Mast Cell Stabilization and Membrane Protection Activity of *Barleria prionitis* L.

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ABSTRACT

Barleria prionitis is a well-known medicinal plant, traditionally used for the treatment of various inflammatory diseases because of its anti-inflammatory activity. But the effect of the extract on inflammatory mediators and cell membrane in response to toxic chemicals was not studied before. Here, we evaluated the membrane stabilization and mast cell protection activity of hydroalcoholic extract of *B. prionitis* whole plant. The hydroalcoholic extract significantly inhibited the hypo-saline induced erythrocyte membrane hemolysis and the compound 48/80 induced mast cells degranulation in a dose dependent manner. The extract at dose concentration of 10 µg/ml, reduced the rat mesenteric mast cells degranulation up to 64.91% and prevented hypotonic solution induced hemolysis of rat erythrocytes by 27.10%. These findings clearly validate the anti-inflammatory activity of *B. prionitis* whole plant extract and provide support for traditional usage for inflammatory disorders.

Key words: *Barleria prionitis*, anti-inflammatory, phytochemical studies

INTRODUCTION

Barleria prionitis L. (Family: Acanthaceae) is a well-known medicinal plant traditionally used in Ayurveda for the treatment of bronchial asthma, rheumatic affections, inflammation, glandular swelling and this plant is found throughout the humid climatic zone of India.[1] The plant reported to contain barlerin, acetylbarlerin, scutellarein-7-neohesperidosideetc, balarenone, pipataline, lupeol, prionisides, barlerinoside, shanzhiside methyl ester, lupulinoside verbascoside, 6-O-trans-p-coumaroyl-8-O-acetylshanzhiside methyl ester and its cis-isomer.[1-3] The plant was reported to show antibacterial, anti-arthritic, anti-inflammatory,[4-5] hepatoprotective,[6] antioxidant,[7] antidiabetic,[8] anthelmintic,[9] antiviral,[2] and antifertility activities.[10] With this scope we have studied the erythrocytes membrane stabilization and mast cell protection activity of hydroalcoholic extract of *B. prionitis* whole plant against toxicants. Inflammation is a complex immunologic reaction frequently associated with pain which leads to increase of vascular permeability, protein denaturation and alteration of membrane integrity.[11] The mast cells plays important role in inflammatory events.[12] Hence, the present work has been highlighted the effect of hydroalcoholic extract of *B. prionitis* on inflammatory events such as membrane stabilization and mast cell protection.

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MATERIALS AND METHODS

Plant material

B. prionitis L. (Acanthaceae) whole plant was procured from local vendor and sample was authenticated from the Department of Botany and Forestry, Vidyasagar University, India. Voucher specimen (no: VBDB/10/17) was preserved in the Department of Botany and Forestry, Vidyasagar University, India.

Preparation of plant extract

The air dried plant material (300 g) was powdered in grinder and extracted with hydro-methanol (70%) by cold maceration process for 15 days to obtain crude extract. The crude extract was filtered through Whatman No. 1 filter paper and filtrate was evaporated and dried in a rotary evaporator followed by lyophilization. The percentage yield of the extract was calculated. The dried extract was dissolved in distilled water before use.

Animals

Male Wistar rats (200-225 g) were obtained from the authenticated vendor and the animal were housed in the animal facilities at Vidyasagar University. The animals were maintained in a laminar air-flow room at a temperature of 22 ± 1 ºC under the relative humidity of 55 ± 10% with standard food and water *ad libitum* throughout the study. The animal studies were done in accordance to the institutional guidelines and the protocols were approved by the Institutional ethical committee for Animal Care and Use at Vidyasagar University before study.

Reagents

Compound 48/80 (C 48/80) was purchased from Sigma-Aldrich (USA), O-toludine blue was obtained from Spectrochem Pvt. Ltd, India, disodium cromoglycate (DSCG) from Cipla, India. Indomethacin (INDO) was obtained from GenPharma International Pvt. Ltd. India. All other analytical grade solvents and reagents utilized in this study were purchased from the local vendors.

Phytochemical screening

The dried extract was subjected for the colour reactions to screen the nature of chemical components present in the extract. Small portion of dried extract was dissolved in purified water. The screening of phytochemical classes was performed according to the standard phytochemical procedures.[13-15]

Tests for alkaloids

The aqueous extract (2 ml) incubated with few drops of dilute hydrochloric acid in four different test tubes and then filtered. Each filtrate was tested with alkaloidal reagents either of the Mayer's (cream precipitate) or Dragendorffs (orange brown precipitate) or Hager's (yellow precipitate) or Wagner's (reddish-brown precipitate) reagents. Development of colour precipitates was observed in each test tube.

Tests for glycosides

The dried extract (50 mg) incubated with concentrated hydrochloric acid for 2 h in a water bath and filtered. Filtrate was subjected for Borntrager's test (pink colour) and Legal's test (pink colour). Development of pink colour in both tests indicated the presence of glycosides.

Tests for saponins

The dried extract (50 mg) dissolved in 20 ml of distilled water and shaken in graduated cylinder for 15 minutes. The formation of a 1-2 cm layer of foam indicated the presence of saponins.

Tests for flavonoids

The dried extract dissolved in distilled water and filtered. 5 ml of dilute ammonia solution was added with some portion filtrate followed by the addition of concentrated H_2SO_4. Formation yellow colouration indicated the presence of flavonoids and colouration disappeared on standing.

Tests for terpenoids

Salkowski test was performed to determine the presence of terpenoids. Five ml of aqueous extract was mixed with 2 ml of chloroform followed by the careful addition of 3 ml concentrated H_2SO_4 to form a layer. A reddish brown colour was formed in the inter face indicated the presence of terpenoids.

Test for tannins

The dried extract (50 mg) was dissolved in 20 ml of distilled water and filtered. With the filtrate few drops of 0.1% ferric chloride was added and the formation brownish-green or a blue-black colouration indicated the presence of tannins.

Test for steroids

The dried extract (50 mg) was dissolved in 2 ml of acetic anhydride. With this, 1-2 drops of concentrated H_2SO_4 was added slowly. The colour of reaction mixture was changed from violet to blue or green colour indicated the presence of phytosteroids.

Membrane stabilization activity

In vitro membrane stabilization was performed using the method of hypotonicity induced rat erythrocyte hemolysis described by Shinde and his co-workers (1999).[16] Whole blood of rats was collected by retro-orbital puncture using heparinized syringe. The whole blood samples were centrifuged at 3000 rpm for 10 minutes. The erythrocytes pellet was collected and washed by re-suspending with isotonic buffered solution (154 M NaCl in 10M phosphate buffer pH 7.4). The procedure repeated three times and each time erythrocytes were centrifuged at 3000 rpm for 10 minutes. The test sample consisted of varying concentrations of extract (10, 100 and 1000 µg/ml) or INDO (10 µg/ml) and 0.50 ml of stock erythrocyte suspension in 4.0 ml of hyposaline solution. The control sample consisted of 0.5 ml of stock erythrocyte suspension with hypotonic buffered saline solution. The reaction mixtures were incubated at 56 ± 1 °C for 30 min and centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured at 540 nm. The inhibition percentage of erythrocyte haemolysis was calculated according to the method of Shinde et al (1999).[16]

$$\text{Inhibition of erythrocyte heamolysis (\%)} = \left[\frac{OD1 - OD2}{OD1}\right] \times 100$$

Where:
OD1 = Optical Density of control
OD2 = Optical Density of test samples

Mast cells protecting activity

The protection of mast degranulation induced C 48/80 was performed using the method describe by Norton (1954).[17] The overnight fasted male Wister rats were anesthetize with excess ether and cut the whole abdomen to expose the intestine. The intestinal mesenteries were collected in Ringer-Locke solution and the mesenteries were cut into small pieces. The pieces of mesentery were placed in different petri dishes consisting of different concentrations of extract (10, 100 and 1000 µg/ml) or DSCG (10 µg/ml) prepared in Ringer Locke solution. The petri-dishes were incubated with toxicant C 48/80 (0.8 µg/ml) at 37 ºC for 30 minutes. Two sets of control were prepared by incubating pieces mesentery with or without toxicant C 48/80 in Ringer-Locke solution. The tissues were placed on a clean microscopic slide and remove the fatty layer carefully. The trimmed tissues were stained with 4% formaldehyde solution containing 0.1% O-toludine blue for 30 minutes. The tissues were then de-stained by successive washing with acetone and xylene for 5 minutes. The stained mesentery pieces were examined under digital light microscope at 100X magnification. The mast cell was considered as degranulated if 4-5 granules were present around the cells. The percentage of degranulated and intact mast cells was calculated on the basis of counting 100 mast cells for each sample. For each set of test sample including control 6-8 pieces of mesentery were observed.

Statistical analysis

All the results were expressed as mean ± SEM of the number of the experiments. Statistical significance was performed by one-way ANOVA followed by Bonferroni's multiple comparison or Dunnett's multiple comparison test wherever applicable. The P values < 0.05 were considered as statistically significant. The data analysis was performed using Graph Pad Prism software.

RESULTS

Phytochemical screening

The vacuum-dried extracts gave 4.15% yield of whole plant extract. The results of preliminary phytochemical analysis of hydroalcoholic extract of whole plant are shown in Table-1. The extract gave positive results for the presence of glycosides, saponins, flavonoids, steriods and tannins.

Effect on protection of mast cells

The C 48/80 at dose concentration of 0.8 µg/ml significantly degranulated rat mesenteric mast cells (93.08 ± 4.59%, $P < 0.001$) in compared to the control (2.65 ± 0.84%). The hydroalcoholic extract showed dose dependent inhibition against C 48/80 induced mast cells degranulation as shown in Figure-1A and 1B. At a dose concentration of 10 µg/ml

Table 1. Phytochemical constituents of hydroalcoholic extract of *B. prionitis* whole plant

Test	Observation	Inference
Tests for alkaloids	No colour formation with Dragendorffs, Hager's, Mayer's, Wagner's alkaloidal reagents.	Absence of alkaloids
Tests for glycosides	Pink colour with Borntrager's and Legal's test	Presence of glycosides
Tests for saponins	Formation of foam upon shaking.	Presence of saponins
Tests for flavonoids	Yellow colouration up on the addition of dilute ammonia and concentrated H_2SO_4 & colouration disappeared on standing.	Presence of flavonoids
Tests for terpenoids (Salkowski test)	No reddish brown colour formation in inter face of $CHCl_3$ & H_2SO_4.	Absence of terpenoids
Test for tannins	Formation of blue-black colour with $FeCl_3$ solution.	Presence of tannin
Test for steroids	Change of violet to blue or green colour with acetic anhydride & H_2SO_4.	Presence of steroids

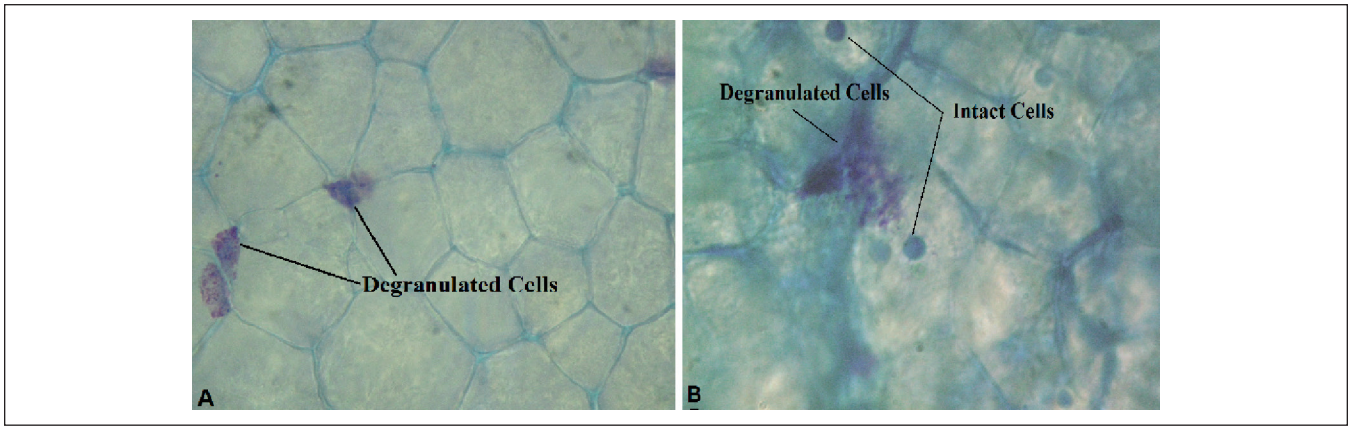

Figure 1: Mast cells protection by *B. prionitis* hydroalcoholic extract
A. Degranulated mast cells in control; B. Intact mast cells in hydroalcoholic extract treated sample.

extract reduced the degranulation of mast cells up to 64.91 ± 2.64% which was comparable with the standard DSCG (19.32 ± 6.92%, 10 µg/ml) with significance of $P < 0.001$, whereas 100 µg/ml of extract reduced the degranulation up to 31.42 ± 6.79% ($P < 0.05$) (Figure 2).

Effect on membrane stabilization

The extract at dose concentration of 10 µg/ml provided significant membrane protection (27.10 ± 3.18%) and the results were comparable with known standard drug, INDO (61.29 ± 6.37%, 10 µg/ml) with significance of $P < 0.01$, whereas 100 µg/ml extract showed membrane protection by 32.48 ± 5.26% ($P < 0.01$) (Figure 3).

DISCUSSION

The cell membrane integrity is essential for the normal growth, development and function of the cells. Exposure of erythrocytes to injurious medium, hyposaline solution, leads to rupture of its membrane followed by haemolysis and oxidation of haemoglobin.[18] The lysis of such bio-membranes leads to the generation of free radicals which enhanced the secondary cellular damage.[19] Thus, the compound with membrane-stabilizing property could offer significant protection of cellular membrane from toxic substances and interfere in early phase inflammatory reactions via inhibiting the formation of inflammatory mediators.[16]

The mast cells have an important role in the development of inflammatory anaphylactic and allergic reactions. During anaphylactic reaction IgE degranulated the mast cells to release histamine, heparin, proteases and other mediators to produce inflammatory effects.[20] These effects can be manipulated therapeutically by regulating the function of these mediators. Constituents from natural resources including plant origin may able to modulate such effects. In this experiment it was found that hydroalcoholic extract of *B. prionitis* whole plant provide significant mast cells stabilization and membrane protection.

CONCLUSSION

In conclusion, the result of the present investigation suggested that hydroalcoholic extract of *B. prionitis* whole plant has significant mast cell stabilizing and membrane protection activities. These effects validate the earlier reported anti-inflammatory activity and the traditional usage in inflammatory disorders. These effects could be the mechanism of action of anti-inflammatory action of this plant and this may due to the presence of glycosides, saponins, flavonoids, steroids, tannins in the extract. Thus, this plant may offer beneficial effects in the management of inflammatory conditions. However, further studies should be performed on isolated chemical compounds to establish the chemical responsible for the activity.

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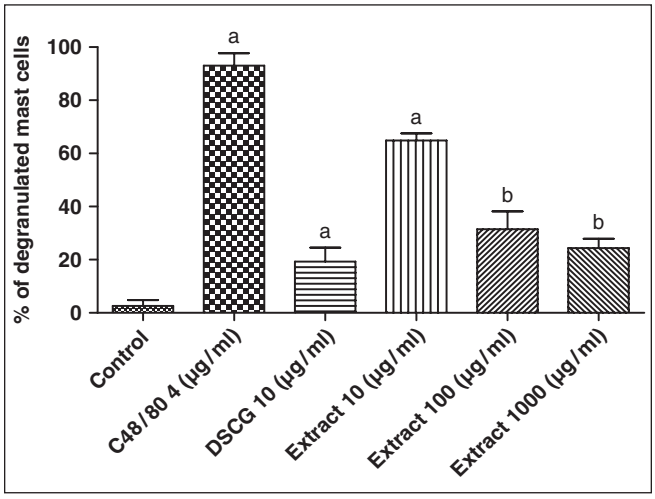

Figure 2: Mast cell protection activity of *B. prionitis* extract
The bars of the graph represents mean ± SEM of three observations (n = 3). Statistical analysis was done through One-way Analysis of Variance (ANOVA) followed by Bonferroni's multiple comparison test. Control Vs C 48/80, a = P < 0.001; C 48/80 Vs DSCG (a = P < 0.001) and extract treated groups (a = P < 0.001; b = P < 0.01).

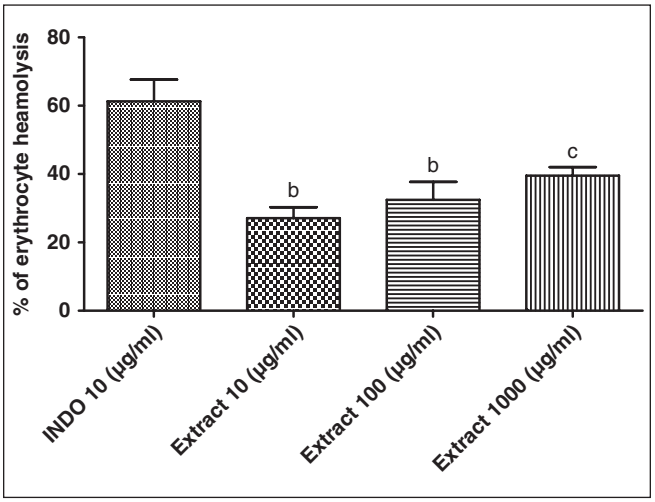

Figure 3: Membrane stabilization activity of *B. prionitis* extract
The bars of the graph represents mean ± SEM of three observations (n = 3). Statistical analysis was done through One-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test. INDO Vs extract treated groups (b = P < 0.01; c = P < 0.05).

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