大韓本草學會誌 제26권 제2호(2011년 6월)
Kor. J. Herbology 2011 ; 26(2) : 39-43

랫드에서 쌍화탕의 급성독성에 관한 연구

김수정[1], 이미영[1], 신인식[1], 서창섭[1], 하혜경[1], 허정임[2], 신현규[1*]

1 : 한국한의학 연구원 한약 EBM 연구센터
2 : 한국 안전성 평가 연구소

Single Dose Acute Toxicity of Ssanghwa-tang in Crl : CD (SD) Rats

Su-Jeong Kim[1], Mee-Young Lee[1], In-Sik Shin[1], Chang-Seob Seo[1], Hyekyung Ha[1], Jung-Im Huh[2], Hyeun-Kyoo Shin[1*]

1 : Herbal Medicine EBM Research Center, Korea Institute of Oriental Medicine, Daejeon 305-811, Republic of Korea,
2 : Division of Toxicology, Korea Institute of Toxicology, Daejeon 305-323, Republic of Korea

ABSTRACT

Objectives : This study was conducted to evaluate the acute toxicity and safety of Ssanghwa-tang (Shuanhetang in Chinese, Sou-wa-to in Japanese) in Crl : CD Sprague-Dawley (SD) rat though the current regulatory guideline.

Methods : In this study, 10 rats of each sex were randomly assigned to two groups of 5 rats each and were administrated singly by gavage at dose levels of 0 and 2000 mg/kg/day of ssanghwa-tang water extract (SHT). After single administration of SHT, mortalities, clinical signs, body weight changes, gross findings were observed for the 15-day period.

Results : Acute toxicity tests revealed that a single oral administration of SHT at dose levels of 2000 mg/kg did not affect clinical signs, body weight, and gross findings, evaluating the safety of SHT. The SHT treatment did not result in any toxicologically significant changes in mortality, clinical signs, body weight changes.

Conclusions : These results showed that the single oral administration of SHT did not cause any toxic effect at the dose levels of 2000 mg/kg/day in rats. In conclusion, the median lethal dose (LD_{50}) of SHT was considered to be over 2000 mg/kg/day body for both sexes.

Key Words : Ssanghwa-tang, Acute toxicity, Safety

Introduction

Traditional herbal medicine has been utilized for treating diseases and promoting the health of humans for thousands of years and is the most important part of complementary and alternative medicine. In addition, it has become a popular alternative choice conventional medicine. Although there are many traditional herbal medicine interventions available, and some have been verified by clinical trials, their efficacy and safety are still questioned by consumers[1].

Ssanghwa-tang (Shuanhetang in Chinese, Sou-wa-to in Japanese) is a herbal formula that includes nine herbs : *Paeonia lactiflora, Rehmannia glutinosa, Astragalus membranaceus, Angelica gigas, Cnidium officinale, Cinnam omumcassia, Glycyrrhiza glabra, Zingiber officinale, Zizyphus jujube*[2]. Ssanghwa-tang water extract (SHT) is commonly used herbal prescription medicine for fatigue recovery[3], alleviation of fever and headaches in Korea, China and Japan[4-5]. Previous studies were proved various efficacies of Ssanghwa-tang. According to various

*Corresponding Author : Hyeun Kyoo Shin, Herbal Medicine EBM Research Center, Korea Institute of Oriental Medicine, 483 Expo-ro, Yuseong-gu, Daejeon 305-811, Republic of Korea.
· Tel : +82-42-868-9464. · Fax : +82-42-864-2120. · E-mail : hkshin@kiom.re.kr.
· 접수 : 2011년 4월 27일 · 수정 : 2011년 6월 4일 · 채택 : 2011년 6월 10일

researchers, it was reported that the various efficacy of Ssanghwa-tang including anti-inflammatory[6], smooth muscle relaxation[7], the effect of osteoporosis improvement[8], increased secretion of sex hormones[9-10], anticonvulsant effect and improvement of liver function. The efficacy of SHT was related to various active ingredients in each herbs of SHT. Previous studies showed that active ingredients of individual herbs in SHT were identified paeoniflorin, albiflorin in *Paeonia lactiflora*, 5-hydroxymethyl-2-furaldehydein in *Rehmannia glutinosa*, decursin, decursinolangelate, decursinolin in *Angelica gigas*, cinnam aldehydecinnamyl acetate, cinnamicacidin in *Cinnamomum cassia*, glycyrrhizin, glycyrrhetic acid in *Glycyrrhiza glabra*, oleanolicacid, maslinicacid in *Zizyphus jujube*[11].

Despite various efficacy of Ssanghwa-tang, few scientific studies have explored the safety of this herbal medicine. Therefore, we investigated the acute toxicity of SHT in compliance with the current regulatory guideline. Acute toxicity are rapid procedures used to measure the concentration that will affect the test organisms, that is make them harmful. Data from these tests can be used to screen for toxicity to determine if the compound is toxic[12]. As results of the study, the contents of residual pesticides and sulfur dioxide after decoction in SHT were not detected. However, there were not carried out the acute toxicity study to provide basic toxicological information of SHT until now[13].

Therefore, in a series of our study on establishment of safety and toxicity of SHT, we investigated the potential acute toxicity of SHT in Crl : CD Sprague-Dawley (SD) rats. The present study was carried out in compliance with good laboratory practice (GLP) and the test guidelines of the Organization for Economic Cooperation and Development (OECD) and Korea Food and Drug Administration (KFDA)[14-15].

Materials and methods.

1. Preparation of SHT

A formation of SHT was prepared in our laboratory from a mixture of chopped crude herbs purchased from Omniherb (Yeongcheon, Korea) and HMAX (Chungbuk, Korea). SHT was prepared as described in Table 1 and extacted in distilled water at 100 ℃ for 2 h. The extract was evaporated to dryness and freeze-dried (yield ; 25.5 %).

Table 1. The composition of medicinal herbs of SHT

Herbal Medicine	Original location	Amount (g)
Paeonia Radix	Hwasun, Korea	9.38
Rehmannia Radix Preparata	Jangheung, Korea	3.75
Astragali Radix	Jeongseon, Korea	3.75
Angelicae Gigantis Radix	Yeongcheon, Korea	3.75
Cnidium Rhizoma	Yeongyang-gun, Korea	3.75
Cinnamomi Cortex	YenBai, Vietnam	2.81
Glycyrrhizae Radix et Rhizoma Crudus	China	2.81
Zingiberis Rhizoma Crudus	Seosan-gun, Korea	3.75
Zizyphi Fructus	Yeongcheon-gun, Korea	3.75
Net Amount (g)	-	37.5

2. Animals husbandry and maintenance

Twenty 5-week-old SD specific pathogen-free rats of each gender were obtained from a Orient Bio Co. (Seoul, Republic of Korea) and used after a week of quarantine and acclimatization. The animals were housed in a room maintained at a temperature of 23 ± 3℃ under a relative humidity of 50 ± 10%, artificial lighting from 08 : 00 to 20 : 00, 10 - 20 air changes per hour and a light intensity of 150-300 Lux. Animals were housed in a stainless wire cage at ≤2-3 animals per cage for the observation period. Pelleted food was purchased from PMI Nutrition International (USA) and was gamma-ray irradiated. Tap water was given *adlibitum*, following UV irradiation and filtration. The water was analyzed at the Daejeon Regional Institute of Health and Environment (Daejeon, Korea).

3. Experimental groups and treatment of SHT

The preliminary study showed that the single oral administration of SHT did not induce any toxic effect

at a dose level of 500, 1000 and 2000 mg/kg/day. Based on the results, a dose of 2000 mg/kg/day was selected as the toxicological limited dose which was recommended by OECD guideline (2001). Healthy male and female rats were assigned to two experimental groups using the A-module of Path/Tox System (Ver. 4.2.2, Xybion Medical System Corporation, USA) : 2000 mg/kg/day of SHT and a vehicle control group. Each group consisted of 5 rats of each sex. Because the oral gavage is clinically intended route for the SHT, the oral administration was selected in the present study. Rats were starvation overnight before drug administration and were fed after 3-4 hours. The SHT was suspended in distilled water and was freshly prepared before treatment. The application volume (10 ml/kg body weight) of SHT was calculated. The SHT was once administered by oral gavage to rats. The vehicle control rats received equivalent volume of distilled water alone.

4. Mortality and clinical observation

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

5. Body weights

Body weights of each rat were measured before SHT administration (day 1) and on day 2, 4, 8 and 15 after the treatment thereafter.

6. Gross findings

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

7. Statistical analysis

Body weight values were presented by mean ± standard deviation (SD). All statistical analyses were conducted with Path/Tox System (Ver 4.2.2). The significance of the differences 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.

Result

1. Mortality

No mortality was observed all groups of both genders during the experiment. Therefore, it was considered that the LD_{50} of the SHT is more than 2000mg/kg/day in both genders (Table2).

Table 2. Mortality in male and female rats treated with SHT

Sex	Dose (mg/kg)	Days on 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
	2000	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
	2000	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

(*Numberofdeadanimals/numberofanimalspergroup)

2. Clinical signs

We consistently observed clinical signs during this study. There was not observed clinical signs in all group of this study (Table 3).

Table 3. Clinical signs in male and female rats treated with SHT

Sex	Dose (mg/kg)	Days after treatment														
		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
	2000	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
	2000	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

(*Numberofdeadanimals/numberofanimalspergroup)

3. Body weights

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 both sexes.

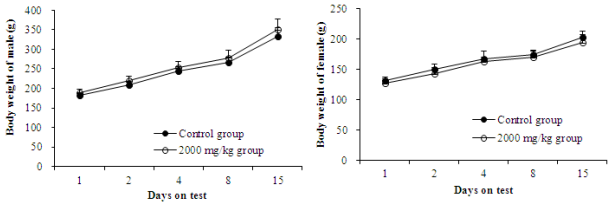

Figure 1. Mean body weight changes animals treated with SHT at dose levels of 0 (●) and 2000 (○) mg/kg. Values are presented as mean ± SD.

4. Gross findings

At the time of scheduled autopsy, a hernia of liver was observed in vehicle control group (n=1). The other was not observed gross findings in internal organs including lung, heart, thymus, stomach, liver, adrenal, spleen in treatment groups regardless of sex (Table 4).

Table 4. Gross findings in male and female rats treated with SHT

Sex	Dose (mg/kg)	Gross finding	Frequency
Male	0	No remarkable findings	5/5
	2000	No remarkable findings	4/5
		Hernia, Median lobe (L)	1/5
Femal e	0	No remarkable findings	5/5
	2000	No remarkable findings	5/5

Discussion

Ssanghwa-tang is one of the most commonly used herbal prescription for anti-fatigue in Korea, China and Japan and is widely used in traditional oriental medicine for the cold such as fever in Korea, China and Japan[11]. Because herbal medicine has been used for disease prevention and treatment for a long time. Therefore, present study was conducted to investigate the potential acute toxicity of SHT, administered orally to SD rats at dose level of 2000 mg/kg/day body weight. There were no treatment-related effects with regards to the body weight, food and water consumption in any treatment groups, regardless of sex. Also, abnormal clinical signs were not observed in the treatment groups.

Current medical therapeutics are evaluated in a series of scientific studies ; however, herbal medicines are often not subjected to toxicity tests before being administered to humans. Herbal preparation is suitable for human body and has les side effects. As the use of herbal medicine prescriptions increases, safety has become an important issue[16].

After administration, animals between both control and SHT groups showed no significant difference in body weight changes. All tested animals showed the increase in body weight with time. There were no increasing or decreasing body weights by SHT. In addition, total food and water intakes had no relevance to oral administration of SHT in both of control and treatment group. And specific clinical signs associated with SHT treatment were not observed. At the time of scheduled autopsy, a hernia of liver were observed in male control rats (n=1). This change was not considered an SHT induced abnormality. Because this finding occurred infrequently and were not related to dose, therefore it was considered to be sporadic findings. And macroscopic examination did not reveal any obvious differences in the size and appearance of visceral organs between experimental group and control group. Based on the results, it was concluded that a single oral dose of SHT did not cause any adverse effects at doses of up to 2000 mg/kg/day. Under these experimental conditions, the LD_{50} of SHT was considered to more than 2000 mg/kg/day body weight, regardless of sex. Further, to establish the safety information on SHT, we will conduct additional toxicity study including repeated oral toxicity and genotoxicity studies.

Achnowledgements

This research was part of a project (The Evidence Based Medicine for Herbal Formula) funded by the Herbal Medicine EBM Research Center of Korea Institute of Oriental Medicine.

Reference

1. Tang JL, Liu LB, Ma KW. Traditional Chinese medicine. Lancet. 2008 ; 372 : 1938-40.
2. Um YR, Park HY, Lee JH, Shim KS, Ma JY. Acute toxicity study on Ssanghwa-tang extract fermented with *Ganoderma lucidum* in mice. Korean Journal of Oriental Medcine. 2010 ; 16 : 135-40.
3. Lee JK, Lee NH, Ha HK, Lee HY, Seo CS, Jung DY, Lee JA, Shin HK. Analysis of studies on ssanghwa-tang for establishment of evidence based medicine. Korean Journal of Oriental Medcine.

2008 ; 14 : 41-7.
4. Lee JH, Um YR, Ma JY, Acute toxicity study on Ssanghwa-tang fermented with Nuruk in ICR mice. Korean Journal of Oriental Medcine. 2010 ; 16 : 149-54.
5. Won JB, Ma JC, Ma JY, Um YR. Simultaneous determination of five marker constituents in Ssanghwa tang by HPLC/DAD. Pharmacognosy Magazine. 2010 ; 6 : 111-5.
6. Jung JC, Park DK. Studies on CNS - Depression and antinflammatory action of Ssangwhatang. Journal of Pharmaceutical Ivestigation. 1986 ; 16 : 24-30.
7. Kim JK, Shim HN, Lee SH. Smooth muscle relaxation by the herbal medicine Ssanghwatang associated with nitric oxide synthase activation ad nitric oxide production. Korean Journal of Oriental Medcine. 2006 ; 27 : 744-83.
8. Shim KS, Lee JH, Lee JH, Ma JY. Effect of Ssangwha-tang fermented by *Lactobacillus fermentum* on osteoclast differentiation and osteoporosis of ovariectomized rats. Korean Journal of Oriental Medcine. 2010 ; 16 : 149-155.
9. Cho TY, Shim CK, Lee MH, Kim SK. Effects of blended Chinese traditional medicine, Ssang wha tang, on hepatic clearance of sulfobromophthalein in rats. Journal of Pharmaceutical Investigation. 1987 ; 17 : 89-93.
10. Kim YB, Kwon KB, Park JS, Park KH, Kim BR, Lee HS, Ryu DK. Gugissanghwatang water extract on the sexual behavior and serum testosterone concentration in male rats. Korean Society of Oriental Pathology. 1999 ; 13 : 152-7.
11. Kim DS, Um YR, Yang MC, Yun NY, Ma JY. Polyphenol contents and antioxidant activities of fractions from Ssanghwa-tang and fermented Ssanghwa-tang. Korean Journal of Oriental Medcine. 2010 ; 16 : 175-8.
12. Shin IS, Yu YB, Seo CS, Ha HK, Lee MY, Huang DS, Kim JH, Shin HK. Subchronic toxicity of Sipjeondaebo-tang (SDT) in Sprague-Dawley rats. Regulatory Toxicology and Pharmacology. 2010 ; 59 : 375-84.
13. Kim JH, Seo CS, Shin HK. The comparative study on decoctions of Ssanghwa-tang (Shuanghe-tang) extracted by different extraction conditions. Korean Journal of Oriental Medcine. 2010 ; 18 : 125-34.
14. Guidelines for Toxicity Tests of Drugs and Related Materials Notification. KFDA. 2009 ; 116 : 1-5.
15. Good Laboratory Practice Regulation for Non-Clinical Laboratory Studies. KFDA. 2009 ; 183 : 1-74.
16. Watanabe S, Imanishi J, Satoh M, Ozasa K. Unique place of Kampo (Japanese traditional medicine) in complementary and alternative medicine : a survey of doctors belonging to the regional medical association in Japan. The Tohoku Journal of Experimental Medicine. 2001 ; 194 : 55-63.