ORIGINAL ARTICLE

13-Week repeated oral dose toxicity study on mixture of Korean red ginseng and deer antler extract in Sprague-Dawley rats

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Abstract

Owing to an increase in the consumption of herbal products as supplementary diets or functional foods, their safety has become an important issue. Repeated oral administration to rats for 13-week was performed to evaluate the potential toxicity of a mixture of Korean red ginseng and deer antler extract, the most popular traditional herbal ingredients. Three test groups for the mixture of Korean red ginseng and deer antler extract were administered at 500, 1000, and 2000 mg/kg/day in addition to a control group (water for injection). 10 male and 10 female rats were included in each group, and we evaluated the clinical, clinicopathological, and histopathological changes in the rats. One male rat in the test group at 1000 mg/kg/day died; however, it was considered a spontaneous death unrelated to the administration of the test substance. No test substance-related toxic effects were noted in rats in terms of body weight, food consumption, ophthalmological findings, urinalysis, hematological parameters, blood biochemical parameters, organ weights, gross postmortem findings, and histopathological findings. The present results suggest that the no observed adverse effect level of the mixture of Korean red ginseng and deer antler extract was greater than 2000 mg/kg/day in all rats after repeated oral administration for 13-week under the present study conditions.

Keywords Korean red ginseng extract · Deer antler extract · 13-Weeks repeated oral dose toxicity study · No-observed-adverse-effect level

Introduction

Ginseng (*Panax ginseng* C.A. Meyer) has been used as traditional herbal medicine in Asian countries including Korea for several thousand years. Ginseng has been consumed to improve overall health and boost the immune system. In particular, red ginseng, which is steamed and then dried, has been used as a medicinal, cosmetic, and dietary supplement in many countries [1].

Numerous studies have reported the various effects of red ginseng through in vitro analyses, experiments with animal models, and clinical trials. Ginseng has been described as

an adaptogen that maintains homeostasis by normalizing overall physiological functions by nonspecifically increasing the resistance to external stress [2, 3]. Moreover, The various effects of Korean red ginseng including improvement of blood circulation by inhibiting platelet aggregation [4, 5], immune function [6, 7], blood glucose level regulation [8] and amelioration of memory and cognitive function of brain [9, 10]. It is also reported to have anti-fatigue [11, 12], anti-oxidation [13, 14] and anti-cancer effect [8].

Deer antler extract has long been believed to benefit human health in many Asian countries, especially China. The effect of deer antler extract was believed to strengthen the body, improve immune function, and regulate cardiovascular health and function. In particular, recent studies have reported that the biological effects of deer antler extracts include promotion of hair growth and hematopoietic properties [15–18].

Although Korean red ginseng and deer antler have been considered relatively safe herbs on the basis of historical usage among humans, limited information is available regarding intake safety [19, 20]. Several toxicological studies

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have evaluated the effects of the toxicity of Korean red ginseng upon its repeated administration [21, 22]; in addition, only one study has investigated the 13-week repeated oral toxicity of deer antler extract [23].

Moreover, although several studies on the safety of Korean red ginseng extract, including the 13-week repeated oral toxicity study, have been reported, the potential toxicity of the mixture of Korean red ginseng and deer antler extract has not been investigated. Because co-administration of various herbal sources is becoming more common, it is important to identify the interaction or potential toxicity of this mixture.

Hence, the present study, the 13-week repeated oral administration toxicity study, aimed to investigate the general toxicity including clinical, clinicopathological, and histopathological effects of dietary supplementation of herbal products in rats, using a mixture of Korean red ginseng and deer antler extract at doses of 0, 500, 1000, and 2000 mg/kg.

Table 1 The content of analytical markers of Korean red ginseng and deer antler extract mixture

Active ingredient	mg/g
Ginsenoside Rg1	1.32
Ginsenoside Rb1	6.39
Ginsenoside Rg3s	2.36
Sialic acid	0.34

Materials and methods

Regulatory guidelines and ethics

This study was conducted in accordance with the Good Laboratory Practice Regulation, “standards and regulation for chemical testing laboratories” and OECD Guidance No. 408, “Repeated dose 90-day oral toxicity in rodents.” The experimental protocol was reviewed and approved by the

Fig. 1 Body weight changes of rats in the 13-week oral administration toxicity test

Institutional Animal Care and Use Committees (IACUC) (2016, Approval No.: 160163).

Test substance

The test substance was a mixture of Korean red ginseng and deer antler extract, a yellowish-brown powder, provided by Korea Ginseng Corporation (Korea).

Korea red ginseng extract was consecutively extracted 7 times with distilled water for 12 h at 87 °C. Thereafter, it was filtered and concentrated. Deer antler (New Zealand) powder was extracted twice with distilled water: first extract, 4 h; second extract, 6 h at 87 °C. Thereafter, the extract was filtered and concentrated.

The recommended daily intake of Korea red ginseng extract is 3 g/day. Although limited information is available regarding the recommended daily intake of deer antler powder, some clinical studies have reported that consumption of 2 g/day of deer antler powder (approximately equal to 1.5 g/day deer antler extract) or greater has beneficial effects [24, 25]. Therefore, the test substance, Korean red ginseng extract and antler extract, were mixed in a 2:1 ratio and converted to a powder via spray-drying.

The test substance was validated by analyzing the content of analytical markers via high-performance liquid chromatography (HPLC) using YMC-Pack pro C18 column (250×4.6 mm, 5 µm, YMC, Japan) (results summarized in Table 1). Distilled water was used as the vehicle.

Fig. 2 Food consumption of rats in the 13-week oral administration toxicity test

A Male

B Female

Table 2 Hematological data of male SD rats (mean $\pm$ SD)

Sex: male	Group/dose (mg/kg/day)			
	G1/0	G2/500	G3/1000	G4/2000
No. of animals	10	10	9	10
RBC ($\times 10^6$ cells/ μ L)	8.79 $\pm$ 0.27	8.78 $\pm$ 0.42	8.76 $\pm$ 0.18	8.78 $\pm$ 0.26
HGB (g/dL)	15 $\pm$ 0.6	14.9 $\pm$ 0.6	14.7 $\pm$ 0.7	15.1 $\pm$ 0.5
HCT (%)	46.1 $\pm$ 1.7	45.4 $\pm$ 1.9	45.3 $\pm$ 2.1	46.1 $\pm$ 1.6
RBC indices				
MCV (fL)	52.4 $\pm$ 1.6	51.7 $\pm$ 1.5	51.6 $\pm$ 2	52.5 $\pm$ 0.9
MCH (pg)	17 $\pm$ 0.6	16.9 $\pm$ 0.8	16.8 $\pm$ 0.6	17.2 $\pm$ 0.5
MCHC (g/dL)	32.5 $\pm$ 0.4	32.8 $\pm$ 0.8	32.5 $\pm$ 0.3	32.8 $\pm$ 0.6
PLT ($\times 10^3$ cells/ μ L)	922 $\pm$ 105	969 $\pm$ 106	953 $\pm$ 92	922 $\pm$ 114
Reti (%)	2.68 $\pm$ 0.3	2.7 $\pm$ 0.76	2.6 $\pm$ 0.49	2.33 $\pm$ 0.42
WBC ($\times 10^3$ cells/ μ L)	9.24 $\pm$ 2.29	8.76 $\pm$ 3.23	9.07 $\pm$ 1.67	9.4 $\pm$ 2.25
WBC differential counting (%)				
NEU	23.5 $\pm$ 9.8	19.4 $\pm$ 8.5	19.5 $\pm$ 9.5	21.7 $\pm$ 7.7
LYM	72.2 $\pm$ 9.8	75.7 $\pm$ 8.7	75.8 $\pm$ 9.3	73.8 $\pm$ 7.9
MONO	2.3 $\pm$ 0.7	2.6 $\pm$ 0.7	2.4 $\pm$ 0.9	2.0 $\pm$ 0.8
EOS	1.2 $\pm$ 0.4	1.4 $\pm$ 0.6	1.3 $\pm$ 0.5	1.2 $\pm$ 0.5
BASO	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1
PT (s)	17.9 $\pm$ 0.6	18.2 $\pm$ 0.7	17.8 $\pm$ 0.6	17.9 $\pm$ 0.7
APTT (s)	18.3 $\pm$ 1.5	18.1 $\pm$ 1.0	18.0 $\pm$ 0.8	17.4 $\pm$ 1.6

Test animals and environmental conditions

Five-weeks-old male and female, specific pathogen-free (SPF) Sprague-Dawley (SD) rats were purchased from ORIENT BIO Inc. (Korea). The rats ($n=2$ or 3) were housed in stainless steel wire mesh cages (26.0 cm wide $\times$ 35.0 cm long $\times$ 21.0 cm high) and acclimatized for 7 days at 21.3–27.0 °C and 44.2–78.1% humidity with a 12 h light (150–300 lx)/dark cycle. Pellet rodent chow (Teklad Certified Irradiated Global 18% Protein Rodent Diet 2918C) obtained from Harlan Laboratories, Inc. (Indianapolis, IN, USA) was provided ad libitum.

After the quarantine-acclimation period, 40 males and 40 females were selected and randomly assigned to 4 groups (10 animals of each sex per group) with equalization of the mean group body weight. The rats were housed individually cages (1 rat per cage) in stainless steel wire mesh cages during the study period.

Group designation and dose levels

Individual doses were determined on the basis of the most recently measured individual body weight at a dose volume of 10 mL/kg body weight (b.w.). Animals were dosed once a

Table 3 Hematological data of female SD rats (mean $\pm$ SD)

Sex: female	Group/dose (mg/kg/day)			
	G1/0	G2/500	G3/1000	G4/2000
No. of animals	10	10	10	10
RBC ($\times 10^6$ cells/ μ L)	8.17 $\pm$ 0.47	8.09 $\pm$ 0.22	8.23 $\pm$ 0.18	8.16 $\pm$ 0.33
HGB (g/dL)	14.7 $\pm$ 0.7	14.8 $\pm$ 0.3	14.7 $\pm$ 0.4	14.7 $\pm$ 0.5
HCT (%)	44.3 $\pm$ 2.3	44.2 $\pm$ 0.9	43.9 $\pm$ 0.9	43.8 $\pm$ 1.2
RBC indices				
MCV (fL)	54.2 $\pm$ 1.4	54.8 $\pm$ 1.2	53.4 $\pm$ 1.4	53.7 $\pm$ 1.1
MCH (pg)	18.1 $\pm$ 0.5	18.3 $\pm$ 0.5	17.9 $\pm$ 0.5	18.1 $\pm$ 0.5
MCHC (g/dL)	33.3 $\pm$ 0.6	33.4 $\pm$ 0.5	33.5 $\pm$ 0.2	33.7 $\pm$ 0.6
PLT ($\times 10^3$ cells/ μ L)	922 $\pm$ 105	969 $\pm$ 106	953 $\pm$ 92	922 $\pm$ 124
Reti (%)	2.37 $\pm$ 1.26	2.09 $\pm$ 0.46	1.7 $\pm$ 0.25	2.08 $\pm$ 0.63
WBC ($\times 10^3$ cells/ μ L)	5.78 $\pm$ 1.58	4.89 $\pm$ 0.95	5.39 $\pm$ 1.46	5.74 $\pm$ 1.52
WBC differential counting (%)				
NEU	18.9 $\pm$ 6	17.8 $\pm$ 8.7	13.4 $\pm$ 4.6	13.3 $\pm$ 7.4
LYM	76.6 $\pm$ 6.8	77.6 $\pm$ 9.1	81.5 $\pm$ 4.9	82.4 $\pm$ 7.2
MONO	2.0 $\pm$ 0.9	2.1 $\pm$ 0.5	2.3 $\pm$ 0.9	1.9 $\pm$ 0.6
EOS	1.4 $\pm$ 0.3	1.5 $\pm$ 0.7	1.6 $\pm$ 0.4	1.4 $\pm$ 0.3
BASO	0.2 $\pm$ 0	0.2 $\pm$ 0.1	0.2 $\pm$ 0	0.2 $\pm$ 0
PT (s)	18.4 $\pm$ 0.8	18.0 $\pm$ 0.4	17.9 $\pm$ 0.7	18.2 $\pm$ 0.8
APTT (s)	15.1 $\pm$ 1.2	15.5 $\pm$ 1.5	15.7 $\pm$ 1.2	14.9 $\pm$ 1.4

RBC Total erythrocyte count, HGB hemoglobin, HCT hematocrit, MCV mean corpuscular volume, MCH mean corpuscular hemoglobin, MCHC mean corpuscular hemoglobin concentration, PLT platelet count, Reti reticulocytes, WBC total leukocyte count, NEU neutrophils, LYM lymphocytes, MONO monocytes, EOS eosinophils, BASO basophils, PT Prothrombin time, APTT activated partial thromboplastin time

day for thirteen weeks via gastric intubation with 3-, 5-, and 10-mL disposable syringes fitted with an intubation tube.

In the general acute toxicity test, the highest dose level was selected as 2000 or 5000 mg/kg [26], based on which the highest dose level of the subacute toxicity test was determined to range from 1000 to 3000 mg/kg [27–29]. When determining the 4-week repeated dose range, no test substance-related effects were observed at 500, 1000, and 2000 mg/kg/day. Hence, the dose for the test substance group was selected as 2000 mg/kg/day as the high dose level. Thereafter, the middle and low dose levels were selected at 1000 and 500 mg/kg/day, respectively. The control group was dosed with the vehicle, with the same dose volume as those of the test substances.

Administration and observation

Clinical signs

All animals were observed daily for mortality and the clinical signs.

Body weights

Body weights were measured just before first administration, once a week during the study period, at the day of the last administration and on the day of necropsy.

Food consumption

Food consumption was measured weekly from the day of group isolation to the first day of administration. During the study periods, the mean daily food consumption was calculated by dividing the weekly consumption amount by 7 days. Six-day food consumption was calculated at week 13.

Ophthalmological examination

Ophthalmological examinations were performed for both eyes of 5 rats in each group on week 13. The examinations for the pupil light reflex and the anterior segment of the eye were conducted with naked eyes before instillation of mydriatic agent (ISOPTOATROPINE® STERILE OPHTHALMIN PREPARATION, Alcon®, Lot No.: 14B06Q) and then the anterior segment of the eye, transparent media and ocular fundus were observed using an ophthalmoscope (ALL PUPIL II, Keeler, U.K.) after instillation of mydriatic agent.

Urinalysis

Fresh, 3 h and 24 h urine samples were collected from 5 males and 5 females in the each group at week 13. When fresh urine was collected, Except for drinking water, feeding and dosing were not performed. Protein, Glucose, PH, Ketone body, Occult blood and Bilirubin were measured by Combur10Test®M stick (Roche, Germany). Urine color, turbidity and sediment were checked by visual inspection or Optical microscope. Urine volume and specific gravity were measured from 24 h urine. The specific gravity was measured by a Gravimeter (VET 360, Reichert Technologies, New York, USA).

Hematology

All rats were fasted overnight for 18 h before necropsy for blood collection under isoflurane anesthesia. About 1 mL of blood samples were collected from the posterior vena cava and placed in a EDTA-vacutainer. Autoanalyzer (ADVIA 2120i,

Table 4 Blood biochemistry data of male SD rats (mean ± SD)

Sex: male	Group/dose (mg/kg/day)			
	G1/0	G2/500	G3/1000	G4/2000
No. of animals	10	10	9	10
ALT (U/L)	28.3 ± 6.5	26.6 ± 4.3	25.8 ± 3.2	28.1 ± 5.7
AST (U/L)	88.8 ± 18.9	82.5 ± 16	87.8 ± 12.9	88.8 ± 20
ALP (U/L)	281.9 ± 53.6	262.1 ± 48	271.6 ± 47.5	281.3 ± 85.1
GGT (U/L)	0.36 ± 0.17	0.37 ± 0.13	0.35 ± 0.14	0.32 ± 0.13
Glu (mg/dL)	137 ± 19	134 ± 13	129 ± 8	130 ± 9
BUN (mg/dL)	12.1 ± 1.1	12.4 ± 0.4	13.7 ± 1.6	12.5 ± 1.4
Crea (mg/dL)	0.46 ± 0.46	0.45 ± 0.45	0.48 ± 0.48	0.46 ± 0.46
T-Bili (mg/dL)	0.04 ± 0.04	0.05 ± 0.05	0.06 ± 0.06	0.05 ± 0.05
T-Chol (mg/dL)	74 ± 21	85 ± 22	87 ± 25	85 ± 25
TG (mg/dL)	58 ± 26	80 ± 33	59 ± 25	72 ± 43
TP (g/dL)	6.2 ± 0.2	6.1 ± 0.3	6.0 ± 0.1	6.0 ± 0.3
Alb (g/dL)	2.5 ± 0.1	2.5 ± 0.2	2.4 ± 0.1	2.4 ± 0.2
A/G	0.65 ± 0.04	0.68 ± 0.04	0.66 ± 0.02	0.66 ± 0.05
P (mg/dL)	6.17 ± 0.48	6.04 ± 0.48	6.06 ± 0.6	5.89 ± 0.41
Ca (mg/dL)	10.1 ± 0.2	10.1 ± 0.4	10.1 ± 0.2	10.1 ± 0.5
Na (mmol/L)	139 ± 1.1	138.7 ± 1	138.9 ± 1	139.5 ± 0.6
K (mmol/L)	4.28 ± 0.13	4.19 ± 0.26	4.25 ± 0.2	4.18 ± 0.17
Cl (mmol/L)	106.2 ± 1.5	105.2 ± 1.3	105.8 ± 1.4	106.8 ± 1.2

SIEMENS, Germany) was used for analyzing the hematology indexes. Plasma was obtained from collected blood mixed with 3.2% sodium citrate by centrifugation at 3000 rpm for 10 min and used to measure the Prothrombin time (PT) and Activated partial thromboplastin time (APTT) by an automatic coagulation time meter (Coapresta 2000, SEKISUI, Japan).

For clinical biochemistry analyses, serum was obtained from collected blood in a EDTA-free vacutainer by centrifugation at 3000 rpm for 10 min. Biochemical automatic analyzer (7180, HITACHI, Japan) and electrolyte analyzer (EasyLyte, MEDICA, USA) were used for analyzing the biochemical indexes to evaluate the liver and kidney function, and lipid metabolism as well as an electrolyte balance.

Necropsy (gross pathology)

At the end of test, all animals were sacrificed by exsanguination from the abdominal aorta under isoflurane anesthesia. Postmortem macroscopic observation for internal organs was performed and all abnormalities were recorded. Absolute and relative weight of organs including the liver, kidney, lung, spleen, brain, pituitary, adrenal gland, testis, prostate, ovary, and uterus were measured. For histopathological examination,

Table 5 Blood biochemistry data of female SD rats (mean $\pm$ SD)

Sex: female	Group/dose (mg/kg/day)			
	G1/0	G2/500	G3/1000	G4/2000
No. of animals	10	10	10	10
ALT (U/L)	24.5 $\pm$ 7.9	24.6 $\pm$ 11.2	23.6 $\pm$ 3.6	24.9 $\pm$ 4.8
AST (U/L)	80.1 $\pm$ 17.9	83.6 $\pm$ 26.7	78.7 $\pm$ 12.3	78.0 $\pm$ 12.4
ALP (U/L)	130.7 $\pm$ 33.8	153.7 $\pm$ 44.0	133.8 $\pm$ 33.4	146.1 $\pm$ 22.5
GGT (U/L)	0.23 $\pm$ 0.16	0.33 $\pm$ 0.16	0.29 $\pm$ 0.21	0.31 $\pm$ 0.18
Glu (mg/dL)	131 $\pm$ 14	132 $\pm$ 13	126 $\pm$ 11	135 $\pm$ 15
BUN (mg/dL)	14.4 $\pm$ 1.5	14.3 $\pm$ 1.2	14.5 $\pm$ 1.6	15.3 $\pm$ 2.0
Crea (mg/dL)	0.51 $\pm$ 0.05	0.53 $\pm$ 0.05	0.51 $\pm$ 0.04	0.51 $\pm$ 0.04
T-Bili (mg/dL)	0.09 $\pm$ 0.02	0.08 $\pm$ 0.02	0.07 $\pm$ 0.03	0.07 $\pm$ 0.03
T-Chol (mg/dL)	84 $\pm$ 18	82 $\pm$ 8	85 $\pm$ 18	88 $\pm$ 11
TG (mg/dL)	40 $\pm$ 40	30 $\pm$ 15	25 $\pm$ 11	22 $\pm$ 6
TP (g/dL)	6.3 $\pm$ 0.5	6.5 $\pm$ 0.2	6.5 $\pm$ 0.4	6.3 $\pm$ 0.3
Alb (g/dL)	2.8 $\pm$ 0.3	2.9 $\pm$ 0.2	2.9 $\pm$ 0.3	2.8 $\pm$ 0.1
A/G	0.82 $\pm$ 0.07	0.79 $\pm$ 0.04	0.82 $\pm$ 0.05	0.80 $\pm$ 0.05
P (mg/dL)	5.07 $\pm$ 0.65	4.67 $\pm$ 0.79	5.17 $\pm$ 0.89	4.77 $\pm$ 0.92
Ca (mg/dL)	10.1 $\pm$ 0.4	10.2 $\pm$ 0.3	10.3 $\pm$ 0.4	10.1 $\pm$ 0.3
Na (mmol/L)	138.6 $\pm$ 1.3	139.3 $\pm$ 0.8	139.0 $\pm$ 0.5	139.1 $\pm$ 1.1
K (mmol/L)	3.96 $\pm$ 0.27	4.03 $\pm$ 0.27	3.99 $\pm$ 0.24	4.00 $\pm$ 0.23
Cl (mmol/L)	107.2 $\pm$ 1.3	107.1 $\pm$ 1.4	106.7 $\pm$ 0.8	107.0 $\pm$ 1.3

ALT alanine aminotransferase, AST aspartate aminotransferase, ALP alkaline phosphatase, GGT gamma glutamyl transpeptidase, BUN blood urea nitrogen, Crea creatinine, T-Bili total bilirubin, TP total protein, Alb Albumin, A/G ratio albumin/globulin ratio, T-Chol total cholesterol, TG triglycerides, P phosphorus, Glu glucose, Ca calcium, Cl chloride, Na sodium, K potassium

the removed organs including the testes, eyes and optic nerve were fixed in Davidson fixative and the others were fixed in 10% neutral buffered formalin.

Histopathology

Each fixed organ was processed according to the previous guideline [30]. Trimmed organs were infiltrated by paraffin and embedded into paraffin blocks which were cut into 4 μ m tissue sections. The tissues were stained with hematoxylin and eosin solution, and the histopathological changes were evaluated under light microscopy (BX51, Olympus, USA).

Statistical analysis

SAS Program (version 9.3, SAS Institute Inc., USA) was used for statistical analysis. Bartlett's test was used for homogeneity of variance ($p < 0.05$). One-way analysis of variance (ANOVA) was used for homogeneous data; if statistical significance was observed, Dunnett's test was applied for multiple comparisons. Kruskal–Wallis test was used for heterogeneous data; if statistical significance was observed, Steel test was applied for multiple comparisons.

Results

Clinical signs

Other than the death of one male rat in the test substance group (1000 mg/kg/day) on day 74 (Animal No. 1308), no significant signs were observed. This death was considered to result incidentally and spontaneously as a rare case and not due to toxic effects of the test substance, with no clear evidence of dose dependency of the test substance in the mid dose group.

Body weights and food consumption

Body weights (Fig. 1) and food consumption (Fig. 2) did not differ significantly between the test groups and the control group.

Ophthalmological examination

No ocular abnormalities were observed in all test groups.

Table 6 Absolute organ weights of SD rats (mean $\pm$ SD)

Sex: male	Group-dose (mg/kg/day)			
	G1-0	G2-500	G3-1000	G4-2000
N	10	10	9	10
B.W.	517.7 $\pm$ 40.3	546.5 $\pm$ 41.2	560 $\pm$ 41.9	515.9 $\pm$ 40.1
Brain	2.13 $\pm$ 0.1	2.12 $\pm$ 0.07	2.13 $\pm$ 0.11	2.11 $\pm$ 0.07
Pituitary	0.0122 $\pm$ 0.0014	0.0132 $\pm$ 0.0013	0.0134 $\pm$ 0.002	0.0127 $\pm$ 0.0022
Heart	1.42 $\pm$ 0.1	1.5 $\pm$ 0.1	1.57 $\pm$ 0.18	1.45 $\pm$ 0.12
Lung	1.61 $\pm$ 0.16	1.66 $\pm$ 0.12	1.7 $\pm$ 0.12	1.61 $\pm$ 0.1
Liver	13.18 $\pm$ 1.51	14.29 $\pm$ 1.71	14.53 $\pm$ 1.33	13.8 $\pm$ 1.63
Spleen	0.85 $\pm$ 0.08	0.84 $\pm$ 0.14	0.97 $\pm$ 0.18	0.83 $\pm$ 0.12
Kidney	3.07 $\pm$ 0.24	3.13 $\pm$ 0.21	3.25 $\pm$ 0.26	3.15 $\pm$ 0.18
Adrenal	0.0609 $\pm$ 0.0103	0.0662 $\pm$ 0.0102	0.0676 $\pm$ 0.008	0.0575 $\pm$ 0.0071
Testis	3.31 $\pm$ 0.26	3.41 $\pm$ 0.33	3.66 $\pm$ 0.29*	3.6 $\pm$ 0.34
Prostate	0.49 $\pm$ 0.19	0.5 $\pm$ 0.09	0.57 $\pm$ 0.13	0.62 $\pm$ 0.15
Sex: female	Group-dose (mg/kg/day)			
	G1-0	G2-500	G3-1000	G4-2000
N	10	10	10	10
B.W.	303.5 $\pm$ 37.5	288.8 $\pm$ 23.8	280.7 $\pm$ 26.8	290.8 $\pm$ 26.7
Brain	1.95 $\pm$ 0.09	1.93 $\pm$ 0.04	1.99 $\pm$ 0.09	1.97 $\pm$ 0.09
Pituitary	0.0161 $\pm$ 0.0046	0.015 $\pm$ 0.0023	0.0147 $\pm$ 0.002	0.0159 $\pm$ 0.0011
Heart	0.94 $\pm$ 0.12	0.94 $\pm$ 0.08	0.92 $\pm$ 0.09	0.9 $\pm$ 0.05
Lung	1.21 $\pm$ 0.1	1.2 $\pm$ 0.07	1.21 $\pm$ 0.07	1.23 $\pm$ 0.08
Liver	7.35 $\pm$ 1.45	7.17 $\pm$ 0.71	6.94 $\pm$ 0.79	7.03 $\pm$ 0.34
Spleen	0.56 $\pm$ 0.11	0.53 $\pm$ 0.08	0.52 $\pm$ 0.06	0.53 $\pm$ 0.07
Kidney	1.86 $\pm$ 0.26	1.8 $\pm$ 0.08	1.84 $\pm$ 0.14	1.84 $\pm$ 0.23
Adrenal	0.0701 $\pm$ 0.0089	0.0718 $\pm$ 0.0105	0.0705 $\pm$ 0.01	0.067 $\pm$ 0.0091
Ovary	0.08 $\pm$ 0.02	0.09 $\pm$ 0.01	0.08 $\pm$ 0.01	0.08 $\pm$ 0.01
Uterus	0.69 $\pm$ 0.25	0.7 $\pm$ 0.22	0.66 $\pm$ 0.26	0.77 $\pm$ 0.29

Significantly different from control by Dunnett's *t* test: **p* < 0.05

Hematology and blood biochemistry

Hematological (Tables 2 and 3) and blood biochemical parameters (Tables 4 and 5) did not differ significantly between the test and control groups.

Organ weights

The absolute weight of the testis was significantly higher in the test substance group (1000 mg/kg/day) than in the control group; however, the absolute organ weight of organs except the testis did not differ significantly (Table 6).

No treatment-related effects were observed on relative organ weights in both sexes in test groups (Table 7).

Urinalysis

Urine parameters did not differ significantly in all test groups (Table 8). Minor changes in urinary parameters were not dose-related, and there were no changes in other related parameters. Therefore, they were not considered to have toxicological significance.

Necropsy and histopathological findings

Macroscopic examination at necropsy revealed no changes in all test substance groups (Fig. 3). No treatment-related changes were observed in histopathological findings in all test groups. All the minor changes were within the normal physiological thresholds and were not dose-related, being considered incidental or spontaneous and to have no toxicological significance.

Table 7 Relative organ weights of SD rats (mean $\pm$ SD)

Sex: male	Group-dose (mg/kg/day)			
	G1-0	G2-500	G3-1000	G4-2000
N	10	10	9	10
B.W.	517.7 $\pm$ 40.3	546.5 $\pm$ 41.2	560 $\pm$ 41.9	515.9 $\pm$ 40.1
Brain	0.41 $\pm$ 0.02	0.39 $\pm$ 0.03	0.38 $\pm$ 0.03	0.41 $\pm$ 0.03
Pituitary	0.0024 $\pm$ 0.0003	0.0024 $\pm$ 0.0004	0.0024 $\pm$ 0.00004	0.0025 $\pm$ 0.0004
Heart	0.28 $\pm$ 0.02	0.27 $\pm$ 0.02	0.28 $\pm$ 0.03	0.28 $\pm$ 0.01
Lung	0.31 $\pm$ 0.03	0.31 $\pm$ 0.03	0.31 $\pm$ 0.02	0.31 $\pm$ 0.02
Liver	2.55 $\pm$ 0.17	2.61 $\pm$ 0.17	2.6 $\pm$ 0.19	2.67 $\pm$ 0.22
Spleen	0.17 $\pm$ 0.02	0.15 $\pm$ 0.03	0.17 $\pm$ 0.04	0.16 $\pm$ 0.02
Kidney	0.6 $\pm$ 0.04	0.57 $\pm$ 0.04	0.58 $\pm$ 0.03	0.61 $\pm$ 0.05
Adrenal	0.0118 $\pm$ 0.0018	0.0122 $\pm$ 0.0018	0.0121 $\pm$ 0.002	0.0112 $\pm$ 0.0014
Testis	0.64 $\pm$ 0.06	0.63 $\pm$ 0.07	0.65 $\pm$ 0.07	0.7 $\pm$ 0.08
Prostate	0.1 $\pm$ 0.03	0.09 $\pm$ 0.02	0.1 $\pm$ 0.02	0.12 $\pm$ 0.03
Sex: female	Group-dose (mg/kg/day)			
	G1-0	G2-500	G3-1000	G4-2000
N	10	10	10	10
B.W.	303.5 $\pm$ 37.5	288.8 $\pm$ 23.8	280.7 $\pm$ 26.8	290.8 $\pm$ 26.7
Brain	0.65 $\pm$ 0.07	0.67 $\pm$ 0.05	0.71 $\pm$ 0.07	0.68 $\pm$ 0.07
Pituitary	0.0053 $\pm$ 0.0012	0.0052 $\pm$ 0.0008	0.0052 $\pm$ 6E-04	0.0055 $\pm$ 0.0005
Heart	0.31 $\pm$ 0.03	0.33 $\pm$ 0.01	0.33 $\pm$ 0.03	0.31 $\pm$ 0.03
Lung	0.4 $\pm$ 0.05	0.42 $\pm$ 0.03	0.44 $\pm$ 0.04	0.42 $\pm$ 0.04
Liver	2.41 $\pm$ 0.27	2.48 $\pm$ 0.08	2.48 $\pm$ 0.18	2.43 $\pm$ 0.17
Spleen	0.19 $\pm$ 0.04	0.18 $\pm$ 0.02	0.18 $\pm$ 0.02	0.18 $\pm$ 0.02
Kidney	0.61 $\pm$ 0.06	0.63 $\pm$ 0.05	0.66 $\pm$ 0.07	0.64 $\pm$ 0.1
Adrenal	0.0234 $\pm$ 0.0042	0.025 $\pm$ 0.0039	0.0254 $\pm$ 0.004	0.0232 $\pm$ 0.0038
Ovary	0.0253 $\pm$ 0.0073	0.0311 $\pm$ 0.0041	0.0287 $\pm$ 0.006	0.0278 $\pm$ 0.0043
Uterus	0.23 $\pm$ 0.08	0.24 $\pm$ 0.07	0.24 $\pm$ 0.09	0.27 $\pm$ 0.11

Discussion

Various populations worldwide are aging, health issues have received increasing interest, and quality of life has become more important, it has become desirable to prevent the occurrence of diseases. Therefore, dietary supplements and functional foods have increasingly been consumed gradually, which has also led to an increase in the number of cases of adverse reactions [31].

Repeated oral administration toxicity study is one of most general tests in the field of toxicology, and its findings are used to predict and determine the safe doses in humans in accordance with the no observed adverse effect level (NOAEL) [32, 33].

To ensure confidence among the general public, toxicity test can be performed by a GLP-approved laboratory in accordance with guidelines such as those of the OECD

and the ICH. In particular, the OECD guidelines have been adapted for toxicity test of dietary supplements and functional foods; thus, the present study was performed in accordance with the OECD guidelines.

Korean red ginseng, produced by steaming *Panax ginseng*, reportedly has various pharmacological properties. Deer antler has also been extensively used as traditional medicine owing to its high bioavailability. Although several safety analyses have been conducted for each individual component [21, 23], those studies are limited owing to their inability to identify whether a mixture of these ingredients is safe to consume. Moreover, relevant regulatory authorities now require safety reports on herbal formulations or mixtures of herbal ingredients to identify any potential adverse reactions and interactions among the ingredients. In particular, it is important to evaluate the toxicity of herbal mixtures

Table 8 Urinalysis results of SD rats (mean $\pm$ SD)

Sex	Male				Female			
Group	G1	G2	G3	G4	G1	G2	G3	G4
Dose (mg/kg/day)	0	500	1,000	2,000	0	500	1,000	2,000
No. of animals	5	5	5	5	5	5	5	5
Volume (mL)								
Mean $\pm$ SD	12 $\pm$ 6.9	8.4 $\pm$ 2.4	14.2 $\pm$ 7.5	7.5 $\pm$ 4.9	8.3 $\pm$ 8	5.3 $\pm$ 2.8	4.1 $\pm$ 2.9	5.4 $\pm$ 1.8
Color								
Pale yellow	1		2		1		1	
Yellow	4	5	3	5	4	5	4	5
Transparency								
Clear	3	1	2		4	3	3	2
Mild turbidity	2	4	2	3	1	2	2	2
Turbidity			1	2				1
pH								
5								
6					1			
6.5	1							
7					1	2	1	
8	3	2	3	2	3	3	4	3
9	1	3	2	3				2
Protein (mg/dL)								
–	2	1	3	1	2	3	4	5
25	2	4	1	4	3	2	1	
75	1		1					
150								
500								
Glucose (mg/dL)								
Normal	5	5	5	5	5	5	5	5
50								
100								
300								
1,000								
Ketone body (mg/dL)								
–	1		1		2		1	
5	3	2	1		3	5	3	4
15	1	3	2	2			1	1
50			1	3				
150								
Bilirubin (mg/dL)								
–	5	4	4	5	4	4	4	4
1		1	1		1	1	1	1
3								
6								
Occult blood (Ery/ μ L)								
–	4	4	4	5	5	5	5	5
10	1	1	1					
25								
50								
150								
250								

Table 8 (continued)

Sex	Male				Female			
Group	G1	G2	G3	G4	G1	G2	G3	G4
Dose (mg/kg/day)	0	500	1,000	2,000	0	500	1,000	2,000
No. of animals	5	5	5	5	5	5	5	5
Cast ^a								
0	5	5	5	5	5	5	5	5
Epithelial cell ^a								
0	5	5	5	5	5	5	5	5
Leukocyte ^a								
0	5	5	5	5	5	5	5	5
Erythrocyte ^a								
0	5	5	5	5	5	5	5	5
Specific gravity								
1.000–1.010					1			
1.011–1.020								
1.021–1.030	1		1					
1.031–1.040			1			1	2	3
1.041–1.050	2		1	1	3	1		1
1.051–1.060	1	2		1		2		1
> 1.060	1	3	2	3	1	1	3	

containing various components other than the well-known analytical or potent substances, which can cause more severe liver and kidney damage than individual compounds. Several toxicity studies have reported that 20% of cases of hepatotoxicity in the United States resulted from previously reported herbal dietary supplements, probably characteristically attributable to liver function and structure as a portal location within the circulatory system [34]. The kidneys are also highly susceptible to toxicity from exogenous substances owing to a high volume of blood flow, thereby increasing toxin exposure, and filtered and concentrated toxins can damage the kidney tubules [35]. Consequently, liver and kidney damage reportedly induced systemic toxicity owing to the accumulation of the toxin, which is the most important indicator of toxicity from herbal products.

Therefore, we performed a safety study including analyses of liver and kidney toxicity using a mixture of Korean red ginseng and deer antler extract, which can potentially be used as an ingredient in supplementary diets or functional foods.

In the present study, the toxicity of a mixture of Korean red ginseng and deer antler extract in SD rats after 13-week of repeated oral administration was evaluated on the basis of clinical, clinicopathological, and pathological parameters. The test substance was orally administered at 0, 500, 1000, or 2000 mg/kg/day. The maximum dose was selected to be 2000 mg/kg/day in accordance with the results of the 4-week subacute toxicity study.

In a previous study [21], Korean red ginseng extract was administered orally at 0, 500, 1000, and 2000 mg/kg/day, to SD rats for 13-week. Significant toxic effects were not observed in clinical, clinicopathological, and pathological characteristics. Therefore, the NOAEL of the test substance was considered to be 2000 mg/kg/day.

In another previous study [23], 13-week oral repeated administration of 1000 mg/kg/day deer velvet powder to rats did not induce toxicological changes in body weight, food consumption, clinical signs, hematological parameters, blood biochemical parameters, and pathological findings.

The present study shows that 13-week repeated oral administration of a mixture of Korean red ginseng and deer antler extract did not reveal any toxicological effects with respect to body weight, food consumption, hematological parameters, blood biochemical parameters, ophthalmological findings, urinalysis findings, and pathological findings. Although one male rat in the 1000 mg/kg/day treatment group died, this death was considered to result incidentally and spontaneously and not because of the dose–response relationship of the test substance, being unrelated to the administration of the test substance.

In addition, the weight of the testis of the 1000 mg/kg/day treatment group increased; however, it was not considered to result from the test substance because there was no dose-dependent response or toxicological change in the histopathological data.

Fig. 3 Histopathological changes of rats in the 13-week oral administration toxicity test. There were no significant histopathological lesions both control and high dose groups. H&E stain, $\times 200$ (a male, b female)

Based on these results, the NOAEL of the mixture of Korean red ginseng and deer antler extract was considered to be greater than 2000 mg/kg/day in male and female rats subjected to repeated oral administration for 13-week.

NOAEL determined from nonclinical experiments has been considered fundamental for risk assessment or to determine the safe dose similar to the maximum recommended starting dose (MRSD) in a previous clinical trial [33]. In particular, the MRSD was determined on the basis of the human equivalent dose (HED), which is the translated dose from animals to humans on the basis of the body surface area of each species. A NOAEL of 2000 mg/kg in rats in the present study was hence converted to an HED of 324 mg/kg, which may be of potential use in future clinical studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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