Hepatoprotective and Antioxidant Role of Flower Extract of *Abutilon indicum*.

P. Revansiddaya,1 Biradar Kalyani,2 A. Veerangouda1, H. Shivkumar1, Payghan Santosh3

1) Department of pharmacology SCS College of pharmacy Harapanahalli Karnataka
2) HKES’S Matoshree Taradevi Rampure Institute of Pharmaceutical Sciences Gulbarga, Karnataka
3) Dr. V.K. Patil Pharmacy College, Aurangabad, (M.S).

Received 18 Dec 2010; Revised 16 Jan 2011; Accepted 28 Jan 2011

ABSTRACT

The hepatoprotective activity of the 70% Ethanolic extract of *Abutilon indicum* flowers was assessed using CCl4 induced hepatotoxicity in albino rats. The degree of protection against liver toxicity was determined by measuring the biochemical markers such as SGPT, SGOT, ACP, ALP and Bilirubin (Direct and Total). In addition morphological changes of liver like wet liver volume and wet liver weight were recorded. Further, histopathological examination of the liver was also studied. Silymarin at the dose of 25 mg/kg, p.o. was used as reference standard drug and it exhibited significant protection. In addition to the screening of hepatoprotective activity, in vitro antioxidant activity was studied by reducing power activity, superoxide anion scavenging activity and hydroxyl radical scavenging activity methods. Sodium meta bisulphate was used as a reference standard drug.

Keywords: *Abutilon indicum*; Hepatoprotective; Antioxidant; CCl4.

INTRODUCTION:

Liver has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. Liver diseases are among the most serious ailments. They may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). Liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon-tetrachloride, paracetamol, chlorinated hydrocarbons, etc.), excess consumption of alcohol, infections and autoimmune disorder. So it has become very much necessary to protect the liver from all these agents.

In spite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available till date. Plant drugs are known to play a vital role in the management of liver diseases. In India, more than 87 medicinal plants are used in different combinations in the preparation of 33 patented herbal formulations1-4. In the traditional system of medicine there are numerous plants and polyherbal formulations have been used in liver diseases. But only a small portion of them have been pharmacologically evaluated for their efficacy. Still more number of medicinal plants is needed to be investigated for their antihepatotoxic effect. *Abutilon indicum* (Malvaceae) is distributed throughout the hotter parts of India. It has been reputed in Siddha system of medicine as remedy for jaundice, piles, ulcer and leprosy5. The plant is reported to be having analgesic6 and antifertility7 properties. The flowers of the *Abutilon indicum* are known to contain flavonoids8. The leaf extract of *Abutilon indicum* has been already reported for the hepatoprotective activity9,10. However, the literature survey afforded no scientific investigation on hepatoprotective property of flowers of the title plant so far. Hence, the present study was undertaken.

MATERIALS AND METHODS:

The plant material and preparation of extracts:
The flowers of *Abutilon indicum* were collected during the month of Feb-March, 2007 from the surrounding fields of Harapanahalli. The plant was authenticated by Professor K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, and Harapanahalli. A voucher specimen has been deposited at the museum of our college. The dried powder of flowers was extracted by using 70% ethanol in soxhlet apparatus (Yield 12.52%). The preliminary phytochemical screening was carried out on 70% Ethanolic extract of *Abutilon indicum* flowers for the detection of photo chemicals present by following the literature reported methods. The experimental animals and acute toxicity studies:
The male albino rats weighing 150 – 200 g and female albino mice 20 – 30 g was used for the experimentation. The animals were procured from Venkateshwara associates, Bangalore, Karnataka. After randomization into various groups, animals were acclimatized for the period of 10 days under standard husbandry conditions.

- Room temperature 27 ± 3°C.
- Relative humidity 65 ± 10%.
- 12 hrs. Light / dark cycle.

All the animals were fed with rodent pellet diet (Gold Mohr, Lipton India Ltd.) and water was allowed *ad-labium* under strict hygienic condition. Ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC) before initiation of the experiments.

The acute toxicity of 70% Ethanolic extract of *Abutilon indicum* flowers was determined in female albino mice weighing 25 – 30 g. The animals were fasted over night prior to the dosing.

Fixed dose (OECD Guideline No. 420) method of CPCSEA was adapted for toxicity studies.

**CCl4 induced hepatotoxicity**

Albino rats weighing 150 – 200 g were divided into six groups of each containing six animals.

Group I – Negative control (received vehicle, distilled water 1 ml/kg, p.o.),

Group II – Positive control (CCl4 1 ml/kg, i.p.),

Group III – Standard (Silymarin 25 mg/kg, p.o.),

Group IV – EEAIF (100 mg/kg, p.o.),

Group V – EEAIF (250 mg/kg, p.o.) and

Group VI – EEAIF (500 mg/kg, p.o.)

Animals were treated as shown above for a period of 10 days. At the end of every 72 hrs. i.e. 4th day, 7th day and 10th day CCl4 (30% in liquid paraffin 1 ml/kg, i.p.) was administered to all groups other than group I. Group III received standard drug silymarin 25 mg/kg p.o. once a day and CCl4 as mentioned above. Whereas group IV, V and VI were treated with test extract dose of (100, 250 and 500 mg/kg, p.o.) respectively. During this period of treatment, the rats were maintained under normal diet and water. The biochemical parameters were determined after 24 hrs. After the last dose of CCl4 i.e. on 11th day. All the animals were sacrificed.

Blood was collected by carotid bleeding under mild ether anesthesia using disposable syringe and needle. Blood was allowed to clot at room temperature for 30 min. then subjected to centrifugation (3000 rpm for 15 min.) and estimation of biochemical parameters namely SGPT, SGOT, ALP, ACP, Bilirubin (Total and Direct).

The liver was dissected out and subjected for morphological study such as wet liver weight and wet liver volume. The volume of wet liver was measured by displacement method and further the livers were placed in 10% formalin solution for histopathological study.

**In vitro antioxidant activity**

Reducing power

Different doses of Ethanolic extract of *Abutilon indicum* flowers were mixed in 1 ml of Distilled water so as to get 10 µg, 25 µg, 50 µg and 100 µg concentration. This was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 ml) of trichloroacetic Acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%), and the absorbance (OD) was measured at 700 nm.

% inhibition was calculated by using the following formula

\[
\% \text{ Inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100
\]

**Superoxide anion scavenging activity**

About 1 ml of nitro blue tetrazolium (NBT) solution (156 µM NBT in 100 mM phosphate buffer, pH 7.4). 1 ml NADH solution (468 µM in 100 mM phosphate buffer, pH 7.4) and 0.1 ml of sample solution of ethanolic extract of *Abutilon indicum* flowers in water was mixed. The reaction was started by adding 100 µl of Phenazine Methosulphate (PMS) solution (60 µM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 minutes and the absorbance at 560 nm was measured against blank. Increased absorbance of
Intoxication of rats with CCl₄ resulted in enlargement of liver which was pale reddish brown. Rats subjected to the CCl₄ challenge developed significant increase in the morphological parameters like wet liver weight and wet liver volume when compared to negative control group (Table-2). Oral administration of the test extract exhibited dose dependent significant reduction in the morphological parameters. Treatment with reference standard, silymarin (25 mg/kg, p.o.) also reversed increased morphological parameters significantly. Organ protective potency of the test extract at the dose of 500 mg/kg was found closer to that of standard.

Histopathological profile of liver in CCl₄ (Group-II) intoxicated rats showed the fatty degeneration of hepatocytes, hepatic cell necrosis, portal tract fibrosis and presence of fatty cyst. The sinusoids of liver were congested and the central vein of globule was constricted. Administration of test extract at the dose of 500 mg/kg showed a significant recovery in the hepatic architecture. The sinusoids are recovered, The globule was normal and hepatocytes are improved. However, there was a improvement in the hepatic architecture observed in rats treated with 100 mg/kg and 250 mg/kg of test extract.

Ethanolic extract of Abutilon indicum flowers demonstrated concentration dependent (10, 25, 50 and 100 µg) significant antioxidant activity against reducing power, superoxide anion scavenging and hydroxyl radical scavenging activity. The antioxidant potential of the higher concentration (100 µg) of the test extract was seems to be more potent than that of reference standard drug in case of superoxide anion scavenging activity and hydroxyl radical scavenging activity. But the antioxidant effect of the test extract was found to be less potent than the standard drug in reducing power activity (Table-3,4 & 5).

Table 1: Effect of EEAIF on biochemical parameters in CCl₄ induced hepatotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>Biochemical parameters Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGPT IU/L</td>
</tr>
<tr>
<td>I</td>
<td>105.70 ± 3.251</td>
</tr>
<tr>
<td>II</td>
<td>247.77 ± 9.423</td>
</tr>
<tr>
<td>III</td>
<td>147.60 ± 3.530***</td>
</tr>
<tr>
<td>IV</td>
<td>228.13±3.536*</td>
</tr>
<tr>
<td>V</td>
<td>221.21 ± 2.950**</td>
</tr>
<tr>
<td>VI</td>
<td>169.39 ± 0.713***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 6)

P < 0.05*, 0.01** and 0.001*** as compared to +ve control.
Values are mean ± SEM (n = 6) 
p < 0.05*, 0.01** and 0.001*** as compared to +ve control.

Table 3: Effect of EEAIF on reducing power activity

<table>
<thead>
<tr>
<th>Group</th>
<th>Absorbance Mean ± SEM</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.146 ± 0.0005</td>
<td>-</td>
</tr>
<tr>
<td>Standard 25 µg</td>
<td>0.250 ± 0.004***</td>
<td>75%</td>
</tr>
<tr>
<td>Ethanol extract 10 µg</td>
<td>0.040 ±0.0003*</td>
<td>16%</td>
</tr>
<tr>
<td>Ethanol extract 25 µg</td>
<td>0.093 ±0.0004**</td>
<td>16%</td>
</tr>
<tr>
<td>Ethanol extract 50 µg</td>
<td>0.121 ±0.0003***</td>
<td>48%</td>
</tr>
<tr>
<td>Ethanol extract 100 µg</td>
<td>0.166 ±0.0004***</td>
<td>66%</td>
</tr>
</tbody>
</table>

Values are mean ± SEM

Table 4: Effect of EEAIF on superoxide scavenging activity

<table>
<thead>
<tr>
<th>Group</th>
<th>Absorbance Mean ± SEM</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.585 ±0.004</td>
<td>-</td>
</tr>
<tr>
<td>Standard 25 µg</td>
<td>0.287 ±0.003***</td>
<td>71%</td>
</tr>
<tr>
<td>Ethanol extract 10 µg</td>
<td>0.390 ±0.004*</td>
<td>62%</td>
</tr>
<tr>
<td>Ethanol extract 25 µg</td>
<td>0.339 ±0.005*</td>
<td>66%</td>
</tr>
<tr>
<td>Ethanol extract 50 µg</td>
<td>0.295 ±0.003***</td>
<td>70%</td>
</tr>
<tr>
<td>Ethanol extract 100 µg</td>
<td>0.274 ±0.004***</td>
<td>73%</td>
</tr>
</tbody>
</table>

Values are mean ± SEM

Table 5: Effect of EEAIF on hydroxyl radical scavenging activity

<table>
<thead>
<tr>
<th>Group</th>
<th>Absorbance Mean ± SEM (after 1 hr.)</th>
<th>Percentage inhibition</th>
<th>Absorbance Mean ± SEM (after 4hr.)</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.078 ±0.001</td>
<td>-</td>
<td>0.069 ±0.005</td>
<td>-</td>
</tr>
<tr>
<td>Standard (SMBS)</td>
<td>0.029 ±0.001***</td>
<td>66.95%</td>
<td>0.027 ±0.005</td>
<td>69.15%</td>
</tr>
<tr>
<td>Ethanol extract 10 µg</td>
<td>0.053 ±0.002*</td>
<td>40.6%</td>
<td>0.047 ±0.004</td>
<td>41.2%</td>
</tr>
<tr>
<td>Ethanol extract 25 µg</td>
<td>0.049 ±0.001**</td>
<td>49.3%</td>
<td>0.041 ±0.006</td>
<td>52.1%</td>
</tr>
<tr>
<td>Ethanol extract 50 µg</td>
<td>0.033 ±0.002***</td>
<td>58.79%</td>
<td>0.030 ±0.005</td>
<td>59.54%</td>
</tr>
<tr>
<td>Ethanol extract 100 µg</td>
<td>0.024 ±0.001***</td>
<td>69.9%</td>
<td>0.022 ±0.004</td>
<td>71%</td>
</tr>
</tbody>
</table>

Values are mean ± SEM

DISCUSSION:

Liver injury induced by CCl₄ is a commonly used model for the evaluation of hepatoprotective agents. Administration of CCl₄ elevated the serum levels of SGOT, SGPT, ALP, ACP and bilirubin (Total and direct) significantly due to its enzymatic activation of CCl₃ free radical, which in turn alters the structure and function of liver cells. The results of the present study reveal that 70% Ethanolic extract of Abutilon indicum flowers (100,250 and 500 mg / kg, p.o.) exhibited protective action against both CCl₄ induced liver damage in a dose related fashion. The amelioration of liver toxicity by the test extract was evident from its significant effect on serum enzyme levels and morphological parameters. These findings were further supported by histopathological observations.

The literature reports indicate that the antioxidant activity is claimed to be one of the mechanisms of the hepatoprotective property of indigenous drugs. Since, in the present investigation the test extract showed significant in vitro free radical scavenging activity in a concentration dependent manner, this could exert a beneficial effect against liver toxicity in experimental animals.

Further, preliminary photochemical investigation revealed that the extract showed presence of flavonoids, tannins, alkaloids, saponins and glycosides. The literature has already documented the hepatoprotective value of flavonoids. Thus, it appears that the hepatoprotection offered by Abutilon indicum flowers extract may be due to its flavonoid content.

REFRANCESES:

4. Sharma A, Shing RT, Sehgal V, Handa SS. Antihypertensive activity of some...
Biradar Kalyani et al. / Hepatoprotective and Antioxidant Role of Flower Extract of Abutilon indicum