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THE ANTIOXIDANT ACTIVITY OF COCOA

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ABSTRACT

In this study we aimed to determine the antioxidant effects of Cocoa (*Theobroma cacao* L.), which is commonly used in both chocolate and cocoa drinks all over the world , on lipid peroxidation, alanine transaminase (ALT), aspartate transaminase (AST), glutathione (GSH) and protein oxidation levels in carbon tetrachloride (CCl_4) treated for male Wistar rats. Two control groups and one treatment group of rats were formed. The control groups were nourished with a standard diet, while the Cocoa group was nourished with standard diet which was enriched with % 6 by weight dried Cocoa powder. After three months, a single dose of carbon tetrachloride (CCl_4) was performed in Control II (CCl_4) and Cocoa groups (1ml/kg, as 20% in olive oil) intraperitoneally and single dose of olive oil was administered (1ml/kg,i.p.) in the same way as rats in Control I group. They were sacrificed two hours later. Lipid peroxide levels in liver, protein oxidation in liver, glutathione levels in liver, ALT and AST in plasma were measured. Cocoa decreased liver lipid peroxide, liver glutathione levels and plasma ALT and AST activities previously increased by CCl_4 treatment, to the Control I levels The protein oxidation levels in the rats in the Cocoa group compared with the rats in CCl_4 treated control group were found to have significantly lessened. These findings suggest that cocoa has strong antioxidant activity because of the fact that cocoa inhibits liver injury.

KEYWORDS: Cocoa, protein oxidation, ALT, AST, lipid peroxidation, glutathione

INTRODUCTION

Cocoa is the seed of cocoa tree, *Theobroma cacao*. The seeds undergo fermantation involving bacterial oxidative processes. The resulting product is roasted and then ground into powder, which has been used world wide as a major ingredient of chocolate and cocoa drinks. For the past few years, the antioxidant and healthpromoting properties of cocoa and cocoa-related products have been investigated. Cocoa seeds are rich in polyphenols, such as (−) epicatechin (EC), (+)catechin, quercetin, clovamide, deoxyclovamide and procyanidin. These compounds are known to possess antioxidant, anticarcinogenic effects (1–6). There are studies about antioxidant effect of Cocoa polyphenols. Most of these were exctractted from cocoa powder and cocoa beans (7–10). However, it remains unclear whether this protective effect is attributable to the phenols or to other agents in the diet. On the other hand several studies have been reported the antioxidant effect of cocoa on lipid peroxide, ALT and AST activities (11–12), but nothing is known the antioxidant effect of cocoa on protein oxidation and glutathione levels. The aim of this study was to determine the antioxidant effects of cocoa on lipid peroxide, ALT and AST activities, protein oxidation and glutathione levels.

MATERIALS AND METHODS

All chemicals were purchased from Merck, Fluka Chemika and Sigma. Cocoa powder was purchased from Nestle. Male adult Wistar rats aged 24–25 weeks, weighing 250–300g. were used in this study. They were accommodated

at Experimental Research and Animal Laboratory Unit, Faculty of Medicine, University of Marmara, Istanbul. Wistar rats were controlled under light conditions (12 hours of dark / light cycle). Three groups of rats were used: Control I, Control II and Cocoa group. Eight rats per group were used. Control I and Control II groups were nourished with the standard diet and Cocoa group was nourished with the standard diet enriched with 6 % Cocoa powder for three months. Rats were fasted 18 hours prior to experiments. A single dose of carbon tetrachloride (CCl_4) was performed in Control II and Cacao groups (1ml/kg, as 20% in olive oil) intraperitoneally and single dose of olive oil was administered (1ml/kg, i.p.) in the same way as the rats in Control I group. Two hours later, the rats were killed humanely in accordance with sanctions approved by the Institutional Animal Care and Use Committee (IACUC) appropriate to the species. Livers of rats were quickly removed and washed in 0.9 % NaCl. Liver parts were homogenized in ice-cold 0.15M KCl (10% w/v) (13). Plasma AST and ALT activities were measured by auto-analyzer. Lipid peroxide levels in liver were measured by the thiobarbituric acid (TBA) test (14). 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. Subsequently, the mixture was heated in water bath at 95°C for 60 minutes. Once being cooled, 1.0 ml of distilled water and 5.0 ml of the mixture of n-butanol pyridine (15:1, v/v) were added. Following the centrifugation at 4000 rpm for 10 min, the upper phase was taken and its absorbance was measured at 532 nm. 1,1,3,3-tetraethoxy-propane (TEP) was used as an external standard. Liver glutathione levels were measured by the method of Ellman (15). 0.5 ml of 10% (w/v) tissue homogenate was added to 1.5 ml of M KCl and 3.0 ml of the mixture of non-proteinization solution. After centrifugation, 0.5 ml of upper phase was taken and 2ml 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. Protein oxidation levels in liver were determined by Levine et al (16) and Lowry (17) methods. Statistical analysis of plasma ALT and AST activities were performed by one way ANOVA and Duncan's test. Statistical analysis of liver lipid peroxide glutathione levels and protein oxidation levels were carried out by the Kruskal Wallis one way ANOVA and Mann Whitney U test.

RESULT AND DISCUSSION

When Cocoa group was compared with the Control II group, the ALT and AST activities were decreased in the Cocoa group. What's more, cocoa notably decreased the ALT and AST levels to that of Control I levels ($p< 0.005$), (Table 1). On the other hand, Sugiyama et al. demonstrated that cocoa could significantly suppress *D*-galactosamine-induced enhancement of plasma ALT and AST activities in rats. These results suggest that cocoa possess preventive effects on hepatic injury, Sugiyama et al. (12).

In the Cocoa group, the liver lipid peroxide levels were lowered compared to the Control II group ($p< 0.005$). Furthermore, Cocoa decreased liver lipid peroxide levels to the Control I levels (Table 1). As a matter of fact, E. Lecumberri et al. (11) recently demonstrated that Cocoa fiber inhibited lipid peroxidation.

In the Cocoa group, the liver glutathione levels were notably decreased compared to the control II group ($p< 0.005$), (Table 1). Cocoa decreased the liver glutathione levels previously increased by CCl_4 treatment, to the Control I levels. For this reason, it is suggested that cocoa lessened the lipid peroxide levels in the liver, and because of this lowering effect the glutathione levels remained low as well. On the other hand, in the study which was made E. Lecumberri et al., has been shown that cocoa fibers to the cholesterol-free or cholesterol-rich diets did not result in differences statistically significant in terms of glutathione levels when compared with the corresponding control groups (11). When Cocoa group was compared with the Control II group, protein oxidation levels were decreased in the Cocoa group ($p<0.005$). These results

Table 1. Effect of Cocoa on ALT, AST activities, lipid peroxide, protein oxidation and glutathione levels in male Wistar rats treated with CCl_4

Parameters	Control I (n=8)	Control II (n=8)	Cocoa (n=8)
Plasma ALT (U/L)	23.1 ± 5.9	44.7 ± 4.6*	28.1 ± 3.1[a]
Plasma AST (U/L)	124.8 ± 9.6	162.2 ± 8.4*	127.1 ± 9.6[a]
Liver lipid peroxide (nmol MDA/ g wet wt)	121.8 ± 16.2	384.3 ± 70.1*	114.9 ± 29.9[a]
Liver protein carbonyl content (nmol carbonyl/ mg protein)	4.8 ± 0.6	11.0 ± 2.6*	7.9 ± 1.7*[a]
Liver glutathione (µmol GSH/ g wet wt)	5.1 ± 1.0	6.5 ± 1.0*	5.2 ± 0.8[a]

Means ± SD, n=8.
*p<0.05, in comparison to Control I group
ap<0,05, in comparison to Control II group

showed that cocoa powder has suppressive effect on liver protein oxidation levels (Table 1). According to these results, it is suggested that cocoa has an antioxidant effect on CCl_4 induced oxidaton.

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