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Original article

Study of Single-dose Toxicity of *Guseonwangdo-go* Glucose Intramuscular Injection in Sprague-Dawley Rats

Su-jeong Jo[1], Sung-chul Kim[2], Yu-jong Kim[1], Eun-jung Kim[1], Kap-sung Kim[1], Seung-deok Lee[1]*

[1] Department of Acupuncture & Moxibustion Medicine, Dongguk University College of Oriental Medicine, Goyang, Korea
[2] Department of Acupuncture & Moxibustion Medicine, Wonkwang University College of Oriental Medicine, Gwangju, Korea

Key Words
Guseonwangdo-go, intramuscular toxicity, pharmacopuncture, single-dose toxicity

Abstract

Objectives: This study was performed to analyze single-dose intramuscular toxicity of *Guseonwangdo-go* glucose pharmacopuncture.

Methods: Eighty six-week-old Sprague-Dawley rats were divided into two large groups of forty rats; *Guseonwangdo-go* glucose 5% and *Guseonwangdo-go* glucose 20% groups. Each group was sub-divided into four smaller groups of five males and five females, with the following dosages of pharmacopuncture being administered by intramuscular (IM) injection in each group: group 1 (G1, control group): 1.0 mL of normal saline solution, group 2 (G2, low-dose group): 0.1 mL, group 3 (G3, mid-dose group): 0.5 mL, and group 4 (G4, high-dose group): 1.0 mL.

Results: No mortalities or clinical signs were observed in any group. Also, no significant changes in body weights or in hematological/biochemical analyses were observed between the control and the experimental groups during necropsy or histopathology.

Conclusion: The above findings suggest that the lethal dose of *Guseonwangdo-go* glucose 5% and 20% pharmacopuncture administered via IM injection is more than 1.0 mL per animal in both male and female rats. Further studies on the repeated-dose toxicity of *Guseonwangdo-go* glucose should be conducted to yield more concrete data.

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1. Introduction

Guseonwangdo-go is a kind of food which allows people to boost their energy. It is usually made in the form of rice cakes or soups by grinding Nelumbinis Semen, Dioscoreae Rhizoma, Hoelen, Coicis Semen, Hordei Fructus Germinatus, Dolichoris Semen, Euryales Semen, and Kaki Mannosum.

It was first recorded in *Manbyeonghwechun* published during the Ming dynasty [1]. Afterward, in *DonguiBogam* and *Bangyak Happyeon*, it was prescribed for stomach conditions as a tonifying medicine for various diseases and internal damage and was described as being in strengthening the spirit, boosting energy, helping the digestive system, improving appetite, tonifying deficiency, growing muscles and removing

***Corresponding Author**
Seung-deok Lee. Department of Acupuncture & Moxibustion, Dongguk University International Hospital 814, Siksa-dong, Ilsandong-gu, Goyang, Gyeonggi 410-773, Korea.
Tel: +82-31-961-9122 Fax: +82-31-961-9009
E-mail: chuckman@dongguk.edu

dampness-heat [2,3]. Similar records can be found in *The Daily Records of the Royal Secretariat of the Joseon Dynasty* for the ruling periods of Injo, Youngjo, and Soonjo, who were kings of Joseon Dynasty [4].

Ju *et al*. reported that *Guseonwangdo-go* had a positive effect on preventing obesity and reinforcing the immune system. In this study, *Guseonwangdo-go* had no effects other than marginal ones and no cytotoxicity when a concentration of over 500 μg/mL, IC_{50}, was administered orally [5]. However, it should be tested for toxicity before clinical application because *Guseonwangdo-go* has never before been used as a base formula of pharmacopuncture. In this experiment, a single-dose of *Guseonwangdo-go* injected intramuscularly in different volumes was tested for toxicity in order to analyze the acute toxicity of *Guseonwangdo-go* pharmacopuncture and to determine its lethal dose.

Therefore, this experiment was conducted not only to study the single-dose toxicity of intramuscular administration of *Guseonwangdo-go* glucose 5% and 20% to both sexes of 6-week-old Sprague-Dawley (SD) rats but also to determine its approximate lethal dose. This experiment was conducted in compliance with the toxicity test guidelines of the Korea Food and Drug Administration (KFDA, KFDA Notification No. 2012-86), and significant results were obtained [6].

2. Materials and Methods

The substances used in the experimental groups of this study were prepared by making *Guseonwangdo-go* glucose 5% and 20% pharmacopuncture in a facility at the Korean Pharmacopuncture Institute under the Good Manufacturing Practice guidelines. According to *Bangyak Happyeon*, *Guseonwangdo-go* consists of 160 g of each of the following constituents: lotus seed (Nelumbinis Semen), yam rhizome (Dioscoreae Rhizoma, stir-baked), poria (Hoelen), and Job's tears seed (Coicis Semen), 80 g of each of the following constituents: malt (Hordei Fructus Germinatus, stir-baked), dolichos bean seed (Dolichoris Semen, stir-baked), fox nut seed (Euryales Semen), and 40 g of persimmon frost (Kaki Mannosum) [3]. Since glucose is the main ingredient of persimmon frost, persimmon frost was adjusted to 5% and 20% in the pharmacopuncture preparation.

Six-week-old SD rats were used in this experiment. Twenty male rats (body weights: 180.4-199.7 g) and 20 female rats (body weights: 144.0-162.3 g) were assigned to the *Guseonwangdo-go* glucose 5% administration group, and 20 male rats (body weights: 190.4-212.9 g) and 20 female rats (body weights: 138.9-157.3 g) were assigned to the *Guseonwangdo-go* glucose 20% administration group. A visual inspection and measurements of body weight by using electronic scales (CP3202S, Sartorius, Germany) were conducted on all animals when they were brought into the experiment. The general symptoms were observed once a day prior to the start of the experiments with both groups being weighed and examined for general symptoms and body weight changes on the last day of acclimatization. No abnormalities were found.

All animals in both the *Guseonwangdo-go* glucose 5% and 20% groups were separated on the last day of acclimatization. Twenty rats of each sex whose body weights were close to the mean weight was selected. They were randomly distributed into four different groups with five individuals of each sex per group so that the means weight of the groups were approximately the same. The four different groups were labeled as follows: Group 1 (G1, control group): normal saline solution, Group 2 (G2): low-dose group, Group 3 (G3): mid-dose group, and Group 4 (G4): high-dose group (Table 1).

In a pilot test (Biotoxtech Study No.: B12878P), 1.0 mL/animal, referring to 1.0 mL/time of clinically applied dose, was administered by intramuscular (IM) injection (left thigh) to one male and one female rat and resulted in no deaths. From this result, the doses for *Guseonwangdo-go* glucose pharmacopuncture in this study were set as follows: 1.0 mL/animal as high-dose (G4), 0.5 mL/animal

Table 1 Composition of the *Guseonwangdo-go* glucose group

GSWG*	group	Dose (mL/animal)	Total injection (mL/animal)	Numbers of animals			
				GSWG 5%		GSWG 20%	
				Male	Female	Male	Female
G1	Control group	0	1.0	5	5	5	5
G2	Low-dose group	0.1	0.1	5	5	5	5
G3	Mid-dose group	0.5	0.5	5	5	5	5
G4	High-dose group	1.0	1.0	5	5	5	5

*GSWG: *Guseonwangdo-go* glucose Grade - minimal

as mid-dose (G3), and 0.1 mL/animal as low-dose (G2). The same amount of normal saline solution (Choongwae Pharma Corp., Korea) as that of *Guseonwangdo-go* glucose pharmacopuncture for the high-dose group was injected into the animals in the control group (G1), and the results were observed and compared with those of the experimental groups. Using a disposable syringe (1 mL, 26 g), IM injection was administered to animals in the low-dose and the mid-dose groups once on in the left thigh, and to those of the high-dose and the control groups one 0.5-mL injection was administered in each thigh.

All experiments were conducted at Biotoxtech, an authorized institution for non-clinical studies, under the regulation of Good Laboratory Practice of KFDA Notification No. 2012-61 (Test Guidelines for Non-clinical Studies, Aug 24, 2012) [7].

The general symptoms (types of toxic symptoms, expression time, recovery time, etc.) and mortality were observed after 30 minutes, and then after 1, 2, 4, and 6 hours on the day of injection (day 0). From the next day to the 14th day after the injection, the general symptoms were examined once a day.

The body weights were measured on the day of the injection (immediately before the injection) and then on the 3rd, 7th, and 14th days after the injection.

All animals were fasted during the 18 hours before autopsy. They were then anesthetized with isoflurane, after which blood was collected from the abdominal aorta on the day of autopsy (15 days after the injection). For the hematological test, approximately 1 mL of the collected blood was placed in an ethylene-diamine-tetra-acetic acid tube and was analyzed using a hematology analyzer (ADVIA® 120, Siemens, Germany). For the coagulation test, approximately 2 mL of the collected blood was placed in a tube with 3.2% sodium citrate and centrifuged at 3,000 rpm for 10 minutes. Blood plasma was then collected. Different laboratory tests were conducted using a coagulation time analyzer (Coapresta® 2000, Sekisui, Japan).

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

During the necropsy performed on all the animals, organs and tissues from the entire body were checked thor-

Table 2 Mean hematology parameters of Sprague-Dawley rats in the *Guseonwangdo-go* glucose 5% group

GSWG group/dose (mL)		Male				Female			
		G1/0	G2/0.1	G3/0.5	G4/1.0	G1/0	G2/0.1	G3/0.5	G4/1.0
RBC (x10^6 cells/$\mu\ell$)		6.76 ± 0.36	6.75 ± 0.12	6.88 ± 0.36	6.78 ± 0.26	6.64 ± 0.14	6.77 ± 0.47	7.04 ± 0.1	3.78 ± 0.33
HGB (g/dL)		14.1 ± 0.6	13.8 ± 0.2	14.1 ± 0.7	13.8 ± 0.4	13.9 ± 0.5	13.7 ± 0.8	14.3 ± 0.5	13.7 ± 0.6
HCT (%)		43.6 ± 2.0	42.2 ± 0.9	43.0 ± 2.6	42.4 ± 1.4	41.0 ± 1.8	40.8 ± 1.7	42.3 ± 1.2	41.1 ± 1.5
	MCV (fL)	64.5 ± 2.6	62.6 ± 1.8	62.5 ± 1.2	62.6 ± 1.1	61.7 ± 2.2	60.4 ± 1.8	60.0 ± 1.0	60.7 ± 1.1
RBC	MCH (pg)	20.9 ± 0.8	20.4 ± 0.2	20.5 ± 0.4	20.4 ± 0.4	20.8 ± 0.6	20.3 ± 0.3	20.3 ± 0.5	20.3 ± 0.5
	MCHC (g/dL)	32.3 ± 0.2	32.7 ± 0.6	32.8 ± 0.4	32.5 ± 0.2	33.8 ± 0.3	33.6 ± 0.6	33.8 ± 0.4	33.4 ± 0.6
PLT (x10^3 cells/$\mu\ell$)		1201 ± 123	1199 ± 87	1299 ± 246	1280 ± 132	1219 ± 22	1293 ± 152	1224 ± 229	1290 ± 172
Reti (%)		4.8 ± 0.3	5.0 ± 0.1	4.7 ± 0.9	4.9 ± 0.6	3.1 ± 0.5	3.2 ± 0.5	2.9 ± 0.5	3.4 ± 1.0
WBC (x10^3 cells/$\mu\ell$)		7.02 ± 0.99	7.98 ± 3.13	8.05 ± 2.31	7.85 ± 2.58	3.75 ± 0.65	3.57 ± 1.3	3.69 ± 0.67	4.22 ± 1.05
	NEU	16.5 ± 2	16.2 ± 3.7	17.0 ± 5.4	17.9 ± 5.8	17.5 ± 7.4	14.5 ± 3.1	14.7 ± 1.1	18.0 ± 8.6
	LYM	79.5 ± 1.8	78.9 ± 4.4	79.1 ± 5.7	78.6 ± 6.1	79.5 ± 7.6	82.0 ± 2.8	82.3 ± 0.8	78.8 ± 8.7
WBC Differential	MONO	2.2 ± 0.4	2.8 ± 1.2	2.2 ± 0.3	1.7 ± 0.3	1.4 ± 0.3	1.8 ± 0.2	1.7 ± 0.5	1.4 ± 0.3
Counting (%)	EOS	0.4 ± 0.1	0.5 ± 0.2	0.4 ± 0.2	0.7 ± 0.7	0.8 ± 0.4	0.8 ± 0.2	0.7 ± 0.4	1.0 ± 0.4
	BASO	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
PT (sec)		17.4 ± 0.6	17.9 ± 0.4	17.6 ± 0.8	17.6 ± 1.0	18.1 ± 0.6	18.4 ± 0.9	18.3 ± 0.4	18.2 ± 0.5
APTT (sec)		13.5 ± 0.4	13.9 ± 0.7	14.2 ± 0.7	14.0 ± 0.6	13.5 ± 1.3	12.8 ± 2.3	13.9 ± 1.3	13.2 ± 1.5

The values are means ± SD.

The number of animal in each group is 5.

oughly by visual inspection.

Tissues at the injection sites for all the animals were sampled and fixed in 10% neutral buffered formalin. Routine histological methods, such as trimming, dehydration, and paraffin embedding, were conducted on the fixed organs and tissues. These were then sliced using a microtome and stained with hematoxylin & eosin (H&E).

All the results obtained were analyzed by using STATA/SE 9.2 for Windows (StataCorp LP, College Station, TX, USA). The equal variance was tested using Bartlett's test. If the sample variances were equal, significant results were obtained using the one-way analysis of variance. Dunnett's multiple range *t*-test was conducted. If the sample variances were not equal, a Kruskal-Wallis test was performed. The *P*-value for statistical analysis was 0.05.

3. Results

During the observation, no mortalities or clinical signs were observed in any of the experimental or control groups.

Weight gains were observed in both the experimental and the control groups, but comparison of the experimental and the control groups showed no significant changes in body weight.

No significant changes were noted in the hematological test results, comparison of the experimental and the control groups (Tables 2, 3).

No significant biochemical changes in the experimental groups were observed when the blood was analyzed (Tables 4, 5).

On necropsy, no abnormalities were observed when the visual inspection was conducted on all of the animals in all groups.

No histopathological abnormalities were observed on the local tolerance tests on the injection sites of all but one out of the 80 animals. Infiltration of inflammatory cells was observed in one animal of the *Guseonwangdo-go* glucose 20% high-dose group (Table 6).

4. Discussion

The main goal of a toxicity study is to ensure the safety of clinical medicine by evaluating the stability of the new

Table 3 Mean hematology parameters of SD rats in the *Guseonwangdo-go* glucose 20% group

GSWG group/dose (mL)		Male				Female			
		G1/0	G2/0.1	G3/0.5	G4/1.0	G1/0	G2/0.1	G3/0.5	G4/1.0
RBC (x10^6 cells/$\mu\ell$)		3.83 ± 0.2	7.01 ± 0.4	6.72 ± 0.34	6.9 ± 0.27	7.02 ± 0.18	7.24 ± 0.33	7.1 ± 0.19	6.77 ± 0.41
HGB (g/dL)		14.4 ± 0.4	14.1 ± 0.3	14.1 ± 0.6	13.8 ± 0.6	14.1 ± 0.4	14.5 ± 0.4	14.3 ± 0.3	13.8 ± 0.6
HCT (%)		44.5 ± 1.7	43.6 ± 1.3	43.3 ± 1.6	43 ± 1.5	41.9 ± 1.2	42.9 ± 1.4	42.5 ± 0.7	40.4 ± 2.2
	MCV (fL)	65.2 ± 0.9	62.3 ± 4.6	64.5 ± 1.5	62.4 ± 2	59.7 ± 0.9	59.4 ± 2.5	59.9 ± 0.8	59.7 ± 0.8
RBC	MCH (pg)	21.0 ± 0.4	20.2 ± 1.0	20.9 ± 0.5	20.0 ± 0.6	20.1 ± 0.3	20.1 ± 1.0	20.2 ± 0.2	20.4 ± 0.6
	MCHC (g/dL)	32.3 ± 0.5	32.5 ± 1	32.4 ± 0.3	32.1 ± 0.4	33.7 ± 0.4	33.8 ± 0.4	33.6 ± 0.2	34.1 ± 0.6
PLT (x10^3 cells/$\mu\ell$)		1242 ± 119	1267 ± 75	1187 ± 90	1311 ± 32	1213 ± 114	1238 ± 114	1254 ± 127	1325 ± 68
Reti (%)		5.0 ± 0.4	5.6 ± 0.9	5.2 ± 0.8	5.1 ± 0.5	3.4 ± 0.5	2.8 ± 0.5	3.3 ± 0.9	4.9 ± 4.0
WBC (x10^3 cells/$\mu\ell$)		9.41 ± 2.47	9.0 ± 2.8	8.74 ± 2.12	8.25 ± 1.48	4.29 ± 1.21	5.25 ± 1.67	4.85 ± 0.45	3.96 ± 0.43
	NEU	17.1 ± 3.6	17.5 ± 6.0	17.7 ± 5.8	15.2 ± 3.8	15.8 ± 4.8	17.0 ± 3.3	15.7 ± 6.2	16.5 ± 7.5
	LYM	78.4 ± 4.3	78.2 ± 6.2	77.7 ± 6.4	80.2 ± 4.7	80.4 ± 5.2	79.0 ± 3.8	81.2 ± 6.2	80.3 ± 7.3
WBC Differential	MONO	2.4 ± 0.6	2.5 ± 0.7	2.6 ± 0.7	2.6 ± 0.9	1.9 ± 0.8	1.9 ± 1	1.5 ± 0.4	1.5 ± 0.3
Counting (%)	EOS	0.7 ± 0.4	0.4 ± 0.2	0.6 ± 0.1	0.4 ± 0.1	1.1 ± 0.3	0.9 ± 0.3	0.6 ± 0.3	0.8 ± 0.3
	BASO	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1
PT (sec)		17.8 ± 0.8	17.3 ± 0.5	16.8 ± 1.5	17.4 ± 1.1	17.7 ± 0.6	17.9 ± 1.2	17.9 ± 0.6	17.4 ± 1.1
APTT (sec)		14.6 ± 0.7	14.4 ± 0.8	13.0 ± 2.7	12.4 ± 2.1	13.2 ± 1.3	12.6 ± 2.1	12.1 ± 2.4	11.8 ± 2.7

The values are means ± SD.

The number of animal in each group is 5.

Table 4 Mean clinical chemistry of SD rats in the *Guseonwangdo-go* Glucose 5% group

GSWG group /dose (mL)	Male				Female			
	G1/0	G2/0.1	G3/0.5	G4/1.0	G1/0	G2/0.1	G3/0.5	G4/1.0
ALT (U/L)	26.5 ± 3.6	26.2 ± 4.5	23.1 ± 3.5	27.6 ± 2.8	24.9 ± 4.9	22.1 ± 2.7	22.0 ± 1.2	24.2 ± 2.0
AST (U/L)	75.5 ± 7.4	77.1 ± 7.1	76.7 ± 6.1	75.4 ± 11.2	78.1 ± 8.8	77.2 ± 6	77.6 ± 8.4	72.8 ± 11.6
ALP (U/L)	940.5 ± 273.3	776.6 ± 121.9	855.5 ± 163.4	764.9 ± 129.8	492.4 ± 87.6	498.2 ± 121.1	530.9 ± 81.6	473.1 ± 75.7
GGT (U/L)	0.51 ± 0.26	0.43 ± 0.09	0.28 ± 0.09	0.39 ± 0.07	0.50 ± 0.21	0.49 ± 0.14	0.59 ± 0.17	0.46 ± 0.14
Glu (mg/dL)	140 ± 22	121 ± 17	130 ± 23	132 ± 16	123 ± 11	123 ± 12	120 ± 18	120 ± 13
BUN (mg/dL)	11.9 ± 0.5	10.5 ± 1.7	13.3 ± 0.9	10.6 ± 1.2	12.9 ± 1.8	11.8 ± 1.7	11.8 ± 0.5	12.2 ± 0.7
Crea (mg/dL)	0.40 ± 0.02	0.39 ± 0.02	0.39 ± 0.01	0.40 ± 0.02	0.42 ± 0.03	0.42 ± 0.02	0.41 ± 0.01	0.42 ± 0.03
T-Bili (mg/dL)	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.03 ± 0.01
T-Chol (mg/dL)	73 ± 13	73 ± 7	59 ± 7	69 ± 14	76 ± 9	67 ± 10	80 ± 25	86 ± 17
TG (mg/dL)	58 ± 38	51 ± 25	55 ± 20	49 ± 15	14 ± 4	12 ± 3	15 ± 3	14 ± 6
TP (g/dL)	5.3 ± 0.2	5.3 ± 0.2	5.3 ± 0.1	5.3 ± 0.2	5.5 ± 0.2	5.6 ± 0.4	5.7 ± 0.1	5.7 ± 0.2
Alb (g/dL)	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.4 ± 0.0	2.6 ± 0.2	2.5 ± 0.14	2.6 ± 0.1
A/G ratio	0.75 ± 0.05	0.75 ± 0.04	0.78 ± 0.07	0.74 ± 0.04	0.79 ± 0.03	0.85 ± 0.06	0.79 ± 0.02	0.81 ± 0.04
P (mg/dL)	8.68 ± 0.36	8.53 ± 0.21	8.43 ± 0.49	8.77 ± 0.38	7.59 ± 0.66	7.59 ± 0.46	7.42 ± 0.61	7.12 ± 0.43
Ca (mg/dL)	10.3 ± 0.3	10.3 ± 0.2	10.4 ± 0.3	10.4 ± 0.2	10.3 ± 0.3	10.1 ± 0.3	10.3 ± 0.3	10.2 ± 0.1
Na (mmol/L)	140 ± 2	140 ± 1	140 ± 1	140 ± 1	139 ± 0	140 ± 0	141 ± 1	140 ± 2
K (mmol/L)	4.8 ± 0.4	4.5 ± 0.3	4.3 ± 0.2	4.7 ± 0.3	4.6 ± 0.3	4.4 ± 0.4	4.5 ± 0	4.4 ± 0.3
Cl (mmol/L)	103 ± 1	103 ± 2	104 ± 1	103 ± 1	106 ± 2	104 ± 1	105 ± 1	105 ± 2

The values are means ± SD.
The number of animal in each group is 5.

Table 5 Mean clinical chemistry of SD rats in the *Guseonwangdo-go* glucose 20% group

GSWG group /dose (mL)	Male				Female			
	G1/0	G2/0.1	G3/0.5	G4/1.0	G1/0	G2/0.1	G3/0.5	G4/1.0
ALT (U/L)	28.5 ± 5.5	28.5 ± 5.8	27.7 ± 1.3	28.6 ± 3.3	21.1 ± 3.9	22.9 ± 2.7	22.5 ± 2.1	20.1 ± 2.7
AST (U/L)	67.6 ± 6.5	67.2 ± 9.5	70.9 ± 3.5	35.0 ± 6.2	65.4 ± 6	64.1 ± 2	63.9 ± 5.1	65.2 ± 6.5
ALP (U/L)	786.6 ± 117.6	867.1 ± 199.2	873.4 ± 103.7	883.9 ± 154.3	476.9 ± 76.3	379.3 ± 36.2	467.9 ± 9.2	461.0 ± 74.4
GGT (U/L)	0.31 ± 0.09	0.39 ± 0.08	0.42 ± 0.12	0.38 ± 0.09	0.62 ± 0.11	0.55 ± 0.13	0.59 ± 0.2	0.5 ± 0.22
Glu (mg/dL)	133 ± 16	127 ± 16	138 ± 13	115 ± 10	125 ± 15	127 ± 11	128 ± 4	130 ± 10
BUN (mg/dL)	13.1 ± 2.4	11.1 ± 1.8	12.2 ± 1.4	11.2 ± 1.2	13.4 ± 1.8	13.2 ± 1.8	12.2 ± 1.1	12.3 ± 1.5
Crea (mg/dL)	0.36 ± 0.02	0.37 ± 0.04	0.35 ± 0.01	0.37 ± 0.02	0.4 ± 0.02	0.37 ± 0.03	0.37 ± 0.03	0.38 ± 0.03
T-Bili (mg/dL)	0.05 ± 0.02	0.07 ± 0.03	0.05 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.02	0.03 ± 0.01	0.06 ± 0.05
T-Chol (mg/dL)	69 ± 17	80 ± 14	77 ± 24	71 ± 9	81 ± 22	81 ± 27	67 ± 22	85 ± 9
TG (mg/dL)	49 ± 21	52 ± 19	61 ± 27	52 ± 11	17 ± 10	12 ± 4	10 ± 3	16 ± 6
TP (g/dL)	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.2	2.2 ± 0.2	5.5 ± 0.3	5.4 ± 0.3	5.3 ± 0.2	5.5 ± 0.3
Alb (g/dL)	2.3 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.3 ± 0.2	2.5 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.6 ± 0.2

A/G ratio	0.77 ± 0.01	0.75 ± 0.03	0.77 ± 0.04	0.79 ± 0.06	0.83 ± 0.03	0.87 ± 0.04	0.85 ± 0.05	0.87 ± 0.07
P (mg/dL)	8.55 ± 0.41	8.48 ± 0.46	8.70 ± 0.32	8.40 ± 0.38	6.86 ± 0.41	7.06 ± 0.39	7.24 ± 0.21	7.08 ± 0.41
Ca (mg/dL)	10.1 ± 0.2	10.0 ± 0.2	9.9 ± 0.2	9.9 ± 0.3	10.1 ± 0.3	9.9 ± 0.3	9.8 ± 0.2	9.9 ± 0.3
Na (mmol/L)	140 ± 1	139 ± 1	139 ± 1	139 ± 1	138 ± 1	138 ± 1	138 ± 1	139 ± 1
K (mmol/L)	4.5 ± 0.1	4.6 ± 0.2	4.3 ± 0.2	4.5 ± 0.2	4.6 ± 0.5	4.6 ± 0.2	4.4 ± 0.2	4.4 ± 0.3
Cl (mmol/L)	103 ± 1	103 ± 2	101 ± 1	104 ± 2	104 ± 3	104 ± 1	105 ± 1	102 ± 2

The values are means ± SD.

The number of animal 5 in each group is 5.

Table 6 Summary of histopathological findings

GSWG group	Sex	Group/Dose (mL/animal)	Number of Animals	Unremarkable Findings	Infiltration, Inflammatory Cells
5%	Male	G1 / 0	5	5	0
		G2 / 0.1	5	5	0
		G3 / 0.5	5	5	0
		G4 / 1.0	5	5	0
	Female	G1 / 0	5	5	0
		G2 / 0.1	5	5	0
		G3 / 0.5	5	5	0
		G4 / 1.0	5	5	0
20%	Male	G1 / 0	5	5	0
		G2 / 0.1	5	5	0
		G3 / 0.5	5	5	0
		G4 / 1.0	5	5	0
	Female	G1 / 0	5	5	0
		G2 / 0.1	5	5	0
		G3 / 0.5	5	5	0
		G4 / 1.0	5	4	1

drug. Toxicity can be classified mainly into acute toxicity, sub-acute toxicity and chronic toxicity study [8].

Guseonwangdo-go consists of Nelumbinis Semen, Dioscoreae Rhizoma, Hoelen, Hordei Fructus Germinatus, Dolichoris Semen, Euryales Semen, and Kaki Mannosum [3]. Lee, in an oral injection study, reported that Nelumbinis Semen had no toxicity [9], but no studies about toxicity with oral administration of the other constituents of *Guseonwangdo-go* are available in Korea.

Acute toxicity test results have been reported in Korea for Scolopendra [10], Juglandis Semen [11], Ginseng Radix (wild) [8], Carthami Tinctor-Fructus [12], Triglii Semen [13], Armeniacaeamarum Semen [14], Scutellariae Baicalensis Radix [15], Houttuyniae Herba [16], Salviae Radix [17], Vermilionum [18], Ephedrae Herba [19] and Ginseng Radix [20] as single drugs; and for Bovis calculus + Felursi [21], Bovis calculus + Felursi + Moschus [22] and Hwangryeonhaedoktang [23] as combination drugs. Sweet Bee venom pharmacopuncture [24] has also been tested for toxicity.

From the experimental data obtained in this study, the following conclusions can be drawn: First, the LD_{50} value could not be obtained as no deaths or abnormalities were observed in any of the groups. In previous studies, Sweet Bee venom [24] had an LD_{50} value of 0.17 mg/kg and showed abnormalities such as hyperemia and abnormal gait. Ephedrae Herba [19] had an LD_{50} value of 3885.112 mg/kg, and Triglii Semen [13] had an LD_{50} value of 0.30

mL/kg. Based on these results, *Guseonwangdo-go* can be considered as being relatively safe compared to Sweet Bee Venom, Ephedra, and Triglii Semen and as safe as any other pharmacopuncture, except as noted above above.

Second, no significant changes in body weights between the experimental groups and the control group were observed during the observation period. In previous studies involving Carthami Tinctor-Fructus [12] and Triglii Semen [13] weight gains were observed.

Third, the variance between measurements from the hematological test was not accepted. This represents a similarity in the toxicity results for pharmacopuncture formulations mentioned above, so *Guseonwangdo-go* should demonstrate similar safety.

Fourth, some statistically significant results were found on some parts of the biochemical test. However, they were not meaningful in terms of toxicity because of the small variance and the lack of a dose dependency. In the toxicity test for herbs above, Scolopendra [10], Juglandis Semen [11], and Ginseng Radix (wild) [8] showed a significant increase in the total cholesterol, and Armeniacaeamarum Semen [14] and Scutellariae Baicalensis Radix [15] showed increases in glucose.

Fifth, no effects of *Guseonwangdo-go* injection were apparent at autopsy, but infiltration of inflammatory cells was observed in one individual in the 1.0-mL dose group of *Guseonwangdo-go* glucose 20% on the tolerance tests on the injection sites. However, the degree was minimal, and it was observed in only one individual, so it is considered to be the result of using a syringe needle for the injection.

A single-dose of *Guseonwangdo-go* glucose 5% and 20% was administered through IM injection to SD rats, and no toxicity was observed in any of the groups. Thus, the approximate lethal dose is considered to be more than 1.0 mL/animal in both male and female rats. In addition, *Guseonwangdo-go* glucose 5% and 20% is shown to be relatively safer than Sweet Bee Venom, Ephedrae Herba, Triglii Semen, Carthami Tinctor-Fructus, Juglandis Semen, Ginseng Radix (wild), Armeniacaeamarum Semen, and Scutellariae Baicalensis Radix. However, before clinical application, further studies with repeated IM injection tests and sub-acute and chronic toxicity tests should be conducted in order to prove the safety of *Guseonwangdo-go* pharmacopuncture.

5. Conclusion

This study was designed to investigate the safety of *Guseonwangdo-go* glucose 5% and 20% pharmacopuncture. A single-dose IM injection was administered to six-week-old SD rats of both sexes in order to analyze its toxicity and determine a lethal dose. The following results were found:

1. No mortalities or clinical signs were observed in either the experimental groups or the control group during the observation period.
2. No significant differences in body weight or in the results from hematological and biochemical analyses were observed between the control and the experimental groups.
3. No necropsy findings were observed in any of the groups.

Therefore, the approximate lethal dose of *Guseonwangdo-go* glucose 5% and 20% pharmacopuncture is considered to be more than 1.0 mL/animal in both male and female rats.

Disclosure Statement

The authors do not have any conflicts concerning this paper.

References

1. Jin JP. [Manbyeonghwechun]. Seoul: Bubin publishers; 2007. 233 p. Korean.
2. Heo J. [DonguiBogam]. Hadong: Donguibogam publishers Co.; 2005. p. 1238-41. Korean.
3. Hwang DY. [Bangyak Happyeon]. Seoul: Younglimsa; 2002. 137 p. Korean.
4. The Daily Records of Royal Secretariat of Joseon Dynasty [Internet]. Seoul: National Institute of Korean History; c2005 [cited 2013 July 29] Available from: http://sjw.history.go.kr/.
5. Ju YS, Choi YH, Kim HK, Ko BS. Effects of *Kuseonwangdogo* on the proliferation of preadipocyte 3T3-L1 cells, the anti-complementary and the cytotoxic effects. J Korean Oriental Med. 2000;20(3):105-14.
6. Korea Food and Drug Administration. Guidelines for toxicity tests of drugs and related materials (KFDA Notification No. 2012-86, 2012 Aug 24) [Internet]. Seoul: The National Legal Information Center of the Ministry of Government Legislation; c1997-2011. [cited 2012 Oct 1]. Available from: http://www.law.go.kr/.
7. Korea Food and Drug Administration. Good laboratory practice regulation for non-clinical laboratory studies (KFDA Notification No. 2012-61, 2012 Aug 24) [Internet]. Seoul: The National Legal Information Center of the Ministry of Government Legislation; c1997-2011. [cited 2012 Oct 1]. Available from: http://www.law.go.kr/.
8. Kwon KR, Cho AL, Lee SG. [The study on acute and

subacute toxicity and anti-cancer effects of cultivated Wild Ginseng herbal acupuncture]. Pharmacopuncture. 2003;6(2):7-27. Korean.
9. Lee HJ. Subchronic oral toxicity of nelumbinis semen in rats and beagle dogs. [dissertation]. [Seoul]: Kyunghee University; 2012. 37 p.
10. Lim SI. A study on safety of scolopendrid aqua-acupuncture.Wonkwang Univ Oriental Med J. 2003.
11. Kang KS, Kwon KR. [The study on acute and subacute toxicity of Juglandis Semen herbal-acupuncture(JsD)]. Pharmacopuncture. 2001;4(3):85-92. Korean.
12. An CS, Kwon KR, Lee SG. [The study on acute and subacute toxicity and sarcoma-180 anti-cancer effects of carthami tinctor-fructus herbal-acupuncture(CF)]. Pharmacopuncture. 2002;5(1):7-26. Korean.
13. Yoo CK, Kwon KR, Yu BG. [The study on acute and subacute toxicity and sarcoma-180 anti-cancer effects of triglii semen herbal-acupuncture]. Pharmacopuncture. 2002;5(1):27-42. Korean.
14. Kim O. The Study on Acute and Subacute Toxicity and Sarcoma-180 Anti-cancer Effects of Armeniacaeamarum semen Herbal-Acupuncture (Haeng-In). Sangji Univ Oriental Med J. 2002.
15. Byun BH, Seo BI. [Safety study on acute toxicity of scutellariae baicalensis radix herbal acupuncture solution (SBRHA)]. Kor J Herbology. 2003;18(4):47-51. Korean.
16. Lim SBN, Kim SH, Park SD. [Acute toxicity of aqua-acupuncture with houttuyniaeherba in mice and rats]. The Journal of Jehan Oriental Medical Academy. 1995;1(1):106-17. Korean.
17. Kim HS, Lee YH, Choi YT. Studies on the skin test, subcutaneous and muscle Irritation test, and antigenicity test of Salviae radix extract for herb - acupuncture]. The Acupuncture.1994;11(1):25-48. Korean.
18. Kwon KR. [The study on acute and subacute toxicity and sarcoma-180 anti-cancer effects of vermilionum]. Pharmacopuncture. 2003;6(3):39-47. Korean.
19. Youn KS, Nam SS, Lee JD, Choi DY, Ahn BC, Park DS, *et al*. [Experimental studies on the safety of Ephedrae Herba extract solution used for herbal - acupuncture]. The Acupuncture. 1997;14(1);361-82. Korean.
20. Lee HJ. [Study for the toxicity of kinds of ginseng radix aqua-acupuncture extract]. The Acupuncture. 1993;10(1);167-73. Korean.
21. Soh KS, Jeong CG, Lee SW, Park PM, Kim JH, Kang DI, *et al*. [Experimental studies on the acute toxicity of Bostaurus, Urusthibetanus extract solution (BU) for herbal-acupuncture]. Pharmacopuncture. 2001;4(3):69-83. Korean.
22. Lee SW, Jeong CG, Kim KH, Kang DI, Soh KS. [Experimental studies on the acute toxicity of bos taurus. ursus thibetanus. moschus extrct solution(BUM) for herbal-acupuncture]. Pharmacopuncture. 2002;5(2):6-24. Korean.
23. Kim YS, Kim CH, Kim YS, Kim NJ. [Experimental studies on the safety of whangryunhaedok-tang extract solution for herbopuncture]. Journal of Korean Medicine. 1999;20(2):54-62. Korean.
24. Kim YJ, Lim CS, Kwon KR. [Study of single dose test of sweet bee venom in rats]. Pharmacopuncture. 2009;12(4):5-32. Korean.