Journal of Ethnopharmacology 191 (2016) 1–8

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Ethnopharmacological communication

Therapeutic effects of *Caragana pruinosa* Kom. roots extract on type II collagen-induced arthritis in rats

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ARTICLE INFO

Article history:
Received 7 September 2015
Received in revised form
4 June 2016
Accepted 8 June 2016
Available online 10 June 2016

Keywords:
Caragana pruinosa Kom.
Rheumatoid arthritis
Anti-arthritic effect
Pro-inflammatory cytokines
Type II collagen

ABSTRACT

Ethnopharmacological relevance: *Caragana pruinosa* Kom. is a deciduous shrub belonging to the genus of *Caragana* (Leguminosae), and *Caragana* plants exhibit a wide range of interesting pharmacological properties including anti-inflammatory, analgesic, and anti-arthritis activity, *etc*.
Aim of the study: This study was aimed to investigate the anti-arthritic effect of 80% EtOH extract from the roots of *C. pruinosa* (ERCP) on arthritis and explore the potential pharmacological mechanism.
Materials and methods: After collagen induced arthritis (CIA) were established in rats, the animals were orally administered with ERCP (130, 260 and 520 mg/kg) for 30 days. During the treatment, the rats' body weights, arthritis indices and paw volumes were measured every 5 days. Subsequently, rats' blood samples were collected to determine TNF-α, IL-1β, IL-6, IL-10, and C-reactive protein (CRP) contents in serum. Then, rats were sacrificed and the hind paws and knee joints were collected for histopathological examination.
Results: Our results indicated that ERCP significantly suppressed the inflammatory reactions and destructions in joints and synovial tissues. ERCP inhibited the paw swelling and arthritis index in CIA rats. Additionally, it decreased the levels of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) and CRP, whereas increased that of IL-10.
Conclusion: Our results suggested ERCP has significant anti-arthritic effect on CIA rats, and the pharmacological mechanisms are related to the down-regulation of TNF-α, IL-1β, IL-6 and CRP and the up-regulation of IL-10.

1. Introduction

Rheumatoid arthritis (RA), also called "immortal cancer", is one of the most intractable chronic destructive diseases of articular cartilage and bone, and characterized by inflammatory cell infiltration and proliferation of synovial tissue (Feldmann et al., 1996; Imboden., 2009). Furthermore, it's also demonstrated that RA could result in some serious organ damages, such as kidney, heart, and lung, *etc*, leading to disability and compromised quality of human being (Brooks, 2006; Silman and Hochberg, 2001). In recent years, commonly used strategies for RA patients include the disease-modifying anti-rheumatic drugs (DMARDs), steroid hormone, non-steroidal anti-inflammatory drugs (NSAIDs), and biologic agents (Silman and Hochberg, 2001; Rossi et al., 2015). However, most of these drugs could result in some severe adverse effects, such as gastrointestinal disorders, cardiovascular complications, and reproductive toxicities, *etc*. (Kremers et al., 2004; Strand et al., 2015; Kim et al., 2015). Thus, finding novel and reliable treatment strategy for RA with low toxicity is needed urgently.

Increasing evidences have demonstrated that natural plant-derived monomers/extracts are potential resources for discovering candidate drugs for treating various diseases with low side-effects

Abbreviations: AIA, adjuvant-induced arthritis; CFA, Complete Freund's Adjuvant; CIA, collagen induced arthritis; CII, type II collagen; CRP, C-reactive protein; DMARDs, disease-modifying anti-rheumatic drugs; ERCP, EtOH extract from the roots of *C. pruinosa*; ESI, electrospray ionization; IFA, Incomplete Freund's Adjuvant; MS-TIC, mass spectrometry-total ions chromatogram; MTX, Methotrexate; NSAIDs, non-steroidal anti-inflammatory drugs; PGE2, prostaglandins E2; TOF, time of flight-mass spectrometry; RA, Rheumatoid arthritis

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http://dx.doi.org/10.1016/j.jep.2016.06.028
0378-8741/

(Patwardhan, 2005; Li and Vederas, 2009). Recently, it's reported that plants of the genus *Caragana* Fabri. (Leguminosae) displayed a wide range of interesting pharmacological properties including anti-inflammatory, analgesic, anti-arthritis and anti-tumor activities (Meng et al., 2009; Niu et al., 2014), *etc*. Phytochemical investigations revealed that *Caragana* species possessed abundance of bioactive flavonoids, stilbenoids, and terpenoids, *etc* (Meng et al., 2009; Sun et al., 2015). *Caragana pruinosa* Kom., an unexploited *Caragana* medicinal plant, is a deciduous shrub mainly distributed in Sinkiang area (Habahe, Hoboksar, Zhaosu, Barkol, Kuqa, Baicheng, Keping, Aheqi, Wuqia, Kashgar) of China, according to our recent ethnobotanical survey and others (Pan et al., 2013; Zhou et al., 2005). It's also distributed in Middle Asia countries, such as Kazakhstan and Kyrgyzstan. *C. pruinosa* is a reliable folk medicine, possessing the ability of invigorating blood circulation, dispelling rheumatism, and inducing diuresis to reduce edema, traditionally used for the treatment of RA and other inflammatory diseases in Sinkiang of China (Pan et al., 2013). However, to the best of our knowledge, no phytochemical and pharmacological studies have been conducted and reported on this species. Thus, as a part of our continuing investigation on *Caragana* species (Jin et al., 2011; Zheng et al., 2013) and searching for potential plant-derived anti-RA drugs (Zheng et al., 2014; Lin et al., 2014), *C. pruinosa* was selected to evaluate its potential therapeutic effect on type II collagen-induced arthritis (CIA) in rats.

2. Materials and methods

2.1. Plant material

C. pruinosa were collected from Urumuchi, Sinkiang, P.R. China, and authenticated by Prof. Xiao-Guang Jia, Chinese Medicine Research Institute of the Sinkiang Uygur Autonomous Region (Urumuchi, China). The voucher specimen of this plant was kept at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, Shanghai, PR China (No. *201203).

2.2. Animals

Male Wistar rats (170–180 g) were purchased from the Shanghai Laboratory Animal Center (Shanghai, China). They were housed at 21 ± 1 °C under a 12 h light/ dark cycle and had free access to standard pellet diet (Purina chow) and tap water. The animals were deprived of food for 12 h before oral administration of the tested drugs, with free access to water. All animal experiments in our present study accorded with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and our experimental protocols were approved by the Animal Care and Use Committee of the Second Military Medical University (2014LY044).

2.3. Chemicals and reagents

Bovine type II collagen (CII) was purchased from the Chondrex, Inc. (Shanghai, China), while Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA) were purchased from the Sigma-Aldrich Co. (Shanghai, China). Rat IL-10 ELISA Kit, Rat TNF-α ELISA Kit, Rat IL-1β ELISA Kit, Rat IL-6 ELISA Kit were purchased from the Wuhan gene beauty Biotechnology Co., Ltd. (Wuhan, China), while rat C-reactive protein (CRP) ELISA Kit was purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Methotrexate (MTX) was purchased from Shanghai Sine Pharmaceutical Co., Ltd. (Shanghai, China). All other chemicals used in this study were of analytical reagent grade.

2.4. Preparation of extract from the roots of C. pruinosa

The preparation of extract from the roots of *C. pruinosa* (ERCP) was carried out as following. Briefly, the air-dried and powdered roots were extracted with 80% EtOH three times by reflux, each extraction period lasting 2 h. After removal of the solvent *in vacuum*, the residue was evaporated to dryness and stored as ERCP (yielding 10.76%).

2.5. Qualitative analysis of ERCP by HPLC-ESI-TOF-MS

The Chromatographic separation was performed on an Agilent 1100 HPLC system (Agilent Technologies), equipped with a binary pump, a micro degasser, Hi-performance well-plate autosampler, thermostated column compartment and diode-array detector (DAD). UV spectra were recorded between 190 and 400 nm, and the UV detector was set at 254 nm. Separation was performed on a SHISEIDO MG-C_{18} (100×3.3 mm; i.d. 3.0 μm) column using a gradient elution [acetonitrile (**A**)/ water (+0.1% HCOOH) (**B**)]. The gradient program was 0–20 min, 10–50% A; 20–35 min, 50–80% A; 35–40 min, 80% A; the flow rate was kept at 0.5 mL/min, and the sample injection volume was 3 μL and the column temperature was set at 20 °C.

All MS experiments were conducted on an Agilent 6220 Time-of-Flight mass spectrometry (TOF) equipped with an electrospray ionization (ESI) interface (Agilent Technologies, USA). Both the auxiliary and nebulizer gases were nitrogen with a flow rate of 10 L/min. The MS analysis was performed in both positive and negative scan modes under the following operation parameters: the dry gas temperature was set at 350 °C, the fragmentor voltage was 160 V/260 V/360 V and the nebulizer pressure was set at 45 psi. Full scan data acquisition and dependent scan event data acquisition were performed from *m/z* 100–1200.

2.6. CIA animal model preparation and experiment protocols

In order to evaluate the anti-arthritic activity of ERCP, a total of 60 rats were divided at random into six groups (n = 10): **A**. Normal: Rats not immunized and treated with saline (10 mL/kg/day); **B**. Control: CIA rats treated with saline (10 mL/kg/day); **C**. MTX: CIA rats treated with MTX (positive drug, 1 mg/kg, three times a week); **D**. ERCP (130 mg/kg/day): CIA rats treated with ERCP at a dose of 130 mg/kg; **F**. ERCP (260 mg/kg/day): CIA rats treated with ERCP at a dose of 260 mg/kg; **E**. ERCP (520 mg/kg/day): CIA rats treated with ERCP at a dose of 520 mg/kg. All the drugs were administered orally, and dosage is expressed as mg/kg.

CIA rat model was prepared according to the described method (Wang et al., 2007) with minor modifications. Briefly, after dissolved in 0.1 mM acetic acid, the CII solution (4 mg/mL) was emulsified with an equal volume of CFA. Rats were firstly immunized by subcutaneously injection of CII emulsion at the tail root (100 μL/rat). After 7 days, the rats were second immunized by CII emulsified by an equal volume of IFA at the same places (100 μL/rat). After approximately 10 days of the initial immunization, rats appeared obvious RA symptoms at the toe joint, including inflammatory reactions, erythema and swelling.

At 10 days after the initial CII immunization, rats were orally treated with saline, MTX and different doses of ERCP, respectively. During our experiment, the rats' body weights and paw volumes were measured every 5 days. In addition, the arthritis indices of rats were measured every 5 days by using the ordinal scale as follows: 0): no obvious signs of arthritis, 1): one joint affected (swelling and erythema), 2): two joints affected, 3): three joints affected, 4): three joints affected and maximal erythema and swelling (Zheng et al., 2014). After 30 days' drug treatment, rats were sacrificed by decapitation. Then, blood was collected from