

Single Oral Dose Toxicity Evaluation of *Samul-tang*, a Traditional Herbal Formula, in Crl:CD (SD) Rats

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Background: *Samul-tang* (*Si-Wu-Tang*, *SMT*) is a traditional herbal formula, which has been widely used to treat various diseases such as menstrual irregularity, bleeding and leucorrhea. Although many studies have investigated the pharmacological properties of *SMT*, its toxicity information has not yet been fully elucidated.

Methods: Five Sprague Dawley (SD) rats of each sex were given a single dose (5000 mg/kg) of *SMT* by gavage; control rats received the vehicle only. After the single administration, mortality, clinical signs, body weight changes and gross findings were monitored for 15 days in accordance with Good Laboratory Practice (GLP) principles.

Results: In a single oral dose toxicity study, there was no adverse effect on mortality, clinical sign, body weight change or gross finding in any treatment group.

Conclusions: The results indicate that *SMT* did not induce toxic effects at a dose level up to 5000 mg/kg in rats and its median lethal dose (LD_{50}) was considered to be over 5000 mg/kg/day body weight for both genders.

Key Words : *Samul-tang*; acute-toxicity; Sprague Dawley rat; Good Laboratory Practice

Introduction

Samul-tang (*SMT*, *Si-Wu-tang* in Chinese; *Shimotsu-to* in Japanese) is traditional herbal formula composed of 4 different crude herbs; *Angelicae Gigantis Radix*, *Cnidii Rhizoma*, *Rehmanniae Radix Preparata* and *Paeoniae Radix*. *SMT* has been used for treatment of gynecological diseases such as postmenopausal syndrome, menstrual irregularity, infertility and toxemias of pregnancy in traditional medicine (TM). To date, many researchers have reported its various pharmacological effects including anti-inflammation^{1,2)}, anti-neural damage³⁾ and hematopoiesis^{4,5)}. Despite various efficacy of *SMT*,

few scientific works have explored the safety of this herbal medicine.

Toxicology is a subject to study safety and the adverse effects of chemicals or physical agents⁶⁾. Recently, drug toxicology studies have not been applied only to single compounds but to crude extracts as well as cocktail drugs like herbal formulas. Safety evaluation of the drugs includes acute, subacute, subchronic, chronic and genetic toxicity tests. Among them, acute toxicity refers to the adverse effects occurring after oral administration of single dose or multiple dose of substance given within 24 hours⁷⁾. The purposes of acute toxicity tests are to obtain biochemical and pathological

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Table 1. Crude Composition of SMT

Scientific name	Amount (g)	Source
Angelicae Gigantis Radix	4.69	Yeongcheon, Korea
Cnidii Rhizoma	4.69	Yeongcheon, Korea
Paeoniae Radix	4.69	Hwasun, Korea
Rehmanniae Radix Preparata	4.69	Jangheung, Korea
Total amount	18.76	

changes, which provide information for sub-acute toxicity tests. Thus, acute toxicity studies in animals are an essential step for single-dose-administered pharmaceuticals that are intended for human use.

We here investigated the acute toxicity of SMT in Sprague Dawley (SD) rats, to evaluate its safety and toxicity profiles. Due to the results of our toxicity study, we have established scientific evidence that SMT is harmless.

Materials and Methods

1. Preparation of SMT

A decoction of SMT was prepared as previously described⁸. Briefly, a mixture of four ground herbal medicines (Table 1) was boiled in distilled water at 100°C for 2 h. The extract was condensed using freeze-drying (yield: 33.3%), dissolved in distilled water and mixed well. The solution was filtered through a SmartPor GHP syringe filter (0.2 μm pore size, Woongki Science, Seoul, Korea).

2. Animals and maintenance

Twenty specific pathogen-free 5-week-old Sprague Dawley (SD) rats of each sex were obtained from Orient Bio Co. (Seoul, Korea). All the rats were housed at a constant temperature (23±3°C) and humidity (50±10%) with a 12 h light/12 h dark cycle, 1020 air changes per hour and a light intensity of 150-300 Lux. The animals were kept in stainless steel cages at ≤2-3 animals per cage for the observation period. They were fed a rodent chow diet (PMI Nutrition International, Shoreview, MN,

USA) and sterilized tap water *ad libitum*. This study was performed in the Korea Institute of Toxicology (earned AAALAC International accreditation in 1998) under the Good Laboratory Practice Regulations (GLPR) for Nonclinical Laboratory studies⁹. The present study was monitored by the Organization for Economic Cooperation and Development (OECD) and the Korea Food and Drug Administration (KFDA).

3. Experimental groups and treatments

To determine a proper dosage of SMT for acute toxicity tests, we performed a test of a single oral administration of SMT at a dose level of 1250, 2500 or 5000 mg/kg/day. SMT did not induce any toxic effect at any of these doses. Based on these results, the highest dose level of 5000 mg/kg/day was chosen for the acute toxicity study. Healthy animals were divided into four groups (n = 5 per group) using Path/Tox System (Version 4.2.2, Xybion Medical System Corporation, Morris Plains, NJ, USA). Because oral administration is the clinically intended route for SMT, it was administered by oral gavage in the present study. Prior to each daily administration, SMT was suspended in distilled water. The daily application volume (10 mL/kg body weight) of SMT was calculated based on the recorded body weights of individual animals. The vehicle groups received an equal volume of distilled water.

4. Clinical signs and mortality

Clinical signs and mortality were recorded

Table 2. Mortality in Male and Female Rats Treated with SMT

Sex	Dose (mg/kg)	Day of test														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Male	0	*0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	5000	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Female	0	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	5000	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

* Number of dead animals / number of animals per group

continuously until 6 h after treatment and then once a day from the following day throughout the study period.

5. Body weights

Body weights were measured before SMT treatment (day 1) and on days 2, 4, 8 and 15 after treatment.

6. Gross findings

All the animals were anesthetized by carbon dioxide and then sacrificed by exsanguination from aorta at the end of the experiments. Gross examinations were observed with naked eyes to all vital and tissues.

7. Statistical analyses

Body weights were presented as mean \pm standard deviation (SD). Statistical analyses were performed F-test using the Path/Tox System 4.2.2. Differences from the normal control were regarded as significant if $p < 0.05$. Statistical analyses for calculating the median lethal dose (LD₅₀) value were not performed because no mortality was observed.

Results

1. Mortality and clinical signs

Over the 15 days of the experimental period, no deaths occurred in SMT-untreated or -treated groups of either sex (Table 2). Oral administration of SMT had no toxic effect on the treatment-related clinical signs compared with the vehicle control group (Table 3).

2. Body weights

In both sexes, there was no adverse effect in the SMT-administered group compared with the vehicle control group (Fig. 1).

3. Gross findings

No treatment-related gross pathological changes in internal organs including lung, heart, thymus, stomach, liver, adrenal, spleen were observed in any groups (Table 4).

Discussion

Traditional herbal medicines including herbal formulas and medicinal herbs have been used for

Table 3. Clinical Signs in Male and Female Rats Treated with SMT

Sex	Dose (mg/kg)	Days after test														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Male	0	*0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	5000	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Female	0	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	5000	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

* Number of animals with sign / total number of animals observed

Table 4. Gross Findings in Male and Female Rats Treated with SMT

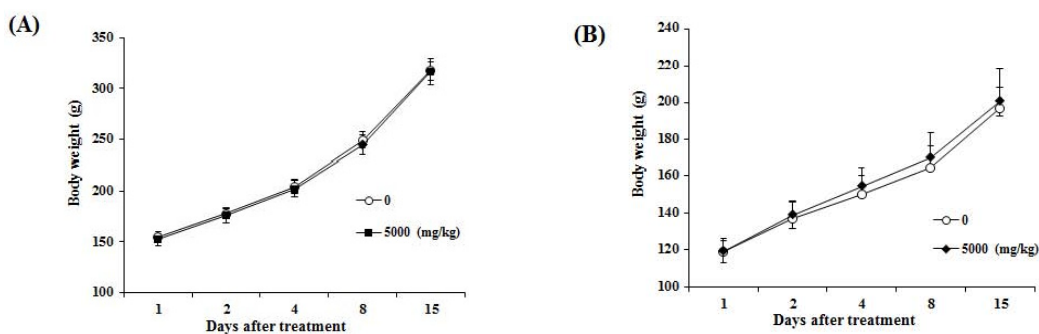
Sex	Dose (mg/kg)	Gross findings	Frequency
Male	0	No remarkable findings	5/5
	5000	No remarkable findings	5/5
Female	0	No remarkable findings	5/5
	5000	No remarkable findings	5/5

centuries in Korea, China and Japan. In general, herbal formulas are made up of several different medicinal herbs that contain various active compounds. Many researchers have worked to establish scientific evidence of the efficacies of herbs and herbal prescriptions^{10,11,12}). However, recent studies have led to increasing concern about the safety and toxicity of herbal prescriptions^{13,14}). Contamination of herbs with metals and dosage miscalculation can lead to an increased risk of side effects¹⁰). Thus, control of herb quality and evaluation of herb toxicity are urgently required to provide essential information for their clinical use.

Several previous studies indicated safety of SMT using animal models. Ma *et al.* reported that SMT didn't have any toxic effect on ICR mouse at the doses of up to 5000 mg/kg¹⁵). Yu *et al.* also reported that there was no adverse effect on SD rats at a dose of 1500 mg/kg during repeated administration of SMT for 28 days¹⁶). These studies suggested that SMT could be a safe prescription for humans.

However, these studies were not performed under GLP standards. GLP refers to a system of quality management controls for process and condition in non-clinical laboratory studies⁹). The toxicity evaluation of a chemical mixture is necessary to guarantee its safety, therefore toxicity tests must be conducted in compliance with GLP guidelines to ensure reliability. Our study was conducted under GLP guidelines and confirmed the safety in male and female SD rats fed a single dose (5000 mg/kg) of SMT by GLP practices.

Consistent with previous results, the present study using SD rats showed no abnormal changes in mortality, body weight or clinical signs in rats of either sex (Tables 2-4 and Fig. 3). These results imply that SMT did not induce any adverse effects at an oral dose of up to 5000 mg/kg/day. Thus, the LD₅₀ was considered to be greater than 5000 mg/kg regardless of sex. Moreover, a single dose of SMT in humans is about 18.76 g dried herb, which is equivalent to 6.25 g of SMT extract (yield = 33.3%).

**Fig. 1.** Effect of SMT on body weight in SD rats.

(A) Male or (B) female rats were fed with SMT extract (0 or 5000 mg/kg) and body weight changes were monitored for 15 days. Results presented as mean \pm SEM. SMT: *Samul-tang*.

Considering that the average weight for an adult is 60 kg, this dose for human is equal to 104.1 mg of SMT extract/kg. This dosage is 48-fold lower than the LD₅₀ of SMT.

In conclusion, we confirmed SMT shows no toxicity in SD rats after treatment with a single oral dosage. Since traditional herbal formulas are usually taken for a longer period, further studies will be needed to establish the safety information on SMT including repeated oral toxicity and genotoxicity.

Conflicts of Interest

The authors declare that they have no competing interests.

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