

ORIGINAL ARTICLE

Intake of purple sweet potato beverage affects on serum hepatic biomarker levels of healthy adult men with borderline hepatitis

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Objective: To examine the effect of purple sweet potato (PSP) beverage rich in acylated anthocyanins on serum hepatic biomarkers in healthy Japanese men.

Design: A randomized, double-blind, placebo-controlled, parallel study.

Setting: Kumamoto in Japan.

Subjects: Healthy adult men (30–60 years) with borderline hepatitis who had one or more of serum γ -glutamyl transferase (GGT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels over normal ranges, and who were negative for hepatitis virus were openly recruited by an advertisement. Of the 48 persons enrolled, 38 (mean age 43.0 years (30–54 years)) completed the study.

Methods: The subjects were randomly assigned to the PSP group and the placebo group. During the 8-week intervention, the subjects in the PSP group consumed two bottles of the PSP beverage with acylated anthocyanins (200.3 mg anthocyanins per 125 ml per bottle) per day, and the subjects in the placebo group, two bottles of a placebo beverage (1.7 mg anthocyanins per 125 ml per bottle). All of the data measured were analyzed by two-way repeated measures analysis of variance (ANOVA) with groups and times. The data of the hepatic markers were analyzed using the Dunnett multiple comparison among the time points and *t*-test between groups at the same time point. Two-sided $P < 0.05$ were defined as the level of significance.

Results: Serum GGT, AST and ALT levels showed interactions ($P < 0.05$) between the beverage groups and time; the others were not affected. The PSP beverage group showed lower hepatic marker levels than the placebo group during the ingestion period, particularly the GGT level (–14.1 IU/l, 95% Confidence interval (CI) –25.4 to –2.7, $P = 0.017$ at 2 weeks; –16.8 IU/l, 95% CI –36.2 to 2.5, $P = 0.081$ at 4 weeks; –26.7 IU/l, 95% CI –47.6 to –5.7, $P = 0.014$ at 6 weeks and –27.9 IU/l, 95% CI –49.9 to –5.9; $P = 0.014$ at 8 weeks). No correlation between alcohol consumption and each hepatic biomarker level before and after the ingestion was observed.

Conclusion: The intake of the PSP beverage significantly decreased the serum levels of hepatic biomarkers, particularly the GGT level, in healthy men with borderline hepatitis.

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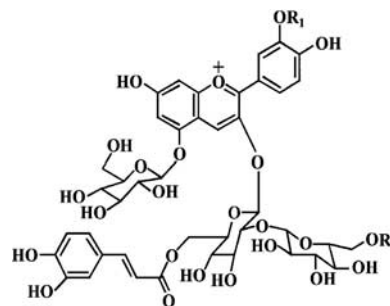
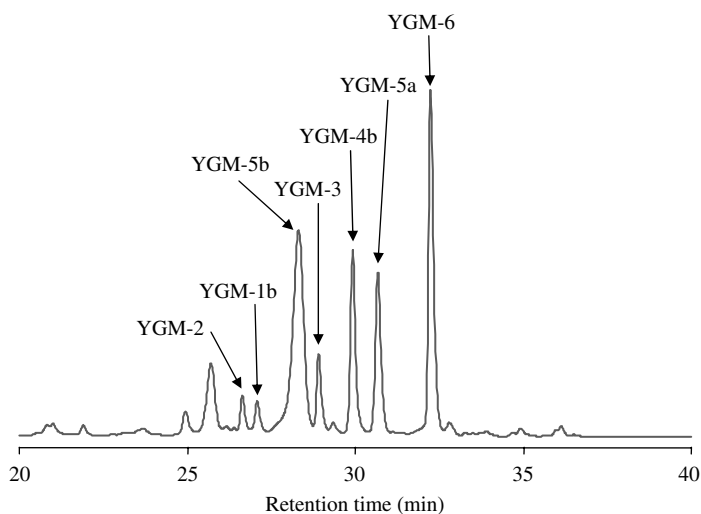
Introduction

Epidemiological studies indicate an inverse correlation between the intake of polyphenols and the incidence of chronic diseases, such as cardiovascular disease (CVD) and cancer (Negri *et al.*, 1991; Hertog *et al.*, 1993; Keli *et al.*, 1996). Fruits and vegetables rich in polyphenols have been

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Cyanidin-type (R ₁ =H)	Peonidin-type (R ₁ =CH ₃)	R ₂
YGM-1b	YGM-4b	caffeic acid
YGM-1a	YGM-5a	p-hydroxy benzoic acid
YGM-2	YGM-5b	H
YGM-3	YGM-6	ferulic acid

Figure 1 High-performance liquid chromatogram of the PSP beverage and chemical structure of the acylated anthocyanins detected in the beverage.

expected to be important materials for the maintenance of health.

Polyphenols are non-nutritive components; however, they have various biological functions, such as antioxidative, anti-inflammatory and antimutagenic activities.

Anthocyanins of purple sweet potato (PSP), *Ipomoea batatas* cultivar Ayamurasaki (Yamakawa *et al.*, 1998), which are mono- and di-acylated forms of cyanidin (YGM-1a, -1b, -2 and -3) and peonidin (YGM-4b, -5a, -5b and -6) (Figure 1), have a higher antioxidative activity than other anthocyanins. Despite the complex chemical structure of PSP anthocyanins, they are rapidly absorbed into the body, detected in blood and rapidly excreted in the urine of rats and humans (Suda *et al.*, 2002; Harada *et al.*, 2004).

Oral feeding of PSP anthocyanins increased the 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity in the urine of rats and humans, increased the resistance of low-density lipoprotein (LDL) to oxidation in rats and the suppressed carbon tetrachloride (CCl₄)-induced liver injury in rats (Suda *et al.*, 1997; Kano *et al.*, 2005).

Liver injury is induced by oxidative stress. For example, experimental acute liver injury with CCl₄ is characterized by centrilobular necrosis resulting from the formation of free radicals and reactive oxygen species during the metabolism of CCl₄ by cytochrome P450 and the activation of Kupffer cells by free radicals (Recknagel and Glende 1973; Edwards *et al.*, 1993). The damage of liver cells accompanies the release of liver function enzymes, such as γ -glutamyl transferase (GGT; EC 2.3.2.2), alanine aminotransferase (ALT; EC 2.6.1.1), and aspartate aminotransferase (AST; EC 2.6.1.2) into the blood (Skinner *et al.*, 1984; Shaper *et al.*, 1985). Clinically, these enzymes are often used as biomarkers

for liver injury or liver diseases. Recently, several epidemiological reports have shown that serum GGT is associated with risk factors of CVD, suggesting the possibility of the close relationship between oxidative stress and chronic diseases (Perry *et al.*, 1998; Lee *et al.*, 2003; Nakanishi *et al.*, 2003; Yamada *et al.*, 2003).

We have previously reported in a preliminary open study, that the serum levels of hepatic biomarkers in healthy volunteers were depressed after ingesting a PSP beverage rich in the acylated anthocyanins (Suda *et al.*, 1998). The ameliorative effect due to PSP beverage intake is clearly exerted in men who had one or more of GGT, AST and ALT over the normal range in their medical checkup, and who had a need to recover their liver function.

In light of these findings, we have examined the effect of the intake of a PSP beverage on the biomarkers of healthy men with the above conditions in this double-blind placebo-controlled study.

Methods

Test beverages

We used the fleshed PSP beverage (trade name 'AYAMUR-ASAKI') obtained from Yakult Honsha Co, Ltd (Tokyo, Japan) as the test beverage. The PSP beverage was a mixture of the concentrated PSP extract prepared from the tuber of *I. batatas* (Suda *et al.*, 2002) and taste adjusting materials (lemon juice and flavors); one bottle of beverage (125 ml) contained 0.7 g protein, no lipid, 14.8 g carbohydrates, 0.4 g dietary fiber, 5 mg sodium, and 200.3 mg anthocyanins (see below on analysis method). Using the same manufacturing

method, the placebo beverage was made from a PSP extract equivalent to 1/100 the volume of the PSP beverage, lemon juice, flavors and glucose solution to adjust to the same Brix value; the anthocyanin content was 1.7 mg/125 ml. Each of the PSP and the placebo beverage was packed in a similar white vessel, so as to be indistinguishable by their outward appearance.

To measure the anthocyanin content of the PSP and the placebo beverages according to the previous report (Suda et al., 2002), each beverage was evaporated to dryness under reduced pressure at 35°C, and the dried extract was re-dissolved into a 1.0% trifluoroacetic acid (TFA) aqueous solution. The absorbance of the sample solution was measured at 530 nm. The anthocyanin content was calculated from a calibration curve for YGM-5b and expressed as the YGM-5b equivalent per 1 ml of beverage. To determine the composition of anthocyanins in the PSP beverage, high-pressure liquid chromatography (HPLC) analysis was performed under the same conditions, which could detect the eight major peaks of the anthocyanins (YGM-1a to YGM-6) in PSP (Figure 1).

Subjects

Healthy adult male volunteers were recruited from Kumamoto city and the surrounding area by an advertisement of SAKURA Inc. (Kumamoto, Japan). The content and methods of the study were fully explained to all participants, and their written informed consent was obtained in writing before enrollment. The study was carried out in accordance to the Declaration of Helsinki, and was approved by the Kumamoto Clinical Examination Ethics Committee.

Forty-eight candidates were selected from applicants satisfying the following eligibility criteria: men between 30 and 60 years old; those with borderline levels of one or more hepatic function markers (over 80 IU/l of GGT, 42–99 IU/l of AST and 42–99 IU/l of ALT) excluding those with a more than 6-year history of impaired hepatic function according to medical diagnosis in those negative for hepatitis virus; those not taking abundant medication or dietary supplements that may affect hepatic function, such as those made from PSP, turmeric, a soft-shelled turtle, sesame or oyster; those without a history of food allergies, diabetes, cardiovascular, gastrointestinal or renal disease; and those who did not donate more than 200 ml of blood during the 4 weeks before the start of the study; those willing to complete a complex diet diary and to maintain their conventional lifestyle with regard to dietary and physical habits.

Study design

A double-blinded comparative study with two parallel groups was performed to test the effect of the PSP beverage in subjects with borderline hepatitis.

Following the entry period, the study was carried out for a period of 15 weeks, which included a 3-week pre ingestion period, an 8-week beverage-ingestion period and a 4-week

post-ingestion period. In the entry period and pre-ingestion period, a pre-clinical examination and a dietary survey were carried out to screen the qualified subjects as mentioned above. After the clinical examination, twice (at week -2 (-2 weeks) and 0 (0 weeks)) during the pre-ingestion period, the 48 subjects were randomly assigned to either the PSP beverage-ingestion group (PSP group) or the placebo beverage-ingestion group (placebo group) with stratification according to age, body mass index (BMI), and serum GGT, AST and ALT levels, and subjected to beverage-ingestion examination. The men in each group were instructed to drink two bottles of the beverage (125 ml/bottle) daily, every 10 ± 2 h. The total number of beverages ingested during this study period was 112 bottles per subject. After the beverage-ingestion period, the post-ingestion period immediately followed.

The subjects were advised not to change their current dietary habits or lifestyle during the study period. The subject was instructed to keep a diary about items as follows: (1) the number of bottles and time of ingesting the beverages, to obtain information as to whether the beverage was drunk at regular intervals; (2) the type, volume, alcohol content and beginning and finish time of ingesting alcoholic drinks, to calculate the amount of alcohol intake and (3) the lack of sleep, feelings of fatigue during the hour of rising, loss of appetite, feelings of nausea, diarrhea, bowel movements and so on to observe the changes in physical condition. Furthermore, the subjects were instructed to record the contents of all foods consumed for 7 consecutive days in every pre-ingestion period, beverage-ingestion period and post-ingestion period. These were filled out referring to the Standard Tables of Food Composition in Japan (5th edition).

Clinical examination for physical, blood and urine parameters was performed every 2 weeks. Blood was drawn between 08:00 and 11:00 hours after the subjects had fasted for at least 10 h. The first spontaneous urine was collected at home of the each subject. Systolic and diastolic blood pressures were measured on the left arm using a standard mercury sphygmomanometer while the subjects were seated. BMIs were calculated using the body weight and height.

During the 15-week study period, the 38 subjects (mean age 43.0 years (30–54 years)) completed the study according to the protocol. However, 10 subjects who were forced to discontinue the study due to the job transfer, who neglected the conditions of clinical examination, who had been forgetting to drink the test beverages and who did not maintain a regular dietary lifestyle were excluded from the final study analysis.

Biochemical analysis of blood and urine

Blood and urine samples were transported to the Clinical Research Center of the Kumamoto City Medical Association (Kumamoto, Japan). Blood samples were used for the analysis of total protein, albumin, globulin, albumin/globulin ratio, total bilirubin, direct bilirubin, indirect

bilirubin, ALT, AST, GGT, alkaline phosphatase, cholinesterase, lactate dehydrogenase, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, urea nitrogen, creatinine, uric acid, sodium, chloride, potassium, magnesium, calcium, glucose, hemoglobin A_{1C}, red blood cell count, white blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, neutrophil, eosinophil, basophil, lymphocyte and monocyte. Urine was used for the analysis of glucose, protein and creatinine.

Dietary survey

Nutrient intake (energy, protein, fat, carbohydrate, ash, sodium, potassium, calcium, magnesium, phosphorus, iron, zinc, vitamin A (retinal equivalent), vitamin E, vitamin C, cholesterol, total dietary fiber, salt (as NaCl), protein ratio, fat ratio, carbohydrate ratio and alcohol intake) was calculated on the basis of the dietary record for 7 consecutive days in three periods for this study, using nutritional software (The Eiyoushidou PRO ver1.0, Access Intelligent, Ltd, Kumamoto, Japan) based on the Standard Tables of Food Composition in Japan (5th edition).

Statistical analysis

The average of the clinical data at -2 and 0 weeks was calculated and used as a baseline value in the preingestion period before the beginning of the beverage ingestion. Data were expressed as the means ± standard error of mean (s.e.m.). Variations in the clinical parameters and food intake in the study were analyzed by two-way repeated measures analysis of variance (ANOVA) for two groups (PSP and placebo) × 7 time points (baseline, 2, 4, 6, 8, 10 and 12 weeks) using SPSS software for Windows (version 12.0J, SPSS Japan, Tokyo, Japan). For the changes in the GGT, AST and ALT levels in the study period, differences between the baseline value and the following time points within the group, and differences between the PSP and the placebo groups for the same period, were analyzed using the Dunnett multiple comparison among the times for the paired data and with the unpaired *t*-test using the software of the SAS Preclinical Package for Windows (version 5.0, SAS Japan, Tokyo, Japan). The correlation between both distances of each hepatic parameter level and the amount of alcohol consumed before and after the ingestion of the beverages was analyzed by linear correlation using Kyplot software for Windows (Kyence Inc, Tokyo, Japan). Two-sided *P*-values < 0.05 were considered to be significant.

Results

Baseline characteristics of the subjects

The baseline clinical characteristics of the subjects are shown in Table 1. Overall, there was no difference between the PSP

Table 1 Baseline clinical characteristics of subjects

Items	PSP group	Placebo group
No of subjects	20	18
Age (year)	42.1 ± 1.4	43.9 ± 1.2
Height (cm)	172.3 ± 1.1	172.0 ± 1.0
Weight (kg)	76.1 ± 1.8	75.1 ± 2.6
BMI (kg/cm ²)	25.6 ± 0.5	25.3 ± 0.7
GGT (IU/L)	103.6 ± 17.3	91.6 ± 11.1
AST (IU/L)	35.5 ± 2.2	32.5 ± 2.3
ALT (IU/L)	51.3 ± 5.0	46.5 ± 5.1

Values are mean ± s.e.m. There were no significant differences between the groups.

The average of clinical data at week -2 and week 0 is used as baseline value. Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; BMI, body mass index; GGT, γ -glutamyl transferase.

and placebo groups in any of the parameters of age, height, weight, BMI, GGT, AST and ALT.

Changes in physical, urine and blood parameters, and dietary status

Physical and urine parameters were not changed during the study period within the same group, and there was no difference between both the PSP and placebo groups for the same period.

Most of the blood parameters were also unchanged. However, in the serum GGT, AST and ALT levels, the interactions between the groups and times were observed by two-way repeated measures ANOVA. These parameters were further analyzed as described below. The dietary status, such as nutrition and alcohol consumption, was not changed throughout the pre-observation, beverage-ingestion and post-observation periods within the same group, and there was no difference between the PSP and placebo groups.

Changes in GGT, AST and ALT

The PSP group (*n* = 20) constituted 11 subjects with hyper GGT over the normal range (> 80 IU/l), five with hyper-AST (> 42 IU/l), and 13 with hyper-ALT (> 42 IU/l) at the start point (Figure 2). The placebo group (*n* = 18) contained 12-hyper GGT subjects, 4 hyper-AST, and 10 hyper-ALT subjects.

The changes in the GGT, AST and ALT value after ingesting PSP and placebo beverages are shown in Figures 3–5. GGT values against the baseline value in the PSP group were decreased at 4 weeks (mean difference, -19.0 IU/l; CI₉₅, -35.3 to -2.7; *P* = 0.016), 6 weeks (-19.9 IU/l; CI₉₅, -36.2 to -3.6; *P* = 0.010), 8 weeks (-20.9 IU/l; CI₉₅, -37.2 to -4.6; *P* = 0.006) and 12 weeks (-20.12 IU/l; CI₉₅, -36.4 to -3.8; *P* = 0.009) in all subjects (Figure 3a) and at 4 weeks (-37.0 IU/l; CI₉₅, -64.8 to -9.3; *P* = 0.004), 6 weeks (-37.7 IU/l; CI₉₅, -65.4 to -10.0; *P* = 0.004), 8 weeks (-37.3 IU/l; CI₉₅, -65.0 to -9.6; *P* = 0.004), 10 weeks (-30.0 IU/l; CI₉₅, -57.8 to

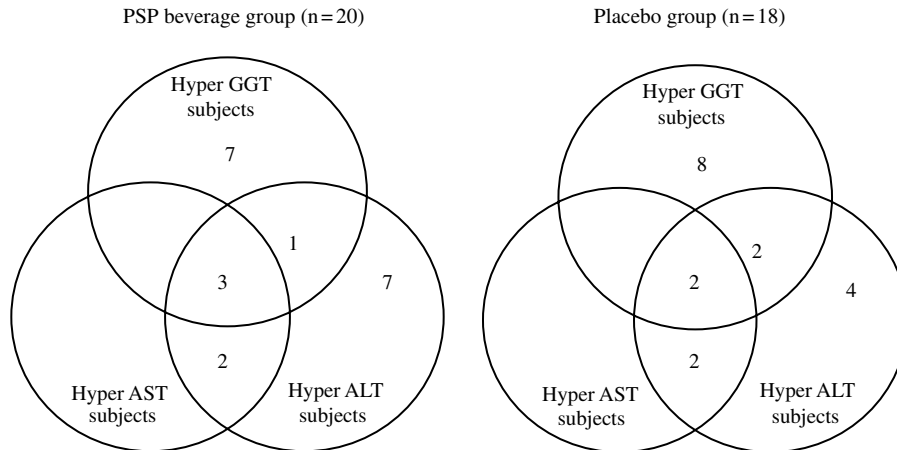


Figure 2 Composition of the subjects classified hepatic biomarkers. Numbers in circles indicate numbers of subjects showing values over the normal range. Hyper-GGT, hyper-AST and hyper-ALT subjects had serum levels of over 80, 42 and 42 IU/l, respectively.

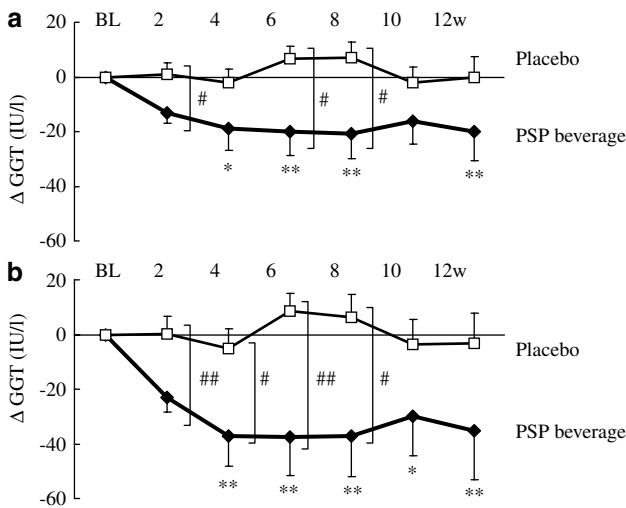


Figure 3 Change in GGT level in the PSP beverage-ingestion and the placebo-ingestion groups. (a) GGT pattern of all subjects; (b) GGT pattern of the hyper-subjects. Baseline level (BL) is set to zero. #*P* indicate significant #*P*<0.05 and ##*P*<0.01, differences in the GGT levels between the two groups. **P*<0.05 and ***P*<0.01 indicate significant differences in the GGT levels after ingestion against the baseline level. Data are shown as means ± s.e.m.

–2.3; *P*=0.029) and 12 weeks (–35.1 IU/l; CI₉₅, –62.9 to –7.4; *P*=0.007) the hyper-subjects (Figure 3b). GGT values in the placebo group, however, were not changed throughout the entire period. A significant difference of the GGT values between both PSP and placebo groups are seen at 2 weeks (–14.1 IU/l; CI₉₅, –25.4 to –2.7; *P*=0.017), 4 weeks (–16.8 IU/l; CI₉₅, –36.2 to 2.5; *P*=0.081), 6 weeks (–26.7 IU/l; CI₉₅, –47.6 to –5.7; *P*=0.014) and 8 weeks (–27.9 IU/l; CI₉₅, –49.9 to –5.9; *P*=0.014) in all subjects, and at 2 weeks

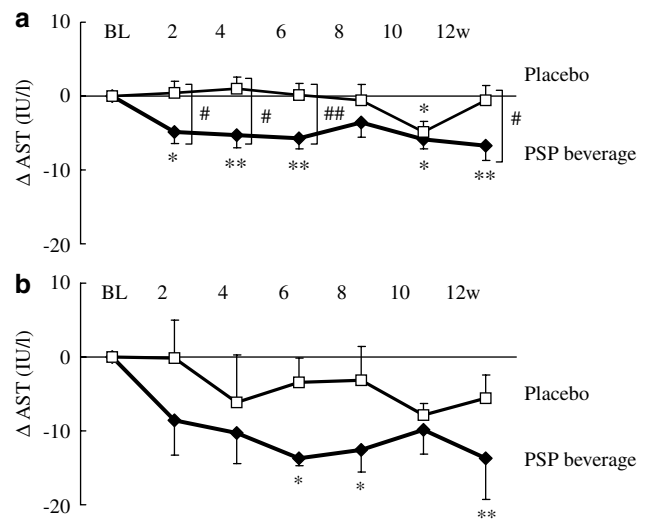


Figure 4 Change in the AST level in the PSP beverage-ingestion and the placebo-ingestion groups. (a) AST pattern of all subjects; (b) AST pattern of the hyper-subjects. Baseline level (BL) is set to zero. **P*<0.05 and ***P*<0.01, indicate significant differences in the AST levels between the two groups at **P*<0.05 and ***P*<0.01 indicate significant differences in the AST levels after the ingestion against the baseline level. Data are shown as means ± s.e.m.

(–23.5 IU/l; CI₉₅, –40.4 to –6.6; *P*=0.009), 4 weeks (–31.8 IU/l; CI₉₅, –59.3 to –4.4; *P*=0.025), 6 weeks (–46.3 IU/l; CI₉₅, –77.7 to –14.9; *P*=0.006) and 8 weeks (–43.8 IU/l; CI₉₅, –77.9 to –9.6; *P*=0.014) in the hyper-subjects.

AST values against the baseline value in the PSP group were decreased at 2 weeks (–4.9 IU/l; CI₉₅, –9.0 to –0.8; *P*=0.012), 4 weeks (–5.4 IU/l; CI₉₅, –9.4 to –1.3; *P*=0.005), 6 weeks (–5.7 IU/l; CI₉₅, –9.7 to –1.6;

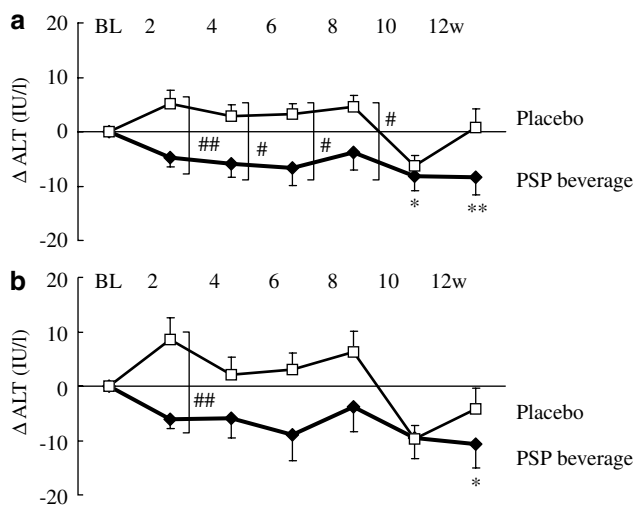


Figure 5 Change in ALT level in the PSP beverage-ingestion and the placebo-ingestion groups. (a) ALT pattern of all subjects; (b) ALT pattern of the hyper-subjects. Baseline level (BL) is set to zero. $P < 0.05$ and $**P < 0.01$ indicate significant differences in the ALT levels between the two groups at $P < 0.05$ and $**P < 0.01$ indicate significant differences in the ALT levels after the ingestion against the baseline level. Data are shown as means \pm s.e.m.

$P = 0.003$), 10 weeks (-5.9 IU/l; CI_{95} , -9.9 to -1.8 ; $P = 0.002$) and 12 weeks (-6.7 IU/l; CI_{95} , -10.8 to -2.6 ; $P < 0.001$) in all subjects, and at 6 weeks (-13.7 IU/l; CI_{95} , -25.5 to -1.9 ; $P = 0.019$), 8 weeks (-12.5 IU/l; CI_{95} , -24.3 to -0.7 ; $P = 0.035$), and 12 weeks (-13.7 IU/l; CI_{95} , 25.5 to -1.9 ; $P = 0.019$) in the hyper-subjects (Figure 4). AST values in the placebo group were not changed throughout the entire period, except for a decrease at 10 weeks (-4.8 IU/l; CI_{95} , -9.3 to -0.3 ; $P = 0.033$) in all subjects, and were not changed in the hyper-subjects. Significant differences in the AST values between both the PSP and placebo groups were seen at 2 weeks (-5.4 IU/l; CI_{95} , -10.0 to -0.8 ; $P = 0.024$), 4 weeks (-6.3 IU/l; CI_{95} , -11.3 to -1.4 ; $P = 0.014$), 6 weeks (-5.8 IU/l; CI_{95} , -9.8 to -1.8 ; $P = 0.005$) and 12 weeks (-6.2 IU/l; CI_{95} , -11.5 to -0.8 ; $P = 0.025$) in all subjects, and were not observed in the hyper-subjects.

ALT values against the baseline value in the PSP group were decreased at 10 weeks (-8.2 IU/l; CI_{95} , -15.0 to -1.4 ; $P = 0.011$) and 12 weeks (-8.5 IU/l; CI_{95} , -15.3 to -1.7 ; $P = 0.008$) in all subjects and at 12w (-10.7 IU/l; CI_{95} , -20.6 to -0.9 ; $P = 0.027$) in the hyper-subjects (Figure 5). The ALT values in the placebo group were not changed during whole period. A significant difference of the ALT value between both the PSP and placebo groups was seen at 2 weeks (-9.9 IU/l; CI_{95} , -15.9 to -4.0 ; $P = 0.002$), 4 weeks (-8.7 IU/l; CI_{95} , -15.3 to -2.0 ; $P = 0.012$), 6 weeks (-9.8 IU/l; CI_{95} , -17.6 to -1.9 ; $P = 0.016$) and 8 weeks (-8.4 IU/l; CI_{95} , -16.2 to -0.5 ; $P = 0.037$) in all subjects, and at 2 weeks (-14.5 IU/l; CI_{95} , -23.1 to -5.9 ; $P = 0.002$) in the hyper-subjects. The

differences of the change in the ALT might be explained by the tendency to decrease in the PSP group and to increase in the placebo group during the intake period.

Correlation between hepatic biomarkers and alcohol intake

Hepatic marker levels are affected by the intake of alcohol as well as liver diseases (Skinner *et al.*, 1984; Shaper *et al.*, 1985). In particular, the GGT levels rise with alcohol consumption, even in the absence of chronic liver diseases (Whitehead *et al.*, 1978; Chick *et al.*, 1981). We analyzed the correlation between alcohol consumption and each marker level before and after the ingestion of the beverages. The difference of the GGT level was not associated with that of alcohol consumption in the PSP group ($r = 0.195$, $P = 0.411$) or the placebo group ($r = -0.126$, $P = 0.617$) in all subjects. It was irrelevant in the case of the hyper GGT subjects ($r = 0.146$, $P = 0.668$ in the PSP group and $r = -0.157$, $P = 0.646$ in the placebo group). Furthermore, there was no association between the difference of the serum AST and ALT levels with that of alcohol consumption before and after the ingestion of the beverages (data not shown).

Discussion

The PSP beverage contains at least eight anthocyanins, each of which has a high radical scavenging activity (Kano *et al.*, 2005). In the case of ingesting the beverage, two major components, YGM-2 (cyanidin 3-*O*-(2-*O*-(6-*O*-(*E*)-caffeoyl- β -*D*-glucopyranosyl)- β -*D*-glucopyranoside)-5-*O*- β -*D*-glucopyranosyl)- β -*D*-glucopyranoside) and YGM-5b (peonidin 3-*O*-(2-*O*-(6-*O*-(*E*)-caffeoyl- β -*D*-glucopyranosyl)- β -*D*-glucopyranoside)-5-*O*- β -*D*-glucopyranosyl)- β -*D*-glucopyranoside)), are selectively detected in the blood and rapidly excreted in the urine of rats and humans (Suda *et al.*, 2002; Harada *et al.*, 2004; Kano *et al.*, 2005). The urinary recovery rate of PSP anthocyanins is very low (0.01% in humans and 0.11% in rats); however, the radical-scavenging activity in the urine is elevated after the ingestion of the PSP beverage (Kano *et al.*, 2005). These facts imply that even if the absorbability is low, the PSP anthocyanins in the body are sufficient to exert some physiological effects.

Since serum GGT, AST and ALT activities reflect damage to the cells, particularly liver cells, they are clinically used as biomarkers for liver injury or liver diseases. In rats, the oral feeding of PSP anthocyanins suppressed the increase in serum hepatic enzyme levels in rats due to CCl_4 administration (Suda *et al.*, 1998; Kano *et al.*, 2005), showing the efficacy of PSP anthocyanins against chemical-induced hepatotoxicity. In this human study, the ingestion of the PSP beverage decreased the serum levels of these hepatic markers in healthy men with these borderline levels. This was the case in subjects whose levels exceeded the normal range of each marker. The previous animal experiments and this human study indicate that acylated anthocyanins rich

in PSP beverages possess the potential capacity for hepatoprotection.

In this study, we instructed the subjects not to change their current dietary habits and conventional lifestyle, including alcohol intake. Consequently, alcohol consumption was not statistically different during the whole period or between the groups. As the hepatic marker levels are probably affected by alcohol consumption on an individual basis, the association between those factors was analyzed using the differences of alcohol consumption and of each marker level before and after the ingestion of the beverages. We observed no correlation between alcohol consumption and the marker levels, indicating that the intake of PSP beverage, but not alcohol, was closely related to the decreases in these marker levels.

A recent cohort study showed that GGT was inversely associated with fruit intake, but directly so with alcohol and meat consumption (Lee *et al.*, 2004). GGT, widely distributed in the human body, plays a key role in antioxidation systems, while maintaining intracellular glutathione recycling in the cells (Kugelman *et al.*, 1994, Takahashi *et al.*, 1997, Karp *et al.*, 2001). The elevation of the serum GGT level is induced by an increase in the production of reactive oxidative species after ethanol treatment (Cederbaum, 2002). Furthermore, serum GGT, AST and ALT levels might be a marker of the progression of lifestyle-related diseases due to oxidative stress (Perry *et al.*, 1998; Falck-Ytter *et al.*, 2001; Lee *et al.*, 2003). Our observation that serum hepatic marker levels, particularly the GGT level, were reduced by the ingestion of PSP beverage might indicate the contribution of PSP anthocyanins to the alleviation of oxidative stress.

In conclusion, the ingestion of PSP beverage decreased the serum levels of the hepatic markers in healthy men with borderline hepatitis in this double-blind placebo-controlled study. There was no correlation between the alcohol intake and each marker level. This study suggested that the PSP beverage may have a potential capacity for hepatoprotection against oxidative stress.

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