Effect of *Evodiae fructus* extracts on gene expressions related with alcohol metabolism and antioxidation in ethanol-loaded mice

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Abstract

The effects of *Evodiae fructus* on alcohol concentration in blood plasma and the relative expression of alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), Cu–Zn superoxide dismutase (Cu–Zn SOD), glutathione peroxidase type 5 (GPX5), and catalase (CAT) in mice were assessed 1, 2, 3, and 4 h after acute alcohol consumption. *Evodiae fructus* extracts (2.5, 5, and 10 mL/kg-bw), a commercial hangover removal drug (2.5, 5, and 10 mL/kg-bw), and saline solution, referred to as treatment, positive control, and negative control group, respectively, were orally given at the same time as alcohol administered to mice (20% and 40% concentration). Alcohol concentrations were the lowest in all of the *Evodiae fructus* treatment groups, as well as one (10 mL/kg-bw) of the positive control groups, 4 h after ethanol exposure (0–5 mg/dL and 60–110 mg/dL for 20% and 40% ethanol-loaded mice, respectively). Overall, the relative expression of ADH and Zn–Cu SOD were higher in treatment groups than in positive controls; whereas, the relative expression of GPX5 was higher in positive control groups than in treatment groups. The relative expression of ALDH and CAT was unchanged between treatments and positive controls. This study suggests that *Evodiae fructus* extracts can be used as a possible therapy for alcohol-induced hangover symptoms by stimulating the expression of hepatic alcohol metabolizing and antioxidant enzymes.

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Keywords: *Evodiae fructus*; Ethanol; cDNA; ADH; ALDH; Cu–Zn SOD; GPX5; CAT

1. Introduction

Ethanol is readily absorbed from the gastrointestinal tract, circulated rapidly, and distributed uniformly throughout the body (Godde and Agarwal, 1989). Thereafter, 80–90% of the ethanol absorbed is rapidly oxidized to acetaldehyde and acetate by enzymes in the liver such as alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH), respectively (Lieber, 1985; Gill et al., 1996). Ethanol, or its metabolites, can prompt a sharp increase of free radicals in a human body (e.g., hepatic cells in liver) by acting as a prooxidant or by reducing antioxidant levels and contributing to the progression of a variety of chronic diseases (Crawford and Balakenhoan, 1991). Alcohol-induced oxygen radicals can be attenuated by converting the superoxide anions to hydrogen peroxide and hydrogen radicals...
peroxide to water, via superoxide dismutase (SOD) and catalase (CAT), respectively (McDonough, 2003). These mechanisms result in the prevention of peroxidative deterioration in the body (Thampi et al., 1991).

The negative results of alcohol-induced hangovers have recently become a cause of concern primarily due to the loss of human health. Hangovers generally occur among social drinkers 8–16 h after alcohol intake (Hamburg et al., 1993) and result in headache, nausea, diarrhea, anorexia, and fatigue (Span and Earleywine, 1999). Acetaldehyde, a toxic alcohol metabolite, has been well characterized as the main cause of hangover symptoms and has been shown to strongly bind sulfur compounds such as cysteine and glutathione in liver microsomes resulting in liver damage (Murray et al., 1999). Extensive studies searching for an effective hangover-reducing compound have previously been performed and have so far proven relatively unsuccessful. Pittler et al. (2003), for example, concluded that aristolochic acid was not effective in preventing the symptoms of alcohol-induced hangovers compared to placebo treatment after 15 volunteers were investigated 1 h before and 10 h after alcohol exposure.

*Evodiae fructus* is the dried, unripe fruit of *Evodia rutaecarpa* belonging to the family Rutaceae and has been cultivated in East Asian nations including China, Korea, and Japan (Chang and But, 1986). Bioactive components from *Evodiae fructus* include evodiamine, rutaecarpine, dehydroevodiamine, 5-methoxy-N, N-dimethyltryptamine, synephrine, and evocarpine (Chang and But, 1986). *Evodiae fructus* has been used as an analgesic, antiemetic, astringent, and antihypertensive agent in oriental medicines (Yu et al., 2000) and has also been recommended as a treatment for gastric inflammation, a diuretic, and an antidote because of the ability of *Evodiae fructus* to prevent oxidation. Additionally, the seed oil of *Evodiae fructus* has been shown to inhibit various diseases such as dermatitis and scabies, and to reduce abdominal pain, acid regurgitation, nausea, and diarrhea (Yu et al., 2000). Although several studies have also raised the possibility of using *Evodiae fructus* to alleviate hangover symptoms, little research has been performed in this area. Therefore, the decision was made to investigate the effect of *Evodiae fructus* treatments on the expression of alcohol metabolizing enzymes as well as enzymatic antioxidants in vivo after alcohol intake.

The objectives of this study were to assess the effect of *Evodiae fructus* extract on: (1) the alcohol concentration in blood plasma from mice 1, 2, 3, and 4 h after administration of 20% or 40% alcohol; and (2) the relative expression of the genes encoding alcohol metabolizing and antioxidant enzymes (ADH, ALDH, Cu/Zn SOD, GPX5, and CAT) in liver tissue from ethanol-loaded mice.

### 2. Materials and method

#### 2.1. Preparation of *Evodiae fructus* extract

*Evodiae fructus* was purchased from an herb market in Seoul, Korea and voucher specimens were deposited at the Herbarium of Natural Products Research Institute, Seoul National University, Korea. Dried plant material (1 kg) was sliced into pieces and extracted three times with 100% MeOH (10 L) at room temperature. The extract was diluted to 90% MeOH, concentrated with n-Hexane/90% MeOH (1:1, v/v) at 30–40 °C, and then re-extracted with n-hexane/ethyl acetate (1:1, v/v). Nitrogen gas was gently applied to evaporate organic solvents prior to administration to mice.

#### 2.2. Animal treatment

ICR male mice (20–25 g) were obtained from the Animal Center of Seoul National University, Korea. Five individuals were kept in each cage and housed in a temperature and light control environment (25 °C; 12-h light/dark cycle). Mice were maintained on stock rodent chow and tap water ad libitum for 1 week. Three cages were randomly assigned to one of 14 different groups as following: Negative control groups (C20, C40), Positive control groups (P20A, P20B, P20C, P40A, P40B, P40C), and Treatment groups (T20A, T20B, T20C, T40A, T40B, T40C). Each group is described according to both alcohol dose (20%, 40%) and concentration of treatment (A: high dose, 10 mL/kg-bw; B: medium dose, 5 mL/kg-bw; C: low dose, 2.5 mL/kg-bw). Mice fasted overnight, and then were orally exposed to either 20% or 40% of ethanol at the same time as given saline solution, commercial hangover removal drug, and *Evodiae fructus* extract for negative control (C), positive control (P), and treatment group (T), respectively. The commercial hangover removal drug (Condition, CJ corp.) had a recommended consumption of 200 mL for 70 kg-bw and was made of glutamate and ADH. *Evodiae fructus* extract was dissolved in CMC (Carboxymethyl cellulose) for oral administration.

#### 2.3. Alcohol concentration in plasma

Blood from orbital bleeding was collected in an Eppendorf tube treated heparin 1, 2, 3, and 4 h after given alcohol. Blood samples were then centrifuged at ×1000 g and 4 °C for 15 min. Plasma was stored at −70 °C until the experiment. Alcohol concentrations in blood plasma were determined using an enzyme-based colorimetric assay (Sigma chemical Co. St. Louis, Mo., USA) and absorbance was measured at 340 nm by spectrophotometer (V-550, Jasco, Japan).
2.4. Quantitative RT-PCR

Liver tissue in a mouse from each cage for each group was collected every hour (1, 2, 3, and 4 h) after alcohol administration. Mice were under anesthesia throughout the time liver tissue was obtained. Liver tissue samples were snap-frozen in liquid nitrogen and then stored at −70°C until the experiment. Total RNAs of ADH, ALDH, Cu–Zn SOD, GPX5, and CAT from liver tissue were prepared by using TRI reagent (Sigma Co., St. Louis, Mo. USA) according to the manufacturer’s protocol. RT-PCR was performed using a commercial RT-PCR kit (Promega, Madison, WI, USA), and oligo-primers (Bionia, Korea) for PCR were synthesized based on previously published DNA sequences. Primer sequences corresponding to the enzymes used in this study are listed in Table 1. The reactants for RT-PCR were composed of 1 AMV/Tf1 reaction buffer, 0.2 mM dNTP mix, 1 μM downstream primer, 1 μM upstream primer, 1 mM MgSO4, 0.1 μL AMV reverse transcriptase, 0.1 μL/L TfL DNA polymerase, 1 μg RNA sample, and brought to 50 μL with deionized water.

RT-PCR conditions were as follows: (1) 94°C for 2 min for denaturation; (2) 94°C for 30 s, 53–60°C for 1 min annealing, and 68°C for 2 min extension, 40 cycles; and (3) 68°C for 7 min final extension (Robocycler gradient 96, Stratagene, USA). PCR products were electrophoretically run on a 2% agarose gel and stained with ethidium bromide in order to visualize the intensity and profile of DNA amplicons.

b-actin genes constitutively expressed were used as a positive control of cDNA amplification.

2.5. Statistical analysis

Statistical analysis was conducted using the SAS program (SAS Institute Co., Cary, NC, USA). Data was expressed as means with standard deviations and analyzed by paired t-tests, LSD with a significance level of α = 0.05.

3. Results

3.1. Effect of Evodiae fructus extracts on alcohol concentrations

Alcohol concentrations in blood plasma from ethanol-loaded mice were measured 1, 2, 3, and 4 h after oral administration of 20% or 40% ethanol, simultaneously given with Evodiae fructus extract (treatment group), a commercial hangover removal drug (positive control group), or a saline solution (negative control group) (Fig. 1). For 20% ethanol-loaded mice, alcohol concentrations ranged from 68 to 72 mg/dL 1 h after ethanol exposure at both the positive control group (10 mL/kg-bw) and treatment group (10 mL/kg-bw), approximately 2.5 times lower than alcohol concentrations observed in a negative control (≈169 mg/dL) (Fig. 1a). The lowest alcohol concentrations (≈5 mg/dL) were observed 4 h after 20% ethanol exposure in all the treatment groups (2.5, 5, and 10 mL/kg-bw), and a high dose of the positive control group (10 mL/kg-bw) (Fig. 1a). Whereas, two positive control groups (2.5 and 5 mL/kg-bw) and a negative control group had alcohol concentration of ≈19 mg/dL and 40 mg/dL, respectively, 4 h after 20% ethanol exposure (Fig. 1a). Serum alcohol concentration from the control group significantly decreased between 1 and 2 h after alcohol administration. In contrast, alcohol concentrations in plasma from treatment group were continuously reduced every hour after alcohol-loaded. Similar results were observed in mice loaded with 40% ethanol (Fig. 1b). One hour after ethanol exposure, alcohol concentration observed in both a positive control (173 mg/dL) and a treatment group (181 mg/dL) at high dose (10 mL/kg-bw) were lower than that in negative control group (Fig. 1b). These values were approximately 2.5 times lower than the alcohol concentration (≈425 mg/dL) of the negative control group. Four hours after ethanol treatment, all the treatment groups (2.5, 5, and 10 mL/kg-bw) and one positive control group (10 mL/kg-bw) had alcohol concentrations of 61–112

<table>
<thead>
<tr>
<th>Primer</th>
<th>Length (bp)</th>
<th>Sequence</th>
<th>Reference</th>
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<tr>
<td>ADH 1</td>
<td>562</td>
<td>Up: 5’-GAAAGCAACTTTTGTAGCCGA-3’ Down: 5’-ACTCTCACGAGCGCTTACA-3’</td>
<td>Zhang et al. (1987)</td>
</tr>
<tr>
<td>ALDH</td>
<td>454</td>
<td>Up: 5’-AAAGGAGTGTTGACAGCGCTTACA-3’ Down: 5’-AGACATCTTGGATCCTAACCAGA-3’</td>
<td>Bond and Singh (1994)</td>
</tr>
<tr>
<td>Cu–Zn SOD</td>
<td>487</td>
<td>Up: 5’-CTGAACCGTCAATGGGTCGATGAA-3’ Down: 5’-ACACAGGAATGATCTGCGC-3’</td>
<td>Bewley (1988)</td>
</tr>
<tr>
<td>GPX 5</td>
<td>502</td>
<td>Up: 5’-GCTGCTCATCGAGAATGTCCG-3’ Down: 5’-AAATCGGTGTTTCTCATTGCAA-3’</td>
<td>Ghyselinck et al. (1993)</td>
</tr>
<tr>
<td>CAT</td>
<td>247</td>
<td>Up: 5’-AGGGCTTCTTGAGCAATGACCACG-3’ Down: 5’-CAGTCTAGAGCCGATTATCG-3’</td>
<td>–</td>
</tr>
</tbody>
</table>
were not greatly different between positive controls and (Fig. 2). For 40% ethanol load mice, ADH expression groups than for positive groups 3 h after ethanol intake (Fig. 3). ALDH expression was higher for treatment 

One hour after 20% of alcohol administration, the expression of Cu–Zn SOD was higher in all treatment groups (2.5, 5, 10 mL/kg-bw) and a positive control group (10 mL/kg-bw) than a negative control group (Fig. 4). However, both GPX5 and CAT were not significantly different among the groups (p > 0.05). After 2 h, Cu–Zn SOD, GPX, and CAT were highly expressed in both treatment and positive control group. Remarkable gene expression was observed in the treatment group rather than positive control and negative control group after 3 h (Fig. 4). Overall, a treatment group at high dose (10 mL/kg-bw) revealed a relatively high gene expression related with antioxidation (Fig. 4). One hour after 40% alcohol intake, the expression of Cu–Zn SOD in both the positive group and treatment group were higher than the negative control group with no significant difference between positive control and treatment group (Fig. 5). Difference in the expression of Cu–Zn SOD and CAT were not observed after 2 h, while the expression of GPX at high dose of treatment and positive control group were higher than a negative control group (Fig. 5). After 3 h, a relative cDNA density of Cu–Zn SOD, GPX5, and CAT at both treatment and positive control group were higher than a negative control group, but it was not dose-dependent relationship (Fig. 5). After 4 h ethanol-load, the same trend of Cu–Zn SOD, GPX5, and CAT as 20% of ethanol-load mice was found (Fig. 5).

4. Discussion

Ethanol and its metabolites, such as acetaldehyde, are the primary causes of alcohol-induced hangovers. Therefore, control of hangover symptoms would necessitate metabolism in the liver. For example, the ADH and ALDH enzymes are considered to be essential for the metabolism of alcohol and the hypothesis has been made that these enzymes can be induced by the pharmaceutical action of some natural plant extracts. As is shown in Fig. 1, treatment with Evodiae fructus extracts is likely to stimulate alcohol metabolism in the blood due to the increased activity of both ADH and ALDH in the livers of both 20% and 40% ethanol-loaded mice. The reduction of blood alcohol concentration at the lowest concentration (2.5 mL/kg-bw) of Evodiae fructus extracts was comparable to that of the highest concentration (10 mL/kg-bw) of a commercial hangover removal drug 4 h after ethanol exposure. The cDNA intensities of ALDH were higher after treatment with both 10 mL/kg-bw of Evodiae fructus extracts and a commercial

mg/dL, while two other positive control groups (2.5 and 5 mL/kg-bw) and a negative control group showed alcohol concentrations of 158 and 170 mg/dL (Fig. 1b).

3.2. Effect of Evodiae fructus extracts on the expression of ADH, ALDH, Cu–Zn SOD, GPX5, and CAT

The relative expression of ADH and ALDH from liver tissue was determined for 20% ethanol-loaded mice (Fig. 2) and 40% ethanol-load mice (Fig. 3) at the same time as oral exposure of each treatment during 4 h period. The expression of ADH in 20% ethanol loaded mice highly increased at two treatment groups (5 and 10 mL/kg-bw) 2 h after ethanol intake and was abruptly reduced for all treatments after alcohol intake (Fig. 2). The relative expression of ALDH was higher for treatment groups than for positive groups 3 h after ethanol intake (Fig. 2). For 40% ethanol load mice, ADH expression were not greatly different between positive controls and treatment groups during 4 h period with the exception of the two treatment groups (5 and 2.5 mL/kg-bw), which showed unexplained low cDNA intensities of ADH 4 h after ethanol intake (Fig. 3). ALDH expression also provided the similar results between positive controls and treatment groups during 4 h period (Fig. 3).
Fig. 2. cDNA intensities of ADH and ALDH in 20% ethanol-loaded mice (A: 1 h, B: 2 h, C: 3 h, D: 4 h). C20: negative control, saline; P20A: positive control, commercial hangover removal drug, 10 mL/kg-bw; P20B: positive control, commercial hangover removal drug, 5 mL/kg-bw; P20C: positive control, commercial hangover removal drug, 2.5 mL/kg-bw; T20A: treatment, *Evodiae fructus* extract, 10 mL/kg-bw; T20B: treatment, *Evodiae fructus* extract, 5 mL/kg-bw; T20C: treatment, *Evodiae fructus* extract, 2.5 mL/kg-bw. *Presents significant difference between positive control and treatment group at $\alpha = 0.05$.

Fig. 3. cDNA intensities of ADH and ALDH in 40% ethanol-loaded mice (A: 1 h, B: 2 h, C: 3 h, D: 4 h). C40: negative control, saline; P40A: positive control, commercial hangover removal drug, 10 mL/kg-bw; P40B: positive control, commercial hangover removal drug, 5 mL/kg-bw; P40C: positive control, commercial hangover removal drug, 2.5 mL/kg-bw; T40A: treatment, *Evodiae fructus* extract, 10 mL/kg-bw; T40B: treatment, *Evodiae fructus* extract, 5 mL/kg-bw; T40C: treatment, *Evodiae fructus* extract, 2.5 mL/kg-bw. *Presents significant difference between positive control and treatment group at $\alpha = 0.05$. 

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Fig. 4. cDNA intensities of Cu–Zn SOD, GPX5, and CAT in 20% ethanol-loaded mice (A: 1 h, B: 2 h, C: 3 h, D: 4 h). C20: negative control, saline; P20A: positive control, commercial hangover removal drug, 10 mL/kg-bw; P20B: positive control, commercial hangover removal drug, 5 mL/kg-bw; P20C: positive control, commercial hangover removal drug, 2.5 mL/kg-bw; T20A: treatment, *Evodiae fructus* extract, 10 mL/kg-bw; T20B: treatment, *Evodiae fructus* extract, 5 mL/kg-bw; T20C: treatment, *Evodiae fructus* extract, 2.5 mL/kg-bw. *Presents significant difference between positive control and treatment group at $\alpha = 0.05$.

Fig. 5. cDNA intensities of Cu–Zn SOD, GPX5, and CAT in 40% ethanol-loaded mice (A: 1 h, B: 2 h, C: 3 h, D: 4 h). C40: negative control, saline; P40A: positive control, commercial hangover removal drug, 10 mL/kg-bw; P40B: positive control, commercial hangover removal drug, 5 mL/kg-bw; P40C: positive control, commercial hangover removal drug, 2.5 mL/kg-bw; T40A: treatment, *Evodiae fructus* extract, 10 mL/kg-bw; T40B: treatment, *Evodiae fructus* extract, 5 mL/kg-bw; T40C: treatment, *Evodiae fructus* extract, 2.5 mL/kg-bw. *Presents significant difference between positive control and treatment group at $\alpha = 0.05$. 
hangover removal drug than in other groups 1 h after ethanol exposure. This data is consistent with observation that after ethanol-loading, blood alcohol concentrations were lowest in mice treated with either 10 mL/kg-bw of *Evodiae fructus* extracts or a commercial hangover removal drug. This study also suggests that treatment with *Evodiae fructus* extracts may result in high antioxidation activity based on the relatively high expression of antioxidative enzymes (Cu–Zn SOD, GPX5, and CAT) associated with recovering from alcohol-induced oxidative stress. Rajagopal et al. (2003) reported that *Cassia auriculata* leaf extracts elevated the activities of SOD and catalase in the liver, brain, kidney, and intestine of alcohol treated rats. They subsequently suggested that treatment with *Cassia auriculata* leaf extract could protect against free radical mediated oxidative stress.

The main components of *Evodiae fructus* extracts, which may induce expressions such as ADH, ALDH, Cu–Zn SOD, GPX5, and CAT, were not specifically determined in this study. Fei et al. (2003) reported that evodiamine, a major alkaloidal component of *Evodiae fructus* extracts, inhibited proliferation of tumor cell lines, such as HeLa cells. Similarly, alkaloids or other natural occurring components may affect alcohol metabolism and antioxidation. Further studies are needed to identify the active components from *Evodiae fructus* extracts and assess the effects on alcohol metabolizing and/or antioxidant enzymes.

In conclusion, *Evodiae fructus* extracts were more effective in reducing alcohol concentration in the blood, as well as in inducing the expression of ADH and Zn–Cu SOD mRNA, compared to a commercial hangover removal drug 4 h after ethanol administration. The results of this study therefore suggest the possibility of developing a new effective hangover removal drug using *Evodiae fructus* extracts to prevent hangover symptoms. To confirm these findings, larger and more rigorous clinical studies should be performed to investigate the toxicological effects of *Evodiae fructus* extracts as well as the effect of chronic/acute alcohol consumption on hangover symptoms.

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**References**


