Therapeutic potential of heat-processed *Panax ginseng* with respect to oxidative tissue damage

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ABSTRACT: *Panax ginseng* has been reported to exhibit a wide range of pharmacological and physiological actions. A method of heat-processing to enhance the efficacy of ginseng is well established in South Korea based on a long history of ethnopharmacological evidence. We investigated the increase in free radical-scavenging activity of *Panax ginseng* as a result of heat-processing and its active compounds related to fortified antioxidant activity. In addition, the therapeutic potential of heat-processed ginseng (HPG) with respect to oxidative tissue damage was examined using rat models. Based upon chemical and biological activity tests, the free radical-scavenging active components such as less-polar ginsenosides and maltol in *Panax ginseng* significantly increased depending on the temperature of heat-processing. According to animal experiments related to oxidative tissue damage, HPG displayed hepatoprotective action by reducing the elevated thiobarbituric acid reactive substance (TBA-RS) level, as well as nuclear factor-kappa B (NF-κB) and inducible nitric oxide synthase (iNOS) protein expressions, while increasing heme oxygenase-1 in the lipopolysaccharide-treated rat liver, and HPG also displayed renal protective action by ameliorating physiological abnormalities and reducing elevated TBA-RS, advanced glycation endproduct (AGE) levels, NF-κB, cyclooxygenase-2, iNOS, 3-nitrotyrosine, N^{ε}-(carboxymethyl)lysine, and receptors for AGE protein expression in the diabetic rat kidney. Therefore, HPG clearly has a therapeutic potential with respect to oxidative tissue damage by inhibiting protein expression related to oxidative stress and AGEs, and further investigations of active compounds are underway. This investigation of specified bioactive constituents is important for the development of scientific ginseng-derived drugs as part of ethnomedicine.

Key Words: *Panax ginseng*, heat-processing, less-polar ginsenosides, maltol, oxidative tissue damage, advanced glycation endproducts

1. Introduction

Herbs have been used for centuries to treat illness and improve health and still account for about 80% of medical treatments in the developing world, with approximately one-third of drugs being derived from plant sources (*1-3*). Since free radical-induced damage and subsequent lipid, protein, and DNA peroxidations are implicated in many kinds of human pathologies, great efforts have been made to examine various herbs or nutraceuticals in the search for therapeutic agents that safely and effectively act against oxidative stress-induced disease (*4,5*).

Panax ginseng C.A. Meyer (Araliaceae) is a medicinal herb that is mainly cultivated in Korea and Northeast China. Considered a valued medicine, it has been used in the Orient for more than 2000 years. The genus name *Panax* means 'cure-all' in Greek and as the plant is considered to be the lord or king of herbs (*6-8*). Ginseng and its components have been reported to exhibit a wide range of pharmacological and physiological actions, such as antiaging, antidiabetic, anticarcinogenic, analgesic, antipyretic, antistress, antifatigue, and tranquilizing properties, as well as the stimulation of DNA, RNA, and protein synthesis (*9-18*). These medicinal properties of ginseng have been suggested to be linked, although not totally, to ginseng's action to protect against free radical attack (*19-23*).

Although traditional Chinese medicine and many current research studies often use products that combine ginseng with other medicinal herbs or vitamins, a method of heat-processing to enhance the efficacy of ginseng is well established in Korea based

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Received June 2, 2007
Accepted June 16, 2007

on its long history of ethnopharmacological evidence (*24-28*). In his book, Xu-Jing, an attendant to a special envoy of the Chinese Emperor to Korea, described two kinds of ginseng products in Korea, sun-dried and steamed ginseng, in 1123. *Panax ginseng* cultivated in Korea is harvested after 4 to 6 years of cultivation and is classified into three types depending on how it is processed. Fresh ginseng can be consumed in an unprocessed state. White ginseng (WG, harvested when 4-6 years old) is dried ginseng root after peeling. Red ginseng (RG, harvested when 6 years old) is ginseng root steamed at 98-100°C without peeling (Figure 1) (*6-8,29*).

Generally, RG is more common as a medicinal herb than WG in Asia because steaming induces changes in the chemical constituents and enhances the biological activity of ginseng (*8,29-31*). On the other hand, a novel heat-processing method in which ginseng is autoclaved at a higher temperature than RG was recently developed to achieve an even stronger activity than that of RG, and this ginseng product was termed heat-processed ginseng (HPG, Figure 1) (*25,32,33*). HPG has been reported to exhibit more potent pharmacological activities, such as vasorelaxation, anxiolytic-like activity, antioxidant activity, and antitumor activity, than conventional WG or RG (*32,34-37*).

Based upon this evidence, this study investigated the increase in free radical-scavenging activity of *Panax ginseng* as a result of heat-processing and its active compounds related to enhanced antioxidant activity. In addition, the therapeutic potential of HPG with respect to oxidative tissue damage was examined using rat models. The aim of this paper was to review scientific evidence underlying the therapeutic potential of HPG with respect to oxidative tissue damage, which should prove helpful in understanding the complex efficacy changes of ginseng brought about by heat-processing.

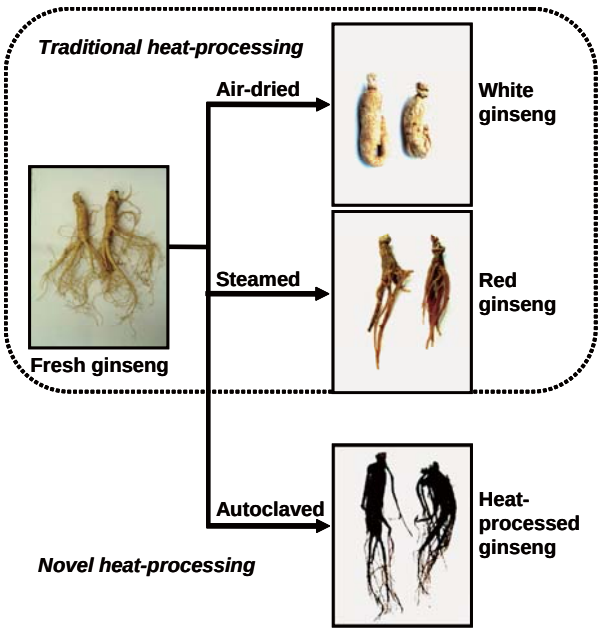


Figure 1. Classification of *Panax ginseng* by heat-processing methods.

2. Increase in the free radical-scavenging activity of ginseng as a result of heat-processing

Reactive oxygen metabolites, including free radicals such as nitric oxide ($^{\bullet}NO$), superoxide anion ($O_2^{\bullet-}$), hydroxyl radical (•OH), and peroxynitrite ($ONOO^-$), are toxic and play an important role in tissue injury (*38-41*). $O_2^{\bullet-}$ reacts rapidly with $^{\bullet}NO$ to produce the more toxic $ONOO^-$. $ONOO^-$ is protonated and forms peroxynitrous acid (ONOOH) under physiological conditions, and ONOOH easily decays to yield strong oxidants such as nitrogen dioxide, nitryl cations, and •OH. $ONOO^-$ and its decomposition products contribute to antioxidant depletion, alterations of protein structure, and oxidative damage observed in human disease (*42-45*). As antioxidants may attenuate oxidative damage by free radical-scavenging or metal chelation, many studies have been performed to identify medicinal herbs' potential action to protect against free radical attack.

Ginseng extract is known to exhibit free radical-scavenging activity with respect to radicals such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), carbon-centered radical, $O_2^{\bullet-}$, $ONOO^-$, and •OH. Although there have been many reports on ginseng's action to protect against free radical attack, ginseng has been found to possess no $^{\bullet}NO$-scavenging activity (*20,32,46*). According to the authors' own *in vitro* studies of free radical-scavenging activity with WG, RG, and HPG (*37,47*), WG had no or little effect on $^{\bullet}NO$-scavenging activity but RG and HPG displayed stronger inhibitory effects than WG, as shown in Figure 2A. In addition, HPG exhibited greater $O_2^{\bullet-}$-scavenging activity than WG and RG; however, there was no difference in the $O_2^{\bullet-}$-scavenging activity of WG and RG (Figure 2B). In particular, HPG effectively scavenged $ONOO^-$ and •OH in a concentration-dependent manner, as shown in Figure 2C and D. Therefore, the free radical-scavenging activity of ginseng increased as a result of heat-processing (*37,47*), and HPG is suggested to have stronger action to protect against reactive oxygen species (ROS) related to tissue damage than conventional ginseng products.

3. Identification of free radical-scavenging active components of HPG

Although the beneficial effects of ginseng with respect to free radical attack and their enhancement by heat-processing have been well documented, the quality of ginseng is important to ensure its safety and efficacy (*48*). The contents of ginseng change with the cultivation period, location, harvesting time, or the parts utilized, and there are wide variations in pharmacological activity and components such as ginsenoside in the ginseng (*49-51*). The employment

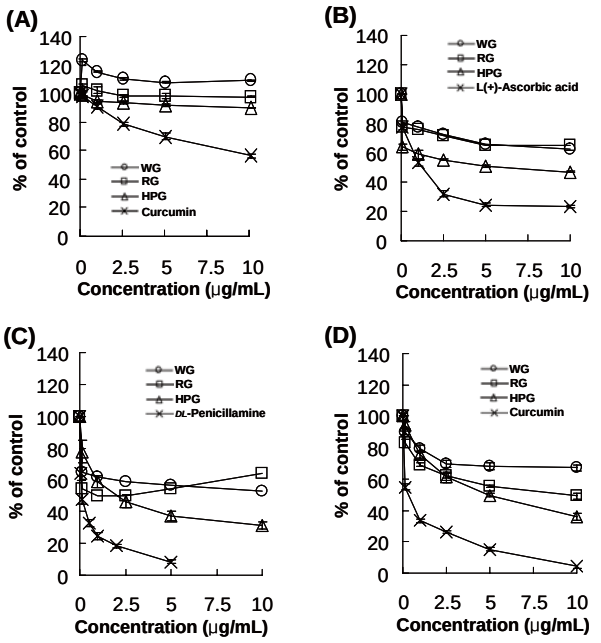


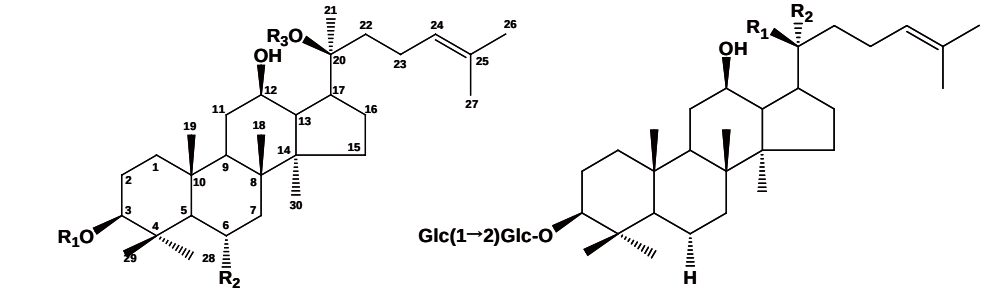
Figure 2. Effects of ginsengs on (A) $^{\bullet}NO$, (B) $O_2^{\bullet-}$, (C) $ONOO^-$, and (D) •OH production.

of standardized extracts of ginseng with specified bioactive constituents has been suggested to be the first step in assessing its efficacy and safety and ensuring optimal quality control of ginseng products (*23*). Therefore, the active components of ginseng produced by heat-processing were investigated by elucidating changes in chemical and free radical-scavenging activity.

3.1. Changes in the chemical and free radical-scavenging activity of ginsenosides

Ginsenosides, unique constituents of ginseng, are glycosides of thirty carbon derivatives of the triterpenoid dammarane, as shown in Figure 3. They have a hydrophobic four-ring steroid-like structure with hydrophilic sugar moieties. About 30 different types of ginsenosides have been isolated and identified from the root of *Panax ginseng*. Each also has at least two (carbon-3 and -20) or three (carbon-3, -6 and -20) hydroxyl groups, which are free or bound to monomeric, dimeric, or trimeric sugars (*6*,*52*).

The HPLC profile of each prepared ginseng extract is illustrated in Figure 4. WG shows typical ginsenosides consisting of Re, Rg_1, Rb_1, Rc, and Rb_2. In the case of RG, the contents of polar ginsenosides (peaks 1 and 2) decreased slightly and less-polar ginsenosides (peaks 5-8) increased. Moreover, major polar ginsenosides (peaks 1-4) in WG decreased greatly and less-polar ginsenosides (peaks 5-10) became major constituents in HPG. This elevation in the content of less-polar ginsenosides was also shown in a heat-processing model experiment using glycine-Rb_2 (Figure 5 and 6), that is, Rb_2 was gradually changed into 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rk_1, and Rg_5 by heat-processing at 100 and 120°C (Figure 5) (*53*). 20(*R*)-ginsenosides and 20(*S*)-



Rb_1 : R_1 = Glc(2←1)Glc, R_2 = H, R_3 = Glc(6←1)Glc
Rb_2 : R_1 = Glc(2←1)Glc, R_2 = H, R_3 = Glc(6←1)Ara(p)
Rc : R_1 = Glc(2←1)Glc, R_2 = H, R_3 = Glc(6←1)Ara(f)
Rd : R_1 = Glc(2←1)Glc, R_2 = H, R_3 = Glc
Re : R_1 = H, R_2 = O-Glc(2←1)Rha, R_3 = Glc
Rg_1 : R_1 = H, R_2 = O-Glc, R_3 = Glc

20(*R*)-Rg_3 : R_1 = CH_3, R_2 = OH
20(*S*)-Rg_3 : R_1 = OH, R_2 = CH_3

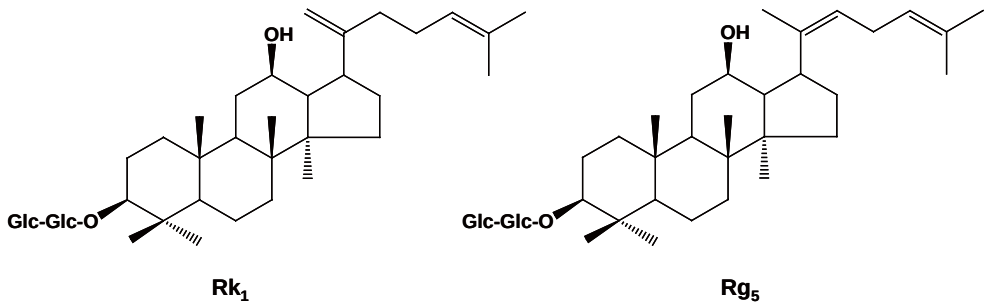


Figure 3. Structures of ginsenosides. –Glc: *D*-glucopyranosyl, -Rha: *L*-rhamnopyranosyl, -Ara(p): *L*-arabinopyranosyl, -Ara(f): *L*-arabinofuranosyl.

ginsenosides are epimers of each other depending on the position of the hydroxyl group on carbon-20. This epimerization is known to occur by the selective attack of the hydroxyl group after the elimination of a glycosyl residue at carbon-20 during the steaming process (*6*,*52*). In addition, more less-polar ginsenosides such as Rk_1 and Rg_5 are known to be easily produced by the elimination of H_2O at carbon-20 of Rg_3 under high pressure and temperature conditions, like in autoclaving

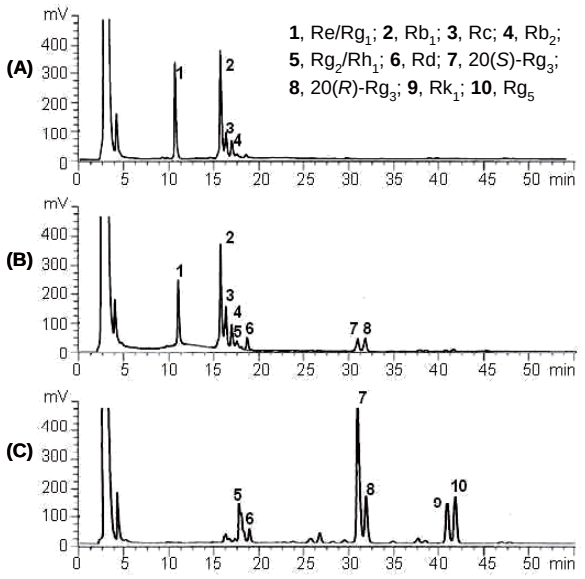


Figure 4. HPLC-ELSD chromatogram of the total ginsenoside fractions of (A) WG, (B) RG, and (C) HPG.

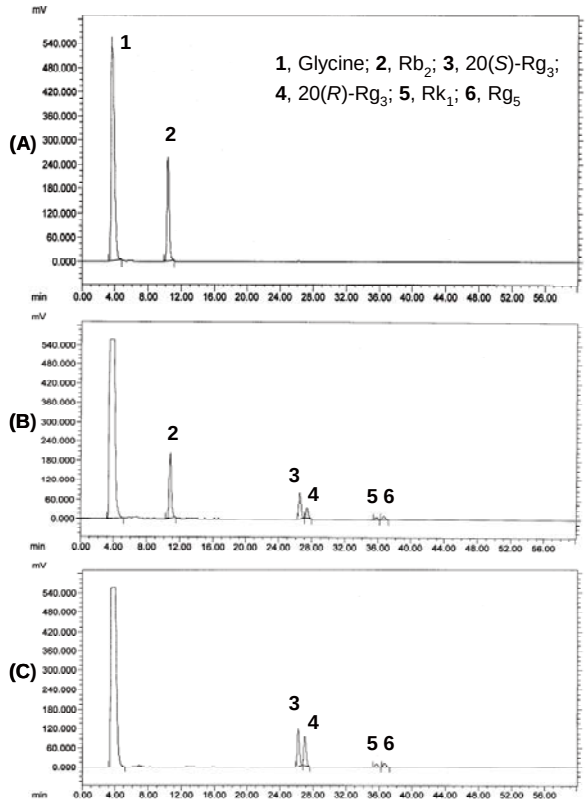


Figure 5. HPLC chromatograms of (A) glycine-Rb_2 mixture, (B) glycine-Rb_2 mixture steamed at 100°C for 3 h, and (C) glycine-Rb_2 mixture steamed at 120°C for 3 h.

(Figure 6) (*25*).

Despite the long history of ginseng research, there have been virtually no *in vitro* reports describing the direct free radical-scavenging mechanisms of ginsenosides. Table 1 shows the •NO-scavenging activity of ginsenoside-Re, Rg_1, Rb_1, 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rk_1, and Rg_5, but no ginsenosides displayed •NO-scavenging activity or increased •NO generation in high concentrations (*47*). However, the *n*-BuOH fraction, mainly consisting of ginsenosides, displayed the strongest activity based on •OH-scavenging activity-guided fractionation of HPG when determined with an electron spin resonance spectrometer (ESR) (Figure 7). The *n*-BuOH fraction was analyzed by HPLC (Figure 8A), and relatively high contents of four ginsenosides, 20(*S*)-Rg_3 32.8%, 20(*R*)-Rg_3 7.3%, Rk_1 15.7%, and Rg_5 18.6%, were detected and isolated, all known to be major ginsenoside constituents of HPG (*32*,*33*). Among them, 20(*S*)-Rg_3 displayed the strongest activity, and the next were, in decreasing order, Rg_5, 20(*R*)-Rg_3, and Rk_1 at a concentration of 0.5% (Figure 8B). Therefore, 20(*S*)-Rg_3 and Rg_5 were suggested to be the main •OH-scavenging active components of HPG (*53*,*54*).

According to the correlation between the structure

Table 1. Effects of ginsenosides on •NO production

Material	Concentration (μg/mL)	•NO (μM)	Production %
Re	12.5	13.6 ± 0.1	101.9
	25	13.5 ± 0.3	101.3
	50	13.6 ± 0.3	102.4
	125	14.6 ± 0.2[c]	109.9
	250	15.1 ± 0.1[c]	113.5
Rg_1	12.5	13.7 ± 0.2[b]	103.1
	25	13.9 ± 0.2[c]	104.6
	50	14.9 ± 0.1[c]	111.9
	125	15.5 ± 0.1[c]	116.8
	250	16.7 ± 0.1[c]	125.8
Rb_1	12.5	13.1 ± 0.1	98.5
	25	13.6 ± 0.1	102.2
	50	14.0 ± 0.1[c]	105.1
	125	15.3 ± 0.2[c]	115.2
	250	16.6 ± 0.1[c]	124.7
20(*S*)-Rg_3	12.5	12.5 ± 0.2[c]	94.0
	25	13.1 ± 0.1	98.8
	50	13.7 ± 0.3[c]	102.9
	125	14.4 ± 0.1[c]	108.4
	250	15.5 ± 0.1[c]	112.5
20(*R*)-Rg_3	12.5	12.9 ± 0.2	96.7
	25	13.0 ± 0.1	97.5
	50	14.1 ± 0.1[c]	106.4
	125	14.7 ± 0.1[c]	110.4
	250	15.5 ± 0.2[c]	116.9
Rk_1	12.5	14.4 ± 0.2[c]	108.2
	25	14.1 ± 0.2[c]	105.8
	50	14.3 ± 0.4[c]	107.7
	125	14.2 ± 0.3[c]	106.4
	250	13.9 ± 0.4[a]	104.2
Rg_5	12.5	13.7 ± 0.1[a]	102.8
	25	13.0 ± 0.3	97.9
	50	13.0 ± 0.1	97.8
	125	13.5 ± 0.1	101.5
	250	14.4 ± 0.1[c]	108.1
Control	-	13.3 ± 0.3	100.0

Significance: [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ compared with control values.

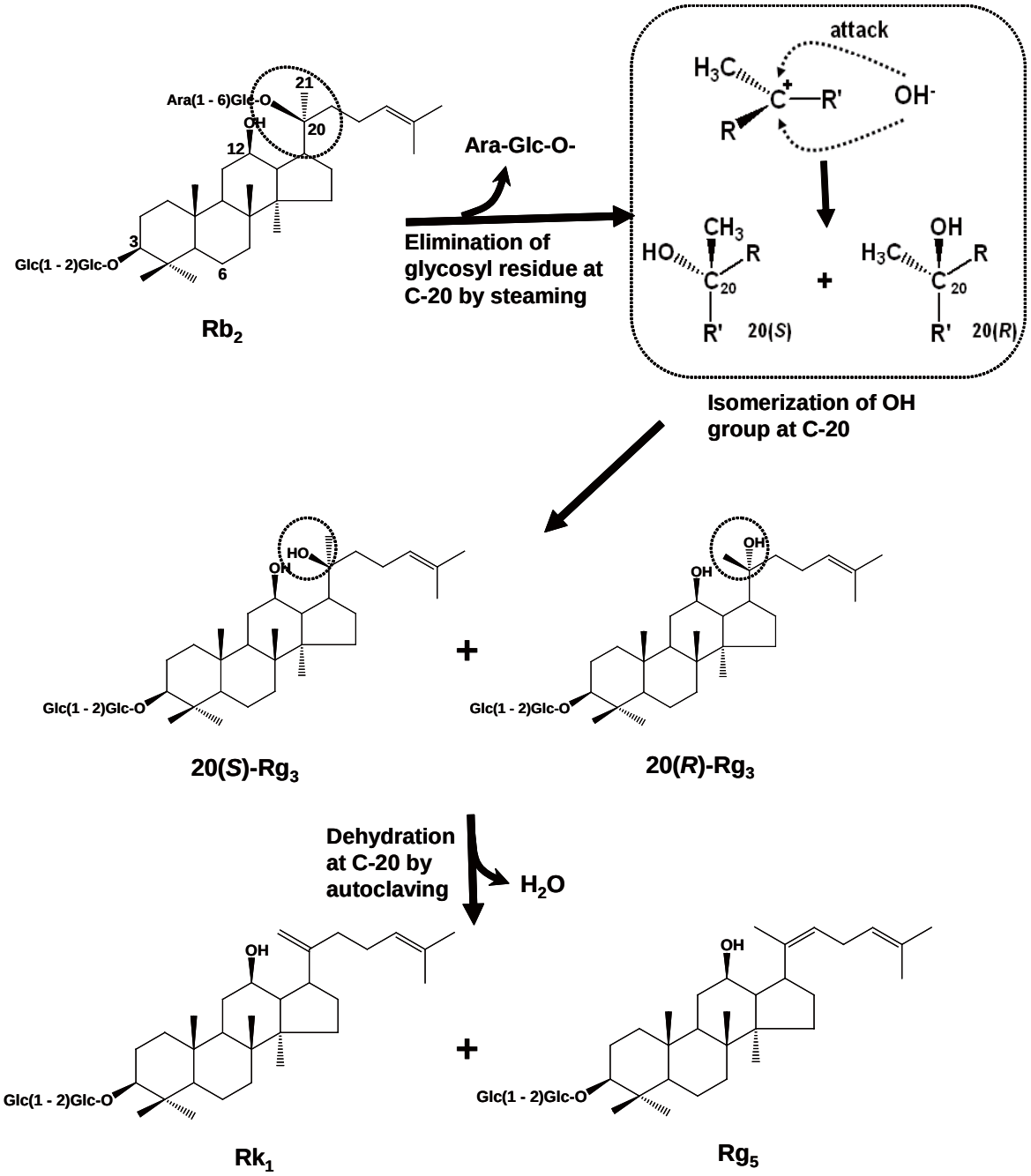


Figure 6. Structural changes of ginsenoside Rb_2 brought about by heat-processing. –Glc, *D*-glucopyranosyl; -Ara, *L*-arabinopyranosyl.

and •OH-scavenging activity of ginsenosides (Rb_1, Rb_2, Rc, Rd, Re, Rg_1, 20(*S*)-Rg_3, 20(*R*)-Rg_3) isolated from *Panax ginseng*, 20(*S*)-Rg_3 displayed the strongest activity, and the next were, in decreasing order, Rb_1, Rg_1, and Rc (Figure 9A). These ginsenosides (2 mM) displayed inhibitory activity with respect to •OH generation 50% greater than that of the control. The other ginsenosides such as Rb_2 and Rd displayed comparably lower activity, and Re and 20(*R*)-Rg_3 exhibited no significant inhibition (Figure 9A). The difference in the structures of ginsenosides is solely due to the position and type of sugar moieties connected to the ring of the triterpenoid dammarane, and this mutual interaction was suggested to play an important role in the antioxidant effects of ginsenosides (*55*).

•OH-scavenging can be accomplished by direct scavenging or *via* the prevention of •OH formation through the chelation of free metal ions or conversion of H_2O_2 to other harmless compounds (*56*). Several ginsenosides used in this study displayed no or weak H_2O_2- and DPPH radical-scavenging activity at a concentration of 2 mM based on preliminary studies (unpublished). Based on the ferrous metal ion-chelating activity tests of several ginsenosides, the •OH-scavenging mechanism of ginsenosides was related more to their transition metal-chelating activity than *via* the direct scavenging of free radicals (Figure 9) (*57*). As weakly •OH-scavenging Rb_2 was gradually changed into strongly •OH-scavenging 20(*S*)-Rg_3 and Rg_5 by heat-processing (Figure 6), the

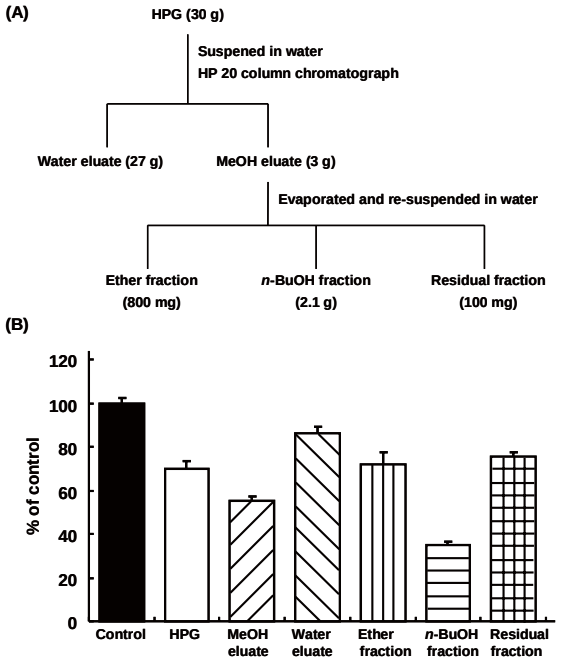


Figure 7. (A) Fractionation of HPG extract. (B) The graph compares the effects of HPG and its fractions (1 mg/mL) on •OH production.

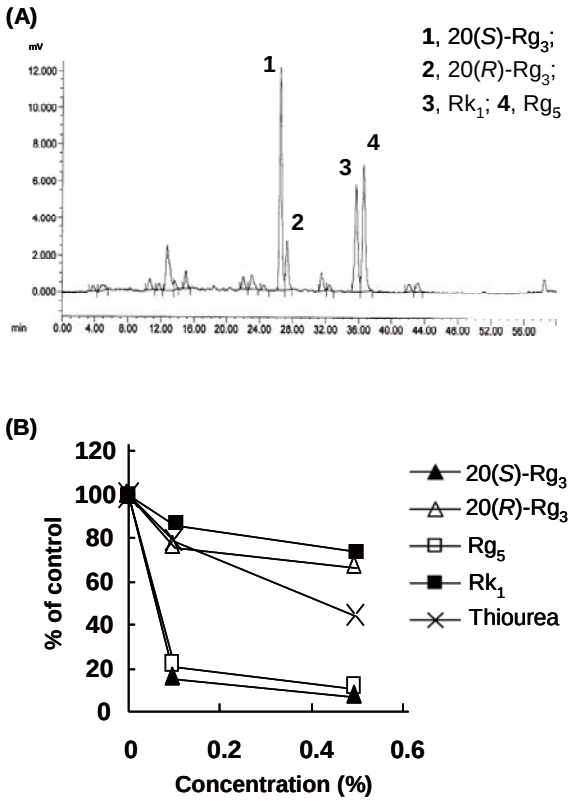


Figure 8. (A) HPLC chromatogram of *n*-BuOH fraction of HPG. (B) The graph compares the effects of less-polar ginsenosides on •OH production.

chemical changes of ginsenosides are thought to be pivotal to enhancing the antioxidant activity of ginseng by ferrous metal ion-chelating activity, and dietary nutrients containing metal chelators have received much attention because of their preventive antioxidant activity (*38*,*41*,*58*).

3.2. Changes in the chemical and free radical-scavenging activity of phenolic acids and maltol

In general, the main pharmacologically active constituents of ginseng are believed to be ginsenosides, but researchers also have investigated phenolic compounds as bioactive constituents of ginseng (*59*,*60*). Phenolic compounds are commonly found in plants, and they have been reported to exhibit multiple biological effects, including antioxidant activity. Many studies have revealed that the phenolic content of plants can be correlated with their antioxidant activity (*61*,*62*). In addition, the free radical-scavenging activity of ginsenosides was, according to previous research, limited to •OH by ferrous metal ion-chelating activity (*53*,*54*,*57*), which fails to explain the increased $^{\bullet}$NO-, $O_2^{\bullet-}$-, and $ONOO^-$-scavenging activity of ginseng as a result of heat-processing with only ginsenosides. However, the highly reactive $ONOO^-$-scavenging ability of HPG was closely related to its ether fraction containing phenolic compounds based on $ONOO^-$-scavenging activity-guided fractionation of HPG (Figure 7A) (unpublished). Therefore, focus was turned to the phenolic compounds of ginseng, *i.e.* non-saponin components such as salicylic acid, vanillic acid, *p*-coumaric acid, and maltol (Figure 10). They are also known to be the principal antioxidant components of WG and RG (*59*,*60*).

Based on GC-MS analyses of ginseng extracts, four compounds were detected in the order of increasing retention time: maltol, salicylic acid, vanillic acid, and *p*-coumaric acid (Figure 11). Maltol was found to be the compound increased most by heat-processing in comparison to the other 3 phenolic acids. In the case of phenolic acids, their contents increased in RG in comparison to those in WG (Table 2). The *p*-coumaric and vanillic acid content is known to be greater in RG than in WG (*63*); however, the contents of these compounds did not continuously increase in HPG. The decreases in some phenolic content in HPG were thought to be caused by thermal decomposition or structural changes in phenolic compounds under high pressure and temperature conditions. In particular, salicylic acid easily decomposes thermally and has a propensity to undergo hydroxylation (*64*,*65*), and the level of total phenolic compounds has been reported to decrease with heat treatment in malt (*66*). Although there were changes in the content of salicylic, vanillic,

Table 2. Quantity of maltol and phenolic acids in WG, RG, and HPG (mg/100 g)

	WG	RG	HPG
Maltol	2.6	10.7	94.0
Salicylic acid	0.1	1.6	0.4
Vanillic acid	0.4	1.0	0.6
p-Coumaric acid	0.5	0.6	0.4

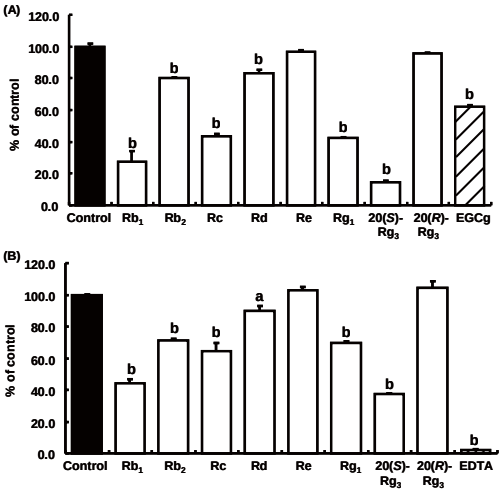


Figure 9. Comparisons of (A) •OH-scavenging and (B) ferrous metal ion-chelating activity of ginsenosides at 2 mM. $^{a}p < 0.01$, $^{b}p < 0.001$ in comparison to control values.

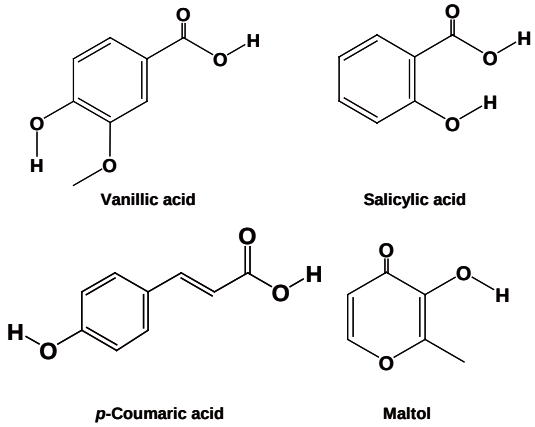


Figure 10. Structures of phenolic compounds and maltol.

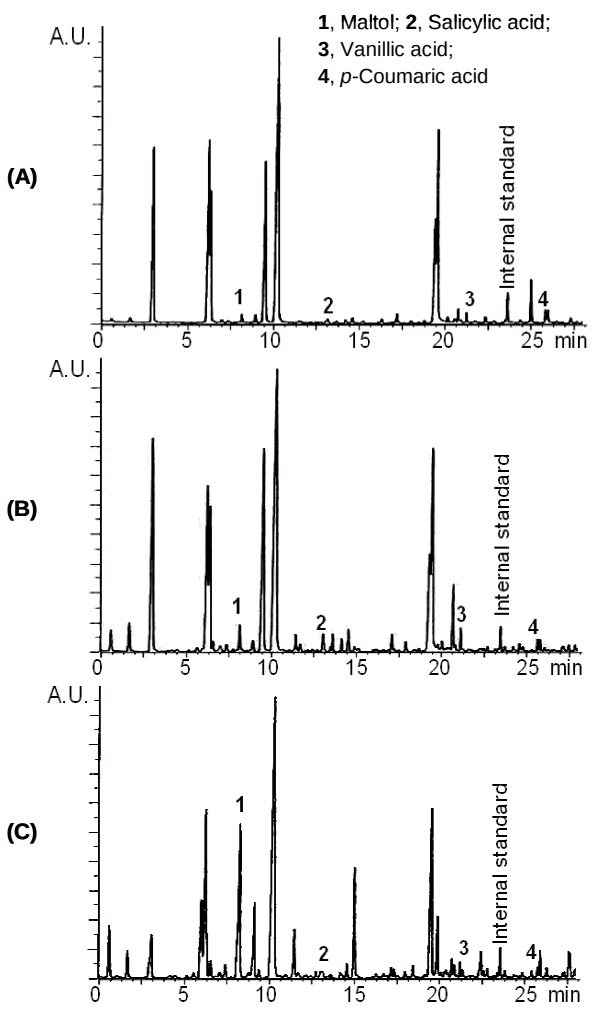


Figure 11. GC-MS chromatograms of phenolic fractions of (A) WG, (B) RG, and (C) HPG.

and *p*-coumaric acids in WG, RG, and HPG, these were considerably fewer than those of maltol (Table 2).

When each phenolic compound and maltol (10 μg/mL) were added, vanillic acid displayed the strongest $^{\bullet}$NO-scavenging activity, and the next was *p*-coumaric acid, but salicylic acid and maltol exhibited no activity (Figure 12A). Similarly, vanillic acid displayed the strongest $O_2^{\bullet -}$-scavenging activity, and the next were, in decreasing order, salicylic acid, maltol, and *p*-coumaric acid (Figure 12B). Vanillic acid, maltol, and *p*-coumaric acid displayed strong $ONOO^-$-scavenging activity, but the effect of salicylic acid decreased with the increase in concentration (Figure 12C). In particular, highly toxic •OH production was reduced to 13.1, 13.2, 39.0, and 74.2% of the control value by maltol, *p*-coumaric acid, vanillic acid, and salicylic acid, respectively (Figure 12D). The free radical-scavenging activity of phenolic compounds in ginseng was in accordance with the reported structure and activity relationships of simple phenolic acids. The number and position of hydroxyl groups or hydrogen-donating groups in the phenolic molecular structures affect their antioxidant activity (*37*,*47*,*65*). On the other hand, the strong $ONOO^-$- and •OH-scavenging activity of maltol was thought to result from ferrous metal ion-chelating action owing to its hydroxyketone structure (*67*).

Based on the quantitative analysis of phenolic contents and radical-scavenging activity tests, maltol was the main free radical-scavenging component of HPG among the 4 principal antioxidant phenolic compounds in ginseng. However, this fails to explain the free radical-scavenging activity of HPG with only phenolic acids and maltol. The most convincing proposal involves Maillard reaction products (MRPs), thought to be the major components in various crude drugs or foods that correlated with enhanced free radical-scavenging activity as a result of heat treatment. MRPs in ginseng were reported to increase as a result of heat-processing; these compounds are arg-fru-glc, arg-fru, maltol, maltol-3-*O*-β-*D*-glucoside, and so on (*68*,*69*). A detailed study of MRPs in HPG has not been conducted yet, but maltol is a typical marker of the Maillard reaction (*70*). Therefore, further studies of free radical-scavenging active components such as MRPs or new products generated during heat-processing are

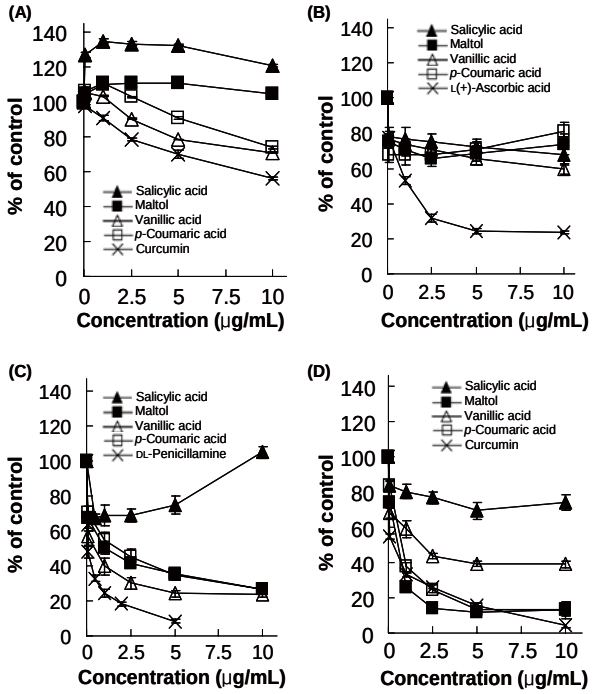


Figure 12. Effects of phenolic compounds on (A) $^{\bullet}NO$, (B) $O_2^{\bullet-}$, (C) $ONOO^-$, and (D) $\bullet OH$ productions.

underway.

4. HPG's protective action against lipopolysaccharide (LPS)-induced hepatic damage

LPS has been shown to increase the constitutive release of $^{\bullet}NO$ by the endothelium and the activity of inducible nitric oxide synthase (iNOS) (*71,72*). In addition, $^{\bullet}NO$ stimulates H_2O_2 and $O_2^{\bullet-}$ production by mitochondria (*73*), and, in turn, these ROS participate in the up-regulation of iNOS expression *via* nuclear factor-kappa B (NF-κB) activation (*74,75*). NF-κB is normally present in the cytoplasm of eukaryotic cells as an inactive complex with the inhibitory protein, IκB. When cells are exposed to various external stimuli, such as ROS or LPS, IκB undergoes rapid phosphorylation with subsequent ubiquitination, leading to the proteosome-mediated degradation of this inhibitor. The functionally active NF-κB exists mainly as a heterodimer consisting of subunits of the Rel family (*e.g.*, Rel A or p65, p50, p52, c-Rel, v-Rel, and Rel B) and translocates to the nucleus, where it binds to specific consensus sequences in the promoter or enhancer regions of target genes, thereby altering their expression (*74,76-78*). ROS also cause the peroxidation of membrane phospholipids, which can alter membrane fluidity and lead to the loss of cellular integrity. Thus, the impaired activity of mitochondrial enzymes leads to decreased energy levels and failure of organs such as of the heart, kidney, lung, and liver (*42,79*). On the other hand, heme oxygenase-1 (HO-1) is believed to play an important role in attenuating tissue injury caused by inflammatory stimuli, and the up-regulation of HO-1 has been shown to protect against LPS-induced cardiovascular collapse or liver damage (*80,81*).

Among the major organs affected by LPS toxicity, the liver plays an important physiological role in LPS detoxification (*82,83*). In addition, there has been growing interest regarding the effects of RG-specific ginsenosides on hepatotoxicity and the antioxidative enzyme activity of the liver because the metabolites of ginsenosides are known to be esterified with fatty acid in the liver without structural variation, and the esterified metabolites accumulate in the liver (*84-87*). Therefore, the effect of HPG, which has increased RG-specific ginsenoside content, on LPS-induced liver injury in rats was examined to identify its hepatoprotective action.

To examine the hepatoprotective action of HPG, male Wistar rats were divided into four groups while avoiding any inter-group differences in body weight. The control groups were given water, while the other groups were orally administered HPG extracts at a dose of 50 or 100 mg/kg body weight daily using a stomach tube. After 15 consecutive days of administration, the rats were given intravenous LPS (from *Escherichia coli* serotype 055: B5, Sigma Chemical Co., USA), at 5 mg/kg body weight. At 6 h after the LPS challenge, the rats were sacrificed, and blood and liver samples were collected.

As shown in Table 3, there was a significantly elevated serum nitrite/nitrate (NO_2^-/NO_3^-) level in LPS-treated control rats. However, HPG administration either failed to ameliorate or only slightly (not significantly) increased this increased level. The fact that the administration of ginseng increases the serum

Table 3. NO_2^-/NO_3^- level of serum

Group	Dose (mg/kg body weight/day)	NO_2^-/NO_3^- (μM)
Normal	-	0.3 ± 0.2
LPS treatment		
Control	-	216.5 ± 1.0[a]
HPG	50	217.4 ± 0.7[a]
HPG	100	223.5 ± 0.1[a]

Values are the mean ± SE, [a]$p < 0.001$ compared with normal rats.

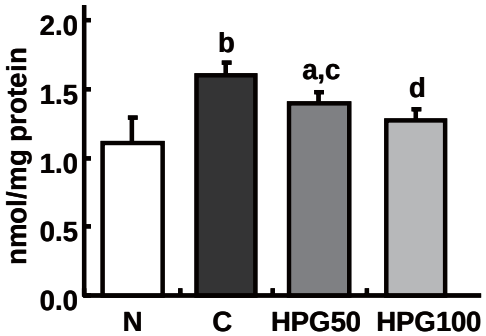


Figure 13. TBA-RS levels in hepatic mitochondria. N, normal rats; C, LPS-treated control rats; HPG50, HPG (50 mg/kg body weight/day)-administered and LPS-treated; HPG100, HPG (100 mg/kg body weight/day)-administered and LPS-treated. [a]$p < 0.01$, [b]$p < 0.001$ *vs.* normal rats; [c]$p < 0.05$, [d]$p < 0.001$ *vs.* LPS-treated control rats.

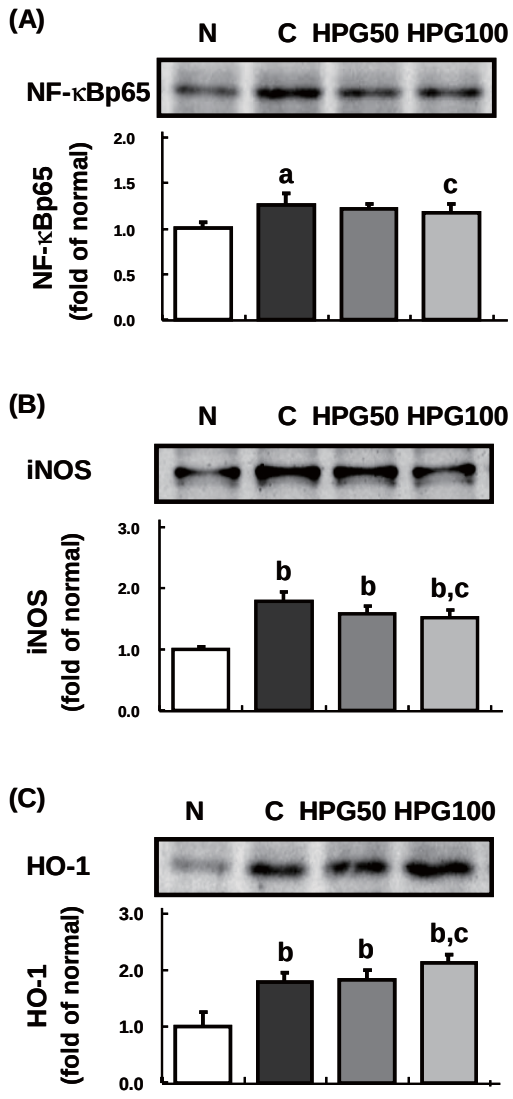


Figure 14. Effects of HPG on (A) NF-κBp65, (B) iNOS, and (C) HO-1 expressions. N, normal rats; C, LPS-treated control rats; HPG50, HPG (50 mg/kg body weight/day)-administered and LPS-treated; HPG100, HPG (100 mg/kg body weight/day)-administered and LPS-treated. $^{a}p < 0.05$, $^{b}p < 0.001$ in comparison to normal rats; $^{c}p < 0.05$ in comparison to LPS-treated control rats.

NO_2^-/NO_3^- level related to vascular relaxation has been well documented (*88,89*), and the slightly increased serum NO_2^-/NO_3^- level of HPG-administered groups in this study was also thought to have been due to the same reason.

Figure 13 shows the thiobarbituric acid reactive substance (TBA-RS) level of hepatic mitochondria, implying an index of endogenous lipid peroxidation caused by oxidative stresses. The TBA-RS level in control rats was significantly higher than that in normal rats, but it was reduced by HPG administrations in a dose-dependent manner. This amelioration of the TBA-RS level suggests the protective effect of HPG on hepatic mitochondria and its function. In addition, Figure 14 shows Western blot analyses in liver tissues related to oxidative stress. The oxidative stress-related proteins NF-κB, iNOS, and HO-1 are up-regulated in the liver during LPS challenge but have opposite roles. That is, down-regulation of NF-κB and iNOS suggests a hepatoprotective effect, but HO-1 is known to exhibit a protective role *via* up-regulation (*72,79,81*). The NF-κBp65 and iNOS levels in the LPS-treated control rats were significantly higher than those of normal rats, but these elevated levels were reduced by HPG administrations in a dose-dependent manner. Although iNOS is well known to be regulated by NF-κB (*75,76*), the deactivation of NF-κBp65 by HPG administration was thought to be too weak to have much of a significant effect on the inhibition of iNOS. Otherwise, the effect of HPG might be involved in transcriptional factors other than NF-κBp65 and post-transcriptional cytokines related to the induction of iNOS (*79*). Therefore, inhibition of the elevated iNOS level was thought to be partially related to the deactivation of NF-κBp65 as a result of HPG administration. On the other hand, the HO-1 level in LPS-treated control rats was significantly higher than that in normal rats, and it was more significantly up-regulated by the 100 mg/kg body weight/day of HPG administration. This differential modulation of iNOS and HO-1 by HPG administration appears to have a protective effect against oxidative stress in accordance with the hepatoprotective effect of ketamine on LPS-induced hepatic injury in rats (*81*). On the other hand, the anti-inflammatory effect of 20(*S*)-protopanaxadiol (20(*S*)-PPD), a metabolite of ginsenoside, was suggested to be mediated by the inactivation of NF-κB, suppression of iNOS, and induction of HO-1 (*90*) based on the study of the effect of 20(*S*)-PPD on LPS-induced RAW 264.7 cells. In addition, maltol was suggested to be a functional agent that prevents oxidative damage in the brain of mice by reducing increased TBA-RS levels (*91*). Although the synergetic antioxidant effects of multiple constituents of HPG cannot be ignored, HPG clearly has an antioxidant effect on liver injury related to acute oxidative stress, and this activity may be related to the increase in antioxidant components as a result of heat-processing (*92*).

5. HPG's action to protect against streptozotocin (STZ)-induced diabetic renal damage

Diabetes mellitus is characterized by hyperglycemia. An abnormally elevated blood glucose level causes oxidative stress and the formation of advanced glycation endproducts (AGEs), which have been closely linked to diabetic complications such as neuropathy, retinopathy, and nephropathy (*78,93*). In particular, diabetics are at increased risk for several types of kidney disease, and the predominant cause of end-stage renal disease in this disorder is diabetic nephropathy (*94,95*). Many attempts have been made to improve the treatment of diabetes. Various kinds of hypoglycemic drugs or insulin are now available for the control of hyperglycemia, but modern medicine offers

no satisfactory therapy without undesirable side effects or contraindications (*96*). Prevention of the occurrence and progression of diabetic nephropathy is crucial. Therefore, great effort has been focused on finding a novel therapeutic agent for diabetic nephropathy in traditional and herbal medicines that produce no toxic effects (*97-100*).

Antioxidants are known to protect against glycation-derived free radicals and may have a therapeutic potential (*78,93*). HPG displayed stronger $^{\bullet}NO$-, $O_2^{\bullet -}$-, $ONOO^-$-, and •OH-scavenging activity than WG and RG, as mentioned above. However, there are virtually no reports concerning the effect of HPG on diabetic oxidative stress, and the enhanced radical-scavenging activity of HPG is thought to be beneficial with respect to diabetic oxidative damage caused by hyperglycemia. Therefore, HPG's protective action against renal damage caused by oxidative stress or the formation of AGEs in diabetes and its molecular biological mechanism were investigated.

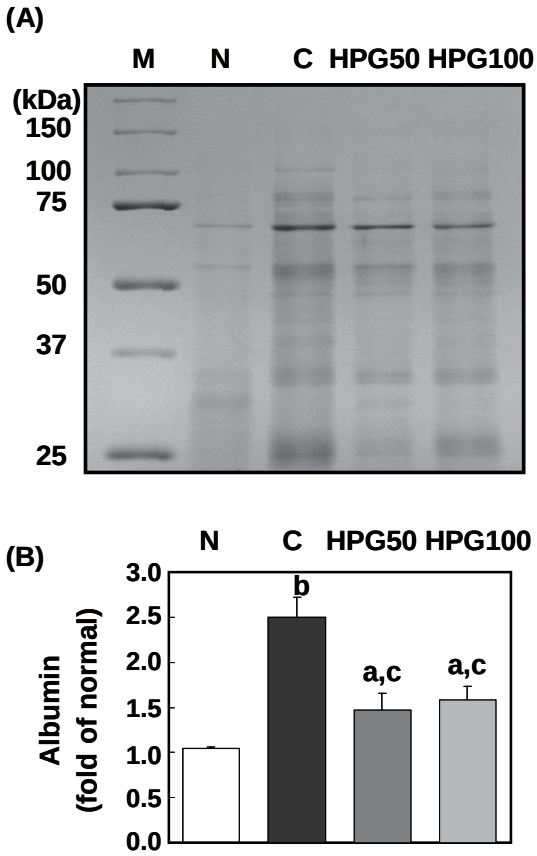


Figure 15. SDS-PAGE pattern of urinary protein. M, protein marker; N, normal rats; C, diabetic control rats; HPG50, diabetic rats treated with HPG (50 mg/kg BW/day); HPG100, diabetic rats treated with HPG (100 mg/kg BW/day). $^{a}p < 0.05$, $^{b}p < 0.001$ in comparison to normal rats; $^{c}p < 0.01$ in comparison to diabetic control rats.

To obtain Type 1 (insulin dependent) diabetic rats, STZ (50 mg/kg body weight) was injected intraperitoneally into male Wistar rats. Ten days after the injection, the glucose level of blood from the tail vein was determined and the rats were divided into three groups, avoiding any inter-group differences in blood glucose levels and body weights. The control group was given water, while the other groups were given an HPG extract orally at a dose of 50 or 100 mg/kg body weight daily using a stomach tube. After administration for 15 consecutive days, urine, blood, and kidneys were collected.

The destruction of β-cells and disorder of insulin secretion in the diabetic state causes physico-metabolic abnormalities such as a decrease in body weight gain and increases in food and water intake and urine volume. The STZ-induced diabetic rats in this study also displayed these changes. However, HPG administration significantly reduced water intake and urine excretion levels, suggesting that HPG would improve the physiological abnormalities associated with diabetes (Table 4).

Hyperglycemia, a primary characteristic of diabetes, is mainly attributed to diabetic oxidative stress caused by several factors. Hyperglycemia leads to the overproduction of free radicals by the nonenzymatic glycation of proteins through the Maillard reaction, and these free radicals exert deleterious effects on the function of β-cells vulnerable to oxidative stress (*101,102*). In addition, hyperglycemia can degrade antioxidant enzyme defences, thereby allowing ROS to cause cellular and tissue damage. As shown in Table 5, the blood glucose levels in diabetic rats significantly decreased in rats fed HPG at a dose of 100 mg. In addition, among the renal function parameters, HPG administration reduced the abnormally increased urinary albumin level in diabetic rats (Figure 15). The urinary protein level slightly increased in diabetic rats but was significantly reduced by HPG administrations. On the other hand, there were no remarkable ameliorations in serum urea nitrogen and creatinine clearance (Ccr) levels (Table 5). The sign of early diabetic nephropathy is an increased urinary albumin level, and advanced diabetic nephropathy is characterized by proteinuria and decreasing Ccr levels (*103*). Therefore, early

Table 4. Physico-metabolic symptoms

Group	Dose (mg/kg BW/day)	Body weight (initial, g)	Body weight (final, g)	Body weight (gain, g)	Food intake (g/day)	Water intake (mL/day)	Urine volume (mL/day)
Normal	-	239.2 ± 3.6	313.3 ± 11.0	72.8 ± 6.6	18.8 ± 0.8	35.9 ± 3.1	15.2 ± 1.3
Diabetic							
Control	-	196.9 ± 4.6	227.0 ± 5.8[a]	27.2 ± 5.0	29.1 ± 0.9[a]	147.1 ± 6.0[a]	120.2 ± 5.2[a]
HPG	50	196.7 ± 7.6	222.8 ± 11.5[a]	26.1 ± 5.1	28.1 ± 0.8[a]	134.8 ± 4.2[a,b]	107.9 ± 3.1[a,c]
HPG	100	196.5 ± 5.6	219.0 ± 9.1[a]	19.9 ± 5.2	26.3 ± 0.9[a,c]	124.9 ± 3.0[a,c]	98.8 ± 2.8[a,c]

$^{a}p < 0.001$ compared with normal rats; $^{b}p < 0.01$, $^{c}p < 0.001$ compared with diabetic control rats.

Table 5. Biochemical features of serum and urine

Item	Normal	Diabetic rats		
		control	HPG (50 mg/kg BW/day)	HPG (100 mg/kg BW/day)
Serum glucose, mg/dL	112 ± 4	558 ± 22[c]	526 ± 26[c]	501 ± 14[c,e]
Serum glycosylated protein,nmol/mg protein	15.5 ± 0.5	20.9 ± 0.9[c]	21.6 ± 0.4[c]	15.6 ± 0.7[f]
Serum urea nitrogen, mg/dL	15.0 ± 0.7	26.0 ± 0.6[c]	24.5 ± 1.0[c,d]	25.3 ± 0.9[c]
Serum creatinine, mg/dL	0.31 ± 0.01	0.32 ± 0.01	0.28 ± 0.01[b,f]	0.28 ± 0.01[b,f]
Urinary protein, mg/day	11.2 ± 2.3	13.0 ± 0.6	9.9 ± 1.0[a,f]	8.9 ± 0.7[b,f]
Ccr, mL/kg BW/min	7.73 ± 0.06	7.29 ± 0.38	7.89 ± 0.36	7.86 ± 0.43

[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ compared with normal rats; [d]$p < 0.05$, [e]$p < 0.01$, [f]$p < 0.001$ compared with diabetic control rats.

diabetic renal changes were thought to have occurred in this study but not advanced nephropathy. Furthermore, hyperglycemia results in irreversible tissue damage caused by the protein glycation reaction, which leads to the formation of glycosylated protein and AGEs (*104,105*). The glycosylated serum protein level increased in the present diabetic animal model, which implies that it stimulates the oxidation of sugars, enhancing damage to both sugars and proteins in circulation and the vascular wall, continuing and reinforcing the cycle of oxidative stress and damage. In addition, accumulation of AGEs in the kidney was also observed. Excessive formation and accumulation of AGEs in tissues can alter the structure and function of tissue proteins. In people with diabetes and/or chronic renal failure, AGEs that accumulate in the kidney are responsible for pathological changes including increased kidney weight, glomerular hypertrophy, glomerular basement membrane thickening, and progressive albuminuria (*106*). Moreover, AGEs stimulate free radical mechanisms and induce membrane peroxidation, which in turn increases membrane permeability. Therefore, AGE accumulation in the kidney is regarded as an index of progressive renal damage in diabetic nephropathy. HPG decreased the levels of glycosylated serum protein and renal AGEs significantly (Table 5 and 6), suggesting that it would inhibit oxidative damage and irreversible renal damage caused by protein glycation reaction in diabetes.

A significant increase in TBA-RS, an index of endogenous lipid peroxidation, has been shown in diabetes (*107,108*). Therefore, the measurement of TBA-RS is frequently used to determine the level of oxidative stress in diabetic patients. In addition, the increased lipid peroxidation in the kidney implies the level of susceptibility to diabetic oxidative stress, which then leads to diabetic complications. From this view point, the prevention of lipid peroxidation resulting from oxidative stress is considered to play a crucial role in protection from disorders associated with diabetes. The administration of HPG reduced the renal TBA-RS level significantly and dose-dependently (Table 6). These results suggest that HPG may alleviate oxidative stress associated with diabetic pathology through the inhibition of lipid peroxidation.

To then consider hyperglycemia-induced renal function parameters and tissue damage, Western blot analyses in the kidney related to oxidative stress and AGE formation were performed. NF-κB, cyclooxygenase-2 (COX-2), and iNOS are known to be involved in the pathogenesis of many chronic diseases associated with oxidative stress. COX-2 and

Table 6. Renal AGE and TBA-RS levels

Group	Dose (mg/kg BW/day)	AGEs (AU)	TBA-RS (nmol/mg protein)
Normal	-	0.77 ± 0.02	0.83 ± 0.01
Diabetic			
Control	-	0.96 ± 0.03[a]	1.45 ± 0.11[a]
HPG	50	0.81 ± 0.03[b]	0.90 ± 0.04[b]
HPG	100	0.80 ± 0.01[b]	0.76 ± 0.05[b]

[a]$p<0.001$ compared with normal rats; [b]$p < 0.001$ compared with diabetic control rats.

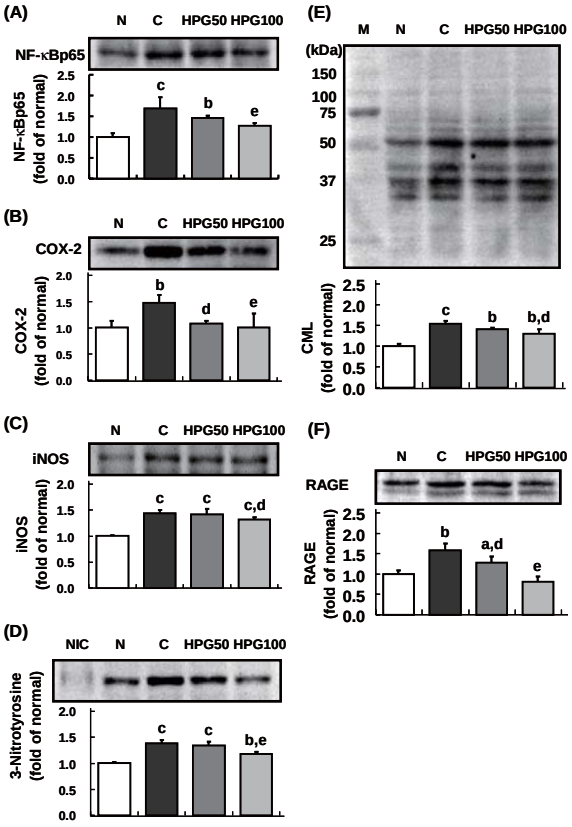


Figure 16. Effects of HPG on (A) NF-κBp65, (B) COX-2, (C) iNOS, (D) 3-nitrotyrosine, (E) CML, and (F) RAGE expressions. NIC, nitrotyrosine immunoblotting control; M, protein marker; N, normal rats; C, diabetic control rats; HPG50, diabetic rats treated with HPG (50 mg/kg BW/day); HPG100, diabetic rats treated with HPG (100 mg/kg BW/day). [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ in comparison to normal rats; [d]$p < 0.05$, [e]$p < 0.01$ in comparison to diabetic control rats.

iNOS expression is known to be significantly enhanced in the kidneys of STZ-induced diabetic rats or mice (*96*). NF-κB is involved in the regulation of COX-2 and iNOS expression. As the current results show, NF-κBp65, COX-2, and iNOS were overexpressed in the diabetic rat kidney, and these overexpressions were concentration-dependently inhibited by HPG administrations (Figure 16A, B, and C). These findings imply that HPG inhibits COX-2 and iNOS expression as a result of the deactivation of NF-κB. Surh *et al*. (*77*) also reported that the anti-tumor promoting activity of HPG and its ingredient 20(*S*)-Rg_3 are mediated through the suppression of intracellular signaling cascades responsible for the activation of NF-κB and subsequent induction of COX-2.

3-Nitrotyrosine, a by-product of the reaction between $ONOO^-$ and proteins, is a potential biomarker for reactive-nitrogen species and increases in diabetic renal tissue. In addition, $ONOO^-$ induces the formation of N^{ε}-(carboxymethyl)lysine (CML) as a result of the oxidative cleavage of Amadori products (*109-111*). CML is a major AGE in human tissues, is known to be a marker of cumulative oxidative stress, and is involved in the development of diabetic nephropathy (*110,112*). Moreover, CML's activation of receptors for AGE (RAGE) results in the activation of NF-κB and the production of proinflammatory cytokines (*78,113*). As shown in Figure 16D, the significantly elevated 3-nitrotyrosine level in diabetic rats was reduced by HPG administrations, suggesting that HPG alleviates oxidative stress by inhibiting the generation of reactive-nitrogen species such as $ONOO^-$. In addition, the elevated renal accumulation of CML and RAGE expression in diabetic rats were significantly reduced in HPG-administered groups (Figure 16E and F). These findings imply that HPG can prevent diabetic nephropathy by inhibiting $ONOO^-$ generation, AGE formation, and RAGE activation.

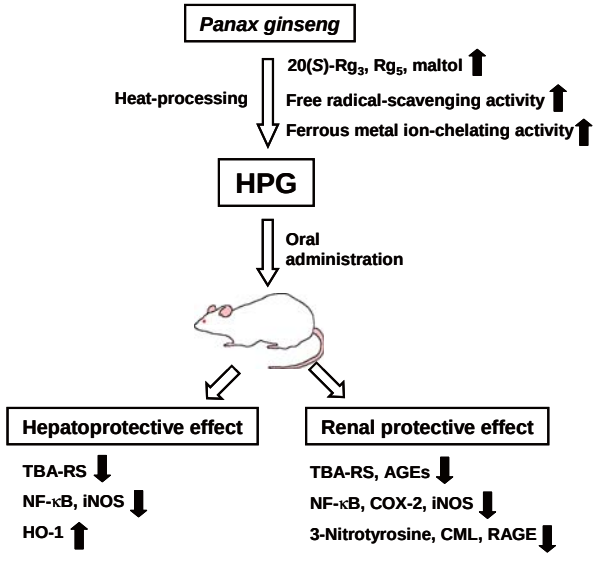


Figure 17. Schematic representation of the therapeutic potential of HPG with respect to oxidative tissue damage.

The current findings strongly suggest that the inhibition of oxidative stress and AGE formation may improve and prevent diabetes-induced tissue damage and diabetic complications. Although the anti-hyperglycemic effect of HPG was not a strong one, HPG displayed considerable action to protect against hyperglycemia-induced renal damage by inhibiting AGE formation and oxidative stress. In particular, HPG suppresses RAGE activation related to NF-κB activation by inhibiting AGE formation. HPG also protects the kidney against diabetic oxidative stress induced by expression of COX-2 and iNOS *via* the deactivation of NF-κB. Furthermore, HPG alleviates oxidative stress in diabetes through the inhibition of lipid peroxidation and the generation of reactive-nitrogen species such as $ONOO^-$. All of this evidence is thought to relate to the enhanced free radical-scavenging activity of ginseng brought about by heat-processing (*114*).

6. Conclusion and perspectives

Chemical and biological activity tests have elucidated the scientific evidence underlying the therapeutic potential of HPG with respect to oxidative tissue damage. Free radical-scavenging active components such as less-polar ginsenosides and maltol in *Panax ginseng* significantly increased depending on the temperature of heat-processing. Based on animal experiments related to oxidative tissue damage, HPG displayed hepatoprotective action by reducing the elevated TBA-RS level, and NF-κB and iNOS protein expression, while increasing HO-1 in LPS-treated rat livers, and HPG also displayed renal protective action by ameliorating physiological abnormalities and reducing elevated TBA-RS, AGEs, NF-κB, COX-2, iNOS, 3-nitrotyrosine, CML, and RAGE protein expression in the diabetic rat kidney (Figure 17). Therefore, HPG clearly has a therapeutic potential with respect to oxidative tissue damage by inhibiting protein expression related to oxidative stress and AGEs. Thus, further investigations with active compounds are underway. This investigation of specified bioactive constituents is important for the development of scientific ginseng-derived drugs as part of ethnomedicine.

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