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Original Articles

The Anti-nociceptive and Anti-inflammatory Effect of *Achyranthes Japonica Nakai*

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Objective : *Achyranthes japonica Nakai* (AJ) has been classified as a herb that activates blood flow and clears the stagnated blood. In this study, we evaluated its anti-nociceptive and anti-inflammatory activity in animals to clarify the effect of AJ on pain or inflammation.

Methods : ICR mice and Sprague-Dawley rats were pretreated with an ethanolic extract of AJ with two dosages of 200 mg/kg (p.o.) and 400 mg/kg (p.o.). Nociceptive responses of acute pain were determined by hotplate and tail-flick tests. The effects of AJ on inflammation were evaluated by flexion/extention test and mechanical hyperalgesia test in models induced by both carrageenan and Complete Freund's Adjuvant (CFA).

Results : AJ showed significant analgesic effects in both hotplate and tail-flick tests at the dose of 400 mg/kg. It also produced a significant inhibition of carrageenan-induced paw edema and CFA induced arthritis in rats at the dose of 400 mg/kg.

Conclusion : We have demonstrated the analgesic and anti-inflammatory properties of an 80% ethanolic extract of AJ in animals. This suggests the application of AJ in relief of pain or inflammatory disease.

Key Words: Analgesia; Inflammation; *Achyranthes japonica Nakai*

Introduction

The root of *Achyranthes japonica Nakai* (AJ) has been used in traditional medicine in Korea for the treatment of various diseases of joint and blood circulation. *Achyranthes japonica Nakai* is classified as a herb that activates our blood flow and clears the stagnated blood. Although it has been used for the treatment of inflammatory diseases, including arthritis in clinics, experimental studies have not yet clarified the effect of AJ on pain or inflammation. Recently, there has been an increase of interest in herbal medicines as the treatment for intractable diseases such as rheumatoid arthritis[1,2]. Based on its use in traditional medicine, we evaluated the possible anti-nociceptive and anti-inflammatory properties of the extract of AJ.

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Materials and Methods

1. Plant Material

The root of AJ was obtained from the department of herbal pharmacy in Kyung Hee Medical Center, and was identified and authenticated by Dr. N.J. Kim (Research Institute of East-West Medicine, Kyung Hee University).

2. Chemicals

The following components were used: -carrageenan and indomethacine were purchased from Sigma. Complete Freund's Adjuvant (CFA) was purchased from Difco Labs.

3. Extraction of AJ

The contents of 300 g Acyranthes japonica Nakai (root) was placed into 3 liter-round bottomed flask fitted with condenser and a heated mantle. The content was refluxed with 80% v/v ethanol (200 ml distilled water+800 ml ethanol) for 3 hrs. The resulting slurry was filtered through Whatman No. 1 filter paper and the residue was again refluxed with fresh solvent as above. The two volumes of the combined filtrate was reduced using an evaporator (Buchi, Switzerland) under reduced pressure. Then, the concentrated extract was dried at the freezing vacuumed drier (EYELA, Japan), and then, it was stored in a vacuumed dessicator until use. The final weight from natural product was 53.85 g (17.95%).

4. Animal

Adult male ICR mice (25-30 g, n = 60) and Sprague-Dawley rats (180-200 g, n = 40) were conditioned at standard temperature (22±3C) and under the standard 12 hr light/dark cycle (lights on at 07:00 hr) with free access to food and water. The experimental procedures were carried out in accordance with the animal care guidelines of NIH and the Korean Academy of Medical Sciences.

5. Anti-nociceptive effect

Nociceptive responses to acute pain were determined by hotplate and tail-flick tests at 1, 3 and 5 hr after the administration of extract (200 and 400 mg/kg, p.o.) in mice. Saline was given to control group (p.o.).

1) Hot plate test

For hotplate nociceptive test, mice were placed on 53 ±0.5℃ hotplate apparatus (Ugo Basile, Italy). A glass cylinder (18 cm height and 20 cm diameter) was used to maintain the mice on the hotplate. Animals were placed on the heated surface and removed immediately after they licked the footpad of any paw. The time spent on the hotplate was recorded[3,4]

2) Tail-flick test

Nociceptive responses to acute pain were determined by tail-flick test[3,4]. Mice were retained in specially designed holders with the hind limbs protruding out and the tail hanging freely[5]. The tail-flick responses were determined by immersing the distal 1/3 of the tail in heated water (51±0.5℃) which was used as the nociceptive stimulus, and the time elapsed till the tail flicking was measured.

6. Anti-inflammatory effect

We used two kinds of inflammatory models, carrageenan- and CFA-induced inflammation, in rats.

1) Acute Inflammation (carrageenan induced paw edema)

Acute inflammation was induced in rats by injection 50㎕ of carrageenan (1% w/v) solution in distilled water into the subplantar region of right hind paw. The volume of paws was measured using a water

displacement plethysmometer (UGO BASIL, Italy) at the time point of 0, 1, 3, and 5 hr after inoculation[6]. AJ extract (200 and 400 mg/kg, p.o.) was given to experimental group 1 hr before testing. Saline or indomethacine (10 mg/kg) was also administered to the control groups.

2) Chronic inflammation (CFA induced chronic arthritis)

(1) CFA induced arthritis and drug administration

To induce arthritis using CFA, the right foot of the rat was held and the fossa of the lateral malleolus of the fibula was located. A 26 1/2-gauge needle was vertically penetrated through the skin, and turned distally to insert into the articular cavity from the gap between the tibiofibular and tarsus bone until a distinct loss of resistance was felt. A volume of 50 μl CFA was then injected[7,8]. Animals receiving CFA injection were then randomly divided into 2 groups: the control (saline, p.o.) group and AJ (400 mg/kg, p.o.), respectively. These treatments were continued for 25 days from the day 0 to 24, after the injection.

(2) Evaluation of ankle joint edema

The ankle circumference was measured 30 min after the rats were retained in specially designed holders with the hind limbs protruding and tail hanging freely. The circumferences of the ankles were measured around the lower edge of lateral and medial malleolus with a scaled soft ruler without elasticity to a precision of 1 mm. The circumferential difference in mm (CD) between the arthritic (ipsilateral) and the normal (contralateral) leg was calculated (CD = ipsilateral - contralateral)[9].

(3) Flexion and extension test

Arthritis-induced hyperalgesia was evaluated by quantifying the total number of vocalization evoked by ankle flexion or extension. The rat was held comfortably and gentle extension was applied to the ankle joint of each hind limb. The number of vocalizations emitted during both the flexion and extension periods was then quantified. A flexion and an extension stimulus was repeated every 5 s until a total of five stimuli each had been delivered to each hind limb. Vocalization rating of 0 (no vocalization) or 1 (vocalization) was given in response to each flexion or extension stimulus, depending on whether or not the animal vocalized. Thus, for each animal the vocalization rating ranged from 0 to 5 at each hind limb both in extension or flexion test.

(4) Mechanical hyperalgesia test

The Randall-Selitto test was applied to evaluate mechanical hyperalgesia in arthritic animals. A graded mechanical force was delivered through an analgesia meter (Dae Jong, Korea) onto the convex surface of the paw. Rats showed withdrawal of their hind paw or vocalization, when the applied force reached the nociceptive threshold[10].

7. Statistical procedure

Continuous data were expressed as mean ± SEM, and tested for statistical significance with two way repeated measures ANOVA, taking the treatment as the between groups measure and times as the repeated measure, followed by Dunn's multiple comparison test. Discrete data were expressed as median ± median-derived absolute deviation (MAD). The Mann-Whitney U test was used for comparison between the groups.

Results

1. Analgesic activity of AJ

1) Hot plate test

In the hotplate test, the extract of AJ (400 mg/kg, p.o.) showed significant analgesic effect at all the time points. Extract at the dose of 200 mg/kg displayed increased analgesic activity, but the significance only appeared at 3 hr after the administration (Fig. 1).

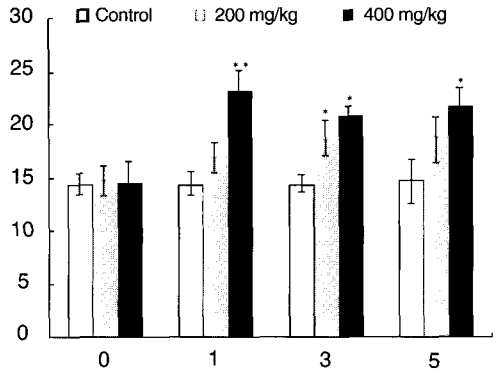

Fig. 1. The analgesic effect of the root of Achyranthes japonicain Nakai in hotplate test of mice. 400 mg/kg ethanol extract of Achyrantes japonica Nakai (p.o.) showed significant anti-nociceptive effects at 1, 3 and 5 hr after administration. The dose of 200 mg/kg displayed increased analgesic activity at only at 3 hr after the injection. Each column represents the mean±SEM. *, $p<0.05$ and **, $p<0.01$ compared with control group.

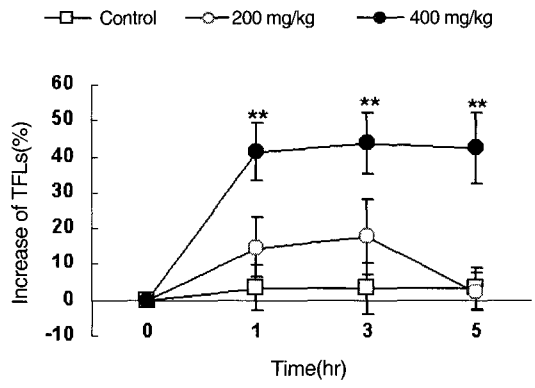

Fig. 2. The analgesic effect of the root of Achyranthes japonicain Nakai tail-flick test of mice. The 400 mg/kg (p.o.) ethanol extract of Achyrantes japonica Nakai displayed significantly increased latencies at all time point (each $P<0.01$). The dose of 200 mg/kg displayed increased analgesic activity, but there is no statistical significance. Each represents the mean±SEM. *,$p<0.05$ and **,$p<0.01$ compared with control group.

2) Tail-flick test

In the tail-flick test, the extract of AJ (400 mg/kg, p.o.) showed significantly increased latencies at all the time point (each $p < 0.01$). Extract at the dose of 200 mg/kg displayed increased analgesic activity, but there's no significance at all the time point (Fig. 2).

2. Anti-inflammatory activity of AJ

1) Anti-inflammatory activity in carrageenan-induced arthritis

The effect of Acyranthes japonica on carrageenan-induced edema is shown on Fig. 3. It was evident that the extract demonstrated a significant anti-inflammatory effect against carrageenan-induced inflammatory activity at the doses of 400 mg/kg, and the anti-inflammatory effect in early phase (1 hr after injection) was much more significant. The extract of 200 mg/kg

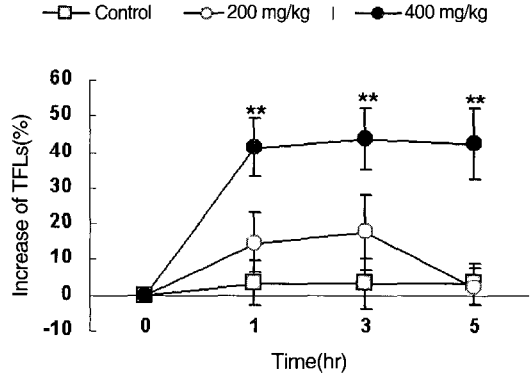

Fig. 3 The anti-inflammatory effect of the root of Achyrantes japonica Nakai on carrageenan-induced edema. The extract (400 mg/kg, p.o.) demonstrated a significant anti-inflammatory effect against carrageenan-induced inflammatory activity, especially at 1 hr after injection. Each represents the mean SEM. *,$p<0.05$ and **,$p<0.01$ compared with control group.

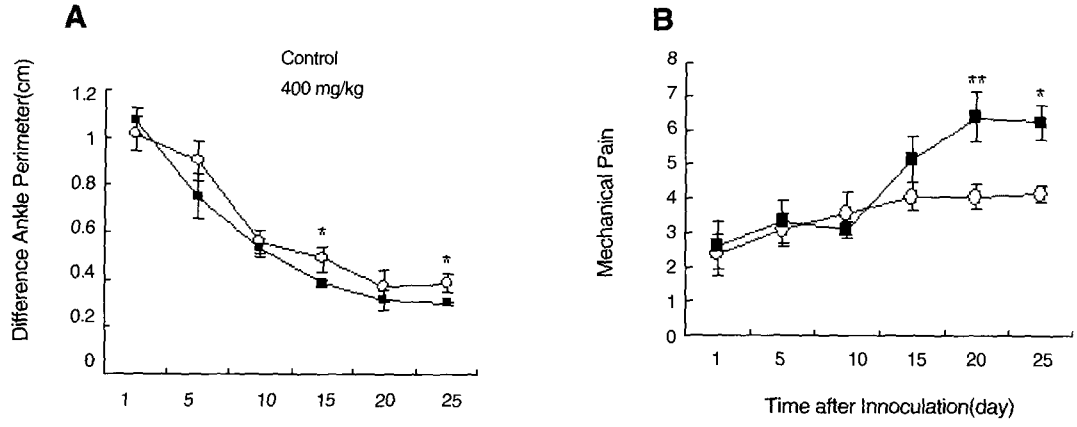

Fig. 4. The anti-inflammatory and anti-hyperalgesic effect of the root of Achyrantes japonica Nakai on complete Freund's adjuvant (CFA) induced chronic monoarthritis in rats. (A) The extract of Achyrantes japonica Nakai (400 mg/kg, p.o.) induced a significant decrease in ankle perimeter compared with those of the control group at 15 and 25 days after inoculation (B) Achyrantes japonica Nakai (400 mg/kg, p.o.) decreased the mechanical hyperalgesia in Randall-Selitto testat 20 and 25 days after inoculation. *,$p<0.05$. **,$p<0.01$ vs control group.

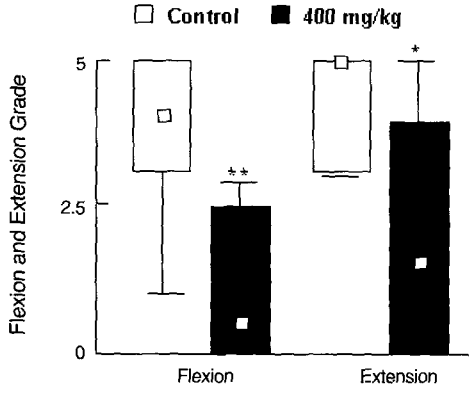

Fig. 5 Gentle flexion and extension of the inflamed ankle elicited vocalizations that were recorded as a measure of hyperalgesia. The number of vocalizations caused by ipsilateral hind limb flexion or extension was decreased significantly in the Achyrantes japonica Nakai group at 25 days after CFA injection. The data were expressed as median ±median-derived absolute deviation. *,$p<0.05$. **,$p<0.01$ vs control group.

also showed anti-inflammatory effect; however, it was less significant than that of 400 mg/kg.

2) Anti-inflammatory activity in CFA induced chronic monoarthritis

Observation of ankle circumference revealed a noticeable inflammation of the ankle, which appeared as early as 4 hr after the intra-articular injection of CFA and became substantial at 24 hr. The condition of arthritis remained stable at least up to the 25th day. The treatment of AJ induced a significant decrease in ankle perimeter compared with the control group at 15th and 25th day after the inoculation (Fig. 4A) (each $p < 0.05$).

There was a significant decrease in the hind paw mechanical pain threshold of animals in the AJ group as compared to those in control group at 20th and 25th day after injection ($p < 0.01$ and $p < 0.05$, separately; Fig. 4B).

Gentle flexion and extension of the inflamed ankle

elicited vocalizations that were recorded as a measure of hyperalgesia. The number of vocalizations caused by ipsilateral hind limb flexion or extension was decreased significantly in the AJ group at 25th day after the CFA injection ($p < 0.01$ in flexion test and $p < 0.05$ in extension test).

Discussion

In Oriental Medicine, AJ is classified as a herb that activates the blood flow and clears the stagnated blood. It has very moderate character, and tastes bitter and sour. When taken rare, AJ disperses the stagnated blood and extinguishes tumors. On the other hand, when one takes a ripe AJ, it reinforces the function of our liver and kidney, and strengthens our musculoskeletal system. So, it is mainly used in treating low back pains, knee pains, aching pains in the limbs, and so forth.

The present study demonstrated that oral administration (400 mg/kg) of AJ extract was effective in anti-nociceptive and anti-inflammatory tests. The plant extract administrated at the oral dose of 400 mg/kg significantly reduced both carrageenan-induced acute inflammation and CFA-induced chronic inflammation compared to those of saline treated groups. Furthermore, oral administration for 25 days did not induce any toxic properties. These findings justify the traditional use of the plant in the treatment of rheumatism and other inflammatory conditions.

Rheumatoid arthritis (RA) is an autoimmune disorder of unknown etiology that is characterized by progressive joint destruction, deformity, disability and premature death in most patients. At present, non-steroidal anti-inflammatory drugs (NSAIDs) supplemented with steroid hormone remains the major recommended strategy for its treatment[11,12]. While these drugs transiently suppress inflammation and ameliorate symptoms, they do not significantly improve the long-term disease outcome[13]. Furthermore, long term treatment with NSAIDs may result in serious side effects, such as gastrointestinal ulcergenicity and renal morbidity[14]. Such shortcomings call for a more effective and safe therapeutic strategy to treat RA using natural products without any side effects.

Acute and chronic oral administration of AJ extract (400mg/kg) showed significant anti-nociceptive activities in the hotplate test and tail-flick test (Fig. 1 and 2). It is generally accepted that different neuronal systems mediate the behavioral responses in hotplate and tail-flick tests. The hotplate test involves supraspinal processing whereas the tail-flick response is considered to be a spinal reflex[3] Therefore, our results suggest that AJ may be related to peripherally, as well as centrally, mediated anti-nociceptive properties.

In conclusion, we have demonstrated, using conventional pharmacological models, the analgesic and anti-inflammatory properties of 80% ethanolic extract of Achyranthes Japonica Nakai. These observations support some of the traditional uses of the plant for medicinal purposes. More studies will be required for the purification of active chemical groups from the crude extracts and to ascertain the mechanisms of action of these crude extracts.

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