

## Anti-Inflammatory Effects of an Herbal Medicine (Xuan-Ju Agent) on Carrageenan- and Adjuvant-Induced Paw Edema in Rats

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### **Abstract:**

Xuan-Ju agent is an herbal formula containing aqueous extract of *Formica fusca*, *Herba epimedii*, *Fructus cnidii*, and *Fructus lycii*, all of which are reputed for their beneficial effects in the treatment of the immunodeficient diseases such as rheumatoid arthritis. We performed a study on the anti-inflammatory effects of this agent using carrageenan- and adjuvant-induced paw edema in rats. Xuan-Ju agent showed a marked inhibitory effect on edema in two models of inflammation in rats, at the dose of 0.20, 0.40 and 0.80 g/kg. Based on this study, Xuan-Ju agent is considered to be a potentially useful drug suitable for further evaluation for rheumatoid arthritis.

### **Article:**

#### **INTRODUCTION**

Anti-inflammatory drugs, presently available for the treatment of joint inflammation of various kinds, have undesirable side effects such as causing peptic ulcers (Pascucci, 2002, Corley et al., 2003 and Flower et al., 1980). Therefore, plant remedies have become increasingly popular and are often preferred to synthetically derived pharmaceuticals.

An attempt was made in our laboratory to search for herbal-based anti-inflammatory products known to have beneficial effects for rheumatic disorders in traditional medicines. A new drug formula, Xuan-Ju agent, was prepared using aqueous extract of *Formica fusca*, *Herba epimedii*, *Fructus cnidii*, and *Fructus lycii*. In this combination formula, *Formica fusca* is dried ant, which has been used in Chinese folk medicine for the treatment of rheumatoid arthritis (Tang, 2003). Other three herbal medicines have been listed in Chinese Pharmacopoeia 2000 edition. *Herba epimedii* is dried branch and leaf of *Epimedium sagittatum* (Sieb. Et Zucc.) Maxim., family Berberidaceae, and traditionally used for impotence due to deficiency of the kidney; *Fructus lycii* is the mature fruit of *Cycium barbarum* L., family Solanaceae, and traditionally used for deficiency of liver-yin and kidney-yin and diabetes; *Fructus cnidii* is the dried ripe fruit of *Cnidium onnieri* (L.) cusson, family Umbelliferae, and traditionally used for deficiency of

kidney-yang, and pudendum itching caused by dampness and eczema (Tang, 2003). We performed a pharmacological investigation on the anti-inflammatory activity of this agent using carrageenin- and adjuvant-induced animal models.

## MATERIALS AND METHODS

### *Animals*

Male Wistar rats, 4–6 weeks old were obtained from the Experiment Center of Beijing Medical University, China. They were housed in air-conditioned room at 25 °C, and fed with a standard laboratory feed and tap water throughout the experiments. Rats weighing 130–160 g were used in the study.

### *Materials*

Xuan-Ju agent was prepared by mixing 220 g of dry ant (10%), 666 g of *Herba epimedii* (30%), 666 g of *Fructus cnidii* (30%), and 666 g of *Fructus lycii* (30%) and decocting the mixture with 1:1 (v/v) boiling water three times for 1 h. The decoction was filtered, mixed, concentrated, and dried into powder. Each gram of Xuan-Ju agent is equivalent to 5 g of dried starting materials (mixed with the same ratio as described above). The powder of Xuan-Ju agent was mixed with distilled water upon oral administration.

### *Carrageenin-induced paw edema in rats*

This anti-inflammatory test was performed according to the method of Winter et al. (1962). Edema in the left hind paw of rat was induced by injection of 0.1 ml of 1% (w/v) carrageenan (Sigma, St. Louis, MO) in saline into the footpad, subcutaneously. The perimeter of the paw was measured before injection and then at 1, 2, 4, 6 h, and the edema value was expressed with the difference between the perimeter of paw at certain time point after injection and the one before injection. The Xuan-Ju agent at three dose levels (0.8, 0.4, and 0.2 g/kg, p.o.) and distilled water were given 30 min to the treatment groups and control group, respectively, before carrageenan injection. Another group of rats was orally administered with acetylsalicylic acid (10 mg/kg) as a standard reference. The edema inhibition rate was calculated as follows:

$$\text{Inhibition rate (\%)} = \frac{V_c - V_t}{V_c} \times 100$$

where  $V_c$  is the edema value of the control group; and  $V_t$  is the edema value of treated group.

### *Adjuvant-induced arthritis in rats*

Experimental arthritis was induced in rats according to the method of Newbould (1963). The right footpad of each rat was injected subcutaneously with 0.1 ml of complete-Freund's adjuvant agent (CFA, Sigma). The Xuan-Ju agent at three dose levels (0.8, 0.4, and 0.2 g/kg, p.o.), distilled water, and acetylsalicylic acid at 10 mg/kg were given daily to the treatment groups, control group, and reference group, respectively, starting at 8 days after CFA injection for 14 consecutive days. The edema of the left and right hind paws was evaluated at 2 h, 8, 12, 16, and 24 days post-injection of CFA. The inhibition rate was calculated with the same method as described above.

### Statistical analysis

Data were expressed as mean, standard deviation (S.D.) and statistically assessed by one-way analysis of variance (ANOVA). Difference between drug-treated groups and control group was evaluated by Dunnett's *t*-test.  $P < 0.05$  was considered significant.

## RESULTS

### Effect on carrageenan-induced rat paw edema

The rat's footpad became edematous soon after injection of carrageenan. Edema value of the injected footpad reached its peak at 4 h (the perimeter of paw increased by 0.67 cm). Administration of Xuan-Ju agent at 0.2, 0.4, and 0.8 g/kg significantly inhibited the development of pad swelling from 1 to 6 h after carrageenan injection ( $P < 0.01$ , Table 1). The results showed that Xuan-Ju agent at a dose of 0.8 g/kg has the most potent anti-inflammatory effects. The reference drug, acetylsalicylic acid at 10 mg/kg, had significant anti-inflammatory effect, similar to the treatment group with the highest dose, 0.8 g/kg.

**Table 1:** Effects of Xuan-Ju agent on carrageenan-induced paw edema in rats ( $n=10$ )

| Group                | Dose (g/kg) | Edema value (cm, $\bar{X} \pm S.D.$ ) |                          | Inhibition rate (%)      |                          |
|----------------------|-------------|---------------------------------------|--------------------------|--------------------------|--------------------------|
|                      |             | 1 h                                   | 2 h                      | 4 h                      | 6 h                      |
| Control              | –           | 0.49 $\pm$ 0.11                       | 0.64 $\pm$ 0.14          | 0.67 $\pm$ 0.12          | 0.63 $\pm$ 0.12          |
| Xuan-Ju agent        | 0.8         | 0.35 $\pm$ 0.15* (28.6)               | 0.40 $\pm$ 0.16** (37.5) | 0.40 $\pm$ 0.16** (40.3) | 0.38 $\pm$ 0.15** (39.7) |
|                      | 0.4         | 0.37 $\pm$ 0.13* (24.5)               | 0.46 $\pm$ 0.13* (28.1)  | 0.50 $\pm$ 0.11** (25.4) | 0.49 $\pm$ 0.13* (22.2)  |
|                      | 0.2         | 0.38 $\pm$ 0.16* (22.4)               | 0.48 $\pm$ 0.19* (25.0)  | 0.51 $\pm$ 0.16* (23.9)  | 0.48 $\pm$ 0.14* (23.8)  |
| Acetylsalicylic acid | 0.01        | 0.36 $\pm$ 0.13* (26.5)               | 0.42 $\pm$ 0.14** (34.4) | 0.45 $\pm$ 0.15** (32.8) | 0.42 $\pm$ 0.19** (33.3) |

Values represent the mean  $\pm$  S.D. of 10 animals for each group. Values in parentheses indicate the percentage inhibition rate.

\*Statistically significant from control:  $P < 0.05$ .

\*\*Statistically significant from control:  $P < 0.01$ .

### Effect on adjuvant-induced rat paw edema

Table 2 showed the time course of edema and inhibition rate after the administration of CFA and Xuan-Ju agent. The left hind paw also developed edema in addition to the right footpad. Edema value of the injected footpad and the left hind paw increased and reached a peak at 12 days. Administration of Xuan-Ju agent at a dose of 0.2 g/kg significantly inhibited the development of the swelling induced by CFA. Three different doses of Xuan-Ju agent all exhibited anti-inflammatory activity which was maintained until the experiment was terminated on day 24.

**Table 2:** Effects of Xuan-Ju agent on CFA-induced paw edema in rats, ( $n=10$ )

| Days           | Group           |                          |                          |                          |                         |
|----------------|-----------------|--------------------------|--------------------------|--------------------------|-------------------------|
|                | Control         | Reference                |                          | Xuan-Ju agent            |                         |
|                |                 | 0.1 g/kg                 | 0.8 g/kg                 | 0.4 g/kg                 | 0.2 g/kg                |
| 8 <sup>a</sup> | 0.85 $\pm$ 0.11 | 0.74 $\pm$ 0.13 (12.9)   | 0.74 $\pm$ 0.14 (12.9)   | 0.78 $\pm$ 0.09 (8.2)    | 0.76 $\pm$ 0.14 (10.6)  |
| 12             | 0.93 $\pm$ 0.11 | 0.75 $\pm$ 0.15* (19.4)  | 0.72 $\pm$ 0.12** (22.6) | 0.76 $\pm$ 0.10** (18.3) | 0.77 $\pm$ 0.19 (17.2)  |
| 16             | 0.90 $\pm$ 0.11 | 0.70 $\pm$ 0.19* (22.2)  | 0.68 $\pm$ 0.12** (24.4) | 0.69 $\pm$ 0.14** (23.3) | 0.70 $\pm$ 0.18* (22.2) |
| 20             | 0.87 $\pm$ 0.11 | 0.66 $\pm$ 0.20* (18.9)  | 0.65 $\pm$ 0.16** (25.3) | 0.66 $\pm$ 0.15** (24.1) | 0.69 $\pm$ 0.16* (20.7) |
| 24             | 0.80 $\pm$ 0.12 | 0.62 $\pm$ 0.17* (22.5)  | 0.59 $\pm$ 0.17** (26.3) | 0.60 $\pm$ 0.12** (25.0) | 0.66 $\pm$ 0.19 (17.5)  |
| Left foot      |                 |                          |                          |                          |                         |
| 8 <sup>a</sup> | 0.39 $\pm$ 0.09 | 0.34 $\pm$ 0.12 (12.8)   | 0.31 $\pm$ 0.11* (20.5)  | 0.34 $\pm$ 0.08 (12.8)   | 0.32 $\pm$ 0.09 (17.9)  |
| 12             | 0.51 $\pm$ 0.09 | 0.38 $\pm$ 0.14* (25.5)  | 0.37 $\pm$ 0.12** (27.5) | 0.39 $\pm$ 0.07* (23.5)  | 0.41 $\pm$ 0.11* (19.6) |
| 16             | 0.48 $\pm$ 0.09 | 0.35 $\pm$ 0.12* (27.1)  | 0.36 $\pm$ 0.13* (25.0)  | 0.37 $\pm$ 0.09 (22.9)   | 0.38 $\pm$ 0.11 (20.8)  |
| 20             | 0.46 $\pm$ 0.07 | 0.34 $\pm$ 0.11** (26.1) | 0.32 $\pm$ 0.10** (30.4) | 0.32 $\pm$ 0.09** (30.4) | 0.38 $\pm$ 0.11 (17.4)  |
| 24             | 0.40 $\pm$ 0.08 | 0.31 $\pm$ 0.11 (22.5)   | 0.29 $\pm$ 0.12 (27.5)   | 0.30 $\pm$ 0.08* (21.5)  | 0.34 $\pm$ 0.13 (15.0)  |

Values in parentheses indicate the percentage inhibition rate.

<sup>a</sup>Edema value was measured at 2h after the drug administration, which was performed at 8 days after the CFA injection.

\*Statistically significant from control,  $P < 0.05$ .

\*\*Statistically significant from control,  $P < 0.01$ .

## DISCUSSION

Our results showed that in tests of short duration, such as carrageenan-induced edema in rats, as well as in tests of longer duration, such as CFA-induced arthritis in rats, Xuan-Ju agent at 0.2, 0.4, and 0.8 g/kg exhibited significant anti-inflammatory effects. Based on these preliminary observations, Xuan-Ju agent is considered a drug candidate to be chosen for further evaluation including clinical investigations for rheumatoid arthritis. Further studies on identification and isolation of active components in Xuan-Ju agent as well as mode of action are under investigation.

Rheumatoid arthritis is an autoimmune disease characterized by chronic inflammation, hyperproliferation of the synovial lining and cartilage destruction (Schiff, 1997). Although many plant extracts have been clinically used as anti-inflammatory remedies in the treatment of rheumatoid arthritis, their mode of action remains unclear (Wang and Zhang, 2002). The interaction between the compounds from medicinal plants and the diseased state of rheumatism might be far more complex than merely the result of an anti-inflammatory activity exerted by a single phytochemical molecule or a group of isolates. We believe traditional formulas containing combination herbal medicines hold the potential to become the therapeutics of choice in future due to the synergistic effect achieved by the multiple ingredients that may relieve the inflammation and pain, strengthen the impaired immune system, restore the damaged bone and cartilage, and improve the overall symptoms associated with the arthritis.

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