



Asian Journal of **Biochemistry**

ISSN 1815-9923



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Protective Effects of *Camellia sinensis* Leaf Extract against Carbon Tetrachloride-Induced Liver Injury in Rats

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ABSTRACT

The present study appraised the hepatoprotective effects of aqueous leaf extract of *Camellia sinensis* against carbon tetrachloride (CCl₄)-induced liver injury in rats. The 100 and 200 mg kg⁻¹ b.wt. of *Camellia sinensis* and 100 mg kg⁻¹ b.wt. Silymarin was administered orally to separate groups of rats for 7 days prior to CCl₄ administration. A significant decrease (p<0.05) was observed in both the groups treated with 100 and 200 mg kg⁻¹ b.wt. of the leaf extract and Silymarin on the levels of the enzymes and non-enzyme markers of tissue damage and lipid peroxidation, with no significant changes in the relative organ weight of the treated groups. This result shows that the leaf extract of *Camellia sinensis* contains some phytochemical(s) that can protect the liver against CCl₄-induced injury in rats.

Key words: *Camellia sinensis*, carbon tetrachloride, hepatotoxicity, liver, rats

INTRODUCTION

Camellia sinensis (Green tea) from the genus *Camellia* of flowering plant in the family Theaceae is a native to mainland of China, South and Southeast Asia but it is today cultivated across the world in tropical and subtropical regions. It is an evergreen shrub that is usually trimmed and the leaves and leaf buds are used to produce tea that is processed differently to attain a different level of oxidation (Mahmood *et al.*, 2010) and it contains certain minerals and vitamins which increase the antioxidant potentials of this type of tea (Cabrera *et al.*, 2003). *Camellia sinensis* extract is fast becoming ubiquitous supplement in consumer products such as shampoos, creams soaps, cosmetics, vitamins, drinks, lollipops and ice creams (Mukhtar and Ahmad, 2000).

In recent times more attention has been paid to the protective effects of natural antioxidants against drug-induced toxicities (such as CCl₄) especially whenever free radical generation is involved (Wakawa and Hauwa, 2013) and several plants and other animal products have been shown to protect the liver against such damages (Dahiru *et al.*, 2005, 2010; Obidah *et al.*, 2010; Hamad *et al.*, 2011; Khan and Alzohairy, 2011; Al-Fartosi *et al.*, 2012). Also medicinal plants are used in preparations of herbal remedies and these herbal remedies which are perceived to be a cheaper means of treatment have often attained popularity for historic and cultural purposes (WHO., 1999), thus a large number of the world's population depends on plants to treat many common ailments (Shri, 2003). The search for new and effective antioxidants in plants has given a new dimension to the antioxidant research (Guzdek and Nizankowska, 1996).

Fresh tea leaves are rich in flavanol monomers known as catechins, the major component of green tea extract which have various physiological effects and have been found to play important roles in the non-enzymatic protection against oxidative stress and body fat reduction (Nagao *et al.*, 2005). Several studies suggest that *Camellia sinensis* may contribute to a reduction in the risk of cardiovascular disease and some forms of cancer (Sakanaka, 1997; Kao *et al.*, 2000; Dulloo *et al.*, 2000; Levites *et al.*, 2003; Cabrera *et al.*, 2006) and traditional uses include treating flatulence, regulating body temperature and blood sugar, promoting digestion and improving mental processes (Choi *et al.*, 2004; Tsuneki *et al.*, 2004; Wu and Yu, 2006).

This study is undertaken to appraised the hepatoprotective effect of aqueous leaf extract of *Camellia sinensis* on liver injury, a highly specialized organ that regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions in humans, against carbon tetrachloride (CCl₄), a chemical model commonly used for animal studies to induce reactive oxygen formation and depletion of glutathione.

MATERIALS AND METHODS

Chemicals: Diagnostic kits for serum alanine aminotransferase (ALT) and aspartate amino transferase (AST), alkaline phosphatase (ALP) and bilirubin were purchased from Randox Laboratories Ltd. and other chemicals and solvents were of highest grade commercially available.

***Camellia sinensis* leaves:** Freshly harvested leaves of the *Camellia sinensis* were used for the preparation of the crude extract. It was collected from highland tea farm land in Sarduana Local Government area of Taraba State-Nigeria (in the month of June). It was authenticated in the plant science department of Modibbo Adama University of Technology Yola and then it was then dried under room temperature.

Preparation of aqueous extract of *Camellia sinensis*: The freshly dried leaves of *Camellia sinensis* were grounded into fine powdered form using laboratory mortar and pestle. Twenty gram of the powder was weight and mixed with 250-300 mL of distilled water and boil for 5-7 min. The extract was filter through Whatman filter paper (No. 4) and the filtrate obtain was concentrated using rotary evaporator at 40°C. It was then stored under frozen condition until use.

Animals: A total number of twenty five male albino rats weighing between 110-150 kg were purchased from the animal farm, National Veterinary Research Institute Vom, Jos Plateau state-Nigeria. They were all housed in standard cages at room temperature under 12/12 night/dark and were fed with pelleted standard laboratory feed (Vital Feeds, Grand cereals and oil mills Jos) and water *ad libitum*. They were allowed to stay for 7 days to acclimatize before the commencement of the work.

Experimental design and treatment: The rats were randomly divided into five groups of five rats per group and were given the extract as follows:

Group 1: Control

Group 2: Rats were given single dose of CCl₄+diet/water

Group 3: (Silymarin) rats were given 100 mg kg⁻¹ b.wt., Silymarin+CCl₄+diet/water

Group 4: (Treated) rats were given 100 mg kg⁻¹ b.wt., leaf extract+CCl₄+diet/water

Group 5: (Treated) rats were given 200 mg kg⁻¹ b.wt., leaf extract+CCl₄+diet/water

Groups 3 and 4 and 5 were pre-treated with Silymarin and the aqueous leaf extract of *Camellia sinensis* for 7 days, respectively prior to CCl₄ administration.

The CCl₄ was dissolved in olive oil and administered intraperitoneally (1:1) 2 mL kg⁻¹ b.wt., to induce liver injury.

Blood and tissue collection: All the rats from the various groups were sacrificed 48 h after the CCl₄ administration using standard laboratory procedures and then blood samples were collected via ocular vein into clean containers and allow to stand for 10 min. It was then centrifuged at 3000 rpm for 15 min to obtain serum. The serum was then separated for the estimation of liver marker enzymes (transaminases and alkaline phosphatase) and total bilirubin.

The liver of the rats were quickly excised, weighed and then used for the determination of lipid peroxidation. The hepatic lipid peroxidation was determined as Thiobarbituric Acid Reactive Substance (TBARS) and expressed as the amount of malonaldehyde (MDA) (Uchiyama and Mihara, 1978).

Statistical analysis: Results were expressed as Means±Standard Error of Mean (SEM). The significance of differences was calculated by using student t-test, p<0.05 was considered statistically significant.

RESULTS

The result of the effect of pretreatment against CCl₄-induced liver damage in rats with aqueous leaf extract of *Camellia sinensis* was examined on serum ALT, AST, ALP and TB levels as shown in Table 1.

The result in Table 1 showed a significant increase (p<0.05) in the serum levels of ALT, AST and ALP in group 2 (CCl₄ group) as compared to group 1 (control). However there was an observed significant decrease (p<0.05) in the 100 and 200 mg kg⁻¹ b.wt. extract treated groups prior to the CCl₄ administration as compared to group 2. The result observed in treated groups is not statistically different from the group treated with Silymarin and it was observed to be dose dependent.

The result also show a significant increase (p<0.05) in the level of bilirubin concentration in group 2 (CCl₄ group) as compare to group 1 rats. A pattern of statistical decrease was also observed in the pretreated groups in the level of the bilirubin concentration as compared to the Silymarin group and group 2.

The result in Table 2 shows the effect of pretreatment with the leaf extract on lipid peroxidation. It showed a significant increase in the MDA levels in group 2 as compared to group 1 (control) with a corresponding decrease in the pretreated groups (3 and 4) and the Silymarin treated group.

Table 1: Effects of pretreatment with aqueous leaf extract against CCl₄ induced liver damage on enzymes and non enzyme markers of liver damage

Groups	ALT (μ L ⁻¹)	AST (μ L ⁻¹)	ALP (μ L ⁻¹)	TB (mg dL ⁻¹)
1	70.40±1.12	49.5±4.1	249.5±18.2	3.20±0.40
2	84.50±1.25	60.6±1.2	486.9±31.6	7.52±2.19*
3	72.65±1.12	49.2±6.1	328.4±16.2	4.50±0.40
4	75.00±0.65	53.4±5.0	267.7±17.8	5.65±0.21
5	73.65±1.35	52.3±5.1*	255.3±24.1	5.41±0.42**

Results are Mean±SEM (n = 5), *Significantly higher than control group (p<0.05), **Significantly lower than group induced with CCl₄

Table 2: Effects of pretreatment with aqueous leaf extract against CCl₄ induced liver damage on liver lipid peroxidation

Groups	MDA (mmol mg ⁻¹)
1	6.1±0.5
2	12.8±1.3
3	7.1±0.5
4	8.4±0.7**
5	8.6±0.7

Results are Mean±SEM (n = 5), **Significantly lower than group induced with CCl₄

Table 3: Effects of pretreatment with aqueous leaf extract against CCl₄ induced liver damage on relative organ weight

Groups	Mean final body weight (g)	Relative organ weight (g/100 g b.wt.)
1	4.80±0.40	1.23±4.37
2	5.20±0.58	1.38±9.35
3	4.74±0.46	1.19±7.25
4	4.69±0.65	1.21±1.32
5	4.70±0.17	1.18±1.24

Results are Mean±SEM (n = 5)

The result in Table 3 showed a relative increase in relative organ weight and the mean final weight in the CCl₄ group as compared to group 1 and a relative decrease was observed in the relative organ weight of the pretreated groups as compared to the CCl₄ group.

DISCUSSION

Hepatotoxicity resulting from exposure to environmental chemicals is now a major global public health problem and a number of these toxicants have been documented. CCl₄ is one of the most commonly used hepatotoxins in the experimental study of liver diseases (Johnston and Kroening, 1998). The hepatotoxic effects of CCl₄ are largely due to its active metabolite, trichloromethyl radical (Srivastava *et al.*, 1990), 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 lipid peroxidative degradation of biomembrane is one of the principal causes of hepatotoxicity of CCl₄ (Cotran *et al.*, 1994). Hepatotoxin can injure the hepatocytes via free radicals that initiate and propagate tissue damage.

Several studies have provided a considerable support for evidencing the protective effects of camel milk on liver damage (Hamad *et al.*, 2011; Khan and Alzohairy, 2011; Al-Fartosi *et al.*, 2012). Also, these studies declared that the protective effect of camel milk against CCl₄-induced oxidative stress in the rat is due to its antioxidant properties. Camel milk was found to contain high concentrations of vitamins A, B₂, C and E and is very rich in magnesium and other trace elements, these vitamins act as antioxidants and have been found to be useful in preventing toxicant-induced tissue injury (Yousef, 2004). The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin. Camel milk decreased (p<0.05) CCl₄ induced elevated enzyme levels in tested groups, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells (Palanivel *et al.*, 2008).

The magnitude of hepatic damage is usually assessed by the measure of the levels of the cytosolic transaminases (alanine amino transferase (ALT) and aspartate aminotransferase (AST)), alkaline phosphatase, lipid peroxide and bilirubin which are reliable markers of liver function, in circulation (Perez Gutierrez and Solis, 2009) and from the result obtained in this study,

hepatotoxicity was observed in the rats treated with CCl₄ as shown by the increase in the levels of the serum marker of hepatic injury, this could be attributed to the damage caused by CCl₄ on the structural integrity of the liver (Dahiru *et al.*, 2005; Galati *et al.*, 2005; Perez Gutierrez and Solis, 2009; Dahiru *et al.*, 2010).

The result obtained in this study showed that pretreatment with the extract of *Camellia sinensis* caused a relative difference in the concentration of the marker enzymes and bilirubin concentration which is an index of jaundice (a condition indicating liver injury), possibly due to either increase production or decrease uptake by the liver, decrease conjugation and secretion from the liver or blockage of bile duct which might be as a result of CCl₄ administration, as compared to the CCl₄ treated group.

The difference in the result was also observed in the levels of malonaldehyde (MDA) in the pretreated group as compared to the CCl₄ group, this was shown to be elevated after CCl₄ administration, a toxicant that is known to increase lipid peroxidation. There was also an observed difference in the relative organ weight, a condition that normally reflect the pathological state of the liver.

The findings in this study revealed that the leaves of *Camellia sinensis* provided a protection the liver against hepatotoxicity due to CCl₄ administration, as demonstrated by the significant difference in the levels of the marker enzymes, the non enzyme marker, bilirubin as well as the decrease liver susceptibility to lipid peroxidation and invariably oxidative stress as seen in the marked levels of MDA in the pretreated group as compared to the CCl₄ induced liver damage group.

Thus the leaves of *Camellia sinensis* can be said to possess phytochemicals that have the capability of protecting the liver against CCl₄ induced hepatotoxicity as well as cellular degeneration and fatty liver development though the mechanism is not understood.

CONCLUSION

The result of this study showed that the leaves of *Camellia sinensis* could block or minimized adipogenesis to a greater extend thus having a potent hepatoprotective agent against CCl₄ induced liver damage in rats.

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