Hepatoprotective Activity of *Camellia sinensis* and its Possible Mechanism of Action

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**ABSTRACT**

The present study appraised the hepatoprotective activity of aqueous extract of *Camellia sinensis* leaves and its possible mechanism of action. Liver damage was induced by intraperitoneal administration of carbon tetrachloride/olive oil (50 % v/v, 0.5 ml/kg ) in male Wistar rats (150-220g) once daily for 7 days and the extent of damage was studied by assessing biochemical parameters such as alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total protein and albumin in serum and concentrations of lipid peroxides (LPO), glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) in liver. The aqueous extract of *Camellia sinensis* (100 mg and 200 mg/Kg) were administered orally to the animals with hepatotoxicity induced by carbon tetrachloride and its effects on biochemical parameters were compared with those in animals treated with vitamin E (100 mg/Kg). Histopathological studies were also done. *Camellia sinensis* 100 and 200mg/kg results in significant reduction in serum hepatic enzymes and liver lipid peroxide which were increased by carbon tetrachloride. There was significant increase in serum total protein, albumin and liver GSH, SOD and CAT when compared to those in rats treated by carbon tetrachloride. The antioxidant activity of *Camellia sinensis* (100 and 200mg/Kg) were comparable with the effects of vitamin E (100mg/Kg). Histopathological changes (congestion of central vein, centrilobular necrosis and sinusoidal congestion) induced by carbon tetrachloride were reduced to a moderate extent in *Camellia-sinensis*-treated rats. Taking together, *Camellia sinensis* protectes the liver from carbon-tetrachloride-induced damage. Probable mechanism of its action is its anti-oxidant property.

**Keywords:** *Camellia sinensis*, Antioxidant, Carbon tetrachloride, Hepatoprotective
Table 1. Effect of *Camellia Sinensis* on serum ALT, AST, ALP, Total protein and Albumin in CCl₄-treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drug treatment</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>Total Protein g / dl</th>
<th>Albumin g / dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Distilled Water (1ml/kg p.o.)</td>
<td>107.6 ± 9.5</td>
<td>59.5 ± 4.1</td>
<td>249.5 ± 18.2</td>
<td>8.5±0.6</td>
<td>6.2±0.5</td>
</tr>
<tr>
<td>II</td>
<td>CCl₄ (0.5ml/kg i.p.)</td>
<td>378.9 ± 23.7</td>
<td>254.9±19.3</td>
<td>586.9 ± 31.6</td>
<td>5.1±0.4³</td>
<td>2.4±0.3³</td>
</tr>
<tr>
<td>III</td>
<td>CCl₄ + <em>Camellia sinensis</em> (100mg/kg)</td>
<td>131.6 ± 10.6</td>
<td>71.4±5.6</td>
<td>267.7 ± 17.8</td>
<td>6.9±0.8¹</td>
<td>4.7±0.3²</td>
</tr>
<tr>
<td>IV</td>
<td>CCl₄ + <em>Camellia sinensis</em> (200mg/kg p.o.)</td>
<td>117.6 ± 6.7</td>
<td>66.2±6.1</td>
<td>258.4 ±16.2</td>
<td>7.6±0.6³</td>
<td>5.4±0.5³</td>
</tr>
<tr>
<td>V</td>
<td>CCl₄ + Vitamin E (100mg/kg p.o.)</td>
<td>111.7 ± 8.7</td>
<td>62.3±5.1</td>
<td>255.3 ±24.1</td>
<td>7.8±0.4³</td>
<td>5.9±0.5³</td>
</tr>
</tbody>
</table>

Values are in Mean ± SEM. Number of animals in each group = 6. *p < 0.001 Vs Group I. b p < 0.01 Vs Group II. c p < 0.05 Vs Group II. (CCl₄: carbon tetrachloride, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase)

MATERIALS AND METHODS

Drugs and chemicals

Carbon tetrachloride (CCl₄) was obtained from CPCSEA. Merck Ltd., Mumbai, India; Thiobarbituric acid (TBA), 5,5-dithio-bis-2-nitrobenzodic acid (DTNB) and glutathione (GSH) were obtained from Sigma, USA. Vitamin E was obtained from Hi Media Pvt. Ltd., Mumbai. All chemicals used in the study were of analytical grades.

Plant material

The aerial parts of *Camellia sinensis* were collected from the hills of Ootacamund, South India, in the month of February. The plant samples were identified and authenticated by the botanist, Botanical Survey of India, Coimbatore, India. The voucher specimen (A 2459) has been deposited in Herbarium.

Extraction Preparation

The collected aerial parts of *Camellia sinensis* was washed, air dried, powdered and boiled in sufficient quantity of distilled water for 2 hours and the aqueous extract was filtered, concentrated in vacuo and lyophilized to give a dry extract [11].

Experimental procedure

Male Swiss albino mice weighing between 20–25 gm and male wistar Albino rats weighing between 150–230 gm were used. The animals were obtained from IRT Perundurai Medical College, Erode, Tamilnadu, India. On arrival, the animals were placed at 22°C for 3 days. Group–I received equal mixture of CCl₄ and olive oil along with *Camellia sinensis* (100 mg/Kg, p.o.) simultaneously once daily for 7 days. Group–IV received equal mixture of CCl₄ and olive oil (50 % v/v, 0.5 ml/kg i.p.) once daily for 7 days [13].

Acute Toxicity Studies

Acute toxicity studies were performed according to Organization for Economic Co-Operation and Development (OECD)-423 guidelines [12]. Male Swiss mice selected by random sampling technique were employed in this study. The animals were fasted for 4 hours with free access to water only. *Camellia sinensis* was administered orally at a dose of 5 mg/kg initially. Mortality if any was observed for 3 days. If mortality was observed then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then higher doses (50, 300, 2000 mg/kg) of *Camellia sinensis* were employed for further toxicity studies.

Control and Supervision on Experiments on Animals

All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (Regd no: 688/2/C-CPCSEA) and were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).
Table 2. Effect of *Camellia sinensis* on liver LPO, GSH, CAT and SOD in CCl₄-treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drug treatment</th>
<th>LPO nmol of MDA/mg protein</th>
<th>GSH nmol/mg tissue</th>
<th>CAT nmol of H₂O₂ decomposition/min./mg protein</th>
<th>SOD Units/g protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Distilled Water (1ml/kg p.o.)</td>
<td>4.1 ±0.5</td>
<td>22.5 ±1.9</td>
<td>189.8 ± 11.3</td>
<td>84.6 ± 6.8</td>
</tr>
<tr>
<td>II</td>
<td>CCl₄ (0.5ml/kg i.p.)</td>
<td>14.8 ±1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.2 ±1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.2 ±5.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.5 ±3.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>CCl₄ + <em>Camellia sinensis</em> (100mg/kg)</td>
<td>8.4 ±0.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.6 ±1.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>152.4 ±13.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>67.7 ±5.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>CCl₄ + <em>Camellia sinensis</em> (200mg/kg p.o.)</td>
<td>6.1 ±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.3 ±1.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>160.3 ±15.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.3 ±6.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>V</td>
<td>Vitamin E (100mg/kg p.o.)</td>
<td>5.4 ±0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.9 ±2.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>168.4 ±11.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>77.1 ±5.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are in Mean ± SEM. Number of animals in each group = 6. *p < 0.001 Vs Group I. *p < 0.01 Vs Group II.*p < 0.05 Vs Group II.( CCl₄: carbon tetrachloride, LPO: lipid peroxide, GSH: glutathione, CAT: catalase and SOD: superoxide dismutase)
which was significantly \( (p<0.01) \) reversed with the treatment of *Camellia sinensis*. 

Lipid peroxidation

The LPO level in liver was significantly increased \( (p<0.001) \) in CCl\(_4\)-treated animals when compared to control. Treatment with *Camellia sinensis* at 200 mg/kg showed significant increase in SOD and CAT levels when compared to control. Treatment with *Camellia sinensis* (200 mg/kg) showed significant \( (p<0.01) \) increase in GSH, SOD and CAT levels when compared to control. Treatment with vitamin E (100 mg/kg) showed significant \( (p<0.01) \) decrease in LPO level when compared to CCl\(_4\)-treated animals.

Glutathione, catalase and super oxide dismutase

In order to find the possible mechanism by which *Camellia sinensis* prevents hepatic damage caused by CCl\(_4\), investigation on levels of GSH, SOD and CAT was carried out. The levels of GSH, SOD and CAT in liver homogenate were significantly decreased \( (p<0.001) \) in CCl\(_4\)-treated animals when compared to control. Treatment with *Camellia sinensis* at 200 mg/kg showed significant \( (p<0.01) \) increase in GSH, SOD and CAT levels when compared to CCl\(_4\)-treated groups. Vitamin E (100 mg/kg) treated showed significant \( (p<0.01) \) rise in GSH, SOD and CAT levels when compared to CCl\(_4\)-treated groups.

Fig 1. Histopathological studies of *Camellia sinensis* and vitamin E on CCl\(_4\)-treated rats.

(A) Transverse section of the liver of control rats, showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus and central vein.

(B) Transverse section of the liver of CCl\(_4\) treated animals showing hydropic changes in centrilobular hepatocytes with single cell necrosis surrounded by neutrophils, congestion of central vein and sinusoids were seen with acute inflammatory cells infiltrating sinusoids mainly in central zone.

(C) Transverse section of the liver, after simultaneous treatment of *Camellia sinensis* (100 mg/kg) and CCl\(_4\) treated animals showing mild fatty change and mild sinusoidal congestion.

(D) Transverse section of the liver, after simultaneous treatment of *Camellia sinensis* (200 mg/kg) and CCl\(_4\) treated animals showing residual hepatocellular necrosis with cords of regeneration hepatocytes.

(E) Transverse section of the liver, after simultaneous treatment of Vitamin E and CCl\(_4\) treated animals showing mild central venous congestion and mild fatty vacuolation.
The results of histopathological studies of *Camellia sinensis* on CCl₄-treated rats are shown in Fig 1. In control rats, liver sections showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus and nucleolus and central vein. In CCl₄-treated rats, liver there were hydropic changes in centrilobular hepatocytes with single cell necrosis surrounded by neutrophils. Congestion of central vein and sinusoids were seen with acute inflammatory cells infiltrating sinusoids mainly in central zone. In *Camellia sinensis* (100 mg/kg) and CCl₄-treated rats, liver sections showed mild fatty change and mild sinusoidal congestion. In *Camellia sinensis* (200 mg/kg) and CCl₄-treated rats, liver sections showed residual hepatocellular necrosis with cords of regenerating hepatocytes. In Vitamin E and CCl₄ treated rats, there was mild central venous congestion and mild fatty vacuolation.

**DISCUSSION**

CCl₄ is one of the most commonly used hepatotoxins in the experimental study of liver diseases. [20]. Protein and albumin which accelerates the regeneration process and the protection of liver cells. The increased activities of serum and liver tissue enzymes originate from the over-utilization of glutathione peroxidase. In fact, decreased significantly (p<0.05) in group IV, animal after treatment with *Camellia sinensis* extracts. The administration of *Camellia sinensis* during severe liver damage condition has elevated the GSH levels, in turn gives products like melanodialdehyde (MDA) that cause damage to the membrane. This lipid peroxidative degradation of biomembrane is one of the principle causes of hepatotoxicity of CCl₄ [24, 25].

In our study, elevation in the levels of end products of lipid peroxidation in liver of rats treated with CCl₄ was observed. The increase in LPO level in liver suggests enhanced lipid per oxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radical [26]. Pretreatment with *Camellia sinensis* reversed these changes. Hence, it is possible that the mechanism of hepatoprotection of *Camellia sinensis* is due to its antioxidant effect. GSH plays a protective role in tissue by detoxification of xenobiotics and is essential to maintain structural and functions integrity of the cell. The significant decrease in liver GSH in *Camellia sinensis* treated rats in the present study may be due to enhanced substrate utilization by glutathione peroxidase. In fact, there is a direct correlation between GSH depletion and enhanced lipid peroxidation. Significant reduction of LPO was observed in *Camellia sinensis*-treated animals. The administration of *Camellia sinensis* during severe liver damage condition has elevated the GSH levels, in turn helps in maintaining the liver tissue damage. This indicates the additional antioxidant property of *Camellia sinensis*.
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REFERENCES

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