

# A Study on Single Dose Toxicity of Intravenous Injection of Mecasin Herbal Acupuncture<sup>※</sup>

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## [Abstract]

### A Study on Single Dose Toxicity of Intravenous Injection of Mecasin Herbal Acupuncture<sup>※</sup>

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**Objectives :** This study was conducted to analyze the single dose toxicity of Mecasin (*Gami-Jakyak Gamcho buja* Decoction) herbal acupuncture administered in the vein of Sprague-Dawley rats.

**Methods :** All experiments were performed at the Medvill, an institution licensed to conduct non-clinical studies, under the Good Laboratory Practice (GLP) regulations. Sprague-Dawley rats were chosen in this pilot study. In the experiment, Sprague-Dawley rats were divided into four groups of five male and five female animals per group. Doses of Mecasin herbal acupuncture, at 0, 500, 1,000, and 2,000 mg/kg, were given to the experimental groups, and a dose of normal saline solution, at 2 ml/kg, was administered to the control group. Mecasin herb acupuncture and normal saline were injected into the vein at once, and we observed mortality, clinical signs, weights, and gross findings for 14 days after injection. This study was conducted under the approval of the Institutional Animal Ethics Committee.

**Results :** There is no death or abnormality in any of the four groups. All groups put on weights favorably. There are no significant gross findings in necropsy examinations.

**Conclusions :** The above results showed that intravenous injection of 500-2,000 mg/kg of Mecasin herb acupuncture did not cause any changes in weight or, in the results of necropsy examinations, in mortalities. Therefore, the toxicity of Mecasin herb acupuncture was not confirmed, and the presumptive lethal dose of Mecasin herb acupuncture was higher than 2,000 mg/kg. The outcomes suggest that treatment with Mecasin herbal acupuncture is relatively safe. Further evaluations on this subject are needed to yield more concrete evidence.

#### Key words :

Mecasin ;  
 Herbal acupuncture ;  
 Intravenous injection ;  
 Toxicity test

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## I. Introduction

Mecasin(*Gami-Jakyak Gamcho buja* Decoction) herbal acupuncture was developed for treating amyotrophic lateral sclerosis patient with pain, muscular weakness, muscular atrophy and joint contracture<sup>1)</sup>. Amyotrophic lateral sclerosis(ALS) is a progressive and fatal disease that causes degeneration of the motor neurons of the brain stem and the spinal cord<sup>2)</sup>. About half of ALS patients reported pain ; described as nagging, annoying, boring and exhausting<sup>3)</sup>.

The components of Mecasin herbal acupuncture are *Paeonia Radix*, *Glycyrrhizae Radix*, *Aconiti Lateralis Preparta Radix*, *Curcuma longa*, *Gastrodiae Elata*, *Radix Salivae Miltriorrhizae*, *Atraylodes japonica*, *Chaenomelis fructus*, *Polygala tenuifolia Willd.* There have been several studies conducted about those compositions of toxicity and effectiveness. And there are toxicity testing of Mecasin about oral taking and intramuscular injection. But toxicity testing of Mecasin herbal acupuncture about intravenous injection has not been conducted yet. Therefore, this study was performed to analyze the single-dose toxicity and fatal dose of intravenous injection of Mecasin herbal acupuncture in rats.

To study acute and subacute toxicity through Good Laboratory Practice(GLP) regulations(Ministry of Food and Drug Safety notification No.2014-67) is the current research trend for single-dose toxicity testing of extracts. All the experiments for this study were performed at Medvill.

## II. Materials and Methods

The Mecasin herbal acupuncture was prepared in a sterile room at the Medvill. Mecasin was provided from Nervous & Muscular System Disease Clinical Research Center of Wonkwang University Gwangju Korean Medical Hospital. The day of injection, it was taken a matter of its needs by using pipette in clean bench, and the Mecasin herbal acupuncture concentration was controlled to 1000 : 1.

The animals used in this study were 8-week-old Sprague-Dawley rats. The weight ranges of the rats were 321.4-367.6 g and 204.7-235.2 g for the male and the female rats at the time of injection in intravenous dose toxicity test.

For all animals, a visual inspection was conducted : all animals were weighed at the beginning. During 7 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. Abnormalities were not found.

Groupings were done after acclimatization period. 20 male rats and 20 female rats were selected in total. The animals were randomly distributed into 4 groups (5 male and 5 female rats per group) by calculating average weight and standard deviation as shown in Table 1.

The temperature of the lab was 19.7-22.6 °C, and the humidity was 48.1-75.6 %. Enough food (Lab Diet 5053) and UV-filtered water were provided.

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

Table 1. Groups of animals

| Group              | Injection dose (ml/kg) | Mecasin administration (mg/kg) | Number of animals (serial number) |                 |
|--------------------|------------------------|--------------------------------|-----------------------------------|-----------------|
|                    |                        |                                | Male                              | Female          |
| G1 control group   | 2                      | 0                              | 5 (11001~11005)                   | 5 (21001~21005) |
| G2 low-dose group  | 2                      | 500                            | 5 (12001~12005)                   | 5 (22001~22005) |
| G3 mid-dose group  | 2                      | 1,000                          | 5 (13001~13005)                   | 5 (23001~23005) |
| G4 high-dose group | 2                      | 2,000                          | 5 (14001~14005)                   | 5 (24001~24005) |

group, 2 ml/kg of normal saline solution was administered. Injection dose was same for all groups (2 ml/kg). Mecasin herbal acupuncture and normal saline were injected into vein of the rats by disposable syringes once. This study was conducted under the approval of the Institutional Animal Ethic Committee of Medvill Co., Ltd.

From the 1st day to 14th day after treatment, the general symptoms were examined once a day. On the day of dosing (day 0), the general symptoms (adverse effects, revealing time, recovery time, etc.) were examined at 30 min and at 1, 2, 4 and 6 hours after injection. The weights were measured immediately before injection and at 1, 3, 7 and 14 days after injection. After observations had been terminated, we conducted necropsies of the rats after cutting these abdominal aorta and vein under anesthetization by using CO<sub>2</sub>. All organs of surviving animals were visually inspected.

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 ANOVA test was conducted when the homogeneity of the variance was recognized. And the Kruskal-Wallis's H-test was conducted when the homogeneity of the variance was not recognized. A significance level of  $p < 0.05$  was used throughout.

### III. Results

In this study, no deaths or abnormalities occurred in any of the groups (Table 2, 3).

In this study, there were no statistically significant changes between the control groups and the experimental groups.

In weight examinations, slight decreases in the weights of some rats were observed. In intravenous dose toxicity test, weights of one male rat in the control group, one male rat in the 500 mg/kg group, one male rat in the 2,000 mg/kg group, one

Table 2. Mortality in toxicity test

| Group /Dose(mg/kg) | No. of animals | Mortality (dead / total) |         |
|--------------------|----------------|--------------------------|---------|
|                    |                | Male                     | Female  |
| G1<br>0            | 5              | 0 %                      | 0 %     |
|                    |                | (0 / 5) <sup>a</sup>     | (0 / 5) |
| G2<br>500          | 5              | 0 %                      | 0 %     |
|                    |                | (0 / 5)                  | (0 / 5) |
| G3<br>1,000        | 5              | 0 %                      | 0 %     |
|                    |                | (0 / 5)                  | (0 / 5) |
| G4<br>2,000        | 5              | 0 %                      | 0 %     |
|                    |                | (0 / 5)                  | (0 / 5) |

<sup>a</sup>: Number of dead animals / Number of tested animals.

Table 3. Clinical signs in intravenous dose toxicity test

| Group /Dose (mg/kg) | Sex    | Number of animals | Clinical signs |
|---------------------|--------|-------------------|----------------|
| G1<br>0             | Male   | 5                 | NAD            |
|                     | Female | 5                 | NAD            |
| G2<br>500           | Male   | 5                 | NAD            |
|                     | Female | 5                 | NAD            |
| G3<br>1,000         | Male   | 5                 | NAD            |
|                     | Female | 5                 | NAD            |
| G4<br>2,000         | Male   | 5                 | NAD            |
|                     | Female | 5                 | NAD            |

NAD : no abnormalities detecte.

female rat in the control group, one female rat in the 500 mg/kg group, two female rats in the 1,000 mg/kg group, three female rats in the 2,000 mg/kg group decreased temporarily at 1 day after injection. At 3 days after injection, weights of four female rats in the control group, three female rats in the 500 mg/kg group, two female rats in the 1,000 mg/kg group, two female rats in the 2,000 mg/kg group decreased temporarily. At 7 days after injection, weights of one male rat in the 2,000 mg/kg group, one female rat in the control group decreased temporarily. At 14 days after injection, one female rat in the 500 mg/kg group and one female

rat in the 1,000 mg/kg decreased temporarily. But increases in the weight were observed in the other rats (Table 4).

In both control and experimental groups, no meaningful changes in the necropsy were noted (Table 5).

Table 4. Body weights in toxicity test

| Group & Dose(mg/kg) | Sex    | Days after administration |       |       |       |       | Final weight gain |      |
|---------------------|--------|---------------------------|-------|-------|-------|-------|-------------------|------|
|                     |        | 0                         | 1     | 3     | 7     | 14    |                   |      |
| G1<br>0             | Male   | Mean                      | 344.2 | 348.4 | 357   | 376.1 | 421               | 76.8 |
|                     |        | S. D.                     | 14.5  | 17.4  | 18.9  | 19    | 20.7              | 9.7  |
|                     |        | N                         | 5     | 5     | 5     | 5     | 5                 | 5    |
|                     | Female | Mean                      | 219   | 221.2 | 219.3 | 225.3 | 248.6             | 29.6 |
|                     |        | S. D.                     | 10    | 10.4  | 7.3   | 6.7   | 17.6              | 9.3  |
|                     |        | N                         | 5     | 5     | 5     | 5     | 5                 | 5    |
| G2<br>500           | Male   | Mean                      | 342   | 345.9 | 353.7 | 377.4 | 421.7             | 79.7 |
|                     |        | S. D.                     | 12.6  | 14.4  | 13.7  | 11.3  | 21                | 18.6 |
|                     |        | N                         | 5     | 5     | 5     | 5     | 5                 | 5    |
|                     | Female | Mean                      | 215.9 | 218   | 219.7 | 229.9 | 247.3             | 31.4 |
|                     |        | S. D.                     | 9.1   | 13.4  | 5.9   | 7.5   | 12.4              | 4.5  |
|                     |        | N                         | 5     | 5     | 5     | 5     | 5                 | 5    |
| G3<br>1,000         | Male   | Mean                      | 346.4 | 350.8 | 362.3 | 387.4 | 432.4             | 86   |
|                     |        | S. D.                     | 13.5  | 16.4  | 17.6  | 20.5  | 27.1              | 20.9 |
|                     |        | N                         | 5     | 5     | 5     | 5     | 5                 | 5    |
|                     | Female | Mean                      | 212.3 | 210.7 | 218.5 | 222.1 | 238.6             | 26.3 |
|                     |        | S. D.                     | 3.5   | 9.9   | 5.5   | 6.2   | 12.1              | 12.6 |
|                     |        | N                         | 5     | 5     | 5     | 5     | 5                 | 5    |
| G4<br>2,000         | Male   | Mean                      | 343.9 | 347.7 | 358.9 | 379.2 | 429.3             | 85.4 |
|                     |        | S. D.                     | 17.9  | 19.4  | 20    | 24    | 32.9              | 28.5 |
|                     |        | N                         | 5     | 5     | 5     | 5     | 5                 | 5    |
|                     | Female | Mean                      | 212.6 | 211.7 | 213.6 | 222.9 | 246.6             | 34   |
|                     |        | S. D.                     | 4     | 4.2   | 11.1  | 11.5  | 14.7              | 14.9 |
|                     |        | N                         | 5     | 5     | 5     | 5     | 5                 | 5    |

N : Number of animals, S.D. : Standard deviatio.

Table 5. Necropsy findings in toxicity test

| Findings                | Group           |        |                   |        |                     |        |                     |        |
|-------------------------|-----------------|--------|-------------------|--------|---------------------|--------|---------------------|--------|
|                         | G1<br>(0 mg/kg) |        | G2<br>(500 mg/kg) |        | G3<br>(1,000 mg/kg) |        | G4<br>(2,000 mg/kg) |        |
|                         | Male            | Female | Male              | Female | Male                | Female | Male                | Female |
| Number of rats examined | 5               | 5      | 5                 | 5      | 5                   | 5      | 5                   | 5      |
| Unre-markable findings  | 5               | 5      | 5                 | 5      | 5                   | 5      | 5                   | 5      |

## IV. Discussion

Mecasin herbal acupuncture has been utilized in clinics to relieve pain, muscular weakness, muscular atrophy and joint especially for amyotrophic lateral sclerosis patients<sup>1</sup>. 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<sup>4</sup>. In addition, *Jakyak Gamcho buja* Decoction, 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 lessen pain with histopathological efficacy<sup>5</sup>. Because of these facts, Mecasin has been studied for treating patients with joint pain, muscle spasms, and arthralgia due to cold<sup>1</sup>. No clinical review on the effects of Mecasin herbal acupuncture is published. But, there have been many studies on the ingredient herbs of this herbal acupuncture.

*Paeonia Radix* herbal acupuncture has inflammatory effect. This herbal acupuncture restrained inflammatory reaction and reduce the number of WBC significantly, affect Globulin and Albumin of in the blood serum. Moreover, muscular tissue necrosis in SD rats plantar was restrained by Freund's complete adjuvant arthritis<sup>6</sup>.

*Glycyrrhizae Radix* extract should be beneficial in the inhibition of allergic inflammatory response by inhibiting secretion of  $\beta$ -hexosaminidase and formation of IL-4 TNF- $\alpha$ <sup>7</sup>.

*Jakyak Gamcho* Decoction-herbal acupuncture, which consists of *Paeonia Radix* and *Glycyrrhizae Radix*, has a analgesic effect and could be used safely. Cheon YS et al. showed that the *Jakyak Gamcho* Decoction-herbal acupuncture has more significant analgesic effect than the control group. And they reported that there was not an example of expired mouse for 2weeks after this experiment<sup>8</sup>.

*Aconiti lateralis preparata Radix* has been widely

used for thousands of years to improve symptoms of heart failure, inflammation, pain, and diarrhea. However, the toxicity of *Aconiti lateralis preparata Radix* has been the subject of controversy<sup>9</sup>. *Aconiti Lateralis Preparata Radix* herbal acupuncture has the painkilling and anti-inflammatory effect on the arthritis induced by Freund's complete Adjuvant in rats. Jeong SH et al. measured the levels of plasma serotonin, WBC, RBC, Hb, ESR after injecting *Aconiti Lateralis Preparata Radix* herbal acupuncture. In this experiment, the levels of plasma serotonin, WBC, RBC, Hb, and ESR are decreased and the paw edema was also decreased<sup>10</sup>. The herbal acupuncture with *Radix aconiti* at an early stage can cause better movement recovery in patients with spinal cord injuries<sup>11</sup>.

*Curcuma longa* L<sub>INNE</sub> herbal acupuncture at ST36 has a suppressing inflammation effect on Freund's adjuvant arthritis in rats<sup>12</sup>.

*Gastrodiae Elata* herbal acupuncture at GB20 showed anti-apoptotic and neuroprotective effects on cholinergic neurons in focal cerebral ischemia caused by stroke in SD rats<sup>13</sup>.

Herbal acupuncture of *Radix Salviae Miltriorrhizae* injected into LR3 has an anti-ischemic effects through the improvement of cerebral hemodynamics<sup>14</sup>.

*Atractylodes japonica*-herbal acupuncture in ST40 SP9 is effective on body weight, food efficiency ratio, the level of serum lipid, protection of liver function and prevention of cardiovascular risk by obesity induced by high fat diet<sup>15</sup>.

*Chaenomelis fructus* herbal acupuncture at the ST36 has an important role to control the immune reactions and suppress inflammatory response on the collagen induced rheumatoid arthritis<sup>16</sup>.

*Polygala tenuifolia Willd* herbal acupuncture study has not conducted yet, but *Polygala tenuifolia Willd* is used widely as an anti-inflammatory, sedative that is thought to exert a variety of neuropsychiatric effects<sup>17</sup>.

Although Mecasin has been used in clinics, safety studies on Mecasin are inadequate, so more safety studies are needed. Toxicity tests provide

important data and are essential for evaluating the safety of test substances in medications<sup>18</sup>. 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 injected to the experimental groups, and a dose of 2 ml/kg of normal saline solution was administered to the control group. In all four groups, no deaths arised, 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 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 essential and basic way to perform safety assessments<sup>19</sup>. The results of our study showed that injection of 2,000 mg/kg of Mecasin herbal acupuncture 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.

## V. Conclusion

The results showed that administration of 500–2000 mg/kg Mecasin herbal acupuncture did not casue 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.

## VI. References

1. Jeong HH, Lee JC, Cha EH et al. A Study on the Oral Toxicity of Mecasin in Rats. *Journal of Pharmacopuncture*. 2014 ; 17(4) : 61–5.
2. Han TR, Bang MS. *Rehabilitation medicine*. Seoul : Gun-ja. 2011 : 697.
3. Pagnini F, Lnetta C, Banfi P et al. Pain in Amyotrophic Lateral Sclerosis : a psychological perspective. *Neurological Science*. 2011 ; 33(5) : 1193–6.
4. 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.
5. Lee JM, Hong SY, Oh MS. Effects of Jakyakkamchobuja-tang on Papain-induced osteoarthritis in mice. *Journal of Korean Medicine*. 2013 ; 34(1) : 116–35.
6. Park HS, Sohn SH. Effect of Paeonia Radix Rubra aqua-acupuncture on Adjuvant Arthritis in rats. *The Acupuncture*. 2000 ; 17(1) : 153–68.
7. Lee HD, Song CH. Effect of Glycyrrhizae Radix Herbal acupuncture on PCA and Secretion of  $\beta$ -Hexosaminidase and Cytokines in RBL-2H3 Cells. *Korean J Acupunct*. 2011 ; 28(3) : 77–83.
8. Cheon YS, Kim JK. Study of the analgesic effect of JakYak GamChoTang-herbal acupuncture. *The Kyung Hee University Oriental Medical Journal*. 1995 ; 18(1) : 117–22.
9. Tong P, Wu C, Wang X et al. Development and assessment of a complete-detoxi arthritis cation strategy for fuzi (lateral root of aconitum car-michaeli) and its application in rheumatoid therapy. *J Ethnopharmacol*. 2013 ; 146(2) : 562–71.
10. Jeong SH, Park DS. The analgesic and anti-inflammatory effect of A.I.P.R aqua-acupuncture on the arthritis induced by Freund's complete Adjuvant in rats. *The Acupuncture*. 1997 ; 14(1) : 334–46.
11. Lee GM, Hwang WJ, Cho KY, Kim KY. The Ef-

- fects of Aqua-acupuncture with Radix aconiti on the Contraction of Rats Spinal Cord Injury. *The Acupuncture*. 1999 ; 16(1) : 283-96.
12. Lee OJ, Lee DG, Lee JH et al. Effects of Curcuma longa L<sub>INNE</sub> Herbal acupuncture at ST36 on Adjuvant Induced Arthritis in Rats. *The Acupuncture*. 2014 ; 30(4) : 95-105.
  13. Na GH, Youn DH, Na CS, Chae WS. Anti-apoptotic and Neuroprotective Effects of Gastrodiae Elata Pharmacupuncture at GB20 on Focal Brain Ischemic Injury Induced by Intraluminal Filament Insertion in Rats. *The Acupuncture*. 2008 ; 25(1) : 1-14.
  14. Lee DH. The effect of salviae radix pharmacupuncture injected into LR3 on cerebral blood flow and transient brain injury in rats [dissertation]. Jeollanam-do : Dongshin Univ. 2007. Korean.
  15. Youn DH, Kang JD, Joo JS, Chae WS, Na CS. Effects of herbal acupuncture(Atratyloides japonica, Coix lachrymajobi, Ephedra sinica, Atratyloides japonica mixed with Coix lachrymajobi and Ephedra sinica mixed with Green tea) at Pungnyung(ST40) and Umnungchon (SP9) in obese Rats induced by high fat diet. *Journal of Pharmacopuncture*. 2004 ; 7(1) : 87-100.
  16. Shin DS, Kim YI, Lee H. Effect of Herbal-acupuncture with Chaenomelis Fructus Solution at the Joksamni(ST36) of Mice with Collagen-induced Aarthritis. *The Acupuncture*. 2007 ; 24(3) : 127-43.
  17. Shin IJ, Son SU, Park HW et al. Preclinical evidence of rapid-onset antidepressant-like effect in Radix Polygalae extract. *PLoS One*. 2014 ; 9(2) : e88617.
  18. Kim YW. The principle and test method, Toxicology. Seoul : Donghwagisul. 1994 : 15-8
  19. Kim YG. Toxicology. Seoul : Donghwagisul. 1984 : 15-8.