Single-dose Toxicity of ShinYangHur Herbal Acupuncture

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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.

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 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 radixis, Alismatis rhizoma, Cinnamomum cassia, Aconitum kansuense root and Cervus elaphus sibericus [4].

These were extracted at low temperature and low...
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°C), 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 was 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 hematometry analyzer (ADVIA 120, SIEMEMS, Germany). A blood sample of about 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-

| 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 endocrinical 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, Eucommiae 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 hepatotoxicity [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 |
|-------|-----------------|--------------------------|------|--------|
|       |                 |                          |      |        |
|       |                 | Male                     |      |        |
| G1    | 0               | 0% (0 / 5)               | 0%   | 0%     |
| G2    | 0.25            | 0% (0 / 5)               | 0%   | 0%     |
| G3    | 0.5             | 0% (0 / 5)               | 0%   | 0%     |
| G4    | 1.0             | 0% (0 / 5)               | 0%   | 0%     |

### 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.
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]. Aconitum kusnezoffii reichb. pharmacopuncture is a relatively safe treatment [20] and has a distinct antidiabetes effect in type-Ⅱ diabetes mellitus model [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 4 Mean body weights

| Group | Dose (mL/animal) | Sex | Mean | S. D. | Days after administration |
|-------|------------------|-----|------|-------|---------------------------|
|       |                  |     | Mean | S. D. | N 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.
Table 5 Mean hematology parameters

| Group | Dose (mL/animal) | Sex     | Mean S. D. N | RBC (x10⁶ cells/μL) | HGB (g/dL) | HCT (%) | RBC Indices | PLT (x10³ 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 5 5 5 5 5 5 5 5 |
|       |                  | 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 (x10³ cells/μL) | WBC Differential Count (%) | PT (sec) | APTT (sec) |
|-------|------------------|---------|-------------|---------------------|---------------------------|----------|------------|
|       |                  |         |             |                     | NEU  LYM  MONO  EOS  BASO |          |            |
| 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)
Table 6 Mean clinical chemistry

| Group | Dose (mL/animal) | Sex | Mean | S. D. | 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 |
|       |                  |     | 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 |
|       |                  |     | 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 |
|       |                  |     | N 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
|       |                  | Female| Mean 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 |
|       |                  |     | 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 |
|       |                  |     | 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 |
|       |                  |     | 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 |
|       |                  |     | N 5 | 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 7 Summary of necropsy findings

| 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  | S. D. 21     | 5.2        | 0.2       | 0.06       | 0.20       | 0.4       | 1          | 0.2         | 1           | 0.2         |
|       |                 | Male  | Mean 28      | 5.6        | 2.6       | 0.84       | 7.39       | 10.0      | 139        | 4.6         | 105         |             |
|       |                 | Female| S. D. 16     | 0.2        | 0.1       | 0.03       | 0.16       | 0.4       | 1          | 0.3         | 1           |             |
|       |                 | Female| N 5          | 5          | 5         | 5          | 5          | 5         | 5          | 5           | 5           |             |
| G2    | 0.25            | Male  | S. D. 25     | 0.2        | 0.0      | 0.05       | 0.26       | 0.3       | 1          | 0.3         | 1           |             |
|       |                 | Male  | Mean 19      | 5.6        | 2.6       | 0.84       | 7.39       | 10.0      | 139        | 4.6         | 105         |             |
|       |                 | Female| S. D. 8      | 0.3        | 0.2       | 0.07       | 0.29       | 0.6       | 2          | 0.3         | 1           |             |
|       |                 | Female| N 5          | 5          | 5         | 5          | 5          | 5         | 5          | 5           | 5           |             |
| G3    | 0.5             | Male  | S. D. 10     | 0.2        | 0.1       | 0.05       | 0.46       | 0.5       | 1          | 0.3         | 3           |             |
|       |                 | Male  | Mean 15      | 5.7        | 2.6       | 0.85       | 7.80       | 10.0      | 139        | 4.5         | 105         |             |
|       |                 | Female| S. D. 4      | 0.3        | 0.1       | 0.06       | 0.42       | 0.1       | 1          | 0.2         | 2           |             |
|       |                 | Female| N 5          | 5          | 5         | 5          | 5          | 5         | 5          | 5           | 5           |             |
| G4    | 1.0             | Male  | S. D. 26     | 0.2        | 0.1       | 0.03       | 0.37       | 0.3       | 1          | 0.1         | 1           |             |
|       |                 | Male  | Mean 18      | 5.6        | 2.6       | 0.86       | 7.48       | 9.9       | 138        | 4.6         | 105         |             |
|       |                 | Female| S. D. 6      | 0.2        | 0.1       | 0.05       | 0.54       | 0.3       | 1          | 0.2         | 2           |             |
|       |                 | Female| N 5          | 5          | 5         | 5          | 5          | 5         | 5          | 5           | 5           |             |

^1Datum was excluded because animal (1303) was not fasted. ^2Significantly 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 glutamyltransferase; 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 8 Histopathological findings

| Findings | 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 |
| 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.

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