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Original Article

Analgesic and anti-inflammatory effect of the aqueous extract of *Angelica dahurica*

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Background: *Angelica dahurica* has been used in various clinical cases. Its taste is hot and its property is warm, dry and nonpoisonous. Its efficacy is to remove wind-damp, cure swelling and edema, exhaust pus, stop itching, rhinitis and leukorrhea.
Object: To test through experiment *Angelica dahurica*'s analgesic and anti-inflammatory efficacy.
Method: Inject acetic acid as a pain-inducing substance to the mice and measure visceral pain bywrithing reflex. Inject carrageenan that is an edema-inducing substance to the rat's paw and measure volume of edema. Take thermal pain to mice with plantar test and measure paw withdrawal latency. Normal group is non *Angelica dahurica*-treated group and treated group is *Angelica dahurica*-treated group.
Results: In acetic acid-induced visceral model, treatment with *Angelica dahurica* suppressed writhing reflex significantlyand dose-dependently. In carrageenan-induced paw edema model, treatment with *Angelica dahurica* suppressed carrageenan-induced paw edema. In plantar test model, no significant effect on the withdrawal latency of thermal stimulation-induced nociception was observed.
Conclusion: *Angelica dahurica* has analgesic and anti-inflammatory efficacy.

Key Words : *Angelica dahurica*, analgesic, edema, carrageenan edema, writhing reflex, the plantar test

Introduction

The aromatic medicinal plant *Angelica dahurica* (umbelliferae) grows wild in thickets in China, Japan, Russia, and Korea. The root of this species has been used to treat the headache of common cold, supraorbital neuralgia, painful swelling on the body, nasal stuffiness, leukorrhea and arthralgia due to wind-dampness in Korean traditional medicine[1)].

Biological activity has been reported from agents such as protection against sepsis of the *Angelica dahurica* roots[2)], as has the anti-staphylococcal activity on *Angelica dahurica*[3)]. And the other research reported that pharmacological activities of this natural remedy include the protective activity against dexamethasone-induced disorders, liver protective activity, antimicrobial activity, anti-inflammatory activity and antimutagenic activity[4)]. and several coumarins that are constituents of *Angelica dahurica* have been extensively studied for their chemical structure[5,6)] and pharmacological effects[7-9)].

Pain is the most common symptom encountered in clinical practice. It is believed that

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current analgesic drugs such as opiates and nonsteroidal antiinflammatory drug (NSAID) are not useful in all cases because of their side effects and potency. As a result, the search for other alternatives seems necessary and beneficial.

In acetic acid-induced abdominal writhing which is the visceral pain model, the releases of arachidonic acid via cyclooxygenase induces prostaglandin biosynthesis, and it plays a role in the nociceptive mechanism[10]. It is useful in studying antinociceptive effects. Carrageenan-induced local inflammation is a commonly used method to evaluate the effects of NSAID. Therefore, carrageenan induced local inflammation (paw edema) is a useful model to asses the contribution of mediators involved in vascular changes associated with acute inflammation[11]. Also the plantar test (Hargreaves method)is used to determine thermal pain threshold by exposing the animals to the heat[12].

In the present study, we evaluated the anti-nociceptive and anti-inflammatory activities of the aqueous extract of *Angelica dahurica* in mice and rats that using several experimental pain models: acetic acid-induced writhing reflex, carrageenan-induced edema, and plantar test.

Materials and methods

1. Preparation of the aqueous extract of *Angelica dahurica* radix

To obtain the aqueous extract of *Angelica dahurica* that was purchased from Omniherb (Daegu, Korea) , 50 g of *Angelica dahurica* was added to distilled water, and extraction was performed by heating at 90°C for 2 h, concentrating with a rotary evaporator, and lyophilization (Eyela, Tokyo, Japan). The 12.4 g of resulting powder was dissolved in saline solution and filtered through a 0.45 μm syringe before use.

2. Animals and treatments

Male ICR mice that was purchased from the Dae-han Experimental Animal Center (Eumsung, South Korea) weighing 28-30 g and Male Sprague-Dawley rats that was purchased from the Dae-han Experimental Animal Center (Eumsung, South Korea) weighing 150-160 g were used for the experiments. The experimental procedures were performed in accordance with the animal care guidelines of the National Institute of Health (NIH) and the Korean Academy of Medical Sciences. The animals were housed under laboratory conditions at a controlled temperature (20 ± 2oC) and maintained under light-dark cycles, each consisting of 12 h of light and 12 h of darkness (lighting from 07:00 to 19:00 h) with food and water made available *ad* libitum.

3. Acetic acid–induced writhing reflex in mice

The animals were divided into five groups: the normal group, the acetic acid-injection group, the acetic acid-injection and 50 mg/kg *Angelica dahurica*-treated group(*ad* group), the acetic acid- injection and 100 mg/kg *ad* group, and the acetic acid-injection and 200 mg/kg *ad* group (n = 10 in each group). *Angelica dahurica* and control vehicle were orally administered at 1 h before acetic acid injection. Mice were injected intraperitoneally with 0.15 ㎖ of 1.0% acetic acid as an irritant stimulus and placed in an individual plastic cage (20 × 30 × 12 cm high) for observation. The number of writhing

reflex was counted for 30 min after acetic acid injection.

4. Carrageenan-induced edema in rats

The volume of the paw edema in each animal was measured using a plethysmometer (Ugo Basile, Italy) with a precision of two decimal places. To induce edema in the experimental animals, a single subplantar injection of carrageenan (1%, 0.05 ㎖; Sigma Chemical Co, St. Louis, MO, USA) was given to each animal, and the animals in the normal group received injections of equivalent doses of normal saline[13] as a same method.

The animals in the normal group received equivalent amount of drinking water at 1 h before normal saline injection and the animals in the *ad* groups received orally with 1 ㎖ of the aqueous extract of *Angelica dahurica* at the respective doses at 1 h before carrageenan injection, and the animals in the carrageenan-induced edema group received equivalent amount of drinking water at 1 h before carrageenan injection. The paw volume was measured immediately at 1 h, 2 h, 3 h, 4 h, and 5 h after carrageenan injection. The animals were divided into five groups: the normal group, the carrageenan-induced edema group, the carrageenan-induced edema and 100 mg/kg *ad* group, the carrageenan-induced edema and 200 mg/kg *ad* group, and the carrageenan-induced edema and 400 mg/kg *ad* group (n = 10 in each group). The percentage of edema was calculated as follows:

Percentage of edema (%) = (Vt-Vn)/Vn × 100

Vt = The paw volume of each time after the injection of carrageenan

Vn = The paw volume before the injection of carrageenan

5. The plantar test (Hargreaves's method)

To assess nociceptive responses to thermal stimuli, paw withdrawal latency was tested using the procedure previously described by Hargreaves[11]. The centre of a focused beam of radiant heat was applied to the plantar surface of the hind paw in rats and the withdrawal latency time recorded. The infrared intensity of the heat stimulus was 60 and stimulation was adjusted. because the baseline latency was 6 s and 20 s cut-off time that impose avoid tissue damage.

Three minutes was allowed between each test. The animals in the *ad* groups received orally with 1 ㎖ of the aqueous extract of *Angelica dahurica* at the respective doses at 1 h before test, and those in the normal group received equivalent amount of saline at 1 h before test. The withdrawal latency time was measured immediately at 1 h and 2 h after drug administration. The animals were divided into four groups: thermal stimulation-induced nociception and drinking water-treated group, thermal stimulation-induced nociception and 50 mg/kg *ad* group, thermal stimulation-induced nociception and 100 mg/kg *ad* group, and thermal stimulation-induced nociception and 200 mg/kg *ad* group (n = 6 in each group).

6. Data analysis

Results are presented as the mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way ANOVA followed by Duncan post-hoc test. Difference was considered significant at $P < 0.05$.

Results

1. Effect of *Angelica dahurica* acetic acid–induced writhing reflex in mice

The number of the writhing reflex in the normal group was zero. The number of writhing reflex in the acetic acid injection group was 47.60 ± 7.47. The number of writhing reflex in the acetic acid injection and *ad* groups(100 , 200 , and 400 mg/kg) was 34.44 ± 5.74, 25.57 ± 4.83, and 18.80 ± 3.00, respectively.

The present results showed that acetic acid injection into the abdominal cavity induced writhing reflex. The treatment with the aqueous extract of *Angelica dahurica* suppressed acetic acid-induced writhing reflex as a significant and dose-dependently. (Fig. 1)

2. Effect of *Angelica dahurica* on the volume of carrageenan–induced paw edema

After 1 h, paw volume in the normal group was 2.87 ± 1.16%. Paw volume in the 1% carrageenan-induced edema group was increased to 14.62 ± 1.01%. Paw volume in the 1% carrageenan-induced edema and *ad* groups(100 , 200 , and 400 mg/kg) was decreased to 4.44 ± 0.68, 8.90 ± 2.69, and 6.71 ± 2.17%.

After 2 h, paw volume in the normal group was 3.58 ± 1.37%. Paw volume in the 1% carrageenan-induced edema group was increased to 16.72 ± 1.30%. Paw volume in the 1% carrageenan-induced edema and *ad* groups(100 , 200 , and 400 mg/kg) was decreased to 5.34 ± 0.87, 7.53 ± 4.27, and 7.73 ± 2.52%.

After 3 h, paw volume in the normal group was 1.94 ± 1.14%. Paw volume in the 1% carrageenan-induced edema group was increased to 18.33 ± 1.17%. Paw volume in the 1% carrageenan-induced edema and *ad* groups(100 , 200 , and 400 mg/kg) was decreased to 8.61 ± 2.05, 10.07 ± 3.07, and 5.93 ± 2.61%.

After 4 h, paw volume in the normal group was 1.47 ± 1.17%. Paw volume in the 1% carrageenan-induced edema group was increased

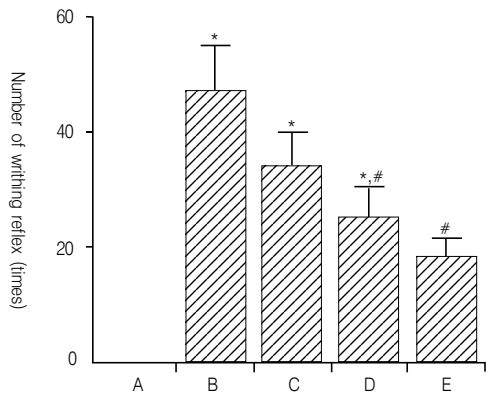

Fig. 1. Effect of *Angelica dahurica* on the number of writhing reflex. (A) normal group, (B) 1% acetic acid-induced writhing reflex group, (C) 1% acetic acid-induced writhing reflex and 50 mg/kg *ao* group, (D) 1% acetic acid-induced writhing reflex and 100 mg/kg *ao* group, (E) 1% acetic acid-induced writhing reflex and 200 mg/kg *ao* group. * represents $P < 0.05$ compared to the normal group. # represents $P < 0.05$ compared to the 1% acetic acid-induced writhing reflex group.

to 24.07 ± 0.99%. Paw volume in the 1% carrageenan-induced edema and *ad* groups(100 , 200 , and 400 mg/kg) was decreased to 16.53 ± 3.63, 19.00 ± 5.52 , and 9.25 ± 2.88%.

After 5 h, paw volume in the normal group was 0.36 ± 0.42%. Paw volume in the 1% carrageenan-induced edema group was increased to 36.97 ± 3.17%. Paw volume in the 1% carrageenan-induced edema and *ad* groups(100 , 200 , and 400 mg/kg) was decreased to 26.29 ± 3.85, 22.68 ± 6.84, and 13.19 ± 3.40%.

The present results showed that the paw volume in the normal group maintained constant level, while carrageenan injection increased paw volume as time-dependently. Treatment with the aqueous extract of *Angelica dahurica* suppressed carrageenan-induced paw edema. (Fig. 2)

3. Effect of *Angelica dahurica* on the plantar test (nociceptive thermal stimulation)

After 1 h, paw withdrawal threshold of the pre-treated value was considered as 1.00. The withdrawal latency of thermal stimulation-induced nociception and drinking water-treated group was 0.99 ± 0.02. The withdrawal latency of thermal stimulation-induced nociception and 50 mg/kg *ad* group was 1.01 ± 0.01. The withdrawal latency of thermal stimulation-induced nociception and 100 mg/kg *ad* group was 0.98 ± 0.05. The withdrawal latency of thermal stimulation-induced nociception and 200 mg/kg *ad* group was 0.91 ± 0.08.

After 2 h, the withdrawal latency of thermal stimulation-induced nociception and drinking water-treated group was 0.91 ± 0.06. The withdrawal latency of thermal stimulation-induced nociception and 50 mg/kg *ad* group was 0.87 ± 0.01. The withdrawal latency of thermal stimulation-induced nociception and 100 mg/kg *ad* group was 0.71 ± 0.08. The withdrawal latency of thermal stimulation-induced nociception and 200 mg/kg *ad* group was 0.77 ± 0.09.

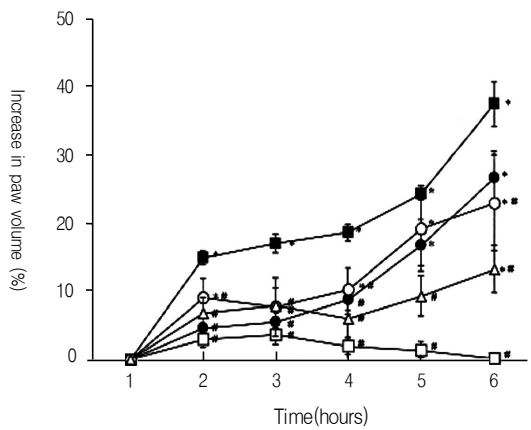

Fig. 2. Effect of *Angelica dahurica* on carrageenan-induced paw edema. (□) normal group, (■) 1% carrageenan-induced edema group, (●) 1% carrageenan-induced edema and 100 mg/kg *ao* group, (○) 1% carrageenan-induced edema and 200 mg/kg *ao* group, (△) 1% carrageenan-induced edema and 400 mg/kg *ao* group. * represents P < 0.05 compared to the normal group. # represents P < 0.05 compared to the 1% carrageenan-induced edema group.

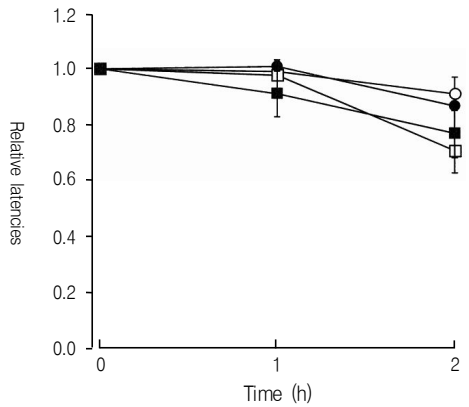

Fig. 3. Effect of *Angelica dahurica* in response to doses and time on thermal pain. (○) Thermal stimulation-induced nociception and drinking water-treated group (control), (●) thermal stimulation-induced nociception and 50 mg/kg *ao* group, (□) thermal stimulation-induced nociception and 100 mg/kg *ao* group, (■) thermal stimulation-induced nociception and 200 mg/kg *ao* group.

The present results showed that *Angelica dahurica* exerted no significant effect on the withdrawal latency of thermal stimulation-induced nociception. (Fig. 3)

Discussion

Angelica dahurica has been used to treat headache, toothache, aching eyes, abdominal pain, hysteria, bleeding, menstrual disorder and neuralgia.[14] This study evaluated the scientific basis for the use of *Angelica dahurica* on inflammation.

Angelica dahurica contains several coumarins and furanocoumarins. The coumarins have been found to inhibit multiplication of bacteria, fungi, and viruses[15-16], and they demonstrated anti-allergy[17], anti-inflammation[18] and immunosuppression activities[19]. The furanocoumarins also been found a variety of biological properties such as an inhibitory effect on prostaglandin E production[20], inhibition of acetylcholinesterase[21], inhibition of NO generation in RAW 264.7 cells[22]. These evidences indicate that coumarins and furanocoumarins can modulate the prostanoid biosynthetic pathway.

The analgesic effect of *Angelica dahurica* was evaluated by acetic acid-induced writhing reflex. The results of the present study showed that the mice pre-treated with administration of the aqueous extract of *Angelica dahurica* revealed a dose-dependent analgesic effect on acetic acid-induced writhing reflex and this effect may be due to inhibition of the synthesis of the arachidonic acid metabolites.

The carrageenan test was selected in this study, because of its sensitivity in detecting acute phase of inflammatory response[10]. The intraplantar injection of carrageenan in rats leads to increase of paw edema. Its first phase (0-2.5 h after injection of carrageenan) results from the increase of vascular permeability and concomitant release of mediators: histamine, serotonin and kinins. The second phase is correlated with the elevated production of

prostaglandins, oxygen-derived free radicals, and production of inducible cyclooxygenase[23]. In the present results, administration of the aqueous extract of *Angelica dahurica* suppressed the edematous response at 2 h after carrageenan injection and this effect continued up to 5 h.

Thermal hyperalgesia can be explained by central convergence of afferents from deep tissues and the skin[24]. Central sensitization and inhibition can be evaluated by administering an agonist such as morphine, which preferentially attenuates in put to the spinal cord from C nociceptors[25]. Because slow temporal summation of pain depends upon N-methyl-D-aspartate (NMDA)receptor activation by C nociceptor input[26]. In the present results, the aqueous extract of *Angelica dahurica* did not preferentially attenuate pain sensitivity.

Here in this study, we have demonstrated that the aqueous extract of *Angelica dahurica* has anti-nociceptive and anti-inflammatory activities, and it can be wide used to treat different types of pain. Further studies are necessary to elucidate the mechanisms behind its effects.

In this case, we know *Angelica dahurica* has Anti-inflammatory efficacy , analgesic efficacy and cures edema. So we can make the pills and tablet that contains *Angelica dahurica*. It will be conducive develop oriental medicine. And we will do more research and clinical trial for that indeed.

Conclusion

Based on the present study, we get to the conclusion like this. The experimental result are as follow:

1. In acetic acid - induced visceral model, the treatment with *Angelica dahurica* suppressed writhing reflex as a significant and dose- dependently
2. In carrageenan - induced paw edema model, the treatment with *Angelica dahurica* suppressed carrageenan - induced paw edema as a significant.
3. In plantar test model, no significant effect on the withdrawal latency of thermal stimulation - induced noiception.

These results demonstrate that *Angelica dahurica* has Anagesic and anti-inflammatory efficacy.

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