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Effects of the herb mixture, DTS20, on oxidative stress and plasma alcoholic metabolites after alcohol consumption in healthy young men

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ABSTRACT

Background: This study was designed to investigate the effect of a herbal mixture extract (DTS20) on the attenuation of oxidative stress and hangover after alcohol consumption in healthy volunteers.

Methods: DTS20 consists of *Viscum album* L. (40%), *Lycium chinense* L. (30%), *Inonotus obliquus* (20%), and *Acanthopanax senticosus* H. (10%). We recruited healthy, nonsmoking, adult men volunteers aged between 21 years and 30 years to participate in a crossover trial. Twenty participants received either one package of placebo with 200 mL water or DTS20 with 200 mL water. Thirty minutes later, the volunteers ingested one bottle of Soju, which is a commercially available liquor (19% alcohol in 360 mL).

Results: Volunteers received the opposite treatment after a 1-week washout period. DTS20 is mainly composed of sugars (564.5 mg/g) and polyphenol (28.2 mg/g). Alcohol levels in the DTS20 group were significantly lower than the control group at 2 hours after drinking Soju ($p < 0.05$). Acetaldehyde levels in the DTS20 group tended to be lower than the control group at 2 hours after drinking Soju, but was not significantly different. The antioxidant activity level was also significantly different between the control and DTS20 group 2 hours after drinking Soju ($p < 0.05$). No differences in plasma alanine transaminase or aspartate transaminase levels were observed between plasma levels before drinking and 2 hours after drinking Soju in the control group.

Conclusion: It was concluded that DTS20 reduced oxidative stress and hangover by mitigating plasma alcohol concentrations and elevating antioxidative activity in healthy male adults.

1. Introduction

Overindulgence of alcoholic beverages causes a hangover, which is characterized by unpleasant physical and psychological signs. Major hangover signs include thirst, vomiting, fatigue, dizziness, and headache, which may be due to toxicity, dehydration, and malabsorption of essential nutrients induced by alcohol and its metabolite acetaldehyde.[1,2] Most of the ethanol that is consumed is metabolized

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to acetaldehyde by nicotinamide adenine dinucleotide-dependent alcohol dehydrogenase and further to acetic acid by nicotinamide adenine dinucleotide-dependent aldehyde dehydrogenase in the liver. These metabolites induce cytotoxicity, headache, abdominal pain, and osmotic changes that lead to dehydration as well as lipid peroxidation-mediated tissue injury.[3,4]

Diverse natural products, including Oriental medicinal preparations, have been screened as candidates for alleviating the symptoms of a hangover. Traditional plant medicines for hangover symptoms include the use of *Evodiae fructus* extracts,[5] root extracts of *Pueraria lobata*,[6] and a mixture of Korean medicinal herbs (*Panax ginseng*, *Liriope platyphylla*, and others).[7]

Previously, we investigated the effect of herbal mixture extract (DTS20), that contains substances from *Viscum album* L., *Lycium chinense* L., *Inonotus obliquus*, and *Acanthopanax senticosus* H., on alcohol degradation.[8] Orally administered DTS20 results in decreased gastric mucous membrane damage in mice. In addition, intraperitoneal administration of DTS20 results in anti-inflammatory effects on vascular permeability inhibition tests. DTS20 also reduces the concentration of nitric oxide and tumor necrosis factor-α in lipopolysaccharide-activated macrophages. Thus, DTS20 possesses the potential to stimulate alcohol degradation and inhibit inflammatory effects in mice.

The purpose of this study was to evaluate antioxidant activities and ability to reduce plasma alcohol concentration of DTS20 in human volunteers. We tested the effects of DTS20 after healthy students consumed alcohol.

2. Methods

2.1. *Herb mixture (DTS20) preparation*

The herb mixture was consisted of *V. album* L. (40%), *L. chinense* L. (30%), *I. obliquus* (20%), and *A. senticosus* H. (10%). The air dried herb mixture (1,000 g) was extracted with water (10 L, repeated three times) in a percolator (specify) at 100 °C for 4 hours. The aqueous extract of herb mixture was filtered twice by vacuum filtration on a Buchner funnel with Whatman No. 2 filter paper, concentrated with an evaporator (Lab extreme, Inc., Kent city, MI, USA), and then lyophilized (IlShin Lab Co. Ltd., Namyangju, Korea). The concentrate was lyophilized and packaged in a stick pack, with about 2.5 g of extract packaged in a stick.[5,8,9]

2.2. *Chemical components*

Total sugar, uronic acid, and reducing sugar were determined by phenol-sulfuric acid,[10] m-hydroxydiphenyl,[11] and 3,5-dinitrosalicylic acid[12] using glucose, galacturonic acid, and glucose as standards, respectively. Total polyphenol was determined using the Folin–Ciocalteu method[13] with garlic acid as a standard. All samples were analyzed in triplicate.

2.3. *Radical scavenging activity*

The scavenging activities on 2, 2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals were measured according to the method of Re et al[14] and Cheung and Cheung,[15] respectively. The radical scavenging activities (%) were calculated with the following equation:

$$\text{Radical scavenging activity}(\%) = (1 - \text{Abs}_{\text{sample}}/\text{Abs}_{\text{control}}) \times 100 \quad (1)$$

where $\text{Abs}_{\text{sample}}$ is the absorbance in the presence of sample, and $\text{Abs}_{\text{control}}$ is the absorbance in the absence of sample.

2.4. *Volunteers and study design*

We recruited healthy adult volunteers aged between 21 years and 30 years to participate in a crossover trial. Twenty participants received either one package of placebo with 200 mL water or DTS20 (2.5 g) with 200 mL water. Thirty minutes later, the volunteers ingested one bottle of Soju, which is a commercially available liquor (19% alcohol in 360 mL). Volunteers received the opposite treatment after a 1-week washout period. The study was approved by the Ethical Committee for Human Experimentation of the Korea University, Seoul (KU-IRB-09-09-A-3) and was conducted in accordance with its rules and regulations.

2.5. *Plasma thiobarbituric acid reactive substances and ascorbic acid equivalent antioxidant capacity*

Blood was withdrawn just before drinking alcohol and at 2 hours after drinking alcohol. The blood sample was centrifuged at 3,000 *g* for 10 minutes at 4 °C to obtain plasma. Lipid peroxidation was estimated by measuring the concentration of thiobarbituric acid reactive substances (TBARS) according to the method of Quintanilha et al,[16], using malondialdehyde as the standard. Plasma (1 mL) was added to 2 mL of 0.017 mm thiobarbituric acid plus 3.36 μm butylated hydroxy toluene and incubated for 15 minutes at 100 °C. After cooling, the absorbance was measured at 535 nm. Data were expressed as TBARS/mM of plasma.

After ABTS radical scavenging activity was measured, ascorbic acid equivalent antioxidant capacity (AEAC) was estimated calculated as the following equation:

$$\text{AEAC} = (\Delta A_{\text{sample}}/\Delta A_{\text{aa}}) \times C_{\text{aa}} \times V \times (100/W_{\text{sample}}) \quad (2)$$

where ΔA_{sample} is the absorbance change in the presence of sample, ΔA_{aa} is the absorbance change after adding ascorbic acid standard solution, C_{aa} is the concentration of ascorbic acid standard solution, V is the volume of sample, and W_{sample} is the weight of sample.[17]

2.6. *Plasma alcoholic metabolites and diagnostic transaminases activity*

The alcohol and acetaldehyde concentrations were measured by using a commercially available kit (Megazyme, Wicklow, Ireland) according to the manufacturer's instructions. Assessments of diagnostic transaminases [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)]

Table 1 – Chemical components of DTS20

Component	Concentration (mg/g)
Total sugar	564.46 ± 15.70
Uronic acid	41.09 ± 0.87
Reducing sugar	310.60 ± 1.21
Polyphenol	28.22 ± 0.45

activities were performed using a biochemistry autoanalyzer (Hitachi Ltd., Tokyo, Japan) equipped with user interface operability features.

2.7. *Statistical analyses*

All statistical analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). A paired *t* test was performed to assess the differences between the placebo treatment and DTS20 treatment. All data were two-sided at the 5% significance level and were reported as means ± standard error of the mean.

3. Results

DTS20 was composed of 564.5 mg/g total sugar, 41.1 mg/g uronic acid, 310.6 mg/g reducing sugar, and 28.2 mg/g total polyphenol (Table 1). The ABTS and DPPH radical scavenging activities were increased linearly according to DTS20 concentration (Fig. 1). The IC_{50} of DTS20 was estimated as 1.75 mg/mL and 1.02 mg/mL for the ABTS and DPPH radicals, respectively.

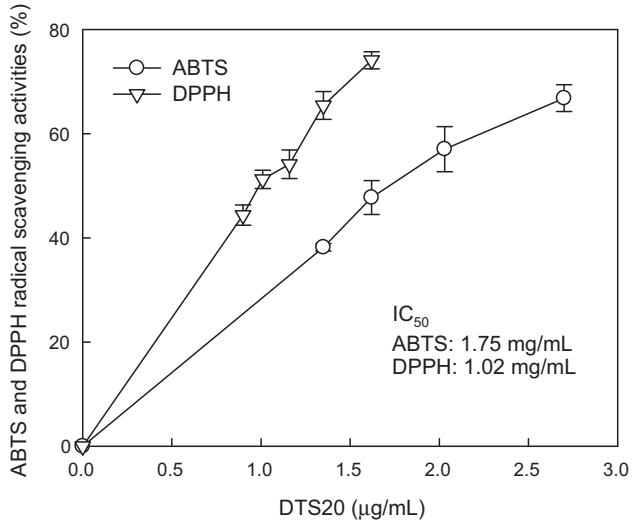

Fig. 1 – Radical scavenging activities of DTS20 on the 3-ethylbenzothiazoline-6-sulfonic acid and 1, 1-diphenyl-2-picrylhydrazyl radicals. The IC_{50} value is the effective concentration at which 1, 1-diphenyl-2-picrylhydrazyl or 3-ethylbenzothiazoline-6-sulfonic acid radicals were scavenged by 50%.
*** Values are the means ± standard error of the mean for triplicate determinations.**
ABTS, 3-ethylbenzothiazoline-6-sulfonic acid; DPPH, 1, 1-diphenyl-2-picrylhydrazyl.

However, plasma alcohol from the alcohol-loading was measured at 0 hours and 2 hours after drinking alcohol (Fig. 2). The plasma alcohol of volunteers before and after drinking alcohol in the placebo treatment group was 0.19 mg/mL and 0.27 mg/mL, respectively. The plasma alcohol of volunteers before and after drinking alcohol in DTS20 treatment group was 0.20 mg/mL and 0.24 mg/mL, respectively. A significant difference in plasma alcohol was observed between plasma levels before and after drinking alcohol in both placebo and DTS20 treatment groups ($p < 0.05$). The change of plasma alcohol in the DTS20 treatment group was lower significantly than that in placebo treatment group ($p < 0.05$).

Fig. 3 shows plasma acetaldehyde in volunteers who consumed the placebo or DTS20 before drinking alcohol. Plasma acetaldehyde in the placebo treatment group was increased significantly after drinking alcohol (26.2 mg/L at 0 hours vs. 55.1 mg/L at 2 hours, $p < 0.05$). Plasma acetaldehyde in the DTS20 treatment group was also increased significantly from 24.3 mg/L before drinking alcohol to 52.1 mg/L after drinking alcohol ($p < 0.05$). The change of plasma acetaldehyde in the DTS20 treatment group had a tendency to be lower than that in the placebo treatment group, but this difference was not significant.

However, DTS20 significantly increased plasma AEAC after drinking alcohol ($p < 0.05$). The change of plasma AEAC was also different significantly between the placebo and DTS20 treatment groups ($p < 0.05$), indicating that DTS20 might have influenced oxidative stress (Fig. 4). It is notable that an independent increase of serum AEAC by DTS20 could be prominent evidence of not only the effect on a hangover but also a possible use of DTS20 as a supplement with functionalities other than alcohol mitigation.

To determine the antioxidant effect of DTS20, Soju-induced lipid peroxidation was estimated by measuring the level of TBARS in plasma (Fig. 5). Plasma TBARS levels in the control and DTS20 groups were not significantly different before and 2 hours after drinking Soju. No significant difference in TBARS levels was observed between the control and DTS20 groups 2 hours after drinking Soju (Fig. 5).

As circulating AST and ALT activities are used as sensitive markers in the diagnosis of hepatic diseases, these enzymes are hepatic damage indicators. Thus, we evaluated AST and ALT activities to determine the effects of DTS20 against the alcohol challenge (Fig. 6). No significant differences in AST and ALT activities were observed between before and after drinking alcohol in both of the placebo and DTS20 treatment groups. The changes of AST and ALT activities were also not different between placebo and DTS20 treatments.

4. Discussion

Alcohol or its metabolites can prompt a sharp increase in free radicals in the human body by acting as a prooxidant or by reducing antioxidant levels and contributing to the progression of a variety of chronic diseases.[18] Alcohol-induced oxygen radicals can be attenuated by conversion of the superoxide anions to hydrogen peroxide and hydrogen peroxide to water via superoxide dismutase and catalase, respectively. These mechanisms prevent peroxidative deterioration in the body.[19]

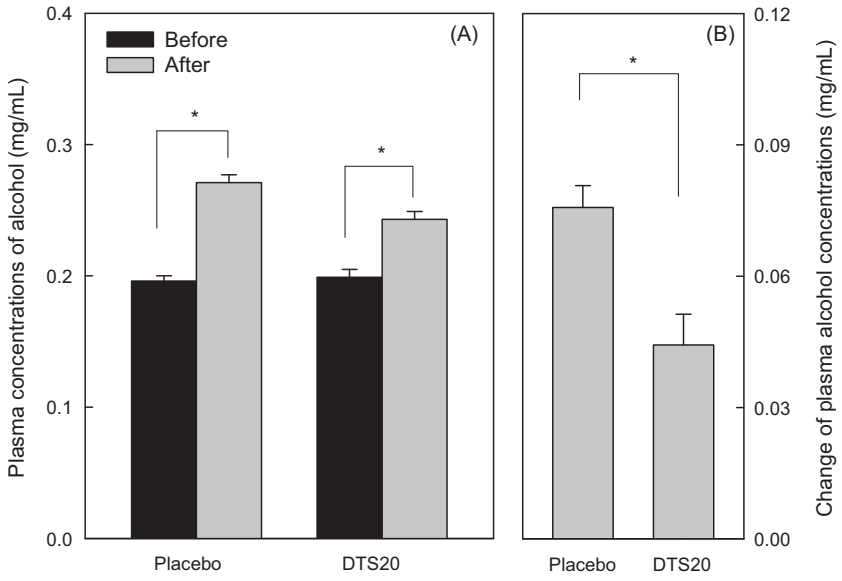

Fig. 2 – (A) Plasma concentrations of alcohol and (B) change of plasma alcohol concentrations by the Soju intake in the control and DTS20 groups. The change of plasma alcohol concentrations was determined by subtracting the concentration of alcohol in water intake group from that of the Soju intake group. The control and DTS20 groups received one package of placebo with 200 mL water or DTS20 with 200 mL water, respectively. Thirty minutes later, the volunteers ingested one bottle of Soju (19% alcohol in 360 mL). Blood was withdrawn just before drinking alcohol and 2 hours after drinking alcohol.
*** Significant difference ($p < 0.05$) between the control and DTS20 groups by the paired *t* test.**
† Values are the means ± standard error of the mean for 10 volunteers, respectively.

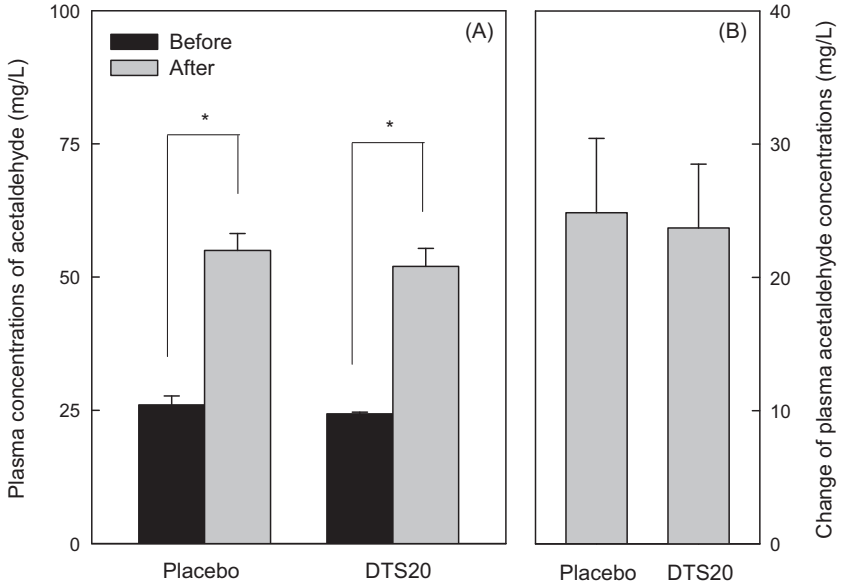

Fig. 3 – (A) Plasma concentrations of acetaldehyde and (B) change of plasma acetaldehyde concentrations by the Soju intake in the control and DTS20 groups. The change of acetaldehyde concentrations was determined by subtracting the concentration of alcohol in water intake group from that of Soju intake group. The control and DTS20 groups received one package of placebo with 200 mL water or DTS20 with 200 mL water, respectively. Blood was withdrawn just before drinking alcohol and 2 hours after drinking alcohol.
*** Significant difference ($p < 0.05$) between the control and DTS20 groups by the paired *t* test.**
† Values are the means ± standard error of the mean for 10 volunteers, respectively.

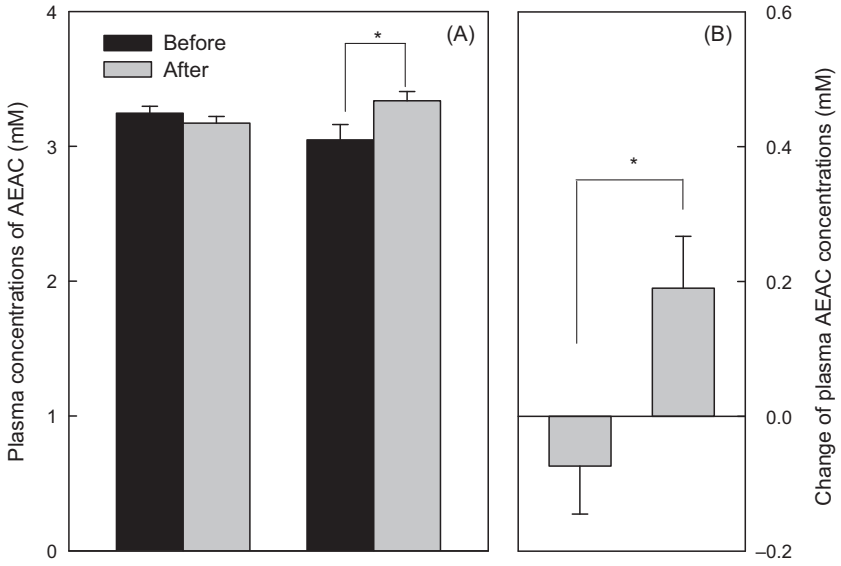

Fig. 4 – (A) Plasma concentrations of ascorbic acid equivalent antioxidant capacity (AEAC) and (B) change of plasma AEAC concentrations by the Soju intake in the control and DTS20 groups. The change of AEAC concentrations was determined by subtracting the concentration of alcohol in water intake group from that of Soju intake group. The control and DTS20 groups received one package of placebo with 200 mL water or DTS20 with 200 mL water, respectively. Blood was withdrawn just before drinking alcohol and 2 hours after drinking alcohol.

*** Significant difference ($p < 0.05$) between the control and DTS20 groups by the paired *t* test.**

† Values are the means ± standard error of the mean for 10 volunteers, respectively.

AEAC, ascorbic acid equivalent antioxidant capacity.

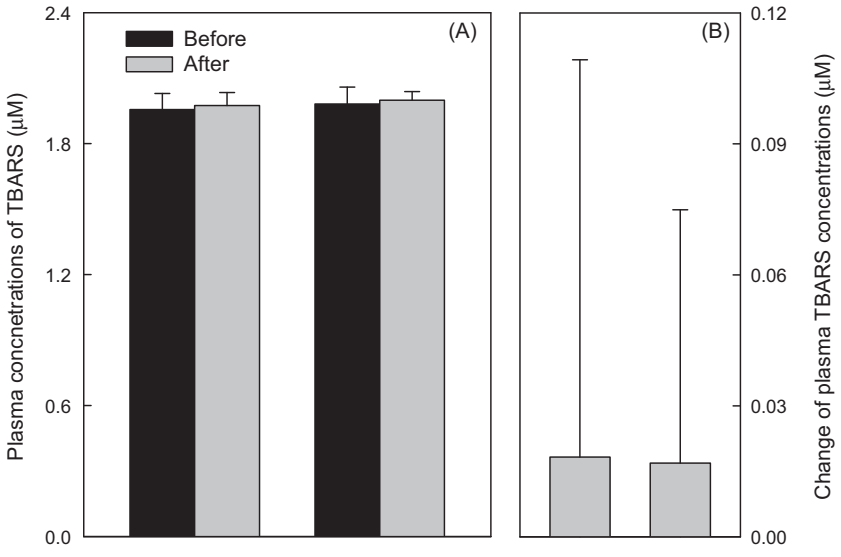

Fig. 5 – (A) Plasma concentrations of thiobarbituric acid reactive substances (TBARS) and (B) change of plasma TBARS concentrations by the Soju intake in the control and DTS20 groups. The change of TBARS concentrations was determined by subtracting the TBARS level of water intake group from that of Soju intake group. The control and DTS20 groups received one package of placebo with 200 mL water or DTS20 with 200 mL water, respectively. Blood was withdrawn just before drinking alcohol and 2 hours after drinking alcohol.

† Values are the means ± standard error of the mean for 10 volunteers, respectively.

TBARS, thiobarbituric acid reactive substances.

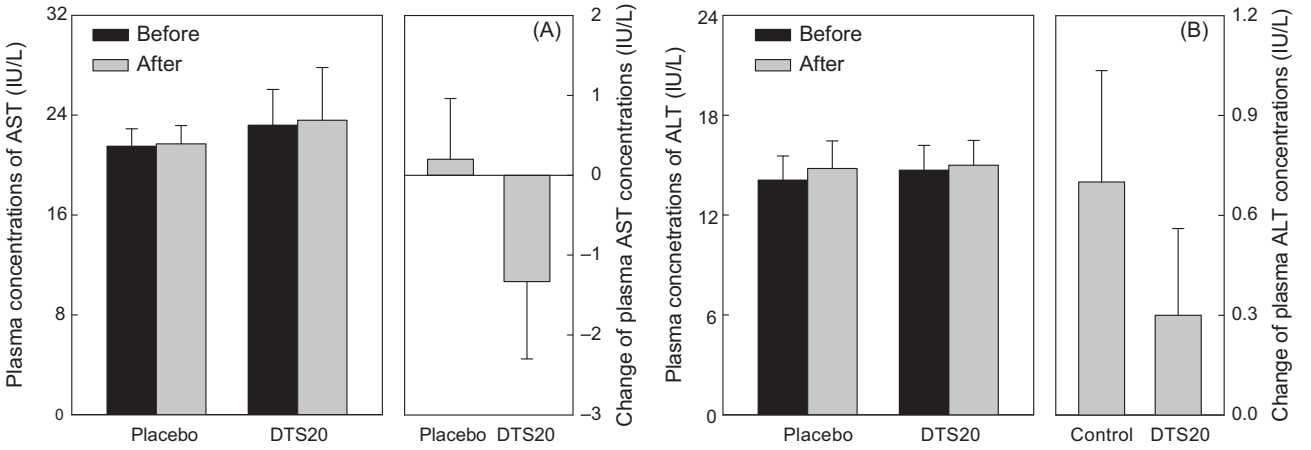

Fig. 6 – (A) Plasma concentrations of alanine aminotransferase (AST) and change of plasma AST concentrations, and plasma concentrations of aspartate aminotransferase (ALT) and (B) change of plasma ALT concentrations by the Soju intake in the control and DTS20 groups. The change of AST and ALT thiobarbituric acid reactive substances concentrations was determined by subtracting the thiobarbituric acid reactive substances level of water intake group from that of Soju intake group. The control and DTS20 groups received one package of 200 mL water or DTS20 with 200 mL water, respectively. Blood was withdrawn just before drinking alcohol and 2 hours after drinking alcohol.
† Values are the means ± standard error of the mean for 10 volunteers, respectively.
ALT, aspartate aminotransferase; AST, alanine aminotransferase.

The body's natural defenses against radicals are inhibited by consuming alcohol, resulting in increased liver damage.[20] Consequently, the generation of excess radicals from alcohol induces breakdown of the natural defense system.[21] Because of radical production during alcohol metabolism and their putative role in alcohol-induced diseases,[22] the presence of active natural radical scavenging antioxidants in DTS20 may be beneficial (Fig. 1, 4). Especially, it is notable that an independent increase of plasma AEAC by DTS20 treatment could be prominent evidence of not only the effect on hangovers but also a possible use of DTS20 as a supplement with functionalities other than alcohol mitigation.

DTS20 decreased the elevation of blood alcohol induced by alcohol intake (Fig. 2). Alcohol is readily absorbed from the gastrointestinal tract, circulated rapidly, and distributed uniformly throughout the body.[23] Thereafter, 80–90% of the alcohol absorbed is rapidly oxidized to acetaldehyde and acetate by enzymes in the liver such as alcohol dehydrogenase and aldehyde dehydrogenase.[24] Acetaldehyde is a toxic alcohol metabolite and has been well characterized as the main cause of hangovers. Acetaldehyde strongly binds sulfur compounds such as cysteine and glutathione in liver microsomes resulting in liver damage.[25] DTS-20 tended to decrease acetaldehyde compared with the placebo, but it did not reach a significant difference (Fig. 3).

A great deal of interest has been paid to the role of complementary and alternative medicines for treating various acute and chronic diseases.[26] Several hundred kinds of herbs that modulate oxidative stress have been examined for treatment of a wide variety of liver disorders.[27,28] Additionally, the combinations of these herbs like DTS 20 have a synergistic effect.[29] A major feature of herb medicine is that there are unique combinations of herbs for treating various diseases and significant advantages by combining specific medicinal herbs. In general, medicinal herbs may act differently to produce synergistic effects. The combination of multiple herbs can minimize side-effects by decreasing the amount of each component. DTS20 contains medicine herbs as follows: *V. album* L., *L. chinense* L., *I. obliquus*, and *A. senticosus* H., and is regarded as an important foodstuff showing radical scavenging activity and alcohol detoxification *in vivo* as well as *in vitro*.[30,31]

V. album L. and *I. obliquus* also reduce alcohol-induced liver toxicity, and inhibit the effects of alcohol-induced lipid accumulation in male Sprague–Dawley rats.[32,33] *A. senticosus* H. prevents hepatic injury by accelerating alcohol metabolism due to increases in alcohol metabolizing enzyme activities, increasing antioxidant system activities against oxidative stress, and by decreasing fat accumulation through inhibiting lipogenic enzyme activities, which is evidenced by decreased hepatotoxic indices in serum/liver tissue and morphological observations in the liver.[5]

The administration of DTS20 (200–500 mg/kg) had beneficial actions toward alcohol degradation in acute alcohol treated mice model. The oral administration of DTS20 showed decreased gastric mucous membrane damage produced in ethanol treated mice. In addition, intraperitoneal administration of DTS20 showed anti-inflammatory effects in inhibition tests of vascular permeability produced by acetic acid. DTS20 also reduced the concentration of nitric oxide, tumor necrosis factor-α in macrophages that were activated by lipopolysaccharide.[10] In this context, the combination of *V. album* L., *L. chinense* L., *I. obliquus*., and *A. senticosus* H. might show a synergistic antioxidative effect.

Plasma AST and ALT activities were decreased significantly in an herb complex treated group compared with those in an alcohol-fed group.[34] These results suggest that these herb complexes can be used as a remedy for alcoholic fatty liver. Taken together, DTS20 decreased blood alcohol level and enhanced plasma antioxidant activities in alcohol intake volunteers without affecting the plasma level of liver enzymes, ALT and AST.

DTS20 enhanced the antioxidant activity and decreased the plasma alcohol level in alcohol-loaded volunteers without affecting the plasma levels of hepatic enzymes, ALT and AST. Therefore, these results suggest that this herb mixture has potential for a hangover related alcohol metabolism.

Conflicts of interest

The author has no conflicts of interest to declare.

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