Journal of Pharmacopuncture

ISSN 2093-6966(Print), **ISSN 2234-6856**(Online)
Journal of Pharmacopuncture 2012;15(3):048-052
DOI: http://dx.doi.org/10.3831/KPI.2012.15.007

Original Article

Study of Single-dose Toxicity of *Aconitum Kusnezoffii* Reichb. Pharmacopuncture in Rats

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Key Words

Aconitum kusnezoffii Reichb. (AKR); pharmacopuncture; toxicity test; *Aconitum ciliare* Decne.; *Aconitum triphyllum* Nakai.; *Aconitum pseudo-proliferum*

Abstract

Objective: This study was performed to analyze the single-dose toxicity of *Aconitum kusnezoffii* Reichb. pharmacopuncture (AKRP).

Methods: All experiments were conducted at the Korea Testing & Research Institute (KTRI), an institute authorized to perform non-clinical studies, under the regulations of Good Laboratory Practice (GLP). Twenty (20) Sprague-Dawley rats were chosen for the pilot study. The animals were divided into four groups of five animals per group: group 1 (G1) being the control group with each animal receiving an injection of 0.3 ml of saline and groups 2, 3, and 4 (G2, G3, and G4) being the experimental groups with each animal receiving an injection of 0.1, 0.2 or 0.3 ml of AKRP, respectively. This study was conducted with the approval of the Institutional Animal Ethics Committee.

Results: No deaths occurred in any of the 4 groups, and the LD_{50} of AKRP administered via IV was higher than 1.77 ml/kg. Some changes in the weights of the male rates were observed between the control group and the experimental groups, but no significant differences were noted in the weights of the female rats. To check for abnormalities in organs and tissues, we stained representative sections of each specified organ with Hematoxylin & Eosin for light microscopic examination. The results showed no significant differences in any of the organs or tissues.

Conclusions: The above findings suggest that *Aconitum kusnezoffii* Reichb. pharmacopuncture is a relatively safe treatment. Further studies on the subject should be conducted to yield more concrete evidence.

Received: July 27, 2012 **Accepted:** Sept 12, 2012

1. Introduction

A traditional Korean herbal medicine, Aconitum *kusnezoffii* Reichb. (AKR), has been used as it exhibits cardiotonic, anti-inflammatory and analgesic effects [1]. AKR contains various toxic alkaloids, such as hypaconitine, aconitine, mesaconitine, and talatisamine in the aconitine category and atisine, songorine, kobusine, ignavine, and napilline in the atisine category [2]. The LD_{50} of aconitine for mice is 0.3 mg/kg. Diester diterpene alkaloids (DDAs) such as mesaconitine and hypaconitine from AKR. can cause respiratory muscle paralysis, and aconitine can increase the Ca^{2+} in intracellular fluids, causing arrhythmia [3, 4]. In addition, if it is overdosed, it may cause ventricular ectopic beats, ventricular tachycardia, and ventricular fibrillation. AKR is also toxic to the liver, so it must not be used with people who have liver disease or are pregnant [5]. It was known that the toxicopathy due to Radix Aconiti was 3-30 g for adult and Aconiti Ciliare Tuber was 1-9 g but only using aconitine alkaloid to oral feeding, the toxicopathy due to 0.2 mg/kg and lethal dose is 3-4 mg [6]. We need more variable studies about toxicopathy and lethal dose in order to use it for treating.

The current research trend for single-dose toxicity testing of a pharmacopuncture is to study the acute and sub-acute toxicity through Good Laboratory Practice (GLP). All the

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experiments for this research were conducted at the Korea Testing & Research Institute (KTRI), an institute authorized to perform non-clinical studies, under the GLP.

2. Materials and methods

Aconitum kusnezoffii Reichb. is from the mountains in Chungwoong-myun, Imsil-gun, Jeonbuk. The pharmacopuncture was made in a clean room by using steam distillation in a laboratory at Korean Pharmacopuncture Institute (KPI) amlording to the following procedure, for which a diagram is given in Fig. 1: Pills were made by grinding 500 g of AKR and cleansing it in ultra-pure water. The AKR and ultra-pure water were put in a reactor and soaked for 80-90 mins. After the leaching process, the AKR was boiled in water at 100~107℃ for 120 mins, after which a condenser was used to turn the vapor into a liquid. After the extraction process, the inorganic salt was precipitated by storing the liquid in a refrigerator at 10℃ for one day. After the precipitation of the inorganic salt, the supernatant liquid was removed, the pH was controlled to be between 7.25~7.35, and a 0.9% isotonic solution was made by adding NaCl; the supernatant liquid was then filtered to 0.1 ㎛ Finally, after the liquid had been put in 10-ml vials, it was sterilized for 30 mins by using high-pressure sterilizing equipment. The completed pharmacopuncture was stored in a refrigerator, as it should have been, until it was used.

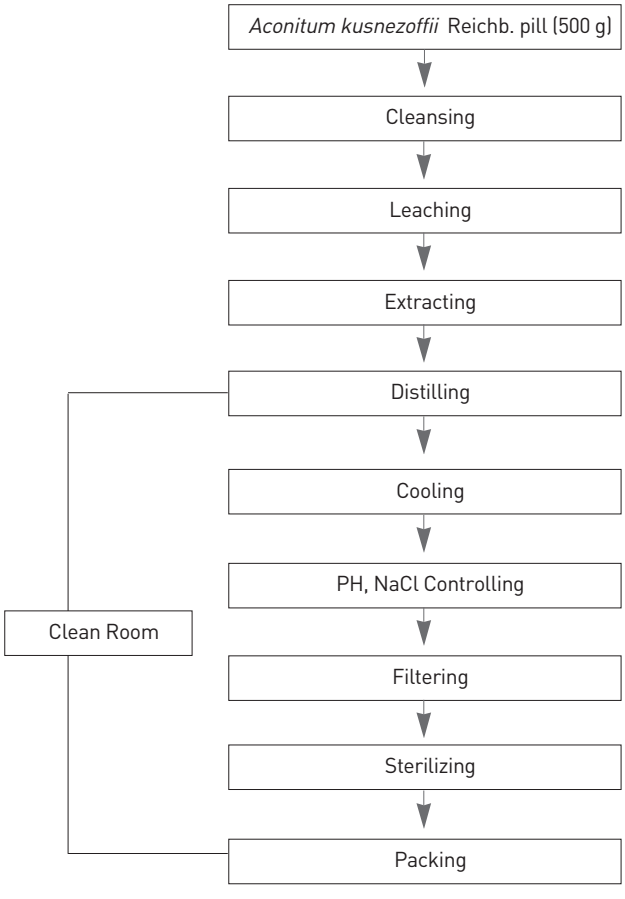

Figure 1 Process for making the AKR pharmacopuncture.

Figure 2 *Aconitum kusnezoffii* Reichb. from the mountains in Chungwoong-myun, Imsil-gun, Jeonbuk.

Figure 3 *Aconitum kusnezoffii* Reichb. pills.

The Animals used in this study were 6-week-old Sprague-Dawley rats. The mean weight of the male rats was 194.6 ± 5.8 g, and that of the female rats was 144.9 ± 7.3 g. For all animals, a visual inspection and weighing were done using a CP3202S system (Sartorius, Germany). After seven days of amllimatization, the rats' general symptoms and changes of weight were recorded. No abnormalities were found. The temperature of the laboratory where the animals were kept was 21.6~23.7℃, and the humidity was 38.7~59.3%. The animals had amless to sufficient food (Teklad Certified Irradiated Global 18% Protein Rodent Diet 2918C) and water.

After the seven days of amllimatization, animals were selected and grouped by using the criteria of their weights being close to the mean weight. A total of 20 male rats and 20 female rats were selected, and the animals were distributed into 4 groups with 5 mice per group, as shown in Table 1.

Table 1 Groupings of the rats

Group	Injection (ml/head)	Number of animals (serial number)	
		Male	Female
G1: control group	0.3	5 (1101~1105)	5 (2101~2105)
G2: low-dose group	0.1	5 (1201~1205)	5 (2201~2205)
G3: mid-dose group	0.2	5 (1301~1305)	5 (2301~2305)
G4: high-dose group	0.3	5 (1401~1405)	5 (2401~2405)

The dose for the AKR pharmacopuncture (AKRP) which was 0.1~0.3 ml/ head, was determined from "The Study on Acute and Sub-acute Toxicity and Anti-cancer Effects of Cultivated Wild Ginseng Herbal Acupuncture" [7]. In the control group, 0.3-ml of normal saline solution was injected through IV at one point of the tail vein. This study was conducted under the approval of the Institutional Animal Ethics Committee.

On the day of dosing (day 0), the general symptoms (types of toxic symptoms, recovering time, etc.), and the mortality was examined at after 30 mins, and 1, 2, 4, and 6 h. From the 1st day to the 14th day of treatment, the general symptoms were examined once a day. The weights were measured immediately before treatment and at 1, 3, 7 and 14 days after treatment. After the termination of observation, all surviving animals were anesthetized with CO_2 gas and phlebotomized at the abdominal aorta. The rats were then subjected to terminal necropsy. Principle organs (brain, liver, lungs, kidneys and spinal cord) and tissues were sampled at terminal necropsy and were fixed in 10% NBF (neutral buffered formalin). After fixation, paraffin embedding was conducted, and 3- to 4-㎛ sections were prepared by using routine histological methods. Representative sections of each specified organ were stained with Hematoxylin & Eosin for examination under a light microscope.

The weight results from the experiment were analyzed by using SAS (version 9.1.3, SAS Institute Inc., U.S.A.), and the *p*-value was analyzed using the Bartlett test, with $p < 0.05$ being considered statistically significant. When the *p*-value obtained by using the Bartlett test was under 0.05, a one-way analysis of variance (ANOVA) and Dunnett's t-test were conducted, with $p < 0.05$ and $p < 0.01$, respectively, being considered statistically significant..

3. Results

In this study, no deaths occurred by injecting AKRP, and the LD_{50} of AKRP administered via IV was found to be over 0.3 ml /head. In addition, no deaths or abnormal changes in clinical signs occurred in any of the groups (Tables 2 and 3); neither did any changes in weights, which were evaluated on the 3rd, 7th and 14th days after the injections (Table 4). No meaningful changes were noted on necropsy, and histopathological examination of all Group (0.3-ml saline/animal) showed no significant changes related to injections in the brain, lungs, liver, kidneys and spinal cord (Table 5).

Table 2 Mortality

Group	Dose	Mortality (%) (dead/tested)	
Group	(ml/head)	Male	Female
G1	0.3	0%	0%
G1	0.3	(0/5)[a]	(0/5)
G2	0.1	0%	0%
G2	0.1	(0/5)	(0/5)
G3	0.2	0%	0%
G3	0.2	(0/5)	(0/5)
G4	0.3	0%	0%
G4	0.3	(0/5)	(0/5)

[a] Number of dead animals /number of tested animals

Table 3 Clinical signs

Group	Dose (ml/head)	Sex	Number of animals	Clinical signs
G1	0.3	Male	5	NAD
G1	0.3	Female	5	NAD
G2	0.1	Male	5	NAD
G2	0.1	Female	5	NAD
G3	0.2	Male	5	NAD
G3	0.2	Female	5	NAD
G4	0.3	Male	5	NAD
G4	0.3	Female	5	NAD

NAD: No abnormalities detected.

Table 4 Body weights

Group	Dose (ml/head)	Sex	Weight in grams at 0, 7, and 14 days after dose			
Group	Dose (ml/head)	Sex		0	7	14
G1	0.3	Male	Mean	210.1	270.9	336.7
G1	0.3	Male	S. D.	7.0	13.2	18.2
G1	0.3	Male	N	5	5	5
G1	0.3	Female	Mean	146.4	172.3	196.8
G1	0.3	Female	S. D.	12.3	9.5	7.5
G1	0.3	Female	N	5	5	5
G2	0.1	Male	Mean	208.6	265.7	331.2
G2	0.1	Male	S. D	6.2	12.3	18.7
G2	0.1	Male	N	5	5	5
G2	0.1	Female	Mean	148.5	171.3	195.8
G2	0.1	Female	S. D.	10.6	13.7	19.0
G2	0.1	Female	N	5	5	5
G3	0.2	Male	Mean	210.3	267.2	333.9
G3	0.2	Male	S. D.	4.1	7.3	7.5
G3	0.2	Male	N	5	5	5
G3	0.2	Female	Mean	144.8	168.8	189.2
G3	0.2	Female	S. D.	5.0	6.7	13.7
G3	0.2	Female	N	5	5	5
G4	0.3	Male	Mean	206.6	262.0	324.1
G4	0.3	Male	S. D.	9.9	12.0	17.6
G4	0.3	Male	N	5	5	5
G4	0.3	Female	Mean	145.5	166.4	188.1
G4	0.3	Female	S. D.	8.0	8.7	10.4
G4	0.3	Female	N	5	5	5

N: number of animals; S.D.: standard deviation

Table 5 Necropsy findings

Group	Group							
Findings	G1 (0.3 ml/head)		G2 (0.1 ml/head)		G3 (0.2 ml/head)		G4 (0.3 ml/head)	
Findings	Male	Female	Male	Female	Male	Female	Male	Female
Number examined	5	5	5	5	5	5	5	5
NGF	5	5	5	5	5	5	5	5

NGF: No gross findings

4. Discussion

Aconitum kusnezoffii Reichb. is a diterpene alkaloid that contains aconitine, mesaconitine, hypaconitine, and jesaconitine, which have a strong toxicity but also have a pain-killing effect; it also contains the non-alkaloids higenamine and coryneine which have a cardiotonic effect [8]. There are other effects such as fervescence, an increase in the peripheral circulation and an anticancer effect [8-9]. A study on the analgesic and the anti-inflammatory effects of A.I.P.R.(Aconiti laterali Preparata Radix) aqua-acupuncture on arthritic rats showed a significant effect during the treatment [10]. Lee et al. [11] did a study on rats whose spinal cord injuries had been treated using aqua-acupuncture with Radix aconite (RA) at Zusanli (ST36) recovering the ability to move their hind limbs and showed that the aqua-acupuncture with RA at an early stage could bring about better movement recovery in patients with spinal cord injuries from traffic amlidents or industrial disasters, but slight increases in ALT, ALP, and AST were noted. A study on the effects of Cinnamomum Cassia and Aconitum Carmichaeli's pharmacopuncture administered orally on blood sugar in type-II diabetic mice showed that Cinnamomum cassia and Aconitum carmichaeli had a distinct anti-diabetic effect in the type-II diabetes-mellitus model [12]. A body-weight study using Aconiti tuber on normal rats showed that the Aconiti tuber could be used for the treatment of obesity, but apparent side effects, such as increased ALT and AST, were observed in the

Aconiti-tuber-treated groups [5]. A study on the toxicity and the biological activities of Aconiti ciliare tuber pharmacopuncture in rats (original articles) showed that the LD50 of Radix aconitum with Semen Glycine and Radix Glycyrrhizae was 9.0 g/kg; on the other hand, the LD50 of Aconiti ciliare tuber was more than 15 g/kg [13]. Thus, the Aconiti ciliare tuber was found not to be highly toxic, and Aconiti ciliare tuber pharmacopuncture was found to have a small antioxidant effect.

If the toxicity of general material is to be tested, the acute and the chronic harmful effects, as well as the relations to the capacity reaction, need to be studied more. To this end, animal testing is the most fundamental and basic way to assess safety. For the study of toxicity, we used the testing protocol guidelines from the Korea Food & Drug Administration [14], and all the experiments were conducted following the Good Laboratory Practice (GLP) regulations. All experiments were conducted at KTRI, an institute authorized to perform non-clinical studies.

No deaths occurred in any of the four groups, and the LD_{50} of AKRP administered via IV was higher than 1.77 ml/kg. Some changes in the weights of male rats were noted between the control group and the injection groups but no significant differences in weights were noted for the female rats. To check for abnormalities in organs and tissues, we stained representative sections of each specific organ with Hematoxylin & Eosin for examination under a light microscope. The results showed no significant differences in any organs or tissues.

The above findings suggest that *Aconitum kusnezoffii* Reichb. pharmacopuncture is a relatively safe treatment. Further studies on the subject should be conducted to yield more concrete

5. Conclusion

The object of this study was to analyze the single-dose toxicity of *Aconitum kusnezoffii* Reichb. pharmacopuncture (AKRP). In this study, the LD_{50} of AKRP was higher than about 1.77 ml/kg in both male and female rats, which demonstrates that it is safer than indicated in previous studies; in addition, its use caused no histological abnormalities. The results obtained in this study suggest that the *Aconitum kusnezoffii* Reichb. pharmacopuncture is a relatively safe treatment.

Acknowledgments

This work was supported by a Korean Pharmacopuncture Foundation grant funded by the Korean Pharmacopuncture Institute (KPI-2011-0104) and by a grant for the Traditional Korean Medicine R&D Project, Ministry for Health & Welfare, Republic of Korea (B110076).

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