Single-dose Toxicity of Water-soluble Ginseng Pharmacopuncture Injected Intramuscularly in Rats

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Key Words
aqua acupuncture, ginseng, herbal medicine, intramuscular injection, intramuscular toxicity, pharmacopuncture

Abstract
Objectives: Radix Ginseng has been traditionally used as an adaptogen that acts on the adrenal cortex and stimulates or relaxes the nervous system to restore emotional and physical balance and to improve well-being in cases of degenerative disease and/or old age. Radix Ginseng has been used for a long time, but the safety of ginseng pharmacopuncture needs testing. This study was done to analyze the single-dose toxicity of water-soluble ginseng pharmacopuncture (GP) intramuscular injections in rats.

Methods: All experiments were performed at Biotech, an institution authorized to perform non clinical studies under the regulations of Good Laboratory Practice (GLP). Each group contained 10 Sprague-Dawley rats, 5 males and 5 females. GP was prepared in a sterile room at the Korean Pharmacopuncture Institute under regulations of Good Manufacturing Practice (GMP). GP dosages were 0.1, 0.5 and 1.0 mL for the experimental groups; normal saline was administered to the control group. The animals general condition was examined daily for 14 days, and the rats were weighed on the starting day and at 3, 7 and 14 days after administration of the pharmacopuncture. Hematological and biochemistry tests and autopsies were done to test the toxicological effect of GP after 14 days. This study was performed with approval from the Institutional Animal Ethics Committee of Biotech.

Results: No deaths were found in this single-dose toxicity test of intramuscular injections of GP, and no significant changes in the general conditions, body weights, hematological and biochemistry tests, and autopsies were observed. The local injection site showed no changes. Based on these results, the lethal dose was assumed to be over 1.0 mL/animal in both sexes.

Conclusion: These results suggest that GP is relatively safe. Further studies, including a repeated toxicity test, are needed to provide more concrete evidence for the safety of GP.

1. Introduction
Pharmacopuncture or herbal acupuncture is a totally different modality of treatment from the traditional methods used in Korean medicine. Its therapy was derived by combining two traditional therapeutic methods, herbal medicine and acupuncture therapy. Pharmacopuncture treatment is performed by injecting small amounts of herbal medicinal materials at acupuncture points or affected areas in order to achieve the effects of both herb medicine and acupuncture [1]. In the Korean clinical environment, pharmacopuncture is frequently used on a daily basis. Nowadays, its safety and efficacy are important issues.
Panax ginseng (Korean ginseng) has been used as a traditional medicine for boosting Qi energy and tonifying the spleen and lungs [2]. It has also been used as a traditional medicine for the treatment of cancer and has been shown to inhibit tumor cell proliferation and tumor growth, to induce differentiation and apoptosis, and to inhibit cancer cell invasion [3-6]. The major important components of Panax ginseng are saponin glycosides, which are known as the ginsenosides, a group of steroidal saponins. Until now, over 50 ginsenosides have been isolated from ginseng saponins [7-9]. Ginsenosides are characterized by a steroidal like skeleton consisting of 4 trans rings, with modifications that depend on the type (e.g., glucose, maltose, and fructose) and the number of sugar moieties, as well as the sites of attachment of the hydroxyl group (e.g., C-3, C-6, or C-20). Based on their chemical structural characteristics, ginseng saponins can be divided into protopanaxadiol and protopanaxatriol groups, except for ginsenoside Ro, which is derived from an oleanolic group. In the protopanaxadiol group, sugars are attached to the β-OH at C-3 and another –OH at C-20, as found in Rb1, Rb2, Rc, Rd, Rg3, and Rh2. In the protopanaxatriol group, sugar residues are attached to the α-OH at C-6, with another –OH at C-20, examples being Re, Rg1, Rg2, Rh1, and Rf.

Generally, ginseng is a representative herb that increases immunity, reduces fatigue, increases blood circulation, increases memory, and helps with anti-oxidation. Also, special ginseng that consists of low molecular weight ginsenosides (ginsenosides Rg3, Rh1, Rh2, Compound K, etc.) have been synthesized by using an enzyme treated technique. This increases absorption in patients lacking the body enzymes necessary to degrade ginseng, thus yielding superior efficacy [10, 11]. However, low molecular weight ginsenosides in ginseng are not soluble in water, so we must use homogenized technology for make a water-soluble ginseng pharmacopuncture. Because we want to know its safety in vivo, in this article, we report the results from our toxicological tests on the ginseng pharmacopuncture (GP).

2. Materials and Methods

The water soluble ginseng pharmacopuncture was prepared in a sterile room at the Korean Pharmacopuncture Institute (Korea-Good Manufacturing Practice, K-GMP). After the mixing process with pure water had been completed, the pH was controlled to between 7.0 and 7.5; then, NaCl was added to make a 0.9% isotonic solution. The completed extract was stored in a refrigerator (2.1 — 6.6°C). A high performance liquid chromatography (HPLC) analysis was performed to determine the changes in the chemical constituents in ginseng saponin following the enzyme treatment. HPLC results showed the appearance of new peaks (Rh1, Rg3, protopanaxtriol (PPT), Compound K, and Rh2), indicative of ginseng saponin metabolites (Fig. 1, Table 1).

The animals used in this study were 6 week old Sprague-Dawley (SD) rats (Orientbio Inc., Korea). The rats were received at an age of 5 weeks, and they were kept for 1 week at room temperature. The mean weights of the rats were 189.5 — 209.2 g (male) and 145.1 — 167.6 g (female) at the time of injection. For all animals, a visual inspection was conducted; all animals were weighed using a...
Table 1 Clinical characteristics of the subjects

| Group               | Rg1  | Re  | Rf  | Rh1 | Rb1 | Rc  | Rb2 | F1  | Rd  | Rg3(S) | Rg3(R) | PPT  | ComK | Rh2 |
|---------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|--------|--------|------|------|-----|
| Regular ginseng     | 9.19 | 7.21| 2.54| 7.65| 4.68 | 2.32| 2.02| 0.61| 0.94| 8.58   | 4.73   |      |      |     |
| Reinforced ginseng  | 0.61 | 1.02| 2.74| 2.65| 0.94| 8.58| 4.73| 2.65| 0.61| 0.94   | 8.58   | 4.73 | 2.65 |

PPT, protopanaxtriol; ComK, Compound K.

Figure 2 Body weights in male Sprague-Dawley rats.

Figure 3 Body weights in female Sprague-Dawley rats.
CP3202S system (Sartorius, Germany). During the 7 days of acclimatization, the general symptoms of the rats were observed once a day. The weights of the rats were recorded on the last day of acclimatization. No abnormalities were found. The temperature of the laboratory was 21.0 — 23.2°C, and the humidity was 40.9% — 59.4%. Enough food (Teklad Certified Irradiated Global 18% Protein Rodent Diet 2918C) and ultra violet (UV)-filtered water were provided. The lights were on for 12 hours/day (from 7 am to 7 pm). Groupings were done after 7 days of acclimatization. Animals were selected if their weights were close to the mean weight. In total, 20 male rats and 20 female rats were selected. The animals were randomly distributed into 4 groups (5 male and 5 female rats per group, Table 2).

The administration route was intramuscular because of clinical considerations, and the administered volume was 1.0 mL/animal of normal saline in the control group and high dose group, 0.1 mL/animal in low dose group, 0.5 mL/animal in mid dose group. The syringe used in the experiment was 1 mL disposable 26G syringe. For the low dose group and mid dose group, the rats were administrated on the Lt. thigh, single injection. But for the control group and high dose group, the rats on the both thigh, 0.5 mL on each thigh.

The expected volume of GP administered in clinical use is 1.0 mL per treatment. No death occurred in a pilot test in which 1.0 mL of GP was injected into each male and female rat. In this study 1.0 mL/animal was set as a high dose, and 0.5 mL and 0.1 mL were set as the mid and the low doses, respectively. In the control group, 1.0 mL of normal saline solution was administered. This study was conducted under the approval of the Institutional Animal Ethic Committee of Biotoxtech.

From the 1st day to the 14th day of treatment, the general symptoms were examined once a day. On the day of injection (day 0), the general symptoms (toxicological effects, manifestation time, recovery time, etc.), as well as mortality, were examined at 30 minutes and 1, 2, 3, and 4 hours after injection. Body weights were measured immediately before treatment and at 3, 7 and 14 days after treatment. After the rats had fasted for more than 18 hours, they were anesthetized by using isoflurane. A blood sample was taken from the abdominal aorta on necropsy day (15 days after injection) and was inserted into an ethylenediaminetetra acetic acid (EDTA) coated tube. The 1 mL of blood was analyzed by using an automatic hematology analyzer (ADVIA 120, SIEMEMS, Germany). The items measured were RBC (erythrocytes), hemoglobin, hematocrits, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), leucocytes (WBC), WBC differential counting (neutrophils, lymphocytes, monocytes, eosinophils), and reticulocytes. A 2.0 mL blood sample underwent centrifugation for the blood coagulation test (3,000 rpm, 10 minutes), and serum was taken. The results were measured by using an automated coagulation analyzer (Coapresta 2000, SEKISUI, Japan). The items measured were the prothrombin time (PT) and the activated partial thromboplastin time (APTT).

Blood taken from the abdominal aorta was used in the blood biochemical test. The results were measured by using an automatic analyzer (7180, HITACHI, Japan) and an electrolyte analyzer (AVL9181, Roche, Germany). The items measured were alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), blood urea nitrogen (BUN), creatinine (Crea), total bilirubin (T-Bili), total protein (TP), albumin (Alb), albumin/globulin (A/G) ratio, total cholesterol (T-Chol), triglyceride (TG), phosphate (P), glucose (Glu), calcium (Ca), chloride (Cl) and potassium (K).

After the termination of all observations, organs and tissues of all surviving animals were visually inspected and were examined under a microscope after they had been stabilized using 10% neutral buffered formalin. For the injection site, tissue slices were stained with hematoxylin & eosin (H&E).

The body weights and the results from the hematologic examinations and the blood biochemical tests were analyzed by using statistical analysis system (SAS) software (version 9.3, SAS Institute Inc., U.S.A.). The Bartlett test was conducted to evaluate the homogeneity of the variance and the significance. The significance level was 0.05. The one-way analysis of variance (ANOVA) test was conducted, and when homogeneity of the variance was recognized, Dunnett’s t test was conducted; if homogeneity was rejected, then the Kruskal-Wallis test was conducted post-hoc.

### 3. Results

In this study, no deaths occurred in experimental rats of either sex based on this result, the LD$_{50}$ of GP was assumed to be over 1.0 mL/animal. In addition, no meaningful

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**Table 2 Summary of mortality**

| Group          | GP Injection (mL/animal) | Number of animals |
|----------------|--------------------------|-------------------|
| G1: Control group | 0                         | Male: 5  Female: 5 |
| G2: Low-dose group | 0.1                      | Male: 5  Female: 5 |
| G3: Mid-dose group | 0.5                      | Male: 5  Female: 5 |
| G4: High-dose group | 1.0                      | Male: 5  Female: 5 |
changes in the body weights or abnormalities in the general conditions of the rats were noticed (Tables 3, 4, Figs. 2, 3). Furthermore, no meaningful changes in the results of the hematological tests and the biochemical tests were found (Tables 5, 6). The necropsy and histopathological findings showed no abnormalities (Tables 7, 8, Figs. 4-7).

4. Discussion

Radix ginseng (Panax ginseng) has been traditionally used as an adaptogen that acts on the adrenal cortex and stimulates or relaxes the nervous system to restore emotional and physical balance and to improve well-being in patients suffering from degenerative disease and old age. Its components mostly are triterpenoid saponins, panax acid, glycosides, sterols and essential oil. Trials indicate hypoglycemic, cardiovascular [12], antiviral [13], and psychomotor enhancement [14], as well as blood pressure normalization and asthma control, properties. It appears to have antioxidant and anti-carcinogenic effects [15, 16]. In addition, we found that the enzymatic processing of ginseng saponin could increase the content of active constituents and enhance its anti-cancer activity, presumably because of the production of minor saponins, such as Rh1, Rg3, Compound K, and PPT constituents in ginseng saponin [10, 11].

Although ginseng (Panax ginseng) has often been used in clinics for a long time, the safety of ginseng pharmacopuncture still needs to be tested, especially that of enzyme enforced ginseng. GP was made, and toxicity tests were performed using 0.1-, 0.5-, 1.0-mL doses of GP; the same dose of normal saline was administered to the animals in the control group. In four groups of rats in this experiment, no deaths or abnormalities on the hematological and biochemical tests were found, as was the case for the results of the necropsies and the histopathological tests. In this study, the LD<sub>50</sub> of GP in rats was above 1.0 mL/animal, which indicates that this dose is safe.

5. Conclusions

The administering of water-soluble ginseng pharmacopuncture via a venous route in SD rats did not cause any changes in the weights, in the hematological and biochemical test and the necropsy results, or in the number of mortalities. These results indicate that venous administration of water soluble ginseng pharmacopuncture is the safe modality of treatment.

Acknowledgements

Table 3 Summary of clinical signs

| Sex     | Group/Dose (mL/animal) | No. of animals | Clinical Signs | Hours (Day 0) after dosing |
|---------|------------------------|----------------|----------------|---------------------------|
|         |                        |                |                | 0.5 | 1 | 2 | 4 | 6 |                     |
| Male    | G1 (0)                 | 5              | NOA            | 5   | 5 | 5 | 5 | 5 |                     |
|         | G2 (0.1)               | 5              | NOA            | 5   | 5 | 5 | 5 | 5 |                     |
|         | G3 (0.5)               | 5              | NOA            | 5   | 5 | 5 | 5 | 5 |                     |
|         | G4 (1.0)               | 5              | NOA            | 5   | 5 | 5 | 5 | 5 |                     |
| Female  | G1 (0)                 | 5              | NOA            | 0   | 0 | 0 | 0 | 0 |                     |
|         | G2 (0.1)               | 5              | NOA            | 0   | 0 | 0 | 0 | 0 |                     |
|         | G3 (0.5)               | 5              | NOA            | 0   | 0 | 0 | 0 | 0 |                     |
|         | G4 (1.0)               | 5              | NOA            | 0   | 0 | 0 | 0 | 0 |                     |

Table 4 Summary of clinical signs

| Sex     | Group/Dose (mL/animal) | No. of animals | Clinical Signs | Days after Dosing |
|---------|------------------------|----------------|----------------|-------------------|
|         |                        |                |                | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| Male    | G1 (0)                 | 5              | NOA            | 0   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|         | G2 (0.1)               | 5              | NOA            | 0   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|         | G3 (0.5)               | 5              | NOA            | 0   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|         | G4 (1.0)               | 5              | NOA            | 0   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

NOA, no observable abnormality.
Table 4 Mean body weights

| Sex     | Group/Dose (mL/animal) | Days after Dosing | Gain 0—14 |
|---------|------------------------|-------------------|-----------|
|         |                        | 0                 | 3         | 7         | 14        |
| Male    |                        |                   |           |           |           |
| G1 (0)  | 199.7 ± 6.4            | 226.2 ± 6.9       | 262.2 ± 6.0 | 323.6 ± 13.3 | 125.9 ± 13.7       |
| G2 (0.1)| 198.8 ± 6.8            | 229.1 ± 4.0       | 267.2 ± 5.2 | 332.7 ± 8.3  | 133.9 ± 7.0 |
| G3 (0.5)| 197.7 ± 7.6            | 226.3 ± 8.5       | 262.6 ± 9.6 | 324.6 ± 10.4| 127.0 ± 9.0|
| G4 (1.0)| 198.4 ± 7.9            | 229.5 ± 9.2       | 270.0 ± 11.2| 336.1 ± 11.8| 137.7 ± 6.5|
| Female  |                        |                   |           |           |           |
| G1 (0)  | 155.9 ± 1.9            | 172.5 ± 3.9       | 186.5 ± 4.3| 216.1 ± 12.9| 60.2 ± 13.6 |
| G2 (0.1)| 156.7 ± 7.2            | 169.8 ± 6.2       | 182.6 ± 7.6| 209.3 ± 13.3| 52.6 ± 8.0 |
| G3 (0.5)| 153.1 ± 7.7            | 171.2 ± 12.1      | 186.7 ± 14.9| 213.7 ± 16.3| 60.6 ± 10.6|
| G4 (1.0)| 158.6 ± 5.6            | 173.3 ± 10.2      | 185.3 ± 11.3| 215.4 ± 15.0| 56.9 ± 10.8|

Table 5 Mean hematology parameters in male, female Sprague-Dawley rats

(male)

| Group/Dose (mL/animal) | RBC (×10⁶ cells/μL) | HGB (g/dL) | HCT (%) | MCV (FL) | MCH (pg) | MCHC (g/dL) | WBC (×10⁹ cells/μL) | Reti (%) |
|------------------------|---------------------|------------|---------|----------|----------|-------------|---------------------|----------|
| G1 (0)                 | 7.03 ± 0.31         | 14.4 ± 0.2 | 44.9 ± 0.8 | 63.9 ± 1.9 | 20.5 ± 0.9 | 32.1 ± 0.6 | 1405 ± 233          | 5.0 ± 0.5 |
| G2 (0.1)               | 7.02 ± 0.18         | 14.2 ± 0.2 | 44.3 ± 0.7 | 63.2 ± 1.4 | 20.2 ± 0.5 | 32.0 ± 0.1 | 1089 ± 332          | 5.0 ± 0.5 |
| G3 (0.5)               | 7.00 ± 0.16         | 14.3 ± 0.4 | 45.0 ± 1.9 | 64.1 ± 2.2 | 20.4 ± 0.6 | 31.8 ± 0.4 | 1237 ± 281          | 5.1 ± 0.6 |
| G4 (1.0)               | 7.15 ± 0.20         | 14.3 ± 0.4 | 44.5 ± 1.0 | 62.3 ± 2.1 | 20.0 ± 0.8 | 32.2 ± 0.3 | 1186 ± 137          | 4.8 ± 0.7 |

(female)

| Group/Dose (mL/animal) | RBC (×10⁶ cells/μL) | HGB (g/dL) | HCT (%) | MCV (FL) | MCH (pg) | MCHC (g/dL) | WBC (×10⁹ cells/μL) | Reti (%) |
|------------------------|---------------------|------------|---------|----------|----------|-------------|---------------------|----------|
| G1 (0)                 | 6.62 ± 1.79        | 15.2 ± 5.1 | 80.3 ± 5.7 | 2.5 ± 0.8 | 0.4 ± 0.1 | 0.2 ± 0.1 | 17.4 ± 0.3          | 12.6 ± 0.8 |
| G2 (0.1)               | 6.95 ± 1.31        | 16.2 ± 4.5 | 79.2 ± 3.7 | 2.4 ± 0.7 | 0.8 ± 0.3 | 0.2 ± 0.0 | 15.9 ± 1.3          | 12.0 ± 2.1 |
| G3 (0.5)               | 8.14 ± 1.86        | 13.5 ± 3.7 | 82.8 ± 3.8 | 1.9 ± 0.3 | 0.6 ± 0.4 | 0.2 ± 0.0 | 16.8 ± 0.3          | 12.6 ± 1.7 |
| G4 (1.0)               | 8.27 ± 0.92        | 13.5 ± 3.1 | 82.3 ± 3.0 | 2.6 ± 1.0 | 0.5 ± 0.2 | 0.2 ± 0.0 | 16.8 ± 0.2          | 11.7 ± 2.2 |

| Group/Dose (mL/animal) | RBC (×10⁶ cells/μL) | HGB (g/dL) | HCT (%) | MCV (FL) | MCH (pg) | MCHC (g/dL) | WBC (×10⁹ cells/μL) | Reti (%) |
|------------------------|---------------------|------------|---------|----------|----------|-------------|---------------------|----------|
| G1 (0)                 | 7.25 ± 0.74        | 14.6 ± 0.8 | 44.3 ± 2.7 | 61.3 ± 2.9 | 20.2 ± 1.0 | 32.9 ± 0.5 | 1245 ± 146          | 3.0 ± 1.0 |
| G2 (0.1)               | 7.18 ± 0.19        | 14.3 ± 0.3 | 43.2 ± 0.8 | 60.3 ± 1.2 | 20.0 ± 0.4 | 33.2 ± 0.1 | 1455 ± 253          | 3.2 ± 0.6 |
| G3 (0.5)               | 7.23 ± 0.28        | 14.3 ± 0.5 | 43.8 ± 1.7 | 60.7 ± 2.2 | 19.9 ± 0.6 | 32.8 ± 0.3 | 1416 ± 120          | 3.3 ± 0.4 |
| G4 (1.0)               | 7.28 ± 0.19        | 14.4 ± 0.4 | 43.4 ± 1.1 | 59.6 ± 0.9 | 19.7 ± 0.4 | 33.1 ± 0.3 | 1387 ± 109          | 2.7 ± 0.4 |

| Group/Dose (mL/animal) | RBC (×10⁶ cells/μL) | HGB (g/dL) | HCT (%) | MCV (FL) | MCH (pg) | MCHC (g/dL) | WBC (×10⁹ cells/μL) | Reti (%) |
|------------------------|---------------------|------------|---------|----------|----------|-------------|---------------------|----------|
| G1 (0)                 | 6.62 ± 1.69        | 12.5 ± 4.1 | 83.8 ± 4.8 | 1.7 ± 0.6 | 0.9 ± 0.3 | 0.1 ± 0.1 | 17.4 ± 1.3          | 10.3 ± 2.9 |
| G2 (0.1)               | 5.33 ± 0.89        | 10.2 ± 2.1 | 85.7 ± 3.3 | 1.9 ± 1.1 | 1.1 ± 0.4 | 0.1 ± 0.1 | 17.9 ± 0.8          | 11.1 ± 1.5 |
| G3 (0.5)               | 4.57 ± 1.46        | 16.7 ± 7.7 | 79.9 ± 7.1 | 1.5 ± 0.7 | 1.1 ± 0.2 | 0.2 ± 0.1 | 17.7 ± 1.2          | 11.3 ± 1.5 |
| G4 (1.0)               | 3.78 ± 1.33*       | 12.2 ± 4.8 | 84.2 ± 5.1 | 1.5 ± 0.7 | 1.0 ± 0.6 | 0.2 ± 0.1 | 18.3 ± 0.7          | 11.7 ± 2.2 |

*Significantly different from control by Steel test: P < 0.05.

RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; Reti, reticulocytes; WBC, white blood cell; NEU, neutrophils; LYM, lymphocytes; MONO, monocytes; EOS, Eosinophils; BASO, basophils; PT, prothrombin time; APTT, activated partial thromboplastin time.
Table 6 Mean clinical chemistry in male, female Sprague-Dawley rats

(male)

| Group/ Dose (mL/animal) | ALT (U/L) | AST (U/L) | ALP (U/L) | GGT (U/L) | Glu (mg/dL) | BUN (mg/dL) | Crea (mg/dL) | T-Bil (mg/dL) | T-Chol (mg/dL) |
|-------------------------|-----------|-----------|-----------|-----------|-------------|-------------|-------------|--------------|---------------|
| G1 (0)                  | 32.2 ± 2.8| 76.7 ± 5.9| 932.7 ± 316.2| 0.31 ± 0.14| 117 ± 11 | 10.7 ± 1.2 | 0.36 ± 0.02 | 0.03 ± 0.02 | 72 ± 11     |
| G2 (0.1)                | 31.0 ± 4.2| 75.0 ± 8.3| 790.2 ± 174.5| 0.40 ± 0.10| 116 ± 13 | 11.5 ± 0.8 | 0.38 ± 0.04 | 0.03 ± 0.01 | 91 ± 16     |
| G3 (0.5)                | 35.0 ± 5.1| 80.3 ± 12.9| 840.7 ± 212.6| 0.43 ± 0.09| 123 ± 14 | 11.4 ± 2.0 | 0.40 ± 0.03 | 0.04 ± 0.01 | 75 ± 11     |
| G4 (1.0)                | 32.0 ± 5.5| 80.8 ± 2.8| 868.9 ± 110.2| 0.35 ± 0.16| 124 ± 20 | 11.7 ± 0.9 | 0.37 ± 0.01 | 0.04 ± 0.02 | 75 ± 19     |

| Group/ Dose (mL/animal) | TG (mg/dL) | TP (g/dL) | Alb (g/dL) | A/G ratio (mg/dL) | P (mg/dL) | Ca (mg/dL) | Na (mmol/L) | K (mmol/L) | Cl (mmol/L) |
|-------------------------|------------|-----------|------------|-------------------|------------|------------|-------------|-----------|-------------|
| G1 (0)                  | 40 ± 15    | 5.3 ± 0.2 | 2.3 ± 0.1  | 0.79 ± 0.03       | 8.58 ± 0.48| 10.2 ± 0.1 | 138 ± 2     | 4.8 ± 0.3 | 103 ± 2     |
| G2 (0.1)                | 51 ± 18    | 5.4 ± 0.2 | 2.3 ± 0.1  | 0.76 ± 0.01       | 8.56 ± 0.42| 10.3 ± 0.3 | 139 ± 1     | 4.6 ± 0.2 | 103 ± 2     |
| G3 (0.5)                | 49 ± 24    | 5.3 ± 0.2 | 2.3 ± 0.1  | 0.76 ± 0.03       | 8.70 ± 0.55| 10.1 ± 0.2 | 138 ± 2     | 4.5 ± 0.3 | 104 ± 2     |
| G4 (1.0)                | 53 ± 23    | 5.3 ± 0.1 | 2.3 ± 0.1  | 0.77 ± 0.03       | 8.57 ± 0.46| 10.3 ± 0.2 | 139 ± 1     | 4.4 ± 0.2 | 103 ± 1     |

(female)

| Group/ Dose (mL/animal) | ALT (U/L) | AST (U/L) | ALP (U/L) | GGT (U/L) | Glu (mg/dL) | BUN (mg/dL) | Crea (mg/dL) | T-Bil (mg/dL) | T-Chol (mg/dL) |
|-------------------------|-----------|-----------|-----------|-----------|-------------|-------------|-------------|--------------|---------------|
| G1 (0)                  | 23.8 ± 3.1| 77.6 ± 11.4| 502.9 ± 105.1| 0.43 ± 0.23| 126 ± 5 | 13.0 ± 0.5 | 0.44 ± 0.05 | 0.03 ± 0.00 | 91 ± 26     |
| G2 (0.1)                | 23.3 ± 3.5| 71.6 ± 5.2 | 551.3 ± 41.8 | 0.59 ± 0.11| 121 ± 8 | 13.9 ± 1.9 | 0.43 ± 0.01 | 0.02 ± 0.01 | 87 ± 17     |
| G3 (0.5)                | 21.6 ± 2.7| 69.2 ± 6.0 | 446.7 ± 95.2 | 0.59 ± 0.18| 131 ± 17 | 13.3 ± 1.8 | 0.43 ± 0.01 | 0.03 ± 0.02 | 89 ± 15     |
| G4 (1.0)                | 23.6 ± 3.5| 81.0 ± 13.6| 532.9 ± 88.1 | 0.62 ± 0.13| 117 ± 8 | 12.8 ± 2.4 | 0.43 ± 0.04 | 0.03 ± 0.01 | 90 ± 12     |

| Group/ Dose (mL/animal) | TG (mg/dL) | TP (g/dL) | Alb (g/dL) | A/G ratio (mg/dL) | P (mg/dL) | Ca (mg/dL) | Na (mmol/L) | K (mmol/L) | Cl (mmol/L) |
|-------------------------|------------|-----------|------------|-------------------|------------|------------|-------------|-----------|-------------|
| G1 (0)                  | 22 ± 8     | 5.9 ± 0.3 | 2.8 ± 0.2  | 0.88 ± 0.07       | 7.29 ± 0.64| 10.4 ± 0.2 | 139 ± 2     | 4.4 ± 0.3 | 105 ± 1     |
| G2 (0.1)                | 15 ± 5     | 5.6 ± 0.2 | 2.5 ± 0.1  | 0.83 ± 0.02       | 7.25 ± 0.33| 10.0 ± 0.2 | 138 ± 1     | 4.8 ± 0.4 | 105 ± 1     |
| G3 (0.5)                | 15 ± 6     | 5.8 ± 0.4 | 2.7 ± 0.2  | 0.85 ± 0.02       | 7.08 ± 0.70| 10.3 ± 0.6 | 138 ± 1     | 4.7 ± 0.2 | 105 ± 2     |
| G4 (1.0)                | 14 ± 5     | 5.7 ± 0.3 | 2.6 ± 0.1  | 0.85 ± 0.06       | 7.23 ± 0.45| 10.1 ± 0.2 | 139 ± 1     | 4.8 ± 0.3 | 105 ± 2     |

*Significantly different from control by Steel test; \textit{P} < 0.05.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transpeptidase; Glu, glucose; BUN, blood urea nitrogen; Crea, creatinine; T-Bil, total bilirubin; T-Chol, total cholesterol; TG, triglycerides; TP, total protein; Alb, albumin; A/G ratio, albumin/globulin ratio; P, phosphorus; Ca, calcium; Na, sodium; K, potassium; Cl, chloride.
Table 7 Summary of necropsy findings

| Sex          | Male | Female |
|--------------|------|--------|
| Group/ Dose (mL/animal) | G1 (0) | G2 (0.1) | G3 (0.5) | G4 (1.0) | G1 (0) | G2 (0.1) | G3 (0.5) | G4 (1.0) |
| No. of animals | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| No. of Unremarkable findings | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

External surface and all organs in body cavity were unremarkable.

Table 8 Summary of histopathological findings

| Sex          | Male | Female |
|--------------|------|--------|
| Group/ Dose (mL/animal) | G1 (0) | G2 (0.1) | G3 (0.5) | G4 (1.0) | G1 (0) | G2 (0.1) | G3 (0.5) | G4 (1.0) |
| No. of animals | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| No. of Unremarkable findings at injection site | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

Figure 4 The tissue of the liver from an intramuscular single-dose toxicity study of ginseng pharmacopuncture in Sprague-Dawley rats. No histopathological change was detected by hematoxylin & eosin staining (×200, ×200).

Figure 5 The tissue of the brain from an intramuscular single-dose toxicity study of ginseng pharmacopuncture in Sprague-Dawley Rats. No histopathological change was detected by hematoxylin & eosin staining (×200, ×200).
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Conflict of interest

The authors declare that there are no conflicts of interest.

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