Journal of Pharmaceutical Biology

www.jpbjournal.com

e-ISSN - 2249-7560
Print ISSN - 2249-7579

EVALUATION OF *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC WHOLE PLANT EXTRACT OF *BARRINGTONIA ACUTANGULA (L.)*

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ABSTRACT

In the present study, the ethanolic extract of *Barringtonia acutangula* (L.) Gaertn. (Family: Lecythidaceae) were investigated for anti-inflammatory activity by HRBC method. The prevention of hypotonicity induced HRBC membrane lysis was taken a measure of anti-inflammatory activity. These extracts show biphasic effects. Their activities were compared with standard drug diclofenac. *Barringtonia acutangula* a widely growing plant has been reported to possess number of medicinal properties. The various parts of *Barringtonia acutangula* such as root, whole plant and seeds are documented to possess various medicinal properties. The findings of the study indicate that the ethanolic extract of *Barringtonia acutangula* possesses anti-inflammatory activity which is probably related to the inhibition of prostaglandin synthesis. This is a possible rationale for its folkloric use as an anti-inflammatory agent.

Keywords: Barringtonia acutangula, Antiinflammatory activity, Leukocytes, Exudate, HRBC membrane.

INTRODUCTION

There is an increasing demand for the medicinal plants in developing countries like India. Attention needs to be given to assess the medicinal value of such plants to explore the potential drugs out of it. Inflammation is the condition associated with many of the disease states and this review elaborate the medicinal plants, their parts used in the effective management of Inflammation and its associated conditions. Medicinal plants constitute important components of flora and are widely distributed in different region of India [1].

Barringtonia acutangula (L.) Gaertn. (*Family*: Lecythidaceae) an evergreen tree of moderate size is called as Hijja or Hijjala in Sanskrit. The fruit is spoken of as samudra-phala and various part of this plant used as a folklore medicine for curing various ailments like hemiplegia, pain in joints, eye diseases, stomach disorders, anthelmintic, diarrhoea, cough, dyspnoea, leprosy, intermittent fever, and spleenic disorders. An aqueous extract of the bark is found hypoglycemic and is reported to be used in pneumonia, diarrhea, asthma and whole plant juice is given for diarrhea. Fruit is bitter, acrid, anthelmintic, emetic, expectorant and vulnerary. It is prescribed in gingivitis, as an astringent and tonic. Whole plant was reported to possess flavonols, phenolic acids, triterpenoids, tannins and steroidal compounds such as barringtogenic acid, tangulic acid and acutangulic acids. The fruit possessed saponins based on barringtogenol B, C and D. The therapeutic potential of this plant were reported to be antitumor, antibiotic, inhibit growth of Helicobacter pylori and antifungal activities [2-8].

MATERIAL AND METHODS

Plant material

The whole plant of *Barringtonia acutangula* was collected from Tirumala hills, Tirupati, Andhra Pradesh. India. The plant was identified and authenticated by Dr.K.Madhava Chetty, Department of botany, S.V.University, Tirupati. The voucher specimen of the plant was deposited at the college for further reference.

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The whole plant were dried under shade, powdered and stored in an air tight container.

Preparation of extract

The collected whole plant were dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 120g of powdered materials were extracted with ethanol (90%) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in normal saline and used for the experiment. The percentage yield of prepared extract was around 10.5%w/w.

Preparation of Human Red Blood Cells (HRBC) Suspension

Fresh whole human blood was collected and mixed with equal volume of sterilized Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05% citric acid and 0.42 % sodium chloride in water). 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 isosaline [9].

Heat Induced Hemolysis

The principle involved here is stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 0.5 ml HRBC suspension [10 % v/v] with 0.5 ml of plant extracts and standard drug diclofenac sodium of various concentrations (50, 100, 250, 500, 1000, 2000 µg/ml) and control (distilled water instead of hypo saline to produce 100 % hemolysis) were incubated at 37oC for 30 min and centrifuged respectively. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm [10].

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

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

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

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

Statistical analysis

All results were expressed as ± S.E.M. The differences between experimental groups were compared by one way ANOVA (Control vs. treatment, Bonferrioni's method) (Using Jindal Scientific Sigmastat Statistical software, Version1.0) and were considered statistically significant when $P<0.005$.

Table 1. Effect of *Barringtonia acutangula* and Standard on HRBC membrane hemolysis and membrane stabilization

Conc. (µg/ml)	% Hemolysis of *Barringtonia acutangula*	% stabilization of *Barringtonia acutangula*	% Hemolysis of Diclofenac	% Stabilization of Diclofenac
50	35.46	65.22	46.85	56.18
100	22.14	79.41	24.12	77.52
250	14.59	82.64	18.57	88.41
500	11.46	87.12	15.32	84.22
1000	9.23	91.69	7.62	96.44
2000	4.39	95.44	2.51	97.54

RESULTS AND DISCUSSION

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

The inhibition of hypotonicity induced HRBC membrane lysis i.e, stabilization of HRBC membrane was taken as a measure of the anti inflammatory activity. The percentage of membrane stabilization for ethanolic extracts and Diclofenac sodium were done at 50, 100, 250, 500, 1000, 2000 µg/ml. Ethanolic extracts of *Barringtonia acutangula* are effective in inhibiting the heat induced hemolysis of HRBC at different concentrations (50-2000µg/ml) as shown in Table 1. It showed the maximum inhibition 95.44% at 2000µg/ml. With the increasing concentration the membrane hemoslysis is decreased and membrane stabilization/protection is increased. Hence anti inflammatory activity of the extracts was concentration dependent.

CONCLUSION

The results from the HRBC method may favour the possible % Hemolysis and % stabilization of *Barringtonia acutangula* as one of the possible mechanisms of antiinflammatory activity of the ethanolic whole plant extract of *Barringtonia acutangula*. On the basis of this study, it is concluded that the ethanolic extract of *Barringtonia acutangula* possesses anti-inflammatory activity, which is probably related to the inhibition of prostaglandin synthesis.

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