Evaluation of hepatoprotective activity of *Clerodendrum serratum* L.

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The ethanol extract of *C. serratum* roots and ursolic acid isolated from it were evaluated for hepatoprotective activity against carbon tetrachloride induced toxicity in male Wistar strain rats. The parameters studied were estimation of liver function serum markers such as serum total bilirubin, total protein, alanine transaminase, aspartate transaminase and alkaline phosphatase activities. The ursolic acid showed more significant hepatoprotective activity than crude extract. The histological profile of the liver tissue of the root extract and ursolic acid treated animal showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration as similar to the controls. The results when compared with the standard drug silymarin, revealed that the hepatoprotective activity of the constituent ursolic acid is significant as similar to the standard drug.

**Keywords**: *Clerodendrum serratum*, Hepatoprotective activity, Root extract, Ursolic acid.

*Clerodendrum serratum* L. (Verbenaceae) is a deciduous shrub distributed in the forests of the Western Ghats of India. In Indian system of medicine, the plant is well-known as Bharangi (Sanskrit) and commonly known as Blue glory (English) and Gantu Bharangi (Kannada). As per the traditional claims roots are the potential source of drugs for ailments such as asthma, bodyache, bronchitis, cholera, dropsy, eye diseases, fever, inflammations, malaria, ophthalmia, rheumatism, snakebite, tuberculosis, ulcers and wounds. Leaves are used as appetizer and expectorant, young shoots, leaves and flowers are eaten as vegetables. It is one of the few shrub that antagonizes the effect of histamine. Ethanolic extract of the root is reported for antinociceptive, anti-inflammatory and antipyretic activities.

Phytochemically the root bark extract contains D-mannitol, stigmasterols and three triterpenoids—oleanolic acid, quercetaric acid and cerratagic acid. Leaf extract contains stigmasterol, α-spinasterol, luteolin, luteolin-7-0 glucuronide, apigenin, baicalin and scutellarin 7-0 glucuronide. In the Western Ghats region of Karnataka the roots of this species are being used by the traditional practitioners for the treatment of jaundice. This communication reports the hepatoprotective activity of the ethanol extract of the roots and the isolated constituent ursolic acid against CCl₄ induced hepatic damage in rats.

**Materials and Methods**

*Collection of plant material*—Roots of *C. serratum* were collected from the Lakkavalli reserve forest range of the Western Ghats region of Karnataka. Taxonomic authenticity was confirmed by referring to herbarium specimen at Madras herbarium, Botanical Survey of India, Southern Circle, Coimbatore and a voucher specimen (FDD-53) is deposited at Kuvempu University herbaria, Shankaraghatta.

*Extraction*—Roots were shade dried for a week and powdered mechanically (Sieve No. 10/44). Powdered material was extracted using soxhlet apparatus with 70% ethanol for about 48 hr. The extract was filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator (Buchi, Flawil, Switzerland) till the complete evaporation of the solvent. The yield was 28.9% (w/w). The active constituent ursolic acid was isolated from the ethanol extract following the method of Suresh and Sastry.

*Isolation and characterization*—The ethanol extract was made alkaline by adding 2% sodium hydroxide and filtered. The filtrate was acidified with dilute HCl and extracted with diethyl ether. The ether soluble portion was separated and concentrated. This

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compound was recrystallised in ethanol. The melting point was determined using melting point apparatus (Jindal, NEW Delhi). The characterization of the compound was done by IR, \(^1\)HNMR, \(^{13}\)C NMR and MASS spectroscopic studies.

**Drug formulation**—Oral suspensions of the root extract (20 mg/ml) and the isolated constituent ursolic acid (10 mg/ml) were prepared in gum tragacanth (1% w/v).

**Animals**—Male Wistar albino rats weighing 150-200 g were procured from the National College of Pharmacy, Shimoga and were maintained at standard housing conditions. The animals were fed with commercial diet (Pranav Agro Industries Ltd., Sangli) and water *ad libitum* during the experiment. The Institutional Animal Ethical Committee (Reg.No. 144/1999/CPCSEA/SMG) permitted the study. Acute toxicity studies were conducted according to "staircase" method\(^8\) following OECD guidelines 2002. The LD\(_{50}\) of root extract and ursolic acid was found to be 200 and 100 mg/kg body weight respectively. One tenth of these doses (20 and 10 mg/kg, body weight respectively) was selected as the therapeutic dose for the evaluation of hepatoprotective activity\(^9\).

**Evaluation of hepatoprotective activity**—The animals were divided into 5 groups of 6 each. The animals of group I (control) received the vehicle gum tragacanth (1 ml/kg/day; 1% w/v). Carbon tetrachloride (E-Merck, Mumbai) with olive oil (1:1) was administered to all the animals of groups II to V in the dose of 0.1 ml/kg/day, ip for 14 days\(^10\). Group III animals were treated with the standard drug silymarin (Ranbaxy Lab, Dewas; 100 mg/kg/day, po). The animals of group IV received ethanol extract (20 mg/kg/day, po) and the animals of group V received ursolic acid (10 mg/kg/day, po). The drugs were administered concomitantly for 14 days. The animals of all the groups were sacrificed on 14\(^{th}\) day under light ether anaesthesia. 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 by centrifugation at 3000 rpm for 10 min and subjected to biochemical investigation viz., total bilirubin (TB)\(^11\), total protein\(^12\), serum alanine transaminase (ALT), aspartate transaminase (AST)\(^13\) and alkaline phosphatase (ALP)\(^14\). Results of biochemical estimations were reported as mean ± SE of six 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.

**Histology**—The liver samples were excised from the animals of each group after draining the blood and washed with the normal saline. Initially the materials were fixed in 10% buffered neutral formalin for 48 hr. They were processed for paraffin embedding. The sections were taken at 5 μm thickness, processed in alcohol-xylene series and were stained with alun-haematoxylin and eosin\(^15\). The sections were examined microscopically for the evaluation of histological changes.

**Results**

The triterpenoid compound isolated from ethanol extract of roots of *C. serratum* showed the following spectral characteristics. The IR (KBr) spectrum showed peaks at cm\(^{-1}\) 3411, 2931, 2862, 1714, 1382 and 1045. The \(^1\)H-NMR analysis showed proton peaks at δ 11.0 (1H); 2.0 (1H); 1.44 (2H); 0.88 (3H). \(^{13}\)C NMR showed ppm related to TMS, shift at 180 (C from 1corboxyl,); 144 (C from 1-ethylene); 122-47 (CH from cyclohexane); 42 (C from cyclohexane); 35 (CH\(_2\) from cyclohexane); 22 (CH\(_3\) aliphatic) and in MASS spectral analysis molecular ion peak observed at m/Z 457 indicating the molecular weight of the compound and fragments at 412, 382, 365, 270. These spectral data were similar with that of the constituent isolated by Suresh and Sastry\(^8\) and the compound was identified as ursolic acid (Fig. 1).

At the end of 14 days treatment, biochemical analysis of blood samples of CCl\(_4\) treated animals showed significant increase in the levels of total bilirubin (5.17-fold), alanine transaminase (16.23-fold), aspartate transaminase (24.47-fold) and alkaline phosphatase (2.73-fold) activities. But the total
protein level (39%) was decreased reflecting the liver injury due to the toxic effect of CCl₄. The blood samples of the animals treated with the root extract and the constituent ursolic acid showed significant reduction in the levels of liver function serum markers. The effect was more pronounced in the animals treated with ursolic acid as similar to that of the standard drug silymarin (Table 1).

Histological profile of control animal showed normal hepatocytes (Fig. 2). The section of liver of the animals treated with CCl₄ exhibited intense centrilobular necrosis, vacuolisation and macrovesicular fatty changes (F) (Fig. 3). The liver sections of silymarin treated animals showed normal hepatic architecture (Fig. 4). Moderate accumulation of fatty lobules (F) was observed in the liver sections (Fig. 5) of root extract treated animals. The liver sections of the animals treated with ursolic acid 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. 6).

Discussion

The injury and dysfunction of liver caused by CCl₄ in experimental animals simulates the human viral hepatitis¹⁶. The toxic effect of CCl₄ is due to its conversion to highly reactive toxic free radical CCl₃O⁻ by cytochrome P₄₅₀. The free radicals produced locally, cause autooxidation of polyenic fatty acids present within membrane phospholipids and oxidative decomposition of lipid is initiated. The organic peroxides formed after reacting with oxygen leads to swelling of smooth endoplasmic reticulum and dissociation of ribosomes from the rough endoplasmic reticulum. Accumulation of lipids ensues due to inability of the cells to synthesize lipoprotein from triglycerides and lipid acceptor proteins leading to the fatty liver. Further, release of products of lipid peroxidation causes damage to plasma membrane owing to increased permeability of plasma membrane. This is followed by progressive swelling of the cell, massive influx of calcium leading to cell death¹⁷. The increase in the levels of AST, ALT, TB and ALP was the clear indication of cellular leakage and loss of functional integrity of the cell membrane¹⁸.

Plant constituents like triterpenoids and flavonoids are well known for their antioxidant and hepatoprotective activities¹⁹,²⁰. Phytochemical analysis of ethanol root extract of C. serratum showed the presence of flavonoids, glycosides, triterpenoids, tannins, quinones and saponins. In the present study a triterpenoid ursolic acid was isolated and characterized. The concomitant treatment of CCl₄ with the root extract or the constituent ursolic acid showed significant reduction in the level of serum bilirubin and liver function marker enzymes. The test drugs mediated restoration in levels of AST, ALT and ALP towards respective normal value is an indication of stabilization of plasma membranes as well as repair of hepatic tissue due to damage caused by CCl₄. In all the parameters studied, the hepatoprotective activity of ursolic acid was significant as similar to that of silymarin. However, the silymarin is slightly effective than ursolic acid. Hence, hepatoprotective potency of C.serratum may be attributed to ursolic acid which is known to normalize the disturbed antioxidant status either by maintaining the levels of glutathione and by inhibiting the production of malondialdehyde²¹ or may be due to

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<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>
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<tr>
<td>Control</td>
<td>0.46±0.02</td>
<td>9.33±0.77</td>
<td>142.23±0.38</td>
<td>57.70±0.33</td>
<td>185.16±1.68</td>
</tr>
<tr>
<td>CCl₄</td>
<td>2.38±0.14</td>
<td>5.65±0.25</td>
<td>2327.84±11.42</td>
<td>1412.34±3.29</td>
<td>451.42±1.81</td>
</tr>
<tr>
<td>CCl₄ + Silymarin</td>
<td>0.53±0.01</td>
<td>9.00±0.02</td>
<td>199.27±0.69</td>
<td>83.03±1.19</td>
<td>214.44±1.21</td>
</tr>
<tr>
<td>CCl₄ + root extract</td>
<td>1.03±0.01</td>
<td>8.00±0.01</td>
<td>282.32±3.30</td>
<td>156.80±2.07</td>
<td>285.92±1.12</td>
</tr>
<tr>
<td>CCl₄ + Ursolic acid</td>
<td>0.89±0.01</td>
<td>8.23±0.04</td>
<td>273.98±0.88</td>
<td>93.57±0.73</td>
<td>243.67±1.48</td>
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ANOVA

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<td></td>
<td>156.1</td>
<td>4.25</td>
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*P≤0.01 indicates significant when compared to control.

AST = Aspartate transaminase; ALT = Alanine transaminase; ALP = Alkaline phosphatase.
Figs 2-6—Section of the liver tissue of 2: control animal showing normal histology, portal triad showing portal vein (V), portal artery (arrow head), hepatic duct (arrow); 3: animal treated with CCl₄ showing fatty vacuole (F) and central vein (V); 4: silymarin treated animal showing normal hepatocytes, portal vein (V), portal artery (arrow head), hepatic duct (arrow) of portal triad; 5: root extract treated animal showing normal arrangement of hepatocytes around the central vein (V), portal artery (arrow head), hepatic duct (arrow), absence of necrosis and few fatty vacuoles (F); 6: ursolic acid treated animals showing normal arrangement of hepatocytes around the portal triad vein (V), portal artery (arrow head), hepatic duct (arrow), absence of necrosis and fatty vacuoles (40×; H&E)
the inhibition of toxicant activation and the enhancement of body defense system. The present findings provide a pharmacological evidence to the ethnomedicinal property of *C. serratum* in treating acute jaundice.

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**References**

2. Keshavamurthy K R, Medicinal plants of Karnataka, (Karnataka Forest Department, Bangalore, India), 1994, 92.