Effect of *Solanum trilobatum* against carbon tetrachloride induced hepatic damage in albino rats

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**Background & objectives:** Many hepatoprotective herbal preparations have been recommended in alternative systems of medicine for the treatment of hepatic disorders. No systematic study has been done on protective efficacy of *Solanum trilobatum* to treat hepatic diseases. Protective action of *Solanum trilobatum* extract (STE) was evaluated by us in an animal model of hepatotoxicity induced by carbon tetrachloride (CCl₄).

**Methods:** Wistar albino rats were divided into five groups. Group I was normal control group; Group II, the hepatotoxic group was given CCl₄; Groups III-V received different doses of plant extract with CCl₄. Liver marker enzymes were assayed in serum and antioxidant status was assessed in liver tissue.

**Results:** Levels of marker enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were increased significantly in CCl₄-treated rats (group II). STE brought about a significant decrease in the activities of all these enzymes. Lipid peroxidation (LP) was increased significantly in liver tissue in the CCl₄-treated rats (group II) while the activities of glutathione peroxidase (GPₓ), catalase (CAT) and superoxide dismutase (SOD) were decreased. STE treatment led to the recovery of these levels to near normal.

**Interpretation & conclusion:** The present observations suggested that the treatment with *S. trilobatum* extract enhance the recovery from CCl₄-induced hepatic damage due to its antioxidant and hepatoprotective property.

**Key words** Antioxidant status - CCl₄ - *Solanum trilobatum* - liver maker enzymes

Liver an important organ actively involved in metabolic functions, is a frequent target of number of toxicants. In absence of a reliable liver protective drug in the modern medicine, there are number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders. The principle causes of carbon tetrachloride (CCl₄) induced hepatic damage is lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals. The antioxidant activity or the inhibition of the generation of free radicals is important in providing protection against hepatic damage. A number of plants have been shown to possess hepatoprotective property by improving antioxidant status. Thus the efficacy of the drug would be preventive and passive for defending against damages. Indian medicinal plants belonging to about 40 families were investigated as liver protective drugs. *Solanum trilobatum* Linn (Family: Solanaceae) is used in the Siddha system of...
medicine as an expectorant and in the treatment of respiratory diseases, asthma, chronic febrile infections, tuberculosis, cardiac and liver diseases. \( \text{Solanum, } \beta \text{-solamarine, solaine, solasodine, glycoalkaloid}\) and diosogenin and tomatidine are the constituents isolated from this plant (personal communication). This plant possesses a broad spectrum of antibiotic, antibacterial, antimitotic and anticancer activity. No detailed study has been conducted on the hepatoprotective activity of \textit{S. trilobatum}. Therefore, the present study was carried out to explore hepato protective efficacy of \textit{S. trilobatum} extract (STE) against \( \text{CCl}_4 \) induced hepatotoxicity in animal model.

**Material & Methods**

**Plant materials:** The whole plant of \textit{S. trilobatum} was collected from and around Chennai. The plant was authenticated by Captain Srinivasa Murthy Drug Research Centre for Ayurveda, Arumbakkam, Chennai.

**Preparation of plant extract:** One kg of plant material (whole plant) was shade dried, coarsely powdered and allowed to soak in 2 l of 90 per cent alcohol for 48 h at room temperature. The extract was filtered and concentrated on a water bath to 20 ml. The inorganic material was precipitated and filtered off. The filtrate was again concentrated in a china dish and dried in vacuum. The yield of the extract was 10 per cent w/w of powdered methanol extract, which was stored in refrigerator for further use.

The doses of STE were selected on the basis of acute toxicity study and the \( \text{LD}_{50} \) of the extract was found to be 5g/kg body weight (bw). STE administration did not produce any abnormalities such as atoxic, circling, lacrimation, labowed breathing etc., in the animals throughout the experimental period. The dose level selected for the present study was non-toxic and safe.

**Chemicals:** Propylene glycol, \( \text{CCl}_4 \), were purchased from SD Fine Chemicals, Chennai, India. All the other chemicals and reagents were used of Analytical grade.

**Animals:** Wistar strain albino rats weighing 120-150g were obtained from TANUVAS-LAMU Madhavaram, Chennai. Animals were fed with normal pelleted (rat chow) diet and water \textit{ad libitum}. The study protocol was approved by the Institutional Animals Ethics Committee (IAEC).

**Experimental design:** The animals were divided into 5 groups of 6 animals each and treated as follows: Group I animals served as normal control and received olive oil (vehicle) 1.0 ml/kg b wt intraperitoneal (ip). Group II animals constituted the hepatotoxic group, which received 30 per cent \( \text{CCl}_4 \) suspended (in olive oil 1.0 ml/kg b wt ip) after every 72 h for 10 days. Groups III, IV and V received STE (150, 200 and 250 mg/kg b wt/day) suspended in propylene glycol for 10 days and \( \text{CCl}_4 \) was given as in group II rats.

At the end of the experimental period, animals were sacrificed by cervical decapitation. Blood was collected and serum was separated. The liver tissue was excised, homogenized in ice-cold buffer and utilized for biochemical analysis.

Marker enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were assayed in serum. Level of total serum protein (TSP) was also estimated.

Antioxidant status was assessed from the levels of glutathione (GSH), lipid peroxidation (LP) and from the activities of glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD), in liver tissue.

**Statistical analysis:** Student’s ‘t’ test was used for statistical significance between groups.

**Results & Discussion**

The involvement of free radicals in the pathogenesis of liver injury has been investigated for many years by using acute poisoning with \( \text{CCl}_4 \). \( \text{CCl}_4 \) an extensively studied liver toxicant, and its metabolites such as trichloromethyl peroxo radical (\( \text{CCl}_4 \text{O}_2 \)) are known to be involved in the pathogenesis of liver damage.
Levels of all marker enzymes increased significantly in group II rats after CCl$_4$ administration ($P<0.001$), as compared to normal controls (Table I). STE treatment caused significant decrease in the activities of all these enzymes though the decrease was maximum ($P<0.001$) in group V rats which received the highest dose of STE. The increased activities of liver marker enzymes such as ALT, AST, ALP and LDH in the serum of CCl$_4$ induced rats indicate damage to hepatic cells.$^{21}$ Damage to the cell integrity of the liver by CCl$_4$ is reflected by an increase in the activity of AST, which is released into circulation after cellular damage. ALP is an ectoenzyme of the hepatocyte plasma membrane. CCl$_4$-mediated acute

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal (group I)</th>
<th>CCl$_4$ induced (group II)</th>
<th>CCl$_4$+STE (150 mg/kg) (group III)</th>
<th>CCl$_4$+STE (200 mg/kg) (group IV)</th>
<th>CCl$_4$+STE (250 mg/kg) (group V)</th>
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<tbody>
<tr>
<td>ALT (Units/ml)</td>
<td>45.12±7.22</td>
<td>119.2±10.79$^*$</td>
<td>85.2±6.51***</td>
<td>81.5±7.21***</td>
<td>61.73±3.91***</td>
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<tr>
<td>AST (Units/ml)</td>
<td>53.24±5.02</td>
<td>171.05±11.0$^*$</td>
<td>131.5±9.71**</td>
<td>69.7±9.41***</td>
<td>47.51±1.90***</td>
</tr>
<tr>
<td>ALP (KA Units)</td>
<td>50.18±12.0</td>
<td>103.63±7.69$^*$</td>
<td>69.25±1.91*</td>
<td>59.21±6.89***</td>
<td>57.57±11.70***</td>
</tr>
<tr>
<td>LDH IU/dl</td>
<td>101.07±5.93</td>
<td>153.25±27.0$^*$</td>
<td>143.12±12.1</td>
<td>127.19±12.1*</td>
<td>117.19±7.23***</td>
</tr>
<tr>
<td>TSP (mg protein/ml serum)</td>
<td>53.57±3.37</td>
<td>46.75±3.13$^*$</td>
<td>51.35±3.89*</td>
<td>51.65±4.11**</td>
<td>52.59±4.37***</td>
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Values are mean ± SEM for 6 animals in each observation

$^* P<0.05; ** P<0.01; *** P<0.001$; as compared with CCl$_4$ induced group

$^*<0.001$ as compared with normal group

ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; TSP, total serum protein

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<tr>
<td>GSH</td>
<td>15.13±1.26</td>
<td>11.47±0.21$^*$</td>
<td>13.1±0.99*</td>
<td>14.6±1.07**</td>
<td>15.09±1.11***</td>
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<tr>
<td>LP</td>
<td>119.9±21.5</td>
<td>185.2±11.69$^*$</td>
<td>144.31±6.91**</td>
<td>108.5±10.93***</td>
<td>120.2±10.02***</td>
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<tr>
<td>GP$_X$</td>
<td>16.89±0.13</td>
<td>14.91±0.09$^*$</td>
<td>15.03±0.11*</td>
<td>16.01±0.14***</td>
<td>16.87±0.15***</td>
</tr>
<tr>
<td>CAT</td>
<td>35.83±5.43</td>
<td>15.73±0.69$^*$</td>
<td>20.69±0.65**</td>
<td>30.23±0.77***</td>
<td>34.31±0.79***</td>
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<tr>
<td>SOD</td>
<td>2.04±0.05</td>
<td>1.79±0.06$^*$</td>
<td>1.75±0.05</td>
<td>1.99±0.13*</td>
<td>2.02±0.08**</td>
</tr>
</tbody>
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Values are mean ± SEM for 6 animals in each observation

$^* P<0.05; ** P<0.01; *** P<0.001$; as compared with CCl$_4$ induced group

$^*<0.001$ as compared with normal group

Values are expressed as GSH, glutathione (µg/mg protein); LP, lipid peroxidation [nmoles of malonaldehyde (MDA) formed/mg protein/h]; GP$_X$, glutathione peroxidase (µg of glutathione consumed/min/mg protein); CAT, catalase (unit/min/mg protein); SOD, superoxide dismutase (unit/min/mg protein)
toxicity increased permeability of the hepatocyte membrane and cellular leakage\textsuperscript{22}. The present findings concur with the above reports. The STE mediated suppression of the increased AST, ALT, ALP and LDH activities suggested the possibility of the extract to give protection against liver injury upon CCl\textsubscript{4} induction.

The decrease in TSP observed in CCl\textsubscript{4} treated rats (Table I) may be associated with the decrease in the number of hepatocytes which in turn, may result into the decreased hepatic capacity to synthesize protein, but the restoration of the level of TSP after the administration of STE confirmed the hepatoprotective nature of \textit{S. trilobatum}.

Lipid peroxidation has been implicated in the pathogenesis of hepatic injury by compounds like CCl\textsubscript{4} and is responsible for cell membrane alterations\textsuperscript{23}. In the present study, significantly elevated level of LP (\textit{P}<0.001) observed in CCl\textsubscript{4} administered rats (group II) indicated excessive formation of free radicals and activation of LP system resulting in hepatic damage (Table II). The significant decline in the LP content in the liver tissue of CCl\textsubscript{4} + STE administered rats (group III-V) indicated antilipid peroxidative effect of \textit{S. trilobatum} (Table II).

The GSH antioxidant system consists of an array of non-enzymic and enzymic reaction pathways involving the neutralization of free radical species. Perturbation of the GSH status of a biological system has been reported to lead to serious consequence\textsuperscript{24}. GP\textsubscript{x} utilizes it for the decomposition of lipid hydroperoxides and other reactive oxygen species (ROS) and glutathione-S-transferase (GST) maximizes the conjugation of free radicals and various lipid hydroperoxides to GSH to form water-soluble products that can be easily excreted out\textsuperscript{25}. Ohta et al\textsuperscript{26} have reported the decreased activities of SOD and CAT after the administration of single dose of CCl\textsubscript{4}. In our study, decline in the activities of SOD, CAT, GP\textsubscript{x} and GSH levels in CCl\textsubscript{4} administered rats (group II) and recovery to near normalcy in groups III-V revealed that oxidative stress elicited by CCl\textsubscript{4} intoxication has been nullified due to the antioxidant effect of STE (Table II). Mohanan et al\textsuperscript{27} suggested that sobatum, an active constituent of \textit{S. trilobatum} possesses free radical scavenging activity. The phytocannabinoids of \textit{S. trilobatum} such as solasodine exert antioxidative property, supporting the present findings.

\textbf{In conclusion, the present findings demonstrated the hepatoprotective and antioxidant activities of \textit{S. trilobatum} extract in the experimental rat model. Further work need to be done to isolate and purify the active principle involved in the hepatoprotective activity of this plant.}

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\textbf{References}


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