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

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Received March 26, 2007; Revised May 28, 2008; Accepted June 16, 2008

This paper is available online at [http://ijpt.iums.ac.ir](http://ijpt.iums.ac.ir)

**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* protects the liver from carbon-tetrachloride-induced damage. Probable mechanism of its action is its anti-oxidant property.

**Keywords:** *Camellia sinensis*, Antioxidant, Carbon tetrachloride, Hepatoprotective
MATERIALS AND METHODS

Drugs and chemicals

Carbon tetrachloride (CCl4) was obtained from 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, Calcutta. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee and were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Extraction Preparation

The collected aerial parts of Camellia sinensis were 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]. The experiment was carried out after obtaining clearance from Institutional Animal Ethics Committee.

Animals

Male Swiss albino mice weighing between 20–25gm and male wistar Albino rats weighing between 150–220 gm were used. The animals were obtained from Tamilnadu, India. On arrival, the animals were placed at 25°C for 3 days. If mortality was observed, then higher doses (50, 300, 2000 mg/kg) of Camellia sinensis were administered orally at a dose of 5 mg/kg initially. Mortality if any was observed for 3 days. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals 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.

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 in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals 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.

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±0.4</td>
<td>2.4±0.3</td>
</tr>
<tr>
<td>III</td>
<td>CCl4 + Camellia sinensis (100mg/kg)</td>
<td>131.6 ± 10.6</td>
<td>71.4 ±5.6</td>
<td>267.7 ± 17.8</td>
<td>6.9±0.8</td>
<td>4.7±0.3</td>
</tr>
<tr>
<td>IV</td>
<td>CCl4 + Camellia sinensis (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±0.6</td>
<td>5.4±0.5</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±0.4</td>
<td>5.9±0.5</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, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase)
**Hepatoprotective activity of Camellia sinensis**

**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 ±1.3ᵃ</td>
<td>11.2 ±1.7ᵃ</td>
<td>46.2 ±5.6ᵃ</td>
<td>42.5 ±3.2ᵃ</td>
</tr>
<tr>
<td>III</td>
<td>CCl₄ + Camellia sinensis (100mg/kg)</td>
<td>8.4 ±0.7ᶜ</td>
<td>17.6 ±1.6ᶜ</td>
<td>152.4 ±13.7ᶜ</td>
<td>67.7 ±5.7ᶜ</td>
</tr>
<tr>
<td>IV</td>
<td>CCl₄ + Camellia sinensis (200mg/kg p.o.)</td>
<td>6.1 ±0.5ᵇ</td>
<td>18.3 ±1.9ᵇ</td>
<td>160.3 ±15.1ᵇ</td>
<td>75.3 ±6.9ᵇ</td>
</tr>
<tr>
<td>V</td>
<td>Vitamin E (100mg/kg p.o.)</td>
<td>5.4 ±0.6ᵇ</td>
<td>20.9 ±2.2ᵇ</td>
<td>168.4 ±11.3ᵇ</td>
<td>77.1 ±5.4ᵇ</td>
</tr>
</tbody>
</table>

Values are in Mean ± SEM. Number of animals in each group = 6.ᵃ p < 0.001 Vs Group I.ᵇ p < 0.01 Vs Group II.ᶜ p < 0.05 Vs Group III. (CCl₄: carbon tetrachloride, LPO: lipid peroxide, GSH: glutathione, CAT: catalase and SOD: superoxide dismutase)

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**RESULTS**

**Acute Toxicity Studies**

All the doses (5, 50, 300, 2000 mg/kg, p.o.) of Camellia sinensis (200 mg/Kg, p.o.) simultaneously once daily for 7 days. Group-V received equal mixture of CCl₄ and olive oil along with vitamin E (100 mg/Kg, p.o.) simultaneously once daily for 7 days [14]. On 8th day, the blood was collected by direct cardiac puncture under light ether anesthesia and the liver was dissected out, washed in the ice cold saline and homogenate was prepared in 0.05 M sodium phosphate buffer (pH 7.0) and centrifuged. The supernatant was used for the estimation of lipid peroxide (LPO), glutathione (GSH), catalase and superoxide dismutase (SOD). The activities of serum hepatic marker enzymes namely aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assayed in serum using standard kits from Lupin Laboratories and Pointe Scientifics. The results were expressed as units/litre (U/L). The levels of proteins i.e., total proteins and albumins were estimated in serum of experimental animals by earlier method reported [15]. The LPO in the liver was determined according to previous report [16]. GSH content was estimated in the liver homogenate using DTNB [17]. CAT and SOD activity were measured in the liver homogenate by the reported method [18,19].

**Biochemical estimations**

The hepatic enzymes ALT, AST and ALP in serum of hepatic enzymes when compared to CCl₄-treated animals. Vitamin E (100 mg/kg)-treated animals also showed significant (p<0.01) increase in the levels of hepatic enzymes when compared to CCl₄-treated animals. Vitamin E (100 mg/kg)-treated animals did not produce any mortality even at the highest dose (2000 mg/kg, p.o.) employed. Two sub-maximal doses (100 and 200 mg/kg, p.o.) which were found to be safe were employed for further pharmacological investigations.

**Statistical analysis**

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet’s t-test. P values <0.05 were considered significant.

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**Statistical analysis**

There was a significant (p<0.001) decrease in the serum total protein and albumin levels with CCl₄ treatment in group II when compared to control group I;
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 ($p<0.01$) decrease in LPO level when compared to CCl$_4$-treated groups. Treatment with *Camellia sinensis* (200 mg/kg) showed significant ($p<0.01$) rise in GSH, SOD and CAT levels of LPO 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 less significant ($p<0.05$) decrease in LPO level and significant ($p<0.01$) increases in GSH, SOD and CAT when compared to CCl$_4$-treated groups. Vitamin E (100 mg/kg)- treated showed significant ($p<0.01$) rise in GSH, SOD and CAT.

(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* (100mg/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* (200mg/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.

Fig 1. Histopathological studies of *Camellia sinensis* and vitamin E on CCl$_4$-treated rats
Hepatoprotective activity of *Camellia sinensis*

222 levels when compared to CCl₄-treated groups. Treat-227 oxidative degradation of membrane lipids of endoplasm-224 with *Camellia sinensis* (100 mg/kg) dose showed 228 mic reticulum which are rich in polyunsaturated fatty 225 less significant (*p*<0.05) rise in GSH, SOD and CAT 228 acids. This leads to formation of lipid peroxides, which 226 levels when compared to CCl₄-treated groups. Vitamin 229 in turn gives products like melanodialdehyde (MDA) 227 E (100 mg/kg)-treated animals also showed significant 228 that cause damage to the membrane. This lipid peroxi-229 (*p*<0.01) rise in the levels of GSH, SOD and CAT when 228 dative degradation of biomembrane is one of the princi-229 compared to CCl₄-treated animals.

230 Histopathological studies

231 The results of histopathological studies of *Camellia 232 sinensis* on CCl₄-treated rats are shown in Fig 1. In con-233 trol rats, liver sections showed normal hepatic cells with 234 damage and failure of antioxidant defense mechanisms 230 well-preserved cytoplasm, prominent nucleus and nu-235 295 to prevent formation of excessive free radical [26]. Pre-236 cleus and central vein. In CCl₄-treated rats, liver there 235 treatment with *Camellia sinensis* reversed these 236 were hydropic changes in centrilobular hepatocytes with 237 297 changes. Hence, it is possible that the mechanism of 237 single cell necrosis surrounded by neutrophils. Conges-238 hepatoprotection of *Camellia sinensis* is due to its anti-239 tional of central vein and sinusoids were seen with acute 238 oxidant effect. GSH plays a protective role in tissue by 239 inflammatory cells infiltrating sinusoids mainly in cen-239 detoxification of xenobiotics and is essential to maintain 237 tral zone. In *Camellia sinensis* (100mg/kg) and CCl₄-240 structural and functions integrity of the cell. The sig-237 treated rats, liver sections showed mild fatty change and 240 nificant decrease in liver GSH in *Camellia sinensis*- 239 mild sinusoidal congestion. In *Camellia sinensis* 241 treated rats in the present study may be due to enhanced 239 (200mg/kg) and CCl₄ treated rats, liver sections showed 240 (200 mg/kg) dose showed significant decreases in all 239 residual hepatocellular necrosis with cords of regener-242 300 substrate utilization by glutathione peroxidase. In fact, 239 ating hepatocytes. In Vitamin E and CCl₄ treated rats, 241 there is a direct correlation between GSH depletion and 239 there was mild central venous congestion and mild fatty 240 enhanced lipid peroxidation. Significant reduction of 239 vacuolation.

248 DISCUSSION

249 CCl₄ is one of the most commonly used hepatotox-250 ins in the experimental study of liver diseases. [20]. 251 *Camellia sinensis* enhanced the synthesis of total 252 protein and albumin which accelerates the regeneration 253 Assessment of liver function can be made by estimating 254 the activities of serum and liver tissue enzymes origi-255 nally present or absent in cytoplasm. During hepatic 256 damage, there may be imbalance in these enzyme levels 257 study, the SOD activity is significantly reduced in CCl₄- 258 treated animals. The SOD activity was reversed close to 259 normal after treatment with the *Camellia sinensis* 260 corresponded to the extensive liver damage induced by 259 extract in CCl₄-treated animals. Decreased activity of CAT 261 was observed in animals treated with CCl₄. Presumably 262 The serum ALT, AST and ALP are reliable markers 263 a decrease in CAT activity could be attributed to cross 260 of liver function. They were significantly increased in 262 linking and inactivation of the enzyme protein in the 260 CCl₄-treated groups. On the other hand, in group III 262 lipid peroxides. Decreased CAT activity is linked to 262 animals which were treated with *Camellia sinensis* (100 263 321 level of total protein in serum indicates the hepatopro-264 nm/kg, p.o.). the activity of ALT, AST and ALP had 264 minally present or absent in cytoplasm. During hepatic 264 decrease significantly (*p*<0.05) and in group IV, ani- 264 321 protective activity of *Camellia sinensis*. In the present 264 321 mal after treatment with *Camellia sinensis* extracts 264 321 dently; which shows the antioxidant property of the ex- 264 321 mals which were treated with *Camellia sinensis* (200 264 321 tacts against oxygen free radicals. All the effects on 264 321 mg/kg, p.o.), the activity of ALT, AST and ALP had 264 321 decreased significantly (*p*<0.01). Simultaneous treat-264 321 264 was observed. The increase in LPO level in liver sug-264 321 264 mals after treatment with CCl₄ caused significant 264 321 264 suggests enhanced lipid per oxidation leading to tissue 264 321 264 effects of CCl₄ are largely due to its active metabolite, 264 321 264 changes. Hence, it is possible that the mechanism of 264 321 264 protection against CCl₄-induced hepatotoxicity, which 264 321 264 was hydropic changes in centrilobular hepatocytes with 264 321 264 vacuolation.

265 recovery from the damage induced by CCl₄ treatment. 265 Histopathological studies showed that CCl₄ caused 267 The fall in serum enzymes suggests a protective effect 330 centrilobular necrosis, congestion of central vein and 267 on the liver against CCl₄-induced sinusoids. *Camellia 267 sinusensis* administration exhibited toxicity. Previous reports shown that yellow tea [21] protection against CCl₄-induced hepatotoxicity, which 269 330 sinusoids. *Camellia sinensis* protective activity of CCl₄. The hepatotoxic *Camellia sinensis* in CCl₄-treated rats protects liver damage. The 269 330 effects of CCl₄ are largely due to its active metabolite, 269 330 Biochemical evaluation indicates the hepatoprotective 269 330 trichloromethyl radical [23]. These activated radicals effects of *Camellia sinensis* may be due to its antioxi- 269 330 269 330 bind covalently to the macromolecules and induce per-330 dant property.
ACKNOWLEDGEMENT

Authors are thankful to Shri.V.Shanmugan, Chair-
man, Nandha College of pharmacy, Erode, India for
providing infrastructural facilities to carry out this pro-
ject.

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