Food and Chemical Toxicology 47 (2009) 530–535

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Saponins isolated from the root of *Platycodon grandiflorum* protect against acute ethanol-induced hepatotoxicity in mice

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ARTICLE INFO

Article history:
Received 9 October 2008
Accepted 5 December 2008

Keywords:
Platycodon grandiflorum
Ethanol
Alcoholic steatosis
Liver injury
CYP2E1

ABSTRACT

The protective effects of saponins isolated from the root of *Platycodon grandiflorum* (Changkil saponins: CKS) against alcoholic steatosis in liver injury induced by acute ethanol administration were investigated. Pretreatment with CKS prior to ethanol administration significantly prevented the increases in serum alanine aminotransferase activity, hepatic TNF-α level, hepatic lipid peroxidation and hepatic triglyceride level. CKS prevented ethanol-induced steatosis and necrosis, as indicated by liver histopathological studies. Additionally, CKS protected against ethanol-induced depletion of hepatic glutathione levels. CYP2E1 has been suggested as a major contributor to ethanol-induced oxidative stress and liver injury. The concurrent administration of CKS efficaciously abrogated the CYP2E1 induction and CYP2E1-dependents hydroxylation of aniline as compared to the individual treatment at higher doses. These findings suggest that CKS may prevent ethanol-induced acute liver injury, possibly through its ability to block CYP2E1-mediated ethanol bioactivation and its free radical scavenging effects.

1. Introduction

Alcohol is one of the oldest drugs that humans have used since the beginning of civilization. High alcohol consumption results in critical problems in the body including alcohol liver diseases (ALD) (Pari and Karthikesan, 2007). ALD is the most common liver disease in Great Britain, causing over 20,000 deaths per year. Many pathways are thought to be involved in ALD, including oxidative stress and mitochondrial damage (Stewart et al., 2001). Ethanol is metabolized to acetaldehyde in the body by enzyme catalysis; acetaldehyde is further oxidized to acetate and then changed into carbon dioxide via the citric acid cycle (Rajagopal et al., 2003). Ethanol also affects the immune system and alters cytokine production, in turn increasing the levels of hepatic triglycerides and lipid peroxidation and decreasing hepatic glutathione (GSH) content. GSH acts as a free radical scavenger and a regenerator of α-tocopherol, and plays significant role in sustaining protein sulfhydryl groups (Wang and Cederbaum, 2007; Dey and Cederbaum, 2006). Decreased hepatic GSH content results in increased susceptibility for hepatic injury via induction of TNF-α (Fernandez-Checa et al., 1997; Colell et al., 2001). TNF-α is a critical factor in the progression of alcoholic liver disease because it induces cell death due to apoptosis and necrosis and stimulates the generation of toxic superoxide anion.

Oxidative stress is known to play an important role in the pathogenesis of ethanol-induced liver injury. Induction of CYP2E1 by ethanol is a key pathway, and the pathological changes in ethanol-induced liver injury are associated with CYP2E1 levels (Stewart et al., 2001). CYP2E1 expression levels are induced during alcohol-dependent metabolic changes in early liver injuries such as steatosis and steatohepatitis (Bondy, 1992; Tsukamoto et al., 1995). CYP2E1 is also well recognized for its role in the activation of many chemicals to toxic and carcinogenic agents (Guengerich et al., 1991; Koop, 1992; Eaton et al., 1995; Jeong and Park, 1998; Jeong, 1999). Natural compounds that reduce the enzymes that activate such chemicals could be considered good candidates for protection against chemically induced toxicities. Herbs have recently attracted attention as health-beneficial foods (physiologically functional foods) and as source materials for drug development. Herbal medicines derived from plant extracts are being utilized increasingly to treat a wide variety of clinical diseases, although relatively little is known regarding their modes of action.

Platycodi Radix is the root of *Platycodon grandiflorum* A. DC (Campanulaceae). It is used as a food additive and in traditional oriental medicines as a sedative and to treat various adult diseases (e.g., bronchitis, asthma and pulmonary tuberculosis) and inflammatory diseases (Lee, 1973; Kim et al., 1995). In our previous studies, aqueous extract from the roots of *P. grandiflorum* A. DC (Campanulaceae), Changkil (CK), exhibited protective effects against acetaminophen- and carbon tetrachloride-induced hepatotoxicity in mice and inhibited the progression of hepatic fibrosis in rats (Lee et al., 2001, 2004b; Lee and Jeong, 2002). In addition,

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doi:10.1016/j.fct.2008.12.009

saponins isolated from the root of *P. grandiflorum* (CKS) had potent antioxidant effects, such as superoxide radical scavenging activity, and inhibited reactive oxygen species (ROS) production by tert-butyl hydroperoxide in hepatocytes and in liver (Lee et al., 2004a). Although we previously reported that CKS prevented chemicals-induced hepatotoxicity and that CKS had *in vivo* and *in vitro* antioxidant effects, the physiological functions and the nature of the protective mechanisms of CKS in ethanol-induced liver injury are not clear. In the present study, we investigated the protective effect of CKS on ethanol-induced liver injury and also elucidated the mechanisms underlying its protective effects in mice.

2. Materials and methods

2.1. Chemicals

Ethanol, thiobarbituric acid (TBA), dithionitrobenzoic acid, L-(−)-ascorbic acid, phenylmethoxysulfonyl fluoride, reduced glutathione (GSH), Bradford solution, and diagnostic kits to measure serum alanine aminotransferase (ALT) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Antibodies against CYP2E1 and β-actin were purchased from Abcam (UK) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively. All other chemicals were of the highest commercial grade available.

2.2. Preparation of CKS

The CK used in this study was an aqueous extract obtained from the roots of *P. grandiflorum* (22 years old) and was supplied by Jang Saeng Doraji Co., Jinju, South Korea. The composition of the root of CK has been previously published (Kim et al., 1995, 2005). The composition of the root of CK were deapioplatycoside E, platycoside E, deapioplatycodin D3, platycodin D3, polygalacin D2, platycodin D2, deapioplatycodin D and platycodin D (Kim et al., 2005). CK was subjected to column chromatography over Amberlite XAD-2, Diaion MCl Gel HP20 or Kogel BG4600. After removing saccharides and amino acids from the column with water, the extract was eluted with methanol to obtain CKS, the saponin fraction of CK, as described previously (Tada et al., 1975).

2.3. Animals and treatments

Male C57BL/6 mice, weighing 23–25 g, were obtained from DAE HAN BIOLINK CO., LTD. (Chungbuk, Korea). The use of animals was in compliance with the guidelines established by the Animal Care Committee of Chosun University. Animals were acclimated to temperature (22 ± 2 °C) and humidity (55 ± 5%) controlled rooms with a 12-h light/dark cycle (light: 0700–1900, dark 1900–0700) for 1 week prior to use. Laboratory chow and tap water were allowed *ad libitum*. The binge drinking mouse model developed by Carson and Pruett (1996) was utilized for ethanol challenge. This model was layout to achieve blood alcohol levels, behavioral effects, and physiological changes comparable to those seen in human binge drinking. Mice were allocated for treatment with 0.5, 1, or 2 mg/kg CKS for 7 days before ethanol challenge until sacrifice. Ethanol diluted with normal saline (50%) was administered orally to mice at a dose of 5 g/kg every 12 h for a total of 3 doses. Mice were sacrificed 10 h after the final ethanol dose.

2.4. Serum biochemistry

ALT was measured to assess hepatotoxicity. The ALT activity was measured using spectrophotometric diagnostic kits (Sigma Chemical Co.) as previously described (Lee et al., 2001). Briefly, samples were centrifuged at 1000*g* for 10 min within 1 h after collection. The serum was stored in the −80 °C freezer before analysis. ALT activity in the serum was evaluated by a fluorescence reader (Varioskan, Thermo Electron Co.).

2.5. Hepatic TNF-α determination

TNF-α was measured in 100 mg of liver that was homogenized in 1 ml (1×) PBS. After incubation on ice for 30 min, samples were centrifuged twice at 15,000 r.p.m. for 15 min at 4 °C. The resulting supernatants were used for the assay. TNF-α levels were detected by a fluorescence reader using a mouse TNF-α ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA) and expressed as nmol per g of liver.

2.6. Histological examinations

Fresh liver tissues were trimmed to a thickness of 3 mm, placed in plastic cassettes and immersed in neutral buffered formalin for 24 h. Fixed tissues were processed routinely, and then embedded in paraffin, sectioned, deparaffinized, and rehydrated using standard techniques. The extent of ethanol-induced necrosis was evaluated by assessing the morphological changes in liver sections stained with hematoxylin and eosin. Tissue was stained with hematoxylin and eosin to assess inflammation, steatosis, and necrosis and was scored using the method described by Nanji et al. (1989). The number of cells containing fat was estimated as follows: steatosis <5%, 1; 25–50%, 2; 50–75%, 3; >75%, 4; areas of inflammation and necrosis were scored as 1 + for 1 foci per low magnification field or 2 + for 2 or more foci per field.

2.7. Determination of lipid peroxidation and GSH levels

Hepatic lipid peroxidation levels were determined by measuring a thiobarbituric acid reactive substance (TBARS) levels (Lee and Jeong, 2002). In brief, samples were mixed with TBA reagent consisting of 0.375% TBA and 15% trichloroacetic acid in 0.25 N hydrochloric acid. The reaction mixtures were placed in a boiling water bath for 30 min and then centrifuged at 2000*g* for 5 min. The absorbance of the supernatant was measured at 535 nm. Hepatic GSH levels were estimated by a colorimetric method using Ellman's reagent and glutathione reductase (Lee and Jeong, 2002). Samples were mixed with 0.1 M sodium phosphate buffer (pH 7.5) containing 5 μM EDTA, 0.6 mM 5,5-dithiol-bis (2-nitrobenzoic acid), 0.2 mM NADPH and glutathione reductase. The mixture was incubated for 2 min at room temperature. The absorbance of the product was measured at 412 nm. GSH content was determined from a standard curve generated with known concentrations of GSH.

2.8. Hepatic triglyceride content determination

For determination of total triglyceride content, 100 mg of liver was homogenized in 4 ml of chloroform:methanol (2:1). A total of 0.8 ml of 50 mM NaCl was added to each sample. Samples were then centrifuged and the organic layer was removed and dried. The resulting pellet was dissolved in phosphate-buffered saline containing 1% Triton X-100, and the triglyceride contents were determined using an enzymatic reagent kit (Sigma Chemical Co.).

2.9. CYP2E1 enzyme activity and expression

Livers were removed quickly, weighed, perfused with ice-cold 0.15 M KCl, and then homogenized in a Potter–Elvehjem homogenizer with 4 vol. (w/v) of 10 mM Tris–HCl (pH 7.4) containing 0.15 M KCl, 0.1 mM EDTA, 1.0 mM dithiothreitol and 0.01 mM phenylmethoxysulfonyl fluoride. Hepatic microsomal fractions were obtained by differential centrifugation as described previously (Lee et al., 2001). Microsomal fractions were used to determine CYP2E1-specific oxidative activities. Aniline hydroxylase activity was determined by measuring *p*-aminophenol formation as described previously (Dicker et al., 1990), and microsomal protein levels were determined using the Bradford method with bovine serum albumin as the standard (Bradford, 1976). CYP2E1 was detected immunochemically as in the previous study (Lee et al., 2008).

2.10. Statistical analysis

All experiments were performed in duplicate. Mean ± SD were calculated for each group and the Tukey–Kramer test was used to calculate statistical significance at the $p < 0.05$ level.

3. Results

3.1. Effect of CKS on ethanol-induced hepatotoxicity

The effects of pretreatment with CKS on the ethanol-induced elevation of serum ALT and hepatic TNF-α activity are shown in Figs. 1 and 2, respectively. Three doses of ethanol (5 g/kg) caused hepatotoxicity in mice, as indicated by the increases in serum ALT and hepatic TNF-α levels after ethanol administration. CKS pretreatment prevented the ethanol-induced elevation of serum ALT and hepatic TNF-α levels in a dose-dependent manner. Low doses of CKS (0.5 mg/kg) partially prevented the elevation of serum ALT and hepatic TNF-α levels, and medium (1 mg/kg) or higher doses of CKS (2 mg/kg) almost completely prevented hepatotoxicity. Liver histology was normal (Fig. 3A) except for the presence of faint microvesicular steatosis in the ethanol-treated group (Fig. 3B). Acute ethanol exposure caused degenerative morphological changes in the liver. These alcohol-induced hepatic pathological changes were significantly inhibited in CKS-pretreated mice (Fig. 3C).

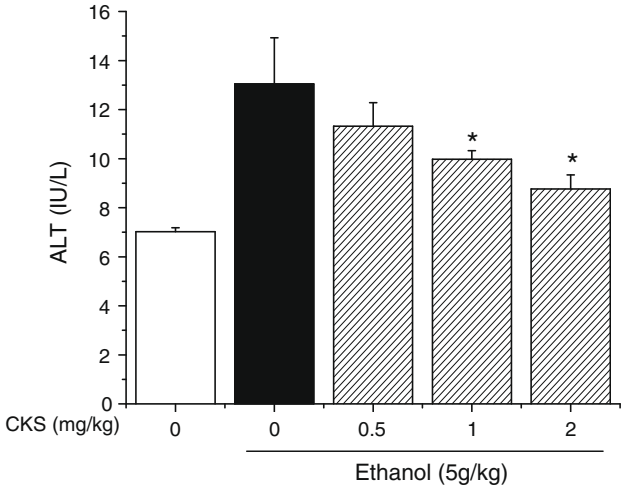

Fig. 1. Effects of CKS on ALT activity. Mice were pretreated with CKS (0.5, 1 or 2 mg/kg, i.g.) once daily for 7 consecutive days. Control mice were given saline. After 7 days, ethanol diluted with normal saline was administered orally to mice at a dose of 5 g/kg every 12 h for a total of 3 doses. Hepatotoxicity was determined 10 h later by quantifying the serum activities of alanine aminotransferase (ALT). Values are expressed as mean ± SD for four mice. *, Significantly different from ethanol at $p < 0.05$.

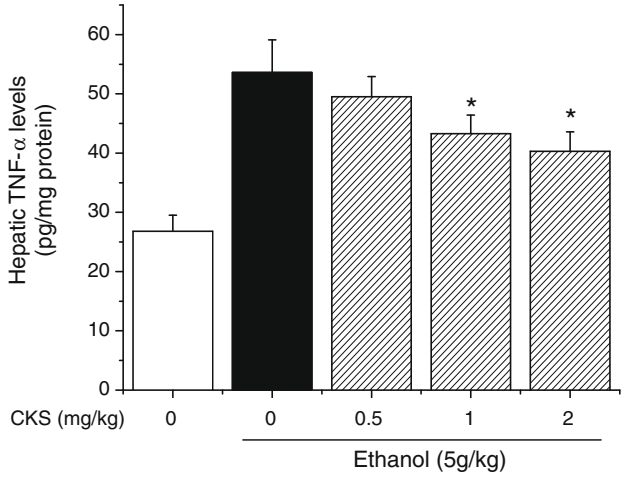

Fig. 2. Effects of CKS on hepatic TNF-α level. Mice were pretreated with CKS (0.5, 1 or 2 mg/kg, i.g.) once daily for 7 consecutive days. Control mice were given saline. After 7 days, ethanol diluted with normal saline was administered orally to mice at a dose of 5 g/kg every 12 h for a total of 3 doses. Mice were sacrificed 10 h after the final ethanol administration. Hepatic TNF-α was measured as described in Section 2. Values are expressed as mean ± SD for four mice. *, Significantly different from ethanol at $p < 0.05$.

3.2. Effects of CKS on hepatic lipid peroxidation

Ethanol was administered to two groups of mice, with or without CKS pretreatment, and livers were removed and homogenized 10 h after the last dose of ethanol. In order to evaluate the effect of CKS pretreatment on ethanol-induced liver lipid peroxidation, we monitored the levels of MDA, an indicator of oxidative damage and one of the principal products of lipid peroxidation. MDA concentration in liver homogenate was determined using the thiobarbituric acid method. As shown in Fig. 4, MDA production in the ethanol-treated group increased 3-fold compared to the control. Consistent with the serum levels of ALT and hepatic TNF-α, CKS pretreatment significantly decreased the ethanol-induced hepatic lipid peroxidation in a dose-dependent manner.

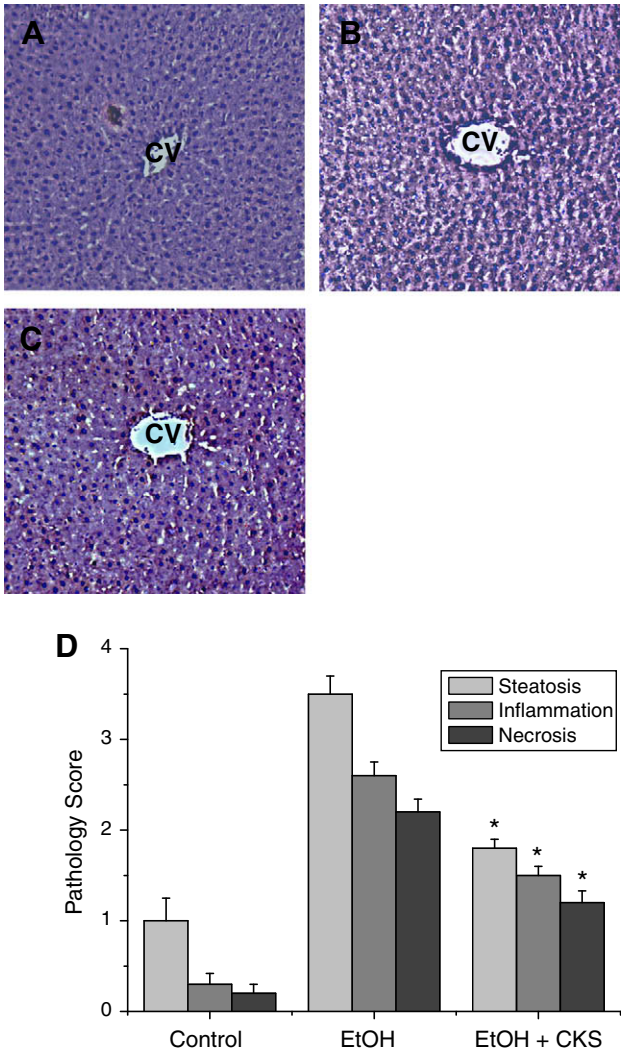

Fig. 3. Effects of CKS on histopathological changes in the liver. Mice were pretreated with CKS (2 mg/kg, i.g.) once daily for 7 consecutive days. Control mice were given saline. After 7 days, ethanol diluted with normal saline was administered orally to mice at a dose of 5 g/kg every 12 h for a total of 3 doses. Mice were sacrificed 10 h after the final ethanol administration. Livers were harvested, formalin fixed, and stained with hematoxylin–eosin for evaluation of pathological changes due to feeding of saline (A), ethanol (B), or ethanol plus CKS (2 mg/kg, i.g.) (C). Microphotographs show views of the liver sections: central vein (CV). X100. (D) Pathology was scored as described in Section 2. Values are expressed as mean ± SD for four mice. *, Significantly different from ethanol at $p < 0.05$.

3.3. Effects of CKS on hepatic GSH levels

Ethanol-treated mice exhibited significant decreases in hepatic GSH concentrations compared to control mice. Pretreatment with CKS significantly and dose-dependently protected against the GSH depletion produced by ethanol (Fig. 5).

3.4. Effects of CKS on hepatic triglyceride levels

Ethanol administration induced significant accumulation of triglyceride in the liver; however, this accumulation was attenuated by CKS (Fig. 6). These results indicate that CKS at doses of 0.5, 1 or 2 mg/kg might be effective against alcoholic steatosis.

3.5. Effects of CKS on CYP2E1 activity and protein expression

In mice, CKS pretreatment resulted in a dose-dependent protective effect against ethanol-induced hepatotoxicity. By immunoblot

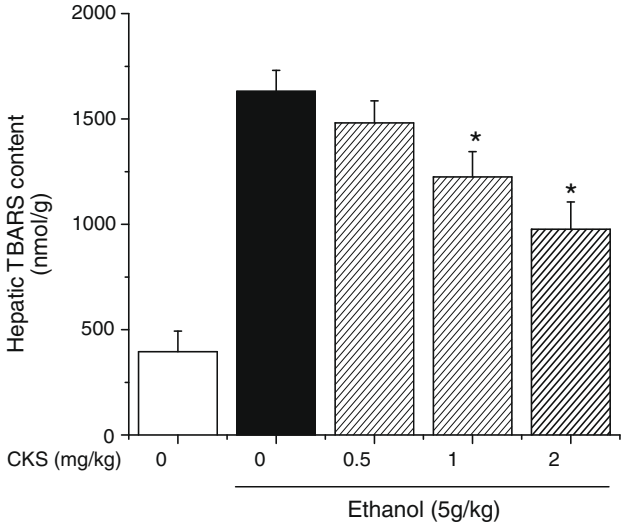

Fig. 4. Effects of CKS on hepatic lipid peroxidation. Mice were pretreated with CKS (0.5, 1 or 2 mg/kg, i.g.) once daily for 7 consecutive days. Control mice were given saline. After 7 days, ethanol diluted with normal saline (50%) was administered orally to mice at a dose of 5 g/kg every 12 h for a total of 3 doses. Mice were sacrificed 10 h after the final ethanol administration. Hepatic lipid peroxidation was measured as described in Section 2. Values are expressed as mean ± SD for four mice. ∗, Significantly different from ethanol at $p < 0.05$.

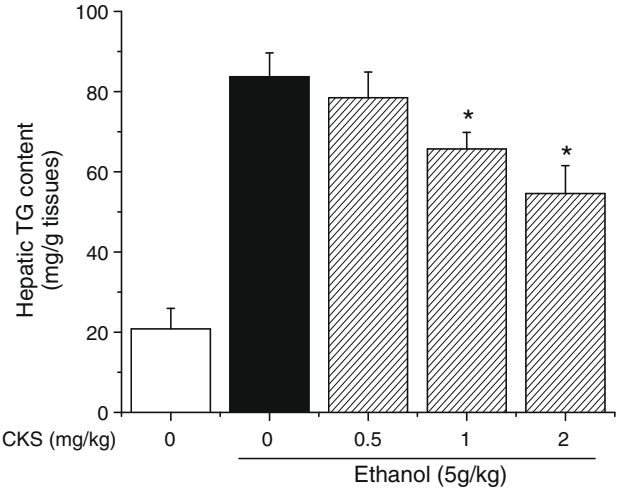

Fig. 6. Effects of CKS on hepatic triglyceride content. Mice were pretreated with CKS (0.5, 1 or 2 mg/kg, i.g.) once daily for 7 consecutive days. Control mice were given saline. After 7 days, ethanol diluted with normal saline was administered orally to mice at a dose of 5 g/kg every 12 h for a total of 3 doses. Mice were sacrificed 10 h after the final ethanol administration. Hepatic triglyceride content was measured as described in Section 2. Values are expressed as mean ± SD for four mice. ∗, Significantly different from ethanol at $p < 0.05$.

analysis, we examined the effects of CKS on CYP2E1 protein expression. Hepatic microsomes from CKS-treated mice were resolved by SDS-PAGE and immunoblotted with anti-CYP2E1 antibodies. As shown in Fig. 7A, ethanol administration increased the CYP2E1 content by 2.5-fold compared to control at 10 h after the final dosing. CKS intake for 7 days did not affect the expression of Cyp2E1 (data not shown). But the elevation of CYP2E1 expression in alcoholic liver was inhibited significantly by CKS in a dose-dependent manner. Therefore, we examined the effects of CKS on the hepatic microsomal CYP2E1-specific monooxygenase activities. As shown in Fig. 7B, hepatic microsomal fractions from mice treated with CKS showed significant decreases in hydroxylation of the CYP2E1-specific substrate, aniline. The inhibitory

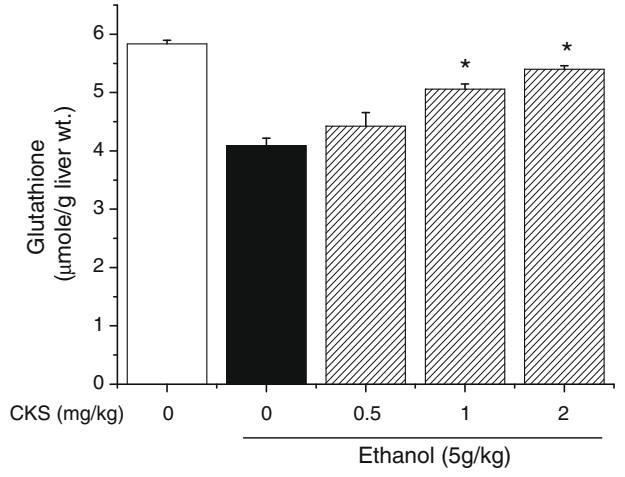

Fig. 5. Effects of CKS on hepatic GSH concentrations. Mice were pretreated with CKS (0.5, 1 or 2 mg/kg, i.g.) once daily for 7 consecutive days. Control mice were given saline. After 7 days, ethanol diluted with normal saline (50%) was administered orally to mice at a dose of 5 g/kg every 12 h for a total of 3 doses. Mice were sacrificed 10 h after the final ethanol administration. Hepatic glutathione was measured as described in Section 2. Values are expressed as mean ± SD for four mice. ∗, Significantly different from ethanol at $p < 0.05$.

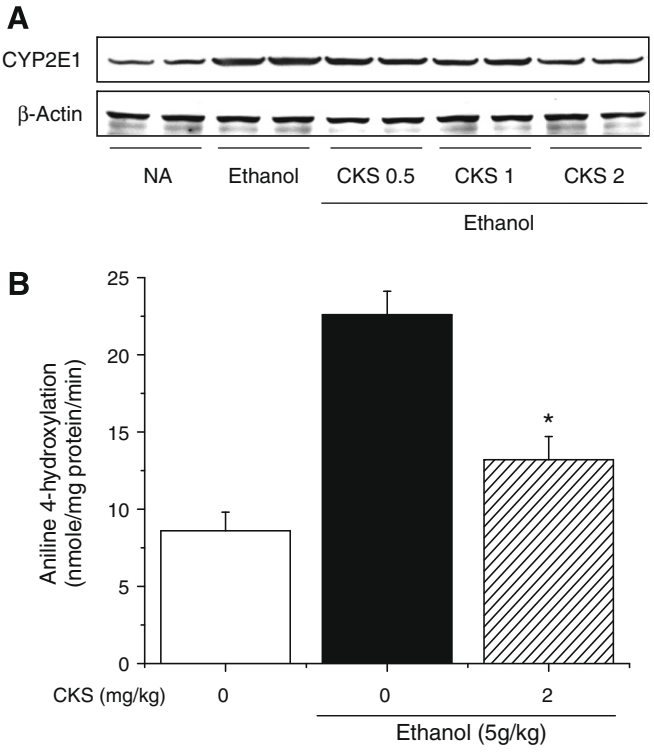

Fig. 7. Effects of CKS on CYP2E1 protein expression and activity. (A) Liver microsomes were obtained from mice pretreated with CKS (0.5, 1 or 2 mg/kg, i.g.) once daily for 7 consecutive days. Control mice were given saline. After 7 days, ethanol diluted with normal saline was administered orally to mice at a dose of 5 g/kg every 12 h for a total of 3 doses. Mice were sacrificed 10 h after the final ethanol administration. The liver CYP2E1 protein levels were determined by immunoblotting of each liver protein sample with specific antibodies. β-actin protein was used as a loading control. (B) CYP2E1 enzyme activity was determined as described in Section 2. Values are expressed as mean ± SD for four mice. ∗, Significantly different from ethanol at $p < 0.05$.

activity of CKS on hepatic microsomal CYP2E1-specific monooxygenase activity was confirmed in pyridine-induced hepatic

microsomal incubations. The results suggest that the suppression of CYP2E1 by CKS in mice is an important aspect of the hepatoprotective effect of CKS against ethanol.

4. Discussion

Ethanol-induced liver damage progresses through alcoholic fatty liver, alcoholic hepatitis, fibrosis and cirrhosis. It is well-established that ethanol susceptibility inhibits the mitochondrial electron transport chain, resulting in increased ROS production (Song et al., 2008). Liver damage in animals due to ingestion of ethanol is a well known phenomenon. One sign of hepatic injury is the leakage of cellular enzymes into plasma (Baldi et al., 1993). In the current experiments, we used CKS in an *in vivo* animal model of acute binge drinking and acute EtOH toxicity. Our results showed that supplementation of CKS attenuated acute EtOH-induced liver injury.

Increased levels of serum enzymes such as ALT and hepatic TNF-α have been observed in alcohol-treated mice, indicating increased permeability, damage and necrosis of hepatocytes (Goldberg and Watts, 1965). Ethanol also affects the immune system and alters cytokine production, which in turn increases the levels of ALT and TNF-α. GSH acts as a free radical scavenger and regenerator of α-tocopherol and plays a significant role in sustaining protein sulfhydryl groups (Wang and Cederbaum, 2007; Dey and Cederbaum, 2006). Decreased hepatic GSH contents result in increased susceptibility to hepatic injury via induction of TNF-α (Colell et al., 2001). TNF-α is a critical factor in the progression of alcoholic liver disease because it induces cell death due to steatosis and necrosis and stimulates the generation of toxic superoxide anion. Our results show that CKS pretreatment significantly inhibited the ethanol-induced ALT activity. Also CKS pretreatment decreased the ethanol-induced TNF-α level.

Reduced GSH is the main antioxidant found in liver cells and plays a protective role in the metabolism of a large number of toxic agents, including ethanol. Many studies assessing the status of hepatic GSH in response to ethanol exposure have shown that both acute and chronic exposure to ethanol cause time-dependent and dose-dependent decreases in hepatic GSH content. Enhanced ethanol toxicity has been associated with the GSH decrease, which may reflect the consumption of GSH by the overproduction of ROS and subsequent oxidative stress caused by ethanol (Fernandez-Checa et al., 1998; Iimuro et al., 2000). The results from our study also confirmed previous studies (Song et al., 2003, 2006). Acute ethanol administration significantly decreased the hepatic GSH level. Our results showed that CKS pretreatment significantly inhibited the ethanol-induced depletion of hepatic GSH. However, CKS pretreatment alone did not affect hepatic GSH levels (data not shown). These findings suggest that the hepatoprotective effect of CKS against ethanol is related to the increase in cellular GSH content. Maximal depletion of GSH by ethanol occurs at 4–6 h; by 10 h, GSH levels have partially recovered, although not to control levels (Nishida et al., 1996). In addition, our results showed that CKS dramatically prevented the ethanol-induced elevation of inflammatory infiltrates in histopathological analysis. We have already reported that CKS exerts a suppressive effect on inflammatory cytokines (Kim et al., 2006). These anti-inflammatory effects of CKS may play an important role in protecting against ethanol-induced hepatotoxicity.

Lipid peroxidation is accepted as one of the principal causes of ethanol-induced liver injury mediated by the production of free radical derivatives of ethanol. Acute ethanol administration has often been studied in both animal models and in human clinical trials to assess oxidative stress associated with increases in hepatic lipid peroxidation (Meagher et al., 1999; Zhou et al., 2002). Our results showed that CKS pretreatment significantly decreased the ethanol-induced lipid peroxidation.

CYP2E1 plays a critical role in the metabolism of many carcinogens, including nitrosamines, which require metabolic activation to exert their carcinogenic effect (Yang et al., 1990; Yoo et al., 1990; Guengerich et al., 1991). CYP2E1 can generate ROS during its catalytic circle, and CYP2E1 levels are increased by acute treatment with ethanol. CYP2E1 has been suggested to be a major contributor to ethanol-induced oxidative stress and to ethanol-induced liver injury. CYP2E1 catalyzes the metabolic conversion of small organic molecules to reactive intermediates, which are frequently capable of covalently binding to tissue macromolecules and thereby cause liver injuries. Initial suggestions of a role for CYP2E1 in alcoholic liver injury came from studies in the intragastric model of ethanol feeding in which prominent CYP2E1 induction occurs (Lu and Cederbaum, 2008). It has been accepted that a major mechanism of CYP2E1 induction by ethanol is post-transcriptional, which protects CYP2E1 from its rapid degradation via proteolytic pathways (Roberts et al., 1995). In view of the importance of CYP2E1 for the bioactivation of toxicants, the selective inhibition of CYP2E1 may represent protective effects against toxic injuries and possibly fat accumulation. The results of the present study indicate that CKS administration itself did not affect the CYP2E1 expression. But CKS supplementation depressed the ethanol-induced elevation of CYP2E1 in this study, which may have a role in the inhibition of lipid peroxidation and liver injury. The mechanism of inhibition of the ethanol-induced CYP2E1 elevation by CKS warrants further studies.

In conclusion, the present results indicate that CKS supplementation may antagonize the development of oxidative liver injury induced by acute ethanol exposure. CKS supplementation significantly attenuated the ethanol-induced liver injury. In association with the hepatocyte injury, acute ethanol administration induced marked decreases in hepatic GSH levels along with enhanced lipid peroxidation. CKS supplementation attenuated hepatic GSH depletion following acute ethanol exposure and inhibited ethanol-associated lipid peroxidation. Furthermore, CKS supplementation prevented the acute ethanol-induced enhancement of hepatic TNF-α production. Also, CKS supplementation depressed the ethanol-induced elevation of CYP2E1 expression. The hepatoprotective effects of CKS may be due to its ability to block the bioactivation of ethanol by inhibiting CYP2E1 activity, in combination with its ability to scavenge free radicals. Taken together, our present study suggests that CKS may be an effective therapeutic agent in ethanol-induced liver injuries.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors are grateful to Jangsaeng Doraji Co. Ltd., Jinju, South Korea for providing the Changkil, aqueous extract from the root of *P. grandiflorum*.

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