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Original Article

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

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Objective: *Leejung-tang* (*Rechu-to* in Japanese) is a traditional Korean herbal formula used for treatment of gastrointestinal disorders such as vomiting, stomach pain, chronic gastritis and gastrointestinal ulceration. The present study was carried out to investigate the potential acute toxicity of *Leejung-tang* water extract (LJT) by a single oral dose in Crl:CD (SD) rats in compliance with current guidelines.
Methods: In the preliminary study, there were no adverse effects such as death, clinical signs, and body weight changes at dose levels of 500, 1000, and 2000 mg/kg/day body weight. Based on the results, a dose of 2000 mg/kg was selected as the toxicological limited dose. LJT was administered once by gavage to male and female rats at dose levels of 0 and 2000 mg/kg bodyweight. During the study period, mortalities, clinical findings, and body weight changes were observed for 14 days following the administration. On day 14 after the treatment, the animals were sacrificed by carbon dioxide overdose and complete gross postmortem examinations were performed.
Results: In present study, no treatment-related deaths were observed. There were no adverse effects on clinical signs and body weight changes. In addition, there were no observed gross findings in all groups except for a kidney cyst in the 2000 mg/kg/day female group.
Conclusion: The results indicated that LJT did not induce toxic effects at a dose level up to 2000 mg/kg in rats and its median lethal dose (LD_{50}) was considered to be over 2000 mg/kg/day body weight for both genders.

Key Words : traditional Korean herbal formula; *Leejung-tang*; single oral dose toxicity

Introduction

Leejung-tang (*Rechu-to* in Japanese and *Lizhong-tang* in Chinese) is a Korean traditional herbal formula composed of 4 different crude herbs; *Ginseng Radix*, *Atractylodis Rhizoma*, *Glycyrrhizae Radix*, *Zingiberis Rhizoma*. *Leejung-tang* water extract (LJT) has been used for treatment of gastrointestinal disorders such as vomiting, stomach pain, chronic gastritis and gastrointestinal ulceration in Korea, Japan, and China for a long time. These properties of LJT have been proved by various *in vivo* and *in vitro* experiments[1-4]. It is reported that LJT increases gastrointestinal motility[1]. Other previous studies showed that LJT possesses immunomodulation and anti-cancer activity[2,3]. In addition, a recent study reported that LJT attenuated atopic dermatitis in NC/Nga mice via suppression of Th2 differentiation by IFN-gamma response in

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immunoglobulin (Ig) E hyperproduction[4].

As mentioned above, studies on the efficacies of LJT have been extensively conducted by several researchers, but no studies have been published on the toxicity of LJT performed in compliance with the current regulatory guidelines. Toxicity studies of other herbal formulas such as *Palmul-tang*, *Ssanghwa-tang*, *Sipjeondaebo-tang*, *Ojeok-san*, *Bangpungtongsung*, and *Gumiganghwal-tang* have been conducted. These toxicity studies revealed that oral administration of herbal formulas did not cause any adverse effects[5-14]. Due to the results of toxicity studies, we have established scientific evidence that herbal formulas are harmless.

According to OECD guidelines (2001)[15], acute toxicity is the toxicity produced by a pharmaceutical when it is administered in one or more doses during a single period that does not exceeding 24 h. To evaluate the acute toxicity of a pharmaceutical, many researchers perform an acute toxicity test in compliance with regulatory guidelines. Data from acute toxicity tests can be used to screen for toxicity of a pharmaceutical or to determine if a compound is toxic. Thus, acute toxicity studies in animals are usually necessary for single-dose-administered pharmaceuticals that are intended for human use.

To establish the safety and toxicity of LJT, we conducted to evaluate concentration changes of heavy metal, residual pesticides and sulfur dioxide in LJT[16]. As a result of the study, residual pesticides and sulfur dioxide after decoction in LJT were not detected. However, little is known about toxicity and safety of LJT, and acute toxicity study has not been done until now.

Therefore, as a series of our study on establishment of safety and toxicity of LJT, we investigated the potential acute toxicity of LJT in Crl:CD (SD) rats.

Materials and Methods

1. Preparation of LJT

A formulation of LJT was prepared in our laboratory from a mixture of chopped crude herbs purchased from Omniherb (Yeongcheon, Korea) and HMAX (Chungbuk, Korea). LJT was prepared as described in Table 1 and extracted in distilled water at 100°C for 2 h. The extract was evaporated to dryness and freeze-dried (yield; 24.8%).

2. Animals

Specific pathogen-free SD rats of each sex were purchased from Orient-Bio (Seoul, Korea) at 5 weeks old and used after one week of quarantine and acclimatization. Only rats which remained in good physical condition during acclimatization in the animal room were selected for the study. The rats were housed in a room maintained at a temperature of 23±3˚C, a relative humidity of 50±10%, an air ventilation frequency of 10-20 times/h, and a light intensity of 150-300 Lux with artificial lighting from 08:00 to 20:00. The animals were kept in stainless

Table 1. The Combination of Crude Drugs in *Leejung-tang*

Latin name	Amount (g)
Ginseng Radix	7.50
Atractylodis Rhizoma	7.50
Glycyrrhizae Radix	3.75
Zingibris Rhizoma	7.50
Total amount	26.25

wire cages and were allowed a commercial pellet diet (PMI Nutrition International, Richmond, USA) and sterilized tap water was provided *ad libitum*. This study was performed in compliance with the test guidelines from the Institutional Animal Care and Use Committee in the Korea Institute of Toxicology (earned AAALAC International accreditation in 1998) under the Good Laboratory Practice Regulations for Nonclinical Laboratory Studies[17].

3. Selection of doses

In the preliminary study, there were no adverse effects such as death, clinical signs, or body weight changes at dose levels of 500, 1000, and 2000 mg/kg/day body weight. Based on the results, a dose of 2000 mg/kg was selected as the toxicological limited dose which was recommended by the OECD guideline (2001).[15] Because oral gavage is the clinically intended route for LJT, oral administration was selected in the present study. Experimental grouping was carried out using the A-module of Path/Tox System (Ver. 4.2.2, Xybion Medical System Corporation, USA) and assigned to 0 and 2000 mg/kg/day groups. Each group consisted of 5 rats of each sex. Each animal was identified by tail staining with marker pens, tail tattoos and cage cards. Distilled water was given to the animals in the vehicle control group. The animals were fasted overnight prior to dosing and LJT was administered by gavage. The application volume (10 mL/kg/day body weight) was measured using the Path/Tox System.

4. Mortality and clinical observation

All animals were observed daily for clinical signs of toxicity and mortality every hour until 6 h after dosing, and then once a day thereafter up to the end day of the study. Abnormal signs were recorded individually for type, observation day/time and duration using the Path/Tox System.

5. Body weight changes

Body weight of each rat was measured before LJT administration (day 1) and thereafter on days 2, 4, 8 and 15 after the treatment.

6. Gross findings

At the scheduled end of the experiments, all surviving animals were anesthetized by carbon dioxide and then sacrificed by exsanguination from the aorta. Complete gross postmortem examinations were performed with special attention to all vital organs and tissues.

7. Statistical analysis

Body weight values are presented as mean ± standard deviation (SD). All statistical analyses were conducted with the Path/Tox System (Ver 4.2.2). The statistical significance between the groups was analyzed by means of an analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The significance of the difference from the vehicle control group was estimated at the probability levels of 1% and 5%. In addition, statistical analysis for calculating the median lethal dose (LD_{50}) value was not performed because no mortality was observed in the present study.

Results

1. Mortality

In present study, no animals in either gender were observed to suffer death by oral administration of LJT during the study period (Table 2). Thus, LD_{50} of LJT is considered to be over 2000 mg/kg/day in both sexes.

2. Clinical signs

As shown in table 3, there was no observed the LJT-treatment related clinical signs in either sex

Table 2. Mortality in Male and Female Rats after Single Oral Administration of *Leejung-tang*

Sex	Dose(mg/kg)	Days after treatment														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Male (n=5)	0	*0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Female (n=5)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Number of dead animals

Table 3. Clinical Signs in Male and Female Rats after Single Oral Administration of *Leejung-tang*

Sex	Dose(mg/kg)	Days after treatment														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Male (n=5)	0	*0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Female (n=5)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Number of animals with clinical sign

during the study period.

3. Body weight changes

The results of body weight changes are summarized in Fig 1. In both sexes, there were no statistically significant changes between 2000 mg/kg/day group and vehicle control group in either sex.

4. Gross findings

At the scheduled necropsy, a cyst in the kidney was observed 1 case in 2000 mg/kg/day body group of female (Table 4) and the rest animals were not observed with gross findings.

Discussion

The present study was conducted to investigate the potential acute toxicity of LJT, a traditional Korean herbal formula, administered orally to SD

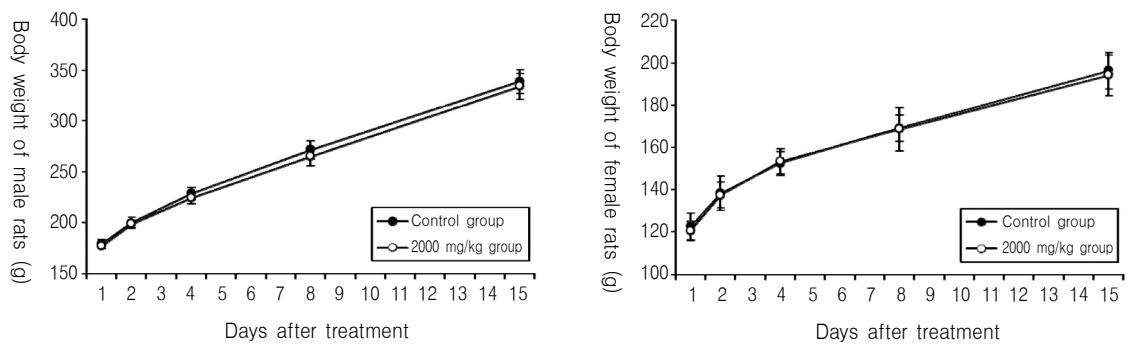

Fig. 1. Changes of body weights in male and female rats after single oral administration of LJT at dose levels of 0 (○) and 2000 mg/kg (●) in males and females. There were no significant differences in body weight between the LJT-treated and control groups.

Table 4. Gross Findings in Male and Female Rats after Single Oral Administration of *Leejung-tang*

Sex	Dose(mg/kg)	Gross finding	Frequency
Male	0	No gross findings	
	2000	No gross findings	
Female	0	No gross findings	
	2000	Cyst in kidney	*1

* Number of animal with gross finding

rats at dose level of 2000 mg/kg/day body weight. The results demonstrated that a single oral dose of LJT did not induce any adverse effects on mortality, clinical signs, body weight changes, or gross findings in rats of both sexes.

At the scheduled autopsy, a kidney cyst was observed in one 2000 mg/kg/day group female. However, this finding was not considered to be related to LJT administration because it occurred in a low incidence and did not exhibit a dose-response relationship. Also, this finding is commonly observed in normal SD rats[18-20]. A renal cyst observed in this study may progress to carcinoma, which rarely occurs. In other parameters including mortality, clinical sings, and body weight changes, we did not observed treatment-related effects.

Many traditional Korean herbal medicines have been used for prevention and therapy of disease. Most herbal medicines generally have few side effects and are very effective[21,22]. Due to these properties, herbal medicines have become increasingly popular in modern societies around the globe[23]. Indeed, use of herbal medicines has been increasing gradually in many countries[24,25]. As the use of herbal medicine increases, concerns have been raised over the lack of both quality control and scientific evidence of the efficacy and safety of herbal formulas[26]. Thus, many researchers using the protocols of evidence-based medicine have extensively researched to establish scientific evidence of safety of the herbal medicines. However, so far toxicity data on herbal medicines are insufficient and particularly few scientific studies have explored the safety and toxicity of traditional Korean herbal formulae in compliance with current guidelines. Considering the current research trends, this study is very important to provide basic toxicological information of a traditional Korean herbal formula. Actually, many researchers have conducted toxicity studies including acute toxicity, repeated toxicity and genotoxicity[5-14,27-29]. Based on the result of these studies, many researchers have announced that Korean traditional herbal formulae are harmless. For instance, *Sipjeondaebo-tang* was the subject of acute and subchronic toxicity studies[7,13]. In addition, genotoxicity studies including bacterial reverse mutation test, chromosomal aberration test and micronucleus test were performed[27-29]. Therefore, these studies provide an important reference for the safety of *Sipjeondaebo-tang* as a tradition Korean herbal formula for humans because *Sipjeondaebo-tang* was established not only from data on long-term therapy but also data on genotoxic profiles including carcinogenicity and mutagenicity.

Based on the results, we conclude that a single oral dose of LJT does not cause any adverse effects at doses of up to 2,000 mg/kg/day. Under these experimental conditions, the LD_{50} of LJT was considered to more than 2,000 mg/kg/day body weight, regardless of sex. Additionally, in humans, the single dose of LJT is about 26.25 g dried herb, which is equivalent to 6.51 g of the LJT extract (yield = 24.8%). Considering an average body weight of an adult of 60 kg[30-31], this dose for a 60

kg human is equal to 108.5 mg of LJT extract/kg. This dosage is about 20-fold lower than the LD_{50} of LJT. Further, to establishing the safety information on LJT we will conduct additional toxicity studies including repeated oral toxicity and genotoxicity studies, since these reflect how most take Korean traditional herbal formulas in the long-term.

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