Journal of Pharmacopuncture

ISSN 2093-6966 [Print], **ISSN 2234-6856** [Online]
Journal of Pharmacopuncture 2014;17[4]:027-035
DOI: http://dx.doi.org/10.3831/KPI.2014.17.034

Original article

A 4-Week, Repeated, Intravenous Dose, Toxicity Test of Mountain Ginseng Pharmacopuncture in Sprague-Dawley Rats

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

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

Key Words

intravenous injection, mountain ginseng, pharmacopuncture, toxicity test

Abstract

Objectives: Mountain ginseng pharmacopuncture (MGP) is a pharmacopuncture made by distilling extract from mountain cultivated ginseng or mountain wild ginseng. This pharmacopuncture is injected intravenously, which is a quick, lossless way of strongly tonifying Qi function. The present study was undertaken to evaluate a 4-week, repeated, intravenous injection, toxicity test of MGP in Sprague-Dawley (SD) rats.

Methods: Twenty male and female 6-week-old SD rats were used as subjects. We divided the SD rats into 4 groups: the high-dosage (10 mL/kg), medium-dosage (5 mL/kg), low-dosage (2.5 mL/kg) and control (normal saline) groups. MGP or normal saline was injected intravenously into the caudal vein of the rats once daily for 4 weeks. Clinical signs, body weights, and food consumption were monitored during the observation period, and hematology, serum biochemistry, organ weight, necropsy, and histological examinations were conducted once the observations had been completed.

Results: No mortality was observed in any of the groups during the observation period. No changes due to MGP were observed in the experimental groups regarding clinical signs, body weights, food consumption, hematology, serum biochemistry, organ weight and necropsy. No histological changes due to MGP were observed in any of the male or female rats in the high-dosage group.

Conclusion: During this 4-week, repeated, intravenous injection, toxicity test of MGP in SD rats, no toxic changes due to MGP were observed in any of the male or female rats in the high-dosage group. Thus, we suggest that the high and the low doses in a 13-week, repeated test should be 10 mL/kg and 2.5 mL/kg, respectively.

Received: Oct 23, 2014 **Accepted:** Nov 06, 2014

1. Introduction

Mountain ginseng pharmacopuncture (MGP) was developed using the aromatic substances found in mountain ginseng, and those substances serve to provide a mechanism for survival in a harsh environment. This pharmacopuncture is injected intravenously and is used in treating patients with intractable disorders such as terminal-stage cancer, patients undergoing chemotherapy, patients with immune disorders, and other patients not responding to conventional treatments. Also, injections of MGP at high doses ranging from 20 mL to 60 mL are possible [1].

Mountain wild ginseng refers to ginseng (Panax ginseng C. A. Meyer) of the Araliaceae family that is natu-

***Corresponding Author**
Chungsan Lim. Department of Korean Medicine, Seil Hospital, 528-31, Namwon-ro, Wonju, Gangwon 220-944, Korea.
Tel: +82-33-765-2211 Fax: +82- 33-732-2124
E-mail: cslim-own@daum.net

rally grown in the mountains. Ginseng has been artificially cultivated from the 14th century, and ginseng used prior to that time is referred to as mountain ginseng. Mountain cultivated ginseng is defined as a young root of mountain wild ginseng that has been replanted in the mountain s or as ginseng cultivated from the seeds of mountain wild ginseng [2]. Because of the high cost of mountain wild ginseng and problems verifying its authenticity, mountain cultivated ginseng that has been grown for about 10 years in an environment similar to that of mountain wild ginseng is used for pharmacopuncture [1].

Groups treated with MGP have been reported to show increased immunoprotein CR2-C3d, which counteracts pathogenic organisms; anti-trypsin, which plays a role in anti-oxidation of proteins; proapolipoprotein and apolipoprotein of high density lipoprotein (HDL) cholesterol, which prevents arteriosclerosis; and vitamin D binding protein, which protects the lungs against inflammation. Also, 20 types of proteins with actions similar to those of ginseng were more than doubled after MGP treatment while proteins that induce mental impairment were suppressed [3, 4].

No significant changes were observed in a randomized, controlled trial of MGP [5], and an intravenous, single-dose toxicity test of MGP in Sprague-Dawley (SD) rats showed no side effects [6]. Thus, we conducted a 4-week, repeated, intravenous dose, toxicity test of MGP in SD rats as part of a safety evaluation of using MGP in SD rats.

2. Materials and Methods

Twenty-four 5-week-old male and female SD rats were purchased from Orientbio Inc. (Gyeonggi, Korea). At the time of purchase, the male and female SD rats had weights in the ranges of 121.3 — 134.17 g and 105.3 — 124.6 g, respectively. Twenty SD rats of each gender were selected based on average weights and were used as the subjects of this test after a week of quarantine and acclimatization. The Twenty 6-week-old male and female SD rats had weights in the ranges of 192.1 — 207.9 g and 153.2 — 174.4 g at the first injection. The animals were housed in a room maintained at 20.4 — 24.5℃ under a relative humidity of 31.9% — 57.9%. The room was illuminated with artificial lighting from 07:00 to 19:00 hours and had 10 — 15 air changes per hour. The animals were housed in suspended stainless-steel wire-mesh cages and were allowed sterilized tap water and commercial rodent chow (Teklad Certified Irradiated Global 18% Protein Rodent Diet 2918C, Harlan Laboratories, Inc., U.S.A.). This study's protocol was approved by the Institutional Animal Care Committee at Biotoxtech Co (No. 110027, Ohchang, Korea).

The MGP was manufactured in a pathogen-free facility at the Korean Pharmacopuncture Institute, Seoul, Korea, following a previously-described procedure [7]. Mountain ginseng (MG) that had been cultivated from the seeds of mountain wild ginseng for over 10 years was selected for use in producing the MGP. First, we washed the MG with running water and dried it in air. Second, the dried MG was ground into a powder with a particle size below 10 ㎛ and was then decocted for 2 hours in distilled water. Residues were then removed, and the decoction was distilled, yielding the desired herbal acupuncture. Then, the pharmacopuncture was filtered using 0.1-㎛ filter paper and kept in a container. The pharmacopuncture was sterilized before being used.

Five healthy male and five healthy female rats were assigned to each of four groups: the control (normal saline), the low-dosage (2.5 mL/kg), the medium-dosage (5 mL/kg), and the high-dosage (10 mL/kg) groups. No toxicity had been observed during 10- or 20-mL/kg intravenous-injection, single-dose tests of MGP in SD rats [6], so in this 4-week, repeated study, we set the new maximum single dose at 10 mL/kg. MGP was administered to the rats by intravenous injection in the caudal vein at a rate of 2 mL/min once daily for 4 weeks. The control group was administered an equivalent volume of normal saline (Lot No. GAJ0071, GAJ0086, Choogwae Pharma Corp., Seoul, Korea).

All animals were observed daily for clinical signs for 4 weeks from the first injection day. The body weight of each rat was measured at the initiation of treatment and twice a week during the treatment period. The amounts of food and water ingested by the rats prior to MGP injection were measured from the day the rats were divided into groups until the day of the first injection while the amounts food and water ingested during the treatment period were averages for a week. At the 4th week after the initiation of treatment, the amounts food and water ingested were the average amounts for 6 days.

The animals were fasted for 18 hours prior to necropsy and blood was collection. Blood samples were drawn from the abdominal aorta by using a syringe needle under isoflurane anesthesia. Blood samples were collected into tubes containing ethylene diamine tetraacetic acid (EDTA), and were analyzed using a blood counting analyzer (ADVIA 120, SIEMENS, Eschborn, Germany) to determine the red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular cell hemoglobin concentration (MCHC), platelet count (PLT), and white blood cell count (WBC).

The serum biochemistry analysis was performed using an auto-analyzer (7180, HITACHI, Tokyo, Japan). Blood samples were centrifuged at 3,000 rpm for 10 minutes. Serum biochemistry parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (Crea), total protein (TP), albumin (Alb), albumin/globulin ratio, (A/G ratio) total cholesterol (T-Chol), triglycerides (TG), and glucose (Glu) were examined.

The organs and tissues of all the animals were visually examined. Net weights were measured for the following organs: the brain, heart, liver, spleen, and kidneys. A relative weight ratio was calculated based on the fasting weight. The following tissues were obtained from all the animals and were fixed with 10% neutral buffered formalin solution: the brain, thymus, thyroid and parathyroid, heart, lungs with bronchi, heart, liver, spleen, kidneys, adrenals, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, epididymis, ovaries, uterus, and spinal nerves. Testis was fixed with Davidson solution. These

tissues were routinely processed, embedded in paraffin, and sectioned. The sections were stained with hematoxylin-eosin (H&E) dye for microscopic examination, and bone tissue was decalcified with Calci-Clear-Rapid™ (National Diagnostics, Atlanta, U.S.A.) for microscopic examination. All organs and tissues taken from all animals were examined microscopically.

Data on the weights, food consumption, hematology, serum biochemistry, and organ weights were tested using SAS (version 9.2, SAS Institute Inc, Cary, USA). The variance was checked by using the Bartlett test. If the variance was homogeneous, the data were subjected to a one-way analysis of variance (ANOVA, $P < 0.05$). If significant changes were observed using the one-way ANOVA, the data were analyzed by using the multiple comparison procedure of the Dunnett's test ($P < 0.05$, $P < 0.01$). If not, they were analyzed using the Kruskal-Wallis test ($P < 0.05$).

3. Results

No mortality was observed in any of the groups of SD rat during the 4-week observation period. No clinical signs

Table 1 Hematological parameters (group summary)

Sex: Male

Group / Dose (mL/kg)	Mean S.D. N	RBC ($\times 10^6$ cells/μL)	HGB (g/dL)	HCT (%)	RBC Indices MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT ($\times 10^3$ cells/μL)	WBC ($\times 10^3$ cells/μL)
G1	Mean	7.74	15.8	45.0	58.2	20.4	35.1	1122	11.08
Saline:	S.D.	0.29	0.5	1.1	1.3	0.7	0.6	94	3.05
10	N	5	5	5	5	5	5	5	5
G2	Mean	7.56	15.2	44.0	58.2	20.1	34.6	1086	11.54
MGP:	S.D.	0.41	0.6	1.9	1.5	0.4	0.5	121	3.26
2.5	N	5	5	5	5	5	5	5	5
G3	Mean	7.76	15.5	44.3	57.1	19.9	34.9	1093	8.35
MGP:	S.D.	0.20	0.5	1.7	1.2	0.3	0.3	96	2.39
5	N	5	5	5	5	5	5	5	5
G4	Mean	7.62	15.5	44.5	58.5	20.3	34.7	1137	9.12
MGP:	S.D.	0.25	0.7	1.6	0.7	0.3	0.4	50	2.03
10	N	5	5	5	5	5	5	5	5

Sex: Female

Group / Dose (mL/kg)	Mean S.D. N	RBC ($\times 10^6$ cells/μL)	HGB (g/dL)	HCT (%)	RBC Indices MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT ($\times 10^3$ cells/μL)	WBC ($\times 10^3$ cells/μL)
G1	Mean	7.44	15.1	42.3	56.9	20.3	35.7	1189	4.88
Saline:	S.D.	0.46	0.7	2.0	2.1	0.7	0.6	177	1.39
10	N	5	5	5	5	5	5	5	5
G2	Mean	7.56	15.2	42.5	56.2	20.1	35.9	1133	4.27
MGP:	S.D.	0.28	0.3	1.2	1.0	0.5	0.4	98	1.85
2.5	N	5	5	5	5	5	5	5	5
G3	Mean	7.41	15.2	42.5	57.4	20.6	35.9	1141	4.18
MGP:	S.D.	0.18	0.4	1.4	1.6	0.5	0.3	73	1.82
5	N	5	5	5	5	5	5	5	5
G4	Mean	7.58	15.6	43.6	57.6	20.5	35.6	1183	4.46
MGP:	S.D.	0.21	0.3	0.9	2.1	0.7	0.3	76	0.95
10	N	5	5	5	5	5	5	5	5

S.D., standard deviation; N, number of animals; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular cell hemoglobin concentration; PLT, platelet; WBC, white blood cell.

Table 2 Blood chemistry values (group summary)

Sex: Male

Group / Dose (mL/kg)	Mean S.D. N	ALT (U/L)	AST (U/L)	ALP (U/L)	BUN (mg/dL)	Crea (mg/dL)	TP (g/dL)	Alb (g/dL)	A/G ratio	T-Chol (mg/dL)	TG (mg/dL)	Glu (mg/dL)
G1	Mean	32.9	73.7	630.2	13.2	0.40	5.7	2.4	0.71	84	49	163
Saline:	S.D.	5.0	9.2	63.0	1.1	0.05	0.1	0.1	0.03	17	18	28
10	N	5	5	5	5	5	5	5	5	5	5	5
G2	Mean	33.7	83.6	644.8	13.5	0.42	5.6	2.3	0.71	84	43	156
MGP:	S.D.	5.5	21.2	108.3	2.5	0.06	0.5	0.1	0.08	16	35	16
2.5	N	5	5	5	5	5	5	5	5	5	5	5
G3	Mean	31.1	85.7	493.2	12.6	0.42	5.6	2.4	0.72	74	35	150
MGP:	S.D.	4.0	11.0	59.0	1.5	0.07	0.3	0.1	0.04	16	13	17
5	N	5	5	5	5	5	5	5	5	5	5	5
G4	Mean	33.6	88.5	614.0	12.4	0.44	5.7	2.3	0.70	87	40	147
MGP:	S.D.	2.6	10.2	217.5	1.9	0.07	0.2	0.1	0.05	7	23	15
10	N	5	5	5	5	5	5	5	5	5	5	5

Sex: Female

Group / Dose (mL/kg)	Mean S.D. N	ALT (U/L)	AST (U/L)	ALP (U/L)	BUN (mg/dL)	Crea (mg/dL)	TP (g/dL)	Alb (g/dL)	A/G ratio	T-Chol (mg/dL)	TG (mg/dL)	Glu (mg/dL)
G1	Mean	28.0	96.2	414.5	14.6	0.42	6.3	2.7	0.78	97	22	148
Saline:	S.D.	3.3	16.3	50.9	1.4	0.05	0.3	0.1	0.02	10	12	15
10	N	5	5	5	5	5	5	5	5	5	5	5
G2	Mean	21.8	92.3	398.7	14.6	0.46	6.1	2.6	0.74	92	14	122
MGP:	S.D.	4.4	11.7	72.3	2.7	0.04	0.4	0.1	0.06	17	4	7
2.5	N	5	5	5	5	5	5	5	5	5	5	5
G3	Mean	26.7	95.0	359.2	16.0	0.46	6.1	2.7	0.78	83	12	138
MGP:	S.D.	6.1	21.2	36.3	1.8	0.05	0.4	0.1	0.02	16	3	20
5	N	5	5	5	5	5	5	5	5	5	5	5
G4	Mean	28.8	93.6	431.9	14.4	0.44	6.0	2.6	0.77	98	20	138
MGP:	S.D.	9.1	22.1	85.8	2.2	0.02	0.3	0.1	0.03	23	9	16
10	N	5	5	5	5	5	5	5	5	5	5	5

S.D., standard deviation; N, number of animals; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; Crea, creatinine; TP, total protein; Alb, albumin; A/G ratio, albumin/globulin ratio; T-Chol, total cholesterol; TG, triglycerides; Glu, glucose.

Table 3 Absolute organ weights (group summary)

Sex: Male (g)

Group / Dose (mL/kg)	Mean S.D. N	B.W.	Brain	Heart	Liver	Spleen	Kidney
G1	Mean	329.8	1.90	1.21	9.78	0.68	2.45
Saline:	S.D.	26.9	0.13	0.09	0.85	0.17	0.18
10	N	5	5	5	5	5	5

(Continued)

Group / Dose (mL/kg)	Mean S.D. N	B.W.	Brain	Heart	Liver	Spleen	Kidney
G2	Mean	342.5	1.87	1.19	9.99	0.74	2.43
MGP:	S.D.	15.3	0.04	0.12	0.57	0.10	0.12
2.5	N	5	5	5	5	5	5
G3	Mean	337.2	1.94	1.15	10.13	0.66	2.40
MGP:	S.D.	18.2	0.05	0.11	0.66	0.09	0.06
5	N	5	5	5	5	5	5
G4	Mean	339.6	1.99	1.10	10.05	0.67	2.47
MGP:	S.D.	21.9	0.06	0.09	1.13	0.04	0.17
10	N	5	5	5	5	5	5

Sex: Female (g)

Group / Dose (mL/kg)	Mean S.D. N	B.W.	Brain	Heart	Liver	Spleen	Kidney
G1	Mean	225.5	1.90	0.86	6.83	0.48	1.79
Saline:	S.D.	18.4	0.10	0.06	0.63	0.05	0.13
10	N	5	5	5	5	5	5
G2	Mean	217.6	1.87	0.83	6.41	0.45	1.74
MGP:	S.D.	19.4	0.07	0.08	0.99	0.04	0.18
2.5	N	5	5	5	5	5	5
G3	Mean	223.3	1.89	0.85	6.49	0.50	1.77
MGP:	S.D.	28.0	0.09	0.10	0.79	0.12	0.16
5	N	5	5	5	5	5	5
G4	Mean	217.9	1.94	0.84	6.49	0.45	1.72
MGP:	S.D.	16.3	0.10	0.08	0.74	0.07	0.07
10	N	5	5	5	5	5	5

MGP, mountain ginseng pharmacopuncture; S.D., standard deviation; N, number of animals; B.W., body weight.

Table 4 Relative organ weights (g/100 g of body weight), (group summary)

Sex: Male (g)

Group / Dose (mL/kg)	Mean S.D. N	B.W	Brain	Heart	Liver	Spleen	Kidney
G1	Mean	329.8	0.58	0.37	2.96	0.20	0.74
Saline:	S.D.	26.9	0.02	0.02	0.13	0.05	0.06
10	N	5	5	5	5	5	5
G2	Mean	342.5	0.55	0.35	2.92	0.21	0.71
MGP:	S.D.	15.3	0.03	0.03	0.11	0.03	0.04
2.5	N	5	5	5	5	5	5
G3	Mean	337.2	0.58	0.34	3.01	0.19	0.71
MGP:	S.D.	18.2	0.04	0.03	0.12	0.03	0.06
5	N	5	5	5	5	5	5

(Continued)

Group / Dose (mL/kg)	Mean S.D. N	B.W	Brain	Heart	Liver	Spleen	Kidney
G4	Mean	339.6	0.59	0.32	2.95	0.20	0.73
MGP:	S.D.	21.9	0.04	0.02	0.18	0.02	0.05
10	N	5	5	5	5	5	5

Sex: Female (g)

Group / Dose (mL/kg)	Mean S.D. N	B.W.	Brain	Heart	Liver	Spleen	Kidney
G1	Mean	225.5	0.85	0.38	3.03	0.21	0.80
Saline:	S.D.	18.4	0.10	0.02	0.09	0.02	0.03
10	N	5	5	5	5	5	5
G2	Mean	217.6	0.86	0.38	2.93	0.21	0.80
MGP:	S.D.	19.4	0.07	0.03	0.20	0.02	0.03
2.5	N	5	5	5	5	5	5
G3	Mean	223.3	0.86	0.38	2.91	0.22	0.80
MGP:	S.D.	28.0	0.10	0.03	0.09	0.03	0.05
5	N	5	5	5	5	5	5
G4	Mean	217.9	0.89	0.39	2.98	0.21	0.79
MGP:	S.D.	16.3	0.05	0.02	0.18	0.02	0.04
10	N	5	5	5	5	5	5

MGP, mountain ginseng pharmacopuncture; S.D., standard deviation; N, number of animals; B.W., body weight.

Table 5 Necropsy findings (group summary)

Sex	Male				Female			
Group	G1	G2	G3	G4	G1	G2	G3	G4
Dose (mL/kg)	Saline		MGP		Saline		MGP	
	10	2.5	5	10	10	2.5	5	10
No. of animals	5	5	5	5	5	5	5	5
Unremarkable findings	5	5	5	5	5	5	5	5
No. examined	5	5	5	5	5	5	5	5

MGP, mountain ginseng pharmacopuncture. External surface and all organs in the body cavity were unremarkable.

occurred in the male and the female SD rats, and no significant differences in weights or food consumptions were observed between the experimental groups and the control group during the 4 weeks. No significant differences in hematology, serum biochemistry and organ weights were observed between the experimental groups and the control group (Tables 1-4). No abnormal macroscopic features were observed during necropsy on the rats in the control and the experimental groups (Table 5).

No effects due to MGP were observed in any of the animals. All groups, including the control group, showed microgranuloma, and female rats in the experimental group showed vacuolation in periportal hepatocytes at the tissue of the liver. In addition, all groups showed basophilic tubules and mineralization in the outer tissue of the kidney, but those changes were minimal and had occurred naturally. No changes were observed in the lungs, hearts, brains, spinal nerves and spleens of the control and the experimental groups. Some organs showed changes, and an inflammatory lesion was observed at an injection site: such changes are common in SD rats of similar age and seem to have occurred naturally or sporadically (Table 6).

4. Discussion

Table 6 Summary of histopathological findings

Organ / Findings	Sex		Male				Female			
	Group		G1	G2	G3	G4	G1	G2	G3	G4
	Dose (mL/kg)		Saline		MGP		Saline		MGP	
			10	2.5	5	10	10	2.5	5	10
	No. of animals		5	5	5	5	5	5	5	5
Heart	-Murine progressive cardiomyopathy	±	1	-	-	1	0	-	-	0
		No. of examined	5	-	-	5	5	-	-	5
Injection site, Tail	-Inflammation	±	5	4	3	4	4	4	5	2
		No. of examined	5	5	5	5	5	5	5	5
Kidney	-Basophilic tubules	±	0	1	2	1	1	1	0	0
	-Mineralization, outer medulla	±	0	0	0	0	2	0	0	1
		No. of examined	5	5	5	5	5	5	5	5
Liver	-Microgranuloma	±	1	3	3	0	2	1	0	2
	-Vacuolation, hepatocyte, periportal	±	0	0	0	0	0	1	3	1
		No. of examined	5	5	5	5	5	5	5	5
Lung	-Cell infiltration, eosinophil, perivascular	±	0	0	1	0	0	0	0	0
	-Inflammation, alveolar, focal	±	0	1	0	0	0	0	0	0
		No. of examined	5	5	5	5	5	5	5	5

Grade - ±: minimal. Unremarkable changes were observed in the brains, spinal nerves and spleens of the rats in groups 1, 2, 3 and 4.

In cancer, MGP has been an effective treatment for maintaining tumor size and enhancing the quality of life of cancer patients [8]. These effects of MGP are thought not to result from the MGP directly attacking the cancer cells but rather from an increase in physical stamina and the stimulation of immune cells, which help the body to suppress the growth of the cancer cells. MGP has a superior efficacy in treating general deficiency patterns, such as cancer, compared to other treatment methods, because of the strong tonifying Qi function of mountain ginseng [1]. Intravenous injection is a quick, lossless method of administering MGP with its strong tonifying Qi function [9].

MGP, in *in-vivo* and *in-vitro* studies, has also been to have anti-oxidant [10-14], anti-hypertensive [15], anti-diabetic [16], anti-obesity [17] and anti-depressant [18] effects. MPG-related case reports have mostly been about studies involving patients with severe diseases such as cancer [19-24] and amyotrophic lateral sclerosis [25], and except for one case, no risk factors associated with the intravenous injection of MGP have been reported Jo *et al* [26]. Reported a drug-induced liver injury (DILI) that may have been caused by injection of MGP; however, in that case, verifying the direct correlation between MGP treatment and DILI was difficult, so we cannot be sure that MGP actually caused the DILI.

The present study was designed to evaluate a 4-week, repeated, intravenous toxicity test of MGP in SD rats. Our study showed no significant changes in the weights, food consumptions, hematology, serum biochemistry, organ weights, necropsy, and histopathology of the rats. Also, no mortality was observed. Although some changes were observed in several organs and at an injection site, those changes seemed to have occurred naturally or sporadically. The results of this study are consistent with those of an earlier study, which will to be published in the near future; that study found intravenous injection of MGP to be a safe treatment in SD rats.

Treating patients by orally administering the pharmacopuncture to them presents difficulties that are not present when the pharmacopuncture, especially MGP, is administered by intravenous injection. Because MPG administered through intravenous injection can provide rapid and useful effects, additional studies, such as randomize, controlled trials, to validate the safety and the usefulness

of MGP are necessary.

In summary, in this 4-week, repeated, intravenous injection, toxicity test of MGP in SD rats, no changes due to MGP were observed in high-dosage group. Consequently, we suggest that high dose should be 10 mL/kg and low dose should be 2.5 mL/kg in the upcoming 13-week, repeated test.

Conflict of interest

The authors declare that there are no conflict of interest.

References

1. Korean Pharmacopuncture Institute. [Pharmacopuncturology]. Seoul: Elsevier Korea; 2012. p. 191-3. Korean.
2. Kwon KR, Wi JS, Kim SW. [Literature study of wild ginseng]. J Pharmacopunct. 2003;6(2):67-75. Korean.
3. Kwon KR, Kang TS, Lee SK. [Analysis of serum proteom before and after intravenous injection of wild ginseng pharmacopuncture]. J Pharmacopunct. 2004;7(3):5-25. Korean.
4. Lee DH, Kwon KR. [Analysis of serum proteom before and after intravenous injection of cultivated wild ginseng herbal acupuncture]. J Pharmacopunct. 2006;9(2):17-37. Korean.
5. Kwon KR. [A clinical study on the effects of intravenous wild ginseng herbal acupuncture on the human body]. J Pharmacopunct. 2004;7(1):15-26. Korean.
6. Lee KH, Sun SH, Yu JS, Lim CS, Kwon KR. Intravenous single-dose toxicity of mountain ginseng pharmacopuncture in sprague-dawley rats. J Pharmacopunct. 2014;17(3):50-6.
7. Kwon KR. Anticancer effect of mountain ginseng pharmacopuncture to the nude mouse of lung carcinoma induced by NCI-H460 human non-small cell lung cancer cells. J Pharmacopunct. 2010;13(1):5-14.
8. Kwon KR, Kim HD, Kim JS, Yoo HS, Cho CK. Case series of non-small cell lung cancer treated with mountain ginseng pharmacopuncture. J Acupunct Meridian Stud. 2011;4(1):61-8.
9. Park SW, Kim YS, Hwang WD, Kim GC. [Effect of pulse-wave factors in middle aged women by mountain cultivated ginseng pharmacopuncture]. J Pharmacopunct. 2011;14(1):35-49. Korean.
10. Choi EJ, Lee JM, Won SH, Kwon KR. [Effects of cultivated wild ginseng pharmacopuncture on lowering lipid and oxidative capacity in biochemical and molecular biological study in obese rats]. J Pharmacopunct. 2006;9(1):5-20. Korean.
11. Ahn YM, Park HS, Kwon KR. [Anti-cancer and anti-oxidant efficacies of wild ginseng and cultivated wild ginseng of korea and china]. J Pharmacopunct. 2007;10(1):5-16. Korean.
12. Kim HS, Park HS, Kwon KR. [Protective effect of wild ginseng extract against t-BHP-induced oxidative damage in HepG2 cells using DNA chip]. J Pharmacopunct. 2007;10(1):121-35. Korean.
13. Jang HY, Park HS, Kwon KR, Rhim TJ. [A study on the comparison of antioxidant effects among wild ginseng, cultivated wild ginseng, and cultivated ginseng extracts]. J Pharmacopunct. 2008;11(3):67-78. Korean.
14. Rhim TJ, Jeong HS, Kim YJ, Kim DY, Han YJ, Kwon HY, *et al*. [A study on the comparison of antioxidant effects among cultivated ginseng, and cultivated wild ginseng extracts]. J Pharmacopunct. 2009;12(2):7-12. Korean.
15. Hong MH, Lim HK, Park JE, Jun NJ, Lee YJ, Cho MJ, *et al*. [The antihypertensive and vasodilating effects of adventitious root extracts of wild ginseng]. J Korean Soc Appl Biol Chem. 2008;51(2):102-7. Korean.
16. Park WP, Kwon KR, Lee E. [Effects of distilled cultivated wild ginseng herbal acupuncture in rats with diabetes induced high fat diet]. J Pharmacopunct. 2005;8(2):97-108. Korean.
17. Kim BW, Kwon KR. [The effect of cultivated wild ginseng extract on preadipocyte proliferation]. J Pharmacopunct. 2007;10(3):29-35. Korean.
18. Kwon SO, Choi SM, Kim MH, Lee BB, Park MW, Lee HJ, *et al*. [Antidepressant effect of the subchronic administration of the methanolic extract of wild-ginseng and cultivated-ginseng in mice tail suspension test]. The Acupuncture. 2009;26(4):99-106. Korean.
19. Kwon KR, Park CW, Ra MS, Cho CK. [2005 clinical observation of multiple metastatic cancer patient with hepatocellular carcinoma treated with cultivated wild ginseng herbal acupuncture]. The Acupuncture. 2005;22(2):211-7. Korean.
20. Park BK, Cho CK, Kwon KR, Yoo HS. [A case report for stage ⅢB squamous cell lung carcinoma patient treated with mountain ginseng pharmacopuncture]. J Pharmacopunct. 2007;10(3):143-7. Korean.
21. Bang SH, Kwon KR, Yoo HS. [Two cases of non small cell lung cancer treated with intravenous cultivated wild ginseng pharmacopuncture]. J Pharmacopunct. 2008;11(2):13-9. Korean.
22. Kwon KR, Kim HD, Kim JS, Yoo HS, Cho CK. Case series of non-small cell lung cancer treated with mountain ginseng pharmacopuncture. J Acupunct Meridian Stud. 2011;4(1):61-8.
23. Lee JH, Kwon KR, Cho CK, Han SS, Yoo HS. Advanced cancer patients treated with cultivated wild ginseng phamacopuncture. J Acupunct Meridian Stud. 2010;3(2):119-24.
24. Lee YH, Kim CW, Lee KH. [A case report of monitoring PSA level changes in two prostate cancer patients treated with mountain ginseng pharmacopuncture and sweet bee venom along with western anticancer therapy]. J Pharmacopunct. 2011;14(4):81-8. Korean.
25. Ryu YJ, Lee KH, Kwon KR, Lee YH, Sun SH, Lee SJ. [Mountain ginseng pharmacopuncture treatment on three amyotrophic lateral sclerosis patients]. J Pharmacopunct. 2010;13(4):119-28. Korean.
26. Jo HG, Jung PS, Kim HY, Bae SY, Jo MJ, Shin JH, *et*

al. [Case of suspected drug-induced liver injury after intravenous wild ginseng pharmacopuncture]. Korean J Orient Physiol Pathol. 2014;28(1):102-6. Korean.