
- Resealing of electrically perforated erythrocytes membrane is then affected by incubation at 37°C in osmotically balanced system.

Endocytosis method
One volume of washed erythrocytes added into a nine volumes of buffer solution (2.5mM, ATP, 2.5 mM MgCl2, and 1mM CaCl2) then incubated for 2 min at room temperature. The pores created by this method are resealed of erythrocytes by using 154 mM of NaCl and incubate for 2 min at 37°C. Then washing of resealed erythrocytes by 5mM imidazole-glyglycine buffer, pH 7.4 containing 154 mM NaCl. The entrapment of drug occurs by endocytosis. Then washed resealed erythrocytes incubate with buffer containing the entrapped material for 30 min at 37°C.

Membrane perturbation method
By the use of certain chemical increases he membrane permeability of erythrocytes ex. Like exposure of polyene antibiotics such as Amphotericine B, this allowed low molecular weight substances to get entrapped. In1980, this method was used successfully by Kitao and Hattori to entrap the antineoplastic drug daunomycin in human and mouse erythrocytes. However, these methods induce irreversible destructive changes in the cell membrane due to this in vivo survival of loaded erythrocytes are very poor and hence are not very popular.

Lipid fusion method
Lipid vesicles containing a drug can be directly fused with human erythrocyte, which leads to an exchange of the entrapped drug. To incorporate inositol monophosphate improves the oxygen carrying capacity of cells. Just 1% encapsulation efficiency of this method.

In-vitro characterization of loaded erythrocytes
Cell counting and cell recovery
This involves counting the number of red blood cells per unit volume of whole blood, usually by automated counting. Red cell recovery may be calculated on the basis of the differences in the hematocrit and the volume of the suspension of erythrocytes before and after loading. The goal is to minimize the loss during the encapsulation procedure to maximize cell recovery. Percent cell recovery can be calculated by assuming the intact erythrocytes remaining per cubic mm with the help of haemocytometer.

Morphological aspect
The morphological examination of these drug loded erythrocytes was compared with un-treated erythrocytes using either transmission (TEM) or scanning (SEM) electron microscopy. Any change occurs when they are placed to solutions of different osmolality.
In isotonic solution (300 mosM/Kg) place the rat erythrocytes shape was found to be biconcave, when in 200mosM/Kg change to uniconcave and when it place in 150 mosM/Kg change to spherocytic shape (the most fragile of the three).

**Drug content**
Loaded erythrocytes (0.5 ml) deproteinized with acetonitrile (2.0 ml) then erythrocytes are centrifuge at 2500 RPM for 10 min take clear and supernated liquid and analyzed drug content.

**Osmotic fragility**
Osmotic fragility is a test to detect the effect of loaded drug content on survival of red blood cells. When drug are loaded in erythrocytes varying the toxicities, there shape due to osmotic imbalance. To evaluate effect of toxicities drug loaded red blood cell are incubated with saline solution from isotonic to hypotonic (0.9% w/v to 0.1% w/V) at 37°C for 10 min, after this take 30g and centrifuge for 15 min and assayed for drug and hemoglobin release which should be acceptable in range.

**Osmotic shock**
In this we will evaluated the ressealed erythrocytes withstand the stress and appearances when it sudden exposed to resealing environment. Resealed erythrocytes (1 ml 10-15% haematocrit) was incubated with water (5 ml) for 15 min then centrifuge at 3000 RPM for 15 min, may causes the releases of hemoglobin to varying degree which could estimated spectrophotometrically.

**Turbulence shock**
When ressealed erythrocytes are injected, check the effect of shear force and pressure on loaded red blood cell. Loaded erythrocytes are passed through 23 gauge hypodermic needle at a flow rate 10 ml/min. after every pass aliquote the suspension and centrifuge at 3000 RPM at 15 min and determine hemoglobin content and leach out are estimated spectrophotometrically.

**In-vitro drug release**
The encapsulation of many drugs in erythrocytes can give rise to a sustained release of the drug that influences the pharmacokinetic behavior in vivo of the loaded erythrocytes. Normal and drug loaded erythrocytes are incubated at 37°C ± 2°C in phoasphate buffer (pH 7.4) at 50% haematocrit in metabolic wheel rotating incubator bath. The sample was withdrawn with the help of hypodermic syringe and assases through 0.8 micron filter paper , the sample was deproteinized with actonitrile and estimated drug releases. Percent hemoglobin can similarly be calculated at various time intervals at 540 nm spectrophotometrically.

Another parameter which evaluates the haemoglobin disposition after the resealing is mean corpuscular haemoglobin. It is the mean concentration of haemoglobine per 100 ml of cell. It express as:

\[
\text{Mean corpuscular haemoglobin} = \frac{\text{haemoglobin (g/100 ml) \times 10}}{\text{erythrocytes count (per mm}^3)}
\]

**In vitro stability**
The stability of the loaded erythrocytes is assessed by means of the incubation of the cells in autologous plasma or in an iso-osmotic buffer, setting the hematocrit between 0.5% and 5% at temperatures of 4 and 37°C.

**Bio-medical application of resealed erythrocytes**
- Erythrocytes encapsulate different enzyme like urease, arginase, asperginase, etc in erythrocytes using hypotonic lysis method has prove that erythrocytes could operate as enzyme carrier.
- Various antineoplastic agent like bleomycine, aderamycin, cytosine circulated through erythrocytes and give a sustain release properties
- It also delivered a protein. Bird et. al.1983 propose erythrocytes as a carrier for delivery of insulin for its sustain release Erythrocyte defense against hydrogen peroxide
- Drug-loaded erythrocytes are safe and useful carriers of new and conventional therapeutics and can be advantageous delivery systems for new clinical applications where proteins and oligonucleotides are therapeutic agents.
- In COPD patients, pharmacokinetic analyses have shown that a single administration of drug-loaded erythrocytes (2.4mg) was able to maintain detectable dexamethasone concentrations in blood for up to 7 days (longer time was not evaluated).
- Antibody coated resealed drug erythrocytes useful to targeting RES organ
- In the case of oxygen deficiency resealed erythrocytes improve oxygen supply

Future prospective
- Magnetite-loaded RBCs respond to an attached permanent magnet for MRI studies
- Nanoerythrosomes are vesicles prepared by the extrusion of RBC ghost, the average diameter of these vesicles being 100 nm. The process gave small vesicles with the size of liposomes. These spheroid particles were named ‘Nanoerythrosomes’ and appear to be stable and maintain both the cytotoxic and antineoplastic activity of daunorubicin (DNR) against mice leukemia P338-D-cell. Antiviral drugs can be pretreated to deliver drug directly to macrophages
- Erythrosome: Erythrosomes are specially engineered vesicular systems in which chemically cross-linked human erythrocytes cytoskeletons are used as sport upon which a lipid bilayer is coated. This can be achieved by a modification procedure normally adopted for reverse phase evaporation. Erythrosomes are proposed as useful encapsulation system for drug delivery particularly for macromolecular drugs.
- Genetic engineering aspect can be coupled to give a newer dimension to existing cellular drug carrier concept.

Conclusion
Human erythrocytes life span 120 days so it can provided an efficient way of delivering of drug to targeted site with long time sustainability, now a day not any marketed product available in market lot of patented technology available for drug loading in erythrocytes and prove that it biocompatible to our body. So we try to develop a product with god gifted erythrocytes which have long life for sustain properties in our body.

References


43. T. S. RAJESWARI, “ Non invasive insulins: advanced insulin therapy over this decade”, Journal of Applied Pharmaceutical Science 01 (08); 2011: 12-20


Abstract:
A simple, sensitive, precise and accurate reversed phase liquid chromatographic method has been developed for the estimation of E and Z guggulesterone from dosage form. The method was developed using a Phenomenex C-18 Waters Column 150 x 4.6mm, 5μ silica column with a mobile phase consisting of Methanol : TDW (0.5% Formic Acid) – 65 : 35, at a flow rate of 1.0 mL min⁻¹. The UV detection was achieved at 240 nm, over a wide dynamic range of 0.312 to 10 μg mL⁻¹. The retention time of E and Z guggulesterone was 15 & 20 minute respectively. The method was successfully validated in accordance to ICH guidelines acceptance criteria for specificity, linearity, accuracy, precision, robustness, ruggedness and system suitability. Intra-day and inter-day assay accuracy and precision of the E and Z guggulesterone were less than 2%, and the average recovery were in the range of 98–102%. E and Z guggulesterone were to the stress conditions of oxidation, acid and base hydrolysis and thermolysis. The method was successfully applied for analysis of E and Z guggulesterone in the presence of excipients in commercially available bulk dosage form.

Introduction
Gum resin of Commiphora mukul is used in Ayurveda for various ailments such as atherosclerosis, hyperlipidemic, obesity and other lipid disorders. The standardized ethyl acetate extract of this gum resin (Guggul) was reported to have marked antihyperlipidemic activity. In 1986, with proven efficacy and safety, Guggul was approved for marketing in India as hyperlipidemic drug and currently in United State and western world it is marketed as dietary supplement.

Guggulsterone, characterized as cis (E)- and trans (Z)-stereo isomers of 4,17(20)-pregnadiene-3,16-dione (Fig-1). It was reported to act by FXR antagonism and inhibition of platelets aggregation. These two isomers also exhibited potential antioxidant, anti-arthritis, anti-inflammatory, memory enhancer and anti-cancer activities. The pharmacokinetic profiles of two active isomers guggulsterone have not been fully explored.

Method validation is the process of proving that an analytical method is accepted for its intended purpose. For pharmaceutical methods, guidelines from the United States of Pharmacopeia (USP), International Conference on Harmonization (ICH) and the Food and Drug Administration (FDA) provides a frame work for performing such validations.

Therefore aim of the present work was to develop simple, precise and accurate RP-HPLC method for determination of E and Z guggulesterone in pharmaceutical and bulk dosage form. The established method was validated with respect to specificity, linearity, precision, accuracy and ruggedness. In addition, forced degradation studies were performed in order to prove the suitability of the method for the stability-indicating assay of E and Z guggulesterone.

Experimental

Chemicals and Materials
Premixed E- and Z-Guggulsterone at 1:1 ratio was kindly given from Sami Lab Limited (Bangalore, India). HPLC grade acetonitrile and methanol were from Sigma Aldrich Chemicals Pvt Ltd (Mumbai, India). Sodium acetate AR, glacial acetic acid AR, and ammonia solution (25%) were purchased from E Merck Pvt Ltd (Mumbai, India). Ultrapure water was obtained from a Milli-Q PLUS PF water purification system. E & Z guggulesterone in Shudha guggule Batch by Himalaya Ltd, (India) were purchased from local pharmacy within their shelf-life period.

Instrumentation and Chromatographic Conditions
The method was developed using a Waters HPLC system consisted of a binary pump , auto sampler and UV detector. The separation was achieved on phenomenex C18 (250 mm × 4.6 mm, 5.0 μ) column with a mobile phase consisting of Acetonitrile: Sodium acetate buffer, pH 5.5 (38: 62 v/v); at a flow rate of 1.0 mL min⁻¹. Detection was carried out with ultra-violet detector at 240 nm. The total run time was 24 min. The volume of injection was 40 μL, prior to injection of analyte, the column was equilibrated for 30-40 min with mobile phase. The analysis was performed at ambient temperature.

Preparation of Standard Stock Solution and Calibration Curves
The stock solutions of 1.0 mg mL⁻¹ of E & Z guggulesterone were prepared in methanol. Calibration samples and quality control samples (QC) were prepared by diluting working standard solution with methanol to give concentrations in the range of 0.039–20 μg mL⁻¹ for E & Z guggulesterone. QC samples at four different
concentrations (0.039, 0.312, 2.5 and 20 µg mL\(^{-1}\)) as LLOQ, low, medium and high, respectively) were prepared separately in five replicates, independent of the calibration standards. Test samples and quality control samples were then interpolated from the calibration curve to obtain the concentrations of the respective analyte. Calibration curves were plotted as concentration of drugs versus peak area response.

**Analysis of Marketed Formulation**

Three replicates of the required dilutions were prepared from Capsules stock solution and sonicated for 10 min. For analysis of suspension dosage form of E & Z guggulesterone was filtered through whatman filter paper.

**Method Validation**

The method was validated in compliance with ICH guidelines; in terms of specificity, selectivity, linearity, precision, accuracy, limit of quantification, limit of detection, robustness and other aspects of analytical validation.

**Specificity and Selectivity**

The specificity of the method was checked by comparison of chromatograms obtained from standard, sample and the corresponding placebo. The selectivity of the method was established from the resolution of the drug peak from the nearest and also among all other peaks. The analytes with a resolution factor greater than 4.2 shows the selectivity of the method.

**Linearity**

The linearity of the method was determined at seven concentration levels ranging from 0.039 to 20 µg mL\(^{-1}\) for E & Z guggulesterone. The calibration curves were established by plotting the peak area versus concentration. The regression parameters of slope, intercept and correlation coefficient were calculated by fitted to the \(y = mx + c\) using weighing factor \((1/x^2)\).

**Precision and Accuracy**

The precision (% RSD) and accuracy (% bias) of this analytical method were determined using QC samples \((n = 5)\) in five replicates of LLOQ, LOQ, MQC and HQC 0.034, 0.312, 2.5 and 20 µg mL\(^{-1}\), respectively. The accuracy of each sample preparation was determined by injection of calibration samples and four QC samples in five replicate for 5 days. The criteria for acceptability of the data included accuracy within ± 2% standard deviation (SD) from nominal values and precision of within ± 2% relative standard deviation (RSD).

**Recovery**

The recovery of the method was assessed by adding known amounts of E & Z guggulesterone to commercial available capsule containing a known amount of the drug (standard addition method). the average recovery obtained were in the range of 98–102% for all with %RSD below 2%. The recovery of the added pure drug was calculated as:

\[
\% \text{ Recovery} = \left( \frac{C_v - C_u}{C_a} \right) \times 100
\]

where \(C_v\) is the total amount of drug measured after standard addition, \(C_u\) the amount of drug in the formulation, and \(C_a\) the amount of drug added to the formulation.

**Limit of Detection and Limit of Quantification**

The limit of detection (LOD) and limit of quantification (LOQ) were calculated from the standard deviation of responses and slopes using signal-to-noise ratio as per ICH guidelines. This study was carried out to determinate the limit of detection (LOD, S/N = 3) and limit of quantitation (LLOQ, S/N = 10) in order to apply this method for the quantification of E & Z guggulesterone.

**Robustness**

Robustness of the method was studied by changing the extraction time of E & Z guggulesterone from dosage form by ± 5 min, composition of mobile phase by ± 2% of organic solvent, flow rate by ± 0.1 mL min\(^{-1}\) and buffer pH by ± 0.2. The changes in the response of the analyte were noted.

**System-Suitability Test**

System suitability testing was done by CDER (Centre for drug and evaluation research) guideline. It is used to verify that the resolution and repeatability of the system were adequate for the analysis intended. The parameters used in this test were asymmetry of the chromatographic peak, peak resolution, tailing factor, theoretical plates and repeatability, as %RSD of peak area for replicate injections.

**Forced Degradation Stability Studies**

The forced degradation of the drug molecule can help to identify the likely degradation pathway and the intrinsic stability of the analyte. Specificity is the ability of the method to measure the analyte response in presence of its potential impurities. All forced degradation studies were performed at an initial drug concentration of 20 µg mL\(^{-1}\).
Method Development
The chromatographic conditions were optimized with respect to specificity, resolution, and time of analysis. Hence we started the development activity with C18 stationary phase of various manufacturers such as Zorbax, ODS (250 × 4.6 mm, 5 µm), Spherisorb ODS (250 × 4.6 mm, 5 µm) Symmetry shield C18 (250 mm × 4.6 mm, 5.0 µ), and Phenomenex Luna C18 (250 × 4.6 mm, 5 µm). The last two columns were found to be suitable. However, Symmetry shield C18 (250 mm × 4.6 mm, 5.0 µ, column was used as a good resolution and minimum elution time were obtained. But the stationary phase is not only the parameter which can give better resolution. Mobile phase, pH and organic modifies also plays very important role which leads the best separation.

Different mobile phases containing acetonitrile, methanol, water and buffer were examined. Initially the methanol was used as an organic modifier which gives the poor baseline with baseline drift. Hence the response for the E & Z guggulesterone was reduced. To improve the resolution and response, acetonitrile was tried as an organic modifier. The base line was found good and response E & Z guggulesterone was improved. Effects of pH (3–7) and ionic strength (5–50 mM) were investigated using phosphate and acetate buffer and it was found that at higher and lower pH the tailing of the E & Z guggulesterone peak was more and also resolution was poor of the analyte. The effect of buffer concentration on the retention of E & Z guggulesterone was also studied. The mobile phase containing acetonitrile: sodium acetate buffer (38: 62 v/v, pH 5.5), was selected as optimal for obtaining well-resolved peaks with acceptable system suitability parameters. Flow rates from 0.8 to 1.5 mL min⁻¹ were tested. Low flow rates led to an increase in retention times increasing the time of analysis for the studied analyte. High flow rates led to a remarkable increase in column pressure and decrease in resolution. It was found that 1.0 mL min⁻¹ was optimal as it compromised between resolution and run time.

Effect of the wavelength on the response factor was observed over the wavelength range 303–240 nm. The detection wavelength, 240 nm was found optimal due to the high absorbtivity at this wavelength for E & Z guggulesterone. Complete separation was achieved in ~24 min at ambient temperature. The average retention times ± RSD % for E & Z guggulesterone was found to be 15.04 ± 0.13 (n = 10) and 20.05 ± 0.18 (n = 10) respectively.

Method Validation
Specificity
There was no cross interference from impurity, excipients or additives at the retention time of E & Z guggulesterone was found. Representative chromatograms of blank and E & Z guggulesterone are shown in Fig. 2. The retention time of the analyte showed less variability with a relative standard deviation (R.S.D.) well within the acceptable limit of 5%.

Linearity
The calibration plot for the method was linear over the con concentration in the range of 0.039 to 20 µg mL⁻¹ for E & Z guggulesterone. The correlation coefficient (r²) of the regression was 0.9996. The best-fit linear equation obtained was y = 100537.2x - 13435. This result demonstrates linearity of this method over the specified range.

Accuracy and Precision
Accuracy and precision (intra- and inter-day) were calculated at four different concentration levels of LLOQ, LOQ, MQC and HQC (n = 5), for analyte on five days are presented in Table 1. The results showed that the analytical method is accurate, as the % bias is within the acceptance limits of ± 2.0% of the theoretical value. The precision around the mean value was never greater than ± 2.0 % at any of the concentrations studied.

Recovery
The Value of recovery (%), standard deviation and %coefficient of variance (%COV), indicating method accuracy, is listed in Table 2.

Limit of Detection and Limit of Quantification
The LOD for E & Z guggulesterone was found to 0.025 µg/ml at a signal to noise ratio of 3:1, while the limit of quantification on was 0.039 ng/ml.

Robustness & System-Suitability Test
The method is found to be robust as the results were not significantly affected by slight variation in composition of mobile phase, extraction time, flow rate and Buffer pH (data not shown).
The SST measured from six replicate injections of E & Z guggulesterone was capacity factor, theoretical plates column efficiency and tailing factor and the results are tabulated in Table 3. For all six injections the tailing factor was less than 1.5.

Stability
The stability of E & Z guggulesterone was investigated thoroughly under auto-sampler storage and bench-top storage (Table 4). The results obtained were well within the acceptable limits. At 4 °C, E & Z guggulesterone showed no degradation for a 24 hr period.

Analysis of E & Z guggulesteronemycin Dosage Form.
The proposed validated RP-HPLC method was successfully applied to determine E & Z guggulesterone in marketed capsule (Shudha guggule). Three replicates of the required dilutions were prepared from suspension stock solution and sonicated for 10 min. These solutions (50 µL) were injected for quantitative analysis. The amounts of E & Z guggulesterone were calculated by extrapolating the peak area from the calibration plot. The mean percentage of E & Z guggulesterone were found to be 100.46 ± 0.028, which are comparable to the corresponding labeled amounts.

Results of Forced Degradation Studies
Degradation in acidic solution: The E & Z guggulesterone was exposed to 0.1 N HCl at 60 °C temperature for 1 h. The E & Z guggulesterone rapidly underwent degradation with time in acidic condition. The major degradation product was observed at RT 4.6, 6.5, 48.5 and 10.5 min.

Degradation in basic solution: The E & Z guggulesterone was exposed to 0.1 N NaOH at 60 °C temperature for 1 h, prominent degradation was observed.

Oxidative conditions: No degradation was observed after 1 h at 60 °C temperature.

Thermal degradation: The E & Z guggulesterone powder was exposed to dry heat at 60 °C for 7 days and the E & Z guggulesterone solution was kept at room temp for 7 days. No significant degradation (~4 %) was observed.

Degradation in neutral (water) solution: No degradation was observed after 1 h at 60 °C temperature.

Conclusion
The validated RP-HPLC method employed here proved to be simple, specific, accurate, precise, sensitive and robust. The validated method showed satisfactory data for all the validation parameters tested. The optimum retention time allows the analysis of a large number of samples and is therefore more cost effective. The information presented here could be very useful for quality monitoring of bulk samples and as well as employed to check the quality of drug during stability studies in pharmaceutical formulations.

References


18. ICH (2006), International conference on Harmonization, IFPMA.


Figures

1a. $R_1 = CH_3, R = H \quad E$-Guggulsterone

1b. $R_1 = H, R_2 = CH_3 \quad Z$-Guggulsterone

Fig. 1. The chemical structure of guggulsterone (1) guggulsterone (Z-isomer (1a) and E-isomer (1b))

Fig. 2.1 Representative chromatogram of blank mobile phase

Fig. 2.2 Representative chromatogram of E and Z guggulsterone
### Table 1. The Accuracy (% bias) and precision (% R.S.D.) of E and Z Guggulesterone

<table>
<thead>
<tr>
<th>Concentration (ng/ml)</th>
<th>E LLOQ</th>
<th>E LQC</th>
<th>E MQC</th>
<th>E HQC</th>
<th>Z LLOQ</th>
<th>Z LQC</th>
<th>Z MQC</th>
<th>Z HQC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical</td>
<td>0.039 µg/mL</td>
<td>0.312 µg/mL</td>
<td>2.5 µg/mL</td>
<td>20 µg/mL</td>
<td>0.039 µg/mL</td>
<td>0.312 µg/mL</td>
<td>2.5 µg/mL</td>
<td>20 µg/mL</td>
</tr>
<tr>
<td>%Bias$_{\text{intra-assay}}$</td>
<td>-1.70</td>
<td>0.003</td>
<td>0.67</td>
<td>0.81</td>
<td>-1.81</td>
<td>0.0039</td>
<td>0.63</td>
<td>0.755</td>
</tr>
<tr>
<td>%Bias$_{\text{inter-assay}}$</td>
<td>1.32</td>
<td>0.45</td>
<td>1.13</td>
<td>1.17</td>
<td>1.22</td>
<td>0.48</td>
<td>1.04</td>
<td>1.176</td>
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<tr>
<td>%RSD$_{\text{intra-assay}}$</td>
<td>2.10</td>
<td>0.16</td>
<td>1.78</td>
<td>0.21</td>
<td>1.98</td>
<td>0.19</td>
<td>1.63</td>
<td>0.213</td>
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<tr>
<td>%RSD$_{\text{inter-assay}}$</td>
<td>1.87</td>
<td>1.05</td>
<td>0.52</td>
<td>0.78</td>
<td>1.93</td>
<td>1.02</td>
<td>0.59</td>
<td>0.75</td>
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</table>

### Table 2. The recovery of E and Z Guggulesterone

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>E %Recovery ± S.D</th>
<th>E % COV</th>
<th>Z %Recovery ± S.D</th>
<th>Z % COV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.039</td>
<td>98.87 ± 0.032</td>
<td>0.89</td>
<td>98.85 ± 0.12</td>
<td>0.88</td>
</tr>
<tr>
<td>0.312</td>
<td>102.70 ± 0.001</td>
<td>0.375</td>
<td>99.70 ± 0.001</td>
<td>0.366</td>
</tr>
<tr>
<td>2.5</td>
<td>99.28 ± 0.026</td>
<td>1.03</td>
<td>98.18 ± 0.023</td>
<td>1.05</td>
</tr>
<tr>
<td>20</td>
<td>99.45 ± 0.216</td>
<td>1.09</td>
<td>99.75 ± 0.213</td>
<td>1.08</td>
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</table>
### Table 3. System suitability study (SST)

<table>
<thead>
<tr>
<th>Guggulestrone</th>
<th>E</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>SST limits</td>
<td>CDER guidelines</td>
<td>Calculated</td>
</tr>
<tr>
<td>Repeatability of peak response</td>
<td>≤1.0% for 5 replicates</td>
<td>0.778</td>
</tr>
<tr>
<td>Resolution</td>
<td>&gt;2.0 general</td>
<td>5.43</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>≤2.0</td>
<td>1.39</td>
</tr>
<tr>
<td>Column efficiency</td>
<td>&gt;2000 (plate count)</td>
<td>2594.74</td>
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<tr>
<td>Capacity factor</td>
<td>&gt;2</td>
<td>5.01</td>
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### Table 4. Bench top and auto sampler stability of E and Z Guggulesterone

<table>
<thead>
<tr>
<th>Storage Conditions</th>
<th>Nominal Conc. (µg/ml)</th>
<th>Initial amount (µg/ml)</th>
<th>Amount after 20 hrs (µg/ml)</th>
<th>% deviation</th>
<th>Nominal Conc. (µg/ml)</th>
<th>Initial amount (µg/ml)</th>
<th>Amount after 20 hrs (µg/ml)</th>
<th>% deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auto-sampler stability (4°C, 24 h)</td>
<td>0.312</td>
<td>0.318</td>
<td>0.3226</td>
<td>-1.361</td>
<td>0.312</td>
<td>0.323</td>
<td>0.3266</td>
<td>-1.261</td>
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<tr>
<td></td>
<td>2.5</td>
<td>2.462</td>
<td>2.468</td>
<td>-0.230</td>
<td>2.5</td>
<td>2.422</td>
<td>2.468</td>
<td>-0.210</td>
</tr>
<tr>
<td>Bench-top stability for 20 hrs at ambient item</td>
<td>0.312</td>
<td>0.317</td>
<td>0.315</td>
<td>0.630</td>
<td>0.312</td>
<td>0.317</td>
<td>0.315</td>
<td>0.630</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>2.475</td>
<td>2.433</td>
<td>1.710</td>
<td>2.5</td>
<td>2.475</td>
<td>2.433</td>
<td>1.685</td>
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</table>

### Table 5. Forced degradation stability studies of E and Z Guggulesterone

<table>
<thead>
<tr>
<th>Guggulestrone</th>
<th>E</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress condition</td>
<td>Time</td>
<td>% Assay</td>
</tr>
<tr>
<td>Acid/0.1 N HCl/60°C</td>
<td>10 min</td>
<td>41.891</td>
</tr>
<tr>
<td>Process</td>
<td>Condition</td>
<td>Time</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>----------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Base/0.1 N NaOH/60°C</td>
<td></td>
<td>10 min</td>
</tr>
<tr>
<td>Oxidation/3% H₂O₂/RT</td>
<td>60 min</td>
<td></td>
</tr>
<tr>
<td>Oxidation/30% H₂O₂/RT</td>
<td>60 min</td>
<td></td>
</tr>
<tr>
<td>Thermal degradation</td>
<td>Powder</td>
<td>1 Week</td>
</tr>
<tr>
<td></td>
<td>Solution</td>
<td></td>
</tr>
<tr>
<td>Water hydrolysis</td>
<td>60 min</td>
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</table>
HEPATOPROTECTIVE ACTIVITY OF LUFFA CYLINDRICA LEAF EXTRACT AGAINST CARBON TETRACHLORIDE-INDUCED DAMAGE IN RATS

Abstract:
The methanolic extract of Luffa cylindrica leaves was evaluated for its hepatoprotective effect against CCl₄ induced hepatotoxicity in rats. Alteration in the levels of biochemical markers of hepatic damage like SGPT, SGOT, ALP, Total bilirubin and tissue LPO, GSH, catalase & SOD were tested in treated groups. Administering of CCl₄ (10ml/kg body weight) significantly enhanced the SGPT, SGOT, ALP, Total bilirubin and tissue level of varied enzymes. Treatment with extract of Luffa cylindrica leaves (50 mg/kg) brought back the altered levels of biochemical markers to the near normal levels.

Introduction
Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. The chemical constituents present in the herbal medicine or plant are a part of the physiological functions of living flora and hence they are believed to have better compatibility with human body. Natural products from plants are a rich resource used for centuries to cure various ailments.

The use of bioactive plant-derived compounds is on the rise, because the main preoccupation with the use of synthetic drugs is the side effects which can be even more dangerous than the diseases they claim to cure. In contrast, plant derived medicines are based upon the premise that they contain natural substances that can promote health and alleviate illness and proved to be safe, better patient tolerance, relatively less expensive and globally competitive. So, in respect of the healing power of plants and a return to natural remedies is an absolute requirement of our time.

Luffa [Luffa cylindrica (L.) syn Luffa aegyptiaca Mill] commonly called sponge gourd, loofa, vegetable sponge, bath sponge or dish cloth gourd, is a member of cucurbitaceous family. The fruits of Luffa cylindrica are smooth and cylindrical shaped. One mature Luffa sponge will produce at least 30 seeds. Some will produce many more.

Luffa cylindrica has alternate and palmate leaves comprising petiole. The leaf is 13 and 30 cm in length and width respectively and has the acute-end lobe. It is hairless and has serrated edges. The flower of Luffa cylindrica is yellow and blooms on August-September. Luffa cylindrica is monoecious and the inflorescence of the male flower is a raceme and one female flower exists. Its fruit, a gourd, is green and has a large cylindrical shape and grows climbing on other physical solid materials.

Luffa cylindrica is a sub-tropical plant, which requires warm summer temperatures and long frost-free growing season when grown in temperate regions. It is an annual climbing plant which produces fruit containing fibrous vascular system. It is summer season vegetable. It is difficult to assign with accuracy the indigenous areas of luffa species. They have a long history of cultivation in the tropical countries of Asia and Africa. Indo-Burma is reported to be the center of diversity for sponge gourd. The main commercial production countries are China, Korea, India, Japan and Central America.

Material and Methods
Reagents and chemicals
Carbon tetrachloride was procured from Krishna Chem. Industry Vadodara (India), silymarin was obtained as gift sample from Micro labs limited India, standard kits of SGPT, SGOT, ALP and bilirubin were obtained from Erba Diagnostics, India. All other reagents used were of analytical grade.

Collection and authentication of plant material
The leaves of Luffa cylindrica plant were collected from local area of Moradabad, Uttar Pradesh, India in the month of August 2010. Plant was authenticated by Dr. Beena Kumari (taxonomist) professor, department of botany, Hindu college (PG) Moradabad, Uttar Pradesh where a voucher specimen (HC.MBD/HAP/BK/2010/7/168) had been deposited for future reference. Leaves were washed with tap water and dried in shade. Dried leaves were ground to coarse powder and stored in an airtight container.
Preparation of extract
The successive extraction of powdered material was carried out in several batches using different solvents in increasing order of polarity in a soxhlet apparatus by hot percolation technique. The solvents used were petroleum ether, chloroform, acetone, methanol and distilled water. The powdered material of Luffa cylindrica leaves was evenly packed in a soxhlet extractor for about 36 hours with different solvents. The temperature was maintained (25°C-100°C) on an electric heating mantle with thermostat control. The extracts were then concentrated by evaporating the solvent under reduced pressure. Preliminary phytochemical studies were carried out on methanolic extract to assess the presence of various phytoconstituents and total phenolic determination.

Experimental animals
Wistar albino rats of either sex, weighing 150 to 200 gm, were housed in groups of four per cage under controlled light (12:12 light: dark cycle) and temperature (25 ± 2°C). Environmental and behavioral assessment was conducted during the light cycle. Food (Golden feed, New Delhi, India) and water ad libitum was provided. The animals were acclimatized to laboratory conditions for seven days before commencement of experiments. All the procedure described, were reviewed and approved by Institutional Animal Ethical Committee.

Carbon tetrachloride induced hepatotoxicity
Hepatoprotective study was carried out using CCl₄ as toxicant and Silymarin was used as standard reference. Methanolic extract at a dose of 50 mg/kg was used as test and compared with the results obtained with standard and control.

Grouping of animals
Animals were divided into 4 groups (n=6/group).

Group 1 - Toxic control: The animals received distilled water for 7 days and given CCl₄ in olive oil (1:9 ratio) single dose, 10ml/kg body weight i.p. on day 8th.

Group 2 - Normal control: The animals received saline for 7 days

Group 3 - Standard: Pre-treated with standard drug Silymarin (100mg/kg body weight/day p.o.) for 7 days followed by a single dose of CCl₄ on day 8th.

Group 4 - Test: Pre-treated with extract (50 mg/kg/day p.o.) for 7 days followed by a single dose of CCl₄ on day 8th.

16 h after administration of last dose of drugs, the blood was collected by retro orbital artery bleeding. Blood samples were centrifuged for 10 minutes at 2000 rpm to separate the serum. Alkaline phosphatase (ALP), serum glutamicoxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and bilirubin levels were estimated from the serum by using standard kits. After collection of blood samples the rat were sacrificed and their liver was excised, rinsed in ice cold normal saline followed by 0.15 M Tris-HCL buffer and processed for the estimation of lipid peroxidation (LPO). A part of homogenate after precipitating proteins with trichloroacetic acid (TCₐ) was used for the estimation of glutathione (GSH) [12]. The rest of the homogenate was centrifuged at 15000 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of superoxide dismutase (SOD) and catalase (CAT).

Histopathology
Tissue samples from liver lobules were dissected using precision microtone (buchi type). Sections of 50 micron thickness were cut by embedding tissue in wax. Sections were stained by Haematoxylene and Eosine. Stained sections were observed at 40x and 100x magnification. Constitution of normal components, presence of inflammation and necrosis were considered during observation.

Biostatistical Interpretation
The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett’s Test. The values were expressed as mean ± SEM and P<0.05 was considered significant.

Results
Preliminary phytochemical screening indicates the presence of carbohydrates, steroids, flavonoids, tannins, phenolic compounds, saponins and terpenoids. The extract was found to contain 53.78 µg/mg total phenolics expressed as gallic acid equivalents.

Acute toxicity studies
Methanolic extract of *Luffa cylindrica* leaves did not produce any toxic symptoms or mortality up to the dose level of 2000mg/kg body weight in rats, and hence the extract was considered to be safe and non-toxic for further pharmacological screening.

**Protective effect of *Luffa cylindrica* on biochemical parameters**

Estimation of the serum total bilirubin and alkaline phosphatase activity is one of the most widely used means of measuring hepatocellular injury. In this study, a significant increase in the levels of bilirubin, AST, ALT and ALP was observed in CCl₄ intoxicated group. In the present study, *Luffa cylindrica* methanolic extract (LCME) administration possesses significant effect on CCl₄ induced hepatotoxicity. Decreased levels of serum bilirubin, the activities of AST, ALT and ALP in LCME pretreated group indicated the effectiveness of the extract against CCl₄ induced hepatotoxicity.

**Table 1: Effect of *Luffa cylindrica* on biochemical parameters in CCl₄-induced hepatotoxicity in rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>SGPT (IU/L)</th>
<th>SGOT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Total bilirubin mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxic control</td>
<td>10 ml/kg</td>
<td>130.35 ± 1.65</td>
<td>172.45 ± 2.78</td>
<td>128.07 ± 3.39</td>
<td>4.72 ± 0.25</td>
</tr>
<tr>
<td>Normal control</td>
<td>1 ml</td>
<td>45.55 ± 1.58***</td>
<td>58.82 ± 2.71***</td>
<td>49.79 ± 2.90***</td>
<td>± 0.98 ± 0.16***</td>
</tr>
<tr>
<td>Standard</td>
<td>100 mg/kg</td>
<td>51.18 ± 1.46***</td>
<td>64.28 ± 1.18***</td>
<td>53.53 ± 1.75***</td>
<td>± 1.32 ± 0.28***</td>
</tr>
<tr>
<td>Test</td>
<td>50 mg/kg</td>
<td>93.23 ± 3.87***</td>
<td>111.40 ± 3.55***</td>
<td>79.25 ± 2.91***</td>
<td>± 2.05 ± 0.24***</td>
</tr>
</tbody>
</table>

All values expressed as Mean ± SEM (n=6) ***P<0.001 as compared with toxic control group (One way ANOVA followed by Dunnett’s test)

**Effect of *Luffa cylindrica* on carbon tetrachloride induced changes in enzyme levels.**

Analysis of LPO levels by thiobarbituric acid reaction showed a significant (*P<0.001*) increase in the CCl₄ treated rats. Treatment with LCME (50 mg/kg) significantly (*P<0.001*) prevented the increase in LPO level which was brought to near normal. The effect of *Luffa cylindrica* was comparable with that of standard drug silymarin. CCl₄ treatment caused a significant (*P<0.001*) decrease in the level of SOD, Catalase and GSH in liver tissue when compared with control group (Table 2). The treatment with *Luffa cylindrica* at the doses of 50mg/kg resulted in a significant increase (*P<0.001*) of SOD, Catalase and GSH levels when compared to CCl₄ treated rats. The liver of silymarin treated animals also showed a significant increase in antioxidant enzymes levels compared to CCl₄ treated rats.

**Table 2: Effect of *Luffa cylindrica* on antioxidant level in CCl₄-induced hepatotoxicity in rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>CATALASE (U/mg protein)</th>
<th>SOD (%inhibition of NBT)</th>
<th>LPO (nM/mg protein)</th>
<th>GSH (mM/gm tissue wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxic control</td>
<td>10 ml/kg</td>
<td>14.22 ± 1.65</td>
<td>26.56 ± 1.29</td>
<td>7.43 ± 0.29</td>
<td>7.82 ± 0.63</td>
</tr>
<tr>
<td>Normal control</td>
<td>1 ml</td>
<td>33.92 ± 1.58***</td>
<td>68.93 ± 2.11***</td>
<td>2.69 ± 0.25***</td>
<td>31.87 ± 1.57***</td>
</tr>
<tr>
<td>Standard</td>
<td>100 mg/kg</td>
<td>30.49 ± 1.46***</td>
<td>61.32 ± 1.52***</td>
<td>2.99 ± 0.26***</td>
<td>27.02 ± 1.01***</td>
</tr>
<tr>
<td>Test</td>
<td>50 mg/kg</td>
<td>21.13 ± 3.87***</td>
<td>46.99 ± 1.84***</td>
<td>4.68 ± 0.34***</td>
<td>19.47 ± 1.56***</td>
</tr>
</tbody>
</table>

All values expressed as Mean ± SEM (No=6) ***P<0.001 as compared with toxic control group (One way ANOVA followed by Dunnett’s test)
Histopathology of liver

Histopathological images of:
Fig. (a): Normal control group, showing normal architecture.
Fig. (b): Toxic control group, showing necrosis of central vein.
Fig. (c): Standard group, showing normal appearance with no evidence of necrosis.
Fig. (d): *Luffa cylindrica* methanolic extract showing less evidence of necrosis compared to toxic group.

Histological profile of the animals treated with *Luffa cylindrica* supported the results obtained by the biochemical studies and enzymatic assays. Recovery against CCl$_4$ induced necrosis in their compact arrangement of hepatic cells was observed; whereas the section of liver of animals treated with silymarin showed that extent of liver damage was lesser in magnitude as compared to the CCl$_4$ treated animals.

Discussion
Liver plays an important role in metabolism of drug and nutrients. Because of its central role in drug metabolism, it is the most vulnerable tissue for drug toxicity. According to the reports published by USFDA, more than 900 drugs, toxins, and herbs have been reported to cause liver injury, and drugs account for 20-40% of all instances of hepatic failure. Approximately 75% of the idiosyncratic drug reactions result in liver transplantation or death. Drug-induced hepatic injury is the most common reason cited for withdrawal of an approved drug. The manifestations of drug induced hepatotoxicity are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic
failure. Various environmental toxicants and clinically useful drugs, like acetaminophen and gentamycin, can cause severe organ toxicities through the metabolic activation to highly reactive free radicals including the superoxides and oxygen reactive species. One of the most extensively studied of the environmental toxicants is carbon tetrachloride (CCl₄). CCl₄ is known to undergo reductive metabolism by CYP2E1 into a highly reactive trichloromethyl radical (·CCl₃) and phosgene that initiates lipid peroxidation, disrupts membrane integrity and causes cell death. Evidence suggests that various enzymatic and non-enzymatic systems have been developed by the cell to cope with the oxidative stress that is associated with reactive oxygen species (ROS) and other free radicals generated. The methanol extract of Luffa cylindrica leaf reduced the elevated levels of all the biochemical parameters caused by CCl₄. CCl₄-induced liver necrosis was inhibited significantly by Luffa cylindrica leaf extract, which confirms the protective action of the methanolic extract of Luffa cylindrica leaf against experimentally induced liver damage in rats. SGOT SGPT, ALP, TBL, LPO GSH, Catalase and SOD are the most sensitive tests employed in the diagnosis of hepatic disease. The elevated levels of these parameters were significantly reduced by the treatment of Luffa cylindrica leaf extract. Decrease the level of SGOT SGPT ALP and total bilirubin after treatment with extract in liver damage indicates the effectiveness of the extract in normal functional status of the liver. It can be concluded from this investigation that leaf of Luffa cylindrica possess hepatoprotective activity. This effect may be due to the presence of polyphenols and other antioxidants in the extract, as the toxicity produced by CCl₄ is due to oxidative stress.

References
Abstract:
In classical theories it is assumed that the strength of geometrically similar structure is not dependent on size of the structure. This assumption is based on strength criteria and is generally applicable to the material of homogenous character such as glass or metal structure. Concrete structures, which are quasibrittle in nature, however do not follow this trend. The size effect implies the dependence of various parameters of structure on its size. The size effect involves the variation of nominal strength $\sigma_N$ with size $D$; where $D$ implies the depth of the specimen. The mechanical properties of engineering materials are routinely determined by testing. In practice, the dimensions of structural elements are often much larger than those of the samples that has been tested in the laboratory. Consequently, testing is usually conducted on reduced scale models for technical and economic reasons of material samples. Nowadays, available theories of material behaviour that predict size effects are receiving increasing attention in the technical literature. However, literature survey does not show any significant study on size effect incorporating polypropylene fibres in RC structures. Hence in the present work, experimental investigations were carried out on both reinforced concrete beam specimen and RC beams strengthened with polypropylene to observe the size effect phenomena. In each type three geometrically scaled down specimens were tested to study various parameters with respect to size effect. Also, the gain in various parameters due to strengthening have been worked out. The specimens were tested with four point bending. The setup for the testing was fabricated in the laboratory. The experimental result reveals that it followed the size effect law proposed by Bazant. The percentage gain in ultimate load carrying capacity due to fibre (polypropylene) strengthening ductility also followed the size effect trend.

Introduction
In classical theories of solid mechanics it is assumed that material properties, such as the tensile or compressive strengths, are not scale dependent. In engineering practice such properties, measured on standard samples of the material, are usually assumed applicable to structural elements whose size greatly differs from those of the test samples. The explicit consideration of size is still far from being common practice in structural design. The size effect is rigorously defined through a comparison of geometrically similar structures of different sizes. It is conveniently characterized in terms of the nominal strength $\sigma_N$, representing the value of the nominal stress $\sigma_N$ at maximum (ultimate) load $P_u$. The nominal stress which serves as a load parameter, may not represent any actual stress in the structure and may be defined simply as $\sigma_N = P/bD$ when the similarity is in two dimensional structure where $b$ = thickness of the two dimensional structure and $D$ = characteristic dimension of the structure. This may be chosen by any dimension like the depth of the beam or the span or half of the span. The nominal strength is then $P_u/bD$ for two dimensional or $P_u/D$ for three dimensional structure. According to the classical failure theories such as the elastic analysis with allowable stress or plastic limit analysis, or any other theory that uses some type of a strength limit or failure surface in terms of stress or strain, $\sigma_N$ is constant, i.e., independent of the structure size for any given geometry. Thus if we plot $\log \sigma_N$ vs. $\log D$, we find the failure states, according to the strength or yield criteria exhibit no size effect. By contrast failure governed by linear elastic fracture mechanics exhibit a rather strong size effect.

Cracks play an important role as it changes concrete structures into permeable elements and consequently with a high risk of corrosion. Cracks not only reduce the quality of concrete and make it aesthetically unacceptable but also make structures out of service. Therefore, it is important to reduce the crack width and this can be achieved by adding polypropylene fibres to concrete. Thus addition of fibres in cement concrete matrix arrest these cracks and restrain them from further opening. In order to achieve more deflection in the beam, additional forces and energies are required to pull out or fracture the fibres. This process, apart from preserving the integrity of concrete, improves the load-carrying capacity of structural member beyond cracking. The major reasons for crack formation are shrinkage, plastic settlement, freeze thaw damage, fire damage etc.

Polypropylene is a plastic polymer, of the chemical composition $C_3H_6$. It is used in many different settings, both in industry and in consumer goods. It can be used both as a structural plastic and as fibres. Polypropylene (PP) is a versatile thermoplastic material, which is produced by polymerizing monomer units of polypropylene molecules into very long polymer molecules or chains in the presence of a catalyst under carefully controlled heat and pressure. Propylene is an unsaturated hydrocarbon, containing only carbon and hydrogen atoms. The chemical formula of polypropylene may be represented as:
Thus in this paper we shall explore the size-effect on reinforced concrete structural specimens by strengthening with polypropylene fibre and its effect in various structural parameters. The RC specimens chosen for analysing size-effect are discussed below along with test setup fabricated to evaluate the ultimate load carrying capacity and ductility which are discussed subsequently.

Methodology, Materials and Methods
In this study two sets of geometrically similar but scaled down specimens, full-scaled, two third and one-third scaled (1:2/3:1/3) were chosen considering the size-effect study in the present work. Two identical sets were casted, one set was treated as control and other set was fibre (polypropylene) strengthened. Both control set (RCBC) and fibre (polypropylene) strengthened set (PFBC) containing one large beam specimen, one medium beam specimen and one small beam specimen each. Scale of reduction in terms of dimensions of the specimen, reinforcements used and aggregate sizes have been kept constant in order to maintain full geometrical similarity. All specimens were designed so that the failure occurs in flexure.

The cross section of the beam specimens were chosen as

i) 120 mm × 150 mm for the large beam

ii) 80 mm × 100 mm for the medium beam

iii) 40 mm × 50 mm for the small beam

maintaining the gradual reduction of scale (1:2/3:1/3). In the large beam, four numbers of high yield strength deformed bars were used out of which two numbers of 8 mm diameter bars at top longitudinal reinforcement and two numbers of 12 mm diameter bars were used as bottom longitudinal reinforcement bars. Stirrups for shear reinforcement of 6 mm diameter mild steel coil were used maintaining a spacing of 120 mm c/c. Similarly, the medium beam specimens and small beam specimens were chosen maintaining the two-third scale and one-third scale. The beams were designed following the provisions of IS: 456-2000. Moreover, the beam was idealised as a simply supported beam for arriving at the failure load of beam. Adequate shear reinforcement was provided to avoid shear failure. In Fig.1 detailing of reinforcement for large beam, medium beam, and small beam has been presented.

Fibres (Polypropylene fibres) were provided by 2% volume fraction of cement in a particular mix. The unit weight of polypropylene fibre is 0.90 gm/cc. Fibres were mixed with cement base matrix before pouring water. When casting of RCBC specimen and PFBC specimen 20% of volume was increased because this excess volume was casted for cube testing. Compressive strength of concrete has been tested, before testing the specimen. Size of the testing cube was 150×150 mm as per IS: 456-2000. Compressive strength of concrete cube has been tested at 28 days curing. In Table 2.1 and Table 2.2, it has been showed the observation value of cube test without fibre and with fibre respectively.

<table>
<thead>
<tr>
<th>S.No. of Cube</th>
<th>Failure load (kN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>610.20</td>
</tr>
<tr>
<td>2</td>
<td>635.18</td>
</tr>
<tr>
<td>3</td>
<td>663.75</td>
</tr>
<tr>
<td><strong>Average value</strong></td>
<td><strong>636.38</strong></td>
</tr>
</tbody>
</table>

\[
\text{Stress} = \frac{636.38 \times 10^3}{(150 \times 150)} = 28.28 \text{ N/mm}^2
\]
Table 2.2 Compressive strength of concrete cube on 28 days curing (with fibre)

<table>
<thead>
<tr>
<th>Sl.No. of Cube</th>
<th>Failure load (kN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>622</td>
</tr>
<tr>
<td>2</td>
<td>650</td>
</tr>
<tr>
<td>3</td>
<td>680.28</td>
</tr>
<tr>
<td><strong>Average value</strong></td>
<td><strong>650.76</strong></td>
</tr>
</tbody>
</table>

Stress = $650.76 \times 10^3 / (150 \times 150) = 28.92 \text{ N/mm}^2$

---

**Figure 1. Schematic Diagram of Specimens**

- **A) Large Specimen**
  - Effective Span $L=1200\text{mm}$, $c/s=bxd=120\text{mm} \times 150\text{mm}$
  - 2-8mm dia main bar top
  - Clear cover = 25mm
  - 2-8mm
  - 120mm : 150mm
  - 2-12mm dia main bar bottom
  - 6mm dia stirrups @ 120mm c/c
  - 2-12mm

- **B) Medium Specimen**
  - Effective Span $L=800\text{mm}$, $c/s=bxd=80\text{mm} \times 100\text{mm}$
  - 2-6mm dia main bar top
  - Clear cover = 16mm
  - 2-8mm main bar bottom
  - 4mm dia stirrups @ 80mm c/c
  - 2-8mm

- **C) Small Specimen**
  - Effective Span $L=400\text{mm}$, $c/s=bxd=40\text{mm} \times 50\text{mm}$
  - 2-4mm dia main bar top
  - 2-40mm c/c
  - 40mm
  - 2-4mm
  - 2-6mm dia main bar bottom
  - Clear cover = 8mm
  - 2-6mm

---

*Figure 1. Schematic Diagram of Specimens*
Test Setup and Instrumentation:

**Four-point bending Test**

In four-point bending, the simply supported beam is supported on two outer points, and deformed by driving two concentrated loads. The maximum stresses are located at the loads. In order to evaluate the ultimate load carrying capacity, corresponding bending stress etc., four-point-bending tests are generally conducted. The loading are generally applied at the middle 1/3rd or 2/3rd length of the specimen such as to get a shear free zone between two point loads and thus the purely bending stress within the shear free zone can be obtained as per our requirement.
The test was conducted using the set up available in the solid mechanics lab of NIT Silchar. A test frame was fabricated, and a Universal Testing Machine (UTM) was used to carry out the tension test of beam specimen.

The beam specimens are placed in the machine between the grips above a steel I-section joist. The steel I-section joist is fabricated and introduced above the upper cross head just to accumulate the overall length of the large beam of 1.4m and thus the other medium beam specimen of overall length 1m and small beam specimen of overall length 0.6m are automatically adjusted. Further a roller support at both the ends of the beam specimen are provided just underneath the specimen and over the joist in such a way that the effective length maintained in every case which is 1.2m in case of large beam, 0.8m in case of medium beam and 0.4m in case of small beam. It is obvious that due care was taken such that the joist is also capable of withstanding dead load of the large beam having length 1.4m and thus it automatically serves the purpose for medium and small specimen of lesser weight. Further two point loads are applied over the beam specimen by a roller arrangement connected to the lower cross head. The loads are applied at the middle one-third of the effective length of the specimen and goes up to failure load. The deflections were properly recorded by the available software in UTM.

**Interpretation of results:**

The typical load displacement curve showing the pattern of the gradual increment of displacement corresponding to the gradual increment of load for the large control and fibre (polypropylene) strengthened beam specimens i.e PFBCL and RCBCL. It can be observed from the graph that the ultimate failure load of 50.02 KN for the PFBCL is more than RCBCL of 45.03 KN. Similar observations were made for scale down specimens.
The failure mode of both control and fibre strengthened specimens under consideration was in flexure. Hence, ultimate bending stress denoted by $\sigma_u$ for all the specimens were calculated. Using $\sigma_u$, bi-logarithmic plots were drawn for both control and retrofitted specimens. For the purpose of statistical regression of data, the size effect law proposed by Bazant was used. This law can be expressed as

$$\sigma_u = \frac{f'_c}{D_0} + B \left( \frac{1}{D_0} \right)^2$$

Where, $f'_c$ is the tensile strength of the material, $B$ is dimensionless constant, $D_0$ is a constant with the dimension of length. The unknown constants $B$ and $D_0$ were determined by statistical regression analysis. The value of tensile strength of concrete was adopted $f'_c = 3.647\text{N/mm}^2$ for large control specimen, $3.719\text{N/mm}^2$ for medium control specimen and $3.802\text{N/mm}^2$ for small control specimen. Similarly, $f'_c = 3.680\text{N/mm}^2$ for large polypropylene fibre strengthened specimen, $3.762\text{N/mm}^2$ for medium polypropylene fibre strengthened specimen and $3.849\text{N/mm}^2$ for small polypropylene fibre strengthened specimen calculated as per IS: 456-2000[4]. A linear regression was achieved by Eq. (3.1a) as follows:

Squaring Eq.3.1.a and rearranging we get

$$\sigma_u^2 = \frac{f'_c^2}{D_0^2} + \frac{1}{B^2} \left( \frac{1}{D_0^2} \right)^2$$

This equation is in the form:

$$Y = AX + C$$

Where $X = D$, $Y = \sigma_u$, $C = \frac{1}{B^2}$ and $A = \frac{f'_c}{D_0^2}$

The values of $A$ and $C$ thus obtained was substituted in Eq. (3.1 d) to find the value of $D_0$ and $B$ as $D_0 = C/A$ and $B = 1/\sqrt{C}$

The calculated value of bending stress and other parameters necessary to carry out regression analysis for both control as well as fibre strengthened cases are furnished in the Table 3.1. The result of regression analysis for RCBC specimens is shown in Fig. 8. From this figure, the value of $B$ and $D_0$ were found to be 22.361 and 500 respectively. Using these values, the bi-logarithmic plot was drawn with Log $(D/D_0)$ in the X axis and Log $(\sigma_u/B)$ in the Y axis as shown in Fig.9 for RCBC specimen. It is observed from the plot that it follows a horizontal line at the initial part, indicating no size effect. The curve approaches a straight line with slope of about $-1/2$ towards the end. In the intermediate zone there is a smooth curved transitional part. Thus, it can be concluded that the plot shows presence of size effect in accordance with Bazant’s size effect law.

Similarly, the value of $B$ and $D_0$ was found as 223.61 and 0.333 respectively for fibre strengthened specimens same as that of control specimen. The bi-logarithmic plot for fibre strengthened specimens is shown in Fig. 9. This plot also shows presence of significant size effect and follows Bazant’s size effect law.
Table 3.1 Parameters for regression plotting

<table>
<thead>
<tr>
<th>Type of Specimen</th>
<th>Name of Specimen</th>
<th>Bending Stress, $\sigma_{tu}$ (N/mm$^2$)</th>
<th>Depth of Specimen, D (mm)</th>
<th>$\left(\frac{f_t}{\sigma_{tu}}\right)^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre strengthened (PFBC)</td>
<td>PFBCL</td>
<td>44.64</td>
<td>150</td>
<td>.00824</td>
</tr>
<tr>
<td></td>
<td>PFBCM</td>
<td>50.34</td>
<td>100</td>
<td>.00559</td>
</tr>
<tr>
<td></td>
<td>PFBCS</td>
<td>73.50</td>
<td>50</td>
<td>.00274</td>
</tr>
<tr>
<td>Control (RCBC)</td>
<td>RCBCL</td>
<td>40.21</td>
<td>150</td>
<td>.00823</td>
</tr>
<tr>
<td></td>
<td>RCBCM</td>
<td>43.58</td>
<td>100</td>
<td>.00728</td>
</tr>
<tr>
<td></td>
<td>RCBCS</td>
<td>59.21</td>
<td>50</td>
<td>.00414</td>
</tr>
</tbody>
</table>

Figure 8. Regression Plot for RCBC Specimen

$$y = 4 \times 10^{-5}x + 0.0025$$
$$R^2 = 0.9127$$
The slope of the bi-logarithmic plot for RCBC is found very close to -1/2, which shows presence of size effect in accordance with Bazán’s size effect law.

The displacement ductility for all the specimens was calculated from the respective Load – displacement curve. The displacement ductility of the control as well as fibre strengthened specimen is shown in Fig.11. It can be concluded from the figure that the displacement ductility increase marginally as the specimen size decreases for both control as well as fibre strengthened specimens. The maximum load designated as the ultimate load carrying capacity of a specimen has been recorded for each control specimens and fibre strengthened specimens. The percentage gain in ultimate load carrying capacity of fibre strengthened specimens with respect to control specimen for all sizes were calculated. It is observed that the variation in percentage gain of ultimate load carrying capacity is maximum in small size specimens and minimum for large specimens which also follows the size effect principle.

Result and Conclusion:
In this paper, the two sets of geometrically scaled down specimens (one set treated as a control specimen and other set treated as a fibre (polypropylene) strengthened specimen) were analyzed and interpreted for various parameters. Total six specimens covering both control and fibre strengthened specimens were tested. Various graphs like load displacement curve, regression plot, bi-logarithmic plot etc for all the six specimens were plotted. The bi-logarithmic plot for both control and fibre strengthened specimens follows the size effect law proposed by Bazant. The variation of the gain in load carrying in capacity due to fibre strengthening is presented in Fig.10. It is observed that this parameter vary from 11.08% for large specimen to 24.32% for small specimen. This clearly indicates that the gain in strength increases as the specimen size decreases supporting the size effect principle.
Displacement ductility varies from 2.133 to 3.11 for the control specimens. Large specimen has the least value and small specimen has the largest value. Further, it can also be noted from the figure that the ductility gets enhanced due to fibre strengthening for the specimens of all sizes. Displacement ductility varies from 2.99 to 3.85 for the fibre strengthened specimens and trend to the variation is similar to that of control specimens. Thus, it can be concluded that the displacement ductility also follows the principle of size effect.

![Figure 11: Displacement ductility improvement in case of fibre strengthened specimen](image)

Based on the interpretation of results, the following conclusions were drawn—

1. The bi-logarithmic plots of stresses for both control and fibre strengthened specimens followed closely the size effect law proposed by Bazant.
2. The gain in ultimate load carrying capacity due to fibre strengthening increased with decrease in size of the specimens ranging from 11% to 24% and it also follows the size effect principle.
3. The displacement ductility followed the principle of size effect in most of the cases studied and enhancement of ductility was observed ranging from 2.99 to 3.85 with gradual reduction of size of the fibre strengthened specimens.

References
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THE END
Among various methods of multiplexing wireless users, WCDMA is a commonly used method of multiplexing. WCDMA is a third-generation technology which provides better data speeds, to mobile and portable wireless devices than commonly offered today and gives the ability to work with various 3G technologies and networks being used worldwide. W-CDMA can support mobile/portable voice, images, data, and video communications at up to 2 Mbps (local area access) or 384 Kbps (wide area access). WCDMA supports highly variable user data rates, in other words the concept of obtaining Bandwidth on Demand (BoD) is well supported. The user data rate is kept constant during each 10 ms frame. However, the data capacity among the users can change from frame to frame. WCDMA supports two basic modes of operation: Frequency Division Duplex (FDD) and Time Division Duplex (TDD). In the FDD mode, separate 5 MHz carrier frequencies are used for the uplink and downlink respectively, whereas in TDD only one 5 MHz is timeshared between the uplink and downlink. Uplink is the connection from the mobile to the base station, and downlink is that from the base station to the mobile. WCDMA is a wideband technology based on the Direct sequence spread spectrum transmission scheme. In this scheme user information bits are spread over a wide bandwidth by multiplying the user data with quasi random bits called, chips, derived from CDMA spreading codes. In order to support very high data rate (2 mbps), the use of a variable spreading factor and multicode connection is supported. Signal processing plays a crucial role in wireless communication for variety of applications. Due to intensive use of FIR filters in video and communication systems, high performance in speed, area and power consumption is demanded. The group delay plays a crucial role in pulse shaping digital finite impulse response filter. The value of group delay should be minimum for efficient performance of digital pulse shaping filter. Pulse shaping is the process of changing the waveform of transmitted pulses. Its purpose is to make the transmitted signal suit better to the communication channel by limiting the effective bandwidth of the communication.

Need of Pulse Shaping
In communications systems, two important requirements of a wireless communications channel demand the use of a pulse shaping filter. These requirements are:

1) Generating band limited channels, and
2) Reducing inter symbol interference (ISI) arising from multi-path signal reflections.

Both of the above requirements can be accomplished by using a pulse shaping filter which is applied to each symbol. In fact, pulse shaping filter serves the purpose of generating signals such that each symbol period does not overlap.

Pulse shaping filter
The ideal pulse shaping filter has two properties:

1) A high stop band attenuation to reduce the inter channel interference as much as possible.
2) Minimized inter symbol interferences (ISI) to achieve a bit error rate as low as possible.

Pulse-shaping can be used to change the waveform of transmitted pulses so the signal bandwidth matches that of the communication channel. In modern digital communication; pulse shaping filters allow the transmission of pulses with negligible Inter symbol Interference (ISI).This means that pulse shaping digital filter is a useful means to shape the transmitted signal and avoid interference of Ultra Wideband (UWB) to other legacy narrow band signals.
System Model for WCDMA

In this we have an information signal which is being modulated with PN-sequence for spreading the signal. After that, it is encoded by Differential encoder. The modulation process is done by using OQPSK modulator. After modulation the Modulated signal is passed through Pulse shaping filter. We are using ‘Transmitting Gaussian filter’. It transmits signal over channel merged with ‘Gaussian Noise’, which shows the noise in Gaussian form. At receiver side it passes through ‘Receiving Gaussian filter’. After filtering we have demodulated and decoded the signal. It will then despreaded to find actual frequency range. Comparator provides the actual or desired signal. Error counter counts errors in total no. Of bits transmitted. Then we calculate the BER (Bit error rate).

Results and Discussion

The simulation study has been carried out at different values of D. The table shown below summarizes the results obtained using the simulation model for Gaussian filter and raised cosine filter. Total Transmitted bits are 4000 at Simulation Time = 0.000065 Sec.

<table>
<thead>
<tr>
<th>Group Delay D</th>
<th>Raised cosine Filter</th>
<th>Gaussian Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of Error Bits</td>
<td>BER</td>
</tr>
<tr>
<td>2</td>
<td>2053</td>
<td>0.51325</td>
</tr>
<tr>
<td>4</td>
<td>2041</td>
<td>0.51025</td>
</tr>
<tr>
<td>6</td>
<td>1977</td>
<td>0.49425</td>
</tr>
<tr>
<td>8</td>
<td>2025</td>
<td>0.50625</td>
</tr>
</tbody>
</table>

As we can see from the graph plotted using the table content that when we increase the value of Group delay from 2 to 6, the no. of error bits decreases, but when we further increase the value of D from 6 to 8, no of error bits also increases again.
Conclusion

We are getting the minimum value of BER at D=6,
For RRC, BER = 0.49425
For Gaussian Filter, BER = 0.48775
Therefore we can say that Gaussian filter is providing an improved BER of 13%.

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Noise in Indoor Power Line Communication Channel

Abstract
The power line communication technology is now considered as a good alternative for the implementing communication network. Digital networks can be established using the same set of wires that is use to distribute the power signal through the power-line channel (PLC) because power line networks are excellent infrastructure for broadband data transmission however various noise exist due to stochastic change in the network load impedance. This paper is an attempt to identify different type of noise in PLC channel and investigate the performance of indoor channel of PLC system. The noise seen in the power-line channel varies with frequency, time and from line to line. In this paper we classify different type of noises its characteristics and the process to remove it from power line channel.

Introduction
Powerline communication (PLC) is a term used to identify technologies, equipment, application and services that allow user to communicate over existing powerline. The most attractive advantage of this technology is that the powerline network is the most accessible network that reach every power socket in every home. Since the powerline network is already installed there is no need to lay new cables. It has established specification heading for data rate as high as 200 MBPS.

The birth and growth of the Internet accelerate the demand for digital telecommunications services to almost every premise. If such services can be carried over electricity distribution networks, a truly universal information superhighway might be realized, with the capability of providing interconnection to every home, factory, office, and organization. Although PLC technology has advantage of requiring no new wire. The major obstacle is its widespread used in broadband communication which results electromagnetic interference (EMI) and noise. Noise in PLC channel is classified into three main categories that are colored background noise, narrowband noise and impulsive noise. Colored background noise results from the simulation of different noise source of low power present in the network and usually characterized with a PSD decreasing with frequency. Narrowband noise seems from the existing from radio broadcasting from long, middle and sort wave ranges. Impulsive noises generated significant among the noise type present in PLC networks.

Although some noise model proposal can be found in literature, there practical value generally varies limited, because most of them describe bottom up approach describing the behavior of network. Only the work reported in present noise model which is based on measurements. However like the other models mentioned above it restricted to frequency range below 150 KHz.

The paper has been organized in 5 sections. Section 2 review the power line channel model. A detailed characteristics of different type of noises found in power lines is describe in section 3 and in section 4 we describe the channel coding technique for indoor powerline communication channel.

Powerline Channel Model
The power line channel model can be considered as a series of discrete stationary state that need to be understand for successful PLC. In general power line can be modeled as time varying frequency selective fading channel with numerous noise source. More simply the power line transfer function can be given as

\[ H(f) = \sum_{k=1}^{L} g_k \cdot \exp(-j2\pi f \cdot \alpha_k \cdot d_k / v) \]  \hspace{1cm} (1)

Where \( L \) is the total number of reflecting path, \( g_k \) is the complex tap factor for each path, \( \alpha_0 \) and \( \alpha_1 \) are attenuation factor, \( d_k \) is the path length \( v \) is the velocity of the propagation.
Noise Model

In time domain, the received signal of power line channel is represented by a standard linear convolution operation that is in discrete form it is given as [1, 2]

\[ r(k) = \sum_{i=0}^{N} s(k-i) d_n + n_{\text{background}}(k) + n_{\text{arrowband}}(k) + n_{\text{impulse}}(k) \]  

Where \( r(k) \) represent the received signal, \( s(k) \) represent spread spectrum signal to be transmitted, \( d_n \) and \( N \) represent individual delay for multipath and number of significant multipath component respectively. Only the work reported by O.hoojen[4] present the noise model which is based on measurement are:

a) Colored noise: it is no stationary noise.it is also known as fluctuation noise because can rise to considerable levels when certain appliances are switched on. The model of the model of the colored noise is similar to the Additive White Gaussian noise (AWGN)

Fig. 3- Voltage of noise \( n(t) \)

a) white noise  
b) colored noise
b) Background Noise: Background noise is caused by assembling of multiple sources of noise with low power. It represents high level noise that exponential decay to spectrally flat Gaussian white noise at approximate 3mhz. in a high frequency limit it can be assumed as spectrally flat despite of its low amplitude relevant in PLC due to significant signal attenuation experience in channel. It can be described by a PSD

\[ s_n(f;N_0,N_1,f_1) = N_0 + N_1 \exp(-f/f_0) \]

Where \(N_0\) is the constant noise power density, \(N_1\) and \(f\) is the parameter of exponential function.

To model the background noise characteristics in PLC channel long term measurement were carried out from 1-30Mhz. The probability distribution of time domain noise amplitude resembles Nakagami-m distribution function

\[ F_n(r) = \frac{2}{\Gamma(m)(m\Omega)}^m r^{2m-1} \exp(-mr^2/\Omega) ; r>0 \]

Where \(r\) is the random variable, \(\rho\) is the probability of corresponding random variable \(\Gamma(m)\) is the gamma function, \(\Omega\) is the mean power of random variable and \(m\) is the shaping parameter of the Nakagami- \(m\) distributed random variable \(n=n_1 + jn_0\). While \(\Omega=E(r^2)\) denote the power of the same. The argument \(\theta=\tan^{-1}(n_Q/n_I)\) is also random and distributed randomly over a complex phase plane \(\theta-U(-\Pi, \Pi)\).

It is well known that if \(m=1\) Nakagami-m PDF reduces to Rayleigh PDF

\[ F_n(r) = 2r/\Omega \exp(-r^2/\Omega) ; r>0 \]

Where \(r\) is the random variable, \(\rho\) is the probability of corresponding random variable \(\Gamma(m)\) is the gamma function, \(\Omega\) is the mean power of random variable and \(m\) is the shaping parameter of the Nakagami- \(m\) distributed random variable \(n=n_1 + jn_0\). While \(\Omega=E(r^2)\) denote the power of the same. The argument \(\theta=\tan^{-1}(n_Q/n_I)\) is also random and distributed randomly over a complex phase plane \(\theta-U(-\Pi, \Pi)\).

\[ F_n(r) = 2r/\Omega \exp(-r^2/\Omega) ; r>0 \]

(5)

c) Narrowband Noise: Narrowband noise is caused by external RF pickup of numerous radio systems. In the discrete sampling space narrowband noise can be modeled as the output of the bandpass filter driven by white Gaussian noise. It can be expressed as

\[ n_{nb}(k) = \sum_{n=-\infty}^{\infty} W_n(k) \cos(2\pi f k + \phi) \]

(6)

Where \(N\) is the number of wave at different frequency \(f\), \(W_n\) is amplitude and phase \(\phi\) is randomly established from interval \([0,2\Pi]\). It is found that \(n_{nb}\) is 30dB greater power level than 1Mhz. Therefore this type of noise can be a source of degradation.

d) Impulsive Noise: Non stationary noise is represented as impulsive noise. Impulsive noise is generated from connected electric appliances. It causes bit or burst error in the data transmission. Middleton's class 'A' noise model is one of the appropriate model for impulsive noise environment [1]. It can be classified into three groups according to their behavior w.r.t main cycle

1) Periodic Synchronous with main
2) Periodic asynchronous with main
3) Aperiodic

Periodic synchronous with the main is a cyclostationary noise synchronous with the main and with a frequency of 50Hz/100Hz. It is commonly originated by silicon controlled rectifier (SCR) in power supply. Periodic asynchronous with the main has been traditionally considered to be formed by a periodic impulse with rate between 50Khz and 200Khz. In addition to its high repetition frequency this noise type also exhibits lower periodicity equal to the main and so, it can also be categorized as cyclostationary noise. Aperiodic impulse noise has a sporadic nature mainly due to transient cause by the connection and disconnection of electric devices. This noise may cause bit or burst error in data transmission. Middleton class 'A' noise model is one of the appropriate model for impulsive noise environment.
Based on the model the combination of impulsive noise and background noise is a sequence of i.i.d complex random variable with the probability density function (PDF) of class ‘A’ noise given by

\[ P_z(z) = \sum_{m=0}^{\infty} \alpha_m 2 \pi \sigma_m^2 \exp(-z^2/2\sigma_m^2) \] ..........................(7.1)

\[ \sigma_m = e^{-A} \frac{A^m}{m} \] ..........................(7.2)

\[ \sigma_m^2 = \sigma_g^2 \frac{((m/A)+\tau)/\tau}{m} \] ..........................(7.3)

\[ \sigma_z^2 = E\{z^2\} = \frac{e^{-A} \sigma_g^2}{\tau} \sum A^m/m!(m/A+\tau) \] ..........................(7.4)

where \( m \) is the number of impulsive noise sources and is characterized by Poisson distribution with mean parameter \( A \) called impulse index (which is the product of the average rate of impulsive noise and mean duration of typical impulsive). \( \Gamma \) is the Gauss impulse power ratio (GIR) which represent the ratio between the variance of Gaussian noise component \( \sigma_g^2 \) and the variance of impulsive component \( \sigma_m^2 \).the variance of noise \( \sigma_z^2 \) is given in.

**Channel Coding Technique**

The impulsive noise and frequency selective behavior of power line networks cause burst noise which hampers high speed communication. To overcome burst noise interleaved coding has been adopted. These include interleaved block code and interleaved convolution code. Recently low density parity check (LDPC) codes were proposed [7]. In few paper concatenated Reed Solomon codes (RS) and interleaved vertebri channel coding used due to its effectiveness of burst error correction.

These concatenated coding scheme consist of an outer block code over GF(2^B) and inner binary convolution code. Assuming Interleaving between vertebri decoder and Reed soloman decoder is sufficiently long to breakup long burst of error out of the decoder, the Reed soloman symbol error Probability (\( p_b \)) for symbol in GF(2^B) can be upper bounded by simple union bound as in eqn

\[ P_b \leq Bp^*_b \] ..........................(8)
Where parameter $p'_b$ is the probability ever of the output of the viterbi decoder which can be expressed by eqn .

$$R_e = R_e \sum_{k=0}^{n-1} p_k (1-p_k)^{n-1}$$

The parameter $d_{free}$ and $R_e$ are the free distance of convolution code and code Rate and B is total number of bit error that occur in All of the incorrect path in their end differ from the correct path in exactly "$d" position. The total error probability of the RS code Account for decoder failure probability or decoder error probability is given by Eqn (10)

$$P_e = 1/n^2 \sum(n_b)p_b(1-p_b)^{n_b-1}$$

Conclusion

In this paper, an innovative approach is applied to impulsive noises which are studied directly at their sources. Measuring Noise at the source led us to analyse much less noises compared to noise measurements at the receiver side, and to establish a correction with effective in device noise generators Characterizing noise at source had made it possible to propose an impulsive noise model for each electrical device. And a random generator of impulsive noise at receiver was also proposed. A model for PLC channel based on the time-variant linear-filter channel. The additive noise on the channel was shown to be collection of four noise types these are spectrally flat noise with a power- spectral density that decreases for increasing frequency, colored noise, background noise, narrowband noise and Impulsive noise. This paper also attempt to find a channel coding technique suitable for indoor powerline communication channel.

References


DISCRETE WAVELET TRANSFORM AND FAST WAVELET TRANSFORM BASED FACE RECOGNITION

Abstract:
Face recognition is one of the challenging applications of image processing. Robust face recognition algorithm should possess the ability to recognize identity despite many variations in pose, lighting and appearance. Principal Component Analysis (PCA) method has a wide application in the field of image processing for dimension reduction of the data. But these algorithms have certain limitations like poor discriminative power and ability to handle large computational load. This dissertation proposes a face recognition techniques based on Principal component Analysis (PCA) with Discrete Wavelet Transform (DWT) and Fast Wavelet Transform (FWT) for recognition. The classification for the proposed system is done using Mahalanobis Distance (MHD) methods and the recognition rate are compared for different distance measures. The proposed method has been successfully tested on ORL and Yale face data base with 400 and 165 frontal images corresponding to 40 and 15 different subjects which are acquired under variable illumination and facial expressions. It is observed from the results that use of Discrete Wavelet Transform and Fast Wavelet Transform recognition rate is improved when using Mahalanobis distance.

Introduction
The issues associated with identity usurpation are currently at the heart of numerous concerns in the modern society. Three approaches are available to prove a person’s identity and to provide “the right person with the right privileges the right access at the right time” Woodward et al. (2003). One of the identity proving approaches to establish the genuineness of the identity is something you are the access to a service can be achieved through the presentation of measurable biometric traits, such as biometric measures. This approach has some significant advantages over the others. Without sophisticated means, biometrics is difficult to share, steal or forge and cannot be forgotten or lost. This latter solution provides thus a higher security level in identity prove. Identity documents are tools that permit the bearers to prove or confirm their identity with a high degree of certainty. In response to the dangers posed by fraudulent use of identity documents, a wide range of biometric technologies is emerging. Biometric identifiers which are conceptually unique attributes, are today portrayed as the panacea to verify someone’s identity.

Biometric System
Biometrics is a term that encompasses “the application of modern statistical methods to the measurements of biological objects”. Hence, biometric recognition refers to the use of distinctive physiological and behavioral characteristics (e.g. Face, fingerprint, hand geometry, iris, gait, signature), called biometric identifiers or simply biometrics, for automatically recognizing a person. This has been used in several domains, such as person authorization examination in e-Banking and e-Commerce transactions or within the framework of access controls for security areas.
A biometric system is essentially a pattern-recognition system. Such system involves three aspects: data acquisition and pre-processing, data representation, and decision making. It can thus compare a specific set of physiological or behavioral characteristics extracted from a person with a template/model acquired beforehand, and recognize the individual. The digital representation recorded in a database as a description of a physical trait is defined as a template and is obtained by feature extraction algorithms. Among different traits the motivations behind using face and fingerprint for person authentication are manifold. To explain the impetus behind the use of any biometric system, some important applications are described in the following section.

Proposed System
We propose a face recognition system based on PCA DWT, FWT and Mahalanobis distance. The main challenge for a face recognition system is of effective feature extraction. The proposed system utilizes the Eigen face method is information reduction for the images. There is an incredible amount of information present even in a small face image. A method must be able to break down pictures so as to effectively represent face images rather than images in general. Base faces are generated and then image being analyzed can be represented by the system as a linear combination of these base faces. Each face that we wish to classify can be projected into face-space and then analyzed as a vector. A Mahalanobis distance measure can be used for classification. The proposed system uses Principal Component Analysis for feature extraction and Mahalanobis distance. The technique used here involves generating the ‘Eigen faces’ then projecting training data into face-space to be used with a predetermined
classification method and evaluation of a projected test element by projecting it into face space and comparing to training data.

**Materials and Method**

**Proposed Method**

How do humans identify individuals with remarkable ease and accuracy? This question has haunted psychologists, neurologists and, recently, engineers in biometry for a long time. The human face is different from any other natural or manmade objects, but has similar structural features across different races, sex and regions. The subtle variations in the face structure are captured by the human brain and help in discriminating one face from the other human brain is able to filter out the common visual features of a face and retain only those suitable to exhibit the unique characteristics (discriminatory evidence) of an individual. An efficient face-based biometric system must possess these properties (as in those in the retina and visual cortex), to perform efficiently like a human being.

Wavelet-based features have already been used to obtain a better face representation in (Ekenel et al.2005; Garcia et al.2000, Zhang et al. 2004). The use of wavelet packet for face recognition was reported in Ekenel et al., (2005). Here, 2D-DWT is used to fully decompose the face image and simple statistical features such as mean and variance are extracted from the decomposed coefficients, and used as a feature vector for representation. Proposed multiresolution face recognition with fusion at data, feature and decision levels to test on face images that differ in expression or illumination separately, obtained from Yale databases. Significant performance gains are reported against illumination perturbations. They selected a number of sub-bands on the basis of performance on testing set and termed them as successful sub-bands. None of these approaches have attempted to exploit the fact that, if some of the common visual features of a face exist in a certain sub-band and are suppressed during reconstruction, we obtain a sub-band face with only the discriminatory information. The retina of the human visual system has been observed to perform local multi-resolution, multi-channel processing of the signal using a bank of tuned band-pass filters (Kulikowsk et al.1982; Marcelja 1980; Pollen and Ronner 1982). The visual cortex uses second order relational differences in structure with respect to an average face for perception. Hence, a unified computational model consisting of DWT/IDWT (signal processing) and PCA/LDA (statistical processing) will be able to closely imitate the human visual system, more effectively than what statistical processing does alone. The use of sub-band face as a new representation for the face recognition task. Only the discriminatory information of a face is retained or captured in a sub-band face. Discrete wavelet transform is used to decompose the original face image into approximation and detail sub-bands. We perform multi-level dyadic decomposition of a face image using the Daubechies filters the sub-band face may be reconstructed from selected sub-bands by suppressing the approximation at a suitable higher level and retaining the details.

**Sub-band Face Representation**

A set of suitable wavelet sub-bands are selected to generate the sub-band face using IDWT, for recognition using DWT. The method of reconstructing a sub-band face is described in the following.

**Wavelet Decomposition**

High-high (HH) sub-bands, which are also known as A (approximations), H (horizontal details), V (vertical details), D (diagonal details), respectively. Fig. 3.2 shows the 2D-DWT performed using low pass h(.) and high pass g(.) filters by Mall.(1989). The decomposition process can be recursively applied to the low frequency channel (LL) to generate dyadic decomposition at the next level.

**Sub-band Face Reconstruction**

A face image of a person contains common (approximations) as well as discriminatory (details) information with respect to faces of all other persons. The discriminatory information is due to structural variations of the face which are acquired as intensity variations at different locations of the face. The location and degree of intensity variations in a face of an individual are unique features which discriminate one from the rest of the population. The similarity of a face with respect to another is in the global appearance and structure of the face. This information (similar and discriminatory) is segregated at different sub-bands using different levels of decomposition of the face image. Wavelet decomposition helps to split the features of a face in different sub-bands with “approximations” containing the common (smooth) parts of the face and “details”, at certain levels of decomposition, containing the discriminatory (variation “approximations” containing the common (smooth) parts of the face and “details”, at certain levels of decomposition
Figure 3.6 (a) Original face, (b) the level-1 wavelet decomposition of the face images into sub-bands A, H (top) and V, D (bottom) and (c) level-3 dyadic wavelet decomposition.

Mathematics of PCA
A 2-D face image can be represented as 1-D vector by concatenating each row (or column) into a long thin vector.

- Assume the training sets of images represented by Γ1, Γ2, Γ3,…, Γm, with each image Γ(x,y) where (x,y) is the size of the image represented by p and m is the number of training images. Converting each image into set of vectors given by (m x p).

- The mean face Ψ is given by:

\[ \Psi = \frac{1}{m} \sum_{i=1}^{m} \Gamma_i \]  \hspace{1cm} (3.2)

- The mean-subtracted face is given by (Φi):

\[ \Phi_i = \Gamma_i - \Psi \]  \hspace{1cm} (3.3)

where i = 1, 2, 3….m. and A = [Φ1, Φ2 ... Φm] is the mean-subtracted matrix with size Amp.

- By implementing the matrix transformations, the vector matrix is reduced by:

\[ C_{mn} = A_{mp} \times A^T_{mp} \Psi_i = \Gamma_i - \Psi \cdot C \]  \hspace{1cm} (3.4)

where C is the covariance matrix.

- Finding the eigen vectors Vmm and eigen values lm from the C matrix and ordering the Eigen vectors by highest eigen values.

- With the sorted eigen vector matrix, Φm is adjusted. These vectors determine the linear combinations of the training set images to form the eigen faces represented by Uk as follows:

\[ U_k = \sum_{n=1}^{m} \Phi_n V_{kn}, \text{ where, } k = 1, 2, m. \]  \hspace{1cm} (3.5)

- Instead of using m eigen faces, me eigen faces (me<< m) is considered as the most significant eigen vectors provided for training of each individual.

- With the reduced eigen face vector, each image has its face vector given by

\[ W_i = U_k^T \left[ \Gamma - \Psi \right], k = 1, 2, M \]  \hspace{1cm} (3.6)

- The weights form a feature vector given by

\[ W_i = U_k^T \left[ \Gamma - \Psi \right], k = 1, 2, M \]  \hspace{1cm} (3.7)

These feature vectors are taken as the representational basis for the face images with reduced dimension.

- The reduced data is taken as the input to the next stage for extricating discriminating feature out of it.
The flow chart of PCA

Calculating Principal Components

Jolliffe (2002) states that principal components (PCs) can be found using purely mathematical arguments – they are given by an orthogonal linear transformation of a set of variables optimizing a certain algebraic criterion.

An overview how to perform principal components analysis:
- Organize data as an \( m \times n \) matrix, where \( m \) is the number of measurement types and \( n \) is the number of samples
- Subtract off the mean for each measurement type
- Calculate covariance matrix
- Calculate the eigenvectors and eigen values of the covariance matrix

Orl (AT & T) face database

A standard face database known as AT & T (formerly ORL database) has been used for the present study apart from the one we developed. AT & T database contains face images of 40 distinct persons. Each person has ten different images, taken at different times, totaling to 400. Figure 4.1 shows the 40 individuals in the AT & T Face database. Each face image in the database has size 112x92 pixels. There are variations in facial expressions such as open/closed eyes, smiling/ non-smiling, and facial details such as glasses/no glasses etc. All the images were taken against a dark homogeneous background with the subjects in an up-right, frontal position, with tolerance for some side movements. There are also some variations in scale. Though the database has been used in many face recognition researches, it is clear that the number of samples or database size is too small to prove and establish/reason the eventual results. A database with higher size is essential for proving the correctness or accuracy of the face recognition researches. Therefore, a larger database has been developed for this research. The succeeding sections give the details of this face database that has been developed for the present research.

Yale face database

The Yale face database has 15 subjects with 11 samples each, having variations in expression and illumination for each subject, one per different facial expression or configuration: center-light, w/glasses, happy, left-light, w/no glasses, normal, right-light, sad, sleepy, surprised, and wink.

Training set and Test set

In this section we present and discuss each of the aforementioned face recognition techniques using two deferent databases. Because of the specifics that we wanted to test about the relative performance of the considered algorithms, many of the standard databases were inappropriate. So we have used a database from AT&T. Secondly, we have use Yale face database that includes variation in both facial expression and lighting. Which in include 9 training set for orl and 10 Training set for Yale and 9 and 10 test set per pose for both Orl and Yale face Database four experiment were conducted each with different portioning scenario .and performance is evaluated against the test set of the training samples

Training set and Test set for Orl face database
To develop the training set and test set we have taken the images randomly selected as follows:
- Training set sample 1 and test set which contains 40 and 40 images for test set.
- Training set sample 2 and test set which contains 180 and 40 images for test set.
- Training set sample 3 and test set which contains 120 and 40 images for test set.
- Training set sample 4 and test set which contains 160 and 40 images for test set.
- Training set sample 5 and test set which contains 200 and 40 images for test set.
- Training set sample 6 and test set which contains 240 and 40 images for test set.
- Training set sample 7 and test set which contains 280 and 40 images for test set.
- Training set sample 8 and test set which contains 320 and 40 images for test set.
- Training set sample 9 and test set which contains 360 and 40 images for test set.

Training set and Test set for Yale Face Database
To develop the training set and test set for Yale we have selected the images randomly as follows:
- Training set sample 1 and test set which consist 15 and 15 images for test set.
- Training set sample 2 and test set which consist 30 and 30 images for test set.
- Training set sample 3 and test set which consist 45 and 30 images for test set.
- Training set sample 4 and test set which consist 60 and 30 images for test set.
- Training set sample 5 and test set which consist 75 and 30 images for test set.
- Training set sample 6 and test set which consist 90 and 30 images for test set.
- Training set sample 7 and test set which consist 105 and 30 images for test set.
- Training set sample 8 and test set which consist 120 and 30 images for test set.
- Training set sample 9 and test set which consist 135 and 30 images for test set.
- Training set sample 10 and test set which consist 150 and 30 images for test set.

Face Recognition
A test set of images for recognition is tested by comparing to training set. Fig: 7 and 8.

Result and Conclusion
The observed performance of proposed method on two public face databases: Yale, and Orl. Yale samples per class respectively have 15 subjects with 11 samples per class. ORL consist of 40 with 10 subjects. More details about observed result is discussed in this chapter.

Performance Analysis on Two Standard Face Databases
To select subject-specific optimal sub-bands we split the image database into two disjoint sets, namely training set, and test set. Training set along with test set are used for sub-band selection, and then the performance of selected sub-bands is observed on the testing set. For sub-band selection, the image size has been kept as 92x112 for all. Table 1 and 2 shows the performances of subspace methods on original images for two databases testing and recognition of Orl and Yale of PCA technique. Table 3 and 4. shows the testing and recognition (in terms of Peak Recognition Accuracy or PRA) performance of sub-band face representation integrated with subspace methods (DWT) on Orl and Yale database. The results in the column labelled positionwise the result is collected as shown in the table. Table 4 and 5 show the performance of testing and recognition (in terms of Peak Recognition Accuracy) that we have tested only once per level -1 for Orl and Yale database. Table 6 and 7 show the performance of our proposed approach that we have proposed in this approach we have chosen only the best performing training set, and only the best result is shown in both table for Orl and Yale.

- PCA Peak recognition Accuracy of original gray-level face image for Orl database.

The performance of PCA on Orl database is tabulated in table 1. For 9 subspace methods namely, test set, we obtained maximum accuracies are 61.38%, 85.04%, 91.06%, 94.16%, 95.7%, 98.22%, 98.80%, 99.21%, 99.4% respectively.

- Peak Recognition Accuracy (PRA) of original gray-level face image with PCA, for Yale database.

The performance of PCA on Yale database is tabulated in Table 2. for all subspace namely as test set the maximum accuracy are 79.99%, 88.88%, 91.66%, 94.28%, 95.10%, 94.88%, 98.33%, 97.49%, 97.0% and 98.00% respectively.

<table>
<thead>
<tr>
<th>Training set</th>
<th>Test set 2</th>
<th>Test set 3</th>
<th>Test set 4</th>
<th>Test set 5</th>
<th>Test set 6</th>
<th>Test set 7</th>
<th>Test set 8</th>
<th>Test set 9</th>
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<tr>
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<td>100</td>
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<td>100</td>
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<td>93.33</td>
<td>93.33</td>
<td>94.28</td>
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<td>96</td>
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</table>
The performance of DWT on ORL database is tabulated in Table 4.3. And we have chosen the best performing training set to test criteria provide same PRA are 93.75%, 96.54%, 98.33%, 99.10%, 99.68%, 99.64%, 99.52%, 100%.

The performance of DWT on ORL database is tabulated in Table 4.4. And we have chosen the best performing training set to test criteria provide same PRA are 94.07%, 95.65%, 96.66%, 97.33% 99.52%, 99.52%, 99.44%, 100%, 100%.

Table 1 PCA result for Yale database
- Peak Recognition Accuracy (PRA) of original gray-level of face image with DWT for ORL database.

Table 2 DWT result of Orl database
- Peak Recognition Accuracy (PRA) of original gray-level of face image with DWT, for Yale database.
Table 3: DWT result for Yale database

<table>
<thead>
<tr>
<th>Training Samples</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
<th>P7</th>
<th>P8</th>
<th>P9</th>
<th>P10</th>
<th>P11</th>
<th>PRA</th>
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</thead>
<tbody>
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<td>100</td>
<td>100</td>
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<td>96.66</td>
<td>94.07</td>
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</tr>
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<td>97.77</td>
<td>95.65</td>
<td></td>
</tr>
<tr>
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<td>100</td>
<td>81.66</td>
<td>100</td>
<td>100</td>
<td>98.33</td>
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</tr>
<tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Peak Recognition Accuracy (PRA) of original gray-level of face image with FWT, for Orl database.

The performance of FWT on Orl database is tabulated in Table 4.5. And in this we have tested the half of the images of the Orl database of the highest peak recognition accuracy rate are 97.7%, 97.8%, 97.9%, 80.5%, 80.5%, 80.5%, 80.5%, respectively.

Table 4: FWT result for selected sub-band for Orl database

<table>
<thead>
<tr>
<th>Selected Sub-band</th>
<th>P2</th>
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<th>P4</th>
<th>P5</th>
<th>P6</th>
<th>PRA</th>
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<td>97.5</td>
<td>99.00</td>
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<td>80.5</td>
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</tr>
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<td>80.5</td>
<td>80.5</td>
<td>80.5</td>
<td>80.5</td>
</tr>
<tr>
<td>D1</td>
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<td>80.5</td>
<td>80.5</td>
<td>80.5</td>
<td>80.5</td>
<td>80.5</td>
</tr>
</tbody>
</table>

- Peak Recognition Accuracy (PRA) of original gray-level of face image with FWT, for Yale database.

The performance of FWT on Yale database is tabulated in Table 4.6. And in this we have tested the half of the images of the Yale database peak recognition accuracy rate are 98.88%, 98.88%, 97.88%, 80.5%, 80.5%, 80.5%, 80.5%, respectively.
### Table 5  FWT result of selected sub-band for Yale database

<table>
<thead>
<tr>
<th>Training samples</th>
<th>P6</th>
<th>P7</th>
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<th>P9</th>
<th>P10</th>
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</thead>
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<td>100</td>
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<td>97.33</td>
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</tr>
<tr>
<td>H</td>
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<td>100</td>
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<tr>
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<td>81.33</td>
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<td>81.33</td>
<td>81.33</td>
</tr>
</tbody>
</table>

- Peak Recognition Accuracy (PRA) of original gray-level of face image with FWT, for Orl database.

The combine performance of DWT and FWT on Orl database is tabulated in Table 4.7. And in this we have tested the best performing sub-band of the images of the Orl database highest the peak recognition accuracy rate are 93.06%, 96.30%, 98.33%, 99%, 9.79%, 99.64%, 99.68,100, the respectively.

### Table 6 DWT FWT result table for Orl database

<table>
<thead>
<tr>
<th>Training sample</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
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<td></td>
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<td></td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

- Peak Recognition Accuracy (PRA) of original gray-level of face image with FWT, for Yale database.

The combine performance of DWT and FWT on Yale database is tabulated in Table 4.8. And in this we have tested the best performing sub-band of the images of the Yale database the highest rate are 93%, 95.27%, 96.38, 97.10%, 99.55%, 99.52%, 99.44%, 99.62%, 100% respectively.
Table 7: DWT FWT for Yale database

<table>
<thead>
<tr>
<th>Training samples</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
<th>P7</th>
<th>P8</th>
<th>P9</th>
<th>p10</th>
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Table 8: Comparison of PCA, DWT, FWT with proposed approach for Orl.

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<th>P5</th>
<th>P6</th>
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- Comparative table for Yale database.
Table 9: Comparison of PCA, DWT, FW with proposed approach for Yale database

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Conclusion

In this paper the three face recognition techniques for face recognition has been used the databases used in experiment are small and contain a large number of faces per subject which makes the task of recognition easier. They do not contain large face occlusion such as scarves or hats, using a large database with more varied images of each subject, might be result with different outcomes.

References


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**Fig 1:** Block Diagram of PCA

**Fig 2:** Face images in AT&T face database.
Figure 3.3 Face images in Yale face database

Figure 4: Sample Faces for Training set of Orl database.
Figure 5

Figure 6: Test set 1X1 as input
Figure 7: 2X40 Training set.
Figure: 8 comparison chart of PCA, DWT, FWT with Proposed approach for Orl

Figure: 9 Comparison of PCA, DWT, FWT with proposed approach for Yale.
STORAGE ARCHITECTURE AND SOLUTIONS FOR BIG DATA

Mr. Rishabh Shukla  
Member of Computer Society of India, Amity University, Lucknow

Abstract:
Data around the world is exploding. This exponential growth in data is driven by several factors - the mass scale utilization of the internet, the movement of paper records to digital environment, and more lately, the advent of the digital world, where online takes on a new meaning. The online experience is akin to a virtual world with virtual communities and virtual markets. To say that people live their lives online, would be a little farfetched yet extremely probable/possible now than it has ever been.

Introduction
We tend to treat the growth of data as a continuous process; however in reality, apart from the continuous growth in data, there have been certain specific factors which have in fact caused Information/Data Explosions at different points in time in the history of data growth. For example, the digital revolution, online e-commerce and later Cloud have all contributed to the growth of data in a big way.

Compliance Requirements are additionally driving requirements for retaining data for longer timeframes which means data destruction rate is much slower than the data generated.

Data is important, however with the increasing volumes of data, making sense out of the voluminous data is sometime similar to hunting for a needle in a Haystack. The context in which one may require or look for information would itself change the perspective of what could be derived from the data. Also, this data needs to be accessed in real time to help take some business critical decisions. This gives rise to the problem of Big Data.

The Unstructured Data Explosion
According to IDC, the total amount of digital information created and replicated surpassed 1.8 zettabytes (a zettabyte is one thousand exabytes) in 2011. The size of the digital universe is more than doubling every two years, and is expected to grow to almost eight zettabytes by 2015.

What’s fueling the explosion? A majority is being driven by individuals creating content using mobile devices (smart phones, tablets, cameras, etc.). The McKinsey Global Institute estimated that in 2010, there were over five billion mobile phones on the planet, 12 percent of which are smart phones capable of generating increasing amounts of digital content and growing by 20 percent per year. The explosion is also being driven by information created around individuals as they go about their daily lives (e.g., surveillance videos, motion sensors tracking location and traffic/inventory movement).

Although most of this data is generated by individuals, IDC estimates that enterprises have liability for 80 percent of the information in the digital universe at some point in its lifecycle. That means organizations take on the responsibility for architecting, delivering and maintaining information technology systems and data storage systems to meet the demand.

Traditional Storage Approaches Won’t Work
Technology advances are helping with this challenge to some degree. Computing is getting faster and cheaper. Virtualization is driving up efficiency and utilization. Storage devices are growing in terms of capacity while declining in price (more bits per device at a lower cost) and recently getting faster with the advent of solid state technologies (although not currently at a suitable price point for all workloads). Delivery mechanisms such as cloud computing are also helping to lower costs and drive efficiencies. But in some cases the advances in technology—specifically the capacity expansion of storage devices—are putting a strain on traditional methods of protecting and preserving digital information. Traditional storage protection technologies such as RAID are simply inadequate when it comes to protecting digital information from data loss at petabyte and above scale.

Traditional storage architectures can’t scale. They’re not secure. They’re not reliable. And they’re expensive.

Consider the following challenges that traditional storage systems face once they reach petabyte scale:

- Data integrity suffers when system size is 10,000,000,000 times larger than the bit error rate of a hard drive.
- Data availability suffers when hundreds of drives fail every day and require a week to rebuild.
- Data security suffers with millions of devices and multiple copies in multiple locations.
- Enterprises that need to store large volumes of unstructured data must look beyond their current storage solutions and evaluate new approaches.
Why RAID Fails at Scale

RAID schemes are based on parity, and at its root, if more than two drives fail simultaneously, data is not recoverable. The statistical likelihood of multiple drive failures has not been an issue in the past. However, as drive capacities continue to grow beyond the terabyte range and storage systems continue to grow to hundreds of terabytes and petabytes, the likelihood of multiple drive failures is now a reality.

Further, drives aren't perfect, and typical SATA drives have a published bit rate error (BRE) of 10^14, meaning that once every 100,000,000,000,000 bits, there will be a bit that is unrecoverable. Doesn't seem significant? In today's big data storage systems, it is.

The likelihood of having one drive fail, and encountering a bit rate error when rebuilding from the remaining RAID set is highly probable in real world scenarios. To put this into perspective, when reading 10 terabytes, the probability of an unreadable bit is likely (56%), and when reading 100 terabytes, it is nearly certain (99.97%).

Replication, Makes RAID More Expensive

As a result, IT organizations address the big data protection shortcomings of RAID by using replication, a technique of making additional copies of data to avoid unrecoverable errors and lost data. However, those copies add additional costs: typically 133% or more additional storage is needed for each additional copy, after including the overhead associated with a typical RAID 6 configuration.

Organizations also use replication to help with failure scenarios, such as a location failure, power outages, bandwidth unavailability, and so forth. Having seamless access to big data is key to keeping businesses running and driving competitive advantages.

As storage grows from the terabyte to petabyte range, the number of copies required to keep the data protection constant increases. This means the storage system will get more expensive as the amount of data increases.

Storage Architectures For Big Data

These requirements have broken the traditional data storage models and created the need for new architectures to effectively store and deliver this data to the analytics systems that do the analysis. Older storage architectures couldn’t scale to the size required or hold the diverse data types that are being created. Limitations on the amount of data that could be stored in an array was in the 100s of terabytes range but the file systems they provided could not scale beyond 16 terabytes. This meant that a user with 50 terabytes of data from a single source would be forced to create at least 3 file systems for the data. This created extra work on the part of storage administrators and made finding all the related data more difficult. This big data requirement has forced existing storage vendors to look at systems in a new way and provided a breeding ground for new startup companies founded by tech savvy entrepreneurs.

This architecture required a number of things to make it work from both a technology and financial aspect. Some of these factors include:

- **Clustered architecture** – for this model to work the entire grid needed to work as a single entity and each node in the grid would need to be able to pick up a portion of the function of any other node that may fail.
**Distributed/parallel file system** – the file system must allow for a file to be accessed from any one or any number of nodes to be sent to the requesting system. This required different mechanisms underlying the file system: distribution of data across multiple nodes for redundancy, a distributed metadata or locking mechanism, and data scrubbing/validation routines.

**Commodity hardware** – for these systems to be affordable they must rely on commodity hardware that is inexpensive and easily accessible instead of purpose built systems.

### Market size

The big data solution is in early stages and there is still relatively little market analysis data available. However you can gain some indication of the market size by examining what percent of all workloads is moving from enterprise premises to public or virtual private cloud service providers, and applying those trends to the Big Data market figure. If you average out the different analysts’ predictions, it is expected that about 15 percent of all IT spending will move to the cloud by 2015, growing to about 35 percent by 2021. Provided that the big data market reaches the $17 billion target by 2015, the big data solution market therefore be 15 percent of the figure, or $2.55 billion. With an estimated big data market of about $88 billion in 2021, big data service market will be 35 percent of that, or about $30 billion. In other words roughly 4 percent of all IT spending will go into big data solutions and services.

Apache Hadoop is an open-source software framework that supports data-intensive distributed applications, licensed under the Apache v2 license. It supports the running of applications on large clusters of commodity hardware. The Hadoop framework transparently provides applications both reliability and data motion. Hadoop implements a computational paradigm named map/reduce, where the application is divided into many small fragments of work, each of which may be executed or re-executed on any node in the cluster. In addition, it provides a distributed file system that stores data on the compute nodes, providing very high aggregate bandwidth across the cluster. Both map/reduce and the distributed file systems are designed so that node failures are automatically handled by the framework. It enables applications to work with thousands of computation-independent computers and petabytes of data. Hadoop was derived from Google's MapReduce and Google File System (GFS).

This solution enables the service providers to offer Hadoop as a service that is, allowing data scientist user to store unstructured data in a persistent Hadoop Distributed File System (HDFS) and process it using MapReduce jobs.

### Hadoop, Why?

- **Need to process Multi Petabyte Datasets**
- **Data may not have strict schema**
- **Expensive to build reliability in each application.**
- **Nodes fail every day**
  - Failure is expected, rather than exceptional.
  - The number of nodes in a cluster is not constant.
- **Need common infrastructure**
An Example Scale-Out System: Typical Big Data Storage Solution using Hadoop

As depicted in the following figure, a Hadoop cluster consists of the Name Node and the N Data Node, which are connected through an IP network. The Hadoop framework has the following components:

- Hadoop Distributed file system, which stores files in Hadoop across different nodes and maintains fault tolerance.
- Map Reduce Engine. This engine consists of the following:
  - Job Tracker - receives requests from applications to process the data.
  - Task Tracker - focuses on performing work assigned on the data that is stored in data node.

While architecting solutions, the major benefits achieved using the Hadoop framework are:

**Better Scalability**: Scalable storage as nodes with compute / storage can be added on the fly i.e. without bringing down the system or even affecting the ongoing operations and is easily able to scale to multi petabyte environment.

**Cost Effectiveness**: It uses commodity hardware and does not require any specialized hardware, which usually increases the costs of the compute and storage.

**Improved Performance**: Analytics applications demanding greater performance are able to achieve better results, as the processing of the data is done on the data node itself.

**Who uses Hadoop?**
1. Amazon/A9
2. Facebook
3. Google
4. IBM
5. Joost
6. Last.fm
7. New York Times
8. PowerSet
9. Veoh
10. Yahoo!

**Storage Architecture Solution Providers**

- IBM offers WebSphere eXtreme Scale (formerly ObjectGrid) which includes two styles of the HADOOP map-reduce pattern in its "agents" a.k.a. DataGrid APIs. Together with its scalable distributed data cache capability, it
gives both map-reduce's ability to parallelize function and the ability to store plenty of data (in memory) for the function to quickly access. It’s transactional and highly available, too.

- IBM offers InfoSphere BigInsights based on Hadoop in both a basic and enterprise edition.
- EMC released **EMC Greenplum Community Edition** and **EMC Greenplum HD Enterprise Edition** in May 2011. The community edition, with optional for-fee technical support, consists of Hadoop, HDFS, HBase, Hive, and the ZooKeeper configuration service. The enterprise edition is an offering based on the MapR product, and offers proprietary features such as snapshots and wide area replication.
- Dell added Pentaho Business Analytics to the Dell Apache Hadoop solution for big data analytics which consists of Dell servers, Dell networking components, Dell's Crowbar cloud deployment framework open source software, and Cloudera Distribution including Apache Hadoop (CDH).

**Conclusion**

Enterprises that need to store large volumes of unstructured data must look beyond their current storage solutions and evaluate new approaches. The Big Data problem is best addressed at a Storage Level by Scale-Out Storage Systems - which can be proprietary or open source. These build up scalable, efficient, robust and high performance systems.

**References**

Abstract:
Wireless Local Area Network (WLAN) has become the virtually standard for wireless networking, providing mobility and connectivity at relatively low cost. However, the key concern with the 802.11 WLANs has been security. Wireless signals can travel long distances and are not bounded by physical Boundaries such as walls and perimeters. Since the Radio Frequency (RF) is a shared medium, wireless signals can also be picked up by unauthorized recipients such as potential attackers. WPA suffers from a few flaws that can allow attackers to tamper with the deployed network and its availability. WLAN security technology has shown great advantages in many fields, the research on WLAN security technology has received a very high degree concern. With flexible and easy access, wireless LAN networking has a wide range of application. These can adjust the confidentiality, availability, integrity and mutual authentication, hence respecting the user's privacy in a network. So we introduced multi-layered security for client to secure with encryption, authentication and access control with logging of each and every client in the network.

However, the key concern with the 802.11 WLANs has been security. Wireless signals can travel long distances and are not bounded by physical Boundaries such as walls and perimeters. Since the Radio Frequency (RF) is a shared medium, wireless signals can also be picked up by unauthorized recipients such as potential attackers. These can adjust the confidentiality, availability, integrity and mutual authentication, hence respecting the user's privacy in a network. So we introduced multi-layered security for client to secure with encryption, authentication and access control with logging of each and every client in the network.

Previous Work
In wireless mesh network secure group communication problems has been researched in a variety of ways. But, group head authentication is not considered in such a way. This is a very mature research area in which lots of work has been done in wireless and in wired networks respectively.
In sensor networks CGR schemes are used for secure group communication. These are efficient and secure in group communication. But, group head communication is not discussed. SGL provides data confidentiality in WAN environments. SGL deals with inter group communication. We can check its performance on intra group communication with decentralized group membership. In the mentioned paper authors did not discuss the robustness and efficiency features. Also the authors does not show that how SGL deals with intra group communication. A reliable and scalable distributed model for secure group communication is practically implemented in WAN but in distributed environment of group communication how can we leave the security issue like authenticity and authorization of group heads. Secure inter and intra group information sharing in a network consisting of multiple node groups.

A mechanism developed addresses this issue. Additional research is also required to study the impacts of group head authorization in mesh networks and then check its performance and communication overhead.

The secure group protocol in a decentralized system is providing the key material to all the members in the group is the main problem of secure group communication. Tree parity machines are use to establish a common group key. This does not mostly used in ad-hoc networks where no server is present and secure group head communication takes place. In future, this protocol must be compared with others.

A sliding window protocol for group communication decreases the time in storing and accessing key entries when nodes enter and leave group. This protocol improves the performance of the ad-hoc network but still security is an issue in this. Before the node enter in the group check whether this is a secure group head
where data has been sent. In secure group communication scalability is a major concern. Broadcast encryption scenario can be used for secure group communication.

An authenticated scheme is proposed for secure group communication. But attacks are not considered in the scheme. An analysis is necessary to check the efficiency and reliability in the case of unicasting and multicasting. A research may be conducted in secure group head authorization. Many distributed applications require a secure reliable group head communication. Secure group oriented applications have grown very rapidly. In wireless mesh network, due to their low cost and rapid access, security is a big issue. To provide secure group communication an agreement protocol is introduced which is very efficient reliable and secure.

Group head communication and also member’s events such as join, leave or update are not discussed in this paper. Security in dynamicity is the major aspect for group communication as the members can join or leave the group at their will. The existing protocol fails to address scalability, authenticity, authorization of the groups.

Data confidentiality in group communication in a decentralized manner is discussed but when the gateway distribute the data to the different group heads how can this group head is secure, may be this is a malicious group head. So, group head authentication, secure inter-group communication via their group heads and group head repairing is a problem in mesh networks.

According to the above literature we come to know that there are serious issues in group head communication and group head authentication. In wireless mesh networks, group head authentication is a crucial issue. In group head communication, a malicious node may try to be a group head and it wants to share and view the sensitive information from the authenticated groups. This intruder node may be a group head or its member or the other group head or its own members. This malicious node may tries to access the sensitive data. Here, group head authentication is required to authenticate the data from these malicious nodes so that authentic, reliable and efficient communication between different group heads will takes place.

Network LANE and Preferred Topology
The preferred topology shown in fig.1. consists of one gateway and thirty nine nodes with two local managers. Gateway provides the internet access. Local managers have a list of all legitimate mesh clients including group heads. Local managers can be deployed at various layers. Different local managers can exchange their databases among each other to share their responsibilities. At the start local managers populate its list of authenticated mesh clients and share it among each other. Group heads sends a request for authentication if it belongs to that particular local manager then it became authenticated otherwise request is forwarded to the other local managers. As group heads are responsible to receive and send the data on behalf of group members. So, it is the most important node among a group. At the same time it is more vulnerable node because internal and external intruders may want to seize its communication. As the preferred topology is based upon the field based routing so the group member nearer to the local manager became the group head. Local manager authenticates the group head while the study makes an assumption that routing nodes are secure.

In field based routing scalar value is assigned to every node in the group. Destination node has the highest value. When data packet is send from the gateway, gateway checks the scalar value and forwards it through the steepest gradient. Field based routing is very simple and robust, in this no routing table is maintained and routing is based on scalar values of the nodes.

When new group heads wants to join, local manager checks whether it is in the list or not. If not in the list then reject its request and update the list and share it with other local manager and declare it as an intruder. Otherwise the group head is authenticated. In the case of crash the group heads must be replaced and updated.

Time stamps are maintained for each session. After every 2 min group head send the keep alive messages to the local managers. If it fails to send then the next nearest node will be the group head and local manager update its list. Local manager also send these updated list to other local managers as well.

Group Head Authentication
In fig.1. the flow chart described that how group heads are authenticated. When network populates, local manager get the list of all group heads. When a group head send a join request, local manager check the list if it is not in the list then reject it and put it to the block list. Else the group head is authenticated.
Inter Group Communication
The flow charts in fig. 2 shows the stepwise layout of the secure inter group communication between different group heads. When a group head send data or it wants to share data to the other group head. The destination group head first verifies the authentication of the sender. If it is in the list of local managers then destination group head accept the data else reject the data as declare it as an intruder.

Performance evaluation
The sample topology is implemented in a network simulator OMNET++. We have analyzed the architecture with a network topology consists of a gateway and 39 wireless nodes with 2 local managers. Fig.4. shows that when we send 320 packets in the network RAGHC (Reliable Authenticated Group Head Communication) delivered 84% of packets, SFBR (Secure Field Based Routing) 49% are delivered and rest is lost. In NR (Normal Routing) just 41% of packets are delivered. As we saw that when the no of packets are increased RAGHC performance is good than the other two techniques. RAGHC delivered more number of packets and the delivery ratio is higher than the normal techniques, hence it is good for heavy traffic.
Fig. 3. Packet Delivery Ratio

Fig. 4. shows the secure inter group communication between different group heads via their local manager levels. There may be many local managers within the network at various levels. Level may be defined as the distance (hop count) from the group head. When local manager is at one level away (in hierarchy) from the groups then the time delay will be 7.8 on packet 160. When it is two level away then delay will be 11.7 and up to so on.

Fig. 4. Secure Inter Group Communication

Fig. 5. shows the packet delay. In RAGHC, 0.12 delay when the no of packets are 20 and in SFBR, 1.44 and in NR 0.586 delay ratio. As the no of packets are increased RAGHC delay ratio is much less than SFBR and NR. RAGHC is reliable and secure.

Fig. 5. Packet Delay
All the above results show that the performance of the RAGHC is good and reliable than the SFBR and NR. RAGHC packet delivery ratio is high and packet loss is much less than the other two techniques. RAGHC is reliable, secure and efficient for the group head communication. Secure group head communication can now easily be done through our sample topology.

Conclusion and Future Work
The problem of performing authentication analysis problems For example, the system could analyze the IEEE 802.1x protocol messages to determine the point at which authentication failed. Increase in security level may increase connectivity time slot. The architecture is organized into three parts i.e. group head authentication, secure inter-group communication via group heads and group head maintenance. Based upon the sample topology various data flows has been analyzed and compared with the previous techniques.

References

THE END
EFFECT OF URBANIZATION ON WATER RESOURCES IN JAIPUR, INDIA

Nidhi Gupta¹ & Dr. Rita Gupta²
¹Sr. Lecturer Department of Civil, Amity University of Rajasthan, Jaipur, ²Research Awardee, Department of Chemistry, University of Rajasthan, Jaipur, INDIA

Abstract:
The status of the water in the environment is unique. Ever since the birth of earth from day one, the need for water is always on the increase, not just because of increase in human population but because all living beings are multiplying at a fast rate. Urbanization has led to immense pressure on ground water resources and has resulted in deterioration of ground water. Jaipur, the capital of Rajasthan is facing rapid urbanization resulting in rapid depletion of ground water level. Uncontrolled urbanization and the growing population pressure are the essential challenges for water management in urbanized regions of the emerging and developing countries. This paper analyses the growth in population and urbanization resulting in lowering of ground water table. Further changes in land use pattern due to urbanization also risks ground water recharging. Significant Water Table Depletion has raised serious concerns over the future development and sustainable development.

Introduction
Jaipur District is one of the 32 districts in the state of Rajasthan in western India. The city of jaipur which is Rajasthan’s capital and largest city is also the district headquarters. The district is situated in the north eastern part of Rajasthan state. It is located between the north latitude of 26°23’N to 27°51’N and east longitude 74°55’E to76°50’E. It is bounded by sikar district in north west, alwar district in the north east, dausa in east, tonk in south, Ajmer in south west and nagaur in west. The district has an area of 11151sq Km and occupies 3.3% area of the state. It ranks ninth in comparison to the other district of the Rajasthan in term of the area. The district imbibes 2131 villages of which 2077 are inhabited and 57 are uninhabited.

Jaipur is probably the first planned city of modern India. Its features of beautiful architecture, planned growth and cosmopolitan character have endowed it with uniqueness in India’s urban setting. Jaipur, being capital of Rajasthan is the focus of socio-economic and political life of state. The slope land in the jaipur district varies from less than 10metres/kilometer to 300 meters /kilometer. The district is distinctly divided diagonally. The lower part of it has a slope less than 10 meters /kilometer and the higher part has 10-20 meters /kilometer. The top most part of the district has a slope again below10 meters/kilometre. Accelerated groundwater exploitation over the past few decades has resulted in great social and economic benefits by providing low-cost, drought-reliable and high quality water supplies for urban areas, rural populations and crop irrigation. The rapidly increasing large cities in semiarid and semi-humid regions raise many problems in water resources management.

Results and Discussions
Jaipur district has shown an increase in the population over the past decades. The population was 15, 22,591 in the year 1961 which has increased to 52,51,071 in the year 2001. This has led to tremendous pressure on the available limited resources. The decadal growth rate of population ranges between 30.9% to 39.8% with an average annual growth rate of 3.6%.

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Source: Derived from Census of India (JDA Master Plan)

Table 2 shows the decadal growth of rural and urban Jaipur district. The table shows that there is growth in both rural and urban population. The growth rate for rural population is between 23.77 % to 25.82 % between the years 1961 to 2001. However there is significant decadal growth in urban population which ranges between 46.07 % to 65.32% signifying that there is tremendous pressure on the urban environment.
Table 2. Decadal population growth of rural and urban Jaipur district

<table>
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<tr>
<th>Year</th>
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</table>

Source: Derived from Census of India (JDA Master Plan)

The rural-urban decadal percentage of growth shows that the ratio of Rural to the Urban is continuously changing. There has been increased urbanization over the decades with agriculture on the decline and increase in service sector.

Table 3. Decadal population growth of Jaipur district

<table>
<thead>
<tr>
<th>YEAR</th>
<th>RURAL%</th>
<th>URBAN%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1961</td>
<td>67.90*</td>
<td>32.10*</td>
</tr>
<tr>
<td>1971</td>
<td>64.19*</td>
<td>35.81*</td>
</tr>
<tr>
<td>1981</td>
<td>57.66*</td>
<td>42.34*</td>
</tr>
<tr>
<td>1991</td>
<td>54.36*</td>
<td>45.64*</td>
</tr>
<tr>
<td>2001</td>
<td>50.64*</td>
<td>49.36%</td>
</tr>
</tbody>
</table>

*Excluding district Dausa in 1992 Jaipur was then re-constituted with the creation of Dausa district.

In 1961, the level of urbanization was 32% which has increased to 49% in 2001. The national average of urbanization is 32%. The increased level of urbanization in the district is on account of the Jaipur city which is a primate city and also the major employment provider for the state of Rajasthan, besides the Jaipur region covers almost 1/3 part of the district.

The water level depletion due to increase level of urbanization in the Jaipur city

Table 5. Water level depletion due to increase in population

<table>
<thead>
<tr>
<th>Blocks under Jaipur city</th>
<th>Water Level (m)</th>
<th>Water level depletion (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMER</td>
<td>39.15</td>
<td>29.89</td>
</tr>
<tr>
<td>BAIRATH</td>
<td>25.38</td>
<td>21.29</td>
</tr>
<tr>
<td>BASSI</td>
<td>47.80</td>
<td>26.34</td>
</tr>
<tr>
<td>DUDU</td>
<td>19.65</td>
<td>19.49</td>
</tr>
<tr>
<td>GOVIND GARH</td>
<td>41.14</td>
<td>28.78</td>
</tr>
<tr>
<td>PHAGI</td>
<td>18.68</td>
<td>17.67</td>
</tr>
<tr>
<td>SANGANAR</td>
<td>49.85</td>
<td>36.03</td>
</tr>
</tbody>
</table>
From the Graph above, it can be concluded/stated that the urbanization in some way has resulted in the Water level depletion and high value of water depletion in dense population area (Bassi and Sanganar) are found. This is clearly shown in Graph 2 that there is significant depleting in water table in Jhotwara, sanganer and Bassi blocks of Jaipur district.

**Conclusions**

This study shows that ground water table and quantity in the urban areas have changed greatly. Rapid urban and industrial developments and extensions of irrigated farmland have doubled the water demand. The consequences were over use of surface water resources and excessive exploitation of ground water in the area. Urbanization does have a marked and detrimental effect on the water resources on a developed area. These findings are generally accepted within the scientific and engineering community. There is significant increase in urban population and rapid urbanization and industrialization has further led to depletion of water table. Further research and resource mapping is required for proper planning and resource mapping.

**References**

1. Data collected from Ground water department, Jaipur (Rajasthan)
2. www.unfpa.org (2010 September 08)
3. Master Plan, JDA
4. Our cities, our health, our future, Report of the WHO commission for health equity in urban settings, WHO
Abstract:

Leaf extract and leaf powder was used to evaluate allelopathic effects of Tagetes minuta L. for the control of important weeds, Avena fatua L., Rumex dentatus L. and Phalaris minor Retz. in wheat. In filter paper method different concentrations (50, 75 and 100%) of leaf extract of Tagetes minuta was applied to determine their effect on weed seed germination and growth. All the extract concentrations significantly reduced the germination of Phalaris minor and radical growth however the shoot length of the surviving seeds was not affected. In Avena fatua the shoot growth inhibition was concentration dependent. The germination of Rumex dentatus reduced only at 100% concentration. Leaf extract of Tagetes minuta significantly reduced the germination of tested weeds and their germination reduction was in the order of 90.69%, 81.81% and 19.14% for Phalaris minor, Avena fatua and Rumex dentatus respectively. Direct incorporation of leaf powder into the soil at the dose of 2.4 and 6g had no effect on shoot and root growth and germination% of all tested weeds except the root growth of Rumex dentatus at 6g/300g of soil mixture. Results indicate that Tagetes minuta leaf extract can be used as an environmentally safe bioherbicide.

Introduction

The inhibition of one species by another is referred to as allelopathy. This inhibition is governed by allelochemicals which can be present in any part of the plant such as in leaves, flowers, roots, fruits, or stems. These chemicals may also be present in the surrounding soil released by the plant (Marwat and Khan, 2006). Allelochemicals act as biocommunicators. These may also act as attractants or repellents, nutrients or toxins to phytophagous insects. Toxic compounds of crop plant origin accumulate in agricultural soils. Various plant species release a variety of organic compounds with specific active life in soil. Active life is determined by factors such as leaching, volatility, microbial action and adsorption (Khalid et al., 2002). Allelopathy is playing its important role in natural ecosystems, plant invasion, exclusion of related species, vegetation patterning and reduction in productivity of many plants in agroecosystem.

Asteraceae family has members that are rich in complex structured secondary compounds. Basic compounds that are present in this family are terpenoid based sesquiterpene lactones and polysaccharide fructans (Khalid, 1995). Tagetes, a member of Asteraceae family, is a genus of flowering marigold. Three species Tagetes erecta L., T. petula L., T. minuta L. of this genus are reported from Pakistan (Stewart, 1972). In tropical regions, oil of T. minuta is used as insect repellent and reported to be use for the treatment of smallpox, ear ache, colds and fever. Additionally this oil possesses hypotensive, anti-inflammatory, spasmylytic, antifungal, antimicrobial and nematicidal properties (Tereschuk et al., 1997). It is also used as a refreshing beverage, as a condiment, and for various medicinal purposes. A chemical characterization of volatile components of Tagetes minuta was done and a total of 27 compounds were identified that constituted 92% of essential oil of aerial parts (Meshkatalsadat et al., 2010). Tagetes species are found in various countries and are also grown as ornamental plants (Kil et al., 2002). Wild Marigold (Tagetes minuta) is widely grown over a range of climatic conditions starting from 3000 to 11000 feet of altitude in the north and northwestern parts of Pakistan (Shehzadi et al., 2010). This species is competitive in nature so it is resistant to the natural drought tolerant soils and can easily survive on poor soils as weed (Hulina, 2007). Tagetes minuta has affinity to grow on disturbed sites and may colonize many areas around the world (Gil et al., 2000). This species is native to South America and since the period of Spanish Conquest, this has been introduced into Europe, Africa, India and Hawaii (Meshkatalsadat et al., 2010). Leaf extracts of Tagetes minuta significantly...
inhibited germination while root extracts did not show any effect on germination. Besides leaf extracts showed the strongest radical growth inhibition resulting in burning of the radicle tips (Alhammadi, 2008). The seeds of test species (*Lotus corniculatus* var. japonicus and *Lactuca sativa*) were inoculated in Petri dishes containing 0, 10, 50 and 100 % *T. minuta* aqueous extracts. At 5th day, it was observed that seed germination of *L. corniculatus* var. japonicas was significantly inhibited, however *L. sativa* was not inhibited (Kil et al., 2002).

Potential utilization of dried powder of *Tagetes minuta* as a natural herbicide for managing rice weeds was investigated. It was observed that leaf powder of *T. minuta* applied to soil of rice field significantly reduced emergence and growth of weeds (*Echinochloa crus-galli* and *Cyperus rotundus*) in pot experiment under greenhouse and in rice field plots (Batish et al., 2007). This species is growing as invasive in natural habit and may possess certain allelopathic potentials. Therefore present study was planned to test allelopathic potential of *T. minuta* by applying aqueous extracts and leaf powder on different weed seeds for effect on germination and growth.

**Materials and Methods**

Experiments were conducted in the Weed Management Programme, Institute of Plant and Environmental Protection at National Agriculture Research Centre Islamabad and in Taxonomy Lab; Pir Mehr Ali Shah Arid Agriculture University Rawalpindi to study the allelopathic activity of *Tagetes minuta* against *Avena fatua*, *Phalaris minor* and *Rumex dentatus*.

**Collection of plant material**

Mature green leaves were collected from naturally growing populations of *Tagetes minuta* from Murree hills and Lehtrar. Fresh leaves were brought into laboratory and thoroughly washed with distilled water and then were placed in paper bags separately that were subjected to oven drying at 60 °C. After appropriate drying, the dried plant material was crushed, sieved with sieve of 200mm mesh size and kept in glass bottles till further use.

**Aqueous extract method**

This method was conducted to investigate the growth inhibitory effect of water soluble constituents (Nasir et al., 2005). Ten grams of oven dried leaves were soaked in 100ml of distilled water in a flask and were agitated for 24h on orbital shaker at room temperature. The extract was stained through two layer of chees cloth and was filtered with Watman’s No 2 filter paper. This filtrate was considered as a stock solution. Further concentrations of this extract were prepared.

Two layers of filter paper were placed in each glass Petri dish (9cm size). Seeds of weeds were added to each glass Petri dish and 5ml of different concentrations of extracts was added per dish. The distilled water was used as control.

The Petri dishes were then sealed with squash tape and incubated in the growth chamber for 10 days. Experiments were performed in the replica of five. This whole set of experiment was repeated twice for each weed species.

**Direct incorporation of leaf material into soil:**

This method was used to find out the effect of leaf mulch on the growth and germination of tested weeds in soil. The following treatments were used for this experiment;

1) T0= Control (Soil only)
2) T1= 2 grams leaf powder + soil
3) T2= 4 grams leaf powder + soil
4) T3= 6 grams plant powder + soil

In this method, soil was collected from NARC Research farm. The collected soil was crushed, air dried and sieved through 20mm sieve in order to remove impurities. Sand was collected from the same area and was air dried. Soil (33%) and sand (67%) was mixed in pots in order to make the final mixture of 300g. 2g, 4g and 6g of leaf powder was thoroughly mixed with the mixture of soil and sand (300g). Weed seeds were sown in pots. For control no plant powder was added. Three replications of each dose for each species were used. Pots were placed in glass house and watered according to daily requirement. Emergence of seedlings was recorded after four days of planting.

**Parameters**

The following parameters were recorded after the completion of experiment.
Root and Shoot length (cm)
Root and shoot length of all the germinated weeds were recorded in centimeter with the help of graduated ruler. Then average per dish/pot was calculated.

Percentage Growth of Root and Shoot
Percentage growth of root/shoot was calculated by following formula:
\[
\text{Percentage growth} = \frac{\text{Average length of root/shoot in particular treatment}}{\text{Average length of root/shoot in control}}
\]

Percentage Germination
Number of plants germinating in each dish/pot was recorded after four days and then daily for 15 days. After that germination (%) was calculated.

Statistical analysis
Data on the parameters namely root length, shoot length, fresh weight, dry weight and percentage germination in weeds was calculated in the different screening methods and then statistically analyzed in Complete Randomized Design. Multiple Comparison Tests for means was also applied for those variables that showed statistically significant difference in the ANOVA. Statistix 9 software was used for this purpose.

Results and Discussion

Aqueous extract method:

Germination:
The highest extract concentration (100%) has highest suppressing potential for germination of all tested weed species. Germination of *Avena fatua* and *Rumex dentatus* was only reduced at 100% concentration. Whereas germination of *Phalaris minor* was suppressed at all three concentration (50%, 75% and 100%). Maximum germination inhibition was observed in case of *P. minor* and minimum germination inhibition was observed in case of *R. dentatus* (Table 1). This may indicate that *T. minuta* is more effective towards small sized seeds as compared to larger seeds or it has differential effect on monocot and dicot weeds. Similar phytotoxic concentration dependent effect was obtained in a study carried out by Takao et al. (2011) which showed that four concentrations of leaf extracts of *Ipomoea carica* negatively affected the germination, early development and morphology of *Bidens pilosa*, *Echinochloa crus-galli*, *Ipomoea grandifolia* and *Euphorbia heterophylla*.

Reduction in germination:
At highest concentration, 90.69% reduction in germination was observed for *P. minor* followed by 81.81% for *A. fatua* and 19.14% for *R. dentatus*. So *P. minor* was found highly vulnerable to germination as compared to other weeds (Fig 1). Here it is evident that *T. minuta* leaf extract is more effective on grass seed germination than broad leaf weed.

Shoot growth:
All the concentrations non-significantly reduced the shoot growth of *P. minor* seeds that survived where as significantly decreased the shoot length of *A. fatua*. In case of *R. dentatus* 75% and 100% concentrations showed a significant decrease in its shoot length. Maximum inhibition of shoot growth was observed in case of *A. fatua* (Table 2).

Overall the maximum plumule length was observed in control treatment (7.53cm) where as minimum length was observed in 100% concentration (0.92cm). It means that extract was more active in allelopathic potential and shoot growth suppression was concentration dependent. One such study was conducted by Fikreyesus et al. (2011) to observe the allelopathic effect of *Eucalyptus camaldulensis* on tomato crop. Root, fruit, bark and leaf extracts of *E. camaldulensis* were used in lab and field experiments respectively. Extracts significantly inhibited the germination and root and shoot elongation of tomato plants. Highest concentration (5-10%) had strong inhibitory effect.

Reduction in shoot growth:
*Avena fatua* shoot growth was reduced by 64.67, 64.80 and 87.87% in 50, 75 and 100% concentration of leaf extract. In *Rumex dentatus* and *Phalaris minor* reduction of shoot growth occurred at higher concentrations although it is non significant in case of *Phalaris minor* (Fig 2).

Root growth:
All the concentrations significantly decreased the root growth of *P. minor, A. fatua* and *R. dentatus* (Table 3). In *R. dentatus* decrease in root growth was concentration dependent. Maximum reduction in root growth at 100%
concentration was also observed in *R. dentatus*. While Renjun *et al.* (2011) in a study found that growth of *Alexandrianum tamatense* was significantly inhibited by the distilled water extracts of *Sargassum thunbergii* at different concentrations but it did not show lethal effects on *A. tamarense* even at the highest concentration.

**Reduction in root growth:**
At 100% leaf extract concentration 79.36% root growth reduction was observed in *R. dentatus* (Fig 2). Whereas at 75% concentration 67% reduction in root growth was observed in *P. minor* followed by 58.10% and 56.94% root growth reduction in *A. fatua* and *R. dentatus* respectively. Minimum root growth reduction was observed in the range of 30-50% whereas maximum root growth reduction was observed in the range of 70-80%.

**Direct incorporation of leaf powder into soil:**

**Germination:**
Direct incorporation of leaf material into the soil non-significantly affected germination % of all the tested weeds (Table 4). It means that a long period of time is required for the degradation of *Tagetes* plant material and for release of phytochemicals. So a set of experiment for a longer period of time could be focused in further studies. As compared to this when Tesio *et al.*, 2011 conducted pot experiments to check allelopathic potential of *Helianthus tuberosus* L. against lettuce, pea and *Digitaria sanguinalis*. More than 30% germination reduction was observed in *D. sanguinalis*. This was the best result as compared to the other species considered. 75.45 and 57.18% increase in germination was observed for *Avena fatua* in 4 and 6g of leaf powder respectively. In *Phalaris minor* 2g of leaf powder decreased the germination (25%) whereas 34.99 and 24.99% increase was observed at 4 and 6g of leaf powder. In *Rumex dentatus* 16.67% decrease occurred at 2 and 6g while 23.33% decrease was observed at 6g of leaf powder. All these effects were statistically non significant (Table 4).

**Shoot growth:**
None of the concentrations of leaf powder is successful in significantly reducing the shoot growth of all the tested weeds species (Table 5). In a previous study on the allelopathic herbicidal effect of *T. minuta* leaf powder on two invasive weeds of rice field (*Echinocloa crus-galli* and *Cyperus rotundus*) it was revealed that *T. minuta* leaf powder significantly reduced the emergence and growth of both of weeds without affecting the growth and yield attributes of rice crop (Batish *et al.*, 2007). All the doses non significantly increased the shoot growth of *A. fatua* and decreased the shoot growth of *P. minor*. 4g of leaf powder non significantly increased shoot growth of *R. dentatus* and 6g of leaf powder non significantly decreased the shoot growth (21.21%).

**Root growth:**
The result of root growth was variable in all the three weeds, only 6g of plant material significantly reduced the root length of *R. dentatus* (Table 6). Results for the root growth were more or less similar to those that were obtained for the shoot growth. All the three doses 2g, 4g and 6g of leaf powder non significantly decreased the root growth of *A. fatua* and non significantly increased the root growth of *P. minor*. In *R. dentatus* 4g of powder showed non significant increase whereas 6g of powder significantly reduced root growth of *R. dentatus* (52.82%).

**Conclusion**
Results suggest that *Tagetes minuta* leaf extract has a great potential to reduce germination and growth of *Avena fatua*, *Phalaris minor* and *Rumex dentatus*. So this can be used for the management of these weeds in crop system. On the other hand additional work is required to test the efficacy of leaf powder against weeds because from results of this experiment it is observed that leaf material required a longer period of time for its degradation in soil. These results may also allow the development of environmentally safe bioherbicide and biologically based weed management systems.

**Table 1: Effect of Tagetes minuta L. leaf extract on germination of different weeds.**

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Avena fatua</th>
<th>Phalaris minor</th>
<th>Rumex dentatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22 A</td>
<td>86 A</td>
<td>94 A</td>
</tr>
<tr>
<td>50%</td>
<td>22 A</td>
<td>34 B</td>
<td>90 AB</td>
</tr>
<tr>
<td>75%</td>
<td>10 AB</td>
<td>8 B</td>
<td>82 AB</td>
</tr>
<tr>
<td>100%</td>
<td>4 B</td>
<td>8 B</td>
<td>76 B</td>
</tr>
</tbody>
</table>

Note: The values in the table are germination percentages. The letters (A, B) in the table indicate significant differences.
Table 2: Effect of Tagetes minuta L. leaf extract on the shoot growth of different weeds.

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Avena fatua</th>
<th>Phalaris minor</th>
<th>Rumex dentatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.53 A</td>
<td>5.84 NS</td>
<td>2.66 A</td>
</tr>
<tr>
<td>50%</td>
<td>2.66 B</td>
<td>3.56</td>
<td>2.65 A</td>
</tr>
<tr>
<td>75%</td>
<td>2.65 B</td>
<td>3.84</td>
<td>2.10 B</td>
</tr>
<tr>
<td>100%</td>
<td>0.92 B</td>
<td>2.34</td>
<td>2.01 B</td>
</tr>
</tbody>
</table>

Table 3: Effect of Tagetes minuta L. leaf extract on the root growth of different weeds.

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Avena fatua</th>
<th>Phalaris minor</th>
<th>Rumex dentatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.54 A</td>
<td>2.97 A</td>
<td>2.81 A</td>
</tr>
<tr>
<td>50%</td>
<td>3.71 AB</td>
<td>1.45 B</td>
<td>1.85 B</td>
</tr>
<tr>
<td>75%</td>
<td>2.74 B</td>
<td>0.98 B</td>
<td>1.21 C</td>
</tr>
<tr>
<td>100%</td>
<td>1.42 B</td>
<td>0.82 B</td>
<td>0.58 D</td>
</tr>
</tbody>
</table>

Table 4: Effect of Tagetes minuta L. leaf powder on germination of different weeds.

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>% germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avena fatua</td>
<td>Phalaris minor</td>
</tr>
<tr>
<td>Control</td>
<td>23.33 NS</td>
</tr>
<tr>
<td>2g</td>
<td>23.33</td>
</tr>
<tr>
<td>4g</td>
<td>40</td>
</tr>
<tr>
<td>6g</td>
<td>36.67</td>
</tr>
</tbody>
</table>

Table 5: Effect of Tagetes minuta L. leaf powder on the shoot growth of different weeds.

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Shoot length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avena fatua</td>
<td>Phalaris minor</td>
</tr>
<tr>
<td>Control</td>
<td>5.85 NS</td>
</tr>
<tr>
<td>2g</td>
<td>6.33</td>
</tr>
<tr>
<td>4g</td>
<td>8.85</td>
</tr>
<tr>
<td>6g</td>
<td>9.54</td>
</tr>
</tbody>
</table>

Table 6: Effect of Tagetes minuta L. leaf powder on the root growth of different weeds.

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Root length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avena fatua</td>
<td>Phalaris minor</td>
</tr>
<tr>
<td>Control</td>
<td>1.68 NS</td>
</tr>
<tr>
<td>2g</td>
<td>1.74</td>
</tr>
<tr>
<td>4g</td>
<td>2.85</td>
</tr>
<tr>
<td>6g</td>
<td>3.24</td>
</tr>
</tbody>
</table>

Fig. 1: Percentage reduction in germination of weeds at 100% concentration of Tagetes minuta leaf extract.
**Fig. 2:** Percentage reduction of shoot and root growth of weeds at 100% concentration of Tagetes minuta leaf extract.

**Plate 1:** Effect of different concentrations of T. minuta leaf extract on growth of Avena fatua.

**Plate 2:** Effect of different concentrations of T. minuta leaf extract on growth of Phalaris minor.
Plate 3: Effect of different concentrations of T. minuta leaf extract on growth of Rumex dentatus.

Plate 4: Effect of different doses of T. minuta leaf powder on Avena fatua.

References

THE END
IMPACT OF BIOLOGICALLY MODIFIED COAL FLY ASH WITH WATER HYACINTH IN CULTIVATION OF ALLIUM SATIVUM L.

Jaywanti Punjwani¹, K.K. Gupta² & Kalpana S.²
P. G. Department of Chemistry, Govt. College, Kota

Abstract:
In order to provide a cost effective and ecofriendly method for correction of agricultural lands spoiled due to spread of coal fly ash (CFA) around Thermal Power Stations few pot and field experiments were carried out. To find out the application impact of biologically modified coal fly ash with water hyacinth in cultivation of Allium sativum L., various physicochemical parameters were determined for different composts obtained by successive replacement of CFA for soil (loam) in constituents of reference compost. CFA, a byproduct from Kota Super Thermal Power Station, located near Kota city of Rajasthan and rooted out water hyacinth from Kishore Sagar Talab situated in center of Kota city were used. Increase in rate of growth and improvement in quality of produce was observed with the favourable changes in physicochemical parameters of soil with respect to productivity. Best results in terms of plant growth, maturation period, quality and quantity of produce were obtained with compost containing 30% (v/v) CFA replacement of soil. Similar pot experiments were carried out for three consecutive years 2009, 2010 and 2011. Field experiments were carried out in the year 2011 with the compost giving best result in pots.

Introduction
Coal constitutes one of the prime sources of energy in number of countries where huge coal reserves are available. Large quantity of coal is burnt to generate steam in Thermal Power Stations (TPSs) producing huge quantity of coal fly ash (CFA) as a byproduct. The problem becomes challenging when available land area for CFA disposal is limited and lies in the vicinity of urban and potential agricultural belts. Further the inherent nature of ash brings adverse effects to the environment and ecological features of the surrounding regions. Coal being abundantly available, is expected to remain major source of energy in near future also. Indian coals, though low in sulphur, contain higher amount of ash (about 35-45%). As per an estimate of Fly Ash Utilization Program, the annual ash generation figures are expected to reach about 225 million tonne by 2017.

CFA is a heterogeneous mixture of amorphous and crystalline phases and in general is considered a ferro aluminosilicate mixture with Al, Ca, Fe, K, Na and Si as predominant elements. It was realized that CFA consists practically all the elements present in soil except organic carbon and nitrogen and can be utilized as a resource material. The potential of the CFA has been understood and brought forward for various utilization fields. It has been demonstrated that CFA can be utilized in major construction projects such as dams, ash dykes, landfills, roads as pavement, agriculture and related fields and for other purposes such as soil stabilization, brick manufacture, cement industry, tiles and paint industry etc.

Agriculture and waste land management have emerged as prime bulk utilization areas for CFA in the country. Addition of CFA with soil alters its physical and chemical characteristics mostly in such a way that it becomes more fertile i.e. having positive impact on growth and yield therefore for better productivity and yield it can be used as an additive or amendment material after deciding the quantity of CFA required for best results (CFA dose) by scientifically designed trials following standard procedures and protocols.

Biomodification further increases the productivity of soil. Agriculture plays a major role in the global fluxes of greenhouse gases. In a study it was demonstrated that 1 tonne of fly ash can sequester up to 26 kg of CO₂ i.e. 38.18 tonnes of flyash per tonne of CO₂ sequestered and confirmed the possibility to use this alkaline residue for CO₂ mitigation.¹¹

The chemical and physical properties of CFA are determined by several variables such as coal source, degree of pulverization, design of boiler unit, loading and firing conditions, handling and storage methods. Thus higher degree of variation can occur in ash not only between TPSs but within a single Thermal Power Station also.

Therefore as a part of our investigations present studies, “Impact of biologically modifies coal fly ash with water hyacinth in cultivation of Allium sativum L.” have been initiated determining various physicochemical parameters for composts obtained by successive replacement of CFA for soil (loam) in constituents of original and reference compost and yield parameters of Allium sativum L.

Material and Methods
Amount of seeds, nitrogenous fertilizer (urea), Potash fertilizer, constituents of reference compost and time chosen for cultivation were following:

(A) Amount of seeds used – 50 kg/hectare

¹¹
Nitrogenous fertiliser (urea) – 120 kg/hectare (half of the dose as basal dose and remaining after 1 month)

Potash fertiliser – 150 kg/hectare

Constitution of reference compost – (2 part soil(loam) + 1 part sand +2 part rotten farmyard manure/waste+ 1 part chopped Eichornia )

Time chosen for cultivation-Third week of October(2009, 2010 and 2011)

Allium sativum L. (Garlic) a widely used herb( leaves and bulbs) of family Alliaceae (Amarylidaeae). Allium sativum L. is one of the important bulb crops. The compound bulb of garlic consists of several small bulblets or cloves. Green leaves of the plant are used , to garnish Indian dishes and clove is a common spice in Indian curries, chutneys and pickels .

Allium sativum L. has been widely recognized as a valuable spice and probably one of the earliest known medicinal plants, used from ancient time to cure different disease conditions in human. Garlic's principal medicinal uses are to lower blood pressure and cholesterol, fight infections, and prevent cancer. The active constituents are sulfur-containing compounds that are rapidly absorbed and metabolized. Garlic has been proved to have antihypertensive potential, wound healing potential, anti diabetic potential, hepatoprotective potential, antiinflammatory potential, antihelmentic potential, anticoagulant and fibrinolytic potential, antimicrobial potential, antioxidant potential, immunomodulatory potential, anticancer potential, antiatherosclerosis and hypolipidemic potential.

G-282 variety of Allium sativum L. was chosen for the study. Bulb of this variety is rigid and white coloured and also in use for export purpose.

CFA utilized in experiments is collected from Kota Super Thermal Power Plant, one of the leading Super Thermal Power Stations of India with the existing installed capacity in different stages1240MW,located at just upstream of Kota Barrage, by the Chambal River, left main canal and approach road to Jawahar Sagar via Nanta Village and abandoned ash dyke. Water hyacinth (sp. of Eichornia of family Pontederaceae) rooted out from Kishore Sagar Talab of Kota City, Rajasthan, is added as organic manure, an aquatic weed is rich in moisture and good source of potash, lime and phosphorus with near about 0.04% biotic fraction is used for biomodification of CFA with respect to improvement in physicochemical properties. The water hyacinth (Eichornia crassipes) is one of the noxious fresh water plants. Its rate of proliferation under optimum conditions is extremely rapid and it can spread to cause infestations over large areas of water causing a variety of problems.

For pot experiments CFA samples were air dried and stored at room temperature before mixing it for converting into composts. 1part water hyacinth was added in organic manure. Different composition of composts were prepared by gradual replacement of soil by CFA (from 10% to 60%) in reference compost. For conversion into composts left these admixtures in separate damp pits (approx. 4 feet in depth) for about 1.5 month. Density, texture, water holding capacity, porosity were determined by adopting standard techniques for soil analysis and pH was measured potentiometrically using glass calomel electrode and conductivity measured with conductivity bridge.

Radioactivity tests obtained from ESL, Nuclear Power Corporation, Rawatbhata for Thorium-232, Uranium-238 and Potassium-40 elements in a sample of CFA used for the purpose were within limits not hazardous to human health. Toxic heavy metal analysis for compost, giving best results of produce and for produce obtained was carried out and the results showed that uptake of heavy metals was within permissible range.

Chemical analysis for nitrate, phosphate, sulphate, potassium, calcium, manganese, magnesium, copper, zinc, iron have been carried out following standard chemical analysis methods ( Titrimetrically / Spectrophotometrically / Colorimetrically / Flame photometrically ). All chemicals used for analysis were of A.R. grade.

Experiments for study of plant growth, quality and yield of produce were carried out in pots. Seeds were sown in reference composts in third week of October of each year 2009, 2010 and 2011. Seedlings were transplanted in the pots of identical dimensions packed with composts of different constitution after reaching definite height (approx 15cm). In field experiment planted at 10-15cm distance. Chosen variety of Garlic was grown according to its requirements of water, support and climatic conditions as referred in standard agriculture literature. As Garlic is a shallow rooted crop, frequently irrigated leaving no moisture scarcity during growth period. At the time of maturity,
irrigation is stopped. Weeding followed by fertilizer application & earthing up was done at one & two months after transplanting. Hoeing at the time of bulb formation (approx two and half month after planting) helps in setting of bigger well filled bulbs. Observations regarding pests (especially stem and bulb Nematodes) and disease attack were recorded time to time.

Five pots were prepared for each composition of composts (0%, 10%, 20%, 30%, 40%, 50% and 60%). The plants were allowed to grow till maturity and harvested when the top turned yellowish or brownish showing sign of drying. It took about 4-5 months after planting to reach maturity. After harvesting, bulbs were dried in shade and stored in cool dry place having proper aeration.

To find out utility of composts preparation (biomodification) carried out simultaneous experiments under similar conditions by growing chosen variety directly in similar mixtures of soil, sand, CFA, farmyard manure and water hyacinth. Field experiments were also carried out in 10m x10m area for this vegetable adopting similar method for cultivation as in pots.

Field prepared by spreading compost (given best results in pots) in the field, after ten days of it turning surface soil down six inches of soil depth & covering the field for next fifteen days with perforated polythene sheet. Field experiments were carried out only once in the year 2011.

Results and Conclusion
The preparation of different composts by application of different percentage of CFA and Eichornia resulted in favourable physicochemical changes with biomodification of soil properties, with chosen variety of Allium sativum L. an improvement in fertility was observed up to 30% replacement of soil by CFA.

Tables-1A, 1B and 1C contain results obtained for texture, density, porosity and WHC determined for different composts prepared for studies. The porosity & WHC increased and density decreased with the increase in percentage of CFA in reference compost (C1 to C6) while from C3 to C6 the trend has got reversed. Texture changed from loamy to silty loamy and organic matter decreased with the increase in percentage of CFA. Tables-1A,1B and 1C also show pH and electrical conductivity increased with the increase in percentage of CFA in compost mixture due to the increase in salt content like calcium oxide (CaO) and magnesium oxide (MgO), their dissociation possibilities contributed from the alkaline CFA added and increased quantity of soluble macro and micro nutrients. Organic matter (in percentage) decreased from C1 to C6 and increased from C3 to C6. It has been explained as during the composting process, Carbon dioxide emits from the composting mass as one of the metabolic end product. Thus the total Carbon content of the composting mass decreases as composting proceeds and probably after maturation most of the organic matter converted in the form of stable humic substances. (Table-2A, 2B, 2C & 3A, 3B, 3C, 4A, 4B, 4C) released by the added CFA or by the interaction of inorganic constituents of CFA with organic matter.

With the increase in dose of CFA the availability of most of the macronutrients was found to increase up to certain ratio. The release of the nutrients in the ionic form increasing their bioavailability which can be considered favourable results of chain of chemical reactions among constituents of composts at more suitable pH and physical conditions i.e. better texture, reduced density, increased porosity with appropriate water holding capacity.

The available nitrogen was measured in terms of NO3-. The percentage of nitrates increased. The available phosphorous was measured in terms of phosphate. The increase (firstly increase than decrease) in concentration of phosphate may be attributed to the available phosphorus present in CFA. Plants take phosphate in HPO42- form. The increase is due to the hydrolysis of iron, aluminum and magnesium compounds in CFA and released inorganic acids by CFA. The liberated acids help in the release of available phosphate from the unavailable form without affecting the pH, as organic matter present in the soil has a buffering capacity in maintaining the pH.

Potassium is measured in terms of K+ . It increased upto C1 to C3 then decreased C3 to C6. The percentage of calcium and magnesium increased up to C3 and then decreased. It is reported by some researchers that at higher concentration of CFA some heavy metals become more active and hinder the microbial activity. pH plays a vital role in the release of specific nutrients. The availability of nutrients is maximum at pH 5.5 to 6.5.

Most of the heavy metals play a vital role in plant physiology. The availability of iron is more than manganese. The results reveal that as the concentration of CFA increases the availability of iron and manganese increases but at the level where iron did not impart any toxicity to plants. It may be attributed to the presence of manganese which oxidizes excess ferrous (soluble) into ferric (insoluble) reducing the availability of soluble iron which may cause toxicity or to the reaction taking place between the carbonate ion and iron which reduces the
excess availability of iron to the plants. The application of different percentage of CFA and biomodification with water hyacinth resulted in an increase in available nutrients (N, P, K, Ca, Mg, S, Fe, Mn, Zn and Cu) in the soil, which altered the physicochemical properties of soil. It is reported that copper helps in the accumulation of calcium and magnesium. In CFA, Cu is present in the form of tenorite (CuO). It reacts with inorganic acids released during the hydrolysis of various compounds and helps in the release of available copper. Copper acts as an "electron carrier" in enzymes which bring about oxidation reduction and regulates respiratory activity in plants.

Table 4A, 4B, 4C indicates that copper is increased with the increase in concentration of CFA up to C3 and then decreased but did not impart any toxicity as excess availability was hindered due to slightly alkaline pH, organic matter and clay content. Tables 6 show the yield of Allium sativum L. in various composts. Yield increased on increasing percentage of CFA up to 30% of CFA & then decreased. Tables 5 show the heavy metal analysis of coriander obtained on cultivation with the compost giving best results.

Maximum percentage increase in yield was 75.39% in the year 2009 in compost having 30% CFA for the cultivation of Allium sativum L. as shown in Table 7. S analysed in different plant parts (in percentage) obtained on cultivation in different composts prepared in different years (2009, 2010 and 2011). S increased from C1 to C3 then decreased from C3 to C6 as shown in Table 8A, 8B and 8C.

Present studies clearly reveal that CFA worked as soil modifier and nutrients supplier in cultivation of Allium sativum L. Results obtained for different parameters indicate that CFA improved physical and morphological properties of soil. Water retention capacity of soil together with increased release of nutrient elements such as calcium, magnesium, sulphur, potassium, copper, phosphorous zinc etc.

Addition of constant amount of water hyacinth helped in increasing potassium, calcium, phosphorous, organic carbon in the mixture. Resistance to diseases increased and attack of insects decreased in plants cultivated in different composts and was maximum in compost having 30% (w/v) replacement of soil with CFA which may be due to presence of Sulphur in CFA.

Best results in terms of plant growth, maturation period, quality and quantity of produce were obtained with compost containing 30% (w/v) replacement of soil with CFA. Therefore, it can be concluded that CFA can be applied (dose 30% w/v) for soil advantageously in cultivation of Allium sativum L. of family Alliaceae. The plants and the edible parts of the plant were observed five times less prone to the diseases and insect attacks.

Reported data will help in generating general acceptability towards application of these two local wastes in agriculture and related fields enhancing the productivity of soil and gainful utilization of CFA, on sustainable basis. The method can also be applied in correction of CFA spoiled lands for particular crops/vegetables around Thermal Power Plants.

References
1. Fly-Ash Utilization Program (FAUP), The Circular of Department of Science and Technology (DST), New Delhi, India, 2007.

**Tables (Experimental Results)**

[% of CFA : $C_0 = 0\%$, $C_1 = 10\%$, $C_2 = 20\%$, $C_3 = 30\%$, $C_4 = 40\%$, $C_5 = 50\%$, $C_6 = 60\%$.]

**Table 1(A): Physical properties of different composts prepared for *Allium sativum L.* in the year 2009**

<table>
<thead>
<tr>
<th>Parameter → % of Compost ↓</th>
<th>Texture</th>
<th>Organic Matter (%)</th>
<th>Density (gm cm$^{-3}$)</th>
<th>pH</th>
<th>WHC (%)</th>
<th>Porosity (%)</th>
<th>Conductivity (μ mho cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_0$ Loamy</td>
<td>0.660</td>
<td>1.380</td>
<td>5.82</td>
<td>52.30</td>
<td>41.95</td>
<td>198.50</td>
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</tr>
<tr>
<td>$C_1$ Loamy</td>
<td>0.643</td>
<td>1.250</td>
<td>5.95</td>
<td>54.80</td>
<td>42.60</td>
<td>209.20</td>
<td></td>
</tr>
<tr>
<td>$C_2$ Loamy</td>
<td>0.622</td>
<td>1.170</td>
<td>6.20</td>
<td>56.50</td>
<td>44.20</td>
<td>220.50</td>
<td></td>
</tr>
<tr>
<td>$C_3$ Silty Loamy</td>
<td>0.617</td>
<td>1.130</td>
<td>6.30</td>
<td>57.20</td>
<td>44.80</td>
<td>230.60</td>
<td></td>
</tr>
<tr>
<td>$C_4$ Silty Loamy</td>
<td>0.638</td>
<td>1.210</td>
<td>6.20</td>
<td>59.42</td>
<td>47.50</td>
<td>210.80</td>
<td></td>
</tr>
<tr>
<td>$C_5$ Silty Loamy</td>
<td>0.618</td>
<td>1.140</td>
<td>6.44</td>
<td>62.60</td>
<td>44.00</td>
<td>230.20</td>
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</tr>
<tr>
<td>$C_6$ Silty Loamy</td>
<td>0.590</td>
<td>1.050</td>
<td>6.80</td>
<td>62.60</td>
<td>46.80</td>
<td>250.10</td>
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</tr>
</tbody>
</table>

**Table 1(B): Physical properties of different composts prepared for *Allium sativum L.* in the year 2010**

<table>
<thead>
<tr>
<th>Parameter → % of Compost ↓</th>
<th>Texture</th>
<th>Organic Matter (%)</th>
<th>Density (gm cm$^{-3}$)</th>
<th>pH</th>
<th>WHC (%)</th>
<th>Porosity (%)</th>
<th>Conductivity (μ mho cm$^{-1}$)</th>
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</thead>
<tbody>
<tr>
<td>$C_0$ Loamy</td>
<td>0.644</td>
<td>1.170</td>
<td>6.3</td>
<td>48.20</td>
<td>42.60</td>
<td>176.50</td>
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<tr>
<td>$C_1$ Loamy</td>
<td>0.613</td>
<td>1.050</td>
<td>6.4</td>
<td>50.80</td>
<td>43.60</td>
<td>184.20</td>
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<tr>
<td>$C_2$ Loamy</td>
<td>0.602</td>
<td>1.020</td>
<td>6.6</td>
<td>53.60</td>
<td>45.20</td>
<td>196.80</td>
<td></td>
</tr>
<tr>
<td>$C_3$ Silty Loamy</td>
<td>0.594</td>
<td>0.950</td>
<td>6.8</td>
<td>54.40</td>
<td>46.80</td>
<td>216.20</td>
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<tr>
<td>$C_4$ Silty Loamy</td>
<td>0.623</td>
<td>1.020</td>
<td>6.6</td>
<td>56.20</td>
<td>44.20</td>
<td>205.70</td>
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<tr>
<td>$C_5$ Silty Loamy</td>
<td>0.583</td>
<td>0.870</td>
<td>6.8</td>
<td>55.10</td>
<td>45.10</td>
<td>220.10</td>
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<tr>
<td>$C_6$ Silty Loamy</td>
<td>0.562</td>
<td>0.760</td>
<td>7.2</td>
<td>55.10</td>
<td>48.80</td>
<td>260.20</td>
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**Table 1(C): Physical properties of different composts prepared for *Allium sativum L.* in the year 2011**

<table>
<thead>
<tr>
<th>Parameter → % of Compost ↓</th>
<th>Texture</th>
<th>Organic Matter (%)</th>
<th>Density (gm cm$^{-3}$)</th>
<th>pH</th>
<th>WHC (%)</th>
<th>Porosity (%)</th>
<th>Conductivity (μ mho cm$^{-1}$)</th>
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<tr>
<td>$C_0$ Loamy</td>
<td>0.743</td>
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<td>6.8</td>
<td>59.80</td>
<td>46.20</td>
<td>196.20</td>
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<tr>
<td>$C_1$ Loamy</td>
<td>0.724</td>
<td>1.163</td>
<td>6.9</td>
<td>57.30</td>
<td>48.30</td>
<td>210.20</td>
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<tr>
<td>$C_2$ Loamy</td>
<td>0.726</td>
<td>1.120</td>
<td>7.2</td>
<td>55.20</td>
<td>50.80</td>
<td>250.20</td>
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</tr>
<tr>
<td>$C_3$ Silty Loamy</td>
<td>0.704</td>
<td>1.086</td>
<td>7.4</td>
<td>53.70</td>
<td>52.10</td>
<td>260.10</td>
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<tr>
<td>$C_4$ Silty Loamy</td>
<td>0.726</td>
<td>1.172</td>
<td>7.2</td>
<td>51.20</td>
<td>51.30</td>
<td>230.80</td>
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<tr>
<td>$C_5$ Silty Loamy</td>
<td>0.704</td>
<td>1.151</td>
<td>7.4</td>
<td>48.10</td>
<td>54.70</td>
<td>250.60</td>
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<tr>
<td>$C_6$ Silty Loamy</td>
<td>0.684</td>
<td>1.132</td>
<td>7.6</td>
<td>48.10</td>
<td>56.20</td>
<td>260.20</td>
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</tbody>
</table>
Table - 2(A): Primary nutrients (in %) in different composts prepared for Allium sativum L. in the year 2009

<table>
<thead>
<tr>
<th>% of Compost</th>
<th>Primary Nutrients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K^{+}</td>
</tr>
<tr>
<td>C_0</td>
<td>0.650</td>
</tr>
<tr>
<td>C_1</td>
<td>0.730</td>
</tr>
<tr>
<td>C_2</td>
<td>0.820</td>
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<tr>
<td>C_3</td>
<td>0.910</td>
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<td>C_4</td>
<td>0.810</td>
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<tr>
<td>C_5</td>
<td>0.805</td>
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<td>C_6</td>
<td>0.790</td>
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</table>

Table - 2(B): Primary nutrients (in %) in different composts prepared for Allium sativum L. in the year 2010

<table>
<thead>
<tr>
<th>% of Compost</th>
<th>Primary Nutrients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K^{+}</td>
</tr>
<tr>
<td>C_0</td>
<td>0.705</td>
</tr>
<tr>
<td>C_1</td>
<td>0.720</td>
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<td>C_2</td>
<td>0.732</td>
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<tr>
<td>C_3</td>
<td>0.751</td>
</tr>
<tr>
<td>C_4</td>
<td>0.742</td>
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<tr>
<td>C_5</td>
<td>0.762</td>
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<tr>
<td>C_6</td>
<td>0.770</td>
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</table>

Table - 2(C): Primary nutrients (in %) in different composts prepared for Allium sativum L. in the year 2011

<table>
<thead>
<tr>
<th>% of Compost</th>
<th>Primary Nutrients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K^{+}</td>
</tr>
<tr>
<td>C_0</td>
<td>0.0820</td>
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<tr>
<td>C_1</td>
<td>0.0852</td>
</tr>
<tr>
<td>C_2</td>
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<tr>
<td>C_3</td>
<td>0.0892</td>
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<td>C_4</td>
<td>0.0872</td>
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<td>C_5</td>
<td>0.0883</td>
</tr>
<tr>
<td>C_6</td>
<td>0.0920</td>
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</table>
### Table 3 (A): Secondary nutrients (in %) in different prepared composts for Allium sativum L. in the year 2009

<table>
<thead>
<tr>
<th>% of Compost</th>
<th>Ca$^{2+}$</th>
<th>Mg$^{2+}$</th>
<th>SO$_4^{2-}$</th>
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</thead>
<tbody>
<tr>
<td>$C_0$</td>
<td>0.80</td>
<td>0.58</td>
<td>0.075</td>
</tr>
<tr>
<td>$C_1$</td>
<td>0.94</td>
<td>0.74</td>
<td>0.081</td>
</tr>
<tr>
<td>$C_2$</td>
<td>1.02</td>
<td>0.80</td>
<td>0.085</td>
</tr>
<tr>
<td>$C_3$</td>
<td>1.12</td>
<td>0.90</td>
<td>0.092</td>
</tr>
<tr>
<td>$C_4$</td>
<td>0.98</td>
<td>0.68</td>
<td>0.071</td>
</tr>
<tr>
<td>$C_5$</td>
<td>0.94</td>
<td>0.59</td>
<td>0.062</td>
</tr>
<tr>
<td>$C_6$</td>
<td>0.92</td>
<td>0.48</td>
<td>0.054</td>
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### Table 3 (B): Secondary nutrients (in %) in different prepared composts for Allium sativum L. in the year 2010

<table>
<thead>
<tr>
<th>% of Compost</th>
<th>Ca$^{2+}$</th>
<th>Mg$^{2+}$</th>
<th>SO$_4^{2-}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_0$</td>
<td>0.58</td>
<td>0.22</td>
<td>0.030</td>
</tr>
<tr>
<td>$C_1$</td>
<td>0.64</td>
<td>0.24</td>
<td>0.040</td>
</tr>
<tr>
<td>$C_2$</td>
<td>0.69</td>
<td>0.28</td>
<td>0.052</td>
</tr>
<tr>
<td>$C_3$</td>
<td>0.78</td>
<td>0.35</td>
<td>0.062</td>
</tr>
<tr>
<td>$C_4$</td>
<td>0.63</td>
<td>0.33</td>
<td>0.058</td>
</tr>
<tr>
<td>$C_5$</td>
<td>0.74</td>
<td>0.44</td>
<td>0.062</td>
</tr>
<tr>
<td>$C_6$</td>
<td>0.78</td>
<td>0.56</td>
<td>0.078</td>
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</tbody>
</table>

### Table 3 (C): Secondary nutrients (in %) in different prepared composts for Allium sativum L. in the year 2011

<table>
<thead>
<tr>
<th>% of Compost</th>
<th>Ca$^{2+}$</th>
<th>Mg$^{2+}$</th>
<th>SO$_4^{2-}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_0$</td>
<td>0.94</td>
<td>0.59</td>
<td>0.100</td>
</tr>
<tr>
<td>$C_1$</td>
<td>0.94</td>
<td>0.70</td>
<td>0.110</td>
</tr>
<tr>
<td>$C_2$</td>
<td>0.98</td>
<td>0.78</td>
<td>0.110</td>
</tr>
<tr>
<td>$C_3$</td>
<td>1.02</td>
<td>0.84</td>
<td>0.120</td>
</tr>
<tr>
<td>$C_4$</td>
<td>0.99</td>
<td>0.75</td>
<td>0.110</td>
</tr>
<tr>
<td>$C_5$</td>
<td>1.01</td>
<td>0.81</td>
<td>0.120</td>
</tr>
<tr>
<td>$C_6$</td>
<td>1.09</td>
<td>0.84</td>
<td>0.130</td>
</tr>
</tbody>
</table>
### Table 4 (A): Micro nutrients (in ppm) in different prepared composts for Allium sativum L. in the year 2009

<table>
<thead>
<tr>
<th>% of Compost</th>
<th>Micro Nutrients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe</td>
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<tr>
<td>C₀</td>
<td>21</td>
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<tr>
<td>C₁</td>
<td>26</td>
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<tr>
<td>C₂</td>
<td>32</td>
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<tr>
<td>C₃</td>
<td>38</td>
</tr>
<tr>
<td>C₄</td>
<td>30</td>
</tr>
<tr>
<td>C₅</td>
<td>30</td>
</tr>
<tr>
<td>C₆</td>
<td>28</td>
</tr>
</tbody>
</table>

### Table 4 (B): Micro nutrients (in ppm) in different prepared composts for Allium sativum L. in the year 2010

<table>
<thead>
<tr>
<th>% of Compost</th>
<th>Micro Nutrients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe</td>
</tr>
<tr>
<td>C₀</td>
<td>6.52</td>
</tr>
<tr>
<td>C₁</td>
<td>6.82</td>
</tr>
<tr>
<td>C₂</td>
<td>6.98</td>
</tr>
<tr>
<td>C₃</td>
<td>7.22</td>
</tr>
<tr>
<td>C₄</td>
<td>7.04</td>
</tr>
<tr>
<td>C₅</td>
<td>7.14</td>
</tr>
<tr>
<td>C₆</td>
<td>7.27</td>
</tr>
</tbody>
</table>

### Table 4 (C): Micro nutrients (in ppm) in different prepared composts for Allium sativum L. in the year 2011

<table>
<thead>
<tr>
<th>% of Compost</th>
<th>Micro Nutrients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe</td>
</tr>
<tr>
<td>C₀</td>
<td>4.6</td>
</tr>
<tr>
<td>C₁</td>
<td>4.9</td>
</tr>
<tr>
<td>C₂</td>
<td>5.3</td>
</tr>
<tr>
<td>C₃</td>
<td>5.8</td>
</tr>
<tr>
<td>C₄</td>
<td>5.2</td>
</tr>
<tr>
<td>C₅</td>
<td>6.0</td>
</tr>
<tr>
<td>C₆</td>
<td>6.4</td>
</tr>
</tbody>
</table>
### Table 5: Heavy metals (in ppm) analysed in the plants obtained from compost giving best results in Allium sativum L. in different years (2009, 2010, 2011)

<table>
<thead>
<tr>
<th>Names of heavy metals</th>
<th>Year 2009</th>
<th>Year 2010</th>
<th>Year 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu (Copper)</td>
<td>7.94</td>
<td>9.98</td>
<td>11.96</td>
</tr>
<tr>
<td>Zn (Zinc)</td>
<td>26.68</td>
<td>28.72</td>
<td>30.74</td>
</tr>
<tr>
<td>Fe (Iron)</td>
<td>136.00</td>
<td>137.00</td>
<td>141.01</td>
</tr>
</tbody>
</table>

### Table 6: Yield of selected plants cultivated (in kg /hc²) in different composts in Allium sativum L. (clove) in different year (2009, 2010, 2011)

<table>
<thead>
<tr>
<th>Year</th>
<th>C₀</th>
<th>C₁</th>
<th>C₂</th>
<th>C₃</th>
<th>C₄</th>
<th>C₅</th>
<th>C₆</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>18700</td>
<td>23600</td>
<td>27700</td>
<td>32800</td>
<td>24500</td>
<td>22500</td>
<td>21600</td>
</tr>
<tr>
<td>2010</td>
<td>25700</td>
<td>27600</td>
<td>32800</td>
<td>37900</td>
<td>31400</td>
<td>29400</td>
<td>28500</td>
</tr>
<tr>
<td>2011</td>
<td>27500</td>
<td>31400</td>
<td>33600</td>
<td>38700</td>
<td>32300</td>
<td>28300</td>
<td>27400</td>
</tr>
</tbody>
</table>

### Table 7: Percentage increase (in kg / m²) in yield of Allium sativum L. cultivated different composts in comparison to reference compost in different year (2009, 2010, 2011)

<table>
<thead>
<tr>
<th>% of composts</th>
<th>% increase in year 2009</th>
<th>% increase in year 2010</th>
<th>% increase in year 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁</td>
<td>26.20</td>
<td>7.39</td>
<td>14.18</td>
</tr>
<tr>
<td>C₂</td>
<td>48.12</td>
<td>27.62</td>
<td>18.54</td>
</tr>
<tr>
<td>C₃</td>
<td>75.39</td>
<td>47.56</td>
<td>26.54</td>
</tr>
<tr>
<td>C₄</td>
<td>31.01</td>
<td>22.27</td>
<td>45.08</td>
</tr>
<tr>
<td>C₅</td>
<td>20.32</td>
<td>14.49</td>
<td>21.81</td>
</tr>
<tr>
<td>C₆</td>
<td>15.51</td>
<td>10.99</td>
<td>18.54</td>
</tr>
</tbody>
</table>

### Table 8A: Sulphur (in %) in different composts prepared for Allium sativum L. in the year 2009

<table>
<thead>
<tr>
<th>Composts Used for cultivation</th>
<th>Sulphur in plant part (%)</th>
<th>Sulphur in produced seed (in mgkg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₀</td>
<td>0.25</td>
<td>11.50</td>
</tr>
<tr>
<td>C₁</td>
<td>0.30</td>
<td>17.96</td>
</tr>
<tr>
<td>C₂</td>
<td>0.35</td>
<td>25.87</td>
</tr>
<tr>
<td>C₃</td>
<td>0.35</td>
<td>24.65</td>
</tr>
<tr>
<td>C₄</td>
<td>0.34</td>
<td>23.45</td>
</tr>
<tr>
<td>C₅</td>
<td>0.33</td>
<td>22.76</td>
</tr>
<tr>
<td>C₆</td>
<td>0.32</td>
<td>21.65</td>
</tr>
</tbody>
</table>
### Table 8B: Sulphur (in %) in different composts prepared for Allium sativum L. in the year 2010

<table>
<thead>
<tr>
<th>Composts Used for cultivation</th>
<th>Sulphur in plant part (%)</th>
<th>Sulphur in produced seed (in mgkg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₀</td>
<td>0.35</td>
<td>25.54</td>
</tr>
<tr>
<td>C₁</td>
<td>0.40</td>
<td>31.98</td>
</tr>
<tr>
<td>C₂</td>
<td>0.45</td>
<td>39.88</td>
</tr>
<tr>
<td>C₃</td>
<td>0.45</td>
<td>38.67</td>
</tr>
<tr>
<td>C₄</td>
<td>0.44</td>
<td>37.47</td>
</tr>
<tr>
<td>C₅</td>
<td>0.43</td>
<td>36.78</td>
</tr>
<tr>
<td>C₆</td>
<td>0.42</td>
<td>35.67</td>
</tr>
</tbody>
</table>

### Table 8C: Sulphur (in %) in different composts prepared for Allium sativum L. in the year 2011

<table>
<thead>
<tr>
<th>Composts Used for cultivation</th>
<th>Sulphur in plant part (%)</th>
<th>Sulphur in produced seed (in mgkg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₀</td>
<td>0.45</td>
<td>32.56</td>
</tr>
<tr>
<td>C₁</td>
<td>0.50</td>
<td>38.96</td>
</tr>
<tr>
<td>C₂</td>
<td>0.55</td>
<td>46.84</td>
</tr>
<tr>
<td>C₃</td>
<td>0.55</td>
<td>45.69</td>
</tr>
<tr>
<td>C₄</td>
<td>0.54</td>
<td>44.49</td>
</tr>
<tr>
<td>C₅</td>
<td>0.53</td>
<td>43.80</td>
</tr>
<tr>
<td>C₆</td>
<td>0.52</td>
<td>42.69</td>
</tr>
</tbody>
</table>
Abstract:
Globalization has paved the way to increased competition and has re-established the fact: “survival of the fittest”. Survival and sustainability emerge as key issues to ponder in a competitive environment. Firms are now concentrating in designing activities and practices that give them additional competitive advantage. Human Resource Management has emerged as one such area with the belief that management of people gives a cutting edge for establishing a competitive advantage for a firm. Effective HR practices result in committed workforce, which can contribute towards firms’ growth. Adequate addressing of employee needs reduces attrition rate and hence dedicated and talented employees are retained, which become an asset for the firm. Retention of talented workforce and designing of good HR practices have greatly assisted organization to achieve competitive advantage over other firms. This paper will highlight few core HR practices undertaken by firms that have resulted in achieving competitive advantage. It will also aim to indicate drivers to achieving competitive advantage through effective HR practices. This study has been conducted in some Organizations that have devised HR practices and have gained positive results.

Economic liberalization and policies of globalization have created a hyper-competitive environment, innovation and fierce competitiveness have raised issues of survival, effectiveness, sustainability, etc. Increasing demand for skilled performers and increasing high attrition of capable workforce forced the companies to shift focus on attracting and retaining high-performing employees in the extremely competitive business environment. Companies have recognized the need to enhance the employee’s opportunity to develop skills and abilities for full performance within the position and for career advancement and growth which would lead to retention of talented workforce in return increase firms performance in terms of profitability and productivity. Companies have realized that in today’s competitive business milieu, the quality of people one employs will make all the difference.

Introduction
People are the assets on which competitive advantage is built, whether in the public or private sector, whether in the corporate world or in the world of education. Human resources are considered the most important asset of an organization, but very few organizations are able to fully harness its potential. Focus on people has become the new mantra for success. The latest theory in human resource management holds that people are an “inimitable” asset. Workforce and their skills are the one thing that competing organisations cannot imitate. Effective and efficient workforce has been shown to enhance company’s performance by contributing to customer satisfaction, innovation, productivity, and development of a favorable reputation of the firm. Therefore a need to retain these talents.

Literature Review
Lately, human resource management has emerged as an essential factor for sustained competitive advantage. Research highlights that organizations develop sustained competitive advantage through management of scare and valuable resources (Barney, 1991). The human resource enables organizations to achieve optimization of resource, effectiveness, and continuous improvement consistently (Wernerfelt, 1984). An organization takes time to nurture and develop human capital in the form of knowledge, skills, abilities, motivation, attitude, and interpersonal relationship, and makes it difficult for competitors to imitate (Becker & Gerhart, 1996).

Pfeffer (1994) stressed that human resource has been vital for firm sustained performance. In knowledge economy, the human resource has been recognized as a strategic tool, essential to organizational profitability and sustainability. This realization has led to the new role of human resource managers as strategic partners in formulation and implementing organizational strategy (Myloni et al., 2004). Organizations are pursuing proactively human resource management (HRM) practices and systems to capitalize on strength of this vital asset for sustained competitive advantage in knowledge economy (Jackson & Schuler, 2000; Mac Duffie,1995).

Huselid (1995) examined HR practices of high performance companies and found that attracting and selecting the right employees increase the employee productivity, boost organizational performance, and contribute in reducing turnover. In meta-analysis of 104 articles, Boselie et al., (2005) concluded that the top four HRM practices are efficient recruitment and selection, training and development, contingency and reward system, and performance management that have been extensively used by different researchers.

Researchers have used financial and non financial metrics to measure organizational performance. The financial measures include profit, sales, and market share. Non-financial measures include productivity, quality, efficiency, and the attitudinal and behavioural measures such as commitment, intention to quit, and satisfaction. (Dyer & Reeves, 1995).
In the changing global market competitiveness has created new challenges for organisations as well as individuals (Schuler 1992, Cascio 1993, Pfeffer 1994, Stavrou-Costeia 2002). For firms to maintain or create competitiveness it is necessary that they build and retain their skilled and talented human resources.

Changing business environment in knowledge economy has made adoption of human resource management (HRM) imperative for competitive advantage. The impact of HRM practices on business performance has been extensively studied in the recent past. Consequently, the role of human resource management becomes strategic and proactive (Brewster & Suutari 2005) to create a more dynamic workplace environments.

Huselid (1995) has identified the link between HRM practices and turnover, productivity and financial performance. Indeed, high performance work practices are likely to have an effect on firm performance. Huselid and Delaney (1996) contended HRM practices, particularly selection and training, are associated with perceived firm performance in profit and nonprofit organisations. Similar results are reported by Goodeham, Ringdal, and Parry (2006) who found a positive impact of HRM practices on firm performance. In a study in Greece, Katou and Bedhwar (2006) found that HRM practices of staffing, training and promotion, involvement of employees, incentives, and safety and health have positive relationship with firms’ performance. In a study in Taiwanese high technology firms, Chang and Chen (2002) established that HRM practices of workforce planning, training and development, benefits, teamwork, and performance appraisal significantly affected productivity. In another study Sing (2003) found that strategic use of HRM practices positively affect performance of organizations in India. In Korea, Bae and Lawler (2000) concluded that HRM practices significantly affect organizational performance. Lee and Lee (2007) established that workforce planning, teamwork, training development, compensation and incentives, performance appraisal, and employees’ security are important HRM dimensions that affect productivity, product quality, and business performance.

It is argued that HRM practices enhance employees’ competency and motivation that affect organizational performance. (Harel & Tzafrir, 1996) contended that HRM practices based on quality hiring, development, and retention boosts firms’ capability. Researchers (Chiu et. al., 2002; Tepstra & Rozell, 1993) have established that HRM practices of extensive recruitment and selection, training and development, and compensation systems have positive association with firms’ performance. 

Lam and White (1998) established that effective recruitment, competitive compensation, and efficient training and development have relationship with financial dimensions of performance Green et al., (2006) concluded that integrated approach to HR practices exhibited satisfied and committed employees who demonstrated remarkable individual and team performance.

Studies established that HRM practices aimed at acquisition and development of employees is an essential investment that develops valuable and rare human assets. (Becker & Huselid, 1998). Delaney and Huselid (1996) confirmed that selective staffing; compensation and incentive, and training had positive influence on performance of organization.

Strong evidence exists in literature about different HRM practices and their effects on superior firms’ performance. Researchers found a positive relationship between effective recruitment and selection practices and top-class performance (Harel & Tzafrir, 1996; Delany & Huselid, 1996); training and development (Bartel, 1994; Fey et al., 2000); compensation and reward (Chie et al., 2002; Batt, 2002); performance appraisal (Boselie et al., 2001, Bjorkman & Xiucheng, 2002); employee relations (Kuo, 2004).

HRM practitioners are striving to meet the emerging challenges of new values of knowledge workers who have necessitated a new paradigm of peoples’ management characterized by heavy investment in human capital and innovative use of HRM practices for attraction and retention of talents for organizational sustainability.

Increasingly, firms are considering the adoption of new work practices, such as problem-solving teams, enhanced communication with workers, employment security, flexibility in job assignments, training workers for multiple jobs, and greater reliance on incentive pay.

Performance-based compensation is the dominant HR practice that firms use to evaluate and reward employees’ efforts (Collins and Clark, 2003). Evidently, performance-based compensation has a positive effect upon employee and organizational performance). Several studies identified self-managed teams and decentralization as important high-performance.
According to Lado and Wilson (1994, p. 701) human resource system are a set of distinct but interrelated activities, functions, and processes that are directed at attracting, developing, and maintaining (or disposing of) a firm’s human resources.

Today more and more companies are interested in using intangible assets and human capital as a way to gain an advantage over competitors. A company’s value includes three types of assets that are critical for the company to provide goods and services: financial assets (cash and securities), physical assets (property, plant, equipment), and intangible assets. Intangible assets include human capital, customer capital, social capital, and intellectual capital. Intangible assets are equally or even more valuable than financial and physical assets but they are difficult to duplicate or imitate. The source of value in a company is in intangible assets and even more human intangible assets have been shown to be responsible for a company’s competitive advantage.

**Competitive Advantage**

Competitive advantage is defined as a condition which enables a firm to operate in a more efficient or otherwise higher quality manner than its competitors, and which results in benefits accruing. Competitive advantages usually originate in a core competency. A company’s core competency is the one thing that a company can do better than its competitors. A competitive advantage can entail a variety of company characteristics; for example, customer focus, brand equity, product quality, research and Development focus, workforce capabilities and innovation. Competitive advantage is simply defined as anything that gives an organization an edge over the competitors in its market. To be effective a competitive advantage must be: Difficult to mimic., Applicable to multiple situations, Unique, Sustainable and Superior to the competition (Porter, 1998).

A competitive advantage is created when a firm implements a strategy that increases its value whereas the competitors do not implement this strategy. A sustainable competitive advantage is a competitive advantage when other firms are not able to duplicate this strategy. Firm resources can be imperfectly imitable for one or a combination of three reasons:

1. The ability of a firm to obtain a resource is dependent upon unique historical conditions.
2. The link between the resources possessed by a firm and firm’s sustained competitive advantage is causally ambiguous.
3. The resource generating a firm advantage is socially complex.

An organization requires the utilization of a complex array of resources to grow, survive and achieve the ultimate mission or objectives for its existence. The mobilization and deployment of the resources such as human, financial and material – in the right resource-mix, gives the organization leverage toward the desired end. Of these resources, the human resource is the most potent and central, contributing significantly to corporate bottom line and competitiveness. The organization therefore gains sustained competitive advantage through people, the organization workforce.

The Resource-Based-View holds the view that sustainable competitive advantage results from implementing strategies that exploit their internal strengths, through responding to environmental opportunities, while neutralizing external threats and avoiding internal weaknesses.

The resource-based perspective offers that human resources can be defined as the pool of human capital under the firm’s control in a direct employment relationship. HRM can be valued not only for its role in implementing a given competitive scenario but for its role in generating strategic capability for its potential to create firms which are more intelligent and flexible than their competitors over the firms which exhibit superior levels of co-operation and operation.

By hiring and developing talented staff and synergizing their contribution within the resource bundle of the firm. HRM may lay the basis for sustained competitive advantage. The resource-based view of the firm, quite clearly, provides a basis for asserting that key human resources are sources of competitive advantage. It helps to lay the intellectual basis for capabilities. HR practices offers advantages because it forces us to think more clearly about the quality of the workforce skills at various levels and the quality of the motivation climate to be created and maintained to keep the workforce happy and satisfied. Competitive advantage can best be achieved by seeking improvement in the better utilization of human resources.
According to Porter (1985), the unique talents among employees, including flexibility, innovation, superior performance, high productivity and personal customer service are ways employees provide a critical ingredient in developing a firm’s competitive position.

**Human Resources Practices**

A firm’s HRM refers to the policies, practices, and systems that influence employees’ behaviour, attitudes, and performance. It is sometimes referred to as involving “people practices”. HRM practices include analyzing and designing work, determining human resource needs, attracting potential employees, choosing employees, teaching them how to perform their jobs and preparing them for the future, rewarding employees, evaluating their performance and creating a positive work environment. It can also be explained as a practice that is devised to increase the competence, commitment and improve the culture. Johnson (2000) details, best practices or high performance work practices are described as HR methods and systems that have universal, additive, and positive effects on organizational performance. These practices maximize their influence on Company’s performance through increased customer satisfaction, productivity, innovation and development of favourable reputation, HR planning, Recruitment & Selection, Training and development, compensation, Performance management and employee relations are some such practices.

Why are employees in some companies happy to stick with the company while others look for a change? The reason is that some companies know how to take good care of their employees and provide a working environment that helps them retain their identity, while proving themselves and growing along with the company.

HR practices encourage the growth of the organization as employees after all play a major role in the well-being of a company they deal with enhancing competencies, commitment and culture building.

Pfeffer (1998) proposed HR practices which according to the literature, can be expected to influence the firm’s performance. In his seminal work, Pfeffer (1998) proposed the following seven HRM practices:

1. Employment Security
2. Selective Hiring,
3. Self-managed teams and Decentralization of Decision making
4. Comparatively high compensation contingent on Organizational performance,
5. Extensive Training,
6. Reduced status distinctions and barriers, including dress, language, office arrangements, and wage differences across levels, and
7. Extensive sharing of financial and performance information throughout the organization.

A number of contemporary and good HR practices have resulted in effectively managing human resources, managing attrition problems and keeping them happy. Some of the best HR practices that are worth mentioning that have helped organizations in the creation of a highly satisfied and motivated work force may be identified as:

Selectively Hiring- practice can ensure that the right people, with the desirable characteristics and knowledge, are in the right place.

**Training and Development**- may be related to firm performance in many ways. Firstly, training programmes increase the firm specificity of employee skills, which, in turn, increases employee productivity and reduces job dissatisfaction that results in decrease in employee turnover

**Job Security**- creates a climate of confidence among employees which cultivates their commitment on the company's workforce. This develops a feeling of confidence and employees commit themselves to expend extra effort for the company’s benefit.

**Flexi-time**- Flexi-time is a practice, wherein an organisation gives its employees the opportunity of a flexible working hours arrangement.
**Performance-linked Incentives and Bonus**- Every good performance is appreciated in the form of a pat on the back, bonuses or giving some other compensation for a job well done. The incentives can be implemented at the individual as well as the team level and it has been seen that this works wonders in getting the best out of the employees and boosts their morale.

**Performance Evaluation**- A good evaluation and a fair evaluation system links the employees’ performance to the business goals and the priorities of an organization.

Highlight Performers- High performers get better results, faster and closer to the targets. Organizations create profiles of their top performers and make them visible through their intranet, display boards etc.

**Open House Discussions & Rewarding Ideas**- Employees are the biggest source of ideas. Successful organizations nurture ideas and they understand that employees who are actually working and know the business can provide the best ideas. Through this system, talented employees are identified and developed. DLF and Infosys have implemented the 1st Teams (Ingenious, Idea, Incubation & Implementation).

**Rewards and Recognition**- recognition of talent is highly important, the primary goal of rewards and recognition is to improve performance of employees. Non-monetary recognition can be very motivating, helping to build feelings of confidence and satisfaction. Another important goal is increased employee retention and encourages others to give their best.

**Coaching and Mentoring** - Organisations use coaching and mentoring to train managers to address specific skill deficits, enhance performance and grow into expanded leadership roles.

**Cross-Functional Training**- Organisations foster and harness talent by imparting in-house cross functional training to make their professionals multi-skilled and multitalented. Many organizations encourage their employees to be multi-skilled and add value to other functional aspects as well.

**Employees Assistance Programmes**- These programmes are offered by many organizations in the corporate sector for the employees’ benefit. Every employee is challenged by personal problems and concerns. The EAPs are intended to help employees deal with their personal problems that might adversely impact their work performance.

**Clearly Defined and Measurable KRAs**- KRA is an acronym for ‘Key Results Area’. Knowledge Sharing - Knowledge sharing sessions are periodically organized by the organizations to widen knowledge base of their employees and improve their effectiveness.

**Work Environment**- A safe and happy workplace makes the employees feel good about being there. Each one is given importance and provided the security that gives them the motivation and incentive to stay.

Providing equal employment opportunities for all, value of trained human resource, providing flexibility to employees specially to women at various life stages, sabbatical from corporate life, and extended maternity leave promoting practice of entrepreneurship by employees within the organization. Human Resource Management has taken a leading role in encouraging CSR activities at all levels. Companies like Wipro inculcate CSR values amongst its workforce right at the beginning during the induction process.

The focus and attention of HR practices today are on creating HR policies based on trust, openness, equity and consensus .Creating conditions in which people are willing to work with zeal, initiative and enthusiasm; make people feel like winners. Fair treatment of people and prompting redressal of grievances would pave the way for healthy work-place relations, prepare workers to accept technological changes by clarifying doubts.

**Research Methodology**

The present study investigates HR practices namely; training and development, recruitment and selection, compensation and reward, performance appraisal, performance linked incentives , highlighting good performance and rewarding suggestions and ideas understanding the effects of these practices on retention of workforce to achieve competitive advantage.

The researchers collected data through Primary and Secondary methods.

Primary data was collected through distribution of questionnaire to employees of private companies in the city of Lucknow.
The questionnaire contained 10 questions, each question were divided into five sub parts. The answers were mostly close ended. The last question was open ended seeking suggestions and HR practices that they would like their organisations to implement.

The objective is to understand the HR practices in the private companies in the city.

Also understand how these HR practices are effective in retention of talented workforce and HR issues leading to competitive advantage.

Analysis and Findings
The collected data has undergone a descriptive understanding and study by the researchers in identifying the critical areas and how firms can achieve competitive advantage through people resource.

HR Practices Identified
The respondents were asked to highlight the HR practices being followed in their organizations. 95% of the respondent ticked Selective hiring, training and development, knowledge sharing, fair performance evaluation as HR practices being followed in their organisations. 15% of the respondent also ticked job security and performance linked rewards and incentives as being practiced along with the above mentioned HR practices.

Coaching and mentoring as a practice in their organization got the least rank.

Selective Hiring
The respondents were also asked to rank these practices in order of importance given to them by the organisations to each of them. 85% of the respondent agreed that Selective hiring was given most importance by their employers. Selectivity in recruitment is an important practice and is undertaken by almost every organization. This perhaps is the first step in achieving edge over others. Organisation follow the approach of selecting the right person with the right attitude and personality make up to provide the right kind of abilities and capabilities to take the organization toward higher growth. This is an aspect of talent retention as it implies matching a person's inherent skills, traits and personality to the jobs. Moreover when employees go through a rigorous selection process, they feel that their knowledge, experience, abilities and capabilities have got them the jobs in the organization. They feel valuable and high responsibility for performance is created, and the message is that person matters. They are selected as they possess the desire to succeed and have the capacity for growth, learning and develop skills and capabilities beneficial to the organization.

Training and Development
60% rated training and development as next important activity followed by knowledge sharing and job security in the order. However all the respondent agreed that training was extremely essential for their growth and development. 92% respondent that training should be need based and specific to the employees need and requirements. Worker autonomy, self managed teams, and even a high salaries are practices that equip and provide employees to make changes and improvements in products and processes. However, training will produce good results only if the trained workers are permitted to employ their skills. If employees are to be given more information about operations, they need skills in employing that information to diagnose problems and suggest improvements. Training is an attempt to improve current or future employee performance by increasing an employee’s attitude or increasing their capabilities. Training provides the opportunity to raise the profile development activities in the organization. Training increases the commitment levels of employees and reduces the perceived growth inequality. Development reduces the turnover rate of employees provides advancement opportunities and also reduces absenteeism.

Inviting Ideas and Suggestions
96% of the respondent agreed that their organisations invited ideas from employees. However 73% of the respondents stated that only sometimes their suggestions and ideas were rewarded it was not a regular practice. 18% felt that their ideas were never rewarded even when they are implemented and resulted in achieving higher performance. 9% of respondent replied that that they were mostly rewarded in non monetary forms. 85% of the respondents reported that when their suggestions and ideas were accepted they felt more committed and motivated to accomplish the tasks given to them. They felt satisfaction towards their employers. They stated their intention to remain in their present jobs. This develops participation in employees and they readily assume responsibility for completing tasks with utmost precision and accuracy. Sharing and seeking information is necessary precondition to encouraging the decision making amongst the employees. Seeking ideas and suggestions develops ownership and citizenship, commitment and loyalty amongst employees. These are aspects to retention of talented workforce.
Rewarding Performance
99% of the respondents agreed that good performance should be highlighted as it is motivating and leads to better performance. People are motivated by recognition and fair treatment. Higher the motivation higher will be the performance. When this performance is clubbed with adequate rewards employees are further motivated to perform. Such practices induce other employees to traverse on the same route of performance and reward, taking their firm on a path of growth and gaining competitive advantage. This dictates that people are responsible for enhanced levels of performance and profitability. However rewarding performance should be fair and equitable based on and linked to the performance of an employee.

Employment Security
Signals a long-standing commitment by the organization to its work force. Increased trust between the organization and its employees helps keep and attract talent and enhances employee empowerment. It contributes to training and employer make greater investments in training. One concern managers sometimes express is that guaranteed employment will foster a — civil service mentality and a lack of emphasis on performance. Security and reliance on the workforce for competitive success mean that one must be careful to choose the right people, in the right way therefore selective hiring.

Intention To Stay
Intention to remain in their present job 80% of the respondent agreed that if their jobs contained potential for growth and career advancement they would not quit. They showed a preference towards performance linked incentives adding towards their intention to stay. 20% of the respondent agreed upon work environment as another aspect for retention. Organisations need to cater to retention tools such career advancement, growth and salaries for gaining competitive advantage. Career advancements and growth are factors that develop job satisfaction and have been rated highly by employees. A satisfied employees tends to be more committed and involved with his assignments adding to the growth of the firm.

In the open ended part of the questions the respondent showed their preference for HR practices such as Flexi timing, Open Door Policy, Discussion with the Boss, Entertainment Activities, Open Book Management Style, Delighting Employees with the Unexpected. The women employees indicated their preference towards flexitiming and equality at workplace.

Conclusion
To perform well under highly competitive conditions and gain competitive advantage is perhaps the greatest single challenge for the firms, how best to develop human resources in a way that will enable them to out-perform other firms. Successful companies in both manufacturing and service sectors have achieved (and more to the point, they attribute) their growth through the ways in which they managed to organize and work with their people to produce competitive advantage.

For achieving competitive advantage it is necessary that employees who are good performers and exhibit potential for even better performance will be assets to the firm. They will be instrumental in not only achieving higher goals and objectives at individual levels but will also induce their fellow colleagues to follow in their path. To maintain this attitude to is necessary for the firms to devise means and ways to keep the employees satisfied and committed. Here begins the role of HR practices for retention of their talent as means of achieving competitive advantage.

Prefer has issued sixteen practices of competitive advantage through people such as:- Employment Security, Incentive Pay , Participation and Empowerment, Selectivity In Recruiting, Symbolic Egalitarianism, Long-term Perspective, Employee Ownership, Teams And Job Redesign, Wage Compression, Measurement Of Practices, Cross-utilization & Cross-training, High Wage, Information Sharing, Training And Skill Development, Promotion From Within and Overarching Philosophy.

The only worry about achieving competitive advantage via people is that it inevitably takes time to accomplish. But once achieved, competitive advantage obtained through the above practices is likely to be more enduring and more difficult to duplicate. Best practices can also be defined as the most efficient (least amount of effort) and effective (best results) way of accomplishing a task, based on repeatable procedures that have proven themselves over time.

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TALENT MANAGEMENT DEVELOPMENT: ANALYTICAL APPROACH

Abstract:
Talent Management (TM) has attracted increasing attention from academics and practitioners in recent years, but there are many gaps and omissions left for further theoretical and empirical development. In all organizations, there are many driving and restraining forces influencing the talent management process, which make a challenging field around it. This study investigated driving and restraining forces in execution of talent management in Pars Company. We have documented top managers’ experiences in this regard. Our study results include some new cultural factors in Iranian organizations such as dishonesty in information expression, mistrust among employees and jealousy attitudes towards colleagues besides factors such as lack of support of top management, and cost-benefit analysis and lack of motivation among employees, which all restrict a succession plan progress. Needless to say, there are also many favorable forces for this plan’s success such as improvement of managers’ level of knowledge and human resources development which will be explained in this article.

Introduction
A more symmetric view of human behavior is provided by ecological psychology which prompts the question: what adaptive purposes underlie human performance and its development? (See Gibson & Pick, 2000). Many managers have learned that one of the most important characteristics of flourishing organizations is their ability to identification, development and effective employment of persons who are likely to be future leaders (Barner, 2006). Such leaders are able to understand organization’s strategic goals and to reach them (Romejko, 2008). Thus, in some organizations, as their top managers believe that talent management guarantees their future successes, some sorts of talent management are considered in order to ensure continuity of successful leadership (Haworth, 2005).

Although at its simplest definition talent management is defined as “determining future leaders”, in fact, it is a voluntary process for ensuring continuity of leadership in key positions, maintaining and developing knowledge capital for future, and encouraging people to develop, and arises from strategic human resource planning of the organization (Hellton & Jackson, 2007).

In this systematic process, personal and professional development are combined with strategy, and result in the organization’s preparedness to occupy any key position with proper person and in proper period of time as soon as it is vacated (Christie, 2005). In this process, first, necessary competencies are identified, and then, talent resource pool is obtained in order to ensure continuity of leadership for key positions (Hellton & Jackson, 2007). This way, the organization is sure that for filling any important roles within it, some capable employees are gradually recruiting and developed.

Given new work methodologies and their fast changing behavior, in addition to multigenerational labor force and lack of human talent in today world, human capital market is encountering some new challenges which did not exist in previous decades. This situation has made talent management very serious for today’s organizations. Actually, this kind of planning is a tool which fulfills the organization’s future needs, provides some candidates for key positions, concentrates on these candidates’ improvement of competencies and skills, and prepares individuals for leadership.

Talent management as a strategy provides organizations with leaders who have the ability to transfer and distribute knowledge within the organization, and reduces probability of knowledge loss due to retirement or turnover of organization’s employees (Hellton & Jackson, 2007). Talent management should be designed so that it covers all important situations of the organization in the way through development of job promotion candidate responsibilities. Organizations are committed to cultivate their internal talents through their employee development. The process should be managed in a way that employees feel they are developing and they will be awarded for this development. This way, finding a suitable person with suitable skills in a suitable period of time within the organization is probable. In addition, people in the organization believe that top managers have not occupied top positions over night, and they have been carefully trained during years of experience.

We should be cautious about differences between talent management and replacement planning. The main goal of replacement planning is to decrease the risk of unplanned missing of some of the organization’s top managers. In other words, replacement planning initiates some activities which limit the damage of unpredicted events. However, talent management is a proactive planning and tries to facilitate continuity of leadership through talent cultivation and planned activities (Kim, 2006).

Identifying and developing talented people consist of a formal talent management system which identifies talented employees and cultivate them through their playing leadership roles in future. This approach emphasizes cultivating of a group of capable individuals for leadership in order to ensure that strategic system will lead to the organization’s strategic goals. While formal succession management aims to prepare a systematic plan for finding potential capabilities and conducting talented people to assigned goals, informal succession management insists on the necessity of growth opportunities development (Jerusalem, 2008).
Force Field Analysis

Levin (1951) considered force field analysis to explain movement from actual situation to ideal situation. This theory highlights driving and restraining forces in front of any change. According to force field analysis, driving forces are willing to facilitate the change, while restraining forces act in a way that decrease and limit driving forces (Baulcomb, 2003). Therefore, three conditions of force field are assumable (see table 1).

Table 1: Definition of different force field conditions (Cronshaw & McCulloch, 2008)

<table>
<thead>
<tr>
<th>No.</th>
<th>Definition of environment situation</th>
<th>Field condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Resources availability, Strategic opportunities which facilitate organization’s internal and external abilities. Organization should replace current situation with the ideal one.</td>
<td>Driving</td>
</tr>
<tr>
<td>2</td>
<td>Limitation in resources, processes, structures and organizations abilities. These limitations restrict organization from moving to ideal situation.</td>
<td>Limiting</td>
</tr>
<tr>
<td>3</td>
<td>Structures, processes, custom, resistance and organization’s limited internal and external abilities are all against the change. All these unfavorable forces restrict the organization’s capabilities and keep it in current situation.</td>
<td>Restraining</td>
</tr>
</tbody>
</table>

Before talent management implementation, the organization is at equilibrium point and by talent management hope to move to point “b”. Some internal and external forces facilitate this movement. However, some forces which are against talent management resist organization movement to point “b” (See Fig. 1).

![Fig. 1: force field presentation of talent management](image)

Smooth movement to point “b” requires some driving forces which are stronger that restraining ones. This movement will happen in case of increase in favorable forces for talent management or decrease in unfavorable ones. In this research, we have tried to identify driving and restraining forces in talent management force field. Therefore, research questions are as follows:

1- What are driving factors for talent management in Iran Pars Company?  
2- What are restraining factors for talent management in Iran Pars Company?

Driving Factors of Talent management

There are some different factors which facilitate talent management in organizations. We have studied six driving factors which act in favor of talent management and facilitate it:
a) Top management support: Support from top levels of the organization will result in other levels motivation. This support will ensure employees that the management is determined to implement talent management within the organization.

b) Systematic approach: The organization should enjoy regular and formal processes for succession plans development throughout the organization with attention to its subsystems and their relationships.

c) Specific development programs: Individuals should believe that they can be prepared for future leadership, if they participate in developments programs. Three steps may be considered for such programs:

Step 1: A team of inexperienced participants who are capable for future is selected. In this step some kinds of general skills are taught to the participants.

Step 2: Trained individuals experience some important positions in the real world.

Step 3: These persons occupy key positions because they have been trained for some higher level positions.

d) Organizational culture: While the organization does not enjoy a suitable environment for growth, future leaders will not be cultivated within it. In talent management, learning knowledge and skills and over estimation of organizations’ abilities should be emphasized. In this process cultural capacity of the organization should be considered by the trainees.

e) Emphasis on quality improvement: Talent management highlights the importance of quality in order to facilitate individuals’ growth to higher positions. Succession programs should prepare employees for higher positions, and build up required competencies within them.

f) Emphasis on seniority: Mentors and coaches usually provide people with some advices for solving personal, organizational and political challenges. Seniority will take place when genuine newcomers believe in an insightful senior person. An insightful person is someone who has the ability to make effective communications with others. These seniors are usually selected by followers. Thus, although some organizations support formal seniority, it usually happens in an informal manner (Rothwell, 2005).

Restraint Factors of Talent management

Managers and supervisors may encounter some restraining forces which are made by some middle managers. These forces prevent succession plans from being effective and influence talent management and management adversely. We have studied six of such factors in this research:

a) Loss of support: If top managers are not willing to support talent management systematic approach, it cannot be successful.

b) Organization’s policies: Organizations’ policies may be arranged to develop only managers’ friends or colleagues instead of all employees. Such policies may significantly damage the level of employees’ performance and talents.

c) Quick-Fix attitudes: Traditional approach toward talent management may encourage quick-fix behaviors. Talent management effectiveness should not be sacrificed for hurry. Future leaders cannot be cultivated overnight; instead, they should be trained during the time.

d) Late return: Top managers cannot often observe fast direct results of talent management advantages. Human resource managers are willing to design and implement various succession programs. Top managers, however, fire HR managers because they cannot see any quick return of such programs. Thus, we need to clarify the talent management advantages to the organization at first.

e) Fast organizational changes: Traditional replacement planning was working in stable organization and environments. However, today managers need to go beyond simple traditional methods. Using talent management software may result in higher organizational capabilities which will synchronize the organization with employees’ needs and changes.

f) Useless administrative protocols: Organizations’ top managers often do not tolerate useless administrative protocols and formalities. Therefore, talent management should be kept from such useless protocols.

In fact, talent management is not a simple process to plan and implement. There are some restrictions which may slow down or even destroy the process, if they are not identified and analyzed carefully. These unfavorable factors depend on organizations’ cultural, strategic and economic situations (Mandi, 2008).

Research Methodology

In this research, we used content analysis, a qualitative research method, for analyzing research data. Usually, sample size for interview depends on our purposes of study (Kvale, 1996). In this research, statistical population was
Pars Company’s employees and 13 persons were selected for interview out of the company’s managers using snowball method. Each person was interviewed based on their access to information and after thirteenth interview, saturation was reached; thus, other interviews were cancelled. In samples selection process, we considered some factors including time, availability of interviewees, and their cooperation with our team, most important of which was their access level to information. To select the samples, we used random sampling method.

For data gathering, at first, three test interviews were done and then, research questions were designed. In order for interviewees to get familiar with principles of talent management, some general aspects of the topic were presented for them, just before the interview began. Each interview took about two hours and we had to write notes of interviews points, due to interviewees’ disapproval of our recording voice.

We used content analysis in order to analyze the research data which was gathered out of the interviews. For content analysis, all interviews were systematically reviewed and analyzed and their key concepts and categories were extracted.

**Research Findings**

At first, all interviews were reviewed and analyzed; then, using primary coding, a set of concepts were retrieved and to find suitable categories, different concepts sets were analyzed and each of them was assigned to its related category. Finally, categories were divided into two main factors: driving factors for talent management and restraining factors for talent management. Knowledge richness or knowledge experience, managers support, individuals’ interest and willingness, are considered to be driving factors and loss of job security feeling, dishonesty in information presentation, expense viewpoint, bureaucratization and jealousy attitudes are considered to be restraining factors.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Category</th>
<th>Concept</th>
<th>Codes</th>
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<tbody>
<tr>
<td>Knowledge richness or knowledge experience</td>
<td></td>
<td>Evaluation oriented vs. experience oriented</td>
<td>5, {8, M9}, {7, M8}, {7, M5}, {3, M2}, {2, M1}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Promotion based on competencies</td>
<td>{5, M13}, {3, M12}, {M11}</td>
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<tr>
<td></td>
<td></td>
<td>Importance of employee’s performance in job rotation</td>
<td>{4, M8}, {6, M7}, {5, M6}, {2, M4}, {5, M1}</td>
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<tr>
<td>Managers support</td>
<td></td>
<td>Support of talent management itself</td>
<td>{9, {5, M9}, {4, M5}, {6, M4}, {4, M3}, {7, M2}}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Give opportunity to employees</td>
<td>{5, M12}, {8, M11}, {M10}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Support of internal promotion within the organization</td>
<td>{4, M13}, {3, M9}, {7, M7}, {4, M4}, {7, M1}</td>
</tr>
<tr>
<td>Willingness and interest</td>
<td></td>
<td>Interest for being manager</td>
<td>{2, 6, M11}, {3, M10}, {2, M7}, {5, M3}, {8, M6}</td>
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<tr>
<td></td>
<td></td>
<td>Willingness to take responsibility</td>
<td>{5, M13}, {12, M6}, {6, M5}, {11, M3}</td>
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<tr>
<td>Organizational commitment</td>
<td></td>
<td></td>
<td>{5, M10}, {9, M6}, {10, M3}, {12, M1}</td>
</tr>
<tr>
<td>Attention to future of organization</td>
<td></td>
<td></td>
<td>{1, M13}, {9, M12}, {4, M11}, {2, M6}, {7, M3}</td>
</tr>
<tr>
<td>Loss of job security feeling</td>
<td></td>
<td>Fear of missing one’s job</td>
<td>{9, {8, M5}, {10, M4}, {3, M3}, {10, M2}}, {11, M1}</td>
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<td></td>
<td></td>
<td>Feeling of being useless</td>
<td>{10, {12, M9}, {12, M8}, {11, M7}}, {13, M6}, {M5}</td>
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<td></td>
<td></td>
<td></td>
<td>{7, M13}, {9, M11}, {M10}</td>
</tr>
<tr>
<td>Dishonesty in information</td>
<td></td>
<td>Practical science</td>
<td>{11, {18, M8}, {14, M8}, {2, M5}}, {5, M2}, {3, M1}</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>{11, M11}, {M10}</td>
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Knowledge richness or knowledge experience: Although many organizations engage in talent management, all of these plans are not successful. The aim of talent management is to make some individuals more capable so that they can be employed as one of top level managers in future. After selection of these potential successors, succession plan will be prepared and the selected individuals will be cultivated based on their capabilities during a specific period of time. An example of this point is mentioned by one of interviewees:

We can’t say that every organization is able to do talent management. For example, when we see that a person whose position is near to managerial position is selected as the next manager without any skill or competency, how can we design and implement such a meritocratic based planning system? (5, M6)

Of course, success of such plans and regular evaluation of these potential successors need time and budget. Therefore, taking care in the selection process is vital. Knowledge richness or knowledge experience category concerns that in selection process, candidates’ level of knowledge and skills should be considered more important that seniority or job experience. Also, job promotions should be designed based on competencies. This means that an employee has cultivated himself more than what he has required in that position. The organization’s employees should be ensured that they will be promoted based on their competencies. This principle should be followed by organizations when they are considering any promotion and replacement.

Manager’s support: any program in the organization needs managers approval to become effective. Top management support and cooperation should be completely clear. Their personal cooperation ensures employees that organization management team members are spending their time and energy on talent management development (Rothwell, 2005). An example of this issue is stated in one of our interviews:

In my opinion, the first thing that an organization needs to start talent management is its managers and all influential people in it pay attention to talent management importance and approve it as a whole (5, M12).

Top managers support is considered to be a vital basis for talent management effectiveness (Smilansky, 2007). In other word, while top managers do not support the program, success of it is not probable. With opportunities they create for their employees, managers can provide the organization with a valuable basis for talent management. Giving such opportunities is a reason for supporting systematic talent management within the organization.

Individual’s willingness and interest: one of important factors which affects success of the planning is the attractiveness of the next job for its potential candidates and their willing to occupy that position. According to expectancy theory, value of a goal and interest to reach it is one of factors which influence behavior towards reaching that goal (Robins & Judge, 2007). An example of this point is mentioned by one of the interviewees:

Always it is said that if management is corrected, all the things will be corrected. In Iran, managers are great losers. They are under pressure from two sides: from top managers and from employees. Such positions are very stressful and require special persons with specific attitudes. As long as we don’t have such persons within our organizations, talking about talent management is useless (6, M5).

<table>
<thead>
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<th>Description</th>
<th>Examples</th>
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Talent management process should be designed in a way that individuals who are interested in managerial positions are identified and selected through the process. Thus in “Willingness and Interest” category, the organization should search for individuals who are ready for growth in order to be able to reach its goals (Metz, 1998). Employees are often the best resource of information about themselves, their interests and motives. For extracting such information, some systems are required which are simple and easily applicable.

**Loss of job security feeling:** this category is less considered by other countries’ researchers. Different organizational situation may result in this attitude. An example of this point is pinpointed in one of the interviews:

> It is our culture that we say don’t teach anything to anybody; he may replace you in future. For example, when we wanted to implement information management systems, people, afraid of their replacement by machines, were resisting it (13, M6).

Unfavorable feeling of missing ones job is a signal of their ambiguity of their situation after leaving the company. It seems that unemployment insurances and clients’ responsibilities toward employees in western countries have resulted in less attention to this category in those countries. However, in this research, we encountered this point many times. The interviews present an important point that as long as organizations do not ensure their employees that talent management will not lead to their firing from the organization, they should not expect a considerable success from the process. In some interviews, interviewees claimed that the organization needs managers’ information and experience and will keep them while nobody else has those resources of seniority. This means that delivering experience and knowledge to others will increase the probability that the organization does not need to someone any more.

**Dishonesty in information presentation:** Any activity requires foundations which base on their truth reaches the considered goal. Talent management is a procedure that long term survival of the organization is one of its main aims. In this way, the gathered information from individuals and units which are supposed to be true and reliable would be the foundations of planning. This argues that if the environment of developing information truth doesn’t exist in the organization, the confident planning wouldn’t be possible. In other words, it would be considered as an obstacle that causes many problems in talent management. Following is an example of this issue in the interviewees’ responses:

> You know, I can’t say that someone is not suitable for a job. When you see he’s got a degree you can’t say that he’s not knowledgeable and if say so, you would never be able to prove it! You have to keep some people in a position anyway, no matter if they are completely ignorant! (19, M11).

Interviewees are kind of unconfident about the people’s true knowledge and rather emphasize on the importance of real science. Bearing in mind that the organization can plan succession when enjoys enough worthy staff. These interviewees had some doubts about the practicality of some people’s knowledge and considered this as an important concern. Individuals’ incapability in practicing their knowledge would be a significant problem in execution of talent management. Stressing on the importance of information correctness, the individuals’ dishonesty in information presenting indicates that individuals’ knowledge is useful for the organization when they put in to practice in the direction of organization’s aims.

**Expense viewpoint:** Senior managers not always can realize the importance of the succession plan. Human resources managers try to convince these managers, but this try ends up with their own elimination in some cases (Rothwell, 2005). Below is an example of the interviewees’ responses:

> As a result of unawareness, managers would find such a plan only an expense when they don’t know its obvious importance and benefit in short term. On the other hand, it contradicts with what’s common in private companies and this means an expense without receiving any benefit. (17, M1)

The majority of the interviewees somehow imply that organizations consider cost as a crucial basis for decision making. These people pessimistically find the profit or cost the main basis of the decisions. Nevertheless, these companies are obliged to finance their budgets and have to consider the cost and profit of the projects in some levels. Rothwell believes that most of the managers wouldn’t agree to execute a plan unless they find it profitable or realize its effect in the short run (Rothwell, 2005).

In general, cost viewpoint can be considered as a significant barrier in talent management, since most of the human resources development plans don’t put in the organizations’ agenda as those plans are expenses and aren’t profitable for the companies.

**Bureaucratization:** Bureaucratization is also an issue which considers as a serious obstacle in talent management. If the staff growth face with a barrier like bureaucracy, it would simply broke. Succession plan should be designed
based on the future requirements and people's potential power. Consequently, the environmental changes and transitions should be taken into account probabilistically. An example in this regard has been illustrated in the interviewees' responses:

In different stages of these sort of plans, there are interfering protocols which prevent the main goal. From upgrading plans to recognition of suitable people to choose, in all of them, protocols cause so many troubles. (15, M9)

Generally speaking, regarding the bureaucratization can argue that most of the interviewees consider the talent management through the administrative correspondences and requirements, regarding the mental background about bureaucracy, argue that these requirements sometimes seem so big that dominate the main succession plan.

**Jealousy attitude:** Apart from the poor performance, low experience and such things, jealousy attitude is one of the other factors which prevents from individuals’ growth. Jealous people won’t be feeling good about their colleagues progress and sometimes they prevent it as much as they can. If people are not ready to expense to upgrade themselves, never would be able to help themselves and their organizations. Some unsuitable characters in people, avoid the proper communication and this can damage the plans and missions. A sample of this has been mentioned in interviewees’ responses:

More importantly in the people succession is that sometimes some people don’t act properly. For example the guy finds it downgrading to learn from a colleague. So doesn’t try to communicate properly {14, M1}.

In the process of talent management, the individuals and their attitudes are determinant factors. If the staff act only based on their own opinion, never can be hopeful to execute such a succession plan in the organization. In general, spread of jealousy is a restraining factor and having such an attitude, it is not easy to hope on the execution of the main succession plan.

**Discussion and Conclusion**

In a conclusion, two main questions of the research can be answered: in reply to the first question, we should introduce driving forces in talent management in Pars Company and in response to the second one, we should introduce restraining factors.

As you can see in Fig. 2, the research results show that the company to execute a proper succession plan needs to develop a set of new conditions. Staff considers their managers as their mentors. On the other hand, execution of different plans would be successful in the case of staff acceptance. One of the cooperation requirements is
establishing trust in staff towards fairness in the organization. Another driving factor is considering knowledge richness instead of work experience. Organizations should keep in mind that not always people with more experience or seniority are more suitable.

Research and studies conducted by Rothwell (2005), Conger & Fulmer (2003), Kim (2006), and Romejko (2008) about talent management emphasize on the important role of senior organizational managers to execute succession plan successfully. They also consider personal desires effects and in some research mention the knowledge richness and meritocracy.

Fig. 2 considers restraining forces in talent management as well. In this figure with respect to cultural differences and other discriminations, some factors which previously were not taken into account in the literature review are considered, though Rothwell mentions bureaucratization and express it as one of the barriers in talent management. Apparently other restraining factors such as feeling job insecurity, dishonesty in information presenting, cost viewpoint and jealousy attitude were not acknowledged by the other researchers. However, these factors are completely observable in Iranian companies’ organizational culture.

In studying the mentioned factors willingness and interest, managers support, feeling job insecurity and jealousy attitude consist the set of individual characters and knowledge richness against work experience, dishonesty in information presentation, cost viewpoint and bureaucratization consist the set of organizational characters which are influential in talent management. It can be seen that among both individual and organizational factors, there are some driving as well as restraining factors. One could be hopeful that in the coming researches, other dimensions of driving and restraining factors in talent management in Iranian organizations would be explored.

For being successful in talent management, managers should know their working requirements and be aware of their organization’s strengths and weaknesses. In addition, choosing the individuals to fill the future’s positions is one of the vital factors in successive talent management. Organizational culture has to be developed and an optimistic attitude towards capable people should be increased instead of only focusing on their seniority and experience. To specify fitting individuals in succession plan could be referred to the experts’ point of view. This method causes managers contribution in the process of designing the succession plan. The human resources department can suggest the sufficient individuals according to the information regarding the work experience, academic qualification, received educations, performance valuation and other individual characters. Moreover, to increase accuracy and reduce the effect of personal desires, it is possible to apply more than one method simultaneously to choose the individuals.

Managers’ support implies their awareness of the importance of the succession plan (Contor, 2005). In talent management specialized consultations, valuation tools and training are provided by human resources department, but senior managers should be executives of the plan. To be successful in this regard, it’s not enough to only talk about the importance of these plans. These matters should be followed continuously and should be talked in this regard (Contor, 2005). Running training courses prior to the succession plan execution is an effective step to familiarize the senior managers with their importance.

Individuals’ willingness to participate in talent management will facilitate the execution of it. Some researchers have highlighted the favorable effect of individuals’ willingness to participate in the program (Smilansky, 2007; McElwain, 1991). This is because people are central to succession plans and their desire plays an important role. Therefore, we suggest that with some educational courses and meetings for employees, their attention and cooperation can be caught in favor of talent management. Considering some sorts of encouraging and rewarding mechanisms for employees who participate in the program and making people aware of career paths will be helpful as well.

Given feeling job insecurity as the first barrier in front of talent management, individuals resist talent management due to their ambiguity of their future job and fearing from missing their current position. To overcome this barrier, we suggest that enough care should be taken during selection process. In addition, various career paths should be considered for people in the organization in order to ensure who are less self-confident of their interesting future career paths.

Organizations which are willing to design and implement talent management should enjoy enough resources of time, energy and budget. Expense category emphasizes that the plan execution costs should be feasible. Managers approach to talent management needs to be improved. They should consider long-run benefits of the planning and cost reduction which will occur after the plans implementation. These benefits include maintaining experienced employees and filling their positions as soon as some employees leave the organization.
In order to obtain competitive advantage and to survive in long-run, organizations consider talent management (Mandi, 2008). The plan designers update it according to the organization’s information resources and their changes. According to “dishonesty in information presentation” category, if there is not an honest climate within the organization, it will be a great barrier in front of talent management. This problem can be solved mainly in selection step and by human resources department. Designing a comprehensive human resources system is an effective solution for this problem. Pars Company does not enjoy such a system. Analyzing job candidates’ certificates and their primary evaluation before their employment are some advantages of such a system which can solve dishonesty problem.

Employees’ attitudes towards one another and types of their relationships are other important factors in a successful succession plans. In these plans, individuals interactions play a significant role. Mentoring relationship is vital for succession plans success. This kind of relationship will be facilitated within the organization, if employees have fair attitudes towards one another's abilities and characteristics without jealousy. If organizations can provide all employees with equal growth opportunities, individuals will try hard and pay attention to their own performance instead of others. In addition, having some recreational programs in employees’ schedules will result in a friendship climate within the organization. Also, organizations need to have a mechanism to realize their employees’ opinions.

“Bureaucratization” refers to paper work, useless administrative protocols, and time consuming decision making process. Many people are upset with situations they have experienced when they have been in some organizations. In this special case, the organization is a barrier in front of talent management. We suggest that with a special department for organization’s processes reengineering which is responsible for designing and implementing proper computer systems in order to enter and save information on them and reduce paper work, talent management success will be probable. Finally, the most important point is that organizations’ environments are changing very fast, and they have no choice but adapting to these changes. Therefore, talent management should be designed according to the organization’s future probable needs with attention to different growth opportunities.

References
Evidence suggests that demand-side barriers may be as important as supply factors in deterring patients from obtaining treatment. Yet relatively little attention is given, either by policy makers or researchers, to ways of minimizing their effect. These barriers are likely to be more important for the poor and other vulnerable groups, where the costs of access, lack of information and cultural barriers impede them from benefiting from public spending. Demand barriers present in low- and middle-income countries and evidence on the effectiveness of interventions to overcome these obstacles are reviewed. The most efficient and effective way of overcoming any demand barrier then becomes Total Quality Management. The 5-S mode of working within the resources and constraints and meeting the performance standards leads to infusing survivalism and sustenance.

Demand barriers are also shown to be important in richer countries, particularly among vulnerable groups. This suggests that while barriers are plentiful, there is a dearth of evidence on ways to reduce them. Where evidence does exist, the data and methodology for evaluating effectiveness and cost-effectiveness is insufficient. An increased focus on obtaining robust evidence on effective interventions could yield high returns. The likely nature of the interventions means that pragmatic policy routes that go beyond the traditional boundaries of the public health sector are required for implementing the findings. The yield curve shall improve the performance of such measures is taken to be internationally acceptable. The paper tries to establish a link between what demand barriers are and how to overcome such barriers by establishing the quality certification that is based on 5-S.

**Demand-side barriers**

One illustration of the importance of demand-side barriers is provided by a survey of obstetric choices in Bangladesh (Barkat et al. 1995 reported in Piet-Pelon et al. 1999) (Table 1). In this survey, the majority of the most important reasons for not seeking emergency obstetric care were found to be demand factors. Most of the standard economic frameworks of health care utilization model both supply and demand sides (Table 2). In this paper, demand-side determinants are defined as those factors that influence demand and that operate at the individual, household or community level.

In a market system, prices signal availability and quality. Within a public distribution system, the role of prices is a little different since they usually do not rise in response to shortage. Rather, scarcity is signalled by actual delays in the supply chain and variations in the quality of supplies. Factors are combined subject to available technology and management capability of the provider. The supply price also helps determine the level of production. In a public system this may be replaced by plans for a required level of production, which is in turn constrained by available budget. On the demand side, the economic literature is dominated by adaptations of the Grossman model that analyze individual investment and consumption decisions to improve health and utilize health care (Grossman 2000). Demand is influenced by factors that determine whether an individual identifies illness and is willing and able to seek appropriate health care. The model leads to a demand for health care of a given quality that is determined by individual and community factors as well as the price of medical care and other similar goods.

**Supply side**

1. Input prices and input availability Wages and quality of staff Absenteeism, staff not attracted to the area Price and quality of drugs and other consumables Scarcity of supplies, weak cold chain
2. Technology Inability to treat disease with given technology
3. Management/staff efficiency Poor quality of management training, lack of management systems and education and knowledge about the characteristics of, and need for, medical treatment. Community factors include cultural and religious influences and other social factors that affect individual preferences. Price is a complex variable and includes the direct price and distance cost, opportunity (time) cost of treatment – since treatment can be time consuming – and any informal payments made to the facility for commodities or to staff. Also included are prices for substitute commodities that impact on health (PH), since individuals have some scope for choosing healthy lifestyles, safer employment or better nutrition in order to improve health or reduce the probability of ill health. The determinants of demand and supply may in turn generate 'barriers' to utilization that arise when factors influence these determinants in a way that reduces utilization of services. In the paper, we focus on those demand factors that can be controlled at the community, household or individual level and are amenable to policy intervention. Individual characteristics that determine need, such as age and sex, are not considered since they cannot be controlled. The effect of gender on access to household resources is discussed however. The analysis does examine the effect of education, information and non-supply user costs of services since these are amenable to interventions. It may also be possible to influence community and cultural factors if they arise as a result of misinformation or inappropriate service configuration. We do not consider the role of income, although this is one of the most important factors in determining health spending and seeking behaviour (Gerdtham and Jonsson 2000). In principle, incomes are amenable to control but they are assumed to be mainly affected by wider economic policies outside the specific control of the health sector. We do not consider the direct price of health services, the price of alternative health services and the relative prices of other health-enhancing inputs since these are mostly related to supply of services.

Education and information
The effect of education and information can be divided into two categories. First, there is the impact of basic education on the demand for services. Education, which is often measured by level or duration of schooling, has been shown to be the most important correlate of good health (Grossman and Kaestner 1997). A study in Pakistan, for example, found that maternal schooling was the most important factor in determining child survival (Agha 2000). A recent comparative article examining pre-requirements for successful development suggested that a high education base is a major determinant of above-average social development (Mehrotra 2000). Education as a determinant of health care utilization is a more complex variable. To some extent, education can improve the ability of individuals to produce health themselves through better lifestyles rather than relying on health services. Yet there is also much evidence that better basic education can, through general improvements in literacy and specific health studies, increase desired and actual use of health services. Studies across a number of countries have, for example, indicated the importance of maternal education on the use of obstetric services (Cleand and van Ginneken 1988; Raghupathy 1996). Education provides the consumer with the basis for evaluating whether they or a dependent require treatment. Information on the best places to seek care is additionally required. While it is sometimes suggested that individuals are unable to assimilate information on treatment options, this assumption is challenged by recent work in Tanzania (Leonard et al. 2001; Leonard 2002). These studies suggest that, far from being passive consumers, patients actively seek out not only the best-known provider but the best facility for a particular illness. Perceptions of quality do, in fact, accord quite well with technical evaluations. The second knowledge effect is the specific impact of information on health and health care. Both education and information may be interlinked since the ability to assimilate health messages is likely to be determined in part by the level of general education. The impact of information on treatment options and desirable health seeking behaviour is also important in determining demand. One study finds that lack of information on the malignant effects of excessive antibiotic use has a substantial positive effect on a preference for self-medication over use of health facilities (Okumura et al. 2002). There is also a substantial literature indicating that demand for family planning services is impeded by a lack of correct knowledge of contraceptive choices and side-effects (for example DeClerque et al. 1986; Donati et al. 2000).

### DEMAND
- Price (official, unofficial charge, travel cost, lost work)
- Quality
- Income
- Social, household, cultural characteristics
- Knowledge of health care available
- Education (general and health)

### SUPPLY
- Official price
- Input prices (staff, capital equipment, buildings)
- Knowledge of technology of treatments
Management efficiency by staff

- TQM
  - cross-functional product design
  - process management
  - supplier quality management
  - customer involvement
  - information and feedback
  - committed leadership
  - strategic planning
  - cross-functional training
  - employee involvement

- 5-S
  - Straightening
  - Systematic Cleaning
  - Standardize
  - Self Discipline
  - Safety

There is much evidence to suggest that distance to facilities imposes a considerable cost on individuals and that this may reduce demand. In studies reviewed for this article, transport as a proportion of total patient costs (including facility costs not financed by the user) was found to be 28% in Burkina Faso, 25% in northeast Brazil and 27% in the United Kingdom (Sauerborn et al. 1995; Frew et al. 1999; Terra de Souza et al. 2000). Another study in Bangladesh suggested that it was the second most expensive item for patients after medicines (CIETcanada 2000). Location and distance costs are often seen to negatively impact service utilization. A study in Vietnam found that distance is a principle determinant of how long patients delay before seeking care (Ensor 1996). Another, in Zimbabwe, suggested that up to 50% of maternal deaths from haemorrhage could be attributed to the absence of emergency transport (Fawcus et al. 1996). At the same time, distance is also cited as a reason why women choose to deliver at home rather than at a health facility; see, for example, studies in the Philippines (Schwartz et al. 1993), Uganda (Amooti-Kaguna and Nuwaha 2000) and Thailand (Rachupathy 1996). The impact of location is not confined to low-income countries. One US study found that patients living more than 20 miles away from a hospital are much less likely to visit ambulatory services for after-care following myocardial infarction (Piette and Moos 1996). In Japan, one study found that access to follow-up treatment after treatment for cerebrovascular disease was considerably influenced by access to suitable transportation (Tamiya et al. 1996). Distance may also have a differential impact across income groups. A study in Australia found that the impact of costs fell most heavily on the poor (Rankin et al. 2001). Qualitative evidence in Vietnam suggests that poorer households usually have access to inferior transport in the event of illness (Segall et al. 2000). Consuming health care can be time intensive. Both patients and relatives may have to give up long periods of work (or leisure) in order to receive treatment. This represents an important cost to individuals, particularly during peak periods of economic activity such as harvest time. A study in Australia found that indirect costs account for 60% of the costs of treatment for surgery for patients from rural areas (Rankin et al. 2001). Convenience of opening hours, an indicator of the importance of taking time off work, was found to be important in both Vietnam and Ghana in determining service use (Bosu et al. 1997; Segall et al. 2000). Opportunity costs vary for different groups. A recent study in Pakistan, for example, found that compliance is more easily improved in those who are not economically active since they are more likely to have time to attend for treatment (Khan et al. 2002). In Uganda it was found that poorer patients were willing to travel considerable distances searching for better facilities, perhaps because their opportunity costs (see below) were lower (Akin and Hutchinson 1999). Similar results are borne out in studies of the private sector in India, where the search for quality (a supply variable) is often seen to override the distance cost and leads to complex and lengthy search strategies (Shenoy et al. 1997; Bhatia and Cleland 1999). These findings must, however, be balanced by the other effects of lower income that are often a consequence of lower opportunity costs. Financial barriers may also interact with other demand barriers. One study in Kazakhstan, for example, found that the education of the household head or the care-seeker was an important determinant of the willingness to travel long distances to obtain treatment (Thompson et al., forthcoming).

Community and household barriers

The Voices of the Poor cross-country study found general agreement that men were invariably given preferential access to health care over women (Narayan 1997). Studies in Bangladesh, India and Côte d’Ivoire (although not in Peru, where the opposite result is reported) found that girls were much more likely to visit health care facilities and
benefit from public and household health care expenditure (Gertler and van der Gaag 1990; Booth and Verma 1992; Begum and Sen 2000). Another study in India found that while a bias to boys existed, this was reduced when the household head was more highly educated. The reason for these differences is related to both cultural patterns and social factors within the household and wider community. Cultural norms, such as purdah restrictions, can prevent women from seeking health care outside the home for themselves and their children (Rashid et al. 2001). This barrier is often raised still further when men provide services, and has been offered as one reason why Asian women living in Western countries often make little use of health services (Whiteford and Szeg 2000). Such restrictions may also interact with other barriers. One study in India found that distance was a much greater barrier to women than to men with similar incomes (Vissandjee et al. 1997).

A related issue is that men often make decisions on care-seeking for women. In Senegal, for instance, a study found that more than 50% of decisions regarding female treatment were made by men (Post 1997). This is particularly important since, as one study in Bangladesh, South Africa, Indonesia and Ethiopia found, male decision-makers often spend less than women on social items (Quisumbing and Maluccio 1999). In a number of South Asian societies, the mother-in-law dominates decisions on childbirth and care related to pregnancy, particularly in early marriage. In these circumstances, whether a woman is delivered at home by a family member, by a traditional birth attendant (TBA) or at a health facility much depends on the beliefs of the mother-in-law (Piet-Pelon et al. 1999).

Increasing demand is therefore far more complex than simply the provision of health education advice or information, but is also strongly related to the relative position and education of family members. As suggested by one Indian study, when women cannot contribute through superior education or through income earning, their position is maintained through household chores (Ramasubban and Rishyasringa 2000). The completion of these duties may mitigate against them receiving care in the event of illness. This reinforces opportunity cost as a factor in reducing demand, not so much through any significant effect on total household earnings but in the lost position within the household hierarchy.

3. Intervening to reduce barriers In this section we review available literature on the nature and impact of actual demand-side interventions. A review was carried out based on a structured search of key electronic databases, websites of international agencies and nongovernment organizations and a series of key informant contacts with researchers working in related fields. More details of the strategy are provided in Ensor and Cooper (2002).

Why intervene? Justification to intervene to reduce demand barriers can be divided into market failures and pursuance of social equity (Hurley 2000). Even once an efficient competitive provider network has been established, two key market failures may impede effective demand for health care. A key assumption for the efficient functioning of a market is that adequate and symmetric information is available to both buyers and sellers of the commodity. Yet often in health care markets, the lack of information or inability to assimilate and utilize the information on health care means that consumers are unable to make informed decisions. Providing education and information to individuals, households and communities is a way of dealing with informational gaps. A further market failure issue is that health care markets are underpinned by considerable uncertainty, which means that consumers often find it difficult to spread risk and make sufficient resources available at the time of ill-health. It is well established that even in countries where insurance and capital markets are well developed, market failure that prevents the adequate coverage of catastrophic costs often persists. The problem is generally more acute in low- and middle-income countries where these markets are underdeveloped or nearly non-existent. The second main justification to intervene is where other means to allocate economic resources to individuals on an equitable basis have failed. In this case, some groups in society will be unable to meet their health care needs because of either the underlying income distribution or differences in intra-household bargaining power. This might lead to interventions to target resources at those in need but unable to access services.

References


A COMPARATIVE STUDY OF THE ATTITUDE OF PUPIL-TEACHERS OF AIDED AND SELF-FINANCING COLLEGES OF NCR REGION TOWARDS TEACHING AS PROFESSION

Introduction
Attitude is a word having no precise technical meaning when used to describe human beings psychologically. It refers in a general way to inclinations, presumed to be enduring to react in certain way in response to certain kinds of situation, to see and interpret events according to some predisposition and to organize opinions into coherent interrelation clusters. The term attitude has been extensively used in psychological investigations during the last twenty years. At present, the word attitude has come to include a wider meaning that was originally the case when it was used to denote the muscular neural set of an organism. Attitude is a hypothetical construct that represents an individual's like or dislike for an item. Attitude are positive, negative or neutral views of an 'attitude objects' i.e. person, behaviors or event etc. Attitude involves what people think about and how they would like to behave towards an object.

Thurstone said, "Attitude is the degree of positive or negative effect associated with same psychological object."

According to Jung, "Attitude is a readiness of the psyche to act or react in a certain way."

Teaching is noble profession confronting teacher pupil relationship in which one's personality, usually that of the teacher acts on the personality of students to ensure the highest ideals of life.

"Teaching is the art of human development." According to the New Encyclopedia Britanica (1985). According to Jacolot, teaching is "to teach is to cause to learn." Teaching is a process that aims to increase or improve knowledge, skills, attitude and behaviors in a person to accomplish a variety of goals.

According to New Webster's Dictionary, profession is, "vocation requiring specialized training in field of learning, art or science; a leading vocation or business; the body of persons engaged in a calling or vocation."

The welfare of a country depends on the proper education of its citizens. The government of India is fully conscious of its responsibility and is trying to introduce reforms in the present educational system. However, successful implementation of any scheme in education rests with teachers.

Teachers are the real architect of the nation for it is they who provide future citizens. "Without good teachers, even the best of system is bound to fail, with good teachers even the defects of a system, can be largely overcome." Kabir Humayun.

Significance of The Study
In the present study, the investigator has tried to study the attitude of the pupil-teachers towards teaching. Many students who like to take admission in B.Ed. course may or may not be interested in the teaching profession. They are selected through competition and expected that only those are qualified and highly motivated for teaching profession get admission in this course. But it has been noticed that only few of them are more serious in doing B.Ed. From the studies conducted in the past it is evident that a large number of pupil-teachers complete the course with boredom in their minds. So unless and until the pupil-teachers or would be teachers realize that their attitude can enhance their teaching ability, they would not develop a positive attitude towards teaching. Thus, the present study, according to the investigator would bring about a substantial change in the attitude of the pupil-teachers community towards teaching.
Objective of The Research
The Purpose of this research study was to find out the attitude of pupil-teachers towards teaching profession and also to find out whether there is any difference in the attitude of pupil-teachers of self-financing and aided college of N.C.R. Region towards teaching as profession. The objective of the study is
- To compare the attitude of pupil-teachers of self-financing and aided colleges of NCR Region towards teaching as profession.

Hypothesis
- There is no significant difference in the attitude of pupil-teachers of self-financing and aided colleges of NCR Region towards teaching as profession.

Methodology and Design of The Study
The method adopted for the present study was descriptive and statistical in nature.
Sampling: The sample consisted of 100 Pupil teachers selected from identifying self-financing college UIIMT, Muradnagar and aided college NAS P.G. College, Meerut. Out of 100 pupil-teachers 50 were male and 50 were female pupil-teachers. Purposive sampling method was applied in selection of sample. the sample chosen for the study is indicated through table.

<table>
<thead>
<tr>
<th>Table 1: Name of institutions with their number of pupil-teachers.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. UIIMT, MURADNAGAR</td>
</tr>
<tr>
<td>2. NAS, MEERUT</td>
</tr>
<tr>
<td>Total Students</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2: Distribution of sample according to the type of educational institute and gender.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of College</td>
</tr>
<tr>
<td>-----------------------------------</td>
</tr>
<tr>
<td>Aided College</td>
</tr>
<tr>
<td>Self financial College</td>
</tr>
</tbody>
</table>

The Tool Used:
Teacher Attitude Scale’ developed and standardized by Dr. Sudhakar Shastri was used to collect data to fulfill the objective of the present study. It is scale of 22 items. It has been developed on Thrustone technique of attitude scale construction. It measures the attitude towards the profession of teaching. Reliability of the scale was determined by the split-half method. It was found to be 0.90 by the Pearson Product-Moment Correlation method. Content validity of the scale was ensured by the judges who carefully rated each item. The validity of the scale was also determined by self-rating by subjects on a graphic continuum of the scale. It was found to be 0.78 Scoring:

Statistical Techniques employed-
The following formula was used for calculating the value of t:

\[ t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \]

Analysis of Data and Interpretation of the Results
From the result obtained for attitude towards teaching as profession of pupil-teachers of self financing colleges, it can be seen that out of 50 male and female pupil-teachers 6 pupil-teachers which is 12% of the total have most favourable attitude towards teaching as profession where as 43 i.e. 86% of the pupil-teachers lie in the category of having favourable attitude towards teaching as profession. I pupil-teacher or 2% of the total pupil-teacher possess
neutral attitude towards teaching profession. No pupil-teachers was found to have unfavourable and most unfavourable attitude towards teacher as profession. The result has been depicted through table 3.

Table 3: Attitude towards teaching as Profession of Pupil-teachers of Self Financing Colleges

<table>
<thead>
<tr>
<th>Attitude Score</th>
<th>Tally</th>
<th>f</th>
<th>% of the total</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2.9</td>
<td>///</td>
<td>6</td>
<td>12</td>
<td>Most favourable</td>
</tr>
<tr>
<td>3-4.9</td>
<td>/////</td>
<td>43</td>
<td>86</td>
<td>Favourable</td>
</tr>
<tr>
<td>5-5.9</td>
<td>/</td>
<td>1</td>
<td>2</td>
<td>Neutral</td>
</tr>
<tr>
<td>6-6.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Unfavourable</td>
</tr>
<tr>
<td>7-8.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Most unfavourable</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N=50</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 4: Significance of the difference between Mean attitude score of pupil-teachers of aided B.Ed. College and pupil-teachers of self-financing B.Ed. College.

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of Pupil-teachers</th>
<th>Mean attitude</th>
<th>SD</th>
<th>SEDM</th>
<th>df</th>
<th>t-value tabulated</th>
<th>t-value calculated</th>
<th>Level of sig.</th>
<th>Null hypothesis R/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aided B.Ed. College P.T.</td>
<td>50</td>
<td>4.00</td>
<td>0.67</td>
<td>0.136</td>
<td>98</td>
<td>1.98</td>
<td>0.677</td>
<td>0.05</td>
<td>A</td>
</tr>
<tr>
<td>Self financing B.Ed. College P.T.</td>
<td>50</td>
<td>4.09</td>
<td>0.68</td>
<td>0.136</td>
<td>98</td>
<td>1.98</td>
<td>0.677</td>
<td>0.05</td>
<td>A</td>
</tr>
</tbody>
</table>

P.T. = Pupil Teachers

The calculated t-value is 0.677 which was found to be not significant at any level with 98 degree of freedom. Therefore it can be asserted that, there really exist no difference in the attitude of pupil-teachers of aided and self-financing B.Ed. College of NCR Region towards teaching as profession.

Thus the null hypothesis was accepted.

Discussion

When the data was analyzed to make comparative study of the attitude of pupil teachers of aided and self-financing college of NCR Region towards teaching as a profession, it was observed that there was no significant difference in their attitude towards teaching as a profession. Thus, this result indicates that the type of institution has no influence on the attitude of pupil-teachers towards teaching profession. As pupil-teachers of both colleges had a view that there is no other profession better than teaching and also teaching makes one imaginative and creative, motivated and develops the personality of the person. Another reason for showing the favorable attitude was that this was mainly due to reason that both male and female pupil teachers shown favorable attitude towards teaching as they were of the view that teaching is means for mediocre and it is the best profession for person from poor socio-economic background.
Delimitation
The limitations of this study are as follows:
- The study was determined only for NCR Region due to scarcity of time and economic resources.
- The study is limited to pupil-teachers only.
- In the present study, the researcher had confined her study to self-financing and aided colleges of NCR Region. This was due to the limited time and money. It was not possible to pursue the work outside of the NCR Region.

Conclusion
The aim of the present study was to find out what pupil-teachers of aided colleges and self-financing colleges feel about teaching as a profession. The investigator has concluded the following findings on the basis of the investigation.
- Most of the pupil-teachers including male and female of self-financing colleges show a favourable attitude towards teaching as a profession.
- In the aided colleges of NCR Region most of the male and female pupil-teachers possess a favorable attitude towards teaching as a profession.
- Most of the female pupil-teachers of self-financing colleges show a favourable attitude towards teaching as a profession in NCR Region.
- There is no significant difference in the attitude of pupil-teachers of aided colleges and self-financing colleges of NCR Region teaching as a profession.

Areas for Further Researches
Some of these research problems related to the present area may be taken by the researchers in future which are as follows:
- Pupil-teacher’ socio-economic status and their attitude towards teaching profession.
- Influence of teaching methods on pupil-teachers' attitude towards teaching profession.
- Student-teachers' attitude towards teaching and its correlation with their achievement, skills and interests.

References

THE END
Abstract:

Biodiversity represents the diversities of plants and animals existing on the planet earth. With UNO’s declaration of 2011-20 as the Decade on Biodiversity, much emphasis is being given to its conservation at national and International level. In order to promote concern and inculcate positive attitude for biodiversity conservation among the young generation, secondary students were taught the topic “Biodiversity” through inquiry based approach in which students collected different plants and animals from local environment (school complex), documented those and searched for their utilitarian values from primary and secondary sources. With the support of ICT and concept map use, different conservation approaches being followed including different categories of threatened species was explained to students. The overall intervention was based on the fundamental premise that the teaching-learning process takes place in an atmosphere that is participatory, exploratory, active, empowering and above all enjoyable. Through pre-test and Post-test design, it was found that there was not only increase in knowledge and understanding of concepts relating to biodiversity among students but also the learning could become become durable. The implications of the study in the light of Education for Sustainable Development have been discussed.

Introduction

The world Commission on Environment and Development (WCED,1987) in its report “Our Common Future” defined the objectives of Sustainable Development (SD) as to conserve the natural resource base for the well being of the present and future generations besides maintaining a healthy environment and life support system. The sustainable development framework entails thinking far into the future and how our present actions might affect our ability to live a wholesome and fulfilling life.

Education for sustainable development is not the same as environmental education (Satapathy & Dash, 2007) as the latter is concerned with quality of the environment whereas the former besides ecological dimension looks into economic and social issues (Desinger, 1990) with the ultimate objective of improving the quality of life of people (Satapathy, 2007) living on the planet. As such there is a shift from awareness and understanding to involvement, participation and problem solving. Under this background and realizing the spirit of NCERT (2004) document “Environmental Education in Schools” that tells implementation of Environmental Education (EE) in schools needs to be undertaken on a mission mode to prepare young minds to appreciate the importance of environment in a holistic manner not only for human survival but also for all life forms, the present study was conceptualized. With UNO’s declaration, 2011-2020 as the “Decade of Biodiversity” in order to provide an understanding of and concern and respect for bio-diversity, the present study was formulated to teach biodiversity through inquiry based approach in local context at secondary level with the following objectives.

(i) To develop sensitivity to and appreciation of the tremendous diversity existing in the natural world and
(ii) To help students to acquire social values including strong feelings of concern for the biodiversity and the motivation for actively participating in its conservation and improvement.

Conceptual Background

Bio-diversity includes the variety of living forms – plants, animals and micro-organisms existing on the planet earth. It also includes the genes they contain and the ecosystem they form. It deals essentially with dynamic processes and increases, when new genetic variation is produced and decreases with the loss of genetic variation or species extinction. The biological variations in nature over time and space form the basis of evolutionary process.

Biodiversity is described on the basis of three levels of biological organization – ecosystem diversity, species diversity and genetic diversity (variation within a species) which are all interrelated. It is estimated that there exists 5-50 million species of living forms on our earth and of these 1.75 million has been identified (Singh, 2002). These include 30,000 species of green plants and fungi, 800,000 species of insects, 40,000 species of vertebrates and 360,000 species of micro-organisms. The Indian sub-continent with a geographical area of 329 million ha (2.4% of the world area) is very rich in biodiversity with a sizable percentage of endemic flora and fauna (Botkin and Keller, 1995). It contributes to about 7.0% of the World’s biodiversity. Survey conducted so far have invented over 49,000 species of plants and 81,000 species of animals of which 30% are insects (Table-1). There are a total of 12 bio-geographical provinces in India. India stands 10th among the plant rich countries, eleventh in the number of endemic species of higher vertebrates, amphibian, birds and mammals and 10th in the world (WCMI, 1993) as far as its richness in mammals is concerned. India has four hot spots out of eighteen identified in the world. Two namely...
Eastern Himalayas and Western Ghats have 5332 endemic species of higher plants, mammals, reptiles, amphibians and butterflies (WCMC, 1993).

Biodiversity carries immense importance (Swaminathan, 1984). It has crucial value (Fig.1) as it (i) provides food, construction materials, raw material for industries, medicines etc. (ii) provides the basic genetic material for all important of domestic plants and animals (iii) maintains ecosystem functions including evolutionary processes, (iv) stores and recycles nutrients, carbon, nitrogen and oxygen essential for life, (v) absorbs and break down pollutants including organic wastes, pesticides and heavy metals, (vi) recharge ground water, protects catchment basins, (vii) produces soil and protects it from excessive erosion and (viii) provides a variety of ecosystem services. Further, there are potential benefits of natural enemies being used as biological control agents for pests.

India’s biodiversity is one of the most significant in the world (Singh, 2002). But we are loosing these nature treasure at a fast rate because of several problems such as over population, large number of cattle herds, growing demand for land and water supply, unplanned developmental work and over exploitation of resources. As Gandhiji once said, “The mother nature has everything to meet our needs and not to satisfy our greed”. Failure to consider this aspect has resulted in the threat to biodiversity as a whole.

Biodiversity is an irreplaceable resource; its extinction is forever. It could be possible to create a building or monument but there is no way at present to recreate an extinct plant or animal species (Rana, 1999) what so ever way it is attempted. Realizing the importance of biodiversity, UN has declared 2011-20 as the “Decade on Biodiversity” in order to promote concern for biodiversity and its conservation. In the present study an attempt has been made to create a good understanding and positive attitude among students about biodiversity keeping the local context in view.

**Methodology**

In the present study, inquiry based approach was used to teach biodiversity and its conservation at secondary level at Jawahar Navodaya Vidyalaya (JNV), Konark, Orissa during the session 2012-13. A normal citizen used to give attention to glamorous mega-vertebrates such as tiger, elephant, rhino, whales, panda etc. as far as biodiversity is concerned. However, in the present study, emphasis was given to local plants and animals (Rajput, 2003) and their usefulness to common people. To study the effectiveness of the teaching through inquiry based approach, Pre-test/Post-test design (Best and Khan, 1996) was used. A total four periods of one hour duration, each at an interval of one day was used to teach the subject as reflected at secondary level, in class X. CBSE curriculum (NCERT, 2006). The study was carried out with a sample population of 30 students.

Before teaching, a multiple choice objective type questionnaire having 30 items (10 factual & 20 conceptual) were given to students to answer at an interval of 30 minutes. Factual questions included, items such as National animal, National Bird, state flower, biodiversity day, specific local plants used for medicinal purpose, percentage of land under forest cover, total number of national parks, red data book etc. The conceptual questions covered four dimensions such as distribution of biodiversity, relevance of biodiversity, conservation of biodiversity, local biodiversity and its usefulness.

After collecting the answer sheets, the students were taken to the school backyard and garden surrounding the school with some glass tubes, plastic jars, poly bags, knife etc. to collect available plants and animals. Students were asked to collect plant/plant parts, small animals and not to touch big animals like lizards, if any. Students were also taken to the small water body in the school campus and were showed the plants and animals there. Taking back to the class, students were told to display the plants and animals collected by each of them and were asked to write each of its the local name/English name, along with its usefulness and draw the diagrams of those animals. Wherever they failed, they were asked to collect information from other sources (secondary sources) such as, seniors, hostel workers, and people staying in the campus including parents. Students were encouraged to collect the picture of big animals which they like and get into the following class.

In the next day, plants, animals and pictures of big animals collected by students were received in the class. The teacher prepared a table with different columns (Table-2) and ask each student to write the name of the organism/plant, plant part, place of collection and its usefulness one after the other. While writing the names of the organism(s), teacher helped the students who could not write the usefulness and discussed it as well. Some fungi growing on the rotten orange and mushrooms was shown to the students. After completing the table, students were taken to the biology laboratory/museum and shown the collected and preserved specimens and discussed their
In the following class, after collecting different information, data and pictures from diverse sources keeping the curricular guidelines in view, a power point presentation was given on different issues of biodiversity such as its present status, distribution and importance. Further through concept map (Patankar et al., 2005) important conservation measures being taken at national and international level was discussed (Fig. 2) with students. While discussing the subject, emphasis was given to local resources with special reference to plants and animals seen in the state. Different categories of threatened species such as extinct species (Example Indian Cheetah, Dodo bird, Wild goat, Mammoth et); endangered species (Example; Great Indian Bustard, One horned Rhino, Pitcher plant); vulnerable species and rare species (Example Golden Langur). Students were encouraged to participate in the discussion and ask questions. One day after completion of the topic, a post-test was administered with multiple choice factual and conceptual questions to assess students change in knowledge/understanding about biodiversity. Further after an interval of 10 days, again the students knowledge on biodiversity and its conservation was tested through short answer questions to measure its retention in the mind of the learners. The result of the study has been presented in Fig. 3.

Result and Conclusion

Science is the spirit of inquiry (NCERT, 2005). According to the National Policy on Education (MHRD, 1986) science should be taught in such a way that it promotes curiosity, creativity, inquisitiveness and ability to ask questions. As living organisms very often create curiosity and interest among children, in the present study students have been put into natural environment (school garden) to know the local plants and animals in order to understand the biodiversity under the over all curricular objectives as reflected in the secondary science text books (NCERT, 2006).

In a total, children have collected four types of lower plants such as algae, fungi, moss and ferns (pteridophytes) and 22 types of higher plants (Table 2). Among lower animals they have collected (Table 3) earth worms, and a variety of insects and located higher animals such as fish, birds, cats, dogs, cattles in and around the campus. Besides these they have collected samples of snails, pila, unio and small crabs from the nearby locality. During discussion in the laboratory, some preserved specimens such as snake, coral, dog fish etc. were shown to the students. During presentation/discussion in the classroom, children were shown different types of animals such as whales, dolphins, tigers, white beer, rhinos, deers, cobras, different types of crop plants, ornamental plants, national parks etc collected from the web sites. Interest and curiosity were noticed from the different types of questions raised by the students during presentation. Further while writing the importance of each organism, the students were found to have noted it either from their own experience or collected from secondary sources (Dash, 2013) by interacting with seniors, workers, parents, school employees etc. After discussing the observations made by the students on the importance of living organisms in terms of food, fodder, fuel wood, shelter (Table 2 & 3) other uses such as role in food chain/food web, source for development of new plants and animals, role in biological control, combating pollution, etc. were discussed with suitable examples from daily life experiences. The students were explained through pictures, the importance of national animal (tiger) national bird (peacock), state animal (deer), state bird (blue bird) and different days celebrated such as world biodiversity day (22nd May), Earth day (22nd April), world forest day (21st March), environment day (5th June) etc. to sensitize students about biodiversity conservation. The overall intervention was based on the fundamental premise that the teaching-learning process takes place in an atmosphere that is participatory, exploratory, active, empowering and above all enjoyable.

In the pre-test conducted on the students, the average score was 28.9 percent (Fig.3) and it reached at 72.8 percent (Post-test score) after having participated in collection followed by demonstration and discussion in the class room. Thus there is an enhancement of more than 150 percent in achievement score which reflected that there is significant increase in knowledge and understanding about biodiversity among students. Curiously, post-test conducted 10 days after teaching, students were found to carry a good amount of knowledge on bio-diversity (average score being more than 70%) which reflected that there is retention of learning (Fig.3).

As such, it appeared that the learning and experience gained while interacting with live plants and animals---seeing, touching, collecting, hearing and drawing those can not be replaced by other modes of education. It was not only a joyful time but also a memorable learning experience. Following inquiry approach, students learn and understand the concepts through active involvement in collection of information from primary as well as secondary sources (Dash, 2013). Thus they constructed their own knowledge (Bhaskar and Sivakumar, 2011) on the importance of biodiversity as natural resource and its conservation and it was further strengthened by demonstration in the laboratory followed by discussion with ICT support (Yasothapriya, 2010) in the classroom. Further, the knowledge of the outside world in the local context was linked to the concepts reflected in the textbooks. For example, studies on local biodiversity in terms of collection, identification, classification of plants, animals and their uses has
implications for understanding of units such as diversity of living organisms and conservation of natural resources that have been included in science text books at secondary level (NCERT, 2006).

Because of involvement, participation and thinking, the learning outcome becomes memorable and long lasting (Dash and Satapathy, 2003) as reflected in the present study (Fig.3). These feelings and concerns are likely to grow over the years and could strengthen the attitude of students for living organisms and wild life and favor conservation as reflected in Indian constitution, article 51(A) which laid emphasis on protection of environment as one of the fundamental duties of every citizen. Further they could remember the endangered plants and animals.

In cities, zoos maintain a collection of various living animals from different parts of the country and sometimes also other parts of the world. As students from rural schools have very limited scope to see the mega vertebrates and big animals, with little creativity and innovations, teachers can organize zoo visit to help students aware of the wonders of nature, relationships and the balance of the living world leading to influencing their attitude and change in behavior. Sometimes studies could be organized to botanical gardens, natural history museums, water bodies etc.wherever available to strengthen the school learning experience.

Though Hon’ble Supreme Court of India has made environmental education a compulsory subject at every level of school and college education, it is being taught in an inefficient manner due to lack of well trained teachers. Further being a graded subject, not much importance is given to it at the institutional level. Hence in order to generate interest, we need to actively integrate the biodiversity conservation issues in school and college curricula as the ultimate objective of EE is to formulate a responsible attitude (Rao,1999) in the young minds towards sustainable development. The development of “sharing and caring habits” along with the attitude to “ live together and allow others to live” through EE could help them to grow as responsible, broad minded, and kind hearted human beings. As such, youth and especially children who are the leaders of tomorrow in their role as green Ambassadors (Yasmeen, 2009) could be major agents of change for a sustainable future.

References

9. NCERT. Environmental Education in Schools. NCERT, New Delhi, 2004
Conservation of Biodiversity

**Live organisms**

- In-situ conservation
  - National Parks
  - Sanctuaries
  - Sacred grooves
  - Wilderness
  - Biosphere reserves

**Dead organisms**

- Ex-situ conservation
  - Gene Banks
  - Bio Banks
  - Museums
  - Herbarium
  - Seed bank
  - Embryo bank
  - Sperm bank
  - Organ bank

**Preservation**

- by
  - Gene Banks includes Botanical garden
  - Bio Banks includes Zoological park
  - Aquaria

**Materials for Industry**

**Ecosystem functions**

- Food and Fodder
- Nutrients
- Pollutants
- Soils erosion
- Ground water

Genetic support for crop & animal improvement

Fig. 1: Concept Map showing major utilitarian values of Biodiversity.

Fig. 2: Concept Map reflecting different approaches followed for Conservation of Biodiversity.
Fig. 3: Average Achievement Scores (Pre-test and Post-test) on Teaching of Biodiversity.

Table 1: Plant/Animal group with a total number of species so far Described from India

<table>
<thead>
<tr>
<th>Plant/Animal group</th>
<th>Total number of species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>850</td>
</tr>
<tr>
<td>Algae</td>
<td>6500</td>
</tr>
<tr>
<td>Fungi</td>
<td>14500</td>
</tr>
<tr>
<td>Lichen</td>
<td>2000</td>
</tr>
<tr>
<td>Bryophytes</td>
<td>2850</td>
</tr>
<tr>
<td>Pteridophytes</td>
<td>1100</td>
</tr>
<tr>
<td>Gymnosperms</td>
<td>64</td>
</tr>
<tr>
<td>Flowering plants</td>
<td>17500</td>
</tr>
<tr>
<td>Protista</td>
<td>2577</td>
</tr>
<tr>
<td>Other invertebrates</td>
<td>8329</td>
</tr>
<tr>
<td>Arthropods</td>
<td>60383</td>
</tr>
<tr>
<td>Mollusca</td>
<td>5050</td>
</tr>
<tr>
<td>Protochordata</td>
<td>166</td>
</tr>
<tr>
<td>Fishes</td>
<td>2546</td>
</tr>
<tr>
<td>Amphibia</td>
<td>206</td>
</tr>
<tr>
<td>Reptiles</td>
<td>485</td>
</tr>
<tr>
<td>Birds</td>
<td>1228</td>
</tr>
<tr>
<td>Mammals</td>
<td>372</td>
</tr>
</tbody>
</table>
Table 2: Plant/Plant parts collected by the students along with their uses as noted by them along with remark from the teacher, if any

<table>
<thead>
<tr>
<th>Common Name of the plant</th>
<th>Plant part collected</th>
<th>Uses</th>
<th>Remark from the teacher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casuarina</td>
<td>Branch/leaf</td>
<td>Firewood</td>
<td>Root helps in soil formation</td>
</tr>
<tr>
<td>Coconut</td>
<td>Small fruit</td>
<td>Food, source of oil</td>
<td>Leaves for thatching</td>
</tr>
<tr>
<td>Papaya</td>
<td>Leaf</td>
<td>Fruit as vegetable</td>
<td></td>
</tr>
<tr>
<td>Tagetes</td>
<td>Flower</td>
<td>Ornamental</td>
<td></td>
</tr>
<tr>
<td>Tridax</td>
<td>Leaf</td>
<td>Medicinal use</td>
<td></td>
</tr>
<tr>
<td>Mutha grass (Cyperus)</td>
<td>Plant</td>
<td>Cattle fodder</td>
<td>Medicinal rake</td>
</tr>
<tr>
<td>Mushroom (Nadi chhatu)</td>
<td>Fruity body</td>
<td>Vegetables</td>
<td></td>
</tr>
<tr>
<td>Fern plant</td>
<td>Leaf</td>
<td>Ornamental purpose</td>
<td></td>
</tr>
<tr>
<td>Algae (Spirogyra)</td>
<td>Thallus</td>
<td>Food for fish</td>
<td>Manure</td>
</tr>
<tr>
<td>Moss plant</td>
<td>Entire plant</td>
<td>Soil formation</td>
<td></td>
</tr>
<tr>
<td>Betelnut</td>
<td>Fruit</td>
<td>Mastication</td>
<td></td>
</tr>
<tr>
<td>Kansir (Commelina)</td>
<td>Leaf</td>
<td>Leaf vegetable</td>
<td>Medicinal</td>
</tr>
<tr>
<td>Banana</td>
<td>Leaf</td>
<td>Fruit as vegetable</td>
<td>Leaf as plates</td>
</tr>
<tr>
<td>Ginger</td>
<td>Rhizome</td>
<td>Spices</td>
<td></td>
</tr>
<tr>
<td>Bougainvillea</td>
<td>Flower</td>
<td>Ornamental</td>
<td></td>
</tr>
<tr>
<td>China rose</td>
<td>Flower</td>
<td>Ornamental</td>
<td></td>
</tr>
<tr>
<td>Pokasungia</td>
<td>Entire plant</td>
<td>Ornamental</td>
<td>Breed</td>
</tr>
<tr>
<td>Capsicum</td>
<td>Fruit</td>
<td>Spices</td>
<td></td>
</tr>
<tr>
<td>Pumpkin</td>
<td>Leaves</td>
<td>Fruits as vegetables</td>
<td>Green leave as vegetables</td>
</tr>
<tr>
<td>Pistia</td>
<td>Entire Plant</td>
<td>Manure</td>
<td></td>
</tr>
<tr>
<td>Tulsi</td>
<td>Leaf</td>
<td>Used for Medical and religious functions</td>
<td></td>
</tr>
<tr>
<td>Mango</td>
<td>Leaf</td>
<td>Fruits are eaten</td>
<td>Firewood</td>
</tr>
</tbody>
</table>
Table 3: Animals collected from school garden and their uses noted by students.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Usefulness</th>
<th>Remarks from the Teacher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ant</td>
<td>Eat upon dead insects</td>
<td>Scavenger</td>
</tr>
<tr>
<td>Termite</td>
<td>Eats the wooden parts</td>
<td>Cleans old wood and dried leaves</td>
</tr>
<tr>
<td>Earthworm</td>
<td>Soil fertility</td>
<td>Decompose dead plants/ organisms</td>
</tr>
<tr>
<td>Snail</td>
<td>Eaten by ducks</td>
<td></td>
</tr>
<tr>
<td>Frog</td>
<td>Controls insects</td>
<td>Food for snake in food chain</td>
</tr>
<tr>
<td>Cockroach</td>
<td>Eats waste vegetables</td>
<td>Scavenger</td>
</tr>
<tr>
<td>Pigeon</td>
<td>Ornamental bird</td>
<td></td>
</tr>
<tr>
<td>Butterfly</td>
<td>Beautiful to look</td>
<td>Pollination</td>
</tr>
<tr>
<td>Black spider</td>
<td>....</td>
<td>Insect control (Bio-control)</td>
</tr>
<tr>
<td>Mosquito</td>
<td>Causes Malaria</td>
<td>Larva acts as food for fishes (food chain)</td>
</tr>
<tr>
<td>Dung beetle</td>
<td>Make drug ball</td>
<td>Prevent decomposition</td>
</tr>
<tr>
<td>Honeybee</td>
<td>Provides honey</td>
<td>Pollination</td>
</tr>
<tr>
<td>Grasshopper</td>
<td>Eats grass</td>
<td>Act as food for frogs and birds</td>
</tr>
<tr>
<td>Water bugs</td>
<td>....</td>
<td>Eat small fishes</td>
</tr>
<tr>
<td>Dragon fly</td>
<td>Ornamental</td>
<td>Eats small insects</td>
</tr>
<tr>
<td>Garden centipede</td>
<td>--</td>
<td>Kills plant roof</td>
</tr>
<tr>
<td>Mealy bugs</td>
<td>--</td>
<td>Eats on plants</td>
</tr>
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</table>
GIS BASE MAPPING OF SPATIO-TEMPORAL DISTRIBUTION OF HEALTHCARE SERVICES IN NASIK DISTRICT, (MAHARASHTRA) INDIA

Abstract:
The study of geographical aspects of disease, nutrition and healthcare services are the part of geography of health. In India, some geographers carried out the work in the field of disease ecology, nutrition, environment & health and healthcare services. Akhtar (1978) have examined the spatial distribution and growth of healthcare services in Rajasthan and Maharashtra respectively. For the study of GIS base mapping of spatio-temporal distribution of healthcare services at micro level in Nasik district of Maharashtra state is selected as a study area. The time span taken into account for this study is from 1970-71 to 2010-11. Data is acquired from the socio-economic abstract of the Nasik district. Variables like number of hospitals, dispensaries, maternity homes, P.H.Gs., doctors, nurses and beds are taken into consideration by calculating the average. It is found that at the beginning of the study period healthcare services in the Nasik district were meager, and in tribal part they are very rare. There is a huge disparity in western part. Planning and rational decision should be required to achieve the balanced of development of healthcare facility in the Nasik district.

Introduction
Regional disparity becomes prominent characteristics in Indian economy and development. Healthcare sector not remain exception for that. Healthcare in India is the responsibility of constituent states and territories of India. The constitution charges every state with "raising of the level of nutrition and the standard of living of its people and the improvement of public health as among its primary duties". The National health policy was endorsed by the Parliament of India in 1983 and updated in 2002. The healthcare services in any location at which medicine are practiced regularly. Medical services range from small clinics and doctor's offices to urgent care centers and large hospitals with elaborate emergency rooms and trauma centers. After independence lot of efforts have been made to develop the network of healthcare services in India. But initially the resources were very few, so only few and largely populated settlements were targeted. Suryawanshi (2002) have studied the spatial patterns of healthcare services at tribal and rural level in Maharashtra. In the booklet published by National Coordination Committee, Jan Swasthya Abhiyan, India, the details of public health system, in India was discussed. This work is focused on the distribution and development of healthcare services in tribal and non tribal part of Nasik district. The main objectives of the present paper are as follows,

I. To find out the present distribution of healthcare services in Nasik District.
II. To identify the regional disparities of healthcare services in the study region.
III. To understand the factors responsible for the imbalances of healthcare services in the district.

Study Region
Nasik district is situated in Tapi and Godavari basin. It lies between 19° 45’ to 20° 45’ north latitude and 73° 30’ to 74° 45’ east Longitude (Fig. No.1). There are 15 tahsils included in the Nasik District. The main system of hills is the Sahyadries, which run from north- south in the western part of the district. All spurs of Sahyadrian range stretch out to the east. Area between the spurs and the eastern part of the district is comparatively plain. Godavari, Girna are the major rivers were draining the area. 23 % of total district population is belong to schedule tribe and it is mainly concentrated in the western hilly part. According to the data of 2011 census, the district population is around 61.1 million. About 43 % population is living in urban areas, Sex ratio is 927 female per 1000 male and literacy is about 80%.
Material and Methodology
The present study is based on the secondary source of data. Secondary data obtained from socio-economic abstract of the Nasik district (1971 & 2011), District census handbook, District Gazetteers and different websites of internet. The Tahsil has been taken as a unit for analysis of the levels of healthcare services in the study region. Statistical technique like percentage and average has been used for study. Data is processed and represented with the GIS Ilwis software. The distribution of healthcare services has been determined on the basic of seven variables like number of hospitals, Dispensaries, Maternity homes, Primary health centers, Doctors, Nurses, and Beds etc.

Results and Discussion
There are seven variables are selected for determine the level of development in government healthcare services in the study region. Table no. 1 is showing the change in different govt. healthcare services from 1970-71 to 2010-11

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Source: Socio-economic Abstract of Nasik District-1971-71, 2010-11 *Newly formed Tahsil
Hospitals
This is an important variable to determine the levels of healthcare development in the region. A hospital is an institution for healthcare providing patient treatment by specialized staff and equipment, and often, but not always providing for longer-term patient stays. In 2010-11, Number of hospitals are highest in Nasik tahsil (23.26 %) & Malegaon tahsil (16.28 %), whereas it is low in other tahsil ranging from 2.33% to 7.0% of the total hospitals in the districts. Western tribal part of the study region is having less hospitals due to rugged topography, steep slope and tribal nature. It is also found that total number of hospitals are increase from 3 in 1971 (2 in Nasik and 1 in Malegaon) to 43 in 2011. This is great achievement but this growth is not uniform. It is slow in many tahsil, only Nasik & Malegaon tahsil records good increase in the number of hospitals. It is because of two major cities of the district are located in these tahsil and population concentration is also high. Comparison of tribal and non tribal region shows that in both the region the growth is uniform. In both the case the increase of hospital facility is same. (93%).

Dispensaries
A dispensary is an office in a school, hospital or other organization that dispenses medications and medical supplies. In a traditional dispensary set-up a pharmacist dispenses medication as per prescription or order form. The development of the dispensaries are showing decrease in numbers as campier to the hospitals. There are 37 dispensaries in 1971 which were decreases to 28 in 2011. The main reason is that many dispensaries are converted in to hospitals so the numbers of hospitals are increase considerably and numbers of dispensaries were decrease. Nasik, Malegaon, Baglan and tribal tahsils like Dindori, Sinner, and Igatpuri shows good increase. Whereas this development is very poor in tribal tahsils like Peth, Surgana, Trambak. Only one new dispensary is open in these 3 tahsil in the span of 40 years. This is again because of social, economical, political backwardness of these tahsils and the policy of government is to promote hospitals and P.H.C. 20 percent tahsils of Nasik district are tribal but they have only 11 percent dispensaries.
Maternity Homes
This is one of the important medical facility, and also one of the criteria of determining the healthcare development of the region. Table no.1 shows that in 1970 there are only 25 maternity homes in the region and in 2011, their number increases to 323.

Campier to the growth of hospitals and dispensaries the development of maternity home is more widespread and balanced. The proportions of maternity homes are more in tahsil like Nasik, Baglan, Malegaon and Niphad. Whereas Chandwad, Nandgaon, Sinnar, Yeola, Trambak and Deola tahsil are yet have a scope to increase the numbers of maternity homes. Most of these tahsil are drought prone. Tribal tahsil like Peth, Dindori shows good increase in maternity homes. Interesting fact is that, the increase in maternity homes is more in tribal tahsil than the non tribal tahsil. This is good achievement of the government. It indicates that government is more focusing on tribal areas.

Primary Health Centers
Primary health centers are the cornerstone of rural healthcare. Primary health centers and their sub-centers are supposed to meet the healthcare needs of rural population. Nasik district shows phenomenal growth in P.H.Cs. There are 18 in 1971 to 103 in 2010.

The speed of this growth is rapid in many tahsil except Nasik, Chandwad, Yeola, Trambak and Deola. The reason behind this, slow increase in the numbers of P.H.C. is different in these tahsil. Nasik has slowest development rate in this regard, because it have more urban population and P.H.Cs. are mainly for rural population. Yeola, Nandgaon, Chandwad are drought prone and less populated region. The numbers of P.H.C. are less. Whereas Deola and Trambak are new formed tahsil, so they have less number of P.H.Cs. Tribal P.H.Cs. had increase from 8 to 57 in this span. This 86 % increase is superficially looked very impressive, but if we consider a fact that, there are only 8 P.H.Cs of 5 tribal tahsil in 1971, 6 tribal tahsil added 49 P.H.Cs. in 40 years span means the rate of P.H.C. establishment in tribal part is 1 P.H.C. per tribal tahsil per 5 years span. The role of P.H.C. is most important in tribal health because 20 percent of tribal tahsil have 57 percent of district P.H.C.
Doctors
This is again important facture in the development of healthcare facilities. In the district numbers of doctors are increases from 113 to 570. Nasik is at the top in this case. 28% of doctors are located in this tahsil. Peth, Kalwan, are also shows good increase in this regard. Trambak is much behind of the district average. In 1971, 30 percent doctors were serving in tribal region, but inverse to expectation this proportion is decrease by 1 percent in 2011. This is showing the unwillingness of doctors to serve in tribal area and second think is that administration is lacking the policy to increase number of doctors in tribal part.

Nurses
Here also Nasik tahsil is at the top and having 42 % of the total nurses in the district. At district level number of nurses are increases from 399 to 1777. The growth of this element is more uniform then some of the above mention variables. Only Deola and Trambak are well behind to other tahsil in this regard, because they are recently formed.

Beds
This facility is important for indoor patients. Any region which is having a sufficient amount of beds, consider as a well developed in health care facilities. In case of Nasik district number of beds increases from 654 in 1971 to 4915 in 2011. This increase is more than 8 times. Nasik tahsil have 50 % of beds followed by Igatpuri and Malegaon. Other tahsil have the number of beds in the range of 100 to 300, means little less than average. Only Deola and Trambak have very less number of beds.
Conclusions
1. There is remarkable increase in every healthcare service in the period of 40 years
2. The spatio-temporal distribution of healthcare facilities in the study region is not uniform.
3. Highly populated and politically strong tahsil like Nasik and Malegaon having large number of healthcare facilities than others.
4. Western hilly, comparatively inaccessible and tribal tahsil like Surgana, Igatpuri, Trambak, Kalwan and Trambak are showing slow progress in the healthcare services.
5. Proportion of Doctors is decreases in western part of study region due to unwillingness of doctors to serve in tribal area. And administration is lacking the policy of increasing the number of doctors in tribal part.

Recommendations
1. Tribal and hilly tahsil like Peth, Surgana, Kalwan, and Trambak should be provided more healthcare services, because these tahsil have large proportion of schedule tribe population, they are economically backward and many people can’t afford the private medical facilities.
2. Government should strictly implement the policy of compulsion of 2 years of service in rural area to newly passed doctors. So that the deficit of doctors in western part will be fulfill.
3. Decision about the establishment of any healthcare service is not should be affect by any political interferes and proper policy should be prepare in this regard.
4. As the private medical facilities are fevers the populated and economically developed area like Nasik and Malegaon city, more incentives and facilities should be given to private medical practitioners to provide these services in rural area.

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Abstract:
Vikrant had been a devoted army sepoy. Being a Rajput from Rajasthan, the commitment and chivalry was exemplary. However, life took a different turn for him when he found himself under stress and delusion, which was caused by his wife’s fidelity. Vikrant could not bear the brunt of his personal life being ruined and rushed to death in haste. The focus of the case is stress (not related to work /organization), self-esteem and its effect on an individual. A number of organizational aspects would also be brought out in the study. A stressful event(s) is any change in the environment that typically induces a high degree of unpleasant emotion such as anxiety, guilt or shame and affects normal patterns of information processing. It came out to Vikrant that soldiering is stressful and evidences exist regarding the stress consequence in Indian soldiers. The case purport to take evidences to foresee a stress epidemic in the Indian Armed Forces or is there any evidence of increasing stress related illnesses in the last decade. Despite a number of welfare measures institutionalized in the last decade little seem to have an effect in containing the trend.

Introduction
Sepoy Vikrant Singh was a Rajput from Barmer district of Rajasthan. This district has significantly and traditionally contributed many of its sons to the Indian Army and his unit was no exception having a sizeable proportion of soldiers from this region. Sep. Vikrant Singh was a good soldier and an excellent sportsman. He was of quiet nature and fond of reading and studies. He had been a likeable person. He stood first in the inter-unit Marathon Race Competition in February 2007. He met with a girl named Veena in March 2008 during leave at Jodhpur and got enamored with her due to her ‘forwardness’. Though they belonged to same community from Rajasthan but her grandparents had moved out from rural areas of Rajasthan and started living in cities and towns. Her father was working in a PSU Bank. The family had adopted urban ways of living, and followed a mix of traditional and modern value system. They gave good education to their children and encouraged their children even girls to mingle freely with everyone, though within limits. Veena was the second child of four children and had studied in co-ed school. She was a good looking girl with an extrovert and trusting nature. She was a popular student in her school and college due to her pleasing disposition, positive attitude and good manners.

After much consoling, Vikrant married the girl on July, 12th, 2009. After the marriage, the young couple stayed together for two months. Like his elder brother, he had to leave his wife with his parents; she did not protest and quickly endeared herself to everyone in the family. She treated his younger brother as her own brother and started helping him in his studies.

After the marriage, Vikrant came home on leave twice. He had returned to unit after a fortnight’s leave in Feb 2010. However, this time, it was noticed that he had become quiet and withdrawn since his return and had not responded to queries about well-being of his family. He just mentioned that he was not feeling well. People assumed he was homesick and missing his wife as he had applied for a few days casual leave again for a week in March, which had been sanctioned by the sub-unit commander but he could not go on leave due to training commitments in the unit, and committed suicide on 11 March 2013.

Triggering effect
Hailing from a rural background, Sepoy Vikrant Singh got married to a modern woman in July 2009. She was an extrovert. But Sepoy Vikrant Singh could not cope with the openness and frank behavior of his wife with the opposite sex. In February 2010 when he had proceeded on leave, he got suspicious of his wife’s closeness to her brother-in-law. A trivial action of his wife had sown suspicion in his mind. Thereafter, he had started inquiring about her college days and background. Due to her free and frank nature, Vikrant felt his wife had loose morals. Despite her denials, he used to brood over each and every action of hers and relate it to her having illicit relations with her brother-in-law. He did not publish his marriage Part II Order or change the nomination of his ‘Will’ in her name. He used to pen down his conflicting thoughts, questions and justifications in every spare time he got. Being from the proud Rajput caste he could not share his agony with his peers or superiors. On the fateful night, in his last phone call to her, he wanted a final admission of her relations with her paramour and a promise from her to break it off, which never came. He decided to pull the trigger in haste!!
Breaking Point– An Overview

It had been a very delightful and satisfying evening albeit a very late one for Col IM Smart, Commanding Officer of an army unit. He had just retired to bed after an interactive function which was a follow up of a successful culmination of a training event in his unit. He was informed at about midnight by his Officer, “Sir, Sepoy Vikrant Singh has shot himself”.

He rushed to the spot where the ghastly incident had occurred. Sepoy Vikrant Singh was lying dead in a pool of blood with his rifle beside him near the southern guard post of the building. The Military Police and civil Police also had arrived and had started the investigations.

Sepoy Vikrant Singh was on duty from 0001h to 0200h along with Sepoy A B Chandra. Sepoy Chandra said that he had seen Vikrant Singh only a few moments back during change-over of guard and had not found anything wrong. He mentioned he had definitely seen Vikrant Singh speaking on the mobile phone while patrolling. At about 0010h, he had suddenly heard a gunshot and had rushed to the site to find Vikrant Singh lying in a pool of blood.

The Joint family of Sep Vikrant Singh comprised of his grandparents, parents, one elder and one younger brother and a sister younger to him. His father was a farmer, elder brother serving in a Rajput Battalion and younger brother studying. His elder brother was married in a Rajput family, the match arranged by his grandparents. The wife of elder brother was staying in-laws and did not accompany her husband. The sister was educated up to class six and then married. The family had limited exposures to modern values and were rigid in following traditions and put “Honour & Prestige” of the Family above everything else.

The early education of Sep. Vikrant Singh was from a school in his village till class 10th. He did his Class XII from Barmer staying in a hostel. Sep. Vikrant Singh was of sober habits and got a limited exposure to urban life before he joined the Army on 15 March 2005.

Status Report

A court of Inquiry was ordered to investigate the incident. The Presiding Officer started interacting with various people, firstly to ascertain if there was any foul play which was not the case he found. Simultaneously, he started to investigate into the circumstances which may have led to the suicide by Vikrant including the organizational climate, Standing Operating Procedures, of the unit which had some lacunae, he felt. He also found that the guard was not homogenous, i.e. from the same sub-unit but a mix of personnel from all the other subunits.

Vikrant had not been interviewed by the Sub-unit Commander or the Commanding Officer prior to going on leave or on return. Linking the statements of his best buddy and the data of profusion of late night calls by Vikrant on the mobile phone to his wife, the last being about fifteen minutes before his pulling the trigger, Officer conducting the Inquiry concluded something was amiss. He grilled all the persons in the chain of command but was stymied to find there was no information about his marital relations. Even his best buddy who had attended his marriage was unaware of any discord. He had been getting leave as per his choice and requirements. But the Sub-unit Commander had noted that lately the performance of Vikrant Singh had dropped down. He had come 10th in the Inter Unit Marathon race Competition. Also, he had not performed so well in the Promotion Examination despite being studious. The subunit Clerk also mentioned that Vikrant had been dilly dallying with his marriage documentation and had not given the necessary photos and details for publishing the ‘Will’ almost for six months. Therefore, prior to Vikrant departure for a fortnight’s leave in the previous month, he had pointed this to the Sub-unit commander who in turn had instructed Vikrant to do the needful work before coming back from leave. The ‘Will’ was finally published on Vikrant return.

Luckily, for the Investigating Officer, a few days later one person came to meet him. He turned out to be a soldier from Vikrant’s village who was posted to another unit in the same station. He narrated that there was some marital discord between Vikrant and his wife as she was ‘modern’. He had inquired about it from him but no response was forthcoming. The soldier mentioned that Vikrant was fond of writing and used to maintain a diary in which he used to pen down his daily life. The Investigating Officer immediately ordered search for the missing diary. A search of his personal belongings yielded his personal diary, on flicking through them, he found himself staring at heart rending pages filled with Vikrant’s personal mental and emotional turmoil, torment and agony. Vikrant Singh was tortured by suspicions about his wife’s fidelity soon after his marriage which had manifested in outpourings in the register during the free hours.
Table #1
1. 10 May 2003 - Sepoy Vikrant Singh joins XX Regt from Centre.
2. 03 Jan 2007 - Sepoy Vikrant Singh comes first in Inter-unit Marathon race Competition.
3. 14 Mar 2008 - Meets Ms Veena in Jodhpur during leave and gets enamored with her due to her ‘forwardness’ being from an urban family.
4. 12 Jul 2009 - Gets married to Ms Veena despite counsel from his family side, they being from a rural background.
5. 03 Oct 2009 - Proceeds on leave. Gets suspicious of his wife’s closeness to her brother-in-law. Starts inquiring about her college days and background. Does not publish marriage Part II Order or change his ‘Will’ on return.
6. 04 Jan 2010 - Comes 10th in the Inter unit Marathon race Competition. Also does not perform so well in the Promotion Cadre despite being studious.
7. 18 Feb 2010 - Proceeds on leave again. Returns back. Becomes slightly reclusive but still does not express any stress. Gets his marriage Pt II Order published and ‘Will’ nomination changed.
8. 09 Mar 2010 - Senior Officer’s visit to Station. Unit very busy. Sepoy Vikrant Singh detailed for Guard Duty.
9. 11 Mar 2010 - Special Barakhana in the unit. Sepoy Vikrant Singh commits suicide.

References
Case Synopsis
The case talks about Vikrant Singh, an Indian army Sepoy who committed suicide due to stress. Most stress situations, we meet in daily life are minor and easy to cope with. However, at times we face highly stressful situations or accumulated stresses, which are difficult to cope with and result in strain, primarily on the mind. In some individuals, this strain crosses the threshold of tolerance, and they face a breakdown. For personnel in the Armed Forces, stress is of special significance, as a soldier has to confront stress at all times in some form or the other. To this end, the deeper study into the various facets of stress will assist commanders at all levels to understand this psychological factor of human relations. Another factor is the organizational behavior, which plays a major factor in prevention of such kinds of situation.

It was under prolonged stress that Vikrant's response to stress of one type or another resulted in his death. He also had begun behaving illogically and irrationally. To this end, the deeper study into the various facets of stress will assist Leaders, at all levels to understand this psychological factor of human relations. Another factor is the organizational behavior, which plays a major factor in prevention in such kinds of situation. With the help of a case study, this article tries to understand the effect of intrapersonal conflict on performance and behavior pattern, relationship between stresses at home and its psychosocial aspects and systematic analysis of the stress factors to arrive at the root cause of the manifested behavior/attitude.

Learning objectives
a) Effect of intrapersonal conflict on performance and behavior pattern,
b) Relationship between stress at home and its psychosocial aspects,
c) Systematic analysis of the stress factors to arrive at the root cause of the manifested behavior/attitude,
d) Some organizational factors affecting stress.

Intended Courses/Theories
a) Organizational Behavior
b) Human Resources and NLP
c) Psychology and Studies of Human Relations School

Discussion Questions
1. What are the various levels of conflicts an army personnel has to bear within his army life?
2. What are the sources of stress? How could Vikrant cope with such a stress?
3. Explain the optimum stress level in the case of Sepoy Vikrant Singh?
4. What is the relevance of Self Esteem and Social needs with Stress?

Teaching Plan
The case must be handed over to scholars a week in advance with a request to study and develop a deeper understanding on entrepreneurial process, Pharmaceutical industry, innovation and Strategy for Start-ups.

1. (05 min): Instructor-About Case
2. (15 min): Synopsis (Pick Sample 2-3 from Group)
3. (5 min): Instructor-Distribution of groups and Identifying Syndicate Rooms based on Specialisation mix
4. (10 min): Comparing Learning/objectives by individually attending the groups
5. (05 min): Instructor-intervention on Learning process
6. (10 min): Case Chronology (Open Discussions based on group)
7. (10 min): Learning outcome (find out from each group, then discuss in class)
8. (30 min)- Instructor- Discussion on Imbroglio by giving one option to each group
9. (20min)- Responses by each group, slide 5-6 points; 3 minutes each with query session
10. (30min) - Instructor- Brief over view of responses by groups and relaying theory

Discussion Questions
1. What are the various levels of conflicts an army personnel has to bear within his army life?

Truly a conflict represents a gap, a disagreement, contradiction, and incompatibility or, a tense situation created, by the complications in one's life, and partially due, to organizational apathy. Conflict is an inevitable part, of individual and, organizational life having divergent personalities, perceptions, and values. Conflict can, be at three different levels. An intrapersonal conflict occurs within the individual. It normally occurs, when there is a disagreement between how a person feels about his/her behavior versus how they really act. An interpersonal conflict is experienced between individuals in the same location, e.g., co-workers, team, members or as in this case between the husband and wife. It exists, whenever people interact or come together to accomplish, a common goal or objective. However their background, personality and experiences, being different, may make attainment of the goal difficult. An intra-group conflict is, defined as conflict between groups in, the same organization, or command. It occurs when ever, there is contact or interaction between groups. Issues of group cohesion, "sticking together, leadership and status, power or influence and lack of or limited resources" often cause sources of inter group conflict. Lack of goal clarity or intense competition may cause intra-group conflict. This was however absent in this case. Conflict is a, natural condition of human, life and one of the states, of dynamic human personality. A state of harmony is the ideal state. The taxonomy, of conflict as shown below depicts, the conflict to apathy continuum.

During the, analysis of conflict, the first step, is to recognize the level of conflict, from where will, flow out the further course of resolution. Vikrant had been facing with an intrapersonal conflict. Intrapersonal conflict is purely internal, to a person and is, probably the most difficult, type of conflict to analyze. All of us have needs. These needs form, the basis for behavior at work, at home, and every activity we pursue. Intrapersonal conflict arising, due to unfulfilled, expectations, colored, perception and hurt to self-esteem and, image increases the stress level. Also, the three types, of roles, namely, the expected, the perceived and the, actual role can result in role conflict. Individual confronted with role conflict will often experience psychological stress, leading to emotional problems. More often than not, It will be latent stress, which will not be very overtly visible.

Other issues connected with intrapersonal conflict are:-
(a) When there is a threat to a person’s values.
(b) Feeling of unfair treatment, which could be from anybody.
(c) Multiple and contradictory social commitments.
(d) Related to the Theory of Cognitive Dissonance.

Any individual confronted, with role conflict will experience, psychological stress leading to emotional problems. Role conflicts can have a markedly adverse impact on satisfaction, and even on mental or physical health. Sepoy Vikrant Singh was under tremendous stress due to umpteen number of mixed thoughts, which, were created in his mind by his own internal conflicts that he was battling alone throughout. Sepoy Vikrant Singh’s conflict started, with his own personality, deterioration. He was in a total turmoil with his own self as regards his wife and his suspicion of her having illicit relations based on his perception. Hence his personality had overtones of his family life. He displayed the typical character of an early achiever with good performance on the course, and had reached a high satisfaction level pre-marriage, but due to latent stress he was undergoing on his family front along with organizational apathy, he rapidly degenerated on the professional front as well. But his personal emotional turmoil was so much bottled up inside him that he could not take it and committed suicide.
2. What are the sources of stress? How could Vikrant cope with such a stress?

Stressors, related to personality factors, vary widely. Various dimensions of vulnerability form the characteristics, for enduring the personality. These are affected, by genetic, developmental, and social influences. Vulnerability also changes, from day-to-day, mood and individual experiences, in life. One’s perception of the expectations held of him, and one’s perceived ability to control the situation are, important factors in understanding, how life events are interpreted, and what reaction, and coping mechanisms are evoked. In the case of Vikrant Singh, this was very evidently demonstrative in his changed behavior and attitude over a period of only one year that of his marriage. While he wanted to marry a modern woman, he could not cope with the openness and frank behavior of his wife with the opposite sex. These resulted in his subdued performance and rapid drifting towards aloofness, quiet behavior and preference for solitude. His only emotional outlet was penning down his mental conflict and reasoning with self about the correctness of his wife’s attitude.

Frustration is a result, of the thwarting of a motive, either by some obstacles which block our progress towards a desired goal or it can arise by delays lack of resources, and failure or denial of individual needs. Frustration can also occur due to personality traits which prevent an individual from bringing, issues troubling him out in the open. Sepoy Vikrant Singh continued to suffer frustration, as he could not openly question his wife despite being doubtful, (which he ultimately did later just before he committed suicide). He could not speak about his suspicions to his family members as he had defied their sentiments and married against their wishes. Being a Rajput, he could not discuss this sensitive issue with his friends, peers and superiors in the unit, fearing that he would become a laughing stock in the unit.

3. Explain the optimum stress level in the case of Sepoy Vikrant Singh?

When the environment exerts a strain and acts, like a stressor as, in case of Sepoy Vikrant Singh, it poses a challenge, for adjustment. These challenges, can result in both positive and negative forms of stress, being experienced as the individual, tries to cope with these. To understand what would constitute an optimum level of positive form of stress, it would be, best to refer to Yerkes-Dodson Model shown below:

All human reactions are in response to, stress of one type or the other. Stress is, necessary for everyone to function satisfactorily. Normally we, perform best at optimum stress level as shown above. Stress at a very low, level results in zone of, boredom leading to the, decay, of our, psychological system while, a high stress level results in zone of, distress leading to accumulation of fatigue that later manifests itself in the form of physiological or psychological problems. In the initial stages of his service till he got married, Sepoy Vikrant Singh displayed high degree of enthusiasm and interest in whatever job was assigned to him and took active part in various activities of the unit. It was quite evident that he was in the ‘Zone of optimal stress’ in the initial stages. However when he was going through the personal stresses post his marriage saw the sudden downfall to a higher stress level. The manifestation of the said stress was so latent due to the impact of the cognitive dissonance that it was not recognized by the peer group and the relevant personnel present during the stage.

4. What is the relevance of Self Esteem and Social needs with Stress?

The needs are arranged, in a hierarchy of importance. In case of Sepoy Vikrant Singh, he was initially striving to be a satisfied man when he got married and was happy with his ‘modern’ wife. However his conflict with own self began this stage when he suspected his wife of some illicit relationship with her brother-in-law, and the issue got further cemented by Halo Effect. He could never transit to higher need level and remained engrossed in his lower order needs only. His ego was always hurting him and staying aloof, not publishing his marriage documents were adopted by him as a defence mechanism to safeguard the ego.
As per Hans Seyle there are three stages in Coping Behavior as per General Adaptation Syndrome, namely Alarm Stage, Resistance stage & Exhaustion Stager? Explain the symptoms of three stages in the case of Sep Vikrant Singh. Coping behavior as per GAS (General Adaptation Syndrome) Theory by Hans Seyle and shown, below is pertinent to, understand the complexities of Sepoy Vikrant Singh’s behavior and actions. Vikrant Singh, due to high degree of latent stress, initially became agitated and started investigating his wife’s antecedents and present movements. Then he withdrew into his shell and there was a dip in his performance. This is typical of the Alarm stage. In the Resistance Stage, he tried to fight with his wife, tried to establish proof, did not publish his DO Part II Orders and Will. Very rapidly due to no support, empathy from anyone, he moved from the resistance stage to the stage of collapse. Due to the feeling of utter helplessness, frustration, and finally exhaustion, he ended his life by shooting himself.

5. What were the changes in the behavior of Sep Vikrant Singh due to Stress?

There is, a need, to monitor the, existing stress, level and carry, out review periodically. In the case of Sepoy Vikrant Singh it can be inferred that he had experienced high emotional stress, which affected his psyche and had distorted his perceptions and responses. But due the sensitive nature of his problem, he could never share his grief with anyone. This stressful situation made him resort to behavior akin to withdrawal. Had the monitoring been done earlier, the management would have been that much better. Peer groups can play an important role in stress identification as also subunit Commander/ immediate superiors.

6. What is the role of Organisation’s Climate & social support System in containing Stress levels?

It allows the person, to cope successfully, with the inevitable stress and continue, performing efficiently. When the well-established, emotional climate of any, unit/ organization reflects genuine, concern for the legitimate needs, of its people and shows regard for its men, the individual values blend, successfully with the purpose, of the organization despite, having their own problems. The same is more, relevant for any military establishments. In the instant case, when Sepoy Vikrant Singh started showing some signs of changed behavior and drop in performance, it should have sent out the desired signals for corrective efforts. As confirmed by investigations later, the interview system as laid down in standing orders was not being followed by the unit wherein the officers could discuss sensitive issues with their men. Also, despite the sub unit being aware of the changes in his behavioral pattern still allowed him to undertake duties at isolated posts where he used to brood more. Normally, any armed and isolated duty is carried out by a homogenous group such as a section, platoon from the same Company/ subunit wherein everyone knows each other well. In this case, Guard duty was being performed by personnel from different subunits. Usually, a supportive, social, relationship with, seniors, colleagues, and subordinates, at work, will directly reduce levels, of perceived stress. Supportive, and helpful, peer group, are less likely, to create, intrapersonal, pressures. Such support, makes people feel more positively, about themselves and their work, and alters our perceptions of potentiality, threatening events.

Though psychological, investigation may be, the, forte of experts, the routine man management, skills acquired in the army, coupled with experience of, handling, variety of cases, provides, enough basic knowledge, to handle many cases. The Court of Inquiry through sequential analysis, of the, case and its correlation with, the personal diary of the individual could identify, the root cause, of the problem which, was eluding everyone. An approach, focused to get involved, and solve the problem, could have lead to the crux, of the problem and maybe Sepoy Vikrant Singh’s life could have been saved.

Conclusion

The case study has, attempted to examine the, theoretical aspects of, latent stress, caused due to intrapersonal, conflict and its manifestation, though, analysis of a real life, incident, of an individual. The, aspects of severe, stress,
level which had affected, his, behavioral factors and which, ultimately led to his withdrawal, and ultimately death, has been analyzed in detail. Managing, the most important resource of defence, forces i.e. the man himself, assumes great importance, in the overall effectiveness of the organization. As a teaching model, it tends to highlight various facets, of organizational, climate and human personality development as related to onset of stress, and how to cope with, stress and the remedial measures.

The case study, in the, ultimate analysis has been aimed, to exercise the minds of, the participants on the various methods of facing stress in one’s life, to increase the optimal stress level and also to, effectively manage stress arising out of intrapersonal conflict in case of an individual which had got unnoticed and led to the tragic end of a promising young person.

The management of human resources in today's fast changing world has acquired new dimensions due to the complex interplay of social, environmental, biological and cultural factors, affecting human behavior. The family values of yester years are anachronisms today. The concept of modernity being practiced by our society has manifested itself in terms of renewed cultural beliefs, social standards, gender equality, peer pressure, interpersonal conflict and raised aspiration conflict and raised aspiration levels. The men in olive green have been hit the hardest by its effects due to peculiar service conditions. The prolonged separation, inability to be with the family at the time of distress and indifferent attitude of relatives to their genuine problems, adds to their agony. Those who are unable to cope up with this paradigm shift suffer from unbearable stress and cause untoward incidents of different kinds.

In such a situation a well administered, happy, motivated and responsive combat outfit can act as a much needed support system to mitigate stress levels. Well defined instructions and guidelines exist on the subject and problem is being tackled head on by the Armed Forces.
Exhibit #3. General Adaption Syndrome

- **Dip in performance, withdrawing to himself**
- **Investigations, Arguments, Non-publication of Part II Documents and Will**
- **Frustration, helplessness and finally suicide**

**NORMAL COPING LEVEL**

**TIME**

**GENERAL ADAPTATION SYNDROME**

**ALARM STAGE**

**STAGE OF RESISTENCE**

**EXHAUSTION STAGE**

**HOMEOSTATIC ADJUSTMENT**

**BEHAVIOUR REORGANISATION**

**DISORGANISATION**

**EXHAUSTION**

**ZONE OF BOREDOM**

**ZONE OF OPTIMUM STRESS**

**ZONE OF DISTRESS**

- Initial pose of Sepoy Vikrant before marriage
- Final stress pose of Sepoy Vikrant
ICHTHYFAUNAL DIVERSITY OF CAT FISHES (TELEOSTEI: SELURIFORMES) FROM MARATHWADA REGION, MAHARASHTRA, INDIA

Shivoji Ubarandhe 1 & Smita Sonawane 2
1Rajarshi Shahu Art’s, Commerce and Science College, Pathri Aurangabad, 2Department of Zoology, Dr. B. A. M. University, Aurangabad

Abstract:
The present paper deals with the study of Ichthyofaunal diversity of cat fishes (Teleostei:Siluriformes) from Marathwada region. The result of present observation reveals the occurrence of 15 species belonging to 09 genera, 05 family. The family bagridae was found dominant with 09 species. Family bagridae was dominant with (09 species)60 %, followed by Siluridae (02 species) 13 % and Schilbeidae (02 species)13 %, Heteropneustidae(01 species) 7 % and Clariidae with (01 species)7 % constituting of the total fish species. During the study period it was observed that Marathwada region has entire diversity of cat fishes. Many of fish species are having commercial as well as good food value but due to habitat destruction, water pollution, unregulated fishing practices, introduction of exotic species and their dominance and lack of awareness regarding fishing techniques are probably playing an important role in declining fish diversity of cat fish and need to conserve.

Introduction
“The most wonderful mystery of the life may well be the means by which it created so much diversity from so little physical matter” (Wilson, 1992).

There are about 450 families of freshwater fishes globally, about 40 represent in India (warm freshwater fishes) about 25 of these families are commercially important. Day (1878) and Hamilton (1822) were the first modern writers of Indian fishes. Osteichthyan fishes of the order Siluriformes, known by the English common name of cat fishes, are the most diverse group and play an important role on decay and feed on dead organic matter. They are basically carnivorous by habit. Live fishes are naked body often with an adipose dorsal fin and spines at the front end of the dorsal and pectoral fins. They are having barbell around the mouth that acts as sensors just like the whiskers of cat. Maharashtra is the third largest state of the Indian union, both in population and geographical area, surrounded by the Arabian Sea in the west, Andhra Pradesh in the south east, and Karnataka in the south, Gujarat in the north west and Madhya Pradesh in the north. The state has three distinct physiographical regions viz., the coastal belt (Konkan), the Western Ghats and the eastern plateau. 5 major water basins i.e. Painganga-Vardha-Vainganga, Tapi-Purna, Bhima, Godavari, & Krishna are the freshwater fish resource of Maharashtra which constitutes 6 orders, 25 families, and 160 species all under the inland water.

Marathwada region is one of the six divisions of Maharashtra state comprising of eight districts, viz. Aurangabad, Beed, Hingoli, Jalna, Latur, Nanded, Osmanabad and Parbhani. The location of Marathwada is on 19°20’ 56.76’’ E longitude and 76°14’ 44.62’’ N latitude (Google Earth, 2013) of forms the part of the vast Deccan plateau of India. The total area of Marathwada region is 64,813 km. and is bounded by Vidarba region on the north, by Andhra Pradesh on the east and south east, Karnataka on the south and by Western Maharashtra on the west.

Cat fishes have an essential role as indicator of ecological integrity of running waters. Under this concept, there is an increasing emphasis on gathering biological database to serve a broad-spectrum of environment object and plans such as the protection of endangered and threatened cat fish species of Marathwada region.

Material and Methods
To study the ichthyofauna (Cat fish) of Marathwada region from May 2006 to April 2010, fish samples were collected from six sampling sites (site I (Ambadi Dam,Aurangabad), site II (Upper Dudna Dam, Jalna), site III(Manjra Dam, Beed), site IV (Jeevrekha Dam, Jalna), site V(Yeldari Dam, Parbhani) and site VI (Vishnupuri Dam, Nanded) of five districts ( Aurangabad, Jalna, Parbhani, Nanded and Beed) which represent the ichthyofaunal composition of Marathwada region.

Fish samples were collected every week during the study period from the fish landing centers with the help of skilled local fishermen by various fishing crafts, gears with variable mesh size. Sampling points were distributed throughout the site to cover its whole area and location was changed for the collection of fish fauna according to the season.

Identification of fishes was done up to species level at fish landing center to get its natural colour, pattern of scales, fins, mouth pattern, identification marks like black spot, bloach on operculum, paired and unpaired fins and body parts with the help of standard literature by Datta Munshi and Srivastava, (1988); Hamilton (1822); Talwar and Jhingran, (1991); Francis Day vol I&II, (1986); Jayaram (1981); Jayaram, (1991); Jyotirmoy, (1999); Menon (1987); Jayaram and Jeyachandra Das, (2000); Yazdani, (1985); Menon, (1986); Jyoti and Arti Sharma (2006) and etc.
Fish species which were not identified on the field (landing center) were preserved in 10 % formalin or 5cc of formalin was injected in the belly of fish with disposable syringe and packed in polythene bags. These fish samples were brought to Fishery research laboratory, Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad for further identification.

Specimen with doubtful identifying characters was sent to Zoological Survey of India (ZSI) Pune, regional branch (ZSI) Kolkata for identification.

**Result and Discussion**

During the study period the observation revealed the occurrence of 15 species belonging to 09 genera, 05 family. The family bagridae was found dominant with 09 species. Family Bagridae was dominant with (09 species)60 % followed by Siluridae (02 species) 13 % and Schilbeidae (02 species)13 %, Heteropneustidae (01 species) 7 % and Claridae with (01 species)7 % constituting of the total fish species.

The minimum species was recorded at site I (03 species) and maximum species was recorded at Site V and VI (14 species). Species like Mystus *Bleekeri* and *Clarias batrachus* were found at all the site and found abundantly. It was also observed that site V and VI contribute 90 % species contribution from all six site it may be due to because yeldari dam and Visnupuri dam are situated on Purna river and Godavari river respectively. During the study period it was reported that species like *Ompok bimaculatus, Wallago attu, Mystus bleekeri, Mystus cavasius, Sperata seenghala, Eutropicthys goongwaree, Clarias batrachus* are having good food as well as market value.

*Clarias batrachus Mystus bleekeri*, are having experimental value in biological research laboratory. Similar results were reported by Tamboli R.k and Jha Y.N (2012) reported 17species from 12 genera and 6 families from River Kelo and Mand in Raigarh District, CG, India. Amal Kumar Patra (2011) reported 07 species belonging 06 genera and 06 families were Bagridae family was dominant with 02 species from Karala river of Jalalpaiguri District, West Bengal, India. Chandasudha Goswami and Sayad Ali (2012): reported 15 species belonging to 05 families were family Bagridae was a dominant with 06 species from Kuls River.

Chatoan Tesia and Sabitry Bordoloi (2012): reported 37 species from that 08 species of catfishes belonging to 04 families were recorded from Charju River, irap District, and Arunachal Pradesh, India. Ashwani Kumar Dubey (2012): reported 08 species belonging to 05 families were Bagridae family was dominant with 03 species from Lentic Freshwater Catfish at Chhatarpur City M.P, India.

**Table 01 Cat Fish Diversity From Marathwada Region**

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>Site I</th>
<th>Site II</th>
<th>Site III</th>
<th>Site IV</th>
<th>Site V</th>
<th>Site VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siluridae</td>
<td><em>Ompok</em></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td></td>
<td></td>
<td><em>bimaculatus</em></td>
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<tr>
<td></td>
<td><em>Wallago</em></td>
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<td>+</td>
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<tr>
<td>Bagridae</td>
<td><em>Mystus</em></td>
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<td></td>
<td><em>armatus</em></td>
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<td>+</td>
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<tr>
<td></td>
<td><em>bleekeri</em></td>
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<td>+</td>
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<tr>
<td></td>
<td><em>cavasius</em></td>
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<td>+</td>
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<td></td>
<td><em>vittatus</em></td>
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<tr>
<td></td>
<td><em>horai</em></td>
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<tr>
<td></td>
<td><em>montanus</em></td>
<td></td>
<td>+</td>
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<tr>
<td>Sperata</td>
<td>aor</td>
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<tr>
<td></td>
<td>seenghala</td>
<td>+</td>
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<tr>
<td>Rita</td>
<td>gogra</td>
<td></td>
<td>+</td>
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<tr>
<td>Schilbeidae</td>
<td>Proeutropiichthys</td>
<td>taakree</td>
<td>+</td>
<td>+</td>
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<td></td>
<td></td>
<td>taakree</td>
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<tr>
<td></td>
<td>Eutropiichthys</td>
<td>goongwaree</td>
<td>+</td>
<td>+</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Heteropneustidae</td>
<td>Heteropneustes</td>
<td>fossils</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td></td>
<td>Clariidae</td>
<td>Clarias</td>
<td>butachus</td>
<td>+</td>
<td>+</td>
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</tr>
</tbody>
</table>
CAT FISHES OF MARATHWADA REGION

Ompok bimaculatus  Wallago attu  Mystus armatus

Mystus bleekeri  Mystus cavarius  Mystus vittatus

Mystus horai  Mystus montanus  Sperata aor

Sperata seenghala  Rita gogra  Proeuroploichthys taakree

Eutroploichthys goongwaree  Heteropneustes fossilis  Clarias batrachus
Following Cat Fish Species Were Observed and Identified In The Present Study From Marathwada Region

<table>
<thead>
<tr>
<th>Family</th>
<th>Siluridae (Sheat fishes)</th>
<th>Genus</th>
<th>Ompok (Bloch, 1794)</th>
<th>Species</th>
<th>bimaculatus (Bloch, 1794)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic characters</td>
<td>Maxillary barnels longer than head extending upto or beyond anal fin, (Datta Munshi and Srivastava 1988).</td>
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<td></td>
</tr>
<tr>
<td>Fin formula</td>
<td>D.4-5; P.12-15 (1/11-14); V.8; A.52-75(2/50-73); C.17-18; Barbels two pairs, (Datta Munshi and Srivastava 1988).</td>
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<td></td>
</tr>
<tr>
<td>Distribution</td>
<td>India: - Freshwater of India; rivers, ponds, lakes, channels and other water bodies, (Datta Munshi and Srivastava 1988).</td>
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<tr>
<td></td>
<td>Abroad – Freshwater of Pakistan, Nepal, Sri lanka, Bangladesh, Burma, Thailand, Maleyasia, Malaya, Java, Sumatra, Borneo, Siam, Vietam, East India and Indonesia, (Datta Munshi and Srivastava 1988).</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Family</th>
<th>Siluridae (Sheat fishes)</th>
<th>Genus</th>
<th>Wallago (Bleeker, 1851)</th>
<th>Species</th>
<th>attu (Bloch &amp; Scheider, 1801)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Record</td>
<td>1801, Silurus attu Schneider, Syst. Ichth. Bloch: 378, pl.75.</td>
<td></td>
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</tr>
<tr>
<td>Diagnostic characters</td>
<td>Gape of mouth very wide, Extending beyond eyes posteriorly, (Jayaram 1999).</td>
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</tr>
<tr>
<td>Fin formula</td>
<td>D.5; P.15-16(1/14-15); V.9-10; A.86-88(4/82-84); C.17; Barbels are two pairs, (Datta Munshi and Srivastava 1988).</td>
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<tr>
<td></td>
<td>Abroad – Bangladesh, Pakistan, Myanmar and Thailand, Java and Sumatra, (Kapoor, Dayal and Ponniah 2002).</td>
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<td></td>
</tr>
<tr>
<td>Family</td>
<td>Bagridae (Bagrid catfishes)</td>
<td>Genus</td>
<td>Mystus (Bleeker, 1851)</td>
<td>Species</td>
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</table>

<table>
<thead>
<tr>
<th>Family</th>
<th>Bagridae (Bagrid catfishes)</th>
<th>Genus</th>
<th>Mystus (Bleeker, 1851)</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic characters</td>
<td>Maxillary barbles reach anal fin interorbital width less than 3.0 (2.0 to 3.0) in head length. No dark spot at base of dorsal fin. Body with two light longitudinal bands, one each above and below lateral line, (Jayaram 1999).</td>
<td></td>
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<tr>
<td>Fin formula</td>
<td>B.X, D.1/7; P. (1/9-10); V.6; A.9-10(3/6-7); C.17. (Day Vol. I, 1986).</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distribution</td>
<td>India- Generally confined to North India Mahanadi head Waters and West Bengal, (Kapoor, Dayal and Ponniah 2002).</td>
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</tr>
<tr>
<td></td>
<td>Abroad – Bangladesh, Nepal, Myanmar, and Sumatra, (Kapoor, Dayal and Ponniah 2002).</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Family</th>
<th>Bagridae (Bagrid catfishes)</th>
<th>Genus</th>
<th>Mystus (Bleeker, 1851)</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Record</td>
<td>1822, Pimelodus cavasius Hamilton, Fish Ganges pp.203, 379 pl-11Fig 67.</td>
<td></td>
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</tr>
<tr>
<td>Diagnostic characters</td>
<td>Maxillary barbles reach caudal fin base or beyond inter orbital width more than 3.0 (3.0 to 4.0) in head length. A dark spot at the base of dorsal fin. No bands on body, (Jayaram 1999).</td>
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</tr>
</tbody>
</table>
### Mystus vittatus (Bloch, 1794)

<table>
<thead>
<tr>
<th>Family</th>
<th>Bagridae (Bagrid catfishes)</th>
<th>Genus</th>
<th>Mystus (Bleeker, 1851)</th>
<th>Species</th>
<th>vittatus (Bloch, 1794)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic characters</td>
<td>Eye diameter 4.5 to 6.0 in head length pectoral fin with nine rays. Body with 3 or 4 longitudinal colour bands above and below lateral line (Plate XI fig 4). A dark shoulder spot. No spot at base of Caudal fin, (Jayaram 1999).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fin formula</td>
<td>B.X, D.1/7/0; P. (1/6); V.6; A.11-13(3-4/8-9); C.15. (Day Vol.I, 1986).</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td><strong>Abroad</strong> – Bangladesh, Nepal, Myanmar, Sri Lanka, Pakistan and Thailand, (Kapoor, Dayal and Ponniah 2002).</td>
<td></td>
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</tr>
</tbody>
</table>

### Mystus horai (Jayaram, 1954)

<table>
<thead>
<tr>
<th>Family</th>
<th>Bagridae (Bagrid catfishes)</th>
<th>Genus</th>
<th>Mystus (Bleeker, 1851)</th>
<th>Species</th>
<th>horai (Jayaram, 1954)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic characters</td>
<td>Caudal peduncle constricted its least height about 03 times in its length. Vomerine tooth band not continuous, (Jayaram, 1999).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fin formula</td>
<td>D.I, 7; P.I, 8; V.I, 5; A.iii, 8; C.17. (Jayaram, 2003).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distribution</td>
<td><strong>India</strong> - River Indus Kalabagh (Jayaram, 1999).</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td><strong>Abroad</strong> – West Pakistan, (Jayaram, 1999).</td>
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</tr>
<tr>
<td>Family</td>
<td>Bagridae (Bagrid catfishes)</td>
<td>Genus</td>
<td>Mystus (Bleeker, 1851)</td>
<td>Species</td>
<td>montanus (Jerdon, 1849)</td>
</tr>
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</tr>
<tr>
<td>Diagnostic characters</td>
<td>Eye diameter 3.5 to 4.0 in head length. Pectoral fin with six rays body with a bluish shoulder spot and a silvery line along the side ending in a dark spot at base of caudal; one or two light bands along sides above lateral line, (Jayaram, 1999).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distribution</td>
<td><strong>India</strong>- Assam, Kerala State, Wayanad range of hills; Madhya Pradesh, Hosangabad district. Javadi hills; Eastern Ghats, (Jayaram, 1999).</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Family</th>
<th>Bagridae (Bagrid catfishes)</th>
<th>Genus</th>
<th>Sperata aor (Sykes, 1839)</th>
<th>Species</th>
<th>aor (Sykes, 1839)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Record</td>
<td>1822. <em>Pimelodus aor</em> Hamilton, <em>Fish Ganges</em> pp.205, 379, Pl.20 Fig 68.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnostic characters</td>
<td>Snout rounded width of gape of mouth less than ½ of head length caudal fin with 17 rays. Maxillary barbels reach nearly to caudal fin or even beyond. Adipose dorsal fin long its base is about twice as long as rayed dorsal, (Datta Munshi and Srivastava 1988).</td>
<td></td>
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<tr>
<td>Fin formula</td>
<td>D.1/7/0; P. (1/9); V.6; A.11-13(3/8-10); C.17. Barbles four pairs. (Datta Munshi and Srivastava, 1988).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distribution</td>
<td><strong>India</strong>- River Ganga, Yamuna, Brahmaputra and Mahaanadi, (Kapoor, Dayal and Ponniah, 2002). <strong>Abroad</strong> – Pakistan, Bangladesh and Myanmar, (Kapoor, Dayal and Ponniah, 2002).</td>
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</table>

<table>
<thead>
<tr>
<th>Family</th>
<th>Bagridae (Bagrid catfishes)</th>
<th>Genus</th>
<th>Sperata aor (Sykes, 1839)</th>
<th>Species</th>
<th>seenghala (Sykes, 1839)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fin formula</td>
<td>D.1/7/0; P. (1/9); V.6; A.11-12(3/8-9); C.19-21. Barbles four pairs. (Datta Munshi and Srivastava, 1988).</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Distribution</td>
<td><strong>India</strong>- Freshwater of East Punjab, Uttar Pradesh, Krishna, Godavari, Cauvery river, Ganga and Yamuna, (Kapoor, Dayal and Ponniah, 2002).</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Family</td>
<td>Genus</td>
<td>Species</td>
<td><strong>Abroad</strong></td>
<td><strong>India</strong></td>
<td><strong>Abroad</strong></td>
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</tr>
<tr>
<td>Diagnostic characters</td>
<td>Teeth on prevomer mixed with molariform and valliform. Premaxillary band of teeth 3.5 to 4.0 times as long as broad. (Jayaram, 1999).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fin formula</td>
<td>D.i6, P.i 10, V.i6-7, A.iii 8-9, C.13-16.</td>
<td></td>
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</tr>
<tr>
<td>Distribution</td>
<td><strong>India</strong>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Schilbeidae</strong></td>
<td><em>Proeutropiichthys</em> (Sykes, 1839)</td>
<td><em>taakree.taakree</em> (Sykes, 1839)</td>
<td>Bangladesh, Nepal, Myanmar, Pakistan and Yunnan, (Kapoor, Dayal and Ponniah, 2002).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnostic characters</td>
<td>Interoperculum almost rounded without any spurs. Eye diameter 3.5 to 3.6 times in head length. Maxillary barbels extend only up to tip of pectoral fins. (Jayaram, 1999).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fin formula</td>
<td>D.ii 7, P.i 10-11, V.i 5, A.40-44, C.17.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distribution</td>
<td><strong>India</strong>- Peninsular India, Krishna river system, Karnataka and Maharashtra. (Kapoor, Dayal and Ponniah, 2002).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Schilbeidae</strong></td>
<td><em>Eutropiichthys</em> (Sykes, 1838)</td>
<td><em>goongawaree</em> (Sykes, 1838)</td>
<td>Pennisular India up to Krishna river inhabits rivers and canals of Andhra Pradesh and Maharashtra. (Kapoor, Dayal and Ponniah, 2002).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnostic characters</td>
<td>Teeth on palate interrupted in the middle. Nasal barbels longer than head length reaching dorsal fin base. (Jayaram, 1999).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fin formula</td>
<td>D.i 6, P.i 13, V.i 5, A.iii 51, C.17.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distribution</td>
<td><strong>India</strong>-</td>
<td></td>
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</tr>
</tbody>
</table>

**Abroad** – Bangladesh, Myanmar, Nepal, Pakistan and Thailand, (Jayaram, 1999).

<table>
<thead>
<tr>
<th>Family</th>
<th>Siluriformes</th>
<th>Genus</th>
<th><em>Heteropneustes</em> (Muller)</th>
<th>Species</th>
<th><em>fossilis</em> (Bloch, 1794)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Record</td>
<td>1798. <em>Silurus fossilis</em> Bloch <em>Ichth., Hist.Nat.Poiss.</em> 11:36; 370, Fig .02.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnostic characters</td>
<td>Anal fin separated from the caudal fin. A deep notch occipital process not reaching base of dorsal fin. (Jayaram, 1999).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fin formula</td>
<td>D 6;P.1/7;V. 6; A.63-74; C. 19; barbels four pairs. (Datta Munshi and Srivastava, 1988).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distribution</td>
<td><strong>India</strong>- Throughout Indian plains and Andaman’s, (Kapoor, Dayal and Ponniah, 2002).</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**Abroad** – Pakistan, Bangladesh, Sri Lanka, Myanmar, Nepal, Thailand, Laos, and Indus basin, (Kapoor, Dayal and Ponniah, 2002).

<table>
<thead>
<tr>
<th>Family</th>
<th>Claridae (Air Breathing Cat fishes)</th>
<th>Genus</th>
<th><em>Clarias</em> (Linnaeus, 1758)</th>
<th>Species</th>
<th><em>batrachus</em> (Linnaeus, 1758)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic characters</td>
<td>Distance from dorsal fin base to base of occipital process 4.5 to 6.0 times in head length measured along upper median line from tip of snout to base of occipital process, (Jayaram, 1999).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fin formula</td>
<td>D 65-72; P.9 (1/8); V. 6 (1/5); A.47-55; C. 17. (Gupta and Gupta, 2004).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distribution</td>
<td><strong>India</strong>- Freshwater of the plains of India, (Kapoor, Dayal and Ponniah, 2002).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abroad** – Pakistan, Bangladesh, Sri-Lanka, Myanmar, Nepal, Thailand, Indonesia, Philippines. (Kapoor, Dayal and Ponniah, 2002).

**References**


Abstract:
The emergence of electronic banking has been a topic of increasing interest in recent years for both academics and practitioners, as the changes taking place in the field are clearly observable. Although several prior research projects have focused on the factors that impact on the adoption of information technology or Internet, there is limited empirical work which simultaneously captures the success factors (positive factors) and resistance factors (negative factors) that help customers to adopt internet banking. This paper explores and integrates the various advantages of internet banking to form a positive factor named perceived benefit. In addition, drawing from perceived risk theory, five specific risk facets – financial, security/privacy, and performance, social and time risk – are synthesized with perceived benefit as well as integrated with the technology acceptance model (TAM) to propose a theoretical model to explain customers’ intention to use internet banking. The results showed that the intention to use internet banking is adversely affected mainly by the security/privacy risk, as well as financial risk and is positively affected mainly by perceived benefit, attitude and perceived usefulness. The implications of integrating perceived benefit and perceived risk into the proposed internet banking adoption model are discussed.

For example, according to Chang (2002), the cost to process an account transfer is about $1.07 through a branch and $0.27 by an automated teller machine (ATM), but is only $0.01 through the Internet. Second, customer adoption of online banking can reallocate service demand across multiple service channels, affecting optimal capacity and service design decisions in other channels, such as branches or ATMs. Finally, customers using online channels may show profitability-enhancing behaviors such as increased loyalty or product utilization, although there is some question as to whether this is because of behavioral change or simply differences between online and offline customer populations (Hitt and Frei 2002).

In recent years, a variety of theoretical perspectives have been applied to provide an understanding of the determinants of Internet banking adoption and use, including the intention models from social psychology. From this stream of social psychology research, the technology acceptance model (TAM) (Davis, 1989), an adaptation of theory of reasoned action (TRA) (Fishbein & Ajzen, 1975) and the theory of planned behavior (Ajzen, 1991) (TPB), are especially well researched intention models that have proven successful to predict technology acceptance behavior (Chau and Hu, 2001; Gefen & Straub, 2000; Szajna, 1994).

This study enlarges the scope of the adoption decision to explicitly include both negative (perceived risk) and positive factors (perceived benefits) simultaneously. The research may give practitioners an increased understanding of customers’ risk perceptions which can then be used to devise risk-reducing strategies and trust-building mechanisms to encourage online trading adoption, especially in the emerging area of e-payments. The purposes of this study are as follows:

- To investigate whether perceived risk and benefit significantly impact customers’ behavioral intention to use internet banking adoption.
- To clarify which factors are more influential in affecting the decision to use internet banking?

This paper proceeds as follows: Section 1 introduces theoretical foundations. Next Section outlines our research model and hypotheses. After that, section 3 details the methodology and research design, the data analysis and hypotheses testing results. Final Section discusses our research findings, implications, concludes with this paper’s limitations, and potential topics for future research.

Introduction
The evolution of technological innovation has had a major effect in banking industry. This evolution of banking has been driven by changes in distribution channels as evidenced by automated teller machine (ATM), Phone-banking, Telebanking, PC-banking and most recently Internet banking (Nasri and Charfeddine, 2012). Despite most modern banks have deployed Internet banking capabilities in an attempt to reduce costs while improving customer service, the adoption of online banking has been limited and, in many cases, has fallen short of expectations (due to uncertainty and security concerns) (Xue et al., 2011, Wade 2003). Although it is clear that self-service can reduce cost, less is known about how customers utilize self-service channels in a multi-channel service delivery system and the resulting impact on firm performance.

Banks are concerned about managing and optimizing the adoption of online banking for several reasons. First, it has been suggested that online banking reduces service costs.

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Theoretical background
Perceived risk and perceived benefit
Since the 1960s, perceived risk theory has been used to explain consumers’ behavior. Considerable research has examined the impact of risk on traditional consumer decision making (Lin, 2008). Peter and Ryan (1976) defined perceived risk as a kind of subjective expected loss, and Featherman and Pavlou (2003) also defined perceived risk as the possible loss when pursuing a desired result. Cunningham (1967) noted that perceived risk consisted of the size of the potential loss (i.e., that which is at stake) if the results of the act were not favorable and the individual’s subjective feelings of certainty that the results will not be favorable. Most of scholars claimed that consumers’ perceived risk is a kind of a multidimensional construct. Six components or types of perceived risk have been identified: financial, performance, social, physical, privacy, and time-loss (Jacoby and Kaplan, 1972; Kaplan et al., 1974; Roselius, 1971). However, the dimensions of perceived risk may vary according to the product (or service) class Featherman and Pavlou, 2003. Internet banking does not incur any threat to human life; therefore, measures of physical risk were not included in this study. We define perceived risk in internet banking as the subjectively determined expectation of loss by an internet bank user in contemplating a particular online transaction. The dimensions of perceived risk were defined in Table 1.

Table 1: Dimensions of perceived risk

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Performance risk</td>
<td>The possibility of the product malfunctioning and not performing as it was designed and advertised and therefore failing to deliver the desired benefits.</td>
</tr>
<tr>
<td>Social risk</td>
<td>Potential loss of status in one’s social group as a result of adopting a product or service, looking foolish or untrendy.</td>
</tr>
<tr>
<td>Financial risk</td>
<td>The probability that a purchase results in loss of money as well as the subsequent maintenance cost of the product.</td>
</tr>
<tr>
<td>Privacy risk</td>
<td>Potential loss of control over personal information, such as when information about you is used without your knowledge or permission. The extreme case is where a consumer is “spoofed” meaning a criminal uses their identity to perform fraudulent transactions</td>
</tr>
<tr>
<td>Time risk</td>
<td>Consumers may lose time when making a bad purchasing decision by wasting time researching and making the purchase, learning how to use a product or service only to have to replace it if it does not perform to expectations.</td>
</tr>
<tr>
<td>Physical risk</td>
<td>The probability that a purchased product results in a threat to human life.</td>
</tr>
</tbody>
</table>

Perceived risks of internet banking
The concept of risk dates back to as early as the 1920s (Knight, 1921). The role of perceived risk in the business arena is actively examined in consumer behavior researches. In Rao & Farley (1987) and Srinivasan & Ratchford (1991), for example, perceived risk was used as an explanatory variable. Bettman (1973), Cunningham (1967), Sheth & Ventean (1968), and Stone & Gronhaung (1993) viewed perceived risk as a determinant of consumers’ intended and actual behavior. Dowling & Staelin (1993) constructed a descriptive model to depict how perceived risk affected consumers’ information search behavior and their risk-modification strategies. Jacoby and Kaplan (1972) identified five dimensions of perceived risk: financial risk, functional or performance risk, physical risk, social risk, and psychological risk. Their study reported that these five dimensions of perceived risk were capable of explaining 74% of variance in the research data. A study conducted by Stone and Gronhaung (1993) found that, when the dimension of time risk was added to the list mentioned above, the percentage of explained variance was increased to 88.8%. In an exploratory study of consumer acceptance of Internet banking services in a Turkish bank using a structured-undisguised questionnaire, Polatoglu and Ekin (2001) also found that perceived risk was one of the major factors affecting consumer adoption, as well as customer satisfaction, of Internet banking services.

The present research investigated five types of risk—security/privacy, financial, social, time/convenience, and performance loss, and the details of these five risks related to internet banking are described as follows:
Security/privacy risk: This is defined as a potential loss due to fraud or a hacker compromising the security of an internet bank user. Phishing is a new crime skill by which phishers attempt to fraudulently acquire sensitive information, such as usernames, passwords and credit card details, by masquerading as a trustworthy entity in an electronic communication (Reavley, 2005). A phishing attack takes place when a user receives a fraudulent email (often referred to as a spoof email) representing a trusted source that leads them to an equally fraudulent website that is used to collect personal information (Entrust, 2008). Both fraud and hacker intrusion not only lead to users’ monetary loss, but also violate users’ privacy, a major concern of many internet users. Many consumers believe that they are vulnerable to identity theft while using internet banking services (Littler and Melanthiou, 2006).

Financial risk: It is defined as the potential for monetary loss due to transaction error or bank account misuse. According to Kuismma et al. (2007), many customers are afraid of losing money while performing transactions or transferring money over the Internet. At present online banking transactions lack the assurance provided in traditional setting through formal proceedings and receipts. Thus, consumers usually have difficulties in asking for compensation when transaction errors occur (Kuismma et al., 2007).

Social risk: This refers to the possibility that using online banking may result in disapproval of one’s friends/family/work group. It is possible that one’s social standing may be enhanced or diminished depending on how online banking is viewed. It may well be that people have unfavorable or favorable perceptions of online banking that in turn affect their views of its adopters; or, alternatively, not adopting online banking may also have negative or positive connotations.

Time/convenience risk: It may refer to the loss of the time and inconvenience incurred due to the delays of receiving the payment or the difficulty of navigation (finding appropriate services and hyperlinks). Two leading causes of dissatisfying online experiences that may be thought of as a time/convenience risk include a disorganized or confusing Web site and pages that are too slow to download (Forsythe and Shi, 2003). It may also be related to the length of time involved in waiting the website or learning how to operate online banking website.

Performance risk: This refers to losses incurred by deficiencies or malfunctions of online banking websites. Customers are often apprehensive that a breakdown of system servers or disconnection from the internet will occur while conducting online transactions because these situations may result in unexpected losses (Kuismma et al., 2007).

Perceived benefit
Internet banking has come to be considered as one of the most effective banking transaction methods (Huang et al., 2005). Because it possesses many advantages which offline banking channels can not offer. Thus, internet banking managers aim to utilize these advantages to increase the internet banking adoption rate. Based to a certain extent on reasons offered by Lee (2008), there are two main types of perceived benefits, which can be categorized as direct and indirect advantages. Direct advantages refer to immediate and tangible benefits that customers would enjoy by using online banking. For example, customers can benefit from a wider range of financial benefits, faster transaction speed, and increased information transparency. First, this wider range of financial benefits includes the lower transaction handling fees, higher deposit rates, opportunities to win prizes and extra credit card bonus points. Second, the faster transaction speed obviously means that time can be saved since internet banking does not need paper documents, the processing of which can give rise to errors and delays, and which also requires more personnel. Internet banking automates this process by mediating transactions through websites, and can also reduce the need for customers to communicate with bank staff regarding transaction details because they can be obtained at a website. Third, during the transaction, internet banking allows customers to monitor contractual performance at any time, or to confirm delivery automatically. In other words, more relevant information is immediately available and transparent to customers. Indirect advantages are those benefits that are less tangible and difficult to measure. For example, internet banking allows customer to perform banking transactions anywhere in the world and enjoy hour service, as well as offering customers more investment opportunities and services, such as stock quotations and news updates.

Attitude and Intention
Based on certain beliefs, a person forms an attitude about certain objects, on the basis of which one forms an intention as to how one should behave with respect to that object. The intention to behave is the sole determinant of actual behavior (Vijayan, Perumal and Shanmugam, 2005). Davis adapted the TRA by developing two key beliefs that specially account for information system usage. The first of these beliefs is perceived usefulness, defined as the ‘degree to which a person believes that using a particular system would enhance his/her job performance’ (Davis, 1989). The second is perceived ease of use, defined as ‘the degree to which a person believes that using a particular system would be free of effort’ (Davis, 1989). A diagram of the model is presented in Figure 1.
TAM is an adaptation of the theory of reasoned action (TRA) by Fishbein and Ajzen (1975) and was mainly designed for modeling user acceptance of information technology (Davis et al., 1989). This model hypothesizes that system use is directly determined by behavioral intention to use, which in turn is influenced by users’ attitudes toward using the system. Attitudes are also affected by perceived ease of use and the perceived usefulness of the system. These determinants are also easy for system developers to understand and can be specifically considered during system requirement analysis and other system development stages. These factors are common in technology-usage settings and can be applied widely to solve the acceptance problem (Taylor and Todd, 1995).

Research model and hypothesis

Research model

We drew upon two primary research streams, information technology (IT) adoption theory and perceived risk theory, to develop this study’s research model and associated hypotheses. Over the past decade, different model have been widely applied to examine IT usage and e-service acceptance (Davis, 1993; Hsu, 2004; Hsu and et al., 2006). However, neither TAM (technology acceptance model) nor TPB (theory of planned behavior) have been found to provide consistently superior explanations or behavioral predictions (Chen et al., 2007). Recently, a growing body of research has focused on attitude as a powerful item to examine IT usage and e-service acceptance, and the results have showed that the investigation of factors influencing the behaviour had better exploratory power because attitudes form behaviour (Bosnjak et al., 2006; Chen et al., 2007; Wu and Chen, 2005). Since the focus of this study is internet banking service adoption, which is an instance of acceptance of innovative technology intertwined with social systems and personal characteristics, investigation of negative and positive factors that influence attitude toward acceptance of internet banking for our research framework should be comprehensive in order to examine the consumers’ intentions towards, and acceptance of, internet banking. There are 10 constructs in our model, which includes perceived ease of use, perceived usefulness, perceived benefit, performance risk, financial risk, time risk, social risk and security risk as independent variables, attitude as the intervening variable, and intention to use as the dependent variable. We will test the strength of the hypothesized relationships embedded in the theoretical model and the robustness of the model in predicting customers’ intention to adopt internet banking in the Iran business environment. The theoretical model is graphically presented in Fig. 1.
Hypotheses development
Hypotheses about attitude

Based on the theoretical model developed in Section 2, we formulated the following research hypotheses.

H1: Perceived usefulness (PU) positively influences attitudes towards the use of internet banking.

H2: Perceived ease of use (PEOU) positively influences attitudes towards the use of online banking.

H3: Attitude positively influences the intention to use online banking.

Hypotheses regarding performance risk

The performance risk refers to losses incurred by deficiency or malfunction of online banking websites. According to Yiu et al., (2007) sudden breakdown of web servers may lead to unexpected losses while conducting online transactions. Littler and Melanthiou (2006) pointed out that malfunctions of online banking websites would reduce customers' willingness to use online banking, while Featherman and Pavlou (2003) found that a high frequency of website breakdowns and disconnections inhibits e-services evaluation (e.g. perceived usefulness). Therefore, it follows that:

H4: Performance risk negatively influences attitudes towards the use of online banking.

Hypotheses regarding financial risk

Financial risk refers to the potential for monetary loss due to transaction errors or bank account misuse. Kuisma et al. (2007) indicated that many consumers resist using online banking because they fear such losses. Off-line banks generally provide clerical personnel to verify whether the account number of the payee and amount of money to transact is accurate, but such safeguards are rarely available in online banking, and this can generate feelings of insecurity and uncertainty. Accordingly, the following hypotheses are proposed:

H5: Financial risk negatively influences attitudes towards the use of online banking.

Hypotheses regarding social risk

Social risks is being defined as a threat which creates a possible loss of self-image or prestige resulting from the purchase or use of certain products or services (Forsythe and Shi, 2003). Under this definition, in the context of online banking threats can be generated due to the unfavorable perceptions of online banking of consumers’ family, acquaintances, or peers that in turn affect their views of its adopters. Over the past decades, several previous research projects regarding retail purchases have shown that social risk has a negative impact on attitude for consumers (Dowling and Staelin, 1994; Yang et al., 2007). Based on these studies, it is reasonable to expect that social risk could negatively influence customers’ attitude to use internet banking. Thus, it follows that:

H6: Social risk negatively influences attitudes towards the use of online banking.

Hypotheses about time/convenience risk

Steven et al. (1999) reported on the importance of time considerations and found that it was a significant predictor of online buying behavior. Their research found that “harried” consumers were more likely to purchase over the Internet in order to save time. The current research similarly proposes that some consumers are very time oriented and concerned about potential risks of “wasting time” spent implementing, learning how to use, and troubleshooting a new e-service. These time-conscious consumers likely guard against the possible loss of time risk, and are less likely to adopt an e-service that they consider to have high switching, setup and maintenance costs (Featherman and Pavlou, 2003). Forsythe and Shi (2003) indicated that time risk is a significant barrier to online shopping, and it is therefore hypothesized that:

H7: Time risk negatively influences attitudes towards the use of internet banking.

Hypotheses about security/privacy risks

Security is being defined as a threat which creates “circumstance, condition, or event with the potential to cause economic hardship to data or network resources in the form of destruction, disclosure, modification of data, denial of service and/or fraud, waste, and abuse” (Kalakota and Whinston, 1997). Under this definition, in the context of online banking threats can be made either through network and data transaction attacks or through unauthorized access to the account by means of false or defective authentication. According to Milind (1999), security risk is a significant impediment to the adoption of online banking. Further, it has been stated in numerous studies that the
greatest challenge to the electronic banking sector will be winning the trust of customers over the issues of privacy and security (Bestavros, 2000; Furnell and Karweni, 1999). It is therefore hypothesized that:

**H8:** Security/privacy risk negatively influences attitudes towards the use of online banking.

**Hypotheses about Perceived benefit**

Internet banking offers lower transaction costs, faster transaction speed, and better information transparency as well as allow customers to enjoy some free services such as stock quotation, news and 24-hour services (Oh et al., 2007) as mentioned above. Moreover, internet banking often offer an incentive program, e.g. higher deposit rate, reduction or waiver of handling free, extra credit card bonus points, luck draw or joining gifts. Evidence provided by an e-business study shows that perceived benefit significantly affects e-business adoption (Zheng et al., 2006). Along the same lines, Beatty et al. (2001) indicated that perceived benefit has a positive influence on the corporate website adoption. Therefore, it is reasonable to infer that perceived benefits positively influence user attitude and intention to adopt Internet banking, and we hypothesized that:

**H9:** Perceived benefit has a positive effect on attitude to use internet banking.

**Research method**

**Data collection**

In order to collect internet banking users’ information, we first required the permission of a private bank in Iran to express our need for the information research purposes. After that, the private bank helped to email invitation letters to its users with a message explaining the need to understand their (the users) experience in the initial adoption of internet banking services. To reduce the possibility that a respondent participated in the survey more than once, each respondent was required to provide his/her mobile phone number in the survey. Later, duplicate mobile phone numbers were used to filter out multiple responses from the same respondent. Users were free to participate at their own discretion. This online survey, which yielded 420 responses, was conducted for one month, with incomplete responses and missing values deleted, resulting in a sample size of 370 users for an overall response rate of 88%. Sample demographics are depicted in Table 2. 71% of the respondents were male and 29% were female. The majority of respondents (65%) were over 35 years old. Finally, the education levels of respondents were 75.5% college and 25.5% high school.

**Measurement development**

The instrument was designed to include a two-part questionnaire. The first part includes nominal scales, and the remainder includes five-point Likert scales, ranging from “disagree strongly” (1) to “agree strongly” (5). Accordingly, the first part is basic information. This part of questionnaire was used to collect basic information about respondents’ characteristics including gender, age and education. The second part of questionnaire was developed based on the constructs of perceived usefulness, perceived ease of use, perceived benefit, attitude, functional risk, financial risk, time risk, social risk, security risk, and intention to use. Perceived usefulness and attitude were adopted from the measurements defined by Cheng et al. (2006) and Lai and Li (2005), containing four items for each construct. Perceived ease of use and intention to use were adapted from the measurements defined by Cheng et al. (2006) and Lai and Li (2005), containing three items for each construct. Performance risk, financial risk, time risk and social risk were adapted from the measurements defined by Littler and Melanthiou (2006), containing two items for each construct. Security risk was adapted from the measurement defined by Littler and Melanthiou (2006) and Cheng et al. (2006), and included three items. Before conducting the main survey, we performed a pre-test to validate the instrument. The pre-test involved 30 respondents were asked to comment on the length of the instrument, the format, and the wording of the scales. Therefore, the instrument has confirmed content validity.

**Result**

In analyzing the collected data, we followed the two-step procedure suggested by Anderson and Gerbing (1988). First, we examined the measurement model to measure convergent validity. Then, we examined the structural model to investigate the strength and direction of the relationships among the theoretical constructs.

**Analysis of the measurement model**

Cronbach’s alpha scores shown in Table 1 indicated that each construct exhibited strong internal reliability. Convergent validity was assessed based on the criteria that the indicator’s estimated coefficient was significant on its posited underlying construct factor. We evaluated the measurement scales using the three criteria suggested by Fornell and Larcker (1981):

All indicator factor loading (λ) should be significant and exceed 0.5.
Construct reliabilities should exceed 0.8.

Average variance extracted (AVE) by each construct should exceed the variance due to measurement error for the construct (e.g., AVE should exceed 0.5).

All (λ) values in confirmatory factor analysis of the measurement model exceeded 0.5 and were significant at $p = 0.001$. Composite reliabilities of constructs ranged from 0.83 to 0.92 (see Table 1). AVE, ranging from 0.61 to 0.79, was greater than the variance due to measurement error. Therefore, all three conditions for convergent validity were met.

Table 1-Cronbach’s alpha and convergent validity

<table>
<thead>
<tr>
<th>Construct/Indicator</th>
<th>Item</th>
<th>Factor loading</th>
<th>t-value</th>
<th>Composite reliability (CR)</th>
<th>Average variance extracted</th>
<th>Cronbach’s alpha</th>
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<tbody>
<tr>
<td>Perceived usefulness</td>
<td>PI1</td>
<td>0.865</td>
<td>19.410</td>
<td>0.92</td>
<td>0.7515</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>PI2</td>
<td>0.846</td>
<td>18.730</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PI3</td>
<td>0.852</td>
<td>18.546</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>PI4</td>
<td>0.857</td>
<td>19.116</td>
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<tr>
<td>Perceived ease of use</td>
<td>PI1U</td>
<td>0.866</td>
<td>19.318</td>
<td>0.00</td>
<td>0.7514</td>
<td>0.98</td>
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<tr>
<td></td>
<td>PI2U</td>
<td>0.873</td>
<td>19.579</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>PI3U</td>
<td>0.865</td>
<td>19.267</td>
<td></td>
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<tr>
<td>Attitude</td>
<td>AT1</td>
<td>0.858</td>
<td>19.068</td>
<td>0.01</td>
<td>0.7242</td>
<td>0.91</td>
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<tr>
<td></td>
<td>AT2</td>
<td>0.851</td>
<td>18.636</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>AT3</td>
<td>0.846</td>
<td>18.661</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>AT4</td>
<td>0.849</td>
<td>18.764</td>
<td></td>
<td></td>
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<tr>
<td>Perceived benefit</td>
<td>PB1</td>
<td>0.837</td>
<td>12.331</td>
<td>0.89</td>
<td>0.7882</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>PB2</td>
<td>0.845</td>
<td>11.312</td>
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<tr>
<td></td>
<td>PB3</td>
<td>0.844</td>
<td>9.334</td>
<td></td>
<td></td>
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<tr>
<td>Intention</td>
<td>IT1</td>
<td>0.917</td>
<td>21.503</td>
<td>0.05</td>
<td>0.8257</td>
<td>0.92</td>
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<tr>
<td></td>
<td>IT2</td>
<td>0.907</td>
<td>21.100</td>
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<tr>
<td></td>
<td>IT3</td>
<td>0.902</td>
<td>20.506</td>
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<tr>
<td>Performance risk</td>
<td>PR1</td>
<td>0.729</td>
<td>5.963</td>
<td>0.81</td>
<td>0.5978</td>
<td>0.80</td>
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<tr>
<td></td>
<td>PR2</td>
<td>0.815</td>
<td>10.583</td>
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<tr>
<td>Financial risk</td>
<td>FR1</td>
<td>0.816</td>
<td>17.289</td>
<td>0.02</td>
<td>0.6908</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>FR2</td>
<td>0.846</td>
<td>18.179</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social risk</td>
<td>SR1</td>
<td>0.823</td>
<td>10.505</td>
<td>0.02</td>
<td>0.6906</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>SR2</td>
<td>0.839</td>
<td>10.516</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time risk</td>
<td>TR1</td>
<td>0.905</td>
<td>11.191</td>
<td>0.84</td>
<td>0.7311</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>TR2</td>
<td>0.802</td>
<td>10.541</td>
<td></td>
<td></td>
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<tr>
<td>Security risk</td>
<td>SR1</td>
<td>0.758</td>
<td>15.378</td>
<td>0.83</td>
<td>0.5051</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>SR2</td>
<td>0.796</td>
<td>15.390</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>SR3</td>
<td>0.741</td>
<td>14.271</td>
<td></td>
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</tr>
</tbody>
</table>

Analysis of the structural model

We assessed the overall goodness-of-fit using the chi-square test. The chi-square test assesses the adequacy of a hypothesized model in terms of its ability to reflect variance and covariance of the data. Due to its tendency to be sensitive to sample size, other fit indices (e.g., GFI, AGFI, CFI, NFI, and RFI) were considered in conjunction with the chi-square. For the statistical significance of parameter estimates, t values were used. The results of structural equation modeling obtained for the proposed conceptual model revealed a ratio of chi-square to the degree of freedom ($\chi^2$/df) of 2.04 ($p < 0.001$), goodness-of-fit index (GFI) of 0.92, adjusted goodness of fit index (AGFI) of 0.9, comparative fit index (CFI) of 0.96, normed fit index (NFI) of 0.94, relative fit index (RFI) of 0.93, and root mean square error of approximation (RMSEA) of 0.06 (see Fig. 2). Generally, fit statistics greater than or equal to 0.9 for GFI, NFI, RFI, and CFI indicate a good model fit (Bagozzi et al., 1991; Hair and et al., 1998). Furthermore, RMSEA values ranging from 0.05 to 0.08 are acceptable (Hair and et al., 1998); therefore, the RMSEA suggested that our model fit was acceptable. Other fit indices, except AGFI, indicated that our proposed model obtained an adequate model fit. Low AGFI statistics may have resulted from the small sample size used.
A structural equation modeling (SEM) approach was adopted in our data analysis (Bagozzi et al., 1991). Fig. 2 presents the results of the structural model with non-significant paths as dotted lines, and the standardized path coefficients between constructs. Intention to use Internet banking in this study was predicted by attitude ($\beta = 0.31$, Standardized path coefficient, $p < 0.05$), and this variable explained 79% of the variance of intention to use ($R^2 = 0.79$, coefficient of determination). As a result, Hypothesis 3 was supported. Attitude was predicted by PU ($\beta = 0.14$, $p < 0.01$), PEOU ($\beta = 0.12$, $p < 0.05$), financial risk ($\beta = -0.33$, $p < 0.01$), time risk ($\beta = -0.21$, $p < 0.05$), social risk ($\beta = -0.09$, $p < 0.01$), performance risk ($\beta = -0.28$, $p < 0.05$), security risk ($\beta = -0.41$, $p < 0.01$) and perceived benefit ($\beta = 0.21$, $p < 0.05$). Together these variables explained 76% of the total variance. These findings validated Hypotheses 1, 2, 4 up to 9. Our hypotheses results are shown in Fig. 2.

Discussion

The results of this study provide support for the research model presented in Fig. 1 and for the hypotheses regarding the directional linkage among the model’s variables. The explanatory power of our research model had an R-square of 79% for attitude towards internet banking. Several insightful results could be summarized from our research framework, and these are presented below in two categories: positive and negative predictors.

Security, financial, time, social and performance risks all emerged as negative factors in the attitude towards adoption of internet banking.

First, the attitude is adversely affected primarily by security risk ($\beta = -0.41$, $p < 0.01$). Security risk appears to be the most important inhibitor to the adoption of internet banking. This underscores the fact that concerns about fraud and identity theft are foremost in the minds of Internet users. Thus, providing encryption and strong authentication to prevent fraud and identity theft should be a priority in this field.

Second, financial risk also has a significant negative effect ($\beta = -0.33$, $p < 0.01$) on the attitude toward intention to adopt internet banking and was the second most important inhibitor to the adoption of such services. At present, internet banking transactions lack the assurance provided by staff assistance in traditional settings with the use of formal proceedings and receipts, and because of this customers usually have difficulties in asking for compensation when transaction errors occur. Thus, this may explain why many customers resist adopting internet banking.

Third, the results indicate that performance risk has a significant negative influence on attitude ($\beta = -0.28$, $p < 0.05$). Therefore, minimizing the risk of website malfunction might increase the willingness of consumers to conduct transactions online.

Fourth, this study found that the time/convenience risk to has a negative influence on the attitudes towards the intention to adopt internet banking. This implies that online banking users might worry about delays in receiving
online payments and might be concerned with the length of time involved in waiting for the website or learning how to operate it. Thus, reducing the possibility of delays of payment and waiting time is an important issue for internet banking service providers.

The fifth finding reveals that the influence of social risk on attitude was significant. This shows that customers care about social pressure from their friends/family/work group with regard to internet banking. One interpretation is that internet banking is already very common and most respondents have friends or family with favorable perception of it. Another possible explanation is that the decision to use internet banking is voluntary rather than mandatory. This is related to the findings of Venkatesh and Davis (2000), that social norms could significantly determine intention to use in a voluntary-usage context.

On the other hand, compared with the negative factors of perceived risk, the attitude toward the intention to use internet banking is primarily and positively affected by perceived benefit ($\beta = 0.21$) and less so by perceived usefulness ($\beta = 0.14$) and perceived ease of use ($\beta = 0.12$). This implies that the perceived benefit is the most important positive predictor of the intention to use internet banking, which is consistent with Featherman and Fuller (2002) and Lee (2009). Our results also support the idea that the inclusion of perceived benefit significantly improves the prediction about the intention to use online banking. Pikkarainen (2004) and Chan and Lu (2004) investigated the acceptance of Internet banking in Finland and Hong Kong, respectively. Both studies reached the same conclusion that perceived usefulness is more influential than perceived ease of use in explaining the acceptance of internet banking. As we knew from previous research, perceived usefulness was always an important determinant of attitude, and it may mediate the influence of perceived ease of use on attitude. Indeed, perceived ease of use has long been recognized as a basic requirement for system design (Chau, 1996; Davis et al., 1989). Another interpretation is that difficulty in using online systems is becoming less of a concern as they are increasingly user-friendly. In addition, since online systems are more common and standardized nowadays, the public has become increasingly competent in using them. Accordingly, in the planning and development of internet banking, software developers should pay attention to practical functions and extend key features that are frequently required (Chen et al., 2007). On the marketing side, banks should accentuate the full functionality of their systems to cater efficiently to the different banking needs of users.

### Implications

#### Managerial implications

The results of this study shed light on some important issues related to customer intentions toward internet banking that have not been addressed by previous studies. First, although both perceived benefit and risk have a significant influence on intention, this study reveals that the latter is a more influential factor, implying that controlling the risk of internet banking is more important than providing benefits. This finding is particularly important for managers as they decide how to allocate resources to retain and expand their current customer base. However, building a risk-free online transaction environment is much more difficult than providing benefits to customers. Therefore, internet banking companies need to search for risk-reducing strategies that might assist in inspiring high confidence in potential customers. This study suggests that they should consider focusing on the prevention of intrusion, fraud and identity theft. For example, building secure firewalls to avoid intrusion, developing methods for strengthening encryption, and authenticating websites in order to prevent fraud and identity theft are all measures that should be undertaken. In addition, this study suggests that internet banking companies could develop trust-building mechanisms to attract customers, such as statements of guarantee, increased familiarity through advertising, and long-term customer service. It is worth noting that because online banking is a less verifiable and controllable environment, online banking customers usually have difficulty in asking for compensation when transaction errors occur. Thus, we suggest that online banking companies should provide customers with digital receipts or a guarantee for every transaction in order to increase confidence in such services.

#### Academician implications

In terms of theory building, this study attempts to develop a new theory by grounding new variables in an integration of two schools of the nomological structure model (TRA) and applying them into a new context. It is important to note that the two new variables – perceived benefit and risk – are compatible with the TAM and TPB variables that have already been placed within the TRA framework (Davis, 1989). This approach is likely to ensure a stable theory development. Hence, the proposed model makes an important contribution to the emerging literature on e-commerce, especially with regard to internet banking. The present study has many implications for future internet banking research.

First, the empirical results show that the perceived benefit and five risk facets all have significant indirect effects on behavioral intention to use internet banking, where security risk has the biggest negative effect ($\beta = -0.41$) while
perceived benefit has the strongest positive effect ($\beta = 0.21$). This result indicates that the risk factor exerts a stronger effect on customers’ decision making than the benefit factor, implying that risk precedes benefit for internet banking customers when they consider using internet banking. Moreover, according to the risk theory of consumer behavior (Bauer, 1960), Bauer indicated that benefits are often accompanied with risks, and thus, it is worth investigating the causal relationship between these two elements. This study provides an initial blueprint to develop further understanding of this causal relationship.

Second, although consumer perceptions of the risks of adopting internet banking have been studied by many researchers (Liao et al., 1999; Tan and Teo, 2000; Yousafzai et al., 2003), the perceived risk variable has only been modeled as a single construct, which fails to reflect the real characteristics of perceived risk and explain why consumers resist such banking services. This study divides the perceived risk into five facets, consequently providing a more in-depth understanding of the characteristics of such risks regarding internet banking. The technology acceptance model variables and their integration with these risk facets have been theorized and empirically validated in this work. However, there are significantly different effects on attitude and behavioral intention toward internet banking among the five risk facets, and there remains a considerable amount of work to be done investigating these particularly, as with this research, with regards to internet banking or e-payment acceptance.

Conclusions and Future Research
This paper aims to develop an extended model to predict and explain customers’ behavioral intentions with regard to adopting internet banking. The proposed model incorporates five categories of perceived risk to provide a more comprehensive investigation covering both the positive and negative aspects of internet banking. The results show that the proposed model has good explanatory power and confirms its robustness in predicting customers’ intentions to use such services. As with any research, care should be taken when generalizing the results of this study. First, the survey was conducted using web-based forms and employed a non-random convenience sample. Gathering a larger sample using an alternate survey modality and random sampling methods would be costly. The online survey method was appropriate for collecting data from participants with Internet experience and who were free of geographical constraints. However, generalizability could be enhanced if future research is systematically sampled from a more dispersed sample. Second, in essence, causal relationships are likely to exist between perceived benefits and perceived usefulness. However, when we first resurveyed the literature, we did not find any evidence to support the existence of the causal relationships. Secondly, we added two causal paths ("perceived usefulness $\rightarrow$ perceived benefits" and "perceived benefits $\rightarrow$ perceived usefulness"), respectively into our proposed research model and ran the structural equation analysis to validate whether there are possible causal relationships between them, but only obtained two insignificant path coefficients. While to date there is no direct evidence, we believe that the possible causal relationship between perceived benefits and usefulness is worth investigating in future research. Third, while this study has identified three external factors (perceived risk, perceived ease of use and perceived benefits) influencing consumers’ adoption of internet banking, it is important to recognize the cultural and national limitations of these findings. This is because cultural differences have been found with respect to how individuals respond to a potential risk (Bontempo et al., 1997; Weber and Hsee, 1998). Moreover, according to Tse’s research (1988), individual’s cognitive propensity to risk differs across culture and is likely to affect the perceptions of the presence of risks as well as the evaluation of the risks. In other words, the customers’ acceptance of internet banking may be indirectly influenced by cultural differences. However, this phenomenon needs further investigations and validations. Hence, the replication of this study on a wider scale with different national cultures is essential for the further generalization of the findings. Finally, the conclusions drawn from our study are based on cross-sectional data. With our cross-sectional data, we only took a snapshot of this model. A stricter test of our argument, however, could be employed by using a longitudinal study to evaluate this aspect. By using a longitudinal study in the future, we could investigate our research model in different time periods and make comparisons, thus providing more insight into the phenomenon of internet banking adoption.

References


Fodder or animal feed is any agricultural foodstuff used specifically to feed domesticated livestock, such as cattle, goats, sheep, horses, chickens and pigs. Most animal feed is from plants, but some is of animal origin. Agriculture and animal husbandry in India are interwoven with the intricate fabric of the society in cultural, religious and economical ways as mixed farming and livestock rearing forms an integral part of rural living. Although the contribution of agricultural sector in the Indian economy is steadily declining (from 36.4% in 1982-83 to 18.5% in 2006-07), the agriculture and livestock sector still provides employment to 52% of the work force. Livestock provides draught power, rural transport, manure, fuel, milk and meat. Most often, livestock is the only source of cash income for subsistence farms and also serves as insurance in the event of crop failure. Further, global energy crisis will lead to utilization of livestock-based bioenergy as well as waste recycling for organic manure and organic forage production for quality animal products. India supports nearly 20% of the world livestock and 16.8% human population on a land area of only 2.3%. It is leader in cattle (16%) and buffalo (55%) population and has world’s second largest goat (20%) and fourth largest sheep (5%) population. At present, the country faces a net deficit of 61.1% green fodder, 21.9% dry crop residues and 64% feeds.

The notable contribution from Indian perspective is that of Duthie (1888) who described the fodder grasses of northern India. Later (Howard, 1923) studied the improvements of fodder and forage in India, digestibility coefficient and nutritive value of some tree species (Ram & Ray, 1943) seasonal variation in the composition of some edible tree leaves (Momin & Ray, 1943), and use of Bauhinia variegata leaves as cattle feed (Kehar & Goswami, 1950); and nutritive value of some grasses and legumes of Jammu and Kashmir (Chopra et al., 1956; Singh et al., 1959), ‘Kharsoo and Dudhiliya’ (Thapliyal, 1962). Some fodder trees (Prakash & Hockins, 1986) and fodder trees and shrubs of Central Himalaya (Purohit & Samant, 1995), traditional forage resources of India (Singh & Shankar, 1996) and Fodder and fuel wood resources of Central Himalaya (Singh et al., 1998) have added to our wealth of knowledge on the subject. Additionally, the work on Ethnobotany in India has been summarized by Binu et al. 1992 and Radhakrishnan et al. 1996, 2000 and its graphic account given by Jain & Srivastava 2001.

Methodology of Study

The collected plant specimens were dried, preserved and mounted as per the known herborizing practices outlined by Jain & Rao (1977). The specimens were identified using regional floras and various revisionary and monographic works (Chauhan, 1999; Chowdhery & Wadhwa, 1984; Collett, 1902; Dhiman, 1976; Hooker, 1872-1897; Nair, 1977; Polunin & Stainton, 1984; Stainton, 1988) and later got authenticated by carefully matching with the specimens at the herbaria of Botanical Survey of India (BSI), Northern Circle and Forest Research Institute (FRI), Dehradun. Nomenclature of these taxa was confirmed from Bennet (1986) and Wielgorskaya (1995). Excepting for the minor modifications to conform to the present day circumscription, families and genera are delimited mainly after The Flora Of British India (Hooker, 1872-1897). The Voucher specimens were deposited in Ethnobotany and Plant Taxonomy lab. of Himachal Pradesh University, Shimla. Nutritional analysis was got done from Choksi Labs, Indore by using Pearson’s Chemical Analysis of Foods, 1987.

Observations/ Results:

Nutritional analysis (Pearson, 1987) of three most commonly used fodder plants of Hamirpur District of Himachal Pradesh revealed the following results:-
Table 1-Nutritional Analysis of Fodder Leaves of Mallotus philippinensis
(On Dry Weight Basis) (Present Study)

<table>
<thead>
<tr>
<th>Name of Plant</th>
<th>Carbohydrates(%)</th>
<th>Proteins(%)</th>
<th>Fat(%)</th>
<th>Ash Content</th>
<th>Phos- phorus</th>
<th>Calcium(%)</th>
<th>Magnesium(%)</th>
<th>Ash Content</th>
<th>Phosphorus</th>
<th>Calcium</th>
<th>Magnesium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mallotus Philippi- nensis</td>
<td>45.03</td>
<td>15.67</td>
<td>3.0</td>
<td>9.22</td>
<td>0.19</td>
<td>1.53</td>
<td>0.28</td>
<td>12.8</td>
<td>20.93</td>
<td>549.48</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Fig. 1. Nutritional Analysis (%) of Fodder Plant Mallotus philippinensis (on dry weight basis).

Fig. 2. Nutritional Components of Leaves of Mallotus philippinensis.
**Fig. 3. Doughnut Showing Respective Value of Vitamins in Leaves of *Mallotus philippinensis*.**

**Table 2. Few Important Fodder Plants of District Hamirpur**

<table>
<thead>
<tr>
<th>Botanical Name</th>
<th>Local Name/s</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Adhatoda vasica</em></td>
<td>Basuti</td>
<td>Acanthaceae</td>
</tr>
<tr>
<td><em>Agave americana</em></td>
<td>Ramban</td>
<td>Agavaceae</td>
</tr>
<tr>
<td><em>Ageratum conyzoides</em></td>
<td>Neela Fulunu</td>
<td>Asteraceae</td>
</tr>
<tr>
<td><em>Albizia lebbeck</em></td>
<td>Sirin</td>
<td>Fabaceae</td>
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<tr>
<td><em>Albizia stipulata</em></td>
<td>Oyee</td>
<td>Fabaceae</td>
</tr>
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<td><em>Amaranthus viridis</em></td>
<td>Cholaee</td>
<td>Amaranthaceae</td>
</tr>
<tr>
<td><em>Amaranthus spinosus</em></td>
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<td><em>Arachis hypogaea</em></td>
<td>Moogphali</td>
<td>Fabaceae</td>
</tr>
<tr>
<td><em>Argemone maxicana</em></td>
<td>Lea</td>
<td>Papaveraceae</td>
</tr>
<tr>
<td><em>Arisaema tortuosum</em></td>
<td>Kirre-Ri-Chhhali</td>
<td>Araceae</td>
</tr>
<tr>
<td><em>Asparagus racemosus</em></td>
<td>Satavari</td>
<td>Liliaceae</td>
</tr>
<tr>
<td><em>Avena sativa</em></td>
<td>Joi, Javi</td>
<td>Poaceae</td>
</tr>
<tr>
<td><em>Bambusa arundinacea</em></td>
<td>Bainjh</td>
<td>Poaceae</td>
</tr>
<tr>
<td><em>Bambusa nutans</em></td>
<td>Bans</td>
<td>Poaceae</td>
</tr>
<tr>
<td><em>Bauhinia variegata</em></td>
<td>Kachnar, Karale</td>
<td>Fabaceae</td>
</tr>
<tr>
<td><em>Bauhinia vahlii</em></td>
<td>Tor</td>
<td>Fabaceae</td>
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<td><em>Boerhavia diffusa</em></td>
<td>Itsat, Utsata</td>
<td>Nyctaginaceae</td>
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<td><em>Bombax ceiba</em></td>
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<td>Boraginaceae</td>
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<td><em>Bougainvillea spectabilis</em></td>
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<td><em>Brassica campestris</em></td>
<td>Sarson</td>
<td>Brassicaceae</td>
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<td><em>Brassica juncea</em></td>
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<td>Brassicaceae</td>
</tr>
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<td><em>Brassica nigra</em></td>
<td>Rai, Ohari</td>
<td>Brassicaceae</td>
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<tr>
<td><em>Butea monosperma</em></td>
<td>Palah, Palash, Plah</td>
<td>Fabaceae</td>
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<td><em>Canna indica</em></td>
<td>Sudarshan, Hardarshan</td>
<td>Cannaceae</td>
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<td><em>Cardiospermum halicacabum</em></td>
<td>Tikku-Malu</td>
<td>Sapindaceae</td>
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<td><em>Carduus nutans</em></td>
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<td>Asteraceae</td>
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<td><em>Carica papaya</em></td>
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<td><em>Carissa carandas</em></td>
<td>Karonda, Bada Garna</td>
<td>Apocynaceae</td>
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<td><em>Carissa spinarum</em></td>
<td>Chhota Garna</td>
<td>Apocynaceae</td>
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<tr>
<td><em>Cassia fistula</em></td>
<td>Allila, Aahali, Kanyar</td>
<td>Fabaceae</td>
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<tr>
<td><em>Carduus nutans</em></td>
<td>Lea</td>
<td>Asteraceae</td>
</tr>
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<td>Scientific Name</td>
<td>Common Names</td>
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<td>Chenopodium album</td>
<td>Kunnah, Bathu</td>
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<td>Chenopodium ambrosioides</td>
<td>Kah-ajwain</td>
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<td>Cissampelos pareira</td>
<td>Patindu, Bhat</td>
<td>Menispermaceae</td>
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<td>Colebrookia oppositifolia</td>
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<td>Lamiales</td>
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<td>Cynodon dactylon</td>
<td>Doob, Dhruv,</td>
<td>Poaceae</td>
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<td>Dalbergia sisso</td>
<td>Tahali, Shish</td>
<td>Fabaceae</td>
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<td>Daucus carota*</td>
<td>Gajjar</td>
<td>Apiaceae</td>
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<tr>
<td>Dendrocalamus hamiltonii*</td>
<td>Baingh, Magg</td>
<td>Poaceae</td>
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<td>Mahendru</td>
<td>Sapindaceae</td>
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<td>Eclipta prostrata</td>
<td>Bhringraj</td>
<td>Asteraceae</td>
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<td>Emblica officinalis</td>
<td>Aamla</td>
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<td>Eriobotrya japonica*</td>
<td>Lukat, Laukha</td>
<td>Rosaceae</td>
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<td>Eruca sativa*</td>
<td>Taramira</td>
<td>Brassicaceae</td>
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<td>Kathman</td>
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<td>Eugenia jambolana</td>
<td>Jamun</td>
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<td>Ficus carica</td>
<td>Anjir</td>
<td>Moraceae</td>
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<td>Ficus glomerata</td>
<td>Umreyan, Umara</td>
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<tr>
<td>Ficus hispida</td>
<td>Devryan</td>
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<td>Ficus palmata</td>
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<td>Triamble, Tray</td>
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<td>Glycine max*</td>
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<td>Glycyrrhiza glabra*</td>
<td>Malathi</td>
<td>Fabaceae</td>
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<td>Gossypium arboreum</td>
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<td>Malvaceae</td>
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<td>Grewia oppositifolia</td>
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<td>Tiliaceae</td>
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<td>Habenaria pectinata*</td>
<td>Orchid</td>
<td>Orchidaceae</td>
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<td>Helicteres isora*</td>
<td>Marorphali</td>
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<td>Hordeum vulgare*</td>
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<td>Ipomoea carnea</td>
<td>Badi Basuti</td>
<td>Convolvulaceae</td>
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<td>Kehmble</td>
<td>Anacardiaceae</td>
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<tr>
<td>Lens culinaris*</td>
<td>Masar</td>
<td>Fabaceae</td>
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<td>Lepidium sativum*</td>
<td>Hallon</td>
<td>Brassicaceae</td>
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<td>Leucaena leucocephala</td>
<td>Alseenia</td>
<td>Fabaceae</td>
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<tr>
<td>Linum usitatissimum*</td>
<td>Alsi</td>
<td>Linaceae</td>
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<tr>
<td>Madhuca indica*</td>
<td>Mahua</td>
<td>Sapotaceae</td>
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<tr>
<td>Mallotus philippinensis</td>
<td>Kambhal, Kambal</td>
<td>Euphorbiaceae</td>
</tr>
<tr>
<td>Melia azedarach</td>
<td>Draek</td>
<td>Meliaceae</td>
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<tr>
<td>Millettia extensa*</td>
<td>Salangaya</td>
<td>Fabaceae</td>
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<td>Morus alba</td>
<td>Toot</td>
<td>Moraceae</td>
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<td>Oroxylum indicum</td>
<td>Tatpalanga</td>
<td>Bignoniaceae</td>
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<td>Oryza sativa</td>
<td>Dhan, Jari</td>
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<tr>
<td>Oxalis corniculata</td>
<td>Maroli</td>
<td>Oxalidaceae</td>
</tr>
<tr>
<td>Paspalum scrobiculatum*</td>
<td>Kodri grass</td>
<td>Poaceae</td>
</tr>
<tr>
<td>Pennisetum americanum*</td>
<td>Bajra, Barley</td>
<td>Poaceae</td>
</tr>
<tr>
<td>Pennisetum purpureum</td>
<td>Char</td>
<td>Poaceae</td>
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</table>
### Table: Nutritional Analysis of Two Important Fodder Plants of District Hamirpur

<table>
<thead>
<tr>
<th>Name of Plant</th>
<th>Carbohydrates (%)</th>
<th>Proteins (%)</th>
<th>Fats (%)</th>
<th>Starch (%)</th>
<th>Sodium ppm</th>
<th>Calcium (%)</th>
<th>Magnesium (%)</th>
<th>Potassium (%)</th>
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</thead>
<tbody>
<tr>
<td><em>Zizyphus jujuba</em></td>
<td>49.83</td>
<td>16.66</td>
<td>2.54</td>
<td>19.44</td>
<td>254.88</td>
<td>2.06</td>
<td>0.20</td>
<td>1.68</td>
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<tr>
<td><em>Grewia oppositifolia</em></td>
<td>41.21</td>
<td>20.20</td>
<td>3.71</td>
<td>19.91</td>
<td>68.42</td>
<td>2.36</td>
<td>0.26</td>
<td>1.76</td>
</tr>
</tbody>
</table>
Discussion
Species richness in forages predominantly belong to Poaceae (17 species: *Avena sativa, Bambusa arundinacea, Bambusa nutans, Cynodon dactylon, Dendrocalamus hamiltonii, Hordeum vulgare, Oryza sativa, Paspalum scrobiculatum, Pennisetum americanum, Pennisetum purpureum, Phragmites karka, Saccharum arundinaceum, Saccharum officinarum, Saccharum spontaneum, Setaria viridis, Triticum aestivum, Zea mays*), Fabaceae (15 species: *Albizia lebbeck, Albizia stipulata, Arachis hypogaea, Bauhinia variegata, Bauhinia vahlii, Butea monosperma, Cassia fistula, Cicer arietinum, Dalbergia sissoo, Glycine max, Glycyrrhiza glabra, Lens culinaris, Millettia extensa, Trifolium alexandrinum, Vicia pallida*), Moraceae (6 species: *Ficus carica, Ficus glomerata, Ficus hispida, Ficus palmata, Ficus roxburghii, Morus alba*), Asteraceae (5 species: *Ageratum conyzoides, Carduus nutans, Eclipta prostrata, Taraxacum officinale, Vernonia anthelmintica*), Brassicaceae (4 species: *Brassica campestris, Brassica juncea, Brassica niger, Lepidium sativum*), Rosaceae (4 species: *Eriobotrya japonica, Prunus domestica, Prunus pashia, Rubus ellipticus*), Chenopodiaceae (3 species: *Chenopodium album, Chenopodium ambrosioides, Spinacia oleracea*), Euphorbiaceae (3 species: *Emblica officinalis, Mallotus philippinensis, Phyllanthus urinaria*), Lamiaceae (3 species: *Colebrookia oppositifolia, Pogostemon alexandrinum, Vicia pallida*), Asteraceae (3 species: *Ageratum conyzoides, Carduus nutans, Eclipta prostrata, Taraxacum officinale, Vernonia anthelmintica*), Apocynaceae (2 species: *Carissa carandas, Carissa spinarum*), Meliaceae (2 species: *Cedrela toona, Melia azedarach*), Menispermacaeae (2 species: *Cissampelos pareira, Tinospora cordifolia*), Myrtaceae (2 species: *Eugenia cerasoides, Eugenia jambolana*), Nyctaginaceae (2 species: *Boerhavia diffusa, Bougainvillea spectabilis*), Sapindaceae (2 species: *Cardiospermum halicacabum, Dodonaea viscosa*), and rest of the families such as Acanthaceae, Agavaceae, Apiaceae, Araceae, Bignoniaceae, Boraginaceae, Cannaceae, Caricaceae, Celastraceae, Convulvulaceae, Flacourtiaeae, Fumariaceae, Liliaceae, Linaceae, Lythraceae, Malvaceae, Orchidaceae, Oxalidaceae, Papaveraceae, Polygonaceae, Ranunculaceae, Rhamnaceae, Salicaceae, Sapotaceae, Sterculiaceae, Tiliaceae, Urticaceae and Verbenaceae are poorly represented by one species each (Table 2). Notably high used genera in the region for forages are: *Ficus* (5 species), *Brassica, Carissa, Saccharum* (3 species each), *Albizia, Amaranthus, Bambusa, Chenopodium, Eugenia, Pennisetum* (2 species each), etc. As a matter of fact, species marked with an asterisk in Table 2 are new records for Indian forages (Anonymous, 1994; Sood & Kaushal, 2008) and hold considerable scope for popularization.

With regard to data on nutritional analysis of three fodder plants presently investigated, leaves of *Mallotus philippinensis, Zizyphus jujuba*, and *Grewia oppositifolia* justify being given as forages in the region as these are rich in carbohydrates, proteins, fats, calcium and magnesium (Table 1; Figs.1-3) and considered essential for improving quality of milk from lactating animals. Besides, leaves of *Mallotus philippinensis* also possess 549.48 ppm iron, 20.93 mg/100g vit. A, 12.80 mg/100g vit. C (Table 1; Figs.3), and that of *Zizyphus jujuba* and *Grewia*...
oppositifolia in possessing starch, sodium, potassium and magnesium (Table 2). Unlike the earlier nutritional analytic work on forages the present study is unique as details of vitamins and minerals have also been worked out for the first time. The results show the unheard and unknown knowledge of the indigenous people of the area who are using these plants as major fodder crops for their livestocks.

References
Abstract:
For a long time man has been in relation with animals in different ways. These relations have been both positive and negative and has generally been beneficial to mankind. Through the ages, animals have been exploited, and some have also become extinct due to humans. The discourse of animal studies has borrowed not only from the seminal works of Peter Singer and Tom Regan but also been enriched by the ecological and social movements like Great Ape Project (1993) and interdisciplinary approaches like Feminism, Minority Studies/Ethnicity, Visual Studies, Post colonialism, Post humanism etc. The philosophical debates about identity, difference, representation etc. have also challenged the framework of animal studies. The relation between man and animal can be compared to that of the relation between humans especially during the colonial era where the slaves, the colonized were dehumanized, exploited, and treated violently like animals. This paper will show that man has always been of a dominating nature- with other creatures all well as with their own genus. The first part will deal with the evolution of and relation of man with animals. I will also discuss the ethical part of this relation. In the second part I will show how these relations are more or less the same as those among human beings. Men and animals have lived together for a long time and their relation has changed through time and utility. I will argue at the end that it is via ethical bind towards animals and other Others that we can make earth a better place to live in.

Introduction
About 1000 years ago, the transition from hunting to the domestication of animals took place. The first animals to be domesticated were goat and sheep. After animal husbandry was introduced, the animals were generally considered as a source of food which is the same even today. According to a French philosopher, animals were soulless creatures who cannot feel pain or any other emotion. For him they had come on earth to accomplish a certain task which they could perform better than human beings. In the early Christian times it was believed that animals existed for the benefit of humanity. They were even brought to trial in the medieval civilization and by the ancient Germans. Pigs, if they harmed anybody were buried alive, chicken and geese that robbed fields were hanged, mad dogs were sentenced to death. Like human beings, animals can feel pain, pleasure, terror, possess social and imitative instincts and other emotions also.

Animals can be divided into two categories- domestic and wild. The former stay in symbiosis with man and are dependent on him, and the latter are independent creatures who follow their instincts to survive.

A tamed or domesticated animal staying with humans don’t run away looking at humans which should be their natural instinct. On the contrary wild animals, be it a cat or a tiger will run away because their instincts tell them to do so. They will attack only if they are hurt or have to defend their young. Domestic animals are used for entertainment, source of food or as pets.

All animals that stay close or with man lose their instinct. About instincts The Mother says:

What is called instincts in animals is simply obedience to the spirit of the species which always knows what ought and ought not to be done… You put a cow in a meadow; it roams around, sniffs, and suddenly puts out its tongue and snatches a blade of grass. Then it wanders about again, sniffs and gets another tuft of grass, and so it goes on. Has anyone ever known a cow under these conditions eating poisonous grass? But shut this poor animal up in a cow-shed, gather and put some grass before it, and the poor creature which has lost its instinct because it now obeys man, eats the poisonous grass along with the rest of it… And these unfortunate animals, like all animals, have a kind of respect (which I could call unjustifiable) for the superiority of man - if he puts poisonous grass before the cow and tells it to eat, it eats it!

Domestic animals obey their masters without questioning them and they become very dependent on humans. The humans then take advantage of this and take them for granted.

One of the incidents recounts the story about a dog that would lick its master’s hand every time he sensed that the man was going to get fits. The man would then lie down or call his wife. In this way the dog would save his master from getting hurt. Nowadays dogs are trained to do this with people who have epilepsy and they do their work very faithfully. In time there is a strong bond that grows between them and they become dependent of one another. But unlike humans who might betray their companion, dogs are more faithful creatures because the concept of betrayal does not exist in them.
How can we improve man's relationship with animals? This will be possible only if man changes his attitude and realizes the ethical and moral value which should exist between them. Animals have helped in healing human beings. There is a program which takes animals into schools and which helps improve the relation between man and animal. The animals that have undergone traumatic experiences with humans are given a chance to overcome their fear. Even elderly people are brought into the program and are given the opportunity to overcome their feeling of abandonment and rejection.

Dogs and monkeys are trained to assist handicapped people and are therefore called service animals. They do their job very well. Dr. Tom Regan an American animal rights activist and philosopher says:

That life includes a variety of biological, individual, and social needs. The satisfaction of these needs is a source of pleasure, their frustration or abuse, a source of pain. In these fundamental ways, the nonhuman animals in labs and on farms, for example, are the same as human beings. And so it is that the ethics of our dealings with them, and with one another, must acknowledge the same fundamental moral principles. At its deepest level, human ethics is based on the independent value of the individual: The moral worth of any one human being is not to be measured by how useful that person is in advancing the interest of other human beings. To treat human beings in ways that do not honor their independent value is to violate that most basic of human rights: the right of each person to be treated with respect. The philosophy of animal rights demands only that logic be respected. For any argument that plausibly explains the independent value of human beings implies that other animals have this same value, and have it equally. And any argument that plausibly explains the right of humans to be treated with respect, also implies that these other animals have this same right, and have it equally, too. It is true, therefore, that women do not exist to serve men, blacks to serve whites, the poor to serve the rich, or the weak to serve the strong. The philosophy of animal rights not only accepts these truths, it insists upon and justifies them.

Humans should not act according to who is in front. Rather he should act on his moral principles and on what he feels is right. He should put himself in the place of the other and see how it feels. Only then would he experience the true emotions.

Humans started keeping sea animals in captivity since the 1870s and were put on display. One of the Hollywood film companies called Marine Studio set up a marine tank in Florida for filming dolphins and training them was quite simple. Nine centuries ago, a Greek moralist stated that "to dolphins alone, beyond all other, nature has granted what philosophers seek: friendship with no advantage." Barbara Tuffy commented on this saying "dolphins also exhibit a friendly willingness to cooperate with other earth creatures- a rare attribute which another animal, Homo sapiens, has not learnt to do with any consistency."

Dolphins seem to be always grinning because it is said that they understand our language. In several human communities people communicate by whistling and this language is called Mezateco and it is spoken in Mexico. In Plutarch’s book On Cleverness of Animals, he says that Odysseus worshipped dolphins and apparently his son Telimachus was saved by a dolphin when he fell into the sea. Therefore his father wears a ring with a dolphin engraved on it.

In Shakespeare Twelfth Night, Arian, musician and poet was threatened by pirates and was asked to jump overboard. Before doing so he sang a whaling song and jumped but was saved by a dolphin who carried him to the shore.

In Africa, there are people who look for bees to harvest honey. There is a particular bird which guides them to the hives. The men then burn the hive to chase the bees. Before leaving, the men leave a small piece for the bird as a reward.

Even in the Indian epic The Ramayana, the love and admiration between Ram and Hanuman represent the love between man and animal. This relation is that of worship and respect for each other whatever be the other person’s status.

**Animals as a source of food**

From a long time man has been killing animals and eating them. Now here comes the ethical question of killing a living being as source of nutrition and gustatory pleasures. The Mother says,

*I knew a lady, a young Swedish woman, who was doing Sadhana and she was a vegetarian from choice and habit. One day she was invited by some friends who gave her chicken for dinner. She did not want to make a fuss, she ate*
the chicken. But afterwards, during the night suddenly she found herself in a basket with her head between two pieces of wicker-work, shaken, shaken, shaken, and feeling wretched, miserable; and then, after that she found herself head down, feet in the air, and being shaken, shaken, shaken. She felt perfectly miserable; and then all of a sudden, somebody began pulling out things from her body, and that hurt her terribly, and then someone came along with a knife and chopped off her head; and then she woke up. She told me all this; she said she had never had such a frightful nightmare, that she had not thought of anything before going to sleep, that it was just the consciousness of the poor chicken that had entered her, and that she had experienced in her dream all the anguish the poor chicken had suffered when it was carried to the market, sold, its feathers plucked and its neck cut! That's what happens! That is to say, in a greater or lesser proportion you swallow along with the meat a little of the consciousness of the animal you eat.

She says that when one eats meat, it is not only that flesh that is absorbed, but also the animal's spirit. These days there are a lot of people who are turning vegetarians or vegans as they have begun to realize that is against their moral value to kill and eat and animal as it also has the right to live. Humans try to show their power by killing them and considering them as helpless creatures.

The idea of treating other fellow being, either humans or animals is much spoken of especially in the colonial and the postcolonial context. Human beings have been treated like animals by fellow humans and this power relation continue between them.

**Man and animal in the colonial and postcolonial context**

There are lots of theorists such as Frantz Fanon, Michel Foucault, Aimé Cesaire who have dealt with the issue of humans being dehumanized and treated worse than animals without any sense of an ethical behavior. There is a play of power relations between the black colonized native peoples and the European colonizing people. The black are considered as being barbarous and therefore the civilizing mission taken up by the dominant white culture try to transform these ‘animals’ into ‘real’ people. In his book *Discourse on Colonialism*, Cesaire expresses the impact colonization had on the colonized, on culture, on society and directs this to the colonizers. He centralizes his argument on the claim that:

*... no one colonizes innocently, that no one colonizers with impunity either; that a nation which colonizes, that a civilization which justifies colonization- and therefore force- is already a sick civilization, a civilization which is morally diseased, which irresistibly, progressing from one consequence to another, one denial to another, calls for its Hitler, I mean its punishment.*

His primary focus is on the negative effect colonization had on the colonies, an effect which contradicts the colonizers claim of their “civilizing mission” or “the white man’s burden”. He says that colonization is a form of dehumanization by calling then barbarians, backward, uncivilized, the Other. This can be compared to the feeling of superiority in the face of animals who are been exploited to the hard labor in fields and that have been greatly exploited.

In order to regain their identities the colonized must resist the dominating force and must face the colonizer. This interaction and resistance is a violent one and therefore the natives have to use inhumane tactics but only for their freedom. If the colonizer accepts to fight for freedom, for his principles and moral values, there are three elements which he has to go through- first for the past, he must recognize and differentiate between his wrong and right deeds; second for the future he must rectify his mistakes and make sure that they are not repeated; and thirdly in the present he must recognize his wrong doings.

*In vain: in order to give them orders, even the harshest, the most insulting, you have to begin by acknowledging them; and as they cannot be watched over constantly you have to resolve to trust them. Nobody can treat a man ‘like a dog’ if he does not first consider him as a man. The impossible dehumanization of the oppressed turns against the oppressors and becomes their alienation. It is the oppressors themselves who, by their slightest gesture, resuscitate the humanity they wish to destroy; and, as they deny it to others, they find it everywhere like an enemy force. To escape from this, they must harden, give themselves the opaque consistency and impermeability of stone; in short they in turn must dehumanize themselves.*

In the case of the relationship between man and animal there is a resistance but man is too strong as he uses different tools and ultimately the animal has to submit himself in the hands of man.
One of the ways in which the colonized can change his situation is by changing the color of his skin and trying to copy him. He wishes to become like the colonizers and in this way his admiration for the colonizers increases and there is a slight hint of approving colonization. "We [the Indians] all admire them [the British]." But at the same time he is rejecting his self and loving the other. This is one of the steps towards assimilation. During this process the colonizer hides his past and his history becomes ignominious. Assimilation opposes colonization and eliminates the colonial relationship. "... but a party to bridge the gulf between East and West. None of the sides want to totally deny the others existence- all the colonizers are not bad. The colonized at the same time know that their life was happier before the arrival of the colonized and therefore want to get rid of them. "Let us shut them out and be jolly." The native becomes aware that he is not an animal (as the settlers say) but has humanity in him only when the colonizers talk about the animals in the mother country. Only after this do the natives know that they have to prepare to fight back. Although he is treated as a savage, he is conscious that he is not one.

As far as the native is concerned, morality is very concrete; it is to silence the settler's defiance, to break his flaunting violence... to put him out of the picture. The well-known principle that all men are equal will be illustrated in the colonies from the moment that the native claims that he is equal to the settler. (1963:44)

As for the settlers, if they go back to their country, the natives will "go back to the Middle Ages" (1963: 51).

The power relations that exist between the colonizer and the colonized are not legitimate. Michel Foucault explains and develops the idea of power relation in great detail where he says that power can be exercised positively or negatively and only among free subjects. The positive point of view involves governing with a set of goals of human beings. Power relations do not exist when man is in chains as in slavery. The slave does not have (physical) freedom. This relation is that of physical constraint. But slaves were not always free (example), they were not bound in chains or confined to cells. They were allowed to move and do their work. They were free to work. This gives the possibility of a power relation between the master and the slave.

This same power relation exists between humans and animals and it still exists today. We can see this by unnaturally breeding animals for consumption and the degraded fashion in which they are slaughtered. The animals cannot do anything but be enslaved by the act of these human beings. Even in the animal kingdom, they live in more harmony than in the human civilization. The human behavior has become more animal-like because they act without any moral or ethical principles. The power relation existing among humans and with animals shows that humans consider themselves to the most powerful. Colonization like the relation between man and animal is that of violence and as animals cannot resist this power it is in the hands of the human beings to take care of the 'lower' creature and take responsibility towards these animals and their own actions.

References
CAST CRIME IN INDIA: (CRIME AGAINST SCHEDULED CASTES & SCHEDULED TRIBES)

Introduction

The problem India is committed to the welfare and development of its people in general and of vulnerable sections of society in particular. Equality of status and opportunity to all citizens of the constitution of India, which also provides that no individual shall be discriminated against on the ground of religion, race, caste, sex or place of birth. It also guarantees that every citizen shall have equality of status and opportunity.

The problems of social inequality and class divide in a country like India with heterogeneous groups and sub-groups need to be recognized and resolved by all available democratic measures including special legislation to deal with particular acts constituting offences against such weaker sections of the society. ‘Scheduled castes and scheduled tribes’ are two such identified social groups. Article 46 of the constitution of India expressly provides that the state shall promote the educational and economic upliftment of the weaker section of the society, in particular of SCs & STs with special care and shall protect them from injustice and all forms of exploitation.

Legal rights

Special social enactments have come into force from time to time for SCs and STs in order to uphold the constitutional mandate and safeguard the interests of this section of the society.

The major legal enactments at the national level are:

1. Protection of civil rights act, 1955;

The protection of civil rights act, 1955 was enacted in furtherance of article 17 of the constitution to abolish untouchability and its practice in any form.

The scheduled castes and scheduled tribes (prevention of atrocities) act, 1989 was brought into force from 30th January 1990 in order to check and deter crimes against SCs/STs by persons belonging to other communities. These enactments have extended the positive discrimination in favour of SCs and STs to the field of criminal law in as much as they prescribe penalties that are more stringent than the corresponding offences under Indian penal code (IPC) and other laws special courts have been established in major states for speedy trial of cases registered exclusively under these acts.

Classification of crimes

The crimes against scheduled castes/scheduled tribes are broadly categorized under two major heads:

(1) UNDER THE INDIAN PENAL CODE (IPC)

1. Murder
2. Hurt
3. Rape
4. Kidnapping & abduction
5. Dacoity
6. Robbery
7. Others (other classified IPC crimes)
(2) **UNDER SPECIAL LAWS (SL)**

1. Protection of civil rights acts, 1955
2. The scheduled castes and the scheduled tribes (prevention of atrocities) act, 1989.

The crimes under IPC such as ‘murder’, ‘hurt’, ‘rape’, etc. or under special acts such as protection of civil rights act & scheduled castes and scheduled tribes (prevention of atrocities) act are already included in overall crimes reported under IPC and SLLs respectively and have been discussed in detail in the preceding chapters. The specific crimes against SCs/STs discussed in the following paragraphs are part and parcel of total crimes but analysed separately for better comprehension of crimes committed against SCs & STs.

The data on crimes against SCs/STs being compiled with provision for district-wise reporting of these crimes with effect from the year 2001. cases under the protection of civil rights act and the scheduled tribes (prevention of atrocities) act have been segregated for SCs and STs to have a clear picture of all the crimes against each category. Cumulative total of crime statics available on monthly basis were used for analysis till 2000 which did not distinguish true or false cases, therefore, the incidence of crime reported in the year 2001 and later years may not be comparable with figure of earlier years.

**Crime against scheduled castes**

**Incidence of crime – national (incidence (IPC+SLL):33,655)**

The year 2012 has witnessed a decrease in crime against scheduled castes as 33,719 cases reported in the year 2011 have decreased to 33,655 cases in the year 2012. This decrease was observed in all heads except rape, arson, and POA act. The cases of rape, arson and SC/ST (prevention of atrocities) act in the year 2012 have increased by 1.2%, 26.6% and 10.9% respectively over the year 2011. On the other hand, murder, kidnapping & abduction, dacoity, robbery, hurt and protection of civil rights act have shown a decrease of 3.3%, 20.5%, 25.0%, 25.9%, 9.2% and 7.5% respectively during the year 2012 over the year 2011. Uttar Pradesh has accounted for 18.4% (6,202 cases) of the total 33,655 cases reported in the country followed by Rajasthan (16.5%) (5,599 cases), Bihar (14.3%) (4,821 cases) and Andhra Pradesh (9.1%) (3,057 cases).

**Crime rate (crime rate: 16.7)**

The rate of crime against scheduled castes was observed as 16.7 during the years 2012. Rajasthan has reported the highest crime rate of 45.5 in the year 2012 followed by Goa (39.3), Orissa (31.5), Bihar (29.1) and Kerala (26.7) against the national average of 16.7.

**Crime head – wise analysis of crime against scheduled castes**

- **Murder (incidence: 651)**
  A total of 651 cases of murder were reported in the country during the year 2012 compared to 673 cases in the year 2011 thereby reporting a decrease of 3.3% Uttar Pradesh has accounted for 35.9% of the total murder cases reported in the country (234 out of 651 cases) followed by Madhya Pradesh 13.5% (88 out of 651 cases).

- **Hurt (incidence 3,855 rate: 1.9)**
  A total of 3,855 cases of hurt were reported during the year 2012 as compared to 4,247 cases in the year 2011 in the country thereby reporting a decrease of 9.2% during the year 2011. Madhya Pradesh (704 cases), Andhra Pradesh (626 cases), and Rajasthan (568 cases) together accounted for 49.2% of the 3,855 such cases during the year 2012.

- **Rape (incidence: 1,576 Rate: 1.6)**
  A total of 1,576 cases of rape of woman belonging to scheduled castes were reported in the country during the year 2012 as compared to 1,557 cases in the year 2011, thereby reporting a marginal increase of 1.2% Madhya Pradesh has reported 367 cases accounting for 23.3% of the total 1,576 cases reported in the country followed by utter Pradesh 18.1% (235 out of 1,576 cases)

- **Kidnapping & abduction (incidence: 490)**
  A total of 490 cases of kidnapping & abduction of scheduled castes were reported during the year 2012 as compared to 616 cases in the year 2011 thereby reporting a decrease of 20.5% utter Pradesh has reported 52.7% (258 out of 490 cases) of such incidence during the year 2012.
Comparative incidence of crime against scheduled castes

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Crime-head</th>
<th>Year</th>
<th>% Variation In 2012 Over 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>2008</td>
<td>2009</td>
</tr>
<tr>
<td>1</td>
<td>Murder</td>
<td>626</td>
<td>624</td>
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<td>2</td>
<td>Rape</td>
<td>1,457</td>
<td>1,346</td>
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<td>3</td>
<td>Kidnapping &amp; abduction</td>
<td>482</td>
<td>512</td>
</tr>
<tr>
<td>4</td>
<td>Dacoity</td>
<td>51</td>
<td>44</td>
</tr>
<tr>
<td>5</td>
<td>Robbery</td>
<td>85</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>Arson</td>
<td>225</td>
<td>195</td>
</tr>
<tr>
<td>7</td>
<td>Hurt</td>
<td>4,216</td>
<td>4,410</td>
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<td>8</td>
<td>Protection of civil rights act</td>
<td>248</td>
<td>168</td>
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<tr>
<td>9</td>
<td>SC/ST prevention of Atrocities Act</td>
<td>11,602</td>
<td>11,143</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td>14,623</td>
<td>15,082</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>33,615</td>
<td>33,594</td>
</tr>
</tbody>
</table>

Dacoity (incidence: 27)

A total of 27 cases of Dacoity were reported during the year 2012 as compared to 36 cases in the year 2011 thereby reporting a decrease of 25.0% Maharashtra has reported the highest number of 16 cases, accounting for 59.3% of total cases.

Robbery (incidence: 40)

A total of 40 cases of robbery were reported during the year 2012 as compared to 54 cases in the previous year thereby reporting a decrease of 25.9% Gujarat (16) and Maharashtra (13) accounted for 72.5% of total cases reported in the country.

Arson (incidence: 214)

A total of 214 cases of arson were reported during the year 2012 as compared to 169 cases in the year 2011 thereby reporting an increase of 26.6%. Bihar has reported the highest number of 47 cases followed by Uttar Pradesh (39 cases) Rajasthan (32 cases) and Odisha (31 cases). There states accounted 69.6% of total cases reported in the country.

Protection of civil rights act, 1955

A total of 62 cases were reported during the year 2012 as compared to 67 cases in the year 2011 thereby reporting a decrease of 7.5% in 2012 over 2011. Pondicherry has reported the 20 cases of such crimes followed by Karnataka (11 cases). Andhra Pradesh and Tamil nadu (9 cases each) during the year 2012. These four states have accounted for 79% of total cases reported in the country.

SC/ST (prevention of atrocities) act

A total of 12,576 cases were reported under this act during the year 2012 as compared to 11,342 in the year 2011 thereby reporting an increase of 10.9% Bihar has reported 4,436 cases accounting for 35.3% of the total cases reported the country followed by Odisha (15.5%) (1,943 cases), Uttar Pradesh (13.8%) (1,740 cases), and Karnataka (10.6%)(1,334 cases). At 27.0 the highest rate of crime was reported in Odisha as compared to national rate of 6.2.
Crime against scheduled tribes

A total of 5,922 cases against scheduled tribes were reported in the country during the year 2012 as compared to 5,756 cases in the year 2011 showing an increase of 2.9% in 2012 over 2011. This increase was observed under heads ‘murder’, ‘robbery’ ‘arson’, ‘hurt’ and ‘POA, act’. The details are presented in table-7 (B). Rajasthan has reported 22.8% (1,351 cases) followed by Madhya Pradesh 20.6% (1216 cases) of the total 5’922 cases reported in the country during the year 2012. However the crime rate was highest in Kerala at 25.6 as compared to only 5.7 at national level.

Crime head-wise analysis of crimes against scheduled tribes murder

A total of 156 cases of murder of scheduled tribes were reported in the year 2012 as compared to 143 cases in the year 2011, showing an increase of 9.1%. Madhya Pradesh has reported the highest number of cases (62 cases) accounting for 39.7% cases followed by Chhattisgarh (19 cases) of the total 156 cases reported in the country during the year 2012.

Rape

A total of 729 cases were reported in the year 2012 as compared to 722 cases in the year 2011. Thereby, showing a decrease of 5.6% Madhya Pradesh has reported 288 cases accounting for 39.5% of the total cases in the country during the year 2012.

Kidnapping & abduction

The incidents of kidnapping & abduction have decreased by 24.8% in the year 2012 over the year 2011 (137 cases). Madhya Pradesh has reported 45 cases followed by Gujarat 13 cases. These two states together accounted for 56.3% of the total 103 cases reported in the country.

Arson (incidence: 26)

A total of 26 cases were reported in the year 2012 as compared to 24 cases in the year 2011 showing an increase of 8.3% Madhya Pradesh has accounted for 30.8% (8cases) of total 26 such cases reported in the country followed by Odisha and Rajasthan have reported 19.2% (5 cases) each such crime during the year 2011.

Protection of civil rights act (incidence: 2)

A total of 2 cases were reported during the year 2012 as compared to 7 cases in the year 2011. Thereby showing decrease of 71.4% over 2011 Karnataka and Maharashtra have reported 1 case each accounted for 100% of such cases during the year 2012.

SC/ST (prevention of atrocities) act

A total of 1.311 cases were reported during the year 2012 as compared to 1,154 cases in the year 2011 showing an increase of 13.6% Odisha has accounted for 44.2% (579 cases) of total 1,311 cases reported in the country followed by Karnataka (164 cases), Jharkhand (133 cases), Andhra Pradesh (127 cases) and Bihar (118 cases) accounted for 85.5% of such crime during the year 2012.

Disposal of crimes by police courts

The general trend of disposal of IPC and SLL crimes by police and court has already been discussed in detail in chapter 4. The average charge-sheeting rate for the crimes against scheduled castes and scheduled castes and tribes stood at 91.8% and 95.8% respectively in comparison to national level general charge-sheeting rate of 78.8% for IPC crimes and 93.4% for SLL crimes.
### Comparative incidence of crime against scheduled tribes

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<tr>
<td>1</td>
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<td>5,885</td>
<td>5,756</td>
<td>5,922</td>
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</tr>
<tr>
<td>2</td>
<td>Murder</td>
<td>128</td>
<td>118</td>
<td>142</td>
<td>143</td>
<td>156</td>
<td>9.1</td>
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<td>3</td>
<td>Rape</td>
<td>585</td>
<td>583</td>
<td>654</td>
<td>772</td>
<td>729</td>
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<tr>
<td>4</td>
<td>Kidnapping &amp; abduction</td>
<td>93</td>
<td>82</td>
<td>84</td>
<td>137</td>
<td>103</td>
<td>-24.8</td>
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<tr>
<td>5</td>
<td>Dacoity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-28.6</td>
</tr>
<tr>
<td>6</td>
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<td>7</td>
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<tr>
<td>7</td>
<td>Arson</td>
<td>18</td>
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<td>Hurt</td>
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<td>29</td>
<td>39</td>
<td>24</td>
<td>26</td>
<td>1.6</td>
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<td>protection of civil rights act</td>
<td>873</td>
<td>787</td>
<td>941</td>
<td>803</td>
<td>616</td>
<td>-71.4</td>
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<tr>
<td>10</td>
<td>SC/ST (prevention of atrocities) act</td>
<td>6</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>others</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13.6</td>
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<tr>
<td></td>
<td>Total</td>
<td>2,794</td>
<td>2,853</td>
<td>2,869</td>
<td>2,700</td>
<td>2,759</td>
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</table>

The average conviction rate for crimes against scheduled castes and scheduled tribes stood at 23.9% and 22.5% respectively as compared to overall conviction rate of 38.5% relating to IPC cases and 88.6% relating to SLL cases.

**Disposal of persons arrested by police and courts**

56,410 persons (71.3%) out of 79,112 persons (including persons under investigation of previous year previous) arrested for committing crimes against scheduled castes were charge-sheeted by the police. A total of 9,086 persons were convicted by the court, out of 38,507 persons against whom trials were completed representing a conviction rate of 23.6%.

9051 persons out of 10,886 persons arrested for crimes committed against scheduled tribes were charge-sheeted accounting for 83.1% charge-sheeting rate. A total of 1,413 persons were convicted out of 7,117 persons against whom trials were completed representing 19.9% conviction rate.

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5. Data from National Crime Records
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SURVIVOR’S GUILT IN HEMINGWAY’S WRITINGS: AN INSIGHT INTO HIS MAKING

39

Abstract:
Ernest Hemingway (1899-1961) is known as the American writer who wrote of himself in his writings and represented the bitter side of the so-called Lost Generation. His works, The Sun Also Rises, A Farewell to Arms, The Old Man and the Sea have special connotations to understand and discover. It is not just the relationships that Hemingway failed in; he never came into an agreement with the idea of war for the sake of war. He stands for the failed heroes of the States who felt shattered after the brutal encounter with the emptiness of warfare. Ernest contrasts arms in love and arms in war. From his adventure fascinated childhood to his suicide, he remained a mythical figure not only for American Literature, rather for the whole genre of fiction. His works started with short stories and concluded with worldly acclaimed fiction. He wrote as a reporter and brought calculated expressions to his works. He represented the post World War-I guilt ridden generation and the resulting emotional void. He was moved to writing by the Survivor’s Guilt that he suffered from along with millions of young participants of the war.

One of the notable authors of the era, encompassing between the two infamous world wars, Hemingway's early writing portrays the lives of two types of people. The former consists of men and women who have lost faith in moral values, and live with cynical disregard for anything but their own emotional needs. They came to be known as the Lost Generation. The other type is, men of simple character and primitive emotions, such as boxers and bullfighters, who wage courageous and usually futile battles against the circumstances of their lives. About Ernest Miller Hemingway it is said: “When you want to find the truth about Hemingway’s life, look first to his fiction.” Thus Survivor’s Guilt in Hemingway’s writings is to be probed through an inter-relation of his own life and the life of his characters. He has actually brought a catharsis of his own mental trauma.

When the war was going on Hemingway was eager to join it. But, he was denied the selection in the force because of his defective vision. Later, he got an opportunity to be a part of the war, as he volunteered as a Red Cross ambulance driver in December 1917, and was accepted for service. Hemingway once wrote to his close friend, Fitzgerald, about the glory and effect of war, saying, “War groups the maximum of material and speeds up the action and brings out all sorts of stuff that normally you have to wait a lifetime to get.”

Hemingway reached Paris, amid heavy bombardment from the Germans. This left him seriously wounded. According to Jeffery Meyers, this wound was a major turning point of his life. It was his first, direct and personal encounter with the wounds of the war on his body, though he had already experienced the psychological agony of war, on his very arrival. It is believed that he got over 200 wounds in his legs. The truth about war, he later observed, was lacking when he needed it most.

The guilt of being survived, while his mates were not alive, haunted Hemingway like hell. The wounds he had received were more psychological than physical. The question: why the World War happened and why he has been a part of it, killed him from inside. In his letters to his family he told that he was shot twice through his scrotum, and had to rest his testicles. It gave him lifelong nightmares about his masculinity. Meyers informs further:

Others soldiers in his ward had seriously damaged genitals and those who mutilated inspired the wound of Jake Barnes in The Sun Also Rises. Jake’s wound is not described in the novel; but the account of Frederic’s wound in A Farewell to Arms...is close to what actually happened.

One of the first patients to be admitted in the Milan Hospital, Hemingway fell in love with the nurse, Agnes, who was seven years older to him, later rejected by her leaving a devastating effect on him. Agnes was later portrayed as Catherine Barkley in his most famous novel, A Farewell to Arms. After his return from the war, Hemingway went through the haunting effects of his concussion. He suffered long nights of insomnia and it was not possible for him to sleep in the dark, having the lights off.

He returned as a wounded man. He joined writing profession, though the psychological stirs could not be calmed down until he wrote his first novel-the damn tragedy of life- The Sun Also Rises, which appeared in 1926. Literature gave Hemingway a tool to rebuild his shattered world of hope and faith. “Writing is a form of therapy;” says Graham Greene, “sometimes I wonder how all those who do not write compose or paint can manage to escape the madness, the melancholia, the panic fear which is inherent in the human situation.”
His post war writings have mental illness in them, as Hemingway could not save himself from the rampant PTSD (Post Traumatic Stress Disorder). The burden of being a survivor of the worst war ever, made him sick of himself. His mental illness, that was also an inheritance through genes too, turned into a psychological disorder, termed as Bipolar Disorder. To have a better understanding between Hemingway’s life and his writings, his guilt and disorders, his attempt to remove the burden of being survivor, one has to elaborate and understand Hemingway’s writings.

Hemingway began with Nick Adams as hero; the first to be wounded among the Hemingway heroes who declares that he has made a separate peace away from the violent world. The expatriates in The Sun Also Rises are in search of peace and in A Farewell to Arms, Henry and Catherine establish this separate peace for themselves. In A Farewell to Arms, chapter 34 has pessimism:

“If people bring so much courage to this world, the world has to kill them to break them, so of course it kills them. The world breaks everyone and afterward many are strong enough at the broken places. But those that will not break it kills.”

The professional psychologist can use his knowledge of psychological problems and situations to interpret a work of literature, even without any reference to its author’s biography. If Jake Barnes or Frederic Henry or Nick Adams or Jordan or Harry Morgan behaves according to a specific pattern, which, Freud discovered to be characteristic of certain kinds of individuals, this does not mean that Hemingway had in mind the Freudian theories, but it does confirm Hemingway’s remarkable insight into human nature.

The holocausts had previously been damaging the psyche of people, but the World War I was the first danger of its kind that posed a threat to the whole humanity itself. “When people realize how bad it is, they cannot do anything to stop it because they go crazy. There are some people who never realize…. There is no finish to a war.”

The First World War was a long series of inconclusive battles. Hemingway was one of those, who received first hand experiences of the war. The war was destructive, as Encarta states:

"The death of over 10 million men in combat left a gaping chasm in the social and economic life of the postwar world. Many of those who survived the war returned home with physical disabilities that prevented them from rejoining the work force. Others suffered the lasting effects of what in those days was called 'shell shock' and what is today labeled Post-Traumatic Stress Disorder (PTSD), a psychological affliction that prevents a successful adaptation to civilian life.

Depression is what came as a consolidated term for a number of diseases, such as, the feeling of guilt that haunted the survivors, and led them in the dark realms of PTSD (Post-Traumatic Stress Disorder). Artists began to produce works that mocked the self-confident assertions of humanism, and portrayed the sordid realities of modern life. It was the surfacing of guilt that they encountered and remained in a constant clash with it.

To elucidate the feeling of guilt, the Encyclopedia of Psychology suggests, “Guilt is a cognitive or an emotional experience that occurs, when a person realizes or believes- whether justified or not- that he or she has violated a moral standard, and is responsible for that violation.” The Sun Also Rises and A Farewell to Arms are the two works that have their roots in this overt action and his personal guilt. States the Wikipedia Online Encyclopedia:

"When we see another person suffering, we can feel their suffering as if it is our own. This constitutes our powerful system of empathy, which leads to our thinking that we should do something to relieve the suffering of others. If we cannot help another, or fail in our efforts, we experience feelings of guilt.

The helplessness of being a victim of the tragedy and thus feeling responsible for the trauma of others, the survivor’s guilt becomes haunting; it is explained in the following words:

"Survivor guilt/survivor syndrome is the mental condition that results from the appraisal that a person has done wrong by surviving traumatic events such as combat, natural disasters, or even surviving a lay-off in a work place. The effect of the survivor’s guilt depends on the person’s own psychological make-up.”

Survivor’s guilt is common among the world war survivors. People suffered from collective guilt after the war. Critics are also of the view that in Hemingway, there lays both the poles- extreme optimism, and the worst state of pessimism. When he was leaving Hadley for Pauline Pfeiffer in 1926, he wrote to Pauline of how he had thought to remove the sin out of her life and avoid Hadley the necessity of divorce by killing himself. Frederick Busch affirms:
A month before, he had written to someone else that "the real reason for not committing suicide is because you always know how swell life gets again after the hell is over." … He was a nexus for death. After he had killed himself, his sister Ursula committed suicide in 1966 and his brother, Leicester, in 1982.

Hemingway seems to be a different man having the same blood. Bipolar Disorder is what made Hemingway, a guilty survivor and a suicidal case of mental stress. Busch says in his article ‘Message From a Divided Man’

…that both as man and writer he was fearfully divided. There is a Hemingway, mostly young, who is sensitive, brave, generous and extremely intelligent. There is a Hemingway, mostly older, who is coarse, bullying and hostile to the exercise of mind.

Apparently, A Farewell to Arms is a love story in the backdrop of war and is a semi-autobiographical one. The novel ends with the death of the heroine, that's what Hemingway did very often with the ladies his men love. He lets his representative hero live alone, and makes him feel the pain of being a survivor. Hemingway's task was not to suffer in heart, rather he set out to make that pain common, and thus bring out the suppressed anguish and fear:

When he writes very well about love, loss, tenderness or fear, Hemingway works with the assumption that he must cause the reader to share the unstated emotion. That is responsible writing, a writing that is about the essential transaction between writer and reader. It is about being human in a time of despair.

The Hemingway hero, by whatever name he appears, carries both physical and psychic scars. Hemingway's basic pre-occupation was how one should live with these scars. Jake Barnes, in The Sun Also Rises, is made impotent by the cruel war. In A Farewell to Arms, Frederic Henry's physical wound has emotional repercussions and Hemingway's hopeless attitude is evident in him:

I put a log on top of the fire and it was full of ants…. I remember thinking at the time that it was the end of the world and a splendid chance to be a messiah and lift the log off the fire…. But I did not do anything but throw a tin cup of water on the log, so that I would have the cup empty to put whisky…. This is all life is. Death makes mockery of all values.

If it is admissible that the age suffered from paranoia, from PTSD, from the Survivor's Guilt; how a man of intellects like Hemingway can escape these? Obviously, he could not, and he has not. Hemingway suffered from the guilt; he used his writing faculty to express it, and thus universalized his emotions. 'Survivor's Guilt' in Hemingway’s writing is thus perceptible and acknowledged: "True guilt is guilt at the obligation one owes to oneself to be oneself. False guilt is guilt felt at not being what other people feel one ought to be or assume that one is.”

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Swami Vivekananda, a Bengali born sadhu attained world fame through his utterances in the parliament of Religions held at Chicago. During his teens, he had experienced many unfortunate happenings. Out of which the total sweep of family's comfort resulted in the meeting with Shri Ramakrishna. His very first sight itself impressed Swami Ramakrishna and he became the principal disciple of him. As per the advice given by him, he felt relieved from miseries by praying at the mother Kali temple at Dakshineswar. He spent his earlier years in meditation, yoga and had taken a short role in Brahma-Samaj. Also he was attracted towards Theosophical movement. Both his poverty at the youthful days and his experiences from the length and breadth of the country induced him to work for the betterment of India. His visit to the West prospered him with the greatest charity which was utilized to back-up the economically down-trodden. His lectures and discourses have given solace to the listeners in many aspects. The follow-up writing focuses the remedy essential for the Indian society.

Salient Features
Swami Vivekananda’s view on education comprises the meaning of true education. A child observes things and teaches its own mind by itself. Here the work of the mother is to help it to go forward in its own way by taking away the obstacles. It is like planting a sapling which grows naturally when it is protected from grazing animals and with the supply of water. The same process is essential for a child to grow. It is a negative way of teaching the children by constantly taxing them by saying read, write and using some harsh words. On the other hand, they must give positive ideas of using kind words and encouragement. By doing so, they will be getting chances of holding good in the region of higher thoughts.

Swami said that his preaching gave a man an expression and the listener will teach his mind. The Vedanta says that it is a false belief that one teaches the other. Because, within man is all knowledge. On the other hand teachers’ work is to awaken the knowledge which is inbuilt in a boy. At this stage he learns to apply his own intellect to the proper use of their hands, legs, ears, eyes etc.

Next is the fault finding in the writing of literature, poetry, language and arts resulted in wounding man's feelings. Instead a teacher must encourage them by giving supporting points with the view of overcoming their mistakes by themselves. At this stage Swami reminds us that by doing so a man discovers his knowledge which is inherent in him. By this Swami would like to convey to the teachers’ community that education does not mean a mere filling of minds with facts. The ideal of education is to perfect the instrument i.e. concentration in order to get complete mastery of one’s mind. Herein the difference between man and an animal and also differentiating one man from another man does not arise. Thus he said that concentration is the essence of all knowledge.

Another important point taken for discussion is food. Food is essential for everyone’s life. But we must keep in mind that what sort of food is best suited for getting strength of our physique. Killing one’s life just for survival is considered as Sin. But if a man’s physique is not suitable for vegetarian food and also leading an active life, he would have turned to be a meat-eater. Swami’s suggestion is that if a person is the only bread-winner whose survival falls on family earning by cutting the throat of animals need not worry about sinful attitude such as killing animals and eating non-vegetarian food. Also Swami Vivekananda condemned the enforcement of vegetarianism upon the labourers is one of the causes of the loss of our national freedom.

People are under false assumption that one’s happiness lies on him and with his belongings and good environment. Some people say that happiness lies on one’s Karma which connects past and the present. In the words of Swami Vivekananda that true happiness consists in killing one’s selfishness.
In this world, rich man has plenty of food but he could not eat the whole lot because it will affect his digestive system. Sometimes rich man does not have children at all. On the other hand poor man has a whole regiment of children. They may not be properly fed. In Swami's words that happiness and misery are the obverse and reverse of the same coin. So a man must accept both happy situation and unhappy happenings.

Swami has reminded us that every soul is slaves to nature, money, wealth, wife and children who are hanging around here and there with the illusion of getting happiness in the future. People are thinking that happiness lies on human's physical comfort i.e. getting happiness through drinking, eating and merry-making. At this juncture, Swami described that men find a higher plane of happiness in the Atman or Self. For the philosopher, this knowledge of the Self is of the highest utility. Getting knowledge is the highest happiness which must be known by every human.

Swami Explained shortly that people will call a householder a good man when he supports and brings comfort to his own family and to others and perform good works as far as possible. In Swami’s words, the exact rule for a householder about the expenditure of his income must be divided into four to convene his family, charity, savings and for self.

Swami Vivekananda's suggestions for the world to get satisfaction through detachment clearly state the real work of the head of the family. Being good, a man earns and spends everything for the sake of his children. There he never expects anything back from them. Like whatever he wishes to offer to the society or to this world, it must be through his free will. There he should not expect the world to compliment him. Here we are reminded of Kennedy, the former president of the U.S.A. His view is that one must do his duty without expecting the reward. This will bring detachment. Swami’s idea is to bring to the door of the meanest, the noble idea that the human race has developed both in and out of India. They are allowed to think for themselves. If there is no liberty of thought and action which is the only condition of life, all the living beings and the nation must be put down under the earth. That is said to be the house of devils that always do bad deeds.

**Conclusion**

Swami’s ideas can very well bring solution to the present day India, which is overcome by terrorism, corruption, exploitation, regionalism and meaningless slogans. No doubt his ideas usher us to a new world which will pave way for the betterment of human beings. The values of his teachings are to be realized by all us. Swami’s views on service to society convey that a man who wants to serve God must extend his helping hand towards poor. If so, he will be blessed with all the prosperity of the earth. Let us walk on the path of Swami Vivekananda to bring peace to the land.

Shanthi..... Shanthi......

**References**

Abstract:
The research was conducted in the management of networks. In order to achieve the best quality health services, respect is achieved through professional competency. Professional competency is easily reached where the working environment affords minimal workload with maximal achievement, in a comfortable work place and a good teamwork. Managing our work will lead to our enjoyment of life. One of the strategic entry points is the working environment improvement, which can easily be achieved by the implementation of the 5-S concept.

The health regulatory services are important and need to be taken care of while ensuring quality services. There are frequent audit of the processes and procedures to make sure that they are correct, constant risk management by assessing the risk involved in all their undertaking and taking preventive and effective measures, avoid quality degradation by continuous quality improvement including adoption of new inventions, have a good system of command and control by having a system that assures good leadership, good decision-making process as well as effective monitoring and evaluation process, employees are well motivated by the existence of a good rewarding system, migrating decision-making is made possible by the existence of clearly known protocols coupled with good communication system in the organization, back-up system is always in place and known to all pertinent employees in the organization, formal rules and procedures are in place and are observed. Where symptoms of poor quality are seen, it is impossible to provide services with safety. To achieve high quality, systems used in implementation have to constantly be improved, for quality fails when systems fail.

The research was conducted in the management of networks. In order to achieve the best quality health services, respect is achieved through professional competency. Professional competency is easily reached where the working environment affords minimal workload with maximal achievement, in a comfortable work place and a good teamwork. Managing our work will lead to our enjoyment of life. One of the strategic entry points is the working environment improvement, which can easily be achieved by the implementation of the 5-S concept. While there are various models of implementing the planning activities the most important and vital point is the need to always improve on what already exist leading to Continuous Quality Improvement. Implementing working environment improvement together with intellectual activities of planning with CQI will lead to acquiring the TQM framework thus enabling the provision of quality services and high productivity.

Use of 5-S in health facilities
KAIZEN is a process of Continuous Quality Improvement (CQI) by means of a non-stop process to uplift the standard of work environment and services contents to the obtainable best condition and maintain it as user-friendly and convenient as possible. CQI has to be practiced by all categories of staff including the management team. Top management is not an exception and should participate in the process. KAIZEN (CQI) is an approach developed in manufacturing sector in Japan to improve the productivity. Imagine a factory manufacturing vehicles. Here, over 2500 parts are prepared, standardized and supplied timely for the assembly process of one vehicle. Also there is a workable communication system among different sections and offices to control the production process. The production line is perfectly in order since they have to assemble the 2500 parts precisely on time having their outcome target of penalizing 5,000 vehicles per day. If there are many rejected items, the company loses money. It also negatively affects the quality of vehicles and finally loses in the competition in the market. Quality of the end-product, which is handled by various groups of people (production units), cannot be maintained, if there is no mechanism, by which all production units seek higher quality of work throughout the on-going production process. It is this concept, which KAIZEN (CQI) seeks to achieve in the provision of health services in the hospitals and other health facilities.

Principles are your reliable instruments to make a break-through in your work environment and staff attending various types of jobs in your institution. 5-S is not only a concept but also a set of actions, which has to be conducted systematically with the full participation of staff serving the institution. 5-S activities are practiced in a real
participatory movement to improve the quality of both the work environment and service contents, which are delivered to your clients using the improved environment. It is used as a basic, fundamental, systematic approach for productivity, quality and safety improvement in all types of organizations.

Guiding themes for QI
The following themes should be recognized and adopted to guide designing, planning and implementation of QI in India. A culture of safety and improvement that rewards improvement and is driven to improve quality is important. The culture is needed to support a quality infrastructure that has the resources and human capital required for successfully improving quality. QI Committees need to have the right stakeholders involved.

QI Committees and stakeholders need to understand the problem and root causes. There must be a consensus on the definition of the problem. To this end, a clearly defined and universally agreed upon metric is essential. This agreement is as crucial to the success of any improvement effort as the validity of the data itself. Methodologically sound approach without being distracted by the jargon used in QI. The importance given to using clear models, terms, and process is critical, especially because many of the quality tools are interrelated; using only one tool will not produce successful results. Standardizing care processes and ensuring that everyone uses those standards should improve processes by making them more efficient and effective—and improve organizational and patient outcomes. Evidence-based practice can facilitate on-going QI efforts.

Efforts to change practice and improve the quality of care can have multiple purposes, including redesigning care processes to maximize efficiency and effectiveness, improving customer satisfaction, improving patient outcomes, and improving organizational climate. Appropriate use of technology can improve team functioning, foster collaboration, reduce human error, and improve patient safety. Efforts need to have sufficient resources, including protected staff time. Continually collect and analyze data and communicate results on critical indicators across the organization. The ultimate goal of assessing and monitoring quality is to use findings to assess performance and define other areas needing improvement. Change takes time, so it is important to stay focused and persevere. Health system interventions are usually multimodal; concurrently addressing providers, patients and system level interventions. Situation analysis and literature review of the various QI methodologies indicates that although the presentation of various modern QI methodologies seems different, the content and basic principles are very similar and in most cases complement each other. In India, districts and partners involved in QI shall implement evidence based targeted QI models and interventions which apply the principle of an iterative cycle of improvement – Plan, Do, Study, Act (PDSA cycle).

In order to set the best stage for health personnel to make maximal use of their skills and knowledge, the MoH recommends the 5S method as the foundation for all QI initiatives in the country. 5-Ss is a management tool, which originated in Japanese manufacturing sector. It is used as a basic, fundamental, systematic approach for productivity, quality and safety improvement in all types of organizations. Usually, improvement of work processes often is sustained only for a while, and workers drift back to old habits and managers lose the determination and perseverance. 5-S in contrast involves all staff members in establishing new disciplines so that they become the new norms of the organization i.e. internalization of concepts and development of a different culture.

Although the 5-Ss originated in the manufacturing environment, they translate well to other work situations including hospitals, general offices, telecommunication companies etc. 5 Ss are abbreviations of the Japanese words Seiri, Seiton, Seiso, Seiketsu, and Shitsuke. In English, 5Ss were translated as Sort, Set, Shine, Standardize, and Sustain. In practical context, local language words are more effective for people to understand easily thus, facilitators of 5S-CQI-TQM in some countries have translated 5-S English words into local language e.g. in Swahili these are: Sasambua (Sort), Seti (Set), Safisha (Shine), Sanifisha (Standardize) and Shikilia (Sustain). 5-S is the initial step towards establishing TQM. There will be no conflict in the implementation of 5-Activities even though organizations are already implementing other QI interventions. 5-Swill support all QI interventions to move forward.

Implementation Modalities
5S activities will be used as tools to prepare the obtainable best stage for health personnel to make maximal use of their knowledge and skills. The 5S principles will be implemented starting with a few targeted areas and use the results from these areas to win support from the remaining areas to implement them. The following steps will be followed in the implementation of 5S: Elimination of all unnecessary stuff from venue of work and reduce clutter. Go through all tools, materials, and so forth in the work area. Keep only essential items and eliminate what is not required, prioritizing things as per requirements and keeping them in easily-accessible places. Everything else is stored or discarded. Setting in order or Straightening / Stabilize Organize everything needed in proper order for ease
of operation. There should be a place for everything and everything should be in its place. The place for each item should be clearly labeled or demarcated. Items should be arranged in a manner that promotes efficient work flow, with equipment used most often being the most easily accessible.

Workers should not have to bend repetitively to access materials. Each tool, part, supply, or piece of equipment should be kept close to where it will be used — in other words, straightening the flow path. Shining or Cleanliness 

Maintain high standard of cleanliness. Clean the workspace and all equipment, and keep it clean, tidy and organized. At the end of each shift, clean the work area and be sure everything is restored to its place. Set up the above three S’s as norms in every section of the workplace. Work practices should be consistent and standardized. All work stations for a particular job should be identical.

All employees doing the same job should be able to work in any station with the same tools that are in the same location in every station. Everyone should know exactly what his or her responsibilities are for adhering to the first 3 S’s. Sustaining the discipline or self-discipline, train and maintain discipline of the personnel engaged. Once the previous 4 S’s have been established, they become the new way to operate, maintain focus on this new way and do not allow a gradual decline back to the old ways, while thinking about the new way, also be thinking about yet better ways, when an issue arises such as a suggested improvement, a new way of working, a new tool or a new output requirement, review the first 4 S’s and make changes as appropriate, on improvement of the work environment/infrastructure from 5S implementation; then other QI initiatives can now come in to improve various aspects of quality in health services including technical issues, all QI initiatives shall be implemented based on the “Improvement Collaborative” Model ensuring application of the principle of an iterative cycle of improvement (PDSA cycle).

An “improvement collaborative” is shared learning system that brings together a large number of teams to work together to rapidly achieve significant improvements in processes, quality, and efficiency of a specific area of care, with intention of spreading these methods to other sites / units. The collaborative approach combines traditional QI methods of team work, process analysis, introduction of standards, measurement of quality indicators, training, job aids, and coaching with techniques based on social learning and diffusion of innovation.

Initiatives should conduct assessments of the current situation (current quality standards) to identify critical gaps and trace their root causes. By this process, it becomes easier to determine and plan for what is to be done to improve the current quality situation (desired quality standards). Depending on what has been identified as of critical need by the MoH or districts, partnering shall determine what to prioritize on supporting and initiate programs that contribute to providing solutions at any level.

A collaborative will focus on a single technical area (for example prevention of mother-to-child transmission of HIV, ART, family planning, immunization, neonatal- newborn care, etc) and seek to rapidly spread existing knowledge or best practices related to that technical topic to multiple settings, through systematic improvement efforts of a large numbers of teams. Also, interventions may decide on their priorities on any health related problem (e.g. Performance Improvement for Health Workforce, QoC in HIV/AIDS, Systems Strengthening, Occupational safety, etc). By implementing their interventions, programs or agencies shall be creating solutions to quality issues within the health sector, as long as their priorities lie within the national health goals and framework. The teams shall work together for a 10 – 12 month period to achieve significant improvements in a specific area of care and then focus on another technical area. Teams of health care providers will work independently to test out changes in how they deliver care that seek to implement best practices and accepted standards for the collaborative’s priority area. Teams shall use a common set of indicators to measure the quality of the care processes the collaborative is trying to improve and, where possible, the desired health outcomes. The planning process should involve everyone and include the following steps:

1. Creating a vision or goal of the future.
2. Developing innovative ideas about the steps taken to achieve the goal.
3. Grouping ideas into specific strategies.
4. Analyzing the strategies using structured problem solving tools.
5. Formation of cross-functional teams to further investigate the viability of each strategy and to flesh-out an action plan if appropriate.
6. Reviewing and approving the final strategies and action plans.
7. Identifying Action Teams members
8. Implementation of strategies.

The full planning team meet should meet regularly to review and measure progress on each initiative, and to make any adjustments in the plan that are needed. Management and the employees must work together by reporting and providing feedback to one another. This is then followed by a Plan-Do-Study-Act cycle. The cycle of PDSA involves measuring the progress to the goal that was set in the beginning of the year, to record the actual results- to-date, to take note of all the problems between the results and the plan, and lastly state the impact on the strategy for the coming year. **Insert 1**

The delivery of health services in Uganda is by both public and private sectors with GoU being the owner of most facilities. Table 2, shows the number of health facilities by ownership and level in the year 2010 (with a target to have every person house hold accessing a health facility within 5 kms radius).

Public health services in Uganda are delivered through VHTs, HC IIs, HC IIIs, HC IVs, general hospitals, RRHs and NRHs. The range of health services delivered varies with the level of care. In all public health facilities curative, preventive, rehabilitative and promotive health services are free, having abolished user fees in 2001. However, user fees in public facilities remain in private wings of public hospitals. Although 72% of the households in Uganda live within 5km from a health facility (public or PNFP), utilisation is limited due to poor infrastructure, lack of medicines and other health supplies, shortage of human resource in the public sector, low salaries, inadequate accommodation at health facilities and other factors that further constrain access to quality service delivery.

A study conducted in 2008 on user’s satisfaction and understanding of client experiences showed that in general clients were satisfied with physical access to health services (66%), hours of service (71%), availability and affordability of services including the providers’ skills and competencies among other things. However, they were dissatisfied with a wide range of issues such as long waiting times, unofficial fees in the public sector, quantity of information provided during care and other behavioural problems relating to health workers. The clients were also more satisfied with community health initiatives because they provide free services and it gives them an opportunity to participate in health services management. **Insert 2**.

The private sector plays an important role in the delivery of health services in Uganda covering about 50% of the reported outputs. The private health system comprises of the PNFPs, PHPs and the Traditional and complementary Medical Practitioners (TCMPs); however, the contribution of each sub-sector to the overall health output varies widely. The PNFP sector is more structured and prominently present in rural areas. The PHP is fast growing and most facilities are concentrated in urban areas. TCMPs are present in both rural and urban areas. The services provided are not consistent and vary from traditional practices in rural areas to imported alternative medicines, mostly in urban areas. The GoU recognizes the importance of the private sector by subsidizing the health facilities, PNFP training institutions and a few private hospitals.

The Government possesses both service delivery, and stewardship functions in health. The stewardship function is exercised by the management, while the service delivery function is exercised by the facilities, and coordinated by the HSD’s and districts. The management structure of the MoH lays emphasis on responsiveness to the requirements of the NHP and the organization is such that there is a clear communication linkage among the national, regional and district level for ease of planning, operations, monitoring and evaluation.

At the district level the District Health Officer (DHO) is in charge of health services with his/her team, addressing both the management and governance issues at the district. At the proposed regional level, the management function will be held by the Hospital Director of the RRH, where they focus on coordination, support to district planning, supervision and monitoring of health services in the region on behalf of the Director General of Health Services (DGHS).

The DGHS coordinates management functions at the national level with those at the sub- national level. These management structures are in place at district level, and will be established at regional level during the implementation of the HSSIP 2010/11 – 2014/15. The detailed structure at the regional level will be outlined after it has been determined through an authentic and agreed process. District health services are coordinated by the District Health Team (DHT). HSD health services are coordinated by the HSD Team.

The HUMC brings together selected community representatives who are responsible for fostering improved communication with the public, thereby encouraging community participation in health activities within and outside the unit. The HUMC reports to the health facility any plans and decision to improve the quality of care within the
health facility. Sensitizing community on health rights, roles and responsibilities (Patients Charter); Assessing work plans to ascertain that community needs are appropriately addressed; Community HIMS data collection and submission to health facility; Providing correct information to the community regarding performance of the health facility; Providing feedback to the health workers about the performance of the health facility. \textit{Insert 3}

Decentralization: QI initiatives in Local Governments shall be delivered within the framework of decentralization and any future reforms therein. This is because the LGs have the mandate to ensure delivery of quality health services and currently serve as the most appropriate level for coordinating top-down and bottom-up planning for organizing community involvement in planning and implementation; and for improving the coordination between government and private health care. Many key development sectors and partners are represented at this level.

Public Private Partnerships: The private sector shall be seen as complementary to the public sector in terms of increasing geographical access to quality health services, the scope and scale of QI initiatives implemented. In order to ensure standardized quality of services the public sector shall implement QI initiatives as guided by the national QIF. Integrated health care delivery: QI initiatives shall be scaled up from disease specific interventions to an integrated approach aimed at health systems strengthening. Harmonization of QI Initiatives: All QI implementers shall be guided by the National QI Framework. One of the gaps in QI efforts in the country has been that QI initiatives were fragmented and not coordinated. There were uncoordinated multiple initiatives, gaps in reporting and feedback, lack of clarity on roles and responsibilities of the different stakeholders as well as inadequate implementation of supervision and mentoring activities for quality. \textit{Insert 4}.

\textbf{Client-oriented:} The client-oriented principle requires the MoH to design strategies focusing on both the internal and external clients. Most of the QI initiatives in the country have been disease specific mainly because of the urgency in need to improve new interventions like the rapid scale up of HIV care and treatment in the mid 2000s. The current drive is for a health systems strengthening approach which builds on the integrated service delivery approach spelt out in the NHP II.

\textbf{Leadership:} The MoH will provide overall leadership for QI in health care. The MoH will work closely with partners in mapping and defining, on a continuous basis, the roles of different institutions, desired quality outcomes of health care and the values that will guide actions. Leadership needs to empower staff, be actively involved, and continuously drive QI. All partners are to apply the QA principles of focusing on the client, use of data focusing on evidence based outcomes, systems thinking and effective communication with all stakeholders. Without the commitment and support of senior-level leadership, even the best intended projects are at great risk of not being successful. Champions of the quality initiative and QI need to be throughout the organization, but especially in leadership positions and on the team. \textit{Insert 5}.

\textbf{Multidisciplinary Teams:} Due to the complexity of health care, multidisciplinary teams and strategies are essential. Multidisciplinary teams from participating centers/units need to work closely together, taking advantage of communication strategies such as face-to-face meetings, conference calls, and dedicated e-mail list servers. They need to also utilize the guidance of trained facilitators and expert faculty throughout the process of implementing change initiatives when possible.

Country-led monitoring and evaluation plan: The National Strategic Plan (HSSIP) core and program specific indicators shall provide a basis for the development of indicators for various QI initiatives. M&E activities will be guided by the national strategic plan M&E Plan.

\textbf{Human Resources:} The capabilities for implementing QI shall be addressed through a) in-service training, as well as b) pre-service education for all health professions, including physicians, nurses, pharmacists, laboratory personnel, health managers, etc. c) The possibility of embedding QI into job descriptions will be explored so that it is understood that everyone participates. The MoH will spearhead development of a national in-service QI training manual and liaise with training institutions in development of pre-service QI training curriculum. The expectations for facilities should be made clear at all levels of training so that the expectation of minimum QI standards for organizations is well understood and disseminated throughout the entire health sector.

Quality improvement requires active and continuing support from top leadership. At the Ministry level it means the Minister himself/herself, the Permanent Secretary, and the Director General of Health Services (Top Management) give their full support. At district level, the Local Council Chairperson, the Chief Administrative Officer (CAO), the DHO and the Hospital Director / Medical Superintendent are involved in efforts to improve the quality of district health
services by supporting application of quality improvement initiatives. Equally at sub-county level, the political and administrative leaders play an important role in sustaining the culture of quality.

The main responsibilities of decision-makers and managers will be to keep the performance of the whole system under review, and to develop strategies for improving quality outcomes and equity across the whole system. Decision-makers will engage health-service providers, communities, and service users in developing and implementing new strategies for quality using evidence based data.

Providers may be seen as whole organizations, teams, or individual health workers. The core responsibilities of health-service providers for QI will be to ensure that the services they provide are of the highest possible standard and meet the needs of individual service users, their families, and communities. Health service providers also need to operate within an appropriate policy environment for quality, and should have proper understanding of the needs and expectations of those they serve so as to deliver the best results.

Improved quality outcomes are not, however, delivered by health-service providers alone. Communities and service users are the co-producers of health. They have critical roles and responsibilities in identifying their own needs and preferences, and in managing their own health with appropriate support from health-service providers. Communities and service users need to influence both quality policy and the way in which health services are provided to them, if they are to improve their own health outcomes. This should be achieved through established mechanisms to address responsiveness like client satisfaction surveys, suggestion boxes, complaints desk, community meetings/dialogues, etc. Findings related to inequalities and vulnerabilities should be prioritized in subsequent plans. The MoH relationships in improving quality involve a number of stakeholders responsible for; policy and strategy development; health service provision; communities and service users.

Methodology and construct

The productivity of the hospitals in the Lucknow in India is characterised with the 5-SModel, wherein it had been stated that a local price index of the health sector, the ratio of purchasing power parity (PPP) of its currency, will be greater the higher the per capita income. Since this empirical finding can be explained in various ways, the interim part of their model also holds. Specifically, how is there an increase in ratio of health and care services. If growth is in health service delivery is heavily rich biased, it might lead to a fall in the delivery services. Loss in gains could have reflections directly to the growth of health service productivity.

In the Health industry in Lucknow, the productivity of doctors has been estimated based upon their participation in the work culture. Sometimes, it is conventional for the hospital to find work and then administer the drug. The productivity subsamples have been taken based upon the adulthood of the nurses, family size and proximity to the hospitals.

The marginal physical productivity of weaver has been derived based upon the following formulation:

\[
Z_t = \frac{\text{value of health services at international prices}}{\text{GDP at international prices}}
\]

Use of hypothesis and canonical correlation model resolve much of the purpose of stating that the health industry. It is important that we impart due consideration to the Lucas critique. The Lucas critique allows the reader to understand that the expectations from policy promises should be kept lower. It also states that structural analysis of the historical data may be less an invariant of the models.

Hypotheses

Three groups of work hypotheses about the impact of structural reforms on productivity of weavers, the migration of workers because of the capital intensive nature of manufacturing sector and the receding share of agriculture in the national economy.

H1. Perceived structural changes shall improve the health service delivery in Lucknow.
H2. Perceived use of 5-S shall improve the service delivery.
H3. Perceived disenchantment of newer generations to stay over in Lucknow.

Empirical Research Design

According to Indian Health Service Delivery Law, hospitals with an investment capacity of two point five million rupees to two million in equipments would be considered small. The framework of the study required the development of specific methodology for necessary data provision according to the main principles of quota sampling and face-to-face interviewing. Questionnaire data was prepared for further analysis following the standard scheme of decomposition of general concepts to operational measures and extracting indicator variables. The empirical survey was conducted in the period between November 2012 and February 2013 by using a quota sample of eleven active small health facilities.
General Sample Description
The sample covers the relative shares of registered active small-health service delivery mechanism. It should be noted that most of the respondents have some sources of information about their employer earnings out of which they are paid less one tenth. In this line, the knowledge for their business environment gives the entrepreneurs an information basis for outlining expectations about the firm’s future. Insert 10,11, 12,13.

Measurement of Indicator Variables
It is recognized in similar studies that the way weavers perceive their HMC units is more relevant to the chosen research approach than involving official statistical data for the environment. In this line, using perceptual measures allows to evaluate a firm from the perspective of target respondents. Insert 14,15,16.

Questionnaire specifically designed for this study asked respondents to rate on a 7-point scale their perception about each environmental characteristics considered as inherent to Lucknow transition economy. For example, adult make and adult female were segregated also based upon their family size and also the distance they covered to reach the loom. The indicator variable was obtained by averaging the ranks attached by the respondents. Analogous approach was adopted to measure the other two variables mentioned above.

Data Analysis and Statistical Results
The hypotheses outlined in the theoretical model of the study were tested by using canonical correlation model with the following sets of variables:
Right set: a1structural changes + a2migration + a3disenchantment
Left set: b1productivity + b2health education + b3decelerating growth in health.
As seen in the correlation matrix, Having the smallest coefficient of 0.39 it can be assumed that canonical correlation analysis should produce reliable statistical results for all variables of interest integrated in one model.

<table>
<thead>
<tr>
<th>Variables</th>
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<td>0.44</td>
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<tr>
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<td>Disenchantment with work</td>
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<td>0.52</td>
<td>1</td>
<td>0.74</td>
<td>-0.41</td>
<td>-0.76</td>
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<tr>
<td>Productivity</td>
<td>0.44</td>
<td>-0.21</td>
<td>0.74</td>
<td>1</td>
<td>0.59</td>
<td>0.65</td>
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<tr>
<td>Medical education</td>
<td>0.74</td>
<td>0.66</td>
<td>-0.41</td>
<td>0.59</td>
<td>1</td>
<td>-0.12</td>
</tr>
<tr>
<td>Deceleration in growth in</td>
<td>-0.43</td>
<td>0.67</td>
<td>-0.76</td>
<td>0.65</td>
<td>-0.12</td>
<td>1</td>
</tr>
</tbody>
</table>

The Quality Assurance Department
Insert 13, 14, 15 The mandate of the QAD is to ensure that the quality of services provided is within acceptable standards for the entire sector, both public and private health services. This is to be achieved through the departmental strategic objective of “Facilitating the establishment of internal quality assurance capacity at all levels”.

The responsibilities of the QAD will include overall operational oversight and coordination. Specifically the QAD will help build a sound quality program that establishes performance measures and data collection systems; develop written QM plans and annual goals; and oversee the progress of QI activities in the health sector. At the provider level, steps include building capacity and capability for QI among providers through training and technical assistance.

The QAD will coordinate the planning, resource mobilization, monitoring and evaluation of QM/QI interventions within the various MoH departments, programs, projects, health institutions and the entire health care delivery system. Implementation of the various QM interventions including QI initiative will be the responsibility of specific programs/projects, departments and institutions which shall have designated QM/QI officers. Insert 12, 14

The QAD will be the secretariat of the National QI Coordination Committee. QAD will receive, synthesize and present any strategic plans and decisions, to maintain and improve the quality of care within the sector to the Supervision, Monitoring, Evaluation and Research Technical Working Group (SME&R TWG). The SME&R TWG will discuss and
present relevant QI strategies and policy recommendations to the Senior Management Committee and the HPAC for action.

The specific responsibilities for QAD are:

1. Developing national standards and guidelines for QI.
2. Ensuring the dissemination of the guidelines to the regions, districts and stakeholders.
3. Coordinating and supporting training at all levels (national, regional, district, health centers and community); including pre-service and in-service health training institutions in new technological and QI issues.
4. Overseeing execution of the national QI plan.
5. Facilitating implementation of regional and local QI activities.
6. Coordinating support supervision at all levels in regard to QI issues.
7. Working with UNHRO to determine the quality improvement research agenda.
8. Working with training institutions to develop and implement the national QI training curriculum and training manual.
9. Documenting quality of care best practices and share information with other interested stakeholders for adaptation.
10. Compiling and disseminating national reports Convening national QI stakeholders meetings.

Recommendations

The Professional Councils will be responsible for regulation of professional standards, ethics and code of conduct. They should be able to recognize and reward good performance and sanction or institute disciplinary measures for poor performance. Insert 12,13.

Development Partners will be responsible for offering technical and financial support to relevant levels in consultation with the Top Management and guided by the QAD on the existing gaps, priorities and community needs. Also participate in supervision, monitoring and evaluation activities.

Program Managers / Heads of Departments/ Central Level Institutions including Regional Referral Hospitals will be responsible for offering technical support in terms of identifying QI priorities in specific program areas and presenting them to the QAD for discussion by the National QI Coordination Committee. In addition Program Managers / Heads of Departments will be responsible for identifying QI Focal Persons / Officers who will collaborate with the QAD in planning, resource mobilization, implementing, monitoring and evaluating QI activities in their programs. Program Managers / Heads of Departments will compile and submit performance reports to the QAD on a quarterly basis.

Implementing partners may be supported by the Development Partners to offer technical and financial support to public, private and community based service providers. This should be done in consultation with the QAD and guided by the existing gaps, priorities and community needs. Implementing partners will engage the DHOs in the day-to-day QI intervention operations. To ensure that all workplans and reports are submitted through the MoH reporting system with copy to the relevant stakeholders. Implementing partners may facilitate collection and synthesis of additional project data without replacing the MoH Health Management Information System (HMIS).

Health service providers including the private and public sectors and community based organizations will plan, implement, monitor and evaluate QI interventions in line with the national planning guidelines, National QIF and Strategic Plan, National supervision, monitoring and evaluation guidelines. Compile and submit periodic reports including documentation and sharing of best practices.

Communities and Service users will participate in identifying and planning for services needed, utilize the services provided and provide feedback on quality of services using established mechanisms. Communication and reporting mechanisms for QI will follow the same structure.
QI Coordination Structure
The following QI coordination structure has been created to enhance the QI policy, strategy development, communication and capacity building activities in a coordinated manner.

The National QI Coordination Committee brings together major stakeholders such as the priority programs, Development Partners, PNFPs, CSOs and health consumers. The key responsibility of the National QI Coordination Committee is to identify opportunities and potential strategies to coordinate the QI initiatives in Uganda.

The Coordination Committee will report to the Quality Assurance Department any plans and decisions, to maintain and improve the quality of care within the sector.

Responsibilities for the National QI Committee

1. Participating in developing strategies for improving quality outcomes which apply across the whole system.
2. Supporting and participating in the formulation of national QI guidelines and standards.
3. Identifying key priority areas for QI and make recommendations to the SME&R TWG and relevant stakeholders.
4. Facilitating networking with partners in identifying problems and solutions at National, Regional, District and Sub-District levels.
5. Receiving and reviewing QI implementation reports.
6. Discuss recommendations and lessons learnt during implementation of QI initiatives.
7. Participating in building capacity of national, regional, district, HSD and facility personnel in the implementation of QI activities in health services.
9. Attending National QI Coordination Committee meetings.

References


THE END
AN APPRAISAL OF POSSIBILITIES FOR ECOTOURISM IN RAJOURI DISTRICT OF JAMMU AND KASHMIR

Abstract:
Tourism is the largest industry in the world and is growing at a very rapidly. However we must not undermine the visitor effect on ecological resources which consequently set off a plethora of problems leading to global warming, which indeed has become a great threat to the life on earth. However an alternative is there i.e. Ecotourism. Ecotourism is both economically viable and environmentally sustainable activity, which aims at conserving and preserving environmental resources on the one hand and providing economic opportunities to the local people on the other. Rajouri is a district in the State of Jammu and Kashmir, India. The present study is an attempt at analyzing the potential of district for ecotourism, which in due course would improve the socio economic conditions of the local people by providing them with economic opportunities. The study is based on both primary and secondary sources of data. It has been established that Rajouri district has a huge potential for ecotourism. The district is quite rich in natural, religious, cultural and historical sources necessary for ecotourism. Its natural landscape, lingual, cultural and religious multitude with only one of its kind historical heritage provides vast prospects for Ecotourists. Different ecotourism activities and concepts like Rural Tourism, Transhumance activities, Lake Tourism, Trekking etc which are able to be considerable to Poonch district have been pointed out. At the end certain important suggestions have been made to give impetus to ecotourism in the district.

Introduction
Tourism is the largest industry in the world and is growing at a very rapidly. However we must not undermine the visitor effect on ecological resources which consequently set off a plethora of problems leading to global warming, which indeed has become a great threat to the life on earth. However an alternative is there i.e. Ecotourism. Ecotourism is both economically viable and environmentally sustainable activity, which aims at conserving and preserving environmental resources on the one hand and providing economic opportunities to the local people on the other. Rajouri is a district in the State of Jammu and Kashmir, India.

Rajouri has a huge potential for ecotourism. The district is quite rich in natural, religious, cultural and historical sources necessary for ecotourism. Its natural landscape, lingual, cultural and religious multitude with only one of its kind historical heritage provides vast prospects for Ecotourists which has never been utilized so far, for ecotourism.

Study Area
Rajouri is a district in the state of Jammu and Kashmir, India. It is 154 km away from Winter Capital Jammu, of the state. It is located in the foothills of Pir Panjal Range. Dhauladhar range lies on the north eastern frontier of the district. It is surrounded by Districts of Udhampur and Jammu in the East, Poonch on the West and Pulwama on the North and Line of actual control passes on its Southern side. It extends between 32° 58’ to 33° 35’ north latitudes and 70° 00’ to 74° 40’ east longitudes.

Mujahid Ul Islam
Research Scholar, Deptt. of Geography, Aligarh Muslim University, Aligarh, Uttar Pradesh
The climate varies from semi tropical in the Southern part comprising Nowshera, Sunderbani and Kalakote to temperate in the mountainous Northern part comprising the area of Rajouri, Thannamandi and Koteranka Tehsils of the District. The average rainfall is 500 mm and temperature varies from 7.42 degree Celsius to 37.4 degree Celsius. Rajouri has a population of 6,19,266 with Sex ratio 863 females per thousand males and density of 235 persons per km². The only means of connectivity with other parts of the state is road. Rajouri town is about 154 kms away from Jammu, the winter capital of the State. Generally it takes 3 to 4 hours to reach Rajouri by public buses. The Private transport has also been also developed and people prefer to use private vehicles like Tata Sumo, Tempos etc. It is connected with Srinagar (Kashmir) via Historical Mughal Road and also via Jammu.

The District has an area of 2630 Sq.Kms. with peculiar physical features. The District is situated in the west of Jammu Division and is surrounded by the Poonch, Udhampur and Jammu Districts. The District comprise of 6 Tehsils namely Rajouri, Nowshera, Sunderbani, Kalakote, Koteranka and Thannamandi and 7 Blocks of Rajouri, Manjakote, Darhal, Budhal, Kalakote, Sunderbani and Nowshera.

The District drives its name from Rajouri town which itself had been historically known as Rajapuri. Rajouri District had been part of Poonch district prior to 1967. On 22nd September 1967, Government divided district Poonch into two districts namely 'Rajouri' and 'Poonch', to facilitate the process of development and better supervision of economic activities in this area. By this way, Rajouri emerged as a District from 1st January 1968.

District Rajouri is quite diverse in terms of Religion, language, cultures and traditions. People of almost all major religions reside in the district in peace and harmony. Islam, Hinduism, Sikhism, and Christianity are the major religions. They speak different languages like Urdu, Kashmiri, Dogri, Gojri and Pahari.

**Ecotourism; A theoretical Framework**

Tourism is world’s largest industry and is growing very fast. It is an effective instrument for booming economic development in a state like Jammu and Kashmir which is already a tourist hub. However we must not underestimate the visitor effect on environmental resources which subsequently triggers a plethora of problems leading to the global warming, which is a great threat to the life on this planet. However, Ecotourism is an important indeed only feasible alternative. Ecotourism is both economically and environmentally viable activity, which aims at conserving environment on the one hand and providing economic opportunities to the local people on the other.

Ecotourism may be defined as the ecological friendly tour and travelling to the unharmed natural environments, wherein understanding of environment and culture, their conservation and economic activity go hand in hand. It is thus a sustainable form of tourism.

The term ecotourism was coined by Hector Ceballos Lascurian in 1983. The term was initially used to describe the nature based travel with emphasis on education, management and development of sustainable tourism product and activity. World Tourism organisation defines Ecotourism as "the tourism that involves travelling to relatively undisturbed natural areas with specified objective of studying admiring and enjoying the scenery and its wild plants..."
and animals as well as any existing cultural aspects (both of past and present) found in these areas. Ecotourism is the sustainable development tourism which is developed on the ecotourism resources (such as ecological landscape, ecological environment, ecological culture, ecological technology, ecological industry, ecological products, etc.) for content, and based on ecological civilization. Ecotourism is one strategy for supporting conservation and providing income for communities in and around protected areas. It can contribute to economic development and conservation of protected areas by: a) generating revenues that can be used to sustainably manage protected areas, b) providing local employment and c) inculcating a sense of community ownership.

The asset of natural beauty along with rich heritage and cultural mosaic enjoyed by Rajouri is the greatest magnetism for the ecotourists. Rajouri district is quite rich in terms of both, its natural possessions such as its mountains, forests, lakes, rivers, heart touching satisfying weather, the diverse indeed rare flora and fauna which is least explored and its cultural, lingual and religious multitude.

**Objectives of the Study**
The study has been carried out to fulfil the following objectives:

1. To analyze the potential of District Rajouri for both domestic and foreign Ecotourists.
2. To explore the so far unexplored ecotourism destinations in district Rajouri.
3. To apply different concepts and kinds of ecotourism to the study area.
4. To present district Rajouri as an important ecourist destination of Jammu and Kashmir which has been not done so far.

**Data Base and Methodology**
The present study has been carried out with the help of data collected from both primary and secondary sources. The primary sources include in depth discussions with local people and empirical observations of different potential ecotourism destinations. Secondary data has been collected through Census of India and internet apart from published and unpublished research works by different authors.

**Ecotourism Sources/Destinations**

**Natural Sources/Destinations**

**Budhal**
Budhal is a beautiful Valley located amid the central Pir Panjal series of mountains. The Geography of the area is comprised of snow laden beautiful mountain peaks, dense forests, garrulous meandering streams, crystal clear lakes, eye catching meadows and sea green pastures. Proper Budhal is a small town, is situated on the hillock near Gabbar Nallah. The town falls on the Southern range of Pir Panjal. This fine-looking region is situated on the way to Budhal pass or Sedhu pass which leads towards the Kashmir Valley. Sedhu pass is at a distance of about 24 kms from Shopian and 25 kms from Budhal Township. Budhal is also used as a base camp for trekkers to Kashmir Valley.

Budhal records its name on the history in 10th century in Rajatarangini of Kalhan as “Budhiwasa” (place of intellectuals). Later on, this name changed from Budhiwasa to “Budhal”. As per the legend, the Paul Shahi Raja “Budh Paul” established Budhal town in the 10th Century AD. He named this town as Budhiwasa, which subsequently was changed to Budhal. Budhal is an ideal site for nature lovers and ecotourists. The pristine scenic beauty and rich history of the area is unique.

**Nowshera**
Nowshera town is situated at an elevation of 758 metres from the sea level. It is placed on the northern side of a fertile valley of Nowshera, beyond the right bank of Tawi River. The town is 124 kms away from Jammu and 45 kms from Rajouri. It is a Tehsil headquarter and a growth centre of the district. It is located on the Line of Actual Control. Numbers of roads have been started in the Tehsil to link up the remote and border villages. Rajal Canal is the main project existing in the Tehsil.

**Thannamandi**
Thannamandi town is situated at 33.33 degree latitude and 74.25 longitudes, on the foot hill of Rattan Peer (8600') along the right bank of Thanna Nallah. This attractive hill spot is about 24 kms on the North side of Rajouri Town. At present this town is a Tehsil Headquarter. Kashmiri Muslim population is dominating the town. This place is mostly known for Chikhri wood handicrafts.
Dehra ki Gali
Dehra ki Gali is situated at an altitude of around 6600 feet above mean sea level, in the vicinity of Pir Panjal Range and is one of the passes connecting Rajouri and Poonch districts. It experiences heavy snowfall during winter months. Thick vegetation cover attracts and charms the visitor. On the peak of this mount Project Beacon has constructed a one-room cottage, which is one of the finest sightseeing spots of the region. The pass has also been used by Indian TV industry in the recent past according to the local people. A two-room inspection hut has also been constructed recently to facilitate the tourists.

Kalakote
Kalakote is a mountainous track located sandwiched amid Koteranka and Sunderbani. The region is also famous for its coal mines. However it is primarily an agricultural region. The region is endowed with thick vegetation cover and is fairly tranquil and pristine.

Tatta Pani (Hot Spring) is an important destination in the region, which is visited by numerous visitors throughout the year for a medicated bath. However the number of visitors increases sharply during winters. The bathing in the water of the hot spring water is supposed to restore to health. It is believed to be very effective treatment for joint, bone and skin diseases.

Sunderbani
Sunderbani also the tehsil of Rajouri, is another beautiful destination to be visited. It is situated on the Jammu-Poonch highway. Highway itself is very picturesque. Sunderbani earlier was known as “Bhajwal Jagir”. Present settlement was uninhabited and there was nothing apart from shrubbery. Only one Haveli of Rai Ghulam Hussain, the Jagirdar of the area existed on a mound, which was known as Phulwari located on the upper side of present Sunderbani. In 1980, Sunderbani emerged as new Tehsil. The region is geographically, climatically and culturally worth seeing.

Tatta Pani
Tatta Pani is a hot spring, situated in Kalakote tehsil at a distance of around 35kilometres from Rajouri district headquarters. In local terminology Tatapani means “Hot water”/ Hot spring. Owing to the importance of the spring, the village besides it has also been named as Tatta Pani Village. There are various myths and stories attributed to the origin of this hot spring. Some people believe it to be the fruit of blessings of a Muslim Saint in the past, while others believe that during the ancient times, some travellers discovered this place by spotting a gigantic cobra belonging to Lord Shiva, the god of Hindus, at the position from where spring originates. However, regardless of the evidences of authenticity of either of the myths, the place catches the attention of people from all walks of life. Regardless of their religions, regions, castes, creeds and colours people bathe in the spring, in their prefect harmonious and secular manners. It has rather become a symbol of Human brotherhood. Tatta Pani is surrounded by opaque forest cover and is quite peaceful and tranquil. The bathing in spring water is believed to cure numerous bone, joint and skin diseases. The water, is rich in various minerals, is very hot and its degree of hotness sometimes rises around boiling point. People, however adjust them gradually by dipping in part by part. Tatta Pani is a fast growing tourist preference.

Samot Sar
Samot Sar is an oval shaped blue water lake situated at an altitude of 3,550 meters on the northern extremity of Badjari Marg. The lake is reachable in 4 hours from Budhal town.

Chandan Sar
Chandan Sar, a bean shaped lake , dotted with icebergs till the end of July. It is situated at an altitude of 3,800 meters from the mean sea level. It is 2 kilometres in circumference. It is the source of Chamar Nullah which joins Bafliaz Nullah near Behram Gala. It is at a day's trekking from Ravi Wali marg.

Divya Sar
Divya Sar looks like an Indian earthen lamp and hence named as "Divya Sar”. it is Situated at an elevation of 3,600 meters from the mean sea level.

Gum Sar
This is another small lake at the foothills of Dhakyar peak at an elevation of 3,600 meters. Dhakyar peak is the highest peak in this range. It is a pyramidal mountain with height of about 4,660 meters.

Manyal Gali
Manyal gali is a mountainous village situated on the Jammu –Poonch highway via DKG. It is a beautiful sight seeing destination, with exceptionally pleasing climate. One can locate the whole region from there. It is at a distance of half an hour from Dehra Ki Gali (DKG). (self)

Wild life
Fauna
Wild life of Rajouri is diverse and rich owing to its varied climate and geography. As the mountains get higher to around 4600 meters in north eastern side of the district, we find alpine areas where snow falls heavily during winters and prolongs up to April-May. Flora and fauna of alpine regions are those which withstand these altitudes and peculiar climatic conditions. As we come down in the direction of lower elevations, the climate becomes temperate type. Animals here thus have to move towards lower altitudes for the duration of winter months. However the large part of the district experiences sub tropical climate. These areas of the district are rich in wild life. They are known to possess leopard, jungle cats, Felis bengalensis, Hogdeer (parha) Porcupine Black bear, Goral (wild goat), Musk deer, snow leopard, and  Marten etc. Wild Boar, Monkeys Mongoose, squirrel and Hare, Jackal, Bats are also in plenty.

Apart from these, Peacock and other birds like Thrushes, Bablers, Orioles, Bayas, Bee-eaters, Fly-catchers, Shrike, Tailor bird, Wood pecker, Cuckoo, Koel, Doves, Hawk, Shikra, Eagle, Brain feverbird (Papita) owlet, Barn owl, king Fishers and Egrets, Partridge, Quail, Bulbul, Parrots, Hoopoe, Pigeon, Sparrow, Crows, Jungles fowl, Kiter Buzzard, and vultures can be seen in plenty the district.

Reptilian fauna comprises of Lizards and Snakes. Varanus (Kas Karoh), Agama, Calotes and Hemidectles python. Snakes include Cobra, Coral snake, Krait, Viper, Natrix, Zemnis, Lycodon and Typhlops are also found in the district.

The rivers of the district are abounding with fish fauna. Trout is important to mention. Monal and other pheasants are found in Darhal and Budhal.

Flora
Owing to its climate and topography, Rajouri is quite rich and diverse in flora as well. Variety of trees, grasses, crops and flowers which district have is the only of its kind These Wild flowers not only add to the beauty of the region but also have medical importance. The important flora of the district includes NARCICUSS (Nargis), BANAFSHA, VICIA FLOWER HUND FLOWER,DIGITALIS,VERBASCUM OR MULLEIN ,WILD ROSE TRIFOLIUM REPENSE (White Clover and Red Clover), TARAXACUM OFFICINALS.

Trekking Routes of Rajouri
Trekking has come a long way since the dawn of human history. However all trekkers did not trek with same purpose. Trekking is both adventure and exploration. Generally, trekking is a voyage on foot to explore new. To meet new people and their products, to discover new routes, places, topographies and natural environments is all a trekker desires. Trekking is to gratify a man's essential desire i.e. to travel into the world and seek adventure.

Rajouri is situated at the foothills of Pir Panjal Range of the mighty Himalayas. The snow laden peaks of Pir Panjal have their own charm and beauty with many of them unexplored and virgin. Prominent among them are, Dhakyar Peak which elevates up to 15290 feet, Kotoria Peak which ascends up to 15000 feet above, Budhal Pir Peak which rises up to 15170 feet, and Brahma which get higher up to 15400 feet. Apart from these Kagha Alana and Handoo Peaks near Chamcchi Sar Lake are one of the best mountaineering destinations. There are eye catching stupendous
lakes like Simar Sar, Kotoria Sar, Dhakyar Sar etc which are surrounded by beautiful snow covered peaks for most time of the year. There are tens of amazing passes which connect the state with Rajouri. These passes also serve as meadows (Dhoks) of tribal people, like Gujjars and Bakarwals. However most of these passes stay enclosed with snow during winter and the snow starts melting in April or May. The passes are open from the first week of October and the Gujjars and Bakarwal shepherds start moving up along with their sheep. The significant and well-known trekking routes of Rajouri are:

**Base: Rajouri**

**Base: Darhal**

**Base: Budhal**
4. Budhal - Saveri Marg - Khodra - Simar Sar Lake - Bella - Kotoria Sar Lake - Dhakyar Peak

**Base: Rajouri**

**Base: Thannamandi**

**Base: Rajouri**

**Religious Sources**

**ZIARAT (Shrine) SHAHDARA SHARIEF**
Ziarat Shahdara Sharif is located in the Village Shahdara, about 35 kilometres from Rajouri town. The shrine is bounded by beautiful lofty mountain peaks, luxuriant forests, sea green pastures and eye catching springs. Village Shahdara in ancient times was known by different names like Simha Dwara, Shindhara etc. In 1765 AD, a Muslim Saint Syed Ghalam Shah Badshah arrived here, and started preaching the message of love and humanity. The village has thus been named after his name. People of all faiths and ideologies in thousands visit the shrine every year. Community kitchen (Langar) provides food and tea to thousands of people regularly. Shrine complex has the capacity to accommodate approximately 10,000 people. And the same number can offer Prayers in Jamia Masjid Shahdara Shrief. The annual congregation (Urs) of Ghalam Shah Badshah is celebrated on 10th of Muharram every year.
GURUDWARA CHATTI PATSHAHI BANGLA SAHIB, RAJOURI
Rajouri has a number of Gurudwaras (Sikh Temples). Apart from a number of Gurudwaras. However, Gurudwara Chhati Padshahi Rajouri has its own significance owing to its history. It has been constructed in the memory of 6th Guru Hargobind Sahib Ji (1595-1646 AD) who stayed at this place in 1616 AD while he was on the way to Kashmir along with Mughal Emperor Jahangir. As per the legend, once Guru Hargobind Sahib Ji Maharaj had a dream that Mai Bhag Wanti in Kashmir had prepared a Cloak (Cholla) for him and she was praying to God to meet (darshan) of Guru Ji so that she could present it. As she was quite old, she was not in a position to visit Punjab and fulfill her desire. Therefore, Guru Ji decided to visit Kashmir and accept the present from her and to advocate Sikhism in the mountainous belt of Pir Panjal and Kashmir Valley. In 1960, a memorandum was passed by the Sangat for reconstruction of Gurudwara Chhati Padshahi Rajouri and the Committee was asked to work for the construction and collection of funds. In 1964, the blueprint of new Gurudwara was prepared. After the approval of blueprint by the Sangat, the construction was started in 1964. The masons and carpenters were brought from Punjab for giving Sikh architectural touch. By this way, the first phase of the building had been completed. The four storey building of Gurudwara has 15 rooms and a large Diwan Hall. A Pathshala comprising of 8 rooms is being constructed and a school namely “Shri Hargobind Sahib Ji Institute of Education” has been running from April, 2000. The Gurudwara Chatti Padshahi is having great importance for not only Sikhs but Hindus too visit the Gurudwara daily. Congregations are organized on Sundays. However, the Birthday of Guru Hargobind Ji is celebrated with great enthusiasm and zeal both by Sikhs and Hindus every year.

MANGLA MATA
Mangla Mata Dev Asthan is located near village Bhawani in Nowshera Tehsil of District Rajouri. Originally, it was located in Hamirpur after the name of Mangla Mai (Pakistan). A dam has been constructed by Pakistan after her name under the name of Mangla Dam. This remained an unknown place till 1965.

The legend says when the construction of Mangla Dam in Hamirpur was started and the Dev Asthan of Mangla Mai came under the dam, without delay after that, a Brahmin of Bhawani area, Pandit Nihal Chand had a dream in which Mangla Mata had given him darshan and asked that I have shifted my Asthan from Hamirpur to the forests of Bhawani and desired for exploring. Not only this, Mangla Mai also told the exact location to the Brahmin. After some days, the Brahmin came to this place and found the Pindies (a shape of Devta made by natural stone) of Mangla Mai in the cave. After performing the havans and other rituals, he established an Asthan of Mangla Mai at this very place and people started coming for darshan. With time several other myths have also been associated with the temple. Whatever are the facts, but it is a beautiful cave. People visit the site every day in good numbers. Besides these gatherings, two important congregations on a large scale are organized annually during the days of Navratras and in the months of April and September. Apart from main cave, there are two other caves also namely Vishnu Cave and Naina Mata Cave. The pindies of Naina Mata are also installed in the cave. Every month on the night of full moon, the devotees from the adjacent neighbourhood come for Jagrata (nightly prayers of Hindus) and remain engaged in bhajan and kirtan for the whole night. A Yatri Nivas has also been constructed apart from the link road which leads
towards the Asthan. Power and water supply is available at site. Pilgrims cook their meals in the Langar. The Asthan is placed in a beautiful jungle area.

PEER BADESAR
Peer Badesar is a temple, in Veer Vadweshav village, at a distance of 60 kilometres from Rajouri city. It is situated near the present Indo-Pak border. The temple named after Veer Vadweshav, is located on a peak at an altitude of around 5000 feet. Some villages of the Pakistan Administered Kashmir can be seen is quite clearly from here. The temple was constructed by the King Kanishka in 142 in the memory of Veer Vadweshav, the Son Shiva the god of Hindus. The temple is the finest example of Hindu architecture of the antiquity.

It is said that in the historic times, the father of Sati, King Dakasha had once organised a massive congregation where food was provided to the people after the "Havan" service. In this congregation king Dakasha did not invite either Shiva or Sati, but Sati however came to attend the congregation. King Dakasha talked very ill of Shiva as well as Sati, upon seeing her. As She could not bear this disgrace to her and her husband, She preferred to burn herself alive in the burning "Kund". When Shiva was told about the happening, he was so annoyed that he powerfully threw his "Jata" (entangled mass of hairs on his head) on the ground. Veer Vadweshav took birth out of this "Jata" to retaliate the humiliation meted out to his mother. Thus later on he attacked the empire of Dakasha and defeated him. On his way back Veer Vadweshav lived at this position where the temple is constructed. Subsequently this area was called as Peer Badesar.

ZIARAT SAIN GANJI SAHIB
The shrine of Saint Ganji Sahib Qadri located at a distance 10 kilometres, from Rajouri city, overlooking Darhal Nullah, is a centre of spirituality for a number of pilgrims all the year. An annual congregation (Urs) is held in the first week of October every year where people of different faiths come in large numbers. The Shrine is regarded as a symbol of clarity, secularism and tolerance. Born and brought up at Fatehpur, Rajouri, the Saint was a spiritualist who developed into a legend in his own life time. He dedicated all his life for the betterment of human kind. He propagated love, peace and harmony throughout his life.

DERA BABA BEERAM SHAH
During 17th Century, the 6th Guru of Sikhs had sent number of Saints from Punjab towards this region for preaching of Sikhism. Baba Beeram Shah Dutt was one among them. He belonged to Sialkote (Pakistan). He established his Dera at Jhangar and kept visiting Nowshera, Dharamsal, Rajouri and Poonch and NWFP. His Dera is located on a beautiful hillock. A Gurudwara and a pilgrim house have also been constructed now. The place is visited daily by large number of his followers. The main congregation is organized annually on the next Sunday of each Baisakhi. Hindus and Sikhs from all over Poonch and Rajouri visit the place in large numbers to take part in the congregation.

PANJ PIR (five Saints)
Panj Pir is situated at a distance of few kilometres from Rajouri town. It is named after the five saints, who were all brothers and sisters to each other. They had from Punjab in the past. A large number of people visit the shrine round the year.

ZIARAT BABA SAKHI SULTAN
This shrine is situated in the core of Rajouri city. Baba Saki Sultan is known for his message of peace, harmony and tolerance. People of all religions visit the shrine regularly.

NAOGAZI ZIARAT
Naogazi Ziarat is the shrine of Qutab Shah. It is situated at Dhanidhar. Naogazi means nine yards long. The grave of the saint is believed to be of 9 yards and hence the name, Naogazi Ziarat.

CHANI PRAT TEMPLE
It is located at a distance of around10 kilometres from Sunderbani at an remote place near the village Chani Prat. It is believed to have been constructed during the time of Maharaja Gulab Singh, through the efforts of a Saint who had unceremoniously visited the place as a matter of custom wandering. After spending some time, he thought of building a temple at this place. The architecture and of the temple is only one of its kind. Small paintings on the walls portray different episodes of the epics of Mahabharata and Ramayana. An impressive Yagya is organised here, in the month of June.

MANMA MATA TEMPLE
Shrine of Manma Mata is at a distance of 6 kilometres from Kalakote. There is a cave at the site apart from a temple. It is believed that Shiva had gone to Kashmir passing through this cave. Manma Mata Devi had come to this place, later on to meditate. Every year, a congregation is organised on the eve of Navratras.

ZIARAT SAIN WALIDAD
The Shrine of Sain Walidad is located in the village Bhatian in Thannamandi.

Ziarat Rattan Pir
The Ziarat at Rattan Pir also a prominent shrine and receives grand admiration by people of different communities. It is situated on the border of Rajouri and Poonch districts.

Shrine of Manma Mata
Another important place in Kalakote area is "Shrine of Manma Mata". The temple is about 6 kms from Kalakote Town located in the Southern side. There is a cave at site and legend goes that Lord Shiva had gone to Kashmir via this cave. Later on, Manma Mata Devi had come to this place and meditated for several years in the cave.

Historical Sources
Rajouri Temple, Sarai, Mosque and Fort
Rajouri has a rich heritage. Its rich cultural and historical background and its proximity to the Colonial Mughal Road leading to Kashmir have endowed it with the remnants of architectural sophistication of the past. Architectural remains of early medieval and ancient periods of Indian history can be seen at many places in Rajouri. In Andarkot Mohalla, for instance, we find vestiges of ancient Hindu rule and Kashmir style temples. Buildings of Mughal Period, like Sarais, forts and Palaces, Mosques and temples, gardens and other remnants are still in a good position. Many of these structures have now been converted into public buildings like schools, hospitals, police stations etc. The important historical sources of Rajouri include:

MURADPUR: SARAI AND MOSQUE
Muradpur is situated at a distance of 7 Kilometres from Rajouri on Rajouri -Chingus route. Here is the medieval Sarai overlooking the green fields. With bastions at the corners, and the enclosure wall extending around 30 metres in length, it is a great centre of attraction for heritage lovers. One of its sides is made up of rubble stones in lime Surkhi Mortar. It also has residential compartments. At a distance of around 1 kilometre from this edifice and on the bank of the river Tawi one can find remnants of an old Mosque and a planned cell of congregation hall. The mosque is of impressive dimensions with a courtyard ahead of it, which has now been covered with vegetation.

CHINGUS: SARAI AND BAWLI
Chingus is a tiny village, on the right bank of the river Tawi, on Jammu- Poonch highway. Mughal Sarai of the village is a celebrated place for having mortal remains of Mughal Emperor Jahangir. The emperor died on his way to Kashmir. It is a wall planned edifice having two buildings i.e. the residential complex and the open yard. Both the apartments have their own entrances. However they are also linked internally with each other. The residential compound has been built within walled enclosure. It has curved cells with openings in the direction of the courtyard. Its doorway from the western side comprises a central chamber with arched top and a undersized room on either side for the guards. In the construction of the edifice random rubbles, large pebbles and lakhauri have been used in a lime Surkhi Mortar. There is also a mosque. At a distance of about half a kilometre to the north of Sarai there are remains of an old well (Bawli).
KHEORA: KUSHAN SITE
Kheora is a Village, situated at a distance of about 1.5 kilometres from Rajouri city. It is located on the Rajouri-Darhal link road. The village is known for the red wave shreds of Kushan period characterized by profound bowls with razor edged rims and thin sides towering towards the bottom apart from a portion of an enclosure wall built with stones of lime Surkhi Mortar.

SARAI, MOSQUE AND HAMMAM at NOWSHERA
Sarai of the Nowshera town is larger and diverse than other Sarais in its design and outlook. It has two enclosures. The outer enclosure is comprised of double storied bastions at the corners and is crowned by crenellated fortification. It has a row of alcoves on the inside and has machicolations. A magnificent gateway in the middle of the western wall provides an entrance into the Sarai. The gateway is the only of its kind in the region, which has square stone pillars surmounted by bracket with bendent, a feature conspicuously found in the Mughal architecture at Agra and Fatehpur Sikri. The residential is situated in the south western bend of the enclosure wall. It also is comprised of a common courtyard having curved cells facing towards it. Above the eastern front entrance of the Sarai facing river side splendid complex is seen in dilapidated condition. It was built by Emperor Akbar as an inscription on the western gateway. Opposite to main entrance lies a Royal Mosque, which is still in utility. Remnants of a Hammam can also be seen nearby.

NADPUR SARAI
Nadpur Sarai is situated at a distance of about 10 kilometres from Nowshera. It was used as a small halting station during Mughal period. It consists of a large rectangular courtyard enclosed by a walled enclosure with battlement shaped embellishment at the top. It is provided with two rows of machicolations. The entrance is through impressive gateways on the eastern and western sides. The facade of the gateway has alcoves at the bottom and is adorned with recessed panels.

MANGLA DEVI FORT
Mangla Devi Fort is located in the Mangla Devi village which is situated at a distance of around 18 kilometres from Nowshera town, on the pinnacle of a picturesque mount. It is a secure fort and is not easy to access because of its location and well-built fortification. On three of its sides, it is astonishingly surrounded by a river and on the fourth side it has a difficult uphill rise. The main gateway is linked with a flight of abrupt steps. It now however has been a treasury building for long. It has a large tank in dilapidated condition besides a modern temple of Mangla Mata. During the reign of Maharaja Ranjit Dev, Mian Rattan Dev had conducted the siege of fort for some years and then brought it under his possessions. It is said that the Sikh Sardar Sahib Singh of Gujrat and Mohan Singh Sukarchaka invaded Khairi-Kharidi and attacked Mangla fort but without success. Finally Maharaja Ranjit Singh occupied the fort after the death of its master Raja Umar Khan.

Dhanidhar Fort
In the year 1846, Britishers sold Jammu and Kashmir to Maharaja Gulab Singh. At that time Maharaja Gulab Singh appointed Mian Hathu as a Governor of Rajouri by removing Raja Raheem Ullah Khan. Mian Hathu then Governor of Rajouri erected Dhanidhar Fort in the year 1855. The main intention of construction of this fort was to keep the Dogra forces at a safer site. Henceforth the edifice was built at a place from where whole of the Rajouri Valley could be kept under observation. It is situated at a distance of two kilometres from Rajouri city. The fort has been used by Indian Army in the past. The fort is in a dilapidated condition now.

Usman Memorial:
Usman Memorial has been constructed at Jhangar in the memory of Brigadier Usman, of Indian Army who played an important role in recapturing the area around Jhangar. Subsequently Brigadier Usman fell prey to intense
shelling of the Pakistani forces on 3rd July 1948. The memorial is maintained by army at Jhangar. A congregation is organized on 3rd July every year, as Jhangar Day.

Balidan Bhawan:
This Bhawan is a memoir of great sacrifices made by the people of Rajouri during 1947-48.

Cultural Sources
People
Rajouri is a blend of different faiths, religions, castes and tribes etc. People of different religions, castes, tribes speaking different languages with diverse dialects live in harmony and peace. Gujjars, Bakarwals, Paharis, Punjabis, Kashmiris and Dogras etc are all dwelling in different places in the district. They speak Gojri, Pahari, Kashmiri, Dogri and Urdu, with a great diversity of local and sub regional dialects. There are populations who are still transhumant. Temporary migrations are common among many families especially, Paharis, Gujjars and Bakarwals with the changing seasons for food and fodder. Muslims, Hindus, Sikhs and others are integrated by a common culture and language in different parts of the district.

Fair and Festivals
Owing to its diverse socio cultural background, people of the district celebrate a wide range of festivals. Important fairs and festivals, which are celebrated in a unique fashion with a specific and matchless regional touch in the district, since long are

DUSSEHRA
Ram Lila is held at Town Hall in Rajouri town. On the Dussehra day a procession is taken out in the city. The effigies of Ravan, Kumbhakaran and Meghnath are burnt publically. People from different walks of life gather here in large numbers.

BAISAKHI FARE AT BANPURI
At a distance of around 2 kilometres from Sunderbani on Beri-Pattan road, there is a Shiva temple at a village Ban Puri. The temple is sited on the roadside. Thus it serves as a great centre of magnetism for travellers. The village is named as Ban Puri after the name of Ban Ganga (Now in Pakistan). It is believed that around 500 years ago, the great saint, Gosain Baba Kashi Das Ji, after seeing "Light" and achieving great spiritual fulfillment from the great shrine of Amarnath, had decided to stay for the meditation for years, for wellbeing of human kind. The Saint is believed to be spiritually eminent and miraculous. After the partition of India, he built the temple at Ban Puri. A grand congregation is organised at the shrine, which is attended by large numbers of devotees, from all over the state.

Nowshera Day
On February 6, every year locals and Indian military remember the heroism of civilians of Nowshera in repulsing the attack of Pakistani invaders in 1975. A celebratory function is organised, which is attended by large no of people including many civilians, ex-soldiers and army personnel. Rural Sports, Cultural Programmes, stalls of developmental accomplishments in various areas are organised by the local administration. However, tableaus of war heroes are the most important attraction of the festival.

Holi
Holi has its own charm, when it is celebrated in Rajouri. On first day, the tableau of Hanuman is taken out from the Sanatan Dharam Sabha. From next day, the tableau of Bhairav, the Lord of Devil is taken out from the Sabha, which also takes a round of the town. Thousands of people from all walks of life join in the congregations. They throw colours (Gulal) on each other and dance on the drum beats. This presents a unique scenario of social life in Rajouri. The tableau of personified Bhairav walks down to Darhal Nullah, and bows his head before the sacred stone, which is around 3 meters in height, and is prevalently known as Bhairav Pathar, every day.

Folk Songs and Dances
Rajouri is beyond any compare and is only one of its kinds when we talk of its rich and diverse heritage, colourful cultural life and superb folk songs and folk dances. Folk songs and dances of any region portray the life style of its people, their tastes, traditions, costumes, rituals and culture. Rajouri has unique cultural forms and styles of singing and dances.

Gujjars and Bakarwals are one of the most important segments of the population of the district. The main ballads of the region are “Noora” & “Taago”. Folk songs like “Dhindi”, “Baisakhi”, “Kenchi”, “Lucko”, “Chann”, “Dholan”, “Sahiban”, “Mehndi”, “Laari”, “Sail-ul-Malook” and “Bar-Shams” are very famous amongst the people. Kashmiri
songs like “Wanawum”, “Chakri”, Dogri "Chann", and Punjabi "Mahiya" also echo in the mountains. People have their unique myths behind all songs. Apart from these “Kenchi” is a love song of a Pahari belle. In other words it is a true love story of a Pahari girl with "Munshi" of a forest contractor. Kenchi expresses the broken heart of the female lead and brings tears in the eyes of the villagers. It is indeed a melancholy. Similarly Chann & Dholan are Pahari romantic songs.

Folk Dances
Folk Dances in the district are generally connected with some function, celebration or with the devotion of some god or goddess. Important folk dances of Rajouri are Dogri and Punjabi Bhangra, Gojri Dance, Letri, Chowki Naach, Jattar, Jagarana, Keekli and Rouf etc.
In performance, set pattern of clothes and songs, Dogri Bhangra is entirely poles apart from Punjabi Bhangra. These dances are performed on the fairs and festivals and at different occasions like Letri is performed on the event of Mass Grass Cutting or Harvesting.

Gojri Naach
Gojri dance is very illustrious of the Gujjar culture. It is generally performed on marriage ceremonies. It is effortless and delightful. Male dancers dance in their customary costumes forming a line having sticks in their hands. Female dancers dance on the other side adjust pattern on the rhythm of Drum. The dance is always going together with a folk song.

Letri
This dance is performed on grass harvesting in the autumn months.

Chowki Naach
It is a religious dance performed in the honour of the Gram Devta or Kul Devta. People assemble in the temple or in a place of worship on a particular day and perform Jaatar with iron chains in their hands. The dance is accompanied by devotional songs.

Bindloo and Jagarna
The Bindloo and the Jagarna are the folk dances subject matter of which is matrimonial relationships. Only Women perform this dance. Children and men are not allowed even to see the dance. It is performed when the Barat leaves for Bridegrooms' house and is carried on all through the night.

Keekli
No musical instrument is used in it. The girls perform this dance. They catch hold of each other’s opposite hands and take turns in a balanced fashion.

Roff and Chhakri
This is the distinguished dance which is performed by women on the occasion of marriage.

Costumes and Jewellery
1. Self designed beads necklaces woven in coloured silken threads
2. Coins of Indian currency - Key shape Lockets - usually having silver plating
3. Chargul or "loung" - either of gold or plating of gold as a nose ring
4. Jhumka or Kanta - of silver, for ears
5. Kangan or Mareedehis and Kada for arms
6. Haseeri for neck
7. Do - Ladi hear of silver for neck
8. Angoothies or rings of silver
9. Silver - hairpins - joints by a chain at both ends
10. Chippri - a scarf of chheet cloth - They always keep their heads covered with topi and Chippri
Arts and Handicrafts
Rajouri is rich and diverse in handicrafts. Embroidery, Chikhri-wood carving, Tilla work, basket and blanket weaving, Namda and Gabba making etc are the chief handicrafts of the recent past. However with the fast industrial development around the world, some of these handicrafts could not survive. However Embroidery, Namda, Gabba making, Blanket (Patti and Loee) making and Chikhri wood crafts somehow still survive managed to live to tell the tale of a glorious art. The key crafts are:

EMBROIDERY
The main items under embroidery craft consist of Shah Embroidery (both Sojni and Aari).

NAMDA GABBA MAKING
This craft is on the verge of due to poor socio economic conditions of the people.

TILLA (COPPER) WORK
The Tilla (Copper) work used to be the main stay of the cobblers. Darnal, Thannamandi and Budhal are known for it.

BLANKET (PATTI AND LOEE) MAKING
Blanket (locally Patti and Loee) making is one of the oldest crafts. It is still carried on. Pattis and Loees of Rajouri are unparalleled.

CHIKRI WOOD CARVING
The artisans involved in this craft are mainly located at Shahdara Sharief, Thannamandi and Budhal. These artisans of these areas are famous for manufacturing a number of articles for domestic use as well as for decorative purpose.

Rural tourism/ Village Tourism
Any form of tourism that showcases rural life, art, culture and heritage at rural locations, there by benefiting the local community economically and socially as well as enabling interaction between the tourist and the locals for a more enriching tourism experience is termed rural tourism.
Villages in Rajouri have a huge prospective for rural ecotourism. This potential if make use of can be an advantage for local people. Villages can be used for halts. Interaction with locals is not novel to the ecotourism. To know different spaces, folks and their products is not new to those who travel to discover, explore and learn. The religious, cultural and lingual diversity of villages in Rajouri is of immense significance for the ecotourists. As ecotourism is the travelling to observe and admire not only the nature, but to be acquainted with the diversity of flora and fauna, with the identification of the different traditions and cultures, that are unique to different people and places.
**Transhumance Activity**

Paharis, Gujjars and Bakarwals are the prominent ethnic groups of the state. They are essentially animal (cattle, goat, and sheep) keeper. Pahari farmers migrate to Dhoks (meadows) where they find green pastures and fodder for animals in the summers. They make Taras or Kothas (mud houses with thatched roofs) and Bandis (for animals). These Dhoks are located at higher reaches. In winter they return back to their permanent settlements. These people essentially speak Pahari. Paharis are however temporary migrants and are sedentary as well. Like Paharis Gujjars and Bakarwals have become sedentary to a large extent. But a large population among them is still transhumant, especially Bakarwals. They migrate seasonally along with their cattle, sheeps and goats in search of fodder. In summer they also migrate to Dhoks (Meadows). They live in Tamboo (tent) and settle temporarily wherever, they find fodder. Some of them have also constructed Taras and Bandis in these Dhoks. These ethnic groups are the best example of symbiotic relationship of man and environment. They me serve as an important tourist destination.

**Conclusion**

Owing to its untouched natural magnificence, multitude and exceptionality of its cultures and rich legacy Rajouri District has enormous possibilities for ecotourism. However, this vast potential for ecotourism has been unnoticed since long. It can be utilized only with the necessary infrastructure and advertisement. The beyond compare magnificence, natural beauty, unique and rich cultural and religious heritage of Rajouri District must be conserved and preserved and make use of, for ecotourism. In order to do so, the very first step is its advertisement and serious concern of State Govt towards necessary and vital infrastructural facilities. District authorities must consult and ask for cooperation from State and other national ecotourism organisations regarding the problem. Awareness among the masses is of prime importance. Thus local authorities should generate consciousness among local people, especially villagers by educating them about the economic and environmental merits of ecotourism. Local people should be given technical and financial assistance for selling their own products to the tourists. In this way ecotourism apart from providing the eternalness of the natural and cultural possessions, may also offer a side income for the local people.

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Website: [www.cpa2.webnode.in](http://www.cpa2.webnode.in)
Email Id: cpapharmacist@gmail.com

**IMPORTANT PHONE NUMBERS**
Mr. Ankit Tiwari (Founder): +91 8960810411
Mr. Dilip Kumar (Associate): +91 9792656777

**CPA MEMBERS**

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<td>Expert &amp; Controller</td>
</tr>
<tr>
<td>3.</td>
<td>Mr. Akash Kotecha</td>
<td>Moderator</td>
</tr>
<tr>
<td>4.</td>
<td>Ms. Nishigandha Bhausaheb Chalak</td>
<td>Expert</td>
</tr>
<tr>
<td>5.</td>
<td>Ms. K Sai Vyshnavi</td>
<td>Expert</td>
</tr>
<tr>
<td>6.</td>
<td>Mr. Kasutubh Ghule</td>
<td>Advertiser</td>
</tr>
<tr>
<td>7.</td>
<td>Ms. Bharti Jadhav</td>
<td>Registration Controller</td>
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</tbody>
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