Enhancement of Alcohol Metabolism by Sprouted Peanut Extract in SD Rats

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ABSTRACT: Excessive ethanol intake is known to induce a number of physiological symptoms, including headache, dizziness and vertigo. In this study, we investigated the attenuation effect of sprouted peanut extract (SPE) on ethanol-induced hangover in male Sprague-Dawley rats. The animals were divided into five groups: the control group, which was administered ethanol only; the ethanol plus SPE experimental groups, which were administered ethanol and 100, 200, or 400 mg SPE/kg b.w.; and the positive control group, which was administered ethanol plus DAWN808®, a commercial product. SPE-suspended water was delivered to rats via gavage 15 h and 30 min before the administration of ethanol. Blood was collected from the tail 0, 1, 3, and 5 h after ethanol administration. The results showed that serum ethanol concentrations were significantly lower in SPE treated groups than in the control group. Furthermore, hepatic alcohol and acetaldehyde dehydrogenase activities were enhanced by SPE in a dose dependent manner. These results suggest that SPE could be useful in attenuating hangover after alcohol consumption.

Keywords: sprouted peanut, hangover, alcohol metabolism, SD rat

INTRODUCTION

Hangover is featured by adverse physical and psychological problems that occur the morning following intake of excess doses of alcohol. Common symptoms reported by people experiencing hangover are headache, gastro-intestinal complaints, sweating, hyper-excitability, dry mouth, anorexia, diarrhea, tremulousness, dizziness, fatigue, and vertigo (1-3).

Our previous study showed that peanut sprouts have anti-obesity effects in high-fat-diet-induced C57BL/6J obese mice, laxative effects in Sprague-Dawley (SD) rats (4,5), and a neuroprotective effect on glutamate-induced neurotoxicity (unpublished). In addition, sprouted peanut extract (SPE) has an antioxidant effect and stimulates the activity of detoxifying enzymes via Nrf2 activation in dermal fibroblasts (4,6). Several studies have demonstrated that phenolic compounds in sprouted peanuts are responsible for various biological activities (5,7). Furthermore, the sprouting of peanuts results in abundant production of asparagine/aspartic acid, which is believed to help relieve hangover after drinking ethanol.

However, the hangover-relieving effect of SPE and its potential mechanism of action have not been studied so far. This study was conducted to investigate whether SPE has a protective effect against acute alcohol-induced hangover in SD rats.

MATERIALS AND METHODS

Preparation of SPE

The SPE was prepared following a method previously described (5). Briefly, peanut (PoongAn cultivar) sprouts were freeze-dried and powdered. Powdered peanut sprouts were extracted in 40 volumes of an 80% ethanol solution with agitation at 70°C for 90 min. Extracts were filtered, concentrated using a rotary evaporator, and freeze-dried prior to use.

Animals

SD rats (6-week-old) were obtained from Samtako Bio Korea Co., Ltd. (Osan, Korea) and housed in plastic cages at a constant temperature and humidity under a 12-h

light/dark cycle. Animals were given free access to AIN-76A Based Cereal Feed purchased from Feedlab (Guri, Korea) and water. The hangover test was begun after 1 wk of adaptation to the lab environment. The study was conducted in accordance with appropriate institutional regulations provided by the Ministry of Food and Drug Safety. All animal experiments were approved by the "Animal Ethic Committee of Kyungpook National University" (permission number KNU2012-123).

Experimental design
Rats were divided into five groups: (1) the control group, which was administered ethanol only; (2-4) the ethanol plus SPE experimental groups, which were administered ethanol and 100, 200, or 400 mg SPE/kg b.w.; and (5) the positive control group, which was administered ethanol plus DAWN808®. DAWN808® was previously reported to possess an alcohol clearance effect (8). SPE was suspended in water and administered via gavage. At 15 h and 30 min post-SPE administration, rats in each group received a dose of ethanol (40%, 4 g/kg) via gavage. Blood was collected from the tail 0, 1, 3, and 5 h after ethanol administration. Serum was prepared from collected blood by centrifugation at 3,000 g for 15 min and stored until further analysis.

Serum alcohol concentration
An Ethanol Assay Kit (Roche, R-Biopharm GmbH, Darmstadt, Germany) was used, with slight modification, to determine serum ethanol concentration. Briefly, 60 μL of serum was mixed with 1 ml of reaction mixture in a cuvette. After mixing for 3 min, the absorbance (A1) was measured at a wavelength of 340 nm. A suspension was added and the mixture was incubated for 3 additional min. The second absorbance (A2) was measured at the same wavelength as A1. During the entire experiment, the cuvette was capped to prevent alcohol evaporation. The concentration of ethanol was calculated according to the equation provided with the kit.

Alcohol dehydrogenase (ADH) activity
The activity of ADH was determined by Bostian's method with some modifications (9). The reaction mixture was composed of 1.4 mL of distilled water, 0.75 mL of 1.0 M Tris-HCl buffer (pH 8.8), 0.3 mL of 20 mM NAD$^+$, 0.3 mL of ethanol, and 0.1 mL of SPE. The enzyme source was prepared from Aroclor 1245-induced rat liver homogenate (S9 fraction) (10). The reaction mixture was pre-incubated with 0.15 mL of enzyme source for 5 min at 30°C and the change in absorbance at 340 nm was monitored for 5 min to determine the amount of NADH generated. ADH activity was calculated relative to a sample blank.

Aldehyde dehydrogenase (ALDH) activity
ALDH activity was determined by Bostian's method with modifications (9). The reaction mixture was composed of 2.1 mL of distilled water, 0.3 mL of 1.0 M Tris-HCl buffer (pH 8.8), 0.1 mL of 20 mM NAD$^+$, 0.1 mL of acetaldehyde, 0.1 mL of KCl, 0.1 mL of 0.33 M 2-mercaptoethanol, and 0.1 mL of SPE. The enzyme source was prepared from Aroclor 1245-induced rat liver homogenate (S9 fraction) (10). The reaction mixture was pre-incubated with 0.1 mL of the enzyme source for 5 min at 30°C and the change in absorbance at 340 nm was monitored for 5 min to determine the rate of NADH generation. ALDH activity was calculated relative to a sample blank.

Assessment of aspartate aminotransferase (AST) and alanine aminotransferase (ALT)
AST and ALT activities are commonly utilized to assess hepatic function. Serum levels of AST and ALT were assessed using a GOT/GPT Kit (ASAN pharmaceutical, Seoul, Korea). Serum was diluted with phosphate buffered saline (PBS) before the assay. Yellow-colored hydrazones, which are metabolites of AST and ALT, were measured at a wavelength of 505 nm.

Statistical analysis
Values are presented as the mean±standard deviation (SD). Statistical differences among groups were analyzed by one-way ANOVA, followed by Duncan's multiple range post hoc test ($P<0.05$). All statistical analyses were performed using SPSS 20 statistical software (SPSS Inc., Chicago, IL, USA).

RESULTS

Determination of ethanol concentration in serum
Alcohol concentrations in blood serum from ethanol-loaded rats were measured at 0, 1, 3, and 5 h after orogastric administration of 40% ethanol. Serum alcohol concentrations ranged from 0.61 to 0.59 g/L at 5 h after ethanol exposure in the positive control group, which was administered with ethanol plus DAWN808®, and in the ethanol plus 100 mg SPE/kg b.w. experimental group. The serum alcohol concentration of the experimental group fed SPE (100 mg/kg b.w.) was 1.5 times lower than that of the negative control group, which was administered ethanol alone (0.95 g/L). The serum alcohol concentrations were also significantly lower in the groups administered with 200 and 400 mg SPE/kg b.w. (Fig. 1), compared with the negative control group. The lowest serum alcohol concentration (0.41 g/L) was observed in the group treated with SPE (400 mg/kg b.w.). Serum alcohol concentrations of the groups administered
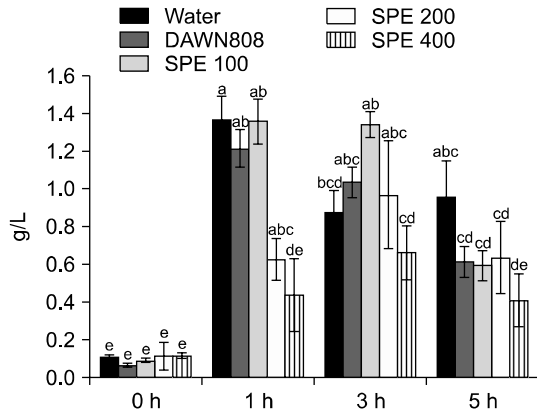
Fig. 1. Effect of SPE administration on serum ethanol concentrations in ethanol-loaded SD rats. Data were expressed as the means±SD (n=7). [a-e]Means with different superscripts are significantly different ($P<0.05$).
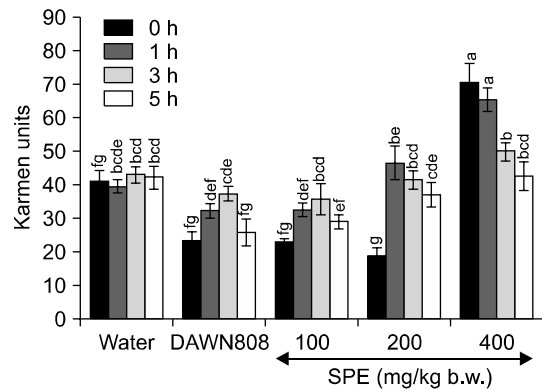

Fig. 3. Effect of SPE administration on serum AST activity in ethanol-loaded SD rats. Data were expressed as the means±SD (n=7). [a-g]Means with different superscripts are significantly different ($P<0.05$).
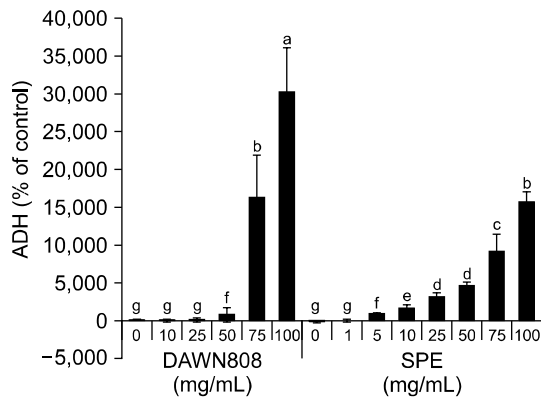

Fig. 2. Effects of DAWN808® and SPE on hepatic ADH activity in ethanol-loaded SD rats. Data were expressed as the means±SD (n=4). [a-g]Means with different superscripts are significantly different ($P<0.05$).
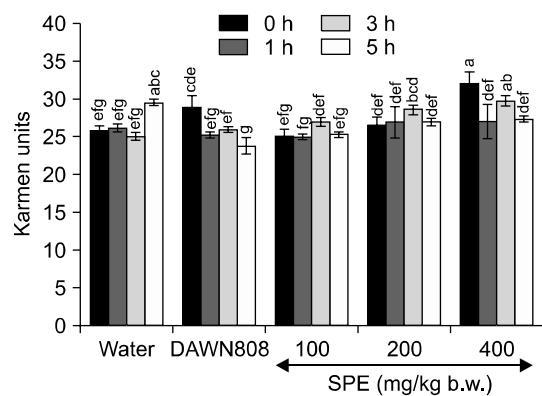

Fig. 4. Effect of SPE administration on serum ALT activity in ethanol-loaded SD rats. Data were expressed as the means±SD (n=7). [a-g]Means with different superscripts are significantly different ($P<0.05$).

Table 1. Effect of DAWN808® and SPE on hepatic ALDH activity

Samples	(mg/mL)	ALDH activity (%)[1]
None		100±39.3[bc]
DAWN808®	10	72.8±5.8[c]
	25	82.9±44[bc]
	100	158.6±47.5[abc]
SPE	1	79.4±37.6[c]
	10	137.3±66.5[abc]
	25	112.8±68.4[bc]
	50	232.2±102.3[a]
	75	141.4±25.4[abc]
	100	174.9±83.1[abc]

[1]Values represent means±SD of four independent measurements.
[a-c]Means with different superscript are significantly different ($P<0.05$)

DAWN808® or SPE (200 mg/kg b.w.) were decreased in a time-dependent manner.

Activation of ADH activity by SPE

SPE increased hepatic ADH activity in rats in a dose-dependent manner (Fig. 2). In particular, 75 mg/mL and 100 mg/mL doses of SPE were associated with higher ADH activity than comparable doses of DAWN808® at the higher doses than 50 mg/mL.

Effect of ALDH activity by SPE

The ALDH activities of groups fed SPE were higher than those of the positive control group. Furthermore, enzyme activity tended to increase in proportion to the dose of SPE used (Table 1).

Effect of SPE on serum AST and ALT activities

The effects of SPE on AST and ALT activities are shown in Fig. 3 and 4. Considering that the normal level of AST is from 8 to 40 Karmen units, it is most likely that treatment with moderate concentrations of SPE (100 mg/kg b.w., 200 mg/kg b.w.) did not cause serious liver damage. However, a high concentration of SPE (400 mg/kg b.w.) was associated with high AST activity, which may be due to liver damage. There was no major difference in ALT activity among experimental and positive control groups.

DISCUSSION

Hangover symptoms are classified into two different types: acute and chronic, depending on the duration of alcohol consumption. Moderate alcohol intake causes acute hangover because alcohol is generally degraded by ADH into acetaldehyde. Alcohol concentration in the blood is reduced over time because acetaldehyde is converted to acetic acid through an oxidation reaction (11).

We measured hepatic ADH activity to determine whether the relieving effect of SPE on alcohol-induced hangover in SD rats was caused by an increase in the activity of enzymes involved in alcohol metabolism.

Acetaldehyde, which is a metabolite produced by hepatic alcohol catabolism, is a major contributor to hangover. Its blood level can be decreased by ADH activation. In the case of delayed elimination of the compound from the body, acetaldehyde accumulates in the liver, leading to severe hangover. Therefore, we determined the activity of ALDH, a direct detoxifying enzyme which converts acetaldehyde into acetate (12).

As shown in Fig. 4 and Table 1, SPE promoted alcohol elimination by increasing ADH and ALDH activities. At present, it is not clear what compounds in SPE are responsible for promoting the removal of alcohol from the blood. Some phytochemicals, including resveratrol, are reported to be present in sprouted peanuts. We have also found that the sprouting of peanuts significantly increases the level of aspartic acid and asparagine (unpublished). Although resveratrol is reported to protect against ethanol-induced tissue, it is unlikely that resveratrol in SPE contributes to the observed lowering of blood alcohol levels because its concentration in SPE is negligible (5,13). Phenolic compounds, which are produced in abundance during the sprouting of peanuts, might play a major role in ethanol metabolism by inducing the activity of alcohol-metabolizing enzymes (5).

In conclusion, our current data demonstrated that SPE slightly attenuated alcohol-induced acute hangover in SD rats. These results suggest that SPE could be used as a functional food ingredient for attenuating hangover after excess alcohol consumption.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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