Activity directed investigation on anti-inflammatory fractions and compounds from flowers of *Trollius chinensis*

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Abstract: The flower of *Trollius chinensis* Bunge is used as an anti-inflammatory drug to treat upper respiratory tract infection, pharyngitis, tonsillitis, and bronchitis. In order to identify the active components, the activity-screen directed compound isolation was carried out, leading to the identification of the major active fraction and 4 compounds thereof. As a result, flavonoids and phenolics were demonstrated to be the major anti-inflammatory components.

Keywords: *Trollius chinensis*; Anti-inflammation; flavonoids; phenolic acids.

INTRODUCTION

The flowers of *Trollius chinensis* Bunge (Ranunculaceae), known as Jinlianhua in China, have been used as an anti-inflammatory drug since ancient times to treat upper respiratory tract infection, pharyngitis, tonsillitis, and bronchitis etc (Jiangsu New College of Medicine, 1977). Related bioactivity investigations have reported that Jinlianhua exhibits significant antibacterial activity against bacteria such as *Staphyloccocus aureus*, *Streptococcus pneumonia*, and *Pseudomonas aeruginosa*, as well as moderate antiviral activity against parainfluenza virus 3 and influenza virus A (Lin *et al*., 2001; Li *et al*., 2002; Wang *et al*., 2004). Both above antibacterial and antiviral activities support the anti-inflammatory use of these flowers. In order to experimenttally trace the active fractions or compounds, the separation of fractions or compounds was carried out under direction of anti-inflammatory screen herein, consequently leading to the isolation and identification of some components in favor of the traditional use of this drug.

MATERIALS AND METHODS

Plant material

The flowers purchased from the drug market of Anguo, Hebei province were identified as *Trollius chinensis* Bunge by Associate Professor Wang Rufeng. A voucher specimen was deposited in the Herbarium of School of Chinese Materia Medica, Beijing University of Chinese Medicine.

Chemicals

Prednisone acetate (PA, positive control) of analytical grade (Batch No. 002239) was the commercial product of Tianjin Pharmaceutical Co. Ltd. All chemical reagents including xylene, ethanol, petroleum ether, ethyl acetate (EtOAc), *n*-butanol, etc. were of analytical grade and purchased from Beijing Chemical Works (Beijing, China). Silica gel of 200-300 mesh was produced by Qingdao Marine Chemical Co. Ltd. (Qingdao, China). Polyamide (50-100 mesh) and Sephadex LH-20 (50 μ) were made by Pharmacia, Fine Chemicals, Inc., Piscataway, N.J., USA.

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Animals

Three hundred and seven male ICR mice (weighing 18-22g) were purchased from Experimental Animals Center, Peking University Health Science Center. The animals were housed under standard laboratory conditions of humidity (50%~60% RH), temperature (25±1°C) and light (12 hours day and 12 hours night cycle), and were supplied with food and water ad libitum.

Extraction and fractionation

The dry flowers of *T. chinensis* (8 kg) were extracted with 95% ethanol under reflux 3 times and 2 hours each. The extract solution was evaporated *in vacuo* to obtain the crude extract (CE, 1.6 kg). About 10 g of CE was used for anti-inflammatory screen; the rest was re-dissolved in water and fractionized with petroleum ether, EtOAc and *n*-butanol successively to yield 4 fractions, namely, petroleum ether fraction (PF, 363g), EtOAc fraction (EF, 190 g), *n*-butanol fraction (BF, 319g), as well as water fraction (WF, 433g).

Isolation

The EF, which was shown to have more potent anti-inflammatory activity by anti-inflammatory tests described in the following text, was further separated by column chromatography including silica gel, polyamide and Sephadex LH-20 to obtain 13 compounds, 5 of which (fig. 1) had been demonstrated to be active by anti-inflammatory test *iv vivo*. The details for separation and identification of these compounds were presented elsewhere (Wang *et al*., 2004; Cai *et al*., 2006).

Xylene Induced Mice Ear Edema (Fan *et al*., 2002)

The test was conducted in 2 stages, and the second stage was designed based on the results obtained from the first one. One hundred and fifty-one male ICR mice were

randomly divided into 13 groups (groups 1-13) of 11 (stage 1) or 12 (stage 2) animals. Except the untreated blank control group, all groups were treated by intragastric administration (ig) of test samples or PA at different dosages twice daily for a total of 5 times. The details of allocation and administration were shown in tables 1 and 2. Then, they were topically applied 40 µl of xylene onto both sides of the left ear after the last dose to induce edema. Thirty minutes later, the mice were sacrificed, both ears of each mouse were removed, and 8 mm punches were made by a borer in the corresponding position of both the right and the left ears. Each disc from the ear was weighed and the difference between the discs from the right and the left ear was calculated and recorded as edema level (W). The edema inhibitory rate of each test sample was calculated according to the formula:

$$\text{Edema inhibitory rate of test sample (\%)} = \frac{(W_{\text{blank control}} - W_{\text{test sample}})}{W_{\text{blank control}}} \times 100\%$$

Acetic acid induced mice vascular permeability (Zhang and Yu, 1999)

Similarly, the test was also conducted in 2 stages, and the second stage was designed based on the results obtained from the first one. One hundred and fifty-six male ICR mice were randomly divided into 13 groups of 12 animals. Except the untreated blank control group, all groups were treated by intragastric administration (ig) of test samples or PA at different dosages twice daily for a total of 5 times. The details of allocation and administration were shown in tables 3 and 4. About 1 hour after the last administration, 0.6% Evan's blue in saline was injected intravenously into the tail vein of each mouse at a dosage of 10 ml/kg body weight. Then, each 0.2 ml of 0.6% acetic acid in normal saline was injected intraperitoneally into each mouse. Thirty minutes later, the mice were euthanized and their abdominal wall was cut open. The abdominal cavity of each mouse was washed using 10 ml of saline and the cleanout fluid was collected and centrifuged at 3000 rpm for 10 minutes. The supernatant was determined colorimetrically at a wavelength of 590 nm on a spectrophotometer. The mice in control groups were treated in the same manner. The inhibitory rate of vascular permeability for each test sample was calculated according to the formula below:

$$\text{Inhibitory rate of vascular permeability} = \frac{(OD_{\text{blank control}} - OD_{\text{test sample}})}{OD_{\text{blank control}}} \times 100\%$$

STATISTICAL ANALYSIS

The data were in the expression of mean ± standard deviation (S.D.) and statistically assessed by one-way variance analysis (ANOVA). Difference between sample-treated groups and untreated blank control group was evaluated using Student's t-test. If $P<0.05$, the difference was considered as significant.

RESULTS

Effect on xylene induced mice ear edema

The results were shown in tables 1 and 2. Compared with the blank control group, CE, WF and EF exhibited inhibitory effect on edema, and the activity of EF was more potent than that of CE and WF after their dosages were converted into the same level. For the major compounds isolated from EF, veratric acid, vitexin, orientin and trollioside had been demonstrated previously to moderately inhibit edema at a dosage of 50 mg/kg body weight (Wang *et al*., 2012); this effect, however, was not shown as the dose decreased to 20 mg/kg body weight. Another major compound trolline did not show any inhibitory effect on edema at the doses of either 50mg/kg or 20 mg/kg body weight.

Effect on acetic acid induced mice vascular permeability

The results obtained from this test (tables 3 and 4) were similar to those obtained from xylene induced ear edema test, which supported that EF was more potent than CE, BF and WF in terms of inhibition of vascular permeability.

DISCUSSION

The results obtained from both xylene induced ear edema test and acetic acid induced vascular permeability test indicated that CE, WF and EF had anti-inflammatory activity. In stage 1, although EF seemed to be less effective in inhibiting edema and vascular permeability than CE and WF, its dosage administrated was only about one-sixth of that of other fractions because of its minor solubility in water. However, it was still more effective than BF even at such dilute concentration. Thus, EF and WF were selected to be subject to further study considering their more potent activity and higher yield. In stage 2, EF was shown to be almost 2-3 times as potent in anti-inflammatory activity as WF at the same dosage, suggesting the active anti-inflammatory components of Jinlianhua were mainly contained in this fraction and the active compounds were of moderate polarity. Total of 13 compounds were isolated from EF, of which 4 compounds including 2 phenolic acids (veratric acid and trollioside) and 2 flavonoids (vitexin and orientin) were reported to be effective in anti-inflammation at the dose of 50 mg/kg body weight (Wang *et al*., 2012). These four compounds were also reported in Jinlianhua in high abundance (orientin: 2.92%; vitexin: 0.93%; veratric acid: 0.87%; trollioside: 0.03%) (Prabhakar *et al*., 1981; Yang *et al*., 2009).

CONCLUSION

This investigation may provide us a clue as to what kinds of compounds mainly contribute to the anti-inflammatory activity of Jinlianhua. It may be concluded that orientin, vitexin, veratric acid and trollioside make an appreciable

Table 1: The effect of different fractions from Jinlianhua on xylene induced mice ear edema (stage 1)

Group No.	Treatment	Dosage (g/kg)	Number of mice (n)	Edema level (mg)	Inhibitory rate (%)
1	-	-	11	6.76±2.66	-
2	CE	3.6	9	3.63±2.49*	46.3
3	EF	0.58	10	4.52±3.00	33.2
4	BF	3.6	11	4.96±3.09	26.7
5	WF	3.6	11	3.56±2.85*	47.4

Notes: group 1 = blank control; in comparison with group 1, *P<0.02; 2 mice in group 2 and 1 mouse in group 3 died accidentally during the test, and this event was unrelated with the administration of the test samples.

Table 2: The effect of different fractions from Jinlianhua on xylene induced mice ear edema (stage 2)

Group No.	Treatment	Dosage (g/kg)	Number of mice (n)	Edema level (mg)	Inhibitory rate (%)
6	-	-	12	11.10±2.28	-
7	PA	0.01	12	5.90±2.39**	47
8		0.4	12	8.08±3.74*	27
9	EF	0.13	12	7.46±2.92**	33
10		0.07	12	9.18±4.01	17
11		2.4	12	8.75±3.02*	21
12	WF	0.8	12	9.15±3.65	18
13		0.4	12	9.53±3.44	14

Notes: group 6 = blank control, group 7 = positive control; in comparison with group 6, *P<0.05, **P<0.01.

Table 3: The effect of different fractions from Jinlianhua on acetic acid induced mice vascular permeability (stage 1)

Group No.	Treatment	Dosage (g/kg)	Number of mice (n)	OD	Inhibitory rate (%)
14	-	-	10	0.243±0.085	-
15	CE	2.4	11	0.167±0.045*	31.3
16	EF	0.39	12	0.170±0.059*	30.2
17	BF	2.4	10	0.177±0.031*	27.1
18	WF	2.4	12	0.131±0.041**	46.3

Notes: group 14 = blank control; in comparison with group 14, *P<0.05,**P<0.01; 2 mice in group 14, 1 mouse in group 15, and 2 mice in group 17 died accidentally during the test, and this event was unrelated with the administration of the test samples.

Table 4: The effect of different fractions from Jinlianhua on acetic acid induced mice vascular permeability (stage 2)

Group No.	Treatment	Dosage (g/kg)	Number of mice (n)	OD	Inhibitory rate (%)
19	-	-	12	0.179±0.047	
20	PA	0.01	12	0.123±0.029**	31
21		0.4	12	0.108±0.041**	40
22	EF	0.13	12	0.110±0.030**	39
23		0.07	12	0.151±0.039	16
24		2.4	12	0.129±0.025**	28
25	WF	0.8	12	0.148±0.027	17
26		0.4	12	0.153±0.030	15

Notes: group 19 = blank control, group 20 = positive control; in comparison with group 19, **P<0.01.

contribution to the anti-inflammatory activity of Jinlianhua although someone might argue that some compounds in low content are sometimes with higher activity.

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