Phytomedicine xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

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

Sadiq Umar[a,g,1], Khalid Umar[b], Abu Hasnath Md. Golam Sarwar[c], Altaf Khan[d], Niyaz Ahmad[e], Sayeed Ahmad[e], Chandra Kant Katiyar[f], Syed Akhtar Husain[g], Haider A. Khan[a,*]

[a] *Clinical Toxicology Laboratory, Department of Medical Elementology & Toxicology, Jamia Hamdard (Hamdard University), New Delhi 110062, India*
[b] *Department of Chemistry, Aligarh Muslim University, Aligarh, U.P. 200202, India*
[c] *CIRBSc, Jamia Millia Islamia, New Delhi 110025, India*
[d] *Research Centre, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia*
[e] *Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi 110062, India*
[f] *Ayurvedic Research Lab., Dabur Research Centre, Ghaziabad, U.P. 201010, India*
[g] *Department of Biotechnology, Jamia Millia Islamia, New Delhi 110025, India*

ARTICLE INFO

Article history:
Received 13 August 2013
Received in revised form
11 December 2013
Accepted 13 February 2014

Keywords:
Boswellia serrata
Collagen induced arthritis
Cytokines
PGE_2

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

* Corresponding author at: Department of Medical Elementology & Toxicology, Jamia Hamdard (Hamdard University), New Delhi 110062, India.
Tel.: +91 9910940516; fax: +91 1126059663.
E-mail addresses: sadiq36@gmail.com (S. Umar), halitox@gmail.com (H.A. Khan).
[1] Present address: Department of Pharmacology, College of Pharmacy, University of Toledo, Toledo, OH 43614, United States.

http://dx.doi.org/10.1016/j.phymed.2014.02.001
0944-7113/

(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

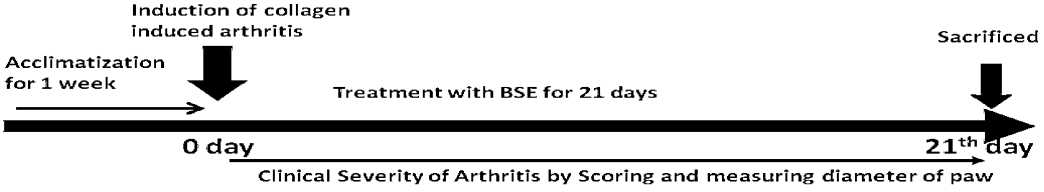
Mass spectrometry was performed on a Waters UPLC–MS/MS ESI-Q-TOF Premier (Micromass MS Technologies, Manchester, UK) mass spectrometer. UHPLC was performed with a Waters ACQUITY UPLC™ system (Waters Corp., MA, USA) equipped with a binary solvent delivery system, an auto-sampler, column manager and a 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.



Please cite this article in press as: Umar, S., et al., *Boswellia serrata* extract attenuates inflammatory mediators and oxidative stress in collagen induced arthritis. Phytomedicine (2014), http://dx.doi.org/10.1016/j.phymed.2014.02.001

Measurement of clinical severity of arthritis

For macroscopic assessment of arthritis, the thickness of each affected hind paw was measured with digital calliper (YAMAYO, Japan) and the measurement was expressed as an average for inflamed hind paws per rats. The severity of the arthritis was quantified daily by a clinical score measurement from 0 to 4 as follows: 0, no macroscopic signs of arthritis; (1) swelling of one group of joints (namely, wrist or ankle joints); (2) two groups of swollen joints; (3) three groups of swollen joints; (4) swelling of the entire paw (Umar et al., 2012).

Preparation of cell-free extract of the knee joints

At the end of experiment, animals were sacrificed by cervical dislocation. Arthritic and nonarthritic joints were removed and cut into small pieces and homogenized in 5 vol. of 50 mM Tris–HCl buffer, pH 7.4 containing 0.1 M NaCl and 0.1% Triton X-100 and 1 vol. of fine glass powder by using a mortar and pestle. The crude extract was then sonicated for 20 s. The homogenate was centrifuged at 3000 × *g* for 5 min to estimate TBARS and GSH and at 12,000 × *g* for 5 min, and resultant PMS was used to carry out elastase, MPO, catalase, SOD and NO assay, the resulting supernatant was stored at −80 °C until further analysis.

Biochemical analyses

Articular elastase (ELA)

ELA levels in the articular joints were evaluated as an index of polymorphonuclear leucocyte (PMNs) accumulation and activation in the inflamed tissue as described earlier (Yoshimura et al., 1994). Briefly, tissue samples were first diluted and homogenized in a solution containing 20 mM potassium phosphate buffer pH 7.0 in a ratio of 1:10 (w/v) and then centrifuged for 20 min at 10,000 × *g* at 4 °C. An aliquot of each sample was incubated for 24 h at 37 °C with 0.1 M Tris–HCl buffer (pH 8.0), containing 0.5 M NaCl and 1 mM N-methoxysuccinyl-Ala-Ala-Pro-Val p-nitroanilide, a high specific synthetic substrate for neutrophil elastase (ELA). The amount of p-nitroanilide liberated was measured spectrophotometrically at 405 nm and was considered as neutrophil ELA activity.

Myeloperoxidase (MPO) assay

Myeloperoxidase activity was analyzed as an index of neutrophils infiltration in the synovial tissue, as it is closely correlated with the number of neutrophils present in the tissue. The assay was carried out by the method described earlier (Umar et al., 2012). Myeloperoxidase activity was expressed as U/g of protein.

Estimation of thiobarbituric acid reactive substances (TBARS)

The assay of TBARS was done according to earlier method (Utley et al., 1967), adapted to microtiter plates by bringing the final volume to 150 μl. In brief, tissue homogenate was prepared in 0.15 M KCl (5%, w/v homogenate) and aliquots of 30 μl were incubated for 0 °C and 37 °C for 1 h. Subsequently, 60 μl of 28% (w/v) TCA was added and the volume was made up to 150 μl by adding 60 μl of distilled water followed by centrifugation at 3000 × *g* for 10 min. The supernatant (125 μl) was taken and colour was developed by addition of 25 μl of 1% (w/v) TBA dissolved in 0.05 N NaOH and kept in boiling water bath for 15 min. The absorbance was read at 532 nm in a plate reader (Bio-Rad, USA).

Reduced glutathione (GSH)

GSH was measured in the groups following the method described earlier (Sedlak and Lindsay, 1968). Homogenized joint tissue (10%, w/v in phosphate buffer pH 7.4) was deproteinized by adding an equal volume of 10% TCA and was allowed to stand at 4 °C for 2 h. The contents were centrifuged at 2000 × *g* for 15 min. 50 μl supernatant was added to 200 μl of 0.4 M Tris buffer (pH 8.9) containing 0.02 M EDTA (pH 8.9) followed by the addition 20 μl of 0.01 M DTNB. The absorbance was read in a microplate reader at 412 nm.

Total superoxide dismutases (SOD) activity

Total SOD were measured in joints as described earlier (Beauchamp and Fridovich, 1971) adapted to microtiter plates by bringing the final volume to 100 μl. Reaction mixture consisted of 0.05 M phosphate buffer (pH 7.4), 1 mM xanthine and 57 μM NBT. After incubation at room temperature for 15 min., reaction was initiated by addition of 50 mU xanthine oxidase mixture without enzyme preparations which served as blank. The SOD activity is expressed in Units/mg protein.

Catalase activity

Catalase activity in the joint tissues was assayed according to method described earlier (Sinha, 1972) using H_2O_2 as substrate. The reaction was adjusted to multiwell flat bottom plates by reducing the final volume to 200 μl. Briefly reaction mixture consisted of phosphate buffer (0.01 M, pH 7.0), distilled water and 10% homogenate (prepared in 0.1 M phosphate buffer). Reaction was started by adding H_2O_2 (0.2 M), incubated at 37 °C for 1 min and reaction was stopped by addition of dichromate: acetic acid reagent (1:3). The tubes were kept in a boiling water bath for 15 min and centrifuged for 10 min at 1500 × *g*. The colour developed was read at 570 nm in a microplate reader. The enzyme activity was expressed as μmol H_2O_2 consumed/min/mg protein.

Measurement of nitric oxide (NO): Griess reaction

After the experiment, animals were sacrificed and the joint tissues were washed with PBS (pH 7.4) and placed on ice as method described earlier (Sajad et al., 2009). Briefly a 50 μl sample was added with 100 μl of Griess reagent and reaction mixture was Incubate for about 5–10 min at room temperature and protects it from light, the optical density was measured at 540 nm in microplate reader according to the reagent manufacturer's protocol. Calculations were done after generating a standard curve from sodium nitrite in the same buffer as used for preparation of homogenate.

Measurement of cytokines level and PGE_2 level

Levels of inflammatory cytokines IL-1β, TNF-α, IFN-γ, IL-6, IL-10 and PGE_2 were measured in the joints. Tissues were homogenized in a pH 7.6 buffer consisting of 20 mM Tris–HCl, 100 mM KCl, 5 mM NaCl, 2 mM EDTA, 1 mM EGTA, 2 mM dithiothreitol and 2 mM PMSF. Homogenates were centrifuged at 4 °C and 12,000 × *g* for 15 min. Supernatants were removed and assayed in duplicate using commercially available cytokine ELISA kits (eBioscience and Cayman Chemical, USA). Tissue cytokine concentrations were expressed as pg/ml of protein.

Histological examinations

Rats were sacrificed on the day 21 by cervical dislocation. Knee joints were removed and fixed in 4% formaldehyde. After decalcification in 5% formic acid, the samples were processed for paraffin embedding (Durie et al., 1993). Tissue sections (5 μm thick) were stained with haematoxylin–eosin for light microscope examination.

Please cite this article in press as: Umar, S., et al., *Boswellia serrata* extract attenuates inflammatory mediators and oxidative stress in collagen induced arthritis. Phytomedicine (2014), http://dx.doi.org/10.1016/j.phymed.2014.02.001

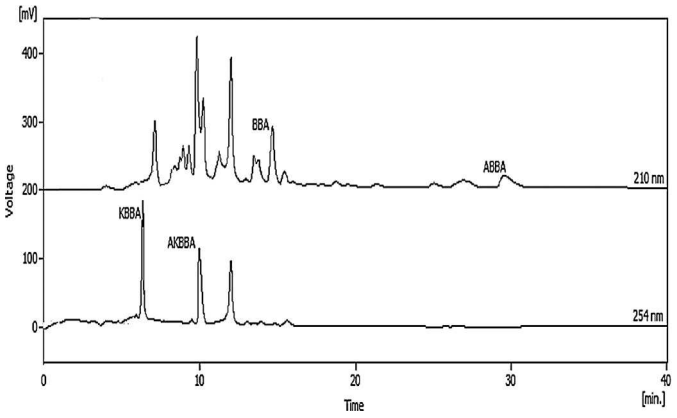


Fig. 1. HPLC chromatogram of KBBA, AKBBA at 254 nm and BBA, ABBA at 210 nm in extract as obtained with LiChroCART® C18 column (BBA = beta boswellic acid, ABBA = acetyl beta boswellic acid, KBBA = keto beta boswellic acid, AKBBA = acetyl keto beta boswellic acid).

Statistical analysis

Results are expressed as mean ± SEM. Statistical analysis of the data was done by applying the analysis of variance (ANOVA), followed by Tukey's test for all parameters. The p-value < 0.05 was considered statistically significant.

Results

Analysis of Boswellia serrata extract by HPLC

The analysis of ethanolic extract of *Boswellia serrata* showed the presence of KBBA (keto beta bowswellic acid), AKBBA (acetyle keto beta bowswellic acid) at 254 nm and BBA (beta bowswellic acid), ABBA (acetyle beta bowswellic acid) at 210 nm in extract (Fig. 1) as obtained with LiChroCART® C_{18} column (250 mm × 4.6 mm, particle size 5.0 μm) with mobile phase acetonitrile: 0.05% acetic acid in the ratio of 90:10 (v/v) in gradient elution mode for 45 min.

UPLC/ESI-Q-TOF-MS/MS analysis

Boswellia serrata extract was dissolved in methanol. MS allows the detection and identification of the target compounds via their typical m/z values. By using the negative SIM mode all boswellic acids revealed comparable signal intensities of their corresponding $[M-H]^-$ ions. The MS full scan spectra for alcoholic extract of *Boswellia serrata* showed deprotonated precursor $[M+H]^-$ ions at m/z (α-Boswellic acid) 455.17 > 437.24, (11-keto-β-Boswellic acid) 469.17 > 391.23, (Acetyl-β-Boswellic acid) 497.19 > 423.21, (3-Acetyl-11-β-Boswellic acid) 511.18 > 441.20 (Fig. 2). Again, similar results were obtained by Kruger et al. (2008). HPTLC analysis showed 3.4% 11-keto-β-boswellic acid (KBA), 2.5% acetyl-11-keto-β-boswellic acid (AKBA) and 25.8% 3-acetyl-11-keto-β-boswellic acids (AKBBA) (unpublished data) in *Boswellia serrata* extracts.

Clinical severity of disease after Boswellia serrata treatment

Arthritis developed rapidly in rats immunized with collagen emulsified with CFA. Clinical signs of the disease were erythema of one or more ankle joints, followed by involvement of the metatarsal and interphalangeal joints that first appeared in the hind paws between 8 and 9 days after CIA immunization and with a 100% incidence by day 13 ± 1. There was no macroscopic evidence of either hind paw erythema or oedema in the control group. Oral BSE administration to collagen-immunized rats reduced the progression of arthritis evidenced by inhibition in arthritis score compared to RA rats. The hind paw swelling reflects both inflammatory and arthritic changes occurring in arthritic rats. Inflammation was assessed by digital calliper and observing changes in their paw diameter (mm) of all group of rats. Fig. 3A demonstrated a time dependent increase in hind paw volume of rats immunized with collagen induces arthritis. *Boswellia serrata* extract significantly suppressed hind paw swelling on day 14 and 21 post immunization compared to arthritic rats.

Effect of Boswellia serrata on articular elastase and myeloperoxidase activity

Articular elastase (Fig. 4A) and myeloperoxidase activity (Fig. 4B) were assayed on the day 21st in the studied groups. Low levels of articular elastase and myeloperoxidase were measured in the joints of control group, while a significant elevated ($p < 0.001$) activity of these enzymes were observed in CIA group. Administration of the BSE at the two doses showed a significant decrease in articular elastase ($p < 0.01$ and $p < 0.001$ at lower and higher doses respectively) and myeloperoxidase levels ($p < 0.01$ and $p < 0.001$ at lower and higher doses respectively) resulting in reduction of neutrophil activation and infiltration in the synovial tissues of the joints.

Boswellia serrata treatment decreased TBARS

The effect of *Boswellia serrata* on TBARS level was measured to demonstrate the oxidative damage on lipids (Fig. 4C). A significant increase ($p < 0.001$) in TBARS level was observed in CIA group as compared to the control group. Treatment with BSE decreased TBARS level at both doses; $p < 0.01$ at low and $p < 0.001$ at high dose, by inhibiting lipid peroxidation in the cartilage tissue.

Boswellia serrata restored GSH and SOD levels

The changes in GSH level and SOD activity evaluated in the joints (day 21) in the experimental groups. The concentration of GSH (Fig. 4D) was evaluated to estimate endogenous defences against hydrogen peroxide formation and SOD activity (Fig. 4E) was measured to estimate endogenous defences against superoxide anions. A marked decrease in GSH and SOD ($p < 0.01$) concentrations were found in the joints of CIA rats. Treatment with BSE significantly inhibited reduction of GSH level at both doses and SOD level ($p < 0.05$ at low and $p < 0.01$ at high) as compared to CIA group.

Effect of Boswellia serrata on catalase activity

The activity of catalase decreased significantly in CIA group on the day 21 in the joints as compared to control group (Fig. 4F). In this case too, treatment with BSE was significantly effective at both doses; $p < 0.05$ at low and $p < 0.001$ at high dose, as compared to CIA group.

Effect of Boswellia serrata on nitric oxide

Analysis of nitrite estimation is summarized in Fig. 4G. A significant increase in nitrite was observed in CIA group as compared to control. The treatment with BSE declined the increase in the nitrite levels significantly at both doses; $p < 0.01$ at low and $p < 0.001$ at high dose, as compared to the CIA group.

Boswellia serrata suppresses IL-1β, TNF-α, IFN-γ and enhance production IL-10 in CIA rats

Proinflammatory cytokines IL-1β, TNF-α, IFN-γ and as well as IL-10 have central role in the perpetuation of chronic inflammation

Please cite this article in press as: Umar, S., et al., *Boswellia serrata* extract attenuates inflammatory mediators and oxidative stress in collagen induced arthritis. Phytomedicine (2014), http://dx.doi.org/10.1016/j.phymed.2014.02.001

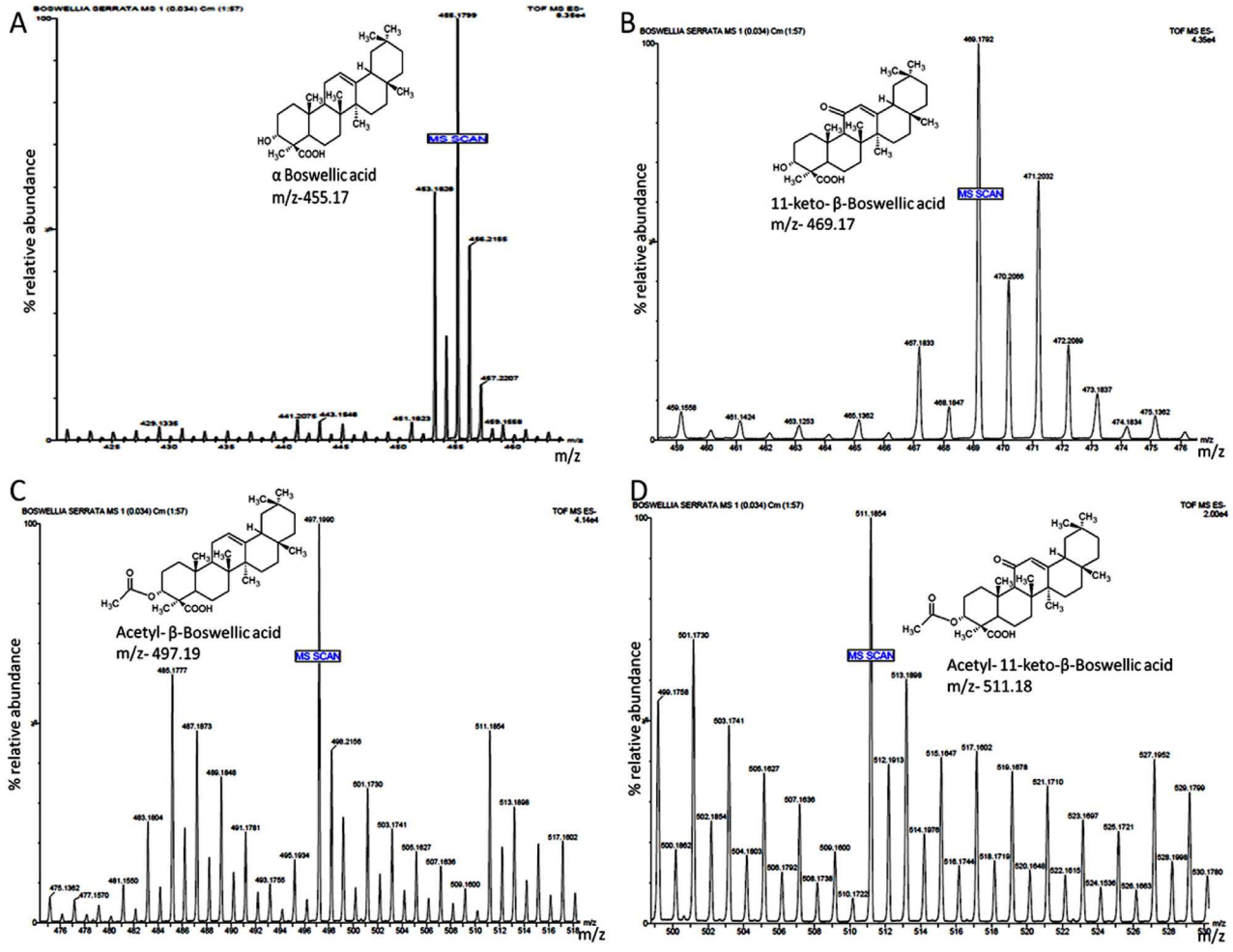


Fig. 2. Mass spectrum of *Boswellia serrata* extract: (A) α-boswellic acid ion (deprotonated precursor $[M+H]^-$ ions at m/z 455.17); (B) keto-β-boswellic acid (deprotonated precursor $[M+H]^-$ ions at m/z 469.17); (C) acetyl-β-boswellic acid (deprotonated precursor $[M+H]^-$ ions at m/z 497.19); and (D) acetyl-11-keto-β-boswellic acid (deprotonated precursor $[M+H]^-$ ions at m/z 511.18).

and tissue damage during progression of RA. As shown in Fig. 5, there was significant increase in the level of TNF-α ($p < 0.001$), IL-1β ($p < 0.001$), IFN-γ ($p < 0.001$), IL-6 ($p < 0.001$) and PGE_2 ($p < 0.01$) in CIA rats compared to the controls while a significant ($p < 0.01$) decrease in IL-10 level was observed. Oral administration of BSE at 200 mg/kg, down regulated the level of IL-1β ($p < 0.001$), IL-6 ($p < 0.001$), TNF-α ($p < 0.01$), IFN-γ ($p < 0.01$) and PGE_2 ($p < 0.01$) while an increase in IL-10 ($p < 0.01$) was observed as compared to CIA group on day 21.

Effect of Boswellia serrata on histopathology

Consistent with the biochemical alterations, the histological findings (Fig. 6) revealed massive cell infiltration in the CIA group.

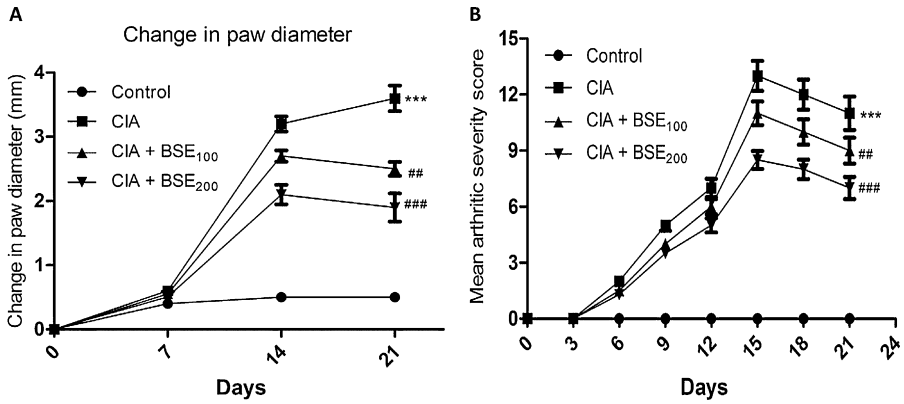


Fig. 3. Effect of *Boswellia serrata* extract (BSE) on time course of change in hind paw diameter (mm) (A) and mean clinical severity score (B) rats immunized with collagen type II. $^{**}p < 0.01$, $^{***}p < 0.001$ vs. Control, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, vs. CIA group

Please cite this article in press as: Umar, S., et al., *Boswellia serrata* extract attenuates inflammatory mediators and oxidative stress in collagen induced arthritis. Phytomedicine (2014), http://dx.doi.org/10.1016/j.phymed.2014.02.001

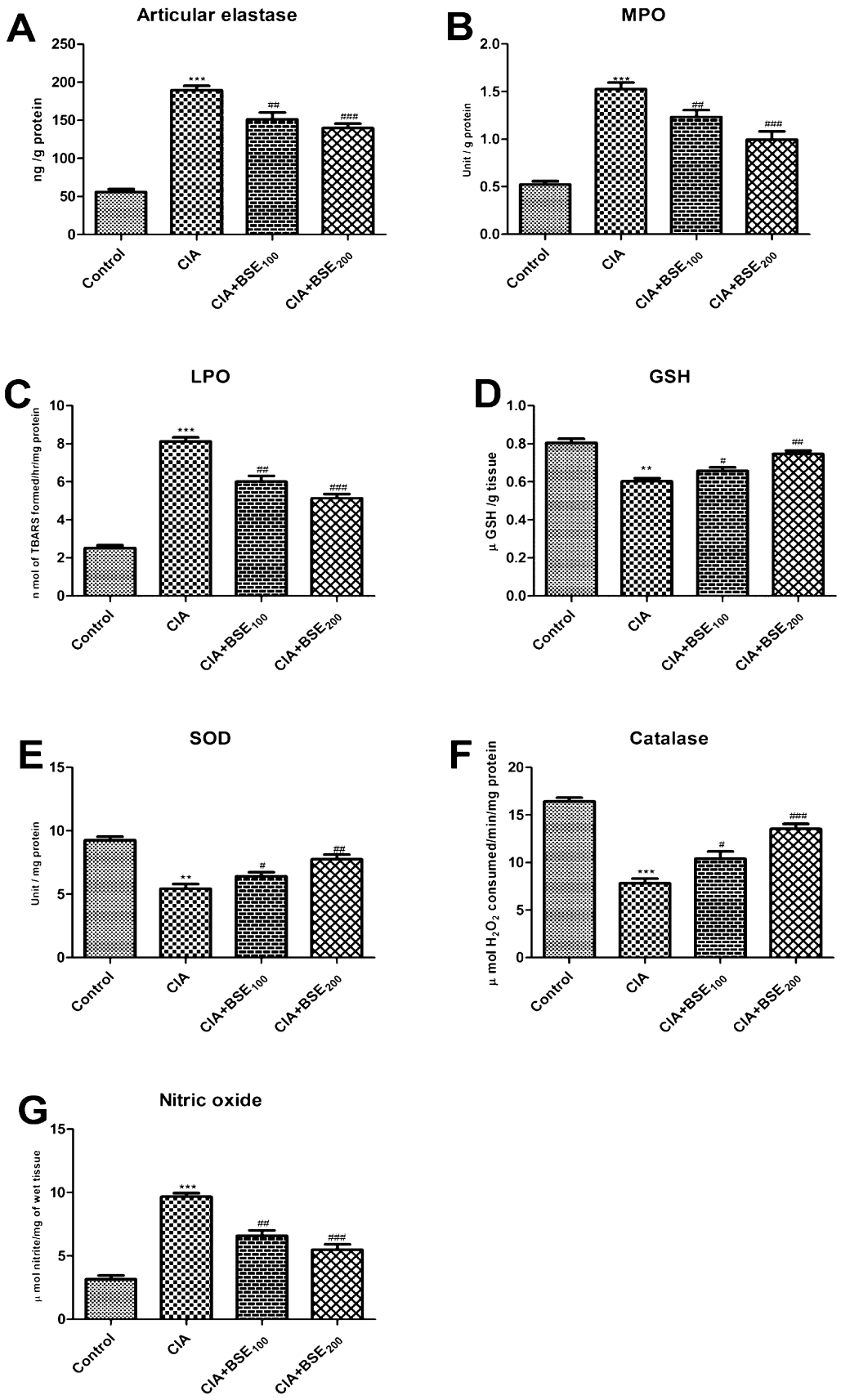


Fig. 4. Effect of *Boswellia serrata* extract (BSE) treatment on articular elastase activity, myeloperoxidase activity, GSH level, SOD activity, Lipid peroxidase, catalase activity and articular nitrite in joints of rats immunized with collagen type II. Data are expressed as mean ± SEM of 6 rats. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. Control, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.01$ vs. CIA.

Please cite this article in press as: Umar, S., et al., *Boswellia serrata* extract attenuates inflammatory mediators and oxidative stress in collagen induced arthritis. Phytomedicine (2014), http://dx.doi.org/10.1016/j.phymed.2014.02.001

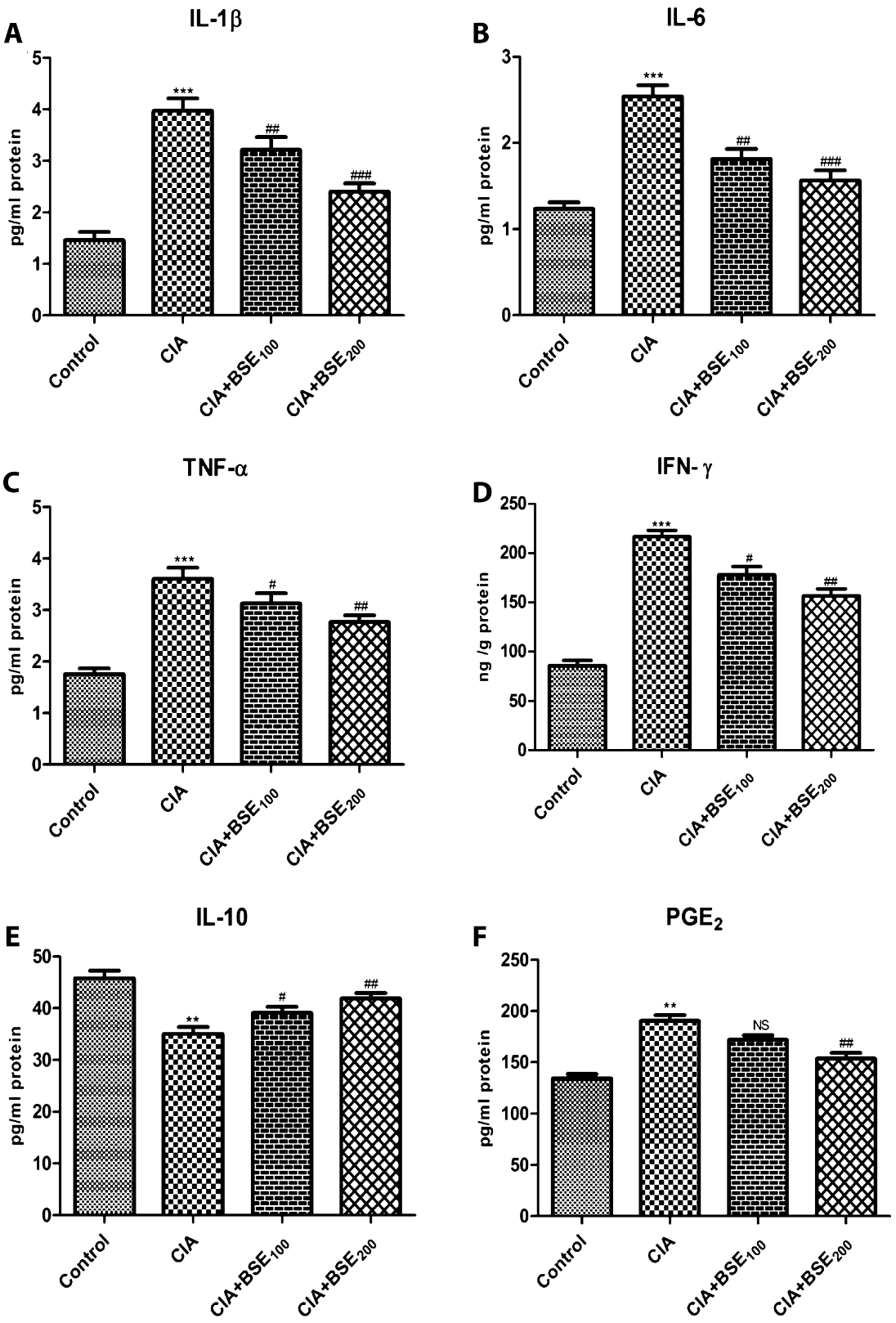


Fig. 5. Effects of *Boswellia serrata* extract (BSE) on cytokine and inflammatory mediators were measured in rat joints and concentration was expressed in pg/ml of (a) interleukin-1 β (IL-1β), (b) interleukin-6 (IL-6), (c) tumour necrosis factor-α (TNF-α), (d) interferon-γ (IFN-γ), (e) interleukin-10 (IL-10) and (f) PGE_2. Data are expressed as mean ± SEM of 6 rats. $^{**}p < 0.01$, $^{***}p < 0.001$ vs. Control, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.01$ vs. CIA group, NS – not significant.

Please cite this article in press as: Umar, S., et al., *Boswellia serrata* extract attenuates inflammatory mediators and oxidative stress in collagen induced arthritis. Phytomedicine (2014), http://dx.doi.org/10.1016/j.phymed.2014.02.001

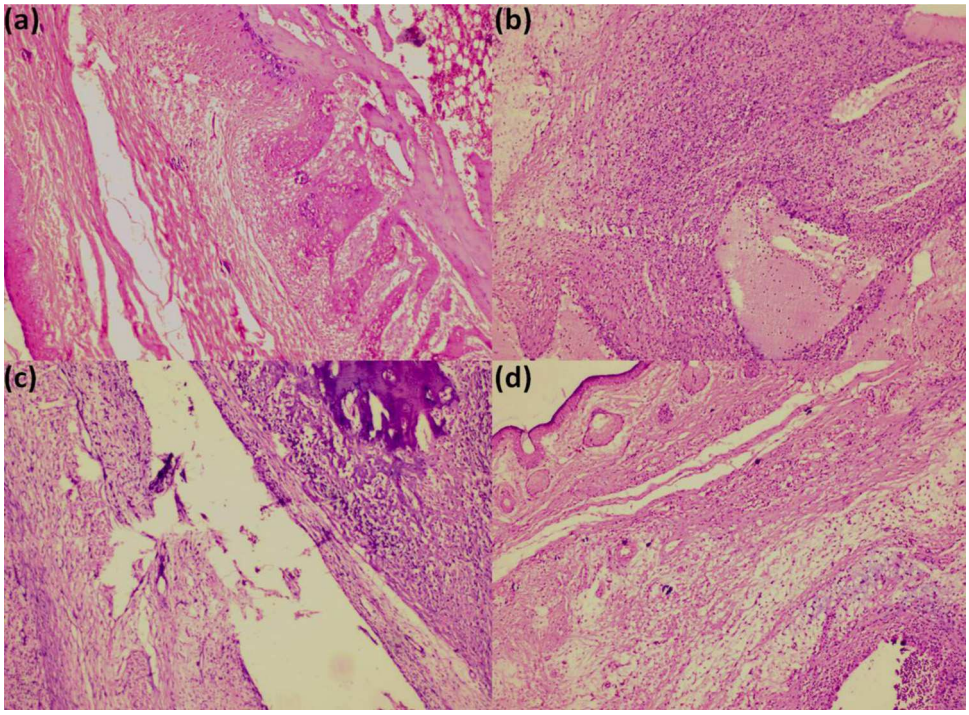


Fig. 6. Histological findings. Massive and diffuse polymorphonuclear cellular flux in the collagen induced arthritic rats (B) in comparison to the control rats (A). Cellular infiltration leads to the joint erosion mainly by inflammatory necrosis (B). Reduction of cellular flux and joint erosion which was evidenced by the minimum necrotic lesions in the rats treated with BSE 100 mg/kg (C) and BSE 200 mg/kg (d). Original magnification 10×.

Bone suffered resorption and pannus formation while synovial hyperplasia was consistent with chronic proliferation of joints. The treatment with BSE ameliorated the changes at histological level and was able to restore the changes to a greater extent at higher dose.

Discussion

We have demonstrated the anti-oxidative and anti-arthritic activity of *Boswellia serrata* extract (BSE) in collagen induced arthritis (CIA), an experimental model of rheumatoid arthritis (RA). The present study was performed to elucidate the effects and the mechanisms of BSE in CIA model. It was found that BSE markedly inhibited clinical sign of joint swelling, significantly decreased the free radical load, modulate inflammatory mediators in arthritic rats.

We evaluated elastase and myeloperoxidase activity which is directly proportional to the accumulation and activation of polymorphonuclear leukocytes in the inflamed tissue as it is released from stimulated granulocytes at the site of injury (Knight, 2000; Umar et al., 2012; van der Vliet et al., 1997). Boswellic acids have been reported as inhibitors of human leucocyte elastase (Safayhi et al., 1997). This could be of help in autoimmune disorders like rheumatoid arthritis. BSE in our study inhibited elastase activity and this decrease in elastase activity might be due to the inhibition of lipid peroxidation and the consequent reduction of chemotactic peroxide (Umar et al., 2012). Lipid peroxidation is considered a critical mechanism of the injury that occurs during RA. The large amount of TBARS found is consistent with the occurrence of damage mediated by free radicals. *Boswellia serrata* extract (BSE) has been reported to possess potential antioxidant and free radical scavenging properties (Kokkiripati et al., 2011; Mothana, 2011), which are thought to initiate cellular damage in cartilage in experimental animals. We found that CIA caused a significant increase in lipid peroxides and depletion in GSH and SOD levels. These results are in agreement with other studies (Campo et al., 2003). Our results clearly indicate that the protective role of BSE was mediated via its antioxidant effect through the suppression of lipid peroxidation and boosting the antioxidant defence system.

Nitric oxide (NO) is an imperative signalling molecule, produced as part of the inflammatory response from activated cells and macrophages (Seo et al., 2001). Therefore, compounds that hamper excessive NO production may have beneficial effects in arthritis by blocking degradation of cartilage (Shukla et al., 2008). In the present study, increased NO level have been detected in CIA rats similar with those previously reported in synovial fluids of patients with rheumatoid arthritis (van der Vliet et al., 1997). Treatment with BSE produced a significant decrease in nitric oxide level.

The inflammatory process is usually tightly regulated, while in the case of rheumatoid arthritis, a relationship between inflammation and bone homeostasis has been attributed to the effects of cytokines such as TNF-α, IL-1β, IFN-γ and IL-6 that are abundantly expressed in patients with RA and in the arthritic joints of rat with collagen-induced arthritis (Juarranz et al., 2005). A reduction of disease severity and bone resorption may be resulted by blockage of these molecules (Schett et al., 2008; Williams, 2004), while IL-4 and IL-10 have potent anti-inflammatory effects and suppress cartilage and bone pathology in RA (Juarranz et al., 2005). Previous studies on the oleo gum resin of Boswellia species showed its anti-inflammatory effect (Duwiejua et al., 1993; Mothana, 2011; Safayhi et al., 1997). Several boswellic acids were isolated from oleo gum resin. Previous work done so far confirmed that these triterpene acids were able to block inflammatory reactions in both acute and chronic inflammation models. Interestingly, the obtained results confirmed that *B. serrata* at the dose 200 mg/kg shift the balance of cytokines towards a bone protecting pattern that acts to both lower levels of TNF-α, IL-1β, IFN-γ and raise the levels of IL-10. Hence, it is plausible to suggest that part of the beneficial anti-inflammatory and cartilage/bone protective effects of *B. serrata* may be mediated through the inhibition of proinflammatory cytokines. Boswellic acids are considered to be the responsible for anti-inflammatory

Please cite this article in press as: Umar, S., et al., *Boswellia serrata* extract attenuates inflammatory mediators and oxidative stress in collagen induced arthritis. Phytomedicine (2014), http://dx.doi.org/10.1016/j.phymed.2014.02.001

activity of the plant (Borrelli et al., 2006). In addition to the anti-inflammatory effect, particularly the extract of Boswellia species showed considerable radical scavenging activity. Probably the two effects are related. Our results are in agreement with previous studies that showed Boswellia inhibits TH1 Cytokines and promoted production of TH2 in DBA/2 splenocytes (Chevrier et al., 2005).

Previous work by Moussaieff et al. (2007) reported that incensole acetate a compound isolated from Boswellia resins inhibits NF-kB activation and Cuaz-Perolin et al. (2008) found that AKBA inhibits activation of NF-kB *in vivo* mice model. NF-kB plays a central role in the regulation of many genes that induce TNF-α, IL-1β, IL-6, iNOS, and COX-2 which are responsible for the generation of mediators or proteins in inflammation (Cuzzocrea et al., 2007; Verma, 2004). It was demonstrated that these triterpene acids were able to block inflammatory reactions, in both acute and chronic inflammation models (Ammon, 2006).

The biochemical alterations were further supported by histopathological observations of the joints. The elevated number of infiltrating cells, extensive bone degradation and synovial hyperplasia which are hallmarks of RA was found in CIA. BSE Treatment was able to reverse the histological findings to normal. This study suggest that the antiarthritic effect of *Boswellia serrata* extract on joints cartilage in CIA rats is probably mediated by the controlling pro- and anti-inflammatory cytokines, nitric oxide and antioxidant enzymes followed by the inhibition of accumulation and activation of PMN cells. Therefore, *Boswellia serrata* extract has significant potential as a phytomedicine and might represent an alternative for classical medicine treatments for chronic inflammatory diseases like rheumatoid arthritis. We believe that our results will contribute to the clinical applications in the treatment of rheumatoid arthritis.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

Author is grateful to Indian council of medical research, Government of India for providing the financial support in the form of senior research fellowship (SRF). We are thankful to CCRUM, Department of AYUSH, Ministry of Health and Family Welfare, Government of India, for providing grant in aid for carrying out research work in arthritis. Help from Herbosin CORPS, Meerut and Dabur Research Centre, Ghaziabad, India for providing a generous gift of *Boswellia serrata* extract is also acknowledged.

References

Abdel-Tawab, M., Werz, O., Schubert-Zsilavecz, M., 2011. *Boswellia serrata*: an overall assessment of in vitro, preclinical, pharmacokinetic and clinical data. Clin. Pharmacokinet. 50, 349–369.

Ammon, H.P., 2002. Boswellic acids (components of frankincense) as the active principle in treatment of chronic inflammatory diseases. Wien. Med. Wochenschr. 152, 373–378.

Ammon, H.P., 2006. Boswellic acids in chronic inflammatory diseases. Planta Med. 72, 1100–1116.

Basch, E., Boon, H., Davies-Heerema, T., Foppo, I., Hashmi, S., Hasskarl, J., Sollars, D., Ulbricht, C., 2004. Boswellia: an evidence-based systematic review by the Natural Standard Research Collaboration. J. Herb. Pharmacother. 4, 63–83.

Beauchamp, C., Fridovich, I., 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44, 276–287.

Borrelli, F., Capasso, F., Capasso, R., Ascione, V., Aviello, G., Longo, R., Izzo, A.A., 2006. Effect of *Boswellia serrata* on intestinal motility in rodents: inhibition of diarrhoea without constipation. Br. J. Pharmacol. 148, 553–560.

Campo, G.M., Avenoso, A., Campo, S., Ferlazzo, A.M., Altavilla, D., Calatroni, A., 2003. Efficacy of treatment with glycosaminoglycans on experimental collagen-induced arthritis in rats. Arthritis Res. Ther. 5, R122–R131.

Chevrier, M.R., Ryan, A.E., Lee, D.Y., Zhongze, M., Wu-Yan, Z., Via, C.S., 2005. *Boswellia carterii* extract inhibits TH1 cytokines and promotes TH2 cytokines in vitro. Clin. Diagn. Lab. Immunol. 12, 575–580.

Choy, E.H., Panayi, G.S., 2001. Cytokine pathways and joint inflammation in rheumatoid arthritis. N. Engl. J. Med. 344, 907–916.

Coussens, L.M., Werb, Z., 2002. Inflammation and cancer. Nature 420, 860–867.

Cuaz-Perolin, C., Billiet, L., Bauge, E., Copin, C., Scott-Algara, D., Genze, F., Buchele, B., Syrovets, T., Simmet, T., Rouis, M., 2008. Antiinflammatory and antiatherogenic effects of the NF-kappaB inhibitor acetyl-11-keto-beta-boswellic acid in LPS-challenged ApoE$^{-/-}$ mice. Arterioscler. Thromb. Vasc. Biol. 28, 272–277.

Cuzzocrea, S., Di Paola, R., Mazzon, E., Crisafulli, C., Genovese, T., Muia, C., Abdelrahman, M., Esposito, E., Thiemermann, C., 2007. Glycogen synthase kinase 3beta inhibition reduces the development of nonseptic shock induced by zymosan in mice. Shock 27, 97–107.

Durie, F.H., Fava, R.A., Foy, T.M., Aruffo, A., Ledbetter, J.A., Noelle, R.J., 1993. Prevention of collagen-induced arthritis with an antibody to gp39, the ligand for CD40. Science 261, 1328–1330.

Duwiejua, M., Zeitlin, I.J., Waterman, P.G., Chapman, J., Mhango, G.J., Provan, G.J., 1993. Anti-inflammatory activity of resins from some species of the plant family Burseraceae. Planta Med. 59, 12–16.

Fan, A.Y., Lao, L., Zhang, R.X., Zhou, A.N., Wang, L.B., Moudgil, K.D., Lee, D.Y., Ma, Z.Z., Zhang, W.Y., Berman, B.M., 2005. Effects of an acetone extract of *Boswellia carterii* Birdw. (Burseraceae) gum resin on adjuvant-induced arthritis in Lewis rats. J. Ethnopharmacol. 101, 104–109.

Firestein, G.S., 2003. Evolving concepts of rheumatoid arthritis. Nature 423, 356–361.

Haqqi, T.M., Anthony, D.D., Gupta, S., Ahmad, N., Lee, M.S., Kumar, G.K., Mukhtar, H., 1999. Prevention of collagen-induced arthritis in mice by a polyphenolic fraction from green tea. Proc. Natl. Acad. Sci. U. S. A. 96, 4524–4529.

Juarranz, Y., Abad, C., Martinez, C., Arranz, A., Gutierrez-Canas, I., Rosignoli, F., Gomariz, R.P., Leceta, J., 2005. Protective effect of vasoactive intestinal peptide on bone destruction in the collagen-induced arthritis model of rheumatoid arthritis. Arthritis Res. Ther. 7, R1034–R1045.

Kimmatkar, N., Thawani, V., Hingorani, L., Khiyani, R., 2003. Efficacy and tolerability of *Boswellia serrata* extract in treatment of osteoarthritis of knee—a randomized double blind placebo controlled trial. Phytomedicine 10, 3–7.

Knight, J.A., 2000. Review: free radicals, antioxidants, and the immune system. Ann. Clin. Lab. Sci. 30, 145–158.

Koelink, P.J., Overbeek, S.A., Braber, S., de Kruijf, P., Folkerts, G., Smit, M.J., Kraneveld, A.D., 2012. Targeting chemokine receptors in chronic inflammatory diseases: an extensive review. Pharmacol. Ther. 133, 1–18.

Kokkiripati, P.K., Bhakshu, L.M., Marri, S., Padmasree, K., Row, A.T., Raghavendra, A.S., Tetali, S.D., 2011. Gum resin of *Boswellia serrata* inhibited human monocytic (THP-1) cell activation and platelet aggregation. J. Ethnopharmacol. 137, 893–901.

Kruger, P., Daneshfar, R., Eckert, G.P., Klein, J., Volmer, D.A., Bahr, U., Muller, W.E., Karas, M., Schubert-Zsilavecz, M., Abdel-Tawab, M., 2008. Metabolism of boswellic acids in vitro and in vivo. Drug Metab. Dispos. 36, 1135–1142.

Lee, D.M., Weinblatt, M.E., 2001. Rheumatoid arthritis. Lancet 358, 903–911.

McInnes, I.B., Schett, G., 2007. Cytokines in the pathogenesis of rheumatoid arthritis. Nat. Rev. Immunol. 7, 429–442.

Mothana, R.A., 2011. Anti-inflammatory, antinociceptive and antioxidant activities of the endemic Soqotraen *Boswellia elongata* Balf. f. and *Jatropha unicostata* Balf. f. in different experimental models. Food Chem. Toxicol. 49, 2594–2599.

Moussaieff, A., Shohami, E., Kashman, Y., Fride, E., Schmitz, M.L., Renner, F., Fiebich, B.L., Munoz, E., Ben-Neriah, Y., Mechoulam, R., 2007. Incensole acetate, a novel anti-inflammatory compound isolated from Boswellia resin, inhibits nuclear factor-kappa B activation. Mol. Pharmacol. 72, 1657–1664.

Safayhi, H., Rall, B., Sailer, E.R., Ammon, H.P., 1997. Inhibition by boswellic acids of human leukocyte elastase. J. Pharmacol. Exp. Ther. 281, 460–463.

Sajad, M., Zargan, J., Chawla, R., Umar, S., Sadaqat, M., Khan, H.A., 2009. Hippocampal neurodegeneration in experimental autoimmune encephalomyelitis (EAE): potential role of inflammation activated myeloperoxidase. Mol. Cell. Biochem. 328, 183–188.

Schett, G., Stach, C., Zwerina, J., Voll, R., Manger, B., 2008. How antirheumatic drugs protect joints from damage in rheumatoid arthritis. Arthritis Rheum. 58, 2936–2948.

Sedlak, J., Lindsay, R.H., 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. 25, 192–205.

Seo, W.G., Pae, H.O., Oh, G.S., Chai, K.Y., Kwon, T.O., Yun, Y.G., Kim, N.Y., Chung, H.T., 2001. Inhibitory effects of methanol extract of *Cyperus rotundus* rhizomes on nitric oxide and superoxide productions by murine macrophage cell line, RAW 264.7 cells. J. Ethnopharmacol. 76, 59–64.

Shukla, M., Gupta, K., Rasheed, Z., Khan, K.A., Haqqi, T.M., 2008. Bioavailable constituents/metabolites of pomegranate (*Punica granatum* L.) preferentially inhibit COX2 activity ex vivo and IL-1beta-induced PGE2 production in human chondrocytes in vitro. J. Inflamm. (Lond.) 5, 9.

Sinha, A.K., 1972. Colorimetric assay of catalase. Anal. Biochem. 47, 389–394.

Stix, G., 2007. A malignant flame. Understanding chronic inflammation, which contributes to heart disease, Alzheimer's and a variety of other ailments, may be a key to unlocking the mysteries of cancer. Sci. Am. 297, 60–67.

Umar, S., Kumar, A., Sajad, M., Zargan, J., Ansari, M.M., Ahmad, S., Katiyar, C.K., Khan, H.A., 2013. Hesperidin inhibits collagen-induced arthritis possibly through suppression of free radical load and reduction in neutrophil activation and infiltration. Rheumatol. Int. 33, 657–663.

Umar, S., Zargan, J., Umar, K., Ahmad, S., Katiyar, C.K., Khan, H.A., 2012. Modulation of the oxidative stress and inflammatory cytokine response by thymoquinone in the collagen induced arthritis in Wistar rats. Chem. Biol. Interact. 197, 40–46.

Please cite this article in press as: Umar, S., et al., *Boswellia serrata* extract attenuates inflammatory mediators and oxidative stress in collagen induced arthritis. Phytomedicine (2014), http://dx.doi.org/10.1016/j.phymed.2014.02.001

Utley, H.G., Bernheim, F., Hochstein, P., 1967. Effect of sulfhydryl reagents on peroxidation in microsomes. Arch. Biochem. Biophys. 118, 29–32.
van der Vliet, A., Eiserich, J.P., Halliwell, B., Cross, C.E., 1997. Formation of reactive nitrogen species during peroxidase-catalyzed oxidation of nitrite. A potential additional mechanism of nitric oxide-dependent toxicity. J. Biol. Chem. 272, 7617–7625.
Verma, I.M., 2004. Nuclear factor (NF)-kappaB proteins: therapeutic targets. Ann. Rheum. Dis. 63 (Suppl. 2), ii57–ii61.
Vierboom, M.P., Jonker, M., Tak, P.P., t Hart, B.A., 2007. Preclinical models of arthritic disease in non-human primates. Drug Discov. Today 12, 327–335.
Williams, R.O., 2004. Collagen-induced arthritis as a model for rheumatoid arthritis. Methods Mol. Med. 98, 207–216.
Yoshimura, K., Nakagawa, S., Koyama, S., Kobayashi, T., Homma, T., 1994. Roles of neutrophil elastase and superoxide anion in leukotriene B4-induced lung injury in rabbit. J. Appl. Physiol. 76, 91–96.

Please cite this article in press as: Umar, S., et al., *Boswellia serrata* extract attenuates inflammatory mediators and oxidative stress in collagen induced arthritis. Phytomedicine (2014), http://dx.doi.org/10.1016/j.phymed.2014.02.001