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Broncho-Relaxant, Anti-Inflammatory and Mast Cells Stabilisation Effects of Successive Extracts of *BarleriaCristata* Whole Plant

VasundharaDAmbalwad[1*], SumanthGTugaonkar[2]

*[1]*Research Scholar, Yeshwant Mahavidayalaya, Nanded, Maharashtra, India*
[2]Department of Botany, Indira Gandhi (Sr.) College, CIDCO, Nanded, Maharashtra, India

****Corresponding Author:-****Vasundhara D Ambalwad*
**Research Scholar, Yeshwant Mahavidayalaya, Nanded, Maharashtra, India*
vasundhara.ambalwad27@gmail.com

Abstract

B. cristata ns L. is a ethno medicinal plant used for various pharmacological potentials. *B. cristata* have been traditionally claimed for its usefulness against cough and bronchitis by means of using of decoction of roots, leaves, or whole plant. In this research an evaluation of successively obtained extracts from whole plant of *B. cristata* were evaluated for the mast cell stabilisation, anti-inflammatory, anti-oxidant and broncho-relaxant effects against Ach-induced contraction. The obtained results revealed that the tracheal contraction induced by the Ach was markedly relaxed by *B. crsitata* with maximum relaxation, at the dose of 0.8 mL. Additionally, extract exhibited mast cell stabilisation effect by decrease in histamine release of 13.96 in compound 48/80 induced destabilisation at 50 mg/ml**.** The BCW-ET extract exhibited not only better relaxant activity on isolated tracheal rings, but also revealed good phytochemical profile, anti-oxidant and anti-inflammatory effect. More detailed studies are needed to identify the target of the inhibition, and to determine precise pharmacological mechanisms of observed biological effects.

Keywords: Asthma, successive extraction, C48/80 induced mast cell destabilization.

Introduction:

There is a dire need and inclination in recent days to correlate the pharmacological activity with its phytochemical compounds of a medicinal plant to establish concrete scientific evidences along with pave new ways to discover newer safe medicines [Gangaram S et al 2021]. Several plant species from Acanthaceae family exhibits valuable pharmacological potential, and have a crucial component in humans as well as animal kingdom as they have been found useful not only for food but also as medicine. Several plant species have been known for their ethnomedicinal properties based on uses and claims by traditional users, with Barleria being one of the most important (3rd largest) genera from Acanthaceae family. Some of the highly valuable species of Barleria include B. *prionitis*, *B. cristata*, *B. grandiflora*, and *B. lupulina* [Kumari R et al 2015; Kumar, V.; Singh, S. 2013]. Almost all parts of plants belonging to this genus are good source phytochemicals which might be considered responsible for its significant pharmacological potential for the

treatment of various pathological conditions. Several scientific reports have mentioned that plants of this genus exhibits vital pharmacological potentials such as analgesic, anti-amoebic, anti-arthritic, antibacterial, anticancer, anti-diabetic, antifungal, antihypertensive, anti-helminthic, anti-inflammatory, antiulcer and antiviral activities along with hepatoprotective, and inhibition of acetylcholinesterase activity (Yoosook C et al 1999; Wang BU et al 2001; Jassim & Naji 2003; Suba V et al 2004; Suba V et al 2004 a; Suba V et al 2005; Chomnawang MT et al 2005; Shukla &Gunjegaokar 2018).

B. cristata have been traditionally claimed for its usefulness against cough and bronchitis, use of decoction of roots, leaves, whole plant individually or in combination are useful to treat asthma, anaemia, pneumonia and eliminates phlegm (Kirthikar and Basu 1985; Sahu T, 1984; Marlesa, & Farnsworth 1995; Kanthale P et al 2012; Harish Kumar et al 2018). However, no one scientifically validated these traditional claims to treat cough and respiratory disorder of bronchitis and asthma. This paper is an attempt to validate these claims in terms of its anti-oxidant, anti-inflammatory, mast cells stabilisation, broncho relaxation effects.

MATERIAL AND METHODS

Plant material authentication letter number

The flowering plant of *Barleria cristata* white was collected from the outfield of Nanded city, Maharashtra, India. The plant material was authenticated by, Department of Botany, Shree Renuka Devi Mahavidayalaya, Mahur, Dist Nanded, MS, India (PhD 2019-20/1015). The plant material was coarsely powdered so as to facilitate further extraction.

Chemicals and reagents:

All the chemicals purchased are of Loba Chemie Pvt. Ltd. For extraction, phytochemical analysis and TLC chemicals were purchased of synthetic grade, while for standardization and other *in vitro* as well as *in vivo* analysis chemicals purchased were of analytical grade. Standard drugs were either used as API or purchased formulation from market.

Instruments:

UV-Visible spectrophotometer (UV-1800; Shimadzu), Rotary evaporator (Superfit- 600) Micro plate reader (iMark, BioRad).

Animal husbandry and ethical committee approvals

Animals were acclimatised to the laboratory conditions for an appropriate period and provided with standard laboratory animal feed and drinking water *ad libitum*. Animals were grouped as per requisite of animal experimentation. Experimental design was approved as Animal Ethical Committee letter number IAEC/ Sangli/2022-23/11.

Preparation of extract of *Barleria cristata* White

The ground powdered material (500g) were exhaustively defatted with n-hexane by Soxhelet extraction followed by successive extraction with ethyl acetate, acetone and finally with absolute ethanol. Obtained extracts were collected, filtered and concentrated over rotary evaporator. Resulting concentrated extracts were marked and labelled as BCW-HE, BCW-EA, BCWP-AC and BCW-ET respectively, these were stored in tightly closed glass container in desiccators for further use.

Evaluation of Phytochemical profile, TLC fingerprinting and Standardization of *Barleria cristata* white whole plant successive extracts

Preliminary phytochemical profile was assessed for the presence of various phytoconstituents present in successively obtained BP extracts using routine test reported previously (Gokhale MS &Kokate CK, 2008).

Standardization of BP extracts for Total flavonoid content (TFC) &Total phenolic content (TPC)

Standardization of BP extracts which shown positive test for presence of flavonoids, tannins or polyphenols were carried out to assess TFC & TPC. The content of TFC from extracts by the aluminum chloride colorimetric method using rutin as standard and expressed as rutin equivalent per g dry weight. While, TPC was determined by the Folin–Ciocalteu colorimetric method using gallic acid as standard and expressed as mg/g gallic acid equivalent (GAE) on dry weight basis by means of method established and reported previously (Ghante M H et al 2012).

Acute toxicity study

The acute toxicity assay was performed by implementing, a limit test which was conducted at 5000 mg/kg of the extract in both male and female mice, following OECD Guidelines 423. Each mouse received a single oral dose of 5000 mg/kg of the extract via gavage, using a feeding tube. Before extract administration, mice were weighed, marked, and subjected to fast for requisite time period, with ad libitum water. After extract administration, mice were observed every 30 minutes during the initial two hours and subsequently twice a

day for 13 days. Observations were mainly based on major clinical signs of toxicity, along with alterations of behaviour, physical, and reflexes responses.

Evaluation of Broncho-relaxant effect:

The effect of extract for bronchorelaxent effect was studied as per previous methods (Boly R et al 2021). Accordingly, the trachea was excised and promptly placed in a Petri dish containing a modified Krebs-Henseleit physiological solution and the pH was adjusted to 7.4. Following excision, the trachea was carefully separated from surrounding tissues and divided into four to five rings, each approximately 5 mm in length. Subsequently, each tracheal ring was placed in an organ bath chamber with a modified KH physiological solution. The organ bath was continuously aerated and kept at a 37°C temperature. The tracheal isolated rings equilibrated under a requisite tension for 1 hour, during which the bathing solution was replaced after 15 minutes interval. The concentration of Ach eliciting maximal contraction in was determined by adding it in cumulative concentrations (10^{-6} to 1.5×10^{-5} M). The maximal contraction effect was observed at concentration of 10^{-5} M. The effects of BC extracts were assessed on tracheal rings against pre-contracted tracheal chain implemented the cumulative addition of the extracts at 0.1, 0.2, 0.4, 0.5 and 0.6 µl.

Anti-inflammatory effects against carrageenan induce inflammation:

The carrageenan suspension (1%) was prepared by soaking in normal saline NaCl, Albino rats of both sexes (150-200 g) were organized into 7 groups, each consisting of 6 animals. The vehicle utilized was gum acacia (3 ml of a 1% solution). Measurements of change in paw volume and percent inhibition were recorded.

Measurement of histamine release from RPMC as mast cell stabilisation effect:

BCW extracts (50 µg/mL) or disodium cromoglycate (DSCG) (50 µg/mL) was mixed with mast cell suspension and incubated at 37◦C for 15 min. Resulting mixture was adjusted up to 3 mL with HBSS, to this an C-48/80 was added and allowed to incubate at 37◦C for 30 min followed by centrifugation at 2500 rpm. Top layer of the resulting solution from each test tube was transferred to a separate tube containing 300 mg NaCl and 1.25 mL n-butanol. This was alkalized by adding 3 M NaOH (1 mL) to extract histamine into n-butanol which was further separated into a tube containing 2 mL of n-heptane and 0.4 mL of 0.12 M HCl. The contents were mixed and allowed for phase separation; 0.5 mL of the aqueous phase was transferred to another test tube. To each tube 1 M NaOH (100 µL) and 0.2% o-phthalaldehyde (100 µL) solution was added immediately under constant stirring. Further 3 M HCl (50 µL) was added after 2 min and finally histamine concentration was determined by using a spectrofluorometer at excitation and emission wavelengths of 350 and 450 nm, respectively (Spectrofluorometer-530, Shimadzu). The control solutions were 1) Spontaneous histamine release: mast cells and solutions used to determine baseline, 2) Histamine release: mast cells and calcium-ionophore (10-6 g/mL), 3) Test compound control: contains solutions and test compound and 4) Solution control: contains only solutions used in the test to determine baseline. % histamine release (HR) inhibition from mast cells was determined by the following formula: (sample-spontaneous HR) / (100% HR - spontaneous HR)* 100.

Results and discussion

By considering need and inclination of scientific community to correlate the pharmacological activity with its phytochemical compounds of a medicinal plants but not explored to establish scientific evidences. *B. cristata* in different forms have been traditionally claimed for its usefulness against cough, bronchitis, asthma and eliminates phlegm (Kirthikar and Basu 1985; Sahu T, 1984; Marlesa, & Farnsworth 1995; Kanthale P et al 2012; Harish Kumar et al 2018). However, scientifically validated data for these traditional claims is not available. There is also lack of scientific information on the toxicology, safety as well as pharmacological potential of extracts of *Barleria cristata*, thus these preliminary evaluations were studies are required.

Successively obtained BC extracts when subjected to phytochemical screening it exhibited presence of essential secondary metabolites which include, alkaloids, flavonoids, terpenoids and tannins. In order to standardize, the RD extracts were quantized for the content of total of phenolics and flavonoids; results of standardization revealed that BCW-EA and BCW-ET shown the presence of 04.42±0.71and 08.45 ±0.83 mg/g of total flavonoids, while polyphenols found were 04.15±1.02and 12.45±2.41 mg/g, respectively. Polyphenols (tannins and flavonoids) were said to exhibit antioxidant, anti-inflammatory, mast cell protective along with bronchorelaxant effects.

Acute toxicity studies of BCW extracts was performed according to OECD guidelines, study revealed safety margin of extracts BCWF as observed by lack of systemic and behavioral toxicity up to 2000 mg/kg (p.o.). No adverse effects were observed during initial half hour, 24 h and even up to 14 days after administration of

BCW extracts. Hence doses selected were 100 and 200 mg/kg for the anti-inflammatory studies. As, the inflammation induction by carrageenan is classically considered into 02 segmental phenomenon based on the release, time along with the type of mediators involved. The 1st hour after carrageenan injection is considered as a preliminary phase which is contributed to the release of histamine and 5-HT. While 3-5 h after carrageenan injection is the lateral phase and attributed by induction of prostaglandins, bradykinins, protease and lysosome, which acts as mediators of oedema formation. In the present study results demonstrates inhibition of carrageenan induced inflammation in both phases by BCW extracts in dose dependent manner as compared to control. The Probable way for the observed anti-inflammatory activity by BCW extracts might be due to ability of extracts to inhibit synthesis, release or action of inflammatory mediators like histamine, serotonin, bradykinins and prostaglandins.

The BCW extracts relaxed the acetylcholine contracted tracheal rings in dose dependent manner. The bronchorelaxant effect of BCW extracts might be by blocking or antagonising an effect on the channels of ACh-induced contraction. However, detailed further studies are needed to establish the mechanism. The important class of photochemical of the BCW extract include alkaloids, flavonoids, steroids, saponins, tannins, and triterpenoids. The anti- inflammatory, mast cell stabilising and anti-asthmatic properties of most of these phytochemicals have been previously reported which helps to bolster the traditional claims.

In conclusion, it can be summarised that BCW extracts exhibits overall anti-asthmatic effects through broncho relaxation, inhibition of inflammation inducers, blocking of cholinergic contractions and inhibition of histamine release from mast cells.

Table 01: Phytochemical qualitative analysis of *Barleria Cristata L* (White) extracts

Sr. no	Chemical test	*Barleria Cristata L* (White)			
		BCW-HE	**BCW-EA**	**BCW -AC**	**BCW -ET**
1.	**Test for Alkaloids**				
	a) Dragendorff's test	-	-	-	+
	b) Mayer's test	-	-	+	+
	c) Wagner's test	+	+	+	+
2.	**Test for Amino acid**				
	a) Ninhydrin test	-	-	-	+
	b) Tyrosine test	-	-	-	+
3.	**Test for Carbohydrate**				
	a) Molish's test	+	+	+	+
	b) Benedicts test	-	+	+	+
	c) Barfoed test	-	-	+	+
	d) Fehling test	-	-	+	+
4.	**Test for Glycosides**				
	a) Keller-killiani test	-	-		
	b) Borntrager's Test	-	-	+	+
	c) Modified Borntrager's Test	-	+	+	+
	d) Legal Test	-	+	+	+
5.	**Test for Flavonoids**				
	a) Lead acetate test	-	-	-	+
	b) Shinoda test	-	+	+	+
	c) Zinc dust & HCL test	-	+	+	+
6.	**Test for Protein**				
	a) Million test	-	-	-	+
	b) Biuret test	-	-	-	+
7.	**Test for Tannins**				
	a) 5% $FeCl_3$ solution	-	-	+	+
	b) Lade acetate solution	-	-	+	+
	c) Dil. HNO_3	-	+	-	+
8.	**Test for Steroid & Terpenoids**				
	a) Salkowski's test	+	+	-	-
	b) Saponin foam test	-	-	+	+

Table 02: Phytochemical standardization and Mast cell stabilization activity of *Barleria Cristata L* (White) extracts

Sr. no	BCW-HE	BCW-EA	BCW -AC	BCW -ET
TFC	ND	04.42±0.71	03.12±0.28	08.45 ±0.83
TPC	ND	04.15±1.02	02.21±0.89	12.45±2.41
% of Histamine release measurement from mast cell (*in vitro*)				
	DSCG*	**BCW-EA**	**BCW -AC**	**BCW -ET**
50 mg/ml/ 50 µg/ml*	21.6±1.17	29.37±1.12	45.00±1.20	22.15±1.18
50 mg/ml/ 100 µg/ml*	12.08 ±1.80	18.76±1.07	32.16±1.71	13.96±1.91

All the values are recorded as mean± SEM

Table 03: Anti-inflammatory activity of *Barleria Cristata L* (White) extracts in carrageenan induced inflammation model

Treatment	Dose (mg/kg)	Measurement of paw volume and % inhibition of inflammation at (h)		
		01	03	05
Control	**----**	5.26 ±0.18	6.93±0.14	7.15±0.11
BCW-EA	**100**	5.10±0.21 [3.04] *	6.11±0.15 [11.83]	5.33±0.10 [25.35] *
	200	4.26±0.25 [19.6 9] **	4.83±0.18 [30.03] **	4.79±0.11 [33] *
BCW –AC	**100**	5.21±0.21 [0.98]ns	6.13±0.25 [11.54]ns	6.08±0.38 [14.96]*
	200	4.75±0.34 [9.75]*	5.98±0.41 [13.80]	6.52±.21 [8.78] **
BCW-ET	**100**	5.07±0.25 [3.17]	6.04±0.41 [07.65]	5.43±0.89 [24.05]*
	200	4.71±0.22 [10.45]*	4.66±0.67 [33.33]*	3.26±0.7 [54.40]*

All the values are recorded as mean± SEM; * indicative of p>0.05 significance level using ANOVA

Table 04: Percent Broncho relaxation effects of *Barleria Cristata L* (White) extracts

Percent Broncho relaxation against tracheal contraction *in vitro*			
Amount of drug/ extract (µg/ml)	**Aminophylline**	**BCW-EA**	**BCW -ET**
0.1	24.02±1.29	12.05±0.80	21.11±1.21
0.2	44.31±1.34	20.07±1.86	44.72±1.02
0.4	72.06±1.40	33.01±1.23	68.22±1.94
0.6	100	53.44±1.81	89.12±1.81
0.8	--	65.81±1.67	99.24± 2.14

All the values are mean ± SEM

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