Research Article
J Ginseng Res Vol. 37, No. 1, 37-44 (2013)
http://dx.doi.org/10.5142/jgr.2013.37.37

JGR Journal of Ginseng Research

Protective effects of Korean red ginseng extract on cadmium-induced hepatic toxicity in rats

Sook Jahr Park[1,2], Jong Rok Lee[3], Mi Jeong Jo[1,2], Sang Mi Park[1,2], Sae Kwang Ku[1,2], and Sang Chan Kim[1,2*]

[1]College of Oriental Medicine, Daegu Haany University, Gyeongsan 712-715, Korea
[2]Medical Research Center for Globalization of Herbal Formulation, Daegu Haany University, Gyeongsan 712-715, Korea
[3]Department of Pharmaceutical Engineering, Daegu Haany University, Gyeongsan 712-715, Korea

Korean red ginseng is known to regulate the immune system and help the body struggle infection and disease. Cadmium is widely distributed in the environment due to its use in industry. Exposure to cadmium is problematic causing organ dysfunction. This study was conducted to evaluate the protective effect of Korean red ginseng extract (RGE) against cadmium-induced hepatotoxicity in rats. In experiments, animals were orally administrated with RGE (25, 50 mg/kg) for 7 d and then intravenously injected with cadmium ($CdCl_2$, 4 mg/kg) to induce acute hepatotoxicity. Cadmium caused the elevated levels of alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase in serum. In contrast, pretreatment with RGE significantly reduced those serum indexes related with liver damage. In histopathological analysis, RGE decreased the centrilobular necrosis around central veins and the peripheral hemorrhage around portal triads. Moreover, RGE restored the deficit in hepatic glutathione level resulting from cadmium treatment. RGE also inhibited the increase in the expression of Bad, a representative apoptosis marker protein, induced by cadmium treatment. Collectively, these results demonstrate that RGE can reduce the cadmium-induced hepatic toxicity, partly via anti-oxidative and anti-apoptotic process.

Keywords: *Panax ginseng,* Korean red ginseng, Cadmium, Hepatic toxicity, Hepatoprotection

INTRODUCTION

Cadmium (Cd) is one of the widely used heavy metals and implicated in many industrial applications like electric batteries, electronic components, pigment, and fertilizer [1]. On the other hand, Cd is an environmental pollutant that is highly toxic to all living organisms. Heavy metals like Cd become toxic when they are not metabolized and can accumulate in most human organs [2,3]. Acute and chronic exposure to Cd in experimental animals causes accumulation of the metal in the liver resulting in hepatotoxicity [4]. The various toxic effects of Cd may be due to increased oxidative stress including lipid peroxidation, depletion of glutathione (GSH), and generation of reactive oxygen species (ROS) [5-7]. It has been also reported that the oxidative stress by Cd exposure may contribute to hepatocellular necrosis and apoptosis [8-10].

Korean red ginseng (*Panax ginseng* Meyer) is a traditional herbal medicine that has been used for maintenance and improvement of human health in Asian countries as well as Korea [11,12]. Red ginseng is prepared through steaming and drying processes from raw ginseng that has been cultivated for 6 yr. Korean red ginseng has

Received 08 May 2012, **Revised** 11 Jul. 2012, **Accepted** 29 Aug. 2012

***Corresponding author**
E-mail: sckim@dhu.ac.kr
Tel: +82-53-819-1863, Fax: +82-2-53-819-1860

been shown to posses more health-related benefits than fresh raw ginseng [13]. It has been reported that the enhanced efficacy of red ginseng is due to production of several therapeutic constituents that are not present in raw ginseng [14]. Ginseng saponins, also known as ginsenosides, are major active compounds found in Korean red ginseng, and it has been well elucidated that ginsenoside may attribute to main pharmacological activity of Korean red ginseng [15,16]. Many studies demonstrated that Korean red ginseng has the pharmacological properties including anti-diabetes [17], anti-oxidants [18], anti-carcinogens [19], anti-aging [20], and immunomodulation [21].

Based on knowledge and information mentioned above, our research interest was focused on the study of heavy metal detoxification by red ginseng extract (RGE) in Cd-treated rats. In the present study, we evaluated hepatoprotective effect of RGE in cadmium treated rats. For this purpose, serum levels of hepatic marker enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST], and lactate dehydrogenase [LDH]) were measured, and histopathological analysis were also conducted. The hepatic oxidant-antioxidant status was assessed by measuring GSH level. Mitochondrial Bad protein that is one of proapoptotic marker proteins was analyzed by Western blot.

MATERIALS AND METHODS

Samples

RGE was kindly supplied by the Korea Ginseng Corporation (Daejon, Korea).

Experimental animals

Rat study was conducted in accordance with the institutional guidelines for the care and use of laboratory animals. Six-week old Sprague-Dawley rats (140 to 160 g) were provided from Hyochang Science (Daegu, Korea), and acclimatized for 1 wk. Rats were caged in an atmosphere of filtered, pathogen-free air, provided with commercial rat chow (Purina, Seoul, Korea) and water *ad libitum*, and maintained at a temperature between 20°C and 23°C with a 12 h light/dark cycle and relative humidity of 50%. To evaluate the hepatoprotective effect of RGE, rats were administrated with vehicle (tap water) or RGE (25, 50 mg/kg) for 7 d, and subsequently exposed to single injection of Cd at 24 h after the last RGE/vehicle treatment. Cd ($CdCl_2$, 4 mg/kg body weight) was dissolved in normal saline, and intravenously injected into the rats to induce acute liver injury. Tissue and blood samples were obtained at 24 h after Cd exposure.

Blood chemistry

ALT, AST, and LDH in serum were analyzed using Photometer 5010, an automatic blood chemistry analyzer (Robert Riele GmbH & Co KG, Berlin, Germany).

Histopathology

The left lateral lobe of the liver was sliced (three slices per rat), and tissue slices were fixed in 10% neutral buffered formalin for 6 h, embedded in a paraplast automatic tissue processor (Shandon Scientific, Cheshire, UK), sectioned (4 μm) and stained with H&E stain. The percentage of the degenerative liver region showing centrilobular necrosis and peripheral hemorrhage were calculated using image analysis (SIS, Munster, Germany) under microscopic examination at 50 magnifications (Zeiss, Jena, Germany) with the result expressed as %/mm^2 of hepatic parenchyma. In addition, the number of degenerative cells showing vacuolation or any necrotic process was also calculated using automated image analysis under microscopic examination at 200 magnifications as *N*/1000 hepatic cells.

Measurement of hepatic glutathione

Hepatic GSH was estimated using commercial assay kit (CS1020; Sigma, St. Louis, MO, USA) according to the manufacturer's instructions.

Mitochondria isolation and Western blot analysis

Mitochondria isolation from liver tissue was conducted using a mitochondria isolation kit (Pierce, Rockford, IL, USA) according to the manufacturer's instructions. Briefly, 150 to 200 mg of liver tissue in 800 μL of phosphate-buffered saline was subjected to Dounce homogenization on ice, and then centrifuged at 1,000 ×*g* for 3 min. The pellet was suspended with the mitochondria isolation reagent provided in the kit and centrifuged at 700 ×*g* for 10 min at 4°C. After centrifugation, the supernatant was transferred into new tube and centrifuged again at 3,000 ×*g* for 15 min at 4°C. The precipitated mitochondrial pellet was used for Western blot analysis. Equal amounts (30 μg) of proteins were resolved on 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred on NC membrane (Schleicher & Schuell GmbH, Dassel, Germany). The membrane was incubated with anti-mouse Bad antibody or anti-goat actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), washed with TBST (10 mM Tris-Cl pH 7.5, 150 mM NaCl, 0.05% Tween 20) and incubated for 1 h with the

appropriate peroxidase conjugated secondary antibodies. Bands corresponding to Bad and actin were visualized using enhanced chemiluminescence western blotting detection reagents (Amersham Biosciences, Piscataway, NJ, USA).

Protein quantitation

The proteins of liver mitochondrial fraction were estimated using the bicinchoninic acid (BCA) method with the BCA protein assay kit (Pierce).

Data analysis

A one-way ANOVA followed by a least significant difference test (SPSS ver. 18; SPSS Inc., Chicago, IL, USA) was used to test the difference among the treatment groups. The criterion for statistical significance was set at $p<0.05$ or $p<0.01$.

RESULTS

Effects of Korean red ginseng extract on serum hepatic marker enzymes

Abnormal increase in serum hepatic marker enzymes such as AST, ALT, and LDH indicates liver damage. Thus, we checked the levels of AST, ALT, and LDH in serum to evaluate the hepatoprotective effect of RGE in Cd treated rats. Intravenous injection of Cd resulted in a significant ($p<0.01$) elevation in the levels of those hepatic enzymes. Administration of RGE (25, 50 mg/kg, per os) for 7 d prior to Cd treatment significantly decreased the levels of ALT, AST, and LDH as compared to Cd alone groups (Fig. 1). The serum levels of ALT, AST, and LDH after Cd treatment were 1,113.8±277.2, 983.6±114.1, and 2,654.4±835.5 IU/L, respectively. However, RGE showed significant ($p<0.01$) inhibitory effects on the serum ALT, AST, and LDH with a value of 124.4±52.5, 193.2±52.0, and 469.6±126.3 IU/L respectively in the concentration of 50 mg/kg. These results indicate RGE possess potent hepatoprotective effect against Cd induced toxicity.

Effect of Korean red ginseng extract on histopathological changes

The liver injuries of Cd treated rats were confirmed by histopathological examinations. Fig. 2. illustrates histological pattern of hematoxylin-eosin stained liver tissues. In case of control, hepatocytes had normal architecture. On the contrary to control, Cd treatment caused alterations in liver histoarchitecture as evidenced by centrilobular necrosis around central veins and peripheral

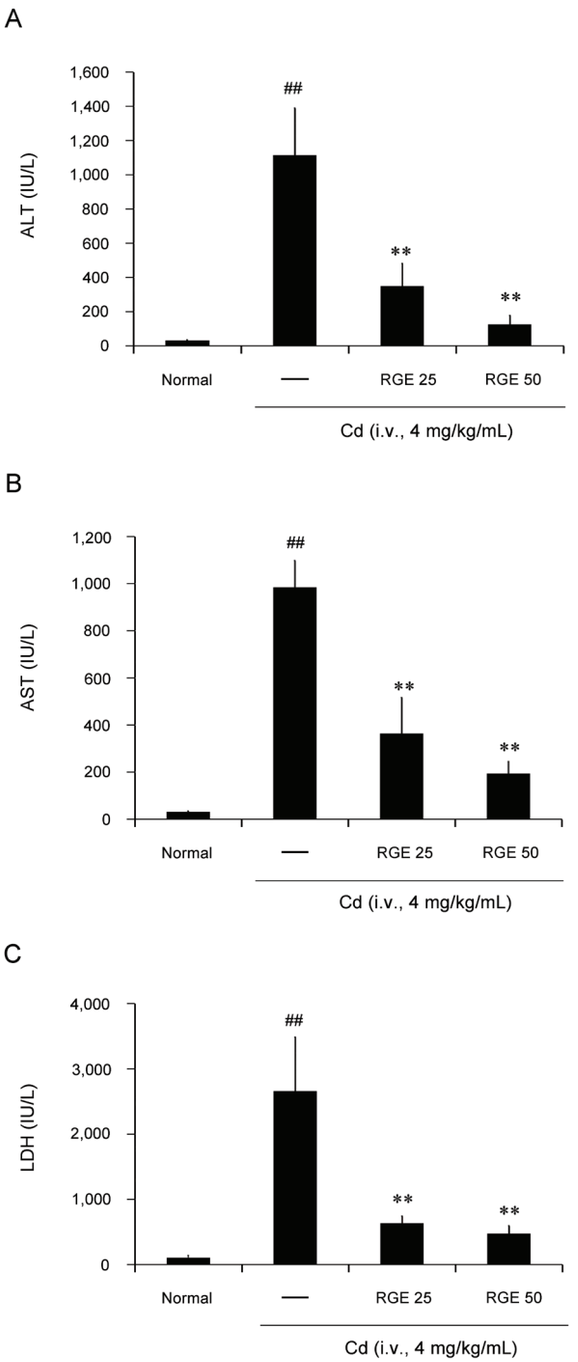

Fig. 1. Inhibitory effects of red ginseng extract (RGE) on cadmium (Cd)-induced elevation of hepatic enzymes. Alanine aminotransferase (ALT, A), aspartate aminotransferase (AST, B) and lactate dehydrogenase (LDH, C) were monitored in the serum of rats that had been orally pretreated with RGE (25, 50 mg/kg body weight for 7 d) and exposed to single injection of Cd (i.v., 4 mg/kg body weight) at 24 h after the last RGE treatment. Blood samples were obtained at 24 h after Cd treatment. Values represent the mean±SE from the 6 rats in each group. $^{\#\#}p<0.01$, significantly different from normal; $^{**}p<0.01$, significantly different from Cd alone. i.v., intravenous.

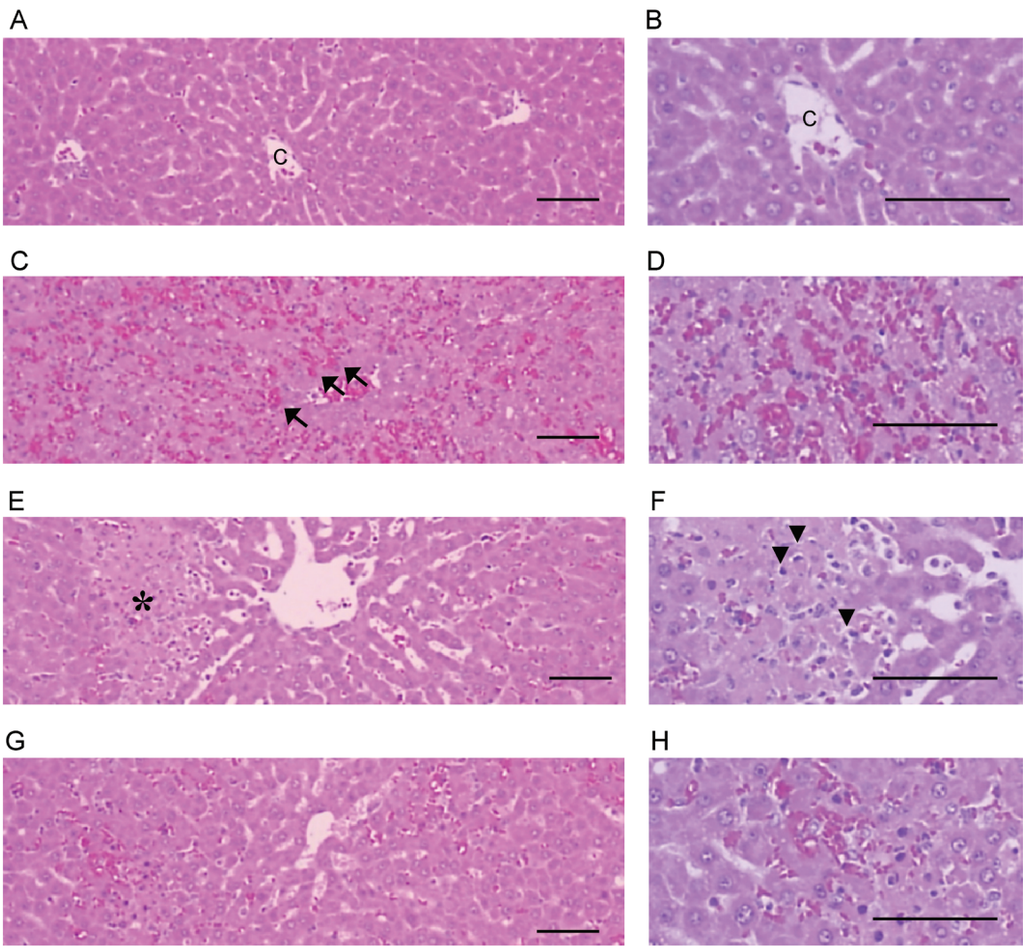

Fig. 2. Histological profiles of the liver from normal and experimental rats. Rats were treated as described in Fig. 1. The liver sections from healthy normal rats (A,B), and rats treated with cadmium (Cd) alone (C,D), Cd+red ginseng extract (RGE, 25 mg/kg) (E,F) and Cd+RGE (50 mg/kg) (G,H) were stained with H&E (×100 or ×200). C, central vein. Scale bars = 160 μm. Asterisk = focal necrotic area. Arrows = congestion of sinusoids. Arrowheads = inflammatory cells infiltrated.

Table 1. Changes on the histomorphometrical analysis of the liver from normal and experimental rats

Group	Percentages of degenerative regions (%/mm^2 of hepatic parenchyma)	Numbers of inflammatory cells infiltrated (cells/1000 hepatocytes)
Normal	2.67±4.09	14.40±11.28
Cd alone	65.48±17.18$^{\#\#}$	645.80±179.33$^{\#\#}$
Cd+RGE (25 mg/kg)	37.84±8.05**	353.80±126.46**
Cd+RGE (50 mg/kg)	25.92±6.33**	286.80±96.45**

Values represent the mean±SE (n=6).
Cd, cadmium; RGE, red ginseng extract.
$^{\#\#}p$<0.01, significantly different from normal; $^{**}p$<0.01, significantly different from Cd alone.

hemorrhage around portal triads. However, the severity of Cd-induced liver intoxication was reduced by the both of two different dosages (25, 50 mg/kg) of RGE (Fig. 2). Furthermore, pretreatment of RGE significantly (p<0.01 or p<0.05) reduced the abnormal increase in the percentages of degenerative regions and numbers of inflammatory cells by the Cd treatment (Table 1). These findings are considered as direct evidence that RGE have favorable hepatoprotective effect against Cd intoxication.

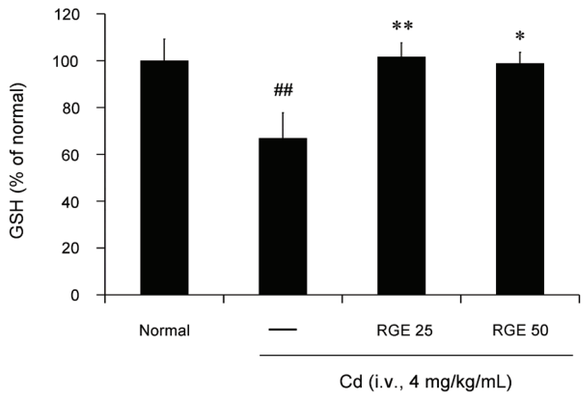

Fig. 3. Effect of red ginseng extract (RGE) on cadmium-induced depletion in hepatic glutathione (GSH) levels. Hepatic GSH content was determined fluorimetrically as described in Materials and Methods section. Values represent the mean±SE from the 6 rats in each group. $^{\#\#}p<0.01$, significantly different from normal; $^{*}p<0.05$, $^{**}p<0.01$, significantly different from cadmium (Cd) alone. i.v., intravenous.

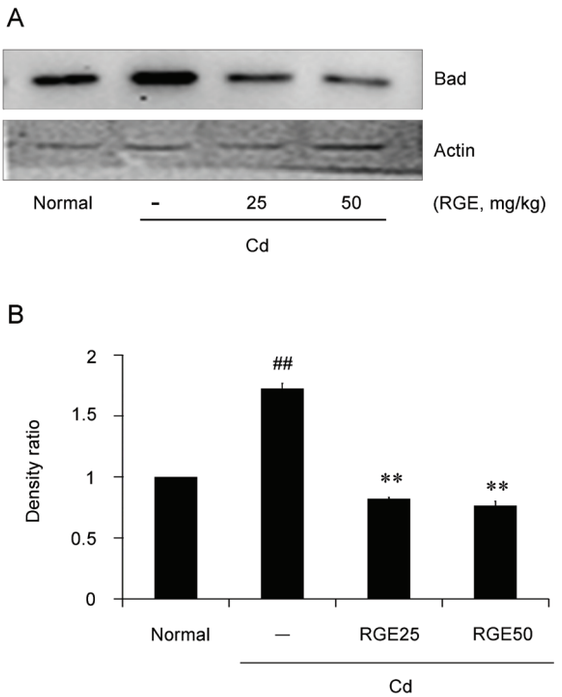

Fig. 4. Effect of red ginseng extract (RGE) on the level of mitochondrial Bad protein in the liver of rats with acute hepatotoxicity induced by cadmium (Cd). (A) Expression of Bad protein was determined by immunoblot analysis using specific antibody. Actin was used as a loading control. (B) The relative density levels of protein bands were measured by scanning densitometry. Data are shown as mean band density normalized to actin. $^{\#\#}p<0.01$, significantly different from normal; $^{**}p<0.01$, significantly different from Cd alone.

Effect of Korean red ginseng extract on hepatic glutathione level and the expression of mitochondrial Bad protein

It is well known that GSH antioxidant defense system plays major roles in various detoxification mechanisms [7]. To demonstrate that the hepatoprotection by RGE is derived from its antioxidant action, we evaluated the effect of RGE on Cd induced depletion of GSH level in liver tissues. Cd treatment caused significant ($p<0.01$) decrease of hepatic GSH level in comparison of normal group. However, pretreatment with 25 and 50 mg/kg of RGE was significantly ($p<0.01$ or $p<0.05$) elevated the hepatic GSH level toward normal (Fig. 3).

Apoptosis is one of mechanisms of hepatotoxicity. In response to apoptotic stimuli, Bad protein migrates to the mitochondria and participates in inducing apoptotic cell death [8,9]. Previously we reported that licorice with hepatoprotective activity reduced expression of mitochondrial Bad protein in Cd intoxicated rats. In this study, we also found that Cd caused increase in expression of mitochondrial Bad protein. However, the bands of Bad protein were markedly disappeared after treatment of 25 and 50 mg/kg of RGE (Fig. 4A). Density values of Cd alone, 25, and 50 mg/kg of RGE were 1.73±0.04, 0.82±0.01, and 0.77±0.03 respectively, when the density value of normal set 1.00 (Fig. 4B).

DISCUSSION

There are approximately 35 heavy metals in our environment, and Cd is one of the most notorious heavy metals that affect human health. Acute exposure to Cd can cause dysuria, polyuria, chest pain, fatigue and headache [22]. Chronic intake of Cd in contaminated food or air is able to produce organ dysfunction as a result of cell death, resulting in pulmonary, hepatic and renal tubular diseases [23].

Liver disorders can be induced by various toxicants including chemical drugs. These toxicants cause liver inflammation and tissue damage, which could finally lead to hepatocellular carcinoma [24,25]. Heavy metals including Cd are also potent liver toxicants, and Cd was reported to exert toxic effects on both acute and chronic hepatic injuries [2,4]. Acute hepatotoxicity induced by Cd is considered to involve two pathways, one for the initial injury produced by direct effects of cadmium and the other for the subsequent injury produced by inflammation [6]. Primary injury is caused by direct toxic effects of the metal, the binding of Ca^{2+} to sulfhydryl groups on critical molecules in mitochondria. Secondary injury from acute exposure of cadmium is thought to be due to the activation of Kupffer cells, which release

a number of inflammatory mediators such as cytokines, chemokines and cytotoxic molecules.

Liver injuries as a result of Cd exposure are characterized by the elevated levels of hepatic marker enzymes in serum. Increment of serum ALT and AST levels is the crucial parameters to detect liver damage [26]. LDH is another index of cell and tissue damage by various toxicants and the increased level of LDH was substantially detected in the experimental animals after exposure of Cd [27]. The present study showed that RGE possess hepatoprotective effect, as evidenced by the significant inhibition in the elevated levels of serum ALT, AST, and LDH induced by Cd.

Histopathological examination conducted in this study also revealed that RGE treatment resulted in significant reduction of hepatic injuries. Generally, liver tissue damages in Cd-intoxicated rats were characterized by centrilobular necrosis and peripheral hemorrhage [28-30]. In the present study, RGE inhibited the severe centrilobular necrosis around central veins and peripheral hemorrhage around portal triads, and reduced the percentage of degenerative hepatic regions (%/mm^2 hepatic parenchyma) and the number of degenerative hepatic cells (N/100 hepatic cells) induced by Cd. The reduction of histopathological changes with RGE treatment provides another evidence of its hepatoprotective activity.

Oxidative stress is a major reason of hepatotoxicity, and the impairment of antioxidant defense system is considered as critical event in Cd induced toxicity [6,7]. It has been reported that Cd induces the generation of ROS and the exhaustion of GSH leading to oxidative stress in hepatocytes. Several studies has been hypothesized that GSH formed complex with Cd for excretion and also scavenged the free radicals produced by Cd, indicating that decreased GSH level may be due to its consumption in Cd detoxification [7,31]. In line with these published reports, we found that drastic depletion of hepatic GSH came from Cd administration in the present study. Rats pretreated with RGE were protected against this acute action of Cd, recovering decreased GSH level toward normal. Our findings suggest that one of the mechanisms underlying the hepatoprotective action of RGE may be associated with its antioxidant properties.

Oxidative stress eventually contributes to the hepatocellular apoptosis and necrosis. Many studies have demonstrated that intracellular GSH depletion and ROS generation by Cd are associated with apoptotic cell death [7-10]. We also founded that micromolar Cd induced apoptosis irrespective of sulfhydryl deficiency in hepatic cell line [32]. In the signaling process associated with apoptosis, mitochondria plays an important role and is regulated by the Bcl-2 family members including anti-apoptotic and pro-apoptotic proteins [33]. Bad protein, one of the pro-apoptotic ligands, may move onto mitochondria and bind to Bcl-2 and Bcl-xL inhibiting their anti-apoptotic actions. The Bad promotes apoptosis through the cytochrome c release from mitochondria [34,35]. Thus, translocation of Bad to mitochondria is an important apoptotic cell death mechanism. Previous *in vivo* and *in vitro* studies, we reported that liver apoptosis followed by Cd exposure was involved in Bad translocation [27,32]. In this work, expression of mitochondrial Bad protein was significantly increased by Cd, and RGE decreased the increment of Bad expression in mitochondrial fraction. Therefore, anti-apoptotic activities might be described as one of possible mechanisms responsible for the hepatoprotective effect of RGE. However, hepatic apoptosis cannot be explained by Bad protein alone. Further studies are needed to clearly investigate the involvement of anti-apoptotic action.

To summarize, the results of the present study indicate that RGE effectively protected liver against Cd-induced acute hepatic toxicity in rats. Hepatoprotective effects of RGE were displayed by decreasing the elevation of blood biochemical parameters and restoring the impairments of histopathology. RGE also ameliorated the depletion of GSH by Cd treatment and inhibited expression of pro-apoptotic Bad protein in liver tissue, indicating the partial involvement of anti-oxidative and anti-apoptotic actions in hepatoprotection by RGE.

ACKNOWLEDGEMENTS

This work was supported by the Korean Society of Ginseng in 2010.

REFERENCES

1. Goyer RA, Clarkson TW. Toxic effects of metals. In: Casarett LJ, Doull J, Klaassen CD, eds. Casarett and Doull's toxicology: the basic science of poisons. New York: McGraw-Hill, 2001. p.811-867.
2. Trinchella F, Riggio M, Filosa S, Volpe MG, Parisi E, Scudiero R. Cadmium distribution and metallothionein expression in lizard tissues following acute and chronic cadmium intoxication. Comp Biochem Physiol C Toxicol Pharmacol 2006;144:272-278.
3. Jarup L, Berglund M, Elinder CG, Nordberg G, Vahter M. Health effects of cadmium exposure: a review of the literature and a risk estimate. Scand J Work Environ Health

1998;24 Suppl 1:1-51.
4. Dudley RE, Svoboda DJ, Klaassen C. Acute exposure to cadmium causes severe liver injury in rats. Toxicol Appl Pharmacol 1982;65:302-313.
5. Waalkes MP. Cadmium carcinogenesis. Mutat Res 2003;533:107-120.
6. Rikans LE, Yamano T. Mechanisms of cadmium-mediated acute hepatotoxicity. J Biochem Mol Toxicol 2000;14:110-117.
7. Sidhu M, Sharma M, Bhatia M, Awasthi YC, Nath R. Effect of chronic cadmium exposure on glutathione S-transferase and glutathione peroxidase activities in rhesus monkey: the role of selenium. Toxicology 1993;83:203-213.
8. Ye JL, Mao WP, Wu AL, Zhang NN, Zhang C, Yu YJ, Zhou L, Wei CJ. Cadmium-induced apoptosis in human normal liver L-02 cells by acting on mitochondria and regulating Ca(2+) signals. Environ Toxicol Pharmacol 2007;24:45-54.
9. Jimi S, Uchiyama M, Takaki A, Suzumiya J, Hara S. Mechanisms of cell death induced by cadmium and arsenic. Ann N Y Acad Sci 2004;1011:325-331.
10. Rana SV. Metals and apoptosis: recent developments. J Trace Elem Med Biol 2008;22:262-284.
11. Park HJ, Kim DH, Park SJ, Kim JM, Ryu JH: Ginseng in traditional herbal prescriptions. J Ginseng Res 2012; 36:225-241.
12. Kim SK, Park JH: Trends in ginseng research in 2010. J Ginseng Res 2011; 35:389-398.
13. Yun TK. Brief Introduction of *Panax ginseng* C.A. Meyer. J Korean Med Sci 2001;16(Suppl):S3-S5.
14. Yun TK, Lee YS, Lee YH, Kim SI, Yun HY. Anticarcinogenic effect of *Panax ginseng* C.A. Meyer and identification of active compounds. J Korean Med Sci 2001;16(Suppl):S6-S18.
15. Park JD. Recent studies on the chemical constituents of Korean ginseng (*Panax ginseng* C. A. Meyer). Korean J Ginseng Sci 1996;20:389-415.
16. Kim MH, Hong HD, Kim YC, Rhee YK, Kim KT, Rho J. Ginsenoside changes in red ginseng manufactured by acid impregnation treatment. J Ginseng Res 2010;34:93-97.
17. Yuan HD, Kim JT, Kim SH, Chung SH: Ginseng and diabetes: the evidences from in vitro, animal and human studies. J Ginseng Res 2012; 36:27-39.
18. Kim GN, Lee JS, Song JH, Oh CH, Kwon YI, Jang HD. Heat processing decreases Amadori products and increases total phenolic content and antioxidant activity of Korean red ginseng. J Med Food 2010;13:1478-1484.
19. Panwar M, Samarth R, Kumar M, Yoon WJ, Kumar A. Inhibition of benzo(a)pyrene induced lung adenoma by *Panax ginseng* extract, EFLA400, in Swiss albino mice. Biol Pharm Bull 2005;28:2063-2067.
20. Kang TH, Park HM, Kim YB, Kim H, Kim N, Do JH, Kang C, Cho Y, Kim SY. Effects of red ginseng extract on UVB irradiation-induced skin aging in hairless mice. J Ethnopharmacol 2009;123:446-451.
21. Spelman K, Burns J, Nichols D, Winters N, Ottersberg S, Tenborg M. Modulation of cytokine expression by traditional medicines: a review of herbal immunomodulators. Altern Med Rev 2006;11:128-150.
22. Wittman R, Hu H. Cadmium exposure and nephropathy in a 28-year-old female metals worker. Environ Health Perspect 2002;110:1261-1266.
23. Patrick L. Toxic metals and antioxidants. Part II. The role of antioxidants in arsenic and cadmium toxicity. Altern Med Rev 2003;8:106-128.
24. Geubel AP, Sempoux CL. Drug and toxin-induced bile duct disorders. J Gastroenterol Hepatol 2000;15:1232-1238.
25. Lata J. Chronic liver diseases as liver tumor precursors. Dig Dis 2010;28:596-599.
26. Honda A, Komuro H, Hasegawa T, Seko Y, Shimada A, Nagase H, Hozumi I, Inuzuka T, Hara H, Fujiwara Y et al. Resistance of metallothionein-III null mice to cadmium-induced acute hepatotoxicity. J Toxicol Sci 2010;35:209-215.
27. Lee JR, Park SJ, Lee HS, Jee SY, Seo J, Kwon YK, Kwon TK, Kim SC. Hepatoprotective activity of licorice water extract against cadmium-induced toxicity in rats. Evid Based Complement Alternat Med 2009;6:195-201.
28. Scheig R. Evaluation of tests used to screen patients with liver disorders. Prim Care 1996;23:551-560.
29. Andersen O, Nielsen JB, Svendsen P. Oral cadmium chloride intoxication in mice: effects of dose on tissue damage, intestinal absorption and relative organ distribution. Toxicology 1988;48:225-236.
30. Thophon S, Kruatrachue M, Upatham ES, Pokethitiyook P, Sahaphong S, Jaritkhuan S. Histopathological alterations of white seabass, *Lates calcarifer*, in acute and subchronic cadmium exposure. Environ Pollut 2003;121:307-320.
31. Rana SV, Verma S. Protective effects of GSH, vitamin E, and selenium on lipid peroxidation in cadmium-fed rats. Biol Trace Elem Res 1996;51:161-168.
32. Kim SC, Byun SH, Yang CH, Kim CY, Kim JW, Kim SG. Cytoprotective effects of *Glycyrrhizae radix* extract and its active component liquiritigenin against cadmium-induced toxicity (effects on bad translocation and cytochrome c-mediated PARP cleavage). Toxicology 2004;197:239-251.
33. Jiang P, Du W, Heese K, Wu M. The Bad guy cooperates

with good cop p53: Bad is transcriptionally up-regulated by p53 and forms a Bad/p53 complex at the mitochondria to induce apoptosis. Mol Cell Biol 2006;26:9071-9082.
34. Willis SN, Adams JM. Life in the balance: how BH3-only proteins induce apoptosis. Curr Opin Cell Biol 2005;17:617-625.
35. Bae J, Hsu SY, Leo CP, Zell K, Hsueh AJ. Underphosphorylated BAD interacts with diverse antiapoptotic Bcl-2 family proteins to regulate apoptosis. Apoptosis 2001;6:319-330.