

IN VITRO ANTIINFLAMMATORY ACTIVITY OF *FICUS BENGHALENSIS* BARK

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ABSTRACT

To evaluate the anti-inflammatory property of the different extract of bark of *Ficus benghalensis*, family Moraceae is a very large, fast growing, evergreen tree up to 30 meters, with spreading branches and many aerial roots. Leaves stalked, ovate-cordate, 3-nerved, entire, when young downy on both sides; petiole with a broad smooth greasy gland at the apex, compressed, downy; Fruit in axillary pairs, the size of a cherry, round and downy. According to Ayurveda, it is astringent to bowels; useful in treatment of biliousness, ulcers, erysipelas, vomiting, vaginal complains, fever, inflammations, leprosy. According to Unani system of medicine, its latex is aphrodisiac, tonic, vulnerary, maturant, lessens inflammations; useful in piles etc. The present study aimed at the evaluation of anti-inflammatory property of the aqueous, chloroform and alcoholic extracts of the bark by *in vitro* methods. *In vitro* method was estimated by human red blood cell membrane stabilization (HRBC) method. Results showed significant anti-inflammatory property of the different extracts tested. The methanolic extract at a concentration of 200 mg/ml. showed potent activity on comparing with the standard drug diclofenac sodium.

Keywords: *Ficus benghalensis*, HRBC, Inflammation and diclofenac.

INTRODUCTION

Inflammation is a reaction of living tissues towards injury and it comprises systemic and local responses¹. In spite of our dependence on modern medicine and the tremendous advances in synthetic drugs, a large number of the world populations (80% of people) cannot afford the products of the pharmaceutical industry and have to rely upon the use of traditional medicines, which are mainly derived from plant material. The fact is well recognized by the WHO which has recently compiled an inventory of medicinal plants listing over 20,000 species. There are several important medicinal plants with wide range of pharmacological, biological activities and interesting phyto chemical constituents. The main action of anti-inflammatory agents is the inhibition of Cyclooxygenase enzymes which are responsible for the conversion of Arachidonic acid to prostaglandins. Since human red blood cell (HRBC) membranes are similar to these lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis was taken as a measure in estimating the anti-inflammatory property of various extracts of *Ficus benghalensis*. Thus, Human red blood cell membrane stabilization (HRBC method)^{2,3} has been used as a method in estimating the anti-inflammatory property. The bark of *Ficus benghalensis* contains leucopelargonidin-3-O-a-L-rhamnoside and leucocynidin galactosylcellobioside, glycoside beta glycoside. Anthocyanidin derivatives 3',5,7-trimethyl ether of delphinidin-3-O-a-L-rhamnoside(VII) and 3',5-dimethyl ether of leucocyanidin-3-O-P-D-galactosylcellobioside. In certain parts of India the bark of this plant was traditionally used in the treatment of inflammation. The present study aimed to authenticate that traditional information by *in vitro* anti inflammatory screening.

MATERIALS AND METHODS**Preparation of extracts**

Fresh bark of *Ficus benghalensis* were collected from FRI, Dehradun, India and were authenticated by botanist. The bark were dried in shade and powdered to a coarse form. It was

then successively extracted with methanol, ethanol, water and hydroalcohol using continuous cold maceration process. The extracts were concentrated under reduced pressure and preserved at low temperature.

Chemicals and instruments

All chemicals used in the estimation were of analytical grade. Reference standard diclofenac sodium was obtained as gift sample from Arbro pharmaceutical, New Delhi, India. Shimadzu 1701 UV Visible spectrophotometer was used for the *in vitro* study.

***In vitro* Anti-inflammatory activity**

The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared (100 and 200 µg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. It was incubated at 37°C 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 560 nm. Diclofenac (100 and 200 g/ml) was used as reference standard and a control was prepared by omitting the extracts.^{4,5}

RESULT***In vitro* anti-inflammatory activity**

Ficus benghalensis extracts at different concentrations (100, 200 mg/mL) showed significant stabilization towards HRBC membranes. The percentage protection of methanolic extract at concentration 200 mg/mL was higher than that of other concentrations. However; the percentage protection was found to be lesser then the reference concentration. The results were tabulated in Table 1.

Table 1: % inhibition of different extracts of the *Ficus benghalensis*

Type of extract	Concentration ($\mu\text{g}/\text{ml}$)	Absorbance	% Inhibition of denaturation
Control		0.18 \pm 0.20	---
Water	100	0.0984 \pm 0.121	33.11 \pm 0.21
Water	200	0.0601 \pm 1.05	59.21 \pm 0.78
Hydroalcohol	100	0.0790 \pm 0.29	42.33 \pm 0.11
Hydroalcohol	200	0.0542 \pm 0.35	62.54 \pm 1.11
Methanol	100	0.0714 \pm 1.31	43.23 \pm 0.10
Methanol	200	0.0497 \pm 1.12	67.24 \pm 1.01
Diclofenac	50	0.0544 \pm 1.20	69.82 \pm 1.20
Diclofenac	100	0.0374 \pm 0.059	79.25 \pm 1.31

DISCUSSION

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. Steroidal anti-inflammatory agents will lyse and possibly induce the redistribution of lymphocytes, which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response. Phytochemical evaluation of the various extracts of *Ficus benghalensis* reveals the presence of flavonoids, glycosides, saponins, steroids, tannins and polyphenols. Here anti-inflammatory activity was performed based on the folk lore information using two methods. HRBC method was selected for the *in vitro* evaluation of anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane⁶ and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release. The result indicated that the bark extract of *Ficus benghalensis* at various concentrations has significant anti-inflammatory property. The present result indicates the efficacy of *Ficus benghalensis* as an effective therapeutic agent in the treatment of acute inflammations. The result of present study authenticates the folk lore information on the anti-inflammatory property of the bark extract of *Ficus benghalensis*. Further and detailed studies are in process for the isolation of active constituent responsible for this

property and to identification of the possible mechanism of its anti inflammatory property.⁷

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