Journal of Pharmacopuncture

ISSN 2093-6966 [Print], ISSN 2234-6856 [Online]
Journal of Pharmacopuncture 2015;18[2]:076-085
DOI: http://dx.doi.org/10.3831/KPI.2015.18.018

Original article

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

Junsang Yu[1], Seungho Sun[2], Kwangho Lee[3], Kirok Kwon[4]*

[1] Department of Sasang Constitutional Medicine, Sangji University College of Korean Medicine, Wonju, Korea
[2] Department of Acupuncture and Moxibustion, Sangji University College of Korean Medicine, Wonju, Korea
[3] Department of Internal Medicine, Sangji University College of Korean Medicine, Wonju, Korea
[4] Research Center of the Korean Pharmacopuncture Institute, Seoul, Korea

**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 Biotoxtech, 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 Biotextech.

**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.

**Received:** Jan 02, 2015 **Reviewed:** Jan 10, 2015 **Accepted:** Feb 04, 2015

## 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.

This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted noncommercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

This paper meets the requirements of KS X ISO 9706, ISO 9706-1994 and ANSI/NISO Z39.48-1992 (Permanence of Paper).

*Corresponding Author
Kirok Kwon. Korean Pharmacopuncture Institute, 4F, Association of Korean Oriental Medicine B/D, 26-27, Gayang-dong, Gangseo-gu, Seoul 157-200, Korea.
Tel: +82-33-744-9304 Fax: +82-33-744-9305
E-mail: drkwon5031@daum.net

ⓒ 2015 Korean Pharmacopuncture Institute

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 steroid 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 $\beta$-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 $\alpha$-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

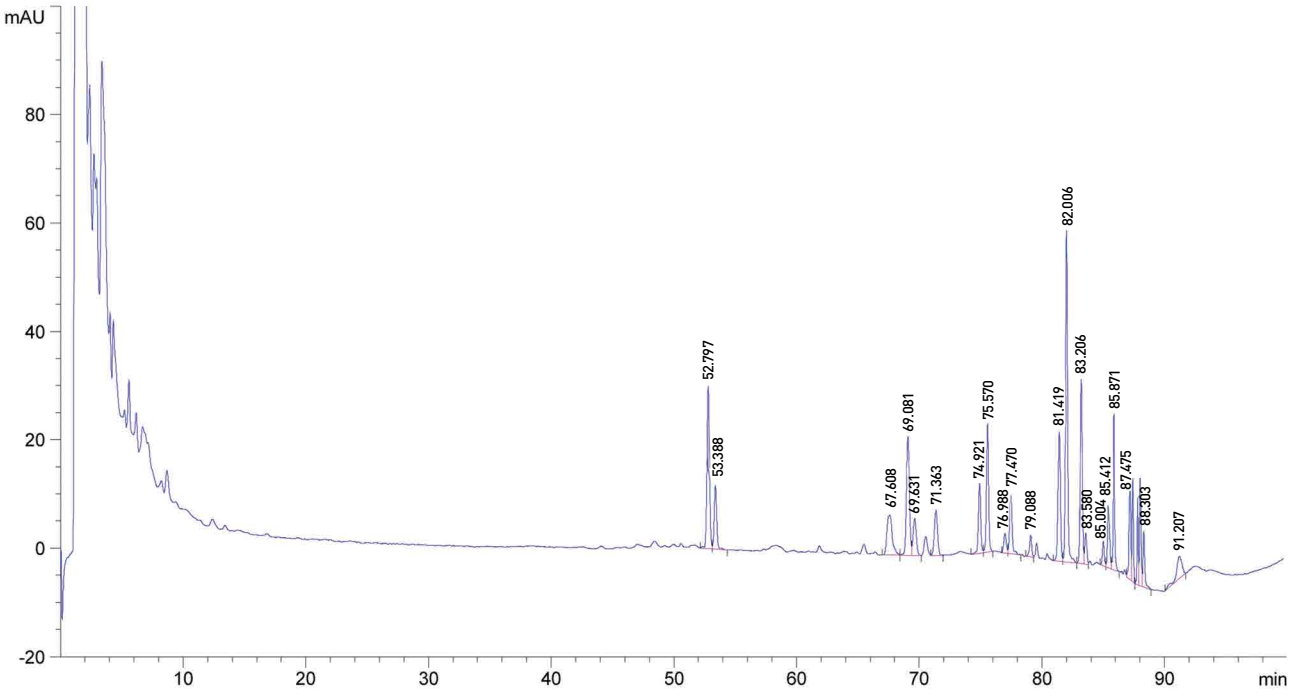


**Figure 1** HPLC-UV chromatograms of ginseng root extracts.
HPLC, high performance liquid chromatography; UV, ultra violet.

**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 | — | — | — | — | — |
| Rein-forced ginseng | 0.61 | 1.02 | — | 2.74 | 2.65 | — | — | — | — | 0.94 | 8.58 | — | 4.73 | 2.65 |

PPT, protopanaxtriol; ComK, Compound K.

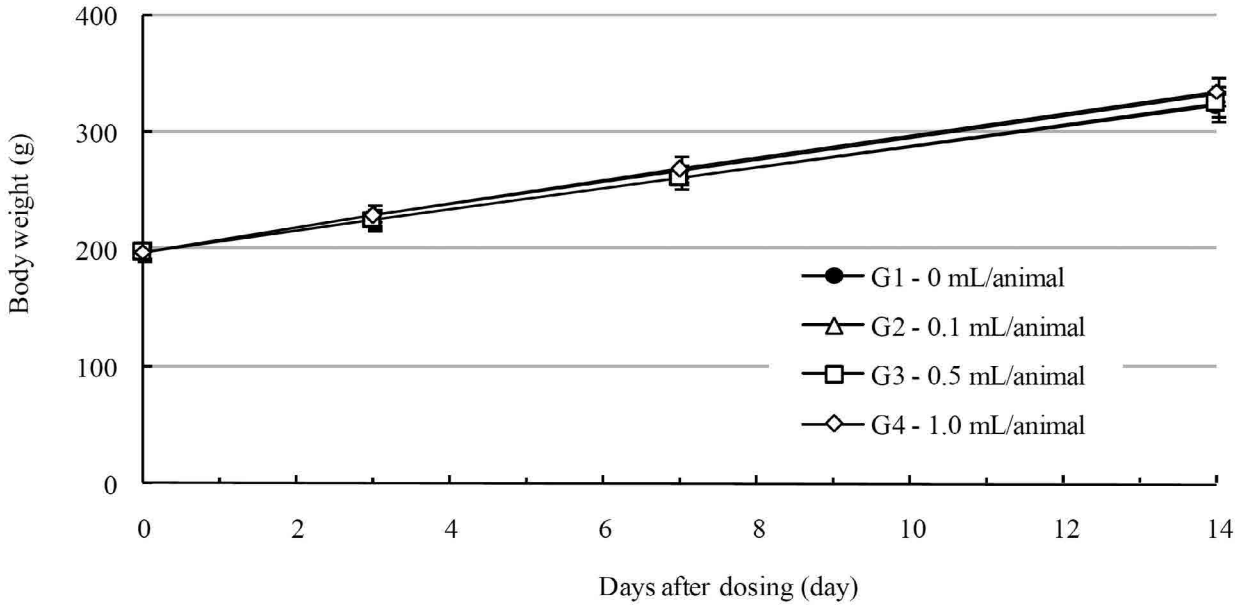


**Figure 2** Body weights in male Sprague-Dawley rats.

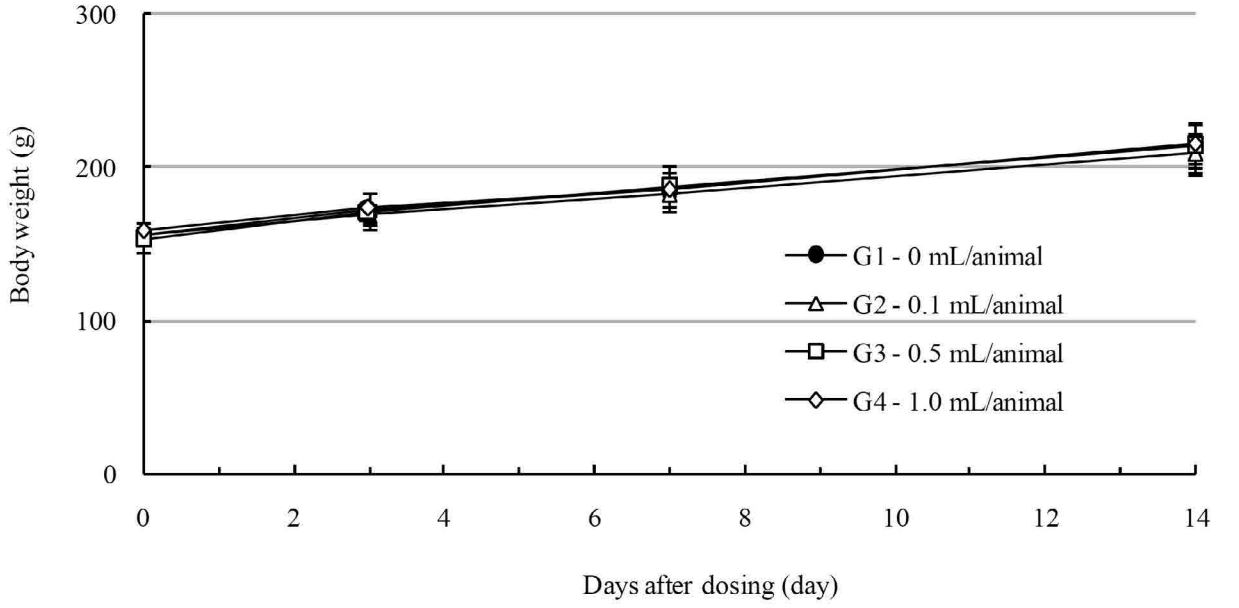


**Figure 3** Body weights in female Sprague-Dawley rats

**Table 2** Summary of mortality

| Group | GP Injection (mL/animal) | Number of animals | |
|---|---|---|---|
| | | Male | Female |
| G1: Control group | 0 | 5 | 5 |
| G2: Low-dose group | 0.1 | 5 | 5 |
| G3: Mid-dose group | 0.5 | 5 | 5 |
| G4: High-dose group | 1.0 | 5 | 5 |

GP, ginseng pharmacopuncture.

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

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 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 $LD_{50}$ 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 | 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 |

| 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 |
| Female | 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 ($\times 10^6$ cells/μL) | HGB (g/dL) | HCT (%) | RBC Indices | | | WBC ($\times 10^3$ cells/μL) | Reti (%) |
|---|---|---|---|---|---|---|---|---|
| | | | | MCV (fL) | MCH (pg) | MCHC (g/dL) | | |
| 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 |

| Group/ Dose (mL/animal) | WBC ($\times 10^3$ cells/μL) | WBC Differential Counting (%) | | | | | PT (sec) | APTT (sec) |
|---|---|---|---|---|---|---|---|---|
| | | NEU | LYM | MONO | EOS | BASO | | |
| G1 (0) | 9.62 ± 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 |

(female)

| Group/ Dose (mL/animal) | RBC ($\times 10^6$ cells/μL) | HGB (g/dL) | HCT (%) | RBC Indices | | | WBC ($\times 10^3$ cells/μL) | Reti (%) |
|---|---|---|---|---|---|---|---|---|
| | | | | MCV (fL) | MCH (pg) | MCHC (g/dL) | | |
| 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) | WBC ($\times 10^3$ cells/μL) | WBC Differential Counting (%) | | | | | PT (sec) | APTT (sec) |
|---|---|---|---|---|---|---|---|---|
| | | NEU | LYM | MONO | EOS | BASO | | |
| 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 ± 0.0` | 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: $P < 0.05$.

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 |

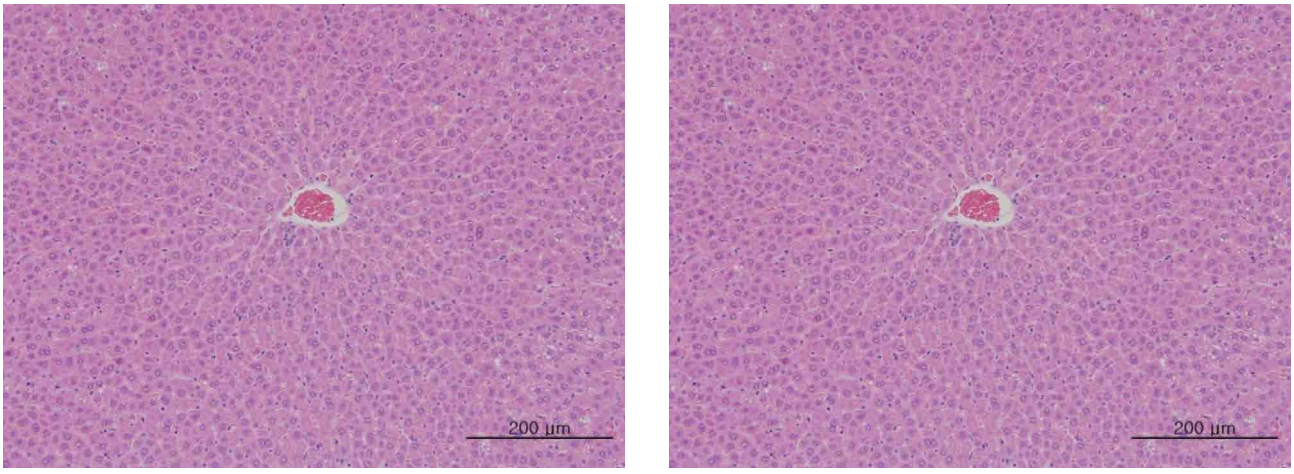


**Figure 4** The tissue of the liver from an intramuscular single-dose toxicity study of ginseng pharmacopuncture in Sprague-Dawley rats. No histopathological change was detected by hematoxylin & eosin staining (× 200, × 200).

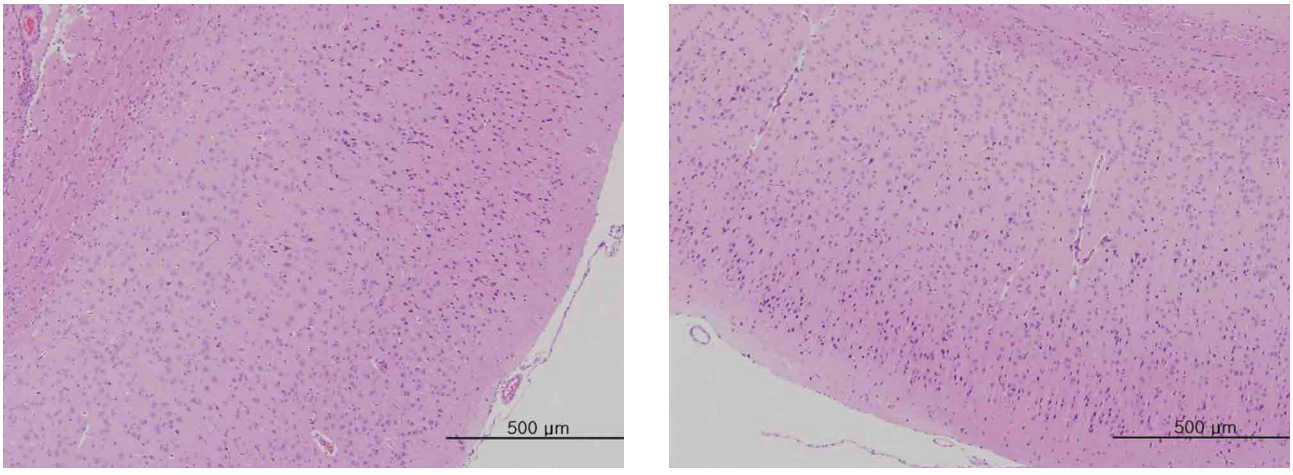


**Figure 5** The tissue of the brain from an intramuscular single-dose toxicity study of ginseng pharmacopuncture in Sprague-Dawley Rats. No histopathological change was detected by hematoxylin & eosin staining (× 200, × 200).

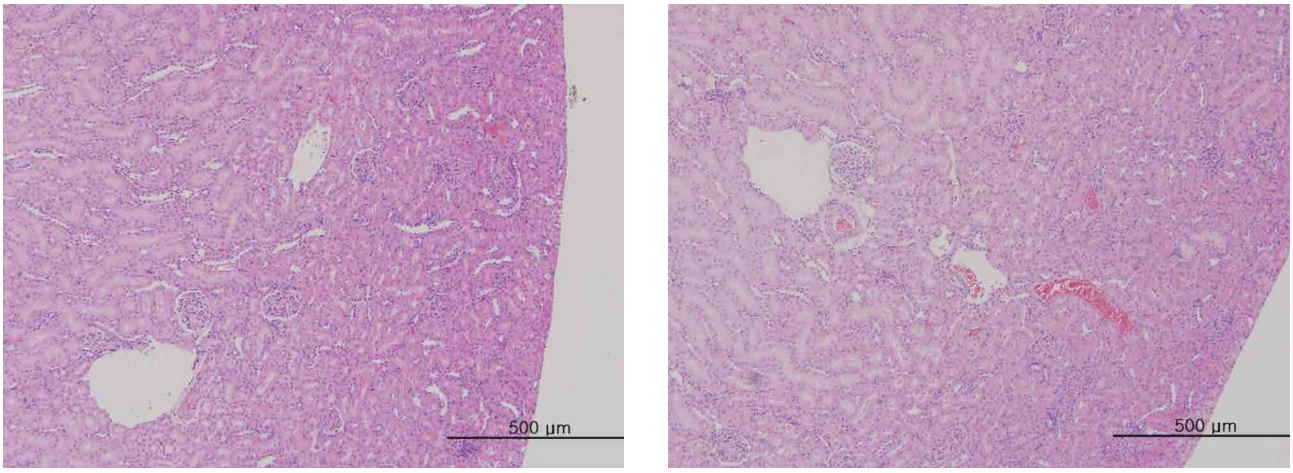


**Figure 6** The tissue of the kidney from an intramuscular single-dose toxicity study of ginseng pharmacopuncture in Sprague-Dawley rats. No histopathological change was detected by hematoxylin & eosin staining (× 200, ×200).

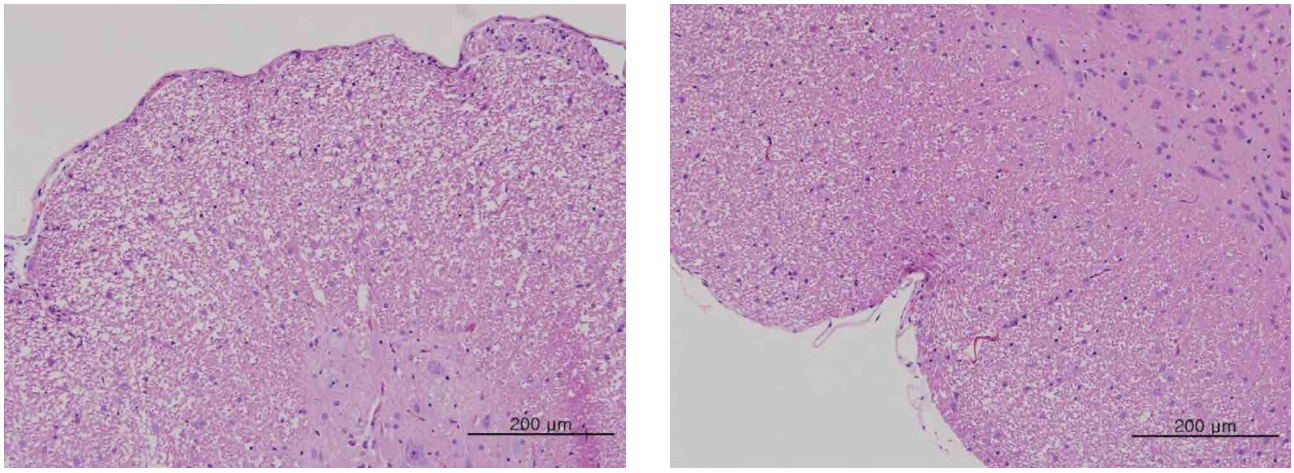


**Figure 7** The tissue of spinal nerves from an intramuscular single-dose toxicity study of ginseng pharmacopuncture in Sprague-Dawley rats. No histopathological change was detected by hematoxylin & eosin staining (× 100, × 100).

This study was supported by funds from the Ministry of Health and Welfare (No. B080009) and by the Sang-ji University Research Fund, 2014.

## Conflict of interest

The authors declare that there are no conflicts of interest.

## References

1. Kim JD, Kang DI. A descriptive statistical approaches to the Korean pharmacopuncture therapy. J Acupunct Meridian Stud. 2010;3(3):141-9.
2. Kang BS, Kang SS, Kang SK, Kang CG, Koh WC, Koh HK, *et al*. [Translated into Korean. big dictionary of traditional Chinese medicine]. Seoul: Jungdam Publishing Co; 1997. p. 4484.
3. An IS, An S, Kwon KJ, Kim YJ, Bae S. Ginsenoside Rh2 mediates changes in the microRNA expression profile of human non-small cell lung cancer A549 cells. Oncol Rep. 2012;29(2):523-8.
4. Kim AD, Kang KA, Zhang R, Lim CM, Kim HS, Kim DH, *et al*. Ginseng saponin metabolite induces apoptosis in MCF-7 breast cancer cells through the modulation of AMP-activated protein kinase. Environ Toxicol Phar. 2010;30(2):134-40.
5. Yuan HD, Quan HY, Zhang Y, Kim SH, Chung SH. 20(S)-ginsenoside Rg3-induced apoptosis in HT-29 colon cancer cells is associated with AMPK signaling pathway. Mol Med Rep. 2010;3(5):825-31.
6. Pan XY, Guo H, Han J, Hao F, An Y, Xu Y, *et al*. Ginsenoside Rg3 attenuates cell migration via inhibition of aquaporin 1 expression in PC-3M prostate cancer cells. Eur J Pharmacol. 2012;683(1-3):27-34.
7. Tanaka O, Kasai R. Saponins of ginseng and related plants. Fortschr Chem Org Naturst. 1984;46:1-76.
8. Jeong HS, Lim CS, Cha BC, Choi SH, Kwon KR. [Component analysis of cultivated ginseng, cultivated wild ginseng, and wild ginseng and the change of ginseno-

side components in the process of red ginseng]. J Pharmacopuncture. 2010;13(1):63-77. Korean.
9. Han YJ, Kwon KR, Cha BC, Kwon OM. [Component analysis of cultivated ginseng, cultivated wild ginseng, and natural wild ginseng by structural parts using HPLC method]. J Pharmacopuncture. 2007;10(1):37-53. Korean.
10. Kim KH, Choi I, Lee YW, Cho CK, Yoo HS, Kwon KR, *et al*. Target genes involved in antiproliferative effect of modified ginseng extracts in lung cancer A549 cells. Acta Biochim Biophys Sin. 2014;46(6):441-9.
11. Hwang JW, Beak YM, Jang IS, Yang KE, Lee DG, Yoon SJ, *et al*. An enzymatically fortified ginswng extract inhibits proliferation and induces apoptosis of KATO3 human gastric cancer cells via modulation of BAX, mTOR, PKB, and IkBa. Mol Med Rep. 2015;11(1):670-6.
12. Dasgupta A, Wu S, Actor J, Olsen M, Wells A, Datta P. Effect of Asian and Siberian ginseng on serum digoxin measurement by five digoxin immunoassays. Am J Clin Pathol. 2003;119(2):298-303.
13. Sung WS, Lee DG. *In vitro* candidacidal action of Korean red ginseng saponins against candidaalbicans(microbiology). Biol Pharm Bull. 2008;31(1):139-42.
14. Lee B, Yang CH, Hahm DH, Lee HJ, Han SM, Kim KS, *et al*. Inhibitory effects of ginseng total saponins on behavioral sensitization and dopamine release induced by cocaine. Biol Pharm Bull. 2008;31(3):436-41.
15. Wang W, Rayburn ER, Hao M, Zhao Y, Hill DL, Zhang R, *et al*. Experiment therapy of prostate cancer with novel natural product anti-cancer ginsenosides. Prostate. 2008;68(8):809-19.
16. Fiona M, Denise T. A-Z of complementary and alternative medicine: a guide for health professionals. London: Churchill Livingstone; 2009. p. 137-8.