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IN-VITRO AND IN-VIVO ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF BOSWELLIA SERRATA

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ABSTRACT
Aim is to explore the *in-vitro* and *in-vivo* pharmacological evaluation of antiinflammatory potential of ethanolic leaf extracts of *Boswellia serrata. In-vitro*HRBC membrane stabilization and *in-vivo*Cotton pelletgranuloma method demonstrates the dose dependent ant-inflammatory activity.

Key Words:ant-inflammatory activity, *Boswellia serrata,* Cotton pelletgranuloma

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INTRODUCTION
Inflammation (Latin, *inflammatio*) is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants[34]Inflammation is a protective immunovascular response that involves immune cells, blood vessels, and molecular mediators. The purpose of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair.The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. Inflammation is a generic response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen[1].Inflammation is tightly regulated by the body. Too little inflammation could lead to progressive tissue destruction by the harmful stimulus (e.g. bacteria) and compromise the survival of the organism. In contrast, chronic inflammation may lead to a host of diseases, such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (e.g., gallbladder carcinoma). Inflammation is therefore normally closely regulated by the body. Inflammation can be classified as either *acute* or *chronic*. *Acute inflammation* is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A series of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as *chronic inflammation*, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process [2-4].

MATERIALS AND METHODS
Preparation of Human Red Blood Cells (HRBC) Suspension

The Fresh whole human blood was collected from healthy human volunteer who had not taken any NSAIDs for 2weeks prior to the experiment and mixed with equal volume of Alseversolution(2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and The blood was centrifuged at 3000 rpm for 10 min and packed cells were washed three times with isosaline (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isotonic saline [5, 6].

Hypotonicity Induced Hemolysis

Various concentrations of extract were prepared (50, 100, 250 & 500 µg/mL) using distilled water.The assay mixture contains 1 mL of phosphate buffer[pH7.4, 0.15M], 2 mL of hypotonic saline[0.36%] and 0.5 mL of HRBC suspension[10% v/v] were addedwith1ml of plant extracts of various concentrations. It was incubated at 37^0C for 30 min and centrifuged at 3,000 rpm for 20 min. and the hemoglobin content of the supernatant solution was estimated on UV spectrophotometer at 540nm. Diclofenac sodium (500µg/mL) was used as reference standard and a control was prepared by omitting the extracts.The percentage of hemolysisand percentage of HRBC membrane stabilization can be calculated as follows[7, 8]:

% Hemolysis = (Optical density of Test sample / Optical density of Control) X 100

The percentage of HRBC membrane stabilisation can be calculated as follows:

% Protection = 100 – [(Optical density of Test sample / Optical density of Control) X 100]

***In-vivo* anti-inflammatory activity by Cotton pellet method**

Wistar rats were divided into five groups of five rats each. Control group rats received mucilage of sodium CMC(0.3% w/v) 1 mL/kg and positive control group of rats received diclofenac-40 mg/kg, orally. The test groups of rats were treated orally with100,200 and 400 mg/kg of the methanolic extracts in the form of suspension. Adsorbent cotton wool was cut into pieces weighing 20±1 mg and made into pellets. The pellets were then sterilized in a hot air oven at 120º for 2 h [99,100]. The animals are anaesthetized with Pentobarbitalsodium (30-50 mg/kg IP).The abdomen was shaved cleanly, swabbed with 70% ethanol and two sterilized cotton pellets were implanted subcutaneously, one on each side of the abdomen of the animal. Test drugs were administered once daily throughout the experimental period of 7 days. On the 8th day after implantation, rats were anaesthetized with thiopental sodium. The pellets were dissected out and theirwet weight is calculated immediately then pellets are dried at 60º for 18 h, weighed after cooling. The mean weight of the cotton pellets of the control group as well as of the test groups was calculated. The transudative weight, granuloma formation and percent granuloma inhibition of the test compound were calculated [9].

RESULTS AND DISCUSSION

The inhibition of hypotonicity induced HRBC membrane lysis

Stabilization of HRBC membrane was taken as a measure of the anti-inflammatory activity. The percentage of membrane stabilization for ethanolic extracts was done at 50, 100, 250& 500µg/mL and Diclofenac sodium 500µg/mL. Ethanolic leaf extracts of *Boswellia serrata* are effective in inhibiting the hypotonic saline induced hemolysis of HRBC at different concentrations (50-500µg/mL). It showed the maximum inhibition 57.783 ± 1.124% at 500µg/mL. With the increasing concentration, the membrane stabilization is increased as shown in Figure-1. Hence anti-inflammatory activity of the extracts was concentration dependent.

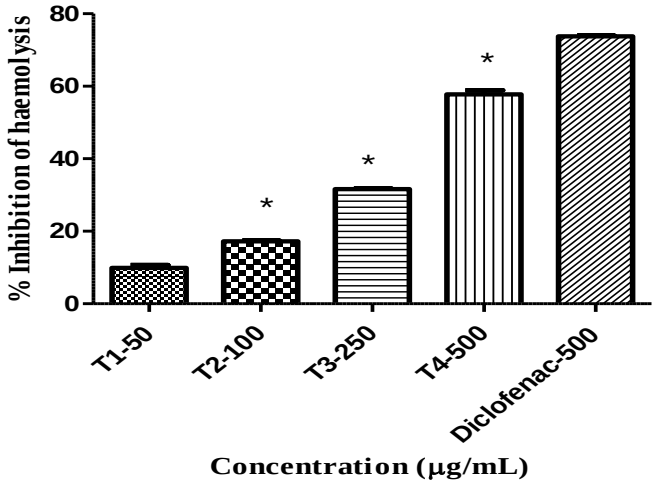

Figure-1 % inhibition of haemolysis of ethanolic leaf extract of *Boswellia serrata*

***In-vivo* study by cotton pellet method**

The granuloma weight of diclofenac and various doses of alcoholic extract after drying were calculated. The percentage inhibition of granuloma weight or antiproliferative effect of diclofenac, alcohol extract 100, 200 and 400 mg/kg were found to be 43.8, 10.9, 14.1 and 29.5%, respectively, when compared with control as shown in Table-1. Hence, the alcohol extract 400 mg/kg has been found to be more significant compared to alcohol extract 100 mg/kg.

These extracts were found to be less significant as compared to diclofenac.As shown in the Table 1, diclofenac showed significant 65.20% inhibition of alkaline phosphatase when compared with disease control. Alcoholic extracts at the dose of 200 and 400 mg/kg showed significant 23.57% and 46.27% inhibition in ALP when compared with standard.

Table-1Effect of *Boswellia serrata*leaf extracts on Transudative weight and alkaline phosphatase by cotton pellet method

Groups	Treatment	Transudative weight (mg) (Wet wt-dry wt)	% inhibition	ALP (IU/L)	% inhibition of ALP
Disease control	vehicle	105±8.32	-	348±24.2	-
Positive control	Diclofenac40 mg/kg	59±7.35	43.8***	121±17.9	65.20***
Test-1	MLEAM-100 mg/kg	93.6±3.97	10.9###	312±13.7	10.33* ###
Test-2	MLEAM-200 mg/kg	90.2±8.35	14.1* ###	266±10.7	23.57***###
Test-3	MLEAM-400 mg/kg	74±4.80	29.5***#	187±6.99	46.27***###

CONCLUSION

Ethanolic leaf extract of *Boswellia serrata* on*In-vitro*HRBC membrane stabilization and *in-vivo*Cotton pelletgranuloma method demonstrates the dose dependent ant-inflammatory activity. The possible mechanism of action underlying the anti-inflammatory activity may be COX-2 enzyme inhibition and inhibition of release of inflammatory mediators by membrane stabilization of storage vesicles.

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