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Anti-inflammatory Properties of Quercetin-3-O-β-D-glucuronide-methyl Ester from *Polygonum perfoliatum* in Mice

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Abstract: *Polygonum perfoliatum* is commonly used in folk medicine to treat inflammatory disorders in China. Present studies on the anti-inflammation effect of quercetin-3-O-β-D-glucuronide-methyl ester from *Polygonum perfoliatum* was evaluated *in vivo* models of both acute inflammations such as xylene-induced ear edema, acetic acid-induced vascular permeability. Quercetin-3-O-β-D-glucuronide-methyl ester showed a significant dose-related inhibition in xylene-induced ear swelling and acid-induced vascular permeability in mice. These results demonstrate that quercetin-3-O-β-D-glucuronide-methyl ester possesses a potent anti-inflammatory function on acute inflammation. These results also support the claims of traditional Chinese medicine practitioners about the use of *Polygonum perfoliatum* in the treatment of inflammatory disease.

Key words: *Polygonum perfoliatum*, quercetin-3-O-β-D-glucuronide-methyl ester, anti-inflammation

INTRODUCTION

Polygoni Perfoliati Herba, the aerial part of *Polygonum perfoliatum* L. also known as traditional Chinese medicine (Guangbangui), is a vine-type weed usually found in Asia, especially in Chinese. It is widely found in Guizhou, Hunan, Anhui, Fujian and other South Provinces in Chinese. In Chinese folk medicine, the whole plant of *Polygonum perfoliatum* is used in the treatment of reliving cough, fever and detoxification, etc. (Delectics Florae Reipublicae Popularis Sinicae Agendae Academiae Sinicae Edita, 1998; National Commission of Chinese Pharmacopoeia, 2010; Xin and Xue, 2000). *P. perfoliatum* has been shown to possess pharmacological properties such as anti-inflammatory (Huang *et al*., 2008), anti-bacterial (Fu *et al*., 2008) and cough relieving activity (Long and Li, 2010). Previous phytochemical investigations on the plant have revealed the presence of diferuloyl esters of sucrose (Sun *et al*., 2000), flavonoids (Zhu *et al*., 2000; Liu *et al*., 1999; Zhao *et al*., 2010; Zhang *et al*., 2008; Wang and Lu, 2004; Li *et al*., 2009), neoflavonoids (Sun and Sneden, 1999), triterpene, steroid (Li *et al*., 2009) etc.

P. perfoliatum is widely used in ethnomedicine for the treatment of inflammation disorders, but its chemical compounds have not yet been pharmacologically evaluated for anti-inflammatory activity. In order to find the chemical compounds of anti-inflammatory potential, this study was undertaken to investigate the bioactivity compounds of *Polygonum perfoliatum*. A flavonoid (Quercetin-3-O-β-D-glucuronide-methyl ester) was isolated from *Polygonum perfoliatum*. The results from animal experiment indicated that the compound had powerful anti-inflammatory activity.

MATERIALS AND METHODS

Plant materials: The whole plants of *Polygonum perfoliatum* were collected at Guiyang city of China in August, 2009 and were identified by macroscopic examination, TLC and HPLC methods compared authentic sample. A voucher specimen (herbarium No. 20090815) of the plant is deposited at the herbarium of the Research Center for Quality Control of Natural Medicine, Guizhou Normal University.

Extraction and isolation: The air-dried powder of whole plant (5.0 kg) was extracted with 85% (v/v) EtOH there times under reflux. The combined EtOH extract was evaporated to dryness in vacuo and the residue was suspended in H_2O and then extracted with EtOAc at room temperature.

The EtOH-soluble fraction (320 g) was subjected to dry column chromatography over silica gel, eluted with $CHCl_3$-MeOH (50: 1 to 2: 1, v/v) and yielded five fractions

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Table 1: ^{1}H and ^{13}C NMR spectral data of quercetin-3-O-β-D-glucuronide-methylester (400 MHz, in CD_3OD, δ in ppm, J in Hz)

Position	δC	δH (m, J Hz)
1	-	
2	159.3	-
3	135.4	-
4	179.2	-
5	162.9	-
6	99.9	6.17 (d, 2.0)
7	166.0	-
8	94.8	6.35 (d, 2.0)
9	158.4	-
10	105.5	-
1'	122.8	-
2'	117.3	7.57 (s)
3'	145.9	-
4'	149.8	-
5'	115.8	6.82 (d, 7.6)
6'	170.7	7.55 (d, 7.6)
1"	104.7	5.22 (d, 7.6)
2"	72.7	3.56 (m)
3"	75.3	3.49 (m)
4"	77.0	3.47 (m)
5"	77.3	3.76 (d, 9.6)
6"	170.7	-
-OCH_3	52.9	3.64 (s)

based on the TLC profiles. Fraction 3 was subjected to column chromatography on silica gel H, eluted with a gradient of $CHCl_3$-MeOH (15: 1 to 5: 1, v/v) and then purified by Sephadex LH-20 ($CHCl_3$-MeOH, 1: 1, v/v) to yield compound 1(50 mg).

Quercetin-3-O-β-D-glucuronide-methyl ester (Fig. 1): amorphous yellow power, m.p. 180-181°C; IR(KBr): 3365, 2932, 1612, 1521, 1452, 1085. ESI-MS: m/z 493 $[M+H]^+$, 515 $[M+Na]^+$; ^{1}H NMR (400MHz, CD_3OD) and ^{13}C NMR (100MHz, CD_3OD): As shown in Table 1.

Apparatus: Melting points were determined on an XT-4 micro melting point apparatus without correction. FT-IR spectra were obtained on a Bruker TENSOR 27 spectrophotometer. UV spectra were acquired on a Shimadzu instrument, UV-1700 UV-visible spectrophotometer. ESI-MS experiment was performed on a Thermo TSQ Quantum Ultra mass spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on an Inova-400 instrument (Varian Inc., Palo Alto, CA) with tetramethylsilane (TMS) as internal standard. TLC was performed on precoated silica gel GF254 plates (Qingdao Haiyang Chemical Co. Ltd.). Column chromatography was carried out with silica gel H (Qingdao Haiyang Chemical Co. Ltd.) and Sephadex LH-20 (Pharmacia, Sweden). Acetic acid (Damao Chemical reagent Factary, Tianjin, China) and dimethyl benzene (Chuandong Chemical Co., Ltd, Chongqing, China) were analytical grade.

Animal: Mice of both sex weighing 18-22 g were obtained from the Animal Laboratory of Medical College of

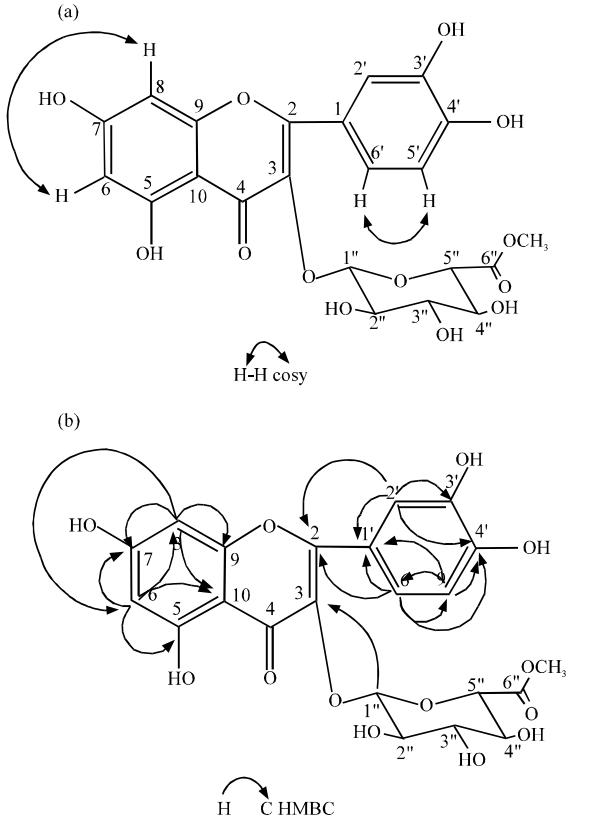

Fig. 1(a-b): (a) HMBC and (b) H1, H1-COSY correlations of Quercetin-3-O-β-D-glucuronide-methyl ester

Guiyang (Guiyang, China). The mice were housed in plastic cages, with food and tap water available *ad libitum*. The experimental animals were randomly divided into four groups of 10 animals each. The animal experiments comply with ethical guidelines for the care of laboratory animals.

Anti-inflammatory activity:

Xylene-induced ear swelling in mice: The effect of quercetin-3-O-β-D-glucuronide-methyl ester on acute topical inflammation was evaluated by the method previously described (Wang *et al*., 2010). Quercetin-3-O-β-D-glucuronide-methyl ester (4 and 8 mg kg^{-1}) was orally administered daily for seven days before the experiments. Blank control and positive control animals were treated with the corresponding distilled water and aspirin (100 mg kg^{-1}). The 45 min preceding the final administration, both surfaces of the mice's right ear were given 40 μL of xylene by topical application. The left ear was used as a control. Mice were sacrificed by cervical dislocation 15 min after xylene application. Following this, both ears were removed for analysis. Circular section were

taken using a cork borer with a diameter of 8 mm and weighed. The ear edema was evaluated as the difference in weight between the right ear section and the left ear section. The anti- inflammatory activity was evaluated in accordance with the following equation:

Edema weight = weight of the right ear-weight of the left ear

$$\text{Inhibition (\%)} = \frac{(\text{Edema weight of control group} - \text{edema weight of treated group})}{\text{Edema weight of control}} \times 100\%$$

Acetic acid-induced peritoneal capillary permeability in mice: The method of Whittle (1964) was used. Quercetin-3-O-β-D-glucuronide-methyl ester (3 and 6 mg kg^{-1}) was orally administered daily for 8 days before the experiments. Blank control and positive control animals were administered with corresponding distilled water and aspirin (100 mg kg^{-1}). The 30 min after the final administration, each mouse was given an intravenous injection of 2% Evans blue saline solution at 0.1 mL/10 g body weight to the tail, immediately followed by an intraperitoneal injection of 0.6% acetic acid at 0.1 mL/10 g body weight was injected intravenously. Mice were sacrificed by cervical dislocation 20 min after acetic acid injection and the peritoneal cavity of each animal was washed with 5 mL normal saline into tubes. Saline washes from the same animal were centrifuged for 5 min at 1000×g. Supernatants were collected and their absorbance at 590 nm was measured with a spectrophotometer. The inhibitory activity was calculated in accordance with following formula:

$$\text{Inhibition (\%)} = \frac{\text{Ec-Et}}{\text{Ec}} \times 100$$

where, Et is average values of absorbance of the treated group and Ec is average values of absorbance of the control group.

RESULTS

Effect of quercetin-3-O-β-D-glucuronide-methyl ester on xylene-induced ear swelling in mice: The effect of quercetin-3-O-β-D-glucuronide-methyl ester on xylene-induced ear swelling in mice was summarized in Table 2. Aspirin (100 mg kg^{-1}, oral) significantly decreased the xylene-induced ear swelling in mice as compared to the control group ($p<0.05$). Quercetin-3-O-β-D-glucuronide-methyl ester showed a significant dose-dependent decrease effects in this test. The 8 and 4 mg kg^{-1} of quercetin-3-O-β-D-glucuronide-methylester showed inhibition at 48.56 and 35.15%, respectively being better than the results obtained by aspirin.

Table 2: Effect of quercetin-3-O-β-D-glucuronide-methylester on xylene-induced ear swelling in mice

Group	Oedema	Inhibitory (%)
Control	7.31±0.77	-
Aspirin (100 mg kg^{-1})	5.51±1.00*	24.62
Quercetin-3-O-β-D-glucuronide-methylester (8 mg mL^{-1})	3.76±1.03***	48.56
Quercetin-3-O-β-D-glucuronide-methylester (4 mg mL^{-1})	4.74±0.71**	35.15

Data presented as Mean±SD for N = 10, *Significantly different from the control group at $p<0.05$, **Significantly different from the control group at $p<0.01$, ***Significantly different from the control group at $p<0.001$

Table 3: Effect of quercetin-3-O-β-D-glucuronide-methylester on vascular permeability induced by acetic acid in mice

Group	Increase in vascular permeability (OD_{590})	Inhibitory (%)
Control	1.92±1.36	-
Aspirin (100 mg kg^{-1})	1.26±0.24*	34.38
Quercetin-3-O-β-D-glucuronide-methylester (6 mg mL^{-1})	0.77±0.06***	59.90
Quercetin-3-O-β-D-glucuronide-methylester (3 mg mL^{-1})	1.01±0.27**	47.40

Data presented as Mean±SD for N = 10, *Significantly different from the control group at $p<0.05$, **Significantly different from the control group at $p<0.01$, ***Significantly different from the control group at $p<0.001$

Effect of quercetin-3-O-β-D-glucuronide ethyl ester on vascular permeability induced by acetic acid in mice: It is well known that the increase of vascular permeability induced by acetic acid corresponds to the early exudative stage of inflammation, one of the most important processes in the inflammatory pathological mechanism (Barros *et al*., 2010). In this study, the effects of aspirin and quercetin-3-O-β-D-glucuronide-methyl ester on acetic acid-induced vascular permeability in mice are shown in Table 3. Aspirin (100 mg kg^{-1}) treated positive control demonstrated inhibitions of dye leakage by 34.38%. Quercetin-3-O-β-D-glucuronide-methyl ester evoked a significant dose-related inhibition of vascular permeability compared to the control group and produced 59.9 and 47.4% inhibition at the respective doses of 6 and 3 mg kg^{-1} compared to the control group.

DISCUSSION

Polygonum perfoliatum is a commonly-use herb in traditional Chinese medicine for treatment of various inflammatory diseases. The present studies verified that quercetin-3-O-β-D-glucuronide-methyl ester from *Polygonum perfoliatum* possesses anti-inflammatory effects in acute inflammation.

Inflammation is a complex process that involves several events, such as: Enzyme action, mediator release,

extravasations of fluid, cell migration, tissue breakdown and repair. This fact makes the use of different experimental models essential when conducting pharmacological trials (Vane and Botting, 1995). In the present study, quercetin-3-O-β-D-glucuronide-methylester significant decrease the edema induced by xylene. Xylene can cause instant irritation to the mice's ear which leads to fluid accumulation and triggers an acute inflammatory response. Suppression of this response is likely an indication of antiphlogistic effect.

Furthermore another well-characterized acute inflammation model, the vascular permeability test, was used to confirm the anti-inflammation activity. Increased vascular permeability occurs as a result of the overexposure of basement membrane due to the concentration and separation of endothelial cells at their boundaries, which allows plasma protein such as immunoglobulins, coagulation factors and fluid to pass the base membrane freely into the injured tissue (Carlson *et al*., 1985). Quercetin-3-O-β-D-glucuronide-methyl ester evoked a significant dose-related inhibition of vascular permeability induced by acetic acid in mice. So quercetin-3-O-β-D-glucuronide-methyl ester may effectively suppress the exudative phase of acute inflammation.

In conclusion, the results of this study provide the evidence for the *in vivo* anti-inflammation activity of quercetin-3-O-β-D-glucuronide- methyl ester in acute inflammation. The anti-inflammation activity may be derived from an inhibition of vascular permeability in addition to the antioxidant activity, just as indomethacin works as a cyclooxygenase inhibitor. Further studies are needed to clarify the mechanisms responsible for the anti-inflammatory activity of quercetin-3-O-β-D-glucuronide-methyl ester. The results also support the claims of traditional Chinese medicine practitioners about the use of *Polygonum perfoliatum* in inflammation disease.

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