Protective effect of black and green tea against carbon tetrachloride–induced oxidative stress in rats

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ABSTRACT

Objectives: To assess the effects of green and black tea on carbon tetrachloride (CCl_4)-induced oxidative stress in rats.

Methods: This study was completed in the period of January to April 2005. Male wistar albino rats (6 weeks old) were randomly assigned to one of 3 diets (12 rats/group), control diet, black tea diet, or green tea diet. At the end of dietary treatment, a single dose of CCl_4 (2.5 ml/kg intraperitoneally) in paraffin (1:1 v/v) was given to 6 rats in each group after overnight fasting. The remaining 6 rats of each group received the same amount of paraffin only intraperitoneally. Thiobarbituric acid reactive substance were used as indicators of oxidative stress. The results were further confirmed by tissue histopathology.

Results: The present study demonstrates that there was a significant ($p<0.001$) increase in alanine aminotransferase (ALT) activity, TBARS, and in vitro hemolysis; and a significant ($p<0.05$) decrease in total plasma antioxidant status by CCl_4 injection. However, green and black tea supplemented groups treated with CCl_4 showed protective effects, as the in vitro hemolysis, ALT, TBARS levels were significantly lowered and total plasma antioxidant activity was significantly raised compared to the control group injected with CCl_4. The endogenous antioxidant component glutathione (GSH) was significantly ($p<0.001$) raised in groups fed with green/black tea prior to CCl_4 injection as compared to controls treated with CCl_4.

Conclusion: The results of this study suggest that both black and green tea possess preventive effects against CCl_4 induced damage in rats.

Saudi Med J 2006; Vol. 27 (12): 1804-1809

Tea, the dried leaves of plant *Camellia sinensis* is one of the most commonly consumed elements of the diet after water in most parts of the world. Green tea, which is prepared from the fresh tea leaves, is quite popular in China and Japan. Black tea, which is popular in many parts of the world including the Middle East and most of the Arab countries, is produced from the fermentation of green tea leaves. The process of fermentation turns the leaves to a deep red brown color and gives the tea its unique rich flavor. In addition to its effect on the color, fermentation of tea may affect the function of the different tea compounds in the body.[1] However, still there is no full agreement on the latter since it was reported that they were equally effective at human equivalent doses.[2] Polyphenolic compounds found in tea, fruits and vegetables[3] have shown to reduce the risks of coronary heart disease, stroke and cancer.[4-6] Tea is also known for its antipyretic, diuretic,[7] constant and astringent actions for generations. However, studies on the antioxidant effects of tea have started recently. Green tea can offer some protection against UV-induced DNA

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Received 21st May 2006. Accepted for publication in final form13th August 2006.

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damage in human cell cultures and also in human peripheral blood samples taken post-tea ingestion.[8] The health benefits of tea are generally attributed to the antioxidant properties of polyphenols due to their ability to scavenge reactive oxygen species[9] and nitrogen species.[10] Polyphenols, thus, reduce the ability of reactive oxygen and nitrogen species that damage lipid membranes, nucleic acids and proteins in cell free systems.[10] In addition, they are antimutagenic,[11] antihypercholesterolemic,[12] and antihypertensive.[13] It has been reported that the soluble polysaccharides and saponins of green tea have antihyperglycemic[14] and anti-inflammatory effects.[15] The in vivo and in vitro antioxidant effects of theaflavins and thearubigens (produced during the manufacturing of black tea) have not been studied extensively. However, recent studies on these molecules showed antioxidant effects on rat liver homogenates,[16] rabbit membrane ghost systems and microsomal systems.[17] Black tea extract has a better protective effect against different types of oxidative stress when compared with free catechins.[18] Many workers have assessed the antioxidant activity of tea in vitro[19-21] and ex vivo after ingestion of tea.[20,21] Tea ingestion has shown to improve the antioxidant status of serum.[20,21] In the present study, the in vivo antioxidant capacity of black and green tea with CCl_4-induced oxidative stress was assessed. Furthermore, the influence of tea on liver injury was examined.

Methods. This study was completed in the Department of Community Health Sciences at the College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia during the period of January to April 2005.

Thiobarbituric acid (TBA) and 5,5-dithio-bis-(2-nitrobenzoic acid) were purchased from BDH Chemical, Poole, UK and Sigma Chemicals Co. St. Louis, Mo, USA. Anhydrous sodium dihydrogen phosphate and disodium hydrogen phosphate were from Fluka, Switzerland. The total antioxidant status kit was from Randox Laboratories, UK. Corn oil, black and green tea were purchased from the local supermarket, all other ingredients of the diet were purchased from Sigma Chemicals Co. St. Louis, Mo, USA. All other chemicals used were of highest grade available.

Diets and feeding regimens. The basal diet (AIN-93)[22] consisted of 14.0% protein free casein, 62.07% corn starch, 10% sucrose, 5% fiber, 3.5% mineral mix, 1% vitamin mix, 0.18% DL-methionine, 0.25% choline barbiturate and 4% corn oil. Control diet consisted of the basal diet without any supplementation. Black tea diet consisted of the basal diet with 3% black tea (substituted for corn oil). Green tea diet consisted of basal diet with 3% green tea (substituted for corn oil). Male wistar albino rats (6 weeks old) were caged individually in a room maintained at 25±2°C with 12 hour light/dark cycle. Rats were randomly assigned to one of the 3 diets (12 rats/group). Animals had free access to food and water. Food consumption was recorded on alternate days and body weight gain was recorded weekly. At the end of dietary treatment, a single dose of CCl_4 (2.5 ml/kg i.p.) in paraffin (1:1 v/v) was given to 6 rats in each group after overnight fasting. The remaining 6 rats of each group received same amount of paraffin only intraperitoneally. The experimental procedure was approved by the Ethics Committee of the College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia.

Blood and tissue sampling. At the end of 60 days of the feeding regimens, rats were fasted overnight, anesthetized with light ether (BDH Chemical Pole, UK) and killed by cardiac puncture. Blood was withdrawn in heparinized vacutainer tubes (Becton Dickinson, Co; Rutherford, NJ). Plasma was separated from blood cells after centrifugation at 2000 rpm for 15 min (Model RT 6000 D&T 6000 D Sorvall table top refrigerated centrifuge, Du Pont Company, Wilmington, USA). Small portion of the blood was immediately processed for the determination of the rate of red blood cell hemolysis. Liver was excised immediately, washed in ice-cold saline, dried and weighed. A portion of the liver tissue was immediately processed for the determination of reduced glutathione.

Determination of the rate of red blood cell hemolysis. Hemolysis of red blood cell was determined using the method of Draper and Csallany[23] modified by Buckingham.[24] Red blood cells, separated from plasma after centrifugation, were washed thrice with 0.9% saline. The washed red blood cells were suspended at a concentration of 0.5% (v/v) in 0.01M sodium phosphate buffer pH 7.0 containing 0.15M sodium chloride (PBS), at 37°C for 20 hours and absorbance was recorded at 540 nm against PBS. Percent hemolysis was measured after completely hemolyzing the same concentration of red blood cells in distilled water.

Analysis of plasma enzymes. Kinetic measurement of plasma ALT, the marker enzyme of liver injury was carried out spectrophotometrically using commercially available diagnostic kit (BioMareuix, France).

Analysis of total antioxidant status. The total antioxidant status of plasma was determined immediately using Randox kit (Crumlin, UK). Results were expressed as millimole/L using 6 hydroxy-

2,5,7,8-tetramethylchroman-2-carboxylic acid as a standard.

Tissue lipid peroxide. TBARS, an index of lipid peroxidation, was considered as an indicator of the extent of the prevailing oxidative stress. Frozen liver was thawed, homogenized and analyzed for TBARS according to the method of Uchingham and Mishara.[25] 1,1,3,3-tetraethoxypropane (Sigma Chemical, St. Louis, Mo) was used as a standard and the results were expressed as nanomoles of malondialdehyde (MDA)/g tissue.

Assay of tissue glutathione (GSH). Tissue GSH was determined by the method of Moron et al,[26] using 5% perchloric acid ($HClO_4$) for deproteinization and 5/,5/ - dithiobis-2-nitrobenzoic acid for the development of color. The results were expressed as μmole GSH/g tissue.

Statistical analysis. Results were expressed as mean ± SD. The data were analyzed using One Way Analysis of Variance followed by Tukey-Kramer multiple comparison test. The differences were considered significant at $p<0.05$.

Results. ***General nutritional status and in vitro hemolysis.*** **Tables 1 and 2** present initial and final body weight, food intake, and liver weights of each group. Sixty days of feeding rats did not affect the major parameters examined (body weight gain, food intake and liver weight). There was a marked increase ($p<0.001$) in percent hemolysis of control group injected with CCl_4 as compared to all other groups (**Table 2**). Percent hemolysis was significantly ($p<0.01$) suppressed in rats fed with green/black tea and injected with CCl_4 as compared to rats on control diet injected with CCl_4.

Plasma ALT. Activity of plasma ALT was dramatically enhanced by CCl_4 injection (**Table 2**). The increase in the enzyme activity was significantly ($p<0.001$) suppressed in green and black tea fed rats injected with CCl_4 as compared to rats on the control group injected with CCl_4.

Plasma antioxidant status. The total antioxidant status of the control group treated with CCl_4 was decreased significantly ($p<0.05$) compared to all other groups (**Table 2**). Antioxidant status of black and green tea fed rats injected with CCl_4 was essentially the same as that of control, black and green tea fed groups.

Table 1 - Effect of feeding tea on body weight and food intake in rats.

Groups	Initial body weight (g)	Final body weight (g)	Food intake g/day/rat
Control	133.3 ± 7.5	293.8 ± 19.7	20.8 ± 5.2
Green Tea	129.7 ± 14.4	299.4 ± 17.0	28.3 ± 6.5
Black Tea	130.2 ± 13.1	274.4 ± 16.5	27.8 ± 6.8
P value	NS	NS	NS

Values represent means ± SD for 12 rats. NS - not significant

Table 3 - Effect of tea on liver malonyldialdehyde (MDA) (nmole MDA/g tissue) and reduced glutathione (GSH) (umole/g tissue) in rats.

Groups	MDA	GSH
Control	255 ± 22.6^{a}	5.5 ± 0.06^{a}
Black Tea	$220 \pm 24.5^{a,b}$	$6.1 \pm 0.35^{a,b}$
Green Tea	$208.3 \pm 31.8^{a,b,c}$	$6.3 \pm 0.47^{b,c}$
Control + CCl_4	491.8 ± 53.4^{d}	3.4 ± 0.34^{d}
Black Tea + CCl_4	$288.3 \pm 34.3^{a,e}$	4.6 ± 0.36^{e}
Green Tea + CCl_4	$270.8 \pm 21.5^{a,b,e,f}$	$4.9 \pm 0.33^{a,e,f}$

Values represent means ± SD for 6 rats. Values in a column with different superscript letters are significantly different ($p<0.05$)

Table 2 - Effect of feeding tea on liver weight, percent hemolysis, plasma alanine amino transferase (ALT) activity and plasma total antioxidant status in rats.

Groups	Liver weight (g)	Hemolysis %	ALT (U/L)	Total antioxidant (mmole/L)
Control	10.4 ± 2.1^{a}	2.2 ± 0.5^{a}	47.2 ± 10.9^{a}	0.5 ± 0.09^{a}
Black Tea	10.9 ± 2.4^{a}	$1.2 \pm 0.7^{a,b}$	$41.7 \pm 7.1^{a,b}$	$0.55 \pm 0.09^{a,b}$
Green Tea	11.2 ± 2.1^{a}	$1.5 \pm 0.7^{a,b,c}$	$38.0 \pm 5.9^{a,b,c}$	$0.55 \pm 0.09^{a,b,c}$
Control + CCl_4	10.1 ± 1.5^{a}	27.5 ± 2.1^{d}	173.0 ± 32.2^{d}	0.3 ± 0.07^{d}
Black Tea + CCl_4	12.9 ± 1.0^{a}	20.7 ± 1.7^{e}	$73.7 \pm 11.6^{a,e}$	$0.45 \pm 0.07^{a,b,c,e}$
Green Tea + CCl_4	11.3 ± 1.5^{a}	16.1 ± 2.7^{f}	$64.5 \pm 9.3^{a,b,f}$	$0.46 \pm 0.06^{a,b,c,e,f}$

Values represent means ± SD for 6 rats. Values in each column with different superscript letter are significantly different ($p<0.05$).

Tissue lipid peroxidation. The TBARS remained unaffected by feeding rats either with control diet or diet supplemented with green/black tea. The rats fed control diet injected with CCl_4 had the highest levels of TBARS (**Table 3**). There was a marked decrease ($p<0.001$) in the level of TBARS of rats fed with black/green tea and given CCl_4 injection as compared to the control group injected with CCl_4.

Tissue glutathione. Rats fed with black tea supplemented diet and injected with CCl_4 had significant ($p<0.001$) reduction in GSH levels as compared to control, green and black tea fed rats. Although, the green tea group injected with CCl_4 showed decrease ($p<0.001$) in GSH levels as compared to rats fed with green and black tea supplemented diets, no significant differences were observed when compared with the control group. However, both black and green tea groups injected with CCl_4 showed significant ($p<0.001$) elevation of GSH levels when compared to the control group injected with CCl_4 (**Table 3**).

Tissue histopathology. The liver of rats fed control, black and green tea supplemented diet remained essentially normal (**Figure 1**). However, the control group treated with CCl_4 showed marked histopathological changes in the liver (**Figures 2a & 2b**). Liver specimens of the control group injected with CCl_4 showed mononuclear cellular infiltration (**Figure 2b**) as evidenced by the inflamed portal area and was commonly seen in intralobular among hepatic cells. Also, the hepatocytes exhibited vacular cytoplasm and precipitation. However, no pathological changes were seen in lobular architecture or hepatocytic nuclei. The black and green tea supplemented groups injected with CCl_4 showed moderate histopathological changes (**Figure 3**).

Discussion. The major constituents of tea (such as flavonols, flavanols and phenols) have been shown to have anticarcinogenic, antimutagenic and antioxidative activity.[27,28] However, the mechanism by which these effects occur is still unclear. Catechins and flavanols are the predominant polyphenols found in green tea,[29] while in the black tea catechins are converted to theaflavins. Tea flavonoids consistently reduce oxidative damage in animals exposed to radiations, chemical oxidants or dietary stress.[9] Rats fed with green tea infusion or tea polyphenols for 2 weeks before exposure to 2-nitropropane had significant low levels of 8-OHdG adducts and lipid peroxide.[30] Tea polyphenols also have potent inhibitory effects on LDL oxidation in vitro[31] and ex vivo.[32] Tea polyphenols prevent the development of arthrosclerosis without changing the plasma lipid

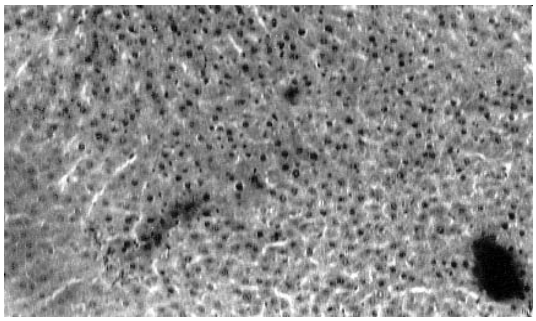

Figure 1 - Histology of the liver from an untreated control rats, showing more or less normal hepatic structure.

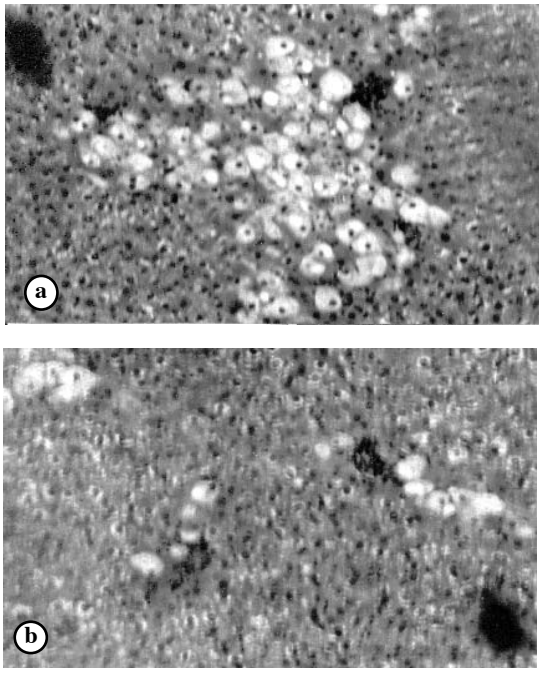

Figure 2 - Histology of the liver from rats fed control diet injected with CCl4 **a)** showing vacuolation of cytoplasm (may be fatty or hydropic degeneration) and **b)** showing mononuclear cellular infiltrations.

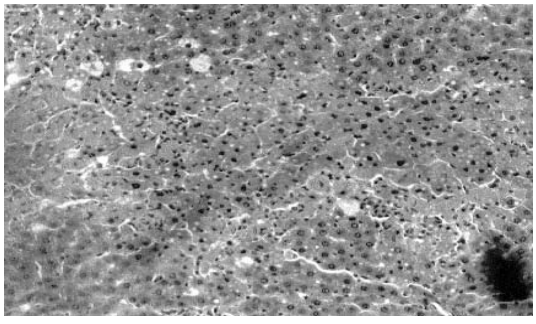

Figure 3 - Histology of the liver from rats fed green and black tea prior to CCl4 injection showing moderate fatty changes.

level probably through the potent antioxidative activity.[33] The in vivo experiments presented in this study demonstrated that the rate of percent hemolysis was significantly lower in black and green tea group in which CCl_4 is used as inducer, indicating the potential free radical scavenging effect of green and black tea. The biochemical mechanisms involved in the hepatotoxic effect of CCl_4 are well documented. It is now well known that the formation of reactive trichloromethyl radicals from the CCl_4 metabolism is a crucial factor in the pathogenesis of CCl_4 induced hepatotoxicity.[34] It seems that tea antioxidants are playing a role in detoxifying trichloromethyl radicals and it may also be suggested that the tea antioxidants were acting as in vivo antioxidants. It was also observed that black and green tea suppress the CCl_4 induced enhancements of plasma ALT activity in rats (**Table 2**). The extent of increase in plasma ALT and AST activities are generally considered to be parallel to the severity of liver damage. The results showed that the black and green tea antioxidants have the potential to suppress the CCl_4 induced hepatotoxicity. The results of this study were in agreement with the findings of Sur-Altiner and Yenice.[35] Sugiyama et al.[36] also observed significant suppression of D-galactose amine induced enhancement of plasma enzyme activities by different beverages including green tea in rats. Ingestion of tea can contribute substantially to the intake of antioxidants including polyphenols.[37] Sano et al,[38] found that the total catechins content of dry green tea leaves were 14.5% and black tea leaves were 1.5% (w/w). They have also reported that the levels of vitamins C and E (antioxidant vitamins) are lower in black tea as compared to green tea. Feeding black tea in the present study resulted in excellent antioxidant effects against liver lipid peroxidation and plasma total antioxidant status. This was similar to that observed after feeding green tea as shown in **Tables 2 and 3**. The results are in agreement with the findings of Sano et al,[38] and Serafini et al,[21] who observed that ingestion of tea increased the total antioxidant capacity of serum. However, the significant increase in total plasma antioxidant status was not seen in the present study. Glutathione is a tripeptide (γ-glu-cys-gly), which is an important non enzymatic antioxidant.[39] The thiol group of reduced GSH helps in many detoxification reactions to protect against free radical injury. Glutathione depletion results in the enhancement of oxidative tissue damage. El-Missiry and El-Gindy[40] reported that the oxidative stress caused a decrease in GSH content of livers of alloxan–diabetic rats. They have also observed that supplementation of Eureca sativa seed oil, known to possess antioxidant potential, caused elevation in liver GSH. It has been observed in the current study that the rats fed with black/green tea for 6 weeks showed resistance to CCl_4 induced injury. This became evident as the GSH content of the liver were restored in these groups. Histopathological examination of liver revealed high incidence of damage due to oxidative stress caused by CCl_4 (Control + CCl_4). However the damage caused by CCl_4 was moderate in rats given black/green tea prior to CCl_4 injection.

It could be concluded that the pretreatment of rats with green/black tea before exposure to CCl_4 reduces the oxidative stress associated with CCl_4.

Acknowledgment. The author would like to thank Dr. Fasih Ahemad, Dr. Zarina Arif, Dr. Sameh E. El-shwemi, and Mr. Tariq Hameed for their help throughout this research.

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