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Boswellia serrata extract attenuates inflammatory mediators and oxidative stress in collagen induced arthritis

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory disease which leads to destruction of joints. Current treatment modalities for RA either produce symptomatic relief (NSAIDs) or modify the disease process (DMARDs). Though effective, their use is also limited by their side effects. As a result, the interest in alternative, well tolerated anti-inflammatory remedies has re-emerged. Our aim was to evaluate the antioxidant and antiarthritic activity of *Boswellia serrata* gum resin extract (BSE) in collagen induced arthritis. Arthritis was induced in male Wistar rats by collagen induced arthritis (CIA) method. BSE was administered at doses of 100 and 200 mg/kg body weight once daily for 21 days. The effects of treatment in the rats were assessed by biochemical (articular elastase, MPO, LPO, GSH, catalase, SOD and NO), inflammatory mediators (IL-1β, IL-6, TNF-α, IL-10, IFN-γ and PGE_2), and histological studies in joints. BSE was effective in bringing significant changes on all the parameters (articular elastase, MPO, LPO, GSH, catalase, SOD and NO) studied. Oral administration of BSE resulted in significantly reduced levels of inflammatory mediators (IL-1β, IL-6, TNF-α, IFN-γ and PGE_2), and increased level of IL-10. The protective effects of BSE against RA were also evident from the decrease in arthritis scoring and bone histology. The abilities to inhibit proinflammatory cytokines and modulation of antioxidant status suggest that the protective effect of *Boswellia serrata* extract on arthritis in rats might be mediated via the modulation of immune system.

Introduction

Inflammation is the first immune-response to body when infected or irritated by external assault. However, when not well regulated, it can result in inflammatory diseases. Clinical evidences have shown that chronic inflammation can contribute to certain kinds of cancers, neurodegenerative disorders and rheumatoid arthritis (Choy and Panayi, 2001; Coussens and Werb, 2002; Koelink et al., 2012; Stix, 2007). Rheumatoid arthritis (RA) is a chronic inflammatory disease which leads to destruction of cartilage and bone within joints by inflammatory cells that migrate to the synovial and periarticular tissue (Firestein, 2003; Lee and Weinblatt, 2001). There has been progress in defining etiology and pathogenesis of this disease but exact mechanism still remains obscure.

In states of chronic inflammation as in RA, the imbalance between pro-inflammatory and anti-inflammatory cytokines determines the degree and extent of inflammation resulting in cellular damage (McInnes and Schett, 2007; Vierboom et al., 2007). Other key modulators in RA are reactive oxygen species (ROS) and reactive nitrogen species (Umar et al., 2012). Current treatment modalities for RA either produce symptomatic relief (non-steroidal anti-inflammatory drugs; NSAIDs) or modify the disease process

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(disease-modifying anti-rheumatic drugs; DMARDs). Though effective, their use is also limited by their side effects including gastrointestinal ulcers and perforation, cardiovascular complications and emergence of opportunistic infections due to immunosuppressant (Umar et al., 2013). In the US, 100,000 hospitalizations and 16,500 deaths per year are linked to NSAID-induced ulcers and gastrointestinal bleeding in arthritic patients (Abdel-Tawab et al., 2011).

As a result, interest in alternative, well tolerated anti-inflammatory remedies has re-emerged. Gum resin extracts of *Boswellia serrata* (BSE) have been found as an anti-inflammatory herbal remedy and used for the treatment of the inflammatory conditions in the traditional Ayurvedic medicine in India for centuries (Kimmatkar et al., 2003). Recent studies from animal and human support the potential of BSE for the treatment of a variety of inflammatory disorders like inflammatory bowel disease, rheumatoid arthritis and osteoarthritis (Ammon, 2002). Fan et al. (2005) showed that acetone extract of *Boswellia carterii* gum resin decreased arthritic scores, reduced paw oedema and significantly suppressed local tissue TNF-α and IL-1β in Lewis rats. Basch et al. reported that in comparison to NSAIDs, administration of BSE is expected to have better tolerability (Basch et al., 2004). Moreover, these extracts are devoid of the typical adverse effects associated with corticosteroids. In last decades, BSE and preparations from gum resins of Boswellia species have attracted increasing popularity in Western countries (Abdel-Tawab et al., 2011). In the present study, we investigated the effect of *Boswellia serrata* gum resin extract (BSE) against collagen induced arthritis in Wistar rats.

Materials and methods

Chemicals

Freund's adjuvant complete (CFA), N-methoxysuccinyl-Ala-Ala-Pro-Val p-nitroanilide and Griess Reagent system were purchased from Sigma Chemical Co. (St Louis, MO, USA). *Boswellia serrata* extract (BSE) was obtained from Herbosin CORPS, Meerut, U.P., India. ELISA kits were purchased from eBioscience and Cayman Chemical USA, Collagen type II from bovine nasal septum was purchased from Elastin Products Co, INC, Owensville, MO, USA. Thiobarbituric acid (TBA), trichloroacetic acid (TCA), 5,5′-dithio-bis-2-nitrobenzoicacid (DTNB), nitrobluetetrazolium (NBT), ethylene diamine tetra-acetic acid (EDTA), xanthine, xanthine oxidase, tris hydrochloride were purchased from SD Fine chemicals India. All other routine chemicals used in this investigation were of research grade.

Animals

Male Wistar rats weighing 150–170 g were used. They were kept in the Central Animal House of Hamdard University in colony cages at an ambient temperature of 25 ± 2 °C and relative humidity 45–55% with 12 h light/dark cycles after initial acclimatization for about 1 week. They had free access to standard rodent pellet diet and water ad libitum. The experimental study was conducted in accordance with the Institutional Animal Ethics Committee of the University, Jamia Hamdard, New Delhi, India.

HPLC analysis

Ethanolic extract of Boswellic acid (BSE) isolated from gum resin of *Boswellia serrata* were separated on a C18 reverse phase column (25 4.6 mm, particle size 5.0 mm, Merck, Germany) maintained at room temperature. The mobile phase consisted of Acetonitrile and 0.05% acetic acid in water in the ratio of 90:10 (v/v) gradient elution for 45 min. The flow rate was 1.0 ml/min; and column was maintained at room temperature. Analysis was performed at a wavelength of 254 for KBBA, AKBBA and BBA, ABBA at 210 nm using 10 mL of injection volume (BBA = beta bowswellic acid, ABBA = acetyle beta bowswellic acid, KBBA = keto beta bowswellic acid, AKBBA = acetyle keto beta bowswellic acid).

UPLC–MS/MS ESI-Q-TOF conditions

Mass spectrometry was performed on a Waters UPLC–MS/MS ESI-Q-TOF Premier (Micromass MS Technologies, Manchester, UK) mass spectrometer. UHPLC was performed with a Waters ACQUITY UPLC™ system (Waters Corp., MA, USA) equipped with a binary solvent delivery system, an auto-sampler, column manager and a tunable MS detector (Synapt; Waters, Manchester, UK). Chromatographic separation was performed on a Waters ACQUITY UPLC™ BEH C18 (100.0 mm × 2.1 mm; 1.7 μm) column. The mobile phase for UHPLC analysis consisted of methanol–water–glacial acetic acid (8:1:0.4, v/v/v), which was degassed. The Q-TOF Premier™ was operated in V mode with resolution over 32,000 mass. Quantitation was performed using Synapt Mass Spectrometery (Synapt MS) with a scan time of 1.0 min, and 0.02 s inter-scan per transition. The accurate mass and composition for the precursor ions and for the fragment ions were calculated using the MassLynx V 4.1 software.

Drug administration (gum resin of Boswellia serrata extracts)

The commercially available ethanolic extract of Boswellic acid (BSE) isolated from gum resin of *Boswellia serrata* Roxb., Family: Burseraceae, a fine white crystalline powder (Batch number: HC/BS/11015) was obtained from Herbosin CORPS, Meerut, U.P., India with a certificate of analysis. The extract was fine powder with creamy colour. The drugs were prepared as a fine homogenized suspension in 2% gum acacia (w/v) for oral administration.

Induction of collagen-induced arthritis (CIA) and experimental protocol

Arthritis was induced in rats as described previously (Haqqi et al., 1999). Collagen Type II from bovine nasal septum was dissolved in 0.05 M acetic acid at a concentration of 2 mg/ml, emulsified with an equal volume of Freund's adjuvant complete (CFA) containing 1 mg/ml Mycobacterium tuberculosis H37 RA, and stored in ice before use. Rats were immunized intradermally at about 1.5 cm distal from the base of the tail. All rats were randomly assigned to four groups of six animals in each group. The first group served as control (C), the second was collagen induced arthritis (CIA), the third was administered with 100 mg/kg body weight *Boswellia serrata* extract (CIA + BSE_{100}) daily and the fourth group was administered 200 mg/kg body weight *Boswellia serrata* extract (CIA + BSE_{200}) for 21 days starting from day 0 followed by CIA.