Journal of Ethnopharmacology 146 (2013) 355–362

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

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

Therapeutic effects of extracts from Radix *Toddaliae* Asiaticae on collagen-induced arthritis in Balb/*c* mice

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ARTICLE INFO

Article history:
Received 17 January 2012
Received in revised form
11 December 2012
Accepted 2 January 2013
Available online 18 January 2013

Keywords:
Radix *Toddaliae* Asiaticae
Toddalia asiatica (L.) Lam.
Collagen-induced arthritis (CIA)
Extract
Arthritis
Cytokines

ABSTRACT

Aim of the study: Radix *Toddaliae* Asiaticae (RTA), also named "Sanbaibang", is the dry root bark of *Toddalia asiatica* (L.) Lam. and has long been used as a traditional ethnic Chinese medicine for its considerable activity to alleviate pain and inflammation for patients suffering from rheumatism. It contains coumarin, alkaloids, triterpenes and volatile oils. Information regarding the anti-arthritis activity of RTA in vivo or in vitro is limited yet. In the present study, the aim is to investigate the therapeutic potential and underlying mechanisms of the ethyl alcohol extract (EtOH) and ethyl acetate fraction (EtOAc) from RTA on collagen II-induced arthritis (CIA) in mice.

Materials and methods: CIA animal model was performed by subcutaneous injection of type II bovine collagen (CII) on the 1st day and the 14th day of the experiment. Ethyl alcohol extract (542.8, 271.4, 135.7 mg/kg), ethyl acetate fraction (260.8, 130.4, 65.2 mg/kg) was orally administrated from the second antigen immunization for 3 weeks. Progression of edema of paws and knee joints was measured using a vernier caliper every 3 days from the 10th day after the first injection to the end of the experiment. The spleen index was measured and the knee joint changes were observed by pathological sections. ELISA was used to measure cytokines including tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and interleukin-10 (IL-10) in mice serum according to the manufacturer's instructions.

Results: Administration of ethyl alcohol extract and ethyl acetate fraction remarkably reduced paws and joints swelling and decreased the spleen indexes. Histopathological examination demonstrated that RTA effectively protected bone and cartilage of knee joint from erosion, lesion and deformation versus those from the control group. Besides, the concentration of cytokines like TNF-α, IL-1β, IL-6 were significantly lower than the ones from the control group respectively, while cytokine like IL-10 was remarkably higher compare with the control group.

Conclusion: In this present study, it is demonstrated that administration of RTA has potential and therapeutic effect on CIA. The data suggests that RTA could have a contributory ethno-pharmacological role in improved management of RA patients.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune and chronic inflammatory disease involving many cytokines that act both in series and in parallel, a phenomenon known as cytokine network redundancy. There are systemic and local clinical manifestations on the RA patients, and the most devastating manifestation is joint destruction (Boissier, 2011). Evidences from animal models and from therapeutic trials in patients with RA have proved an involvement of T cells, B cells, osteoclasts, and several cytokines during the induction, progression and maintain of this disease (Boissier et al., 2009; Kremer et al., 2003; Redlich et al., 2002; Scheinecker et al., 2008; Takagi et al., 1998). The cytokines establish a tangled and complex network whose overall balance depends on various connections. Specifically, pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6), and immunoregulatory cytokines, like interleukin-10 (IL-10), play key roles in RA (Gravallese and Goldring, 2000; Quattrocchi et al., 2001).

TNF-α and IL-1β are produced by activated macrophages in the inflamed synovial membrane tissue in patients with RA. The actions of TNF-α, perceived to be important in the pathogenesis of RA, include its ability to induce the production of other pro-inflammatory cytokines like IL-1β and IL-6 (Brennan and McInnes, 2008). Although TNF-α seems to be the major cytokine involved

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http://dx.doi.org/10.1016/j.jep.2013.01.004

in the inflammatory process, IL-1β is the key mediator with regard to cartilage and bone destruction (Joosten et al., 1999, Van den Berg et al.,1994). Besides, IL-10 has recently been recognized as a significant immune counter regulatory cytokine secreted by Tr1 Regulatory Cells. The various functions of are reported for IL-10 include down-regulation of co-stimulatory molecules and class II MHC molecule, prevention of inflammatory mediators secretion, inhibition of cytokine secretion from T cells, induction of anergy of T cells, and promoting of the differentiation and survival of B cells (Pestka et al., 2004).

Recent study shows that 60–90% people suffering RA tend to use complementary and alternative medicine (CAM) such as chiropractic and herbal therapies (Efthimiou and Kukar, 2010). Thus, there is growing interests and efforts have been put into discovery of new effective drug or therapeutic agents from commercial and easily available herbal therapies. Radix *Toddaliae* Asiaticae (RTA), also named "Sanbaibang", is the root of *Toddalia asiatica* (L.) Lam.. *Toddalia asiatica* (L.) Lam. is the only plant of *Toddalia* genera, Rutaceae family, and widely distributes in China. RTA has a favorable effective treatment for trauma pain, working injured waist pain, and rheumatism with effects including promoting blood circulation for removing blood stasis and promoting bone tissue regeneration (Chinese Folk Medicine to Blog (Jia and Li, 2005); Chinese Materia Medica, 1999; Flora Republicae Popularis Sinicae (China Flora Editing Group, 1997); National Herbal Compendium, 1996; Yunnan Flora (Wu et al., 1995)). It is also known as "Tujia drugs", the traditional rare medicinal herbs used by Tujia residents of Enshi of Hubei (Tujia Medicine (Tian et al., 1994)). Although some pharmacologic researches about analgesic, anti-inflammatory (Hu et al., 2000; Wang et al., 2006, 2007), tumor specific cytotoxicity (Iwasaki et al., 2006), anti-platelet (Tsai et al., 1998), and anti-bacterial (Duraipandiyan and Ignacimuthu, 2009) of RTA are reported, the study about anti-arthritis has not been reported yet. Furthermore, CIA mice model is a widely favorable experimental model of human RA (Myers et al., 1997; Schurgers, Billiau and Matthys, 2011). In this study, collagen-induced arthritis (CIA) animal model was produced, in order to evaluate the anti-arthritis activity of RTA extracts.

2. Materials and methods

2.1. Animals

Male Balb/c (bought from Hubei Provincial Center for Disease Control and Prevention, Wuhan, China) mice weighing 18–22 g each were used and maintained under specific pathogen-free conditions at the experimental animal center of Tongji Medical College, Huazhong University of Science and Technology, China. The experimental protocol was approved by Huazhong University of Science and Technology Committee on Animal Care and Use. The animals were kept in cages at 23 ± 2 °C and fed with standard laboratory diet and tap water throughout the experiments. The experiments were all carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals issued by National Academy of Sciences, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council [2006-04-06].

2.2. Plant material

The dry root barks of *Toddalia asiatica* (L.) Lam. were collected in Enshi Autonomous Prefecture, Hubei Province, People's Republic of China, in Jun 2010, and identified by Pharmacist Congrong Wang (Medicine Inspecting Institution of Enshi Autonomous Prefecture). Avoucher specimen (No.12-01-2011) was deposited at the Faculty of Pharmaceutical Sciences, Tongji Medical College of Huazhong University of Science and Technology, Wuhan, China.

2.3. Preparation of the extract and fractions

The collected root barks were shade dried at room temperature and ground in a mill. The dried power (5.6 kg) was refluxed with warm ethyl alcohol (EtOH) for 40 min (repeated three times). The extract was filtered and evaporated to dryness under pressure using rotary evaporator (YARONG, RE-52AA, China) at 50 °C. Then, the initial EtOH extract was desiccated in the vacuum dryer (BOXUN, DZF-6050, China) at 40 °C for about 72 h to give a viscous mass (759.9 g). The dried EtOH extract was dissolved with 2% HCl to produce acid solvent and insolvent fraction. The acid insolvent fraction was neutralized to pH7, suspended in water and fractionated with ethyl acetate (EtOAc) and normal butanol (*n*-BuOH) to produce three fractions (the EtOAc, *n*-BuOH and the water fractions). The yield of EtOH, EtOAc and *n*-BuOH extracts were 13.57%, 6.52% and 1.30%, respectively. All the three extracts were kept at 4 °C for preparation.

2.4. General toxicity studies of RTA

To evaluate the acute oral toxicity of RTA, 30 male and 30 female mice were assigned randomly to 6 experimental groups. We determined its single-dose toxicity of EtOH on mice at the doses of 6.79, 8.07, 9.59, 11.41, 13.57 g/kg body weight. The control group was treated with 0.5% CMC-Na orally. The single-dose toxicity of EtOAc was also been determined, and the doses were arranged at 3.25, 3.87, 4.61, 5.48, 6.52 g/kg. All mice were kept under standard laboratory conditions for seven days to observe their reaction and activity (The technical research guidelines of acute toxicity of traditional Chinese medicine and natural medicine (State Food and Drug Administration, 2005); Xu et al., 1982).

2.5. Induction of CIA and medicine treatment

Bovine type II collagen (Chondrex, USA) was dissolved in 0.1 M acetic acid at 2 mg/ml. Then, an equal volume of complete Freunds adjuvant (CFA) (Santa Cruz Biotechnology, USA) was slowly added and thoroughly stirred into CII emulsion. On the 1st day of the experience, all mice were intradermally injected with the CII emulsion in several spots at the base of the tail, 0.1 ml per mice, as the primary immunization. Two weeks after the primary immunization (on the 14th day), the mice were challenged again, injected with the same volume of CII emulsion at the same place as the second immunization. The next day of the second immunization, all immunized mice were randomly processed into groups ($n=10$ per group). The normal group was separated randomly before the experiment without any immunization. The control group was treated orally with 0.5%CMC-Na (0.2 ml/10 g). Positive control groups were treated orally with dexamethasone (Dex) (3 mg/kg) (Cuzzocrea et al., 2005) and *Tripterygium* glycosides (TGs) (60 mg/kg) (Wang et al., 2011). The treated groups were orally treated with EtOH and EtOAc, and every extract was designed for three doses: EtOH (542.8, 271.4, 135.7 mg/kg), EtOAc (260.8, 130.4, 65.2 mg/kg), all of them were in the dosage of 4, 2 and 1 g/kg body weight, relative to the dried root bark. All the groups were administrated orally every two days from the second immunization for three weeks.

2.6. Measurement of progression of paw and joint edema

From the 10th day from the primary immunization, the progression of CIA was evaluated by measuring thickness of