



ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(2):13-16
(<http://www.derpharmachemica.com/archive.html>)

Preliminary Phytochemical Screening and Evaluation of Anti-Inflammatory Activity of Methanolic Extract of *Barleria cristata* Linn. Roots in Experimental Animals

Shanaz Banu^{1*}, Ashoka Babu VL², Josephine Leno Jenita J¹, Premakumari KB¹, Manjula D¹

¹Dayananda Sagar University, Bengaluru, Karnataka, India

²M.S.R.U.A.S, Bengaluru, Karnataka, India

ABSTRACT

Barleria cristata Linn. (Acanthaceae) has been widely used for its reported biological activities in indigenous system of medicine. The present investigation was carried out to find the effect of methanolic extract of roots for its anti-inflammatory activity in rats. Oral administration of the extract at the doses 250 and 500 mg/kg b.w. exhibited dose dependent and significant anti-inflammatory activity in acute (carrageenan-induced hind paw edema) and chronic (cotton pellet granuloma models) inflammation. Animals receiving 500 mg/kg exhibited maximum inhibition ($p < 0.001$). Hence, the present investigation established some pharmacological evidences to support the folklore claim of *Barleria cristata* Linn. as an anti-inflammatory agent.

Keywords: *Barleria cristata* Linn., Anti-inflammatory, Methanolic extract, Paw edema, Granuloma

INTRODUCTION

Inflammation is a local response of living mammalian tissues to the injury. It's a defence reaction in order to limit or eliminate the spread of injurious agents. Various components contribute to the tissue injury and the associated symptoms. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation [1]. *Barleria cristata* Linn. (Acanthaceae), commonly known as "Crusted purple Nail Dye" is one of the plants belonging to the genus *Barleria* [2]. It is usually found growing in the forest grounds of Tikauli in Nepal and the Tirupati hills in Andhra Pradesh [3]. Traditionally the plant is used since many years for treatment of inflammation, cough, diabetes and anaemia [4]. Whole plant is used as a stimulant and demulcent [5].

OBJECTIVE

Ancient wisdom is the basis of modern medicine and remains as an important source of future medicine and therapeutics. However, the development of folk remedies from plant origin for various ailments has been painstakingly slow. Hence, in the present study, we were interested in screening the roots of *Barleria cristata* Linn. for anti-inflammatory activity.

MATERIALS AND METHODS

Plant material

Barleria cristata Linn. roots were collected from Tirupati hills, identified and authenticated by the botanist, Dr. Madhava Chetty, Dept. of Botany, S.V. University, Tirupati. Roots were shade dried at a temperature not exceeding 30°C and were cut into pieces and milled. Extraction was then carried out.

Preparation of plant material

Roots of *Barleria cristata* Linn. were extracted in a soxhlet extractor by successive solvent extraction. Preliminary phytochemical screening was carried out for the presence of phytoconstituents [6].

Preparation of 1% suspension of tween 80

Tween 80 suspension was made by trituration of 1 g of Tween 80 with 100 ml of distilled water. It was used for suspending the test compound and standard drug.

The methanolic extract of *Barleria cristata* Linn. roots (MEBC) was suspended in Tween 80. This suspension of extract was prepared daily for oral administration.

Chemicals used

All the chemicals used for the present study were of pharmaceutical grade. Carrageenan was obtained from Sigma Chemicals Company. Pure Indomethacin was obtained from Strides Arcolab Pvt. Ltd., Bangalore, India.

EXPERIMENTAL ANIMALS

Swiss albino mice (25-30 g) and Wister albino rats (180-220 g) of either sex used for the study were obtained from Venkateshwara Enterprises, Bengaluru, and Karnataka. They were randomly distributed into groups and housed in cages and maintained under standard conditions at $26 \pm 2^\circ\text{C}$ and relative humidity 44-56% and 10 h light: 14 h dark cycles each day for one week before and during the experiments. All animals were fed with the standard rodent pellet diet and water *ad libitum*. Institutional Animal Ethical Committee clearance was taken for this project.

Acute toxicity

Acute toxicity studies were carried out as per the OECD guidelines 423 Annex 2C [7].

METHOD

Anti-inflammatory activity of MEBC was carried out by using acute inflammatory model; Carrageenan induced paw edema and chronic model, and cotton pellet induced granuloma [8].

Preparation of carrageenan suspension

Carrageenan suspension (1%) was prepared by soaking in saline NaCl (0.9%) and then homogenous suspension was made by mixing thoroughly with the help of a magnetic stirrer. Albino rats of either sex (150-200 g) in 7 groups with 6 animals each were used and vehicle used was Tween 80 (3 ml of 1% solution). Increase in paw volume and percent inhibition was recorded. Wet weight (wt) and dry weight (wt) of the cotton pellet with percent inhibition was recorded for cotton pellet induced granuloma model [9].

RESULTS AND DISCUSSION

MEBC roots indicated the presence of phenolic compounds, terpenoids, flavonoids, phytosterols, alkaloids, glycosides and carbohydrates.

Acute toxicity studies

No adverse effects or mortality were detected in the mice upto 5 g/kg p.o., during the first 24 h observation period. Based on the results obtained from this study, the dose for the anti-inflammatory activity was fixed to be 250 mg/kg b.w and 500 mg/kg b.w. for the dose dependent activity.

Carrageenan induced rat paw edema model

The effect of methanolic extracts on carrageenan induced paw edema in rats is tabulated in Table 1 and graphically represented in Figure 1. The extracts exerted significant ($p < 0.001$) activity in the experimental animals. Carrageenan induced edema was reduced by both the doses of MEBC (250 and 500 mg/kg b.w.) by 44% and 51% respectively after 3 h, and indomethacin exhibited 54% inhibition in comparison with control group.

Table 1: % Inhibition of paw edema by MEBC

Treatment	Concentration (mg/kg)	Increase in paw volume after 3 h (ml)	% Inhibition of edema
Control	1% Carrageenan suspension	0.65 ± 0.02	-
Indomethacin	10	$0.30 \pm 0.008^{***}$	54.0
MEBC	250	$0.37 \pm 0.01^{***}$	44.0
MEBC	500	$0.32 \pm 0.006^{***}$	51.0

Values are expressed as mean \pm SEM, n=6; *** $p < 0.001$ compared to control group

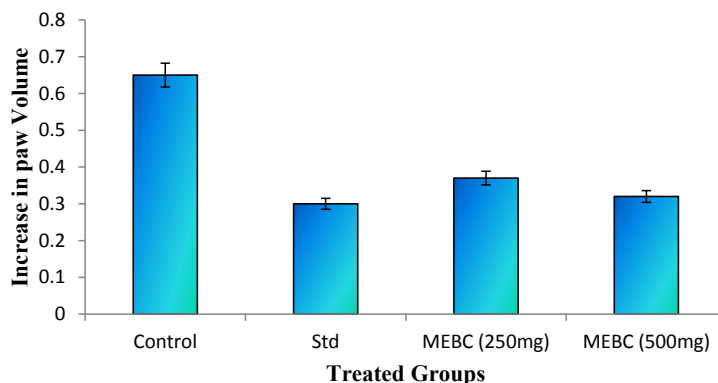


Figure 1: Effect of MEBC treatment on carrageenan induced edema

Cotton pellet induced granuloma formation in rats

The activity exhibited by methanolic extracts is displayed in Table 2. The average wet weight is shown in Figure 2 and dry weight is represented in Figure 3. Inhibition of granuloma formation by MEBC extract was 45.33% and 64% at the doses of 250 and 500 mg/kg b.w respectively, whereas standard Indomethacin showed 78.67% inhibition.

Table 2: % Inhibition of granuloma formation by MEBC

Treatment	Conc. (mg/kg)	Granuloma wet wt. (mg)	% Inhibition (wet wt.)	Granuloma dry wt. (mg)	% Inhibition (dry wt.)
Control	-	236.66 ± 15.84	-	125 ± 7.63	-
Indomethacin	10	98.33 ± 7.03***	58.45	26.66 ± 3.33***	78.67
MEBC	250	171.66 ± 9.09**	27.46	68.33 ± 3.07**	45.33
MEBC	500	143.33 ± 4.21***	39.43	45 ± 2.23***	64.0

Values are expressed as mean ± SEM, n=6; **p<0.01, ***p<0.001 compared to control group. Statistical analysis of data was performed by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test

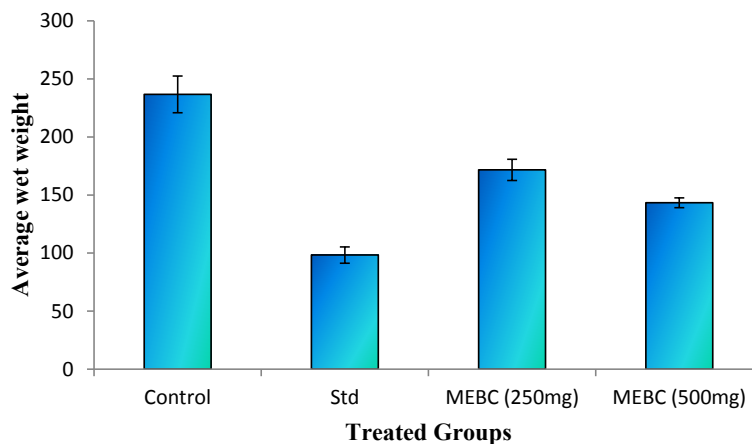


Figure 2: Anti-inflammatory activity of MEBC on cotton pellet induced granuloma pouch model (average wet weight)

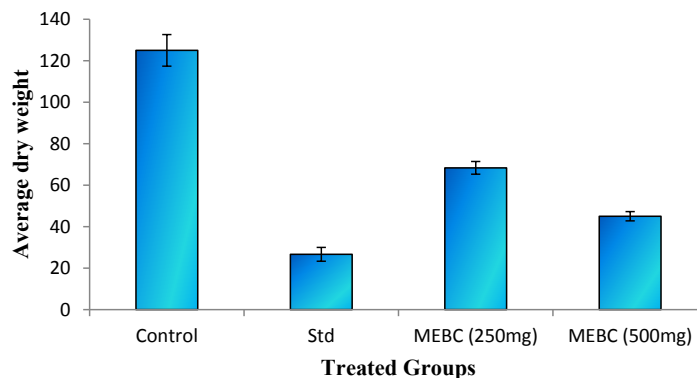


Figure 3: Anti-inflammatory activity MEBC on cotton pellet induced granuloma pouch model (average dry weight)

DISCUSSION

In recent era, in spite of tremendous development of synthetic drugs, lot of side effects are found which has led the plants to still hold a unique place by giving minimal side effects. Thereby, a systematic approach has to be made to find the efficiency of plants as potent anti-inflammatory agents. Phospholipase A2 is the enzyme responsible for the formation of mediators of inflammation like prostaglandins and leukotrienes by attracting polymorphonuclear leucocytes to the site of inflammation and leading to tissue damage probably by releasing the free radicals [10]. In the cell membrane, phospholipids are converted into arachidonic by phospholipase A2, which is highly reactive and rapidly metabolized by cyclooxygenase (prostaglandin synthesis), which are major components that induce pain and inflammation [11].

Anti-inflammatory activity of MEBC were evaluated by using two anti-inflammatory models such as Carrageenan induced paw edema in rats and Cotton pellet induced granuloma pouch model.

Carrageenan induced edema is used as a model for acute inflammation and the reactions involved are biphasic in nature. Chemical mediators like histamine and serotonin play a very important role in the first phase, within 2 hours after Carrageenan injection, whereas kinin and prostaglandins are involved in the second phase, 3-4 h after carrageenan injection [12].

Both the doses of the plant exhibited significant activity. MEBC at 500 mg/kg b.w. showed the highest inhibition (51%) of edema. The wet weight and the dry weight of the cotton pellets were significantly reduced in the treated groups in case of granuloma pouch model. MEBC at the dose level of 500 mg, showed significant reduction in inflammation evidenced by decrease in dry weight (39.43%) and wet weight (64.0%) of cotton pellets as compared to the control group. The extract successfully reduced the inflammation produced by serotonin and histamine. So, the anti-inflammatory activity of the extract may be possibly backed by its anti-5-HT activity which is responsible for the same [13].

The significant reduction in the granular tissue formation reflected the efficacy of MEBC to inhibit the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides which are natural proliferative events in the formation of granulation tissue.

Higher anti-inflammatory activity of MEBC extract could be attributed to the presence of terpenoids which are anti-inflammatory and anti-oxidant in nature. The presence of flavonoids may be contributing for the activity. Further the phenolic constituents of MEBC could also be inhibiting the oxidative burst of activated polymorphonuclear leukocytes effectively.

CONCLUSION

Thus, it can be concluded that the methanolic extract of *Barleria cristata* Linn. roots possess anti-inflammatory activity. 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 low toxicity and better therapeutic index.

ACKNOWLEDGEMENTS

The authors are thankful to the management and Dean, College of Pharmaceutical Sciences, Dayananda Sagar University, for providing necessary facilities to carry out the research work.

REFERENCES

- [1] R.N. Mitchell, R.S. Cotran, Harcourt Pvt. Ltd., **2000**, 7, 33-42.
- [2] M.N. Gambhire, S.S. Wankhede, A.R. Juvekar, *J. Young. Pharm.*, 2009, 1(3): 220-224.
- [3] Madhavachetty, K. Sivaji, K. Tulsi Rao, Students Offset Printers, **2008**, 2, 252-254.
- [4] R.J. Marlesa, N.R. Farnsworth, *Phytomedicine.*, **1995**, 2(2), 170.
- [5] V. Asolkar, K. Kakkar, J.O. Chark, Part-I (a-k) (1965-1981), *Pub. Inform. Dir.*, (CSIR), **1992**.
- [6] C.K. Kokate, Vallabh Prakashan, **1999**, 11, 107-121.
- [7] OECD guideline on acute oral toxicity (AOT), **2001**, 425.
- [8] U.K. Mazumder, M. Gupta, *Phytomedicine.*, **2003**, 10, 185-88.
- [9] M.A. Ebrahimzadeh, M. Mahmoudi, E. Salimi, *Fitoterapia.*, **2006**, 77, 146-148.
- [10] G.A. Higgs, S. Moncada, J.R. Vane, *Ann. Clin. Res.*, **1984**, 16, 287-299.
- [11] J.R. Vane, *Nat. New. Biol.*, **1971**, 231-235.
- [12] J. Castro, H. Sasame, H. Sussman, P. Buttette, *Life. Sci.*, **1968**, 7, 129-136.
- [13] U.K. Mazumder, M. Gupta, L. Manikandan, S. Bhattacharya, P.K. Haldar, S. Roy, *Phytomedicine.*, **2003**, 10, 185-188.