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Original article

Single-dose Toxicity of ShinYangHur Herbal Acupuncture

Eunhye Cha[1,2], Jongcheol Lee[1,2], Seongjin Lee[1,2], Manyong Park[1,2], Sungchul Kim[1,2]*

[1] Department of Acupuncture & Moxibustion Medicine, Wonkwang University Gwangju Korean Medical Hospital, Gwangju, Korea
[2] Nervous & Muscular System Disease Clinical Research Center of Wonkwang University Gwangju Korean Medical Hospital, Gwangju, Korea

Key Words

herbal acupuncture, intramuscular injection, pharmacopuncture, ShinYangHur (SYH), toxicity test

Abstract

Objectives: This study was carried out to analyze the single-dose toxicity of ShinYangHur (SYH) herbal acupuncture injected into the muscles of Sprague-Dawley (SD) rats.

Methods: The SYH herbal acupuncture was made in a clean room at the Korean Pharmacopuncture Institute (KPI, Korea-Good Manufacturing Practice, K-GMP). After the mixing process with sterile distilled water, the pH was controlled to between 7.0 and 7.5. Then, NaCl was added to make a 0.9% isotonic solution by using sterilized equipment. All experiments were conducted at Biotoxtech, an institution authorized to perform non clinical studies under the regulations of Good Laboratory Practice (GLP). SD rats were chosen for the pilot study. Doses of SYH herbal acupuncture, 0.25, 0.5, and 1.0 mL, were administered to the experimental groups, and a dose of normal saline solution, 1.0 mL, was administered to the control group. This study was conducted under the approval of the Institutional Animal Ethics Committee.

Results: No deaths or abnormalities occurred in any of the four groups. No significant changes in weight, hematological parameters or clinical chemistry between the control group and the experimental groups were observed. To check for abnormalities in organs and tissues, we used microscopy was used to examine representative histological sections of each specified organ; the results showed no significant differences in any of the organs or tissues.

Conclusion: The above outcomes suggest that treatment with SYH herbal acupuncture is relatively safe. Further studies on this subject are needed to yield more concrete evidence.

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1. Introduction

Pharmacopuncture is a new acupuncture therapy that can be used along with the more traditional acupuncture and moxibustion. It is a distinctive Oriental treatment in which a herbal extraction is injected into specific acupoints related to the disease. First, highly effective herbs are selected based on the diagnosis; then, the pharmacopuncture fluid is extracted from those selected herbs and injected into the meridian points or sore spots [1]. Thus, a single procedure, can achieve both the effects of acupuncture and herbal medicine [2]. Furthermore, because pharmacopuncture does not pass through the gastrointestinal tract, it work faster and without any loss [3].

The constituents of ShinYangHur (SYH) herbal acupuncture are *Achyranthis radix, Plantaginis semen, Ligustri lucidi fructus, Rehmanniae radix preparata, Dioscoreae rhizoma, Dispaci radix, Eucomiae cortex, Poria cocos, Moutan cortex radicis, Alismatis rhizoma, Cinnamomum cassia, Aconitum kusnezoffii reichb,* and *Cervus elaphus sibericus* [4].

These were extracted at low temperature and low

*Corresponding Author
Sungchul Kim. Department of Acupuncture & Moxibustion Medicine, Wonkwang University Gwangju Korean Medical Hospital, 543-8 Juweol 1-dong, Nam-gu, Gwangju 503-310, Korea.
Tel: +82-62-670-6441 Fax: +82-62-670-6767
E-mail: kscndl@hanmail.net

pressure in an aseptic room at the Korean Pharmacopuncture Institute (KPI). SYH herbal acupuncture is known to be effective for treating deficiency syndrome of yang of the kidneys and has been widely used to elevate kidney function by changing the glomerular filtration rate and, free water clearance [5]. Deficiency syndrome of yang of the kidneys is one of the categories of eight principle pattern identification (EPPI).

Despite this, toxicity testing of SYH herbal acupuncture has not been conducted yet. Therefore, this study was performed to analyze the single-dose toxicity and the lethal dose of SYH herbal acupuncture in rats.

The current research trend for single-dose toxicity testing of extracts is study the acute and the sub acute toxicities through Good Laboratory Practice (GLP) regulations. All the experiments for this study were conducted at Biotoxtech.

2. Materials and Methods

The SYH herbal acupuncture was made in a clean room at the KPI (Korea-Good Manufacturing Practice, K-GMP). After the mixing process with Sterile distilled water, the pH was controlled to between 7.0 and 7.5. Then, NaCl was added to make a 0.9% isotonic solution by using a sterilized equipment. The completed extract was stored in a refrigerator (2.1 — 5.5, until it was used. The date of manufacture on this extract was 2013-5-3, and its expiration date was 2013-11-3.

In this study, 6 week old Sprague-Dawley (SD) rats reared by ORIENTBIO were used. The reason SD rats were chosen is that they have been widely used in safety tests in the field of medicine, so the results could be easily compared with many other data bases. At the time of injection, the range on weights of the male rats were 182.6 — 197.2 g, and that of the female rats was 138.5 — 162.3 g. Upon receipt, all animals were visually inspected and then weighed by using a CP3202S system (Sartorius, Germany). During 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 breeding environment was 22.0 — 23.9°C, the humidity was 50.3% — 70.4%, and the illumination is 150 — 300 Lux. Feedstuff (Teklad Certified Irradiated Global 18% Protein Rodent Diet 2918C) and ultra violet (UV)-filtered water were provided.

After 7 days of acclimatization, animals were selected and grouped by using the criteria of their weights being 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 mice of each sex per group), as shown in Table 1.

In clinical applications the usual dose for SYH herbal acupuncture is 1.0 mL per treatment. No death occurred in the pilot test in which 1.0 mL of SYH herbal acupuncture was injected into each male and female rat. In this study 1.0 mL/animal was set as the high dose, and 0.5 mL/animal and 0.25 mL/animal were set as the mid and the low doses, respectively. In the control group, 1.0 mL/animal, 0.5 mL/animal in each thigh, of normal saline solution was injected. A single dose, 0.25 and 0.5 mL/animal, was injected into the left thigh muscle of the rats in the low and the mid dose groups, respectively, and 0.5 mL of SYH herbal acupuncture was injected into each thigh muscle of the rats in the high dose groups, for a total of 1.0 mL/animal, by using disposable syringes. This study was performed under the approval of the Institutional Animal Ethics Committee of Biotoxtech Co., Ltd.

From the 1st day to 14th day after treatment, the general symptoms were examined once a day. On the day of dosing (day 0), the general symptoms (side effects, revealing time, recovery time, etc.), as well as mortality, were examined at 30 minutes and at 1, 2, 3, and 4 hours after injection. The weights were measured immediately before treatment and at 3, 7 and 14 days after treatment. After fasting for more than 18 hours before autopsy, the rats were anesthetized by using isoflurane.

Blood samples were taken from the abdominal aorta on the day of autopsy (15 days after injection). About 1 mL blood sample was analyzed by using an automatic hematology analyzer (ADVIA 120, SIEMEMS, Germany). A blood sample of about a 2.0 mL was centrifuged for the blood coagulation test (3,000 rpm, 10 minutes). The results were measured by using an automated coagulation analyzer (Coapresta 2000, SEKISUI, Japan). The blood obtained from the abdominal aorta was analyzed using blood biochemical tests. The results were measured by using an automatic analyzer (7180, HITACHI, Japan) and an electrolyte analyzer (AVL9181, Roche, Germany). For all animals, the organs and the tissues of the body were visually inspected and microscopically observed.

The weights and the results of the hematologic examinations and blood chemical tests obtained from the experi-

Table 1 Groups of animals

Group	SYH Injection (mL/animal)	Number of animals (serial number)	
		Male	Female
G1: Control group	0	5 (1101 — 1105)	5 (2101 — 2105)
G2: Low-dose group	0.25	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)

SYH, ShinYangHur.

ments were analyzed by using a statistical analysis system (SAS, version 9.3, SAS Institute Inc., U.S.A.). A Bartlett test was conducted to evaluate the homogeneity of the variance and the significance. The one-way analysis of variation (ANOVA) test was carried out when the homogeneity of the variance was recognized, and the Kruskal-Wallis test was conducted post-hoc.

3. Results

In this study, no deaths or abnormalities were observed in any of the groups (Tables 2, 3). In addition, No changes in weight were observed in any of the groups (Table 4). Finally, no remarkable changes were noted in the results from the hematological examinations, blood chemical tests, necropsies and histopathological examinations (Tables 5, 6, 7, 8).

4. Discussion

SYH herbal acupuncture has been widely utilized in clinics to elevate kidney function [5] and is known to be effective for treating deficiency syndrome of yang of the kidneys. Deficiency syndrome of yang of the kidneys is one of the categories of EPPI. Deficiency syndrome of yang of the kidneys is loss of endocrinal function due to congenital weakness, aging, immoderate sexual life, or physical consumption due to chronic diseases. The symptoms, such as general weakness, loss of body function, impotence, edema, polyuria, nocturia, dawn diarrhea, appear in patients having this condition [6].

Though SYH herbal acupuncture has been widely used to treat such symptoms, no clinical review on the effects of SYH herbal acupuncture has been published. However, many studies have been done to identify and isolate the components of this pharmacopuncture, and SYH herbal acupuncture has been found to consist of *Achyranthis radix, Plantaginis semen, Ligustri lucidi fructus, Rehmanniae radix preparata, Dioscoreae rhizoma, Dispaci radix, Eucomiae cortex, Poria cocos, Moutan cortex radicis, Alismatis rhizoma, Cinnamomum cassia, Aconitum kusnezoffii reichb,* and *Cervus elaphus sibericus* [4].

Recent reports have suggested that *Achyranthis radix* pharmacopuncture has a therapeutic effect on hyperlipidemia [7]. *Plantaginis semen* herbal acupuncture has a protective effect on glycerol induced acute renal failure [8] and can be used in the prevention and the treatment of hepatoxicity [9]. *Ligustri lucidi fructus* water extract has an anti-inflammatory effect and immune modulating ac-

Table 2 Summary of Mortalities

Group	Dose (mL/animal)	Mortality (dead / tested)	
		Male	Female
G1	0	0% (0 / 5)	0% (0 / 5)
G2	0.25	0% (0 / 5)	0% (0 / 5)
G3	0.5	0% (0 / 5)	0% (0 / 5)
G4	1.0	0% (0 / 5)	0% (0 / 5)

Table 3 Summary of clinical signs

Group	Dose (mL/animal)	Sex	Number of animals	Clinical signs
G1	0	Male	5	NOA
		Female	5	NOA
G2	0.25	Male	5	NOA
		Female	5	NOA
G3	0.5	Male	5	NOA
		Female	5	NOA
G4	1.0	Male	5	NOA
		Female	5	NOA

NOA, no observable abnormality.

Table 4 Mean body weights

Group	Dose (mL/animal)	Sex	Mean S. D. N	Days after administration 0	3	7	14
G1	0	Male	Mean	189.8	218.4	257.0	317.0
			S. D.	3.3	7.0	12.5	30.3
			N	5	5	5	5
		Female	Mean	153.1	164.7	181.4	210.4
			S. D.	5.6	5.9	8.5	13.1
			N	5	5	5	5
G2	0.25	Male	Mean	187.6	216.3	252.8	313.1
			S. D.	5.3	8.9	11.4	15.0
			N	5	5	5	5
		Female	Mean	151.1	163.8	178.8	200.6
			S. D.	4.3	5.8	6.4	9.3
			N	5	5	5	5
G3	0.5	Male	Mean	190.0	219.0	256.4	317.5
			S. D.	4.4	5.3	7.8	22.5
			N	5	5	5	5
		Female	Mean	151.6	166.1	180.6	202.6
			S. D.	9.3	9.0	14.1	14.1
			N	5	5	5	5
G4	1.0	Male	Mean	189.0	213.5	246.6	302.7
			S. D.	5.7	8.6	11.0	21.4
			N	5	5	5	5
		Female	Mean	152.5	162.8	177.2	201.0
			S. D.	4.3	6.9	7.2	10.4
			N	5	5	5	5

S.D., standard deviation; N, number of animals.

tivity [10]. *Rehmanniae radix preparata* extract modulates the production of pro-inflammatory cytokines in the human mast cell (HMC) line HMC-1 treated with phorbol 12-myristate13-acetate plus the calcium ionophore A23187 [11]. *Dioscoreae rhizoma* pharmacopuncture does not cause any serious physical responses or subjective symptoms and is safe [12]. Furthermore, it is effective and safe for use in patients with peripheral facial paralysis [13]. Corni fructus pharmacopuncture has useful therapeutic effects on osteoporosis [14]. *Dispaci radix* solution has relevance to the control of synovial cell proliferation by inhibiting of expressions of Interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)-α gene forming synovial cell [15]. *Eucomiae cortex* herbal acupuncture solution has an effect on the control of synovial cell proliferation and cartilage destruction in rheumatoid arthritis, and will be put to practical use rheumatoid arthritis clinics in the future [16]. *Poria cocos* herbal acupuncture improves hyperinsulinemia and hyperlipidemia, and protected against pancreatic destruction induced by streptozotocin [17]. *Moutan cortex radicis* herbal acupuncture has therapeutic effects on hyperlipidemia and related complications in rats with high fat diets [18]. *Alismatis rhizoma* has a therapeutic effect on nephritis [19]. *Cinnamomum cassia* has a distinct antidiabetes effect in type-Ⅱ diabetes mellitus model [20]. *Aconitum kusnezoffii reichb.* pharmacopuncture is a relatively safe treatment [21] and has a distinct antidiabetes effect in type-Ⅱ diabetes mellitus [20]. *Cervus elaphus sibericus* pharmacopuncture has the effects of increasing heart rate variability [22] and body weight [23] and decreasing the osteoporosis induced by an ovariectomy [24].

Thus, component herbs of SYH herbal acupuncture have been reported to have many effects on several disorders. Although it is used in clinics, safety studies on SYH herbal acupuncture are insufficient, so more safety studies are needed. Toxicity studies are an essential data base and are important for evaluating the safety of the test substances in medications [25].

Table 5 Mean hematology parameters

Group	Dose (mL/animal)	Sex	Mean S. D. N	RBC ($\times10^6$ cells/μL)	HGB (g/dL)	HCT (%)	RBC Indices MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT ($\times10^3$ cells/μL)	Reti (%)
G1	0	Male	Mean	7.04	14.6	41.0	58.4	20.7	35.5	1102	4.55
			S. D.	0.54	0.5	1.7	2.2	0.9	0.5	107	0.81
			N	5	5	5	5	5	5	5	5
		Female	Mean	7.45	15.0	41.5	55.7	20.2	36.3	1141	2.59
			S. D.	0.37	0.7	1.4	1.2	0.4	0.6	82	0.60
			N	5	5	5	5	5	5	5	5
G2	0.25	Male	Mean	7.09	14.9	41.8	59.0	21.1	35.7	1131	4.78
			S. D.	0.28	0.4	1.3	1.9	0.8	0.5	61	0.77
			N	5	5	5	5	5	5	5	5
		Female	Mean	7.29	14.9	41.1	56.4	20.5	36.3	1207	2.51
			S. D.	0.18	0.3	0.9	0.7	0.3	0.4	151	0.24
			N	5	5	5	5	5	5	5	5
G3	0.5	Male	Mean	7.29	14.5	41.7	57.3	19.8	34.7	1076	4.56
			S. D.	0.44	0.7	1.8	1.6	0.5	0.9	161	1.26
			N	4*	4*	4*	4*	4*	4*	4*	4*
		Female	Mean	7.35	15.0	41.3	56.2	20.4	36.3	1170	2.43
			S. D.	0.26	0.6	1.3	0.9	0.4	0.5	86	0.49
			N	5	5	5	5	5	5	5	5
G4	1.0	Male	Mean	7.41	15.1	42.5	57.5	20.4	35.5	1161	4.06
			S. D.	0.35	0.3	1.0	1.8	0.7	0.3	101	0.77
			N	5	5	5	5	5	5	5	5
		Female	Mean	7.29	14.9	40.8	56.1	20.4	36.4	1178	2.56
			S. D.	0.37	0.4	1.4	1.8	0.8	0.3	119	0.36
			N	5	5	5	5	5	5	5	5

Group	Dose (mL/animal)	Sex	Mean S. D. N	WBC ($\times10^3$ cells/μL)	WBC Differential Count (%) NEU	LYM	MONO	EOS	BASO	PT (sec)	APTT (sec)
G1	0	Male	Mean	6.23	15.5	81.7	1.6	0.6	0.1	16.6	17.2
			S. D.	1.98	4.1	4.3	0.2	0.1	0.0	0.6	1.7
			N	5	5	5	5	5	5	5	5
		Female	Mean	4.65	15.2	81.8	1.3	0.9	0.1	18.5	15.0
			S. D.	1.58	4.4	4.8	0.5	0.2	0.1	0.5	0.4
			N	5	5	5	5	5	5	5	5
G2	0.25	Male	Mean	7.99	17.1	79.7	1.7	0.4	0.1	16.4	16.0
			S. D.	2.43	6.5	6.8	0.1	0.2	0.1	0.5	1.3
			N	5	5	5	5	5	5	5	5
		Female	Mean	4.89	12.8	84.3	1.3	0.8	0.2	18.9	16.0
			S. D.	2.53	2.2	2.6	0.4	0.2	0.1	0.4	1.4
			N	5	5	5	5	5	5	5	5

(continued)

G3	0.5	Male	Mean	9.82	14.4	82.4	1.8	0.5	0.2	17.0	16.5
			S. D.	3.22	3.8	4.1	0.7	0.3	0.1	0.6	1.8
			N	4*	4*	4*	4*	4*	4*	4*	4*
		Female	Mean	4.22	15.4	81.8	1.3	0.8	0.1	18.8	16.1
			S. D.	0.82	3.8	4.1	0.5	0.1	0.0	0.5	1.5
			N	5	5	5	5	5	5	5	5
G4	1.0	Male	Mean	9.52	15.3	81.9	1.6	0.5	0.2	17.4	17.5
			S. D.	2.38	2.3	2.7	0.6	0.2	0.0	0.9	0.5
			N	5	5	5	5	5	5	5	5
		Female	Mean	4.96	18.0	79.2	1.2	1.1	0.1	19.0	16.6
			S. D.	0.29	5.0	5.1	0.2	0.5	0.1	0.4	0.8
			N	5	5	5	5	5	5	5	5

*Datum was excluded because animal (1303) was not fasted.
NS, normal saline; SP, samjeong pharmacopuncture; 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

Group	Dose (mL/animal)	Sex	Mean S. D. N	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	Male	Mean	30.0	73.9	778.2	0.48	120	12.5	0.38	0.03	92
			S. D.	6.7	18.4	223.5	0.09	7	2.8	0.03	0.02	26
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	22.0	78.3	477.4	0.47	119	12.5	0.41	0.02	87
			S. D.	3.1	16.6	87.1	0.08	10	2.5	0.03	0.01	19
			N	5	5	5	5	5	5	5	5	5
G2	0.25	Male	Mean	25.3	77.4	783.0	0.36	120	11.3	0.38	0.02	81
			S. D.	3.5	14.2	156.2	0.14	8	1.21	0.03	0.02	19
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	24.5	90.0	537.6	0.51	115	12.0	0.41	0.02	76
			S. D.	4.9	13.6	70.4	0.11	6	1.7	0.02	0.01	10
			N	5	5	5	5	5	5	5	5	5
G3	0.5	Male	Mean	26.7	77.3	780.6	0.32	124	10.5	0.36	0.03	66
			S. D.	5.3	3.4	181.7	0.12	17	1.4	0.01	0.01	17
			N	4*	4*	4*	4*	4*	4*	4*	4*	4*
		Female	Mean	28.9	90.8	514.6	0.60	113	13.2	0.41	0.02	72
			S. D.	6.5	16.3	103.9	0.20	5	1.5	0.04	0.01	10
			N	5	5	5	5	5	5	5	5	5
G4	1.0	Male	Mean	24.6	74.2	840.8	0.28†	117	11.2	0.37	0.02	71
			S. D.	4.7	17.9	91.3	0.08	20	1.6	0.02	0.01	21
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	28.9	94.6	541.5	0.57	113	13.3	0.40	0.01	69
			S. D.	5.1	18.1	94.1	0.19	14	1.5	0.02	0.01	17
			N	5	5	5	5	5	5	5	5	5

(continued)

Group	Dose (mL/animal)	Sex	Mean S. D. N	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	Male	Mean	68	5.5	2.4	0.74	8.73	10.2	139	4.6	103
			S. D.	21	0.2	0.2	0.06	0.20	0.4	1	0.2	1
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	28	5.6	2.6	0.84	7.39	10.0	139	4.6	105
			S. D.	16	0.2	0.1	0.03	0.16	0.4	1	0.3	1
			N	5	5	5	5	5	5	5	5	5
G2	0.25	Male	Mean	70	5.4	2.4	0.80	8.56	10.0	139	4.6	103
			S. D.	25	0.2	0.0	0.05	0.26	0.3	1	0.3	1
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	19	5.6	2.6	0.84	7.39	10.0	139	4.6	105
			S. D.	8	0.3	0.2	0.07	0.29	0.6	2	0.3	1
			N	5	5	5	5	5	5	5	5	5
G3	0.5	Male	Mean	54	5.3	2.3	0.75	8.29	10.0	139	4.6	104
			S. D.	10	0.2	0.1	0.05	0.46	0.5	1	0.3	3
			N	4*	4*	4*	4*	4*	4*	4*	4*	4*
		Female	Mean	15	5.7	2.6	0.85	7.80	10.0	139	4.5	105
			S. D.	4	0.3	0.1	0.06	0.42	0.1	1	0.2	2
			N	5	5	5	5	5	5	5	5	5
G4	1.0	Male	Mean	55	5.4	2.3	0.78	8.56	9.9	139	4.6	105
			S. D.	26	0.2	0.1	0.03	0.37	0.3	1	0.1	1
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	18	5.6	2.6	0.86	7.48	9.9	138	4.6	105
			S. D.	6	0.2	0.1	0.05	0.54	0.3	1	0.2	2
			N	5	5	5	5	5	5	5	5	5

*Datum was excluded because animal (1303) was not fasted. †Significantly different from control by Dunnett's *t*-test: $P < 0.05$.
S.D., standard deviation; N, number of animals; 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, chloride.

Table 7 Summary of necropsy findings

Findings	Group							
	G1 (0 mL/animal)		G2 (0.25 mL/animal)		G3 (0.5 mL/animal)		G4 (1.0 mL/animal)	
	Male	Female	Male	Female	Male	Female	Male	Female
Number of rats examined	5	5	5	5	5	5	5	5
Unremarkable findings	5	5	5	5	5	5	5	5

Table 8 Histopathological findings

Findings	Group							
	G1 (0 mL/animal)		G2 (0.25 mL/animal)		G3 (0.5 mL/animal)		G4 (1.0 mL/animal)	
	Male	Female	Male	Female	Male	Female	Male	Female
Number of rats examined	5	5	5	5	5	5	5	5
Remarkable findings	0	0	0	0	0	0	0	0

This study was carried out to provide objective safety data for SYH herbal acupuncture. Doses of 0.25, 0.5, 1.0 mL/animal of SYH herbal acupuncture were injected into the animals in the three experimental groups, and doses of 1.0 mL/animal of normal saline solution were injected into the animals of the control group. In all four groups, no deaths occurred, and no abnormalities were observed. For all animals, the clinical signs, weights, hematologic examination results and blood chemical test results were within normal range. Organ and tissues were checked for abnormalities, and no significant histopathological findings were observed.

To assess the toxicity of SYH herbal acupuncture, we need to study its acute and chronic harmful effects and its relations with capacity reaction more. Animal testing is the best way to conduct safety assessments [26]. The Korea Food & Drug Administration has published testing protocol guidelines for the study of toxicity, and all experiments should be carried out following GLP regulations [27].

The results of our toxicity test showed that treatment with 1.0 mL/animal of SYH herbal acupuncture did not cause any changes in weight or in the results of the hematological, blood chemistry, and autopsy examinations. Because SYH herbal acupuncture had no risks, SYH herbal acupuncture can safely be administered as a treatment.

5. Conclusions

The results of this study suggest that intramuscular injection of 1.0 mL/animal of SYH herbal acupuncture does not cause any changes in weight or in the results of hematological, blood chemistry, and necropsy. Neither does it cause any mortality. Thus, intramuscular injection of SYH herbal acupuncture can be used as a safe treatment.

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Conflict of interest

The authors declare that there are no conflicts of interest.

ORCID

Sungchul Kim. http://orcid.org/0000-0003-3580-5290.

References

1. Yook TH. [Clinical observation about the extent of improvement of low back pain patient through medi-acupuncture therapy]. J Korean Oriental Med. 1995;16(1):184-97.
2. Korean Pharmacopuncture Institute. Pharmacopuncturology: principles and clinical applications. Seoul: Elsevier Korea LLC; 2012. p. 3-4.
3. Joo HJ. [Researches on parmacopuncture]. KIOM; 1995;5:193-210. Korean.
4. Korean Pharmacopuncture Institute. Pharmacopuncturology: principles and clinical applications. Seoul: Elsevier Korea LLC; 2012. p. 166.
5. Jeong WK, Ryu DK, Lee HS. [Effects of palmijihwangtang water extracts on the renal function in rats]. Korean J Orient Physiol Pathol. 1997:12(1):157. Korea.
6. Korean Pharmacopuncture Insitute. [Pharmacopuncturology: principles and clinical application]. Seoul: Elsevier Korea LLC; 2012. p. 152. Korean.
7. Choi JS, Lim YK, Lee BR, Yang KY, Kim JK. [The effect of *achyranthis radix* herbal-acupuncture on hyperlipidemia in rats]. Korean J Acupunct. 2010;27(3):25-46. Korean.
8. Cho SY, Kim CH, Yoon HM, Jang KJ, Ahn CB, Song CH. The effect of *plantaginis semen* herbal acupuncture on acute renal failure in rat. Korean J Acupunct. 2005;22(4):117-27.
9. Kwon SH, Song CH. [The effect of *plantaginis semen* on CCI4 induced hepatoxicity in rats]. The Acupuncture. 2001;18(4):152-60. Korean.
10. Lee YH, Lim EM. [Anti-inflammatory effect of *ligustri lucidi fructus* water extract in raw 264.7 cells induced by LPS]. J Orient Obstet Gynecol. 2013;26(4):66-8. Korean.
11. Park SJ. The study of anti-inflammatory mechanism in mast cells by r*ehmanniae radix preparata* water extract. [dissertation]. [iksan]: Wonkwang University; 2008. p. 1-2. Korean.
12. Ko MK, Hong KE. Clinical study for evaluation of safety of sanyak (dioscoreae rizoma) pharmacopuncture according to extract method - a double-blind randomized controlled trial [master's thesis]. [Daejeon]: Daejeon University; 2011. p. 34. Korean.
13. Sung IS, Hong KE, Kim MJ, Song I. Clinical research of the efficacy and the safety of *dioscoreae rhizoma* (sanyak) pharmacopuncture therapy for peripheral facial paralysis patients. J Pharmacopuncture. 2012;15(4):15-24.
14. Kim KS. [Effects of the herbal-acupuncture with corni fructus extract at eumgok(KI10) on osteoporosis in ovariectomized mice. Korean J Acupunct. 2010;27(1):63-85. Korean.
15. Lee HL. Study on the effects of aqua-acupuncture with *dispaci radix* solution on adjuvant-induced arthritis of rat [master's thesis]. [Daejeon]: Daejeon University; 2000. p. 1-51. Korean.
16. Kang JH, Lee H. [A study on the effect of herbal-acupuncture with *eucomiae cortex* solution at joksamni(ST36) on collagen-induced arthritis]. The Acupuncture. 2006;23(3):129-42. Korean.
17. Seo CW, Seo BK, Kim JI, Kang SK. [*Poria cocos* herbal acupuncture prevents -cell damage on streptozotocin-induced diabetic rat]. The acupuncture. 2009;26(5):39-47. Korean.
18. Ahn YS, Anh TW, Kang HJ, Lee YH, Lim YK. The effect of herbal-acupuncture with *moutan cortex radicis* extract. Korean J Acupunct. 2009;26(1):85-109.
19. Han JK, Kim YS, Kim BS, Lim YK. [The effect of a*lismatis*

rhizoma herbal-acupuncture at KI(10) on LPS-induced nephritis in rats. Korean J Acupunct. 2014;31(1):51-60. Korean.
20. Jeong HS. Effects of *cinnamomum cassia* and aconitum carmichaeli´s phamacopunture and oral administration on blood sugar in type Ⅱ diabetic mice [dissertation]. [Jeonju]: Woosuk University; 2009. p. 1-39. Korean.
21. Kim JK, Kim SH, Lee SM, Jeong HH, Park MY, Kim DW, *et al.* Study of single-dose toxicity of *aconitum kusnezoffii reichb* pharmacopuncture in rats. J Pharmacopuncture. 2012;15(3):48-52.
22. Lee JM, Kim YT, Lee HI, Son YS, Jin SH, Lee HS, *et al.* [The effects of cervus elaphus aquapuncture and ginseng radix aquapuncture on the growth of animals]. J Pharmacopuncture. 2000;3(2):131-52. Korean.
23. Seol H, Song BY, Yook TH. [The effects of panax gingseng radix pharmacopuncture and zizyphi spinosi semen pharmacopuncture on the heart rate variability]. The Acupuncture. 2009;26(5):19–28. Korean.
24. Han SW, Lee YH, Kim CH. [A study on effects of the cervi pantotricuhum cornu herb-acupuncture on the osteoporosis induced by ovariectomy in rats]. J Pharmacopuncture. 2000;3(1):177-91. Korean.
25. Kim YK. [The principle and test method. toxicology]. Seoul: Donghwagisul; 1994. p. 15–8. Korean.
26. Kim YG. [Toxicology]. Seoul: Donghwagisul; 1984. p. 15-8. Korean.
27. Korea Food & Drug Administration. Korea Food & Drug Administration notification [Internet]. Seoul: Korea Food & Drug Administration; 2005 [cited 2015 JAN 2]. Available from: http://www.mfds.go.kr/.