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Antiinflammatory Activity of Aqueous Extract of *Barleria cristata* Leaves

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ABSTRACT

Barleria cristata linn (family acanthaceae) has been used traditionally for the treatment of variety of diseases including anemia, toothache, and inflammatory disorders. Due to lack of sufficient scientific evidence indicating the utility of this plant in the treatment of inflammation, the present study was aimed at investigating the antiinflammatory activity of the plant in different experimental screening methods. Antiinflammatory activity of aqueous extract of *Barleria cristata* leaves (BCW) at doses of 125, 250, and 500 mg/kg was evaluated in acute inflammatory models against carrageenan induced paw edema in rats, prostaglandins inhibitory activity, and acetic acid induced capillary permeability in mice. Results were analyzed by one-way ANOVA followed by Dunnett's test $P < 0.05$ and considered significant compared to control. BCW significantly inhibited edema induced by carrageenan, inhibited significantly prostaglandin activity, and vascular permeability in mice dose dependently. Indomethacin (10 mg/kg) was used as a positive control. It is concluded that, aqueous extract of *Barleria cristata* Linn leaves exhibited significant antiinflammatory activity.

Key words: Acute inflammation, *Barleria cristata*, prostaglandins, vascular permeability

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INTRODUCTION

Inflammation is the complex biological response of vascular tissues to harmful stimuli including pathogens, irritants or damaged cells.[1] It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation, however, if runs unchecked, leds to onset of diseases such as vasomotor rhinnorrhoea, rheumatoid arthritis, and atherosclerosis.[2] It is believed that current drugs available such as opoids and nonsteroidal antiinflammatory drugs are not useful in all cases of inflammatory disorders, because of their side effects and potency.[3] As a result, a search for other alternatives is necessary. Through medicinal plants, having a wide variety of chemicals, novel antiinflammatory agents could be discovered. Research on the biological activities of plants during the past two centuries has yielded numerous compounds for the development of modern drugs.[4]

Barleria Cristata Linn (family acanthaceae) is a shrub found widely in subtropical Himalaya, Sikkim, Khasi Hills, central, and southern India at a height of 1,350 m. The chemical constituents of the plant have been identified as flavonoids type phenolic compounds, especially apigenin, quercetin, quercetin-3-O-β-D-glucoside, naringenin, luteolin, and apigenin glucuronide.[5] Barleria cristata have been used traditionally for the treatment of variety of diseases including anemia, toothache, cough and as a hypoglycemic agent.[5] Leaves were used to reduce swellings

in inflammation.[5] However, there is no systematic scientific report published showing its antiinflammatory activity. Therefore, objective of present study was to evaluate antiinflammatory activity of barleria cristata by different pharmacological screening methods.

MATERIALS AND METHODS

Plant material

Fresh leaves of barleria cristata linn (5 kg) were collected from Mumbai region (India) in August. The plant material was taxonomically identified by Dr. Ganesh Iyer, Botany Professor, Ramnarain Ruia College, Mumbai, India. A voucher specimen, No. 9-1/08, has been preserved in our laboratory for future reference.

Preparation of plant extract

The leaves were dried under shade and then powdered with a mechanical grinder and stored in an airtight container. The dried powder material of the leaves (2 kg) was defatted with petroleum ether (60–80^{0}C) and subsequently extracted with distilled water by hot maceration method. The solvent was completely removed by drying and aqueous extract of barleria cristata leaves (BCW) was obtained (yield 13.2%). Solution of BCW was prepared freshly in distilled water and used for studies.

Phytochemical screening

The BCW extract was screened for the presence of various phytochemical constituents i.e., steroids, alkaloids, tannins, flavonoids, glycosides, etc., by employing standard screening tests.[6]

Chemicals and drugs

Carrageenan was obtained from Sigma Aldrich (US), indomethacin (Recon, Bangalore, India), castor oil (Jayant Agro Organics Limited, Mumbai, India), and all other chemicals used were of analytical grade.

Animals

Wistar albino rats of either sex weighing between 180–200 g and Swiss albino mice of either sex weighing 18–22 g were used for animal studies. The animals were grouped in clean polyacrylic cages and maintained under standard laboratory conditions (temperature 25 ± 2°C) and relative humidity (50 ± 5%) with dark and light cycle (12/12 h). They were allowed free access to standard dry pellet diet and water ad libitum. The rats and mice were acclimatized to the laboratory condition for ten days before commencement of experiment. The Institutional Animal Ethics Committee had approved the experimental protocols and care of animals was taken according to CPCSEA guidelines.

Acute toxicity test

The animals were divided into six groups containing six animals each. BCW was dissolved in distilled water and administered orally as a single dose to mice at different dose levels viz. 500, 750, 1,000, 1,250, 1,500, and 2,000 mg/kg of body weight (bw). Mice were observed periodically for symptoms of any toxicity and death within 24 hours and then daily for next 14 days.[7]

Evaluation of antiinflammatory activity

Carrageenan-induced rat paw edema

This test was followed by the method described by Winter *et al*.[8] Rats were divided into five different groups (n = 6). Group I served as control and received vehicle orally. Group II, III, and IV were received BCW extract (125, 250, and 500 mg/kg, respectively) orally. Group V received indomethacin (10 mg/kg) orally. One hour after the respective treatment, 100 µl of 1% freshly prepared carrageenan in normal saline was injected in sub-plantar region of right hind paw of rats. The paw volume was measured at 0 h i.e., immediately after carrageenan injection and then at 1, 2, 3, and 4 h using plethysmometer. The antiinflammatory effect of BCW was calculated by the following equation.[9]

Antiinflammatory activity (%) inhibition = (1 – D/C) × 100.

Where, D represents the percentage difference in increased paw volume after the administration of test drugs to the rats and C represents the percentage difference of increased paw volume in the control groups.

Prostaglandins inhibitory activity in mice

Prostaglandins inhibitory activity of the BCW extract was evaluated by using castor oil-induced diarrhea model in mice according to method described by Awouters et al[10] with some modifications. Five groups of six mice each were used for the study. The mice were starved for 10 h prior to the experiment. Group I treated as vehicle control, groups II, III, and IV were treated orally with 125, 250, and 500 mg BCW extract/kg, respectively, while group V received indomethacin (10 mg/kg) orally. One hour after the treatment, mice in all the groups were given 0.1 ml

castor oil/10 g orally. The mice in each group were then placed singly in cages having adsorbent paper beneath and examined for the presence and frequency of wet stool every hour for four hours. Absence or delay in production of watery stool was regarded as protective or positive. Percentage inhibition of prostaglandins was calculated by comparing with control group.

Acetic acid-induced vascular permeability in mice

This experiment was done according to the method described by Whittle[11] with minor modifications. Five groups of six mice each were used. Group I treated as vehicle control, groups II, III and IV were treated with 125, 250 and 500 mg BCW extract/kg orally respectively, while group V received indomethacin (10 mg/kg) orally. One hour after the respective treatments, 0.2% Evan's blue in normal saline was injected intravenously through tail vein at a dose of 0.1 ml/10 g. Thirty minutes later, each mouse was injected intraperitoneally with 0.2 ml of 0.6% acetic acid in normal saline. After 1 h, the mice were sacrificed and the abdominal wall was cut to expose the entrails. The abdominal cavity was washed using 5 ml of normal saline to collect pigments in a test tube. After centrifuging the contents of the tube to eliminate contaminants, the solution was subjected to colorimetric analysis using a spectrophotometer at a wavelength of 590 nm. The vascular permeability effects were expressed as the absorbance (A), which represented the total amount of dye leaked into the intraperitoneal cavity. Percentage inhibition of vascular permeability was calculated by comparing with control group.

Statistical analysis

The experimental data was expressed as mean ± SEM, the significance of difference among the various treated groups and control group were analyzed by means of one-way ANNOVA followed by Dunnett's test using Graphad Instat Software (San Diego, CA, USA). The level of significance was set at $P < 0.05$.

RESULTS

In this study antiinflammatory activity of aqueous extract of Barleria Cristata leaves was evaluated by different *in vivo* and *in vitro* screening methods.

Preliminary phytochemical screening of the aqueous extract of Barleria Cristata leaves revealed the presence of flavonoids, alkaloids and glycosides. Further separation of the specific phytochemicals is in progress. In the acute toxicity assay no deaths were observed during the 72 h period at the doses tested. At these doses, the animals showed no stereotypical symptoms associated with toxicity, such as convulsion, ataxy, diarrhea or increased dieresis; thus, the median lethal dose (LD_{50}) was determined to be higher than the dose tested i.e., 2.0 g/ kg.

Evaluation of antiinflammatory activity

Carrageenan-induced rat paw edema

The antiinflammatory activity of BCW at the doses of 125, 250 and 500 mg/kg against paw edema induced by carrageenan is shown in Figure 1. BCW extract at the doses of 125 and 250 mg/kg moderately inhibited paw edema (18.42 and 23.02% respectively) where as at the dose of 500 mg/kg and indomethacin at dose of 10 mg/ kg significantly ($P < 0.05$) inhibited paw edema (55.08 and 62.5% respectively) at the end of 4 h after carrageenan injection.

Prostaglandins inhibitory activity in mice

The BCW extract at higher dose level (500 mg/kg) and indomethacin (10 mg/kg) significantly ($P < 0.05$) (83 % and 100%, respectively) protected the mice against diarrhea at the end of third and fourth hour after castor oil administration [Table 1].

Acetic acid-induced vascular permeability in mice

Results of the study showed that BCW and indomethacin significantly ($P < 0.05$) inhibited acetic acid induced vascular permeability in mice. BCW at the doses of 125, 250 and 500 mg/kg showed 15.61%, 25.94% and 52.74% respectively inhibition of vascular permeability, where as indomethacin (10 mg/kg) showed 68.45% inhibitory activity when results were compared with vehicle control.

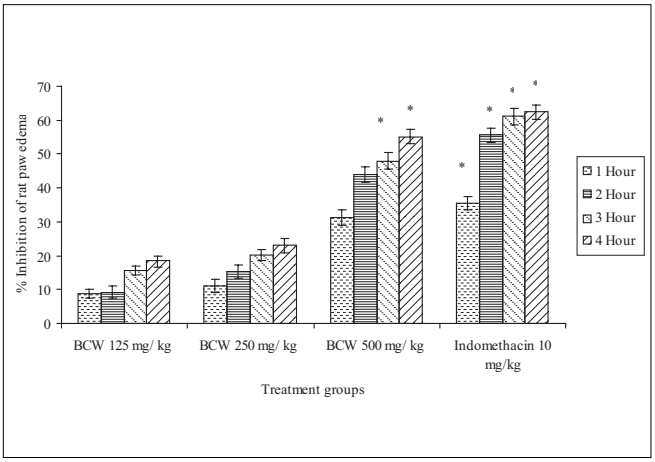

Figure 1: Effect of aqueous extract of Barleria Cristata leaves on carrageenan-induced rat paw edema. Each value represents the mean ± SEM, n = 6. *$P < 0.05$ compared with control, Dunnett's multiple comparison tests after analysis of variance

Table 1: Effect aqueous extract of *Barleria cristata* leaves on castor oil-induced diarrhea in mice

Treatment (s)	Dose (mg/kg)	No. of mice protected from diarrhea (% Inhibition)			
		1h	2h	3h	4h
Control	Vehicle	0	0	0	0
Aqueous extract	125	0 (0%)	0 (0%)	1 (17%)	1 (17%)
of Barleria	250	1 (17%)	2 (33%)	3 (50%)*	3 (50%)*
Cristata leaves	500	3 (50%)*	4 (67%)*	5 (83%)*	5 (83%)*
Indomethacin	10	6 (100%)*	6 (100%)*	6 (100%)*	6 (100%)*

DISCUSSION

In the present study, antiinflammatory activity of aqueous extract of barleria cristata leaves was evaluated. Antiinflammatory activity was tested by different in vivo screening models, representing different phases of inflammation.

Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation study and is believed to be biphasic. The early phase (1–2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The later phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages.[12] Results of the study indicate that the BCW 500 mg/kg acts significantly in later phases probably by inhibiting prostaglandin synthesis involving arachidonic acid metabolites.[13]

The gut wall contains prostaglandins E and F with prostaglandin synthetase activity mainly in the mucosa. Prostaglandins cause intestinal cramps and diarrhea which is due to effect on intestinal smooth muscle and secretion. Ricinoleic acid, the active principle present in the castor oil caused changes in mucosal cell layer permeability, electrolyte transport and intestinal peristalsis, leading to hyper-secretory response and diarrhea. It causes irritation and inflammation of the intestinal mucosa, leading to prostaglandin release, which results in an increase in the net secretion of water and electrolytes into the small intestine.[14] Inhibitors of prostaglandin biosynthesis were observed to delay castor oil-induced diarrhea. BCW showed significant reduction in the castor oil induced diarrhea in dose dependent manner indicating that the extract has the ability to inhibit the synthesis of prostaglandins. The preliminary phytochemical screening shows the presence of flavonoids in the extract. Flavonoids are known to modify the production of cyclooxygenase (COX-1 and 2) and lipooxygenase (LOX) involved in the prostaglandin synthesis.[15,16] Thus, the studies shown that the inhibition of prostaglandins synthesis is a major mechanism by which the BCW exerts antiinflammatory and antidiarrheal properties.

The inflammatory response is a physiological characteristic of vascularized tissues.[17] Increased vascular permeability occurs as results of contraction and separation of endothelial cells at their boundaries to expose the basement membrane, which is freely permeable to plasma proteins and fluid,[18] that leads to exudation of fluid rich in plasma proteins including immunoglobulins (antibodies), coagulation factors[19] and cells[20] into the injured tissues. Exudation, which is a consequence of increased vascular permeability, is considered a major feature of acute inflammation.[21] Chemical-induced vascular permeability (acetic acid) causes an immediate sustained reaction that is prolonged over 24 h[22] and its inhibition suggests that the BCW extract may effectively suppress the exudative phase of acute inflammation in concentration dependent manner showing maximum inhibition (52.74%) at 500 mg/kg.

The antiinflammatory activity of BCW extract found may be due to the presence of therapeutically active flavonoids i.e., apigenin, quercetin, naringenin and luteolin.[5] Flavonoids are known to prevent the synthesis of prostaglandins and have therapeutic applications on inflammation.[23,24]

CONCLUSION

The data obtained from the present study indicated that several factors may contribute to the antiinflammatory action of BCW. Firstly, potent inhibition of rat paw edema and castor oil induced diarrhea in mice shows inhibition of prostaglandins synthesis is major mechanism by which the plant extract exerts antiinflammatory activity. Secondly, BCW reduced the increased vascular permeability in mice, indicating the suppressive effect of BCW on the vascular response in the process of acute inflammation.

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