Amelioration of collagen-induced arthritis by Boswellia serrata through altered Th1/Th2 cytokine production, inflammatory mediators and oxidative stress in Wistar rats
5.1. Introduction

Inflammation is the body's first immune-response 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 (Koelink et al., 2012, Coussens and Werb, 2002, Stix, 2007, Libby, 2002). 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 (Lee and Weinblatt, 2001, Ziff, 1990). There has been progress in defining aetiology and pathogenesis of this disease but exact mechanism still remains obscure.

In RA, the balance between pro-inflammatory and anti-inflammatory cytokines determines the degree and extent of inflammation (McInnes and Schett, 2007, Vierboom et al., 2007). Proinflammatory cytokines like interleukin (IL)-1β, tumor necrosis factor (TNF) α and IL-6 are highly expressed in the rheumatoid joint and play key role in the pathogenesis of RA (Kim et al., 2011). Fibroblast like synoviocytes (FLS), in response to these cytokines produce chemokines, metalloproteinases (MMPs), prostaglandin E₂ (PGE₂) and cyclooxygenase-2 (COX-2) which further promote inflammation, hyperplasia and cartilage destruction (Vaillancourt et al., 2011). Other key modulators of inflammation in RA are reactive oxygen species (ROS) and reactive nitrogen species. They might perpetuate inflammation by facilitating the production of chemotactic factors at the local site (Merry et al., 1989, Woodruff et al., 1986). The hydroxyl radical, superoxide anion radical, and the peroxynitrite anion (de Groot, 1994) are the major ROS generated during the disease condition. Reactive species like nitric oxide (NO) serve as an important messenger molecule in inflammatory conditions (Cuzzocrea, 2006). Decreased production of NO via suppressing or inhibiting iNOS may reduce arthritic symptoms and afford protection (Rostoka et al.).

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 including gastrointestinal ulcers and perforation, cardiovascular complications and emergence of opportunistic infections due to immunosuppressant (Nair et al.). 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. Boswellic acid (BA) is an active component of Boswellia serrata (also known as Salai guggul). Extensive research in the past 30 years identified the active component of this resin as BA (a pentacyclic triterpenic acid) and its derivatives (acetyl-b-boswellic acid, 11-keto-b-boswellic acid and acetyl-11-keto-b-boswellic acid). BA, a mixture comprised of four major pentacyclic triterpene acids: beta-boswellic acid, 3-acetyl beta boswellic acid, 11-keto-beta-boswellic acid and 3-acetyl-11-keto-beta-boswellic acid, isolated from the oleo gum resin of Boswellia serrata (Singh et al., 1996). 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). 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.

5.2. Materials and methods

5.2.1. 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 Ayurvedic Research Lab., Dabur Research Centre, Ghaziabad. 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, Missouri, USA. Thiobarbituric acid (TBA), trichloroacetic acid (TCA), 5,5'-dithio-bis-2-nitrobenzoic acid (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.

5.2.2. 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.

5.2.3. 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 x 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 32000 mass. Quantitation was performed using Synapt Mass Spectrometry (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.

5.2.4. Preparation of BSE solution

Alcoholic extract of Boswellia serrata (BSE) was obtained from Herbosin CORPS, Meerut, UP, India with a certificate of analysis is attached in annexure I. The extract was fine powder with creamy colour. The alcohol extract residue has been shown to comprise a mixture triterpene pentacyclic acid derivative of boswellic acid to the tune of 65 %; Indian frankincense (B. serrata) contains 11-keto-p-boswellic acid (KBA, 3–4.7%) and acetyl-11-keto-p-boswellic acid (AKBA, 2.2–2.9%) (18). The drugs were prepared as a fine homogenised suspension in 2% gum acacia (w/v) for oral administration.

5.2.5. 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 five 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⁻¹ body weight Boswellia serrata extract (CIA + BSE100) daily and the fourth group was administered 200 mg kg⁻¹ body weight Boswellia serrata extract (CIA + BSE200) for 21 days starting from day 0 followed by CIA.

5.2.6. 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 development of arthritis in rats was evaluated daily starting from day 0 after the intradermal injection using macroscopic scoring system (Larsson et al., 1990): 0 = No evidence of erythema and swelling, 1 = Erythema and mild swelling confined to the tarsals or ankle joint, 2 = Erythema and mild swelling extending from the ankle to the tarsals, 3 = Erythema and moderate swelling extending from the ankle to metatarsal joints, 4 = Erythema and severe swelling encompass the ankle, foot and digits, or ankylosis of the limb.

5.2.7. 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 sec. The homogenate was centrifuged at 3,000 × g for 5 min, and the resulting supernatant was stored at -80°C until further analysis.

5.2.8. Articular Elastase (ELA)

Articular elastase levels in the articular joints were evaluated as an index of polymorphonuclear leukocyte (PMNs) accumulation and activation in the inflamed tissue as
described earlier (Yoshimura et al., 1994). The articular elastase activity was expressed as ng/g protein.

5.2.9. Myeloperoxidase (MPO) assay
Myeloperoxidase activity was analysed 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 (Campo et al., 2003; Lefkowitz et al., 1999). Myeloperoxidase activity was expressed as U/g of protein.

5.2.10. Estimation of thiobarbituric acid reactive substances (TBARS)
The assay of TBARS was done according to the method mentioned earlier (Utley et al., 1967). The result was expressed in nmol TBARS formed / h /g tissue.

5.2.11. Reduced glutathione (GSH)
GSH was measured in the groups following the method described earlier (Sedlak and Lindsay, 1968). Results were expressed as μg GSH/g tissue.

5.2.12. Total superoxide dismutase (SOD) activity
Total SOD were measured in joints as described earlier (Beauchamp and Fridovich, 1971). The SOD activity is expressed in Units /mg protein.

5.2.13. Catalase activity
Catalase activity in the joint tissues was assayed according to method described earlier (Sinha, 1972). The enzyme activity was expressed as μmol H2O2 consumed / min / mg protein.

Nitric oxide was determined with Griess method as described earlier (Sajad et al., 2009).

5.2.15. Measurement of cytokines level and PGE\textsubscript{2} production
Levels of inflammatory cytokines IL-1β, IL-6, TNF-α, IFN-γ, IL-10 and PGE\textsubscript{2} in the joints were determined by using commercially available cytokine ELISA kits (eBioscience and Cayman Chemical USA). Supernatants were removed and assayed in duplicate according to the manufacturer's guidelines. Tissue cytokine concentrations were expressed as picograms of antigen per milligram of protein.

5.2.16. Immunohistochemistry of NF-κB
Sections of formalin-fixed, paraffin-embedded joints were obtained on poly-L-lysine coated slides. The sections were fixed in neutral buffered formalin, and embedded in paraffin. The samples were processed according to the manufacturer's protocol recommended for the NF-κB immunohistochemistry with slight modifications. Following deparaffinization and
rehydration, sections were irradiated in 0.1 mol/L sodium citrate buffer (pH 6.0) in a microwave oven (medium low temperature) for 20 min. Thereafter, the sections were exposed to 3% H2O2 for 10 min to bleach endogenous peroxidases, followed by 3 times rinsing in Tris buffer (pH 7.4) for 10 min. Sections were selectively incubated under humid conditions using an anti-NFκB polyclonal antibody (1:200; Thermo Scientific, USA) overnight at 4°C. Next day, the slides were washed three times in Tris buffers (pH 6.0) and incubated with biotinylated Goat Anti-Polyvalent Plus (Thermo Fisher Scientific, USA) for 30 min at room temperature. This step was followed by further washing in Tris buffer and incubation of slides at room temperature with a Streptavidin Peroxidase Plus (Thermo Fisher Scientific, USA) that binds to the biotin present on the secondary antibody. After washing in Tris buffer, the immunostaining reaction product was developed using 3, 3'-diaminobenzidine (DAB Plus substrate, Thermo Fisher Scientific, USA). After immunoreactivity, slides were dipped in distilled water, counterstained with Harris hematoxyline and finally the sections were dehydrated in xylene, mounted with DPX and coverslipped. Placenta acted as a positive control for NFκB. Negative controls included staining tissue sections with omission of the primary antibody whereas positive control slides were also run in parallel in each case. Slides prepared for each case were examined by light microscopy.

5.2.17. 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.

5.2.18. 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.
5.3. Results

5.3.1. 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 (Fig. 1a), (11-keto-β-Boswellic acid) 469.17 → 391.23 (Fig. 1b), (Acetyl-β- Boswellic acid) 497.19 → 423.21 (Fig. 1c), (3-Acetyl-11-β- Boswellic acid) 511.18 → 441.20 (Fig. 1d). Again, similar results were obtained by Kruger et al (34).

![Mass spectrum of Boswellia serrata extract](image)

Figure 5.1. Mass spectrum of *Boswellia serrata* extract: (A) α-boswellic acid ion (deprotonated precursor [M+H]+ ions at m/z 455.17 → 437.24).
Figure 5.1. Mass spectrum of *Boswellia serrata* extract: (B) keto-\(\beta\)-boswellic acid (deprotonated precursor [M+H]- ions at m/z 469.17 — 891.23).

Figure 5.1. Mass spectrum of *Boswellia serrata* extract: (C) Acetyl-\(\beta\)-boswellic acid (deprotonated precursor [M+H]- ions at m/z 497.19 — 823.21).
Fig. 5.1. Mass spectrum of *Boswellia serrata* extract: (D) acetyl-11-keto-β-boswellic acid (deprotonated precursor [M+H]+ ions at m/z 511.18 -> 441.20).

5.3.2. 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 CFA immunization and with a 100% incidence by day 13 ± 1 (Fig. 5.2 a). 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 (Fig. 5.2 b) and paw swelling compared to untreated group.
Fig. 5.2 Effect of *Boswellia serrata* extract (BSE) on time course of change in hind paw diameter (mm) (2 A) and mean clinical severity score (2 B) rats immunized with collagen type II. Values are Mean ± SEM for six animals for each group.

5.3.3. Effect of *Boswellia serrata* on articular elastase and myeloperoxidase activity

Articular elastase (Fig. 5.3 a) and myeloperoxidase activity (Fig. 5.3 b) were assayed on the day 21st in the studied groups. While low levels of articular elastase and myeloperoxidase were measured in the joints of control group, a significant elevated 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 < 0.05 and p < 0.01 at lower and higher doses respectively) resulting in reduction of neutrophil activation and infiltration in the synovial tissues of the joints.
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Fig. 5.3 Effect of Boswellia serrata extract (BSE) treatment on (a) articular elastase activity (b) myeloperoxidase activity in joints of rats immunized with collagen type II. Data are expressed as Mean ± SEM of 6 rats.

"*" (p < 0.01), "**" (p < 0.001) vs. Control, "#" (p < 0.05), "##" (p < 0.01), "###" (p < 0.001) vs. CIA.

5.3.4. Boswellia serrata restored GSH and SOD levels

Fig. 5.4 illustrate the changes in GSH level and SOD activity evaluated in the joints (day 21) in the experimental groups. The concentration of GSH (Fig. 5.4 a) was evaluated to estimate endogenous defences against hydrogen peroxide formation and SOD activity (Fig. 5.4 b) was measured to estimate endogenous defences against superoxide anions. A marked decrease in GSH (p < 0.01) and SOD (p < 0.001) concentrations were found in the joints of CIA rats. Treatment with BSE significantly inhibited reduction of GSH level at both doses (p < 0.05 at low and p < 0.01 at high) and SOD level (p < 0.01 at low and p < 0.001 at high) as compared to CIA group.

5.3.5. Boswellia serrata treatment decreased TBARS

The effect of Boswellia serrata on TBARS level was measured to demonstrate the oxidative damage on lipids (Fig. 5.4 c). 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.

5.3.6. 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. 5.4 d). In this case too, treatment with BSE was significantly effective at both doses; p < 0.01 at low and p < 0.01 at high dose, as compared to CIA group.
Fig. 5.4 Effect of Boswellia serrata extract (BSE) treatment on (a) GSH level (b) SOD activity (c) Lipid peroxidase (d) catalase activity 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.

Fig. 5.5 Effect of Boswellia serrata extract (BSE) treatment on articular nitrite content in joints of rats immunized with collagen type II. Data are expressed as Mean ± SEM of 6 rats.

*** (p < 0.001) vs. Control, ## (p < 0.01), ### (p < 0.01) vs. CIA.
5.3.7. Effect of Boswellia serrata on nitric oxide

Analysis of nitrite estimation is summarised in Fig. 5.6. 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.

Fig. 5.7 Effects of Boswellia serrata extract (BSE) on cytokine levels were measured in rat joints and concentration was expressed in pg/ml of a) Interleukin-1β (IL-1β), b) Interleukin-6 (IL-6), c) Tumor necrosis factor-α (TNF-α), d) Interferon-γ (IFN-γ), e) Interleukin-10 (IL-10), and f) PGE2. Data are expressed as Mean ± SEM of 6 rats.

** (\( p<0.01 \)), *** (\( p<0.001 \)) vs. Control, # \( p<0.05 \), ## \( p<0.01 \), ### \( p<0.001 \) vs. QA group, NS - not significant

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

Proinflammatory cytokines IL-1β, TNF-α, IFN-γ and as well as IL-10 have central role in the perpetuation of chronic inflammation and tissue damage during progression of RA. As
shown in Fig. 5.7, there was significant increase in the level of TNF-α (P<0.01), IL-1β (P<0.001), IFN-γ (P<0.001), IL-6 (P<0.01) and PGE₂ (P<0.001) in RA 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<0.001), IFN-γ (P<0.01) and PGE₂ (P<0.01) while an increase in IL-10 (P<0.01) was observed as compared to CIA group on day 21.

5.3.9. Boswellia serrata inhibited activation of NF-κB and effect on histopathology

Consistent with the biochemical alterations, the histological findings (Fig. 5.8) revealed massive cell infiltration in the CIA group. 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. NF-κB is a master regulator of innate immunity and inflammatory signalling. Thus, inhibition of NF-κB is recognized as a valuable tool to prevent the inflammation. BSE suppressed the activation of NF-κB activity (Fig. 5.9) in dose dependant manner.
Fig. 5.8 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 cartilage erosion mainly by inflammatory necrosis (B). Reduction of cellular flux and cartilage 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 40x.

Fig. 5.9 Immunohistochemical analysis of joint sections with NF-κB immunostaining. (a) Control (b) CIA, showed high expression of NFκB (c) CIA treated with 100 mg/kg of BSE and (d) CIA treated with 200 mg/kg of BSE suppressed NF-κB activity. (Magnification 40x).

5.4. Discussion

We have demonstrated the anti-oxidative and anti-arthritis 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, and inhibits activation of the NF-κB in CIA rats.

A link between inflammation and bone homeostasis has been attributed to the effects of cytokines such as TNF-α, IL-1β, IFN-γ, IL-6 and PGE₂ that are abundantly expressed in
patients with RA and in the arthritic joints of rat with collagen-induced arthritis. Blockade of these molecules resulted in a reduction of disease severity and bone resorption (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 (Juarroz et al., 2005). Previous studies on the oleo gum resin of Boswellia species showed its anti-inflammatory effect (Duwiejua et al., 1993, Safayhi et al., 1997, Mothana, 2011). 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 toward 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 ingredients responsible of the plant anti-inflammatory activity (Borrelii 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). 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). Previous work by Moussaieff et al (Moussaieff et al., 2007) reported that incensol acetate a compound isolated from Boswellia resins inhibits NF-kB activation and Cuaz-Perolin et al (Cuaz-Perolin et al., 2008) found that AKBA inhibits activation of NF-kB in vivo mice model. Our results clearly show that Boswellia inhibits activation of the NF-kB after collagen induced arthritis. This action may be one explanation why BSE hamper production of cytokines.

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 (van der Vliet et al., 1997, Knight, 2000, Wills, 1969). Boswellic acids have been reported as inhibitors of human leukocyte 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 (Wills, 1987). 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 (Mothana, 2011, Kokkairipati et al., 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 important 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 arthritic group 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 biochemical alterations were further supported by histopathological observations of the joints. The higher 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.

5.5. Conclusion

We have demonstrated that Boswellia serrata resin is a major anti-inflammatory agent in herbal medicines as well as a common food supplement. Its anti-inflammatory activity has been attributed to boswellic acid and its derivatives. It is practically non-toxic in rats, with the high margin of safety exhibited by the extract in the present study. The findings also suggest that the administration of Boswellia serrata extract to RA rats markedly inhibited clinical sign of joint swelling, significantly decrease the free radical load, modulate inflammatory mediators and inhibits activation of the NF-κB in RA rats. Therefore, Boswellia serrata extract has significant potential as a phytomedicine in the treatment of rheumatoid arthritis. Inhibition of NF-κB activity by plant resins from species of the
Boswellia family might represent an alternative for classical medicine treatments for chronic inflammatory diseases like rheumatoid arthritis.