

# Single-dose Toxicity of *Guseonwangdo-go* Glucose 20% Intravenous Injection in Sprague-Dawley Rats

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## Key Words

*Guseonwangdo-go*, herbal medicine, intravenous injection, pharmacopuncture, toxicity

## Abstract

**Objectives:** This study was performed to evaluate the single-dose intravenous toxicity of *Guseonwangdo-go* glucose 20% pharmacopuncture.

**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 group 1 (G1, control group); an IV injections of 0.1, 0.5, and 1.0 mL of *Guseonwangdo-go* glucose pharmacopuncture per animal were administered to experimental groups 2, 3, and 4 (G2, G3, and G4), respectively. General symptoms, body weights, hematological and biochemical test results, and necropsy histopathological observation were recorded in all groups. In the statistical analyses, significance was determined by using the one-way analysis of variance (ANOVA). The significance level was 0.05 in all comparisons.

**Results:** For 14 days, no deaths or abnormalities were observed in any of the 4 groups. The body weights of all groups continuously increased during the observation period. In the hematological test, the WBC count was significantly increased in female rats of G4 compared to

the control group, but this difference was considered not to be statistically meaningful. No significant biochemical changes were observed. On necropsy, crust formation was observed in one rat of the control group, and granulation tissues were observed around the injection site in one rat of G4; these changes were concluded to have been caused by injection of the needle into a vein.

**Conclusion:** The findings suggest that the lethal dose of *Guseonwangdo-go* glucose pharmacopuncture is more than 1.0 mL per animal in both male and female rats. Thus, we can conclude that *Guseonwangdo-go* glucose pharmacopuncture injection is relatively safe to use in acute toxicity tests. Further studies are needed to establish more detailed evidences of its toxicity.

## 1. Introduction

Neurofibromatosis type 1 (NF1), known as Von Recklinghausen disease, is one of the most common gene disorders to human. It has an incidence of 1 per 3,500 – 4,000. Pharmacopuncture is a type of neo-acupuncture that treats diseases by injecting herbal fluids at acupoints to gain the simultaneous effects of acupuncture and herbal medicine [1]. *Guseonwangdo-go* was prescribed as a tonifying medicine for treating internal damage in *Donggeui Bogam* [2]. It can be consumed as a functional food because it is usually made into rice cakes. Ju *et al.* (2000) [3] conducted the only

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study on *Guseonwangdo-go* in Korea. In this experimental study on rats and cells, *Guseonwangdo-go* was reported to contribute to keeping homeostasis and to have an effect on anti-obesity and immune-system reinforcement. From these results, *Guseonwangdo-go* pharmacopuncture was devised for the treatment of obesity and a weakened immune system.

The previous experimental study, however, only proved the anti-obesity effect by administrating dried samples of *Guseonwangdo-go* through an oral route to animals. No studies on its toxicity when injected in animals have been performed yet. Therefore, this study was designed to evaluate the safety of administering a single-dose of *Guseonwangdo-go* glucose through an intravenous route.

## 2. Materials and Methods

The *Guseonwangdo-go* glucose pharmacopuncture used in the experimental groups was prepared in the facility at the Korean Pharmacopuncture Institute under the Good Manufacturing Practice guidelines. According to *Bangyak Hapgyeon* [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), fox nut seed (*Euryales Semen*), and 40 g of persimmon frost (*Kaki Mannosum*). Because glucose is the main ingredient of persimmon frost [5], persimmon frost was adjusted to 20% in the pharmacopuncture preparation.

Forty six-week-old Sprague-Dawley (SD) rats were used in this experiment: 20 male rats (body weights: 193.9 — 210.5 g) and 20 female rats (body weights: 144.3 — 178.0 g). Visual inspections 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 males and females within each groups being weighed and examined for general symptoms and body-weight changes on the last day of acclimatization. No abnormalities were found.

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: 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 (Biototech Study No.: B12880P), 1.0 mL/animal was administered through an intravenous route to one male and one female rat, which 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 the high dose (G4), 0.5 mL/animal as the mid-dose (G3), and 0.1 mL/animal as the 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 G1, 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/min (Table 1).

This experiment was conducted at Biototech, an authorized institution for non-clinical studies, under the regulation of Good Laboratory Practice (GLP) of KFDA Notification No. 2012-61 (Guidelines for non-clinical laboratory studies, Aug 24, 2012) [6].

The general symptoms and mortality were observed after 30 minutes, 1, 2, 4, and 6 hours on the day of injection (day 0). From the next day to the 14<sup>th</sup> day after the injection, the general symptoms were examined once a day.

**Table 1** Compositions of the experimental groups

Group	Glucose Dose (mL/animal)	Injection (mL/animal)	Number of animals	
			Male	Female
G1: Control group	0	1.0*	5	5
G2: Low-dose group	0.1	0.1	5	5
G3: Mid-dose group	0.5	0.5	5	5
G4: High-dose group	1.0	1.0	5	5

\*normal saline solution.

The body weights were measured on the day of the injection and on the 3<sup>rd</sup>, 7<sup>th</sup>, and 14<sup>th</sup> day after the injection.

All animals were fasted during 18 hours before autopsy, the 15<sup>th</sup> day after the injection. 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 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. 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, Ger-

many).

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

**Table 2** Hematological Values of SD Rats

Group / Dose (mL/animal)	Male				Female			
	G1 / 0	G2 / 0.1	G3 / 0.5	G4 / 1	G1 / 0	G2 / 0.1	G3 / 0.5	G4 / 1
RBC ( $\times 10^6/\mu\text{L}$ )	7.03 $\pm$ 0.45	6.96 $\pm$ 0.34	6.96 $\pm$ 0.24	6.85 $\pm$ 0.26	6.98 $\pm$ 0.39	7.14 $\pm$ 0.3	7.33 $\pm$ 0.17	7.15 $\pm$ 0.26
HGB (g/dL)	14.3 $\pm$ 0.6	14.2 $\pm$ 0.5	14.2 $\pm$ 0.5	14.1 $\pm$ 0.1	14.1 $\pm$ 0.7	14.1 $\pm$ 0.5	14.7 $\pm$ 0.4	14.4 $\pm$ 0.5
HCT (%)	43.3 $\pm$ 1.8	43.4 $\pm$ 1.2	43.8 $\pm$ 1.6	43 $\pm$ 1	41.4 $\pm$ 1.9	41.2 $\pm$ 1.4	43.2 $\pm$ 0.8	42.5 $\pm$ 1.8
RBC indices								
- MCV (fL)	61.7 $\pm$ 3	62.5 $\pm$ 2.4	63 $\pm$ 1.1	62.9 $\pm$ 1.7	59.3 $\pm$ 1.2	57.8 $\pm$ 0.8	58.9 $\pm$ 1	59.4 $\pm$ 2
- MCH (pg)	20.3 $\pm$ 0.9	20.4 $\pm$ 0.8	20.4 $\pm$ 0.3	20.6 $\pm$ 0.8	20.1 $\pm$ 0.5	19.8 $\pm$ 0.5	20.1 $\pm$ 0.3	20.1 $\pm$ 0.4
-MCHC (g/dL)	33 $\pm$ 0.5	32.7 $\pm$ 0.2	32.5 $\pm$ 0.1	32.8 $\pm$ 0.5	33.9 $\pm$ 0.3	34.3 $\pm$ 1.1	34.1 $\pm$ 0.3	33.8 $\pm$ 0.6
PLT ( $\times 10^3/\mu\text{L}$ )	1212 $\pm$ 136	1190 $\pm$ 131	1260 $\pm$ 154	1140 $\pm$ 77	1199 $\pm$ 183	1437 $\pm$ 106	1299 $\pm$ 116	1339 $\pm$ 232
Reti (%)	5.2 $\pm$ 0.6	4.7 $\pm$ 0.9	5.5 $\pm$ 0.4	4.9 $\pm$ 0.3	3.3 $\pm$ 0.5	4 $\pm$ 1.5	2.7 $\pm$ 0.6	3 $\pm$ 1.1
WBC ( $\times 10^3/\mu\text{L}$ )	8.83 $\pm$ 1.96	11.3 $\pm$ 2.21	8.64 $\pm$ 2.53	8.79 $\pm$ 2.28	3.13 $\pm$ 0.81	3.8 $\pm$ 1.38	3.49 $\pm$ 1.11	5.50 $\pm$ 1.36*
WBC differential counting								
- NEU (%)	17.8 $\pm$ 8.2	15.7 $\pm$ 5.9	16.6 $\pm$ 4	15.8 $\pm$ 4.3	21.3 $\pm$ 8.4	18.8 $\pm$ 4.3	17.7 $\pm$ 6.5	19.7 $\pm$ 6.1
- LYM (%)	77.2 $\pm$ 9	79.8 $\pm$ 5.8	79.3 $\pm$ 4.4	79.3 $\pm$ 5.9	75.4 $\pm$ 8.1	78.1 $\pm$ 4.5	78.7 $\pm$ 6.2	77.1 $\pm$ 6.5
- MONO (%)	2.7 $\pm$ 0.9	2.5 $\pm$ 1.1	2.3 $\pm$ 0.3	2.5 $\pm$ 1.4	1.6 $\pm$ 1	1.7 $\pm$ 0.5	1.7 $\pm$ 0.6	1.4 $\pm$ 0.1
- EOS (%)	0.5 $\pm$ 0.2	0.4 $\pm$ 0.2	0.3 $\pm$ 0.2	0.4 $\pm$ 0.2	1 $\pm$ 0.7	0.9 $\pm$ 0.3	1 $\pm$ 0.2	0.8 $\pm$ 0.5
- BASO (%)	0.2 $\pm$ 0.1	0.2 $\pm$ 0	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1
PT (sec)	17.5 $\pm$ 0.5	17.4 $\pm$ 0.4	17.4 $\pm$ 0.4	17.5 $\pm$ 0.6	18.3 $\pm$ 0.9	18.2 $\pm$ 0.3	18.7 $\pm$ 0.8	18 $\pm$ 0.6
APTT (sec)	14.1 $\pm$ 0.8	14.2 $\pm$ 1.1	13.9 $\pm$ 0.8	14 $\pm$ 1.3	14.8 $\pm$ 1.6	14.6 $\pm$ 1.1	14.4 $\pm$ 1.2	14.2 $\pm$ 0.9

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

### 3. Results

During the observation, no deaths or abnormalities were observed in either the control or the experimental groups. However, crust formations were observed in the left ear, left neck, and left shoulder of one female of the control group.

The body weights in both the control and the experimental groups continuously increased during the observation period, but no significant changes in body weight were observed.

In the hematological test, the WBC counts were significantly increased in the female rats of G4 compared to G1.

However, no significant changes were found between the experimental groups and the control group (Table 2).

No significant biochemical changes were observed when the results of the experimental groups were compared with those of the control group.

On necropsy, crust formation was observed in the posterior neck and the left posterior cranium of one female rat of the control group. However, no abnormalities were observed when visual inspections were conducted on both the control group and the experimental groups (Table 3).

In the tolerance test on the injection sites, the crust found in the one female rat of the control group was confirmed, in the autopsy, to be an ulcer/erosion with crust. Gran-

**Table 3** Summary of necropsy findings

Group / Dose (mL/animal)		Number of animals	Number examined	Unremarka- ble findings	Skin: crust, left posterior neck and left posterior cranium
Male	G1 / 0	5	5	5	0
	G2 / 0.1	5	5	5	0
	G3 / 0.5	5	5	5	0
	G4 / 1.0	5	5	5	0
Female	G1 / 0	5	5	5	1
	G2 / 0.1	5	5	5	0
	G3 / 0.5	5	5	5	0
	G4 / 1.0	5	5	5	0

External surface and other organs in the body cavity were unremarkable.

**Table 4** Summary of Histopathological Findings

Group / Dose (mL/animal)		Number of animals	Skin:		Injection site:	
			ulcer/erosion, with crust		granulation tissue, perivascular, lateral vein	
			Number examined	<+>	Number examined	<+>
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	1	5	0
	G2 / 0.1	5	-	-	5	0
	G3 / 0.5	5	-	-	5	0
	G4 / 1.0	5	-	-	5	1

+ mild grade. <+> presence on "presence or not" basis.

ulation tissues were observed around the injection site - perivascular area of the lateral vein - of one female rat of G4. However, no abnormal histopathological signs were observed in either the control or the experimental groups (Table 4).

#### 4. Discussion

A toxicant refers to any substance that may harm the body in a sufficient concentration. Swiss scientist Paracelsus said, "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy" [7]. Therefore, a toxicity test should be conducted to prove the safety of a drug before its clinical use because any drug with minimum side effects can be toxic, depending on the dose.

There are three different types of toxicity tests: an acute toxicity test injecting a single-dose, a sub-acute toxicity test injecting a dose repeatedly for one month, and a chronic toxicity test injecting a dose repeatedly for over three months [8]. The toxicity test has recently been perceived as being better for focusing on finding the relationship between the dose and toxicity changes before and after the administration than for simply acquiring LD<sub>50</sub> values [9]. The safety of a pharmacopuncture with respect to its toxicity needs to be proven because a pharmacopuncture uses an injection route, which is different from the oral administration route used in previous treatments.

*Guseonwangdo-go* was first prescribed in *Manbyeonghwechun* [10]. In the *Japbyeong-pyeon* Chapter of *Dongui Bogam*, *Guseonwangdo-go*, as one of tonifying remedies for internal damage, was recorded to "nourish essence-spirit, reinforce source-qi, fortify spleen and stomach, digest diets, recover depletion, produce muscles, and eliminate dampness-heat" [2].

*Guseonwangdo-go* is also recorded in *Bangyak Happyeon* [4]. It is usually made in the form of rice cakes due to its starch-rich and mild drug properties. Also, it can be used as functional food in modern society as it can be eaten with pleasure as gruel.

Ju *et al.* (2000) [3] reported that *Guseonwangdo-go* suppressed the proliferation of preadipocytes in the adipocytes-formation process and caused white blood cells, monocytes, and hemoglobin to increase and plasma glutamic-oxaloacetic transaminase (GOT) and cholesterol to decrease. In addition, *Guseonwangdo-go* was reported to be more efficient in the activation of a complement as the glucose content was higher, and it showed no or marginal cytotoxicity, with an IC<sub>50</sub> value of more than 5000 µg/mL.

Therefore, it can be utilized for clinical purposes because it is expected to have effects on immunity reinforcement,

and obesity prevention and to have minimum side effects. However, toxicity tests in animals have not been reported in Korea yet.

In China, injectable formulations of Chinese medicine such as *Houttuyniae Herba*, *Bupleuri Radix*, *Astragali Radix*, and *Radix Salviae Miltiorrhizae* Composita were made and administered through intravenous injection to animals [11]. Many studies have been conducted in various areas such as clinical treatment [12] and side-effect management [13].

However, pharmacopuncture studies in Korea are mainly conducted via subcutaneous or intramuscular injection through an acupoint or a tender point. Studies of intravascular pharmacopuncture injection have rarely been conducted, the only exception being a study on wild Ginseng *Radix* pharmacopuncture [9, 14], because of the limited use of intramuscular injection in medical practice in Korea. Therefore, a toxicity test for *Guseonwangdo-go* IV injection was conducted in this experiment.

SD rats have been widely used in safety tests of drugs and related materials because of the system of their supply is stable and because they have consistent reactions to drugs [15]. Thus, they are considered as appropriate for use as test animals.

No deaths from toxicity occurred as a result of *Guseonwangdo-go* glucose injection into rats of both sexes, and abnormal symptoms were not observed. In regards to changes in weight, no significant differences were observed between the control group and the experimental group.

In the hematological test, female rats of G4 showed a significant increase in WBC counts compared to the control group. However, the result demonstrated a small variance and showed no dose-dependency or consistent variance by sex. Thus, the result was not considered to be statistically meaningful. No significant changes were observed in the biochemical test when the results from the experimental groups and the control group were compared.

On autopsy, a crust was observed with the naked eyes on the posterior neck and the cranium of one female rat of the control group. It was confirmed to be an ulcer or erosion, but it was concluded to be a one-time or accidental change. In the local tolerance test, granulation tissue was observed around the injection site in one female rat of G4. However, the formation of granulation tissue is a general mechanism of wound healing, as are angiogenesis and increased vascular permeability [16]. The formation of granulation tissue was observed in only one individual, so it was concluded to be due to changes caused by insertion of the needle into the vein. No other results were found in the autopsy and the local tolerance test on the injected sites.

A single-dose of *Guseonwangdo-go* glucose was injected

through an IV route into SD rats, and no toxicity was observed in the 0.1-, 0.5-, and 1.0-mL injection groups. Thus, we consider its approximate lethal dose to be over 1.0 mL/animal in both sexes, and we can conclude that it is relatively safe to use in an acute toxicity test. As no toxicity was observed in a maximum 1.0 mL injection for SD rats with body weights of 144.3 — 210.5 g, about 238 — 347 mL of *Guseonwangdo-go* glucose 20% intravenous injection may be a safe dosage in humans weighting 50 kg.

Further studies should be conducted to evaluate the toxicity more accurately for clinical applications. Also, toxicity tests for non-rodents and sub-acute or chronic toxicity tests with repeated administration should be conducted.

## 5. Conclusion

A single-dose of *Guseonwangdo-go* glucose was injected through an IV route into SD rats, and no toxicity was observed in the 0.1-, 0.5-, and 1.0-mL injection groups. Thus, the approximate lethal dose of *Guseonwangdo-go* glucose is considered to be over 1.0-mL/animal.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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