



Analgesic effect of *Clematidis Radix* (CR) herb-acupuncture in a rat model of pain and inflammation

Hye-Jeong Hwang¹, Seung-Tae Kim^{1,2}, Hye Jung Lee^{1,2}, Young-Suk Kim³, In-Sop Shim⁴, Hi-Joon Park^{1,2}, Won-Ju Choi⁵, Jang-Hyun Kim⁵ and Dae-Hyun Hahm^{1,*}

¹Acupuncture & Meridian Science Research Center, Kyung-Hee University, Hoigi-dong, Dongdaemoon-gu, Seoul 130-701; ²Department of Meridian & Acupuncture, College of Oriental Medicine, Kyung-Hee University, Hoigi-dong, Dongdaemoon-gu, Seoul 130-701; ³Department of Oriental Internal Medicine, College of Oriental Medicine, Kyung-Hee University, Hoigi-dong, Dongdaemoon-gu, Seoul 130-701; ⁴Department of Integrative Medicine, The Catholic University of Korea, Seoul, 137-701; ⁵Department of Oriental Medicine, Dongkuk University, Kyeongsangbuk-do, 780-714, Korea

SUMMARY

Anti-nociceptive and anti-inflammatory effects of *Clematidis Radix* (CR)-distillates were investigated in three different pain animal models. Highly purified distillate of CR was injected to Zusanli (ST36) acupoint, called CR herb-acupuncture in the Korean traditional medicine. In tail flick latency test, the CR herb-acupuncture treatment did not show a significant effect of relieving acute phasic pain. To investigate the anti-inflammatory effect of CR herb-acupuncture, inflammatory pain was induced by subcutaneous injection of formalin to the plantar tissue or intra-articular injection of carrageenan to the tibio-tarsal joint in the rats. And the medicinal effect of CR herb-acupuncture was evaluated by analyzing pain behavior such as licking or biting behavior, or by measuring weight distribution ratio between two foot and ankle circumference. In the rat formalin test, the analgesic effect of CR herb-acupuncture was more pronounced in the late phase (for 20 min after the early phase) than in the early phase (for the first 10 min post formalin injection). It also significantly alleviated the carrageenan-induced monoarthritis, in terms of weight distribution ratio and ankle edema. These results revealed that CR herb-acupuncture was effective to treat the inflammatory pain and could be used as an analgesic treatment with an anti-inflammatory effect.

Key words: Pain; Inflammation; *Clematidis Radix*; Herb-acupuncture; Rat

INTRODUCTION

Clematis root (*Radix Clematidis* in Latin and Wei Ling Xian in China) is a root of *Clematis chinensis* Osbeck, which contains anemonin, anemonol,

sterol, saccharides, saponin, lacton, phenols and several amino acids. In the Korean traditional medicine, it has been widely used for treating various kinds of chronic diseases such as rheumatoid arthritis, gout, lower back and limb pain, strokes, headaches, urinal disorders, irregular menstruation, deeply lodged fish bones in the throat, tonsillitis, tetanus, beriberi, etc. (Ahn, 1998). Its property is pungent and salty in taste, warm in nature, and acting on urinary bladder channel.

*Correspondence: Dae-Hyun Hahm, Acupuncture & Meridian Science Research Center, Kyung-Hee University, 1 Hoigi-dong, Dongdaemoon-gu, Seoul 130-701, Korea. Tel: +8229610366; Fax: +8229632175; E-mail: dhahm@khu.ac.kr

Traditionally, this herb has been known to have strong action of dispersing the exopathogens and cold, eliminating the wind-dampness, and clearing stasis in channels to relieve arthralgia, and also to have the effects of elimination phlegm and softening the fish bone. Especially, the herbal extracts have been mainly used as an analgesic and anti-inflammatory agents as a COX inhibitor (Li *et al.*, 2003).

In many Asian countries, acupuncture is widely used as one of the most effective medicinal treatments, especially for the chronic diseases accompanying severe pain such as sprain, osteoarthritis and rheumatoid arthritis (Filshie and White, 1998; Stux *et al.*, 1995). And there are various methods for stimulating an acupuncture point (acupoint) in acupuncture treatments. Although electrical or manual stimulation of an acupoint is the most popular types in acupuncture therapy (Kim *et al.*, 2005), recently the use of herb-acupuncture has a tendency to increase. For the past two decades, a certain group of physicians in oriental medicine have been reported that the acupoint stimulation by subcutaneous injections of distilled herbal extracts, referred to herb-acupuncture or pharmaco-acupuncture in Korea, produced highly enhanced medicinal effects, compared to single treatment of either acupuncture or herbal therapy (Kwon *et al.*, 2001; Yeom *et al.*, 2003; Kim *et al.*, 2005). The herbal extracts, used in the herb-acupuncture, are manufactured by repeated water distillation with water for extreme purity and only tiny amount of the herbal extracts is injected into an appropriate acupoint of the body (Lee *et al.*, 2000). Accordingly, both medicinal benefits of acupuncture and herbal therapy can be attempted by a single treatment of herb-acupuncture. However there have been little systematic and scientific studies on the therapeutic effectiveness of herb-acupuncture, as of yet.

The ST36 acupoint (Zusanli), selected for *Clematidis Radix* (CR) herb-acupuncture in this study, is one of the most effective acupoints with a

wide range of medicinal applications, for example, spasmolytic and analgesic effects on the gastrointestinal tract, and homeostatic effect in endocrine and metabolism. It has been also used for the treatments of aching of the knee joints and legs in the Korean traditional medicine (Ross, 1995; Stux *et al.*, 1995; Filshie and White, 1998; Berman *et al.*, 1999; Kwon *et al.*, 2001).

The aim of the present study is to investigate the antinociceptive and anti-inflammatory properties of the aqueous extracts (distillates) from CR on experimentally induced pain and acute inflammation in the rats.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, weighting 230 - 250 g were used in this experiment. All animals were purchased from Samtaco Animal Corp. (Kyungki - do, Korea). They were kept under controlled environmental conditions ($20 \pm 2^\circ\text{C}$, 12/12 h light-dark cycles with lights on at 7 a.m., free access to food and water) for at least 1 week prior to study. Food and water were available ad libitum.

Preparation of *Clematidis Radix*

The CR was purchased from an oriental drug store (Jungdo Inc., Seoul, Korea). The distilled water extracts of CR for herb-acupuncture treatment were manufactured in an ampoule by the Korean Institute of Herbal Acupuncture (Seoul, Korea). The manufacturing processes were sequentially boiling, evaporation, condensation, and sterilization steps, which were always under the quality control of assuring that there were no suspended particles detectable by visible spectrophotometer. The extraction was started with 200 g of dried CR and finally produced 200 ml of CR distillates for herb-acupuncture. By the regulations of the Korean Institute of Herbal Acupuncture, the CR distillates passed the germ-free, anti-histamine, and endotoxin-free tests; and thus they were accepted as an s.c.

injection grade for humans.

Acupuncture treatment

Herb-acupuncture treatment of acupoint was administered into the right Zusanli (ST36), located at the anterior tibial muscle about 5 mm lower and lateral to the anterior tubercle of tibia, using a 30-gauge syringe for insulin injection. The rats in CON group were treated with saline into the same acupoint. And the rats in non-acupoint treatment group were intramuscularly treated into an arbitrary non-acupoint, located near the midline on the back. 0.5 ml insulin syringe with 30-gauge 5/16 (8 mm) needle (BD Medical Systems, NJ, USA) was used for CR or saline injection.

Antinociceptive study: tail flick latency test

Rats were randomly divided into three groups; normal group (NOR, n = 8), CR herb-acupuncture treatment into the acupoint group (ACU, n = 14), CR herb-acupuncture treatment into the non-acupoint group (NON-ACU, n = 14). The tail of the rat was immersed (5.5 cm from the tip) in a water bath at 50°C and time taken (in seconds) to flick the tail by using a stop watch was determined. Experiments were performed at room temperature (22 ± 1°C). During the test, rats were lightly restrained in a holder. Baseline tail flick latency was defined as the mean of three determinations at 10 min intervals before herb-acupuncture treatment. Only those rats with the baseline latency within the range of 4-7 s were used for further studies. Following herb-acupuncture administration, tail flick latency was measured at 10 min intervals. The antinociceptive effect in this test is presented either as latency or calculated as percentage change of the tail flick latency from the baseline level according to the formula: percentage change (%) = [(post-drug latency - pre-drug latency)/pre-drug latency] × 100.

Anti-inflammatory study: rat formalin test

Rats were randomly divided into three groups; saline-treated control group (CON, n = 10), CR

herb-acupuncture treatment into the acupoint group (ACU, n = 8), CR herb-acupuncture treatment into the non-acupoint group (NON-ACU, n = 8). Five min after acupuncture treatment, 50 µl of 5% formalin was subcutaneously injected under the right hind-paw plantar surface with a 30-gauge syringe (BD Medical Systems, NJ, USA) for insulin injection. After the formalin injection, the rats were immediately placed on clear plastic chamber (20 cm × 20 cm × 20 cm) with a mirror to allow an unimpeded view of the animal's paws and recorded the behavior through the video camera system for 30 min. After video-recording, two experienced researchers independently calculated the paw licking time in every 5 min. The licking behavior means that the rats lick or bit the formalin-injected paw. The paw licking time was classified into two phases: early phase (0-10 min after formalin injection) and late phase (10-30 min after formalin injection). The rats were treated with the aqueous extract.

Anti-inflammatory study: carrageenan-induced ankle arthritis

Rats were randomly divided into three groups; saline-treated control group (CON, n = 11), CR herb-acupuncture treatment into the acupoint group (ACU, n = 8), CR herb-acupuncture treatment into the non-acupoint group (NON-ACU, n = 10). Inflammation was induced by injection of 0.7% of carrageenan lamda (Sigma, St. Louis, MP, USA) in 50 µl of pyrogen-free saline into the right tibio-tarsal joint. To estimate the level of pain in the arthritic ankle, the amount of weight bearing force of the foot and circumference difference (CD) were measured before injection and then at 2, 4, 6 and 8 h. The animals were placed in a test box with slanted plank. The mechano-transducer (CASS Co., BCL-1L, CI-2001A, KOREA) were placed below the hind paw. The bearing force of each hind-paw was estimated at the average for five seconds. And the mean bearing force was calculated from the data obtained by these 4 times estimation:

% WDR = $100 \times (\text{weight borne by ipsilateral paw} / \text{total weight borne by both paws})$.

The circumference of right ankle was measured around the lower edge of lateral and medial malleolus with a scaled soft ruler with elasticity. The CD at 0, 2, 4, 6, 8 and 24 h was indicated as the ratio of differences between formalin-injected and normal ankle circumferences to circumference of normal ankle, using following equation:

$$\% \text{ difference of ankle circumference} = 100 \times (\text{circumference post} - \text{circumference pre}) / \text{circumference pre}$$

Circumference post: circumference at each time point after formalin injection

Circumference pre: circumference of normal ankle before formalin injection

One ankle joint was gently extended for 2 times respectively with an inter-test interval of 5 s.

Statistical analysis

The data were presented as the mean \pm S.E.M. Paw licking time was calculated and analyzed by one-way ANOVA followed by the Tukey post hoc test for further confirmation. Weight distribution ratio (WDR), difference of ankle circumference and tail flick latency were calculated and analyzed by repeated ANOVA followed by the Tukey post hoc test for further confirmation. The criterion for statistical significance was considered to be $P < 0.05$.

RESULTS

Tail flick latency

The nociceptive pain sensitivity of rats was measured with the tail flick test. The effect of CR herb-acupuncture is presented in Fig. 1. The mean baselines of tail flick latency in the three groups of rats were 5.61 ± 0.23 , 5.63 ± 0.24 and 5.75 ± 0.18 s, respectively. There were no significant differences

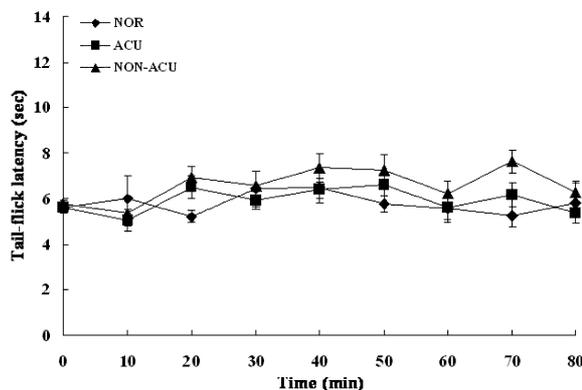


Fig. 1. Relationship between tail-flick latencies after immersion of the rats' tails in a 50 water bath.

($P > 0.05$) among these data. Also, there was no significant increase in the reaction time with CR herb-acupuncture in the tail flick test.

Rat formalin test

In order to investigate the analgesic effect of CR herbal acupuncture, the rat formalin test was used as an acute inflammatory pain model and paw licking time was measured for assessing a pain behavior after the induction of formalin-induced pain. As shown in Fig. 2, paw licking time in each group in the early phase was as follows: CON, 111.13 ± 7.90 s; ACU, 117.19 ± 9.24 s; NON-ACU, 87.37 ± 10.26 s. In the case of late phase, paw licking

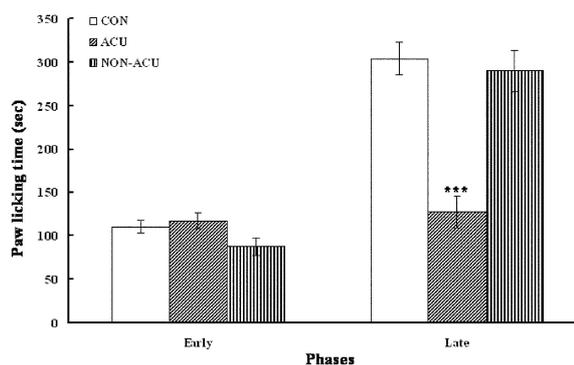


Fig. 2. Analgesic effect of CR herb-acupuncture on formalin-induced pain behavior. Data were calculated and analyzed by one-way ANOVA followed by the Tukey post hoc test for further confirmation. ($P < 0.001$ as compared to CON group)

times were as follows: CON, 303.27 ± 18.82 s; ACU, 127.89 ± 18.35 s; NON-ACU, 289.61 ± 23.48 s. There were no significant differences between groups in the early phase (0 - 10 min after formalin injection). However, in the late phase (10 - 30 min after formalin injection), herb-acupuncture of CR reduced formalin-induced pain behavior (paw licking time) than control group (CON). However, there were no significant differences between CON group and NON-ACU group in the late phase.

Carrageenan-induced ankle arthritis

Weight distribution ratio

In order to investigate the analgesic effect, the carrageenan-induced ankle arthritis was used as an acute inflammatory pain model and WDR as pain behavior was analyzed. As shown in Fig. 3, WDR (%) before carrageenan injection was as follows: CON, 48.82 ± 0.92 ; ACU, 50.09 ± 0.46 ; NON-ACU, 48.09 ± 1.43 . In 2, 4, 6, 8, 24 h, WDR (%) were as follows: CON, 42.13 ± 1.92 , 33.41 ± 2.25 , 19.03 ± 3.08 , 15.39 ± 3.13 , 30.19 ± 3.13 ; ACU, 46.37 ± 2.70 , 31.81 ± 3.63 , 30.71 ± 3.59 , 27.20 ± 4.04 , 38.56 ± 3.33 ; NON-ACU, 44.00 ± 1.82 , 30.51 ± 3.85 , 22.46 ± 1.67 , 18.16 ± 2.02 , 25.22 ± 2.83 . Before the carrageenan injection (0 h), the mean WDR did not differ among

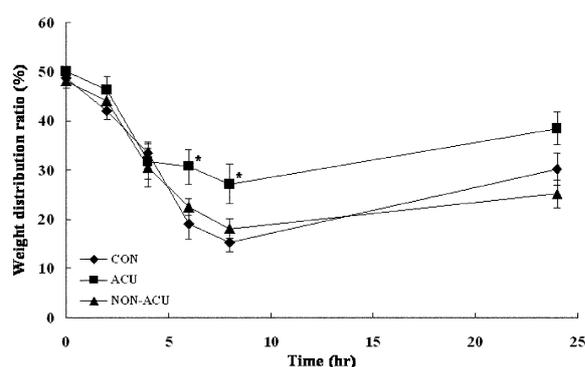


Fig. 3. Effect of CR-herb acupuncture on weight distribution between paws of carrageenan-induced ankle arthritic rats. Data were calculated and analyzed by repeated ANOVA followed by the Tukey post hoc test for further confirmation. ($P < 0.05$ as compared to CON group)

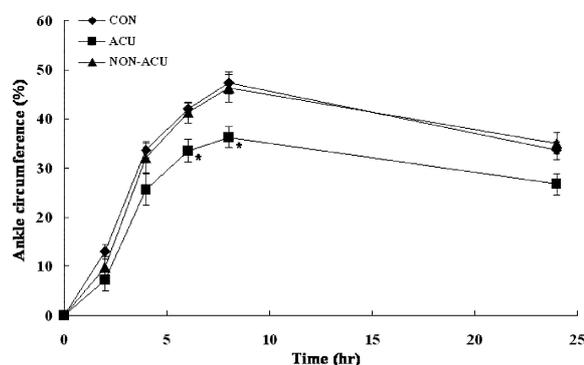


Fig. 4. Effect of CR-herb acupuncture on ankle circumferences of carrageenan-induced ankle arthritic rats. Data were calculated and analyzed by repeated ANOVA followed by the Tukey post hoc test for further confirmation. ($P < 0.05$ as compared to CON group)

the groups. The ratio in CON group was significantly decreased. The maximal ratio was measured at 8 h. ACU group significantly differ from CON group at 6 and 8 h.

Ankle circumference difference

The carrageenan-induced ankle edema in the rat was used to evaluate the anti-inflammatory effect of CR herb-acupuncture. As shown in Fig. 4, the ankle circumference before carrageenan injection (0 h) did not differ significantly among the groups. After arthritis induction, it was significantly increased, reaching a maximum at 8 h. In the CON group, ankle circumference increased to 0, 13.00 ± 1.58 , 33.68 ± 1.68 , 42.14 ± 1.28 , 47.33 ± 2.16 , 33.66 ± 1.83 at 0, 2, 4, 6, 8, 24 h. In the ACU group, the increase rate of ankle circumference was 0, 7.19 ± 2.22 , 25.65 ± 3.13 , 33.46 ± 2.35 , 36.30 ± 2.14 , 26.74 ± 2.13 and in the NON-ACU group, the increase rate of ankle circumference was 0, 9.82 ± 2.27 , 31.99 ± 3.07 , 41.21 ± 2.09 , 46.31 ± 2.82 , 35.10 ± 2.10 . Herb-acupuncture of CR significant inhibited the ankle edema than CON and NON-ACU groups. However, there were no significant differences between CON and NON-ACU groups.

DISCUSSION

The results of this study show that the aqueous extract from CR has an antinociceptive effect against the chemical stimuli provoked by the subplantar injection of formalin or by the injection of carrageenan into tibio-tarsal joint. However, this activity was very slight against thermal stimuli. This plant also presented an important anti-inflammatory effect on acute edema.

In the rat formalin test, it is well established that the early (0 - 10 min) phase of formalin induced-pain behavior is produced by direct activation of primary afferent fibers, while pain behaviors associated with late (10 - 30 min) phase are related to formalin-induced inflammatory reaction (Tjolsen *et al.*, 1992). Therefore, this behavioral test can be used to verify the anti-nociceptive effect of a test compound and to investigate the functional mechanism. Several chemical mediators, such as histamine, kinin, serotonin and prostaglandins are released from damaged cells (Rosland *et al.*, 1990). These mediators take part in the inflammatory response and are able to stimulate nociceptors and thus induce pain. Late phase pain may be blocked by drugs, which are known to reduce the inflammatory response (Hunnskaar *et al.*, 1986; Hunnskaar and Hole, 1987; Shibata *et al.*, 1989). In most cases, stimulation of pain endings in the periphery is chemical in origin. Excessive mechanical or thermal stimuli can obviously cause acute pain, but the persistence of such pain after the stimulus has been removed and the pain resulting from inflammatory or ischemic changes in tissues generally reflect a chemical stimulation of the pain afferents (Nikfar *et al.*, 1997). Many studies have confirmed that inflammation takes place in the late phase (Rosland *et al.*, 1990; Nikfar *et al.*, 1997; Nikfar *et al.*, 1998). From the results that the analgesic activity of CR herb-acupuncture was more pronounced in the late phase than in the early phase, it was suggested that the pain-relieving effect of CR herb-acupuncture might be resulted from the anti-inflammatory

action.

In many animal studies, carrageenan has been used for inducing various types of inflammation, paw edema and acute mono-arthritis. It induces hyperalgesia and inflammation in rodents, which has been widely accepted as an appropriate model to examine the efficacy of anti-inflammatory and / or analgesic drug candidates. In previous reports, the injection of carrageenan into the ankle joint of one leg in rats produced edema and pain-related behaviors, and the WDR was used as one of the main tools to assess the severity of pain (Schött *et al.*, 1994). Decreased WDR was one of the most commonly observed functional disabilities in mono-arthritic animals (Urban and Gebhart, 1999). In this study, the CR herb-acupuncture treatment into the ST36 acupoint significantly inhibited the WDR decrease in the arthritic rats, due to reducing the carrageenan-induced pain and inflammation. It suggested that CR herb-acupuncture could be a strong candidate of analgesic drug, controlling pain and inflammation.

The arthritic edema is a major symptom on arthritic patients (Bennell and Hinman, 2005), it usually resulted from the local action of multiple inflammatory mediators, including prostaglandins (Zhang *et al.*, 1997), bradykinin (Wirth *et al.*, 1992), NO (Handy and Moore, 1998), 5-hydroxytryptamine (Holsapple *et al.*, 1980) and histamine (Stochla and Maslinski, 1982). Acupuncture reduced the severity of the edema by downregulating inflammatory substances such as prostaglandins, IL-1 β (Fang *et al.*, 2007) and TNF- α (Lee *et al.*, 2004). So the inhibition of carrageenan-induced ankle edema in animals by the CR herb-acupuncture treatment gave further support for proving its action as an anti-inflammatory drug.

In conclusion, CR herb-acupuncture significantly reduced formalin-induced pain behavior in the late phase of the rat formalin test, and alleviated pain and inflammation in carrageenan-induced ankle arthritis rat, in terms of weight distribution ratio and severity of the ankle edema. These results

indicated that it was effective to treat the inflammatory pain and could be used as an analgesic treatment with an anti-inflammatory effect.

ACKNOWLEDGEMENTS

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (R11-2005-014), Republic of Korea

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