Crepidiastrum denticulatum Extract Protects the Liver Against Chronic Alcohol-Induced Damage and Fat Accumulation in Rats

Ji-Hye Yoo, Kyungsu Kang, Ji Ho Yun, Mi Ae Kim, and Chu Won Nho

Functional Food Center, Korea Institute of Science and Technology, Gangneung, Korea.

ABSTRACT Alcohol is a severe hepatotoxicant that causes liver abnormalities such as steatosis, cirrhosis, and hepatocarcinoma. *Crepidiastrum denticulatum* (CD) is a well-known, traditionally consumed vegetable in Korea, which was recently reported to have bioactive compounds with detoxification and antioxidant properties. In this study, we report the hepatoprotective effect of CD extract against chronic alcohol-induced liver damage *in vivo*. The rats that were given CD extract exhibited decreased alanine aminotransferase, aspartate aminotransferase, and γ -glutamyl transpeptidase activities, which are liver damage markers that are typically elevated by alcohol consumption. The results were confirmed by histopathology with hematoxylin and eosin staining. Chronic alcohol consumption induced the formation of alcoholic fatty liver. However, treatment with CD extract dramatically decreased the hepatic lipid droplets. Treatment with CD extract also restored the antioxidative capacity and lipid peroxidation of the liver that had been changed by alcohol consumption. Furthermore, treatment with CD extract normalized the activities of the antioxidative enzymes superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase, which had been decreased by alcohol consumption. The results indicate that CD extract has protective effects against chronic alcohol hepatotoxicity in rats by increasing the liver's antioxidant capacity, and has potential as a dietary supplement intervention for patients with alcohol-induced liver damage.

KEY WORDS: • *antioxidative capacity* • *chronic alcohol consumption* • *Crepidiastrum denticulatum* • *hepatoprotection* • *oxidative stress* • *wild vegetable*

INTRODUCTION

ACCORDING TO THE WORLD HEALTH ORGANIZATION (WHO), there are at least 2 billion alcohol users worldwide. Moreover, alcohol consumption results in approximately 2.5 million deaths each year and is the third largest risk factor for disease and disability.¹ Alcohol has been reported as a causal factor related to more than 60 diseases and injuries, including liver cirrhosis, cardiovascular diseases, and cancer. When alcohol is consumed, it is absorbed through the digestive organs and affects a variety of tissues in the human body.² Among these tissues, the liver is the major site of alcohol-induced damage because it directly receives blood containing high concentrations of alcohol and is the major organ of ethanol metabolism, producing toxic metabolites from alcohol.³

Liver injury due to ethanol exposure is mediated by two well-characterized pathways.⁴ The first pathway involves alcohol oxidation by alcohol dehydrogenase and aldehyde dehydrogenase. The increase in alcohol oxidation and the levels of alcohol metabolites results in metabolic stress and

the development of a fatty liver. The second pathway is the ethanol-induced microsomal ethanol oxidation system (MEOS). The major components of this pathway are cytochrome P450 2E1 (CYP 2E1) and the reactive oxygen species (ROS) generated by CYP 2E1. Because ROS generation is a naturally occurring process in the human body, a variety of enzymatic and nonenzymatic mechanisms have evolved to protect cells from ROS.^{5,6} However, the increased oxidative stress caused by chronic alcohol consumption impairs those mechanisms. Antioxidant enzymes involved in the elimination of ROS are inhibited, and glutathione, an essential nonenzymatic antioxidant in cells, is depleted by the oxidative stress from alcohol consumption, resulting in DNA damage, necrosis, and apoptosis of hepatic cells.⁷

As oxidative stress is a key mechanism underlying alcohol-mediated hepatotoxicity, antioxidant therapy for alcoholic liver disease is particularly important. Therefore, several trials involving a variety of antioxidants have been recommended.⁸ Specifically, natural antioxidants such as green tea extracts or silymarin are preferred for long-term therapy. Silymarin is a polyphenolic mixture of flavonoids obtained from milk thistle, and has been used in the treatment of alcoholic liver disease.⁹ Recently, a woody plant native to Asia, *Hovenia dulcis*, and its fruit have been reported to have antioxidant properties that act against alcohol

Manuscript received 7 February 2013. Revision accepted 14 February 2014.

Address correspondence to: Chu Won Nho, PhD, Functional Food Center, Korea Institute of Science and Technology, 290 Daejeon-dong, Gangwon-do 210-340, Republic of Korea, E-mail: cwnho@kist.re.kr

and has been commercialized as a dietary supplement.^{10,11} However, the large-scale cultivation of *H. dulcis* is hindered by the time required to grow the plant and harvest its fruits, which limits the supply of raw materials and makes commercialization difficult.

Crepidiastrum denticulatum (CD; also known as *Youngia denticulata*) is a Korean composite plant whose early sprouts have been traditionally eaten as wild vegetables.¹² CD contains large amounts of phenolic compounds such as dicaffeoylquinic acid, chlorogenic acid, and chicoric acid,¹² all of which have been reported to exhibit antioxidant activity.^{13–15} We recently reported that youngiasides A, B, and C isolated from CD could induce detoxification enzymes, quinone reductase and CYP 1A1, through the activation of Nrf2 and the aryl hydrocarbon receptor (AhR).¹⁶ We also recently reported that CD had a protective effect in HepG2 cells against *tert*-butyl hydroperoxide, a strong inducer of oxidative stress, by increasing the glutathione level.¹² In the present study, we investigated whether CD extract may protect the liver against alcohol *in vivo* and whether there is potential for the development of a dietary supplement to improve liver function against chronic alcohol consumption.

MATERIALS AND METHODS

Preparation of CD extract

Whole plants of CD were collected in August 2010 in Gangneung, Korea. Voucher specimens (D-043) were deposited in the herbarium of the Korea Institute of Science and Technology. Air-dried whole plants were extracted by reflux with 75% ethanol at 70°C for 4 h on a large scale (Bioland, Ansan, Korea). In some cases, the CD extract was mixed with β -cyclodextrin (Wacker, München, Germany), as a pharmaceutical excipient, at a 1:1 ratio. The extracts were added to Lieber DeCali liquid diet (Dyets, Bethlehem, PA) for the animal experiment.¹⁷

Animal experiment design

Animal care and handling were performed following the guidelines of the Institutional Animal Care and Use Committee of Korea Institute of Science and Technology. Five-week-old male Sprague-Dawley (SD) rats weighing between 140 and 160 g were purchased from Orient Bio (Seongnam, Korea). The rats were kept in a temperature-controlled room on a 12 h light/12 h dark schedule with food and water provided *ad libitum* for 5 days before the experiment. The animals were then divided randomly into six groups, with seven or eight rats in each group. They were individually housed and fed the Lieber DeCali liquid diets¹⁷ as control diet or alcohol added diet (6.7% v/v) for 28 days as follows: Group I, control diet, no treatment; Group II, alcohol diet; Group III, alcohol diet with CD extract (75 mg/kg/day); Group IV, alcohol diet with CD extract (18.75 mg/kg/day) and β -cyclodextrin (18.75 mg/kg/day); Group V, alcohol diet with CD extract (37.5 mg/kg/day) and β -cyclodextrin (37.5 mg/kg/day); and Group VI, alcohol diet with CD extract (75 mg/kg/day) and β -cyclodextrin (75 mg/kg/day). At the end of the experiment,

food was withheld overnight, and the rats were sacrificed via inhalation of ethyl ether. Blood samples were collected in tubes containing EDTA (BD Biosciences, Franklin Lakes, NJ, USA), and the livers were collected after perfusion with 0.9% KCl solution.

Sample preparation for biochemical analysis

The serum from the blood samples was prepared by centrifugation at 2660 g, for 10 min and stored at 4°C. The liver tissues were collected and stored at –80°C. The liver tissue was homogenized in ice-cold liver homogenization buffer (0.25 M K_2HPO_4/KH_2PO_4 –0.15 M KCl buffer, pH 7.25) and centrifuged at 2660 g for 20 min to isolate lipid peroxidation and antioxidative enzymes. The supernatant was transferred to a fresh tube and centrifuged at 100,900 g for 30 min. The resulting supernatant contained the antioxidative enzymes, while the pellet was composed of the lipid peroxidation enzymes. The pellet was resuspended in ice-cold liver homogenization buffer and sonicated for 4 sec.

Measurement of serum parameters

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured using spectrophotometric diagnostic kits (Young-Dong Diagnostics, Yongin, Korea). γ -Glutamyl transpeptidase (γ -GT) activity was determined using L- γ -glutamyl-p-nitroanilide, as previously reported.¹⁸

Measurement of liver enzymes activities

Total cellular superoxide dismutase (SOD) activity was measured using the reduction of nitroblue tetrazolium by the xanthine–xanthine oxidase system as previously described with slight modifications.¹⁹ The measurement of catalase (CAT) activity was based on the decomposition of hydroperoxide by CAT as previously described with slight modifications.²⁰ Glutathione peroxidase (GPx) activity was measured by monitoring the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) from glutathione (GSH) as previously described.²¹ Glutathione reductase (GR) activity was evaluated by measuring the oxidation of NADPH via the reduction of oxidized glutathione (GSSG) as previously reported.²²

Measurement of liver parameters

The ORAC (oxygen radical absorbance capacity; Cell Biolabs, San Diego, CA, USA) antioxidant assay kit was used to determine the total antioxidant capacity of the liver tissue. Lipid peroxidation was examined by measuring the formation of thiobarbituric acid reactive substances (TBARS) as previously reported.²³

Histological observation of liver

Liver sections were fixed in 10% formalin solution, dehydrated with a sequence of ethanol solutions, and embedded in paraffin. Fixed tissues were sliced at a thickness of 5–6 μ m and stained with hematoxylin and eosin.

Statistical analysis

Data are expressed as the mean $\pm$ standard deviation, which was calculated from at least five different animals. The statistical significance among the test groups was determined with analysis of variance (ANOVA) and Tukey's honest significance test (HSD) using JMP pro v10.0 (SAS Institute, Inc., Cary, NC, USA). A P value of $<.05$ was considered significant.

RESULTS

Effect of CD extract on chronic alcohol-induced liver injury in rats

The effects of CD extract on alcohol-induced liver injury in rats were investigated by feeding SD rats liquid diets mixed with alcohol and CD extract for 28 days. After the rats were sacrificed, we determined the serum enzyme activities (Fig. 1) and liver weights (Table 1). Rats in the alcohol-treated group showed a slight increase in the relative liver weight (liver weight/body weight) compared to the nontreated group. However, rats that received CD extract had slightly lower liver/body weights compared to the alcohol-treated group.

The serum activities of AST, ALT, and γ -GT, were investigated to evaluate the severity of the liver damage (Fig. 1). Chronic alcohol consumption significantly increased the activities of the serum liver enzymes ($P < .01$, as compared to the nonalcoholic control group), as previously reported in the literature.²⁴ The activity of AST, a primary indicator of hepatic injury, was significantly increased by alcohol treatment compared to the nonalcoholic group (Fig. 1A). However, the CD extract (18.75 and 75 mg/kg/day with β -cyclodextrin) significantly suppressed the elevation of AST activity caused by alcohol consumption. The activity of ALT, another marker of hepatic damage, was also significantly increased by alcohol consumption and was significantly decreased by treatment with CD extract at a dose of 75 mg/kg/day with β -cyclodextrin (Fig. 1B). Remarkably, the activity of γ -GT also increased in animals fed the alcohol diet, and it was significantly lowered when the 37.5 or 75 mg/kg/day of CD extract with β -cyclodextrin was consumed (Fig. 1C).

We confirmed the protective effect of the CD extract through histological observation of the liver tissues (Fig. 2). Lipid accumulation in the livers (alcoholic fatty liver) of the alcohol-treated group increased dramatically (Fig. 2B), whereas in the CD extract-treated group, the amount of hepatic lipid droplets was reduced, consistent with the change in serum enzyme activity. Group V treated with the CD extract (37.5 mg/kg/day) combined with β -cyclodextrin appeared to have mild fatty liver. Notably, livers from the group treated with the highest dose of CD extract (75 mg/kg/day) and β -cyclodextrin were very similar to the livers from the nonalcoholic group (Fig. 2A, C, and F).

Effect of CD extract on antioxidant parameters

The *in vivo* antioxidant levels are shown in Figure 3. Ethanol intake slightly reduced the antioxidant capacity of li-

FIG. 1. Effect of *Crepidiastrum denticulatum* (CD) extract on serum parameters. The activities of (A) aspartate aminotransferase (AST), (B) alanine aminotransferase (ALT), and (C) γ -glutamyl transpeptidase (γ -GT) were measured using blood obtained from Sprague-Dawley (SD) rats after chronic alcohol consumption. ** $P < .01$, compared to the control group; # $P < .05$, ## $P < .01$, ### $P < .001$, and #### $P < .0001$, compared to the alcohol-treated group ($n \geq 5$).

ver tissue (Fig. 3A). CD extract treatment with β -cyclodextrin slightly increased the antioxidant capacity to levels comparable to those of the control group in a dose-dependent manner. However, the CD extract without β -cyclodextrin did not recover antioxidant capacity, which was decreased by

TABLE 1. EFFECTS OF *CREPIDIASTRUM DENTICULATUM* EXTRACT AND ALCOHOL TREATMENT ON BODY AND LIVER WEIGHTS

Group	Diet	Sample	Excipients	CDE (mg/kg/day)	β -cyclodextrin (mg/kg/day)	Initial body weight	Final body weight	Liver weight	Liver weight/body weight
I	Normal	None	None	0	0	179.7 $\pm$ 7.8	287.9 $\pm$ 12.7	17.5 $\pm$ 1.3	0.060 $\pm$ 0.005
II	Ethanol	None	None	0	0	179.6 $\pm$ 7.7	261.6 $\pm$ 10.1**	17.0 $\pm$ 1.4	0.067 $\pm$ 0.005
III	Ethanol	CDE	None	75	0	179.7 $\pm$ 6.4	263.4 $\pm$ 16.3**	17.1 $\pm$ 1.8	0.065 $\pm$ 0.005
IV	Ethanol	CDE	β -cyclodextrin	18.75	18.75	179.8 $\pm$ 6.3	265.5 $\pm$ 13.5*	15.9 $\pm$ 0.8	0.060 $\pm$ 0.005
V	Ethanol	CDE	β -cyclodextrin	37.5	37.5	179.7 $\pm$ 6.2	263.0 $\pm$ 12.7**	16.8 $\pm$ 1.0	0.062 $\pm$ 0.004
VI	Ethanol	CDE	β -cyclodextrin	75	75	179.7 $\pm$ 6.3	257.2 $\pm$ 8.9***	15.6 $\pm$ 3.2	0.064 $\pm$ 0.004

* $P < .05$, ** $P < .01$, and *** $P < .0001$, compared to the control group (Group I; $n \geq 5$).

CDE, *Crepidiastrum denticulatum* extract.

alcohol. The level of malonaldehyde (MDA), an indicator of lipid peroxidation by ROS, was significantly increased by alcohol treatment ($P < .001$, compared to control group) and was lowered by treatment with CD extract (37.5 or 75 mg/kg/day) with β -cyclodextrin (Fig. 3B). The amount of MDA in the group treated with 75 mg/kg/day CD extract without β -cyclodextrin was slightly higher than in the alcohol-treated group.

Effect of CD extract on antioxidant enzymes

Because the oxidative stress associated with chronic alcohol consumption usually weakens the antioxidant defense mechanism, we also evaluated the activities of antioxidant enzymes involved in the antioxidant defense mechanism. The activities of the antioxidant enzymes SOD, CAT, GR, and GPx are shown in Figure 4. A significant decrease in

FIG. 2. Representative liver sections stained with hematoxylin and eosin (200 $\times$). (A) The livers from rats in the normal diet group. (B) The livers from rats that consumed alcohol for 4 weeks. (C) The livers from rats treated with 75 mg/kg/day of CD extract without β -cyclodextrin. The livers from rats treated with (D) 18.75, (E) 37.5, and (F) 75 mg/kg/day of CD extract with β -cyclodextrin (1:1). The hepatic lipid droplets were marked by arrows. Color images available online at www.liebertpub.com/jmf

FIG. 3. The inhibitory effect of CD extract against oxidative stress induced by chronic alcohol consumption in SD rats. **(A)** Antioxidative capacity. **(B)** Malondialdehyde (MDA) levels. * $P < .05$, ** $P < .01$, *** $P < .001$, and **** $P < .0001$, compared to the control group ($n \geq 5$).

SOD activity was observed in the alcohol-fed group compared to the nonalcoholic group (Fig. 4A, $P < .05$). Treatment with CD extract significantly increased SOD activity at even the lowest dosage of CD extract ($P < .0001$, compared to the alcohol-treated group). The CAT activity was also slightly

decreased by alcohol consumption and was significantly increased by treatment with CD extract (75 mg/kg/day without β -cyclodextrin, 37.5 mg/kg/day with β -cyclodextrin; $P < .05$, compared to the alcohol-treated group; Fig. 4B). Similarly, the GR activity was slightly decreased by alcohol

FIG. 4. Effects of CD extract on antioxidant liver enzymes: **(A)** superoxide dismutase (SOD), **(B)** catalase (CAT), **(C)** glutathione reductase (GR), and **(D)** glutathione peroxidase (GPx). * $P < .05$, **** $P < .0001$, compared to the control group; # $P < .05$, ## $P < .01$, #### $P < .0001$, compared to the alcohol-treated group ($n \geq 5$).

consumption and was significantly increased by treatment with CD extract (37.5 or 75 mg/kg/day) with β -cyclodextrin ($P < .05$ and $P < .01$ respectively, compared to the alcohol-treated group; Fig. 4C). The recovery of the GPx activities by CD extract treatment was observed, but it was not as robust as the recoveries of the SOD, CAT, or GR activity (Fig. 4D).

DISCUSSION

The hepatoprotective effects of CD extract against chronic alcohol consumption in rats are explained as follows. First, the CD extract has apparent antioxidative effects and a potent radical scavenging activity. It can protect against oxidative stress-induced hepatic cell death *in vitro* by inhibiting ROS generation and restoring glutathione levels.¹² In the present study, CD extract also showed antioxidative effects *in vivo* by increasing the total antioxidant capacity of liver tissues and decreasing MDA levels, which indicate lipid peroxidation. CD extract also increased the activities of various liver antioxidant enzymes, such as SOD, CAT, GR, and GPx. Second, CD extract can remove toxic metabolites by activating the Nrf2 pathway, which is one of the most important signaling pathways regulating cellular antioxidant and detoxification. The Nrf2 pathway also plays a pivotal role in the protection of the liver against alcohol-induced damage.^{25–27} Similarly, quercetin and *Ca-janus cajan* extracts protect hepatocytes from ethanol-induced liver damage by inducing phase II detoxification enzymes such as heme oxygenase-1 and UDP-glucosyl transferase through the activation of Nrf2.^{28,29} Third, we speculated that the *in vivo* hepatoprotective effects of CD extract originate from the suppression of hepatocyte necrosis via the downregulation of proinflammatory cytokines. Specifically, interleukin-6 (IL-6) and tumor necrosis factor α (TNF- α) are known to be important cytokines linked to hepatocyte damage induced by chronic alcohol consumption.^{30,31} According to our preliminary experiments, CD extract appeared to restore IL-6 and TNF- α levels to normal, after they were initially increased by the chronic consumption of a high-fat diet in rats (unpublished data by Dr. Elizabeth Jeffery, University of Illinois at Urbana-Champaign, Champaign, IL, USA).

We mixed CD extract with the pharmaceutical excipient, β -cyclodextrin, for tablet formulation and to increase the solubility of the CD extract in water, since CD extract was prepared by the extraction using 75% ethanol, not water. We used different amounts of β -cyclodextrin for the animal experiment, since we needed to produce standardized CD extract with cyclodextrin on a large scale for further clinical trials and commercialization. β -Cyclodextrin has been reported to have no toxicity or no effect for AST and ALT until 12,500 ppm (12.5%) after 13 weeks of treatment. Rather, a higher dose of β -cyclodextrin increased AST and ALT.³² Our maximum dose of β -cyclodextrin was 0.5%. For this reason, β -cyclodextrin in our experiment was not high enough to affect AST and ALT levels. Therefore, we believe that the hepatoprotective effects against chronic alcohol consumption are due to the CD extract, not from β -cyclodextrin.

We also compared the hepatoprotective effects of CD extract with and without β -cyclodextrin. Interestingly, in rats fed equivalent doses of CD extract, CD extract without β -cyclodextrin was less effective than CD extract with β -cyclodextrin. AST and ALT data, which are decisive makers for the hepatoprotective effect, as well as most of the other biochemical data (γ -GT, ORAC, TBARS, GR, and GPx), showed that CD extract with β -cyclodextrin had better hepatoprotective effects than CD extract without β -cyclodextrin. Only the activities of SOD and CAT were similarly increased by CD extract (75 mg/kg/day) both with and without β -cyclodextrin. Taken together, we could conclude that CD extract with β -cyclodextrin has better hepatoprotective effect against chronic alcohol consumption than CD extract without β -cyclodextrin.

We believe that these effects may have been caused by the excipient. The excipient was composed of β -cyclodextrin derivatives, and β -cyclodextrins have been used as drug carriers because they enhance the water solubility, stability, and safety of drugs.^{33,34} Thus, the increased effect observed when the excipient was used may be a result of the increased absorption of CD extract mediated by the excipient. Therefore, further pharmacokinetic studies of the major active compounds including chicoric acid of CD extract are needed to explain the effect. Similar results were reported in the case of silymarin, a representative hepatoprotective agent. Silymarin is known to have poor bioavailability due to its extensive metabolism, low permeability across intestinal epithelial cells, low water solubility, and rapid excretion. When silybin, a major active constituent of silymarin, was administered with β -cyclodextrin *in vivo*, the bioavailability of silybin was dramatically increased compared to the administration of silybin alone.³⁵

In conclusion, we have shown that the CD extract has potent hepatoprotective effects against chronic alcohol consumption in rats. To our knowledge, the present study is the first to report on the *in vivo* pharmacological effects of CD. This extract inhibited the hepatic damage accompanied by decreased activity of serum liver enzymes. Treatment with CD extract resulted in restoration of the antioxidant defense system, which was impaired by chronic alcohol exposure, and suppressed lipid peroxidation. Currently, we are also performing a clinical trial of CD extract for patients with alcohol-induced liver damage. We hope that a series of comprehensive studies will encourage the development of CD extract as a new and promising dietary supplement for protection against alcohol-induced liver damage.

ACKNOWLEDGMENT

This study was supported by an intramural grant from the Korea Institute of Science and Technology (2Z03850).

AUTHOR DISCLOSURE STATEMENT

The technologies described in this article were transferred to the Korean pharmaceutical company Arlico Pharm. Co. Ltd. for the further development of CD extract as a hepatoprotective dietary supplement.

REFERENCES

1. WHO: Global status report on alcohol and health. World Health Organization, Geneva, Switzerland, 2011, p. xii.
2. Lieber CS: Alcohol: its metabolism and interaction with nutrients. *Annu Rev Nutr* 2000;20:395–430.
3. Karinch AM, Martin JH, Vary TC: Acute and chronic ethanol consumption differentially impact pathways limiting hepatic protein synthesis. *Am J Physiol Endocrinol Metab* 2008;295:E3–E9.
4. Stewart S, Jones D, Day CP: Alcoholic liver disease: new insights into mechanisms and preventative strategies. *Trends Mol Med* 2001;7:408–413.
5. Chaudiere J, Ferrari-Iliou R: Intracellular antioxidants: from chemical to biochemical mechanisms. *Food Chem Toxicol* 1999;37:949–962.
6. Finkel T, Holbrook NJ: Oxidants, oxidative stress and the biology of ageing. *Nature* 2000;408:239–247.
7. Albano E: Alcohol, oxidative stress and free radical damage. *Proc Nutr Soc* 2006;65:278–290.
8. Mckillop IH, Schrum LW: Alcohol and liver cancer. *Alcohol* 2005;35:195–203.
9. Song Z, Deaciuc I, Song M, Lee DY, Liu Y, Ji X, McClain C: Silymarin protects against acute ethanol-induced hepatotoxicity in mice. *Alcohol Clin Exp Res* 2006;30:407–413.
10. Hase K, Ohsugi M, Xiong QB, Basnet P, Kadota S, Namba T: Hepatoprotective effect of *Hovenia dulcis* Thunb on experimental liver injuries induced by carbon tetrachloride or D-galactosamine/lipopolysaccharide. *Biol Pharm Bull* 1997;20:381–385.
11. Hyun TK, Eom SH, Yu CY, Roitsch T: *Hovenia dulcis*—an Asian traditional herb. *Planta Med* 2010;76:943–949.
12. Kang K, Jho EH, Lee HJ, Oidovsambuu S, Yun JH, Kim CY, Yoo JH, Kim YJ, Kim JH, Ahn SY, Nho CW: *Youngia denticulata* protects against oxidative damage induced by *tert*-butylhydroperoxide in HepG2 cells. *J Med Food* 2011;14:1198–1207.
13. Dalby-Brown L, Barsett H, Landbo AKR, Meyer AS, Molgaard P: Synergistic antioxidative effects of alkamides, caffeic acid derivatives, and polysaccharide fractions from *Echinacea purpurea* on *in vitro* oxidation of human low-density lipoproteins. *J Agric Food Chem* 2005;53:9413–9423.
14. Kim KA, Kang KD, Lee EH, Nho CW, Jung SH: Edible wild vegetable, *Gymnaster koraiensis* protects retinal ganglion cells against oxidative stress. *Food Chem Toxicol* 2011;49:2131–2143.
15. Tsuchiya T, Suzuki O, Igarashi K: Protective effects of chlorogenic acid on paraquat-induced oxidative stress in rats. *Biosci Biotechnol Biochem* 1996;60:765–768.
16. Yun JH, Lee SB, Lee HJ, Kim CY, Kim MA, Sohn YC, Nho CW: Bi-functional induction of the quinone reductase and cytochrome P450 1A1 by youngiasides via Nrf2-ARE and AhR-XRE pathways. *Biol Pharm Bull* 2010;33:1650–1657.
17. Bertola A, Mathews S, Ki SH, Wang H, Gao B: Mouse model of chronic and binge ethanol feeding (the NIAAA model). *Nat Protoc* 2013;8:627–637.
18. Orłowski M, Meister A: Isolation of γ -glutamyl transpeptidase from hog kidney. *J Biol Chem* 1965;240:338–347.
19. Spitz DR, Oberley LW: An assay for superoxide dismutase activity in mammalian tissue homogenates. *Anal Biochem* 1989;179:8–18.
20. Aebi H: Catalase *in vitro*. *Methods Enzymol* 1984;105:121–126.
21. Flohe L, Gunzler WA: Assays of glutathione peroxidase. *Methods Enzymol* 1984;105:114–121.
22. Wheeler CR, Salzman JA, Elsayed NM, Omaye ST, Korte DW: Automated assays for superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity. *Anal Biochem* 1990;184:193–199.
23. Jung SH, Kang KD, Ji D, Fawcett RJ, Safa R, Kamalden TA, Osborne NN: The flavonoid baicalin counteracts ischemic and oxidative insults to retinal cells and lipid peroxidation to brain membranes. *Neurochem Int* 2008;53:325–337.
24. Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S: The current state of serum biomarkers of hepatotoxicity. *Toxicology* 2008;245:194–205.
25. Bae SH, Sung SH, Cho EJ, Lee SK, Lee HE, Woo HA, Yu DY, Kil IS, Rhee SG: Concerted action of sulfiredoxin and peroxiredoxin I protects against alcohol-induced oxidative injury in mouse liver. *Hepatology* 2011;53:945–953.
26. Jin M, Kumar A, Kumar S: Ethanol-mediated regulation of cytochrome P450 2A6 expression in monocytes: role of oxidative stress-mediated PKC/MEK/Nrf2 pathway. *PLoS One* 2012;7:e35505.
27. Lamle J, Marhenke S, Borlak J, Von Wasielewski R, Eriksson CJ, Geffers R, Manns MP, Yamamoto M, Vogel A: Nuclear factor- κ B-related factor 2 prevents alcohol-induced fulminant liver injury. *Gastroenterology* 2008;134:1159–1168.
28. Kundu R, Dasgupta S, Biswas A, Bhattacharya A, Pal BC, Bandyopadhyay D, Bhattacharya S: *Cajanus cajan* Linn. (Leguminosae) prevents alcohol-induced rat liver damage and augments cytoprotective function. *J Ethnopharmacol* 2008;118:440–447.
29. Yao P, Nussler A, Liu L, Hao L, Song F, Schirmeier A, Nussler N: Quercetin protects human hepatocytes from ethanol-derived oxidative stress by inducing heme oxygenase-1 via the MAPK/Nrf2 pathways. *J Hepatol* 2007;47:253–261.
30. Mcvicker BL, Tuma DJ, Kharbanda KK, Kubik JL, Casey CA: Effect of chronic ethanol administration on the *in vitro* production of proinflammatory cytokines by rat Kupffer cells in the presence of apoptotic cells. *Alcohol Clin Exp Res* 2007;31:122–129.
31. Yang SQ, Lin HZ, Yin M, Albrecht JH, Diehl AM: Effects of chronic ethanol consumption on cytokine regulation of liver regeneration. *Am J Physiol* 1998;275:G696–G704.
32. Bellringer ME, Smith TG, Read R, Gopinath C, Olivier P: β -Cyclodextrin: 52-week toxicity studies in the rat and dog. *Food Chem Toxicol* 1995;33:367–376.
33. Brewster ME, Loftsson T: Cyclodextrins as pharmaceutical solubilizers. *Adv Drug Deliv Rev* 2007;59:645–666.
34. Challa R, Ahuja A, Ali J, Khar RK: Cyclodextrins in drug delivery: an updated review. *AAPS PharmSciTech* 2005;6:E329–E357.
35. Javed S, Kohli K, Ali M: Reassessing bioavailability of silymarin. *Altern Med Rev* 2011;16:239–249.