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Subacute Oral Toxicity and Bacterial Mutagenicity Study of a Mixture of Korean Red Ginseng (*Panax ginseng* C.A. Meyer) and *Salvia plebeia* R. Br. Extracts

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Abstract

As various populations are rapidly becoming an aging society worldwide and interest in health issues has increased, demand for functional foods including herbal products has increased markedly to maintain a healthy state which has led to safety issues about their intake as an inevitable result. The objective of this study was to identify the safety profile of a Korean red ginseng and *Salvia plebeia* R. Br. extract mixture (KGC-03-PS) which is a valuable ingredient that can be used as a functional food. In the present study, the subacute oral toxicity and bacterial reverse mutagenicity of KGC-03-PS were evaluated. Sprague Dawley rats were administered KGC-03-PS orally for 28 days by gavage. Daily KGC-03-PS dose concentrations were 0, 500, 1,000, or 2,000 mg/kg body weight (bw) per day. Bacterial reverse mutation test with KGC-03-PS dose levels ranging from 312.5 to 5,000 μg/plate was carried out by OECD test guideline No. 471. Five bacterial strains (*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and *Escherichia coli* WP2) were tested in the presence or absence of metabolic activation by plate incorporation method. There were no toxicological effects related with test substance in the clinical evaluation of subacute oral toxicity test including clinical signs, body weight, and food consumption. Moreover, no toxicological changes related to KGC-03-PS were observed in the hematological and serum biochemical characteristics as well as in the pathological examinations, which included organ weight measurements and in the gross- or histopathological findings. KGC-03-PS did not induce an increase in the number of revertant colonies in all bacterial strains of the bacterial reverse mutation test. The no-observed-adverse-effect level of KGC-03-PS is greater than 2,000 mg/kg bw/day, and KGC-03-PS did not induce genotoxicity related to bacterial reverse mutations under the conditions used in this study.

Key words: Korean red ginseng and *Salvia plebeia* R. Br. extract mixture (KGC-03-PS), Subacute oral toxicity, Bacterial reverse mutagenicity, No-observed-adverse-effect level

INTRODUCTION

As the consumption of natural functional foods has markedly increased, cases of adverse reactions related to intake safety have also increased (1). Moreover, it is more difficult to determine the safety of natural ingredients than that of drugs, which have a single active pharmaceutical ingredient because natural ingredients have various constituents, and most functional foods or supplements are a mixture of various ingredients (2).

Therefore, scientific evidence on the safety of ingredients in natural foods or supplementary diets is required, and experimental studies *in vivo* and *in vitro* have been done according to guidelines established by international organizations such as the Organization for Economic Cooperation and Development (OECD) and the International Council for harmonisation (ICH). In particular, general toxicity and genotoxicity studies are representative of what related authorities require for determining the safety of ingredients.

Korean red ginseng (*Panax ginseng* C.A. Meyer), pro-

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duced by steaming *Panax ginseng* in water vapor, has been traditionally used as medicine and supplementary diet in Asian countries including Korea (3). It contains ginsenosides well known as a main biological component in red ginseng as well as arginine-fructose-glucose, acidic polysaccharides, and poly acetylenes whose biological activities have been demonstrated in previous studies (4-8). It has also been reported that Korean red ginseng has various pharmaceutical potencies including neurological improvement (9), blood pressure regulation (10), anti-inflammatory (11), anti-cancer (12), and liver protection (13).

Salvia plebeia R. Br. belongs to the family Lamiaceae, and is an annual herb widely distributed in many counties including Korea, China, Japan, India, Iran, and Australia. It has been used as a traditional medicine to treat various inflammatory diseases including hepatitis, cough, tumors, diarrhea (14), and atopic dermatitis (15), and it was also shown to have a strong anti-oxidative activity (16,17), anti-obesity effects in mice fed high-fat diets (18), and a respiratory protective effect in ambient particulate matter-induced airway inflammation (19).

Korean red ginseng is often used in mixtures with other herbs which could be used to enhance the effects of red ginseng (20). Because the Korean red ginseng and *Salvia Salvia plebeia* R. Br. extract has strong anti-inflammation and anti-oxidation effects, the mixture could also be developed as a functional food or supplementary diet ingredient with the expectation of a synergistic effect between the two herbs.

Despite the usefulness of the Korean red ginseng and *Salvia plebeia* R. Br. extract mixture, there has been little published scientific evidence on it, and what has been published about the toxicity of Korean red ginseng (21,22) is not sufficient to ensure the intake safety of each ingredient alone or as a mixture.

Therefore, we did repeated-dose oral administration and bacterial reverse mutagenicity studies to identify the potential toxicity of the Korean red ginseng and *Salvia plebeia* R. Br. extract mixture.

MATERIALS AND METHODS

Test substance and anaylsis. The extract powder of two herbs was mixed in a ratio of Korean red ginseng: *Salvia plebeia* R. Br., 1:3 which was determined as optimal composition for anti-oxidant and anti-inflammatory effects *in vitro* and *in vivo* preliminary tests. Korean red ginseng extract powder was provided from Korea Ginseng Corporation and dried *Salvia plebeia* R. Br. was provided from Buan Dongjin Farm (Buan, Korea). *Salvia plebeia* R. Br. were extracted two times with an fifteen-fold volume of 30% alcohol for 4 hr at 80°C. After the extract was filtered through 1 μm pore size, the resultant was concentrated on evaporator and prepared into powder by spray-drying. Quantities of analytical markers in Korean red ginseng and *Salvia plebeia* R. Br. extract mixture (KGC-03-PS) was analyzed by ultra performance liquid chromatography (UPLC) using Waters BEH C18 column (1.7 μm, 2.1 × 50 mm). The composition of KGC-03-PS was showed in Table 1. The distilled water was used for administration vehicle.

Table 1. Contents of test article KGC-03-PS

Component	Content (mg/g)
Rg1	0.45
Rb1	1.96
Rg3(S)	0.65
Nepetin	3.06

Subacute oral toxicity study. Subacute oral toxicity test were based on the Organisation for Economic Co-operation and Development (OECD) - Guideline 407 (23).

• ***Test animals and environmental conditions:*** Five-weeks-old male and female, specific pathogen-free (SPF) Sprague-Dawley (SD) rats were purchased from Koatech Inc. (Pyeongtaek, Korea) and acclimated for 7 days before first administration. The rats were housed in polycarbonate plastic cages (1 rat per cage) with aspen porous GLP bedding (Samtako, Osan, Korea) in a room with controlled temperature (20-23°C) and humidity (40-55%), and a 12 hr light/dark cycle. The rats were fed standard rodent chow (Purina, MN, USA) and filtered water ad libitum. This experiment was approved by Institutional Animal Care and Use Committee of KT&G.

• ***Experimental group:*** At six weeks old, the rats were divided into four groups (6 male and 6 female rats in each group): vehicle control (distilled water), low-dose group (500 mg/kg/day), middle-dose group (1,000 mg/kg/day), and high-dose group (2,000 mg/kg/day). The rats were exposed to KGC-03-PS following the toxicity test guideline from the Korea Food and Drug Administration (KFDA) for Nonclinical Laboratory Studies. The dosing volumes were 10 mL/kg bw and the route of administration was oral. The maximum dose was determined according to the recommendation by the OECD - Guideline 423 (24).

• ***Body weight changes:*** Clinical signs of animals were observed each administration day during the 30 min after dosing. Body weight of animals was measured weekly from the initiation day of administration to the day of necropsy.

• ***Food consumption:*** Food consumption was measured at the initiation day of administration, thereafter weekly, and the day of last administration, and then calculated the daily average food intake.

• ***Biochemistry and hematology:*** Before necropsy, all rats were fasted for overnight and killed by exsanguina-

tions under isoflurane anesthesia after recording the body weights. Blood samples were drawn from the inferior vena cava, collected in EDTA-vacutainers, and analyzed for the white blood cell (WBC), red blood cell (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet counts (PLT) using a blood cell counter (MS9-5, Melet Schloesing laboratories, Osny, France).

For clinical biochemistry analyses, serum was obtained from collected blood in a EDTA-free vacutainer by centrifugation at 3,000 rpm for 10 min. Biochemical automatic analyzer (7100, HITACHI, Tokyo, Japan) were used for analyzing alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (CRE), total protein (TP), albumin (ALB), A/G ratio, total cholesterol (T-CHO), triglyceride (TG), and glucose (GLU).

• ***Organ weights:*** The organ including the brain, heart, lung, liver, spleen, kidneys, adrenal glands, testis, and uterus were dissected and the absolute weights were measured immediately. Relative organ weights were calculated as the ratio between the absolute organ weight and the body weight before necropy.

• ***Histopathology:*** The macroscopic organ observation was checked in all animals. The heart, lung, liver, spleen and kidneys were fixed in 10% neutral buffered formalin. Each fixed organ was trimmed as described in previous guideline (25). After paraffin infiltration by tissue processor, the organs were embedded in paraffin and cut into 4 μm section. The sections were stained with hematoxylin and eosin, and examined under light microscopy.

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" (26).

• ***Tester strains:*** In the test, five strains such as TA98, TA100, TA1535, TA1537 of *Salmonella typhimurium*, and WP2uvrA of *Escherichia coli* were used. These strains are highly sensitive to mutagens, commonly used in mutagenicity studies and recommended in the regulatory guidelines (27). *Salmonella typhimurium* TA100, TA1535 and *E. coli* WP uvrA were used to detect the base-pair substi-

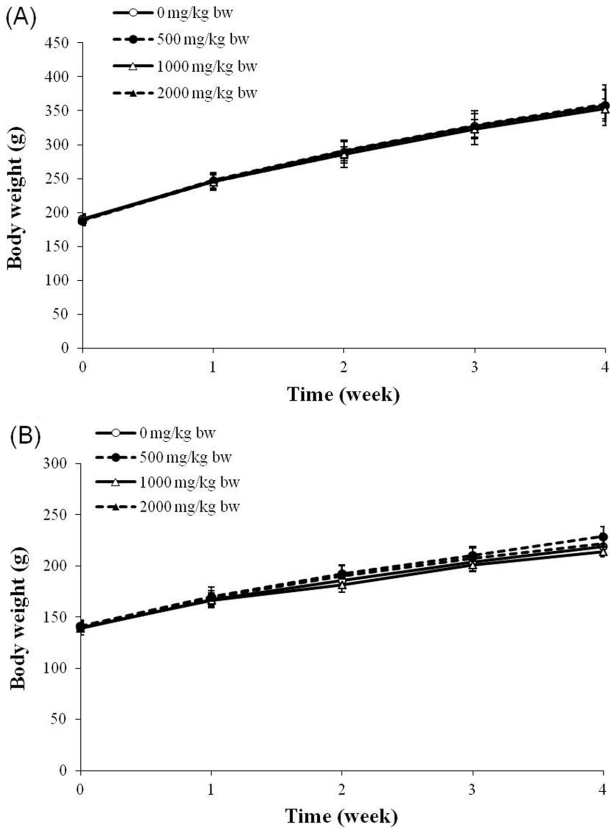

Fig. 1. Body weight changes of male (A) and female (B) rats in the 28 days oral administration toxicity test. The rats exposed to KGC-03-PS showed no significant difference compared to control.

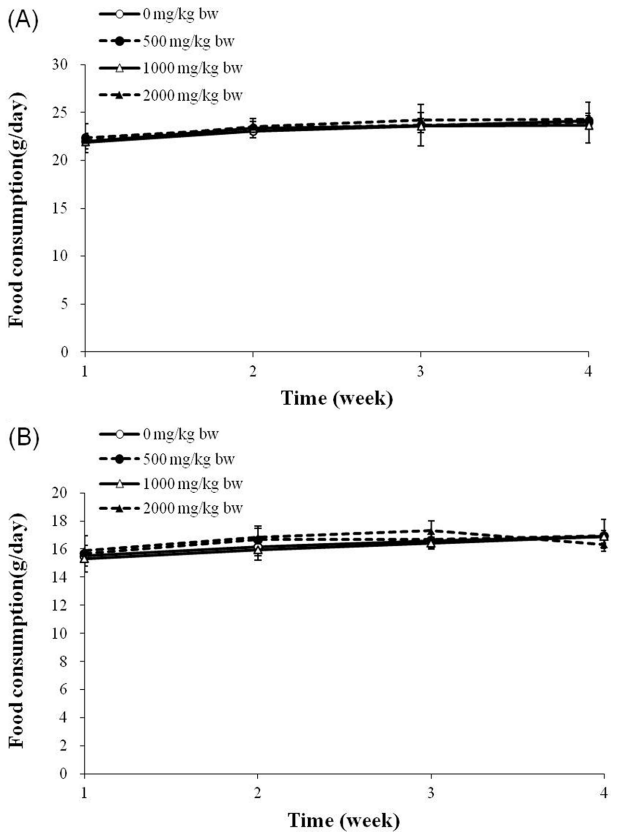

Fig. 2. Food consumption of male (A) and female (B) rats in the 28 days oral administration toxicity test. The rats exposed to KGC-03-PS showed no significant difference compared to control.

tution type mutation, whereas TA98 and TA1537 were used to detect the frame-shift type mutation (28-30). The *Salmonella* strains were purchased from Molecular Toxicology Inc. and *E. coli* WP2 uvrA strain was obtained from Preclinical Research Center (ChemOn, Inc., Gyeonggi, Korea).

• ***Controls:*** With metabolic activation system, Benzo[α]pyrene (Sigma-Aldrich, MO, USA) for TA98, 2-Aminoanthracene (Sigma-Aldrich) for TA100, TA1535, TA1537 and *E. coli*. Without metabolic activation system, Sodium azide (Sigma-Aldrich) for TA100 and TA1535, 4-Nitroquinoline N-oxide (Sigma-Aldrich) for TA98 and *E. coli*, 9-Aminoacridine (Sigma-Aldrich) for TA1537 were used.

• ***Test substance treatment:*** As *Salvia plebeian* leaf extracts in KGC-03-PS was extracted with 30% ethanol, test article was diluted in 30% ethanol. 30% ethanol was used as negative control.

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

For the test, two-fold serial dilutions were performed to yield five concentration (312.5, 625, 1,250, 2,500, and 5,000 μg/plate).

The test was performed based on the plate incorporation method with or out metabolic activation system S9 mixture (31,32). Each sample was assayed in triplicate. Each culture plate was placed in incubator for 48 hr at 37°C. After the incubation, the number of revertant colonies was counted by visual counting.

• ***Evaluation and interpretation of results:*** The test substance was considered as positive in the bacterial reverse mutation assay when there was an increase (≥ two-fold) of spontaneous revertant colonies compared with those in the 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.

Statistical analysis. The data for the body weights, food consumption, and organ weights and the blood biochemistry and hematology indexes were analyzed using an

Table 2. Blood biochemistry data for rats in the 28 days oral administration of KGC-03-PS

	Dose (mg/kg)			
	0 (n = 6)	500 (n = 6)	1000 (n = 6)	2000 (n = 6)
Male				
ALB	2.48 ± 0.05	2.51 ± 0.04	2.55 ± 0.07	2.50 ± 0.10
ALP	395.0 ± 91.2	432.7 ± 31.3	398.7 ± 48.2	371.7 ± 37.9
T-CHO	80.5 ± 10.1	76.0 ± 3.9	74.7 ± 13.4	84.3 ± 5.6
CRE	0.48 ± 0.04	0.45 ± 0.05	0.47 ± 0.05	0.42 ± 0.04
GLU	110.3 ± 13.3	112.8 ± 10.2	102.0 ± 20.4	107.5 ± 24.4
AST	120.5 ± 14.7	120.3 ± 17.7	121.5 ± 14.3	112.8 ± 13.5
ALT	43.8 ± 10.2	43.0 ± 6.1	39.5 ± 3.8	38.3 ± 5.1
TP	5.55 ± 0.10	5.53 ± 0.15	5.62 ± 0.13	5.60 ± 0.14
BUN	19.1 ± 1.5	18.6 ± 2.2	19.4 ± 2.7	17.6 ± 1.0
A/G ratio	0.81 ± 0.02	0.83 ± 0.02	0.83 ± 0.02	0.81 ± 0.04
TG	38.8 ± 7.0	46.7 ± 8.3	39.8 ± 10.5	43.7 ± 9.2
Female				
ALB	2.62 ± 0.09	2.61 ± 0.04	2.65 ± 0.11	2.55 ± 0.11
ALP	269.7 ± 63.0	306.5 ± 61.2	299.0 ± 61.9	243.0 ± 24.2
T-CHO	83.5 ± 10.4	91.5 ± 11.8	88.7 ± 10.8	79.7 ± 13.0
CRE	0.47 ± 0.05	0.50 ± 0.00	0.50 ± 0.06	0.48 ± 0.04
GLU	96.5 ± 7.4	101.7 ± 8.3	110.2 ± 10.9	113.2 ± 13.5*
AST	109.7 ± 14.4	109.5 ± 12.0	109.7 ± 13.2	97.7 ± 13.2
ALT	30.7 ± 1.8	31.2 ± 3.8	30.3 ± 3.8	29.5 ± 2.3
TP	5.57 ± 0.15	5.55 ± 0.14	5.58 ± 0.17	5.43 ± 0.20
BUN	18.3 ± 1.2	18.2 ± 2.9	20.4 ± 2.2	18.9 ± 2.5
A/G ratio	0.89 ± 0.02	0.89 ± 0.02	0.90 ± 0.04	0.89 ± 0.03
TG	23.7 ± 6.8	25.5 ± 11.9	22.0 ± 7.1	21.0 ± 4.7

ALB (g/dL), Albumin; ALP (IU/L), Alkaline phosphatase; CHO (mg/dL), Total cholesterol; CRE (mg/dL), Creatinine; GLU (mg/dL), Glucose; AST (IU/L), Aspartate aminotransferase; ALT (IU/L), Alanine aminotransferase; TP (g/dL), Total protein; BUN (mg/dL), Blood urea nitrogen; A/G ratio; TG (mg/dL), Triglyceride.
Data were expressed as mean ± SD.
*Significant difference vs. control ($p < 0.05$).

one-way analysis of variance (ANOVA) if the variances of the groups were assumed to be equal. When ANOVA showed a statistical significance, post-hoc Dunnett's multiple range test was used to identify the differences between the control and the experimental groups. If the variances of the groups were not assumed to be equal, Kruskal-Wallis test was used to determine the significance of the group differences. The IBM SPSS statistics version 20 software

Table 3. Hematological data for rat in the 28 days oral administration of KGC-03-PS

	Dose (mg/kg)			
	0 (n = 6)	500 (n = 6)	1000 (n = 6)	2000 (n = 6)
Male				
WBC	8.33 ± 1.40	8.44 ± 1.29	8.71 ± 1.81	9.96 ± 1.75
RBC	7.96 ± 0.36	8.66 ± 0.48	8.92 ± 0.39*	8.86 ± 0.73
Hb	17.62 ± 0.57	18.67 ± 1.46	19.48 ± 1.60	19.62 ± 1.46
HCT	43.0 ± 1.2	46.2 ± 3.4	47.7 ± 3.8	47.8 ± 2.8
MCV	54.2 ± 1.1	52.8 ± 0.5	52.9 ± 1.6	53.0 ± 0.6
MCH	22.2 ± 1.1	21.4 ± 0.7	21.4 ± 0.3	21.9 ± 0.8
MCHC	40.1 ± 1.7	40.5 ± 1.0	40.6 ± 1.1	41.2 ± 1.2
PLT	761 ± 53	658 ± 96	653 ± 81	708 ± 76
Female				
WBC	6.08 ± 0.95	7.45 ± 1.40	7.48 ± 1.96	6.90 ± 0.96
RBC	7.47 ± 0.37	7.43 ± 0.28	8.12 ± 0.45	8.24 ± 0.62*
Hb	15.9 ± 0.7	16.2 ± 0.5	16.7 ± 0.8	17.6 ± 1.1*
HCT	42.6 ± 1.5	41.9 ± 2.6	46. 7 ± 1.9	47.8 ± 3.5*
MCV	57.2 ± 2.3	56.4 ± 1.9	57.5 ± 1.6	57.0 ± 1.0
MCH	21.3 ± 0.9	21.9 ± 0.5	20.6 ± 0.6	21.4 ± 0.6
MCHC	37.2 ± 1.0	38.9 ± 1.5	35.8 ± 1.5	37.6 ± 0.7
PLT	915 ± 48	905 ± 35	898 ± 95	774 ± 109*

WBC ($10^3/\mu L$), White blood cells; RBC ($10^6/\mu L$), Red blood cells; Hb (g/dL), Hemoglobin; HCT (%), Hematocrits; MCV (fL), Mean corpuscular volume; MCH (pg), Mean corpuscular hemoglobin; MCHC (g/dL), Mean corpuscular hemoglobin concentration; PLT ($10^3/\mu L$), Platelet.
Data were expressed as mean ± SD.
*Significant difference vs. control ($p < 0.05$).

Table 4. Absolute organ weight for rats in the 28 days oral administration of KGC-03-PS

Absolute weight (g)	Dose (mg/kg)			
	0 (n = 6)	500 (n = 6)	1000 (n = 6)	2000 (n = 6)
Male				
Brain	1.91 ± 0.18	1.87 ± 0.12	1.89 ± 0.10	1.96 ± 0.04
Heart	1.13 ± 0.11	1.14 ± 0.11	1.19 ± 0.06	1.16 ± 0.08
Lung	1.47 ± 0.09	1.50 ± 0.11	1.48 ± 0.12	1.45 ± 0.10
Liver	9.23 ± 1.43	9.64 ± 0.71	9.50 ± 0.31	10.03 ± 0.53
Spleen	0.82 ± 0.13	0.84 ± 0.12	0.76 ± 0.05	0.79 ± 0.09
Kidney	1.13 ± 0.09	1.17 ± 0.12	1.15 ± 0.03	1.18 ± 0.10
Adrenal gland	0.022 ± 0.002	0.022 ± 0.004	0.022 ± 0.003	0.023 ± 0.002
Testis	1.83 ± 0.19	1.93 ± 0.14	1.96 ± 0.11	2.01 ± 0.17
Female				
Brain	1.68 ± 0.05	1.75 ± 0.11	1.71 ± 0.12	1.71 ± 0.08
Heart	0.72 ± 0.06	0.72 ± 0.06	0.72 ± 0.04	0.81 ± 0.03
Lung	1.18 ± 0.05	1.15 ± 0.05	1.14 ± 0.07	1.16 ± 0.10
Liver	5.83 ± 0.24	5.97 ± 0.60	5.72 ± 0.38	5.84 ± 0.44
Spleen	0.58 ± 0.06	0.60 ± 0.05	0.57 ± 0.04	0.60 ± 0.05
Kidney	0.71 ± 0.02	0.72 ± 0.05	0.71 ± 0.03	0.72 ± 0.05
Adrenal gland	0.031 ± 0.004	0.028 ± 0.003	0.031 ± 0.004	0.029 ± 0.004
Uterus	0.52 ± 0.19	0.43 ± 0.07	0.43 ± 0.09	0.47 ± 0.20

Data were expressed as mean ± SD.
There were no significantly differences among the groups.

package was used. A $p < 0.05$ indicated a statistical significance. In the bacterial reverse mutation test, the mean value and standard deviation of each group were measured and compared with the negative control groups.

RESULTS

Subacute oral toxicity study. No mortality and notable clinical signs were observed during the 28 days expo-

Table 5. Relative organ weight for rats in the 28 days oral administration of KGC-03-PS

Relative weight (%)	Dose (mg/Kg)			
	0 (n = 6)	500 (n = 6)	1000 (n = 6)	2000 (n = 6)
Male				
Brain	0.579 ± 0.038	0.566 ± 0.028	0.581 ± 0.014	0.579 ± 0.013
Heart	0.343 ± 0.020	0.345 ± 0.020	0.367 ± 0.026	0.353 ± 0.010
Lung	0.448 ± 0.023	0.456 ± 0.032	0.455 ± 0.023	0.439 ± 0.030
Liver	2.795 ± 0.346	2.923 ± 0.091	2.933 ± 0.159	3.048 ± 0.062
Spleen	0.249 ± 0.024	0.252 ± 0.025	0.235 ± 0.012	0.239 ± 0.019
Kidney	1.129 ± 0.095	1.170 ± 0.117	1.149 ± 0.026	1.183 ± 0.098
Adrenal gland	0.007 ± 0.001	0.007 ± 0.001	0.007 ± 0.001	0.007 ± 0.001
Testis	0.556 ± 0.036	0.583 ± 0.060	0.607 ± 0.029	0.618 ± 0.034
Female				
Brain	0.839 ± 0.031	0.840 ± 0.055	0.871 ± 0.060	0.846 ± 0.041
Heart	0.361 ± 0.029	0.360 ± 0.015	0.368 ± 0.017	0.354 ± 0.019
Lung	0.588 ± 0.030	0.550 ± 0.021	0.581 ± 0.042	0.573 ± 0.027
Liver	2.911 ± 0.130	2.850 ± 0.177	2.909 ± 0.190	2.891 ± 0.167
Spleen	0.291 ± 0.026	0.290 ± 0.029	0.289 ± 0.020	0.296 ± 0.024
Kidney	0.357 ± 0.010	0.343 ± 0.010	0.361 ± 0.010	0.355 ± 0.013
Adrenal gland	0.015 ± 0.002	0.013 ± 0.002	0.016 ± 0.002	0.015 ± 0.002
Uterus	0.258 ± 0.088	0.205 ± 0.028	0.219 ± 0.047	0.231 ± 0.094

Data were expressed as mean ± SD.
There were no significantly differences among the groups.

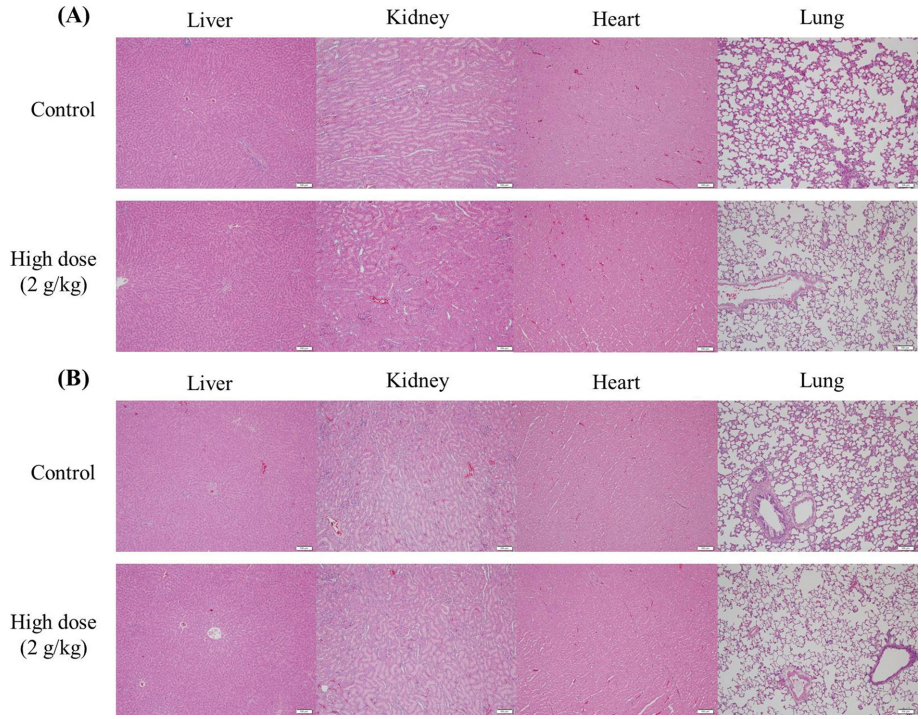

Fig. 3. Histopathological examination of male (A) and female (B) rats exposed with KGC-03-PS. There were no significant toxicological lesions both control and high dose groups. Hematoxylin & Eosin; Magnification: 100×.

sure period and there were no statistically significant changes in body weight and food consumption compared with control group (Fig. 1, 2).

In blood biochemistry analyses, there were statistically significant increase in glucose ($p < 0.05$) in female high-dose group (2,000 mg/kg bw) (Table 2). In hematology analyses, red blood cell significantly increased ($p < 0.05$) in male middle-dose groups (1,000 mg/kg bw) compared to control group (Table 3). In addition, red blood cell count, hemoglobin, hematocrit, and platelet count significantly increased ($p < 0.05$) in female high-dose group compared to control group.

No significant organ weight changes were observed in either the absolute and relative organ weights between the experimental and control animals both sexes (Table 4, 5).

There were no any toxicological changes related with test substances in gross finding and histopathological evaluation of each organ (Fig. 3). Several non-toxicological

Table 6. Summary of histopathological findings

Organ/ Findings	Sex		Male		Female	
	Group		G1	G4	G1	G4
	Dose (mg/kg)		0	2000	0	2000
	No. of animals		6	6	6	6
Kidney	- Hyaline cast, focal, cortex	±	1	1	1	2
		No. of examined	6	6	6	6
Liver	- Fatty degeneration, hepatocyte, focal		0	0	1	1
	- Inflammatory cell infiltration, focal		0	1	1	0
		No. of examined	6	6	6	6
There were unremarkable changes in the heart and lung in Group 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	*E. coli*	TA98	TA1537
0	130 ± 12	10 ± 1	21 ± 3	20 ± 2	13 ± 1
312.5	126 ± 2	9 ± 1	22 ± 2	22 ± 0	9 ± 2
625	119 ± 10	14 ± 4	22 ± 4	22 ± 3	7 ± 0
1250	142 ± 10	10 ± 0	21 ± 4	19 ± 3	9 ± 1
2500	141 ± 10	10 ± 2	23 ± 4	18 ± 1	8 ± 1
5000	145 ± 4	10 ± 2	19 ± 4	19 ± 7	9 ± 1
Positive control (μg/plate)	NaN3 (0.5)	NaN3 (0.5)	4-NQO (0.25)	4-NQO (0.25)	9-AA (50)
	482 ± 12	220 ± 26	95 ± 2	122 ± 3	116 ± 14

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	*E. coli*	TA98	TA1537
0	151 ± 11	9 ± 2	21 ± 2	27 ± 5	10 ± 0
312.5	143 ± 4	12 ± 3	23 ± 2	26 ± 3	10 ± 1
625	127 ± 10	11 ± 3	23 ± 3	29 ± 3	9 ± 1
1250	145 ± 6	8 ± 3	21 ± 3	29 ± 4	10 ± 2
2500	136 ± 3	10 ± 3	24 ± 3	30 ± 2	11 ± 3
5000	126 ± 1	12 ± 3	23 ± 6	26 ± 5	10 ± 0
Positive control (μg/plate)	2-AA (2.0)	2-AA (2.0)	2-AA (6.0)	BaP (1.0)	2-AA (2.0)
	466 ± 24	83 ± 4	167 ± 8	207 ± 12	165 ± 9

changes such as focal tubular hyaline cast in kidney cortex, and minimal macrovesicular fatty changes and inflammatory cells infiltration in liver, were observed in some control and high-dose (2,000 mg/kg bw) treatment animals (Table 6).

Bacterial reverse mutation test. The results of the bacterial reverse mutation test with the five bacterial strains (TA98, 100, 1535, 1537 and *E. coli* WP2) for KGC-03-PS are showed in Table 7 and 8. The test was performed on KGC-03-PS at five different concentrations, from 312.5 to 5,000 μg/plate, with or without S9 mix. There was no increase in the number of revertant colonies compared to its negative control with any dose or strains. The number of colonies of positive control chemicals for each tester stain was significantly higher than the negative control and within the control historical data range of our lab. Background lawn was observed and test substance precipitation and cytotoxicity was not detected all test strains.

DISCUSSION

Korean red ginseng and *Salvia plebeia* R. Br. have been used as an herbal medicine worldwide for a while. The efficacy of Korean red ginseng has been proven by *in vitro* and *in vivo* studies (9-13) as well as by clinical research (33). Moreover, the various effects of Korean red ginseng such as the improvement of the immunity (34), blood circulation (35) and memory (36) as well as fatigue relief (37), anti-oxidation (38), and mitigation of menopausal symptoms in women (39) has been approved by the Korean Food and Drug administration. *Salvia plebeia* R. Br. has also been shown to have potent anti-inflammatory and anti-oxidation effects described in previous studies (15,16). Repeated oral toxicity and genotoxicity studies are representative safety assessments which are required as scientific safety data to register a functional food or supplementary diet ingredients. A repeated oral toxicity study is usually done following the OECD test guideline which suggests clinical, clinicopathological, and pathological examinations be done to evaluate the safety of the test article (23).

In the present study, clinical signs, body weight, and food intake were analyzed in the clinical evaluation. Particularly, body weight is used to identify the toxicological significance of a substance which is used to calculate a maximum tolerated dose (40). Korean red ginseng and *Salvia plebeia* R. Br. extract mixture (KGC-03-PS) did not exhibit any toxicological changes in the clinical signs, body weight, and food intake.

Hematology and serum biochemistry were done as a clinicopathological evaluation. Hematology measures the numbers and morphology of various blood cell component and generally is used to diagnosis and monitor various diseases of the blood and bone marrow as well as of the immunologic, hemostatic, and vascular systems. Serum biochemistry is useful tool to predict the toxicological effects including liver and kidney function, and electrolyte balance. Because the results could be affected by the experimental environment and test machines, it is important to accumulate normal historical data from each institution to increase the confidence level of the test (41).

The increase in the red blood cell related index in the present study could be caused by a low oxygen level due to heart and pulmonary failure or kidney damage. However, no toxicological changes were observed in the weight of related organs, serum biochemistry and histopathological results. Moreover, the levels were within the historically normal range which suggest that KGC-03-PS has no toxicologically significant effect. In addition, decreased platelet was considered to be non-toxicological finding because it was shown in only 2,000 mg/kg treatment of female rat not in male and the count variation is mild within the historically normal range.

The statistically significant changes in glucose for the 2,000 mg/kg treatment of female rat are also considered as no toxicological effect because the changes were within the historically normal ranges and did not show a dose response relationship.

In the pathological evaluation, the necropsy organ weights and the gross and histopathological lesions were analyzed. Organ weight is considered to be an important indicator for the identification of toxicological effects of the test article in toxicology studies. In addition, the Society of Toxicologic Pathology (STP) recommends that the weights of the liver, heart, kidneys, brain, testes, and adrenal glands be measured in all general toxicology studies (42).

Information on gross and histopathological lesions provides target organ toxicity by identifying organ size and color as well as cellular changes (43). Administration of the KGC-03-PS did not induce any pathological changes in the main organs including the liver, kidney, heart, lung, and spleen.

Based on the clinical, clinicopathological, and pathological evaluations, it was shown that the 28 days repeated oral dose of the KGC-03-PS did not induce any toxic effects in the treated animals at the 2,000 mg/kg high dose under the conditions used in this study. This result suggests that the no-observed-adverse-effect level (NOAEL) of the KGC-03-PS is considered to be 2,000 mg/kg for both sexes.

The mutagenicity of KGC-03-PS was evaluated by bacterial mutation test and toxicity was not observed up to a maximum concentration of 5,000 μg/plate in all tested bacterial strains (*S. typhimurium* TA98, TA100, TA1535, TA1537, and *E.coli* WP2) in presence or absence of metabolic activation. There was no difference in the number of

revertant colonies between the KGC-03-PS treatment and negative control groups. The negative and positive control values were within historical control ranges observed in our laboratory, which assured of the test validity. These results showed that KGC-03-PS did not induce genotoxicity through bacterial reverse mutation under the conditions in the study. In conclusion, the results of this study indicate that oral doses of KGC-03-PS repeated daily for 28 days do not produce any detectable toxic effects in SD rats at dose levels up to 2,000 mg/kg bw. Thus, the NOAEL of KGC-03-PS is considered to be greater than 2,000 mg/kg bw in both sexes of SD rats. In addition, int the bacterial reverse mutation test, KGC-03-PS did not have any mutagenicity. Our results, as fundamental information for the evaluation of the subchronic or chronic toxicity of KGC-03-PS, should be useful in designing further studies on the safety and efficacy of KGC-03-PS.

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CONFLICT OF INTEREST

All authors declare no conflicts of interest.

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