The American Journal of Chinese Medicine

# Preventive Effect of Fermented Black Ginseng against Anticancer Drug-Induced Renal Damage in Rats

--Manuscript Draft--

| | |
|---|---|
| **Manuscript Number:** | AJCM-D-15-00018 |
| **Full Title:** | Preventive Effect of Fermented Black Ginseng against Anticancer Drug-Induced Renal Damage in Rats |
| **Article Type:** | Research Paper |
| **Keywords:** | Panax ginseng; Fermented black ginseng; Cisplatin; Oxidative stress; Renal damage. |
| **Corresponding Author:** | Su-Nam Kim, Ph.D.<br>Korea Institute of Science and Technology<br>Gangneung, Gangwon-do KOREA, REPUBLIC OF |
| **Corresponding Author Secondary Information:** | |
| **Corresponding Author's Institution:** | Korea Institute of Science and Technology |
| **Corresponding Author's Secondary Institution:** | |
| **First Author:** | Ki Sung Kang, Prof |
| **First Author Secondary Information:** | |
| **Order of Authors:** | Ki Sung Kang, Prof |
| | Jun Min An |
| | Su-Nam Kim, Ph.D. |
| **Order of Authors Secondary Information:** | |
| **Abstract:** | Research in efforts to develop methods for increasing the pharmaceutical effect of Panax ginseng by conversion of the dammarane-based saponin by thermal processing has been conducted. In line with this notion, fermented black ginseng (FBG) is newly prepared by heat-processing and fermentation of raw ginseng. The contents of polar ginsenosides (Re, Rg1, Rb1, Rc and Rb2) were decreased, whereas the peaks of less-polar ginsenosides (Rg3, Rg5, Rk1 and Rh4) were newly detected in FBG. The objective of this study is to evaluate how FBG shows renoprotective efficacy against cisplatin-induced renal oxidative stress. The free radical-scavenging activity of FBG was stronger than raw ginseng. The improved free radical-scavenging activity was mediated by the generation phenolic compounds. Then, the protective effect of FBG on cisplatin-induced oxidative renal damage was investigated in rats. FBG was orally administered every day at a dose of 150 mg/kg body weight for 10 days, and a single dose of cisplatin was administered intraperitoneally (7.5 mg/kg body weight) in 0.9% saline at day 4. The decreased creatinine clearance levels as a marker of renal dysfunction in cisplatin-treated rats were recovered nearly back to its normal level after administration of FBG. Moreover, FBG showed protective effects against cisplatin-induced oxidative renal damage in rats through the inhibition of NF-κB/p65, COX-2 and caspase-3 activation. These results collectively show that the therapeutic evidence for FBG ameliorates the renal damage via regulating oxidative stress, inflammation and apoptosis. |
| **Suggested Reviewers:** | Eun-Hwa Sohn<br>ehsohn@kangwon.ac.kr |
| | Tong Ho Kang<br>panjae@khu.ac.kr |
| | Dong Wan Seo<br>dwseomb@dankook.ac.kr |

# Preventive Effect of Fermented Black Ginseng against Anticancer Drug-Induced Renal Damage in Rats

Ki Sung Kang[a], Jun Min An[b], Su-Nam Kim[c,*]

[a]*College of Korean Medicine, Gachon University, Seongnam 461-701, Korea*

[b]*GINSENG BY PHARM Co., Ltd., Wonju 220-862, Korea*

[c]*Natural Skinomics Team, Natural Products Research Institute, Korea Institute of Science and Technology, Gangneung 210-340, Korea*

**Running Title:** THE RENOPROTECTIVE EFFECT OF BLACK GINSENG

Numbers of pages: 19

Numbers of figures: 3

Numbers of tables: 0

[*]**Correspondence to:** Su-Nam Kim, PhD., Natural Skinomics Team, Natural Products Research Institute, Korea Institute of Science and Technology, 290, Daejeon-dong, Gangneung-si, Gangwon-do 210-340, Republic of Korea. Tel: +82 33-650-3503; Fax: +82 33-650-3529; E-mail: snkim@kist.re.kr

Abstract: Research in efforts to develop methods for increasing the pharmaceutical effect of *Panax ginseng* by conversion of the dammarane-based saponin by thermal processing has been conducted. In line with this notion, fermented black ginseng (FBG) is newly prepared by heat-processing and fermentation of raw ginseng. The contents of polar ginsenosides (Re, Rg1, Rb1, Rc and Rb2) were decreased, whereas the peaks of less-polar ginsenosides (Rg3, Rg5, Rk1 and Rh4) were newly detected in FBG. The objective of this study is to evaluate how FBG shows renoprotective efficacy against cisplatin-induced renal oxidative stress. The free radical-scavenging activity of FBG was stronger than raw ginseng. The improved free radical-scavenging activity was mediated by the generation phenolic compounds. Then, the protective effect of FBG on cisplatin-induced oxidative renal damage was investigated in rats. FBG was orally administered every day at a dose of 150 mg/kg body weight for 10 days, and a single dose of cisplatin was administered intraperitoneally (7.5 mg/kg body weight) in 0.9% saline at day 4. The decreased creatinine clearance levels as a marker of renal dysfunction in cisplatin-treated rats were recovered nearly back to its normal level after administration of FBG. Moreover, FBG showed protective effects against cisplatin-induced oxidative renal damage in rats through the inhibition of NF-κB/p65, COX-2 and caspase-3 activation. These results collectively show that the therapeutic evidence for FBG ameliorates the renal damage via regulating oxidative stress, inflammation and apoptosis.

*Keywords: Panax ginseng*; Fermented black ginseng; Cisplatin; Oxidative stress; Renal damage.

## Introduction

Nutrition or herbal therapeutics field has become one of the most popular trends because herbal products not only contain important group of multicomponent therapeutics, but are also perceived as being harmless (Gesler, 1992; Wojcikowski *et al*., 2004). Concomitant and appropriate use of traditional Chinese medicines with modern Western medicinal can prevent or ameliorate the development of complications of multiple chronic conditions. Numbers of evidences have reported that many medicinal herbal supplements have the potential to become valuable complementary therapy in the treatment of various renal disorders (Wojcikowski *et al*., 2004; Ceylan-Isik *et al*., 2008).

Kidneys are vital organs that function to keep the blood clean and maintain chemical balance within. That is, kidneys play important roles in excreting waste products and in maintaining electrolyte and water balance in the body. Therefore, kidney injury is considered to contribute to organ dysfunction of the lung, brain, liver, heart, and other organs (Li *et al*., 2009). Recent literature indicates that reactive oxygen species (ROS) play critical roles in the development and progression of kidney damage (Forbes *et al.*, 2008; Nistala *et al.*, 2008). Oxidative stress which is due to the alterations in redox homeostasis can directly exert renal parenchymal damage and may intensify renal microvascular and functional dysregulation (Heyman *et al*. 2011). Then, increased oxidative stress in the kidney leads to deterioration of the renal function, inflammation and apoptosis (Manucha and Vallés, 2012; Ozbek, 2012). Moreover, oxidative stress is closely associated with independent risk factors such as aging, diabetes, hypertension, hyperlipidemia and metabolic syndrome (Li and Shah, 2003; Gökhan, 2010; Chung *et al*., 2012; Lim *et al*., 2012).

Cisplatin, an important chemotherapeutic agent commonly used for the treatment of solid tumors, accumulates in proximal tubules of the kidneys and causes acute renal failure. Although the exact mechanisms of cisplatin nephrotoxicity is not fully understood, multiple studies have shown

that it is associated with DNA fragmentation, ROS, and caspase activation (Matsushima *et al*., 1998; Kaushal *et al*., 2001; Basnakian *et al*., 2005). It has also recognized that apoptosis and inflammation are important factors in cisplatin-induced nephrotoxicity (Mitazaki *et al*., 2011).

*Panax ginseng* C.A. MEYER is one of the most widely used traditional herbal medicines in Asia, and there are a variety of commercial ginseng products such as white, red and black ginseng (dehydrated by sun, steamed at 95-100$^{o}$C for 2-3 hours, and nine cycles of steamed, respectively). Steaming process is known to induce a structural change of ginsenoside and to enhance the biological activities of ginseng (Kitagawa *et al*., 1987; Wang *et al*., 2006; Kang *et al*., 2007). Fermentation is also known as a beneficial tool in increasing the safety and efficacy (Lee *et al*., 2012). On the other hand, based on a large number of scientific studies, ginseng is known to have a wide range of pharmacological and physiological actions such as anti-aging, immunoenhancement, anti-stress, and anti-tumor (Kaneko and Nakanishi, 2004; Kang *et al*., 2008; Byeon *et al*., 2012; Ramesh *et al*., 2012). Although the efficacy of black ginseng is poorly understood, augmentation of antioxidant activity and beneficial on vascular dementia have recently been reported (Park *et al*., 2011; Lee *et al*., 2012). The objective of this study is to evaluate how fermented black ginseng (FBG) shows renoprotective efficacy against cisplatin-induced renal oxidative stress.

## Materials and methods

*Reagents*

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent and cisplatin were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). NF-κBp/65, COX-2, Cleaved caspase-3, GAPDH and horseradish peroxidase (HRP) conjugated anti-rabbit antibodies were purchased from Cell Signaling (Boston, MA, USA). The other chemicals and reagents used were of high quality and obtained from commercial sources.

*Preparation of herbal extract*

FBG in the form of a dried powder extract was supplied from GINSENG BY PHARM Co., Ltd. (Wonju, Korea). Four-year-old white ginseng was purchased from a local ginseng center (Geumsan, Korea). Authenticity of a ginseng was determined based on the ingredient profile. Black ginseng was manufactured via nine cycles of repeated steaming of white ginseng at 85°C for 8 h and drying at 50°C for 48 h. To prepare ginseng extracts, black ginseng was crushed into a powder and extracted one time in 10 volumes of distilled water at 80°C for 72h, then filtered and chilled. Ginseng extract was fermented with *Saccharomyces cerevisiae* (Lallemand, Denmark) at 34°C for 25h. Following fermentation, the FBG extract was sterilized (at 85°C for 22 h) and lyophilized. The ginsenoside content of FBG extracts used in this study was: Rg2 2.86 μg/mL, Rg3 24.52 μg/mL, Rh1 12.62 μg/mL, Rh2 0.63 μg/mL, Rf 1.32 μg/mL (Bak et al., 2014).

*Measurement of total phenolic contents*

The total phenolic contents of samples were determined using the Folin-Ciocalteu method (Singleton *et al.,* 1999). Contents were expressed as milligrams of gallic acid equivalents (GAE) per gram of ginseng extract, and the measurement was repeated three times.

*DPPH radical scavenging activity test*

In microwells, 100 μl of an aqueous solution of the sample (control: 100 μl of D.W.) was added to an ethanolic solution of DPPH (60 μM) according to the method of Hatano *et al.* (1989). Four concentrations were prepared for each sample. After mixing gently and leaving to stand for 30 min at room temperature, the absorbance at 540 nm was determined using a microplate reader (PowerWave XS; Bio-Tek Instruments, Winooski, VT, USA), and a green tea extract was used as DPPH-scavenging positive control.

*Renoprotective effect against cisplatin-induced oxidative damage in rats*

*Treatment of animals*

The Guidelines for Animal Experimentation, approved by the Korea Institute of Science and Technology were followed in these experiments. Male Wistar rats weighing 140-160 g were used for evaluating the protection of the black ginseng against cisplatin-induced nephrotoxicity. The rats were housed under temperature (23 ± 2°C) and humidity (55 ± 5%) condition with a standard light (12 h light/dark). The rats were given free access to water and normal diet (38057, Agribrands Purina Korea, Seongnam, Gyeonggi, Korea) containing 10 kcal% fat for a period of one week after arrival. Then rats were divided into 3 groups based on their body weight and assigned to the vehicle, cisplatin and cisplatin + FBG.

Group 1: Vehicle (n=4) received water (no sample treatment)

Group 2: Cisplatin (n=4) received water (no sample treatment)

Group 3: Cisplatin + FBG (n=4) treated with FBG water extract (150 mg/kg) in aqueous solution orally for 10 days

FBG was orally administered every day at a dose of 150 mg/kg body weight, while vehicle-treated rats were orally given water. The 150 mg/kg of FBG dosage was chosen according to the literature

(Oh *et al*., 2013). After 4 days rats in two groups (cisplatin and cisplatin + FBG) were administered a single dose of cisplatin intraperitoneally (7.5 mg/kg body weight) in 0.9% saline. Animals in the vehicle group received an equivalent amount of normal saline for 10 days. The rats were sacrificed 6 days after cisplatin administration under light ether anesthesia. Twenty four h urine samples were collected using metabolic cage. Blood samples were collected from abdominal aorta and kidneys were removed. All the preparations and analyses of various parameters were performed simultaneously under similar experimental conditions to avoid any day to day variations. During the experimental period, their body weights were measured daily.

*Plasma biomarker analyses*

Blood samples were collected in tubes containing 0.18 M EDTA and centrifuged at 5,000 rpm for 5 min at 4ºC. After centrifugation, plasma was separated for estimation of total cholesterol and creatinine. Creatinine levels were determined by a rate-blanked kinetic Jaffe method. Creatinine clearance was calculated on the basis of the urinary Cr, serum Cr, urine volume, and body weight using the following equation: Creatinine clearance (mL/kg body weight/min) = [urinary Cr (mg/dL) × urine volume (mL)/serum Cr (mg/dL)] × [1,000/body weight (g)] × [1/1,440 (min)].

*Histological analysis of kidney*

Kidney samples were fixed in 10% buffered formalin phosphate (Fisher Scientific, Pittsburgh, PA), dehydrated, embedded in paraffin, sectioned in 3 μm thickness, and stained with periodic acid-Schiff (PAS) reagents for histological examination. Tubular damage in PAS-stained sections was examined under the microscope.

*Preparation of cytosolic and nuclear extracts from tissue*

The frozen kidney tissues, weighing 30 mg, were powdered by grinding thoroughly with a pestle

and mortar in liquid nitrogen. The tissue powders were resuspended in hypotonic buffer (10 mM HEPES [pH 7.9], 10 mM KCl, 0.1 mM EDTA, 1 mM DTT, 1× protease inhibitor cocktail, 1 mM PMSF and 1 mM $Na_3VO_4$) for 15 min on ice. Subsequently, 10% Nonidet P-40 (USB, OH, USA) was added, and the mixture was vortexed and then centrifuged at 13,200 rpm for 30 sec at 4°C. The supernatant containing cytosolic proteins was collected and stored at -80°C until further use. The nuclear pellets were rinsed twice with cold PBS and resuspended in hypertonic buffer (20 mM HEPES [pH 7.9], 0.4 M NaCl, 0.1 mM EDTA, 1 mM DTT, 1× protease inhibitor cocktail, 1 mM PMSF and 1 mM $Na_3VO_4$) by rocking at 4°C for 15 min. The resuspended nuclear fraction was then centrifuged at 13,200 rpm for 5 min at 4°C. The supernatant containing nuclear proteins was collected and stored at -80°C until further use.

*Preparation of whole-cell extracts from tissue*

Whole-cell extracts from kidney tissue were prepared according to the manufacturer's instructions using RIPA buffer (Cell Signaling, MA, USA) supplemented with 1× protease inhibitor cocktail and 1 mM phenylmethylsulfonyl fluoride (PMSF).

*Western blot analysis*

Proteins (whole-cell extracts, 30 μg/lane; nuclear extracts, 10 μg/lane; cytosolic extracts, 20 μg/lane) were separated by SDS-PAGE, transferred to polyvinylidene fluoride (PVDF) membranes for 1 h at semi-dry, and blocked in blocking buffer for 1 h at room temperature. The PVDF membranes were incubated with primary antibody against NF-κB/p65 (1:1000 dilution), COX-2 (1:1000 dilution), cleaved caspase-3 (1:1000 dilution), GAPDH (1:1000 dilution) for overnight at the 4°C, washed three times for 5 min to wash buffer, incubated with HRP-conjugated secondary antibody (1:2000 dilution, anti-rabbit) for 1 h at room temperature, washed three times, and then detected with ECL solution.

*Statistical analysis*

The quantitative data were expressed as means ± S.E. Statistical significance was determined using the analysis of variance (ANOVA) followed by a multiple comparison test with a Bonferroni adjustment. Individual differences between groups were evaluated using the Student's *t*-test, and $p < 0.05$ were considered statistically significant.

## Results

*Total phenolic contents and DPPH radical scavenging activity of FBG extract*

The total phenolic contents of raw ginseng and FBG were 3.9 ± 0.5 and 40.5 ± 2.2 GAE, respectively (Fig. 1(A)). Total phenolic content of FBG was 10 times more compared to that of raw ginseng (Fig. 1(A)). The DPPH radical scavenging activity of FBG ($IC_{50}$=399.5 μg/mL) was increased in a dose dependent manner, and its effect was significantly stronger than that of raw ginseng (Fig. 1(B)). The free radical scavenging activities of raw ginseng and FBG extract were positively correlated with the total phenolic contents.

*Protective effect of black ginseng oncisplatin-induced renal damage in rats*

The body weight gain of rats after cisplatin and cisplatin + FBG were markedly reduced compared to after vehicle treatments (Fig. 2(A)). Similarly, food intake amounts were slightly lowered after cisplatin treatments and gradually recovered to the vehicle-treated groups (Fig. 2(B)). The sharp decrease in the food intake was observed on day 4 in cisplatin and cisplatin + FBG treated groups. These results are in accordance with earlier reports on the decrease of body weight gain after cisplatin injection (Takeda *et al*., 2008).

In the comparison of serum and urine biochemical parameters, cisplatin-injected rats showed increased serum creatinine and decreased creatinine clearance levels than those of the vehicle-treated group (Fig. 2(C), (D)). The elevated serum creatinine level of cisplatin-treated rats was slightly reduced by co-treatment with FBG. In particular, the decreased creatinine clearance level was recovered nearly back to its normal levels by administrations of FBG (Fig. 2(D)).

PAS staining was performed on renal sections to measure tubular damage. As shown in the representative pictures of renal sections, severe tubulointerstitial injuries including tubular epithelial cell detachments, cystic dilatation of tubules and inflammatory cell infiltration were occurred in the

cisplatin-exposed kidneys (Fig. 3(A)). However, the increased tubular damage in cisplatin-treated rats was reduced by co-treatments with FBG (Fig. 3(A)). Figure 3(B) shows the effect of FBG on NF-κBp65, COX-2 and cleaved caspase-3 protein expression in the cisplatin-treated rat kidneys. NF-κBp65, COX-2 and cleaved caspase-3 protein expressions were significantly increased after cisplatin-injection, and its co-treatment with FBG afforded almost complete renoprotection (Fig. 3(C), (D), (E)).

**Discussion**

Recent literature indicates that ROS plays critical roles in the development and progression of kidney damage (Li & Shah, 2003; Forbes *et al*., 2008; Heyman *et al*., 2011). Antioxidants treatment can prevent oxidative damage, and might delay the progression of kidney disease. Therefore, antioxidant as an inhibitor against oxidative stress is considered to be an important therapeutic approach for kidney disease. In the present study, we have investigated the protective effect and mechanism of FBG on the kidney damage caused by cisplatin, oxidative stress and inflammation to evaluate its possible use in renal damage.

*Panax ginseng* contains high concentration of saponins including ginsenosides $Rb_1$, $Rb_2$, Rc, Rd, $Rg_1$, and Re unlike the other plants. Research in efforts to develop methods for increasing the pharmaceutical effect of ginseng by conversion of the dammarane-based saponin by high temperature and high pressure thermal processing has been conducted (Kang *et al*., 2007; Lee *et al*., 2012; Yamabe *et al.*, 2012, 2013). FBG is prepared by heat-processing and fermentation of raw ginseng. The contents of polar ginsenosides (Re, Rg1, Rb1, Rc and Rb2) were decreased, whereas the peaks of less-polar ginsenosides (Rg3, Rg5, Rk1 and Rh4) were newly detected in FBG. Especially, ginsenoside 20(*S*)-$Rg_3$ which is abundantly contained in FBG was found to prevent the progression of renal damage and dysfunction in type 1 diabetic rats via inhibition of oxidative stress and inflammation (Yokozawa *et al.*, 2007; Kang *et al.*, 2008).

DPPH has been widely used to test the ability of compounds or plant extracts to act as free radical scavengers or hydrogen donors (Hatano *et al.*, 1989; Zhu *et al.*, 2002), and the phenolic contents of plants can be correlated to their antioxidant activities (Cai *et al.*, 2004). FBG extract showed stronger free radical scavenging activity than that of raw ginseng. Stronger free radical scavenging activity of FBG than raw ginseng was related with its higher total phenolic contents. Then, we further examined the effect of FBG on cisplatin-induced oxidative renal damage in rats.

In line with our *in vitro* results, FBG ameliorated the cispaltin-induced renal dysfunction and tubulointerstitial injuries in rats. ROS play an important role in mediating apoptosis by inducing the activation of caspases. Among all the caspase members, caspase-3 in particular is an essential apoptotic effector leading to cytoskeletal breakdown, nuclear demise, and other cell changes associated with apoptosis (Bratton and Cohen, 2001). Therefore, caspase inhibitors have the potential to minimize uncontrolled apoptosis in cisplatin-induced nephropathy (Razzaque, 2007). Our results also showed significant increases in cleaved caspase-3 expression levels of the cisplatin-treated rat kidney, and its elevated level was significantly reduced after FBG administration. Similarly, the elevated NF-κB/p65 and COX-2 protein expressions which are reliable markers of inflammation in cisplatin-treated rat kidney were lowered nearly back to its normal levels. These results imply that FBG may alleviate oxidative stress by preventing caspase-3 activation and related inflammation in the kidney.

In summary, the kidney cell damage induced by oxidative stress was significantly inhibited by the treatments with FBG. In addition, the renal dysfunction of cisplatin treated-mice was markedly ameliorated by the FBG extract administration. Renoprotective effect of FBG was associated with caspase-dependent anti-inflammatory pathway. Taken together, these results demonstrate that FBG exerted a renoprotective effect in cisplatin-treated rats, and therefore, it can be considered to be used for the prevention of kidney damage.

**References**


Basnakian, A.G., E.O. Apostolov, X. Yin, M. Napirei, H.G. Mannherz and S.V. Shah. Cisplatin nephrotoxicity is mediated by deoxyribonuclease I. *J. Am. Soc. Nephrol.* 16: 697-702, 2005.

Bratton, S.B. and G.M. Cohen. Apoptotic death sensor: an organelle's alter ego? *Trends Pharmacol Sci.* 22: 306-315, 2001.

Byeon, S.E., J. Lee, J.H. Kim, W.S. Yang, Y.S. Kwak, S.Y. Kim, E.S. Choung, M.H. Rhee and J.Y. Cho. Molecular mechanism of macrophage activation by red ginseng acidic phlysaccharide from Korean red ginseng. *Mediators Inflamm.* 2012: 732860, 2012.

Cai, Y., Q. Luo, M. Sun and H. Corke. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci.* 74: 2157-2184, 2004.

Gesler, W.M. Therapeutic landscapes: medical issues in light of the new cultural geography. *Soc. Sci. Med.* 34: 735-746, 1992.

Ceylan-Isik, A.F., R.M. Fliethman, L.E. Wold and J. Ren. Herbal and traditional Chinese medicine for the treatment of cardiovascular complications in diabetes mellitus. *Curr. Diabetes Rev.* 4: 320-328, 2008.

Chung, H.W., J.H. Lim, M.Y. Kim, S.J. Shin, S. Chung, B.S. Choi, H.W. Kim, Y.S. Kim, C.W. Park and Y.S. Chang. High-fat diet-induced renal cell apoptosis and oxidative stress in spontaneously hypertensive rat are ameliorated by fenofibrate through the PPARα-FoxO3a-PGC-1α pathway. *Nephrol. Dial. Transplant.* 27: 2213-2225, 2012.

Forbes, J.M., M.T. Coughlan and M.E. Cooper. Oxidative stress as a major culprit in kidney disease in diabetes. *Diabetes* 57: 1446-1454, 2008.

Gökhan, S.H. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. Cell 140: 900-917, 2010.

Hatano, T., R. Edamatsu, M. Hiramatsu, A. Mori, Y. Fujita, T. Yasuhara, T. Yoshida and T. Okuda.

Effects of the interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical, and on 1,1-diphenyl-2-picrylhydrazyl radical. *Chem. Pharm. Bull.* 37: 2016-2021, 1989.

Heyman, S.N., S. Rosen and C. Rosenberger. A role for oxidative stress. *Contrib. Nephrol.* 174: 138-148, 2011.

Kaneko, H. and K. Nakanishi. Prood of the mysterious efficacy of ginseng; basic and clinical trials: clinical effects of medical ginseng, Korean red ginseng: specifically, its anti-stress action for prevention of disease. *J. Pharmacol. Sci.* 95: 158-162, 2004.

Kang, K.S., H.Y. Kim, S.H. Baek, H.H. Yoo, J.H. Park and T. Yokozawa. Study on the hydroxyl radical scavenging activity changes of ginseng and ginsenoside-Rb2 by heat processing. *Biol. Pharm. Bull.* 30: 724-728, 2007.

Kang, K.S., N. Yamabe, H.Y. Kim, J.H. Park and T. Yokozawa. Therapeutic potential of 20(S)-ginsenoside Rg3 against streptozotocin-induced diabetic renal damage in rats. *Eur. J. Pharmacol.* 591: 266-272, 2008.

Kaushal, G.P., V. Kaushal, X. Hong and S.V. Shah. Role and regulation of activation of caspases in cisplatin-induced injury to renal tubular epithelial cells. *Kidney Int.* 60: 1726-1736, 2001.

Kitagawa, I., T. Taniyama, H. Shibuya, T. Noda and M. Yoshikawa. Chemical studies on crude drug processing. V. On the constituents of ginseng radix rubra (2): Comparison of the constituents of white ginseng and red ginseng prepared from the same Panax ginseng root. *Yakugaku Zasshi.* 107: 495-505, 1987.

Lee, H.S., M.R. Kim, Y. Park, H.J. Park, U.J. Chang, S.Y. Kim and H.J. Suh. Fermenting red ginseng enhances its safety and efficacy as a novel skin care anti-aging ingredient: in vitro and animal study. *J. Med. Food* 15: 1015-1023, 2012.

Lee, W., S.H. Park, S. Lee, B.C. Chung, M.O. Song, K.I. Song, J. Ham, S.N. Kim and K.S. Kang. Increase in antioxidant effect of ginsenoside Re-alanine mixture by Maillard reaction. *Food*

*Chem.* 135: 2430-2435, 2012.

Li, J.M. and A.M. Shah. ROS generation by nonphagocytic NADPH oxidase: potential relevance in diabetic nephropathy. *J. Am. Soc. Nephrol.* 14: S221-S226, 2003.

Li, X., H.T. Hassoun, R. Santora and H. Rabb. Organ crosstalk: the role of the kidney. *Curr. Opin. Crit. Care.* 15: 481-487, 2009.

Lim, J.H., E.N. Kim, M.Y. Kim, S. Chung, S.J. Shin, H.W. Kim, C.W. Yang, Y.S. Kim, Y.S. Chang, C.W. Park and B.S. Choi. Age-associated molecular changes in the kidney in aged mice. *Oxid. Med. Cell Longev.* 2012: 171383, 2012.

Manucha, W. and P.G. Vallés. Apoptosis modulated by oxidative stress and inflammation during obstructive nephropathy. *Inflamm. Allergy Drug Targets* 11: 303-312, 2012.

Mathushima, H., K. Yonemura, K. Ohishi and A. Hishida. The role of oxygen free radicals in cisplatin-induced acute renal failkure in rats. *J. Lab. Clin. Med.* 131: 518-526, 1998.

Mitazaki, S., S. Honma, M. Suto, N. Kato, K. Hiraiwa, M. Yoshida and S. Abe. Interleukin-6 plays a protective role in development of cisplatin-induced acute renal failure through upregulation of anti-oxidative stress factors. *Life Sci*. 88: 1142-1148, 2011.

Nistala, R., A. Whaley-Connell and J.R. Sowers. Redox control of renal function and hypertension. Antioxid. *Redox Signal*. 10: 2047-2089, 2008.

Oh, J.S., S.R. Lee, K.T. Hwang and G.E. Ji. The anti-obesity effects of the dietary combination of fermented red ginseng with levan in high fat diet mouse model. *Phytother. Res.* In press.

Ozbek, E. Induction of oxidative stress in kidney. *Int. J. Nephrol.* 2012: 465897, 2012.

Park, H.J., H.S. Shim, K.S. Kim and I. Shim. The protective effect of black ginseng against transient focal ischemia-induced neuronal damage in rats. *Korean J. Physiol. Pharmacol.* 15: 333-338, 2011.

Ramesh, T., S.W. Kim, S.Y. Hwang, S.H. Sohn, S.K. Yoo and S.K. Kim. Panax ginseng reduces oxidative stress and restores antioxidant capacity in aged rats. *Nutr. Res.* 32: 718-726, 2012.

Razzaque, M.S. Cisplatin nephropathy: is cytotoxicity avoidable? *Nephrol. Dial. Transplant.* 22: 2112-2116, 2007.

Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventós. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.* 299: 152-178, 1999.

Takeda, H., C. Sadakane, T. Hattori, T. Katsurada, T. Ohkawara, K. Nagai and M. Asaka. Rikkunshito, an herbal medicine, suppresses cisplatin-induced anorexia in rats via 5-HT2 receptor antagonism. *Gastroenterology* 134: 2004-2013, 2008.

Wang, C.Z., B. Zhang, W.X. Song, A. Wang, M. Ni, X. Luo, H.H. Aung, J.T. Xie, R. Tong, T.C. He and C.S. Yuan. Steamed American ginseng berry: ginsenoside analyses and anticancer activities. *J. Agric. Food Chem.* 54: 9936-9942, 2006.

Wojcikowski, K., D.W. Johnson and G. Gobé. Medicinal herbal extracts--renal friend or foe? Part two: herbal extracts with potential renal benefits. *Nephrology (Carlton).* 9: 400-405, 2004.

Yamabe, N., K.I. Song, W. Lee, I.H. Han, J.H. Lee, J. Ham, S.N. Kim, J.H. Park and K.S. Kang. Chemical and free radical-scavenging activity changes of ginsenoside Re by Maillard reaction and its possible use as a renoprotective agent. *J. Ginseng Res.* 36: 256-262, 2012.

Yamabe, N., Y.J. Kim, S.Y. Lee, E.J. Cho, S.H. Park, J. Ham, H.Y. Kim and K.S. Kang. Increase in antioxidant and anticancer effects of ginsenoside Re–lysine mixture by Maillard reaction. *Food Chem.* 138: 876-883, 2013.

Yokozawa, T., K.S. Kang, N. Yamabe and H.Y. Kim. Therapeutic potential of heat-processed Panax ginseng with respect to oxidative tissue damage. *Drug Discov. Ther.* 1: 30-44, 2007.

Zhu, Q.Y., R.M. Hackman, J.L. Ensunsa, R.R. Holt and C.L. Keen. Antioxidant activities of oolong tea. *J. Agric. Food Chem.* 50: 6929-6934, 2002.

**Acknowledgements**

This work was supported by a grant from Korea Institute of Science and Technology, Republic of Korea (Grant No. 2Z04371).

**Legends to figures**

Figure 1. Antioxidant effects of FBG. (A) Total phenolic contents. (B) DPPH radical scavenging activity. $p < 0.05$ compared to the cisplatin-treated control value.

Figure 2. Effect of FBG on body weight, food intake and renal function parameters in the cisplatin-induced renal damage rat model. (A) Changes in body weight. (B) Changes in food intake. (C) Serum creatinine levels. (D) Creatinine clearance. $p < 0.05$ compared to the cisplatin-treated control value.

Figure 3. Histological and protein analysis of renal cortex tissues. (A) PAS staining of representative renal section and its quantitative data for the tubular damage. (B) NF-κB/p65, COX-2 and cleaved caspase-3 protein expressions. (C) Quantitative data for the COX-2 Western blot analysis of renal cortex tissues. (D) Quantitative data for the cleaved caspase-3 Western blot analysis of renal cortex tissues. (E) Quantitative data for the NF-κB/p65 Western blot analysis of renal cortex tissues. $p < 0.05$ compared to the cisplatin-treated control value.

Figure

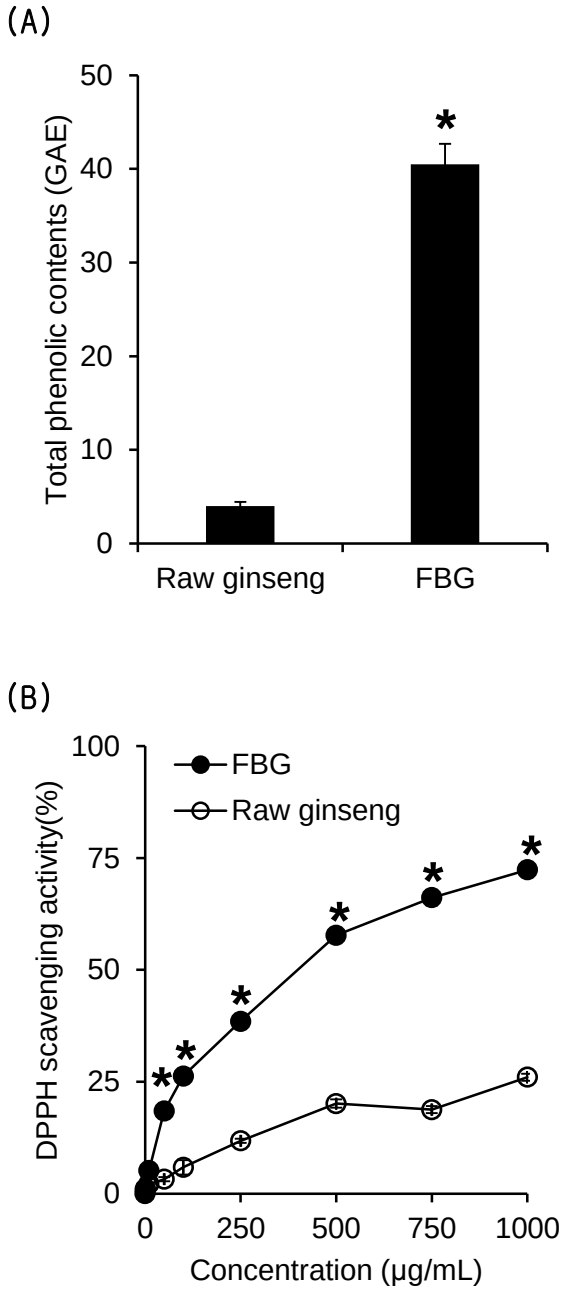


Figure 1

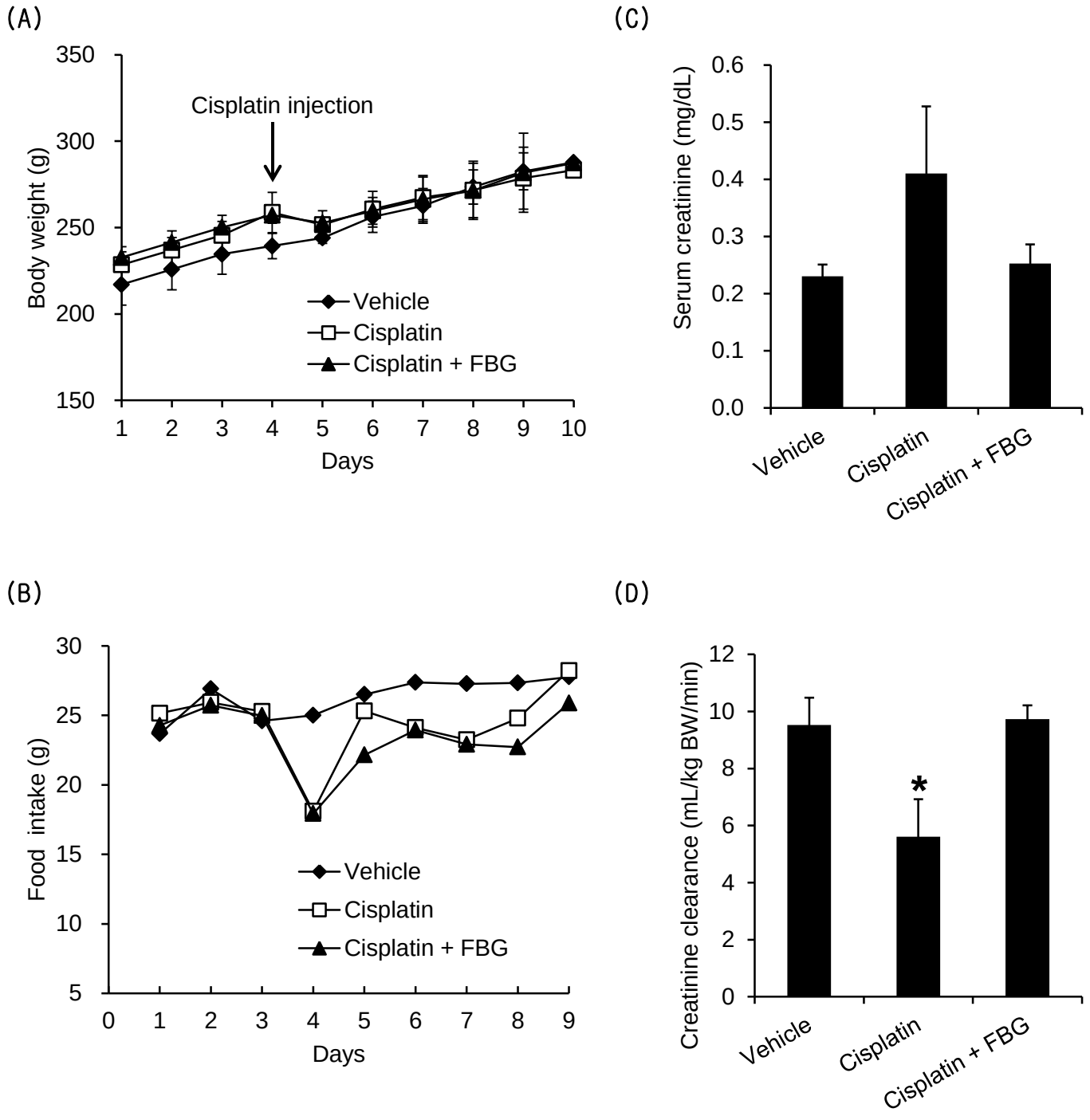
(A)
Body weight (g)
350
300
250
200
150
Cisplatin injection
Vehicle
Cisplatin
Cisplatin + FBG
1
2
3
4
5
6
7
8
9
10
Days
(C)
Serum creatinine (mg/dL)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Vehicle
Cisplatin
Cisplatin + FBG
(B)
Food intake (g)
30
25
20
15
10
5
Vehicle
Cisplatin
Cisplatin + FBG
0
1
2
3
4
5
6
7
8
9
Days
(D)
Creatinine clearance (mL/kg BW/min)
12
10
8
6
4
2
0
*
Vehicle
Cisplatin
Cisplatin + FBG

Figure 2

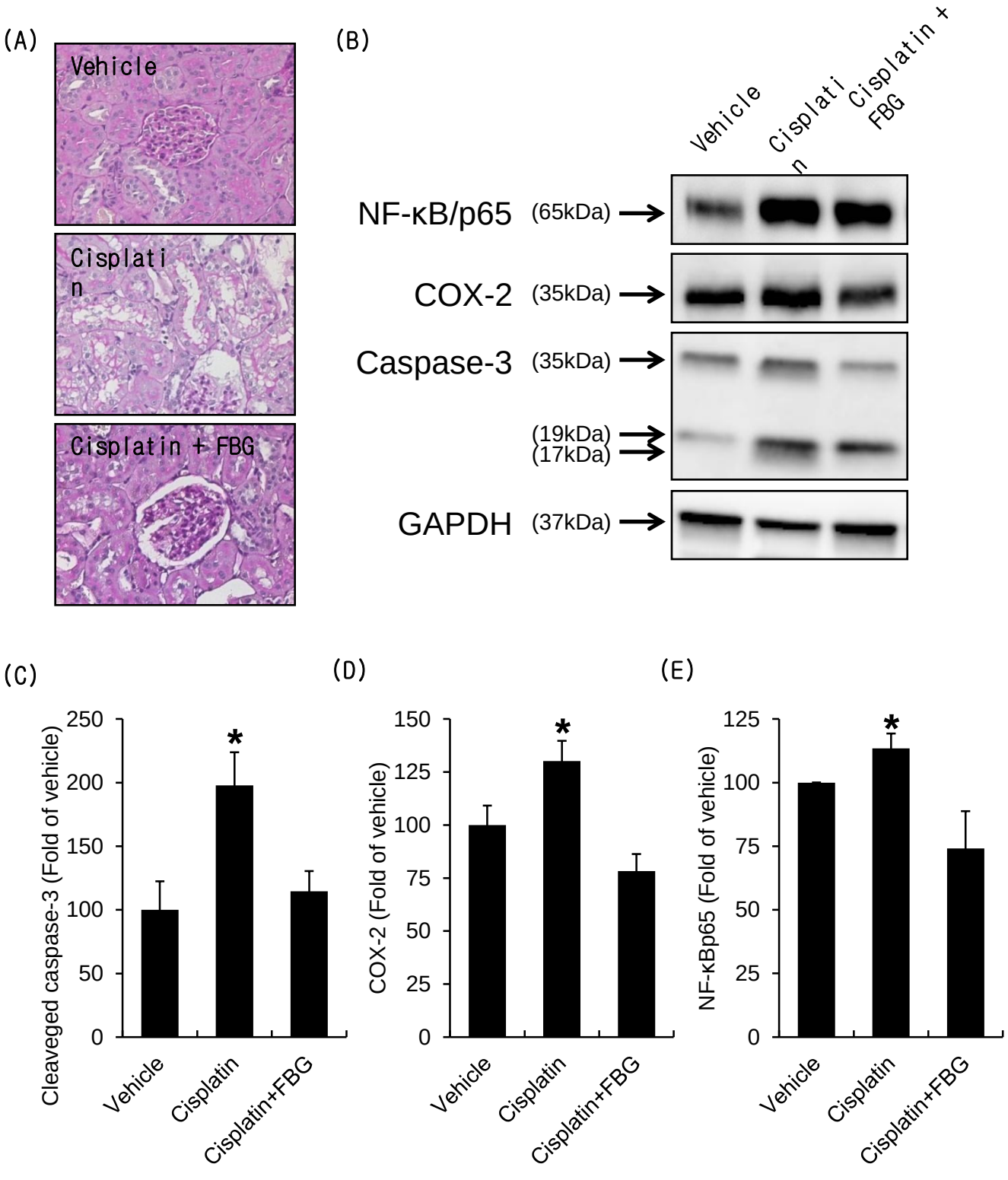
(A)
Vehicle
Cisplati
n
Cisplatin + FBG
(B)
Vehicle
Cisplati
n
Cisplatin +
FBG
NF-κB/p65 (65kDa)
COX-2 (35kDa)
Caspase-3 (35kDa)
(19kDa)
(17kDa)
GAPDH (37kDa)
(C)
Cleaveged caspase-3 (Fold of vehicle)
250
200
150
100
50
0
*
Vehicle
Cisplatin
Cisplatin+FBG
(D)
COX-2 (Fold of vehicle)
150
125
100
75
50
25
0
*
Vehicle
Cisplatin
Cisplatin+FBG
(E)
NF-κBp65 (Fold of vehicle)
125
100
75
50
25
0
*
Vehicle
Cisplatin
Cisplatin+FBG

Figure 3