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Therapeutic effects of Smilax glabra and Bolbostemma paniculatum on rheumatoid arthritis using a rat paw edema model

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ABSTRACT

Smilax glabra Roxb. (Tufuling) and *Bolbostemma paniculatum* (Maxim.) Franquet (Tubeimu) are used as couplet medicine in traditional Chinese medicine for the treatment of arthritis. This study is conducted to provide evidence on their therapeutic effects on rheumatoid arthritis (RA) and to explore its possible mechanisms of action. The identification and quantification of representative components (Astilbin and Tubeimoside I) in the n-butyl alcohol fraction of this couplet medicine (BFCM) were carried out by HPLC-UV assays. The contents of Astilbin and Tubeimoside I in BFCM were 13.13% (15.434 min) and 3.4% (18.619 min) respectively. For the assessment of anti-RA and anti-inflammatory activities, a carrageenan-induced paw edema model in rats was used. The swelling rates of paws and levels of IL-1β, IL-6 and TNF-α in the swelling tissue were determined. We observed that the BFCM exhibited significant inhibitory activity on carrageenan-induced paw edema model ($p < 0.01$). The down regulated levels of IL-1β, IL-6 and TNF-α (all $p < 0.05$) were reported. The results indicate that BFCM possesses significant anti-RA and anti-inflammatory effects, and it has a potential to be developed as a new therapeutic agent against RA.

1. Introduction

Rheumatoid arthritis (RA) is a prevalent form of inflammatory autoimmune disease that affects 1–2% of the world population [1]. RA is characterized by synovial inflammation and hyperplasia, cartilage destruction and bone degradation. Apart from joint damage, there are also extra-articular manifestations including subcutaneous nodules, pulmonary fibrosis, vasculitis and systemic comorbidities [2,3]. The commonly used medications against RA include non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), glucocorticoids and biopharmaceuticals. However, the need of patients with RA is still unmet because of the severe side effects, inability to achieve permanent cure and high expenditure of these drugs. Traditional Chinese medicine might be the preferable approach to treat RA due to its certain efficacy, multi-target functioning on disease pathway, low toxicity and affordability [4,5].

Smilax glabra Roxb. (liliaceae), also known as Tufuling (TFL), is a traditional Chinese medicine used for the treatment of muscular constricture, arthralgia and myalgia. Flavonoids, saccharides and terpenoids are considered to be the active components for TFL [6–8]. Modern studies revealed the anti-inflammatory, immunomodulatory and cardiovascular effects of TFL [9]. *Bolbostemma paniculatum* (Maxim.) Franquet (Cucurbitaceae), with the Chinese name Tubeimu (TBM), is often used for the treatment of tumor and toxication in traditional Chinese medicine. Saponins, sterols and alkaloids are regarded as the active components for TBM [10–12]. Recent research has shown the immunosuppressive, anti-tumor and anti-inflammatory effects of TBM [13,14]. TFL and TBM are used as couplet medicine in the treatment of acute stage of psoriatic arthritis. This couplet medicine can relieve fever, pain, swelling and stiffness of the joints, and also can

Abbreviations: TFL, *Smilax glabra* Roxb. liliaceae; TBM, *Bolbostemma paniculatum* Maxim. Franquet (Cucurbitaceae); RA, rheumatoid arthritis; BFCM, n-butyl alcohol fraction of the couplet medicine; NSAIDs, non-steroidal anti-inflammatory drugs; DMARDs, disease-modifying anti-rheumatic drugs; Dex, dexamethasone; IL, interleukin; TNF, tumor necrosis factor; MMP, matrix metalloproteinase; GM-CSF, granulocyte-macrophage colony stimulating factor; VEGF, vascular endothelial growth factor; FLS, fibroblast-like synoviocytes; TGF-β, transforming growth factor

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attenuate the elevated inflammatory indicators [15].

In the present research, we prepared the n-butyl alcohol fraction from the ethanol extract of this couplet medicine, and the identification and quantification of representative components in the n-butyl alcohol fraction of this couplet medicine (BFCM) were carried out by HPLC-UV assays. We used a carrageenan-induced paw edema rat model to investigate the anti-rheumatoid arthritis and anti-inflammatory activities of BFCM, and intended to elucidate the possible mechanisms involved in the treatment of BFCM.

2. Experimental

2.1. Plant materials and reagents

The rhizomes of *Smilax glabra* Roxb. were collected in Hezhou, Guangxi province, People's Republic of China. The bulbs of *Bolbostemma paniculatum* (Maxim.) Franquet were collected in Anhui province, People's Republic of China. Both plant materials were identified by Professor Chun-Feng Zhang (China Pharmaceutical University). The voucher specimens were deposited at the State Key Laboratory of Natural Products and Functions, China Pharmaceutical University.

Astilbin (> 98%, CAS: 29838-67-3), Tubeimoside I (> 98%, CAS: 102040-03-9) were purchased from Chengdu Must Bio-Technology Co., Ltd. (Sichuan, China). ELISA kits of IL-1β, IL-6 and TNF-α were purchased from Nanjing Dizhao Bio-Technology Co., Ltd. (Jiangsu, China). Creatinine kit, urea nitrogen kit, ALT kit and AST kit were obtained from Nanjing Jiancheng Institute of Bioengineering (Jiangsu, China). All the other reagents used were analytically pure.

2.2. Preparation of the fraction

Dried and powdered rhizomes of *Smilax glabra* Roxb. (125 g) and bulbs of *Bolbostemma paniculatum* (Maxim.) Franquet (375 g) were extracted with 70% ethanol (6 L) for 2 times (2 h per time). The extract was filtered and evaporated on a rotary evaporator under reduced pressure to obtain the viscous residue. The residue was dissolved in water and then extracted with n-butyl alcohol to obtain the n-butyl alcohol fraction of the couplet medicine (BFCM).

2.3. Identification and quantification of representative components in BFCM

Astilbin and Tubeimoside I were selected as representative components for TFL and TBM based on Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission, 2015) [16]. The standard compounds of Astilbin and Astilbin in BFCM were analyzed by Agilent 1100 HPLC with C18 column (250 mm × 4.6 mm, 5 μm). The mobile phase consisted of methanol (A, 39%) and 1‰ acetic acid (B, 61%). The flow rate was 1 mL/min, and the column temperature was maintained at 30 °C. Injection volume was 10 μL and the peaks were detected at 291 nm with UV detector. While the standard compounds of Tubeimoside I and Tubeimoside I in BFCM were analyzed by Agilent 1100 HPLC with C18 column (250 mm × 4.6 mm, 5 μm) and the mobile phase consisted of methanol (A, 65%) and water (B, 35%). The flow rate was 1 mL/min with the column temperature maintained at 35 °C. Injection volume was 10 μL and the peaks were detected at 291 nm with UV detector. Astilbin and Tubeimoside I were detected by comparing with the standard compounds.

Quantification of Astilbin and Tubeimoside I in BFCM were performed by calibration curve equation corresponding to their reference standard compounds. Standard solutions were prepared as follows: Astilbin (0.16, 0.10, 0.08, 0.04, 0.02 and 0.01 mg/mL), Tubeimoside I (1.6, 0.80, 0.40, 0.20, 0.10 and 0.05 mg/mL) in methanol. Injection volume of each sample was 10 μL. The linear regression equations were calculated with $y = ax + b$ (x = concentration, y = peak area). Linearity was established by the coefficient of equation (R^2).

2.4. Biological activity assays

2.4.1. Experimental animals and preparation of test samples for bioassay

Male Sprague-Dawley (SD) rats (150 ± 20 g) were purchased from Shanghai Jiesijie experimental animal Co. Ltd., and housed under standard condition (12 h light / 12 h dark cycle at 25 ± 2 °C) with free access to standard laboratory food and water. Animal welfare and experimental procedures were carried out strictly in accordance with the Guide for the Care and Use of Laboratory Animals and approved by Institutional Animal Ethics Committee in China Pharmaceutical University.

Carrageenan purchased from Sigma Corporation (USA) was suspended in physiological saline at a concentration of 1%. BFCM and Dexamethasone (Zhejiang Xianju pharmaceutical Co. Ltd.) was suspended in 0.5% CMC-Na to the final concentrations at 34.65 mg/mL and 0.0591 mg/mL respectively.

2.4.2. Establishment of carrageenan-induced hind paw edema model and drug administration

The rats were randomly divided into 4 groups (n = 8), namely control, model, BFCM and dexamethasone (Dex) groups. Based on conversion from dose for human, the rats of BFCM group were administered 346.5 mg/kg of BFCM orally per day, and the Dex group rats were given 0.5906 mg/kg of dexamethasone per day. The rats in control and model groups were given equal volume of 0.5% CMC-Na. The above administrations were continued for 7 days and all the rats were weighed once a day from the first day of administration.

One hour after the last administration, rats from model, BFCM and Dex groups were anesthetized and injected with 0.1 mL of Carrageenan saline suspension subcutaneously into plantar fascia of the right hind paw. The rats from the control group were injected with the same volume of saline. Dexamethasone was used as reference drug in this study [17,18].

2.4.3. Biological activity assays

After the injection of Carrageenan, paw edema levels of the rats were measured once every hour for 5 h and the swelling rates of each group were calculated. Blood samples of the rats were collected 5 h after induction of inflammation and centrifuged immediately at 3000 rpm for 10 min. The serum was transferred and stored at 4°C for the determination of creatinine, urea nitrogen, ALT and AST levels. All rats were sacrificed after the collection of blood samples. The right hind legs of the rats were removed, and a small section of the swelling part near the footpad was carefully cut off, soaked in methanol and fixed for histopathological observation. The rest of the parts were weighed and homogenized after adding saline at a ratio of 1:9 and centrifuged at 3000 rpm (4 °C) for 10 min. The supernatant was transferred for the measurement of IL-1β, IL-6 and TNF-α by the ELISA Kits. The main visceral organs of the rats were collected and weighed for the calculation of organ index.

2.5. Statistical analysis

The data were presented as mean ± SE and analyzed by one-way ANOVA in SPSS software. *P* values less than 0.05 were defined to be statistically significant.

3. Results

3.1. Identification and quantification of representative compounds in BFCM

The HPLC analysis results (Fig. 1) confirmed the presence of Astilbin (15.434 min) and Tubeimoside I (18.619 min) in BFCM by comparing the retention time and UV spectra with reference standard compounds

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