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Carrageenan으로 유발한 관절염 쥐에서의 우슬추출물 효과

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Effects of *Achyrantes japonica* on Carrageenan-Induced Arthritis Rat Model

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ABSTRACT : *Achyrantes japonica* (AJ) has been used to treat edema and arthritis in the traditional Korean medicine. To elucidate the anti-inflammatory and anti-nociceptive effects of ethanol extract of AJ, the carrageenan-induced paw edema using a plethysmometer and thermal hypersensitivity using the plantar test were measured. Ibuprofen was used as a control drug. Treatment with AJ (200 ㎎/kg p.o.) significantly reduced paw edema, compared to the carrageenan - treated rats. In the plantar test, the thermal withdrawal latency in AJ - treated group was significantly increased than the carrageenan - treated group. The results indicate that AJ could have be the anti-inflammatory and anti-nociceptive properties.

Key Word **:** *Achyrantes japonica*, Inflammation, Nociception, Paw Edema

INTRODUCTION

Achyrantes japonica (AJ), which belong to the known to Amaranthaceae family, the root of the plant is used in traditional medicine of Korea to treat edema, arthritis menstruation disturbances and as a contraceptive and abortifacient. The root contains triterpenoid saponins, which has been shown to have analgesic, antiallergic (Kosuge *et al* 1985), anti-inflammatory, antispasmodic, diuretic, hypotensive and uterine stimulant properties

In addition, it contains protocatechuic acid, which has antioxidant properties, and also inhibits the aggregation of platelets (Yun-Choi *et al*., 1985).

Recently, there has been an increase of interest in herbal medicines as the treatment for chronic inflammatory diseases such as rheumatoid arthritis. Based on the traditional medicinal values described earlier and lack of exploration on the potential pharmacological properties, the present study was aimed at investigating the anti-nociceptive and anti-inflammatory properties of AJ. (Solano *et al*., 1999) Inflammation is initiated by the infiltration of inflammatory, immune and mesenchymal cells into the inflamed sites (An *et al*., 2009). For the anti-inflammatory effect, the volume of edema was mcasured using a plethysmometer. To test the antinociceptive effect, the thermal withdrawal latency by the plantar test was estimated.

MATERIAL AND METHOD

1. Plant Material

The roots of AJ were collected at Jeju, Korea. The roots were air-dried avoiding sun-light and cut into small pieces for the experiment. The dried roots (400 g) were soaked in 70% ethanol (3 L) at room temperature for 1 day and extracted for 1 h three times with 70% ethanol in an ultrasonic apparatus and filtered with filter paper (Advantec, Toyo Roshi Kaisha, Japan) to remove the debris. The ethanol extract was evaporated under reduced pressure by rotary evaporator (R-205, Büchi, Germany) and lyophilized with freezing dryer (Operon, Seoul, Korea) to give 70% ethanol crude extract (153.31 g, yield 38.33%).

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2. MTT assay of cell viability

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was carried out as per the manufacturer's protocol (Roche, Germany) to investigate the effect of BV on cell viability (Cheon *et al*., 2009) MTT assay is based on the cleavage of the yellow tetrazolium salt MTT and the subsequent formation of purple formazan crystals by metabolically active cells in a reaction involving pyridine nucleotide cofactors NADH and NADPH. The formazan product is then solubilized and spectrophotometrically quantified using an ELISA reader. 5 × 104 cells were grown in each well of a 96-well culture plate with 100 ul of serum-free medium and the vehicle or AJ for 12 hours at the following concentrations: (1) 0, 100 ng/㎖, (2) 1, 10, 100 ug/㎖, (3) 0.54, 1.5 ㎎/㎖. 10 ul MTT solution was added to each well, and cells were then incubated for another 4 hours. The purple formazan salts thus produced were solubilized by adding 100 ul of solubilization solution to each well and incubating overnight, again at 37℃ and in 5%-CO_2 supplemented humidified atmosphere. The solubilized solution was colorimetrically assayed using an ELISA reader (TECAN USA) at a wavelength of 595 ㎚, with a reference wavelength of 690 ㎚. % cell viability was calculated as the absorbance rate of the experimental group over that of the control group.

3. Animal

Healthy, inbred adult male SD rats, weighing between 180 and 200 g, were selected and maintained under standard laboratory conditions. They had free access to food (standard rat feed pellets procured from Jung Ang Ltd., Korea) and water. Animal experiments were carried out after getting clearance from the Institutional animal ethical committee (IAEC No. 09/04/03).

4. Acute oral toxicity studies

The oral toxicity studies were performed. SD ($n = 10$) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. The AJ suspended with (0.5%, w/v, water) was administered orally at a dose of 5 mg/kg initially and mortality was observed for 3 days. If mortality was observed in 2/3 or 3/3 animals, then the dose administered was considered as toxic dose. However, if the mortality was not observed then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher doses such as 100, 300, 1000 and 2000 ㎎/㎏.

5. Induction of paw edema

ICR mice of male were housed in pairs at standard temperature (22 ± 2℃) with standard 12 h light/dark cycle (lights on at 7:00 h). Free access to food and water was allowed. Experimental procedures were carried out according to the animal care guidelines of the National Institute for Health (NIH) Guide and the Korean Academy of Medical Sciences. Acute inflammation was induced by intraplantar injection of 0.1 ㎖ of 1% (w/v) λ-carrageenan into the right hind paw. The λ-carrageenan was purchased from Sigma-Aldrich (Milano, Italy).

6. Evaluation of paw edema

Evaluation of paw edema The volume of the injected paw was measured with a plethysmometer (Ugo Basile, Varese, Italy) at 0.5 h after carrageenan injection. The volume of edema was measured as difference in volume between right and left paws. AJ (200 ㎎/㎏) and ibuprofen (50 ㎎/㎏) were administered orally 1 h before the subplantar injection of carrageenan. The animals of control group were received saline (1 ㎖/㎏). The thickness (㎜) of paw was measured at 0.5 h after the administration of carrageenan. Ibuprofen was used as a positive control (Eddy and Leimback, 1953).

7. Evaluation of thermal hypersensitivity

Thermal hypersensitivity was tested according to the Hargreave's procedure (Hargreaves *et al*., 1988) using the plantar test (Ugo Basile, Comerio, Italy). Briefly, animals were placed in a clear plexiglass box and allowed to acclimatize. A constant intensity, radiant heat source was aimed at the midplantar area of the hind paw. The time from initial heat source activation until paw withdrawal was recorded.

8. Statistical analysis

All results were expressed as mean ± S.E. Data were analyzed using one-way ANOVA followed by Duncan t-test. $P < 0.05$ was considered as statistically significant.

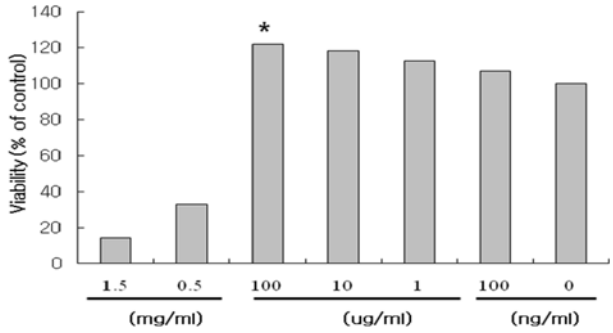

Fig. 1. Cell viability (%) after 12 hours of incubation with AJ at the respective concentrations. BV2 cells were incubated with different concentrations of AJ or the vehicle for 12 hours, and percent viabilities of the treatment groups were calculated by MTT colorimetric assay relative to that of the control group. Values are given as percent (mean ± S.E.). *$P < 0.05$ vs. control.

RESULTS

1. MTT assay of cell viability

12 hours incubation with AJ at the following concentrations produced the respective percent viabilities relative to that of the control group in the BV2 cell line cultures; 21.9% (mean ± S.E.) with 100 ug/㎖ AJ, 18.1% with 10 ug/㎖ AJ, 12% with 1 ug/㎖ AJ, and 6.7% with 100 ng/㎖ AJ (Fig. 1). Percent viability with 100 ug/㎖ AJ was significantly different from that of the control group.

2. Acute oral toxicity test

AJ extract did not produce any mortality even at the highest dose (2000 ㎎/㎏, p.o.) employed. All the doses (100, 300, 1000 and 2000 ㎎/㎏, p.o.) of AJ were thus found to be non-toxic. One doses (200 ㎎/㎏, p.o.) of AJ were selected for further pharmacological studies (data not shown). The selection of the doses for AJ was guided by the results obtained with individual concentration (50, 200, and 1000 ㎎/㎏). The dose (200 ㎎/㎏) used was the best anti-inflammatory effects.

3. Anti-inflammatory activity

Subplantar injection of carrageenan resulted in an increase in ipsilateral hindpaw volume. In AJ treated group (0.42 ± 0.03 ㎜), the volume of edema was markedly reduced, compared to the carrageenan-treated group (1.23 ± 0.03 ㎜). The positive drug, ibuprofen also revealed reduced paw edema (0.64 ± 0.01 ㎜) (Fig. 2).

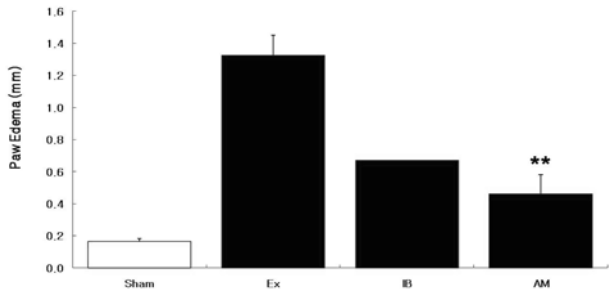

Fig. 2. Effect of AJ on carrageenan-induced edema. Sham, control group; EX, carrageenan treatment group; AM, carrageenan with AJ treatment group (200 ㎎/㎏), IB, carrageenan with ibuprofen treatment group. Values represent the mean ± S.E. ***$P < 0.001$ vs. EX; **$P < 0.05$ vs. EX.

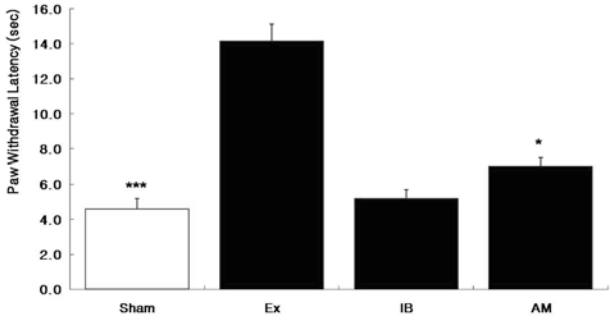

Fig. 3. Effect of AJ by plantar test. Sham, control group; EX, carrageenan treatment group; AM, carrageenan with AJ treatment group (200 ㎎/㎏), IB, carrageenan with ibuprofen treatment group. Values represent the mean ± S.E. ***$P < 0.001$ vs EX.; *$P < 0.05$ vs. EX.

4. Anti-nociceptive activity

In this study, anti-nociceptive effect against thermally induced nociceptive pain stimuli was evaluated. As shown in Fig. 3, AJ (7.04 ± 0.468s) showed increasing inflamed paw thermal withdrawal latency, compared to carrageenan-treated group (14.1 ± 0.990s). Ibuprofen (5.208 ± 0.476s) showed more increased inflamed paw thermal withdrawal latency than AJ (Fig. 3).

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.

Carrageenan-induced paw edema experiment is regarded as one of the best methods for screening of anti-inflammatory properties of herb extract (Winter *et al.,* 1962). The carrageenan-induced paw edema production is related with the presence of kinins and prostaglandins (Damas *et al.,* 1986). The ability of the AJ to reduce the thickness of the edematous hind paw (Joseph *et al.,* 2005) indicates the anti-inflammatory properties of the extract.

The present study, oral administration (200 ㎎/㎏) of ethanol extract of AJ was found to exhibit remarkable anti-nociceptive and anti-inflammatory activities. The observed anti-inflammatory activity could probably use in the treatment arthritis. The anti-nociceptive activity was evaluated by acetic acid-induced writhing response. The writhing response of the mouse to an intraperitoneal injection of noxious chemical is used to screen for both peripherally and centrally acting, anti-nociceptive activity (Chan *et al.,* 1985). Acetic acid causes pain by liberating endogenous substances and many others that excite pain nerve endings (Collier *et al.,* 1968). Recent findings that the acetic acid-induced writhing response was caused by release of prostacyclin, synthesized by the cyclo-oxygenase (COX), within the peritoneal cavity could be attributed to, at least in part, inhibition of the peripheral COX (Ballou *et al.,* 2000).

NSAIDs can inhibit COX in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors. The mechanism of the analgesic action of AJ may be due to the blockade of the effect or the release of endogenous substances that excite pain nerve endings similarly to ibuprofen, which is mediated *via* a peripheral mechanism. This inhibition revealed an anti-nociceptive effect on acetic acid-induced writhing response. However, other than the well known involvement of the opioidergic and non-opioidergic system in the central antinociceptive mechanism, the inhibition of central COX could also be suggested as part of the mechanism that leads to the observed AJ central anticnociceptor activity. This suggestion is based that on the Ballou *et al.* (2000) on the present of central antonociceptive processes and that paracetamol-induced central nociceptive activity involved the central COX inhibition (Pini *et al.,* 1997).

In conclusion, the present report revealed the anti-inflammatory and anti-nociceptive ability of AJ, suggesting AJ may be used for the treatment of inflammation and pain.

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