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Modulation of Oxidative Stress Mediators in Adjuvant Induced Arthritis by Herbal Adaptogen *Boswellia serrata*

R. Divisha[1*], M. Usha Rani[2], B. Kala Kumar[2] and A. Gopala Reddy[3]

[1]JRF, ICAR-NDRI, Southern Regional Station, Adugodi, Bengaluru – 560030, Karnataka, India; vetrd89@gmail.com
[2]Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar, Hyderabad – 500030, Telangana, India
[3]Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Korutla, Kanchikacherla - 505326, Telangana , India

Abstract

A study was carried out to evaluate *Boswellia serrata* on adjuvant induced oxidative stress in rats. Intradermal administration of Freund's adjuvant to induce rheumatoid arthritis consequently produced oxidative damage as indicated by an increase in thiobarbituric acid reducing substances, myeloperoxidase, protein carbonyls and a reduction in superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione in tissue homogenates. Oral administration of methanolic extracts of *Boswellia serrata* at 500 mg/kg body weight re-established the antioxidant profile. Co-treatment with an anti-inflammatory agent, meloxicam showed an enhanced healing effect. Hence, a significant restorative effect was witnessed with the herbal adaptogen *Boswellia serrata*.

Keywords: Adjuvant Arthritis, Antioxidants, *Boswellia serrata*, Oxidative Stress

1. Introduction

The adjuvant activity in experimental rheumatoid arthritis is brought about by a sustained release of antigens from the oily deposit followed by the stimulation of local innate immune response. Subsequently, this builds up an inflammatory reaction at the site of antigen deposition due to entry of inflammatory leukocytes that interact with the antigen[1]. This leads to a full spectrum of cellular and humoral events involving the immune system which collectively contributes to the pathophysiology of rheumatoid arthritis[2,3].

Oxidative stress is described as an important mechanism that underlies the destructive proliferative synovitis[4]. It damages cell membranes, nucleic acids, proteins, lipids and components of the Extracellular Matrix (ECM) such as proteoglycans and collagen[5]. It also influences the functional characteristics of synovial T-lymphocytes[6].

Boswellia serrata (BS), known as Indian Frankincense, is used therapeutically for various inflammatory conditions such as arthritis, colitis, asthma, psoriasis, hyperlipidemia, cancer and so on [7,8]. It principally acts by inhibiting the 5-lipoxygenase (5-LOX) enzyme thereby suppressing the pro-inflammatory leukotriene by-products that promote free radical damage[9].

In view of the above facts, an experimental study was conducted on laboratory rats to assess the effect of *Boswellia serrata* on oxidative stress markers.

2. Materials and Methods

2.1 Animals

A total of thirty male *Wistar* rats of the same age, weighing 150−200 g were procured from Sanzyme Laboratories, Hyderabad. All the rats were housed in polypropyl-

*Author for correspondence

ene cages and were provided *adlibitum* feed and water throughout the experiment. A 12-hour light/dark cycle was followed and the rats were acclimatized for a week before commencing the experiment.

2.2 Plant Extract

A dry resin powder of *Boswellia serrata* was procured from Derex Laboratories, Hyderabad. 100g of the dry resin was macerated with 200ml 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 sun dried, powdered and stored at 4°C until dosing.

2.3 Experimental Design

The rats were randomly divided into five groups containing six rats each and maintained for 21 days. The experiments were conducted with the approval of Institutional Animal Ethics Committee. Freund's Complete Adjuvant (FCA) obtained from M/s Veterinary Biologicals Research Institute, Hyderabad was used for inducing arthritis in the animals. On day '0' all the animals except group 1, were injected intradermally with FCA into their left hind foot pads. The treatment schedule of different groups of rats is as follows: Group 1 - normal control, Group 2 - adjuvant induced arthritic control, Group 3 - treatment with Meloxicam at 1mg/kg bw subcutaneously, Group 4 - treatment with *Boswellia serrata* at 500 mg/kg bw orally, Group 5 - treatment with Meloxicam + *B. serrata* extract.

2.4 Antioxidant Analysis

After completion of the experiment, paw tissue homogenates were prepared for analysing the antioxidant profile. Thiobarbituric Acid Substance (TBARS) was estimated by Balasubramanian *et al.* method[10]. Myeloperoxidase was estimated by the method of Krawisz *et al.*[11]. Protein carbonyls were assessed by Levine *et al.*, method[12]. Superoxide dismutase was determined by Madesh *et al.* method[13]. Catalase was assayed by Asru method[14]. Reduced glutathione and glutathione peroxidase were analyzed by Moron *et al.*[15] and Paglia *et al.*[16] methods, respectively.

2.5 Statistical Analysis

The data were analysed statistically by applying one-way Analysis Of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 17.0. Differences between the means were tested using Duncan's multiple comparison test and the significance level was set at $p<0.05$.

3. Results

At the end of 21 days, the adjuvant induced arthritic control group 2 exhibited significant increase in the levels of TBARS, myeloperoxidase and protein carbonyls compared to the treated groups 3, 4 and 5 (Table 1). A significant reduction in the levels of glutathione peroxidase, reduced glutathione, catalase and superoxide dismutase was also noticed in group 2 compared to other treated groups (Figure 1).

Table 1. Concentration of TBARS, Myeloperoxidase and Protein Carbonyls in paw tissue homogenates of different groups of rats

Groups	TBARS	Myeloperoxidase	Protein Carbonyls
G1: Normal Control	7.44 ± 0.34^{D}	3.07 ± 0.24^{D}	1.51 ± 0.07^{D}
G2: Arthritic control	27.02 ± 2.72^{A}	16.04 ± 0.47^{A}	11.05 ± 0.29^{A}
G3: Meloxicam	17.21 ± 0.39^{B}	8.83 ± 0.2^{B}	5.19 ± 0.21^{B}
G4: *Boswellia serrata* (BS)	13.42 ± 0.5^{C}	7.7 ± 0.33^{C}	4.01 ± 0.37^{C}
G5: Meloxicam + BS	9.56 ± 0.61^{D}	6.64 ± 0.18^{D}	2.02 ± 0.22^{D}

Values are mean ± standard error (n = 6); One way ANOVA
Means with different alphabets as superscripts differ significantly ($p<0.05$)

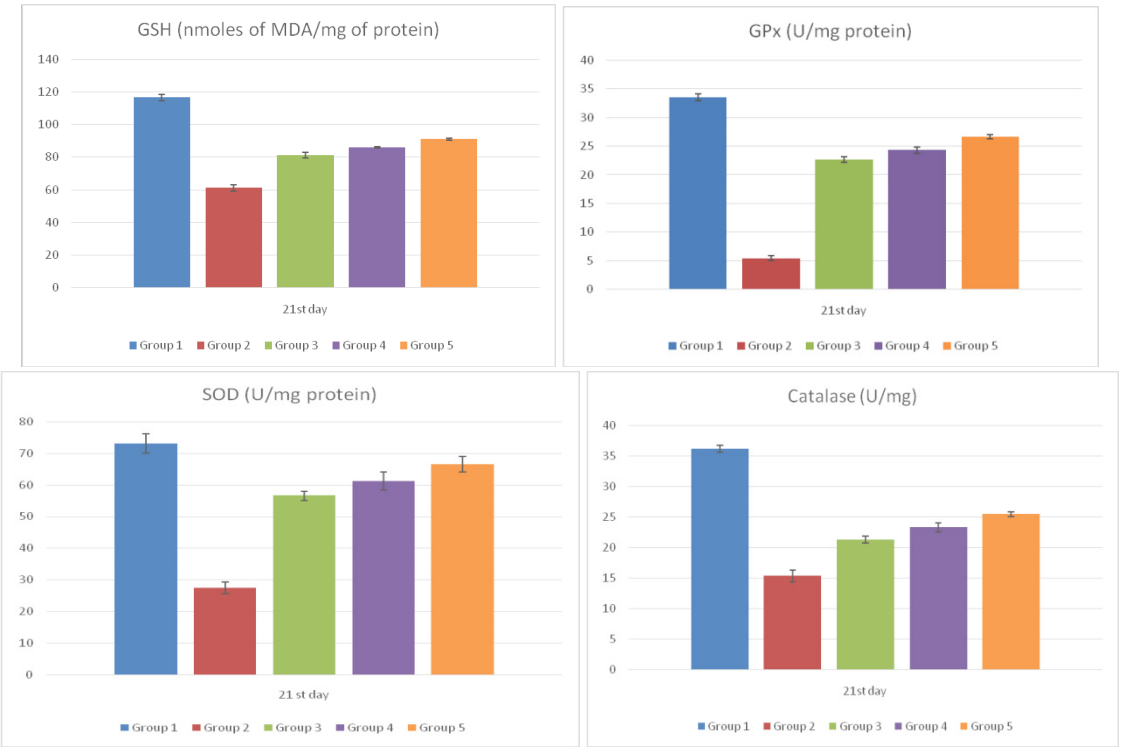


Figure 1. Concentration of GSH, GPx, SOD and Catalase in paw tissue homogenates of different groups of rats.

4. Discussion

Lipid peroxidation is said to be a major contributor for the loss of cell functions under conditions of oxidative stress[17]. It is one of the critical mechanisms of cell injury occurring in rheumatoid arthritis wherein large amounts of free radicals are generated in response to inflammation at the affected joints and surrounding tissues[18].

The superoxide radicals are the initial products of molecular oxygen reduction. Superoxide Dismutase (SOD), which is the first line of defence against free radical damage, catalyzes the dismutation of superoxide radicals into hydrogen peroxide (H_2O_2) and molecular oxygen to protect cells and tissues *in vivo*[19].

Catalase, which decomposes hydrogen peroxide and highly reactive hydroxyl radicals, inhibits other long-chain peroxides too to protect the tissues. A decreased catalase activity in arthritic rats is an indication of H_2O_2 accumulation which in turn inhibits catalase[20].

Glutathione peroxidase (GPx) which is localized in the cytoplasm and mitochondria catalyzes the degradation of various peroxides by oxidizing glutathione. It has more affinity than catalase for H_2O_2. An increase in the GPx activity at joint tissues of arthritic rats indicates an increased H_2O_2 concentration[21,22]. In treatment groups, an adaptive mechanism instigated by the affected cells detoxifies the Reactive Oxygen Species (ROS) thereby reducing the extent of tissue damage[23].

Reduced glutathione plays an important role in protecting cells and tissues by detoxifying free radicals. Its depletion in tissues, promotes oxidative stress and tissue injury[24]. In the present study, the arthritic rats revealed comparatively lower concentrations of GSH, signifying oxidative stress[25].

Thiobarbituric Acid Reducing Substances (TBARS) is an index of lipid peroxidation, which estimates Malondialdehyde (MDA), the major end product of oxidative deterioration of polyunsaturated lipids[26]. In the present study, the treatment groups showing significantly lower MDA levels suggest an improved antioxidant status which is indicative of a modulation in cellular antioxidant defence mechanism[27,28].

Myeloperoxidase (MPO) is an indicator of neutrophilic infiltration in the inflamed paws, which is a major effect of tissue damage[29]. Its concentration in plasma is related to the disease activity[30]. In the present study, all arthritic rats showed a significant rise in MPO activity compared to the treatment groups; this correlates with

neutrophilic infiltration that was evident histopathologically. In treated rats, a reduction of MPO relates to the inhibition of arthritic progression. This could be attributed to the active metabolites present in the plant extracts[31].

Protein carbonyl is the most general indicator and commonly used marker for protein oxidation and its accumulation has been observed in several human diseases including arthritis[32]. An earlier investigation has revealed modulation of altered antioxidant status and amelioration of hepatic oxidative stress and protein carbonylation in hepatic cells with treatment[33].

5. Conclusion

In conclusion, the treatment of experimental arthritis with methanolic extracts of *Boswellia serrata* showed a significant restoration which was apparent by a rise in the antioxidant status. This may be attributed to the various pharmacologically active principles present in the plant. Further, the treatment of arthritic rats with *Boswellia serrata* showed a better ameliorative effect compared to that of the anti-inflammatory drug, Meloxicam. An enhanced curative effect was also evident when both *Boswellia serrata* and meloxicam were administered in conjunction.

6. References

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