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Radiographic Progression and Histopathologic Correlation of Adjuvant Arthritis Ameliorated by *Boswellia serrata*

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ABSTRACT

Boswellia serrata is an Indian herb known for its potent anti-inflammatory and anti-arthritic properties in ancient folk medicine. The present study was undertaken to evaluate the anti-arthritic potential of *Boswellia serrata* on radiographic joint damage and histopathology of adjuvant-induced arthritis. Thirty male *Wistar* rats were randomly divided into 5 groups with six rats each. While group 1 served as normal control, arthritis was induced in animals belonging to groups 2 (arthritic control); 3, 4 and 5 (treatment groups) by injecting 0.1 ml of Freund's complete adjuvant, intradermally into the hind foot pad. Treatment protocol was followed from 3rd to 21st day, with *Boswellia serrata* given orally as methanolic extract @ 500 mg/kg b.wt. to group 3, meloxicam given subcutaneously @1 mg/kg b.wt. to group 4 and both the drugs given concurrently to group 5. The onset and progression of arthritis were assessed weekly by radiographic interpretation. The drug effects were evaluated on the histopathology after completion of the experiment. The extent of paw inflammation before treatment and its subsequent amelioration after treatment were noticed in groups 3, 4 and 5 *Boswellia serrata* showed better amelioration compared to meloxicam with a superior curative effect witnessed when both the drugs were administered together. The significant alleviation of joint damage in adjuvant-induced arthritis may be attributed to the pharmacologically active principles present in the extracts of *Boswellia serrata.*

Keywords: *Boswellia serrata*, adjuvant arthritis, radiography, histopathology

Rheumatoid arthritis (RA) is a systemic, autoimmune and chronic inflammatory disorder of the joints, with an unknown aetiology (Bendele, 2001). Its prevalence increases with age, affecting 1% of the population worldwide and 0.75% in India (Kvien, 2004; Rajendra *et al*., 2019). The pathogenesis of RA involves an interaction between environmental and genetic factors (Tobon *et al*., 2010). A wide range of cellular and humoral events together contribute to the pathology of RA (Cho *et al*., 2002; Mishafiey *et al*., 2005) resulting in cartilage destruction, bone erosion, joint space narrowing and periarticular demineralization (Alexander *et al*., 2013). Oxidative stress has been described as an important mechanism underlying the destructive proliferative synovitis. One of the most common causes for local bone erosion is the process of destruction of the juxta-articluar bone in addition to mild cartilage destruction (McInnes and Schett, 2007).

Currently, the therapy for RA includes non-steroidal anti inflammatory drugs (NSAIDs), corticosteroids, disease-modifying anti-rheumatic drugs (DMARDs) and other biological agents most of which are associated with undesirable adverse effects such as gastrointestinal ulcers or perforation, body fat distribution and emergence of opportunistic infections as a result of immune-suppression

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they produce (Venkatesha *et al*., 2011). Due to these adverse effects, many herbal drugs are currently being used as an alternative to conventional medicines (Ahmed *et al*., 2005). Hence, in view of these facts this study was carried out with an objective to evaluate the ameliorative potential of *Boswellia serrata* on radiographic joint damage and its histopathology associated with adjuvant induced rheumatoid arthritis.

MATERIALS AND METHODS

The present study was conducted at the Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Hyderabad. The experimental procedures were duly approved by the Institutional Animal Ethics Committee (IAEC No. I/4/2014 dated 25.01.2014).

Study animals

A total of thirty male *Wistar* rats of same age, weighing 150-200 g procured from Sanzyme Laboratories, Hyderabad were used in the 21day study. All the rats were housed in polypropylene cages and were provided *ad libitum* water and feed throughout the experiment. A 12 hour light/dark cycle was followed and all the rats were acclimatized for a week before commencing the experiment.

Plant extract

A methanolic extract was prepared with dried gum-resin powder of *Boswellia serrata* procured from Derex Laboratories, Hyderabad. 100 g of the dry resin was macerated with 200 ml of methanol for 72 hours. The acquired solvent was filtered and evaporated until a semi-solid, viscous, brown mass was formed. This crude methanolic extract was dried, powdered and stored at 4°C until further dosing.

Experimental design

The rats were randomly divided into five groups, each containing six rats and were maintained for 21 days till completion of the study. Arthritis was induced with Freund's complete adjuvant (FCA) obtained from Veterinary Biological and Research Institute (VBRI), Hyderabad. On day '0' all the animals except those from group 1, were injected intradermally with FCA onto their left hind foot pads. The treatment schedule followed for different groups of rats is as follows: Group 1 - normal control, Group 2 - adjuvant induced arthritic control, Group 3 - treatment with Meloxicam @ 1 mg/kg b.wt. subcutaneously, Group 4 - treatment with *Boswellia serrata* @ 500 mg/kg body weight orally, Group 5 - treatment with Meloxicam + *Boswellia serrata* extract.

Radiographic analysis

On the 21st day i.e., after completion of the experiment, the rats were anaesthetized and subjected to radiographic imaging. Each rat was placed in an anterio-posterior position on a radiography table at a distance of 90 cm from the focal spot. The arthritic hind paws were imaged using a digital X-ray machine (AGFA-CR30-X) with an exposure of 48 kVp for 0.5 mAs. The resulting radiographs were further examined to evaluate the severity of deformities in the affected joints and surrounding soft tissues (Cuzzocrea *et al*., 2001).

Histopathologic evaluation

Ankle joints were collected from euthanized rats after completion of the experiment and fixed in 10% neutral buffered formalin (NBF) for histopathological studies. The preserved ankles were then decalcified in 10% formic acid for 21 days followed by dehydration, processing and paraffin embedding. Wax embedded specimens were cut into 5 µm sections, mounted and stained with haematoxylin and eosin (H & E) as described by Shealy *et al*. (2002), for further evaluation.

RESULTS AND DISCUSSION

Radiography

Besides measuring the disease activity, it is important to assess the radiographic progression of arthritic joints by detecting bone erosions and joint space narrowing (Bridges *et al*., 2010). Radiographic changes are useful in indicating the severity of inflammation in the affected joints. In the present study, the radiographs of arthritic hind paws in group 2 revealed narrowed joint space, bone erosions and severe soft tissue swelling (Fig. 1b). While treatment groups 3 and 4 showed moderate soft tissue swelling and

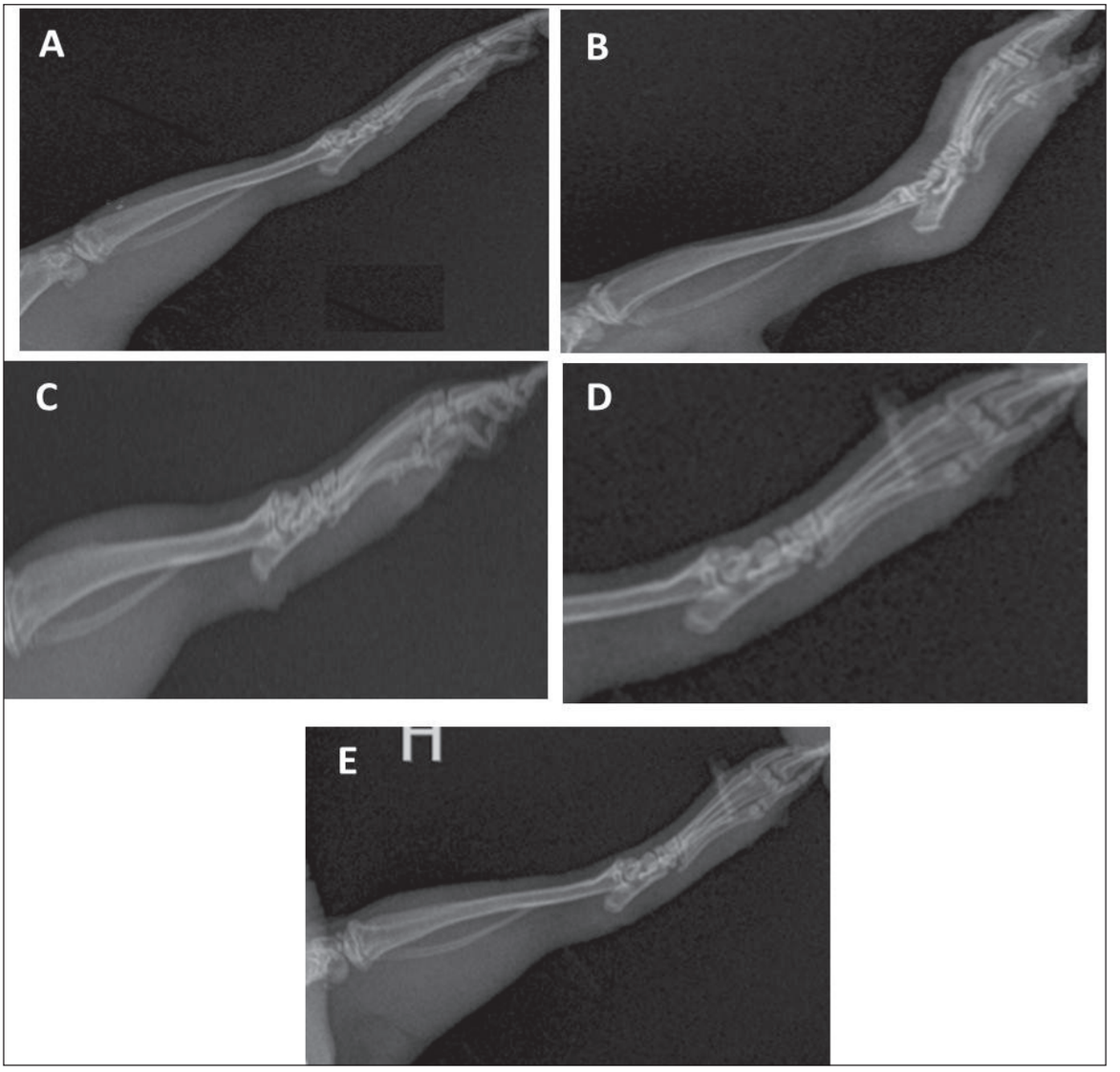

Fig. 1: Radiographic interpretation of ankle joints of different groups of rats. **(A)** Radiograph showing normal bone architecture of hind paw (Group 1); **(B)** Radiograph showing severe soft tissue swelling, narrowed joint space and bone erosion at ankle joint (Group 2); **(C)** Radiograph showing moderate soft tissue swelling and bone erosion around ankle joint (Group 3); **(D)** Radiograph showing mild joint destruction at ankle joint (Group 4); **(E)** Radiograph showing mild joint destruction and reduced soft tissue swelling (Group 5).

joint destruction (Fig. 1c and 1d), mild deviations were noticed in group 5 treated concurrently with both drugs (Fig. 1e). Non-arthritic rats showed normal architecture of the bones and its surrounding tissues (Fig. 1a). Inflammation of the synovial membrane triggers cartilage damage and loss of cartilage components. Some of the notable features of adjuvant arthritis include soft tissue swelling around ankle joints due to edema of peri-articular tissue, narrowed joint space due to articular cytokine-mediated cartilage destruction, bone loss due to increased bone resorption and reduced bone formation, pannus formation in joints and, eventual ankyloses of the joints (Akihisa *et al*., 2002; Makinen *et al*., 2007). Co-treatment targeting the hallmark pannus formation at the cartilage-

bone interfaces may halt, further joint destruction in RA (Lafeber and Van der Laan, 2011).

Histopathology

Joint damage in hind paws was further evaluated microscopically. The histopathologic picture of affected synovium in rheumatoid arthritis varies widely, displaying some of the ascertained features (Baeten *et al*., 2000). In group 2, the ankle joint sections revealed hyperplasia of the synovial membrane, cartilage erosion, neutrophilic and lymphocytic infiltration (Fig. 2b & 2c). Treatment groups 3 and 4 showed moderate to mild edema and synovial membrane thickening with mononuclear infiltration (Fig. 2d and 2e). Group 5 treated concurrently with both the drugs showed mild disruption and hyperplastic changes (Fig. 2f). Non-arthritic rats showed no lesions of pathological significance in the synovium and articular cartilage (Fig. 2a). In RA, the synovium markedly expands with an unusual increase in cellular infiltration (Lylia *et al*., 2019).

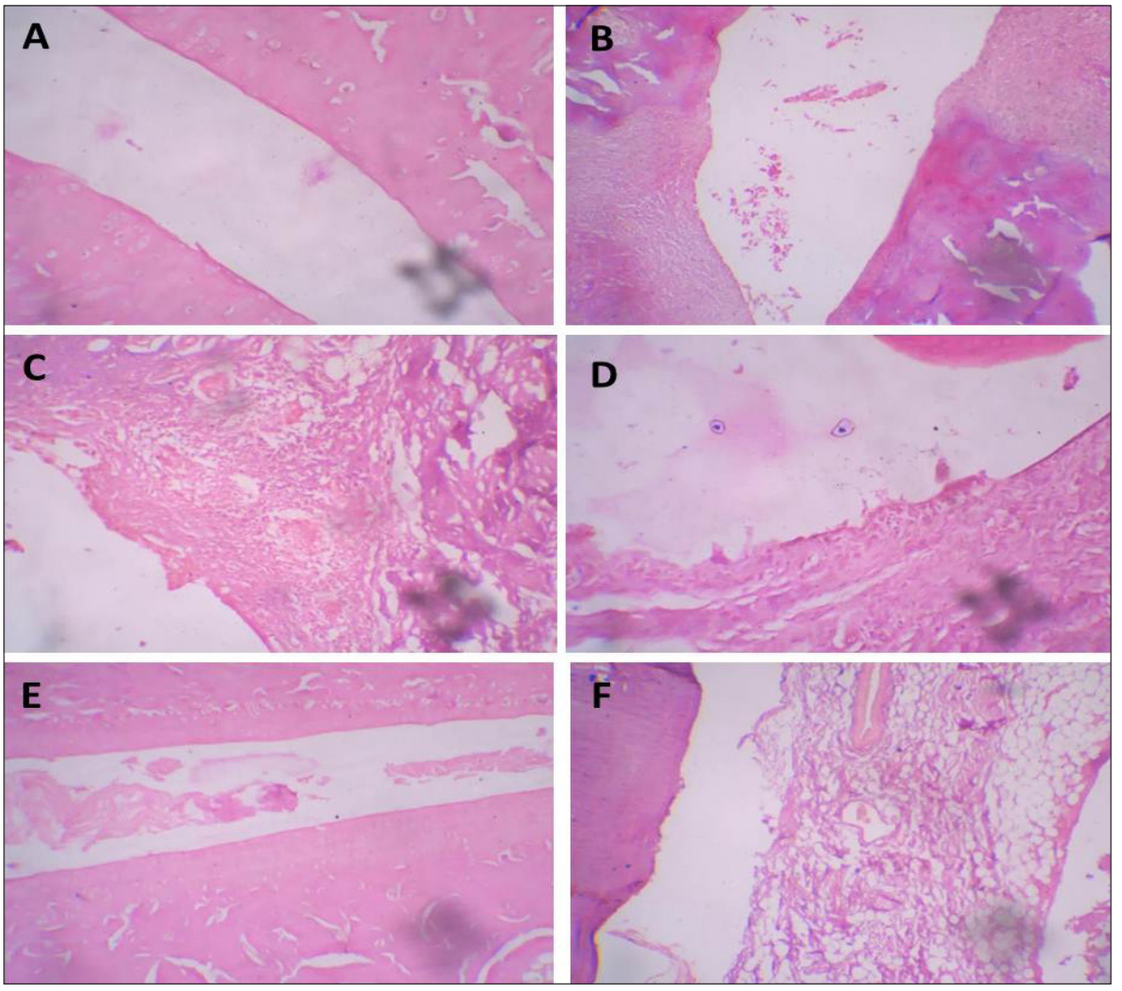

Fig. 2: Histological photomicrographic examination of ankle joints of different groups of rats. **(A)** Ankle joint showing normal histology of synovial membrane and articular cartilage (Group 1); **(B)** Ankle joint showing cartilage destruction and neutrophilic infiltration (Group 2); **(C)** Ankle joint showing synovial hyperplasia, lymphocytic infiltration and cartilage erosion (Group 2); **(D)** Ankle joint showing moderate synovial hyperplasia, moderate edema and mononuclear infiltration (Group 3); **(E)** Ankle joint showing mild lymphocytic infiltration, synovial membrane thickening (Group 4); **(F)** Ankle joint showing restored articular cartilage with mild damage (Group 5).

Some of the prominent histopathological abnormalities of the synovial membrane include neutrophilic infiltration, villous hypertrophy, synovial and periosteal proliferation, erosion and pannus formation composed of macrophages, leukocytes, fibroblast-like synoviocytes (FLS), plasma cells and mast cells in tarsal joint, lymphocyte follicles, plasma cells, iron deposits within macrophages and rarely giant cells (Firestein, 2003). FLS proliferation is the main cause of synovial hyperplasia. It mediates cartilage and bone damage via interaction between inflammatory mediators, adhesion molecules, proteolytic enzymes and pro-osteoclastogenic factors (Bottini and Firestein, 2013). The amelioration of inflammation in treated groups may be accredited to the anti-inflammatory and anti-arthritic potential of *Boswellia serrata* extracts.

CONCLUSION

It is concluded that the architecture of the synovium depends more on the local disease activity than duration of the disease. Adjuvant induced RA causes cartilage destruction, bone damage and alterations in the histology of the affected joints and surrounding tissues. The present study indicates that these changes were significantly alleviated by *Boswellia serrata,* which may be attributed to the pharmacologically active principles such as boswellic acids present in its extract.

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