

Protective Effect of Dandelion Extracts on Ethanol-Induced Acute Hepatotoxicity in C57BL/6 Mice

Xiao-Yu Liu¹, Jie Ma¹, Chung-Mu Park¹, Hee-Kyung Chang², and Young-Sun Song^{1†}

¹BK 21 Center of Smart Food & Drug, Inje University, Gimhae 621-749, Korea

²Department of Pathology, Medical College of Kosin University, Busan 606-701, Korea

Abstract

Dandelion (*Taraxacum officinale*) has been widely used as an anti-inflammatory agent in oriental medicine. In the current study, we investigated the protective effect, and the possible mechanism, of dandelion extracts against ethanol-induced acute hepatotoxicity in C57BL/6 mice. Dandelion water and ethanol extract was administered at 2 g/kg body weight (BW) once daily for 7 consecutive days, whereas control and ethanol groups received water by gavage. Ethanol (50% ethanol; 6 g/kg BW) was administered 12 hr before sacrificing the mice in order to generate liver injury. Significantly increased serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities as well as liver triglyceride (TG) and cholesterol levels were attenuated by dandelion supplementation. In addition, dandelion extracts not only enhanced alcohol dehydrogenase (ADH) and anti-oxidative enzyme activities, but reduced lipid peroxidation. Cytochrome P450 2E1 (CYP 2E1), one of the critical enzymes xenobiotic metabolism, expression was lower with ethanol treatment but restored by dandelion supplementation. These results were confirmed by improved histopathological changes in fatty liver and hepatic lesions induced by ethanol. In conclusion, dandelion could protect liver against ethanol administration by attenuating of oxidative stress and inflammatory responses.

Key words: dandelion (*Taraxacum officinale*), ethanol, hepatotoxicity, oxidative stress, CYP2E1

INTRODUCTION

As much as 80~90% of ingested ethanol is metabolized in the liver, where ethanol is oxidized to acetaldehyde (1,2). Ethanol is metabolized predominantly *via* two well-characterized pathways. The first involves its oxidation to acetaldehyde, by cytosolic alcohol dehydrogenase. The second major pathway responsible for ethanol metabolism is the ethanol-inducible microsomal ethanol oxidizing system. The major component of this system is cytochrome P450 2E1 (CYP2E1), which can also oxidize alcohol to acetaldehyde (3). Since acetaldehyde is much more toxic than ethanol, it is associated with a larger number of the metabolic abnormalities in ethanol induced liver disease (4,5).

Metabolism through the CYP2E1 system generates reactive free radicals. Elevated levels of oxygen radicals can generate a state of oxidative stress, which through various mechanisms leads to cell damage. Oxygen radicals can also interact with fat molecules (lipids) in the cell in a process known as lipid peroxidation (6). The resulting products of lipid peroxidation, reactive oxygen species (ROS) themselves and acetaldehyde, all contribute to the formation of neo-antigenic adducts (3). Adduct

formation has been shown to occur in the livers of humans and animals consuming alcohol, which predominate in those liver regions that show the first signs of damage (6).

Circulating antioxidant enzymes, including superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-px) and glutathione reductase (GSH-red) play important roles in alleviating tissue damage induced by free radical formation (7,8). Therefore, a compound with antioxidant properties can therapeutically ameliorate the progression of lipid peroxidation and hepatocellular injury induced by ethanol (9).

Dandelion (*Taraxacum officinale*) is acclaimed as a nontoxic herb used in traditional Chinese medicine for a variety of health benefits (10) such as choleric, diuretic, antitumor, and anti-inflammatory activities (11,12). The root is known to accelerate elimination of toxins from the liver and kidney (13). The leaves or roots may also dissolve gallstones (13). The flower extract has been reported to suppress ROS and nitric oxide and prevent lipid oxidation *in vitro* (10). The protective effect of dandelion extracts against cholecystokinin-induced acute pancreatitis has been confirmed in an animal study (14). However, the protective effect of dandelion extracts on

†Corresponding author. E-mail: fdsnsong@inje.ac.kr
Phone: +82-55-320-3235, Fax: +82-55-321-0691

acute ethanol-induced hepatotoxicity has not yet been investigated.

The present study was undertaken to evaluate the protective effects of dandelion extracts on ethanol-induced hepatotoxicity and to elucidate the mechanism underlying these protective effects in mice.

MATERIALS AND METHODS

Preparation of dandelion extracts

Dandelion (*Taraxacum officinale*) was obtained from Min-dle-leh Food (Uiryeong, Korea) in early summer. Collected fresh dandelion leaves were washed, dried and ground to a fine mesh powder. DWE (Dandelion Water Extract) and DEE (Dandelion Ethanol Extract) were prepared as follows. One hundred grams of dandelion powder was extracted with 1 L of water at 100°C and ethanol at 80°C for 4 hrs. The extracts were filtered with Whatman No. 4 filter paper (Maidstone, England). DWE and DEE were concentrated by lyophilization and vacuum evaporation, respectively. The recovery rates were 22.5% and 16.3% each.

Animals

This study was conducted in accordance with the Guideline for Animal Experiments approved by Inje University, Gimhae, Korea. Nine-week-old, male C57BL/6 mice (20~25 g) were obtained from Hyochang Science (Daegu, Korea) and used after 1 week adaptation. All animals were housed in polycarbonate cages in a temperature regulated ($22 \pm 2^\circ\text{C}$) and humidity ($60 \pm 5\%$) controlled room with a 12 hr light/dark cycle with free access to standard chow diet and water.

Treatment of experimental groups

C57BL/6 mice were randomly divided into 4 groups, ethanol untreated group (Control), ethanol treated group (Ethanol), ethanol administration after dandelion water extract supplementation (DWE+ethanol), ethanol administration after dandelion ethanol extract supplementation (DEE+ethanol), with 9 mice in each group. Another experiment using a plant extract, polyphenol-rich extract from bamboo leaves, was well tolerated and no mortality occurred at doses of 0, 1.43, 2.87, and 4.30 g/kg body weight (BW) per day (15). Based on this result, dandelion water and ethanol extract concentrations were set at 2 g/kg BW, and were administered once daily for 7 consecutive days by oral gavage while control and ethanol groups were received equal volumes of water. Regardless of different recovery rate, the same amounts of dandelion extracts were administered to compare their anti-inflammatory and antioxidative properties. A single dose of ethanol (50% ethanol; 6 g/kg BW) was ad-

ministered 12 hrs before their sacrifice in order to generate acute liver injury (16). Same doses of glucose were also fed to other groups to adjust calories. The mice were anesthetized by CO₂ and blood samples were collected to determine biochemical parameters. Livers were collected, weighed and a thin slice preserved in 10% buffered formalin solution for histopathological analysis. The remaining livers were frozen in liquid nitrogen and stored at -70°C for biochemical assays.

ALT, AST, and ADH assay

The serum ALT and AST activities were determined by using commercially available reagent kits (YD diagnostics Co., Yong In, Korea). The activity of ADH was determined in a final volume of 1 mL: 100 μL of 1 mM NAD, 100 μL propionaldehyde, 600 μL of 50 mM sodium pyrophosphate buffer (pH 8.5), 200 μL of serum. Absorbance was measured on a spectrophotometer with a water bath (30°C) at 340 nm for 10 min (every 30 sec) for estimation of ADH activity.

Hepatic lipid levels

Hepatic lipid was extracted by the method of Folch et al. (17). Liver samples were homogenized with chloroform/methanol (2/1, v/v) to a final volume 20 times the volume of the liver sample (1 g in 20 mL of solvent mixture). The homogenate was filtered. The solvent was washed with 0.88% potassium chloride solution. After mixing for a few seconds, the mixture was placed in separation funnel. The lower phase was collected and mixed with chloroform/methanol/H₂O (8/4/3, v/v/v) solution. The mixture was placed in separation funnel again, the lower phase collected and evaporated under a nitrogen stream, and the resulting lipid pellet was dissolved in chloroform with Triton X-100 and assayed for triglyceride (TG) and cholesterol levels using colorimetric kits (YD diagnostics Co., Yong In, Korea).

Lipid peroxidation assay

Lipid peroxidation in the liver was quantified by measuring the levels of thiobarbituric acid-reactive substance (TBARS) as described by Fraga et al. using a standard curve for 1,1,3,3-tetraethoxypropane (18) and expressed as nM of malondialdehyde (MDA) equivalents per gram of liver.

Antioxidant enzyme activities assay

The activities of the above isoenzymes of SOD were determined by the Oyanagui method (19). One unit is defined as 50% of inhibition of nitric ion production. Catalase activity was analyzed with the use of Aebi kinetic method (20). The kinetic changes of absorbance were marked at 240 nm. Activity of catalase was determined as the quantity of μM of hydrogen peroxide

decomposed in 1 min per mg of liver protein (U/mg). GSH-px activity in liver was assayed by the Paglia and Valentine kinetic method (21). Decrease in absorbance at 340 nm was measured. Activity of GSH-px was determined as the quantity in μM of NADPH used to recover reduced glutathione in 1 min per mg of liver protein (U/mg). Determination of GSH-red activity in liver sample was also assayed by the kinetic method (22). The decrease in the concentration of NADPH after reduction of oxidized glutathione back to reduced glutathione was measured. Activity of GSH-red was determined as the quantity in μM of NADPH used to recover reduced glutathione in 1 min per mg of liver protein (U/mg). The protein concentration was determined by the Biuret reaction (23), using bovine albumin as the standard.

Protein expression of CYP2E1

The expression of CYP2E1 protein was analyzed by Western blotting. Liver proteins were extracted by PRO-PREP™. Electrophoretic separation of the proteins (200 μg) was performed in NuPAGE 4~12% Bis-Tris Gel using the SE260 Mini-vertical Gel Electrophoresis Unit (Amersham, Cardiff, UK). The separation proteins were electrotransferred to nitrocellulose membranes using a Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (Bio-Rad, Hercules, CA, USA). The membranes were blocked with 5% nonfat dry milk for 1 hr and the immunoblots exposed to rabbit polyclonal antibody against CYP2E1 protein. Immunodetection was performed using alkaline phosphatase-labeled goat anti-rabbit IgG antibody and developed with a mixture of 5-bromo-4-chloroindolylphosphate (BCIP) and nitroblue tetrazolium (NBT). Data were quantified using the Quantity One imaging software (Bio-Rad, Hercules, CA, USA). All signals were normalized to protein expression levels of the housekeeping gene, GAPDH, and expressed as a ratio. Although many diverse functions of GAPDH are reported these days, it is still used as a control in numerous studies due to its well characterized basic cell catabolic processes.

Histopathological examinations

Fresh liver tissues, previously sliced to approximately 2 mm thickness, were placed in plastic cassettes and immersed in neutral buffered formalin for 24 hr. Fixed tissues were processed routinely, and then embedded in paraffin, sectioned, deparaffinized, and rehydrated using standard techniques. The magnitude of acute ethanol-induced liver injury was assessed by visual morphological changes in liver sections stain with hematoxylin and eosin (H&E) (9,24).

Statistical analysis

Data were expressed as the mean \pm SD, and statistical

analyses were performed by the SPSS program. One-way ANOVA and Duncan's multiple-range test were used to examine the difference between groups, statistical significance being considered at $p < 0.05$.

RESULTS AND DISCUSSION

Dandelion ameliorated serum ALT and AST activities

ALT and AST have been used as biochemical markers for acute hepatic damage. Fig. 1 shows the effect of dandelion extracts on these enzyme activities. Serum ALT and AST activities were significantly ($p < 0.05$) increased in ethanol-administered mice compared to the control group. However, this increase was suppressed by the dandelion extract supplementation. Liver is the most common site of damage in laboratory animals administered drugs and other chemicals (25). Since the extent of hepatic damage is assessed by the activities of released cytoplasmic AST and ALT in the circulation (26), AST and ALT were used as sensitive markers in the diagnosis of hepatic diseases (9). In the present study, a single dose of ethanol caused significant increases in serum AST and ALT, which means ethanol induced hepatic injury. However, pretreatment with dandelion extracts prevented these trends, demonstrating reduced liver damage followed by dandelion extracts administration.

Dandelion restored ADH activity

Fig. 2 shows the effect of dandelion extracts supplementation on serum ADH activity in mice fed ethanol. The activity of serum ADH decreased significantly

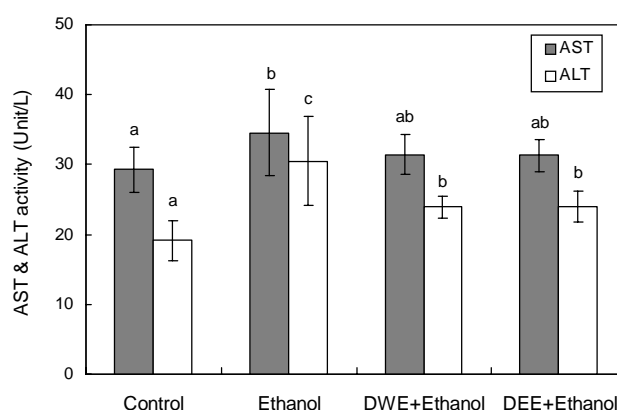


Fig. 1. Effect of dandelion extracts on serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in ethanol-intoxicated mice. Each value represents the means \pm SD of 9 mice for each group. Values sharing the same superscript are not significantly different at $p < 0.05$ by Duncan's multiple range test. Control, ethanol untreated group; Ethanol, ethanol treated group; DWE+Ethanol, dandelion water extract+ethanol treated group; DEE+Ethanol, dandelion ethanol extract+ethanol treated group.

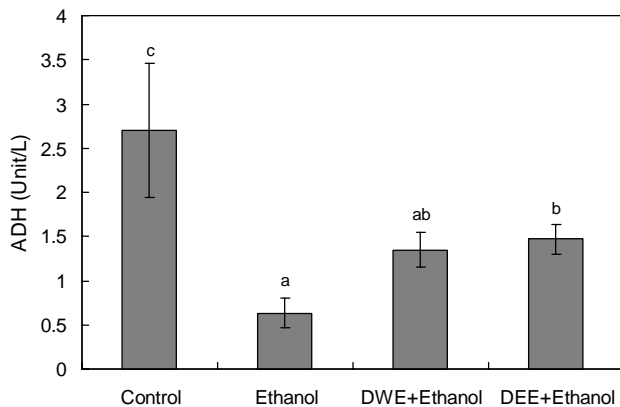


Fig. 2. Effect of dandelion extracts on serum alcohol dehydrogenase (ADH) levels in ethanol-intoxicated mice. Each value represents the means \pm SD of 9 mice for each group. Values sharing the same superscript are not significantly different at $p < 0.05$ by Duncan's multiple range test. Control, ethanol untreated group; Ethanol, ethanol treated group; DWE+Ethanol, dandelion water extract+ethanol treated group; DEE+Ethanol, dandelion ethanol extract+ethanol treated group.

($p < 0.05$) in ethanol treated animals compared to control. Dandelion water extract slightly restored the activity of ADH which was decreased by ethanol administration, while dandelion ethanol extract significantly ($p < 0.05$) restored the ADH activity. Ethanol and its metabolites are the primary causes of alcohol-induced hangovers. Therefore, control of hangover symptoms would necessitate metabolism in the liver. For example, the ADH enzyme is considered to be essential for the metabolism of alcohol and the hypothesis has been made that this enzyme can be induced by the pharmaceutical action of some natural plant extracts (27). Our results showed that in the acute ethanol treatment model, the activity of serum ADH was significantly lower in ethanol treated animals compared to controls. Dandelion water extract slightly restored the activity of ADH reduced by ethanol administration, while dandelion ethanol extract significantly restored the ADH activity.

Dandelion attenuated obstructed liver lipid levels

Ethanol administration induced a significant ($p < 0.05$) accumulation of TG and cholesterol in the liver, however, this accumulation was attenuated by dandelion extract supplementation (Fig. 3). Fat accumulation in hepatocytes leads to the development of fatty liver (steatosis), which is a reversible condition (28). Studies have considered steatosis as the 'first hit', increasing the sensitivity of the liver to a variety of injurious mechanisms, or 'second hit', considered to play a role in alcohol-induced liver injury (29). In our results, levels of TG and cholesterol in liver are significantly higher in ethanol treated mice compared with ethanol-untreated

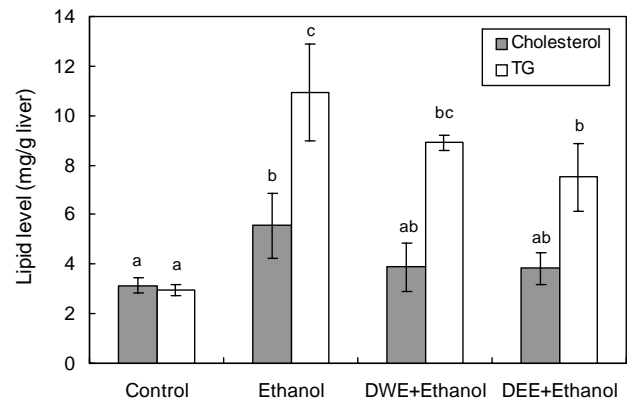


Fig. 3. Effect of dandelion extracts on liver lipid levels in ethanol-intoxicated mice. Each value represents the means \pm SD of 9 mice for each group. Each value represents the means \pm SD of 9 mice for each group. Values sharing the same superscript are not significantly different at $p < 0.05$ by Duncan's multiple range test. Control, ethanol untreated group; Ethanol, ethanol treated group; DWE+Ethanol, dandelion water extract+ethanol treated group; DEE+Ethanol, dandelion ethanol extract+ethanol treated group.

mice. However, dandelion extract treatment prevented TG and cholesterol elevations induced by ethanol administration. These results demonstrate that dandelion extracts can prevent fatty liver induced by acute ethanol consumption.

Dandelion inhibited ethanol induced lipid peroxidation by elevating antioxidative enzyme activities

Fig. 4 shows the effect of dandelion extract pretreatment on lipid peroxidation. Significantly ($p < 0.05$) higher MDA levels resulting from lipid peroxidation were

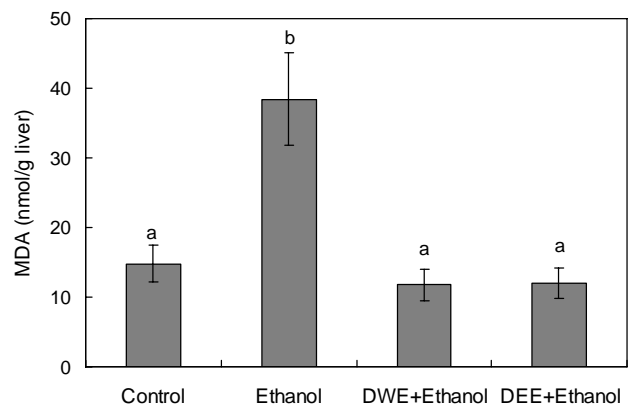


Fig. 4. Effect of dandelion extracts on hepatic lipid peroxidation in ethanol-intoxicated mice. Each value represents the means \pm SD of 9 mice for each group. Values sharing the same superscript are not significantly different at $p < 0.05$ by Duncan's multiple range test. Control, ethanol untreated group; Ethanol, ethanol treated group; DWE+Ethanol, dandelion water extract+ethanol treated group; DEE+Ethanol, dandelion ethanol extract+ethanol treated group.

Table 1. Effect of dandelion extracts on antioxidant enzyme activities in ethanol-intoxicated mice

	Control	Ethanol	DWE+Ethanol	DEE+Ethanol
Catalase (U/mg protein)	0.35 ± 0.02 ^{1)ab2)}	0.33 ± 0.03 ^a	0.36 ± 0.01 ^{ab}	0.37 ± 0.02 ^b
Cu/Zn-SOD ⁴⁾ (NU/mg protein)	73.1 ± 6.17 ^{NS3)}	66.7 ± 14.6	68.8 ± 7.71	92.9 ± 24.6
Mn-SOD ⁵⁾ (NU/mg protein)	1.36 ± 0.16 ^{NS}	1.35 ± 0.10	1.41 ± 0.16	1.37 ± 0.13
GSH-px ⁶⁾ (U/mg protein)	2.85 ± 0.4 ^b	2.26 ± 0.39 ^a	2.85 ± 0.19 ^b	2.74 ± 0.34 ^b
GSH-red ⁷⁾ (U/mg protein)	39.2 ± 3.23 ^b	34.1 ± 3.42 ^a	37.6 ± 4.36 ^b	37.7 ± 3.13 ^b

¹⁾Data represent the means ± SD of 9 mice for each group. ²⁾Values sharing the same superscript are not significantly different at $p < 0.05$ by Duncan's multiple range test. ³⁾Not significant. ⁴⁾Cu/Zn-superoxide dismutase. ⁵⁾Mn-superoxide dismutase. ⁶⁾Glutathione peroxidase. ⁷⁾Glutathione reductase.

Control, ethanol untreated group; Ethanol, ethanol treated group; DWE+Ethanol, dandelion water extract+ethanol treated group; DEE+Ethanol, dandelion ethanol extract+ethanol treated group.

found in the ethanol-intoxicated mice. However, dandelion extracts supplementation for 7 days significantly ($p < 0.05$) inhibited the lipid peroxidation. To support this result, hepatic antioxidative enzyme activities were investigated to understand the mechanism involved in hepatoprotection. As shown in Table 1, significantly ($p < 0.05$) lower catalase, GSH-px and GSH-red activities were observed in ethanol-intoxicated mice when compared to control, however, treatment with dandelion extracts in ethanol-intoxicated mice increased the activities of catalase, GSH-px and GSH-red. There were no significant differences in Mn-SOD and Cu, Zn-SOD activities among the groups. Ethanol causes liver damage by many mechanisms, the generation of lipid peroxidation by free radicals has been proposed as one mechanism for ethanol-induced hepatotoxicity (30). Alcohol administration causes accumulation of ROS, including superoxide, hydroxyl radical, and hydrogen peroxide (31). Reactive oxygen species, in turn, cause lipid peroxidation of cellular membrane and protein and DNA oxidation, which result in hepatocyte injury (32-34). In this study, a marked increase in lipid peroxidation was noted after acute ethanol administration, however, this increase was significantly attenuated by dandelion extract pretreatment, which means dandelion extracts ameliorated lipid peroxidation caused by ethanol, which might be due to antioxidative effects of dandelion extract (35,36). Further study was conducted to investigate the free radical scavenging property of dandelion, which attributed to ameliorated lipid peroxide concentration through antioxidative enzyme activities. Increasing evidence demonstrates that oxidative stress plays an important etiological role in the development of alcoholic liver disease (34,37, 38). Oxidative stress is generally considered the result of an imbalance between pro-oxidants and antioxidants (22) and is largely determined by endogenous enzyme antioxidant systems including SOD, catalase, GSH-px and GSH-red. In this study, there were no significant differences in Mn-SOD and Cu, Zn-SOD activities

among the groups, while the activities of catalase, GSH-px, GSH-red were lower in the ethanol group when compared to control group. However, dandelion extract treated groups had higher activities of catalase, GSH-px and GSH-red, which alleviates deleterious effects induced by ethanol. These data suggest that dandelion extracts could protect against free radical mediated oxidative stress.

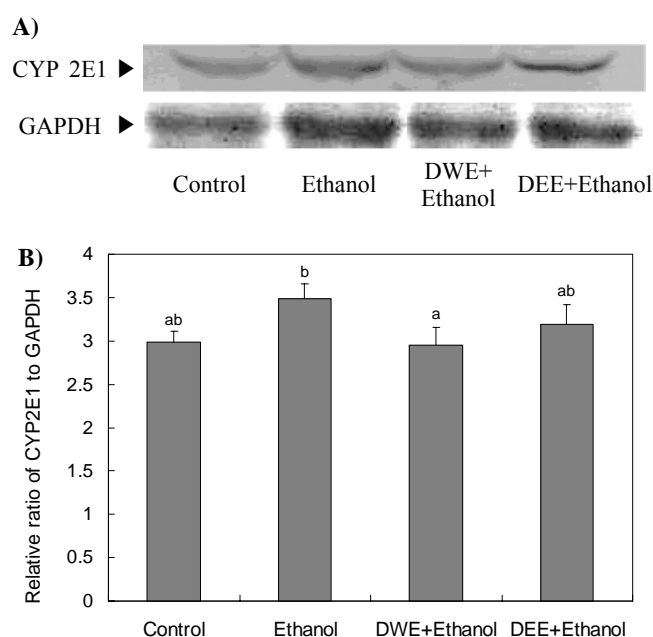


Fig. 5. Effect of dandelion extracts on hepatic cytochrome P450 2E1 (CYP2E1) protein expression in ethanol-intoxicated mice. Panel A) represents levels of CYP 2E1 protein were measured by Western blot analysis. All CYP 2E1 expression levels were normalized to protein expression levels of the housekeeping gene, GAPDH, and expressed as a ratio. Control, ethanol untreated group; Ethanol, ethanol treated group; DWE+Ethanol, dandelion water extract+ethanol treated group; DEE+Ethanol, dandelion ethanol extract+ethanol treated group. Panel B) represents that all signals were normalized to protein levels of the house keeping gene, GAPDH and expressed as a ratio. Data represent the mean SD of 9 mice. Values sharing the same superscripts are not significantly different at $p < 0.05$ by Duncan's multiple range test.

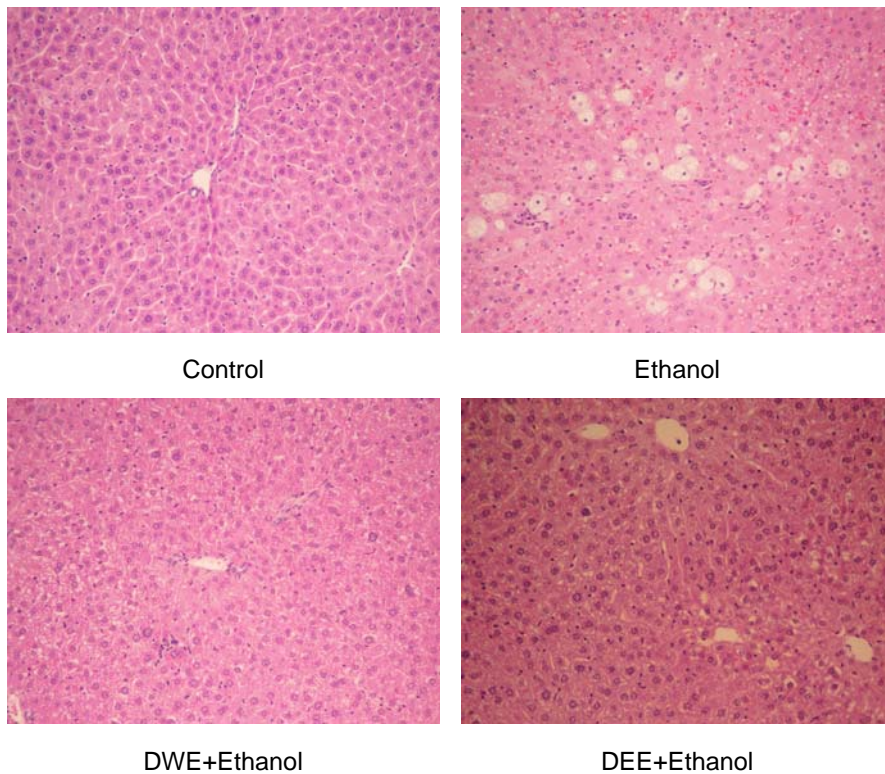


Fig. 6. Effect of dandelion extracts on hepatic morphological changes in ethanol-intoxicated mice. Control, ethanol untreated group; Ethanol, ethanol treated group; DWE+Ethanol, dandelion water extract+ethanol treated group; DEE+Ethanol, dandelion ethanol extract+ethanol treated group.

Dandelion lowered hepatic CYP2E1 protein expression

The CYP2E1 protein expression was higher in the ethanol-intoxicated group, as shown in Fig. 5. However, dandelion water and ethanol extracts supplementation lowered this protein expression increased by ethanol. Although there are many potential sources of ROS in response to acute ethanol exposure, CYP2E1 is one of the major proteins involved in ROS production in the liver responding to alcohol, it has been reported that long-term alcohol exposure increases CYP2E1 activities (32,36). Furthermore, investigations using CYP2E1 inhibitors, including diallyl sulfide or chlormethiazole, have shown that inhibition of CYP2E1 activity inhibits alcohol-induced liver injury, demonstrating the importance of CYP2E1 in alcohol-induced ROS accumulation and liver injury (39,40). In order to investigate the possible mechanisms by which dandelion extracts attenuated acute ethanol-induced liver injury, we evaluated the effect of dandelion extracts on CYP2E1 expression induced by ethanol administration. Our study indicated that ethanol increased CYP2E1 expression and that this increase was diminished by dandelion extracts treatment.

Histopathologic changes in liver

The results in this study were confirmed by histopathological observation. In contrast to the control group, ethanol-intoxicated mice showed mild inflammatory cell infiltration and fatty change, but dandelion

administration for 7 days attenuated this histopathological change (Fig. 6).

In conclusion, dandelion extracts appeared to prevent ethanol-induced acute hepatic injury by ameliorating oxidative stress through elevated antioxidant enzyme activities and suppressed CYP2E1 expression, which is evidenced by decreased hepatotoxic indices in serum/ liver tissue, and morphological observations in the liver.

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