EFFECT OF BLACK TEA ON LIPID PEROXIDE AND GLUTATHIONE LEVELS IN FEMALE RATS

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SUMMARY

The effects of black tea (*Camellia sinensis* L.) on lipid peroxidation and glutathione (GSH) levels in carbon tetrachloride (CCl_4)-treated female Wistar rats were examined. Two control groups and one treatment group were tested. The control groups were fed with a standard diet, while the black tea group was fed the standard diet plus 6% by weight dried black tea leaves. At the end of 2 months, a single dose of CCl_4 (1 ml/kg, i.p.) in olive oil was administered to rats in one of the control groups and the black tea group. They were sacrificed after 2 hours. Rats in the other control group were administered olive oil in a similar fashion. Measurements were made of lipid peroxide levels in liver and plasma, glutathione levels in liver, and alanine transaminase (ALT) and aspartate transaminase (AST) activities in plasma. Liver lipid peroxide levels, plasma ALT and AST activities were significantly decreased in the black tea group compared with the CCl_4-treated control group, while plasma lipid peroxide levels were not. These results are parallel to those previously found with Wistar male rats. Glutathione levels, however, were not significantly affected, in contrast to the data relating to male rats, either after CCl_4 or black tea treatments. The results of our study add to the findings that black tea attenuates CCl_4-induced hepatic injury but also indicates the susceptibility of glutathione levels to endocrinological effects.

KEY WORDS

black tea, lipid peroxidation, glutathione, female rats, carbon tetrachloride

INTRODUCTION

The incidence of heart disease and several types of cancer are low in populations consuming tea obtained from *Camellia sinensis* on a regular basis /1-3/. The protective effects of tea against heart disease and carcinogenesis may be related to its antioxidant properties /4,5/. Much more information is needed about the antioxidant effects of black tea, which is consumed in greater quantities than green tea throughout the world. The terms green tea and black tea both refer to products obtained from the leaves of *Camellia sinensis*, but they are chemically very different. During the black tea production process, polyphenols in green tea undergo fermentation which results in the conversion of catechins into theaflavins and thearubigins /6/. *In vitro* studies have demonstrated the antioxidant effects of these molecules /7,8/. It was reported in one *in vitro* study that black tea extract in comparison to free catechins seem to be a better protective agent against oxidative stress /9/. A study carried out with dietary tea, in order to evaluate its total antioxidant abilities, demonstrated that black tea has antioxidant effects on tissue lipid peroxidation *ex vivo* /10/. There have been only a few *in vivo* studies with black tea.

In our previous *in vivo* study carried out with male rats, we demonstrated that black tea decreases liver lipid peroxide and glutathione levels elevated by CCl_4 and lessens hepatic injury /11/. We reported that normal liver glutathione levels of female rats are significantly lower than those of males /12/. Absorption and metabolism of black tea still remain obscure. It is evident that sex hormones are involved in the process of the development of chronic liver damage /13/. In a study in which the content of secondary lipid peroxidation products, triglycerides and alpha-tocopherol were determined in the liver, plasma and erythrocytes, it was suggested that the parameters studied may be susceptible to endocrinological effects /14/. In order to observe the effect of black tea on lipid peroxide and glutathione levels in female rats, we used Turkish tea leaf powder in the diet of the animals in an *in vivo* study, with CCI_4 as lipid peroxidation inducer.

MATERIALS AND METHODS

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

Commercial black tea leaves (Çayaçelya), collected from Rize, Turkey, were used in this study. Female adult Wistar rats (maintained at the Experimental Research and Animal Laboratory Unit, Faculty of Medicine, Marmara University, Istanbul) weighing 200-220 g were used. They were maintained on a 12-hour light-dark cycle for 2 months.

The rats were assigned to three groups at the beginning of the 2 month period, Control I, Control II and Black Tea, each group containing 12 animals. The Control I and Control II 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. At the end of the dietary regime the animals were fasted for 18 hours. A single dose of CCl_4 (1 ml/kg as 20% in olive oil) was administered by intraperitoneal injection to the rats in Control II and Black Tea groups. The rats in Control I group received 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) /15/. Plasma ALT and AST activities were measured by auto-analyzer. Lipid peroxide levels in plasma were measured by the TBA test according to the method of Yagi /16/. 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 (1.56×10^5 $M^{-1}.cm^{-1}$). Lipid peroxide levels in liver were measured by the TBA test /17/. 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 /18/. 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 was measured at 412 nm. GSH was used as an external standard.

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

This study was approved by the Ethics Committee for Animal Experimentation of Marmara University.

RESULTS AND DISCUSSION

The levels of liver lipid peroxide in the Control II group were significantly higher than in the Control I group ($p<0.001$) (Table 1). 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 Control I group (Table 1). Thus, black tea significantly decreased the liver lipid peroxide levels elevated by CCl_4, just as in our previous study carried out with male Wistar rats /11/ and as in other studies with male Wistar rats and tert-butyl hydroperoxide as inducer /7,10/, and brought lipid peroxide concentrations 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 ($p<0.001$ and $p<0.05$). In the Black Tea group, the ALT and AST activities were significantly decreased compared to the Control II group ($p<0.001$ and $p<0.05$), but not significantly different from the Control I group (Table 1). These results add to the findings that black tea attenuates CCl_4-induced hepatic injury /11/.

TABLE 1

Effect of black tea on ALT, AST, lipid peroxide and glutathione levels in female 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)	20.08±5.43	45.75+18.91*	28.33±6.23[a]
Plasma AST (IU/l)	123.00±19.80	175.58±47.13***	138.92±49.64[c]
Plasma lipid peroxide (nmol MDA/ml plasma)	3.52±0.95	4.00±1.16	3.33±0.81
Liver lipid peroxide (nmol MDA/g wet wt)	317.35±179.79	508.63±78.55*	373.34±237.97[b]
Liver glutathione (μmol GSH/g wet wt)	1.48+1.39	2.10±1.12	1.63±0.91

Mean±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 in olive oil: 1 ml/kg, i.p.
MDA = malondialdehyde.

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). Both according to our previous study /11/ and to other workers /15/, the increase in CCl_4-induced lipid peroxidation was not reflected in plasma concentrations under these experimental conditions.

There was no significant difference caused by CCl_4 in glutathione levels between Control I and Control II groups under our experimental conditions, nor was there any significant difference caused by black tea between the Control II and Black Tea groups (Table 1). Paranich and Chernikova /14/ reported that vitamin E content in liver and in some other tissues of female rats was different from that in males. In our previous study with male rats /11/, we determined that black tea decreases liver glutathione levels elevated by CCl_4 treatment, in contrast to our present study in female rats performed under the same experimental conditions. In another study with male and female rats

/12/, we reported that normal glutathione levels in female rats were significantly lower than in males. Bien and Witt, who examined the effect of different hepatotoxic substances on female rats /19/, reported that after CCl_4 treatment, a significant difference in glutathione levels was not observed, in accord with our present results. In the light of these findings, we hypothesize that glutathione levels are susceptible to endocrinological effects.

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