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In vitro and *in vivo* hepatoprotective effects of the aqueous extract from *Taraxacum officinale* (dandelion) root against alcohol-induced oxidative stress

Yanghee You [a], Soonam Yoo [a], Ho-Geun Yoon [b], Jeongjin Park [a], Yoo-Hyun Lee [c], Sunoh Kim [d], Kyung-Taek Oh [e], Jeongmin Lee [f], Hong-Yon Cho [g], Woojin Jun [a,*]

[a] *Department of Food and Nutrition, Chonnam National University, Gwangju 550-757, Republic of Korea*
[b] *Department of Biochemistry and Molecular Biology, College of Medicine, Yonsei University, Seoul 120-752, Republic of Korea*
[c] *Department of Food and Nutrition, University of Suwon, Gyeonggido 445-743, Republic of Korea*
[d] *Jeonnam Institute of Natural Resources Research, Jeonnam 529-851, Republic of Korea*
[e] *Jeollanamdo Regional Innovation Agency, Jeonnam 534-700, Republic of Korea*
[f] *Research Institute of Medical Nutrition, Kyung Hee University, Republic of Korea*
[g] *Department of Food and Biotechnology, Korea University, Chungnam 339-700, Republic of Korea*

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ABSTRACT

The protective effects of *Taraxacum officinale* (dandelion) root against alcoholic liver damage were investigated in HepG2/2E1 cells and ICR mice. When an increase in the production of reactive oxygen species was induced by 300 mM ethanol *in vitro*, cell viability was drastically decreased by 39%. However, in the presence of hot water extract (TOH) from *T. officinale* root, no hepatocytic damage was observed in the cells treated with ethanol, while ethanol-extract (TOE) did not show potent hepatoprotective activity. Mice, which received TOH (1 g/kg bw/day) with ethanol revealed complete prevention of alcohol-induced hepatotoxicity as evidenced by the significant reductions of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and lactate dehydrogenase activities compared to ethanol-alone administered mice. When compared to the ethanol-alone treated group, the mice receiving ethanol plus TOH exhibited significant increases in hepatic antioxidant activities, including catalase, glutathione-*S*-transferase, glutathione peroxidase, glutathione reductase, and glutathione. Furthermore, the amelioration of malondialdehyde levels indicated TOH's protective effects against liver damage mediated by alcohol *in vivo*. These results suggest that the aqueous extract of *T. officinale* root has protective action against alcohol-induced toxicity in the liver by elevating antioxidative potentials and decreasing lipid peroxidation.

1. Introduction

Taraxacum officinale, known as dandelion, is a member of the Asteraceae/Compositae family. It is a perennial herb, native throughout the Northern hemisphere. Traditionally, dandelion has been used as an herbal medicine due to its antidiabetic, choleretic, antirhematic, and diuretic properties (Schütz et al., 2006). Recent studies have provided evidence that it may reduce the risk of diseases, including inflammation and tumors (Kim et al., 2007; Sigstedt et al., 2008). However, scanty data have been reported in relation to the protective effects of dandelion against alcoholic liver damage.

The long-term heavy consumption of alcohol results in the development of alcohol-related liver disease, which is the second leading cause of death among all liver diseases (Lieber, 2000; Neuman, 2003). Oxidative stress is considered as one of the key mechanisms responsible for alcoholic liver damage (Xu et al., 2003; Caro and Cederbaum, 2004). The excessive intake of ethanol causes elevated reactive oxygen species (ROS) production. These enhanced quantities of ROS, which overwhelm the capacity of the body's defense systems, lead to an imbalance between ROS production and antioxidant defense, which in turn, eventually mediates hepatic tissue damage.

Ethanol is metabolized by several enzyme systems located mainly in the liver. Numerous sources of ROS have been reported following acute and chronic ethanol exposure. One potential

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; CYP2E1, cytochrome P-450 2E1; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GST, glutathione-*S*-transferase; LDH, lactate dehydrogenase; MDA, malondialdehyde; ROS, reactive oxygen species.

* Corresponding author. Tel.: +82 62 530 1337; fax: +82 62 530 1339.
E-mail address: wjjin@chonnam.ac.kr (W. Jun).

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source of ROS in hepatocytes is the microsomal cytochrome P-450 system. Chronic ethanol consumption has been shown to stimulate microsomal ROS generation as a result of the induction of cytochrome P-450 2E1 (CYP2E1) and uncoupling between CYP2E1 and NADPH cytochrome c reductase (Lu and Cederbaum, 2008). The elevation of ethanol-induced CYP2E1 activity is suggested to be a major contributor in generating a state of oxidative stress, which results in hepatotoxicity (Lieber, 1997).

The identification for plant products or alternative medicines that could limit ROS-mediated injuries is necessary to help protect the liver from possible damage. Antioxidants are substances that delay or prevent the oxidation of inter- or intra-cellular oxidizable substrates from oxidative stress. Some bioactive compounds, which are generally found in plants, have been determined to protect cells from oxidative stress by preventing the formation of free radicals or by detoxifying free radicals, resulting in the prevention of a variety of pathophysiological processes (Mates and Sanchez-Jimenez, 2000).

This study aimed to investigate the *in vitro* and *in vivo* protective effects of *T. officinale* root extract supplementation on the liver damage induced by ethanol. Furthermore, the mode of action relevant to the extract's antioxidative activity in alcoholic liver damage was determined. It might aid in the development of effective therapeutics on alcoholic liver damage.

2. Materials and methods

2.1. Sample and chemicals

The *T. officinale* used in this study was grown in Hwasun County (Jeollanamdo, Korea) and authenticated by Dr. Sangin Shim at Gyeongsang National University. A voucher specimen was deposited at the same institute. Iscove's Modified Dulbecco's Medium (IMDM), fetal bovine serum (FBS), and antibiotics were products of Gibco BRL (Grand Island, NY). Sodium salt of (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide inner salt) or XTT, ethanol, 2′,7′-dichlorofluoresceindiacetate (DCFH-DA), reduced glutathione (GSH), catalase (CAT), glutathione peroxidase (GPx), glutathione-*S*-transferase (GST), and glutathione reductase (GR) were purchased from Sigma–Aldrich (St. Louis, MO). All other chemicals were of analytical reagent-grade.

2.2. Cell culture

The HepG2 cell line was obtained from the American Type Culture Collection (Manassas, VA) and transfected with human CYP2E1 (HepG2/2E1). The HepG2/2E1 cells were grown in IMDM supplemented with 20% FBS, penicillin (100 units/mL), and streptomycin (100 μg/mL). Stock cultures were maintained in 100 mm dishes at 37 °C under a humidified atmosphere of 5% CO_2–95% air. When the cells reached at >80% confluency, subculture was conducted at a cell density of 1×10^6 cells/dish. The medium was refreshed approximately three times a week.

2.3. Animals

Male ICR mice (8 weeks, 30 ± 2 g of body weight, bw) were purchased from Orient Bio (Seongnam, Korea) and housed in cages under automatically controlled air-conditions of temperature (22 ± 2 °C), humidity (about 60%), and lighting (12:12-h light–dark cycle). The mice were fed a commercial pelleted chow (AIN-76A rodent purified diet, Orient Bio) and water *ad libitum*. Chonnam National University's Institutional Animal Care and Use Committee approved the protocol for the animal study, and the animals were cared for in accordance with the "Guidelines for Animal Experiments" established by the university.

For the *in vivo* protective test against ethanol-induced hepatotoxicity, 24 mice were divided into three groups ($n = 8$ per group): (i) normal control group; mice received saline, (ii) ethanol dosed group; mice received 5 g/kg bw/day of ethanol, and (iii) sample treated group; mice received 5 g/kg bw/day of ethanol 30 min after 1 g/kg bw/day of sample was administered. Each group received the appropriate vehicle or ethanol with/without sample daily by gastric intubation for 8 days. At the end of the experiment, the mice were sacrificed to collect serum and tissues. These were stored at −70 °C.

2.4. Extraction

The root of *T. officinale* was extracted with 20 volumes of distilled water or 80% ethanol at 100 °C for 3 h. Each extracted solution was then filtered, concentrated, and lyophilized, yielding hot water- (TOH) or ethanol-extract (TOE), respectively.

2.5. Cytotoxicity

The cells were seeded to a culture plate containing 24 wells (5×10^4 cells/well) and grown as described above. After 24 h of incubation, the medium was removed and the cultured cells were washed twice with Hank's balanced salt solution (HBSS). Then, 1 mL of IMDM containing various concentrations of the tested sample was transferred into each well, and incubated for 5 days. This medium solution was refilled every other day. Cell viability was measured according to the method of Rochem et al. (1991) with some modifications. The 0.25 mL of freshly prepared XTT-PMS solution (1 mg XTT and 10 μg PMS/mL of phenol red-free IMDM) was added to each well and incubated for an additional 2 h. After incubation, the culture medium was collected and the absorbance was measured at a wavelength of 450 nm by a spectrophotometer. The cytotoxicity was expressed as the percentage of control, which contained no sample.

2.6. Assay for hepatoprotective activity against ethanol-induced cytotoxicity

The cells were grown in a 24-well plate as described above. Twenty-four hours after seeding, the growth medium was removed and the cells were washed twice with HBSS. Thirty minutes before 300 mM ethanol was added, the cells were incubated with IMDM plus 3% FBS, containing the respective tested sample. The medium solution was freshly replaced after renewing ethanol every day. After 5 days of incubation, cytotoxicity was measured according to XTT assay as described above.

2.7. Determination of intra-cellular ROS

The ROS level was detected using a fluorescence probe, DCFH-DA (Zuo and Clanton, 2002). The cells (5×10^4 cells/well) were incubated with 100 and 500 μg/mL of a tested sample for 24 h. Subsequently, 200 mM ethanol was added to the cells, and they were then incubated with 30 μM DCFH-DA for an additional 30 min. The fluorescence intensity of the cells was measured on a fluorescence microplate reader (BioTek Instruments, Winooski, VT) with an excitation wavelength of 490 nm and an emission wavelength of 526 nm.

2.8. Assay for serum marker enzymes

The enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) in blood serum were evaluated spectrophotometrically, using commercially available diagnostic kits supplied by Asanpharm (Seoul, Korea).

2.9. Assay for hepatic antioxidant activities

For the antioxidant activity assays, liver tissue was homogenized in 50 mM phosphate buffer. The resulting suspension was then centrifuged at 13,000*g* for 15 min at 4 °C, and the supernatant was used for the measurement. The activity of CAT was determined as described by Aebi (1984). Hepatic GST activity was assayed according to the method of Habig and Jakoby (1981). GPx activity was estimated by the method of Paglia and Valentine (1967). GR activity was measured using an adaptation of Carlberg and Mannervik's method (1975). The level of GSH, a key intra-cellular antioxidant, was measured by the method of Akerboom and Sies (1981). The concentration of malondialdehyde (MDA), the end product of lipid peroxidation, was assayed by monitoring thiobarbituric acid reactive substance formation as described by Draper and Hadley (1990). The amount of protein was measured using the Bradford assay (1976).

2.10. Statistical analysis

Data are presented as mean ± S.D. The data were statistically evaluated using the Student's *t*-test or Duncan's Multiple Range test to compare significant differences between the groups at $p < 0.05$.

3. Results

3.1. In vitro effects of T. officinale extracts against alcoholic damage

The dried root of *T. officinale* was extracted with hot water or 80% ethanol, yielding TOH or TOE, respectively. The cytotoxicities of the hot water- and ethanolic-extracts from the root of *T. officinale* were evaluated using an XTT assay. Up to the concentration of 1000 μg/mL, no significant cytotoxic effects were found in either extract (Fig. 1). Based on cell viability and morphological observation, the concentration of 500 μg/mL was chosen as the non-cytotoxic level to carry out the subsequent studies.

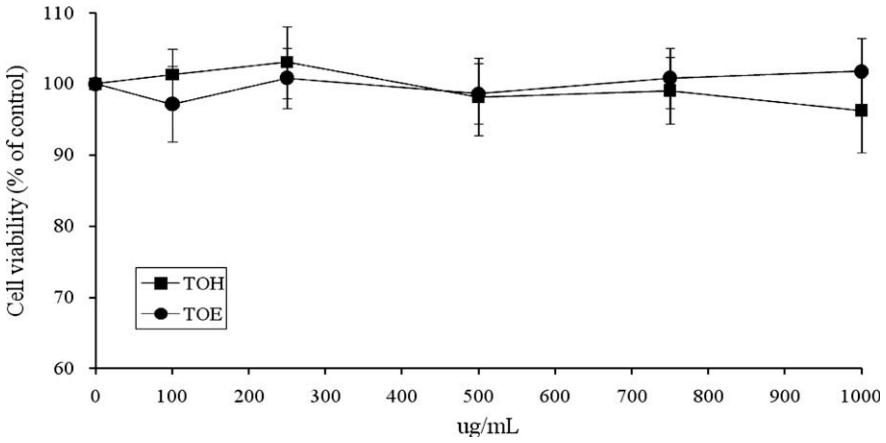

Fig. 1. Cytotoxic effects of various concentrations of extracts from the root of *Taraxacum officinale* on HepG2/2E1 Cells. Data express the mean ± S.D. of three replicates. Values are not significantly different from the respective non-treated control by Student's *t*-test ($p < 0.05$). TOH: hot water extract, TOE: 80% ethanol-extract.

Changes in ethanol-induced cytotoxicities on HepG2/2E1 cells pretreated with TOH or TOE are shown in Fig. 2. Here, the oxidative stress induced by ethanol caused a drastic decrease in cell viability with 61%. This ethanol-induced cellular toxicity, however, was significantly reduced when the HepG2/2E1 cells were pretreated with TOH (98% of cell viability). On the other hand, the treatment of TOE did not prevent cell death by oxidative stress in comparison to the ethanol-alone treated group.

3.2. Effects of T. officinale extracts on free radical generation

ROS formation was evaluated with DCFH-DA after the cells were exposed to 200 mM of ethanol. DCFH-DA is a non-polar compound that enters the cell and is cleaved to form DCFH. Trapped DCFH is oxidized by oxygen free radicals to fluorescent DCF. As revealed in Fig. 3, pretreatment with TOH significantly attenuated an increase in intra-cellular ROS content, which was triggered by ethanol-treatment, from the concentration of 100 μg/mL. At the high concentration of TOH (500 μg/mL), intra-cellular ROS level was noticeably reduced by ~25% compared to the positive control. Its level was similar to that of cells without ethanol-treatment. However, the pretreatment of TOE did not prevent the elevation of ROS formation induced by ethanol.

3.3. In vivo effects of aqueous extract from T. officinale on ethanol-induced hepatotoxicity

The results of biochemical indicators of liver function are summarized in Table 1. The administration of ethanol caused severe hepatotoxicity in the mice, as evidenced by the significant elevations of serum ALT, AST, ALP, and LDH activities. However, pretreatment with TOH significantly prevented ethanol-induced increases in the serum parameters. Noticeably, administration of TOH at the dose of 1 g/kg bw/day almost completely recovered the impaired liver functions, resulting from ethanol-induced toxicity.

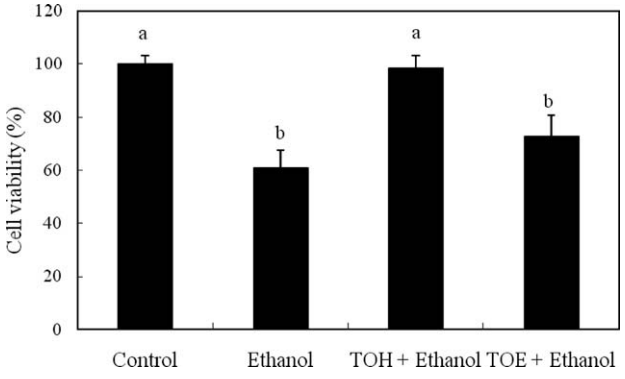

Fig. 2. Effects of extracts from the root of *Taraxacum officinale* on HepG2/2E1 cells induced by ethanol. Data express the means ± S.D. of three replicates. Different letters above the bar indicate statistically significant differences by Duncan's multiple range test ($p < 0.05$). TOH: hot water extract, TOE: 80% ethanol-extract.

3.4. Effects of aqueous extract from T. officinale on hepatic antioxidant enzymes

The antioxidant effects of TOH on the ethanol-induced depletion of antioxidant enzymes are shown in Table 2. In the ethanol dosed group, CAT activity was decreased by approximately 38% when compared to the normal control group. However, the pretreatment of TOH resulted in the prevention of a decrease in CAT activity. Compared to the normal control group, hepatic GST activity was also significantly declined in the ethanol dosed group, while no statistical change in GST activity was found in the TOH-treated mice. Consistent with the CAT and GST activities, pretreatment with TOH significantly protected against the depletion of GPx activity induced by ethanol. On the other hand, a relatively large decrease in GPx activity was observed in the ethanol-alone administered mice. The activity of GR in the TOH-administered mice was significantly enhanced in comparison to the ethanol dosed group.

3.5. Effects of aqueous extract from T. officinale on GSH and MDA levels

As presented in Table 3, the concentration of hepatic GSH in the ethanol dosed group was lower than that in the normal control group. However, the hepatic concentration of GSH was increased in the TOH group compared to the ethanol-alone administered mice. The concentration of MDA, an end product of lipid peroxidation, in the ethanol-treated mice that did not receive TOH was increased by 1.2-fold compared to the normal control mice. On the other hand, the pretreatment of TOH in mice lead to lower MDA levels than the ethanol dosed group (Table 3).

4. Discussion

Recent interest in *T. officinale* extracts has focused on the bioactive potentials. Some investigations of their efficacy with human subjects such as the diuretic effect with the ethanol-extract of *T. officinale* leaves are also reported (Clare et al., 2009). However, natural products are not always intrinsically safe, which needs the information of their potential toxicological effects when consumed. Even though there is the evidence that *T. officinale*

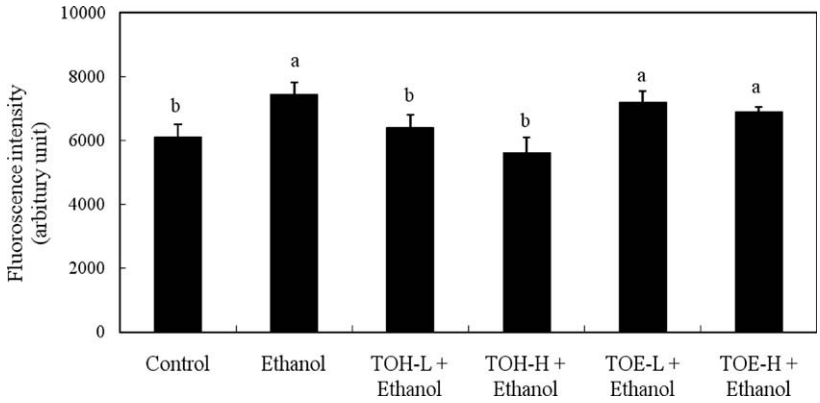

Fig. 3. Inhibitory effects of extracts from the root of *Taraxacum officinale* on intra-cellular reactive oxygen formation in HepG2/2E1 cells induced by ethanol. Data express the means ± S.D. of three replicates. Different letters above the bar indicate statistically significant differences by Duncan's multiple range test ($p < 0.05$). TOH-L: hot water extract (100 μg/mL), TOH-H: hot water extract (500 μg/mL), TOE-L: 80% ethanol-extract (100 μg/mL), TOE-H: 80% ethanol-extract (500 μg/mL).

Table 1
Effects of aqueous extract (TOH) from the root of *Taraxacum officinale* on hepatic markers in the serum of mice.*

Group	AST ** (IU/L)	ALT ** (IU/L)	ALP ** (K-A)	LDH ** (Wroblewski)
Normal control group	26.5 ± 2.1 a***	3.27 ± 0.56 a***	4.87 ± 0.40 a***	1187 ± 79 a***
Ethanol dosed group	31.5 ± 2.7 b	13.23 ± 0.71 b	6.02 ± 0.33 b	1482 ± 62 b
TOH-administered group	22.1 ± 1.9 a	4.32 ± 0.41 a	4.98 ± 0.26 a	1197 ± 73 a

* Data express the mean ± S.D. for eight mice.
** AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase.
*** Values with different letters in a column are significantly different by Duncan's multiple range test ($p < 0.05$).

Table 2
Changes in enzymatic antioxidant activities by the administration of aqueous extract (TOH) from the root of *Taraxacum officinale* to mice.*

Group	CAT** (U/mg protein)	GST** (U/mg protein)	GPx** (U/mg protein)	GR** (U/mg protein)
Normal control group	6.96 ± 0.65 a***	8.67 ± 0.55 a***	4.10 ± 0.02 a***	6.38 ± 0.44 a***
Ethanol dosed group	4.35 ± 0.79 b	7.51 ± 0.42 b	3.38 ± 0.21 b	4.83 ± 0.45 b
TOH-administered group	7.14 ± 0.48 a	8.60 ± 0.41 a	4.09 ± 0.13 a	6.29 ± 0.37 a

* Data express the mean ± S.D. for eight mice.
** CAT: catalase, GST: glutathione-*S*-transferase, GPx: glutathione peroxidase, GR: glutathione reductase.
*** Values with different letters in a column are significantly different by Duncan's multiple range test ($p < 0.05$).

Table 3
Effects of aqueous extract (TOH) from the root of *Taraxacum officinale* on levels of hepatic glutathione and malondialdehyde in mice.*

Group	GSH** (μmol/mg protein)	Malondialdehyde (mmol/g tissue)
Normal control group	13.8 ± 1.3 a***	11.4 ± 0.8 a***
Ethanol dosed group	9.8 ± 0.7 b	13.5 ± 1.0 b
TOH-administered group	14.1 ± 1.2 a	10.4 ± 1.1 a

* Data express the mean ± S.D. for eight mice.
** GSH: reduced glutathione.
*** Values with different letters in a column are significantly different by Duncan's multiple range test ($p < 0.05$).

supplementation leads to some clinical implications (Goksu et al., 2010), the toxicity of hot water extract from *T. officinale* root in human perspective has not been studied. Our toxicity test of TOH in mice revealed that the administration of TOH with 1 g/kg bw/day for 4 weeks did not possess the adverse effect (data not shown).

Approximately 80% of ingested alcohol is metabolized in the liver, so excessive alcohol consumption can lead to acute and chronic liver disease. During the metabolism of ethanol to acetaldehyde in the body, a state of oxidative stress is created by excessive ROS generation, which plays a vital role in the development of alcoholic liver disease (Tuma and Casey, 2003). Therefore, this study focused on determining changes in the hepatic antioxidation defense system in association with oxidative stress, using cell line and animal models challenged with ethanol. In our experiments, TOH was applied before the intoxication by ethanol for the preventive effect because the elevated level of CYP2E1, which increases 4- to 10-fold in liver biopsies of drinking subjects (Lieber, 2000), forms a lot of reactive radicals during the oxidization of ethanol to acetaldehyde.

Cell viability for the TOH co-treated cells was significantly increased over the ethanol-alone treated group, implying that the aqueous extract of *T. officinale* root might have a protective effect against ethanol-induced liver damage. ROS mediates hepatic tissue damage induced by ethanol (Song et al., 2008). The induction of CYP2E1 by ethanol is considered to play a key role in the increased generation of ROS such as $O_2^{\cdot-}$ and H_2O_2 (Lu and Cederbaum, 2008). It usually causes a pro-oxidative state in the cellular environment, which results in oxidative damage. The administration of nutritional regimens can inhibit the excessive existence of intra-cellular ROS (Molina et al., 2003; You et al., 2009). High levels of oxidative stress caused by ethanol-treatment in cells contribute to hepatotoxicity, as evidenced by a significant elevation in cellular ROS, suggesting a role of oxidative stress in ethanol-induced hepatic

damage. Restoration of the intra-cellular ROS level by pretreatment with TOH indicates that its protective effect against ethanol-induced liver damage was mainly due to the suppression of an increase in intra-cellular ROS.

Ethanol-induced hepatic damage is characterized by hepatic marker enzymes such as ALT, AST, ALP, and LDH. The elevation of these enzymes in serum suggests hepatocytic damage (Kasdallah-Grissa et al., 2007). In this study, there were significant decreases in serum enzymes by the co-administration of TOH with ethanol, confirming that the aqueous extract of *T. officinale* root effectively protected the mice livers against severe damage caused by ethanol ingestion.

The excessive ROS generated during ethanol metabolism rapidly react with lipid membranes. This initiates the lipid peroxidation chain reaction, which produces lipid peroxyl radicals (Nordmann et al., 1992). Enhanced hepatic MDA, a major reactive aldehyde resulting from the peroxidation of polyunsaturated fatty acids in the cell membrane, reflects a causal role of lipid peroxidation in ethanol-induced liver damage. The elevation of lipid peroxidation caused by ethanol has been previously reported in a mouse model (Balasubramaniyan et al., 2003), which is in agreement with the results obtained in this study. Our results revealed that pretreatment with TOH exhibited a significant inhibitory role against lipid peroxidation in mice, and thereby diminished ethanol-induced hepatic membrane destruction and hepatic damage. The prevention of lipid peroxidation might, at least in part, be derived from the capability of TOH to scavenge ROS, which was supported by the observation that TOH reduced the level of TBARS formation *in vitro*. When the lipid peroxidation inhibitory potentials of the *T. officinale* extracts were evaluated according to their inhibitory activities on the formation of MDA produced during the oxidation process, TOH revealed a strong inhibitory ability towards lipid peroxidation with an activity of 37.8% (data not shown).

The antioxidant defense system in the body plays an important role in protection against oxidative stress. Administration of ethanol elevates the formation of lipid peroxides and ROS, leading to the inactivation of enzymatic and non-enzymatic antioxidants in the liver. The aqueous extract from the root of *T. officinale* may protect the liver against ethanol-induced oxidative injury *via* its improving effects on cellular antioxidative potential. Hepatic GSH is an important non-enzymatic antioxidant that plays a crucial role in scavenging ROS and maintaining enzymatic antioxidants (Kadiska et al., 2000). Depletion of GSH obtained by ethanol exposure may be attributed to its rapid utilization by the overproduction of ROS and subsequent oxidative stress. This decreased hepatic GSH gives rise to the inactivation of methionine adenosyltransferase (a key enzyme for the formation of *S*-adenosylmethionine) activity and the reduction of *S*-adenosylmethionine (metabolic source of GSH production) level, which in turn aggravates the depleted level of intra-cellular GSH (Choi et al., 2000). In this study, the level of hepatic GSH remained the same by the pretreatment of TOH in ethanol administered mice. This indicates that the detoxification of ROS, which is responsible for ethanol-induced protein oxidation, leads to the prevention of hepatotoxicity.

Ethanol-induced oxidative stress, stemming from the exhaustion of endogenous antioxidant defense mechanisms by excess ROS, may inactivate enzymatic antioxidants. As expected, antioxidant enzymes, including CAT, GST, GPx, and GR, were partially inactivated by ethanol-induced hepatotoxicity, which confirms previous studies (Balasubramaniyan et al., 2003; Sultana et al., 2005). This might be largely due to an overwhelming oxidative modification of the enzymatic proteins by increased ROS production. Our observations revealed that TOH supplementation improved the impaired antioxidative defense system in mice livers challenged with ethanol, as indicated by the restoration of enzyme activities. Many flavonoids act as antioxidants. The *T. officinale* contains several flavonoids such as quercetin, luteolin, and luteolin-7-*O*-glucoside (Schütz et al., 2006). The protective effects of some flavonoids against oxidative stress were reported; luteolin and luteolin-7-*O*-glucoside as ROS scavenger (Rodriguez-Fragoso et al., 2008), and quercetin as protectant against ethanol-induced liver damage (Molina et al., 2003). According to our investigation, TOH possessed 2% of flavonoids and 0.013 mg/g of luteolin (data not shown). Research is currently underway to identify the structure of the hepatoprotectant in *T. officinale* root against ethanol-induced toxicity.

In summary, irreversible liver damage induced by the consumption of excessive ethanol is strongly associated with oxidative stress *via* enhanced lipid peroxidation and ROS production. The aqueous extract from the root of *T. officinale* is implicated as a potentially useful radical scavenger for a host of radicals. TOH supplementation antagonizes the ethanol-induced hepatic injury as evidenced by a reversal of hepatic antioxidant status and lipid peroxidation. It is presumed that the antioxidant-sparing action of the aqueous extract of *T. officinale* could be responsible for the amelioration of oxidative stress during ethanol toxicity. Therefore, the development of dietary supplementation using *T. officinale* could be helpful to protect against alcoholic liver damage mediated by oxidative stress and the further study with human subjects should be performed for the safe administration with potent efficacy.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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