

## **Anti-inflammatory activity of aqueous extract fractions of *Barleria prionitis* L. roots**

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### **ABSTRACT**

*Barleria prionitis* L. (Acanthaceae) roots paste is used traditionally in the treatment of swelling and boils. In the present study roots were extracted successively to obtain various extracts. These extracts were screened for anti-inflammatory activity using caragennan induced rat paw edema at the dose of 200 and 400 mg/kg orally. The aqueous extract was found most active, it was then fractionated into four major fractions (FR) and were screened by the same tests. Result showed that, at a dose of 400mg/kg FR-III & IV showed significant (\*\* $P < 0.01$ ) with 50.64% & 55.76% inhibition of edema respectively at the end of 4 h. as compared with reference drug indomethacin (\*\* $P < 0.01$ ) with 60.25% inhibition of edema. Aqueous extracts fractions showed significant dose dependant anti-inflammatory activity in rat model.

**Keywords:** *Barleria prionitis*, anti-inflammatory, caragennan, rat paw edema.

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### **INTRODUCTION**

Inflammation is a normal protective response shown by living tissue against the injury caused by physical trauma, noxious chemicals or microbiological agents [1-3], which removes pathogens or other stimuli and further help to restore cells to normal state or replace damaged tissue with scar[4] Bacterial infection causes an increased numbers of neutrophils, which leads to the production of oxidative burst at the site of microbial invasion[5]. However inflammation remains unchecked, it leads to the onset of disease such as vasomotor rhinitis and atherosclerosis[6] *Barleria prionitis* (Family: Acanthaceae) is an erect smooth, branched shrubs, 1-2 meters in height, with slender auxiliary spines and yellow coloured flowers [7,8]. Whole plant is found to have anti-inflammatory and antiarrhythmic activity [9]. It is also used in urinary infection, jaundice, hepatic obstruction and dropsy, paste of roots is applied with benefits to boils and glandular swellings[10] Aerial parts of the plants was reported to contains barlenoside, shanzhiside methyl ester, 6-O-trans-P coumaroyl-8-O acetyl shanzhiside methyl ester, barlerin,acetylbalerin, 7-methoxydideroside and lupulinosides[11]. Long term uses of synthetic anti-inflammatory drugs (non steroidal), have shown various problems related to gastrointestinal tract like ulceration, bleeding and perforation [12,13]. It also reduce gastro duodenal prostaglandin, mucosal concentration which leads to loss of protective mechanism against

mucosal injury[14] Anti-inflammatory compounds obtained from natural sources can be used safely to all ages of human being, because of their no side effects. *B. Prionitis* L. plant has traditional claim to have anti-inflammatory activity [15], therefore effort has been made to experimentally determine anti-inflammatory activity of *B. prionitis* L. extract obtained successively with different solvents.

## MATERIALS AND METHODS

### Animals

Wistar albino rats of either sex weighing between 150-200g were used for Anti-inflammatory studies. Animals were grouped in clean polyacrylic cages and maintained at standard laboratory condition (temp  $25\pm 2^{\circ}\text{C}$ ) and relative humidity ( $50\pm 5\%$ ) with dark and light cycles (12/12 hrs). animals were allowed to free access to standard dry pellets diets and water ad libitum for two days. The institutional animal ethics committee has approved the experimental protocols and was performed in accordance with the guidelines for the care and use of laboratory animals as adopted and promulgated by institutional animal ethical committee. (CPCSEA, India Reg. No.1211/ac/08/CPCSEA)

### Chemicals and drugs

Carragennan (Sigma Aldrich US), indomethacin (Loba chem. Mumbai, India), Petroleum ether AR, Chloroform AR, Ethyl acetate AR, Ethanol AR, Methanol AR (SD Fine ,India), Saline water (Claris life sci., India)

### Plant material:

Roots of *Barleria prionitis* L. were collected at Ahmednagar district of Maharashtra [India] and authenticated by Mr. D.L. Shirodkar at botanical survey of India Pune. A voucher specimen (CHADBAP1) was deposited in the herbarium of BSI Pune for further reference.

### Extraction and fractionation:

Dried, coarsely a powder root was extracted by successive solvent extraction in soxhlet extractor with petroleum ether, chloroform, ethyl acetate, and ethanol and lastly marc was reflux with water. All the extracts were vacuum dried, which were labeled as PETE (1.82%), CHME (2.34%), ETAE (2.83%), EOHE (5.27%) and AQSE (3.58%) respectively.

### Column chromatography

On preliminary pharmacological evaluation of anti-inflammatory activity, AQSE was found most active extract and it was then subject to reverse phase chromatography. AQSE (20g) was dissolved in small volume of water and applied to polyamide column (3x60 cm) which was eluted by methanol (FR-I, 19.34% w/w), Methanol: Water (9:1) yielding (FR-II, 18.72% w/w), Methanol: Water (1:1) yielding (FR-III, 28.41% w/w) and lastly with water yielding (FR-IV, 30.84% w/w).

### Phytochemical evaluation

Various roots extracts of *B. prionitis* L. were screened by qualitative phytochemical test[16] and it was found to contain steroid, alkaloids, glycoside, tannins, flavonoids and carbohydrates.

### Acute toxicity

Acute toxicity for different fractions of aqueous extracts was carried out using acute toxic class method as described in OECD [organization of economic co-operation and development]. All the

fractions were found to be safe up to dose of 2000mg/kg body weight, hence 200 and 400mg/kg moderate dose was used for evaluation.

### Evaluation of anti-inflammatory activity

Anti-inflammatory activity [17] was studied for different fraction (FR) of aqueous extract of *B. prionitis* by carrageenan induced paw edema in rat model. Extracts were suspended into 1% carboxymethyl cellulose in saline water and administered orally. The wistar albino rats were divided into ten groups (n=6). Group-I serve as control and orally administered vehicle only. Group II-V administered orally (200mg/kg) of FR I-IV respectively and Group VI-IX administered orally (400mg/kg) of FR I-IV respectively. Group X administered orally with Indomethacin (10mg/kg) as reference drug. One hour after the respective treatment carrageenan (0.1ml of 1% in normal saline) was injected into sub planter side of the right hind paw of rats. The paw volume was measured at 1, 2, 3 and 4 hours using plethysmometer and anti-inflammatory effects of different fractions were calculated by using the following equation [18]

$$\% \text{ inhibition of edema} = (V_c - V_t / V_c) \times 100$$

Where  $V_t$  is paw volume in test group of rats and  $V_c$  is paw volume in control group of rats.

### Statistical analysis

All the experimental data was expressed as mean  $\pm$  SEM, significance of difference among the various groups and control group were carried out using one way ANOVA followed by Dunnett's t test using graphpad Instat software. Where \* $P < 0.05$  was considered as significant, while \*\* $P < 0.01$  was considered as more significant of test group compared with control group.

**Table 1: Anti-inflammatory activity of AQSE fractions of *B. prionitis* L. by carrageen induced rat paw edema**

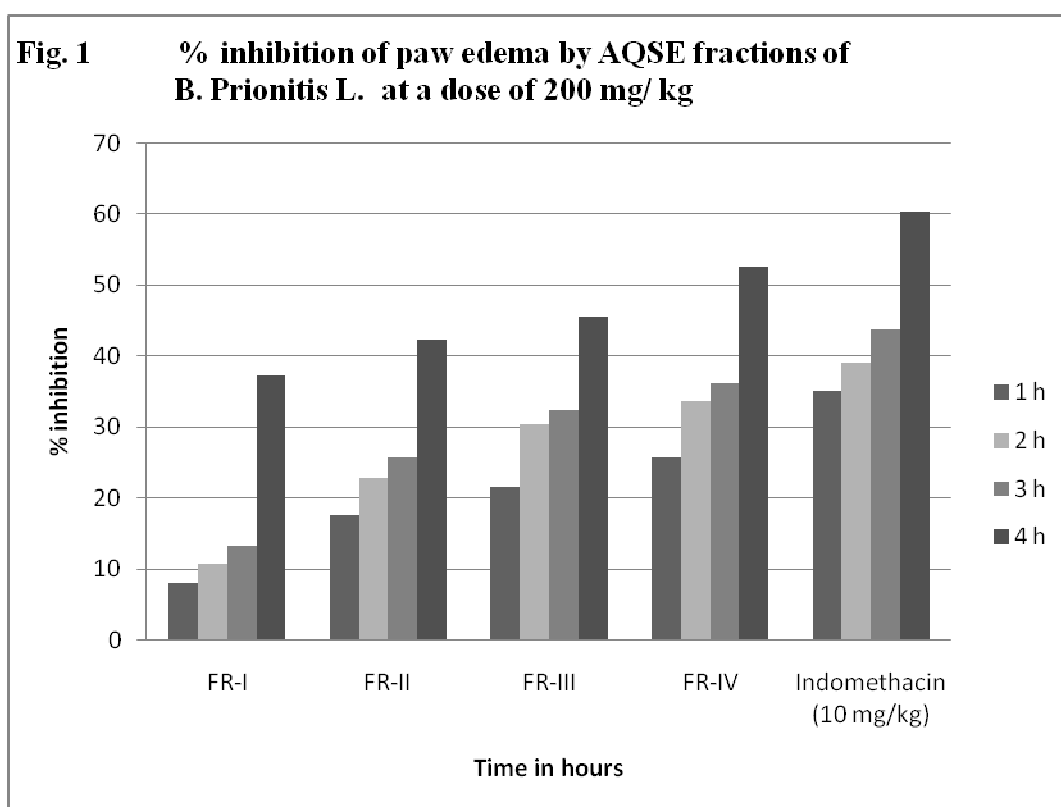
Group		Increase in rat paw volume in ml $\pm$ SEM (% inhibition)			
		1 hr	2 hr	3 hr	4 hr
Control	Dose mg/kg	0.74 $\pm$ 0.01	0.92 $\pm$ 0.01	1.05 $\pm$ 0.02	1.56 $\pm$ 0.01
FR-I	200	0.68 $\pm$ 0.11 (8.10%)	0.82 $\pm$ 0.15 (10.86%)	0.91 $\pm$ 0.11* (13.33%)	0.98 $\pm$ 0.03* (37.18%)
	400	0.63 $\pm$ 0.03 (14.86%)	0.72 $\pm$ 0.02 (21.73%)	0.73 $\pm$ 0.08* (30.47%)	0.83 $\pm$ 0.06* (46.79%)
FR-II	200	0.61 $\pm$ 0.06 (17.56%)	0.71 $\pm$ 0.05 (22.82%)	0.78 $\pm$ 0.07* (25.71%)	0.90 $\pm$ 0.06* (42.30%)
	400	0.62 $\pm$ 0.01 (16.21%)	0.68 $\pm$ 0.02 (26.08%)	0.69 $\pm$ 0.02* (34.28%)	0.80 $\pm$ 0.01* (48.71%)
FR-III	200	0.58 $\pm$ 0.01 (21.62%)	0.64 $\pm$ 0.02* (30.43%)	0.71 $\pm$ 0.02* (32.38%)	0.85 $\pm$ 0.03** (45.51%)
	400	0.53 $\pm$ 0.03 (28.37%)	0.62 $\pm$ 0.04* (32.60%)	0.69 $\pm$ 0.03** (34.38%)	0.77 $\pm$ 0.01** (50.64%)
FR-IV	200	0.55 $\pm$ 0.03 (25.67%)	0.61 $\pm$ 0.02* (33.69%)	0.67 $\pm$ 0.02** (36.19%)	0.74 $\pm$ 0.05** (52.56%)
	400	0.52 $\pm$ 0.03 (29.72%)	0.58 $\pm$ 0.02** (36.95%)	0.64 $\pm$ 0.02** (39.04%)	0.69 $\pm$ 0.08** (55.76%)
Indomethacin	10	0.48 $\pm$ 0.02 (35.13%)	0.56 $\pm$ 0.01** (39.13%)	0.59 $\pm$ 0.07** (43.80%)	0.62 $\pm$ 0.05** (60.25%)

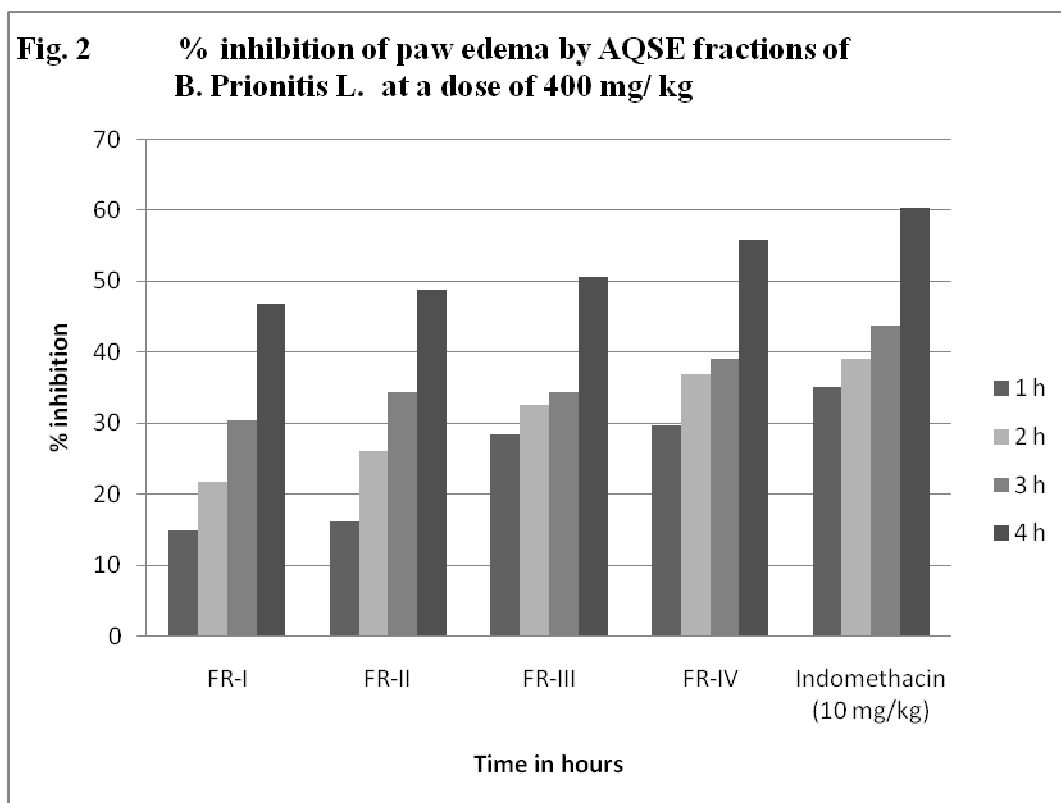
Values are expressed as mean  $\pm$  SEM (n=6) animals in each group

\* $P < 0.05$  & \*\* $P < 0.01$  significant compared to control group by one way ANOVA followed by Dunnett's multiple comparison test.

## RESULTS AND DISCUSSION

Preliminary phytochemical screening of root extracts of *B. prionitis* revealed the presence of flavonoids, steroids, glycosides, alkaloids and carbohydrates. All the extracts were preliminary screened for in-vivo anti-inflammatory activity and AQSE was found to be most active among all extracts. So it was further fractionated by using reverse phase column chromatography. These fractions were carried out for acute toxicity assay, no death was observed during 72 h period at the dose tested also does not shown any symptoms of convulsion, diarrhea or increased diuresis, thus the moderate dose of 200 and 400mg/kg was used in the study. In the caragennan induced rat paw edema test (table-I, fig-I &II) for acute inflammation, FR-III & IV (200 mg/kg) showed significant (\*\*P<0.01), with 45.51% & 52.56% inhibition of edema respectively at the end of 4 h. At a dose of 400mg/kg FR-III & IV showed significant (\*\*P<0.01) with 50.64% & 55.76% inhibition of edema respectively at the end of 4 h as compared with reference drug indomethacin (\*\*P<0.01) with 60.25% inhibition of edema. The caragennan induced rat paw edema is a biphasic process [19,20]. Early phase (1-2 h) of caragennan model is mainly mediated by histamine and serotonin in the mast cells. The later phase is mediated by prostaglandin, the products of cyclooxygenase and lipoxygenase enzymes. Formations of arachidonic acid via cyclooxygenase and lipoxygenase pathway represent two important classes of inflammatory mediator. The product of cyclooxygenase pathway mainly prostaglandin E2 is known to cause cardinal sign of inflammation and the product of lipoxygenase pathway mainly leukotrine B4 is mediator of leukocyte activation in the inflammation. From the result FR-III & IV 400mg/kg showed more percentage of inhibition against caragennan induced paw edema which is comparable with reference standard Indomethacine which is cyclooxygenase inhibitor but anti-inflammatory activity against caragennan induced paw edema also shown by lipoxygenase inhibitor, hence inhibition of caragennan induced paw edema by crude extract may be due to inhibitory activity of lipoxygenase enzymes.





### CONCLUSION

From the present study, it is concluded that AQSE fractions (FR-IV) of *B. prionitis* showed maximum percentage inhibition of rat paw edema (52.56% & 55.76%) at a dose of 200 & 400 mg/kg respectively. Anti-inflammatory activity was found to be dose dependant for all four fractions. These results support the tradition use of this plant as anti-inflammatory activity. Potent inhibition of carragennan induced rat paw edema, showed inhibition of prostaglandins synthesis is major mechanism by which the plant extract may showed anti-inflammatory activity. Further studies are in progress to isolate responsible active constituents for anti-inflammatory activity.

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