HEPATOPROTective ACTIVITY OF INDIGOfera TRIFOLIATA AGAINST PARACETAMOL INDUCED HPATOTOXICITY IN ALBINO RATS

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ABSTRACT

Indigofera trifoliata is a shrub which has been traditionally used to cure Hepatitis, cirrhosis, Jaundice etc. However the scientific evaluation of its hepatoprotective role was lacking. Hence in the present study, hepatoprotective activity of ethanol extract of Indigofera trifoliata was investigated in paracetamol intoxicated albino wistar rats. Paracetamol induced albino rats produced a marked elevation in the level of Biochemical markers (SGOT, SGPT, Bilirubin, ALP, Cholesterol and Total protein), which showed a marked decrease after oral administration of Indigofera trifoliata extract (150mg/kg and 200mg/kg doses). The results were comparable to the effect of the standard drug Silymarin. The histopathological analysis also supported the finding of blood biochemical parameter analysis, thus confirming the hepatoprotective role of Indigofera trifoliata.

KEYWORDS: Hepatoprotective, Indigofera trifoliata, albino rats, herbal drug, paracetamol.

INTRODUCTION

Traditional medicinal practice exists in every part of the world, about 80% of the population relies on traditional system of medicine for primary health care (Akereie, 1992). Herbal drugs have the advantage of being available for patients in the geographical area where the traditional medicine is practiced. It is no wonder that worlds one fourth’ population i.e. 1.42 billion people are dependent on traditional medicine for the treatment of various ailments.
India is endowed with a rich wealth of medicinal plants. India indisputably occupies the top ranking in the use of herbal drugs. Large ethno botanical knowledge exists in India from ancient times. The diverse culture of our country is rich source of traditional medicine, many of which are of plant origin. (Gupta, 1994).

Liver disease is one of the major causes of morbidity & mortality in public, affecting humans of all ages. About 20,000 deaths occur every year due to liver disorders. Some of the commonly known disorder is viral hepatitis, alcohol liver disease, non alcoholic fatty disease, autoimmune liver disease, drug induced liver injury, gallstones etc. (Gupta and Misra, 2006). Unavailability of rational therapy in modern medicine and side effects of synthetic drugs used in liver damage have urged researchers in this field to look for herbal drugs with better hepatoprotective action. There is growing focus to evaluate traditional herbal medicine for hepatoprotective activity. (Karan et al., 1999). Natural products of plant origins with hepatoprotective and antioxidants properties play an important role in treatment of liver toxicity. (Vaidya et al., 1996).

*Indigofera trifoliata* L (Hindi: Diwali) belongs to family Fabaceae, which is a shrub widely distributed in various parts of India. It has been traditionally used to cure Hepatitis, Cirrhosis, Jaundice, Antioxidant etc. However its scientific evaluation is lacking. The objective of the present study was to assess the hepatoprotective activity of *Indigofera trifoliata* on paracetamol induced hepato toxic rats.

**MATERIALS AND METHODS**

**Preparation of plant extract**

The whole plant of *Indigofera trifoliata* was collected from Nimboni village at Amravati District. The plant materials were dried under shade and ground to a coarse powder. Then the air dried coarsely powdered plant material, were extracted with ethanol in a Soxhlet apparatus, concentrated and dried under reduced pressure in a large petridish. The dried extract was stored in airtight container in refrigerator below 10ºC until experimental testing, Thimmaiah (2004).

**Procurement, maintenance and acclimatization of animals**

Wistar albino rats (180-220 gm) used for the experiment were purchased from the animal house of Sudhakarrao Naik Institute of Pharmacy, Pusad (Maharashtra) and maintained in animal house of Government Vidarbha Institute of Science and Humanities, Zoology.
Department, Amravati (Maharashtra). All the rats were kept in standard plastic rat cages with stainless steel coverlids and wheat straw was used as bedding material. The animals were facilitated with standard environmental condition of photoperiod (12:12hr dark: light cycle) and temperature (25±2°C). They were provided with commercial rat feed and water given *ad libitum*. The animals were habituated to laboratory conditions for 15 days prior to the experimental protocols to minimize any non-specific stress.

**Selection of animals for experiments**

In each experiment thirty adult, healthy male albino rats of Wistar strain which were three months of age and weighing about 150-190gm. were selected. The experimental animals were divided into five groups (G₁, G₂, G₃, G₄, and G₅) each containing six animals. As per the treatment plan first group served as a control and the rest served as experimental groups.

**Acute toxicity test**

**Toxicity test**

Adult albino male rat were divided into four group i.e. containing five animal in each group. Acute toxicity study was performed as described by Turner (1971). The rat were fasted for eighteen hours, the prepared drug was administrated orally at three different doses 500,1000 and 1500 mg/kg body weight, respectively to different groups of rat separately. Control rats received the vehicle (distilled water) only. The animals were observed for 72 hrs for behavioral changes and mortally, as there was no mortality seen at this dose level, the procedure was repeated by further increasing the dose (2000 mg/kg) using fresh animals.

**Treatment protocol: (Experimental design)**

Overnight fasted, healthy rats were randomly divided into five groups (6 rats per group)

**Group I:** (Control group) / (Normal control) received oral dose of distilled water (1ml each) for 7 days.

**Group II:** (Toxic control) Paracetamol control group, received Paracetamol dissolved in normal saline (NaCl 0.9%) orally for 15 days (Parthsarthy, *et al.*, 2007).

**Group III:** (Standard group) received Silymarin and in addition received Paracetamol dissolved in normal saline orally for 15 days.

**Group IV:** (Extract 100 group) received 100 mg/kg alcoholic extract of plant parts and in addition Paracetamol dissolved in normal saline orally for 15 days.
Group V: (Extract 200 group) Received 200 mg/kg of alcoholic extract of plant parts and in addition Paracetamol dissolved in normal saline orally for 15 days.

Preparation of samples for biochemical studies
After administration of the last dose of the treatment, blood samples were collected on 15th day in case of chronic liver damage experiments. All rats were sacrificed by cervical dislocation and blood was collected by intracardiac puncture. The blood was kept for 30 minutes without disturbing. The clot was dispersed with glass rod and then centrifuged for 15-20 minutes at 2000 r.p.m. to separate serum.

Assessment of hepatoprotective activity
Biochemical investigation
Biochemical evaluation
The serum of each animal of all experimental groups of rats was used for estimation of various biochemical parameters to determine the functional state of the liver.

Histopathological Study
Liver sample from each rat were removed after dissection and preserved in 10% formalin. Using the standard micro technique (Carleton, 1980), sections were taken at (5 μm thickness) and stained with hemotoxylin and eosin dye. The sections were then observed under microscope for histopathological changes in liver architecture, and their photomicrographs were taken using micro imaging Microscope.

Statistical Analysis
The results were expressed as mean ± SEM (Standard error of mean) for each parameter. The statistical analysis was done by using Student t-test for estimating variation in set of data. Data was analyzed to determine the significant difference of result between the treated and control groups. The statistically significant level was taken as described by Khan and Khanum (2008).

OBSERVATIONS AND RESULT
Table No. 1 Effect of alcoholic extract of Indigofera trifoliata (whole plant) on various Blood parameters in Paracetamol treated rats.
Value in mean ± S.E (Standard Error), n=6,*P<0.05,**P<0.02, ***P<0.01, when compared between groups
Acute toxicity study of *Indigofera trifoliata* whole plants extract

No mortality and changes in behaviour were observed in all the treated and control group of rat up to dose of 2000 mg/kg body weight *Indigofera trifoliata* whole plants extract. Hence 200 mg/kg body weight plant extract was used as the maximum dose for hepatoprotective study.

<table>
<thead>
<tr>
<th></th>
<th>Bili –T mg/dl</th>
<th>Bili –D mg/dl</th>
<th>SGOT Units/ml</th>
<th>SGPT Units/ml</th>
<th>Total Protein mg/dl</th>
<th>Albumin mg/dl</th>
<th>ALP Unit/ml</th>
<th>Cholesterol mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.75±0.3</td>
<td>7.78±0.003</td>
<td>214.00±1.1</td>
<td>83.07±0.7</td>
<td>6.78±4.6</td>
<td>3.46±0.1</td>
<td>79.7±0.92</td>
<td>179.00±1.7</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>1.24±0.05***</td>
<td>12.48±0.28***</td>
<td>310.7±0.61***</td>
<td>116.26± .88***</td>
<td>11.60±0.28***</td>
<td>5.85±0.23***</td>
<td>98.47±0.5***</td>
<td>221.48±2.7***</td>
</tr>
<tr>
<td>Silymarin</td>
<td>0.80±0.04</td>
<td>9.73±0.26***</td>
<td>240.48±0.7***</td>
<td>90.32±0.59***</td>
<td>7.77±0.56</td>
<td>3.82±0.21</td>
<td>79.95±0.46</td>
<td>180.66±0.7</td>
</tr>
<tr>
<td>Ext. 1 150Mg/Kg</td>
<td>0.83±0.05</td>
<td>11.41±0.27***</td>
<td>250.24±1.36***</td>
<td>84.31±0.9</td>
<td>9.63±0.22***</td>
<td>4.28±0.25***</td>
<td>86.20±0.47***</td>
<td>210.69±0.57***</td>
</tr>
<tr>
<td>Ext. 2 200Mg/Kg</td>
<td>0.94±0.4***</td>
<td>10.03±0.24***</td>
<td>240.78±0.61***</td>
<td>92.25±0.41***</td>
<td>8.32±0.18***</td>
<td>4.2±0.25**</td>
<td>82.15±0.44***</td>
<td>189.57±1.25***</td>
</tr>
</tbody>
</table>
Effect on serum enzymatic activity and other biochemical parameters

In this study after treatment with paracetamol, a significant increased level of Cholesterol SGOT, SGPT, Bilirubin T, ALP, total protein were observed (Table 1) While in group of rats pre-treated with Silymarin the level of these blood biochemical parameters were significantly lowered as compared to paracetamol group. The group of rats pre-treated with *Indigofera trifoliata* whole plants extract also demonstrated dose dependent inhibition of elevation of the biochemical parameters.

*Indigofera trifoliata* at dose of 200 mg/kg showed significant inhibition of elevation of the biochemical parameters comparable to standard drug Silymarin. It was evident that this extract dose was able to reduce all the elevation in SGOT, SGPT, Cholesterol, Bilirubin, Total Protein, Albumin and Alkaline phosphatase increased due to hepatotoxic intoxication.

The hepatoprotective effect of ethanolic extract of *Indigofera trifoliata* (whole plant) was confirmed by histopathological examination of the liver tissue of control and treated animals. The histological profile of the liver tissue of control group shows normal hepatocytes, sinusoidal space and central vein. However paracetamol treated liver tissue shows centrilobular necrosis and vacuolization, sinusoidal congestion, and disk like arrangement of hepatocytes. Whereas in rats treated with plant extract show mild degree of necrosis, hepatocytes are compact and sinusoids appear normal, with cluster like arrangement of hepatocytes, comparable to sections of liver showing the normal hepatic architecture taken from silymarin treated animals.

**DISCUSSION AND CONCLUSION**

Paracetamol is one of the most commonly used hepatotoxins in experimental studies of liver diseases (Srivastava *et al.*, 1990). The changes associated with paracetamol induced liver damage are similar to those of acute viral hepatitis. Hence in the present work paracetamol was used to induce liver toxicity in rat models for hepatoprotective studies.

In the present study silymarin was used as standard drug, which is a standardized potent hepatoprotective agent. It reverses hepatotoxin-induced alteration of biochemical parameters (Dehmlow *et al.*, 1996; Gakova, *et al.*, 1992; Saller, *et al.*, 2007). The study of different enzyme activities such as SGOT, SGPT, ALKP, Total protein and Bilirubin have been found to be of great value in assessment of clinical and experimental liver damage (Vaishwanar and Kowale, 1976).
In the present investigation it was found that animals which were treated with paracetamol had significant hepatic damage shown by elevated serum markers when compared with that of control group. The normalization of serum marker level by administration of ethanolic plant extract suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against paracetamol (acetaminophen). The ethanol extracts of present plant may contain pharmacologically active substances with hepato protective properties.
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Group I Control Fig.I

Group II Paracetamol Fig.II

Group III Para.+ Silymarin Fig.III

Group IV Para.+ Extract 150 mg/Kg Fig.IV

Group V Para.+ Extract 200mg/Kg Fig.V
CONCLUSION
These observations suggest that ethanolic extract of *Indigofera trifoliata* exhibited significant hepatoprotective activity. These attributes provides the rational for the use of it in liver disorders by traditional healers in India.

REFERENCES
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