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Research article

Subacute oral toxicity and bacterial mutagenicity study of Korean Red Ginseng oil

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ABSTRACT

Background: Red ginseng oil (RGO) is produced by supercritical CO_2 extraction of secondary products derived from Korean Red Ginseng extract. As the use of RGO has increased, product safety concerns have become more important.
Methods: In the present study, the subacute oral toxicity and bacterial reverse mutagenicity of RGO were evaluated. Sprague–Dawley rats were orally administered with RGO for 28 d by gavage. Daily RGO dose concentrations were 0 mg/kg body weight (bw), 500 mg/kg bw, 1,000 mg/kg bw, or 2,000 mg/kg bw per day. Bacterial reverse mutation tests included five bacterial strains (*Escherichia coli* WP2 and *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537), which were used in the presence or absence of metabolic activation. The plated incorporation method for mutation test was used with RGO concentrations ranging from 312.5 μg to 5,000 μg per plate.
Results: The subacute oral toxicity test results did not reveal any marked changes in clinical characteristics. There were no toxicological changes related to RGO administration in hematological and serum biochemical characteristics in either control or treatment animals. Furthermore, no gross or histopathological changes related to RGO treatment were observed. The bacterial reverse mutation test results did not reveal, at any RGO concentration level and in all bacterial strains, any increase in the number of revertant colonies in the RGO treatment group compared to that in the negative control group.
Conclusion: The no-observed-adverse-effect level of RGO is greater than 2,000 mg/kg bw and RGO did not induce genotoxicity related to bacterial reverse mutations.

1. Introduction

Safety concerns related to the use of herbal products have increased as the worldwide use of herbal ingredients in medicine and dietary supplements has increased markedly [1,2]. Herbal products contain various compounds; thus, it can be more difficult to predict their toxicity than it is to predict that of a single compound used in general medicine [3]. Therefore, an accumulation of safety-related information on herbal products is important to reduce the safety risks associated with side effects.

Ginseng (*Panax ginseng* Meyer) is a widely used traditional herbal product that has been used as a medicinal treatment in many countries for several thousands of years [4]. In particular, Korean Red Ginseng, produced by steaming fresh *P. ginseng* in water vapor, is used as a medicine, cosmetic, and nutritional supplement in Asian countries including Korea. It has been demonstrated that Korean Red Ginseng has various effects including neurological improvement [5], blood pressure regulation [6], anti-inflammatory [7], anticancer [8], and liver protection effects [9]. Most of this efficacy is related to water-soluble saponin components, which are abundant when red ginseng is taken in the form of a hot-water extract, as is traditionally the case. However, studies evaluating the efficacy of lipid-soluble components have increased because of the higher bioavailability characteristics of such components [10,11]. In particular, several studies have investigated the efficacy of red ginseng oil (RGO), a lipophilic nonsaponin component of red

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ginseng. RGO is produced by performing supercritical CO_2 extraction of secondary products generated by water-based extraction of red ginseng. RGO contains various fatty acids, phospholipids, and phytosterols. Among these, the RGO phytosterol component has been reported to have various beneficial effects including anti-inflammatory, antioxidant, and hepatoprotective effects [12,13]. Phytosterols obtained from other plants are also reported to have anticancer and anti-inflammatory effects, and they have been used in the manufacture of nutrient supplements and cosmetics [14,15]. Despite the professed usefulness of RGO in these industries, little has been reported on the health-related safety of RGO. To date, there is only one report describing the results of a single-dose, oral administration toxicity test in rats [16]. Thus, additional safety data, such as data related to repeated-dose oral administration and the mutagenicity of RGO, are needed to elucidate the toxicity potential of RGO. In this study, the 28-d repeated-dose oral administration toxicity and bacterial reverse mutagenicity of RGO were evaluated in order to clarify the health effects of RGO.

2. Materials and methods

2.1. Test substance

RGO was obtained from Korea Ginseng Corporation (Korea), and the composition is shown in Table 1. The vehicle, corn oil, was obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Subacute oral toxicity study

Subacute oral toxicity tests were based on the Organization for Economic Co-operation and Development (OECD) Guideline 407 [17].

2.2.1. Test animals and environmental conditions

Five-wk-old male and female, specific pathogen-free Sprague–Dawley (SD) rats were purchased from ORIENT BIO Inc. (Seongnam city, Korea) and acclimated for 7 d prior to starting the experiments. During the acclimation and experimental periods, the rats were housed in stainless mesh cages (1 rat per cage) in a room with controlled temperature (20.5–23.2°C) and humidity (36.2–56.3%), and a 12-h light/dark cycle. The rats were fed rodent chow (Harlan Teklad, Madison, WI, USA) and filtered water *ad libitum*. This experiment was approved by the Institutional Animal Care and Use Committee of Biotoxtech (approval number, 100301). All procedures in this study have been performed in accordance with the provisions of Good Laboratory Practice.

Table 1
The fatty acids composition of red ginseng oil

Component	Proportion (%)
Linoleic acid	71.41
Palmitic acid	9.39
Linolenic acid	6.24
Oleic acid	5.02
cis-11,14-Eicosatyrienoic acid	1.34
Eucic acid	0.79
Stearic acid	0.76
cis-13,16-Docosadienoic acid	0.64
Pentadecanoic acid	0.6
Nervonic acid	0.52
Lignoceric acid	0.41
cis-11-Eicosenoic acid	0.4
Heptadecanoic acid	0.38
Palmitoleic acid	0.37
Arachidic acid	0.35
r-Linolenic acid	0.3
Arachidonic acid	0.23
Tricosanoic acid	0.23
Behenic acid	0.2
Myristic acid	0.17
Heneicosanoic acid	0.15
cis-10-Heptadecenoic acid	0.13
Total	100

2.2.2. Experimental group

At 6 wk, the rats were divided into four groups (5 rats in each group): vehicle control (corn oil), low-dose group (500 mg/kg bw), middle-dose group (1,000 mg/kg bw), and high-dose group (2,000 mg/kg bw). The rats were exposed to RGO following the toxicity test guidelines of the Korea Food and Drug Administration for Nonclinical Laboratory Studies applying Good Laboratory Practice. The dosing volume used was 5 mL/kg body weight (bw; oral administration). The maximum dose was determined according to the recommendations of the Korea Food and Drug Administration and OECD Guideline 423 [18].

2.2.3. Body weight changes

The body weight of each animal was measured at the initiation of administration, weekly thereafter, and on the day of scheduled sacrifice.

2.2.4. Food consumption

Food consumption was measured for 7 d in the 1st week and for 6 d in the 2nd week to the 4th weeks, then the daily average food intake was calculated.

2.2.5. Biochemistry and hematology

At the end of the 28-d experiment, the rats were 10 wk old. Prior to necropsy, food was withheld overnight. The rats were killed by exsanguinations following isoflurane inhalation after recording the terminal body weight. Blood samples were drawn from the descending aorta, collected in heparinized vacutainers, and analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine, total protein, albumin (ALB), albumin/globulin ratio, total cholesterol, triglyceride, and glucose using a biochemical blood analyzer (Hitachi 7080; Hitachi, Tokyo, Japan). The blood was also analyzed for the red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet counts, and white blood cell count using a blood cell counter (ADVIA 120; Siemens, Erlangen, Germany).

2.2.6. Organ weights

After collecting the blood, brain, heart, liver, spleen, and kidneys were carefully removed and the absolute organ weights were recorded. Relative organ weights were calculated as the ratio between the absolute organ weight and the body weight of fasting.

2.2.7. Histopathology

The gross observation was recorded in all animals. The brain, heart, liver, spleen, kidneys, and lung were removed carefully and fixed in 10% neutral buffered formalin. Each organ was trimmed as described in previous guideline [19]. After paraffin infiltration by tissue processor, the organs were embedded in paraffin and cut into 4-μm sections. The sections were stained with hematoxylin and eosin, and examined under light microscopy.

2.3. Bacterial reverse mutation test

The bacterial reverse mutation test (also called Ames test) was carried out according to the OECD Guideline 471 for the testing of chemicals, “Bacterial reverse mutation test” [20]

2.3.1. Tester strains

The histidine auxotroph strains of *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, and a tryptophan auxotroph strain of *Escherichia coli* WP2 uvrA were used. TA100, TA1535, and *E. coli* WP uvrA were used to identify the mutagenicity of base-pair substitution type, whereas TA98 and TA1537 were used as frame-shift type [21–23]. The *Salmonella* strains were purchased from Molecular Toxicology Inc. (Boone, NC, USA) and *E. coli* WP2 uvrA strain was obtained from Preclinical Research Center (ChemOn, Inc., Gyeonggi-do, Korea). Each strain was added in 2.5% nutrient broth number 2 solution and incubated for about 12 h, 150 rpm at 37°C in a shaking incubator. When bacteria counts exceeded 1×10^9 cells/mL, they were used in the test.

2.3.2. Controls

With metabolic activation system, 2-aminoanthracene (Sigma A38800, Sigma) was used all stains. Without metabolic activation system, sodium azide (Sigma S8032, Sigma) for TA100 and TA1535, 4-nitroquinoline *N*-oxide (Sigma N8141, Sigma) for TA98, 9-aminoacridine (Sigma 92817, Sigma) for TA1537 were used.

2.3.3. Test substance treatment

RGO was water-insoluble, so it was diluted in dimethyl sulfoxide. Dimethyl sulfoxide was used as negative control.

2.3.4. Dose finding test

According to the results of a dose finding test, 5,000 μg/plate was selected as the highest concentration for all test strains in both the absence and presence of the S9 mixture.

There was no precipitation of test substance and cytotoxicity. Background lawn was observed in all test strains. For the test, 2-fold serial dilutions were performed to yield five concentrations (312.5 μg/plate, 625 μg/plate, 1250 μg/plate, 2,500 μg/plate, and 5,000 μg/plate).

2.3.5. Treatment method

The test was performed based on the plate incorporation method within/without the metabolic activation system S9 mixture [24,25]. Each sample was assayed in triplicate. To a 2-mL top agar added test tube, 0.5 mL of 0.1M sodium phosphate buffer (pH7.4) for without metabolic activation or 0.5 mL of S9 mix (10% S9 and salt-Cofactor-I solution) for with metabolic activation was added with 0.1 mL of bacterial culture and 0.1 mL test solution. The mixture was mixed gently and poured onto prewarmed a minimal glucose agar plate. Top agar consisted of 0.05mM histidine–biotin used for the *Samonella* strains and 0.05mM tryptophan for the *E. coli* strain. Each culture plate was placed in an incubator for 48 h at 37°C. After the incubation, the numbers of revertant colonies were counted.

2.3.6. Evaluation and interpretation of results

The test substance was considered positive in the bacterial reverse mutation assay when there was an increase (≥2-fold) of spontaneous revertant colonies compared with those in the

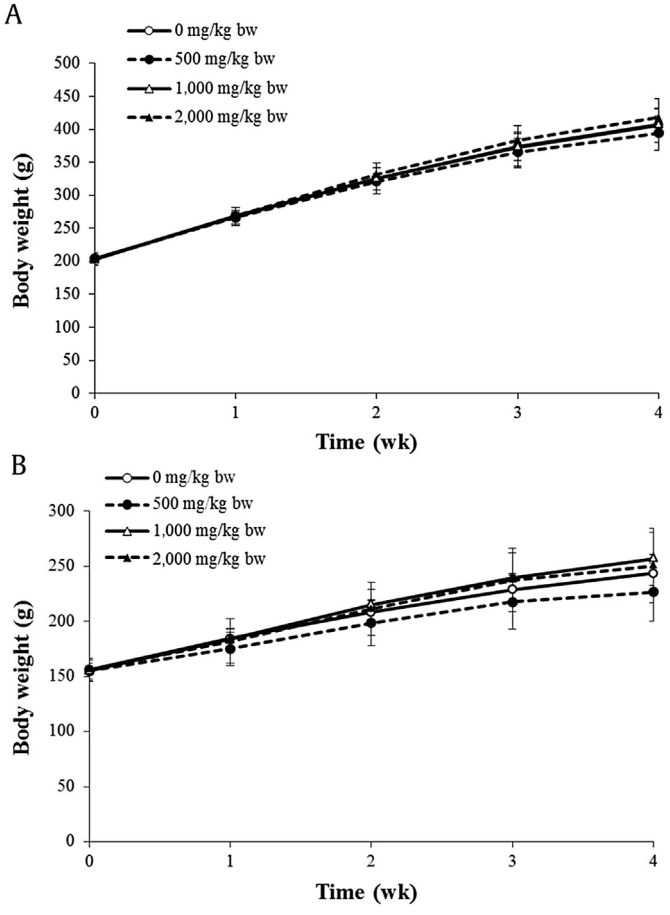

Fig. 1. Body weight changes of rats in the 28-d oral administration toxicity test. (A) Male rats. (B) Female rats. Four groups of five rats were given red ginseng oil at concentrations of 0 mg/kg body weight (bw), 500 mg/kg bw, 1,000 mg/kg bw, and 2,000 mg/kg bw, 7 d/wk for 28 d. The rats exposed to red ginseng oil showed no significant difference compared with the control.

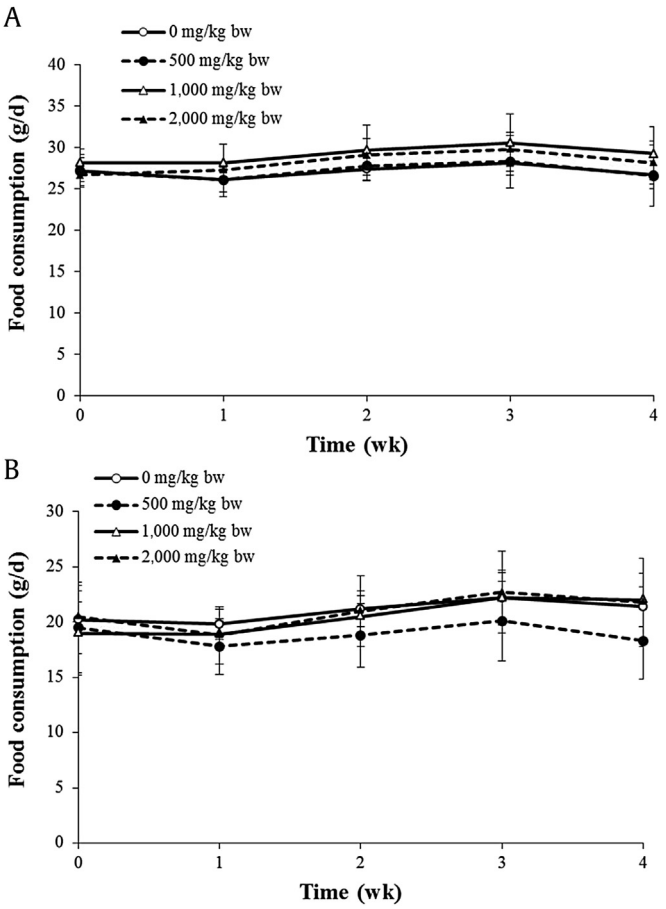

Fig. 2. Food consumption during the 28-d oral administration toxicity test. (A) Male rats. (B) Female rats. The rats exposed to red ginseng oil showed no significant difference compared with the control.

negative control or a concentration-related increase over the range tested and/or a reproducible increase at one or more concentrations in the number of revertant colonies per plate in at least one strain with or without metabolic activation.

2.4. Statistical analysis

The data for the body weights, food consumption, organ weight, value of the blood biochemistry, and hematology were analyzed using one-way analysis of variance, if variances of the groups were assumed to be equal. When analysis of variance showed statistical significance, Dunnett's multiple range test was used to compared the differences between experimental groups and the control group. If variances of the groups were not assumed to be equal, Kruskal–Wallis test was used to determine the significance of the group differences. SAS (version 9.1.3; SAS Institute Inc., Cary, NC, USA) software package was used. A p value of <0.05 indicated statistical significance.

3. Results

3.1. Body weight changes and food consumption

No mortality or notable clinical signs were observed during the 28-d exposure period, and there were no statistically significant changes in body weight or food consumption compared with the control group (Figs. 1, 2).

3.2. Blood biochemistry and hematology

In female rats, there were statistically significant decreases in ALB ($p < 0.05$) in the low-dose group (500 mg/kg bw; Table 2). No significant differences were observed between the experimental and control animals in terms of hematological findings (Table 3).

Table 2
Blood biochemistry data for rats in 28 d of oral administration of RGO (mean ± SD)

	Dose (mg/kg)			
	0 ($n = 5$)	500 ($n = 5$)	1,000 ($n = 5$)	2,000 ($n = 5$)
Male				
ALB	2.5 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.5 ± 0.1
ALP	773.7 ± 63.6	590.0 ± 94.4	771.2 ± 133.3	740.9 ± 130.9
T-CHO	75 ± 6	84 ± 18	78 ± 14	90 ± 10
CRE	0.47 ± 0.02	0.45 ± 0.04	0.45 ± 0.04	0.48 ± 0.03
GLU	144 ± 15	147 ± 34	153 ± 10	138 ± 18
AST	63.5 ± 4.0	70.1 ± 6.7	69.1 ± 10.1	72.8 ± 13.2
ALT	30.5 ± 4.1	31.4 ± 5.5	34.3 ± 6.6	32.1 ± 4.1
TP	5.8 ± 0.2	6.1 ± 0.3	5.8 ± 0.2	5.7 ± 0.3
BUN	11.0 ± 1.6	11.8 ± 1.6	11.4 ± 1.8	11.1 ± 1.6
A/G ratio	0.75 ± 0.04	0.74 ± 0.02	0.74 ± 0.05	0.75 ± 0.07
TG	50 ± 20	55 ± 21	46 ± 9	71 ± 19
Female				
ALB	2.9 ± 0.2	2.6 ± 0.1*	2.8 ± 0.1	2.7 ± 0.1
ALP	329.6 ± 79.6	287.7 ± 56.2	289.9 ± 97.4	368.0 ± 74.2
T-CHO	97 ± 13	81 ± 12	91 ± 28	74 ± 16
CRE	0.46 ± 0.03	0.45 ± 0.05	0.47 ± 0.07	0.49 ± 0.04
GLU	134 ± 11	126 ± 15	143 ± 17	132 ± 18
AST	90.1 ± 28.1	79.3 ± 13.9	65.1 ± 9.2	68.7 ± 7.5
ALT	40.4 ± 24.4	24.3 ± 2.4	24.9 ± 4.2	26.7 ± 3.4
TP	6.2 ± 0.3	6.0 ± 0.2	6.2 ± 0.3	5.9 ± 0.2
BUN	12.9 ± 2.7	12.3 ± 0.6	11.7 ± 1.6	14.1 ± 1.2
A/G ratio	0.86 ± 0.05	0.79 ± 0.06	0.85 ± 0.08	0.83 ± 0.05
TG	26 ± 9	16 ± 6	25 ± 6	20 ± 5

* Significant difference versus control ($p < 0.05$)
A/G ratio, albumin/globulin ratio; ALB, albumin (g/dL); ALP, alkaline phosphatase (IU/L); ALT, alanine aminotransferase (IU/L); AST, aspartate aminotransferase (IU/L); BUN, blood urea nitrogen (mg/dL); CHO, total cholesterol (mg/dL); CRE, creatinine (mg/dL); GLU, glucose (mg/dL); RGO, red ginseng oil; SD, standard deviation; TG, triglyceride (mg/dL); TP (g/dL), total protein

Table 3
Hematological data for rat in the 28-d oral administration of RGO (mean ± SD)

	Dose (mg/kg)			
	0 ($n = 5$)	500 ($n = 5$)	1,000 ($n = 5$)	2,000 ($n = 5$)
Male				
WBC	8.06 ± 1.69	10.59 ± 3.55	9.24 ± 2.30	8.92 ± 2.40
RBC	7.61 ± 0.34	7.78 ± 0.12	7.46 ± 0.27	7.53 ± 0.28
Hb	14.4 ± 0.6	14.6 ± 0.4	14.2 ± 0.3	14.3 ± 0.6
HCT	43.7 ± 1.6	44.5 ± 1.4	42.9 ± 1.1	43.5 ± 1.7
MCV	57.5 ± 0.7	57.1 ± 1.6	57.5 ± 1.7	57.8 ± 0.7
MCH	19.0 ± 0.3	18.7 ± 0.4	19.0 ± 0.5	18.9 ± 0.3
MCHC	33.0 ± 0.4	32.7 ± 0.3	33.1 ± 0.5	32.7 ± 0.2
PLT	1,284 ± 175	1,171 ± 156	1,204 ± 97	1,222 ± 56
Female				
WBC	5.55 ± 0.94	4.00 ± 1.67	5.85 ± 1.95	5.15 ± 2.93
RBC	7.52 ± 0.18	7.50 ± 0.27	7.38 ± 0.49	7.26 ± 0.26
Hb	14.2 ± 0.3	14.2 ± 0.6	14.1 ± 0.8	14.1 ± 0.3
HCT	42.3 ± 0.8	41.9 ± 1.7	41.8 ± 2.5	41.1 ± 0.8
MCV	56.2 ± 0.4	55.9 ± 1.6	56.6 ± 0.5	56.8 ± 1.5
MCH	18.9 ± 0.3	18.9 ± 0.8	19.1 ± 0.2	19.4 ± 0.7
MCHC	33.6 ± 0.4	33.8 ± 0.5	33.7 ± 0.3	34.1 ± 0.4
PLT	1,301 ± 152	1,295 ± 130	1,322 ± 44	1,263 ± 138

There were no significant differences among the groups
Hb, hemoglobin (g/dL); HCT, hematocrit (%); MCH, mean corpuscular hemoglobin (pg); MCHC, mean corpuscular hemoglobin concentration (g/dL); MCV, mean corpuscular volume (fL); PLT, platelet count (K/μL); RBC (M/μL), red blood cells; RGO, red ginseng oil; SD, standard deviation; WBC, white blood cells (K/μL)

3.3. Organ weights

No significant organ weight changes were observed in either the absolute or relative organ weights, except for a decrease in the relative organ weight of the heart for the middle-dose (1,000 mg/kg bw) and high-dose (2,000 mg/kg bw) female rats (Tables 4 and 5).

3.4. Histopathology

There were no toxicological changes related with test substances in the gross finding and histopathological evaluation of each organ (Fig. 3). Several nontoxicological changes such as focal tubular basophilia in the kidney, and focal fatty changes and microgranuloma in the liver were observed in some of the control and high-dose (2,000 mg/kg bw) treatment animals (Table 6).

3.5. Bacterial reverse mutation test

Results of the bacterial reverse mutation test with the five bacterial strains (TA98, 100, 1535, 1537, and *E. coli* WP2) for RGO in the presence or absence of metabolic activation at five different concentrations, from 312.5 μg/plate to 5,000 μg/plate, are shown in

Table 4
Absolute organ weight for rats in 28 d oral administration of RGO (mean ± SD)

	Dose (mg/kg)			
	0 ($n = 5$)	500 ($n = 5$)	1,000 ($n = 5$)	2,000 ($n = 5$)
Male				
Brain	1.93 ± 0.07	1.97 ± 0.08	1.96 ± 0.08	1.96 ± 0.04
Heart	1.19 ± 0.11	1.21 ± 0.08	1.33 ± 0.13	1.26 ± 0.07
Liver	12.33 ± 1.07	12.29 ± 2.03	12.84 ± 1.60	12.91 ± 1.64
Spleen	0.67 ± 0.09	0.78 ± 0.11	0.73 ± 0.08	0.76 ± 0.14
Kidney	2.91 ± 0.30	2.75 ± 0.28	3.11 ± 0.18	2.95 ± 0.17
Female				
Brain	1.85 ± 0.07	1.85 ± 0.06	1.78 ± 0.03	1.85 ± 0.07
Heart	0.90 ± 0.04	0.79 ± 0.10	0.85 ± 0.10	0.85 ± 0.07
Liver	7.33 ± 0.22	6.78 ± 0.98	7.86 ± 1.27	7.58 ± 1.05
Spleen	0.49 ± 0.06	0.47 ± 0.03	0.48 ± 0.10	0.44 ± 0.05
Kidney	1.76 ± 0.08	1.69 ± 0.16	1.87 ± 0.22	1.80 ± 0.14

There were no significantly differences among the groups
RGO, red ginseng oil; SD, standard deviation

Table 5
Relative organ weight for rats in the 28-d oral administration of RGO (mean ± SD)

	Dose (mg/kg)			
	0 ($n = 5$)	500 ($n = 5$)	1,000 ($n = 5$)	2,000 ($n = 5$)
Male				
Brain	0.51 ± 0.04	0.53 ± 0.02	0.51 ± 0.04	0.50 ± 0.02
Heart	0.31 ± 0.02	0.32 ± 0.00	0.34 ± 0.03	0.32 ± 0.01
Liver	3.21 ± 0.15	3.27 ± 0.37	3.33 ± 0.09	3.25 ± 0.32
Spleen	0.17 ± 0.02	0.21 ± 0.03	0.19 ± 0.02	0.19 ± 0.04
Kidney	0.76 ± 0.05	0.73 ± 0.05	0.81 ± 0.05	0.74 ± 0.03
Female				
Brain	0.81 ± 0.05	0.88 ± 0.09	0.74 ± 0.08	0.79 ± 0.08
Heart	0.39 ± 0.02	0.37 ± 0.07	0.35 ± 0.02*	0.36 ± 0.02*
Liver	3.20 ± 0.15	3.19 ± 0.30	3.23 ± 0.30	3.21 ± 0.15
Spleen	0.21 ± 0.02	0.22 ± 0.01	0.19 ± 0.03	0.19 ± 0.02
Kidney	0.77 ± 0.03	0.80 ± 0.06	0.77 ± 0.05	0.77 ± 0.06

* Significant difference versus control ($p < 0.05$)
RGO, red ginseng oil; SD, standard deviation

Tables 7 and 8. There was no increase in the number of revertant colonies compared to the negative control at any dose in all strains. The positive control chemicals for each tester strain induced at least a 3-fold increase in the number of revertant colonies compared to the solvent control. Solvent and positive control values were distributed within the historical control ranges obtained in our laboratory. There was no precipitation of test substance in any strain. Background lawn was observed and no cytotoxicity was detected in any of the test strains.

4. Discussion

Red ginseng, produced by steaming *P. ginseng*, has various pharmacological properties, and the mechanisms associated with its efficacy have been described in many studies [5–8]. It has been used mostly in its water-extracted form. However, potent lipid-soluble compounds in red ginseng have been described in several reports [10,13,26]. In particular, RGO was isolated by performing supercritical CO_2 extraction of secondary products remaining after water-based extraction of red ginseng. The anti-inflammatory and anticancer effects of RGO have been described in previous studies [12,13]. As reports of the health-related usefulness of RGO increase, concerns about its safety need to be resolved; however, there has only been one study evaluating the safety of RGO, a study based on single-dose oral administration of RGO in rats [16].

In the present study, the toxicity of RGO after 28 d of repeated oral administration to SD rats was evaluated by undertaking clinical, clinicopathological, and pathological analyses. The RGO was orally administered at daily dose levels of 0 mg/kg bw, 500 mg/kg bw, 1,000 mg/kg bw, or 2,000 mg/kg bw. In the general acute toxicity test, the highest dose level was set at 2,000 mg/kg bw or 5,000 mg/kg bw [16]; on the basis of this highest dose level, subacute toxicity test levels were set in the 1,000–3,000 mg/kg bw range [27–29]. As the acute oral lethal dose 50% value of RGO was found to be greater than 5,000 mg/kg in a previous study that suggested that RGO was a safe substance, we selected a highest dose level of the subacute toxicity test of 2,000 mg/kg bw.

Mortality, clinical characteristics, food consumption, and body weight results, along with other gross findings, revealed no marked differences among all treatment and control groups for both sexes.

AST, ALT, and ALP are enzymes that are good indicators for predicting the toxic effects of substances on liver function. There were no changes in AST, ALT, or ALP levels, indicating that RGO did not affect liver function. Creatinine and BUN are the main markers of kidney damage associated with glomerular filtration and renal tubule dysfunctions. There were no significant differences in creatinine and BUN levels between the treatment and the control groups, which suggests that RGO did not have a toxic effect on renal function. Moreover, the fact that there were no significant

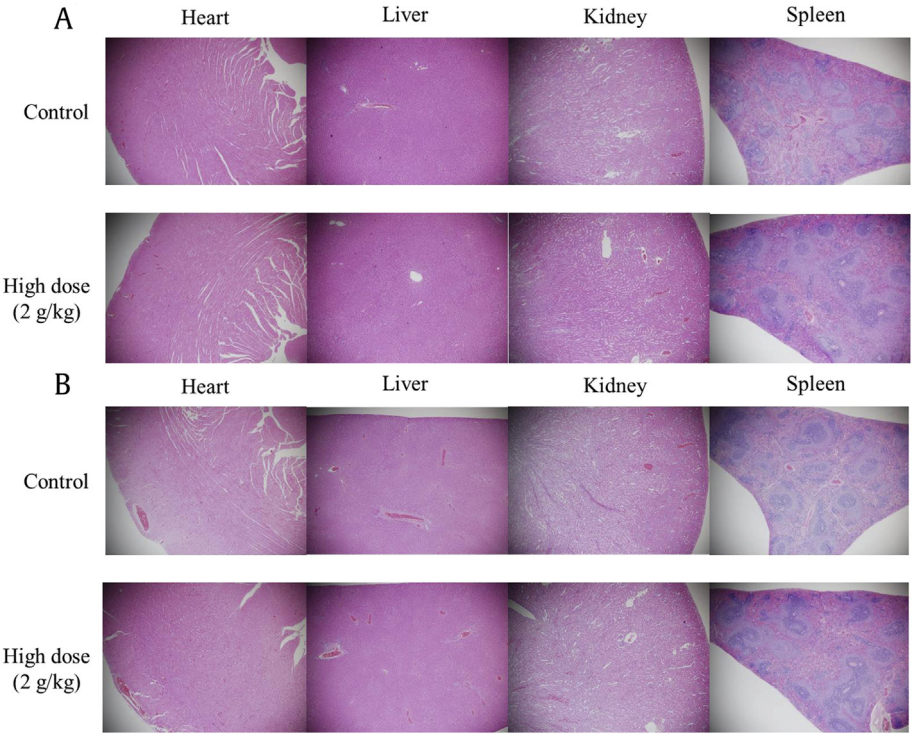

Fig. 3. Histopathological changes in rats exposed to red ginseng oil. (A) Male rats. (B) Female rats. There were no significant toxicological changes both control and high dose groups. Hematoxylin and eosin. Magnification: 40×.

Table 6
Summary of histopathological findings

Organ/findings	Sex		Male		Female	
	Group		G1	G4	G1	G4
	Dose (mg/kg)		0	2,000	0	2,000
	No. of animals		5	5	5	5
Kidney	Basophilic tubules, focal, cortex	±	0	1	0	0
		No. of examined	5	5	5	5
Liver	Fatty degeneration, hepatocyte, periportal	±	0	0	1	2
		+	0	0	2	1
	Microgranuloma	+	0	0	1	0
		No. of examined	5	5	5	5

There were unremarkable changes in brain, heart, and spleen in Groups 1 and 4

Table 7
Result of bacterial reverse mutation assay without S9 activation

Dose (μg/plate)	Number of reverse mutants/plate (mean ± SD)				
	Base substitution			Frame shift	
	TA100	TA1535	*Escherichia coli*	TA98	TA1537
0	112 ± 2	8 ± 1	33 ± 2	22 ± 2	7 ± 2
312.5	108 ± 6	11 ± 4	26 ± 4	18 ± 3	8 ± 2
625	123 ± 11	11 ± 2	29 ± 3	17 ± 6	7 ± 2
1,250	137 ± 16	9 ± 2	24 ± 4	17 ± 2	8 ± 2
2,500	133 ± 12	11 ± 2	25 ± 1	15 ± 1	6 ± 2
5,000	114 ± 10	10 ± 2	32 ± 1	17 ± 3	7 ± 1
Positive control (μg/plate)	NaN_3 (0.5)	NaN_3 (0.1)	4-NQO (0.25)	4-NQO (0.25)	9-AA (50)
	526 ± 18	71 ± 4	157 ± 7	157 ± 12	140 ± 4

9-AA, 9-aminoacridine; 4NQO, 4-nitroquinoline *N*-oxide; SD, standard deviation

histopathological changes in the liver or kidney supports the results of the biochemistry parameters.

Among the blood biochemistry and hematology assessments, the only statistically significant change was a decrease in ALB in the female 500 mg/kg bw dose group. Regardless, that result was considered toxicologically insignificant because the change was within the normal range of biological variation [30]. Moreover, the change did not form part of a dose–response relationship, and it was unsupported by other pathological results. The statistically significant low relative heart weights in female rats in the 1,000 mg/kg bw and 2,000 mg/kg bw groups were clinically insignificant, not associated with other gross findings, and unsupported by pathological findings. In addition, there was no dose-dependent relationship associated with the change in heart weight, and the heart weights were within the normal range.

Table 8
Result of bacterial reverse mutation assay with S9 activation

Dose (μg/plate)	Number of reverse mutants/plate (mean ± SD)				
	Base substitution			Frame shift	
	TA100	TA1535	*Escherichia coli*	TA98	TA1537
0	151 ± 11	9 ± 2	21 ± 2	22 ± 4	10 ± 0
312.5	143 ± 4	12 ± 3	23 ± 2	22 ± 3	10 ± 1
625	127 ± 10	11 ± 3	23 ± 3	25 ± 8	9 ± 1
1,250	145 ± 6	8 ± 3	21 ± 3	31 ± 7	10 ± 2
2,500	136 ± 3	10 ± 3	24 ± 3	25 ± 2	11 ± 3
5,000	126 ± 1	12 ± 3	23 ± 6	28 ± 3	10 ± 0
Positive control (μg/plate)	NaN_3 (0.5)	NaN_3 (0.1)	4-NQO (0.25)	4-NQO (0.25)	9-AA (50)
	466 ± 24	83 ± 4	167 ± 8	125 ± 2	165 ± 9

4NQO, 4-nitroquinoline *N*-oxide; SD, standard deviation

In a previous study, palm oil, a plant oil containing a high proportion of saturated fatty acids, was shown to induce toxicity related to reproductive and liver functions [31]. This type of toxicity has been attributed to the promotion of lipotoxicity by saturated fatty acids, with effects on liver, cardiovascular, endothelial, and gut microbiota systems [32]. Unlike palm oil, RGO contains a high proportion of unsaturated fatty acids (Table 1) and has shown no significant toxic effects; these results are similar to those in previous toxicity studies of plant oils containing a high proportion of unsaturated fatty acids [28,33].

Mutagenicity test by Ames assay has been conducted to screen carcinogens because most carcinogens, with a few exceptions, have mutagenicity. Results of the bacterial reverse mutation test with RGO showed no difference in the number of revertant colonies between the RGO treatment and negative control groups at any RGO concentration, and in all tested bacterial strains (*S. typhimurium* TA98, TA100, TA1535, TA1537, and *E. coli* WP2) in the presence or absence of metabolic activation. The solvent and positive control values were within historical control ranges observed in our laboratory, which assured us of the test validity. These results indicate that RGO did not induce genotoxicity related to bacterial reverse mutation under the conditions present in the study.

In conclusion, the results of this study indicate that oral doses of RGO repeated daily for 28 d do not produce any detectable toxic effects in SD rats at dose levels up to 2,000 mg/kg bw. Thus, the no-observed-adverse-effect level of RGO is considered to be greater than 2,000 mg/kg bw in both sexes of SD rats. In addition, RGO did not display mutagenicity. Our results, as fundamental information in the evaluation of subchronic or chronic toxicity of RGO, should be useful in designing further studies of the safety and efficacy of RGO.

Conflicts of interest

All authors declare no conflicts of interest.

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