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RESEARCH ARTICLE

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*

Camellia sinensis is a perennial tree belonging to the family Theaceae, commonly called as 'Green tea'. It is native from the south of China. It appears as a bush of about 2.5 m in the high areas of Asia and China with warm and humid climates. The leaves are thermogenic, appetizer, digestive, carminative, diuretic, and useful in cardiodynia, hemorrhoids, inflammation and abdominal disorders [1]. It has been previously reported that the leaves have used to treat the cancer of duodenum, lung, liver and mammary gland [2-5]. Camellia sinensis contain many biologically-active polyphenolic flavonol commonly known as catechine which make up 30 % of dry weight of its leaves [6]. Free radicals cause oxidation of nucleic acid proteins. Free radical also damage biomembranes, reflected by increased lipid peroxidation, thereby compromising cell integrity and function. During this process, the ability of the body's defense system to combat the oxidative stress may diminish due to reduced anti-oxidant. Antioxidants are compounds that protect cell against the damaging effect of reactive oxygen species such as singlet oxygen, superoxide, proxy radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidant and reactive oxygen species results in oxidative stress, leading to cellular damage [7]. Catechins are hypothesized to help protect against many disease by contributing along with anti-oxidant vitamin E and enzyme like superoxide dismutase, catalase to the total anti-oxidant defense system. Presence of number of constituents has been reported in *Camellia sinensis* catechin (flavanols) especially epigallocatechin gallate, epigallocatechin, epicatechin gallate and epicatechin which have been identified as active components responsible for antioxidant property [8].

Among the various mechanisms involved in hepatotoxic effect of carbon tetrachloride, one is oxidative damage through free radical generation and antioxidant

Table 1. Effect of *Camellia Sinensis* on serum ALT, AST, ALP, Total protein and Albumin in CCl_4- treated rats

Groups	Drug treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Protein g / dl	Albumin g / dl
I	Distilled Water (1ml/kg p.o.)	107.6 ± 9.5	59.5 ± 4.1	249.5 ± 18.2	8.5±0.6	6.2±0.5
II	CCl_4 (0.5ml/kg i.p.)	378.9 ± 23.7 [a]	254.9±19.3[a]	586.9 ± 31.6[a]	5.1±0.4[a]	2.4±0.3[a]
III	CCl_4 + *Camellia sinensis* (100mg/kg)	131.6 ± 10.6[c]	71.4 ±5.6[c]	267.7 ± 17.8[c]	6.9±0.8[c]	4.7±0.3[c]
IV	CCl_4 + *Camellia sinensis* (200mg/kg p.o.)	117.6 ± 6.7 [b]	66.2± 6.1[b]	258.4 ±16.2[b]	7.6±0.6[b]	5.4±0.5[b]
V	CCl_4 + Vitamin E (100mg/kg p.o.)	111.7 ± 8.7 [b]	62.3 ± 5.1 [b]	255.3 ± 24.1 [b]	7.8±0.4[b]	5.9±0.5[b]

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 II. (CCl_4: carbon tetrachloride, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase)

property is claimed to be one of the mechanisms of hepatoprotective effect of indigenous substance [9].

Camellia sinensis has antioxidant properties [10]. Hence, the objective of the study was to evaluate the hepatoprotective effect of *Camellia sinensis* on carbon-tetrachloride-induced hepatotoxicity.

MATERIALS AND METHODS

Drugs and chemicals

Carbon tetrachloride (CCl_4) was obtained from Merck Ltd., Mumbai, India; Thiobarbituric acid (TBA), 5, 51-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, Agricultural University, Coimbatore, India. The voucher specimen (A 2459) has been deposited in Herbarium.

Extract 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].

Animals

Male Swiss albino mice weighing between 20–25 gm and male wistar Albino rats weighing between 150–220 gm were used. The animals were obtained from animal house, IRT Perundurai Medical College, Erode, Tamilnadu, India. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30–70 %. A 12:12-h light:dark cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). 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).

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.

Experimental procedure

The experiment was carried out after obtaining clearance from Institutional Animal Ethics Committee. The animals were divided in to 5 groups of 6 animals each. Group-I, which served as normal control received distilled water (1ml/kg., p.o.), Group–II received equal mixture of CCl_4 and olive oil (50 % v/v, 0.5 ml/kg i.p.) once daily for 7 days [13]. Group–III received equal mixture of CCl_4 and olive oil along with *Camellia sinensis* (100 mg/Kg, p.o.) simultaneously once daily for 7 days. Group–IV received equal mixture of CCl_4

Table 2. Effect of *Camellia sinensis* on liver LPO, GSH, CAT and SOD in CCl_4-treated rats

Groups	Drug treatment	LPO nmol of MDA/mg protein	GSH nmol/mg tissue	CAT nmol of H_2O_2 decomposition/min./mg protein	SOD Units/g protein
I	Distilled Water (1ml/kg p.o.)	4.1 ±0.5	22.5 ±1.9	189.8 ± 11.3	84.6 ± 6.8
II	CCl_4 (0.5ml/kg i.p.)	14.8 ±1.3[a]	11.2 ±1.7[a]	46.2 ±5.6[a]	42.5 ±3.2[a]
III	CCl_4 + *Camellia sinensis* (100mg/kg)	8.4 ±0.7[c]	17.6 ±1.6[c]	152.4 ±13.7[c]	67.7 ±5.7[c]
IV	CCl_4 + *Camellia sinensis* (200mg/kg p.o.)	6.1 ±0.5[b]	18.3 ±1.9[b]	160.3 ±15.1[b]	75.3 ±6.9[b]
V	CCl_4 + Vitamin E (100mg/kg p.o.)	5.4 ±0.6[b]	20.9 ±2.2[b]	168.4 ±11.3[b]	77.1 ±5.4[b]

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 II.(CCl_4: carbon tetrachloride, LPO: lipid peroxide, GSH: glutathione, CAT: catalase and SOD: superoxide dismutase)

and olive oil along with *Camellia sinensis* (200 mg/Kg, p.o.) simultaneously once daily for 7 days. Group–V received equal mixture of CCl_4 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 serum was separated for various biochemical estimations. All animals were sacrificed by cervical decapitation and immediately, the livers were 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 (CAT) 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 was measured in the liver homogenate by the areported method [18,19].

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.

RESULTS

Acute Toxicity Studies

All the doses (5, 50, 300, 2000 mg/kg, p.o.) of *Camellia sinensis* employed for acute oral toxicity studies were found to be non-toxic. *Camellia sinensis* 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.

Biochemical estimations

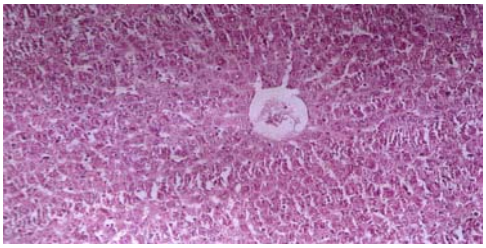
The results of hepatoprotective activity of *Camellia sinensis* on CCl_4-treated rats are shown in Tables 1 and 2.

Serum hepatic enzymes

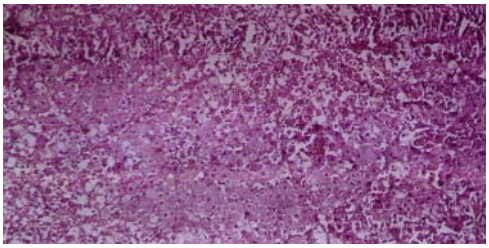
The hepatic enzymes ALT, AST and ALP in serum was significantly increased in CCl_4-treated animals when compared to control ($p<0.001$). The *Camellia sinensis* treatment (200 mg/kg) significantly ($p<0.01$) increased the levels of hepatic enzymes when compared to CCl_4-treated animals. The *Camellia sinensis* treatment (100 mg/kg) less significantly ($p < 0.05$) increased the levels of hepatic enzymes when compared to CCl_4-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 CCl_4-treated animals.

Serum proteins

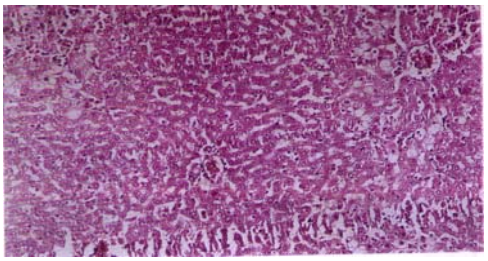
There was a significant ($p<0.001$) decrease in the serum total protein and albumin levels with CCl_4 treatment in group II when compared to control group I;



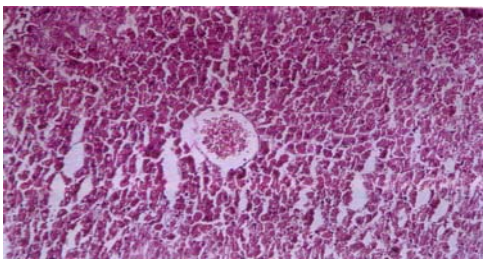
(A) Transverse section of the liver of control rats, showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus and central vein.



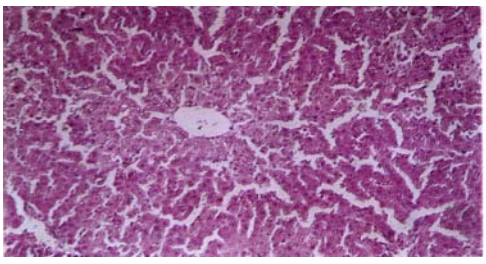
(B) Transverse section of the liver of CCl_4 treated animals showing hydropic changes in centrilobular hepatocytes with single cell necrosis surrounded by neutrophils, congestion of central vein and sinusoids were seen with acute inflammatory cells infiltrating sinusoids mainly in central zone.



(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

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* at 100 mg/kg showed less significant (p<0.05) decrease in LPO level when compared to CCl_4-treated groups. Vitamin E (100 mg/kg)- treated animals also showed significant (p<0.01) decrease in the 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* (200 mg/ kg) showed significant (p<0.01) rise in GSH, SOD and CAT

levels when compared to CCl_4-treated groups. Treatment with *Camellia sinensis* (100 mg/ kg) dose showed less significant (p<0.05) rise in GSH, SOD and CAT levels when compared to CCl_4-treated groups. Vitamin E (100 mg/kg)-treated animals also showed significant (p<0.01) rise in the levels of GSH, SOD and CAT when compared to CCl_4-treated animals.

Histopathological studies

The results of histopathological studies of *Camellia sinensis* on CCl_4-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 CCl_4-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* (100mg/kg) and CCl_4-treated rats, liver sections showed mild fatty change and mild sinusoidal congestion. In *Camellia sinensis* (200mg/kg) and CCl_4 treated rats, liver sections showed residual hepatocellular necrosis with cords of regenerating hepatocytes. In Vitamin E and CCl_4 treated rats, there was mild central venous congestion and mild fatty vacuolation.

DISCUSSION

CCl_4 is one of the most commonly used hepatotoxins in the experimental study of liver diseases. [20]. Assessment of liver function can be made by estimating the activities of serum and liver tissue enzymes originally present or absent in cytoplasm. During hepatic damage, there may be imbalance in these enzyme levels with the extent of liver damage. The altered levels of these enzymes in CCl_4-treated rats in the present study corresponded to the extensive liver damage induced by the toxin.

The serum ALT, AST and ALP are reliable markers of liver function. They were significantly increased in CCl_4-treated groups. On the other hand, in group III animals which were treated with *Camellia sinensis* (100 mg/kg, p.o.), the activity of ALT, AST and ALP had decreased significantly (p<0.05) and in group IV, animals which were treated with *Camellia sinensis* (200 mg/kg, p.o.), the activity of ALT, AST and ALP had decreased significantly (p<0.01). Simultaneous treatment of *Camellia sinensis* and CCl_4 caused significant recovery from the damage induced by CCl_4 treatment. The fall in serum enzymes suggests a protective effect of *Camellia sinensis* on the liver against CCl_4-induced toxicity. Previous reports shown that yellow tea [21] and green tea [22] protects the liver against CCl_4-induced hepatic damage in rats which support hepatoprotective activity of *Camellia sinensis*. The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloromethyl radical [23]. These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum which are rich in polyunsaturated fatty acids. This leads to formation of lipid peroxides, which 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 CCl_4 [24, 25].

In our study, elevation in the levels of end products of lipid peroxidation in liver of rats treated with CCl_4 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, which in turn helps in maintaining the liver tissue damage. This indicates the additional antioxidant property of *Camellia sinensis*.

Camellia sinensis enhanced the synthesis of total protein and albumin which accelerates the regeneration process and the protection of liver cells. The increased level of total protein in serum indicates the hepatoprotective activity of *Camellia sinensis*. In the present study, the SOD activity is significantly reduced in CCl_4-treated animals. The SOD activity was reversed close to normal after treatment with the *Camellia sinensis* extract in CCl_4-treated animals. Decreased activity of CAT was observed in animals treated with CCl_4. Presumably a decrease in CAT activity could be attributed to cross linking and inactivation of the enzyme protein in the lipid peroxides. Decreased CAT activity is linked to exhaustion of the enzyme as a result of oxidative stress caused by CCl_4. The CAT activity was restored to normal after treatment with *Camellia sinensis* extracts evidently; which shows the antioxidant property of the extracts against oxygen free radicals. All the effects on enzymes activities induced by *Camellia sinensis* were comparable with vitamin-E-treated groups.

Histopatholoical studies showed that CCl_4 caused centrilobular necrosis, congestion of central vein and sinusoids. *Camellia sinensis* administration exhibited protection against CCl_4-induced hepatotoxicity, which confirmed the results of biochemical studies. The results of our study indicate that administration of *Camellia sinensis* in CCl_4-treated rats protects liver damage. The biochemical evaluation indicates the hepatoprotective effects of *Camellia sinensis* may be due to its antioxidant property.

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