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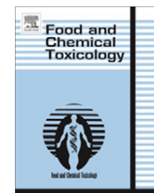

Taraxerone enhances alcohol oxidation via increases of alcohol dehyderogenase (ADH) and acetaldehyde dehydrogenase (ALDH) activities and gene expressions

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ABSTRACT

The present study, taraxerone (D-friedoolean-14-en-3-one) was isolated from *Sedum sarmentosum* with purity 96.383%, and its enhancing effects on alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) activities were determined: EC_{50} values were 512.42 ± 3.12 and 500.16 ± 3.23 μM for ADH and ALDH, respectively. In order to obtain more information on taraxerone related with the alcohol metabolism, 40% ethanol (5 mL/kg body weight) with 0.5–1 mM of taraxerone were administered to mice. The plasma alcohol and acetaldehyde concentrations of taraxerone-treated groups were significantly lowered than those of the control group ($p < 0.01$): approximately 20–67% and 7–57% lowered for plasma alcohol and acetaldehyde, respectively. Compare to the control group, the ADH and ALDH expressions in the liver tissues were abruptly increased in the taraxerone-treated groups after ethanol exposure. In addition, taraxerone prevented catalase, superoxide dismutase, and reduced glutathione concentrations from the decrease induced by ethanol administration with the concentration dependent manner.

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1. Introduction

Alcohol consumption has been continued from the pre-historic times with many reasons; to quench thirst, to get drunk, to participate in social and/or religious ceremonies, and to feed an addiction in many cultures. The effects of acute alcohol consumption on the human body can take several forms; dehydration, fatigue, headache, nausea, vomiting, flushing, diarrhea, elevated body temperature, sensitivity to light and/or noise, and sleeping problem (Lieberman et al., 2005; Pittler et al., 2005). These unpleasant physiological symptoms and/or reactions following excessive alcohol consumption are known generically as hangover (veisalgia).

Most absorbed alcohol is removed mainly via hepatic oxidation by alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) which classified as phase I xenobiotic metabolizing enzymes, and members of the microsomal ethanol oxidizing system as cytochrome P450 (CYP2E1). Alcohol is oxidized into acetaldehyde by ADH in the liver cell's cytosol or fluid, and by CYP2E1 in the liver cell's mitochondria or cytosol. Acetaldehyde, the metabolic intermediate of alcohol oxidation, is further oxidized into acetic acid and water by ALDH.

The accumulation of acetaldehyde by the inactivation and/or disturbance of ALDH leads to serious consequences to the subject. That is, increased and repeated acetaldehyde exposure due to increased alcohol consumption could increase the risk of development of acetaldehyde related pathology to the host, thus acetaldehyde is more toxic to the body than ethanol. Therefore, complications of alcohol intake could be solved by effectively decreasing the plasma acetaldehyde concentration.

Many documents have reported the detoxification effects of plant food components such as theanine, resveratrol, saponin, and cryptotanshinone in the prevention and/or treatment of acute and/or chronic alcoholism and alcohol induced diseases (Li et al., 2012; Yan et al., 2012; Khanal et al., 2009; Yin et al., 2009).

Taraxerone has been identified from higher plants, and it has cytotoxic activity against cancer cell lines (Setzer et al., 2000; Moulisha et al., 2009; Ahmed et al., 2010), inhibitory activity on topoisomerase (Setzer et al., 2000), antimicrobial activity (Manríquez-Torres et al., 2007; Ahmed et al., 2010), antiviral activity (Kuljanabhagavad et al., 2009), antiparasitic activity (Gachet et al., 2011), and insecticidal activity (Wang, 2010). However, its effect on alcohol oxidation has yet to be reported. Thus, the objective of this study was to evaluate the enhancing effects of taraxerone on ADH and ALDH activities via in vitro and in vivo studies.

2. Materials and methods

2.1. Preparation of taraxerone

Taraxerone was separated from the ethyl acetate extract of *Sedum sarmentosum* as described earlier (Mo et al., 2012), and the purity was confirmed by high performance liquid chromatography (HPLC, Shimazu; LC-20AT/CBM-20A/SPD-20A/

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CTO20A, Sidney, NSW, Australia) and gas chromatography (GC, Agilent 6890 N, Santa Clara, CA, USA). Electron impact-mass spectrometry (EI-MS) was performed using a mass spectrometer (5975E MSD, Agilent) under the same GC analytical conditions. Mass spectra were obtained by electron impact ionization at 70 eV; the ion species were of a normal ion (MF-Linear) and the TIC range was from 0 *m*/*z* to 600. The spectrometric data were compared with those from the NIST Hewlett–Packard 59942C original library mass-spectra. The HPLC and GC analytical conditions were described in Table 1.

2.2. ADH and ALDH activities

Effects of taraxerone on the ADH and ALDH activities were determined by commercial assay kits (K-ETOH; K-ACHYD, Megazyme, Wicklow, Ireland) following manufacturer's instructions. Results were expressed as the ethanol and acetaldehyde concentrations in the reaction mixtures after enzyme reactions, respectively. Dihydromyricetin (DHM, purity > 99%; Selleckchem, Houston, TX, USA) was used as a positive control. Taraxerone and DHM were dissolved in dimethyl sulfoxide (DMSO, Sigma–Aldrich, St. Louis, MO, USA), respectively, and used.

2.3. Animal treatments

Male ICR mice (20–25 g) were purchased from Daihan Bio Link Co. (Eumsung, Korea). Mice were kept in stainless steel cages with wire mesh bottoms and housed in a temperature and light control environment (20 ± 2 °C; 12 h light–dark cycle), and maintained standard rodent chow and tap water ad libitum for 1 week. Mice were randomly assigned to five experimental groups, and fasted overnight. All groups were administered 40% ethanol. Soon after, Group 1 received 500 μM taraxerone, Group 2 received 1 mM taraxerone, Group 3 (positive control) received 3 mM DHM, Group 4 received DMSO (control), Group 5 received saline, respectively. Taraxerone and DHM were dissolved in DMSO, and all treated samples (ethanol, taraxerone, DHM, DMSO, and saline) were administered orally (5 mL/kg body weight). Animals were deprived of food and water during the experiments.

Mice were anesthetized, liver tissues in four mice from the each group and blood from the caudal vena cava using heparin vacutainers were collected for 3 h after ethanol administration. All animal procedures described conformed to the principles of Institute of Laboratory Animal Resources (2010).

2.4. Ethanol and acetaldehyde concentration in plasma

Blood samples were centrifuged at 3000 rpm at 4 °C for 15 min. Ethanol concentration was detected by a Roche Cobas Integra 400 analyzer (Roche Diagnostics, Grenzacherstrasse, Basel, Switzerland). Acetaldehyde concentration was determined using a commercial assay kit (Boehringer Mannheim, Mannheim, Germany) following the manufacturer's instructions.

2.5. ADH and ALDH expressions

Liver tissue samples from an each group were snap-frozen in liquid nitrogen and stored at −70 °C until the experiments. Total RNAs for ADH and ALDH were prepared by using TRIzol^R reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. One microgram of total RNA was reverse-transcribed using a one-step RT-PCT kit (Qiagen, Valencia, CA, USA) according to the supplier's instruction. The primers used in this study were Left: 5′-GCAAGCTTTCCGTGAGAAAC-3′ and Right: 5′-TCCACCAAAGGGCATAGAAG-3′ for ADH1 (449 bp), Left: 5′-CAGGTTCCACTGAGGTTGGT-3′ and Right: 5′-ATGCCATCTTTGACGTCTCC-3′ for ALDH2 (448 bp), and Left: 5′-CTGACCGAGCGTGGCTAC-3′, and Right: 5′-CCTGCTTGCTGATCCACA-3′ for beta-actin (505 bp). Beta-actin genes constitutively expressed were used as a positive control of cDNA amplification. RT-PCR conditions were as follows: 95 °C for 3 min for denaturation; 94 °C for 45 s, 56 °C for 45 s annealing, and 72 °C for 1 min extension, 25 cycles; 72 °C for 10 min final extension (MyGenie 96 thermal block, Bioneer, Alameda, CA, USA). Five microliters of PCR products were electrophoretically run on a 1.2% agarose gel and stained with ethidium bromide in order to visualize the intensity and profile of DNA amplicons. The intensity of each band was determined by a densitometer (620 Video densitometer, BioRad, Hercules, CA, USA).

Table 1
Analytical conditions of HPLC and GC.

	HPLC	GC
Stationary phase	C_{18} (250 × 4.6 mm)	DB-1 (30 m, 0.32 mm, 5.0 μm)
Mobile phase	Acetonitrile:water = 60:40 (Flow rate = 1 mL/min)	Nitrogen (Split ratio = 10:1)
Detector	UV (200 nm)	FID
Oven temperature	40 °C	70 °C (5 min)//10 °C/min// 320 °C (5 min)
Detector temperature	Room temperature	320 °C
Injector temperature	Room temperature	300 °C

2.6. Determination of antioxidant components in the liver

The concentrations of catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH) in the liver tissue were evaluated using commercial assay kits, following supplier's instructions (Applied Bioanalytical Labs, Bradenton, FL, USA).

2.7. Statistical analysis

All analyses were performed in triplicated using SPSS (ver. 14, SPSS Inc., IL, USA). Data were expressed as the mean ± standard deviation (S.D.). One-way analysis of variance and Duncan's multiple range test were used to analyze significant differences. The *p*-values less than 0.05 were considered statistically significant.

3. Results

3.1. Preparation of taraxerone

Taraxerone was separated and isolated from *S. sarmentosum* described earlier (Mo et al., 2012). Based on the result of HPLC analysis, the purity of separated taraxerone (D-friedoolean-14-en-3-one) was 96.383% (Fig. 1A). In order to confirm the taraxerone, GC analysis was performed. As presented in Fig. 1B, the only single peak was detected, and it was identified as taraxerone (MW 424) by GC–MS analysis (Fig. 1C). Thus, the separated taraxerone was used in the following experiments.

3.2. Effects of taraxerone on ADH and ALDH activities

According to the manufacturer's instructions, the lower ethanol and acetaldehyde concentrations in the assay systems after enzyme reactions mean higher ADH and ALDH activities. In the present study, tested samples including positive control, DHM, were dissolved in DMSO, thus, the effects of DMSO on the activities of ADH and ALDH were determined (Table 2). The ADH activity was not affected by DMSO, however, ALDH activity was 12.56% decreased compare to the saline-treated group. Therefore, the DMSO-treated group used as a control group.

The ADH and ALDH activities were considerably increased by the taraxerone treatments; ADH activities were increased 4.87, 5.89, and 6.01 times, and ALDH activities were elevated 9.15, 11.46, and 11.51 times at 1, 3, and 5 mM of taraxerone, respectively. Compare to 3 mM of taraxerone-treated group, the ADH and ALDH activities of 5 mM of taraxerone-treated group were narrowly decreased, but significant differences were not detected between them. The DHM-treated groups also showed lower ethanol and acetaldehyde concentrations than that of the control group, however, 1 mM DHM did not affect the ADH and ALDH activities. The enhancing effects of taraxerone on ADH and ALDH activities were approximately 1.5 and 2 times higher than that of DHM at 3 and 5 mM treatments, respectively.

In order to calculate the half maximal efficient concentration (EC_{50}) of taraxerone, the dose–response curves were obtained (Fig. 2). Based on these results, EC_{50} of taraxerone on the elevation of ADH and ALDH activities were 512.42 ± 3.12 and 500.16 ± 3.23 μM, respectively.

3.3. Effects of taraxerone on plasma alcohol and acetaldehyde concentrations

Alcohol and acetaldehyde concentrations in plasma from ethanol-loaded mice were determined for three hours after oral administration of 40% ethanol with tested samples (Fig. 3). At 30 min after ethanol exposure, significant differences of plasma alcohol concentrations were not detected among samples. The highest

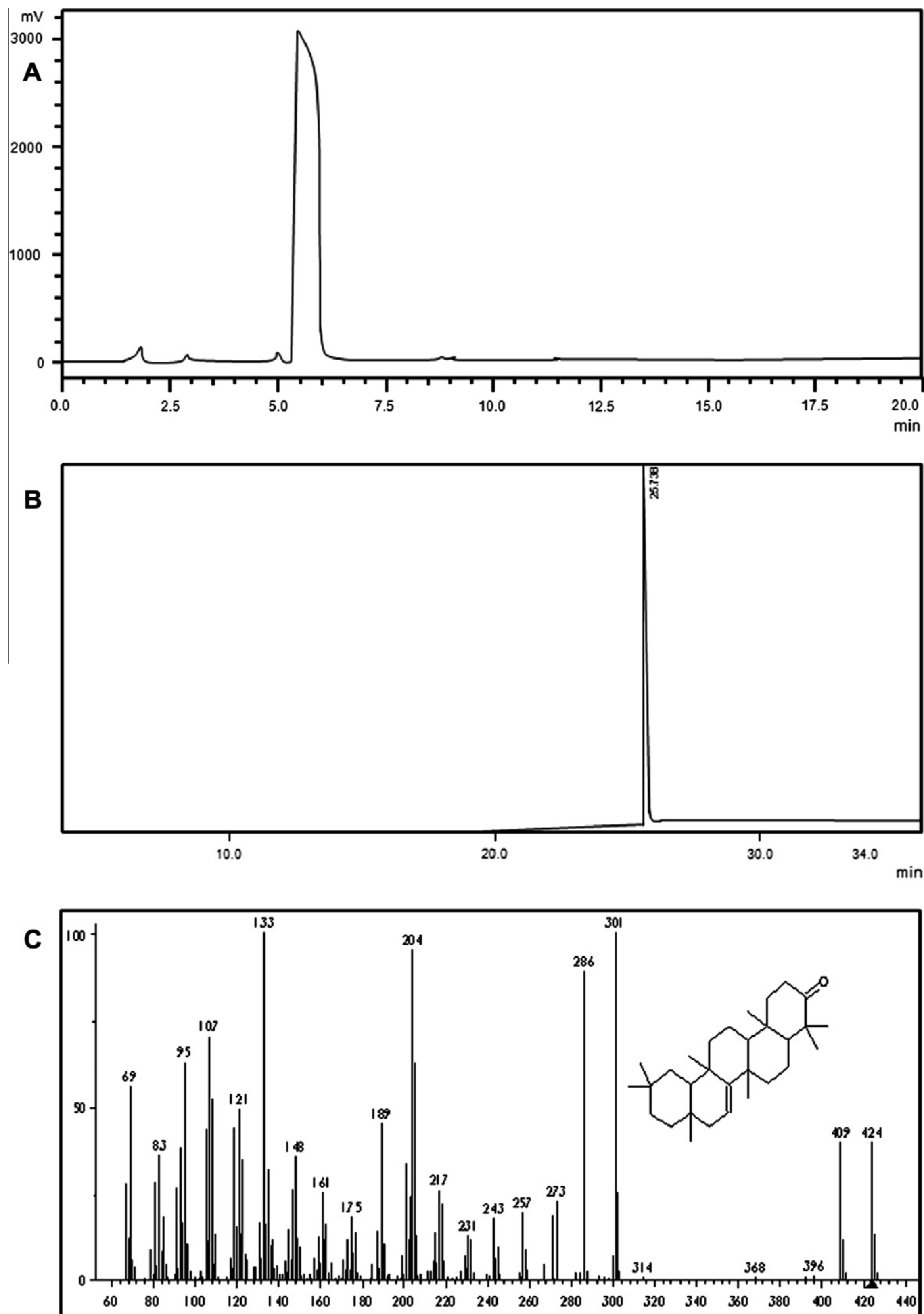

Fig. 1. Chromatograms and mass spectrum of taraxerone isolated from the ethyl acetate extract of *S. sarmentosum*. (A) HPLC chromatogram, (B) GC chromatogram and (C) mass spectrum.

plasma alcohol concentrations were observed after 1 h ethanol exposure, and decreased in all experimental groups. The plasma alcohol concentrations of Group 5 (saline) were slightly lower than that of Group 4 (DMSO, control), however, significant differences were not observed between them. The plasma alcohol concentrations of Group 3 (positive control) were approximately 5% lower

Table 2
Effects of taraxerone on ADH and ALDH activities.

	Ethanol (mM)		Acetaldehyde (mM)	
	Taraxerone	DHM[a]	Taraxerone	DHM
Saline	517.04 ± 19.01[a]	517.04 ± 19.01[a]	584.28 ± 15.83[a]	584.28 ± 15.83[a]
Control (DMSO)	517.04 ± 19.01[a]	517.04 ± 19.01[a]	584.28 ± 15.83[a]	584.28 ± 15.83[a]
1 mM	106.57 ± 2.43[b]	522.03 ± 12.65[a]	73.05 ± 2.45[b]	660.13 ± 10.54[b]
3 mM	88.09 ± 3.43[c]	122.03 ± 10.49[b]	58.29 ± 0.09[c]	106.60 ± 5.87[c]
5 mM	86.32 ± 2.91[c]	121.23 ± 9.93[b]	58.05 ± 2.08[c]	108.08 ± 6.24[c]

[a] DHM, dihydromyricetin. The different letters in a column denote values that were significantly different ($p < 0.05$).

than that of the control group throughout the experiments. Compare to the control group (Group 4), the plasma alcohol concentrations of taraxerone-treated groups (Groups 1 and 2) were obviously decreased; 30.1%, 28.5%, and 20% lowered in Group 1, and 32.8%, 58.6%, and 66.7% lowered in Group 2 at 1, 2, and 3 h, respectively.

The plasma acetaldehyde concentrations at 30 min after ethanol loaded mice were dissimilar: Group 4, Groups 3 and 5, Group 1, and Group 2 in that order, and significant difference was detected between Group 4 and Group 2. The plasma acetaldehyde concentrations of Groups 3, 4, and 5 showed tendencies to the changes of plasma alcohol concentrations. No significant differences between Group 3 and Group 4 were observed until after one hour; plasma acetaldehyde concentrations of Group 3 were significantly lower than that of Group 4 after two hours. Compare to the control group, the taraxerone-treated groups (Groups 1 and 2) showed tendencies to decrease during the whole experimental periods: 6.6%, 23.1%, 38.1%, and 52.2% declined in Group 1, and 20.1%, 57.7%, 54.8%, and 56.5% declined in Group 2, respectively.

Based on these results, the reducing effects of taraxerone on plasma alcohol and acetaldehyde concentration after ethanol exposure were superior to DHM, a positive control.

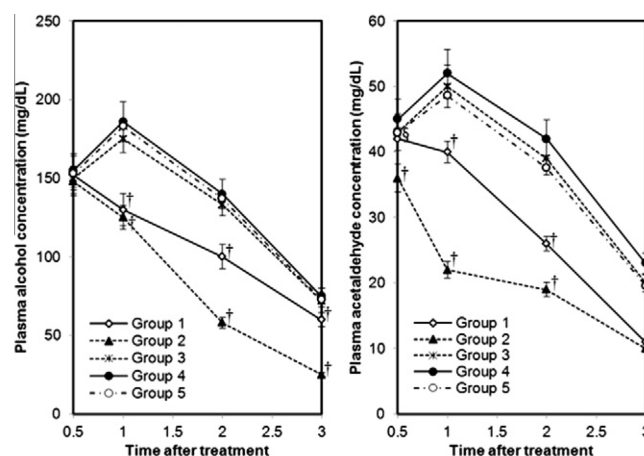

Fig. 3. Changes of plasma alcohol and acetaldehyde concentrations in 40% ethanol loaded mice for 3 hours. Group 1, 500 μM taraxerone; Group 2, 1 mM taraxerone; Group 3, 3 mM DHM (positive control); Group 4, DMSO (control); Group 5, saline. Taraxerone and DHM were dissolved in DMSO, and all treated samples (ethanol, taraxerone, DHM, DMSO, and saline) were administered orally (5 mL/kg body weight). § and † represent significant differences at $p < 0.05$ and $p < 0.01$, respectively. The error bar represents standard deviation.

3.4. ADH and ALDH expressions

The expressions of ADH and ALDH from liver tissues were determined for 40% ethanol loaded mice at the same time as oral administration of each treatment during three hours (Fig. 4). The expressions of ADH ALDH in Groups 3 (positive control), 4 (DMSO, control) and 5 (saline) were highly increased at 1 h after ethanol intake, and gradually declined. The ADH expressions at 1 h and ALDH expressions at 3 h after ethanol exposure were significant differed between Group 4 and Groups 3 and 5 ($p < 0.05$). Compare to Group 5 (saline treated group), ADH expressions were slightly decreased (13–42%) by the DMSO treatment but not significant ($p = 0.672$). The ALDH expression of Group 4 at 30 min after ethanol exposure was considerably lower than that Group 5, and showed lower expressions (approximately 20–40%) at the rest of experimental time.

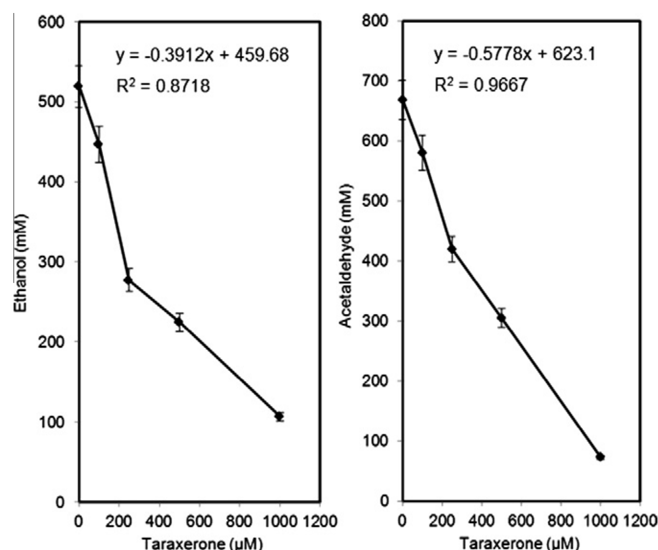

Fig. 2. Dose–response curves of taraxerone on the activities of ADH and ALDH. The error bar represents standard deviation.

The ADH and ALDH expressions were abruptly increased in the taraxerone-treated groups compare to Group 4. In the taraxerone-treated groups, ADH expressions were increased in conformity with the experimental time, and showed the concentration dependent manner based on the cDNA intensities: Group 2 showed approximately 1.2–2.3 times higher ADH expressions than that of Group 1. The highest ALDH expressions of the taraxerone-treated groups were detected at 1 h after ethanol exposure, and gradually decreased. According to cDNA intensities, ALDH expressions of the taraxerone-treated groups were also concentration dependent manner: approximately 1.01–4.02 times higher ALDH expressions were observed in Group 2. Compare to Group 3, the taraxerone-treated groups showed significantly higher ADH and ALDH expressions: the former was 2.24–10.25 times and the latter was 2.23–4.36 times increased.

3.5. Concentrations of hepatic antioxidant components

The hepatic CAT, SOD, and GSH contents of the ethanol unexposed mouse ($n = 3$) were 15.865 ± 0.0019, 12.528 ± 0.0013 U/mg

protein, and 5.153 ± 0.0128 μM/mg protein, respectively. The levels of CAT, SOD, and GSH in the liver tissues were considerably decreased by the ethanol administration in all treated groups at 30 min after ethanol exposure. The lowest contents of CAT, SOD, and GSH were observed in the early period of experiment (0.5–1 h), and these levels were elevated along with the increase of experimental times. However, Groups 4 and 5 could not restore these hepatic antioxidant components to the normal ranges till 3 h.

As presented in Table 3, the taraxerone administration prevented hepatic CAT, SOD, and GSH levels from the decrements induced by ethanol exposure. The taraxerone-treated groups showed approximately 25–50%, 4–21%, and 22–58% higher hepatic concentrations than those of Group 4 for CAT, SOD, and GSH, respectively. These antioxidant components contents of Group 2 were higher than those of Group 1: 1.61–1.68, 1.2–3.12, and 1.1–1.3 times for CAT, SOD, and GSH, respectively.

4. Discussion

Ethanol oxidation is a multistep process: ADH, cytochrome P450 2E1 (CYP2E1), and CAT contribute to oxidative metabolism of ethanol. The predominant ethanol-metabolizing enzyme, ADH, produces acetaldehyde and NADH. Acetaldehyde and other products such as reactive oxygen species (ROS) of ethanol metabolism have been implicated in many pathogenic effects of ethanol. The oxidative stress induced by ethanol plays an important role in the development of ethanol related disease, thus many studies were reported that ethanol intoxication could be alleviated by the treatment of antioxidant compounds such as DHM (Shen et al., 2012; Liu et al., 2009; Yoo et al., 2006).

Taraxerone has been isolated from various plants and its physicochemical properties have been reported described in Section 1, and it has potent antioxidant activity (Mo et al., 2012). No study on the relationship between taraxerone and alcohol metabolism has been reported. Based on in vitro assay, ADH and ALDH activities were considerably enhanced by taraxerone and DHM. Taraxerone maintained its enhancing effects on ADH and ALDH activities, but DHM could not retain its enhancing effects at low concentration (1 mM). Furthermore, the reducing effects of taraxerone on plasma alcohol and acetaldehyde contents after ethanol exposure were superior to DHM. It was considered that taraxerone powerfully promote ADH and ALDH gene expressions as soon as exposure to ethanol. The highest ADH and ALDH gene expressions of the control- and DHM-treated groups were observed at 1 h after ethanol intake, and gradually decreased. However, the highly promoted ADH and ALDH gene expressions in the taraxerone-treated groups persisted throughout experimental period with the concentration dependent manners, and these promotions resulted in the decrease of plasma alcohol and acetaldehyde concentration after ethanol intake.

The acute ethanol exposure is associated with increase of lipid peroxidation, production of ROS, and especially decreases in hepatic GSH content (Arteel, 2003). Thus, the active metabolites such as ROS could be detoxified by the sufficient amount of GSH in the liver. The CAT requires hydrogen peroxide (H_2O_2) to oxidize ethanol, and induces the decomposition of H_2O_2 to water and oxygen. The SOD plays an important role in the antioxidant system in the body by lowering the amount of O_2^-. Therefore, the maintenance of antioxidant mechanism as a normal condition and/or the inhibition of ROS production are important for the protection of liver

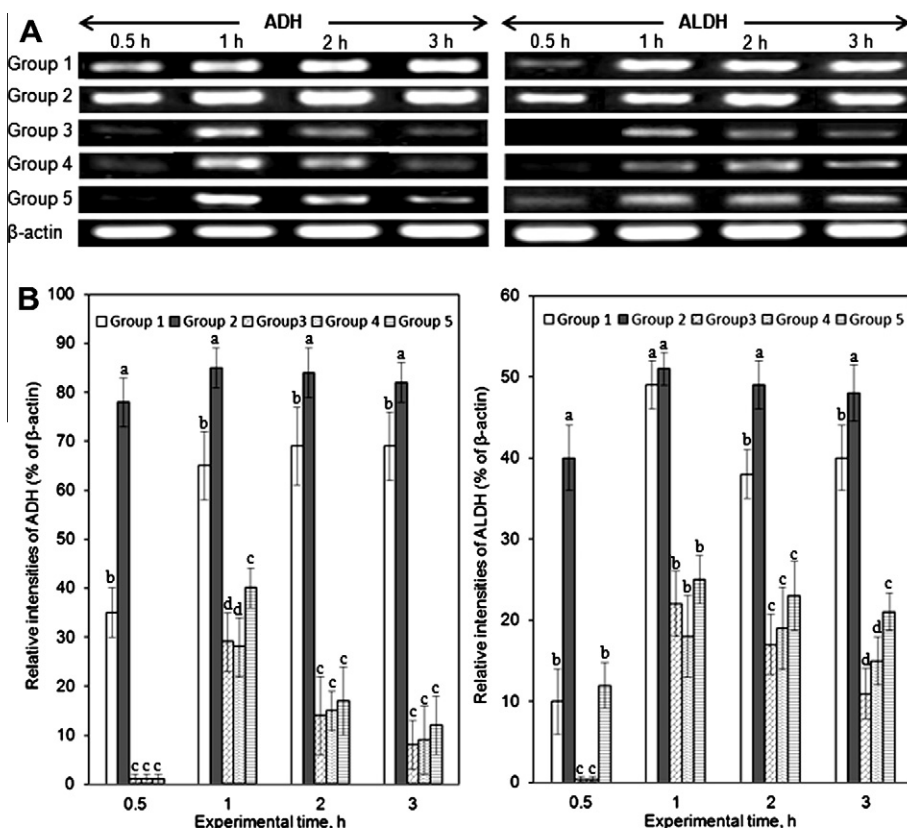

Fig. 4. ADH and ALDH expressions (A) and their cDNA intensities (B) in 40% ethanol loaded mice for three hours. Group 1, 500 μM taraxerone; Group 2, 1 mM taraxerone; Group 3, 3 mM DHM (positive control); Group 4, DMSO (control); Group 5, saline. Taraxerone and DHM were dissolved in DMSO, and all treated samples (ethanol, taraxerone, DHM, DMSO, and saline) were administered orally (5 mL/kg body weight). The same letters denote significant differences at $p < 0.05$, and the error bar represents standard deviation.

Table 3
Effects of taraxerone on the concentrations of hepatic antioxidant components in 40% ethanol loaded mouse.

		Experimental time (h)			
		0.5	1	2	3
CAT (U/mg protein)	Group 1	12.881 ± 0.0916[c]	13.014 ± 0.1172[c]	13.142 ± 0.1183[c]	13.265 ± 0.0132[d]
	Group 2	14.654 ± 0.0201[d]	14.779 ± 0.0188[d]	14.942 ± 0.0189[d]	14.953 ± 0.0144[e]
	Group 3	10.293 ± 0.0089[a]	10.188 ± 0.0097[ab]	10.241 ± 0.0499[ab]	11.635 ± 0.0518[c]
	Group 4	10.282 ± 0.0182[a]	10.124 ± 0.0161[a]	10.139 ± 0.0313[a]	10.529 ± 0.0325[a]
	Group 5	10.849 ± 0.0368[b]	10.269 ± 0.0364[b]	10.275 ± 0.0304[b]	10.673 ± 0.0315[b]
SOD (U/mg protein)	Group 1	10.796 ± 0.0015[a]	10.899 ± 0.0020[b]	11.549 ± 0.0663[b]	12.523 ± 0.0051[bc]
	Group 2	10.798 ± 0.0012[b]	11.837 ± 0.0021[c]	12.529 ± 0.0018[c]	12.528 ± 0.0017[b]
	Group 3	10.797 ± 0.0036[b]	11.535 ± 0.0019[d]	12.371 ± 0.0253[d]	12.502 ± 0.0241[c]
	Group 4	10.791 ± 0.0035[a]	10.463 ± 0.0034[a]	10.361 ± 0.0024[a]	10.367 ± 0.0189[a]
	Group 5	10.799 ± 0.0005[b]	10.462 ± 0.0011[a]	10.372 ± 0.0145[a]	10.374 ± 0.0076[a]
GSH (μM/ g protein)	Group 1	4.532 ± 0.0123[b]	4.685 ± 0.1138[b]	4.852 ± 0.0025[c]	4.893 ± 0.0056[b]
	Group 2	4.769 ± 0.0133[c]	4.854 ± 0.0598[c]	4.985 ± 0.0066[d]	5.034 ± 0.0102[c]
	Group 3	4.833 ± 0.0094[d]	4.685 ± 0.0165[b]	4.775 ± 0.0079[e]	4.798 ± 0.0031[d]
	Group 4	3.704 ± 0.0168[a]	3.125 ± 0.0157[a]	3.165 ± 0.0057[a]	3.2102 ± 0.015[a]
	Group 5	3.711 ± 0.0132[a]	3.139 ± 0.0116[a]	3.179 ± 0.0121[b]	3.2143 ± 0.014[a]

Group 1, 500 μM taraxerone; Group 2, 1 mM taraxerone; Group 3, 3 mM DHM (positive control); Group 4, DMSO (control); Group 5, saline. Taraxerone and DHM were dissolved in DMSO, and all treated samples (ethanol, taraxerone, DHM, DMSO, and saline) were administered orally (5 mL/kg body weight). The different letters in a column denote values that were significantly different ($p < 0.05$).

from ethanol exposure (Lee at al., 2010). Based on our results, the contents of CAT, SOD, and GSH were significantly reduced in the control group, while the hepatic levels of these antioxidant components were substantially increased in the taraxerone- and DHM-treated groups as compared to the control group. From the results of this study, ethanol exposure could considerably exhaust the CAT content in the liver. Taraxerone-treated groups maintained significantly high CAT concentrations throughout the whole experimental period, while DHM could not increase the hepatic CAT content as taraxerone treatment. The taraxerone- and DHM-treated groups recovered the losses of hepatic SOD and GSH contents to the normal range for ethanol exposure at the end of experiments. In addition, low concentration (500 μM) of taraxerone showed comparable capacity to high concentration of DHM (3 mM) toward the normalization of hepatic SOD and GSH contents reduced by ethanol consumption. Therefore, taraxerone could provide its antioxidant capacity against ethanol induced oxidative stress.

Taraxerone and DHM used in this study were dissolved in DMSO which reacts with hydroxyl radicals and inhibits the oxidation rate of ethanol in liver microsomes (Cederbaum et al., 1977). In this study, the amount of DMSO was minimized to dissolve taraxerone and DHM when performed in vitro assay. According to the results of in vitro assay, ADLH activities were slightly decreased by DMSO, while ADH activities were not influenced. Compare to the saline-treated group, the ADH and ALDH gene expressions at 30 min after ethanol intake and hepatic CAT contents were significantly lowered in the DMSO-treated group (control group). It was considered that DMSO could inhibit ethanol oxidation via the reduction of ADH and ALDH gene expressions as well as the decreased of ALDH activity, and the loss of hepatic CAT contents. Considering these results, taraxerone might have more enhancing capacities on ADH and ALDH activities and/or expressions when it existed in plant food, *S. sarmentosum*.

DHM, an active component of *Hovenia dulcis*, is known that it prevents the liver injury from ethanol consumption and enhances ADH and ALDH activities (Shen et al., 2012; Kim et al., 2000). The hepatic CAT contents of DHM-treated group were significantly lowered than that of the taraxerone-treated groups throughout the experiment despite DHM has potent antioxidant power (Liu et al., 2009). Considering the more oxidative condition could be caused by the more exposure time to ethanol, strong ADH and ALDH activities could be prevented the loss of antioxidant component such as CAT from ethanol oxidation. It was considered that the reduction of ROS generating time could be more important than scavenging free radicals produced during ethanol oxidation for the protection of liver from ethanol intoxication.

Triterpenoids are made from six isoprene units (C30), and synthesized in plants from squalene precursor (Spanova and Daum, 2011). It is well known that triterpenoids have multiple biological and/or pharmaceutical activities. It was reported that betulin, betulinic acid, and oleanolic acid decreased ethanol-induced oxidant production such as superoxide anion and hydrogen peroxide, thus these triterpenoids are able to protect the hepatocyte from the ethanol-induced cytotoxicity (Szuster-Ciesielska and Kandefer-Szerszen, 2005). Thus, the administration of triterpenoids protects the liver from the ethanol-induced hepatic diseases. Rogchaponin, a pentacyclic triterpenoid which was isolated from *Camellia sinensis*, showed inhibitory activity against ADH, and no inhibitory activity against ALDH (Varughes et al., 2011). It was considered that the enhancing effects of taraxerone on ADH and ALDH activities originated with its biological characteristics not depend on its chemical structure.

In our previous work (Mo et al., 2012), approximately 7.5 mg of taraxerone was isolated from 1 kg of dried sedum stems and leaves by simple solvent extraction method. Sedum (*Sedum sarmentosum* Bunge, stonecrop, *Dolnamul*) is a type of perennial herb widely distributed in Asia, Europe, and North America. Generally, fresh sedum stems and leaves are consumed as an ingredient in salads, and sedum juice has been used in the making of gelatin jelly (Mo et al., 2007; Corlett et al., 2002). Thus, the intake of sedum could help to prevent the adverse effects of alcohol consumption.

Having studied the effects of taraxerone on ADH and ALDH activities and antioxidant status in acute alcohol intoxication, the effects of taraxerone on physiological and/or psychological changes in chronic alcohol consumption could be investigated. Furthermore, we have studied whether the brain function could be protected from repeated alcohol drinking by the administration of taraxerone.

In conclusion, present study was demonstrated that ADH and ALDH activities were enhanced by taraxerone via increases of AHD and ALDH gene expressions, thus plasma alcohol and acetaldehyde levels were efficiently reduced by taraxerone treatment with the concentration dependent manners. In addition, taraxerone prevented the loss of hepatic antioxidant components from ethanol exposure. Taraxerone showed significantly higher enhancement of ADH and ALDH activities and expressions than

that of DHM. Thus, this study could be presented scientific information to use of taraxerone as a new anti-alcohol intoxication agent, despite further studies that the effects of taraxerone on chronic ethanol consumption and ethanol induced liver injury are needed.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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