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Research Article

Acute and repeated dose 26-week oral toxicity study of 20(S)-ginsenoside Rg3 in Kunming mice and Sprague–Dawley rats

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ABSTRACT

Background: 20(S)-ginsenoside-Rg3 ($C_{42}H_{72}O_{13}$), a natural triterpenoid saponin, is extracted from red ginseng. The increasing use of 20(S)-ginsenoside Rg3 has raised product safety concerns.
Methods: In acute toxicity, 20(S)-ginsenoside Rg3 was singly and orally administrated to Kunming mice and Sprague–Dawley (SD) rats at the maximum doses of 1600 mg/kg and 800 mg/kg, respectively. In the 26-week toxicity study, we used repeated oral administration of 20(S)-ginsenoside Rg3 in SD rats over 26 weeks at doses of 0, 20, 60, or 180 mg/kg. Moreover, a 4-week recovery period was scheduled to observe the persistence, delayed occurrence, and reversibility of toxic effects.
Results: The result of acute toxicity shows that oral administration of 20(S)-ginsenoside Rg3 to mice and rats did not induce mortality or toxicity up to 1600 and 800 mg/kg, respectively. During a 26-week administration period and a 4-week withdrawal period (recovery period), there were no significant differences in clinical signs, body weight, food consumption, urinalysis parameters, biochemical and hematological values, or histopathological findings.
Conclusion: The mean oral lethal dose (LD_{50}) of 20(S)-ginsenoside Rg3, in acute toxicity, is above 1600 mg/kg and 800 mg/kg in mice and rats, respectively. In a repeated-dose 26-week oral toxicity study, the no-observed-adverse-effect level for female and male SD rats was 180 mg/kg.

1. Introduction

Ginseng (*Panax ginseng* Meyer), a perennial herbaceous plant, has been used as a medicinal plant in East Asia for more than 2000 years [1]. Over thirty types of ginsenosides, the main active ingredient of ginseng, have been identified. Bioactive compounds in ginseng exert beneficial effects on cancer-related fatigue in clinical studies [2], anti-angiogenesis in human umbilical vein endothelial cells [3], anxiety, and depression-like behaviors in animal experiments [4]. Recently, ginseng has been a subject of interest for use as a cancer therapeutic and preventive agent [5].

20(S)-ginsenoside-Rg3 ($C_{42}H_{72}O_{13}$), a natural triterpenoid saponin, is extracted from red ginseng (Fig. 1). It has a molecular formula of $C_{42}H_{72}O_{13}$. A variety of *in vitro* studies show that 20(S)-ginsenoside Rg3 slows the growth of malignancies in lung cancer cells, human ovarian cancer cells, human leukemic cells, and human glioma cells [6–9]. 20(S)-ginsenoside Rg3 promotes the cytotoxicity of paclitaxel through inhibiting NF-kB signaling and regulating Bax/Bcl-2 expression on triple-negative breast cancer cells [10]. In hepatocellular cells, 20(S)-ginsenoside Rg3 also has a significant anticancer effect on hepatocellular carcinoma [11–13]. As far as preclinical safety evaluation is concerned, some studies

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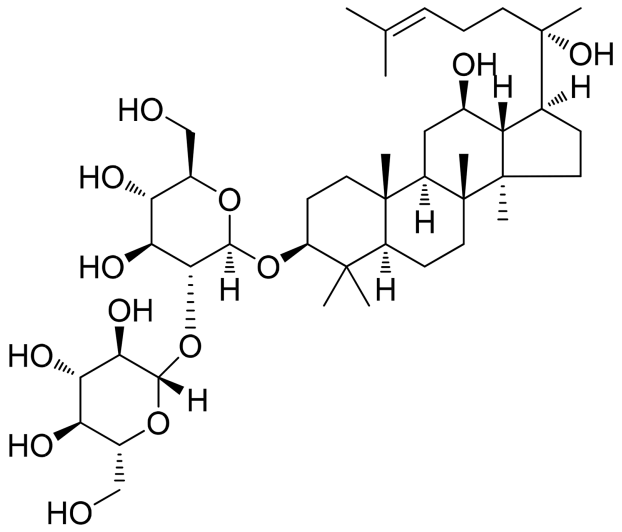

Fig. 1. Chemical structure of 20(S)-ginsenoside-Rg3.

have been conducted on potential subchronic toxicity with repeated intramuscular administration of 20(S)-ginsenoside Rg3 in dogs and rats over a 26-week period [14,15], and the results indicate that the no-observed-adverse-effect levels (NOAELs) for dogs and rats is considered to be 7.2 mg/kg and 4.2 mg/kg, respectively.

However, a different dosing method can directly influence absorption, distribution, metabolism, excretion, and toxicity of the substance. In the current evaluation, we conduct acute and 26-week repeated-dose toxicity studies of 20(S)-ginsenoside Rg3 by oral administration in mice and rats, respectively. The aim of studies is to assay the potential toxicity of 20(S)-ginsenoside Rg3 on clinical signs, body weight, food consumption, urinalysis parameters, hematology blood chemistry, or histopathology. The results will provide information on the safety of 20(S)-ginsenoside Rg3 and guide the selection of a safe dose for further study or human use.

2. Materials and methods

2.1. Materials

The compound 20(S)-ginsenoside Rg3 (CAS No:14197-60-5, purity $>$ 99 %) was extracted from ginseng and kindly provided by Professor Benming Xu (Yantai University, Yantai, China). We established purity with reversed-phase high-performance liquid chromatography (Fig. 2) [16,17].

2.2. Animals and treatment

Kunming mice (4–5 weeks old) and Sprague–Dawley (SD) rats (6–7 weeks old) were provided by the Experimental Animal Center of Shandong Engineering Research Center for Natural Drugs (Yantai, China). Animals were clinically examined 7 days before treatment for general health and parasite infestation, and then, healthy animals were randomly divided into different groups according to the body weight. Rats were housed in a stainless steel cage, and mice were housed in a PVC box. Animals were free access to a commercial standard rat/mouse cube diet (Beijing Keao Xieli, Beijing, China) and water. The animal feeding laboratory was kept at 22 ± 2°C and 50 ± 5% relative humidity with a 12-h light/dark cycle. Animals were observed two times once daily. All studies were conducted in compliance with Good Laboratory Practice Regulations from the State Food and Drug Administration of China [18], the "Guidelines for acute toxicity studies in the rodent" [19], and the "Guidelines for subchronic toxicity studies in the rodent" [20]. All animal protocols were approved by the Institutional Animal Care and Use Committee of Yantai University.

2.3. Acute toxicity study

The aim of this study was to evaluate acute toxicity of 20(S)-ginsenoside Rg3 after a single oral administration in Kunming mice and SD rats. After quarantine period, healthy animals were used for this study. Mice (10 female and 10 male/group) and rats (10 female and 10 male/group) received 1600 mg/kg and 800 mg/kg of 20(S)-ginsenoside Rg3 by a single gavage after an overnight fast,

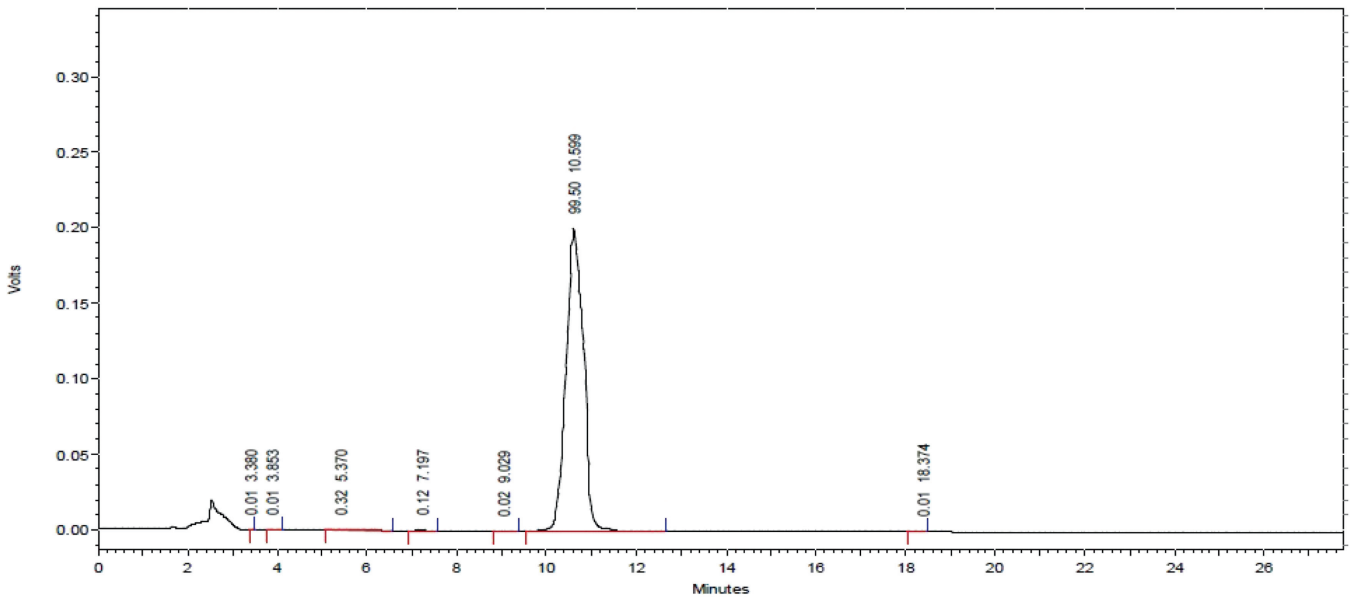

Fig. 2. HPLC chromatograms of 20(S)-ginsenoside-Rg3. Chromatographic conditions: Discovery C18 column, 5 m, 4.6 × 250 mm; mobile phase, V(acetonitrile): V(water): V(orthophosphoric acid) = 46:54:0.05.

respectively. An equal number of animals (mice and rats) served as a control group, and the control animals received an equal volume of vehicle only. Animals were observed for 14 days to monitor changes in the body weight, clinical signs, and food and water consumption. At the end of the study, animals were sacrificed, and major organs were examined macroscopically and microscopically. We performed necropsy and histopathological examinations on any animals that died.

2.4. A 26-week repeated-dose toxicity study

2.4.1. Study design

The study includes a 26-week administration period and a 4-week recovery period. After quarantine period, 120 healthy SD rats were randomly divided into (30 animals/group, half female and half male) control group and three 20(S)-ginsenoside Rg3 groups (20, 60, or 180 mg/kg groups). The total dosage was adjusted according to the body weight change weekly. Eighty rats (10 rats/sex/group) were sacrificed after a continuous 26-week treatment, and the remaining 40 rats (five rats/sex/group) were autopsied after 4 weeks of recovery to observe the persistence, delayed occurrence, and reversibility of toxic effects. The body weights and the food consumption were measured once a week.

2.4.2. Hematology, blood chemistry, and urine analysis

On week 26 of the study, and again after 4-week withdrawal period, animals were fasted 12 h before collection of blood and urine samples. Blood samples from aorta abdominalis were evacuated into blood collection tubes under anesthetic condition (choral hydrate, 300 mg/kg, intraperitoneal injection). Ethylenediaminetetraacetic acid was used as an anticoagulant for the blood coagulation study. Platelet counts, mean corpuscular hemoglobin (MCH), leukocyte counts (WBC), hemoglobin, hematocrit, mean corpuscular volume, MCH concentration, and erythrocyte counts (RBC) were assessed with a hematology analyzer (AITAIK, Japan). The single-channel coagulometer (MC-1000, Germany) was used to analyze the blood coagulation parameters including activated partial thromboplastin time, prothrombin time, and thrombin time. Differential counts of leukocytes (monocytes; neutrophilic leukocyte; and leukomonocyte) were detected under a microscope after staining with hematoxylin and eosin under a microscope. We used an automatic Autolab analyzer (AMS, Italy) to determine blood chemistry parameters (serum). These parameters include glucose, creatine kinase, aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase (ALT), albumin, blood urea nitrogen, total bilirubin, creatinine, total protein, triglyceride, and total cholesterol. We used the Easylyte Plus electrolyte analyzer (Medica, Bedford, USA) to analyze the serum ions of chloride, potassium, and sodium. Urine samples were determined by an autoanalyzer (FA-100, Shan'xi, China) for pH, leukocytes, specific gravity, protein, urobilinogen, glucose, ketones, nitrite, occult blood, bilirubin, and hemoglobin.

2.4.3. Gross observation, organ weight, and histopathological examination

We analyzed the absolute weight and calculated the relative weights (the organ weight/body weight) of major tissues and organs, including the spleen, thymus, uterus, lungs, heart, epididymis, adrenals, testes, brain, kidneys, ovaries, and liver. We collected the following tissues: the heart, skin, nervus opticus, ileum, submandibular lymph nodes, abnormal lesions, spinal cord (cervical, thoracic, and lumbar), esophagus, mammary gland, adrenal glands, kidneys, mesenteric lymph node, spleen, salivary gland, pituitary gland, thyroid (and parathyroid), pancreas, lung, prostate, sternum, stomach, testes, tongue, duodenum, cecum, trachea, ovaries, epididymis, colon, bladder, liver, brain, thymus, uterus, aorta, and sciatic nerve. The neutral buffered formalin solution (10%) was used to fix tissues/organs. The tissues/organs, in control and high-dosage group, were paraffin embedded and cut in to 3–5 μm thickness for further hematoxylin and eosin staining. Pathological examination was blinded and diagnosed by the study pathologist. If treatment-related effects were found in certain tissues, the middle dosage group was examined until no abnormity was noted.

2.5. Statistical analysis

We used Bartlett's test to determine the homogeneity of data. One-way analysis of variance was applied for homogeneous data. For data that were not homogenous, we applied the Kruskal–Wallis's test. We used the Dunnett's test on parameters found significant in the analysis of variance. $P < 0.05$ or $P < 0.01$ was considered significant for pairwise comparisons.

3. Results

3.1. Acute oral toxicity study

During a 14-day acute toxicity testing, no obvious abnormal clinical signs of toxicity or mortality were observed in mice and rat at up to 1600 and 800 mg/kg 20(S)-ginsenoside Rg3 groups, respectively. There was no statistically significant difference in body weight between 20(S)-ginsenoside Rg3 treatment groups and control groups (Table 1). Food consumption did not indicate any treatment-related adverse effects (data not shown).

3.2. A 26-week repeated-dose toxicity study

3.2.1. Clinical signs

All rats survived until scheduled necropsy. No obvious abnormal clinical signs (e.g., changes in behavior patterns, skin color, loss of

Table 1
Body weight changes of mice and rats in acute toxicity studies

Animal	Group	Sex	N	Body weight ($\overline{X}$±SD)		
				Before study	d_7	d_{14}
Mice	Control	♀	10	18.12 ± 0.57	26.61 ± 2.03	32.92 ± 1.74
		♂	10	19.10 ± 0.75	31.14 ± 1.90	33.80 ± 2.21
	20(S)-ginsenoside Rg3 (1600 mg/kg)	♀	10	18.13 ± 0.65	26.57 ± 1.90	32.40 ± 1.81
		♂	10	19.39 ± 0.73	31.35 ± 2.09	33.22 ± 1.96
Rats	Control	♀	10	118.90 ± 8.65	159.10 ± 11.08	178.40 ± 11.72
		♂	10	123.10 ± 10.60	181.80 ± 16.21	211.20 ± 18.19
	20(S)-ginsenoside Rg3 (800 mg/kg)	♀	10	122.20 ± 6.49	154.80 ± 11.57	175.80 ± 8.01
		♂	10	128.78 ± 10.07	183.00 ± 10.98	218.04 ± 19.77

SD, standard deviation.
Data are presented as mean ± SD for N = 10.

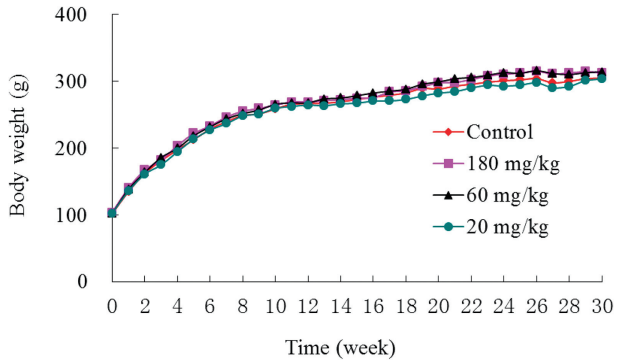

Fig. 3. Body weight change of female rats during administered ginsenoside Rg3 (n = 15, from 0 week to 26th week) and withdrawn from the drug for 4 weeks (n = 5). The drug was withdrawn from 27th week and continuously observed to 30th week.

fur, mucous membranes, or scabbing) were observed in any 20(S)-ginsenoside Rg3 groups. No obvious differences in the animal body weights were found between 20(S)-ginsenoside Rg3-treated groups and control during study periods (Figs. 3 and 4). There was no statistically significant difference in food consumption, urinalysis, and ophthalmoscopic examinations between the three 20(S)-ginsenoside Rg3 groups and control group (data not shown).

3.2.2. Blood chemistry and hematological examination

No toxicologically significant differences were found in hematological examination between the 20(S)-ginsenoside Rg3 groups and the control group. However, some parameters slightly increased or decreased in the 20(S)-ginsenoside Rg3–treated groups (Tables 2 and 3). For example, triglyceride was decreased in 20 mg/kg group (female, $P < 0.05$), total protein was decreased in 20 mg/kg group (male, $P < 0.01$), ALT was decreased in 180 mg/kg group (female, $P < 0.05$), aspartate aminotransferase and creatinine were decreased in 20 mg/kg group (female, all $P < 0.05$), and ALT was increased in 60 mg/kg group (male, $P < 0.05$). Tables 4 and 5 show a summary of biochemical parameters. There were slight increases or decreases in the test substance–treated groups compared with control for the following parameters: platelet count was increased in 180 mg/kg group (male, $P < 0.05$), MCH was increased in 60 mg/kg group (female and male, all $P < 0.05$) and 20 mg/kg group (male, $P < 0.05$), and RBC was decreased in 20 mg/kg group (female, $P < 0.05$).

3.2.3. Macroscopic observation, organ weights, and histopathological examinations

We focused on the related organ in macroscopic and histopathological examinations. Tables 6 and 7 show the relative organ weights. After 26 weeks, in treated animals, the relative weight of the liver in the 60 mg/kg male group and the lung in the 20 mg/kg male group was significantly lower than that in the control group ($P < 0.05$). In addition, the relative spleen weight was obviously higher in the 180 mg/kg female group when compared with the control group ($P < 0.05$) after 26-week treated periods. But this change was disappeared after a 4-week recovery period. In addition, there were no statistically significant differences in other absolute and relative organ weights and tissues (the brain, uterus, heart, ovaries, testes, thymus, kidneys, and epididymides) at any dose levels. On histopathological examination, no abnormal changes were found in all organs after 26-week administration and recovery periods (data not shown). Especially, no obvious macroscopic and microscopic changes were observed in the spleen (180 mg/kg group), liver (60 mg/kg group), and the lung (20 mg/kg group).

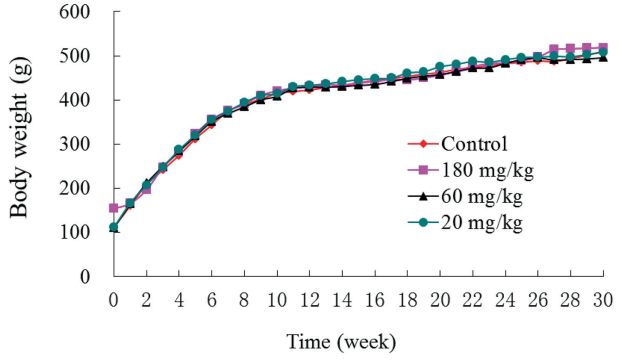

Fig. 4. Body weight change of male rats during administered ginsenoside Rg3 (n = 15, from 0 week to 26th week) and withdrawn from the drug for 4 weeks (n = 5). The drug was withdrawn from 27th week and continuously observed to 30th week.

4. Discussion

Medicinal herbs have traditionally been used to prevent or treat various diseases. With the wide application of medicinal herbs, people tend to believe that natural herbal medicines are free of side effects and harmless [21]. In fact, it is necessary to evaluate the safety of drugs or plant products before human use [22,23]. Recently, the safety of several commercially available herbs has attracted more and more attention because of reports of adverse reactions, such as severe hepatotoxicity, cardiotoxicity, and even death [24]. Ginseng has been widely consumed as a food or used as an ingredient in well-established traditional Chinese medicines, and some toxicity studies have been reported. For example, Soe et al conducted a subacute oral toxicity and bacterial mutagenicity study of Korean Red Ginseng oil, and the results indicated that the NOAEL of red ginseng oil is greater than 2.0 g/kg in SD rats [5]. Also, red ginseng oil did not induce genotoxicity [5]. Park et al also carried out a study to evaluate the repeated oral dose toxicity of Korean Red Ginseng extract, and the outcome suggested that the NOAEL of the test substance is 2000 mg/kg in SD rats [25]. Aleman et al showed that *P. ginseng* is not toxic or tumorigenic in 2-year toxicity and carcinogenicity studies [26].

20(S)-ginsenoside Rg3 is one of the main active components responsible for ginseng's actions and possesses antitumor effects on many cancer cells, such as prostate cancer cells [27], Lewis lung carcinoma cells [28], human gastric cancer cells [29], B16 melanoma cells [30], and different lines of human colon cancer cells [31]. Previous studies have indicated that the NOAEL of 20(S)-ginsenoside Rg3 is 7.2 mg/kg and 4.2 mg/kg for dogs and rats, respectively, after intramuscular administration [14,15]. In the present study, mice and rats were orally administrated 20(S)-ginsenoside Rg3 to further evaluate its safety before clinical use.

In the acute toxicity evaluation, mice and rats survived until the scheduled study under our test conditions. The body weight and food consumption were unaffected by 20(S)-ginsenoside Rg3 treatment in contrast to the control group. The results show that oral administrations of 20(S)-ginsenoside Rg3 to mice and rats at up to 1600 and 800 mg/kg, respectively, are well tolerated.

In the 26-week repeated-dose toxicity study, all rats survived until the scheduled necropsy. During the 26-week administration period and 4-week recovery period, there were no notable abnormal clinical appearances or changes in the 20(S)-ginsenoside Rg3–treated animals (20, 60, and 180 mg/kg). Similarly, there were no significant changes in food consumption or body weight in animals that received 20(S)-ginsenoside Rg3.

Examination of serum biochemical and hematological parameters can indicate potential lesions in organs, such as the liver or kidneys [32]. Under the conditions of this study, we found no

Table 2
Biochemical findings in rats treated with 20(S)-ginsenoside Rg3 for 26 weeks

Dose (mg/kg)	Female				Male			
	0	20	60	180	0	20	60	180
ALT (IU/L)	36.71 ± 9.11	38.29 ± 7.95	40.00 ± 5.34	40.86 ± 4.98	51.14 ± 6.99	46.86 ± 8.49	48.86 ± 8.13	45.43 ± 5.26
AST (IU/L)	170.71 ± 27.15	174.14 ± 31.28	161.00 ± 17.46	177.43 ± 50.06	180.29 ± 44.94	180.14 ± 22.32	207.86 ± 37.20	190.86 ± 43.65
ALP (IU/L)	38.43 ± 12.82	40.14 ± 9.91	33.80 ± 8.98	40.71 ± 23.03	83.57 ± 22.71	86.00 ± 16.50	73.14 ± 17.06	90.14 ± 15.26
TP (g/L)	65.57 ± 3.69	62.29 ± 3.35	64.00 ± 3.39	66.29 ± 3.35	62.57 ± 3.05	56.43 ± 2.44**	58.86 ± 2.91	61.29 ± 3.77
ALB (g/L)	33.86 ± 4.34	33.00 ± 2.31	33.60 ± 2.88	33.00 ± 2.16	27.71 ± 2.06	27.86 ± 1.86	27.43 ± 1.13	26.29 ± 2.81
T-BIL (Mg/dl)	0.27 ± 0.08	0.24 ± 0.08	0.30 ± 0.10	0.29 ± 0.07	0.20 ± 0.00	0.21 ± 0.04	0.21 ± 0.04	0.21 ± 0.04
CHO (mg/dl)	87.14 ± 12.28	75.00 ± 14.56	79.40 ± 14.50	78.57 ± 18.91	51.29 ± 7.80	46.14 ± 5.70	47.57 ± 6.08	46.71 ± 9.69
BUN (mg/dl)	15.71 ± 1.38	15.43 ± 1.62	15.40 ± 2.97	15.86 ± 2.48	17.43 ± 1.99	17.29 ± 2.21	17.00 ± 1.00	17.00 ± 2.08
CRE (mg/dl)	0.73 ± 0.08	0.66 ± 0.05	0.74 ± 0.09	0.67 ± 0.05	0.63 ± 0.08	0.63 ± 0.08	0.67 ± 0.08	0.66 ± 0.08
GLU (mg/dl)	78.86 ± 18.12	65.43 ± 18.82	62.20 ± 10.45	69.86 ± 11.36	77.14 ± 9.94	72.00 ± 11.34	69.71 ± 10.40	75.00 ± 8.29
CK (IU/L)	833.7 ± 238.2	892.0 ± 210.5	724.4 ± 124.7	752.1 ± 185.0	799.3 ± 283.8	895.4 ± 101.3	901.0 ± 177.4	736.4 ± 2133.0
TG (mg/dl)	106.00 ± 15.72	88.86 ± 12.12*	104.60 ± 17.04	109.43 ± 18.48	45.29 ± 4.31	50.57 ± 8.36	48.29 ± 9.20	48.43 ± 7.07
Na (mmol/l)	142.64 ± 1.14	143.17 ± 1.26	143.74 ± 0.17	143.45 ± 1.33	144.13 ± 0.35	144.01 ± 0.55	144.39 ± 1.13	144.29 ± 1.48
K (mmol/l)	5.27 ± 0.46	5.24 ± 0.25	5.21 ± 0.50	5.35 ± 0.69	5.50 ± 0.25	5.40 ± 0.29	5.39 ± 0.51	5.52 ± 0.34
CL (mmol/l)	107.74 ± 1.27	108.69 ± 1.95	109.42 ± 0.86	108.56 ± 2.63	108.76 ± 0.91	109.47 ± 0.67	108.23 ± 1.90	109.21 ± 3.77

Data are presented as mean ± SD.
SD, standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TP, total protein; ALB, albumin; T-BIL, total bilirubin; CHO, cholesterol; BUN, blood urea nitrogen; CRE, creatinine; GLU, glucose; CK, creatine kinase; TG, triglyceride; Na, sodium; K, potassium; CL, chloride.
* Significantly different from the control group at $p < 0.05$
** Significantly different from the control group at $p < 0.01$.

Table 3
Biochemical findings in rats withdrawn from 20(S)-ginsenoside Rg3 for 4 weeks

Dose (mg/kg)	Female				Male			
	0	20	60	180	0	20	60	180
ALT (IU/L)	42.60 ± 2.51	40.80 ± 6.30	42.40 ± 8.44	37.60 ± 4.04*	48.60 ± 4.67	46.80 ± 6.06	56.40 ± 5.13*	49.40 ± 10.01
AST (IU/L)	197.40 ± 25.44	157.60 ± 24.96*	180.40 ± 39.99	190.40 ± 65.40	185.20 ± 24.01	175.60 ± 37.58	189.60 ± 22.79	188.81 ± 21.65
ALP (IU/L)	35.20 ± 9.60	109.00 ± 96.32	44.80 ± 11.34	79.80 ± 65.46	102.80 ± 19.21	87.00 ± 14.88	96.20 ± 22.77	81.60 ± 11.37
TP (g/L)	78.00 ± 4.90	76.20 ± 2.17	76.80 ± 6.38	81.40 ± 8.73	69.80 ± 4.09	71.40 ± 3.44	72.00 ± 2.55	71.20 ± 2.39
ALB (g/L)	36.40 ± 2.97	35.40 ± 2.61	35.60 ± 6.95	30.40 ± 6.15	30.60 ± 1.82	29.80 ± 1.30	29.40 ± 2.30	28.20 ± 2.95
T-BIL (Mg/dl)	0.32 ± 0.08	0.38 ± 0.13	0.30 ± 0.10	0.30 ± 0.10	0.22 ± 0.04	0.26 ± 0.05	0.24 ± 0.05	0.20 ± 0.00
CHO (mg/dl)	71.40 ± 6.07	80.60 ± 13.52	74.60 ± 10.16	73.60 ± 5.73	52.80 ± 9.52	56.20 ± 3.27	53.80 ± 10.16	53.80 ± 5.76
BUN (mg/dl)	16.40 ± 2.51	17.80 ± 1.30	16.80 ± 2.95	17.40 ± 3.21	15.80 ± 1.64	15.40 ± 1.52	15.20 ± 0.84	16.00 ± 1.41
CRE (mg/dl)	0.90 ± 0.07	0.80 ± 0.00*	0.86 ± 0.05	0.84 ± 0.05	0.76 ± 0.05	0.76 ± 0.05	0.80 ± 0.00	0.78 ± 0.04
GLU (mg/dl)	83.20 ± 11.82	89.80 ± 15.12	93.60 ± 11.41	62.20 ± 26.41	89.80 ± 22.22	100.20 ± 19.32	98.00 ± 13.93	77.00 ± 13.21
CK (IU/L)	874.0 ± 284.7	618.4 ± 196.8	745.6 ± 294.8	821.0 ± 500.8	783.6 ± 333.4	725.4 ± 308.8	821.4 ± 446.7	848.6 ± 157.4
TG (mg/dl)	83.00 ± 16.36	129.00 ± 47.91	94.40 ± 52.06	60.80 ± 19.40	62.00 ± 16.43	62.80 ± 8.56	62.40 ± 15.77	67.40 ± 9.26
Na (mmol/l)	144.98 ± 2.52	146.10 ± 0.76	145.14 ± 0.57	144.36 ± 1.70	145.38 ± 2.21	143.76 ± 0.93	144.22 ± 1.44	144.92 ± 0.58
K (mmol/l)	5.27 ± 0.28	4.94 ± 0.32	5.02 ± 0.43	5.23 ± 0.28	5.66 ± 0.32	5.50 ± 0.33	5.35 ± 0.35	5.94 ± 0.36
CL (mmol/l)	110.54 ± 2.49	110.08 ± 0.76	108.70 ± 1.23	109.78 ± 2.62	109.74 ± 1.84	109.58 ± 0.38	108.46 ± 1.83	108.54 ± 2.32

Data are presented as mean ± SD.
SD, standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TP, total protein; ALB, albumin; T-BIL, total bilirubin; CHO, cholesterol; BUN, blood urea nitrogen; CRE, creatinine; GLU, glucose; CK, creatine kinase; TG, triglyceride; Na, sodium; K, potassium; CL, chloride.
* Significantly different from the control group at $p < 0.05$.

Table 4
Hematologic findings in rats treated with 20(S)-ginsenoside Rg3 for 26 weeks

Dose (mg/kg)	Female				Male			
	0	20	60	180	0	20	60	180
WBC ($\times 10^9$/L)	2.34 ± 0.65	2.43 ± 2.09	2.24 ± 1.15	3.13 ± 1.54	4.99 ± 1.82	5.29 ± 1.91	5.37 ± 1.99	4.61 ± 0.94
RBC ($\times 10^{12}$/L)	6.43 ± 0.70	6.77 ± 0.71	6.63 ± 0.50	6.75 ± 0.63	7.85 ± 0.34	7.72 ± 0.40	7.88 ± 0.66	7.65 ± 0.87
HGB (g/L)	132.00 ± 11.05	134.29 ± 4.39	133.60 ± 6.27	134.29 ± 4.46	143.57 ± 6.27	137.71 ± 6.65	140.14 ± 7.78	140.86 ± 8.80
HCT (L/L)	0.35 ± 0.03	0.35 ± 0.04	0.36 ± 0.03	0.37 ± 0.05	0.41 ± 0.02	0.41 ± 0.02	0.40 ± 0.02	0.40 ± 0.04
MCV (f L)	53.79 ± 1.39	52.36 ± 2.46	54.36 ± 3.00	55.00 ± 2.13	51.69 ± 2.16	53.34 ± 1.14	51.17 ± 3.54	53.20 ± 4.85
MCH (Pg)	20.56 ± 1.75	19.94 ± 1.95	20.42 ± 0.73	19.97 ± 1.80	18.26 ± 0.82	17.80 ± 0.90	17.80 ± 1.22	18.47 ± 1.41
MCHC (g/L)	383.00 ± 26.55	381.43 ± 32.75	376.80 ± 14.69	364.57 ± 42.19	354.14 ± 24.49	334.29 ± 12.83	348.57 ± 16.71	348.29 ± 16.91
PLT ($\times 10^9$/L)	845.14 ± 155.54	762.57 ± 82.11	954.60 ± 168.44	875.29 ± 127.59	693.00 ± 48.77	671.29 ± 184.94	760.71 ± 91.10	848.86 ± 138.95*
L (%)	71.29 ± 4.92	68.14 ± 6.74	70.60 ± 5.13	73.43 ± 6.29	71.71 ± 5.41	73.86 ± 3.67	70.00 ± 4.90	68.29 ± 5.74
N (%)	26.14 ± 5.64	29.14 ± 5.96	27.00 ± 5.20	24.14 ± 5.55	26.14 ± 4.95	24.29 ± 3.90	27.57 ± 5.16	29.57 ± 5.38
M (%)	2.57 ± 1.13	2.71 ± 1.11	2.40 ± 0.55	2.43 ± 0.98	2.14 ± 1.07	1.86 ± 0.90	2.43 ± 1.27	2.14 ± 1.07
TT (s)	20.17 ± 1.47	20.44 ± 1.23	21.04 ± 1.24	21.17 ± 0.84	21.23 ± 1.50	20.60 ± 1.22	21.19 ± 0.69	21.11 ± 1.12
PT (s)	12.04 ± 0.92	12.26 ± 1.37	12.52 ± 1.09	12.57 ± 1.11	12.06 ± 0.70	12.29 ± 0.98	12.01 ± 0.92	11.66 ± 1.05
APTT (s)	25.17 ± 1.96	25.64 ± 2.45	25.46 ± 1.97	25.27 ± 0.70	25.96 ± 2.53	24.01 ± 1.48	24.87 ± 1.49	25.30 ± 1.45

Data are presented as mean ± SD.
SD, standard deviation; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet count; L, leukomonocyte; N, neutrophilic leukocyte; M, monocyte; TT, thrombin time; PT, prothrombin time; APTT, activated partial thromboplastin time.
* Significantly different from the control group at $p < 0.05$.

Table 5
Hematologic findings in rats withdrawn from 20(S)-ginsenoside Rg3 for 4 weeks

Dose (mg/kg)	Female				Male			
	0	20	60	180	0	20	60	180
WBC ($\times 10^9$/L)	1.90 ± 0.68	1.45 ± 0.42	2.68 ± 1.29	2.40 ± 1.14	4.06 ± 1.24	4.30 ± 1.94	3.28 ± 0.90	4.40 ± 1.29
RBC ($\times 10^{12}$/L)	7.17 ± 0.30	6.64 ± 0.24*	6.53 ± 0.30	6.80 ± 0.38	8.15 ± 0.49	7.85 ± 0.21	7.50 ± 0.43	8.04 ± 0.37
HGB (g/L)	130.00 ± 6.04	123.50 ± 4.04	125.60 ± 3.91	127.40 ± 9.53	134.80 ± 6.76	135.00 ± 2.74	131.60 ± 8.38	136.20 ± 4.55
HCT (L/L)	0.39 ± 0.03	0.38 ± 0.04	0.36 ± 0.02	0.37 ± 0.04	0.42 ± 0.04	0.40 ± 0.03	0.39 ± 0.02	0.43 ± 0.03
MCV (f L)	54.68 ± 3.69	57.38 ± 3.86	54.48 ± 1.84	54.14 ± 2.58	51.12 ± 3.26	50.60 ± 3.68	51.38 ± 2.23	52.84 ± 1.95
MCH (Pg)	18.10 ± 0.46	18.55 ± 0.39	19.18 ± 0.56*	18.66 ± 0.60	16.50 ± 0.37	17.14 ± 0.34*	17.38 ± 0.68*	16.92 ± 0.33
MCHC (g/L)	332.60 ± 28.38	325.00 ± 23.27	353.60 ± 21.33	345.80 ± 18.46	324.40 ± 25.15	341.00 ± 24.61	338.80 ± 14.17	320.40 ± 13.07
PLT ($\times 10^9$/L)	711.6 ± 134.3	775.5 ± 166.1	665.8 ± 172.8	720.0 ± 157.1	705.2 ± 203.5	683.4 ± 56.7	692.8 ± 175.6	722.8 ± 94.1
L (%)	67.20 ± 4.44	70.25 ± 4.11	72.60 ± 4.62	66.60 ± 7.02	73.60 ± 3.05	72.00 ± 7.52	70.00 ± 6.20	75.00 ± 4.74
N (%)	31.00 ± 5.00	28.00 ± 3.92	25.20 ± 5.17	31.20 ± 7.19	24.00 ± 2.92	25.80 ± 7.09	27.60 ± 5.46	23.20 ± 5.07
M (%)	1.80 ± 0.84	1.75 ± 0.50	2.20 ± 0.84	2.20 ± 0.84	2.40 ± 0.55	2.20 ± 0.84	2.40 ± 1.14	1.80 ± 0.84
TT (s)	21.06 ± 0.91	20.65 ± 0.53	19.88 ± 1.20	21.04 ± 1.13	19.86 ± 1.04	21.06 ± 1.28	20.26 ± 0.82	20.32 ± 0.86
PT (s)	12.48 ± 1.68	11.45 ± 1.10	12.08 ± 0.76	12.46 ± 0.49	11.88 ± 0.90	11.42 ± 0.64	11.32 ± 0.61	11.94 ± 0.70
APTT (s)	25.46 ± 1.00	25.03 ± 1.77	25.08 ± 1.47	25.68 ± 1.56	25.48 ± 1.51	25.14 ± 0.96	25.38 ± 2.62	24.56 ± 1.01

Data are presented as mean ± SD.
SD, standard deviation; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet count; L, leukomonocyte; N, neutrophilic leukocyte; M, monocyte; TT, thrombin time; PT, prothrombin time; APTT, activated partial thromboplastin time.
* Significantly different from the control group at $p < 0.05$.

Table 6
Relative organ weight in rats treated with 20(S)-ginsenoside Rg3 for 26 weeks (‰)

Dose (mg/kg)	Female				Male			
	0	20	60	180	0	20	60	180
Brain	6.82 ± 0.52	6.97 ± 0.57	6.76 ± 0.59	7.06 ± 0.94	4.99 ± 0.60	4.50 ± 0.31	4.43 ± 0.38	4.94 ± 0.59
Heart	3.97 ± 0.47	4.03 ± 0.49	3.67 ± 0.35	4.02 ± 0.48	3.64 ± 0.37	3.46 ± 0.24	3.56 ± 0.18	3.76 ± 0.47
Lung	5.34 ± 0.77	5.35 ± 1.10	5.88 ± 1.08	5.38 ± 0.84	4.22 ± 0.16	3.99 ± 0.20*	4.14 ± 0.43	4.38 ± 0.43
Thymus	1.12 ± 0.10	1.06 ± 0.27	1.02 ± 0.23	0.99 ± 0.23	0.73 ± 0.28	0.61 ± 0.22	0.59 ± 0.07	0.70 ± 0.20
Liver	25.02 ± 2.05	25.39 ± 1.85	24.22 ± 1.79	27.03 ± 4.72	23.14 ± 1.08	22.75 ± 1.41	21.67 ± 1.14*	23.13 ± 1.67
Kidneys	6.36 ± 0.63	6.26 ± 0.50	6.03 ± 0.47	6.58 ± 0.98	6.39 ± 0.53	5.92 ± 0.22	6.03 ± 0.33	6.52 ± 0.69
Adrenals	0.26 ± 0.04	0.22 ± 0.03	0.24 ± 0.05	0.25 ± 0.09	0.13 ± 0.03	0.11 ± 0.02	0.11 ± 0.02	0.13 ± 0.02
Spleen	1.95 ± 0.25	1.95 ± 0.25	1.98 ± 0.28	2.64 ± 0.57*	1.68 ± 0.19	1.67 ± 0.26	1.64 ± 0.26	1.88 ± 0.45
Testes	—	—	—	—	8.12 ± 1.56	7.77 ± 1.12	7.51 ± 0.70	7.68 ± 0.66
Epididymides	—	—	—	—	3.64 ± 0.67	3.32 ± 0.28	3.52 ± 0.53	3.80 ± 0.58
Ovaries	0.47 ± 0.11	0.53 ± 0.14	0.54 ± 0.07	0.52 ± 0.09	—	—	—	—
Uterus	2.07 ± 0.42	2.10 ± 0.80	1.82 ± 0.43	2.10 ± 0.74	—	—	—	—

Date presented as mean ± SD.
SD, standard deviation.
* Significantly different from the control group at $p < 0.05$.

Table 7
Relative organ weight in rats withdrawn from 20(S)-ginsenoside Rg3 for 4 weeks (‰)

Dose (mg/kg)	Female				Male			
	0	20	60	180	0	20	60	180
Brain	6.96 ± 0.74	6.55 ± 0.47	6.70 ± 0.82	7.10 ± 1.65	4.33 ± 0.44	4.51 ± 0.54	4.51 ± 0.35	4.35 ± 0.41
Heart	4.12 ± 0.55	3.72 ± 0.56	3.64 ± 0.30	3.97 ± 0.48	3.52 ± 0.45	3.37 ± 0.21	3.43 ± 0.47	3.27 ± 0.40
Lung	5.73 ± 1.09	4.95 ± 0.79	5.27 ± 0.64	6.14 ± 1.34	4.35 ± 0.78	4.45 ± 0.31	4.22 ± 0.47	3.71 ± 0.37
Thymus	1.06 ± 0.26	0.88 ± 0.12	1.05 ± 0.28	1.14 ± 0.74	0.74 ± 0.25	0.54 ± 0.16	0.56 ± 0.04	0.51 ± 0.19
Liver	23.93 ± 1.50	24.99 ± 1.65	26.49 ± 6.05	26.15 ± 1.28	24.38 ± 1.01	23.37 ± 0.70	22.89 ± 1.91	22.59 ± 2.14
Kidneys	6.02 ± 0.31	6.11 ± 0.40	6.35 ± 0.76	6.77 ± 0.94	5.78 ± 0.52	5.75 ± 0.25	5.72 ± 0.59	5.83 ± 0.49
Adrenals	0.26 ± 0.05	0.23 ± 0.04	0.22 ± 0.04	0.22 ± 0.07	0.10 ± 0.02	0.10 ± 0.02	0.11 ± 0.02	0.11 ± 0.03
Spleen	1.86 ± 0.28	1.72 ± 0.19	2.03 ± 0.34	2.60 ± 1.02	1.51 ± 0.06	1.73 ± 0.22	1.72 ± 0.49	1.67 ± 0.37
Testes	—	—	—	—	6.96 ± 1.01	7.78 ± 1.27	7.46 ± 0.63	7.77 ± 1.18
Epididymides	—	—	—	—	3.18 ± 0.64	3.37 ± 0.65	3.41 ± 0.76	3.27 ± 0.63
Ovaries	0.55 ± 0.10	0.46 ± 0.06	0.52 ± 0.07	0.50 ± 0.07	—	—	—	—
Uterus	2.56 ± 0.82	2.27 ± 0.64	1.84 ± 0.45	1.87 ± 0.79	—	—	—	—

Date presented as mean ± SD.
SD, standard deviation.

toxicological significance in biochemical and hematological parameters in any 20(S)-ginsenoside Rg3 treatment groups. Although some data were increased or decreased after 20(S)-ginsenoside Rg3 administration, there were no dose–response relationships, and data were within the normal range. Relative organ weights can reflect pathological changes in impaired organs [33]. Under the conditions of this study, the relative weight of the spleen was higher in the 180 mg/kg female group, and the relative weights of

the liver in the 60 mg/kg male group and the lung in the 20 mg/kg male group were lower than those in the controls. However, these changes only were found in female or male rats, and the value is within our facility's control range. In addition, no significant change was found in blood chemistry and hematological and histopathological features on examination. Thus, the effect was not considered to be the toxicological effects of the test substance.

In summary, the mean oral lethal dose (LD_{50}) of 20(S)-ginsenoside Rg3, in acute toxicity, is above 1600 mg/kg and 800 mg/kg for mice and rats, respectively. In a repeated-dose 26-week oral toxicity study, the NOEAL for female and male rats is 180 mg/kg. Our results should be useful in determining the chronic toxicity of 20(S)-ginsenoside Rg3 and in designing future studies on its safety and efficacy.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2018.10.001.

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