

Research Paper

Chemopreventive Effects of Korean Red Ginseng Extract on Rat Hepatocarcinogenesis

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Abstract

The objective of this study was to determine a chemopreventive activity of Korean red ginseng extract (KRG) in diethylnitrosamine (DEN) induced hepatocarcinogenesis in rats. After acclimatization for a week, Sprague-Dawley rats were randomized into five groups (n = 15) and fed either KRG (0.5, 1 or 2%) or control diets for 10 weeks. After two weeks of starting of experimental diets, the rats were initiated hepatocarcinogenesis by injection of DEN and were then subjected to two-thirds partial hepatectomy at five-week for developing the medium-term bioassay system. Both 0.5 and 1% KRG diets suppressed the area (55 and 60%; p= 0.0251 and 0.0144) and number (39 and 59%; p= 0.0433 and 0.0012) of glutathione S-transferase placental form (GST-P) positive foci when compared to the DEN-control group. The production of thiobarbituric acid reactive substances (TBARS) was significantly reduced in 0.5 and 1% KRG-treated rats. The supplementation of 1% KRG diet significantly elevated the levels of total glutathione (tGSH) and glutathione-related enzymes including cytosolic glutathione S-transferase (GST) and glutathione peroxidase (GPx) activities. It was also observed in cDNA microarray that the gene expressions (*Cyp2c6*, *Cyp2e1*, *Cyp3a9*, and *Mgst1*) involved in the xenobiotics metabolism via cytochrome P450 signaling pathway were down-regulated in the 1% KRG diet-treated group when compared to the DEN-control. The chemopreventive effects of KRG could be affected by 1) the decrease of lipid peroxidation, 2) the increase of tGSH content and GSH-dependent enzyme activities, and 3) the decrease of the gene expression profile involved in cytochrome P450 signaling pathway. These results suggest that KRG may prove to be a therapeutic agent against hepatocarcinogenesis.

Key words: Korean red ginseng, rat, glutathione S-transferase placental form positive foci, hepatocarcinogenesis, antioxidant.

Introduction

Liver cancer is the second cause of cancer death in men and the sixth in women. The main risk factor for liver cancer is the elevated prevalence of chronic hepatitis B virus infection [1]. It has been shown that a

diet rich in dietary antioxidants and phytochemicals may decrease the risk of hepatocellular carcinoma, a primary malignant cancer of the liver. Hence, identifying the promising chemopreventive agents in diets

and their underlying molecular mechanisms have been considered to be the best strategy to protect against hepatocarcinogenesis [2].

Panax ginseng C.A. Meyer was traditionally used as a medicinal plant in Asian countries, and it has now gained worldwide popularity [3]. Ginseng is identified to contain ginsenosides, phenolic compounds, polysaccharides, and polyacetylenes, which are known to have a chemopreventive effect through antioxidant, apoptotic, and anti-cell proliferation in various cancers [4-8]. Red ginseng is heated *panax ginseng* produced by steaming followed by drying, and contains higher amount of ginsenosides and polyphenolics than white ginseng [9, 10]. The heat processing converts ginsenosides into other types of ginsenosides, including ginsenoside Rh2 and Rg3, and produces the antioxidant agents and phenolic compounds such as maltol [11, 12]. Since Korea red ginseng (KRG) has unique anti-carcinogenic compounds, it has been suggested that KRG has more potent chemopreventive activity than fresh and white ginseng [13]. In fact, it has been demonstrated that KRG extract has a chemopreventive effect on the hepatotoxins-induced liver cancer in rats [13]. Also, KRG, specifically ginsenosides Rg3, Rg5 and Rh2, has been shown to increase apoptosis in human hepatocellular carcinoma cells [14]. Ginsenoside Rh2 exhibited the apoptotic properties through caspase-3 activation in SK-HEP-1 cell lines [15]. Moreover, ginsenoside Rg3 has been shown to induce apoptosis in human hepatocellular carcinoma cells and to inhibit liver cancer growth *in vivo* via alterations of Bcl-2 family proteins [16, 17]. It was reported that compound K [20-O- β -(D-glucopyranosyl)-20(S)-protopanaxadiol], which is an intestinal metabolite of the protopanaxadiol-type ginsenoside, suppressed cell proliferation and induced apoptosis in hepatocellular cancer cell via a Bid-mediated pathway [18].

Although the beneficial effects of KRG are well documented, the administration of high dose of KRG might be detrimental through their toxicity. Several studies have reported that overdose and long-term usage of ginseng are associated with side effects such as hypertension, nausea, diarrhea, insomnia, and headache, known as ginseng abuse syndrome [19, 20]. Ginsenoside Rh2, which is one of active ginsenosides of KRG, is known to have anticancer activities, while it showed cytotoxic effects to human hepatocyte cells [21]. These evidences suggest that comparative studies between various concentrations of red ginseng for chemoprevention of hepatocarcinogenesis need to be performed, and that the proper usage of ginseng on liver cancer has to be established.

The objective of this study is to determine the potential chemopreventive effects of various concen-

trations of KRG extract on hepatocarcinogenesis in rats. We hypothesized that the proper amount of KRG extract may prevent hepatocarcinogenesis through modulation of the liver oxidative environment, but that the chemopreventive effects may differ based on the concentrations. Subsequently, the underlying mechanisms were investigated to determine whether these different concentrations of KRG extract minimize oxidative damage via the modulation of the cellular redox environment on rat hepatocarcinogenesis.

Materials and Methods

Animals

After obtaining Institutional Animal Care and Use Committee (IACUC) approval, male Sprague-Dawley rats (Four week-old) were supplied from the Animal Care Facility (Seoul National University, Seoul, Korea), and were then acclimatized for a week. Rats were randomized into five groups and fed either Korean red ginseng extract (KRG; n = 15/group; 0.5, 1, or 2%) diets or control diet for 10 weeks. Animals were kept in polycarbonate cages under standard conditions (room temperature $23 \pm 2^\circ\text{C}$, relative humidity $55 \pm 5\%$, 12-hr light/dark cycle), given food and water *ad libitum*, recorded daily and weighed weekly. After two weeks of starting of experimental diets, all of the rats except a control group were initiated hepatocarcinogenesis by the injection of DEN (200 mg/kg body weight; Sigma Chemical Co., St. Louis, MO, USA) dissolved in saline. In addition, they were subjected to two-thirds of partial hepatectomy (PH) after 3 weeks according to a modified medium-term bioassay protocol [22]. The control group was treated with saline and a sham operation. This study was terminated at ten weeks, and the liver sections were fixed in 10% neutral buffered formalin. Two aliquots (0.1 and 5g, respectively) of each liver were quickly removed and kept at -80°C for total RNA extraction and glutathione content determination.

Diets

KRG extract was received from Cheong-Kwan-Jang (Seoul, Korea) in high value of commercial concentrated pure extract prepared from Korean red ginseng root (6-year-old *Panax ginseng* C.A Meyer). The moisture of KRG extract was approximately 40%, the amount of crude ginsenoside was 70 mg/g, and the total concentration of ginsenosides was 20 mg/g. The composition of the basal diet is given in Table 1. The KRG extract was substituted for part of cornstarch in the experimental KRG diets. The prepared dietary foods were stored at -20°C .

Table 1. Composition of control and experimental diets (g / 100g)

| Components | Control | 0.5% KRG | 1% KRG | 2% KRG |
|--------------------------|---------|----------|--------|--------|
| KRG extract | - | 0.5 | 1 | 2 |
| Corn starch | 55.2 | 54.7 | 54.2 | 53.2 |
| Casein | 20.00 | 20.00 | 20.00 | 20.00 |
| Corn Oil | 15.00 | 15.00 | 15.00 | 15.00 |
| α -Cellulose | 5.00 | 5.00 | 5.00 | 5.00 |
| Mineral mix ^a | 3.5 | 3.5 | 3.5 | 3.5 |
| Vitamin mix ^a | 1.00 | 1.00 | 1.00 | 1.00 |
| DL-Methionine | 0.30 | 0.30 | 0.30 | 0.30 |

^a Mineral and vitamin mixtures for AIN-76 diet.

Sample preparation

Five gram of liver was homogenized in Tris-HCl buffered solution (pH 7.4) and centrifuged at 10,000×g for 20 min, followed by re-centrifugation of the supernatant at 100,000×g for 60 min. The supernatant was considered as the cytosol, and the pellet as the microsome. The microsomal pellet was re-suspended in 20% glycerol buffered solution. The entire fractionation procedure was conducted at 4°C [23].

Immunohistochemical staining for glutathione S-transferase placental form positive foci (GST-P+ foci)

GST-P+ foci, DEN-initiated lesions, is thought to be a biomarker of hepatocellular carcinoma [24]. At autopsy, 2-3 mm of liver sections were fixed in ice-cold acetone for immunohistochemistry of GST-P+ foci using an anti-mouse GST-P antibody (Medical Biological Laboratories Co, Nagoya, Japan). The avidin-biotin-peroxidase complex method (Vectastain ABC kit, Vector Lab. Inc., Burlingame, CA) was used to visualize GST-P+ foci that reflect putative preneoplastic lesions. The areas and numbers of the GST-P+ foci (> 0.2 mm in diameter) in liver sections were measured using an image analyzer with a microscope (Quantinet 520, Cambridge Instruments, Cambridge, UK) [23].

Determinations of lipid peroxidation

Hepatic lipid peroxidation (thiobarbituric acid reactive substances, TBARS) was determined by the reaction between TBA and malondialdehyde formed from peroxidation of lipids [25]. In short, 200 μ L of 0.375% thiobarbituric acid-15% trichloroacetic acid-0.25 N HCl were added to 100 μ L of rat liver microsomal suspension, and the mixture was incubated in a boiling water bath for 15 min. After that, it was centrifuged at 1,000×g for 10 min. Malondialdehyde in the supernatant was measured at 532 nm. Protein was quantified using a modified Lowry method [26], with bovine serum albumin as a standard.

Determinations of total glutathione (tGSH) contents and GSH-dependent enzymes

tGSH content (both reduced and oxidized glutathione; GSH and GSSG) was measured by the 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and glutathione reductase (GR) recycling procedure. The existing GSSG can be converted to GSH by adding GR and NADPH. After that, tGSH can be determined by measuring the reaction of GSH with DTNB [27]. The tGSH content was expressed as moles of reduced GSH equivalents per liver (g). Glutathione S-transferase (GST) activities in the hepatic cytosolic fraction were determined through monitoring the conjugation of GSH with 1-chloro-2,4-dinitrobenzen (CDNB) at 340 nm by using a dual beam spectrophotometer (Beckman DU650, Beckman Coulter Inc, Miami, FL) [28]. The glutathione peroxidase (GPx) was measured indirectly by a coupled reaction with GR. The GSSG catalyzed by GPx is recycled to GSH by GR and NADPH. Hepatic cytosolic fraction was added in the reaction mixture contained 50 mM Tris-HCl buffer (pH 7.6), 0.1 mM EDTA, 0.25 mM GSH, 1 unit/ml GR, and 0.12 mM NADPH. The reaction was initiated by adding the cumene hydroperoxide, and the oxidation of NADPH to NADP⁺ was monitored at 340 nm [29]. For measuring the cytosolic GR activity, hepatic cytosolic fraction was added in the reaction mixture containing a 0.2 mM potassium phosphate buffer (pH 7.0), 2mM EDTA, 20 mM GSSG, and 2mM NADPH. The oxidation of NADPH to NADP⁺ was monitored at 340 nm [30]. The levels of GPx and GR were defined as the amount of enzyme causing the oxidation of 1 nmol of NADPH (extinction coefficient, 6.22mM⁻¹cm⁻¹) per minute and per mg protein.

cdNA microarray analysis

cdNA microarray analysis was used to examine differential gene expression between 0% and 1% KRG extract group. Total RNA was isolated from 6 tissues randomly selected in each group using the Trizol extraction reagent (Invitrogen, Carlsbad, CA). Digital Genomics Inc. (Seoul, Korea) performed duplicate examinations on GenePloer™twin chip™-Rat 5K containing 4,863 gene probes with mixed total RNA. Global median, intensity/location-dependent normalization was performed to analyze all data. Genes were considered differentially expressed when the log₂ ratio was more than 1 or less than -1. To identify the pathway altered by the 1% KRG extract, differentially expressed gene data underwent further analyses using KEGG pathway. A DAVID (the database for annotation, visualization and integrated discovery) bioinformatics resources (<http://david.abcc.ncifcrf.gov/>) was used to perform a functional analysis for those genes [31].

Statistical analyses

All results were shown as the means \pm SE ($n = 15$) for each group. All data were analyzed using one-way ANOVA followed by Duncan's post-hoc test using SPSS (version 11.5, SPSS). Correlations between variables were calculated using a Pearson correlation.

Results

Body weight and liver weight of rats

Animals of all groups were kept under close observation for intake of diet and fluid, rate of weight gain, and general health. There was no significant difference in final body weight, liver weight, or relative liver weight (percentage liver weight per body weight) among the groups (Table 2).

Table 2. Final body weight, food intake, liver weight, and relative liver weight

| Group | Final body weight (g) | Food intake (g/d) | Liver weight (g) | Relative liver weight (%) ^a |
|---------|---------------------------------|--------------------------------|-------------------------------|--|
| Control | 389.16 \pm 9.24 ^{ns} | 14.91 \pm 0.59 ^{ns} | 9.93 \pm 0.32 ^{ns} | 2.55 \pm 0.02 ^{ns} |
| DEN-Con | 368.81 \pm 12.26 | 14.44 \pm 0.87 | 8.74 \pm 0.45 | 2.36 \pm 0.06 |
| KRG 0.5 | 376.54 \pm 15.47 | 14.56 \pm 0.92 | 8.79 \pm 0.45 | 2.33 \pm 0.03 |
| KRG 1 | 378.74 \pm 9.62 | 14.55 \pm 0.89 | 8.76 \pm 0.45 | 2.31 \pm 0.07 |
| KRG 2 | 373.07 \pm 11.46 | 14.31 \pm 0.92 | 9.15 \pm 0.41 | 2.45 \pm 0.06 |

^aRelative liver weight (%) = [Liver weight (g) / Body weight (g) * 100].

All values are means \pm SE ($n = 15$ per group), and "ns" means "not significantly different" among groups.

KRG extract suppressed the formation of preneoplastic foci

GST-P+ foci were developed in all groups treated with DEN. The area (mm^2/cm^2) and number ($\text{No.}/\text{cm}^2$) of the GST-P+ foci (mean diameter >0.2 mm) in the DEN-control were 1.21 and 9.93, respectively. The values were significantly lower to 0.55 and 6.04 (55% and 39% reduction; $p=0.0251$ and 0.0433 , respectively) in the KRG 0.5% group and 0.49 and 4.03 (60% and 59% reduction; $p=0.0144$ and 0.0012 , respectively) in the KRG 1% group, respectively when compared to the DEN-control (Fig. 1A and B). By contrast, the frequency of GST-P+ foci was not significantly different between the KRG 2%-supplemented group and the DEN-control (Fig. 1A and B).

KRG extract suppressed the lipid peroxidation

The animals supplemented with KRG (0.5 or 1%) showed a lower level of lipid peroxidation compared to the DEN-control (Fig. 2). TBARS was significantly elevated after the DEN treatment followed by PH in a positive correlation with the area of GST-P+ foci ($r = 0.99$, $p = 0.011$).

KRG 1% extract induced the levels of total glutathione and glutathione-dependent enzymes

Hepatic tGSH content of the 1% KRG-supplemented rats was significantly increased when compared to the DEN-control (Fig. 3A). In addition, the cytosolic GST activity of the 1% KRG supplemented rats was also significantly increased when compared to the DEN-control (Fig. 3B). The activity of GPx was significantly elevated by the 0.5% and 1% KRG, when compared to the DEN-control (Fig. 3C). However, there was no significant difference in the activity of GR between groups (Fig. 3D).

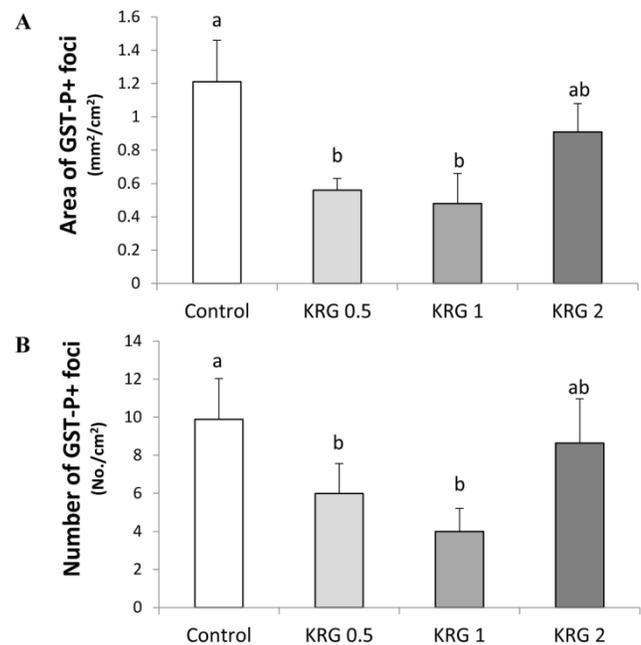


Figure 1. Effects of Korea red ginseng extract on the area and number of placental glutathione S-transferase (GST-P) positive foci in DEN-induced and PH-promoted hepatic carcinogenesis in rat. (A) The area of GST-P+ foci. (B) The number of GST-P+ foci. Values are means \pm SE ($n = 15$) from image reading of stained GST-P+ foci. Means with different letters are significantly different, $p < 0.05$, whereas means with similar letters are not different from each other.

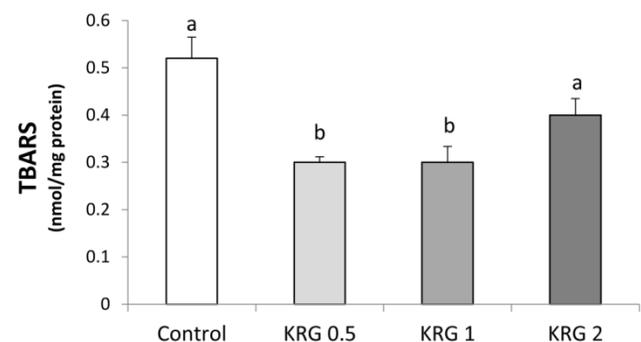


Figure 2. Effects of Korea red ginseng extract on Thiobarbituric acid (TBARS) in DEN-induced and PH-promoted hepatic carcinogenesis in rat. Values are means \pm SE ($n = 15$). Means with different letters are significantly different, $p < 0.05$, whereas means with similar letters are not different from each other.

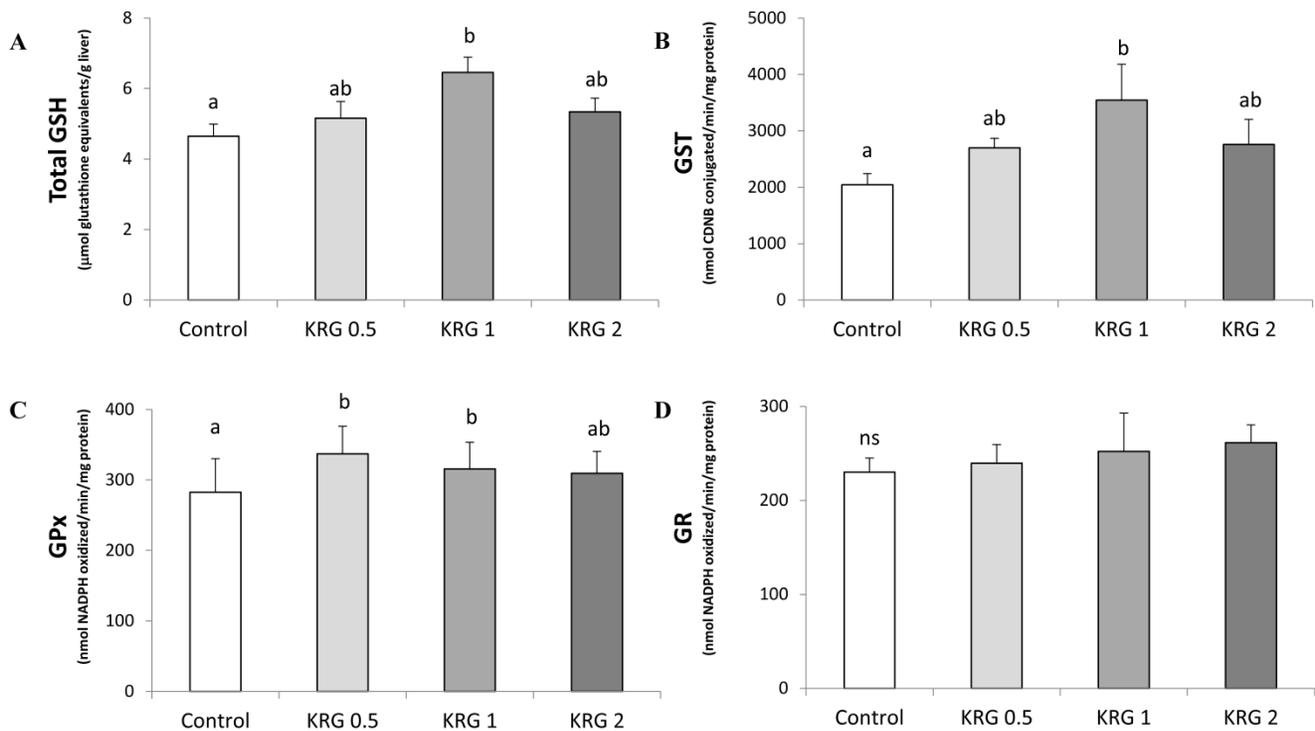


Figure 3. Effects of Korea red ginseng extract on glutathione level and glutathione-dependent enzyme activities in DEN-induced and PH-promoted hepatic carcinogenesis in rat. (A) tGSH Content. (B) GST, (C) GPx, and (D) GR activities. Values are means \pm SE (n = 15). Means with different letters are significantly different, $p < 0.05$, whereas means with similar letters are not different from each other.

Table 3. KEGG analysis with significant enrichment of genes differentially expressed by 1% Korean red ginseng intake on hepatocarcinogenesis

| GO terms | p-value | Up-regulated | Down-regulated |
|---|---------------|--------------|--------------------------------------|
| rno00830:Retinol metabolism | 0.0138 | - | <i>Cyp2c6, Cyp3a9, Cyp4a3, Rdh10</i> |
| rno00980:Metabolism of xenobiotics by cytochrome P450 | 0.0144 | - | <i>Cyp2c6, Cyp2e1, Cyp3a9, Mgst1</i> |
| rno03320:PPAR signaling pathway | 0.0226 | - | <i>Acadm, Acs1, Cyp4a3, Slc27a2</i> |
| rno00982:Drug metabolism | 0.0234 | - | <i>Cyp2c6, Cyp2e1, Cyp3a9, Mgst1</i> |
| rno00591:Linoleic acid metabolism | 0.0322 | - | <i>Cyp2c6, Cyp2e1, Cyp3a9</i> |

KEGG pathways based on differentially expressed genes altered by the 1% KRG diet

Since the KRG 1% extract group has the most protective effects (based on Fig. 1), it was further studied to identify genes using the cDNA microarray. Of 4,863 gene probes on cDNA arrays, it was identified that 19 genes were up-regulated, and 114 genes were down-regulated in the 1% KRG extract diet. When functional analysis of differentially expressed genes was performed using the DAVID web-based program, 5 KEGG pathways were significantly enriched in the 1% KRG extract diet (Table 3). Importantly, the most relevant GO terms was metabolism of xenobiotics via cytochrome P450 (Cyp P450, $p = 0.0144$), in which four genes (*Cyp2c6, Cyp2e1, Cyp3a9, Mgst1*) were significantly down-regulated.

Discussion

In this study, various concentrations of KRG extract were utilized to investigate whether 1) KRG extract may play an important role in modulating redox status, and 2) the optimum intake of KRG may suppress hepatocarcinogenesis in carcinogen-treated rats. It was hypothesized that KRG extract may prevent hepatocarcinogenesis through modulation of the liver redox environment and oxidative stress, but that the chemopreventive effects may differ based on the concentration.

KRG is a traditional medicine to treat a variety of disorders, including cancers [32]. The DEN model for this study is a pre-clinical model of hepatocellular cancer that exhibits many phenotypic characteristics relevant to the liver cancer [22]. We observed that the chemopreventive effects of KRG extract on rat hepa-

tocarcinogenesis initiated by DEN and promoted by PH range between 0.5-1% [33]. These ranges significantly reduced the area and number of GST-P positive foci when compared to the control. However, the KRG 2% diet had no suppressive effect on DEN-induced hepatocarcinogenesis in terms of the area and number of GST-P foci. We previously reported that white *panax ginseng* has a chemopreventive effect on hepatocarcinogenesis in 2% concentration [34]. The average value of ginsenosides in four-year-old white *panax ginseng* was 1.348% [35], whereas the concentration of ginsenosides in six-year-old KRG was 2%. In addition, red ginseng has more active deglycosylated derivatives including ginsenosides Rg3 than white ginseng by the heat processing [11]. Therefore, the different therapeutic ranges for these white and red ginsengs can be explained by higher amount of active ginsenosides in KRG.

High-dose KRG, however, might reduce its chemopreventive effects through induction of its toxicity. It was previously reported that high intakes of well-known chemopreventive compounds, such as indole-3-carbinol, caffeic acid, and ferulic acid were associated with increased risks of cancers [36-39]. A previous study showed similar outcomes that high-dose intake of chemopreventive compounds lost its chemopreventive efficacy. The effects of *pfaffia paniculata* root (Brazilian ginseng) on hepatocarcinogenesis were evaluated at 0.5, 2, and 10% in mice, and a 2% dose was shown to be the most effective on suppressing tumor incidence than 0.5 or 10% dose. Especially, the 10% dose had no suppressive effect on DEN-induced hepatocarcinogenesis in female mice [40]. One potential mechanism is that high dose intake of phytochemicals as xenobiotics may induce the activities of hepatic Cyp P450 and reduce phase II enzymes, which enhance oxidative stress response and hepatocarcinogenesis [38].

In the dosage calculation of this study, the amounts of KRG intake in 0.5 and 1% KRG group were 165.5 and 331 mg/kg/day in rats, and these groups have chemopreventive effects on carcinogen-treated rats. The results were similar to the previous study in which red ginseng extract suppressed skin tumor in rats in a dose-dependent manner at 50-400 mg/kg [41]. Moreover, it has been reported that there was no toxic effect in rats fed on ginseng extract at dose levels of 105-210 mg/kg/day for 25 weeks [42]. It has been shown that human equivalent intake of 0.5-1% KRG is 26.84-53.68 mg/kg/day for human [43], which equals 2-4 g intake of KRG for an individual of 75 kg body weight. It has been shown that KRG at dose of 3-6 g/day for eight weeks improved the antioxidant enzymes and oxidative stress

markers in healthy human [44]. By contrast, there was no chemopreventive effect of the 662 mg/kg/day intake of KRG (2%) on carcinogen-treated rats in this study. However, there were no significant difference in the body weight and relative weight of liver (Table 2). In addition, it has been suggested that the No Observed Adverse Effect Level (NOAEL) of KRG was 2 g/kg/day in rats fed the KRG extract for 4 weeks [45]. Based on our results, even though there are no toxic effects, we suggest that more than the 8 g/day intake of KRG may not improve the redox status of glutathione in human.

Oxidative stress represents an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system [46]. High ROS production has been shown to lead to DNA damage, mutations of tumor suppressors, gene instability, and carcinogenesis, and damage other molecules including the fatty acid side chains of lipids in the membranes of the cell [47]. Direct measurement of ROS has not established well due to their instability, so ROS generation is usually indirectly assayed by detecting specific biomarkers, such as lipid peroxidation (TBARS analysis) [48]. It has been shown previously that KRG has a potent antioxidant activity [49], and red ginseng oil has a protective effect on liver damages by inducing the antioxidant enzymes activity and by inhibiting lipid peroxidation *in vitro* and *in vivo* [50]. In addition, KRG extract has been shown to be a chemopreventive effect via improvement of antioxidant capacity in hepatotoxin-treated rats [51]. Similar to these previous reports, we demonstrated for the first time that 0.5% and 1% KRG suppress the level of TBARS, a lipid peroxidation biomarker compared to control. It suggests that KRG has an antioxidant property, which may contribute to inhibition of lipid peroxidation, and suppress hepatocarcinogenesis.

Liver detoxification process metabolizes carcinogens by Phase I (i.e. Cytochrome P450), Phase II enzymes (i.e. GST), and antioxidant enzymes including GR and GPx [52, 53]. In phase II of detoxification, glutathione (GSH and GSSG) and its related enzymes such as GST, GPx, and GR play an important antioxidant role, preventing damage caused by ROS. Elevations of these enzymes may suppress the process of liver disease development such as liver cancer [54]. The combination of carcinogen injection and PH are vulnerable with an oxidative damage that may lead to lipid peroxidation and hepatocarcinogenesis [55]. In our study, the induction of tGSH level by the 1% KRG extract may be associated with the reduction of oxidative stress. We also observed that the 1% KRG extract increased cytosolic GST and GPx activities when compared to the control, suggesting that the increase

of cytosolic GST activity would improve the cellular detoxifying potential. Moreover, the increase in the activities of GPx in the KRG fed rats would help to speed up the redox cycling. It suggests that KRG-regulated both cytosolic GST and GPx activities may relate to the cell protection against oxidative damage by catalyzing the elimination of peroxide.

To understand the genetic metabolic adaptation, we performed cDNA microarray and identified 133 differentially expressed genes comparing between the 1% KRG diet and the DEN-control group. When the differentially expressed genes underwent KEGG pathway analysis with the DAVID web-based program, we observed that 5 KEGG pathways such as Retinol metabolism and/or Metabolism of xenobiotics by Cyp P450 and PPAR signaling pathway were over-represented. The gene expressions of *Cyp2c6*, *Cyp2e1*, *Cyp3a9* and *Mgst1* in Metabolism of xenobiotics via Cyp P450 were down-regulated in the 1% KRG-treated rats when compared to the control. It has been shown that the Cyp P450 involved in the phase I xenobiotic metabolism is a key enzyme in cancer formation and cancer treatment [56]. It has also been suggested that red ginseng has an inhibitory effect on the Cyp P450 activities in rat liver [56], and ginsenoside, such as Rg3, suppressed the Cyp P450 enzymes activity [57]. It has been shown that CYP2E1 acts as a lipid peroxidation inducer [58]. Also, it was reported that the intake of garlic powder decreased the pre-neoplastic foci formation and contributed to chemoprevention against the rat hepatocarcinogenesis through the suppression of CYP2E1 [58]. Based on these previous reports and our data, we suggest that KRG may have a beneficial effect on the inhibition of lipid peroxidation via modulation of Cyp P450 enzymes during hepatocarcinogenesis.

In conclusion, the 0.5~1% dose of Korean red ginseng has a chemopreventive effect on chemically-induced rat hepatocarcinogenesis by suppression of oxidative stress and modulation of redox-enzymes. Additionally, more than the 2% dose of KRG may lose the chemopreventive efficacy due to the fact that it cannot improve carcinogen detoxifying enzyme. Ginseng is a popular chemopreventive compound; however the lack of the dose ranging trials may result in overdose. We report for the first time to determine the chemopreventive effects of KRG at the certain concentration range. Since we did not look at all the possible signaling pathways that are considered targets of KRG, further *in vitro* and *in vivo* tests are needed to elucidate the role of high-dose ginseng on rat hepatocarcinogenesis. Thus, investigating the detailed molecular mechanisms of the anti-oxidative effects of KRG is essential to further understand the chemopreventive effects of KRG. Despite these limi-

tations, our results have shown that KRG has a chemopreventive effect via the modulation of the cellular redox environment to minimize oxidative damage, and we suggested that KRG could be a potential therapeutic agent against hepatocarcinogenesis.

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Abbreviations

Cyp P450: cytochrome P450; CDNB: 1-chloro-2,4-dinitrobenzen; DAVID: database for annotation, visualization and integrated discovery; DEN: diethylnitrosamine; DTNB: 5,5'-dithiobis-2-nitrobenzoic acid; GPx: glutathione peroxidase; GR: glutathione reductase; GSH: reduced glutathione; GSSG: oxidized glutathione; GST: glutathione S-transferase; GST-P+ foci: glutathione S-transferase placental form positive foci; KRG: Korea red ginseng; NOAEL: no observed adverse effect level; PH: partial hepatectomy; ROS: reactive oxygen species; TBARS: thiobarbituric acid reactive substances; tGSH: total glutathione.

Competing Interests

The authors have declared that no competing interest exists.

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