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Original article

Single-Dose Intramuscular Toxicity of Mahwangcheonoh Pharmacopuncture in a Rat Model

- Toxicity of Mahwangcheonoh Pharmacopuncture in SD Rats -

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Key Words

Cheonoh, Mahwang, pharmacopuncture, single-dose, toxicity

Abstract

Objectives: This study was conducted to analyze the single-dose toxicity and the safety of Mahwangcheonoh pharmacopuncture extracts.

Methods: Six-week-old Sprague-Dawley rats were used for this study. Doses of Mahwangcheonoh pharmacopuncture extracts were set at 0.25 mL (low-dose), 0.5 mL (medium-dose) and 1.0 mL (high-dose) for the test groups. A dose of 1.0 mL of normal saline solution was set for the control group. During 14 days, general symptoms, mortalities, and changes in hematology, blood biochemistry and histopathology of all rats were observed.

Results: No death was observed in all test groups. Any abnormal symptom was not observed in all of the groups. No significant changes in weight between the control group and the test groups were observed. In addition, no significant differences in the hematology signs, the blood biochemistry levels and the histopathological signs related to the Mahwangcheonoh pharmacopuncture extracts injection were observed.

Conclusion: The findings of this study indicate that Mahwangcheonoh pharmacopuncture at doses of 1.0 mL or less may be consider safe and non-toxic. So, it can be used for therapy of obesity sufficiently. But further studies on this subject must be performed to confirm and verify this conclusion.

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1. Introduction

Pharmacopuncture is a new acupuncture therapy based on a combination of meridian theory and herbal pharmacology. In pharmacopuncture, by injecting the extracts of herbs at acupuncture points, doctors gain a synergism of positive effects, i.e., a physical stimulation due acupuncture and a chemical effect due to the herbal medicine [1]. Recently, in Korean medicine, pharmacopuncture has been widely used to treat patients with such diverse diseases as herniated intervertebral discs, arthropathia, skin diseases, and especially obesity. For obesity care, many reports have been published concerning related acupuncture points, such as Jusanli (ST36) and Zhongwan (CV12), and pharmacopuncture extracts, such as *Atractylodes Lancea*, *Panax ginseng*, Sweet bee venom, etc. [2, 3]. In addition, Mahwang (*Ephedra sinica*)- Cheonoh (*Aconitum carmichaeli*) extract is known to be able to inhibit the generation of fat cells, as well as the activation of collateral obese metabolic diseases [4].

As a perennial plant belonging to the family Ephedraceae, Mahwang, in Korean medicine, can perspire, drive out cold, generate diuresis, and reduce edema. Mahwang also contains many alkaloids, such as ephedrine, pseudo-ephedrine, tannin and flavonoids, which can pharmacologically activate sympathetic

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nerves to reduce appetite, inhibit cholesterol absorption, and accelerate the disintegration of body fat [5, 6]. Because of these effects, it has frequently been used as a herb prescription to treat obese patients [7] and as an ingredient in food for short-term weight loss or for elevating exercise performance [8].

As a perennial plant belonging to the Ranunculaceae family, Cheonoh can drive out cold, moisture, and stroke. For this reason, Cheonoh is expected to be able to promote the blood-circulation system and the meridian system on the surface of the body and to stimulate metabolism just as Mahwang can; thus, Cheonoh has frequently been used with Mahwang as a herb prescription for the treatment of obese patients [9].

Reports have been published indicating that Mahwangcheonoh pharmacopuncture is effective in promoting the disintegration of fat cells [4, 9]. However, to the best of the authors' knowledge, no reports on its toxicity have yet to be published. Accordingly, the authors performed such toxicity tests and obtained significant results demonstrating that Mahwangcheonoh pharmacopuncture is a safe, credible pharmacopuncture therapy for treating obese patients.

2. Materials and Methods

This study was conducted at Biotoxtech under the regulations of Good Laboratory Practice. Mahwangcheonoh pharmacopuncture extracts were prepared at the Korean Pharmacopuncture Institute (Seoul, Korea) in a clean room, were stored in a refrigerator at 2.1 - 5.3°C, and remained stable for 6 months following production (Lot No. KPI-2013-03). The normal saline used as a control material was manufactured by Choongwae Pharma Corp. (Seoul, Korea, Lot No. 12133) and stored at room temperature.

The animals used in this study were 6-week-old specific-pathogen-free (SPF) Sprague-Dawley (SD) male (184.7 - 204.0 g) and female (156.1 - 179.5 g) rats purchased from Orientbio Inc. (Gyeonggi-do, Korea). This study was approved by the Institutional Animal Care and Use Committees of Biotoxtech (No. 130364). When the animals were received, a visual inspection was done, and weights were recorded using electronic scales (CP3202S, Sartorius, Göttingen, Germany). The general symptoms and changes were checked once daily during the seven days of acclimatization, after which all animals were checked and found to be normal.

All animals were bred in stainless-steel wire breeding boxes (260 mm (W) × 350 mm (D) × 210 mm (H)) kept at a temperature from 22.1 to 24.1°C, a humidity from 47.7% to 70.8%, and an illuminance from 150 to 300 Lux, with a 12-hour light (7:00 am to 7:00 pm)/dark cycle. Each breeding group was divided by three animals during acclimatization periods and was divided by one animal during observation periods. Feed (Teklad Certified Irradiated Global 18% Protein Rodent Diet 2918C, Harlan Laboratories Inc., USA) and ultraviolet (UV) sterilizer running water were provided freely.

Groupings were done at end of acclimatization. Twenty male and twenty female rats with weights closest to the mean weight were selected. The selected animals were distributed into four groups (five rats of each sex per group). The Mahwangcheonoh pharmacopuncture extracts were injected into the animals *via* a muscular route, and the high dose was set at 1.0 mL per animal because no mortalities had occurred in a preliminary test (Biotoxtech Study No.: B13474P) in which a single dose of 1.0 mL of Mahwangcheonoh pharmacopuncture extract had been injected into the muscles of male and female rats. Based on this high dose, we set 0.5 mL per animal as the medium dose and 0.25 mL per animal as the low dose. For the control group, 1.0 mL of normal saline was injected. In the low-dose group (G2) and the medium-dose group (G3), the Mahwangcheonoh pharmacopuncture extracts were injected into the left femoral muscle. For the control group (G1) and the high-dose group (G4), 0.5 mL of normal saline and 0.5 mL of Mahwangcheonoh pharmacopuncture extract, respectively, were injected into the left and the right femoral muscles, for a total of 1.0 mL per animal. All injections were made using disposable syringes (1.0 mL, 26 G).

On the day of injection (day 0), general symptoms (types of toxic indications, times of toxic expression, recovery times) and any deaths were recorded at 30 minutes and at 1, 2, 4 and 6 hours after injection. From day 1 until day 14 after injection, general symptoms were observed once daily. Body weights were measured on the day of injection (before injection) and on days 3, 7 and 14 after injection.

All animals were denied access to food and water (fasted) for at least 18 hours prior to necropsy. On the day of necropsy (15 days after injection), the animals were anesthetized with isoflurane, and blood was collected from the abdominal aorta. One mL of the collected blood was put into a tube containing ethylene diamine tetraacetic-acid (EDTA), and the hematology parameters, the prothrombin time (PT) and the activated partial thromboplastin time (APTT) were measured using a hematology analyzer (ADVIA 2120i, SIEMENS, Germany). Two mL of the collected blood were put into a tube containing 3.2% sodium citrate; then, the plasma was collected after centrifugation at 3,000 rpm for 10 minutes. A coagulation examination was performed using coagulation time analyzers (Coapresta 2000, SEKISUI, Japan) to measure the PT and the APTT.

The blood biochemistry was determined by using serum taken from blood that had been collected from the abdominal aorta and had been centrifuged at 3,000 rpm for 10 minutes. A blood chemistry analyzer (7180, HITACHI, Japan) and an electrolyte analyzer (AVL 9181, Roche, Germany) were used for the measurements.

For the histopathology, the organs and the tissues collected from all animals after necropsy were inspected. After necropsy, tissues surrounding the injection regions were excised and fixed in 10% neutral buffered formalin solution, after which local resistance tests were performed on those tissues. Fixed organs and tissues sections were produced using a common process: cutting, dehydrating and embedding in paraffin. The tissue sections were stained using hematoxylin & eosin (H&E). The weights, as well as the hematological and the blood biochemical results, were analyzed by using SAS (version 9.2, 9.3, SAS Institute Inc., USA). A Bartlett test was conducted to evaluate equal var-

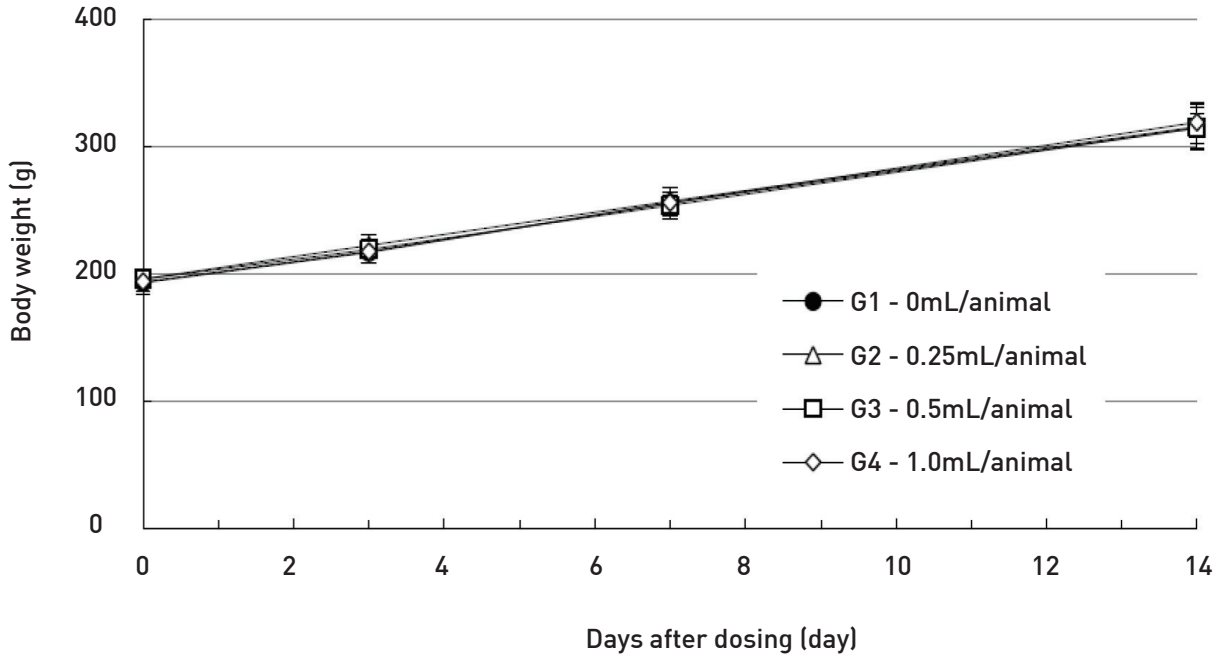

Figure 1 Body weights in Male sprague-dawley rats.

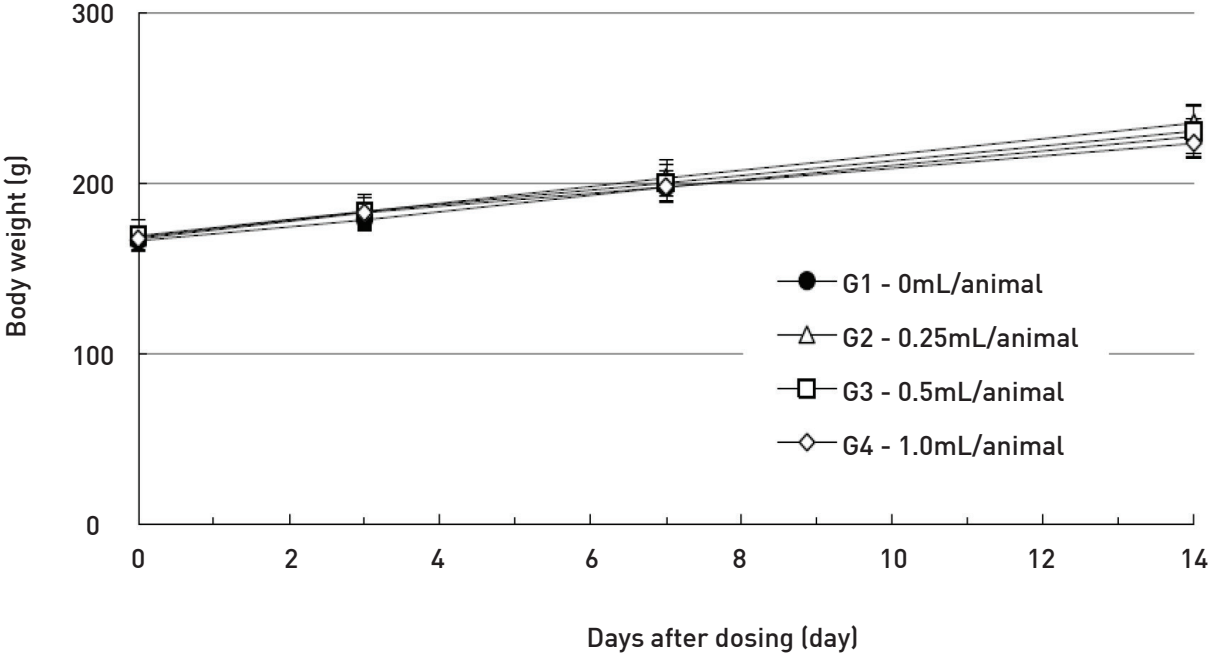

Figure 2 Body weights in Female sprague-dawley rats.

iance between the test and the control groups ($P < 0.05$). The one-way analysis of variation (ANOVA) test was conducted when equal variance was recognized on the Bartlett test ($P < 0.05$), and the Dunnett's *t*-test was conducted when equal variance was recognized on the ANOVA test (both $P < 0.05$, $P < 0.01$). Also, the Kruskal-Wallis test was conducted when equal variance was not recognized on the Bartlett test ($P < 0.05$), and the Steel-test was conducted when equal variance was recognized on the Kruskal-Wallis test (both $P < 0.05$, $P < 0.01$).

3. Results

During the observation period, no abnormal symptoms or deaths were observed in either the male or the female rats

Table 1 Summary of clinical signs and necropsy for the rats used in this study

Sex	Group/ Dose (mL/animal)	No. of animals	Clinical sign	Hours (Day 0) after dosing					Days after dosing													
				0.5	1	2	4	6	0	1	2	4	5	6	7	8	9	10	11	12	13	14
Male	G1 (0)	5	NOA*	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	G2 (0.25)	5	NOA	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	G3 (0.5)	5	NOA	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	G4 (1.0)	5	NOA	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Female	G1 (0)	5	NOA	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	G2 (0.25)	5	NOA	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	G3 (0.5)	5	NOA	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	G4 (1.0)	5	NOA	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

*NOA, no observable abnormality.

in the test and the control groups (Table 1). During that period, the weights of the male rats in the control and the test groups changed from 192.6 ± 8.7 g (G1), 195.6 ± 5.6 g (G2), 195.3 ± 4.3 g (G3), and 193.2 ± 7.4 g (G4) to 314.1 ± 11.9 g (G1), 315.0 ± 18.0 g (G2), 314.2 ± 16.3 g (G3), and 318.5 ± 16.2 g (G4), respectively. Those of the female rats changed from 166.3 ± 6.0 g (G1), 168.3 ± 6.9 g (G2), 169.2 ± 9.1 g (G3), and 167.4 ± 7.4 g (G4) to 227.5 ± 10.1 g (G1), 235.4 ± 9.9 g (G2), 230.5 ± 15.3 g (G3), and 223.4 ± 9.2 g (G4), respectively. However, no statistically significant changes in weights associated with injection were recognized (Figs. 1, 2).

No significant differences were observed in the hematology between the test groups and the control group for either the male or the female rats (Tables 2, 3).

Furthermore, no changes in blood chemistry due to the injections were observed. The change observed in the low-dose female group (G2) was a minor variation because it was observed sporadically and exhibited no a dose dependency. That change was determined to be an accidental change with no statistical significance (Tables 4, 5).

No gross abnormalities in histopathology were observed in any of the rats. The results on the local resistance tests were not affected by the injections. However, a weak inflammation was observed in the region of injection in a rat in the control group. Because the inflammation occurred in the control group, it was determined to have no toxicological significance, but rather to be a spontaneous or accidental change without injection relevance.

4. Discussion

According to National Health Statistics, the obesity rate in Korean adults, based on the body mass index (BMI), has reached 31.5%, being 37.7% in males and 25.3% in females. Also, the teenage obesity rate in Korea has reached 11.5%. Because of this, the people's interest in obesity has also increased. According to a survey, 85.4% of the people were aware of being overweight, and 60% indicated that they were actively trying to lose weight [10]. Moreover, during 2008, the social and economic cost associated with the treatment of obese patients in the country was estimated to be approximately 1.6 billion US dollars. Because of this, many medical workers cannot but be interested in research on treatments for obesity [11].

In Korean medicine, much research has been done on the treatment of obese patients by using acupuncture, electro-acupuncture, moxibustion, herbs, subcutaneous acupuncture, and exercise therapy [11]. Among these possible treatments, pharmacopuncture injection of one-tenth the volume of a medicine dose directly into fatty areas or acupuncture points is expected to be very effective. Moreover, many pharmacopuncture solutions for obesity treatment have already undergone many safety and reliability tests

Table 2 Mean hematology parameters for the male rats used in this study

Group/Dose (mL/animal)		G1 (0) (Mean ± S.D.)	G2 (0.25) (Mean ± S.D.)	G3 (0.5) (Mean ± S.D.)	G4 (1.0) (Mean ± S.D.)
RBC (× 10^6 cells/μL)		7.57 ± 0.33	7.24 ± 0.38	7.46 ± 0.34	7.49 ± 0.37
HGB (g/dL)		14.9 ± 0.5	14.9 ± 0.4	15.1 ± 0.8	14.9 ± 0.7
HCT (%)		42.6 ± 1.4	42.8 ± 1.4	43.2 ± 2.2	43.1 ± 1.7
RBC Indices	MCV (fL)	56.3 ± 2.4	59.1 ± 1.4	57.9 ± 1.0	57.6 ± 1.4
	MCH (pg)	19.7 ± 0.8	20.6 ± 0.5	20.2 ± 0.6	20.0 ± 0.6
	MCHC (g/dL)	35.0 ± 0.6	34.8 ± 0.1	34.8 ± 0.4	34.7 ± 0.3
PLT (× 10^3 cells/μL)		1125 ± 102	1112 ± 144	1074 ± 174	1086 ± 83
Reti (%)		4.54 ± 1.32	4.58 ± 1.09	4.02 ± 1.05	3.99 ± 0.61
WBC (× 10^3 cells/μL)		11.56 ± 3.16	9.49 ± 1.96	10.06 ± 2.54	10.71 ± 1.84
WBC Differential Counting (%)	NEU	12.5 ± 3.3	17.2 ± 5.3	15.2 ± 2.4	13.3 ± 2.3
	LYM	84.7 ± 4.0	80.2 ± 5.3	81.1 ± 2.7	83.7 ± 2.3
	MONO	1.5 ± 0.5	1.6 ± 0.4	2.3 ± 0.5	1.8 ± 0.5
	EOS	0.4 ± 0.2	0.4 ± 0.2	0.6 ± 0.1	0.6 ± 0.2
	BASO	0.2 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
PT (sec)		16.7 ± 0.5	17.2 ± 0.9	17.1 ± 0.5	16.8 ± 0.8
APTT (sec)		13.7 ± 2.2	14.4 ± 2.7	13.8 ± 1.0	14.2 ± 1.3

S.D., standard deviation; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular cell volume; MCHC, mean corpuscular cell hemoglobin concentration; PLT, platelet; Reti, reticulocytes; WBC, white blood cell; NEU, neutrophils; LYM, lymphocytes; MONO, monocytes; EOS, Eosinophils; BASO, basophils; PT, prothrombin time; APTT, active partial thromboplastin time.

Table 3 Hematology parameters for the female rats used in this study

Group/Dose (mL/animal)		G1 (0) (Mean ± S.D.)	G2 (0.25) (Mean ± S.D.)	G3 (0.5) (Mean ± S.D.)	G4 (1.0) (Mean ± S.D.)
RBC (× 10^6 cells/μL)		7.51 ± 0.22	7.53 ± 0.19	7.35 ± 0.27	7.34 ± 0.44
HGB (g/dL)		15.2 ± 0.1	15.3 ± 0.3	15.1 ± 0.5	15.2 ± 0.7
HCT (%)		41.1 ± 0.7	42.0 ± 0.6	41.6 ± 1.5	41.2 ± 2.4
RBC Indices	MCV (fL)	55.1 ± 1.4	55.8 ± 0.8	56.6 ± 2.5	56.2 ± 0.8
	MCH (pg)	20.3 ± 0.5	20.3 ± 0.6	20.6 ± 1.0	20.7 ± 0.3
	MCHC (g/dL)	36.9 ± 0.4	36.4 ± 0.7	36.3 ± 0.7	36.8 ± 0.7
PLT (× 10^3 cells/μL)		1043 ± 46	1093 ± 70	1160 ± 142	1025 ± 90
Reti (%)		2.22 ± 0.31	2.22 ± 0.31	2.38 ± 0.72	2.21 ± 0.22
WBC (× 10^3 cells/μL)		6.15 ± 1.30	5.63 ± 1.65	4.97 ± 1.16	4.88 ± 1.27
WBC Differential Counting (%)	NEU	18.4 ± 10.7	15.8 ± 6.3	15.0 ± 5.9	12.6 ± 4.6
	LYM	77.9 ± 10.7	81.1 ± 6.3	81.3 ± 6.7	84.1 ± 4.5
	MONO	1.6 ± 0.5	1.3 ± 0.4	1.6 ± 0.7	1.4 ± 0.4
	EOS	1.1 ± 0.5	1.1 ± 0.3	1.1 ± 0.2	1.0 ± 0.3
	BASO	0.2 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
PT (sec)		18.6 ± 0.7	18.7 ± 0.8	18.1 ± 1.0	18.0 ± 0.3
APTT (sec)		14.6 ± 1.0	14.7 ± 1.1	14.0 ± 0.9	15.2 ± 1.6

S.D., standard deviation; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular cell volume; MCHC, mean corpuscular cell hemoglobin concentration; PLT, platelet; Reti, reticulocytes; WBC, white blood cell; NEU, neutrophils; LYM, lymphocytes; MONO, monocytes; EOS, Eosinophils; BASO, basophils; PT, prothrombin time; APTT, active partial thromboplastin time.

Table 4 Mean clinical chemistry for the male rats used in this study

Group/Dose (mL/animal)	G1 (0) (Mean ± S.D.)	G2 (0.25) (Mean ± S.D.)	G3 (0.5) (Mean ± S.D.)	G4 (1.0) (Mean ± S.D.)
ALT (U/L)	28.0 ± 4.1	33.4 ± 4.4	31.6 ± 2.6	31.9 ± 2.2
AST (U/L)	80.4 ± 6.6	83.2 ± 10.3	83.1 ± 11.9	82.7 ± 13.7
ALP (U/L)	877.1 ± 189.8	772.9 ± 196.1	725.9 ± 169.0	669.9 ± 79.7
GGT (U/L)	0.41 ± 0.14	0.39 ± 0.19	0.38 ± 0.07	0.44 ± 0.14
Glu (mg/dL)	124 ± 14	111 ± 12	113 ± 13	124 ± 16
BUN (mg/dL)	12.0 ± 1.1	13.1 ± 1.8	13.1 ± 0.9	13.0 ± 2.1
Crea (mg/dL)	0.42 ± 0.03	0.40 ± 0.02	0.43 ± 0.01	0.40 ± 0.02
T-Bili (mg/dL)	0.03 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
T-Chol (mg/dL)	68 ± 21	66 ± 21	69 ± 15	76 ± 12
TG (mg/dL)	32 ± 14	37 ± 8	35 ± 4	42 ± 10
TP (g/dL)	5.4 ± 0.1	5.5 ± 0.1	5.5 ± 0.2	5.5 ± 0.2
Alb (g/dL)	2.3 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	2.3 ± 0.1
A/G ratio	0.74 ± 0.04	0.77 ± 0.03	0.75 ± 0.05	0.74 ± 0.04
P (mg/dL)	8.46 ± 0.38	8.33 ± 0.39	8.17 ± 0.47	8.32 ± 0.67
Ca (mg/dL)	10.2 ± 0.3	10.1 ± 0.3	10.3 ± 0.1	10.2 ± 0.1
Na (mmol/L)	139 ± 1	140 ± 1	140 ± 1	140 ± 1
K (mmol/L)	4.8 ± 0.2	4.6 ± 0.3	4.5 ± 0.1	4.4 ± 0.1
Cl (mmol/L)	102 ± 1	103 ± 1	103 ± 1	103 ± 1

S.D., standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyltranspeptidase; Glu, glucose; BUN, blood urea nitrogen; Crea, creatinine; T-Bili, 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, chlorine.

Table 5 Mean clinical chemistry for the female rats used in this study

Group/Dose (mL/animal)	G1 (0) (Mean ± S.D.)	G2 (0.25) (Mean ± S.D.)	G3 (0.5) (Mean ± S.D.)	G4 (1.0) (Mean ± S.D.)
ALT (U/L)	32.0 ± 11.2	24.0 ± 2.0	28.2 ± 2.8	24.3 ± 3.1
AST (U/L)	87.7 ± 21.2	80.6 ± 10.0	76.0 ± 17.5	66.7 ± 7.7
ALP (U/L)	363.6 ± 115.8	487.1 ± 88.3	473.0 ± 72.9	413.8 ± 131.1
GGT (U/L)	0.42 ± 0.35	0.45 ± 0.23	0.49 ± 0.15	0.43 ± 0.17
Glu (mg/dL)	109 ± 10	111 ± 6	114 ± 4	118 ± 8
BUN (mg/dL)	14.1 ± 1.6	14.5 ± 1.8	14.4 ± 1.8	15.4 ± 1.2
Crea (mg/dL)	0.44 ± 0.04	0.46 ± 0.01	0.45 ± 0.02	0.46 ± 0.03
T-Bili (mg/dL)	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
T-Chol (mg/dL)	78 ± 10	72 ± 16	92 ± 24	100 ± 22
TG (mg/dL)	16 ± 3	16 ± 6	20 ± 7	21 ± 12
TP (g/dL)	5.8 ± 0.3	5.5 ± 0.2	5.8 ± 0.3	5.9 ± 0.5
Alb (g/dL)	2.7 ± 0.1	2.4 ± 0.0*	2.6 ± 0.1	2.7 ± 0.3
A/G ratio	0.88 ± 0.06	0.80 ± 0.04	0.81 ± 0.06	0.83 ± 0.07
P (mg/dL)	7.20 ± 0.39	7.35 ± 0.46	7.63 ± 0.14	7.42 ± 0.27
Ca (mg/dL)	10.2 ± 0.3	10.0 ± 0.1	10.6 ± 0.3	10.4 ± 0.2
Na (mmol/L)	140 ± 1	140 ± 1	139 ± 1	140 ± 1
K (mmol/L)	4.5 ± 0.3	4.5 ± 0.3	4.4 ± 0.2	4.6 ± 0.3
Cl (mmol/L)	104 ± 1	104 ± 2	103 ± 1	104 ± 1

(Continued)

S.D., standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyltranspeptidase; Glu, glucose; BUN, blood urea nitrogen; Crea, creatinine; T-Bili, 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, chlorine.

$^{*}P < 0.05$, which implies the existence of a statistically significant difference from the control group based on the Steel test

in both experimental and clinical studies [2]. Pharmacopuncture extracts known to be effective in the treatment of obese patients are *Ephedra*, *Atractylodes lancea*, *Coix lacryma-jobi* [12], *Astragalus propinquus* [13], *Angelica* [2], Ginseng [14, 15], and the like. All of these have been found through laboratory research to be able to reduce effectively blood lipids or fat cells, and body weight, to have no toxicity, and to result in no abnormalities in the blood levels.

Mahwangcheonoh pharmacopuncture has also been proven to be effective in the treatment of obese patients [4, 9], but its toxicity and safety have not been adequately addressed. Thus, this research was conducted to establish the toxicity, safety, and stability of this pharmacopuncture so that it could be added as another reliable pharmacopuncture for the treatment of obese patients. This study observed the general symptoms, weight changes, and hematological and biochemical blood changes caused by single-dose intramuscular injection of Mahwangcheonoh pharmacopuncture in SD 6-week-old male and female rats. The rats were also subjected to necropsy to identify any changes in tissues or organs due to the injections.

No deaths or abnormalities occurred any of the rats, and no significant differences in body weights were observed during the 14-day observational period following injection of the pharmacopuncture. Furthermore, no hematological or blood biochemistry changes due to the pharmacopuncture were observed. Finally, no abnormal changes due to the pharmacopuncture were observed on the histopathological examinations after the necropsy.

Based on the above results, the lethal single dose of Mahwangcheonoh pharmacopuncture for rats is over 1.0 mL/animal. In addition, the Mahwangcheonoh pharmacopuncture at that dose is stable and safe to use and has no toxic effects on viscera, muscles or functions. Although this study has shown that Mahwangcheonoh pharmacopuncture is safe to use at doses of 1.0 mL or less, no research on its side effects has yet been officially reported. In addition, because this research involved investigating the effect of a single-dose injection over a 14-day observation period, research on its long-term effects and on the effects of multi-dose injections is needed if Mahwangcheonoh pharmacopuncture is to be firmly established as an effective treatment for obese patients.

5. Conclusion

The objective of this study was to analyze the safety and the toxicity of Mahwangcheonoh pharmacopuncture. The results after a single intramuscular dose and observations of the general symptoms over a 14-day period showed no abnormal symptoms or mortalities in either the test groups or the control group. In addition, no significant changes in weight, hematology, blood biochemistry, and histopathology between the test groups and the control group were found. Based on these results, we consider the lethal dose of Mahwangcheonoh pharmacopuncture to be far beyond 1.0 mL/animal; thus, Mahwangcheonoh pharmacopuncture may be considered a safe therapeutic agent for the treatment of patients with obesity.

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Conflicts of interests

The authors declare that there are no conflicts of interest.

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