EFFECT OF BLACK TEA ON LIPID PEROXIDATION IN CARBON TETRACHLORIDE TREATED MALE RATS

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SUMMARY

This study examined the effects of black tea (*Camellia sinensis* L.) on lipid peroxidation and glutathione levels in carbon tetrachloride (CCl_4)-treated male Wistar rats. Three groups of rats formed two control groups and one treatment group. The control groups were fed with a standard diet, while the black tea group were fed the standard diet plus 6% by weight dried black tea leaves. After two months, the rats in the black tea group and in one control group were administered a single dose of CCl_4 (1 ml/kg, i.p.) and sacrificed two hours later. Rats in the other control group were administered olive oil in a similar fashion. Lipid peroxide levels in liver and plasma, glutathione (GSH) levels in liver and alanine transaminase (ALT) and aspartate transaminase (AST) activities in plasma were measured. Rats in the black tea group were found to have significantly decreased liver lipid peroxide levels, and ALT and AST activities compared with the rats in the CCl_4-treated control group. In addition, liver glutathione levels were decreased in the black tea group. These data suggest that black tea attenuates CCl_4-induced hepatic injury.

KEY WORDS

black tea, lipid peroxidation, glutathione, ALT, AST

INTRODUCTION

Camellia sinensis L. (Theaceae) is an evergreen shrub found in about 30 countries and its leaves are used for one of the most widely consumed beverages in the world. Tea has been used for generations as an antipyretic, diuretic /1/, constipant and astringent /2/, and studies on its antioxidative effects have recently begun. Little information about the antioxidative effects of black tea is available. Black tea, which is consumed in greater quantities than green tea throughout the world, is produced from the fermentation of green tea and theaflavins and thearubigin are produced during this process. Studies with rat liver homogenates /3/, rabbit erythrocyte membrane ghost systems and microsomal systems /4/ have demonstrated the antioxidant effects of these molecules *in vitro*. A study carried out with human red blood cells *in vitro* has reported that black tea extract in comparison to free catechins seemed to be a better protective agent against various types of oxidative stress /5/. Therefore it is of interest to examine black tea extracts for their antioxidant effects *in vivo*. Tea is generally consumed as a hot-water infusion containing water soluble anti-oxidants. Besides those components, tea leaves also contain several lipid soluble chemicals such as beta-carotene and tocopherols /6/. A study carried out with dietary tea, in order to evaluate its total antioxidant abilities, demonstrated that black tea had antioxidant effects on tissue lipid peroxidation *ex vivo* /7/. Studies with lipid peroxidation inducers, such as tert-butyl hydroperoxide and bromo-trichloromethane /3,4,7/, $FeCl_3$ and ascorbate /8/ have demonstrated the antioxidative effect of black tea. The antioxidant content of tea differs widely among cultivation areas. We used Turkish tea leaf powder in the diet of the rats in our *in vivo* study carried out with CCl_4 as lipid peroxidation inducer.

MATERIALS AND METHODS

Thiobarbituric acid (TBA) was purchased from Merck. 1,1,3,3-Tetraethoxy-propane (TEP) was purchased from Fluka Chemika. Glutathione was purchased from Sigma. All other chemicals were of analytical grade.

Commercial black tea leaves (Çayaçelya), collected from Rize, Turkey, were used in this study. Male adult Wistar rats (maintained at

the Experimental Research and Animal Laboratory Unit, Faculty of Medicine, Marmara University, Istanbul) weighing 225-250 g were used in this study. They were housed under controlled light conditions (12 hours of dark/light cycle).

Three groups were studied: Control I, Control II and Black Tea, each group containing 12 animals. Control I and Control II groups were fed with the standard diet and the Black Tea group was fed with the standard diet plus 6% black tea for 2 months. The animals were fasted for 18 hours before the experiments. The rats in the Control II and Black Tea groups were treated with a single dose of CCl_4 (1 ml/kg as 20% in olive oil) intraperitoneally and the rats in the Control I group were treated with the same amount of olive oil by the same route. The animals were sacrificed by decapitation 2 hours after treatment. The livers were rapidly removed, and washed in 0.9% NaCl. Liver portions were homogenized in ice-cold 0.15 M KCl (10%, w/v) /9/. Plasma ALT and AST activities were measured by auto-analyzer. Lipid peroxide levels in plasma were measured by the TBA test /10/. Plasma (0.3 ml) was added to 2.4 ml $^1/_{12}$ N H_2SO_4 and 0.3 ml 10% phosphotungstic acid. After centrifugation at 3000 rpm, the upper layer was discarded. Distilled water (4.0 ml) and 0.335% TBA were added to the pellets. They were then heated in a water bath at 95°C for 60 min. After cooling, 1.0 ml of distilled water and 3.0 ml of a mixture of n-butanol and pyridine (15:1, v/v) were added. After centrifugation at 3000 rpm for 10 min, the upper layer was removed and its absorbance was measured at 532 nm. Calculations were made using the extinction coefficient ($1.56x10^5$ $M^{-1}.cm^{-1}$). Lipid peroxide levels in liver were measured by the TBA test /11/. 0.2 ml of 10% (w/v) tissue homogenate was added to 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with NaOH, and 1.5 ml of 0.8% aqueous solution of TBA. Distilled water was used to produce 4.0 ml of mixture, which was then heated in a water bath at 95°C for 60 min. After cooling, 1.0 ml of distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1, v/v) were added. After centrifugation at 4000 rpm for 10 min, the upper layer was removed and its absorbance was measured at 532 nm. TEP was used as an external standard. Liver glutathione levels were measured by the method of Ellman /12/. 0.5 ml of 10% (w/v) tissue homogenate was added to 1.5 ml of 0.15 M KCl and 3.0 ml of the mixture of non-proteinization solution. After centrifugation, 0.5 ml of

upper layer was taken and 2 ml of 0.3 M Na_2HPO_4 and 0.5 ml of Ellman reagent were added. Its absorbance at 412 nm was measured. GSH was used as an external standard.

Statistical analysis of plasma ALT and AST activities and lipid peroxide levels was carried out by one way ANOVA and Duncan's test. Statistical analysis of liver lipid peroxide and glutathione levels was carried out by the Kruskal Wallis one way ANOVA and Mann Whitney U test.

RESULTS AND DISCUSSION

The levels of liver lipid peroxide in the Control II group were significantly higher than in the Control I group ($p<0.001$). In the Black Tea group, the liver lipid peroxide levels were significantly decreased compared to the Control II group ($p<0.01$), and not significantly different from those in the Control I group (Table 1). Thus, black tea significantly decreased liver lipid peroxide levels elevated by CCl_4 (Table 1), just as in the study carried out with tert-butyl hydroperoxide and bromotrichloromethane /7/ and brought them close to normal levels. Black tea also showed a lowering effect on plasma ALT and AST activities (Table 1). The ALT and AST activities in the Control II group were significantly higher than in the Control I group ($p<0.001$). In the Black Tea group, the ALT and AST activities were significantly decreased compared to the Control II group ($p<0.001$), but were still higher than in the Control I group ($p<0.001$) (Table 1). In the light of these findings, we suggest that black tea lessens hepatic injury.

The levels of plasma lipid peroxide in the Control II group were not significantly higher than in the Control I group (Table 1). Although black tea decreased liver lipid peroxide levels, it did not decrease lipid peroxide levels in plasma under our experimental conditions (Table 1). According to a previous study on theaflavins in black tea /4/, it was observed that they showed strong antioxidant activities in the erythrocyte ghost system, but they showed weaker antioxidative activity in the microsomal system.

The levels of glutathione in the Control II group were significantly higher than in the Control I group ($p<0.01$). In the Black Tea group, the liver glutathione levels were significantly decreased compared to the Control II group ($p<0.001$), and not significantly different from the Control I group (Table 1). Thus, black tea decreased the liver

TABLE 1

Effect of black tea on ALT, AST activities, lipid peroxide and glutathione levels in male rats treated with CCl_4

	Control I (olive oil)	**Control II** (CCl_4 in olive oil)	**Black Tea** (CCl_4 in olive oil)
Plasma ALT (IU/l)	24.50±4.94	47.33±12.56*	39.58±8.59*[a]
Plasma AST (IU/l)	101.17±19.12	169.08±24.64*	143.83+33.10*[a]
Plasma lipid peroxide (nmol MDA/ml plasma)	4.13+0.96	4.72+1.20	4.00+0.72
Liver lipid peroxide (nmol MDA/g wet wt)	242.67±41.09	470.03+82.85*	353.25+180.17[b]
Liver glutathione (μmol GSH/ g wet wt)	3.53±1.98	6.24±5.56**	2.62±0.78[a]

Means ± SD. n=12.
*p<0.001, **p<0.01, ***p<0.05 in comparison to Control I group.
[a] p<0.001, [b] p<0.01, [c] p<0.05 in comparison to Control II group.
CCl_4 and olive oil: 1 ml/kg, intraperitoneally.
MDA: Malondialdehyde.

glutathione levels elevated by CCl_4 treatment, bringing them close to normal levels. We speculate that black tea decreased the lipid peroxide levels in the liver, and in response to this decrease, the glutathione levels remained low. In a study with alpha-tocopherol as an anti-oxidant against CCl_4 /13/, a decrease in -SH groups and normalization of glutathione reductase were reported.

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