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Original article

Single Intramuscular-dose Toxicity of *Samgihwalryeok*-Pharmacopuncture in *Sprague-Dawley* Rats

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Key Words
acupuncture, pharmacopuncture, *Samgihwalryeok* pharmacopuncture, single toxicity test

Abstract

Objectives: This study was performed to examine the single-dose toxicity of *Samgihwalryeok* pharmacopuncture.

Methods: Forty six-week-old *Sprague-Dawley* (SD) rats were divided into four groups of 10 rats each; each group was then sub-divided into two smaller groups, one of five males and the other of five females. Group 1 (G1, control) received 1.0 mL of normal saline solution, while group 2 (G2, low-dose group), group 3 (G3, middoes group, and group 4 (G4, high-dose group) received 0.1, 0.5, and 1.0 mL of *Samgihwalryeok* pharmacopuncture, respectively.

Results: No mortalities or clinical signs were observed in the four groups. Also, no significant changes in body weights were observed among the group, and no significant differences in hematology/biochemistry, necropsy, or histopathology results were noted.

Conclusion: The above findings suggest that treatment with *Samgihwalryeok* pharmacopuncture is relatively safe. Further studies on this subject are needed.

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1. Introduction

Pharmacopuncture therapy is a new acupuncture therapy to treat diseases based on herbal medicine, acupuncture & moxibustion medicine, and meridian theories [1]. Pharmacopuncture fluid is extracted from herbs and injected into acupoints or sore spots [2].Through a single procedure, it can achieve both the effects of acupuncture and herbal medicine [3].

The *Samgihwalryeok* pharmacopuncture consists of *Panax ginseng, Cervus elaphus sibericus, Angelica gigas Nakai, Liriope platyphylla,* and *Schisandra chinensis Baillon* and can be used to treat lethargy and chronic fatigue from qi deficiency, blood deficiency or both qi and blood deficiency [4]. This study was performed to examine the single-dose toxicity of *Samgihwalryeok* pharmacopuncture. The testing methods were visual observation of general symptoms, body weight changes, hematological tests, biochemical analyses, necropsy and histopathological observations with 6-week-old Sprague-Dawley (SD) rats. All experiments were conducted at Biotoxtech (Chungwon, Korea), an authorized institution for non-clinical studies, under the regulations of Good Laboratory Practice (GLP) of

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Korea Food & Drug Administration (KFDA) Notification No. 2012-86 (Test guidelines for non-clinical studies, Aug 24, 2012) [5].

2. Materials and Methods

Samgihwalryeok pharmacopuncture solution was prepared in a sterile room at the Korean pharmacopuncture institute (KGMP). After the mixing process with pure water, the pH was controlled to between 7.0 and 7.5. NaCl was added to the pharmacopuncture solution to make a 0.9% isotonic solution. The completed extract was stored in a refrigerator (2.1 − 6.6°C).

The animals used in this study were 6-week-old SD rats. The mean weights were 185.4 − 209.0 g and 153.0 − 174.5 g for the male and the female rats, respectively, at the time of injection of the pharmacopuncture. Visual inspections were conducted for all animals; all animals were weighed using a CP3202S system (Sartorius, Germany). During 7 days of acclimatization, the general symptoms of the rats were observed at the end of the day. The weights of the rats were recorded on the last day of acclimatization. No abnormalities were observed. The temperature of the lab 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 UV-filtered water were provided.

Group separations were done after 7 days of acclimatization. The animals were randomly distributed into 4 groups of 5 male and 5 female rats per group (Table 1): the control, low-dose, mid-dose and high-dose groups.

In a pilot test (Biotoxtech Study No.: B12876P), 1.0 mL/animal, referring to a 1.0 mL dose for each clinical application, was administered by intramuscular (i.m.) injection (left thigh) to one male and one female rat and resulted in no deaths. From this result, the doses for *Samgihwalryeok* pharmacopuncture were set up as follows: animals in group 1 (G1, the control group) were injected with 0 mL/animal of pharmacopuncture and 1.0 mL/animal of normal saline solution (ChoongwaePharma Corp., Korea), animals in group 2 (G2, the low-dose group) were injected with 0.1 mL/animal of pharmacopuncture, animals in group 3 (G3, the mid-dose group) were injected with 0.5 mL/animal of pharmacopuncture, and animals in group 4 (G4, the high-dose group) were injected with 1.0 mL/animal of pharmacopuncture. Using a disposable syringe (1 mL, 26 G), we administered the *Samgihwalryeok* pharmacopuncture solution by i.m. injection in the animals of the low-dose and the mid-dose groups once on the left thigh; for the high-dose and the control groups, we administered one 0.5-mL injection of pharmacopuncture and of normal saline, respectively, in each thigh. All experiments were conducted at Biotoxtech (Chungwon, Korea), an authorized institution for non-clinical studies, under the regulations of GLP of KFDA Notification No. 2012-86 (Test guidelines for non-clinical studies, Aug 24, 2012) [5].

The general symptoms (side effects, revealing times recovery time etc.), as well as the mortalities, were examined for 10 seconds at 30 minutes and at 1, 2, 3, and 4 hours after injection on the day of dosing (day 0).

From the 1st day to the 14th day of treatment, the general symptoms were examined once a day. The body weights were measured immediately before treatment and at 3, 7 and 14 days after injections.

All animals were fasted for more than 18 hours before autopsy. The rats were anesthetized by using isoflurane, and blood samples were collected from the abdominal aorta on the day of autopsy (15 days after injection). An automatic hematology analyzer (ADVIA120, SIEMEMS, Germany) was used to analyze blood for the hematological examinations. For the blood coagulation test (3,000 rpm, 10 minutes), 2-mL samples of blood were placed in a tube with 3.23% sodium citrate to collect blood plasma. The RBC, HGB, HCT, MCV, MCH, MCHC, PLT, etc. were measured for the hematological examinations and the prothrombin time (PT) and the activated partial thromboplastin time (APTT) were determined for the coagulation tests. The results were obtained using an Automated Coagulation Analyzer (Coapresta 2000, SEKISUI, Japan).

Table 1 Groups of animals

Group		*Samgihwalryeok* pharmacopuncture (mL/animal)	Number of animals (serial numbers)	
			Male	Female
G1	control group	0	5 (1101 − 1105)	5 (2101 − 2105)
G2	low-dose group	0.1	5 (1201 − 1205)	5 (2201 − 2205)
G3	mid-dose group	0.5	5 (1301 − 1305)	5 (2301 − 2305)
G4	high-dose group	1.0	5 (1401 − 1405)	5 (2401 − 2405)

For the biochemical tests, the blood remaining after carrying out the hematological tests was centrifuged at 3,000 rpm for 10 minutes, and the serum was collected. Measurements were done using a biochemistry analyzer (7180, HITACHI, Japan) and an electrolyte analyzer (AVL9181, Roche, Germany).

After the observations, organs and tissues from the entire bodies of all animals were visually inspected and examined under an optical microscope. Tissue samples of all the animals were fixed in 10% neutral buffered formalin. Routine histological methods, such as trimming, dehydration, and paraffin embedding, were conducted on the fixed organs and tissues. Fixed samples were then sliced using a microtome and stained with hematoxylin & eosin (H&E).

All the results from the experiments were analyzed by using SAS (version 9.2, 9.3, statistical analysis system [SAS] Institute Inc., USA). The Bartlett test was conducted to evaluate the homogeneity of the variance and the significance. If the sample variances were equal, the significant result was obtained using a one-way analysis of variance (ANOVA) test. If the sample variances were not equal, a Kruskal-Wallis test was conducted post-hoc. Statistical significance was associated with $P \leq 0.05$.

3. Results

3.1. General symptoms

During the observation, no mortality or clinical signs were observed in the all of experimental (0.1, 0.5, and 1.0 mL/animal) or control groups (Tables 2 and 3).

3.2. Body weight changes

There was no significant change in body weight shown by comparison of the experimental (0.1, 0.5, and 1.0 mL/animal) and the control group (Fig. 1).

3.3. Hematological test findings

There was no significant change in the hematological test results observed from comparison of the experimental (0.1, 0.5, and 1.0 mL/animal) and the control group (Table 4).

3.4. Biochemical test findings

There was no significant change in the biochemical test results from experimental and control group (Table 5).

3.5. Necropsy findings

No abnormalities were observed when the visual inspection was conducted on all of the animals in experimental and control group (Table 6).

3.6. Histopathological findings

No abnormalities were observed in the local tolerance test on the injection sites (Table 7).

Table 2 Summary of Mortalities

Sex	Group/dose (mL/animal)	No. of animals	Days after dosing															Mortalities
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	0/5
Male	G1 0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	G2 0.1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	G3 0.5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	G4 1.0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
Female	G1 0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	G2 0.1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	G3 0.5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	G4 1.0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5

Table 3 Summary of clinical signs

Sex	Group/dose (mL/animal)	No. of animals	Clinical sign	Hours (Day 0) after dosing				
				0.5	1	2	4	6
Male	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
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

Sex	Group/dose (mL/animal)	No. of animals	Clinical sign	Days after dosing														
				0	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	0
	G2 0.1	5	NOA	0	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	0
	G4 1.0	5	NOA	0	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	0
	G2 0.1	5	NOA	0	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	0
	G4 1.0	5	NOA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

NOA: no observable abnormality

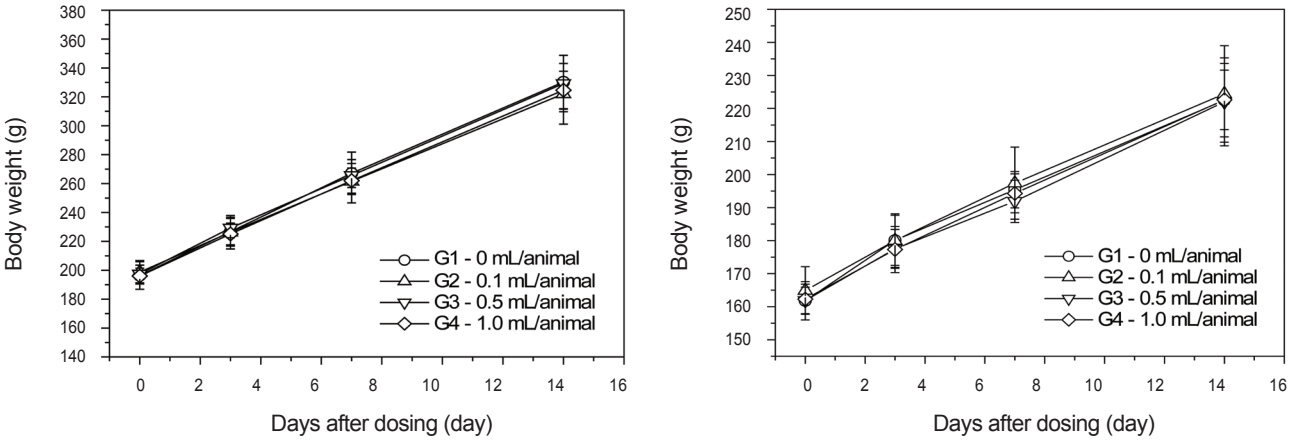

Figure 1 Changes in the mean values of the body weights in male and female SD rats.

Table 4 Mean hematology parameters

Sex: Male

Group/dose (mL/animal)		RBC (× 10^6 cells/μL)	HGB (g/dL)	HCT (%)	RBC Indices			PLT (× 10^6 cells/μL)	Reti (%)
					MCV (fL)	MCH (pg)	MCHC (g/dL)		
G1 0	Mean	6.79	13.8	43.3	63.8	20.3	31.8	1260	4.7
	S.D.	0.26	0.3	1.4	2.7	0.7	0.4	120	0.7
	N	5	5	5	5	5	5	5	5
G2 0.1	Mean	6.90	14.0	44.0	63.8	20.4	31.9	1488	4.7
	S.D.	0.21	0.4	1.3	1.2	0.1	0.5	568	0.5
	N	5	5	5	5	5	5	5	5
G3 0.5	Mean	6.98	14.1	44.0	63.2	20.3	32.1	1221	4.6
	S.D.	0.42	0.6	2.1	0.9	0.4	0.2	120	0.6
	N	5	5	5	5	5	5	5	5
G4 1.0	Mean	7.14	14.4	45.2	63.5	20.3	31.9	1204	4.5
	S.D.	0.33	0.4	1.2	3.5	1.1	0.1	113	0.8
	N	5	5	5	5	5	5	5	5

Group/dose (mL/animal)		WBC (× 10^6 cells/μL)	RBC Indices					PT (sec)	APTT (sec)
			NEU	LYM	MONO	EOS	BASO		
G1 0	Mean	12.38	9.7	85.5	2.8	0.4	0.4	17.4	14.4
	S.D.	2.63	2.6	2.9	1.0	0.1	0.3	0.3	1.6
	N	5	5	5	5	5	5	5	5
G2 0.1	Mean	11.22	11.7	82.8	3.1	0.5	0.3	17.9	14.4
	S.D.	2.60	2.7	2.5	0.4	0.1	0.1	0.4	2.1
	N	5	5	5	5	5	5	5	5

(Continued)

Group/dose (mL/animal)		WBC (× 10^6 cells/μL)	RBC Indices					PT (sec)	APTT (sec)
			NEU	LYM	MONO	EOS	BASO		
G3 0.5	Mean	9.35	10.1	85.3	2.4	0.4	0.3	17.6	15.2
	S.D.	1.24	3.2	3.5	0.6	0.2	0.1	0.4	0.5
	N	5	5	5	5	5	5	5	5
G4 1.0	Mean	9.20	14.3*	81.0	2.6	0.6	0.2	17.3	15.0
	S.D.	2.01	1.6	1.9	0.4	0.2	0.1	0.4	1.0
	N	5	5	5	5	5	5	5	5

Significantly different from control by Dunnett's *t*-test: $^*P < 0.05$
S.D., standard deviation; N, number of male SD rats

Sex: Female

Group/dose (mL/animal)		RBC (× 10^6 cells/μL)	HGB (g/dL)	HCT (%)	RBC Indices			PLT (× 10^6 cells/μL)	Reti (%)
					MCV (fL)	MCH (pg)	MCHC (g/dL)		
G1 0	Mean	7.14	14.4	43.9	61.5	20.1	32.8	1224	2.8
	S.D.	0.13	0.5	1.3	2.1	0.8	0.3	24	0.7
	N	5	5	5	5	5	5	5	5
G2 0.1	Mean	7.06	14.0	42.6	60.3	19.8	32.9	1321	2.7
	S.D.	0.18	0.4	1.4	1.8	0.6	0.2	221	0.5
	N	5	5	5	5	5	5	5	5
G3 0.5	Mean	7.18	14.2	43.4	60.4	19.8	32.8	1355	3.0
	S.D.	0.15	0.4	1.2	1.3	0.4	0.1	205	0.3
	N	5	5	5	5	5	5	5	5
G4 1.0	Mean	7.09	14.4	43.8	61.8	20.3	32.8	1136	2.6
	S.D.	0.43	0.7	2.0	1.2	0.5	0.3	110	0.6
	N	5	5	5	5	5	5	5	5

Group/dose (mL/animal)		WBC (× 10^6 cells/μL)	RBC Indices					PT (sec)	APTT (sec)
			NEU	LYM	MONO	EOS	BASO		
G1 0	Mean	7.05	7.7	88.7	1.7	0.7	0.2	18.1	14.8
	S.D.	3.75	3.2	4.2	0.6	0.2	0.1	0.4	1.3
	N	5	5	5	5	5	5	5	5
G2 0.1	Mean	7.74	8.4	87.6	1.7	0.8	0.2	18.4	14.7
	S.D.	1.98	2.8	3.0	0.3	0.3	0.1	0.7	0.2
	N	5	5	5	5	5	5	5	5

(Continued)

Group/dose (mL/animal)		WBC (× 10^6 cells/μL)	RBC Indices					PT (sec)	APTT (sec)
			NEU	LYM	MONO	EOS	BASO		
G3 0.5	Mean	6.69	12.9	82.8	2.3	0.7	0.2	17.8	14.4
	S.D.	2.03	3.1	2.7	1.2	0.3	0.1	0.8	0.6
	N	5	5	5	5	5	5	5	5
G4 1.0	Mean	5.69	7.6	88.3	1.7	1.2*	0.1	18.3	13.2
	S.D.	0.71	4.2	4.4	0.1	0.3	0.1	0.5	1.2
	N	5	5	5	5	5	5	5	5

Significantly different from control by Dunnett's *t*-test: *$P < 0.05$

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

Table 5 Mean clinical chemistry

Sex: 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-Bili (mg/dL)	T-Chol (mg/dL)
G1 0	Mean	28.4	83.6	693.6	0.47	134	10.6	0.38	0.02	87
	S.D.	7.0	23.6	267.7	0.09	9	1.3	0.03	0.01	11
	N	5	5	5	5	5	5	5	5	5
G2 0.1	Mean	29.8	75.0	799.0	0.75	126	11.4	0.37	0.03	82
	S.D.	3.1	13.2	137.9	0.47	17	1.2	0.03	0.02	14
	N	5	5	5	5	5	5	5	5	5
G3 0.5	Mean	27.1	82.4	659.4	0.52	144	10.7	0.38	0.02	76
	S.D.	2.7	16.7	157.9	0.22	16	0.9	0.04	0.01	11
	N	5	5	5	5	5	5	5	5	5
G4 1.0	Mean	25.8	91.5	770.6	0.45	140	11.0	0.39	0.02	75
	S.D.	1.9	14.0	175.6	0.22	7	1.6	0.03	0.02	11
	N	5	5	5	5	5	5	5	5	5

Group/dose (mL/animal)		TG (mg/dL)	TP (g/dL)	Alb (g/dL)	A/G ratio	P (mg/dL)	Ca (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
G1 0	Mean	43	5.2	2.3	0.77	8.89	10.0	137	4.8	102
	S.D.	10	0.2	0.1	0.05	0.48	0.3	2	0.4	1
	N	5	5	5	5	5	5	5	5	5

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Group/dose (mL/animal)		TG (mg/dL)	TP (g/dL)	Alb (g/dL)	A/G ratio	P (mg/dL)	Ca (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
G3 0.5	Mean	35	5.3	2.2	0.74	9.04	9.9	138	5.1	102
	S.D.	5	0.3	0.1	0.03	0.48	0.4	1	0.6	2
	N	5	5	5	5	5	5	5	5	5
G4 1.0	Mean	43	5.2	2.2	0.75	8.84	10.0	138	4.8	102
	S.D.	12	0.1	0.1	0.03	0.52	0.2	1	0.3	1
	N	5	5	5	5	5	5	5	5	5
G2 0.1	Mean	45	5.2	2.2	0.76	8.87	9.9	138	4.8	102
	S.D.	13	0.2	0.0	0.04	0.45	0.2	1	0.4	1
	N	5	5	5	5	5	5	5	5	5

S.D., standard deviation; N, number of male SD rats

Sex: 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-Bili (mg/dL)	T-Chol (mg/dL)
G1 0	Mean	21.7	79.0	511.2	0.64	130	12.5	0.41	0.03	89
	S.D.	4.0	15.2	68.2	0.32	20	1.5	0.02	0.01	10
	N	5	5	5	5	5	5	5	5	5
G2 0.1	Mean	22.6	74.6	517.2	0.63	134	13.9	0.41	0.03	96
	S.D.	1.9	17.9	99.1	0.33	22	1.6	0.03	0.02	21
	N	5	5	5	5	5	5	5	5	5
G3 0.5	Mean	21.3	71.2	422.3	0.48	136	11.0	0.39	0.02	88
	S.D.	3.1	5.5	112.1	0.14	18	0.8	0.03	0.01	9
	N	5	5	5	5	5	5	5	5	5
G4 1.0	Mean	19.9	76.3	519.6	0.55	129	11.4	0.43	0.02	78
	S.D.	3.3	9.3	53.1	0.14	28	1.0	0.02	0.02	7
	N	5	5	5	5	5	5	5	5	5

Group/dose (mL/animal)		TG (mg/dL)	TP (g/dL)	Alb (g/dL)	A/G ratio	P (mg/dL)	Ca (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
G1 0	Mean	15	5.3	2.4	0.85	7.29	9.9	139	4.7	105
	S.D.	2	0.2	0.1	0.05	0.69	0.3	1	0.2	1
	N	5	5	5	5	5	5	5	5	5

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Group/dose (mL/animal)		TG (mg/dL)	TP (g/dL)	Alb (g/dL)	A/G ratio	P (mg/dL)	Ca (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
G2 0.1	Mean	21	5.4	2.5	0.84	7.50	9.9	138	4.9	103
	S.D.	10	0.1	0.1	0.05	0.41	0.4	0	0.2	1
	N	5	5	5	5	5	5	5	5	5
G3 0.5	Mean	20	5.4	2.6	0.89	7.50	10.0	139	4.8	104
	S.D.	6	0.2	0.1	0.05	0.82	0.2	1	0.3	2
	N	5	5	5	5	5	5	5	5	5
G4 1.0	Mean	17	5.3	2.4	0.85	7.43	9.7	139	4.7	104
	S.D.	6	0.2	0.1	0.05	0.58	0.2	1	0.3	2
	N	5	5	5	5	5	5	5	5	5

S.D., standard deviation; N, number of female SD rats; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltranspeptidase; ALP, alkaline phosphatase; Glu, glucose; BUN, blood urea nitrogen; Crea, creatinine; T-Bili, total bilirubin; T-Chol, total cholesterol; TG, triglycerides; TP, total protein; Alb, albumin.

Table 6 Summary of necropsy findings

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

External surface and all organs in body cavity were unremarkable.

Table 7 Summary of histopathological findings

	Sex	Male				Female			
Organ/findings	Group	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (mL/animal)	0	0.1	0.5	1.0	0	0.1	0.5	1.0
	No. of animals	5	5	5	5	5	5	5	5
Injection site	Remarkable findings	0	0	0	0	0	0	0	0
	No. of examined	5	5	5	5	5	5	5	5

4. Discussion

Pharmacopuncture is a type of new acupuncture technique that combines acupuncture and drug therapies. It can be seen as a unique treatment technique of Korean Oriental medicine that provides the effects of meridian theory in acupuncture therapy and flavor theory in drug therapy [6]. The *Samgihwalryeok* pharmacopuncture consists of *Panax ginseng, Cervus elaphus sibericus, Angelica gigas Nakai, Liriope platyphylla,* and *Schisandra chinensis Baillon* [4] and is prepared in a sterile room at the Korean pharmacopuncture institute (KGMP, pH 7.0% – 7.5%, 0.9% NaCl).

Recent reports have suggested that *Panax ginseng* pharmacopuncture has the effects of increasing body weight [7], heart rate variability [8] and immune response [9],

and of decreasing NOS expression induced by noise stress [10] and anti-cancer effects [11]. *Cervus elaphus sibericus* pharmacopuncture has the effects of increasing heart rate variability [8] and body-weight [7] and decreasing the osteoporosis induced by an ovariectomy [12]. Decreased cerebral infarction or ischemia damage [13] and improvement in hypothyroidism induced by thiourea [14] were reported in *Angelica gigas* pharmacopuncture studies. *Liriope platyphylla* was reported to have anti-inflammation [15] and anti-cancer effects [16], and *Schisandra chinensis Baillon* was reported to have anti-inflammation effects [17]. *Samgihwalryeok* pharmacopuncture made with all of these was reported to affect chronic fatigue and insomnia [4].

This study was performed to determine the safety of using *Samgihwalryeok* pharmacopuncture in SD rats. Animal testing is the most fundamental and basic way to perform safety assessments [18]. The *Samgihwalryeok* pharmacopuncture used in study was prepared in a sterile room at the Korean pharmacopuncture institute (KGMP). The animals were randomly distributed into 4 groups, 5 male and five female rats per group: control (0 mL/animal and 1.0 mL of saline), low-dose (0.1 mL/animal), mid-dose (0.5 mL/animal), and high-dose (1.0 mL/animal) groups. After intramuscular injection of *Samgihwalryeok* pharmacopuncture, general symptoms, body-weights, hematological factors, blood-biochemical factors, necropsy features and histopathological features were observed. These observations produced no significant findings. All conditions of this study followed The Korea Food & Drug Administration's testing protocol guidelines for the study of toxicity, and all experiments were conducted following the GLP regulations [19]. According to the all of the above results, *Samgihwalryeok* pharmacopuncture can be used as a safe treatment, but further studies should be conducted to yield more concrete evidence to support this safety and prove its efficacy.

5. Conclusion

This study was designed to investigate the safety of *Samgihwalryeok* pharmacopuncture for single-dose intramuscular injection (0.1 - 1.0 mL/animal, 5 rats per group). The following results were found: No mortalities or abnormal clinical signs, no changes in body weights, and no differences in hematological and biochemical analyses, necropsy findings and histopathological findings were observed in this study. Therefore, the approximate lethal dose of *Samgihwalryeok* pharmacopuncture must be considered to be more than 1.0 mL/animal in both male and female rats.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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