ANTI-INFLAMMATORY ACTIVITY OF FRACTIONS OF THE *BARLERIA CRISTATA* LEAVES EXTRACT

Gambhire Manoj N[1], Wankhede Shaijesh S[1],
Juvekar Archana R[1]

[1]University Institute of Chemical Technology, Department of Pharmaceutical Science and Technology, Matunga, Mumbai. (India). Pin code 400 019

Summary

Barleria Cristata Linn (family Acanthaceae*)* has been used traditionally in the treatment of anemia, toothache and inflammatory disorders. Aim of the present study was to investigate the anti inflammatory activity of fractions of the methanol extract of *Barleria Cristata* leaves in acute and chronic models of inflammation. Anti-inflammatory activity of pet ether, chloroform and methanol fractions of *Barleria Cristata* extract were studied by carrageenan induced rat paw edema and cotton pellet induced granuloma method at the dose levels of 50, 100 and 200 mg/kg. Indomethacin (10 mg/kg) was used as a positive control. Results of the study showed that chloroform fraction has moderate anti-inflammatory activity where as methanol fraction showed significant and dose dependent anti-inflammatory activity in both the models studied. Methanol fraction at dose of 200 mg/kg and indomethacin (10 mg/kg) significantly (P<0.05) inhibited (65.21% and 69.07 respectively) rat paw edema at the end of 4 h after carrageenan injection. In the cotton pellet induced granuloma method all the three fractions and indomethacin showed significant (P<0.05) activity when compared with control group. Methanol fraction (200 mg/kg) showed maximum inhibition of 62.37 % (wet cotton) and 53.84 % (dry cotton) where as indomethacin (10 mg/kg) showed 68.04 % (wet cotton) and 59.61 % (dry cotton) inhibition of cotton pellet induced granuloma in rats. Results were analyzed by One-way ANOVA followed by Dunnett's multiple comparison test P<0.05 and considered significant as compared to control. It is concluded that methanol fraction of *Barleria Cristata Linn* leaves exhibited significant anti-inflammatory activity.

Key Words: Acute inflammation, *Barleria Cristata,* carrageenan, edema

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 like vasomotor rhinnorrhoea, rheumatoid arthritis and atherosclerosis [2]. It is believed that current drugs available such as opoids and non-steroidal anti-inflammatory 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. Medicinal plants having a wide variety of chemicals from which novel anti-inflammatory 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].

Drug discovery involves many steps which must be carefully carried out. One of the most popular and important procedure for drug discovery is bioguided fractionation of extracts. Bioguided isolation of pharmacologically active plant components stills a valuable strategy for the finding of new lead compounds.

This procedure involves the fractionation of active extracts and fractions till getting pure active ingredients [5]. *Barleria Cristata Linn* (family Acanthaceae*)* is a shrub found widely in subtropical Himalaya, Sikkim, Khasi Hills, central and southern India at height of 1,350 m. The interest in undertaking the study on *Barleria Cristata* is justifiable based on the local uses of the plant for the treatment of variety of diseases including anemia, toothache, cough and swellings in inflammations [6]. The chemical constituents of the plant have been identified as flavonoids type phenolic compounds, especially apigenin, quercetin, quercetin-3-O-β-D-glucoside, naringenin and apigenin glucuronide [6]. Till now there is no any systematic scientific report exists showing its anti-inflammatory activity.

Therefore the objective of the present study was to systematically fractionate and determine the anti-inflammatory activity of various active fractions of *Barleria Cristata* leaves in carrageenan induced paw edema and cotton pellet induced granuloma in rat as model of acute and chronic inflammation respectively.

Materials and Methods

Plant material

Fresh leaves of *Barleria Cristata Linn* were collected from Mumbai region, India. The plant material was taxonomically identified by Dr. Ganesh Iyer, Prof. in Botany, Ramnarain Ruia college, Mumbai, India. A voucher specimen (No. 9-1/08) has been preserved in our laboratory for future reference. The leaves were dried under shade and then powdered with a mechanical grinder and stored in airtight container.

Preparation of the fractions

The dried powder material of the leaves was extracted with methanol in a soxhlet apparatus. Further methanol extract was subjected to the fractionation with different solvents i.e. pet ether, chloroform and methanol (in the order increasing polarity) by maceration technique.

The solvent was completely removed by drying and pet ether fraction (BCP) yield 25.92%, chloroform fraction (BCC) yield 4.14%, and methanol fraction (BCM) yield 59.50% were obtained.

Chemicals and drugs

Carrageenan [Sigma Aldrich (USA)], indomethacin [Recon, (Bangalore) India] and all other chemicals used were of analytical grade.

Animals

Wistar albino rats of either sex weighing 180–200 g were used for animal studies. The animals were grouped in polyacrylic cages and maintained under standard laboratory conditions (temperature 25 ± 2 °C) with dark and light cycle (14/10 h).

They were allowed free access to standard dry pellet diet and water *ad libitum*. The rats were acclimatized to laboratory condition for 10 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.

Evaluation of anti-inflammatory activity

Preparation of test samples for study

Test samples of the fractions were prepared in 0.5% Sodium carboxymethylcellulose (Na CMC) and used for studies. For both carrageenan induced rat paw edema and cotton pellet induced granuloma the study animals were divided in to eleven groups containing six animals each,

Group I, II and III were treated with 50, 100 and 200 mg/kg of BCP respectively, group IV, V and VI were treated with 50, 100 and 200 mg/kg BCC respectively, group VII, VIII and IX were treated with 50, 100 and 200 mg/kg BCM respectively where as group X, XI were treated with indomethacin 10 mg/kg and vehicle (0.5% NaCMC, 5ml/kg) respectively.

Carrageenan-induced rat paw edema

This test was followed by the method described by Winter *et al* [7]. Animals were divided in to eleven different groups as described in the preparation of test samples and respective test samples were administered. One hour after the respective treatment, 100µl 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 anti-inflammatory effect was calculated by the following equation [8].

Anti-inflammatory 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.

Cotton pellet-induced granuloma in rats

The cotton pellets-induced granuloma in rats was studied according to the method described by D'Arcy *et al* [9]. The animals were divided into eleven different groups as described in the preparation of test samples. The rats were anaesthetized and sterile cotton pellets weighing 10 ± 1 mg were implanted subcutaneously into both sides of the groin region of each rat. Each group was treated with respective test samples as described in the preparation of test samples for study for seven consecutive days from the day of cotton pellet implantation. On 8^{th} day the animals were anaesthetized and the pellets together with the granuloma tissues formed were carefully removed and made free from extraneous tissues. The wet pellets were weighed and then dried in an oven at 60 °C for 24 h to constant weight. After that the dried pellets were weighed again. Increase in the wet and dry weight of the pellets was taken as a measure of granuloma formation. The anti-proliferative effect of test samples was compared with the 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 multiple comparison test using Graphat Instat Software (San Diego, CA, USA). The level of significance was set at $P<0.05$.

Results

Evaluation of anti-inflammatory activity

Carrageenan-induced rat paw edema

The anti-inflammatory activity of BCP, BCC and BCM against acute paw edema induced by carrageenan in rats is shown in figure 1. BCC at dose of 200 mg/kg moderately inhibited (42.76%) rat paw edema at the end of 4h after carrageenan injection. BCM showed significant and dose dependent inhibitory activity against carrageenan induced rat paw edema through out the study when results were compared with control

group. BCM at dose of 200 mg/kg and indomethacin (10 mg/kg) showed more significant (P<0.05) inhibition of rat paw edema (65.21 % and 69.07% respectively) at the end of 4h after carrageenan injection. Pet ether extract didn't show any significant activity against carrageenan induced rat paw edema at the dose levels studied.

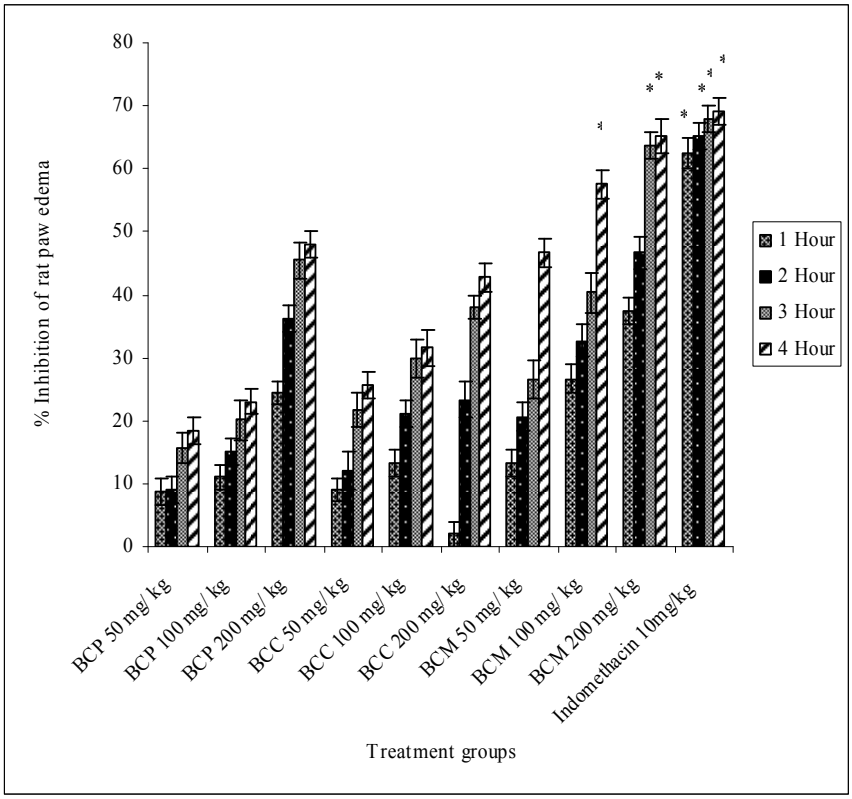

Figure 1. Effect of fractions of the *Barleria Cristata* leaves extract on carrageenan-induced rat paw edema. Where BCP- *Barleria Cristata* pet ether fraction, BCC- *Barleria Cristata* chloroform fraction, BCM- *Barleria Cristata* methanol fraction

Each value represents the mean ± S.E.M., n=6.

*P<0.05, **P<0.01 compared with control, Dunnett's multiple comparison test after analysis of variance.

Cotton pellet-induced granuloma in rats

Effect of different fractions of *Barleria Cristata* extract on cotton pellet-induced granuloma in rats is shown in Table 1. BCP and BCC moderately inhibited cotton pellet induced granuloma at higher dose level where as BCM and indomethacin significantly (P<0.01) inhibited cotton pellet induced granuloma in rats when results were compared with control group. BCM (200 mg/kg) showed maximum inhibition of 62.37 % (wet cotton) and 53.84 % (dry cotton) where as indomethacin (10 mg/kg) showed 68.04 % (wet cotton) and 59.61 % (dry cotton) inhibition of cotton pellet induced granuloma in rats.

Discussion

In the present study extract of the *Barleria Cristata* leaves was fractioned by different solvents with increasing polarities and was studied for its anti-inflammatory activity by using carrageenan induced rat paw edema and cotton pellet induced granuloma method.

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 [10]. The inhibitory activity shown by the BCM fraction at dose of 200 mg/kg over a period of 4 h in carrageenan induced paw inflammation was quite similar to that exhibited by the group treated with indomethacin (10 mg/kg). BCM at higher dose level (200 mg/kg) acts on later phase of inflammation probably involving arachidonic acid metabolites, which produce an edema dependent on neutrophils mobilization [11].

Table 1. Effect of fractions of the *Barleria Cristata* leaves extract on cotton pellet-induced granuloma in rats

Treatment (s)	Dose (mg/kg)	Weight of granulation (mg) (wet)	Percentage inhibition	Weight of granulation (mg) (dry)	Percentage inhibition
Control	Vehicle	194 ± 9.1	-------	52 ± 5.2	------
Barleria Cristata pet ether fraction	50	175 ± 8.5	9.79	45 ± 9.3	13.46
	100	149 ± 7.8	23.19	41 ± 8.6	21.15
	200	104 ± 8.2*	46.39	31 ± 9.2*	40.38
Barleria Cristata chloroform fraction	50	160 ± 8.9	18.55	44 ± 9.4	15. 38
	100	111 ± 7.9*	42.78	38 ± 8.1*	26.92
	200	79 ± 8.2*	59. 27	28 ± 9.2*	46.15
Barleria Cristata methanol fraction	50	159 ± 5.7	20.10	43 ± 5.2	23.07
	100	109 ± 6.9*	44.84	32 ± 5.9*	40.38
	200	72 ± 8.5**	62.37	25 ± 6.3**	53.84
Indomethacin	10	62 ± 6.3**	68.04	21 ± .2**	59.61

Each value represents the mean ± S.E.M., n=6.
*P<0.05, **P<0.01 compared with control, Dunnett's multiple comparison test after analysis of variance.

Cotton pellet granuloma is a chronic inflammation reaction arising when the acute response is insufficient to eliminate proinflammatory agents. It occurs by means of the development of proliferative cells. These cells can be either spread or in granuloma form. The cotton pellet method is widely used to evaluate the transudative (infiltration of neutrophils and exudation) and proliferative (proliferation of fibroblasts) components of the chronic inflammation [12, 13]. The wet weight of the cotton pellets correlates with the transude, whereas the dry weight of the pellets correlates with the amount of the granulomatous tissue [14, 15]. The BCM fraction at higher dose (200 mg/kg) and indomethacin (10 mg/kg) showed significant (P<0.01) inhibitory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in number of fibroblasts, synthesis of collagen and mucopolysaccharides during granuloma tissue formation [16].

BCP didn't showed any significant activity in carrageenan induced rat paw edema but showed significant inhibitory activity in cotton pellet induced granuloma method indicating that fractions containing non polar compounds don't have significant activity against acute inflammation but it possessed significant activity in chronic model of inflammation. As the polarity of the solvent increases significance level of the anti-inflammatory activity in both the model studied were also increased indicating that fractions containing more polar compounds responsible for anti-inflammatory activity.

The anti-inflammatory of BCM fraction found may be due to the presence of more polar flavonoids i.e. apigenin, quercetin and quercetin-3-O-B-D-glucoside, naringenin and apigenin glucuronide [6] in the plant extract. The therapeutic applications of flavonoids on inflammation have previously been reported [17, 18]. Flavonoids are known to prevent the synthesis of prostaglandins. Biochemical investigations on the mechanism of action of flavonoids have shown that these compounds can inhibit a wide variety of enzymes. Linoleic acid and arachidonic acid are indigenous compounds of the cell membrane with a task to protect the cell.

The release of arachidonic acid is closely related to the cyclooxygenase and 5-lipoxygenase enzyme systems. The ability of flavonoids to inhibit both cyclooxygenase and 5-lipoxygenase pathways of the arachidonate metabolism has been suggested to contribute anti-inflammatory action [17].

Conclusion

From the present study it is concluded that the methanol fraction of *Barleria Cristata* leaves extract produced significant anti-inflammatory activities in dose dependent manner on both acute and chronic animal models of inflammation. Further studies involving the purification of the chemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with a low toxicity and better therapeutic index.

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