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The pharmacological potential of *Sorbus commixta* cortex on blood alcohol concentration and hepatic lipid peroxidation in acute alcohol-treated rats

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Abstract

The effect of *Sorbus commixta* cortex, a traditional herbal medicine used for the treatment of bronchitis, gastritis and dropsy, on blood alcohol concentration (BAC) and hepatic lipid peroxidation was examined in acute alcohol-treated rats. A 30-min pretreatment with a methanol extract of *S. commixta* cortex (SC) at concentrations higher than 200 mg kg^{-1} resulted in a significant decrease in BAC and the ethyl acetate fraction (SE) of the extract showed the highest potency, with a maximum of a 46% decrease at 150 mg kg^{-1} 2 h after alcohol administration (3.0 g kg^{-1}) compared with the control group ($P < 0.005$). The rapid reduction in BAC did not appear to be due to the protection or activation of hepatic alcohol dehydrogenase (ADH) activity by SE. Hepatic malondialdehyde (MDA) levels were significantly increased by acute alcohol administration within 6 h, although pretreatment with the SE caused a significant decrease in MDA levels compared with alcohol treatment alone. Hepatic glutathione (GSH) levels and superoxide dismutase (SOD) activity remained unchanged by alcohol, SE alone or by the combined treatment of alcohol and SE. However, catalase activity was significantly reduced by acute alcohol administration and pretreatment with the SE led to significant protection of its activity. These results suggest that pretreatment with SE reduces hepatic lipid peroxidation by decreasing the bioavailability of alcohol and its oxidative metabolites, such as H_2O_2, at least partly, through the protection of hepatic catalase in acute alcohol-treated rats.

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Introduction

Alcohol is the most widely abused chemical substance by society today and long-term heavy alcohol use is the most prevalent cause of illness and death from liver disease in the USA (Puzziferri et al 2000).

Although small amounts of alcohol can be exhaled in the breath or excreted in the urine and sweat (Kalant 1971), the major pathway for alcohol metabolism is NAD^+-dependent cytoplasmic alcohol dehydrogenase (ADH) in the liver, which catalyses the oxidation of alcohol to cytotoxic acetaldehyde. The acetaldehyde is subsequently oxidized to acetate by mitochondrial aldehyde dehydrogenase, giving rise to reactive oxygen species (ROS). The intoxicating effects of alcohol are mostly derived either directly from alcohol and its metabolite, acetaldehyde, or indirectly from the metabolic results of alcohol oxidation, such as the decreased cytoplasmic NAD^+-to-NADH ratio and the involvement of ROS (Lieber 1991).

The liver is particularly susceptible to alcohol-related injury because it is the primary organ for alcohol metabolism. The acute and chronic ingestion of alcohol is known to be one of the major causes of liver injury and development of serious liver diseases (Tuma 2002). The mechanisms of alcoholic liver injury include the effects of alcohol, acetaldehyde and ROS on hepatocytes and the depletion of the endogenous antioxidant system leading to lipid peroxidation and associated membrane damage (Halliwell 1997).

A substance that rapidly and safely lowers the blood alcohol concentration (BAC) by accelerating its metabolism and thereby decreasing its bioavailability, referred to as an amethystic agent, might be useful in the treatment of acute alcohol overdose (Chung et al 1996). In the course of our studies on the identification of a non-toxic amethystic agent

from natural sources, a methanolic extract of *Sorbus commixta* cortex (SM) was found to cause a significant decrease in BAC in acute alcohol-treated rats. *S. commixta* is tree-type of plant belonging to the Rosaceae family and *S. commixta* cortex is used as a folk remedy and herbal medicine for the treatment of bronchitis, gastritis, dropsy and diseases associated with vitamin A and C deficiencies (Bae 2000). In several previous studies, *S. commixta* cortex has been shown to be a vasorelaxant (Kang 2005; Yin et al 2005), an antioxidant (Na et al 2002) and to have anti-atherogenic (Sohn et al 2005a, b) and anti-cancer properties (Lee et al 2002). In this report, we describe the pharmacological potential of SM and its fractions on BAC and hepatic lipid peroxidation in acute alcohol-treated rats.

Materials and Methods

Materials

All chemicals were obtained from Sigma Chemical Co. (St Louis, MO, USA) unless otherwise indicated. Methanol, n-hexane, chloroform, ethyl acetate and n-butanol were HPLC reagent grades purchased from J. T. Baker (Mallinckrodt Baker, NJ, USA). The enzyme assay kits used to determine alanine amino transferase (ALT) and aspartate amino transferase (AST) activity were purchased from AsanPharm (Asan Pharmaceutical Co., Republic of Korea).

Extraction and fractionation

Dried *S. commixta* cortex (1 kg) was sliced into pieces and extracted three times with 80% aqueous methanol (10 L) at room temperature and the methanol extract was then partitioned with n-hexane, chloroform, ethyl acetate, n-butanol and water. The extract and solvent fractions were concentrated under reduced pressure and lyophilized, as described previously (Lee et al 2000). The yields (% w/w) obtained for the methanol extract and the n-hexane, chloroform, ethyl acetate, n-butanol and water fractions were about 25.85, 1.49, 2.38, 2.95, 16.89 and 2.13% of the dry weight, respectively.

From the cortex of *S. commixta*, triterpenoids such as lupenone and lupeol have been isolated (Lee & Lee 1999). Other compounds isolated from the cortex, such as catechin-7-O-β-D-xylopyranoside and catechin-7-O-β-D-apiofuranoside, are known to have antioxidant properties (Na et al 2002).

Determination of total phenolics

The concentration of total phenolics was measured by the method of Singleton et al (1999). Total phenolics were expressed as tannic acid molar equivalents.

Animals and treatment

Six-week-old male Sprague-Dawley rats, initially weighing 160–180 g, were kept in a room maintained at $21 \pm 2^{\circ}C$ under $55 \pm 5\%$ humidity and a 12-h light–dark cycle and acclimatized to the facility for 7 days before the start of the experiments. The rats were allowed free access to a commercial standard rat diet and tap water and were fasted for 12 h before the start of the treatments. SM and its fractions, at the indicated concentrations, were administered by oral intubation in a 1% dimethyl sulfoxide (DMSO) vehicle 30 min before alcohol administration ($3.0\ g\ kg^{-1}$ in a 30% solution with water). The protocols were approved by the institute's animal care ethics committee, and were based on NIH guidelines for animal research.

Determination of blood alcohol concentration

Blood was collected from the tail vein at time intervals of 0.5, 1, 2, 4, 6 and 8 h post alcohol administration. Based on the method of Chung et al (1996), 900 μL of perchloric acid (3.4%) was added to 100 μL of the blood sample with vortexing and the mixture was centrifuged at 12 000 *g* for 1 min. The supernatant was separated and used for the determination of BAC. BAC was determined using a commercial assay kit (Sigma) according to the manufacturer's instructions. The blank sample consisted of 3.4% perchloric acid. For alcohol, area under the curve (AUC) was calculated according to the trapezoidal rule up to 8 h after alcohol administration.

Serum and tissue preparations

Rats were sacrificed under ether anaesthesia at the indicated times after alcohol administration, and blood was collected by heart puncture. The liver was perfused with ice-cold saline through the portal vein until the liver was uniformly pale and was then immediately removed. The collected blood was kept at room temperature for 30 min to allow clotting and serum was then separated by centrifugation at 1500 *g* for 10 min. The removed liver was washed in ice-cold saline, cut into small pieces and homogenized with a glass Teflon homogenizer in ice-cold 0.1 M potassium phosphate buffer (pH 7.5) equivalent to four times the liver weight. This homogenate was used for the determination of malondialdehyde (MDA) and glutathione (GSH) levels. The homogenate was centrifuged at 600 *g* for 10 min, and the resulting supernatant was re-centrifuged at 10 000 *g* for 20 min, in both cases at 4°C. The resulting precipitate, the mitochondrial fraction, was suspended in the above buffer and used as the source of catalase, and the supernatant was further centrifuged at 105 000 *g* for 60 min at 4°C. The supernatant obtained at this point, the cytosolic fraction, was used as the source of the enzymes ADH and superoxide dismutase (SOD).

Biochemical analysis

The MDA content of the liver homogenate was measured by the method of Ohkawa et al (1979) as a marker of lipid peroxidation. A mixture of 0.4 mL liver homogenate, 1.5 mL 8.1% sodium dodecyl sulfate, 1.5 mL 20% acetic acid (pH 3.5) and 1.5 mL 0.8% thiobarbituric acid was

placed in a boiling water bath for 1 h. The mixture was allowed to cool at room temperature and 5.0 mL of n-butanol/pyridine (15:1) was then added, followed by vortexing and centrifugation at 1000 *g* for 10 min. The coloured layer was measured at 532 nm using 1,1,3,3-tetraethoxypropane as a standard. The reduced GSH content of the liver was measured using the method described by Park et al (2004). A mixture of 0.5 mL liver homogenate and 0.5 mL 4% sulfosalicylic acid was centrifuged at 1500 *g* for 10 min. To 0.3 mL of the resulting supernatant, 2.7 mL of disulfide reagent (0.1 mM 5,5′-dithiobis in 0.1 M sodium phosphate buffer, pH 8.0) was added and the mixture was allowed to incubate at room temperature for 20 min. The absorbance was measured at 412 nm using oxidized GSH as a standard.

Enzymatic assay

Serum alanine amino transferase (ALT) and aspartate amino transferase (AST) activity was determined using commercial enzyme assay kits (Asan Pharmaceutical) according to the manufacturer's instructions. Distilled water was used as a blank. The ADH activity of liver was determined by a method based on that of Park et al (2004). A final 4.0-mL volume of the reaction mixture contained 70 mM glycine-NaOH buffer (pH 9.6), 10 mM ethanol, 0.67 mM NAD^+ and 0.1 mL of cytosolic fraction. ADH was measured at 340 nm after 5 min at 37°C using NADH as a standard and the activity was expressed as nM of NADH produced per min per mg protein. The SOD activity of liver was measured by the method of Martin et al (1987). A 1-mL volume of the cytosolic fraction was added to 0.4 mL of ethanol–chloroform (5:3 v/v), vortexed and centrifuged at 8000 *g* for 30 min. The resulting supernatant was used as the source of SOD. A final 3.0-mL volume of reaction mixture contained 0.1 mM EDTA, 50 mM potassium phosphate buffer (pH 7.5) and 10 μL of supernatant. A 60-μL volume of 5 mM haematoxylin was added to this mixture to initiate the reaction. SOD was measured at 560 nm for 4 min at 37°C. One unit of enzyme activity was defined as the amount of enzyme required to inhibit the oxidation of haematoxylin by 50%. Catalase activity of the liver was measured by the method of Aebi (1984). A final 3.0-mL volume of reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 10 mM H_2O_2 and 0.1 mL of cytosolic fraction. Catalase activity was measured at 240 nm for 1 min at 25°C. An extinction coefficient of 0.041 $mM^{-1}\,cm^{-1}$ was used to determine catalase activity.

Protein assay

Protein was determined using a bicinchoninic acid (BCA) protein assay kit (Sigma) according to the manufacturer's instructions, using bovine serum albumin as a standard.

Statistical analysis

The results are expressed as means ± standard deviation (s.d.) and significance was determined using one- or two-way analysis of variance followed by Dunnett's test using SPSS (12.0) statistical software. $P \leq 0.05$ was considered statistically significant.

Results

Effects of SM and its fractions on BAC in acute alcohol-treated rats

To determine the effects of SM and its fractions on BAC, samples were administered orally to fasted rats at the indicated doses 30 min before administration of alcohol. BAC and the activity of hepatic ADH were determined at various times following the alcohol administration. Significant decreases in BAC were found from the 2-h time interval post alcohol administration in response to pretreatment with 200 and 300 $mg\,kg^{-1}$ of SM, and, at a concentration of 300 $mg\,kg^{-1}$, SM caused about a 25% decrease in BAC at the 2-h time point, compared with the control group (Figure 1A; $P < 0.001$). The 200 and 300 $mg\,kg^{-1}$ SM pretreatments significantly reduced the AUCs (0–6 h) of BAC (Figure 1B; $P < 0.05$ and $P < 0.001$, respectively). However, there were no differences in BAC when SM at concentrations lower than 100 $mg\,kg^{-1}$ was used and an appropriate volume of DMSO itself also had no effect on BAC (data not shown). On the basis of the effect of SM on BAC (Figure 1A, B), to obtain further information relative to the characteristics of the active compound(s) responsible for its potency, SM was fractionated using a series of solvents of increasing polarity. Among the solvent fractions, the ethyl acetate fraction (SE) at a level of 100 $mg\,kg^{-1}$ caused significant decreases in BAC and the AUC (0–8 h), while the other fractions had no effect (Figure 1C, D). The contents of total phenolics of SM and its fractions ranged between 65.38 and 595.85 $mg\,g^{-1}$ (Figure 2) and a high degree of correlation ($r^2 = 0.9413$) was observed between the total phenolics content of SM fractions and BAC at the 2-h time point (data not shown). As shown in Figure 3A, SE at 150 mg/kg caused significant decreases in BAC at all time intervals, and SE at 150 mg/kg caused about a 46% decrease in BAC at 2 h, compared with the control group ($P < 0.001$). The 100 and 150 $mg\,kg^{-1}$ SE pretreatments significantly reduced the AUCs (0–8 h) of BAC (Figure 3B; $P < 0.01$ and $P < 0.001$, respectively). However, there were no differences in BAC when SE at concentrations lower than 50 $mg\,kg^{-1}$ were used.

To determine the mechanism for the decrease in BAC mediated by SE, analysis of hepatic ADH was carried out at time intervals of 0.5, 2 and 6 h post alcohol administration in rats. As shown in Table 1, alcohol ingestion resulted in significant decreases in the activity of ADH at the 2- and 6-h time intervals, and the decreased ADH activity was not restored by SE. On the other hand, ADH activity was not altered by SE treatment in rats that had been treated with no alcohol (normal group). These findings suggest that the decreased BAC in SE-pretreated rats is not associated with protection or activation of hepatic ADH directly by SE.

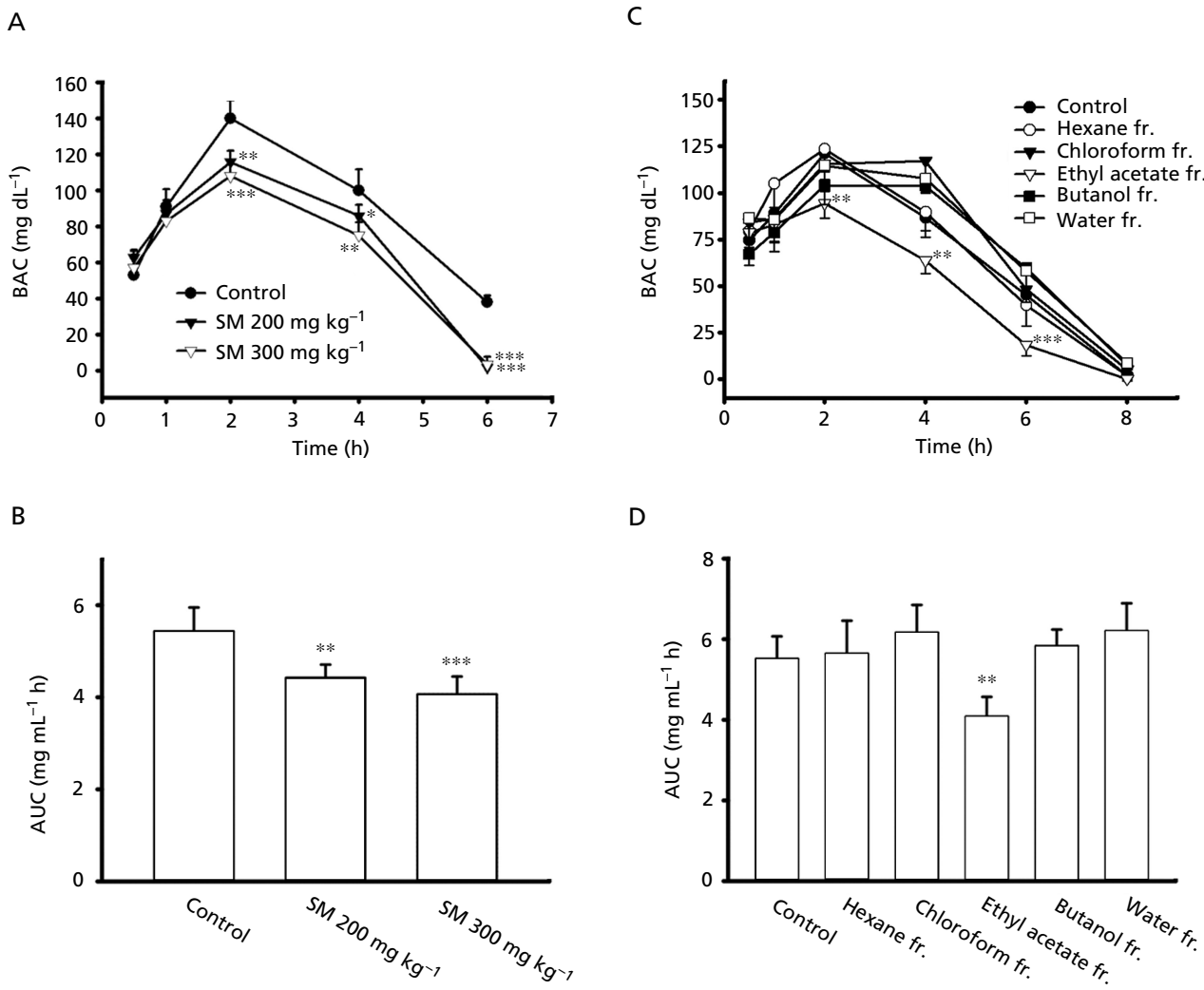

Figure 1 Effects of SM and its fractions on BAC and the AUC in acute alcohol-treated rats. Male Sprague-Dawley rats were fasted for 12 h before an oral administration of alcohol (3.0 g kg^{-1} in a 30% solution with water). SM at various concentrations (0, 200, 300 mg kg^{-1}) (A, B) and its fractions at 100 mg kg^{-1} (C, D) were dissolved in 1% DMSO and each sample was administered to the rats by oral intubation 30 min before alcohol administration. Each value represents the mean ± s.d. for 7–14 rats. *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$ vs control at the same time interval.

Effect of SE on the alcohol-derived acute hepatic toxicity

Hepatocellular necrosis, which results in the release of hepatocellular cytoplasmic enzymes such as ALT and AST into the systemic circulation, and hepatic lipid peroxidation are indicators of hepatotoxicity caused by acute alcohol intoxication (Uysal et al 1989; Jordão et al 2004). To determine whether SE (150 mg kg^{-1}) was able to protect against alcohol-induced hepatotoxicity, serum ALT and AST levels and hepatic MDA levels were determined in rats 6 h after alcohol administration. Alcohol ingestion resulted in a significant increase in serum ALT and AST ($P < 0.001$ and $P < 0.005$, respectively), and the increased enzyme activity was not altered by SE (Figure 4A). Hepatic MDA levels were increased significantly by alcohol ingestion ($P < 0.001$); however, the increased MDA levels were partially and statistically significantly restored by SE (Figure 4B). Serum ALT and AST activity and hepatic MDA levels were not changed by SE treatment in the normal group of rats.

Effect of SE on the antioxidant system

The effect of SE pretreatment on hepatic GSH levels and the enzymatic antioxidant system, SOD and catalase, in rats at time intervals of 0.5 and 2 h post alcohol administration are shown in Table 2. Hepatic GSH levels and SOD activity were not changed by the alcohol treatment, SE alone, or by the combined treatment of alcohol and SE. However, alcohol ingestion resulted in a slight but

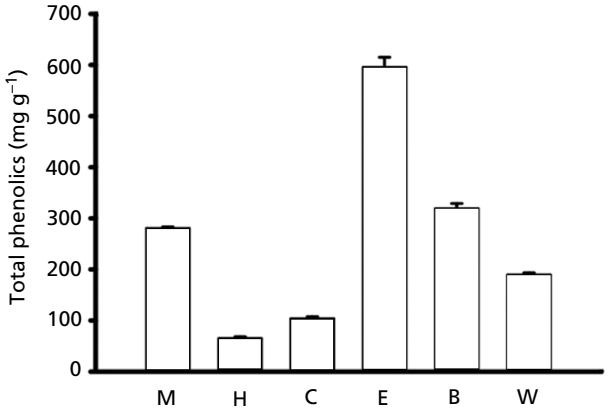

Figure 2 Total phenolics contents of SM and its fractions. Total phenolics contents in SM (M) and the n-hexane (H), the chloroform (C), the ethyl acetate (E), the n-butanol (B) and the water (W) fractions are expressed as tannic acid equivalents and each value represents the mean ± s.d. of three independent experiments.

statistically significant decrease in the activity of catalase at the 2-h time interval ($P < 0.005$), and this decreased catalase activity was completely protected against by SE pretreatment ($P < 0.005$).

Discussion

In the liver, alcohol is oxidized to acetaldehyde by three pathways, each of which is located in a different subcellular location: ADH, located in the cytoplasm; the microsomal ethanol oxidizing system (MEOS), located in the endoplasmic reticulum; and catalase, located in the peroxisomes (Park et al 2004). In these three pathways, ADH, which catalyses the oxidation of alcohol to acetaldehyde in the presence of NAD^+, is generally considered to be the major pathway for alcohol oxidation. On the other hand, the MEOS, which is recognized as an accessory pathway of alcohol oxidation, may account for the major pathway in the case of high BAC, especially during the long-term ingestion of alcohol, because of its higher K_m value compared with ADH (Lieber 1988; Tsutsumi et al 1989). Catalase not only catalyses the decomposition of H_2O_2 but also oxidizes alcohol at high concentrations in the presence of H_2O_2, and is also considered to have a very low or no contribution to hepatic alcohol oxidation under acute alcohol-treated conditions (Lieber 1985; Chen et al 1992).

The continued oxidation of alcohol following acute alcohol ingestion results in a shift in the intracellular redox state to a reduced state (decreased NAD^+-to-NADH ratio) and under such conditions the rate of alcohol oxidation would be reduced and alcoholic hepatotoxicity, such as hepatocellular necrosis and lipid peroxidation, would be increased (Lieber & Savolainen 1984). Indeed, the regeneration of hepatic NAD^+ during alcohol oxidation has been reported to increase the turnover rate of ADH and enhance the rate of alcohol oxidation (Ryle et al 1985; Chung et al 1994).

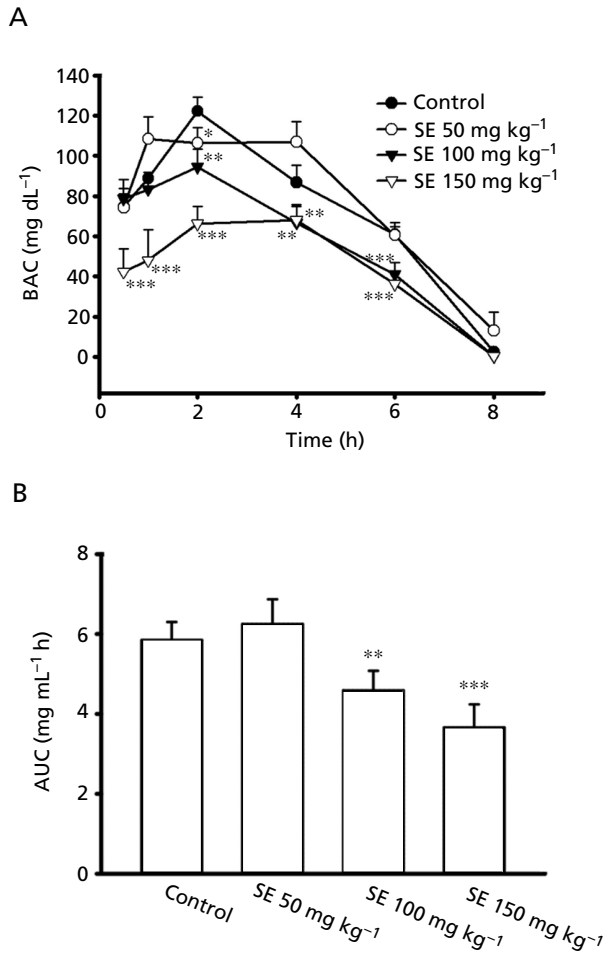

Figure 3 Effect of SE on BAC (A) and the AUC (B) in acute alcohol-treated rats. Male Sprague-Dawley rats were fasted for 12 h before an oral administration of alcohol (3.0 g kg^{-1} in a 30% solution with water). SE at various concentrations (0, 50, 100, 150 mg kg^{-1}) was dissolved in 1% DMSO and each sample was administered to rats by oral intubation 30 min before alcohol administration. Each value represents the mean ± s.d. for 7–14 rats. *$P < 0.05$, **$P < 0.01$, *** $P < 0.001$ vs control at the same time interval.

As an approach to the identification of natural amethystic agents, methanol extracts of various types of medicinal plants were tested for their ability to enhance alcohol oxidation in acute alcohol-treated rats in a preliminary study in comparison with the effect of hot water extract of *Puerariae flos*, which has been reported to show a significant decrease in BAC in the current experimental system (Kim et al 1995). As a result, a 30-min pretreatment with SM (300 mg kg^{-1}) caused a significant decrease (about a 25% decrease) in BAC at 2 h post alcohol administration, compared with the control group and this potency of SM was similar to that of the extract of *Puerariae flos* at the same concentration (data not shown). We therefore investigated its dose- and time-dependent potential on alcohol oxidation and hepatic lipid peroxidation.

Table 1 Effect of SE on hepatic cytosolic ADH activity in rats with or without acute alcohol treatment

Group	ADH activity (nM NADH min^{-1} (mg protein)$^{-1}$)		
	0.5 h post alcohol administration	2 h post alcohol administration	6 h post alcohol administration
Normal[a]	22.42 ± 4.15	22.28 ± 1.16	22.21 ± 0.26
Normal + SE	22.54 ± 1.37	21.74 ± 0.99	23.35 ± 1.23
Alcohol	20.26 ± 2.09	11.53 ± 2.84*	16.93 ± 3.44**
Alcohol + SE	18.58 ± 2.59	15.86 ± 5.26*	17.25 ± 2.87**

Male Sprague-Dawley rats were fasted for 12 h before an oral administration of alcohol (3.0 g kg^{-1} in a 30% solution with water). SE at 150 mg kg^{-1} was dissolved in 1% DMSO and administered to rats by oral intubation 30 min before the alcohol administration. The rats were sacrificed at 0.5, 2 and 6 h after alcohol administration and their livers were excised for the determination of ADH activity in the liver cytosol fraction. Each value represents the mean ± s.d. for 6–8 rats. *$P < 0.05$, **$P < 0.01$ vs normal at the same time interval. [a]Rats without alcohol administration.

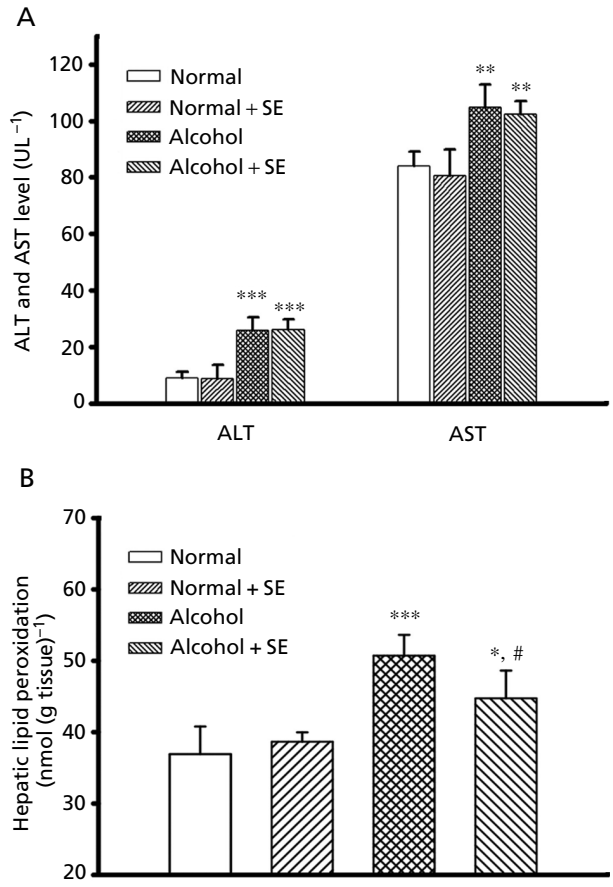

Figure 4 Effect of SE on serum ALT and AST levels (A) and hepatic lipid peroxidation (B) in rats with or without acute alcohol treatment. Male Sprague-Dawley rats were fasted for 12 h before an oral administration of alcohol (3.0 g kg^{-1} in a 30% solution with water). SE at 150 mg kg^{-1} was dissolved in 1% DMSO and administered to rats by oral intubation 30 min before alcohol administration. The rats were sacrificed 6 h after the alcohol administration and blood and livers were collected for the determination of ALT and AST levels in serum and lipid peroxidation in the total liver respectively. Each value represents the mean ± s.d. for 6–8 rats. *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$ vs normal rats; #$P < 0.05$ vs alcohol-treated rats at the same time interval.

There were significant decreases in BAC and the AUC in response to pretreatment with amounts of SM higher than 200 mg kg^{-1} (Figure 1A, B). The only effective fraction at 100 mg kg^{-1} was the ethyl acetate fraction (Figure 1C, D) and strong correlation ($r^2 = 0.9413$) was observed between the total phenolics content of SM fractions and the rate of alcohol oxidation, indicating that the constituents most responsible for the potency of the extract are largely contained in the ethyl acetate fraction and phenolics in SE would be the contributors to the potency. These results would be supported by data in which daidzin, a polyphenolic compound isolated from *Pueraria lobata*, caused a significant decrease in BAC in a similar experimental system (Lin & Li 1998). A 30-min pretreatment with SE at 150 mg kg^{-1} significantly reduced BAC at all time intervals during the absorptive period of alcohol (0–6 h), and caused about a 46% decrease in BAC at 2 h, compared with the control group (Figure 3A). To obtain additional information concerning the action mechanism of SE on BAC, the effect of SE on ADH activity was determined in-vitro and in-vivo. The activity of ADH was not affected by SM in the concentration range of 1–100 μg mL^{-1} in-vitro (data not shown), and the ADH activity of SE-administered rats was also not significantly different from that of the control group (Table 1). This indicates that the effect of SE on BAC cannot be accounted for by enhanced ADH activity. In other words, the decreased BAC in SE-pretreated rats might be due to decreased intestinal absorption, or increased gastric catabolism, rather than to an increased hepatic catabolism by ADH. On the other hand, several plant extracts have been shown to enhance the rate of alcohol oxidation in acute alcohol-treated rats by stimulating hepatic ADH activity directly (Shin et al 1995; Cho et al 2005).

According to a previous report (Sakai et al 1989), a 30-min pretreatment with an Aloe water extract led to a decreased BAC. Chung et al (1996), however, reported that at least a 6 h pretreatment of purified aloin was required to produce a significant reduction in BAC. Based on the results of this report, although the longer time course for SE pretreatment was not examined, a 30-min pretreatment with SE appeared to be sufficient to produce a significant reduction in BAC. Indeed, several plant extracts administered 0–60 min before alcohol ingestion have been shown to decrease BAC in acute alcohol-treated rats (Kim et al 1995; Shin et al 1995; Seo & Kim 2001). Based on the report that any single agent failed to produce a more extensive increase in the alcohol metabolism rate because when one process is accelerated, another becomes rate limiting (Chung et al 1996), combinations of agents that affect the total ADH activity with SE

Table 2 Effect of SE on the hepatic GSH level and the activity of antioxidant enzymes, SOD and catalase, in rats with or without acute alcohol treatment

Group	GSH level (μM (g tissue)$^{-1}$)		SOD activity (U (mg protein)$^{-1}$) (50% inhibition of autoxidation of haematoxylin))		Catalase activity (μM H_2O_2 reduced/min/mg protein)	
	Time post-alcohol administration					
	0.5 h	2 h	0.5 h	2 h	0.5 h	2 h
Normal[a]	4.83 ± 0.44	4.53 ± 0.16	10.86 ± 1.04	10.67 ± 1.41	2.52 ± 0.05	3.06 ± 0.41
Normal + SE	4.69 ± 1.58	4.74 ± 0.24	10.17 ± 0.37	10.63 ± 1.60	2.68 ± 0.21	2.82 ± 0.43
Alcohol	4.70 ± 0.89	4.64 ± 0.54	13.50 ± 2.11	11.37 ± 0.90	2.16 ± 0.21	2.05 ± 0.14*
Alcohol + SE	5.05 ± 1.23	4.86 ± 0.71	11.49 ± 2.01	11.49 ± 0.68	2.59 ± 0.39	3.16 ± 0.42#

Male Sprague-Dawley rats were fasted for 12 h before an oral administration of alcohol (3.0 g kg^{-1} in a 30% solution with water). SE at 150 mg kg^{-1} was dissolved in 1% DMSO and administered to rats by oral intubation 30 min before alcohol administration. The rats were sacrificed at 0.5 and 2 h after the alcohol administration and their livers were excised for the determination of GSH levels in total liver, SOD activity in the cytosol fraction and catalase activity in the mitochondria fraction. Each value represents the mean ± s.d. for 6–8 rats. *$P < 0.005$ vs normal, #$P < 0.005$ vs alcohol at the same time interval. [a]Rats without alcohol administration.

treatment might successfully create a further acceleration in alcohol oxidation.

The liver, the primary organ of alcohol metabolism, is highly susceptible to oxidative events associated with the toxicity of alcohol, and hepatocellular necrosis and hepatic lipid peroxidation are known to be important parameters for alcohol-induced hepatotoxicity. Therefore, the significant reduction in BAC by SE pretreatment (Figure 3A) suggests that acute liver injury induced by alcohol ingestion and the hepatic antioxidant system would be favourably changed by SE pretreatment. It has been reported that the optimum time for an elevation of hepatic lipid peroxidation levels appears to be 6 h after alcohol administration rather than other time intervals tested in similar experimental systems (Oh et al 1997; Scott et al 2000). Therefore, the parameters of alcohol-induced hepatic toxicity were determined at 6 h after alcohol administration. In this experiment, alcohol ingestion led to significant increases in hepatic MDA levels and serum ALT, as well as AST levels, within 6 h, and the increased ALT and AST levels were not altered as the result of SE pretreatment (Figure 4A). SE pretreatment, however, produced a significant level of protection against increased MDA levels (Figure 4B). These results indicate that SE pretreatment prevents the lipid peroxidation in liver that results from acute alcohol ingestion, partly by decreasing the bioavailability of alcohol (Figure 3A, B); however, there was no preventive effect of SE on hepatocellular necrosis induced by acute alcohol ingestion.

ROS, such as superoxide anions, hydrogen peroxide, hydroxyl radicals and lipid peroxides, can contribute to a variety of human diseases (Yokozawa et al 2004; Badenhorst et al 2005; Maharaj et al 2005; Rajasekaran et al 2005; Zhang et al 2005) or are present in toxic conditions such as acute alcohol ingestion (Ashak et al 1991; Fernandez-Checa et al 1993; Halliwell 1997). In normal conditions, ROS are efficiently scavenged by the cellular antioxidant defence system, which includes enzymes such as SOD, catalase, glutathione peroxidase and glutathione reductase, and non-enzymatic antioxidants such as GSH and vitamins A, C, and E (Rajasekaran et al 2004; Jahangir et al 2005; Sood et al 2005). However, when the ROS produced in the tissues exceed the ability of the antioxidant system to eliminate them, oxidative stress results (Jenkins & Goldfarb 1993). Many previous studies have demonstrated the effects of alcohol administration on the major hepatic antioxidant enzymes and substrates, but the reported changes in those parameters after alcohol administration are highly contradictory (Husain & Somani 1997; Schlorff et al 1999; Scott et al 2000; Jordão et al 2004; Uzun et al 2005). These reported discrepancies might be due to variations in experimental design, including the mode of administration, dose, animal species, gender and age. Depletion of hepatic GSH and SOD is generally known to be indicative of hepatic injury induced by acute alcohol ingestion in rats and this depletion is intimately associated with an increased level of hepatic lipid peroxidation (Speisky et al 1985; Scott et al 2000; Uzun et al 2005). On the other hand, several groups have reported no change in GSH levels (Jordão et al 2004) and an increase in SOD activity (Valenzuela et al 1980; Genç et al 1998) following acute alcohol administration. Reported results on the effect of acute alcohol ingestion on catalase activity in rats also appear to be very contradictory (Chen et al 1992; Oh et al 1997; Scott et al 2000). In this experiment, GSH levels and SOD activity were not changed at 0.5 and 2 h after alcohol administration, at which time a clear change in each parameter was observed in the alcohol-treated group compared with the normal group (Schlorff et al 1999; Scott et al 2000). These results appear to be due to either a complete lack of susceptibility of GSH and SOD to alcohol ingestion or time differences in the periods studied. Catalase activity at the same time intervals, however, was decreased by alcohol ingestion and SE pretreatment completely protected the decreased catalase activity. It is known that alcohol metabolism

through catalase is one of the three pathways that operate during high-dose alcohol ingestion. However, it has been shown that the hepatic catalase pathway is not stimulated by acute alcohol treatment (Chen et al 1992). In several previous studies, it has been also reported that catalase activity was reduced in acute alcohol-treated rats, suggesting that the reduced activity was due to oxidative inactivation by ROS generated from alcohol metabolism or inhibition by elevated influx of superoxides, a catalase inhibitor (Pigeolet et al 1990; Schlorff et al 1999). Our results are consistent with the findings of Bykova & Zhukova (1991), Dinu & Zamfir (1991) and Husain & Somani (1997), who reported decreased catalase activity in the liver of alcohol-treated rats. Based on these findings, catalase appears to be used as an antioxidant defence enzyme to catalyse H_2O_2 decomposition rather than as an alcohol oxidizing enzyme in this experiment as well.

Conclusion

In conclusion, a 30-min pretreatment with SM higher than 200 mg kg^{-1} caused significant decreases in BAC and the AUC in acute alcohol-treated rats. The constituents most responsible for the potency of SM were largely contained in the ethyl acetate fraction, which contained a higher content of phenolics than other fractions. Pretreatment with SE, which decreased BAC, reduced hepatic lipid peroxidation by decreasing the bioavailability of alcohol and its oxidative metabolites such as H_2O_2, at least partly, through the protection of hepatic catalase in acute alcohol-treated rats. Therefore, *Sorbus commixta* cortex extract represents a potential agent for the treatment of acute alcohol intoxication. Further studies on the identification of the active compound(s), as well as the mechanism of action, will be needed to develop a better understanding of its potency on BAC and alcohol intoxication.

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