Journal of Pharmacopuncture

ISSN 2093-6966 [Print], **ISSN 2234-6856** [Online]
Journal of Pharmacopuncture 2014;17[4]:061-065
DOI: http://dx.doi.org/10.3831/KPI.2014.17.038

Original article

A Study on the Oral Toxicity of Mecasin in Rats

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Key Words

gami-jakyak gamcho buja decoction (mecasin), toxicity test

Abstract

Objectives: In this study, we investigated the oral toxicity of Gami-Jakyak Gamcho buja Decoction (Mecasin) to develop safe treatments.

Methods: All experiments were conducted at the Medvill, an institution authorized to perform non-clinical studies, under the Good Laboratory Practice (GLP) regulations. In order to investigate the oral toxicity of Mecasin, we administered Mecasin orally to rats. Sprague-Dawley rats were divided into four groups of five male and five female animals per group: group 1 being the control group and groups 2, 3, and 4 being the experimental groups. Doses of Mecasin, 500 mg/kg, 1,000 mg/kg and 2,000 mg/kg, were administered to the experimental groups, and a dose of normal saline solution, 10 mL/kg, was administered to the control group. We examined the survival rate, weight, clinical signs, and gross findings. This study was conducted under the approval of the Institutional Animal Ethics Committee.

Results: No deaths or abnormalities occurred in any of the four groups. Although slight decreases in the weights of some female rats were noted on the third day, no significant changes in weights or gross findings between the control group and the experimental groups were observed. To check for abnormalities in organs, we used microscopy to examine representative histological sections of each specified organ; the results showed no significant differences in any of the organs.

Conclusion: The results showed that administration of 500 – 2,000 mg/kg of Mecasin did not cause any changes in weight or in the results of necropsy examinations. It also did not result in any mortalities. The above findings suggest that treatment with Mecasin is relatively safe. Further studies on this subject are needed to yield more concrete evidence.

Received: Aug 20, 2014 **Accepted:** Sep 17, 2014

1. Introduction

Gami-Jakyak Gamcho buja Decoction (Mecasin) was developed for treating amyotrophic lateral sclerosis patients with pain, joint contracture and muscular weakness. Mecasin, which is the major component of Mecasin, has been used in traditional medicine to relieve pain, muscle spasms and cold syndrome due to blood deficiency [1]. A recent study showed that Jakyak Gamcho Decoction (JGT) and its constituents have a protective effect against tert-butyl hydroperoxide (t-BHP)-induced cytotoxicity in the hippocampal HT22 cell line [2]. In addition, Mecasin, which consists of JGT and Radix aconiti lateralis preparata, is believed to be useful for suppressing the progress of osteoarthritis because of its anti-inflammatory effects and its ability to reduce pain with histopathological efficacy [3]. Because of these facts, Mecasin has been studied for treating patients with joint pain, muscle spasms,

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and arthralgia due to cold. The constituents of Mecasin are Radix paeoniae ALBA, Radix glycyrrhizae, Radix aconiti lateralis preparata, Radix salviae miltiorrhizae, Rhizoma gastrodiae, Radix polygalae, Curcuma root, Fructus chaenomelis, Rhizoma atractylodis japonicae.

Several studies on the relation between the toxicity and the composition of Mecasin have been conducted. studied Radix glycyrrhizae consumption as a causing of severe hypokalemic paralysis Elinav *et al* [4]. conducted a study on the single-dose toxicity of Aconitum kusnezoffii reichb. pharmacopuncture in rats and suggested that Aconitum kusnezoffii reichb. pharmacopuncture was relatively safe Kim *et al* [5]. Studied the cytotoxic effect of Radix salviae miltiorrhizae against L1210 cells Sun *et al* [6]. Conducted a study on the acute oral toxicity of Radix polygalae extract and suggested that water soluble extract of Radix polygalae had no acute oral toxicity and that the oral LD_{50} value was over 4,000 mg/kg in Sprague-Dawley (SD) rats Roh *et al* [7]. Conducted a philological study on the poisoning and the side effects of Rhizoma gastrodiae Kim *et al* [8]. conducted a study on the cytotoxicity and the antimicrobial effects of extracts from the Atractylodes japonica koidzumi Choi *et al* [9]. Nevertheless, objective oral toxicity testing of Mecasin, which is complex combination of herbs, has not been conducted yet.

The current research trend for oral toxicity testing of extracts is to study acute and subacute toxicity through Good Laboratory Practice (GLP) regulations. All the experiments for this research were conducted at Medvill, an institution authorized to perform non-clinical studies, under the GLP regulations. This study was performed to analyze the oral toxicity and the lethal dose of Mecasin in rats.

2. Materials and Methods

The Mecasin was prepared in a sterile room at the Hanpoong Pharm & Food Co., Ltd. (K-GMP). After the mixing process with pure water, the pH was controlled to between 7.0 and 7.5 NaCl was added to make a 0.9% isotonic solution. The completed extract was stored at room temperature (17.6 – 23.5°C).

The animals used in this study were 8-week-old SD rats. The reason SD rats were chosen is that they have been widely used in safety test in the field of medicine, so the results can be easily compared with many other databases. The mean weights of the rats were 229.1 – 246.6 g and 169.7 – 199.0 g, respectively, for the male and the female rats at the time of Mecasin administration. For all animals, a visual inspection was conducted; all animals were weighed at the beginning. During 6 days of acclimatization, the general symptoms of the rats were observed once a day. The weights of the rats were recorded on the last day of acclimatization. No abnormalities were found.

The temperature of the lab was 19.8 – 24.3°C, and the humidity was 47.7% – 68.1%. Enough food (Lab Diet 5053) and UV-filtered water were provided.

Groupings were done after 6 days of acclimatization. Animals were selected if their weights were close to the mean weight. In total, 20 male rats and 20 female rats were selected. The animals were randomly distributed into 4 groups (5 male and 5 female rats per group) as shown in Table 1.

In this study 2,000 mg/kg was set as a high-dose, and 1,000 mg/kg and 500 mg/kg were set as mid and low doses, respectively. In the control group, 10 mL/kg of normal saline solution was administered. Mecasin and normal saline were administered into the mouths of the rats in all groups by using disposable syringes. This study was conducted under the approval of the Institutional Animal Ethics Committee of Medvill Co., Ltd.

From the 1st day to the 14th day after treatment, the general symptoms were examined once a day. On the day of dosing (day 0), the general symptoms (side effects, revealing time, recovery time, etc.,) as well as the mortality, were examined at 30 minutes and at 1, 2, 4, and 6 hours after injection. The weights were measured immediately before treatment and at 1, 3, 7 and 14 days after treatment. After observations had been terminated, we conducted a necropsy of the rats after cutting the abdominal aorta and vein under CO_2 anesthesia, and the organs of all surviving animals were visually inspected and microscopically examined.

The weight data from the experiments were analyzed by using SPSS program (SPSS 16.0). A Levene test was conducted to evaluate the homogeneity of the variance and the significance. The one-way analysis of variance (ANOVA) test was conducted when the homogeneity of the variance was recognized.

3. Results

In this study, no deaths or abnormalities occurred in any of the groups. In weight examinations, slight decreases in the weights of four female rats (one female rat in the control group, two female rats in the 500-mg/kg group, and

Table 1 Groups of animals

Group	Mecasin administration (mg/kg)	Number of animals (serial number)	
		Male	Female
G1: Control group	0	5 (11001 – 11005)	5 (21001 – 21005)
G2: Low-dose group	500	5 (12001 – 12005)	5 (22001 – 22005)
G3: Mid-dose group	1,000	5 (13001 – 13005)	5 (23001 – 23005)
G4: High-dose group	2,000	5 (14001 – 14005)	5 (24001 – 24005)

Table 2 Body weights in grams

Group & Dose (mg/kg)	Sex	Mean S. D. N	Days after administration 0	1	3	7	14	Final weight gain
G1 0	Male	Mean	276.3	306.8	323.4	362.5	423.5	147.2
		S. D.	6.6	8.1	9.4	9.4	16.0	11.2
		N	5	5	5	5	5	5
	Female	Mean	190.8	211.8	215.0	224.6	239.7	49.2
		S. D.	7.6	8.4	7.8	9.8	14.3	9.3
		N	5	5	5	5	5	5
G2 500	Male	Mean	270.6	300.3	314.6	351.9	407.5	136.9
		S. D.	7.0	8.9	6.6	9.6	15.1	11.9
		N	5	5	5	5	5	5
	Female	Mean	190.7	209.9	209.0	223.5	239.9	49.2
		S. D.	6.9	5.3	14.0	9.6	13.7	7.0
		N	5	5	5	5	5	5
G3 1,000	Male	Mean	272.3	302.1	321.6	361.1	421.4	149.1
		S. D.	6.7	4.1	4.5	5.2	15.8	12.5
		N	5	5	5	5	5	5
	Female	Mean	186.2	206.9	209.6	224.4	241.5	55.3
		S. D.	5.0	8.9	9.1	13.6	22.9	18.7
		N	5	5	5	5	5	5
G4 2,000	Male	Mean	272.4	302.8	321.8	361.7	420.2	147.8
		S. D.	6.5	7.3	10.9	10.3	15.5	11.9
		N	5	5	5	5	5	5
	Female	Mean	188.7	208.8	215.7	227.7	238.8	50.2
		S. D.	7.6	9.4	6.9	9.1	9.3	6.3
		N	5	5	5	5	5	5

Significant difference from control by ANOVA test: $P < 0.05$. N, number of animals; S.D., standard deviation.

one female rat in the 1,000-mg/kg group) were observed. However, no significant changes in weight were observed in the other rats (Table 2). Finally, in both the control and the experimental groups, no meaningful changes in the necropsy were noted.

4. Discussion

Radix paeoniae ALBA is a herbal medicine that has been used for treating gastrointestinal disorders for hundreds of years [10]. conducted a study providing *in-vitro* and *in-vivo* evaluations of the use of Radix paeoniae ALBA for peripheral nerve regeneration. They suggested that Radix paeoniae ALBA extract could be a potential nerve growth-promoting factor, being salutary in helping the growth of injured peripheral nerves Huang *et al* [11].

Radix glycyrrhizae has been commonly used in the Orient for treating a variety of diseases. Reported that administration of Radix glycyrrhizae improved spatial learning and memory and reduced stress-induced anxiety. They concluded that Radix glycyrrhizae had the potential to attenuate the behavioral and neurochemical impairments caused by stress Park *et al* [12].

Radix aconiti lateralis preparata has been widely used for the improvement of symptoms such as heart failure, inflammation, pain, and diarrhea for thousands of years in the Orient. However, the toxicity of Radix aconiti lateralis preparata has been the subject of controversy [13]. Some kinds of diester diterpenoid-type aconitum alkaloids, such as aconitine and mesaconitine, are major toxic ingredients. Some processing techniques such as pressure-steaming are known to be able to reduce the toxicity of aconitum alkaloids. These methods use the facts that alkaloids can hydrolyze highly toxic diester-diterpene aconitum alkaloids to compounds of much lower toxicity such as aconine, benzoylaconine and benzoylmesaconine [14]. Recently, Radix aconiti lateralis preparata was found to have the ability to activate the proliferation of mouse bone-marrow mesenchymal stem cells and to induce osteogenic lineage differentiation [15].

Radix salviae miltiorrhizae has been used for treating heart disease for several thousand years in the Orient. It has been found to exhibit an anti-atherosclerosis effect, which improves the patient's heart function recovery. Reported that treatment with Radix salviae miltiorrhizae protected the myocardium against ischemia and reperfusion injury. They also suggested that Radix salviae miltiorrhizae decoction could act as an anti-apoptotic and anti-ion stunning agent to protect hearts against ischemia and reperfusion injury Hu *et al* [16]. Conducted a study of the effects of Radix salviae miltiorrhizae on neuronal apoptosis following intracerebral hemorrhage in rats. They showed that Radix salviae miltiorrhizae reduced neuronal apoptosis Lee *et al* [17].

Rhizoma gastrodiae has been used for treating diseases such as headache, dizziness, paralysis, and convulsion and for enhancing health [18]. It also has been used for balancing the liver and extinguishing wind for the efficacies of moistening viscera, for calming and protecting the human body, and for calming the liver and suppressing liver-yang [19].

Radix polygalae is used as an anti-inflammatory, sedative that is thought to exert a variety of neuropsychiatric effects. reported that Radix polygalae extract could protect against N-methyl D-aspartate (NMDA) neurotoxicity and induced brain-derived neurotrophic factor expression, suggesting modulatory roles at glutamatergic synapses and possible antidepressant action Shin *et al* [20].

Curcuma root is a spice that not only is used in preparing Asian curries dishes but also is a component of some ancient herbal remedies for various diseases such as cardiovascular disease. Nowadays, curcumin, a component of Curcuma root, has been demonstrated to have a variety of beneficial health effects, including anti-atherosclerosis, anti-oxidative, anti-inflammatory and anticancer effects; thus, it has been recently used as a herbal medicine medicine Qin *et al* [21].

Performed a study to investigate the effects of Fructus chaenomelis water extract on the production of inflammatory mediators in RAW 264.7 cell mouse macrophages stimulated with lipopolysaccharide (LPS) Ryu *et al* [22]. They showed that Fructus chaenomelis significantly inhibited increases in IL-2, IL-10, IL-12p70, TNF-α, GM-CSF, M-CSF, LIF, VEGF, NO and Ca in LPS-induced RAW 264.7 cells without causing toxicity. These results suggested that Fructus chaenomelis had an anti-inflammatory effect on controlling the over-inflammatory reaction by the RAW 264.7 cells Ryu *et al* [22]. In addition, Fructus chaenomelis pharmacopuncture at the Joksamni is known to play an important role in controlling immune reactions and suppressing the inflammatory response to collagen-induced rheumatoid arthritis [23].

Although Mecasin has been used in clinics, safety studies on Mecasin are insufficient. Toxicity tests provide important data and are essential for evaluating the safety of test substances in medications [24]. This study was performed to provide objective safety data for Mecasin. Doses of 500 mg/kg, 1,000 mg/kg, and 2,000 mg/kg of Mecasin were administered to the experimental groups, and a dose of 10 mL/kg of normal saline solution was administered to the control group. In all four groups, no deaths occurred, and no abnormalities were found. No significant differences in the clinical signs or weights were noted between the control group and the experimental groups. In necropsy for checking for abnormalities in organs, no significant findings were noted.

To assess the oral toxicity of Mecasin, we need to study its acute and chronic side effects more. We also need more study on hematologic examinations and blood chemistry tests. Animal testing is the most fundamental and basic way to perform safety assessments [25]. The Korea Food & Drug Administration has testing protocol guidelines for the study of toxicity, and all experiments should be conducted following GLP regulations [26]. The results of our study showed that administration of 2,000 mg/kg of Mecasin did not cause any changes in the weights of SD rats or in the results of necropsy examinations. It also did not result in any mortality, which indicates that Mecasin administration can be used as a safe treatment.

5. Conclusion

The results showed that administration of 500 – 2,000 mg/kg of Mecasin did not cause any changes in weight or in the results of necropsy examinations. It also did not result in any mortalities, which indicated that the lethal dose of Mecasin was higher than 2,000 mg/kg. The results obtained in this study suggest that Mecasin administration can be used as a safe treatment.

Acknowledgment

This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Korea (grant number : HI11C2142).

Conflict of interest

The authors declare that there are no conflict of interest.

References

1. Guo L, Cho SY, Kang SS, Lee SH, Baek HY, Kim YS. Orthogonal array design for optimizing extraction efficiency of active constituents from jakyak-gamcho decoction, the complex formula of herbal medicines, paeoniae radix and glycyrrhizae radix. J Ethnopharmacol. 2007;113(2):306-11.
2. Kang TH, Baek HY, Kim YC. Protective effect of jakyak-gamcho-tang extract and its constituents against t-BHP-induced oxidative damage in HT22 cells. Am J Chin Med. 2005;33(2):181-9.
3. Lee JM, Hong SY, Oh MS. Effects of jakyakkamchobuja-tang on papain-induced osteoarthritis in mice. J Korean Oriental Med. 2013;34(1):116-35.
4. Elinav E, Chajek-Shaul T. Licorice consumption caus-

ing severe hypokalemic paralysis. Mayo Clin Proc. 2003;78(6):767-8.
5. Kim JK, Kim SH, Lee SM, Jeong HY, Park MY, Kim DW, *et al*. Study of single-dose toxicity of aconitum kusnezoffii reichb. pharmacopuncture in rats. J Pharmacopunct. 2012;15(3):48-52.
6. Sun JK, Shin MK. Cytotoxic effect of salvia miltiorrhiza root against L1210 cell. J Orient Neuropsychiatry. 1992;3(1):84-90.
7. Roh HS, Jeong JY, Seok Jh, Ha HY. Acute oral toxicity of root of polygala teunifolia wild. extract. J Orient Obstet Gynecol. 2013;26(4):1-13.
8. Kim YH, Seo BI. A philological study on poisoning and side effects of gastrodiae rhizoma. The Journal of Jeahan Oriental Medical Academy. 2012;10(1):137-45.
9. Choi EY, Oh HJ, Park NK, Chun HJ, Ahn JW, Jeon BH, *et al*. [Screening of cytotoxicity and antimicrobial effects of extracts from the atractylodes macrocephala koidz]. J Orient Physiol Pathol. 2002;16(2):348-52. Korean.
10. Fang YS, Shan DM, Liu JW, Xu W, Li CL, Wu HZ, *et al*. Effect of constituents from fructus aurantii immaturus and radix paeoniae alba on gastrointestinal movement. Planta Med. 2009;75(1):24-31.
11. Huang KS, Lin JG, Lee HC, Tsai FJ, Bau DT, Huang CY, *et al*. Paeoniae alba radix promotes peripheral nerve regeneration. Evid Based Complement Alternat Med. 2011;109809:1-8.
12. Park HJ, Shim HS, Kim H, Kim KS, Lee H, Hahm DH, *et al*. Effects of glycyrrhizae radix on repeated restraint stress-induced neurochemical and behavioral responses. Korean J Physiol Pharmacol. 2010;14(6):371-6.
13. Tong P, Wu C, Wang X, Hu H, Jin H, Li C, *et al*. Development and assessment of a complete-detoxi arthritis cation strategy for fuzi (lateral root of aconitum carmichaeli) and its application in rheumatoid therapy. J Ethnopharmacol. 2013;146(2):562-71.
14. J. Singhuber, M. Zhu, S. Prinz, B. Kopp. Aconitum in traditional chinese medicine: a valuable drug or an unpredictable risk?. J Ethnopharmacol. 2009;126(1):18-30.
15. Kim DR, Kim HY, Park JK, Park SK, Chang MS. Aconiti lateralis preparata radix activates the proliferation of mouse bone marrow mesenchymal stem cells and induces osteogenic lineage differentiation through the bone morphogenetic protein-2/smad-dependent runx2 pathway. Evid Based Complement Alternat Med. 2013;586741:1-11.
16. Hu F, Koon CM, Chan JY, Lau KM, Fung KP. The cardioprotective effect of danshen and gegen decoction on rat hearts and cardiomyocytes with post-ischemia reperfusion injury. BMC Complement Altern Med. 2012;12:249.
17. Lee HG, Kim YS. Effects of salvia miltriorrhiza radix on neuronal apoptosis following intracerebral hemorrhage of rats. Kor J Herbology. 2012;27(3):89-94.
18. Miao Q. New advance in identification of chinese medicine tianma. China Medical Herald. 2011;8(20):20-5.
19. Tang XH, Li B, Hu LY, Hu Zuo, YE Q, ZHU H, *et al*. [The compatibility applications of tianma]. Journal of Shanxi College of Traditional Chinese Medicine. 2012;13(3):75-6. Chinese.
20. Shin IJ, Son SU, Park H, Kim Y, Park SH, Swanberg K, *et al*. Preclinical evidence of rapid-onset antidepressant-like effect in radix polygalae extract. Plos One. 2014;9(2):e88617.
21. Qin L, Yang YB, Tuo QH, Zhu BY, Chen LX, Zhang L, *et al*. Effects and underlying mechanisms of curcumin on the proliferation of vascular smooth muscle cells induced by chol:MbetaCD. Biochem Biophys Res Commun. 2009;379(2):277-82.
22. Ryu HW, Kim YS, Lim EM. The Anti inflammatory Effects of Chaenomelis Fructus Herba Water Extract on Mouse RAW 264.7 Cell. J Orient Obstet Gynecol. 2012; 25(3): 1-15.
23. Shin DS, Kim YI, Lee H. [Effect of herbal-acupuncture with chaenomelis fructus solution at the joksamni of mice with collagen-induced arthritis]. The Acupuncture. 2007;24(3):127-43. Korean.
24. Kim YK. [The principle and test method. toxicology]. Seoul: Donghwagisul; 1994(3). p. 15-8. Korean.
25. Kim YG. [Toxicology]. Seoul: Donghwagisul; 1984. p. 15-8. Korean.
26. Korea Food & Drug Administration notification. Seoul: Korea Food & Drug Administration; 2005. p. 60.