Short Communication

Green Tea Suppresses D-Galactosamine-Induced Liver Injury in Rats

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Dietary supplementation with powder of a green tea extract suppressed the enhancement of plasma alanine aminotransferase and aspartate aminotransferase activities induced by D-galactosamine, but not by carbon tetrachloride, in a dose-dependent manner in rats. The minimum dose to cause a significant effect was 1 to 2%. Drinking green tea also suppressed plasma enzyme activities. These results indicate that green tea had a liver injury-preventive effect.

Key words: green tea; liver injury; D-galactosamine; carbon tetrachloride; rat

Green tea is one of the most popular beverages in Japan and Asian countries. A number of studies so far reported have shown that green tea and tea catechins possessed a variety of physiological and biochemical effects, *e.g.*, antioxidation,¹⁾ antimutation,²⁾ anticarcinogenesis,³⁾ antibiotic action,⁴⁾ antihypercholesterolemia,⁵⁾ antihyperglycemia,⁶⁾ and antihypertension.⁷⁾ Since tea is taken habitually, it is interesting to know whether tea drinking, unlike alcohol drinking, would have a liver injury-preventive effect. With regard to this, Hikono *et al.*⁸⁾ have shown that catechins such as (+)-catechin, (–)-epicatechin, epicatechingallate, epigallocatechin and epigallocatechingallate each had a preventive effect on D-galactosamine- and carbon tetrachloride-induced hepatotoxicity when added to a medium of primary cultured rat hepatocytes. However, it is still unclear whether tea or certain tea constituents have an anti-hepatotoxic effect even *in vivo*.

This report describes the effect of dietary supplementation with powder of a green tea extract on D-galactosamine- and carbon tetrachloride-induced liver injury, as assessed by the plasma alanine aminotransferase (glutamic-pyruvic transaminase, GPT) and aspartate aminotransferase (glutamic-oxaloacetic transaminase, GOT) activities, to rats. The effect of tea drinking is also described. The results clearly demonstrate that green tea had a preventive effect on liver injury induced by D-galactosamine, but not by carbon tetrachloride.

Green tea (sen-cha) of upper middle grade was obtained from a market (Shizuoka City, Japan). The green tea was extracted by adding 10 volumes (volume/weight) of boiling water to the tea, standing for 30 min at room temperature, and filtering through five sheets of gauze. The extract was finally lyophilized and powdered with a mixer. The dry matter thus extracted was 23.5 g

per 100 g of green tea. Green tea was also extracted with 60 or 30 volumes of boiling water to test the effect of tea drinking. Male rats of the Wistar strain (Japan SLC, Hamamatsu, Japan) of 5 weeks old (90–100 g) were used as experimental animals. The rats were fed on a stock diet (Type MF, Oriental Yeast, Tokyo, Japan) for 4 or 5 d, and then they were given free access to the experimental diet and drink for 14 d in a temperature (23–25°C)- and humidity (50–60%)-controlled room with a 12 h cycle of light (06:00–18:00 h) and dark. The composition of the basal diet (25C) was as follows (g/100 g): casein, 25; corn starch, 39.9; sucrose, 20; corn oil, 5; AIN-76 mineral mixture, 3.5; AIN-76 vitamin mixture, 1; vitamin C+K mixture (containing 22.5 mg of vitamin K₃ and 2500 mg L(+)-ascorbic acid per 100 g), 0.2; choline bitartrate, 0.4; and cellulose, 5. The powder of the green tea extract was added to the basal diet at the expense of starch. Food and drink (water or the green tea solution) were renewed daily, and the body weight and food consumption of the animals were also measured daily. Four separate experiments were conducted in this study. In experiments 1 and 2, the effects of dietary supplementation with powder of the green tea extract (3%) on D-galactosamine- and carbon tetrachloride-induced liver injury were respectively investigated. In experiments 3 and 4, the dose-dependent effect of the dietary green tea extract (0.5–3.0%) and the effect of green tea drinking on D-galactosamine-induced liver injury were respectively investigated. After feeding the experimental diets and drinks for 14 d, D-galactosamine or carbon tetrachloride was administered to the rats between 14:00 and 14:30 h on the 15th day without starvation before and after administration of the drugs. D-Galactosamine was injected intraperitoneally at a dose of 300 mg/kg of body weight. Carbon tetrachloride was dissolved in olive oil (1:1, volume/volume) and administered to the rats by a stomach tube at a dose of 1 ml/kg of body weight. Untreated control rats were administered with saline or olive oil alone. After 22 h, the rats were killed by decapitation between 12:00 and 12:30 h to obtain the blood and liver. The activities of GPT and GOT were measured with a kit (Transaminase C II-Test; Wako Pure Chemical, Osaka, Japan), the enzyme activity being expressed as I.U. ($\mu\text{mol}/\text{min}$ per l of plasma at 25°C). Data were subjected to an analysis of variance, and the differences between means were considered significant at $p < 0.05$ by Duncan's multiple-range test⁹⁾ when the F value was significant at $p < 0.05$.

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Table I. Effects of Dietary Supplementation with the 3% Green Tea Extract on the Body Weight Gain, Food Intake, Liver Weight and Plasma Enzyme Activities in Rats (Experiments 1 and 2)¹

Expt. no.	Diet	Treatment on 15th day	Body wt. gain (g/14 d)	Food intake (g/14 d)	Liver wt. (g/100 g of body wt.)	Enzyme activity ($\mu\text{mol}/(\text{min}\cdot\text{l})$)	
						GPT	GOT
1	25C	Saline	73 $\pm$ 2 ^a	199 $\pm$ 5 ^a	4.45 $\pm$ 0.08 ^a	25 $\pm$ 2 ^b	75 $\pm$ 5 ^b
	25C	GalN	71 $\pm$ 1 ^a	199 $\pm$ 3 ^a	3.77 $\pm$ 0.08 ^c	1010 $\pm$ 133 ^a	2290 $\pm$ 408 ^a
	+3% tea ext.	GalN	53 $\pm$ 2 ^b	186 $\pm$ 4 ^b	4.18 $\pm$ 0.06 ^b	191 $\pm$ 31 ^b	429 $\pm$ 81 ^b
2	25C	Olive oil	70 $\pm$ 1 ^a	200 $\pm$ 1 ^a	4.42 $\pm$ 0.10 ^a	14 $\pm$ 1 ^b	56 $\pm$ 3 ^b
	25C	CCl ₄	69 $\pm$ 2 ^a	201 $\pm$ 4 ^a	4.01 $\pm$ 0.06 ^b	1360 $\pm$ 142 ^a	2670 $\pm$ 320 ^a
	+3% tea ext.	CCl ₄	52 $\pm$ 2 ^b	187 $\pm$ 5 ^b	3.95 $\pm$ 0.07 ^b	1650 $\pm$ 96 ^a	3420 $\pm$ 354 ^a

¹ Each value is the mean $\pm$ SEM for 8 rats; values in a column of each experiment with different superscript letters are significantly different at $p < 0.05$.

Abbreviations: wt., weight; 25C, basal diet; tea ext., green tea extract; GalN, D-galactosamine; GPT, alanine aminotransferase (glutamic-pyruvic transaminase); GOT, aspartate aminotransferase (glutamic-oxaloacetic transaminase)

Fig. Dose-dependent Effects of the Dietary Green Tea Extract on D-Galactosamine-Induced Enhancement of Plasma Alanine Aminotransferase (A) and Aspartate Aminotransferase (B) Activities in Rats (Experiment 3).

Rats were fed on the basal diet or a diet supplemented with 0.5, 1.0, 2.0 or 3.0% green tea extract for 14 d. The rats were injected with D-galactosamine on the 15th day of feeding, and blood was sampled after 22 h. The circle and its bar represent the mean value and SEM, respectively, for 8 rats. Values with different letters are significantly different at $p < 0.05$. Abbreviations: tea ext., green tea extract; GPT, alanine aminotransferase (glutamic-pyruvic transaminase); GOT, aspartate aminotransferase (glutamic-oxaloacetic transaminase).

Table I summarizes the results of experiments 1 and 2. Dietary supplementation with powder of the green tea extract at the 3% level caused slight decreases in the growth and food consumption of the animals. The relative liver weight was significantly decreased by the administration of D-galactosamine or carbon tetrachloride. Supplementation with the 3% green tea extract partially suppressed the decrease in liver weight caused by D-galactosamine, but not by carbon tetrachloride. The D-galactosamine-induced enhancement of plasma GPT and GOT activities was effectively suppressed by the dietary green tea extract. In contrast, the green tea extract had no effect on carbon tetrachloride-induced enhancement of plasma enzyme activities. The green tea extract failed to suppress liver injury even when the rats were administered with a lower dose of carbon tetrachloride of 0.3 ml/kg of body weight (data not shown). Dietary supplementation with graded levels of the green tea extract suppressed GPT and GOT activities in a dose-de-

pendent manner, as shown in Fig. (Experiment 3). The results also indicate that the minimum doses to bring about a significant effect were 1% and 2% for GPT or GOT activity, respectively. Table II summarizes the results of experiment 4. Drinking a green tea solution extracted with 30 volumes of boiling water had a suppressive effect on the D-galactosamine-induced enhancement of plasma enzyme activities, while a dilute green tea solution extracted with 60 volumes of boiling water had no effect.

The results of the present study clearly demonstrate that green tea could suppress liver injury, as assessed by plasma GPT and GOT activities, induced by D-galactosamine when added to the diet as powder and when given as a solution *in vivo* to rats. Drinking the green tea solution extracted with 30 volumes of boiling water was estimated, on the basis of the volume of green tea solution consumed during 14 d (260 ± 11 ml), to be comparable to the diet supplemented with an approximately 1.4% green tea extract. Therefore, the intensity of the effect of tea drinking seems to be compatible with that of dietary supplementation with the powder of a tea extract. In the present study, we could not detect any preventive effect of green tea on carbon tetrachloride-induced liver injury in rats. On the other hand, it has been found that tea catechins had a preventive effect on carbon tetrachloride-induced hepatotoxicity in cultured rat hepatocytes when added to the medium.⁹ One of the reasons for the discrepancy in the effect between *in vivo* experiments and cells in culture may be that the rate of intestinal absorption of tea catechins is considerably low.¹⁰ Although green tea caused slight decreases in the growth and food consumption of the animals, these decreases are not considered to have been associated with a liver injury-preventive effect. While the caffeine-containing fraction, which had been prepared by extracting green tea with chloroform, had no effect on liver injury, it caused significant decreases in growth and food consumption (unpublished results).

Sanada and his associates have shown that D-galactosamine-induced liver injury could be effectively prevented by dietary supplementation with certain substances such as wheat gluten,¹¹ glutamine,¹² and some oligosaccharides.¹³ Hence, green tea is considered to be another type of liver injury-preventive food. At present, it is unknown whether other types of tea and other

Table II. Effects of Green Tea Drinking on Body Weight Gain, Food Intake, Liver Weight and Plasma Enzyme Activities in Rats (Experiment 4)¹

Diet	Drink	Treatment on 15th day	Body wt. gain (g/14 d)	Food intake (g/14 d)	Liver wt. (g/100 g of body wt.)	Enzyme activity ($\mu\text{mol}/(\text{min} \cdot \text{l})$)	
						GPT	GOT
25C	Water	Saline	70 $\pm$ 2 ^a	190 $\pm$ 2 ^a	4.39 $\pm$ 0.08 ^a	22 $\pm$ 1 ^c	64 $\pm$ 4 ^c
25C	Water	GalN	71 $\pm$ 2 ^a	189 $\pm$ 4 ^a	3.59 $\pm$ 0.05 ^{bc}	2080 $\pm$ 286 ^a	4780 $\pm$ 597 ^a
25C	Tea ($\times$ 60)	GalN	61 $\pm$ 2 ^b	180 $\pm$ 3 ^a	3.51 $\pm$ 0.06 ^c	2380 $\pm$ 180 ^a	4860 $\pm$ 425 ^a
25C	Tea ($\times$ 30)	GalN	46 $\pm$ 4 ^c	167 $\pm$ 6 ^b	3.76 $\pm$ 0.05 ^b	1250 $\pm$ 87 ^b	2330 $\pm$ 185 ^b

¹ Each value is the mean $\pm$ SEM for 8 rats; values with different superscript letters are significantly different at $p < 0.05$.

Abbreviations: wt., weight; 25C, basal diet; tea ($\times$ 60) and tea ($\times$ 30), green tea solution extracted with 60 or 30 vol. of hot water, respectively; GalN, D-galactosamine; GPT, alanine aminotransferase (glutamic-pyruvic transaminase); GOT, aspartate aminotransferase (glutamic-oxaloacetic transaminase)

beverages have a similar effect on liver injury. The mechanism by which green tea exerts its preventive effect on D-galactosamine-induced liver injury is also unclear. Further studies are now in progress.

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