Teas and Other Beverages Suppress D-Galactosamine-Induced Liver Injury in Rats[1]

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ABSTRACT We compared the effects of various types of beverages (teas, coffee, and cocoa) on D-galactosamine-induced liver injury by measuring plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in 7-wk-old male Wistar rats. The effects of five fractions extracted with different organic solvents from green tea, different types of dietary fibers, and some short chain fatty acids were also investigated. All of the beverages tested significantly suppressed D-galactosamine-induced enhancement of plasma enzyme activities when powdered beverages were added to the diet (30 g/kg) and fed to rats for 2 wk. Plasma ALT activities were 1155 ± 82 [μmol/(min·L), control], 289 ± 61 (green tea), 626 ± 60 (roasted green tea), 471 ± 84 (puerh tea), 676 ± 69 (oolon tea), 423 ± 76 (black tea), 829 ± 53 (coffee), and 885 ± 89 (cocoa). The profile of AST activities was similar. The caffeine-containing fraction from green tea had no significant effect, whereas the other four fractions, including the soluble fiber fraction, significantly suppressed liver injury. In addition to tea fibers, many other types of dietary fiber (hemicellulose, chitin, chitosan, alginate, pectin, guar gum, glucomannan, and inulin, but not cellulose) had liver injury-preventive effects when added to the diet (30 g/kg), suggesting that liver injury-prevention may be one of the general effects of dietary fibers. Of three short-chain fatty acids tested (acetate, propionate, and butyrate), only acetate prevented liver injury when added to the diet (15 g/kg), supporting the possibility that the liver injury-preventive effect of dietary fibers may be mediated at least in part by certain organic acids. These results suggest that several beverages possess preventive effects on certain types of liver injury, such as that induced by D-galactosamine, and that different constituents of high and low molecular weights contribute to the liver injury-preventive effects of green tea. J. Nutr. 129: 1361–1367, 1999.

INDEXING KEY WORDS: • D-galactosamine • liver injury • green tea • dietary fiber • rats

Teas and other beverages, such as coffee and cocoa, contain caffeine or caffeine analogues (e.g., theobromine and theophylline) as common constituents. Therefore, the primary importance of these beverages is considered to be as caffeine sources. Indeed, caffeine has a variety of biological and pharmacological effects (Dews 1982). Teas contain relatively large amounts of catechins (tea tannins) in addition to caffeine. A number of studies have shown that teas or tea catechins have a wide range of biological effects, such as antioxidation (Matsuzaki and Hara 1985), antimutation (Kada et al. 1985), anticarcinogenesis (Fujiki et al. 1996), antibiotic action (Toda et al. 1989), antihypercholesterolemia (Muramatsu et al. 1986), antihypertension (Hara and Tonooka 1990), antihyperglycemia (Shimizu et al. 1988) and anti-inflammatory action (Sagesaka et al. 1996). Furthermore, we recently demonstrated that D-galactosamine-induced liver injury could be suppressed by dietary supplementation with a powder of a green tea extract (Sugiyama et al. 1998). Liver injury is caused by different agents, such as viruses, chemicals, alcohol, and auto-immune diseases. It was pointed out that liver injury induced by D-galactosamine resembles that induced by the H-1 strain virus, one of two serologic types of rat virus, in its symptoms (Keppler et al. 1968). D-Galactosamine is thought to induce hepatotoxicity by inhibiting the synthesis of RNA and protein through a decrease in cellular UTP concentration, which finally leads to the necrosis of liver cells (Decker and Keppler 1974). The extent of liver injury can be easily estimated by measuring the activities of certain plasma enzymes, e.g., alanine aminotransferase (ALT)[3] and aspartate aminotransferase (AST). It would be interesting to know what types of beverages are more effective than others in protecting the liver from injury, but little information is available It is also unclear by what constituent(s) and by what mechanism(s) green tea exerts its liver injury-preventive effect.

In this study, we investigated the effects of dietary supplementation with different types of beverages on D-galactosamine-induced liver injury in rats, as assessed by ALT and AST activities. The effects of five fractions, which were prepared from a green tea extract by successive extraction with

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[3] Abbreviations used: ALT, alanine aminotransferase; AST, aspartate aminotransferase; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor α.

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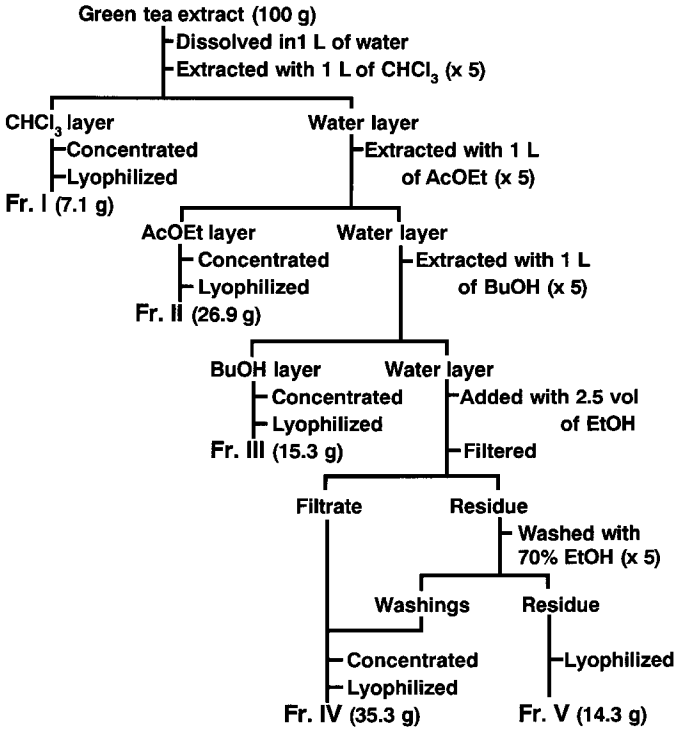

FIGURE 1 The procedure for the fractionation of a green tea extract into five fractions (Fr. I–V) by successive extraction with different organic solvents. EtOH, ethanol; AcOEt, ethyl acetate.

different organic solvents, were also investigated to determine what type of constituents contributes to the effect of green tea. Furthermore, the effects of different types of dietary fibers and short-chain fatty, acids such as acetate, propionate, and butyrate, on D-galactosamine-induced liver injury were investigated to confirm the effect of dietary fibers because the soluble dietary fiber fraction of green tea was found to have a liver injury-preventive effect.

MATERIALS AND METHODS

Materials. Various types of teas, instant coffee and cocoa were obtained from a market (Shizuoka City, Japan). The green tea (Sencha) and roasted green tea (Houji-cha) we used were made in Japan, the puerh tea and oolon tea in China, the black tea in England, and the instant coffee and cocoa in Japan. Sodium alginate, pectin (lemon), and inulin were obtained from Wako Pure Chemical (Osaka, Japan), and cellulose, glucomannan (konjak mannan), and guar gum were obtained from Oriental Yeast (Tokyo, Japan), Shimizu Shokuhin (Shimizu, Japan) and Sigma Chemical (St. Louis, MO), respectively. Corn hemicellulose (water-soluble type) was supplied by Nihon Shokuhin Kako (Tokyo, Japan), and chitin and chitosan supplied by Yaizu Suisan Kagaku (Yaizu, Japan). D-Galactosamine was obtained from Sigma. Authentic tea catechins and caffeine for HPLC analysis were obtained from Funakoshi (Tokyo, Japan) and Sigma, respectively. Sodium acetate, sodium propionate, and sodium butyrate were obtained from Wako. Mineral and vitamin mixtures were obtained from Oriental Yeast.

Extraction of teas, fractionation and analysis of constituents. Teas were extracted as follows: 10 volumes (v/wt) of boiling water was added to each tea, let stand for 30 min at room temperature, and the extract was filtered through five sheets of gauze. The extract was lyophilized and powdered with a mixer. The dry matter thus obtained were 23–24 g per 100 g for five types of teas. The powder of a green tea extract was further fractionated into five fractions by successive extraction with organic solvents, such as chloroform, ethyl acetate, *n*-butanol, and 70% (v/v) ethanol (**Fig. 1**), according to Matsuzaki and Hara (1985) and Sagesaka et al. (1994) with slight modifications. A relatively large amount of soluble fiber fraction (fraction V) was likewise prepared to examine its dose-dependent effect. The concentrations of caffeine and catechins in a green tea extract and fractions I and II (Fig. 1) were measured by HPLC (Model LC 10A; Shimadzu, Kyoto, Japan) using an octadecylsilyl column (6 x 150 mm; Shimadzu) according to Terada et al. (1987). The sugar content of fraction V (Fig. 1) was determined by a phenol-sulfate method using D-glucose as a standard (Dubois et al. 1956).

Animals and diets. Five-wk-old male Wistar rats, weighing 90–100 g, were obtained from Japan SLC (Hamamatsu, Japan). The rats were individually housed in hanging, stainless steel, wire cages and kept in an isolated room at a controlled temperature (23–25°C) and ambient humidity (50–60%). Lights were maintained on a 12-h light-dark cycle (lights on from 0600 to 1800 h). Animals were acclimated to the facility for 4 or 5 d and given free access to water and a commercial stock diet (Type MF; Oriental Yeast). The composition of the control diet was as follows (g/kg): casein, 250; corn starch, 401; sucrose, 200; corn oil, 50; mineral mixture (American Institute of Nutrition 1977), 35; vitamin mixture (American Institute of Nutrition 1977), 10; choline bitartrate, 4; and cellulose, 50. Supplements were added to the control diet at the expense of cellulose. Rats were given free access to the experimental diets, and body weight and food consumption were measured daily.

In this study, six separate experiments were conducted. In experiment 1, 78 rats were divided into nine groups and fed the control diet or diets supplemented with the powder of one of each tea extract, instant coffee, or cocoa at a level of 30 g/kg. In experiment 2, 75 rats were divided into eight groups and fed the control diet or diets supplemented with the powder of a green tea extract (30 g/kg diet) or one of each fraction from a green tea extract. Each fraction was added to the diet to make it comparable to the addition of powdered green tea extract on the basis of percentage distribution of each fraction. In experiment 3, 50 rats were divided into six groups and fed the control diet or diets supplemented with a soluble dietary fiber fraction (fraction V) at levels of 1, 2, 4, 6 or 10 g/kg. In experiment 4, 90 rats were divided into 11 groups and fed the control diet or diets supplemented with different types of dietary fibers at a level of 30 g/kg. In experiment 5, 42 rats were divided into five groups and fed the control diet or diets supplemented with guar gum at levels of 5, 10, 30, or 50 g/kg. In experiment 6, 34 rats were divided into four groups and fed the control diet or diets supplemented with sodium acetate, sodium propionate, or sodium butyrate at levels of 20.8, 19.7, or 19.0 g/kg. These addition levels corresponded to a net amount of short-chain fatty acids of 15 g/kg diet.

After feeding the experimental diets for 14 d, D-galactosamine was injected intraperitoneally at a dose of 350 mg/kg body weight between 1400 and 1440 h. Untreated rats were injected with saline. After the injection (22 h), rats were killed by decapitation between 1200 and 1240 h to obtain blood and liver. Rats were not starved either before and after the injection of saline or the drug. Blood plasma was separated from heparinized, whole blood by centrifugation at 2000 × *g* for 20 min at 4°C; the plasma and liver were stored at -30°C until analyses. The experimental design was approved by the Laboratory Animal Care Committee of the Faculty of Agriculture, Shizuoka University.

Biochemical analysis. The activities of plasma ALT and AST were measured with a kit (Transaminase C II-Test, Wako). The enzyme activity was expressed as μmol/(min·L plasma) at 25°C. In some of the rat groups of experiment 1, several variables of the liver and plasma were measured. Whole liver was minced and divided into two portions. The water content of the liver was determined by lyophilizing one portion of the liver. Another portion was homogenized in 4 volumes (v/wt) of ice-cold saline, and the protein, nucleic acids, and glycogen content of the homogenates were determined. Protein was measured by the method of Lowry et al. (1951) using bovine serum albumin as a standard. RNA and DNA were separated by the method of Schneider (1946) and measured spectrophotometrically. The optical density of 0.286 at 260 nm was assumed to represent nucleic acid concentration of 10 mg/L. Glycogen was measured by the anthrone method (Stifter et al. 1950). Plasma glucose con-

TABLE 1

Body weight gain, food intake, liver weight, and plasma enzyme activities of rats fed the control diet or diets supplemented with different types of beverages (experiment 1)[1]

Diet	Body wt gain	Food intake	Liver wt	Enzyme activity	
				ALT	AST
	g/14 d		*g/100 g body wt*	*μmol/(min · L)*	
Control (−GalN)	74 ± 2[a]	193 ± 3[a]	4.36 ± 0.13[a]	13 ± 1[g]	69 ± 4[e]
Control (+GalN)	74 ± 1[a]	192 ± 3[a]	3.42 ± 0.05[e]	1155 ± 82[a]	2617 ± 219[a]
+Green tea (30 g/kg)	64 ± 1[bc]	180 ± 2[bc]	4.05 ± 0.04[b]	289 ± 61[f]	561 ± 92[d]
+Roasted tea (30 g/kg)	65 ± 2[bc]	191 ± 5[a]	3.83 ± 0.05[c]	626 ± 60[cde]	1134 ± 96[bc]
+Puerh tea (30 g/kg)	48 ± 1[d]	174 ± 2[cd]	3.71 ± 0.05[c]	471 ± 84[ef]	958 ± 152[cd]
+Oolon tea (30 g/kg)	61 ± 2[c]	190 ± 5[ab]	3.74 ± 0.06[c]	676 ± 69[bcd]	1169 ± 131[bc]
+Black tea (30 g/kg)	48 ± 1[d]	167 ± 3[d]	3.69 ± 0.05[cd]	423 ± 76[ef]	804 ± 134[cd]
+Coffee (30 g/kg)	66 ± 2[b]	194 ± 3[a]	4.03 ± 0.06[b]	829 ± 53[bc]	1485 ± 64[b]
+Cocoa (30 g/kg)	72 ± 2[a]	192 ± 3[a]	3.53 ± 0.05[de]	885 ± 89[b]	1570 ± 226[b]

[1] Values are mean ± SEM for 5 (control, −GalN), 10 (control, +GalN), or 9 (the other groups) rats. Values in a column with no common letters are significantly different at $P < 0.05$. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GalN, D-galactosamine.

centration was measured enzymatically with a kit (Glucose C II-Test; Wako).

Statistical analysis. Results were expressed as mean ± SEM . Data were analyzed by one-way ANOVA, and the difference between means were tested at $P < 0.05$ using Duncan's multiple range test (Duncan 1957) when the F value was significant at $P < 0.05$. Simple correlation between variables was calculated by linear regression analysis.

RESULTS

Effect of different types of beverages (experiment 1). Most of the beverages, except for cocoa, depressed growth compared to D-galactosamine-injected control rats, whereas food intake was reduced by green, puerh, and black teas compared to D-galactosamine-injected control rats (**Table 1**). Relative liver weights were significantly lower in D-galactosamine-injected control rats than in saline-injected control rats. Most of the beverages, except for cocoa, partially prevented the decrease in liver weight caused by D-galactosamine injection. Activities of plasma ALT and AST were dramatically enhanced by D-galactosamine injection. The increases in these enzyme activities were significantly suppressed by all of the beverages tested, although the magnitude of the effect varied. There was a significant correlation between the activity of ALT and AST among the nine groups ($r = 0.975$, $P < 0.001$, $n = 9$). As a whole, certain types of teas, such as green, puerh, and black, had a greater effect than coffee and cocoa. The effects of green tea on liver components and plasma glucose concentration were measured (**Table 2**). D-Galactosamine injections, compared to saline injections, significantly enhanced the water and DNA contents of the liver and, conversely, reduced protein, glycogen, and RNA contents; the ratio of protein or RNA to DNA; and plasma glucose concentration. Dietary supplementation with a green tea extract partially prevented or tended to prevent these effects of D-galactosamine.

Effect of each fraction derived from green tea (experiments 2 and 3). The HPLC analysis showed that a green tea extract contained 6.2 g of caffeine and 27.3 g of catechins per 100 g. Fraction I (Fig. 1) contained 87.5 g caffeine and a trace amount of catechins per 100 g, whereas fraction II contained 1.5 g caffeine and 83.2 g catechins per 100 g. These data confirmed that the major constituents of fractions I and II were caffeine and catechins, respectively. The sugar content of fraction V was at least 95 g per 100 g, indicating that this fraction was composed of polysaccharides. Consistent with the results of experiment 1, body weight gain was significantly lower in rats fed a green tea extract than in rats fed the control diet (**Table 3**). Of the five fractions prepared from the green tea extract, only fraction I caused significant depressions of body weight gain and food intake. Relative liver weights were higher in rats fed diets supplemented with a green tea extract or each fraction, except for fraction II, than in D-galactosamine-injected control rats. Fractions II, III, IV, and V had suppressive effects on D-galactosamine-induced enhancement of ALT and AST activities, although the intensity of the effect of fraction II on ALT activity was significantly smaller than those of fractions III and IV. Because fraction V was found to be relatively pure, the dose-dependent effect of this

TABLE 2

Effect of dietary supplementation with green tea extract (30 g/kg) on liver components and plasma glucose concentration in rats fed control diets or diets supplemented with different types of beverages (experiment 1)[1]

	Diet (injection)		
	Control (−GalN)	Control (+GalN)	+Green tea (+GalN)
n	5	10	9
Liver			
Water, *g/100 g liver*	68.1 ± 0.1[c]	73.9 ± 0.3[a]	71.8 ± 0.1[b]
Protein, *mg/g liver*	222 ± 2[a]	206 ± 3[b]	217 ± 2[a]
Glycogen, *mg/g liver*	23.9 ± 0.8[a]	7.8 ± 1.1[c]	11.1 ± 1.0[b]
RNA, *mg/g liver*	10.5 ± 0.1[a]	8.5 ± 0.5[b]	10.0 ± 0.4[a]
DNA, *mg/g liver*	3.1 ± 0.2[b]	3.7 ± 0.1[a]	3.5 ± 0.1[ab]
Protein/DNA, *g/g*	75.6 ± 4.6[a]	52.5 ± 1.1[c]	63.3 ± 2.5[b]
RNA/DNA, *g/g*	3.5 ± 0.2[a]	2.3 ± 0.1[c]	2.8 ± 0.1[b]
Plasma			
Glucose, *mmol/L*	8.7 ± 0.2[a]	5.3 ± 0.2[c]	6.8 ± 0.2[b]

[1] Values are mean ± SEM. Values in a row with no common superscript letters are significantly different at $P < 0.05$. The three groups of rats presented here were the same as those in Table 1. GalN, D-galactosamine.

TABLE 3

Body weight gain, food intake, liver weight, and plasma enzyme activities of rats fed the control diet or diets supplemented with a green tea extract and each fraction (experiment 2)[1]

Diet	Body wt gain	Food intake	Liver wt	Enzyme activity	
				ALT	AST
	g/14 d		*g/100 g body wt*	*μmol/(min · L)*	
Control (−GalN)	71 ± 2^{bc}	189 ± 3^{ab}	4.46 ± 0.08^{a}	23 ± 2^{e}	60 ± 9^{d}
Control (+GalN)	70 ± 1^{bc}	189 ± 2^{ab}	3.50 ± 0.03^{e}	1895 ± 291^{a}	4125 ± 557^{a}
+Green tea (30 g/kg)	58 ± 2^{d}	180 ± 4^{bc}	3.90 ± 0.04^{b}	589 ± 36^{d}	1389 ± 125^{c}
+Fraction I (2.2 g/kg)	49 ± 2^{e}	173 ± 4^{c}	3.70 ± 0.03^{cd}	1548 ± 237^{ab}	3209 ± 473^{ab}
+Fraction II (8.1 g/kg)	67 ± 1^{c}	184 ± 2^{b}	3.58 ± 0.04^{de}	1194 ± 145^{bc}	2287 ± 273^{bc}
+Fraction III (4.7 g/kg)	72 ± 1^{b}	194 ± 3^{a}	3.78 ± 0.05^{bc}	644 ± 69^{d}	1452 ± 201^{c}
+Fraction IV (10.7 g/kg)	77 ± 1^{a}	197 ± 3^{a}	3.76 ± 0.06^{bc}	675 ± 67^{d}	1512 ± 111^{c}
+Fraction V (4.3 g/kg)	68 ± 1^{c}	190 ± 3^{ab}	3.72 ± 0.07^{cd}	783 ± 115^{cd}	1767 ± 263^{c}

[1] Values are mean ± SEM for 5 (control, −GalN) or 10 (the other groups) rats. Values in a column with no common superscript letters are significantly different at $P < 0.05$. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GalN, D-galactosamine.

fraction (soluble tea dietary fibers) was investigated. The minimum dose that had a significant effect was 2 g/kg diet for both ALT and AST activities (**Fig. 2**).

Effects of different types of dietary fibers and short-chain fatty acids (experiments 4, 5, and 6). The growth of rats was slightly depressed only by chitosan, and food intake was slightly lowered by hemicellulose, chitosan, guar gum, and glucomannan compared to D-galactosamine-injected control rats ($P < 0.05$; **Table 4**). None of the dietary fibers tested affected relative liver weight. Most of the dietary fibers, except for cellulose, suppressed the D-galactosamine-induced enhancement of plasma ALT and AST activities, with different amplitudes of the effect. Because guar gum had the strongest effect, this fiber was further investigated. Guar gum suppressed the enhancement of plasma enzyme activities in a dose-dependent manner (**Fig. 3**); the effect was significant, even at a low addition level (5 g/kg diet). Because certain types of dietary fibers are assimilated by intestinal microflora to produce short-chain fatty acids, such as acetate, propionate, and butyrate, the effect of dietary supplementation with these acids on D-galactosamine-induced liver injury was assessed to study the mechanism by which dietary fibers suppress liver injury. None of the short-chain fatty acids tested depressed the growth or food intake of animals (data not shown). The plasma ALT activities were 1325 ± 94 [μmol/(min·L), control, $n = 10$], 864 ± 30 (acetate, $n = 8$), 1365 ± 74 (propionate, $n = 8$), and 1471 ± 98 (butyrate, $n = 8$), and the AST activities were 3412 ± 267 (control), 2847 ± 200 (acetate), 3851 ± 222 (propionate), and 3829 ± 232 (butyrate). In both enzyme activities, only acetate had significantly suppressive effects ($P < 0.05$) compared to the control group.

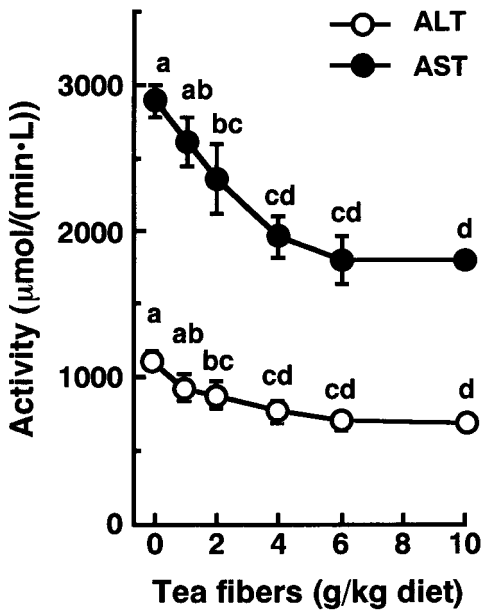

FIGURE 2 Dose-dependent effects of soluble dietary fibers from a green tea extract on D-galactosamine-induced enhancement of alanine aminotransferase and aspartate aminotransferase in rats (experiment 3). The circle and its bar represent mean values ± SEM, respectively, for 10 (control) or 8 (the other groups) rats. Values with different letters are significantly different at $P < 0.05$. ALT, alanine aminotransferase; AST, aspartate aminotransferase.

DISCUSSION

Different types of beverages, which contain caffeine or caffeine analogues, could significantly suppress D-galactosamine-induced enhancement of plasma ALT and AST activities in rats. Because the extent of an increase in these plasma enzyme activities parallels that of liver injury, the results of the present study are taken to indicate that all of the beverages tested have liver injury-preventive effects, although the potency of these effects differed. It was shown that growth and food intake of rats were suppressed by dietary caffeine and caffeine-containing beverages (Naismith et al. 1969). This was also the case for the present study. It should be noted, however, that suppression of growth and food intake, per se, would not be associated with the liver injury-preventive effect of beverages because the caffeine-containing fraction (fraction I) did not suppress liver injury while it did depress the growth of the rats. The reported D-galactosamine-induced changes in the concentration of liver components, such as RNA, protein, and glycogen (Decker and Keppler 1974; Keppler et al. 1968), were also observed in the present study. The fact that green tea normalized, or tended to normalize, the decreased concentrations of these variables also supports that green tea could protect against the hepatotoxicity of D-galactosamine.

In the present study, we focused our attention on the

TABLE 4

Body weight gain, food intake, liver weight, and plasma enzyme activities of rats fed the control diet or diets supplemented with different types of dietary fibers (experiment 4)[1]

Diet	Body wt gain	Food intake	Liver wt	Enzyme activity	
				ALT	AST
	g/14 d		*g/100 g body wt*	*μmol/(min · L)*	
Control (−GalN)	72 ± 2^{a}	180 ± 2^{a}	4.73 ± 0.08^{a}	21 ± 2^{f}	62 ± 8^{e}
Control (+GalN)	70 ± 2^{ab}	177 ± 3^{ab}	3.58 ± 0.04^{bc}	1014 ± 44^{a}	2930 ± 121^{a}
+Cellulose (30 g/kg)	71 ± 2^{ab}	180 ± 2^{a}	3.59 ± 0.03^{bc}	982 ± 91^{a}	2742 ± 196^{a}
+Hemicellulose (30 g/kg)	69 ± 2^{ab}	168 ± 2^{cd}	3.70 ± 0.03^{b}	726 ± 81^{bcd}	2036 ± 189^{b}
+Chitin (30 g/kg)	71 ± 2^{ab}	180 ± 2^{a}	3.57 ± 0.04^{bc}	807 ± 54^{b}	2212 ± 219^{b}
+Chitosan (30 g/kg)	63 ± 1^{c}	167 ± 2^{cde}	3.33 ± 0.02^{d}	736 ± 49^{bc}	2136 ± 127^{b}
+Alginate (30 g/kg)	73 ± 2^{a}	175 ± 2^{ab}	3.63 ± 0.04^{bc}	547 ± 39^{d}	1468 ± 95^{c}
+Pectin (30 g/kg)	69 ± 2^{ab}	172 ± 2^{bc}	3.52 ± 0.03^{c}	678 ± 45^{bcd}	1807 ± 154^{bc}
+Guar gum (30 g/kg)	67 ± 1^{bc}	162 ± 1^{e}	3.61 ± 0.04^{bc}	290 ± 28^{e}	875 ± 82^{d}
+Glucomannan (30 g/kg)	68 ± 1^{ab}	166 ± 1de	3.54 ± 0.05^{c}	712 ± 54^{bcd}	2166 ± 167^{b}
+Inulin (30 g/kg)	70 ± 1^{a}	175 ± 2^{ab}	3.58 ± 0.04^{bc}	602 ± 46^{cd}	1762 ± 119^{bc}

[1] Values are mean ± SEM for 10 (control, +GalN) or 8 (the other groups) rats. Values in a column with no common superscript letters are significantly different at $P < 0.05$. ALT, alanine aminotransfertase; AST, aspartate aminotransferase; GalN, D-galactosamine.

constituents of green tea because green tea was one of the most effective beverages in alleviating D-galactosamine-induced liver injury. For this purpose, a green tea extract was separated into five fractions by successive extraction with organic solvents, and the effect of each fraction was investigated. Several fractions had preventive effects on D-galactosamine-induced liver injury. It is apparent that caffeine had no preventive effect on liver injury. Fraction II, which contained tea catechins as major constituents, had a significant effect, but the extent of the effect generally was smaller than that of some other fractions (Table 3). These results indicate that the major part of the effect of green tea cannot be ascribed to tea catechins. In contrast, Hikino et al. (1985) showed that, when added to a medium of primary cultured rat hepatocytes, catechins had a preventive effect on D-galactosamine- and carbon tetrachloride-induced hepatotoxicity. Recently, Miyagawa et al. (1997) also reported that tea catechins could protect against the cytotoxicity of 1,4- naphthoquinone in primary cultured rat hepatocytes. It seems worthwhile to note that the intestinal absorption of tea catechins is very low in humans (Nakagawa et al. 1997, Unno et al. 1996). Nakagawa et al. (1997) estimated the absorption of (−)-epigallocatechin gallate and (−)-epigallocatechin to be only 0.2–2% and 0.2–1.3% of the amount of administered, respectively. The low absorbabilities of catechins suggest that the effect of tea catechins in vivo may be far smaller than that expected from the results obtained in vitro or in cells in culture. However, the effect of tea catechins on liver injury must be further studied by using pure tea catechins to obtain conclusive results. Fractions III, IV, and V were found to have relatively strong effects on D-galactosamine-induced liver injury. Fraction III, which was prepared by extracting with *n*-butanol, was shown to contain tea saponins and flavonoids (Sagesaka et al. 1994), whereas fraction IV, which was soluble in 70% ethanol, contains water-soluble compounds of low molecular weights, such as free sugars, amino acids, and oligosaccharides. Recently we found that glycosidic flavonoids included in fraction III had a liver injury-preventive effect (Wada et al. 1999), but the entity responsible for the effect of fraction IV has not yet been elucidated. In contrast, fraction V was relatively pure and was composed of soluble dietary fibers (polysaccharides). With regard to this fraction V, Shimizu et al. (1988) have already isolated soluble polysaccharides from an extract of green tea (Ban-cha), which had a hypoglycemic effect in streptozotocin-induced diabetic rats. They showed that the molecular weight was ~40 kDa, and the constituting sugars were arabinose, D-ribose, and D-glucose (5.1:4.7:1.7, wt/wt). These soluble

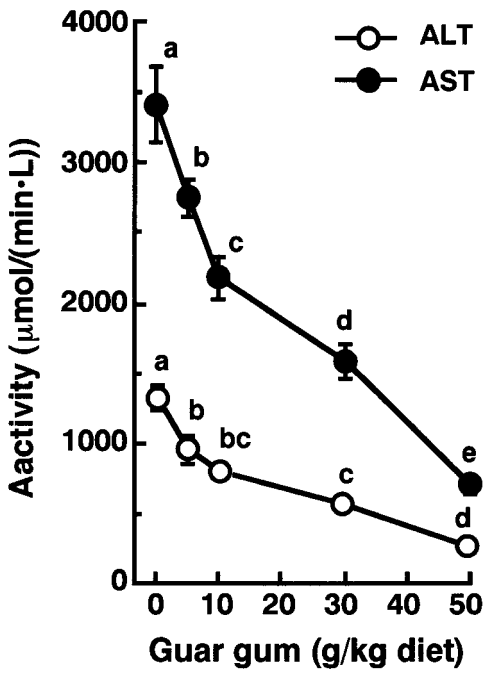

FIGURE 3 Dose-dependent effects of guar gum on D-galactosamine-induced enhancement of plasma alanine aminotransferase and aspartate aminotransferase in rats (experiment 5). The circle and its bar represent mean values ± SEM, respectively, for 10 (control) or 8 (the other groups) rats. Values with different letters are significantly different at $P < 0.05$. ALT, alanine aminotransferase; AST, aspartate aminotransferase.

polysaccharides are considered to be contained in fraction V of the present study.

The present study demonstrated that in addition to tea soluble dietary fibers, many other dietary fibers also have preventive effects on D-galactosamine-induced liver injury. These results suggest that prevention of liver injury may be one of the important physiological effects of dietary fibers. It is interesting that Wang et al. (1995) have shown that dietary supplementation with free galactose or galactose-containing oligosaccharides, such as lactose, lactulose, raffinose, and some other galactooligosaccharides, had a preventive effect on D-galactosamine-induced liver injury in rats. Based on these results, they concluded that the galactose residue of oligosaccharides participates in the effect. This might be applied to the effect of polysaccharides. However, this assumption cannot explain why several dietary fibers, which do not contain galactose, e.g., chitin, chitosan, glucomannan, alginate, and inulin, also suppressed D-galactosamine-induced liver injury. Therefore, it is possible that dietary fibers exert their suppressive effects on D-galactosamine-induced liver injury through both galactose-dependent and galactose-independent mechanisms. In terms of the physiological and nutritional effects of dietary fibers, the latter mechanism appears to be more interesting than the former because D-galactose may alleviate D-galactosamine-induced liver injury simply by competing with D-galactosamine in some metabolic processes.

At present, the mechanism of the galactose-independent liver injury-preventive effect of dietary fibers is unclear. Recently, Hase et al. (1996) have demonstrated that celosian, an acidic polysaccharide isolated from the seeds *Celosia argentea*, had a liver injury-preventive effect in different liver injury models, including that caused by the administration of D-galactosamine and bacterial lipopolysaccharide (LPS). In their study, celosian was administered to mice and rats intraperitoneally or subcutaneously, whereas in the present study fibers were added to the diet and fed to rats. So, the possibility that the mechanism of the liver injury-preventive effect of dietary fibers may be different from that of celosian cannot be ruled out. In our investigation, it seems unlikely that dietary fibers suppressed liver injury simply by preventing the absorption of D-galactosamine because fibers and D-galactosamine were administered by different routes. A number of studies have shown that certain types of dietary fibers can be assimilated by intestinal microflora to produce short-chain fatty acids, such as acetate, propionate, and butyrate (Eastwood 1992). With regard to this ability of intestinal microflora, it is interesting that, in the present study, acetate was found to have a liver injury-preventive effect when added to the diet. This finding can be taken to support the idea that certain dietary fibers may elicit their liver injury-preventive effects, at least in part, via specific short-chain fatty acid, e.g., acetate, one of the results of the fermentation of dietary fibers by intestinal microflora. However, this assumption must be further examined.

D-Galactosamine is usually used in combination with other hepatotoxic substances, such as LPS, in mice. In the present study, we used D-galactosamine alone to cause liver injury because rats are much more sensitive to D-galactosamine than are mice (Galanos et al. 1979). It should be noted, however, that in liver injury caused by D-galactosamine and LPS, an inflammatory response is initiated by LPS, but not by D-galactosamine; D-galactosamine is thought to increase the sensitivity of hepatocytes to LPS by inhibiting the synthesis of acute-phase proteins (Wang and Wendel 1990). It was postulated that tumor necrosis factor α (TNF-α), released mainly from activated macrophages, acts as a mediator for several types of experimental liver injury, including that caused by D-galactosamine and LPS, through the induction of the transcription of proinflammatory genes (Bradham et al. 1998). Actually, TNF-α could substitute for LPS in D-galactosamine-sensitized mice (Tiegs et al. 1989), supporting the central role of TNF-α in LPS-induced hepatic injury. It is unclear whether TNF-α also plays a critical role in the model of rat liver injury caused by D-galactosamine alone, but further studies on the effect on TNF-α should help to elucidate the mechanism of the liver injury-preventive effect of beverages.

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