

Anti-inflammatory effects of leaf and twig of *Tripterygium wilfordii* on paw edema in mice

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Abstract

The root of *Tripterygium wilfordii* (TWH) is a traditional Chinese herb used to treat the immune-related diseases such as rheumatoid arthritis, whereas the leaf and twig of TWH was considered useless and discarded. We performed a study on the anti-inflammatory effects on the leaf and twig portion agent using carrageenan- and adjuvant-induced paw edema in rats. They showed a marked inhibitory effect on edema in both models of inflammation in rats, at the dose of 50, 100 and 200 mg/kg, especially on secondary immunological arthritis. Based on this study, we confirmed that the leaf and twig of TWH is a potentially useful drug suitable for further evaluation for rheumatoid arthritis and can replace root of TWH.

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Keywords: *Tripterygium wilfordii*; Anti-inflammatory effects; Rheumatoid arthritis

1. Introduction

Tripterygium wilfordii is a traditional Chinese herb grown in the south of China. Extract from the root, either by solvents or by water boiling methods, has been widely used in the treatment of some immune-related diseases, especially rheumatoid arthritis (RA) [1–3]. However, the leaf and twig of TWH was considered useless and discarded. The excessive digging for TWH's root have exhausted this resource. It is reported that the leaf and twig of TWH have the same compounds present in the root [4], but to the best of our knowledge, no data are reported on RA. Thus, we performed a pharmacological investigation aimed to evaluate the anti-inflammatory activity of leaf and twig TWH extract using carrageenan- and Freund's complete adjuvant-induced edema in rats.

2. Experimental

2.1. Plant

Tripterygium wilfordii Hook f. collected from Hubei Province of PR China in October 2003 was identified by Prof. Zizhen Chen, Hubei Academy of Traditional Chinese Medicine and Pharmacy. A voucher specimen (Zizhen

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Chen 031011) was deposited in the Herbarium of Hubei Academy of Traditional Chinese Medicine and Pharmacy.

2.2. Animals

Male Wistar rats (Hubei Experimental Animal Center) weighing 200 ± 15 g were used. The animals were maintained under standard laboratory conditions of humidity, temperature (25 ± 1 °C) and light (12 h day:12 h night), and allowed free access to food and water ad libitum for at least 5 days, before the experiments. All experimental designs and procedures had received approval from the Animal Ethics Committee of Huazhong University of Science and Technology.

2.3. Extraction

The *T. wilfordii* air-dried leaf and powdered twigs (1 kg) extracted with hot water for 3 and 2 h, respectively, combined and evaporated to dryness in vacuo gave a residue (91.8 g). The residue dissolved in 1000 ml of H₂O and shaken with petroleum ether to remove chlorophyll fraction was extract with *n*-hexane. After evaporated in vacuo were obtained 22.2 g of *n*-hexane extract.

2.4. Carrageenin-induced paw edema in rats

This anti-inflammatory test was performed according to Winter et al. [5]. Edema in the left hind paw of rat was induced by the subcutaneous injection of 0.1 ml of 1% (w/v) carrageenan (Sigma) in saline into the footpad. The edema was measured (cm) before and then at 1, 2, 4, 6 h, after carrageenan injection. The TWH extract at (200,100 and 50 mg kg⁻¹) were given orally 30 min before carrageenan injection. Another group of rats was orally administered with acetylsalicylic acid (ASA) 10 mg kg⁻¹ as a standard reference. Control group received distilled water.

2.5. Adjuvant-induced arthritis in rats

Experimental arthritis was induced in rats according to the method of Newbould [6]. The right footpad of each rat was injected subcutaneously with 0.1 ml of Freund's complete adjuvant agent (FCA, Sigma). The TWH extract at three dose levels (200,100 and 50 mg kg⁻¹, p.o.), distilled water, and ASA at 10 mg kg⁻¹ were given daily for 16 consecutive days to the treatment groups, control group, and reference group, respectively. Treatment started from day 8th after FCA injection. The edema of the left and right hind paws was evaluated at 2 h, 8, 12, 16, and 24 days post-injection of FCA.

2.6. 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 Student's *t*-test. $P < 0.05$ was considered significant.

3. Results

3.1. 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 TWH extract at 50, 100 and 200 mg kg⁻¹ significantly inhibited the development of pad swelling from 1 to 6 h after carrageenan injection ($P < 0.01$, Table 1).

3.2. Effect on adjuvant-induced rat paw edema

Table 2 showed the time course of edema and inhibition rate after the administration of FCA and TWH hexanic extract. The left hind paw also developed edema in addition to the right footpad. The edema value of the injected

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