Hepatoprotective activity of *Leucas hirta* against CCl₄ induced hepatic damage in rats

B K Manjunatha, S M Vidy, Promilla Dhiman & R Pallavi,
Department of Botany, S.R.N.M.N. College of Applied Sciences, Shimoga 577 201, India

and

K L Mankani
Department of Pharmacology, National College of Pharmacy, Shimoga

Methanol and aqueous leaf extracts of *L. hirta* demonstrated hepatoprotective activity against carbon tetrachloride induced liver damage in rats. The parameters studied were serum total bilirubin, total protein, alanine transaminase, aspartate transaminase and alkaline phosphatase activities. The hepatoprotective activity was also supported by histopathological studies of liver tissue. Results of the biochemical studies of blood samples of CCl₄ treated animals showed significant increase in the levels of serum markers and decrease in total protein level reflecting the liver injury caused by CCl₄. Whereas blood samples from the animals treated with methanol and aqueous leaf extracts showed significant decrease in the levels of serum markers and increase in total protein indicating the protection of hepatic cells. The results revealed that methanol leaf extract followed by aqueous extract of *L. hirta* could afford significant protection against CCl₄ induced hepatocellular injury.

Keywords: Hepatoprotective activity; Leaf extract; *Leucas hirta*

The plant *Leucas hirta* (Roth) Spreng., (Labiateae/Lamiceae) is a herb or undershrub, sparsely distributed in Deccan Peninsula and Western Ghats. The plant is locally known as Kaduthumbe. The plant is under threat because of the anthropological activities and over exploitation of this herb in and around the forest areas by the medical practitioners. The tribal groups of the Western Ghats use leaves as antiseptic and wound healer in septic wounds (handful of leaves are ground into thick paste using cow’s urine and applied externally), treating fever and cough (about 100 g of leaves are boiled in 1000 ml of water, filtered, 20 ml of the extract given orally), snake bite (thick paste of leaf is applied externally and about 30 ml of the water extract is administered orally after every 30 min) and liver disorders (about 100 g of leaves + 10 g of jaggery is ground into thick paste, mixed with few drops of lime juice and made into pills, 2 pills thrice a day for 8 days are given to cure acute jaundice). Review of the literature revealed that though this plant is known for its hepatoprotective activity by the tribal groups of the Western Ghat region, it has not been subjected to scientific evaluation. Hence an attempt has been made to evaluate the hepatoprotective potency of this rare plant genetic resource against CCl₄ induced hepatic damage in rats using aqueous and methanolic extracts.

Materials and Methods

Collection of plant materials—Leaves of *L. hirta* were collected from the Kuduremukha reserve forest of Chikmagalur district, Karnataka State, during December 2003 and identified by the first author. Taxonomic authenticity was confirmed by referring to herbarium specimen at Madras Herbarium, Botanical Survey of India, Southern Circle, Coimbatore and a voucher specimen (BKM-234) is deposited in the departmental herbaria, Department of Biotechnology, Kuvempu University, Shankaraghatta as authentic specimen for future reference.

Preparation of extracts—Leaves were shade dried for a week, powdered mechanically (sieve no. 10/44) and stored in airtight containers. About 250 g of the powdered material was subjected to soxhlation and exhaustively extracted with 70 % methanol for 48 hrs. The solvent was evaporated at low temperature under reduced pressure using rotory flash evaporator (Buchi, Flawil, Switzerland) till the complete evaporation of the solvent. The yield was 30.8 % w/w. Another 250 g of the powdered material was boiled in distilled water...
for 30 min, kept for 3 days with intermittent shaking, filtered and concentrated using rotary flash evaporator to get the aqueous extract. The yield was 20.2% w/w. Both the extracts were subjected to preliminary phytochemical tests.

Drug formulation—Oral suspensions containing 35 mg/ml of the aqueous and methanol leaf extracts were prepared in 1% w/v gum tragacanth.

Animals—Male Wistar albino rats weighing 150-200 g were procured from the National College of Pharmacy, Shimoga. The animals were housed in polypropylene cages and were maintained at 27±2°C, 60±5% RH, 12:12 hr light/dark cycle. They were fed with commercial diet (Hindustan Lever Ltd., Bangalore) and water ad libitum during the experiment. The study was permitted by the Institutional Animal Ethical Committee with Reg No 144/1999/CPCSEA/SMG.

Acute toxicity studies—Acute toxicity study was conducted for both the extracts by stair case method following OECD guidelines 2002. The LD₉₀ of aqueous and methanol leaf extracts was found to be 350 mg/kg, body weight, po. One tenth of this (ie, 35mg/kg, po) was selected as maximum dose for the evaluation of anti hepatotoxic activity.

Evaluation of hepatoprotective activity—The animals were divided into five groups of 6 rats each. The animals in group I served as control and received the vehicle (1ml/kg/day of 1% w/v gum tragacanth, po) for 14 days. All the animals of group II to V received 0.1 ml/kg/day CCl₄, ip (E-Merck, Mumbai, India) for 14 days. Group III animals received the standard drug silymarin (100 mg/kg/day, po, Ranbaxy Lab, Dewas) for 14 days. Aqueous and methanol leaf extracts (35 mg/kg/day, po) of L. hirta were administered to the animals of groups IV and V respectively for 14 days. The CCl₄, silymarin and the extracts were administered concomitantly to the respective groups of animals.

The animals of all the groups were sacrificed by light ether anesthesia on 14th day. The blood sample of each animal was collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 1000 g for 10 min and were subjected to biochemical investigation viz., total bilirubin, total protein, serum alanine transaminase, aspartate transaminase and alkaline phosphatase.

Results of biochemical estimations were reported as mean ± SE of 6 animal in each group. The data were subjected to one way ANOVA followed by Student's t-test. P ≤ 0.01 was considered as statistically significant.

Histopathology—The liver samples were excised from the experimental animals of each group and washed with the normal saline. Initially, the materials were fixed in 10% buffered neutral formalin for 48 hr and then with bovine solution for 6 hr. They were processed for paraffin embedding. The sections were taken at 5 µm thickness using microtome, processed in alcohol-xylene series and were stained with alum-haematoxylin and eosin. The sections were examined microscopically for the evaluation of histopathological changes.

Results
Effects of aqueous and methanol leaf extract of L. hirta on CCl₄ induced liver damage in rats with reference to biochemical changes in serum are shown in Table 2. At the end of 14 days treatment, blood samples of CCl₄ treated animals showed significant increase in the levels of total bilirubin, alanine transaminase, aspartate transaminase and alkaline phosphatase compared to normal control groups but the total protein level decreased reflecting the liver injury caused by CCl₄. Whereas blood samples from the animals treated with aqueous and methanol leaf extracts of L. hirta showed significant decrease in the levels of serum markers and significant increase in total protein to the near normal which are comparable to the values registered in the standard drug treated (silymarin) group of animals, indicating the protection of hepatic cells. Protection against CCl₄ induced hepatic damage was more pronounced in methanol extract treated group of animals.

---

**Table 1—Acute toxicity study**

<table>
<thead>
<tr>
<th>Dose (mg/kg, body weight)</th>
<th>No. of animals died/survived</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous extract</td>
</tr>
<tr>
<td>50</td>
<td>0/10</td>
</tr>
<tr>
<td>100</td>
<td>0/10</td>
</tr>
<tr>
<td>150</td>
<td>0/10</td>
</tr>
<tr>
<td>200</td>
<td>1/9</td>
</tr>
<tr>
<td>250</td>
<td>2/8</td>
</tr>
<tr>
<td>300</td>
<td>4/6</td>
</tr>
<tr>
<td>350</td>
<td>*4/5</td>
</tr>
<tr>
<td>400</td>
<td>8/2</td>
</tr>
<tr>
<td>450</td>
<td>10/0</td>
</tr>
</tbody>
</table>

*50% mortality is considered as lethal dose (LD₉₀)
Table 2—Effect of aqueous and methanol leaf extract of *L. hirta* on CCl₄ induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Group (N)</th>
<th>Total bilirubin (mg/dl)</th>
<th>Total protein (gm%)</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1% w/v gum tragacanth, po)</td>
<td>0.474±0.013</td>
<td>9.213±0.079</td>
<td>148.26±0.450</td>
<td>53.74±1.050</td>
<td>174.17±1.433</td>
</tr>
<tr>
<td>CCl₄ (0.1ml/kg/day, ip)</td>
<td>2.459±0.105&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.704±0.297&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2188.56±5.105&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1390.5±2.243&lt;sup&gt;a&lt;/sup&gt;</td>
<td>493.93±2.680&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCl₄+silymarin (0.1ml/kg/day, ip +100mg/kg/day, po)</td>
<td>0.569±0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.977±0.015&lt;sup&gt;b&lt;/sup&gt;</td>
<td>207.32±0.260&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.01±0.337&lt;sup&gt;b&lt;/sup&gt;</td>
<td>208.18±0.490&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCl₄ + aqueous extract (0.1ml/kg/day, ip + 35mg/kg/day, po)</td>
<td>1.007±0.002&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>8.008±0.002&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>269.50±0.320&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>165.45±0.190&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>274.3±0.290&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCl₄ + methanol extract (0.1ml/kg/day, ip+35mg/kg/day, po)</td>
<td>0.892±0.002&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>8.210±0.017&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>265.80±0.240&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>98.80±0.289&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>248.8±0.350&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

AST=aspartate transaminase
ALT=alanine transaminase
ALP=alkaline phosphatase

P values: <0.01; compared to control; *CCl₄; **silymarin

Histological profile of control animal showed normal hepatocytes (Fig.1), the section of liver of the group II animals exhibited severe intense centrilobular necrosis (N), vacuolisation and macrovesicular fatty changes (F; Fig.2). The liver sections of silymarin treated animals showed normal hepatic architecture (Fig.3). The liver sections of the animals treated with methanol extract exhibited significant liver protection against CCl₄ induced liver damage as evident by the presence of normal hepatic cords, absence of necrosis and fatty infiltration (Fig.4). However, moderate accumulation of fatty lobules (Fig.5) was observed in the liver sections of aqueous extract treated animals.

Discussion

CCl₄ induced hepatic injury is the common model used for hepatoprotective drug screening<sup>14</sup>. In CCl₄ induced toxic hepatitis a toxic reactive metabolite, trichloromethyl (CCl₃) was produced by the microsomal oxidase system cytochrome P₄₅₀. This activated radical binds covalently to the macromolecules of the lipid membranes of the adipose tissue and causes peroxidative degradation. As a result, fats from the adipose tissues are translocated and accumulated in the hepatocytes<sup>15</sup>. Several plants viz., *Sarcostemma brevistigma*<sup>16</sup>, *Murraya koenigii*<sup>17</sup>, *Balanites aegyptica*<sup>18</sup>, *Glycyrrhiza glabra*<sup>19</sup> etc., have been reported for their efficacy in controlling the CCl₄ induced hepatic damage. The extent of hepatic damage is assessed by the elevated level of biochemical parameters which is attributed to the generation of trichloromethyl free radical which in turn causes peroxidation of lipids of cellular membrane<sup>20</sup>. Hepatocellular necrosis leads to very high level of aspartate transaminase and alanine transaminase released from liver to blood. Between the two, alanine transaminase is a better index of liver injury, as its activity represents 90% of total enzyme present in the body. The decrease in serum transaminase concentration indicates the stabilization of plasma membrane and protection of hepatocytes against the damage caused by CCl₄ (ref. 21). ALP activity on the other hand is related to the functioning of hepatocytes and increase in its activity is due to its increased synthesis in presence of increased biliary pressure<sup>22</sup>. The data in Table 2 reveal the decreased level of serum transaminase in animals treated with methanol extract of *L. hirta*, indicating the stabilization of plasma membrane and hepatoprotection against the effect of CCl₄ and decreased ALP concentration evidences the normal functioning of hepatic cells.

Further, in the present investigation, preliminary phytochemical analysis of leaf extracts revealed the presence of flavonoids, alkaloids, tannins, saponins, glycosides, steroids and triterpenoids. Flavonoids<sup>23</sup>, triterpenoids<sup>24</sup> and alkaloids<sup>25-28</sup> are well known for their antioxidant and hepatoprotective activities. In this study methanol extract showed protective effect against toxicity induced by CCl₄, which may be attributed to the individual or combined effect of phytoconstituents present in it. Several phytoconstituents have the ability to induce microsomal enzymes thereby accelerating the excretion of CCl₄ or inhibiting the lipid peroxidation...
Figs 1-5—Section of the liver tissue of 1: control animals showing normal histology, portal triad showing portal vein (V), portal artery (arrow) and hepatic duct (arrow head); 2: animals treated with CCl₄ showing necrosis (N), fatty vacuole (F) and portal vein (V); 3: silymarin treated animals showing normal hepatocytes, portal vein (V), portal artery (arrow) and hepatic duct (arrow head); 4: methanol leaf extract treated animals showing normal arrangement of hepatocytes around the central vein (V), portal artery (arrow), hepatic duct (arrow head), absence of necrosis and fatty vacuoles and 5: aqueous leaf extract treated animals showing normal arrangement of hepatocytes around the central vein (V), portal artery (arrow), hepatic duct (arrow head), absence of necrosis and few fatty vacuoles (F) (Figs 1-5 H&E, 100x).
induced by CCl₄ (ref. 29). Further, though the aqueous extract showed positive tests to only three groups of phytoconstituents viz., saponins, glycosides and triterpenoids, it exhibited considerable liver protection against CCl₄ induced hepatic damage. This may be attributed to the antioxidant and hepatoprotective activity of saponins, triterpenoids and glycosides present in it.

Based on the above results of the pharmacological screening, it can be concluded that the methanol leaf extract of L. hirta possesses more significant hepatoprotective activity. The reason for the variation in the potency of the extracts may be due to the presence of additional phytoconstituents like alkaloids and flavonoids in the methanol extract of the plant. The present finding provides scientific evidence to the ethnomedicinal value of this rare plant genetic resource used by the tribal group of Western Ghats in treating hepatitis.

Acknowledgement

The authors are grateful to Sri Girimaji N Rajgopal, Sri S V Thimmaiah, Prof Darmananda Rao (National Education Society) and Dr T S Ramkumar (Principal, S.R.N.M.N. College of Applied Sciences, Shimoga) for financial support and to Dr V Krishna, Prof B Abdul Rahman (Department of Biotechnology, Kuvempu University) Dr Y N Manohara and S D Jagadeesh Singh (National College of Pharmacy, Shimoga) for support.

References

28 Chung H S & Woo W S, A quinolone alkaloid with antioxidan
tivity from the aleurone layer of anthocyanin-
29 Mehta R S, Shankar M B, Geetha M & Saluja A K, 
Hepatoprotective activity of Trianthema portulacastrum, 
30 Yoshikawa M, Morikawa T, Kashima Y, Ninomiya K & 
Matsuda H, Structure of new dammarane-type triterpeno
saponins from the flower buds of Panax notoginseng and 
hepatoprotective effects of principal ginseng saponins, J Nat 
31 Somova L J, Shode F O, Ramnanan P & Nadar A, 
Antihypertensive, antiatherosclerotic and antioxidan
t activity of triterpenoids isolated from Olea europaea, subspe
cific africana leaves, J Ethnopharmacol, 84 (2003) 299.
32 Quyang M A, He Z D & Wu C L, Antioxidative activity of 
381.