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Inhibition of Ethanol Absorption by *Rhodiola sachalinensis* in Rats

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We used a herbal medicine, roots of *Rhodiola sachalinensis* (RS) to assess whether RS extract can decrease blood ethanol concentrations in rats fed ethanol and if so, to elucidate the mechanism by which RS extract reduces blood ethanol levels. Rats were fed ethanol orally 1 hr after the oral administration of various doses of RS extract. In another experiment, rats were injected intraperitoneally with ethanol following the intake of RS extract via gastric catheter to eliminate possible inhibition of ethanol absorption in the gastrointestine by RS extract. The administration of RS extract remarkably lowered blood ethanol levels in a dose-dependent manner in rats given ethanol orally. However, the intake of RS extract did not reduce ethanol levels in rats injected with ethanol intraperitoneally. The activities of two main hepatic enzymes, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), involved in ethanol metabolism, were not affected by the administration of RS extract in rats fed ethanol. In addition, the intake of RS extract reduced serum triglyceride levels elevated by ethanol to the normal level. We conclude that the administration of RS extract lowers blood ethanol concentrations by inhibition of ethanol absorption in the gastrointestinal tracts of ethanol-fed animals.

Key words : *Rhodiola sachalinensis,* Ethanol, Absorption, Alcohol dehydrogenase, Aldehyde dehydrogenase

INTRODUCTION

Ethanol is rapidly absorbed from the gastrointestinal tracts of the animals after intake. Ethanol after absorption is principally metabolized in the liver. The liver contains three different enzyme systems for ethanol metabolism (Lieber, 1984 & 1985). They are alcohol dehydrogenase (ADH) pathway, catalase, and the microsomal ethanol-oxidizing system. The ADH pathway is the main one. The first step in the metabolism of ethanol is its oxidation to acetaldehyde using NAD as a cofactor by cytosolic ADH (Theorell and Bonnichsen, 1951; Theorell and Chance, 1951). The acetaldehyde is further oxidized by mitochondrial aldehyde dehydrogenase (ALDH) to acetate (Marjanen, 1972).

Blood ethanol concentrations in ethanol-fed animals can be lowered either by promoting ethanol metabolism or by inhibiting ethanol absorption in the gastrointestinal tract. Agents which can lower blood ethanol concentrations rapidly and safely after ethanol intake may alleviate symptoms of hangover. Many attempts have been made to lower blood ethanol concentrations (Ylikahri *et al.*, 1976; Khan *et al.*, 1973; Tygstrup *et al.*, 1965; Thieden *et al.*, 1972; Damgaard *et al.*, 1972; Muir *et al.*, 1973; Kelly *et al.*, 1971; Rawat, 1969 & 1977; Pawan, 1968; Rogers *et al.*, 1987). Fructose has been intensively studied for this purpose. Fructose has been shown to reduce blood ethanol levels by accelerating ethanol metabolism in man and in animals (Ylikahri *et al.*, 1976; Tygstrup *et al.*, 1965; Thieden *et al.*, 1972; Damgaard *et al.*, 1972; Rawat, 1969 & 1977; Rogers *et al.*, 1987; Crownover *et al.*, 1986; Ylikahri *et al.*, 1972; Mascord *et al.*, 1991; Brown *et al.*, 1972). The precise mechanisms by which fructose affects ethanol metabolism are, however, not completely understood. Nevertheless, Keegan and Batey (1993) reported that an increase in ethanol elimination in fructose-fed female rats was not due to a change in hepatic ADH activity. A few remedies composed of traditional medicinal plants have been believed to be effective for alleviating symptoms of hangover since ancient times (Huh, 1971). Recently, a few reports showed that herbal extracts decreased blood ethanol concentrations by stimulating ethanol metabolism (Sakai *et al.*, 1987; Sakai *et al.*, 1989; Kim *et al.*, 1994). However, the herbal extracts blocking ethanol absorption

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have not been reported before.

Rhodiola sachalinensis (RS) grows in China at an elevation of 1700~2500 m. The roots of RS are used, according to the Chinese folkloric tradition, as tonic and antiaging agent. Recently, Cheng *et al.* (1993) reported the hypoglycemic effect of RS. However, little work has previously been carried out on pharmacological effects of RS.

In the present study, we investigated the mechanism by which RS extract affects blood ethanol levels in ethanol-fed animals.

MATERIALS AND METHODS

Materials

Ethanol and perchloric acid were purchased from Merck (Darmstadt, Germany). Acetaldehyde, glycine, KOH, K_2CO_3, Tris, sucrose, pyrazole, bicinchoninic acid, and pyrophosphate were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Ethanol assay kit and NAD were purchased from Boehringer Mannheim (Mannheim, Germany). Total cholesterol and triglyceride assay kits were from Wako Pure Chemical (Osaka, Japan). All other chemicals were reagent grade.

Preparation of RS extract

One hundred g of roots of RS were extracted with 1 l of water at 100°C for 3 hr. The extract was filtered through a few layers of cheese cloth and then lyophilized in a freeze-dryer. Usually, 35 g of dry extract were obtained. The powdered extract was dissolved in a proper amount of water for an administration into animals.

Treatment of animals

Young male Sprague-Dawley rats were purchased from Korea Research Institute of Chemical Toxicology (Daejon, Korea), housed in individual cages, and maintained on a chow diet until use. Rats had free access to water. Rats, weighing 150 g to 200 g, were fasted for 24 hr for the experiments.

For the time course experiment, various doses ranging from 0.375 g to 3 g of RS extract/kg body weight (bw) of rat, in a volume of 10 ml/kg bw, were administered to 24 hr-fasted rats by gastric catheter. Ethanol (3 g/kg bw, as 30% w/v in H_2O) was fed orally 1 hr after the administration of RS extract. For rats fed ethanol only (control), an equal volume of water instead of RS extract was given 1 hr before the ethanol treatment. Blood was collected from tail vein 1, 2, 4, 6, and 8 hr after the ethanol load.

For the experiment with ethanol-injected rats, 24 hr-fasted rats were administered RS extract (2 g/kg bw) and then injected with ethanol at a dose of 1.5 g/kg bw intraperitoneally. Blood was collected 1, 2, 4, and 6 hr from tail vein after the ethanol administration. As a control, 24 hr-fasted rats were fed an equal volume of water instead of RS extract.

For the measurement of activities of ADH and ALDH, rats were fasted for 24 hr and fed RS extract at a dose of 3 g/kg bw orally. Ethanol (3 g/kg bw) was administered 1 hr after the intake of RS extract. Rats were then sacrificed 2 hr after the ethanol intake. As a control, rats were fasted for 24 hr and fed an equal volume of water instead of RS extract, and then sacrificed 2 hr after the ethanol treatment. For normal group, rats were fasted for 24 hr and then sacrificed. Livers were removed right after sacrifice and frozen in liquid nitrogen. For the determination of serum triglyceride levels, blood was collected right before sacrifice.

Determination of blood ethanol levels

Blood ethanol concentration was determined as previously described (Kim *et al.*, 1993). Blood was deproteinized with an equal volume of chilled, 0.7 M perchloric acid right after collection and the supernatant was obtained by centrifugation. A half volume of a buffer solution containing 0.4 M KOH, 0.13 M K_2CO_3, and 0.5 M Tris was added to the supernatant. After incubation on ice for 30 min, the mixture was centrifuged. The supernatant was frozen and stored at -70 °C. The ethanol level in the supernatant was determined using an assay kit (Boehringer Mannheim, Mannheim, Germany) by the method suggested by the manufacturer.

The area under the curve (AUC) was calculated from each time course curve constructed with ethanol levels. Percent reduction in ethanol levels of rats treated with RS extract was determind by comparing AUC with that of control.

Determination of specific activities of ADH and ALDH

A piece of liver was accurately weighed and homogenized in a buffer solution containing 10 mM Tris-HCl, 250 mM sucrose (pH 7.4) using a Potter-Elvehjem type homogenizer. A 10% (w/v) homogenate was obtained and centrifuged at 700×g for 10 min followed by further centrifugation of the supernatant at 10,000×g for 10 min. The supernatant was ultracentrifuged at 105,000×g for 1 hr to obtain the cytosolic fraction for the ADH assay. The precipitate obtained after centrifugation at 10,000×g was washed twice with a homogenization buffer to obtain the mitochondrial fraction for the ALDH assay. The precipitate was frozen in liquid nitrogen and stored at -70°C until use. All the experimental manipulations were carried out at 4°C.

Activities of ADH and ALDH were measured according to Koivula and Koivusalo (1975). The ADH activity was measured in 50 mM glycine (pH 9.6), 0.8 mM NAD, 3 mM ethanol and 50 μl of cytosolic fraction in a final volume of 1.0 ml. ALDH activity was measured in 100 mM pyrophosphate buffer (pH 8.0), 1 mM NAD, 2 mM pyrazole, 15 mM acetaldehyde, and 50 μl of mitochondrial fraction in a final volume of 1.0 ml. All assays were performed at room temperature. Total protein concentration was determined by the method described by Smith *et al.* (1985) using bicinchoninic acid. Specific activity was expressed as units of enzyme activity per mg of protein.

Determination of serum triglyceride and cholesterol levels

Blood was centrifuged at 1,000×g for 10 min to obtain serum. The levels of serum triglyceride and total cholesterol were measured using assay kits (Wako Pure Chemical, Osaka, Japan)) by the method suggested by the manufacturer.

Statistics

All values are presented as the means±SE of 5 animals. Statistical analysis was conducted by Student's *t*-test.

RESULTS

The administration of various doses of RS extract into rats fed ethanol by gavage markedly reduced blood ethanol concentrations throughout 8 h period after the ethanol intake (Fig. 1). The reduction in blood ethanol levels by RS extract was dose-dependent. The decrease was observed as early as 1 hr and the maximum decrease was 66% with 3 g/kg bw of RS extract as calculated by the difference in AUC, a measure of bioavailability of ethanol (Fig. 2).

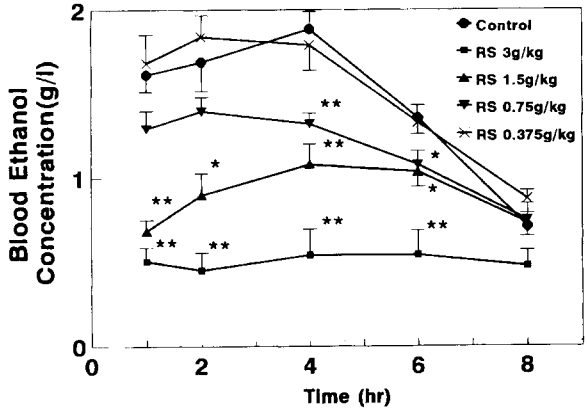

Fig. 1. Effect of RS extract on blood ethanol concentrations in rats fed ethanol orally. Rats were fasted for 24 hr and administered various doses of RS extract (3 g, 1.5 g, 0.75 g, 0.375 g/kg bw) orally. Ethanol at a dose of 3 g/kg bw was fed orally 1 hr after the treatment with RS extract. Blood was collected from tail vein 1, 2, 4, 6, and 8 hr after the ethanol treatment. As a control, rats were given an equal volume of water instead of RS extract. Data are means±SE from 5 rats. * and **, Significantly different from the control with $p<0.05$ and $p<0.01$, respectively.

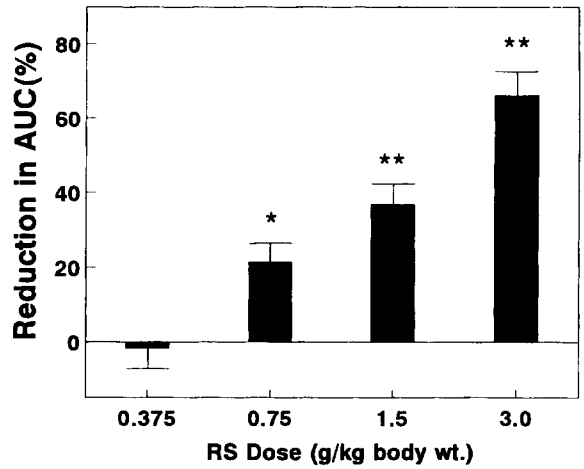

Fig. 2. Reduction in blood ethanol concentrations by RS extract in rats given ethanol orally. Reduction in blood ethanol concentrations by RS extract was expressed as percent reduction in AUC, calculated from each time course curve shown in Fig. 1, by comparing AUC's of RS-treated groups with that of control. * and **, Significantly different from the control with $p<0.05$ and $p<0.01$, respectively.

Blood ethanol concentrations can be reduced by agents which are capable of blocking ethanol absorption or accelerating ethanol metabolism. To study the mechanism by which RS extract lowered blood ethanol levels, we tested the possibility that RS extract inhibits the absorption of ethanol given orally in the gastrointestinal tract. For this purpose, ethanol was injected intraperitoneally instead of an oral intake. The intake of RS extract did not affect blood ethanol levels in rats injected with ethanol (Fig. 3). This result suggests that the reduction in ethanol levels observed in rats fed ethanol orally (Fig. 1) is due to an inhibition of ethanol absorption in the gastrointestinal tracts of rats. It also implies that RS extract may not stimulate ethanol metabolism.

To further provide the evidence that RS extract does not stimulate ethanol metabolism, the activities of two main enzymes involved in ethanol metabolism in the liver were measured in rats treated with RS extract. The intake of RS extract did not affect the activities of both ADH and ALDH in ethanol-fed rats (Fig. 4), suggesting that the decrease in blood ethanol levels by RS extract does not involve the stimulation of ethanol metabolism.

The acute administration of ethanol causes an increase in serum triglyceride levels (Lieber, 1985; Kim

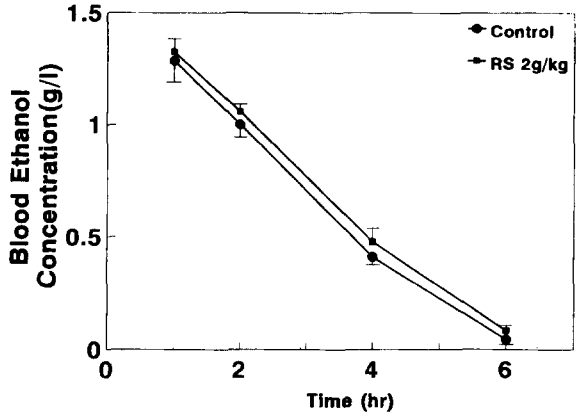

Fig. 3. Effect of RS extract on blood ethanol concentrations in rats injected with ethanol intraperitoneally. Rats were fasted for 24 hr and given RS extract (2 g/kg bw) orally. Ethanol at a dose of 1.5 g/kg bw was injected intraperitoneally 1 hr after the intake of RS extract. As a control, rats were given an equal volume of water instead of RS extract. Data are means±SE from 5 rats.

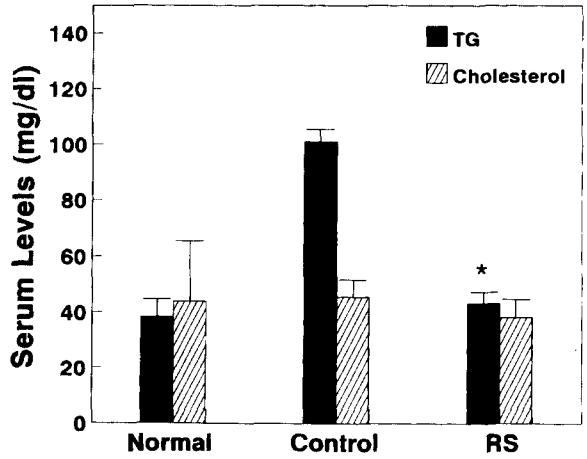

Fig. 5. Effects of RS extract on levels of triglyceride and cholesterol in sera of rats fed ethanol. Rats were fasted for 24 hr and given 3 g/kg bw of RS extract orally. Ethanol at a dose of 3 g/kg bw was administered 1 h after the intake of RS extract. Blood was collected by heart puncture 2 hr after the ethanol intake. As a control, rats were given an equal volume of water instead of RS extract. Data are means±SE of 5 rats. *Significantly different from the control with $p<0.05$.

and Kwon, 1992). To determine whether RS extract can decrease the triglyceride level elevated by ethanol, we measured serum triglyceride concentrations 2 h after ethanol intake. The intake of RS extract decreased the serum triglyceride level by 57% to the normal level (Fig. 5). However, the serum cholesterol level was not affected by ethanol or ethanol plus RS extract (Fig. 5).

DISCUSSION

This study shows that RS extract lowers blood ethanol concentrations by blocking ethanol absorption in the gastrointestinal tracts of ethanol-fed rats, not by stimulating ethanol metabolism. We found that RS extract decreased blood ethanol levels remarkably in a dose-dependent manner in rats fed ethanol orally (Fig. 1 & 2) while such effect was not observed in rats injected with ethanol intraperitoneally (Fig. 3). Furthermore, RS extract did not affect the activities of hepatic ADH and ALDH involved in ethanol metabolism (Fig. 4).

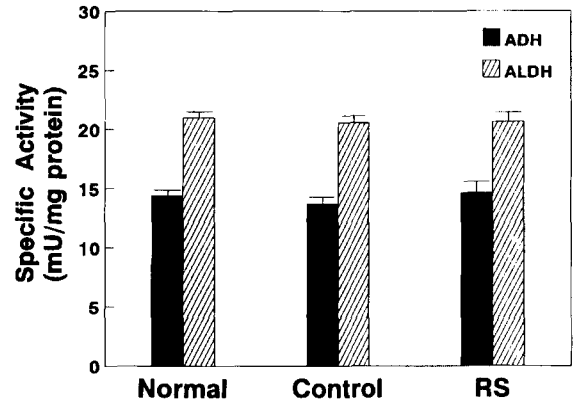

Fig. 4. Effects of RS extract on hepatic ADH and ALDH activities in rats fed ethanol orally. Rats were fasted for 24 hr and fed 3 g/kg bw of RS extract orally. Ethanol at a dose of 3 g/kg bw was given 1 hr after the administration of RS extract. Rats were sacrificed 2 hr after the ethanol treatment. As a control, rats were given an equal volume of water instead of RS extract. For normal group, rats were fasted for 24 hr and then sacrificed. Data are means±SE of 5 rats.

In contrast to our present report, a few other herbal extracts have been shown to decrease blood ethanol concentrations by preventing the reduction in ADH activity caused by an acute administration of ethanol, suggesting the stimulation of ethanol metabolism (Sakai *et al.*, 1987; Sakai *et al.*, 1989; Kim *et al.*, 1994). Those earlier studies report that the acute administration of ethanol inhibited ADH enzyme 1 hr after the ethanol intake and the inhibition of ADH was prevented by herbal extracts. In the present investigation, ADH activity was not inhibited by the ethanol intake 2 hr after the ethanol treatment (Fig. 4). It is assumed that RS extract contains the active ingredient(s), though not determined, which strongly adsorbs ethanol, thus preventing absorption of ethanol in the gastrointestine of animals. Although RS extract seems to decrease blood ethanol concentration by adsorbing specifically to ethanol, the possibility that the non specific binding of high dose of RS extract to ethanol may in part contribute to a reduction in ethanol level can't be ruled out due to the bulky characteristic of RS extract especially at a high dose such as 3 g/kg bw.

The acute administration of ethanol increased the

serum triglyceride level by 2.6 fold and this increase was reduced to the normal level by RS extract (Fig. 5). It is possible that RS extract inhibits the absorption of ethanol, which provides a precursor for the synthesis of fatty acid and triglyceride in the liver. The decreased supply of precursors, in turn, could decrease the levels of hepatic and serum VLDL containing triglycerides as a major lipid.

In conclusion, this study shows that RS extract reduces blood ethanol levels and the blood ethanol-lowering effect of RS extract is due to the inhibition of ethanol absorption in the gastrointestine of rats, not due to the stimulation of ethanol metabolism. These findings imply that RS extract may be used to help alleviate hangover symptoms after the excessive intake of alcohol by reducing blood alcohol levels.

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