

Single-dose Toxicity of *Guseonwangdo-go* Glucose 5% Intravenous Injection in a Rat Model

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Key Words

Guseonwangdo-go glucose 5%, herbal medicine, intravenous, pharmacopuncture, toxicity test

Abstract

Objectives: The purpose of this study was to examine the single-dose intravenous toxicity of *Guseonwangdo-go* glucose 5% pharmacopuncture (GWG5).

Methods: Forty Sprague-Dawley rats were divided into four groups of five males and five females per group: an intravenous (IV) injection of 1.0 mL of normal saline solution per animal was administered to the control group; IV injections of 0.1, 0.5, and 1.0 mL of GWG5 per animal were administered to the experimental groups (G: 0.1, G: 0.5, and G: 1.0). Observation of clinical signs and body weight measurements were carried out for 14 days following the injections. At the end of the observation period, hematological, biochemical, and histopathological tests, as well as necropsy examinations, were performed on the injected parts.

Results: No mortalities or adverse clinical signs were observed in any of the groups. The body weights of all groups continuously increased. In the hematological and the biochemical tests, females in G-0.1 had minimal changes, but those changes were not dose dependent. On necropsy examination, no abnormalities were observed. In the histopathological test, focal inflammatory cell infiltrations were observed in two female rats, one in the control group and one in G-1.0. Also, one female rat in the control group had an epidermis crust. These

changes were concluded to have been caused by the insertion of the needle into a vein.

Conclusion: The above findings suggest that the lethal dose of GWG5 administered via IV injection is more than 1.0 mL per animal in both male and female rats. Further studies are needed to establish more detailed evidence of its toxicity.

1. Introduction

For centuries, traditional herbal medicine has been administered orally in Asia for the treatment of various conditions [1]. Recently, various routes of administration, such as pharmacopuncture, have been used to deliver herbal medicine.

Guseonwangdo-go was first recorded in Manbyeong-hwechun [2] and was prescribed as one of the tonifying remedies for treating internal damage in Dongeuibogam [3]. Its composition, including *Kaki mannosum* (persimmon frost), is recorded in Bangyakhappyeon, and generally, it is made in the form of soups or rice cakes and is used to boost energy [4].

Research has been performed on *Guseonwangdo-go* since 2000 in Korea. Ju *et al* [5] reported that *Guseonwangdo-go* had an anti-obesity effect and reinforced the immune system. In that study, *Guseonwangdo-go* administered orally showed no cytotoxicity. Jo *et al* [6] conducted a study on the single-dose toxicity of *Guseonwangdo-go* glucose (GWG) administered by intramuscular (IM) injection in Sprague-Dawley (SD) rats, and Kim *et al* [7] conducted a study on *Guseonwangdo-go* glucose 20% (GWG20) administered by intravenous (IV) injection in SD rats. These studies concluded that the lethal doses of GWG and GWG20 were greater

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than 1.0 mL/animal for IM and IV injections, respectively [6, 7].

In Western medicine, the use of dextrose water containing glucose differs based on the concentration of glucose in the water. Generally, 5% dextrose water is used to treat dehydration, and in adults, one injection of 500 — 1000 mL via an IV route is used [8]. Thus, if an IV injection of *Guseonwangdo-go* glucose 5% pharmacopuncture (GWG5) is to be used for human, its safety and maximal dose must be established.

Therefore, this study was designed to evaluate the safety of administering a single-dose of GWG5 through an IV route, even though the lethal dose of GWG20 administered via an IV route, as determined in a previous study, is considered to be higher than 1.0 mL/animal.

2. Materials and Methods

The GWG5 used in the experimental groups was prepared in the facility at the Korean Pharmacopuncture Institute under the Good Manufacturing Practice guidelines. According to Bangyakhappyeon [4], *Guseonwangdo-go* consists of 160 g each of lotus seed (*Nelumbinis semen*), yam rhizome (*Dioscoreae rhizoma*, stir-baked), poria (*Hoelen*), and Job's tears seed (*Coicis semen*), 80 g each of malt (*Hordei fructus germinatus*, stir-baked), dolichos bean seed (*Dolichoris semen*, stir-baked), and fox nut seed (*Euryales semen*), and 40 g of persimmon frost (*Kaki mannosum*). Because glucose is the main ingredient of persimmon frost [9], persimmon frost was adjusted to 5% in the pharmacopuncture preparation.

Forty-six (46)-week-old SD rats were used in this experiment: 20 male rats (body weights: 166.5 — 189.7 g) and 20 female rats (body weights: 133.9 — 148.5 g). Visual inspections and measurements of body weights by using electronic scales (CP3202S, Sartorius, Germany) were conducted on all animals. The general symptoms were observed once a day prior to the start of the experiments, and general symptoms and body weight changes were recorded on the last day of acclimatization.

Group separation was conducted on the last day of acclimatization. All animals were randomly distributed into four different groups with five individuals of each sex per group. The four different groups were labeled as follows: Control:

normal saline solution, G-0.1: low-dose group, G-0.5: mid-dose group, and G-1.0: high-dose group (Table 1). In a pilot test (Biototech Study No.: B13482P), 1.0 mL/animal was administered through an IV route to one male and one female rat, which resulted in no deaths. From this result, the doses for GWG5 in this study were set as follows: 1.0 mL/animal as the high dose (G-1.0), 0.5 mL/animal as the mid-dose (G-0.5), and 0.1 mL/animal as the low dose (G-0.1). The same amount of normal saline solution (Choongwae Pharma Corp., Korea) as that of GWG5 for the high dose group was injected into the animals in the control group, and the results were observed and compared with those of the experimental groups. All injections were administered at a caudal vein at a rate of 2 mL/minute. This experiment was conducted at Biototech, an authorized institution for non-clinical studies, under the regulation of Good Laboratory Practice (GLP) of the Korea Food and Drug Administration's (KFDA's) Notification No. 2012-61 (Guidelines for non-clinical laboratory studies, Aug 24, 2012) [10].

The general symptoms and mortality were observed after 30 minutes, and 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 and on the 3rd, 7th, and 14th day after the injection.

All rats had been fasting for more than 18 hours before necropsy. They were then anesthetized with isoflurane, after which blood was collected from the abdominal aorta. For the hematological test, about 1 mL of the collected blood was placed in an ethylene diamine tetra acetic acid tube (EDTA tube) and was analyzed with a hematology analyzer (ADVIA® 120, Siemens, Germany). For the coagulation test, about 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, after which blood plasma was 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).

Visual inspections of all body organs and tissues were performed on all animals after necropsy. Body organs and

Table 1 Compositions of the experimental groups

Group	GWG5 (mL/animal)	Injection (mL/animal)	Number of animals	
			Male	Female
Control group*	0	1.0	5	5
G-0.1 (Low-dose group)	0.1	0.1	5	5
G-0.5 (Mid-dose group)	0.5	0.5	5	5
G-1.0 (High-dose group)	1.0	1.0	5	5

*normal saline solution.

GWG5, *Guseonwangdo-go* glucose 5% pharmacopuncture.

tissues were extracted 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 (Stata Corp LP, College Station, TX, U.S.A.). The equal variance was tested by using Bartlett's test. If the sample variances were equal, significant results were obtained by 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 significance was 0.05.

3. Results

During the observation, no mortalities or adverse clinical signs were observed in any of the control or the experimental groups. Weight gains were observed in both the experimental and the control groups, but no significant changes were observed in the comparisons of the exper-

imental and control groups. In the hematological test, by Dunnett's *t*-test, the prothrombin time (PT) for female rats in G-0.1 was significantly different from that for female rats in the control group (Table 2). In the biochemical test, by Dunnett's *t*-test, the albumin/globulin ratio (A/G ratio) of female rats in G-0.1 was significantly different from that for female rats in the control group (Table 3). On necropsy, no abnormalities were observed visually. In the histopathological test, focal inflammatory cell infiltrations were observed in two female rats, one in the control group and one in G-1.0. Also, one rat in the control group had an epidermis crust (Table 4).

4. Discussion

Guseonwangdo-go, which consists of *Nelumbinis semen*, *Dioscoreae rhizoma*, *Hoelen*, *Hordei fructus germinatus*, *Dolichoris semen*, *Euryales semen*, and *Kaki mannosum* [4], has been described as strengthening the spirit, boosting energy, helping the digestive system, improving appetite, tonifying deficiency, growing muscles and remov-

Table 2 Hematological values of SD rats

Group	Male				Female			
	Control	G-0.1	G-0.5	G 4 / 1	Control	G-0.1	G-0.5	G 4 / 1
RBC ($\times 10^6/\mu\text{L}$)	7.10 \pm 0.27	7.16 \pm 0.23	7.11 \pm 0.21	6.94 \pm 0.15	7.33 \pm 0.15	7.49 \pm 0.34	7.64 \pm 0.40	7.55 \pm 0.18
HGB (g/dL)	14.8 \pm 0.6	15.0 \pm 0.5	14.9 \pm 0.6	14.4 \pm 0.3	14.6 \pm 0.8	15.4 \pm 0.5	15.3 \pm 0.8	15.2 \pm 0.4
HCT (%)	41.0 \pm 1.6	41.6 \pm 1.6	41.1 \pm 1.4	40.4 \pm 0.8	40.0 \pm 1.1	41.3 \pm 1.2	41.1 \pm 2.4	40.9 \pm 0.6
RBC indices								
MCV (fL)	57.8 \pm 1.0	58.1 \pm 0.9	58.1 \pm 1.1	58.2 \pm 0.7	54.6 \pm 1.3	55.2 \pm 1.3	53.9 \pm 2.0	54.2 \pm 1.1
MCH (pg)	20.8 \pm 0.5	20.9 \pm 0.2	20.9 \pm 0.5	20.8 \pm 0.2	20.0 \pm 1.2	20.5 \pm 20.6	20.0 \pm 0.4	20.1 \pm 0.5
MCHC (g/dL)	35.9 \pm 0.7	36.0 \pm 0.3	36.0 \pm 0.5	35.8 \pm 0.2	36.5 \pm 1.3	37.2 \pm 0.3	37.2 \pm 0.8	37.1 \pm 0.3
PLT ($\times 10^3/\mu\text{L}$)	1176 \pm 95	1205 \pm 64	1205 \pm 127	1159 \pm 140	1233 \pm 99	1252 \pm 58	1280 \pm 216	1178 \pm 107
Reti (%)	4.4 \pm 0.5	4.9 \pm 0.6	4.9 \pm 0.4	4.9 \pm 0.4	2.4 \pm 0.1	2.7 \pm 0.5	3.8 \pm 2.3	2.6 \pm 0.3
WBC ($\times 10^3/\mu\text{L}$)	11.88 \pm 1.93	8.33 \pm 2.63	9.42 \pm 2.22	10.94 \pm 5.29	5.18 \pm 0.74	4.58 \pm 1.41	4.24 \pm 1.00	5.75 \pm 1.25
WBC differential counting								
NEU (%)	15.9 \pm 3.8	14.2 \pm 1.6	13.5 \pm 2.5	13.1 \pm 1.4	13.8 \pm 4.3	16.6 \pm 4.8	17.0 \pm 6.4	16.2 \pm 5.5
LYM (%)	80.6 \pm 4.3	82.7 \pm 1.8	83.5 \pm 2.3	83.9 \pm 1.4	83.2 \pm 4.4	80.5 \pm 4.7	80.2 \pm 6.0	80.7 \pm 5.1
MONO (%)	1.7 \pm 0.2	1.5 \pm 0.2	1.3 \pm 0.1	1.5 \pm 0.3	1.3 \pm 0.2	1.4 \pm 0.1	1.1 \pm 0.4	1.3 \pm 0.5
EOS (%)	0.4 \pm 0.1	0.5 \pm 0.2	0.5 \pm 0.1	0.5 \pm 0.3	1.0 \pm 0.3	0.8 \pm 0.1	1.1 \pm 0.3	1.0 \pm 0.3
BASO (%)	0.3 \pm 0.1	0.2 \pm 0.1	0.2 \pm 0.0	0.2 \pm 0.1	0.1 \pm 0.0	0.2 \pm 0.1	0.1 \pm 0.1	0.1 \pm 0.1
PT (sec)	16.7 \pm 1.0	16.9 \pm 0.9	16.4 \pm 0.3	16.4 \pm 0.4	19.1 \pm 0.2	18.5* \pm 0.4	19.2 \pm 0.5	18.7 \pm 0.3
APTT (sec)	15.5 \pm 0.9	15.1 \pm 1.0	15.1 \pm 1.6	14.4 \pm 1.5	16.0 \pm 0.8	16.3 \pm 0.7	15.4 \pm 0.8	14.7 \pm 1.5

Values are presented as mean \pm standard deviation (S.D.). The number of animals in each group is 5. *significantly different from control by Dunnett's *t*-test (*P* < 0.05).

SD, Sprague-Dawley; 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 3 Biochemical values of SD rats

Group	Male				Female			
	Control	G-0.1	G-0.5	G 4 / 1	Control	G-0.1	G-0.5	G 4 / 1
ALT (U/L)	29.1 ± 2.4	25.4 ± 3.2	27.2 ± 2.5	31.7 ± 3.8	23.0 ± 1.6	21.8 ± 2.8	22.5 ± 4.0	20.5 ± 3.5
AST (U/L)	99.7 ± 31.2	85.8 ± 8.6	93.7 ± 19.6	102.8 ± 28.2	86.9 ± 15.7	95.2 ± 9.7	81.4 ± 10.9	86.0 ± 4.1
ALP (U/L)	801.5 ± 176.5	801.6 ± 108.4	756.7 ± 170.4	916.4 ± 173.5	459.1 ± 97.3	525.8 ± 107.9	457.6 ± 79.4	526.2 ± 146.3
GGT (U/L)	0.45 ± 0.12	0.34 ± 0.13	0.44 ± 0.07	0.37 ± 0.15	0.43 ± 0.16	0.63 ± 0.34	0.60 ± 0.23	0.57 ± 0.11
Glu (mg/dL)	125 ± 21	115 ± 14	116 ± 14	131 ± 8	99 ± 10	0.5 ± 7	107 ± 10	113 ± 13
BUN (mg/dL)	11.0 ± 1.0	11.5 ± 2.7	11.8 ± 1.4	11.3 ± 1.2	13.4 ± 2.5	14.0 ± 3.4	13.4 ± 2.5	116 ± 1.0
Crea (mg/dL)	0.38 ± 0.02	0.40 ± 0.03	0.42 ± 0.02	0.41 ± 0.03	0.45 ± 0.02	0.46 ± 0.03	0.45 ± 0.02	0.44 ± 0.03
T-bili (mg/dL)	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.03	0.02 ± 0.01	0.06 ± 0.07	0.03 ± 0.02
T-Chol (mg/dL)	93 ± 21	85 ± 22	90 ± 19	67 ± 17	70 ± 16	73 ± 12	76 ± 9	65 ± 15
TG (mg/dL)	44 ± 18	50 ± 18	39 ± 20	42 ± 22	11 ± 2	11 ± 3	13 ± 4	11 ± 4
TP (mg/dL)	5.2 ± 0.3	5.1 ± 0.1	5.3 ± 0.2	5.1 ± 0.1	5.3 ± 0.2	5.4 ± 0.3	5.1 ± 0.3	5.4 ± 0.2
Alb (mg/dL)	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.0	2.5 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	2.5 ± 0.1
A/G ratio	0.77 ± 0.06	0.82 ± 0.07	0.75 ± 0.06	0.81 ± 0.05	0.85 ± 0.03	0.79 [*] ± 0.04	0.81 ± 0.01	0.88 ± 0.05
P (mg/dL)	8.79 ± 0.47	8.53 ± 0.47	8.70 ± 0.14	8.93 ± 0.23	7.21 ± 0.68	7.30 ± 0.44	7.26 ± 0.36	7.09 ± 0.39
Ca (mg/dL)	9.8 ± 0.4	9.9 ± 0.3	9.8 ± 0.2	9.7 ± 0.2	9.7 ± 0.3	9.5 ± 0.3	9.5 ± 0.3	9.6 ± 0.3
Na (mmol/L)	139 ± 1	140 ± 1	140 ± 1	139 ± 2	139 ± 1	139 ± 1	139 ± 1	140 ± 2
K (mmol/L)	4.7 ± 0.2	4.5 ± 0.2	4.6 ± 0.3	4.6 ± 0.4	4.5 ± 0.2	4.5 ± 0.2	4.5 ± 0.3	4.4 ± 0.2
Cl (mmol/L)	104 ± 1	103 ± 1	104 ± 1	104 ± 1	106 ± 1	106 ± 1	106 ± 1	107 ± 3

Values are presented as mean ± standard deviation (S.D.). The number of animals in each group is 5. *significantly different from control by Dunnett's *t*-test ($P < 0.05$).

SD, Sprague-Dawley; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transpeptidase; Glu, glucose; BUN, blood urea nitrogen; Crea, creatinine; T-Bil, total bilirubin; T-Chol, total cholesterol; TG, tri-glycerides; TP, total protein; Alb, albumin; A/G ratio, albumin/globulin ratio; P, phosphorus; Ca, calcium; Na, sodium; K, potassium; Cl, chloride.

Table 4 Summary of histopathological findings

Group	Inflammatory Cell Infiltration		Crust, Epidermis		
	Number examined	Minimal	Number examined	Minimal	
Male	Control	5	0	5	0
	G-0.1	5	0	5	0
	G-0.5	5	0	5	0
	G-1.0	5	0	5	0
Female	Control	5	1	5	1
	G-0.1	5	0	5	0
	G-0.5	5	0	5	0
	G-1.0	5	1	5	0

ing dampness-heat [3]. Pharmacopuncture is a unique treatment of traditional Korean medicine and combines acupuncture and herbal medicine. The treatment area of pharmacopuncture is the common acupoints, tender points, or responsive points, and it may be administered via subcutaneous or IM injection [11]. The bioavailabilities of medicines differ based on the routes used to administer them, i.e., IV, oral, intraperitoneal, hepatportalvenous, and intrarectal [12]. Because vessels provide the quickest pathway for medicine absorption, IV pharmacopuncture injection may have some advantages over the oral administration of herbal medicines [13]. Also, IV injection is listed as a legal manipulation in Korean classification procedures in medicine [14].

Studies of IV pharmacopuncture injection have been conducted in Korea on wild ginseng [14, 15], Water-soluble Carthami-flos (WCF) [16], *Angelica gigas* [17], *Glycyrrhizae radix* [18], Saeng Maek San [19], and GWG20 [7]. In China, IV injections of several herbal medicines, such as *Houttuyniae herba*, *Bupleuri radix*, *Astragali radix*, and *radix salviae miltiorrhizae composita*, were administered to animals [20]. Generally, the concentration of the medicine is related to the effect or the side effect. Also, the method of administration may affect the therapeutic level in serum. Because IV injection elevates the plasma concentration of the medicine more rapidly than the IM injection [13], the safety of IV injection of GWG5 must be established, even though, from a previous study, the lethal dose of GWG20 is thought to be higher than 1.0 mL/animal [7].

In this study, IV injection of 1.0 mL of normal saline solution per animal was administered to the control group, and IV injections of 0.1, 0.5, and 1.0 mL of GWG5 per animal were administered to the experimental groups (G-0.1, G-0.5, and G-1.0). Observations of clinical signs and body weight measurements were carried out for 14 days following the injections. During the observation, no mortalities or adverse clinical signs were observed in any of the groups. Weight gains were observed in all groups, but no significant differences between the experimental and the control groups were observed.

In the hematological test, by Dunnett's *t*-test, the PT of female G-0.1 rats was significantly different from that of female control rats (Table 2). However, that difference is not considered to be significant because the difference was minimal and dose independent. In the biochemical test, by Dunnett's *t*-test, the A/G ratio of female G-0.1 rats was significantly different from that of female control rats (Table 3). However, this difference is not thought to be significant because it is dose independent. On necropsy, no abnormalities were observed when visual inspections were conducted on the rats in all groups. In the histopathological test, focal inflammatory cell infiltrations were observed in two female rats, one in the control group and one in G-1.0. Also, one rat in the control group had an epidermis crust (Table 4). These changes were concluded to have been caused by insertion of the needle into a vein.

Based on the above data, we consider the approximate lethal dose of GWG5 to be higher than 1.0 mL/animal. Therefore, GWG5 is a relatively safe pharmacopuncture that can be used for treatment. However, further studies should be performed and repeated IV injection tests, as

well as sub-acute and chronic toxicity tests, should be conducted before GWG5 is used for clinical applications.

5. Conclusion

This study was performed to analyze the single-dose IV toxicity of GWG5. No meaningful changes were observed in general symptoms, body weights, hematological and biochemical test results, and necropsy histopathological observations. Therefore, the approximate lethal dose of GWG5 IV injection is considered to be more than 1.0 mL/animal in both male and female rats.

Conflict of interest

The authors declare that there are no conflict of interest.

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