Study on the Single Dose Toxicity of ShinEumHur Pharmacopuncture Injected into the Muscles of Rats

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Abstract

Objectives: This study was carried out to analyze the single dose toxicity of ShinEumHur (SEH) pharmacopuncture injected into the muscles of Sprague-Dawley rats.

Methods: The SEH pharmacopuncture was made in a clean room at the Korean Pharmacopuncture Institute (K-GMP). After the mixing process with sterile distilled water had been completed, the pH was controlled to between 7.0 and 7.5. All experiments were conducted at Biotoxtech, an institution authorized to perform non-clinical studies under the Good Laboratory Practice (GLP) regulations. Sprague-Dawley rats were chosen for the pilot study. Doses of SEH pharmacopuncture, 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. We examined the survival rate, weights, clinical signs, mean hematological parameters, mean clinical chemistry, necropsy and histopathological findings.

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 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 findings suggest that treatment with SEH pharmacopuncture is relatively safe. Further studies on this subject are needed to yield more concrete evidence.

1. Introduction

Pharmacopuncture therapy is a distinctive therapy based on acupuncture & moxibustion medicine, meridian theory, and herbal medicine [1]. Depending on the diseases, highly effective herbs are selected, and pharmacopuncture fluid is extracted from those selected herbs and injected into meridian points [2]. A single procedure, achieve the effects of both acupuncture and herbal medicine. Moreover, as the pharma-
copuncture does not pass through the digestive system, it works faster and has effects that oral administration of the same herbal decoction would not have [3].

The ShinEumHur (SHE) pharmacopuncture, which originated from Yukmijihwang decoction, was developed to treat diseases, such as immune, endocrine, digestive, respiratory, urinary, and circulatory system diseases, caused by kidney-yin deficiency syndromes [4]. SHE herbal acupuncture has been widely used in clinics and is known to be effective for treating kidney-yin deficiency syndromes. Especially, it shows considerable effects on disorders of the musculoskeletal system, such as lumbago, melosalgia, and weakness of the lower limbs [5]. The constituents of SEH herbal acupuncture are Achyranthis Radix, Plantaginis Semen, Rehmanniae Radix Preparata, Ligustri Lucidi Fructus, Corni Fructus, Dioscoreae rhizoma, Poria cocos, Moutan Cortex Radicis, Alismatis Rhizoma. These were extracted at low temperature and low pressure in an aseptic room at the Korean Pharmacopuncture Institute (Seoul, Korea).

Several studies on the relation between the toxicity and the composition of SEH pharmacopuncture have been conducted. Park et al conducted a study on the pharmacological action and toxicity of Rehmanniae Radix Preparata [6]. In a toxicity study, Kim et al conducted stability tests of extracts of Achyranthis Radix and, Moutan Cortex Radicis [7]. Chung et al reported that lyophilized seed extract of Corni Fructus was lethal to experimental mice at doses of 250 – 300 mg/kg and that the toxic component was related to proteins [8]. Seok et al reported that water soluble extract of Alismatis Rhizoma had no acute oral toxicity and that the oral lethal dose (LD)\textsubscript{50} value was over 4,000 mg/kg in Sprague-Dawley (SD) rats [9]. In addition, Jeon et al conducted stability tests of Yukmijihwang decoction and that its LD\textsubscript{50} value for mice was more than 5,000 mg/kg \textit{via} an oral route [10]. Despite its worldwide use for general health promotion, objective toxicity testing of SEH pharmacopuncture has not been conducted yet. Therefore, this study was performed to analyze the single-dose toxicity and the lethal dose of SEH pharmacopuncture in rats.

The current research trend for single-dose toxicity testing of extracts is to study the acute and the subacute toxicities through Good Laboratory Practice (GLP) regulations. All the experiments for this study were conducted at Biototech, an institution authorized to perform non-clinical studies, under the GLP.

### 2. Material and Methods

The SEH pharmacopuncture was made in a clean room at the Korean Pharmacopuncture Institute (K-GMP). After the mixing process with sterile distilled water had been completed, 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. The completed extract was stored in a refrigerator (2.1 - 5.3°C) until it was used. The date of manufacture on this extract was March 5, 2013, and its expiration date was Mar 11, 2013.

The animals used in this study were 6-week-old SD rats. The reason SD rats were chosen is that they have been widely used in safety tests in the field of medicine, so the results of this study could be easily compared with many other databases. The range of weights of the male rats was 177.9 – 198.5 g at the time of injection, and that of the female rats was 153.0 – 182.9 g. For all animals, a visual inspection was conducted; all animals were weighed by using a CP3202S system (Sartorius, Germany). During the seven 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 – 24.1°C, the humidity was 47.7% – 70.8%, and the illumination was 150 – 300 Lux. Sufficient food (Teklad Certified Irradiated Global 18% Protein Rodent Diet 2918C) and ultraviolet (UV)-filtered water were provided.

Groupings were done after the seven days of acclimatization. Animals were selected if their weights were 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 SEH pharmacopuncture is 1.0 mL per treatment. No death occurred in a pilot test in which 1.0 mL of SEH pharmacopuncture 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/site in each thigh, of normal saline solution was injected. A single dose, 0.25 or 0.5 mL/animal, was injected into the left thigh muscle of the rats in the low or the mid-dose groups, respectively. In the high dose-groups, 0.5 mL

### Table 1 Groups of animals

| Group          | SEH 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)               |

SHE, ShinEumHur.
of SEH pharmacopuncture was injected into each thigh muscle of the rats, for a total of 1.0 mL/animal. All injections were done 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 the 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 the mortality, were examined at 30 minutes and at 1, 2, 4, and 6 hours after injection. The weights were measured immediately before administration and at 3, 7 and 14 days after administration. After a fast of more than 18 hours immediately before autopsy, which was conducted 15 days after injection, the rats were anesthetized by using isoflurane. Blood samples were taken from the abdominal aorta on that day, and blood samples of about 1 mL blood sample were analyzed by using an automatic hematology analyzer (ADVIA 2120i, SIEMENS, Germany). A blood sample of about a 2 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 also analyzed using blood biochemical tests. The results were measured by using an automatic analyzer (AVL9181, Roche, Germany). After observations had been terminated, the organs and tissues of all surviving animals were visually inspected and microscopically examined. The weights and the results of the hematologic examinations and the blood chemical were analyzed by using SAS software (version 9.3, SAS Institute Inc., USA.). A Bartlett test was conducted to evaluate the homogeneity of the variance and the significance. The one-way 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 occurred 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 meaningful changes in the hematological examination, blood chemical test, necropsies and histopathology were noted (Tables 5, 6, 7, 8).

4. Discussion

The term, ‘ShinEumHur,’ means Kidney-Yin deficiency syndrome, which is one of the categories of eight-principle pattern identification in Oriental medicine. This syndrome has been recognized to be caused by an imbalance in the kidney function. Its symptoms include pain in the back or the knees, forgetfulness, tidal fever, night sweating, dizziness, tinnitus, insomnia, thirst at night, etc. [11]. In the Orient, Yukmijihwang decoction has been used typically for more than a thousand years for the treatment of this syndrome. Yukmijihwang decoction, a classic six-herb oriental medicine formula, is composed of Rehmanniae Radix Preparata, Corni Fructus, Dioscoreae rhizoma, Poria cocos, Alismatis Rhizoma and Moutan Cortex Radicis. Originally, it was used to improve or restore decreased functions related to aging, such as impairments of vision, hearing, cognition, and memory [12]. Furthermore, a number of studies on the effects of Yukmijihwang decoction have been conducted recently. Yukmijihwang decoction has been reported to be able to protect against β-amyloid toxicity [13], inhibit the development of benign prostatic hyperplasia [14], decrease proteinuria and protect kidney function [15], and lower body weight and improve insulin and leptin sensitivity in obese rats [16]; it can also be used to treat Henoch-Schonlein purpura caused by SEH [17]. SEH pharmacopuncture, which is comprised of compositions of Yukmijihwang decoction and several additional herbal medicines, has been widely utilized in clinics to treat Kidney-Yin deficiency syndrome. In clinics, its effects

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

Table 2 Mortalities

| 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 3 Clinical signs
and functions in clinics have many similarities with those of Yukmijihwang decoction. Until now, several studies on the effects of SEH pharmacopuncture and Yukmijihwang decoction pharmacopuncture have been conducted. Yukmijihwang decoction pharmacopuncture was shown to have an anti-aging effect by reducing the collagenase activity [18]. SEH pharmacopuncture has been reported to be effective for the recovery of chondrocyte phenotype and to be useful for cartilage regeneration in arthritic diseases [19]; it can also be used to recover ankylosing spondylitis functionally to relieve pain and morning stiffness effectively [20]. Kim et al studied the anti-arthritic properties of SEH pharmacopuncture and reported that it exhibited the significant therapeutic efficiency in the treatment of adjuvant-induced monoarthritis in rats [21].

Furthermore, several pharmacopunctures made of components of SEH pharmacopuncture have various effects. *Achyranthis Radix* pharmacopuncture reduces inflammatory reactions and muscular tissue necrosis induced in the paws of SD rats by using Freund’s complete adjuvant [22]. *Corni Fructus* pharmacopuncture is known to significantly increase the level of serum phosphorus and to increases the levels of serum calcium, tibial calcium, and phosphorus in ovariectomized mice. These results suggest that *Corni Fructus* pharmacopuncture may have useful therapeutic effects on osteoporosis in ovariectomized mice [23]. *Alismatis Rhizoma* pharmacopuncture has a therapeutic effects on nephritis in lipopolysaccharide-stimulated rat [24]. Moreover, *Plantaginis Semen* pharmacopuncture is known to increase significantly the glomerular filtration rate and to decreases the serum creatinine and, BUN levels, as well as the fractional excretion of Na + and Cl . These results suggest that *Plantaginis Semen* pharmacopuncture can be used in the prevention and the treatment of acute renal

| Group | Dose (mL/animal) | Sex | Mean S. D. | Days after administration |
|-------|-----------------|-----|------------|--------------------------|
|       |                 |     | N          | 0 | 3 | 7 | 14 |
|       |                 | Male | Mean | 188.5 | 212.2 | 250.4 | 313.2 |
|       |                 |     | S. D. | 4.0  | 4.8  | 6.0  | 14.5 |
| G1    | 0               |     | N      | 5   | 5   | 5   | 5   |
|       |                 | Female | Mean | 163.5 | 172.0 | 188.6 | 219.1 |
|       |                 |     | S. D. | 7.1  | 7.2  | 11.0 | 13.9 |
|       |                 |     | N      | 5   | 5   | 5   | 5   |
|       | 0.25            | Male | Mean | 188.5 | 211.1 | 248.6 | 307.2 |
|       |                 |     | S. D. | 7.7  | 8.3  | 12.4 | 15.5 |
| G2    |                 |     | N      | 5   | 5   | 5   | 5   |
|       |                 | Female | Mean | 163.7 | 175.2 | 193.2 | 220.0 |
|       |                 |     | S. D. | 7.7  | 6.5  | 9.5  | 13.3 |
|       |                 |     | N      | 5   | 5   | 5   | 5   |
|       | 0.5             | Male | Mean | 188.2 | 210.1 | 245.3 | 307.2 |
|       |                 |     | S. D. | 6.7  | 7.7  | 8.5  | 10.0 |
| G3    |                 |     | N      | 5   | 5   | 5   | 5   |
|       |                 | Female | Mean | 163.3 | 179.4 | 198.4 | 231.2 |
|       |                 |     | S. D. | 11.9 | 10.7 | 16.1 | 24.0 |
|       |                 |     | N      | 5   | 5   | 5   | 5   |
|       | 1.0             | Male | Mean | 189.6 | 214.0 | 251.9 | 313.5 |
|       |                 |     | S. D. | 7.9  | 8.9  | 8.3  | 4.7  |
| G4    |                 |     | N      | 5   | 5   | 5   | 5   |
|       |                 | Female | Mean | 163.5 | 174.1 | 192.6 | 222.6 |
|       |                 |     | S. D. | 9.2  | 9.7  | 11.8 | 16.4 |
|       |                 |     | N      | 5   | 5   | 5   | 5   |

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

*Table 4 Mean body weights*
Table 5  Mean hematology parameters

| Group | Dose (mL/animal) | Sex | Mean S. D. N | RBC (× 10⁶ cells/μL) | HGB (g/dL) | HCT (%) | RBC Indices | PLT (× 10³ cells/μL) | Reti (%) |
|-------|------------------|-----|--------------|-----------------------|------------|---------|-------------|----------------------|----------|
|       |                  | Male| Mean 7.25    | 14.9                  | 41.5       | 57.5    | 20.6        | 36.0                 | 1291     | 5.09     |
|       |                  |     | S. D. 0.56   | 0.5                   | 0.9        | 4.7     | 1.3         | 1.6                  | 68       | 1.37     |
|       |                  | N   | 5            | 5                     | 5          | 5       | 5           | 5                    | 5        | 5        |
|       |                  | Female| Mean 7.61  | 15.5                  | 42.4       | 55.7    | 20.4        | 36.5                 | 1150     | 2.26     |
|       |                  |     | S. D. 0.12   | 0.4                   | 0.8        | 0.3     | 0.2         | 0.3                  | 33       | 0.23     |
|       |                  | N   | 5            | 5                     | 5          | 5       | 5           | 5                    | 5        | 5        |
|       |                  | Male| Mean 6.91    | 14.8                  | 41.4       | 60.0    | 21.5        | 35.7                 | 1228     | 4.25     |
|       |                  |     | S. D. 0.44   | 0.2                   | 0.9        | 2.8     | 1.2         | 0.6                  | 130      | 1.32     |
|       |                  | N   | 5            | 5                     | 5          | 5       | 5           | 5                    | 5        | 5        |
|       |                  | Female| Mean 7.86  | 15.6                  | 43.0       | 54.8    | 19.8        | 36.2                 | 1215     | 1.92     |
|       |                  |     | S. D. 0.40   | 0.3                   | 1.0        | 2.3     | 0.9         | 0.2                  | 145      | 0.36     |
|       |                  | N   | 5            | 5                     | 5          | 5       | 5           | 5                    | 5        | 5        |
|       |                  | Male| Mean 7.19    | 15.2                  | 42.7       | 59.5    | 21.2        | 35.6                 | 1235     | 4.16     |
|       |                  |     | S. D. 0.38   | 0.5                   | 1.9        | 1.8     | 0.9         | 0.7                  | 132      | 0.37     |
|       |                  | N   | 5            | 5                     | 5          | 5       | 5           | 5                    | 5        | 5        |
|       |                  | Female| Mean 7.45  | 15.5                  | 41.9       | 56.4    | 20.9        | 37.0                 | 1054     | 2.34     |
|       |                  |     | S. D. 0.50   | 0.6                   | 1.6        | 2.1     | 1.0         | 0.3                  | 97       | 0.63     |
|       |                  | N   | 5            | 5                     | 5          | 5       | 5           | 5                    | 5        | 5        |
|       |                  | Male| Mean 7.11    | 14.9                  | 41.3       | 58.0    | 20.9        | 36.0                 | 1140     | 4.29     |
|       |                  |     | S. D. 0.25   | 0.2                   | 0.9        | 2.0     | 0.8         | 0.7                  | 170      | 0.58     |
|       |                  | N   | 5            | 5                     | 5          | 5       | 5           | 5                    | 5        | 5        |
|       |                  | Female| Mean 7.15  | 15.4                  | 41.6       | 55.9    | 20.6        | 36.9                 | 1175     | 2.58     |
|       |                  |     | S. D. 0.50   | 0.6                   | 1.3        | 2.3     | 0.8         | 0.4                  | 155      | 0.88     |
|       |                  | N   | 5            | 5                     | 5          | 5       | 5           | 5                    | 5        | 5        |

| Group | Dose (mL/animal) | Sex | Mean S. D. N | WBC (× 10³ cells/μL) | WBC Differential Count (%) | PT (sec) | APTT (sec) |
|-------|------------------|-----|--------------|----------------------|---------------------------|----------|------------|
|       |                  | Male| Mean 9.40    | 14.3                  | 83.1                      | 1.5      | 0.2        | 0.2        | 17.3 | 13.9 |
|       |                  |     | S. D. 3.34   | 2.4                   | 2.0                       | 0.4      | 0.1        | 0.1        | 0.9  | 2.3  |
|       |                  | N   | 5            | 5                     | 5                         | 5        | 5          | 5          | 5    | 5    |
|       |                  | Mean| 5.29    | 9.1                  | 87.9                      | 1.0      | 0.9        | 0.2        | 17.7 | 14.8 |
|       |                  | Female| 1.31  | 1.7                  | 1.6                       | 0.2      | 0.3        | 0.1        | 1.1  | 0.9  |
|       |                  |     | N           | 5                     | 5                         | 5        | 5          | 5          | 5    | 5    |
|       |                  | Male| Mean 7.40    | 12.1                  | 85.4                      | 1.0      | 0.4        | 0.2        | 16.9 | 15.1 |
|       |                  |     | S. D. 0.79   | 3.8                   | 3.8                       | 0.2      | 0.1        | 0.1        | 0.3  | 0.9  |
|       |                  | N   | 5           | 5                     | 5                         | 5        | 5          | 5          | 5    | 5    |
|       |                  | Mean| 6.52    | 12.0                  | 84.1                      | 1.7      | 0.9        | 0.1        | 18.0 | 15.8 |
|       |                  | Female| 2.57  | 4.2                  | 5.0                       | 0.7      | 0.1        | 0.1        | 0.9  | 0.6  |
|       |                  |     | N           | 5                     | 5                         | 5        | 5          | 5          | 5    | 5    |

(Continued)
S.D., standard deviation; N, number of animals; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular cell hemoglobin concentration; PLT, platelet; 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 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 | 31.0 | 95.3  | 792.8 | 0.35 | 113 | 12.5 | 0.40 | 0.03 | 68 |
|       | Male             | S. D. | 3.6  | 26.3  | 69.0  | 0.11 | 7 | 0.4 | 0.02 | 0.03 | 12 |
|       | N                | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    |
|       | Female           | Mean | 22.2 | 74.4  | 443.6 | 0.50 | 114 | 11.9 | 0.42 | 0.02 | 93 |
|       | Female           | S. D. | 0.9  | 7.6   | 124.8 | 0.05 | 5 | 1.5 | 0.03 | 0.01 | 20 |
|       | N                | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    |
| G2 0.25 | Male        | Mean | 29.5 | 81.0  | 697.0 | 0.35 | 114 | 11.8 | 0.39 | 0.01 | 74 |
|       | Male            | S. D. | 5.3  | 11.2  | 104.5 | 0.10 | 8 | 0.9 | 0.02 | 0.01 | 8 |
|       | N               | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    |
|       | Female          | Mean | 24.7 | 80.8  | 419.4 | 0.41 | 118 | 13.4 | 0.43 | 0.02 | 82 |
|       | Female          | S. D. | 5.7  | 12.8  | 112.3 | 0.12 | 9 | 1.1 | 0.03 | 0.01 | 9 |
|       | N                | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    |
| G3 0.5 | Male         | Mean | 29.0 | 80.2  | 710.3 | 0.29 | 113 | 13.3 | 0.40 | 0.01 | 77 |
|       | Male           | S. D. | 3.3  | 12.7  | 112.2 | 0.10 | 13 | 1.8 | 0.02 | 0.01 | 21 |
|       | N               | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    |
|       | Female         | Mean | 23.0 | 83.2  | 521.7 | 0.56 | 108 | 13.3 | 0.43 | 0.02 | 88 |
|       | Female         | S. D. | 5.0  | 18.6  | 85.1  | 0.15 | 11 | 1.8 | 0.02 | 0.01 | 30 |
|       | N               | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    |
| G4 1.0 | Male       | Mean | 30.0 | 78.0  | 750.7 | 0.28 | 123 | 13.3 | 0.40 | 0.02 | 64 |
|       | Male            | S. D. | 5.0  | 6.7   | 101.8 | 0.12 | 12 | 1.7 | 0.02 | 0.01 | 7 |
|       | N               | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    |
|       | Female          | Mean | 25.0 | 85.7  | 457.5 | 0.63 | 118 | 14.4 | 0.44 | 0.02 | 80 |
|       | Female          | S. D. | 8.7  | 21.2  | 99.0  | 0.18 | 8 | 1.1 | 0.02 | 0.01 | 14 |
|       | N               | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    |

(Continued)
### Table 7 Necropsy findings

| Group         | Dose (mL/animal) | Sex     | Mean S. D. | 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    | 47         | 5.4        | 2.3       | 0.72       | 8.42       | 10.0      | 140        | 4.8         | 102        |
|               | S. D.            | N       | 5          | 5          | 5         | 5          | 5          | 5         | 5          | 5           | 5          | 5           |
| G2 0.25       | Male             | Mean    | 52         | 5.4        | 2.3       | 0.76       | 8.04       | 9.9       | 141        | 4.6         | 103        |
|               | S. D.            | N       | 15         | 0.2        | 0.1       | 0.05       | 0.42       | 0.3       | 1          | 0.2         | 1          | 1           |
| G3 0.5        | Male             | Mean    | 50         | 5.5        | 2.4       | 0.77       | 8.60       | 10.0      | 140        | 4.8         | 101        |
|               | S. D.            | N       | 16         | 0.1        | 0.1       | 0.04       | 1.11       | 0.3       | 1          | 1.1         | 1          | 1           |
| G4 1.0        | Male             | Mean    | 58         | 5.4        | 2.3       | 0.73       | 8.24       | 10.1      | 140        | 4.6         | 103        |
|               | S. D.            | N       | 24         | 0.2        | 0.0       | 0.04       | 0.30       | 0.2       | 1          | 0.2         | 2          | 2           |

*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 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 | Group                  |
|----------|------------------------|
| G1 (0 mL/animal) | Male Female | G2 (0.25 mL/animal) | Male Female | G3 (0.5 mL/animal) | Male Female | G4 (1.0 mL/animal) | Male Female |
| Number of rats examined | 5 5 | 5 5 | 5 5 | 5 5 | 5 5 | 5 5 |
| Unremarkable findings | 5 5 | 5 5 | 5 5 | 5 5 | 5 5 | 5 5 |
| Remarkable findings | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 | 0 0 |
failure [25]. Although SEH pharmacopuncture is used widely in clinics, safety studies on it are insufficient, so more safety studies are needed. Toxicity studies form an essential database and are important for evaluating the safety of the test substances in medications [26]. To assess the toxicity of SEH pharmacopuncture, we need to study its acute and chronic side effects and its relations with capacity reaction more. Animal testing is the most fundamental way to perform safety assessments [27]. The Korea Food & Drug Administration has published testing protocol guidelines for the study of toxicity, and all experiments should be conducted following GLP regulations [28].

This study was carried out to provide objective safety data for SEH pharmacopuncture. Doses of 0.25, 0.5, and 1.0 mL/animal of SEH pharmacopuncture were administered to the experimental groups, and a dose of 1.0 mL/animal of normal saline solution were administered to 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. Organs and tissues were checked for abnormalities, and no significant histopathological findings were observed. The results of our toxicity test showed that treatment with 1.0 mL/animal of SEH pharmacopuncture did not cause any changes in weight or in the results of the hematological, blood chemistry, and necropsy examinations, which indicates that SEH pharmacopuncture can safely be administered as a treatment. Further studies on the subject should be conducted to yield more concrete evidence to support this finding.

5. Conculsion

The results of this study suggest that an intramuscular injection of 1.0 mL/animal of SEH pharmacopuncture does not cause any changes in weight or in the results of hematological, blood chemistry, and necropsy examinations. It also did not result in any mortality, which indicates that intramuscular injection of SEH pharmacopuncture 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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