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Original article

Intravenous Injection of Saeng Maek San - A Safe Method of Treatment in Rats

Min-ji Choi[1], Sung-chul Kim[2], Seung-Hun Cho[1,3]*

[1] Department of Neuropsychiatry, College of Korean Medicine, Kyung Hee University, Seoul, Korea
[2] Department of Acupuncture & Moxibustion Medicine, Wonkwang Gwangju Oriental Medical Hospital, Gwangju, Korea
[3] Department of Neuropsychiatry, Hospital of Korean Medicine, Kyung Hee University Medical Center, Seoul, Korea

Key Words
acupuncture, pharmacopuncture, Saeng Maek San, toxicity test

Abstract

Objectives: This study evaluated the single-dose toxicity of Saeng Maek San (SMS) in rats.

Methods: All experiments were conducted at Biotoxtech (Chungwon, Korea), an institute authorized to perform non-clinical studies under the regulations of Good Laboratory Practice (GLP). A single-dose intravenous toxicity study was carried out on 40 6-week-old Sprague-Daley rats. The animals were randomly divided into the following four groups of ten animals each: Group 1 (G1) was the control group, with each animal receiving an intravenous injection of 1.0 mL of saline, and Groups 2, 3 and 4 (G2, G3 and G4) were the experimental groups, with the animals in the groups receiving an injection of 0.1, 0.5 and 1.0 mL of SMS, respectively. Mortality, clinical signs, body-weight changes and gross pathological findings were observed for 14 days following a single administration of SMS or saline. Organ weights, clinical chemistry and hematology were analyzed at 14 days. This study was conducted with the approval of the Institutional Animal Ethics Committee.

Results: No deaths occurred in any of the four groups, indicating that the lethal dose of SMS in rats is greater than 1.0 mL/animal. Some changes in weights of male rats between the control group and the experimental groups were observed, but no significant changes in the weights of female rats were noted. To identify abnormalities in organs and tissues, we stained representative sections of each specified organ with hematoxylin and eosin for examination with a light microscope. No significant abnormalities were observed in any of the organs or tissues.

Conclusion: The results suggest that intravenous injection of SMS is a safe method of treatment.

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1. Introduction

Saeng Maek San (SMS), a traditional Korean herbal medicine, composed of *Panax ginseng*, *Ophiopogon japonicas*, and *Schisandra chinensis*. In the theory of traditional Chinese medicine (TCM), it strongly supports energy levels that have been severely tapped. This means the qi and the yin have been compromised, leading to signs of thirst and fatigue that contribute to weakness of the body. Furthermore, it has been used to treat symptoms related to cardiovascular diseases such as heart failure, stroke and shock [1, 2]. Previous studies have suggested that SMS exerts protective effects against oxidative damage in the cells or tissues of the cardiovascular system and the nervous system [3-

***Corresponding Author**
Seung-Hun Cho. Department of Neuropsychiatry, College of Korean Medicine, Kyung-Hee University, Hoegi-dong, Dongdaemun-gu, Seoul, Korea.
Tel: +82-2-958-9188 Fax: +82-2-958-9186
E-mail: chosh@khu.ac.kr

5]. Additionally, earlier studies have shown the preventive effect of SMS against cerebral oxidative damage in rats [6], and Wu *et al* [7]. reported the memory-enhancing potential of SMS in a vascular dementia model.

In an *invitro* model, SMS was reported to protect against amyloid-b-induced cytotoxicity in PC12 cells [8]. Xuejiang *et al*. [9] described the potential of SMS for neuroprotection and neurite outgrowth, which further implicated the applicability of SMS for the treatment of axon-degenerative diseases such as amyotrophic lateral sclerosis (ALS), in which a deficiency of superoxide dismutase (SOD) is thought to be linked to neuronal damage. Also, several studies have established that ginsenoside Rb1, Rg1, schizandrin, and ruscogenin from Sheng-mai San have neuroprotective effects because of their antioxidant, antiapoptosis, and anti-inflammatory actions [10-14]. DT-13, a saponin with ruscogenin as the parent nucleus, also showed significant anti-inflammatory activity with low toxicity [15, 16]. More recently, Guo *et al*. [17] suggested that SMS could regulate the neuronal autophagy and its related AMPK/mTOR and JNK pathways in a MCAO model in mice.

Despite the efficacy and the non-toxicity of SMS, there has to date been no detailed investigation of the acute toxicity of such a commonly used drug. To build upon the above-mentioned studies, the safe concentration of SMS in the blood should be determined. This study provides data regarding both the median lethal dosage (LD_{50}) of SMS in rats through different routes of injection and the largest safe dose for intravenous injection.

2. Materials and Methods

SMS, which is a mixture of three traditional herbs, was prepared under sterile conditions at the Korean Pharmacopuncture Institute (K-GMP). The ratio of the herbal drugs in SMS is 2 : 1 : 1 of Liriopis Tuber, Ginseng Radix and Schisandrae Fructus, respectively (total: 5500 g). SMS extract was prepared by decocting the dried prescription in distilled water. The extract was obtained by decocting for approximately 2 hours (total extracts: 12 L), and the pH was controlled to between 7.0 – 7.5 by adding NaOH to make a 0.9% isotonic solution. The final solution was stored at 4°C.

This study used 5-week-old male and female Sprague-Dawley rats (supplied by Orient Bio Inc., Korea) weighing 103.4 – 134.1 g. Sprague-Dawley rats are used widely for drug-safety testing. The animals' weights at the time of injection were 180.4 – 202.2 g and 135.4 – 165.7 g for the male and the female rats, respectively. The general condition of, and any symptoms displayed by, the rats were observed once per day for 7 days following injection; the weights of the rats on day 7 were recorded. The room in which the rats were kept was maintained at 20.0 – 22.8°C, with a relative humidity of 48.5% to 65.9%, natural illumination, and automated ventilation. The animals were housed individually in cages and allowed free access to solid feed (Teklad Certified Irradiated Global 18% Protein Rodent Diet 2918C) and UV-filtered water. Groupings of the rats were allocated 7 days after injection. Animals with weights close to the mean were selected. In total, 20 male and 20 female rats were selected. The animals were randomly distributed into four groups of five rats each (Table 1).

In clinical applications, the standard dose for SMS is 1.0 mL per treatment. In our study, 1.0 mL/animal was set as the high dose and 0.5 and 0.1 mL as the medium and the low doses, respectively. Normal saline (1.0 mL) was administered to control rats. A single dose of SMS at the prescribed level was injected intravenously into the rats in the low-, the medium-, and the high-dose experimental groups. Clean disposable syringes were used throughout the study. The study was conducted with the approval of the Institutional Animal Ethics Committee (No.120877).

General symptoms were recorded daily at 1 to 14 days following injection. On the day of dosing (Day 0), general symptoms (side effects, revealing time, recovery time, etc.) and mortality were examined at 30 minutes and at 1, 2, 3 and 4 hours after injection. The weight of each rat was measured immediately before injection and at 3, 7, and 14

Table 1 Groups of animals

Group	SMS injection (mL/animal)	Number of animals (serial numbers)	
		Male	Female
G1: control group	0	5 (1101 – 1105)	5 (2101 – 2105)
G2: low-dose group	0.1	5 (1201 – 1205)	5 (2201 – 2205)
G3: mid-dose group	0.5	5 (1301 – 1305)	5 (2301 – 2305)
G4: high-dose group	1.0	5 (1401 – 1405)	5 (2401 – 2405)

days thereafter.

Fifteen days after injection on the day of necropsy, after the rats had been fasted for at least 18 hours, they were anesthetized with isoflurane, and a blood sample was taken from the abdominal aorta). Blood (1.0 mL) was analyzed using an automatic hematology analyzer (ADVIA 120, SIEMEMS, Germany). Blood coagulation was assessed by centrifuging a 2.0-mL blood sample (3,000 rpm, 10 minutes) and taking measurements using an Automated Coagulation Analyzer (Coapresta 2000, SEKISUI, Japan). Biochemical tests were performed on blood taken from the abdominal aorta by using an Automatic Analyzer (7180, HITACHI, Japan) and Electrolyte Analyzer (AVL9181, Roche, Germany).

After completion of the above measurements, all organs and tissues of each rat were inspected visually and were examined under an optical microscope. The organs of each rat were fixed with 10% formalin or Calci-Clear-Rapid™ solution (the latter only for testes showing osseous tissue demineralization), stained with hematoxylin–eosin (H&E) and fixed in paraffin wax. A complete histopathological examination was performed on these tissue samples.

The weight, hematological, and blood biochemical test results were analyzed using the statistical analysis system (SAS) software (versions 9.2 and 9.3, SAS Institute Inc., USA). A Bartlett's test was conducted to evaluate the homogeneity of the variance and significance. A one-way analysis of variance (ANOVA) was conducted when homogeneity of the variance was recognized, and the Kruskal-Wallis test was conducted as a post-hoc analysis.

3. Results

No deaths or abnormalities occurred in any of the groups, indicating that the LD_{50} of the SMS administered via intravenous injection was greater than 1.0 mL/animal in rats (Tables 2 and 3). In addition, no changes in weight were observed (Table 4). In this study, even though computation of the LD_{50} was difficult, all groups presented a sustained increase in body weight, but the differences among the groups were not significant. Finally, no significant changes in the hematological results, blood biochemical parameters or necropsy findings were noted (data not shown). Upon histopathological examination, interstitial-infiltrating macrophages were found in one female rat in the 0.5-mL/animal group. No significant changes in the brain, lungs, liver, kidney or spinal cord were found in any of the other groups (Fig. 1).

4. Discussion

SMS is a traditional medical formula composed of *Panax ginseng*, *Ophiopogon japonicas*, and *Schisandra chinensis* and has been used for almost 1,000 years in China. It was first recorded in "Yixue Qiyuan", which was written by Zhang Yuansu, a famous physician, during the Jin Dynasty. In China, it has long been used for the treatment of Qi and Yin deficiency and in modern times to treat diverse conditions, including cardiovascular and neurological disorders [3, 5]. Previous experimental data have indicated the efficacy of SMS in treating various conditions [5, 18]. SMS is used in the treatment of myocardial infarction, where it

Table 2 Mortalities. A single intravenous injection dose of SMS at the each groups including 1.0 mL/animal group caused no mortalities.

Group	Dose (mL/animal)	Mortality (dead/tested)	
		Male	Female
G1	0	0% (0/5)*	0% (0/5)
G2	0.1	0% (0/5)	0% (0/5)
G3	0.5	0% (0/5)	0% (0/5)
G4	1	0% (0/5)	0% (0/5)

*Number of dead animals/number of tested animals

Table 3 Clinical signs. No abnormal clinical symptoms, such as type of toxicity symptom, time of expression, time of recovery were observed after injection.

Group	Dose (mL/animal)	Sex	Number of animals	Clinical signs
G1	0	Male	5	NOA
		Female	5	NOA
G2	0.1	Male	5	NOA
		Female	5	NOA
G3	0.5	Male	5	NOA
		Female	5	NOA
G4	1	Male	5	NOA
		Female	5	NOA

has a protective effect against the accompanying renal ischemic damage [19, 20]. Some studies have reported that SMS can improve sperm viability and movement parameters *in vitro* [21]. Ginseng has been reported to be effective for tolerable toxicity in the treatment of cancer-related fatigue [22]. Ginseng polysaccharides also displayed anti-fatigue effects in one study in mice [23]. Ginsenosides, the primary active components of ginseng, have been found to have anti-inflammatory and anti-oxidative effects. Neuroprotective effects of ginsenosides were also reported in an AD mouse model [24]. Ginsenoside Rg5 (Rg5) has been suggested as a novel therapeutic agent for treating memory loss [25]. *Ophiopogon japonicas* is used for Yin deficiency according to TCM theory and has displayed potential for treating hypoglycemia and hypolipidemia in type-2 diabetes [26]. Schisandra chinensis is used to benefit Qi energy and promote body-fluid production according to TCM theory and has been reported to have positive effects in viral- and chemical-induced hepatitis [27, 28].

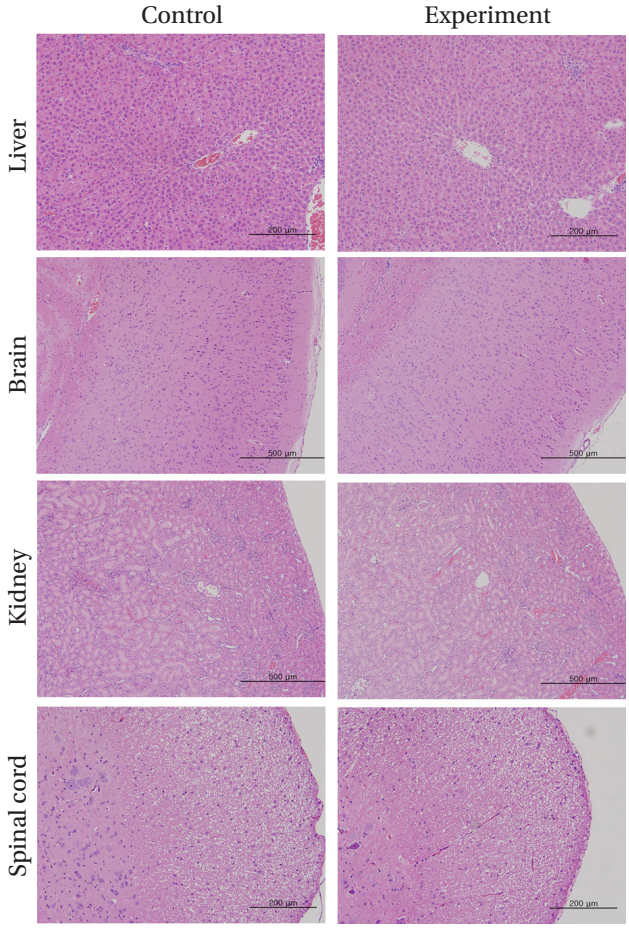

Figure 1 Histopathological observations. H&E, × 200, × 40

Thus, SMS and its component herbs have been reported to have a myriad of positive effects on several disorders. Although it is used widely in clinics, studies on the safety of SMS are lacking, so further research is needed, and toxicity testing is essential for evaluating the safety of medications [29]. This study was performed to provide objective safety data for SMS. Three SMS doses (0.1, 0.5 and 1.0 mL) were administered to the experimental groups, and 1.0 ml of saline was administered to the control group. In all four groups, no deaths occurred, indicating that the LD_{50} of SMS is greater than 1.0 mL/animal in rats. There were no significant differences between the control group and the experimental groups in the clinical signs, weights, hematologic examination results, and blood biochemical parameters. At necropsy, only one rat displayed any significant histopathological abnormalities in the organs and tissues.

Further studies of the acute and the chronic side effects and the reaction capacity are required to rigorously assess the toxicity of SMS. Animal testing is the most important method of performing these safety assessments [30]. The

Table 4 Body weights (g)

Group	Dose	Sex	Days after administration				Gain
			0	3	7	14	0 — 14
G1	0	Male	191 ± 4.3	216.5 ± 5.2	253.1 ± 6.7	307.6 ± 6.8	116.5 ± 6.9
		Female	151.7 ± 5.3	162.7 ± 3.8	176 ± 4.8	199.3 ± 5.7	47.6 ± 7.1
G2	0.1	Male	189.7 ± 4.7	215.8 ± 8.7	252.6 ± 9.8	311.5 ± 14.1	121.9 ± 11.2
		Female	150.8 ± 9.7	162.2 ± 9.8	172.4 ± 11.7	189.9 ± 19.8	39 ± 14.9
G3	0.5	Male	189.3 ± 7.7	215.5 ± 12	252 ± 13.1	310.1 ± 20.1	120.7 ± 14.6
		Female	152.6 ± 11	163.7 ± 13.7	176.3 ± 13.5	199.9 ± 16.5	47.3 ± 6.6
G4	1	Male	191.8 ± 7.9	218.2 ± 10.7	257.2 ± 14.2	310 ± 23.4	118.1 ± 17.9
		Female	151 ± 10.1	163.3 ± 9.8	177.5 ± 13.2	199.1 ± 16.8	48.2 ± 10.7

Korea Food and Drug Administration has testing-protocol guidelines for the assessment of toxicity, and all experiments should be conducted following Good Laboratory Practice (GLP) regulations [31]. In this study, the LD_{50} of SMS in rats was shown to be > 1.0 mL/animal, indicating that this dose is safe in humans and does not cause significant histological abnormalities. Further studies are needed to yield more data to support our findings.

5. Conclusion

In our study, the administration of 1.0 mL/animal SMS did not produce any significant changes in body weight, in the results of hematological, blood biochemistry or necropsy examinations, or in the incidence of mortality. Our findings indicate that SMS administration up to this dose is a safe option for treatment.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Ichikawa H, Konishi T. *In vitro* antioxidant potentials of traditional Chinese medicine, Shengmai San and their relation to *in vivo* protective effect on cerebral oxidative damage in rats. Biol Pharm Bull. 2002;25(7):898–903.
2. Li LH, Wang JS, Kong LY. Protective effects of Shengmai San and its three fractions on cerebral ischemia–reperfusion injury. Chin J Nat Med. 2013;11(3):222–30.
3. Zhou Q, Qin WZ, Liu SB, Kwong JS, Zhou J, Chen J. Shengmai (a traditional Chinese herbal medicine) for heart failure. Cochrane Database Syst Rev. 2014 14;4:CD005052. DOI: 10.1002/14651858.CD005052.pub5.
4. Wang NL, Chang CK, Liou YL, Lin CL, Lin MT. *Shengmai San*, a Chinese herbal medicine protects against rat heat stroke by reducing inflammatory cytokines and nitric oxide formation. J Pharmacol Sci. 2005;98(1):1–7.
5. Seo TB, Baek K, Kwon KB, Lee SI, Lim JS, Seol IC, *et al*. *Shengmai-san*-mediated enhancement of regenerative responses of spinal cord axons after injury in rats. J Pharmacol Sci. 2009;110(4):483–92.
6. Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, Alexander DD, *et al*. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. Science. 1994;264(5166):1772–5.
7. Wu Y, Wen YL, Du L. [Effect of Shengmaisan on learning and memory abilities and hippocampal nitric oxide synthase expression and neuronal apoptosis in rats with vascular dementia]. Nan Fang Yi Ke Da Xue Xue Bao. 2010;30(6):1327–9. Chinese.
8. Xuejiang W, Magara T, Konishi T. Prevention and repair of cerebral ischemia-reperfusion injury by Chinese herbal medicine, shengmai san, in rats. Free Radic Res. 1999;31(5):449–55.
9. Nishida H, Kushida M, Nakajima Y, Ogawa Y, Tatewaki N, Sato S, *et al*. Amyloid-beta-induced cytotoxicity of PC-12 cell was attenuated by *Shengmai-san* through redox regulation and outgrowth induction. J Pharmacol Sci. 2007;104(1):73–81.
10. Cheng HY, Hsieh MT, Wu CR, Tsai FH, Lu TC, Hsieh CC, *et al*. Schizandrin protects primary cultures of rat cortical cells from glutamate-induced excitotoxicity. J Pharmacol Sci. 2008;107(1):21–31.
11. Huang YL, Kou JP, Ma L, Song JX, Yu BY. Possible mechanism of the anti-inflammatory activity of ruscogenin: role of intercellular adhesion molecule-1 and nuclear factor-kappaB. J Pharmacol Sci. 2008;108(2):198–205.
12. Chen Z, Lu T, Yue X, Wei N, Jiang Y, Chen M, *et al*. Neuroprotective effect of ginsenoside Rb1 on glutamate-induced neurotoxicity: with emphasis on autophagy. Neurosci Lett. 2010;482(3):264–8.
13. Liu Q, Kou JP, Yu BY. Ginsenoside Rg1 protects against hydrogen peroxide-induced cell death in PC12 cells via inhibiting NF-κB activation. Neurochem Int. 2011;58(1):119–25.
14. Chiu PY, Lam PY, Leung HY, Leong PK, Ma CW, Tang QY, *et al*. Co-treatment with Shengmai San-derived herbal product ameliorates chronic ethanol-induced liver damage in rats. Rejuvenation Res. 2011;14(1):17-23.
15. Ma ST, Kou JP, Yu BY. Safety evaluation of steroidal saponin DT-13 isolated from the tuber of *Liriope muscari* (Decne.) Baily. Food Chem Toxicol. 2011;49(9):2243–51.
16. Tian YQ, Kou JP, Li LZ, Yu BY. Anti-inflammatory effects of aqueous extract from *Radix Liriope muscari* and its major active fraction and component. Chin J Nat Med. 2011;9(3):222–6.
17. Guo Z, Cao G, Yang H, Zhou H, Li L, Cao Z, *et al*. A combination of four active compounds alleviates cerebral ischemia-reperfusion injury in correlation with

inhibition of autophagy and modulation of AMPK/mTOR and JNK pathways. J Neurosci Res. 2014;7; DOI: 10.1002/jnr.23400.
18. Konishi T. Brain oxidative stress as basic target of antioxidant traditional oriental medicines. Neurochem Res. 2009;34(4):711-6.
19. Xu N, Qiu C, Wang W, Wang Y, Chai C, Yan Y, *et al*. HPLC/MS/MS for quantification of two types of neurotransmitters in rat brain and applications: myocardial ischemia and protection of Sheng-Mai-San. J Pharm Biomed Anal. 2011;55(1):101–8.
20. Mok TS, Yeo W, Johnson PJ, Hui P, Ho WM, Lam KC, *et al*. A double-blind placebo-controlled randomized study of Chinese herbal medicine as complementary therapy for reduction of chemotherapy-induced toxicity. Ann Oncol. 2007;18(4):768–74.
21. Lee IY, Lee CC, Chang CK, Chien CH, Lin MT. Sheng mai san, a Chinese herbal medicine, protects against renal ischemic injury during heat stroke in the rat. Clin Exp Pharmacol Physiol. 2005;32(9):742–8.
22. Zhang F, Xu JX, Ma HG, Zhou LY, Cheng ZL. [Sheng Mai Zhusheye improves the viability and movement parameters of human sperm in vitro]. Zhonghua Nan Ke Xue. 2009;15(5):468–71. Chinese.
23. Barton DL, Soori GS, Bauer BA, Sloan JA, Johnson PA, Figueras C, *et al*. Pilot study of Panax quinquefolius (American ginseng) to improve cancer-related fatigue: a randomized, double-blind, dose-finding evaluation: NCCTG trial N03CA. Support Care Cancer. 2010;18(2):179–87.
24. Blanco M, Rodríguez-Yáñez M, Sobrino T, Leira R, Castillo J. Platelets, inflammation, and atherothrombotic neurovascular disease: the role of endothelial dysfunction. Cerebrovasc Dis, 2005;Suppl 2:S32–9.
25. Fang F, Chen X, Huang T, Lue LF, Luddy JS, Yan SS. Multi-faced neuroprotective effects of Ginsenoside Rg1 in an Alzheimer mouse model. Biochim. Biophys. Acta, 2012;1822(2):286–92.
26. Wang J, Li S, Fan Y, Chen Y, Liu D, Cheng H, *et al*. Anti-fatigue activity of the water-soluble polysaccharides isolated from *Panax ginseng C. A. Meyer*. J Ethnopharmacol. 2010;130(2):421–3.
27. Chen X, Bai X, Liu Y, Tian L, Zhou J, Zhou Q, *et al*. Anti-diabetic effects of water extract and crude polysaccharides from tuberous root of *Liriope spicata var. prolifera* in mice. J Ethnopharmacol. 2009;122(2):205–9.
28. Liu GT. Pharmacological actions and clinical use of fructus schizandrae. Chin Med J (Engl). 1989;102(10):740–9.
29. S. Parasuraman. Toxicological screening. J Pharmacol Pharmacother. 2011;2(2):74–9.
30. Kim YG. [Toxicology]. Seoul (Korea): Donghwagisul; 1984, p. 15–8. Korean.
31. Korea Food and Drug Administration (KFDA), 2005b. Toxicity Test Guidelines for Safety Evaluation of Drugs. Notification No. 2005-60.