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EVALUATION OF ANTIASTHMATIC ACTIVITY OF ROOT EXTRACTS OF B*YTTENERIA HERBACEAE*

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Abstract:

Bytteneria herbaceae is a profusely branched prostrate herb. Traditionally different part of this plant is used for the treatment of cholera, diarrhoea and asthma, but lack scientific evidence. Hence, in the present study aqueous and ethanolic extracts of Bytteneria herbaceae was evaluated for antiasthmatic activity in rats by studying its effect on mast cell stabilization and isolated goat tracheal chain preparation.

In mast cell stabilizing activity male albino rats of Wistar strain weighing 150-200 g were divided into eight groups of six animals each. Except group I (Control), all animals were sensitized by 0.5ml of sheep serum and 0.5ml of triple antigen containing diphtheria, tetanous toxoid and B.pertusis organisms. Group I and II rats received vehicle, group III – VI rats were treated with aqueous and ethanolic root extracts of Bytteneria herbaceae 200 and 400 mg/kg.b.w.p.o., respectively, group VII and VIII rats were treated with standard prednisolone and disodium chromoglycate 10 and 50 mg/kg i.p. respectively. Treatment was given once daily single dose for 14 days. On 14th day intestinal mesentery was isolated under anesthesia and stained with 0.1% toluidine blue. Numbers of intact and degranulated mast cells were counted under high-power microscope (45X). Antagonism of extracts was recorded against histamine induced contraction on goat tracheal chain preparation. Results were expressed as mean ± SEM and analyzed by one way ANOVA, followed by Tukey's multiple comparison test.

Aqueous and ethanolic extracts of Bytteneria herbaceae root significantly ($P<0.001$) reduced the mast cell disruption and increased intact mast cells dose dependently. Ethanolic extract was significantly ($P<0.001$) more potent than aqueous extract. In isolated goat tracheal chain preparation extracts of Bytteneria herbaceae antagonized the histamine induced contraction irreversibly and was dose dependent. Antiasthmatic activity of Bytteneria herbaceae was due to the presence of active constituents such as flavonoids, steroids, carbohydrates and tannins. Results of the study substantiate traditional use of Bytteneria herbaceae in the treatment of asthma.

Keywords: *Bytteneria herbaceae; Antiasthmatic; Prednisolone; Mast cell; Sodium cromoglycate; Antihistaminic.*

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INTRODUCTION:

Asthma is a disease characterized by bronchial airway inflammation resulting in increased mucous production and airway hyper responsiveness. The resultant symptomology includes episodes of wheezing, coughing and shortness of breath. Asthma is a multifactorial disease process with genetic, allergic, environmental, infectious, emotional & nutritional components associated with the etiology of it, other factors like urbanization; air pollution and tobacco smoke contributes more significantly [1]. However there is no complete remedy to cure asthma. Further several classes of synthetic drugs have been adopted in the treatment of asthma. In many conditions the patient has to be administered with allopathic drugs for a prolonged period or even lifelong. Administration of these drugs for long period may result in adverse effects or chronic toxicities or drug - drug interactions. Therefore there are several attempts to explore the possibility of natural drugs for asthma treatment.

Bytteneria herbaceae is a profusely branched prostrate herb, the traditional healers of the Kol tribes of West Bengal, Bihar and Jharkhand (India), widely use the woody stock of *Bytteneria herbaceae* to reduce the swelling of limbs, due to filariasis.[2] Besides filariasis different part of this plant is used for the treatment of cholera, diarrhoea and asthma. However there are no scientific reports in this regard. Methanolic extract of *Bytteneria herbaceae* root was found to be effective against histamine induced inflammation in rodents. Hence in the present study aqueous and ethanolic extracts of *Bytteneria herbaceae* root was evaluated for antiasthmatic activity.

MATERIALS AND METHODS:

Plant Material

Bytteneria herbaceae roots were collected from the Laxmipura village, Ramnagarm district, Karnataka, India in the month of August. The samples were authenticated by National Ayurveda Dietetics Research Institute, (RRCBI-13107) Bangalore 560011. It was washed thoroughly and shade dried. The process of extraction was done at Green chem, Herbal extract and formulations, by Soxhlet extraction. The percentage yield was 15% and 11.4% w/w for aqueous and ethanolic extract respectively.

Experimental Animals

Inbred *albino* rats of *Wistar* strain of either sex, weighing 150 - 200 g were selected. The study protocol was approved by the Institutional Animal Ethical Committee [Reg no.152/99/CPCSEA] and experiments were conducted as per the guidelines of CPCSEA.

Drugs and Chemicals

Histamine, Toludine blue (Rolex Chemical Industries), DTP (Diphtheria, Tetanus and Pertussis vaccine), Prednisolone, Disodium chromoglycate (Strides gift sample).

Anti Asthmatic Activity

Dose selection: *Bytteneria herbaceae* aqueous and ethanolic root extracts of doses 200 and 400 mg/kg. b.w.*p.o.*, was selected based on earlier studies [2].

Mast Cell Stabilization Property by Induction of Active Anaphylaxis In Rats

In anti asthmatic activity 42 *albino* rats of *Wistar* strain were divided into eight groups of six animals each, weighing between 150-200 g was taken. Except group I (Control), all were sensitized by 0.5ml of sheep serum and 0.5ml triple antigen containing diphtheria, tetanous toxoid and B.pertusis organisms. Sensitized rats were divided into following groups and treatment was started from the day of sensitization. Group II was considered as sensitized control received vehicle, group III - VI were treated with aqueous and ethanolic root extracts of *Bytteneria herbaceae* 200 and 400 mg/kg.b.w.*p.o.*, respectively, group VII and VIII were treated with standard prednisolone and disodium chromoglycate 10 and 50 mg/kg i.p. Treatment was given single dose once daily for 14 days. On 14th day, 2 hours after the last dose treatment, rats were sacrificed under anesthesia. Intestinal mesenteries were isolated for the study of mast cells.

Mast Cell Count: A piece of small intestine along with intact mesentery was excised and spread without damage in a Petri-dish containing Ringer-Locke solution at 37^{o}C. The mesentery was challenged with 5% v/v sheep serum for
10 min and then transferred to a wide mouthed bottle containing 10% formalin for 24 hr. The mesenteric fans were fixed, dried and stained with (0.1% toluidine blue).The numbers of intact and disrupted mast cells were counted under high-power microscope 45X [3,4].

Isolated Goat Tracheal Chain Preparation [5].

Goat trachea was brought from slaughter house and kept in Kreb's solution. Trachea was transversely cut between the segments of the cartilage to give a number of rings of the trachea. About 3-4 rings were tied to form a chain of approximately 4-5 cm length, which was kept in Kreb's solution. It was suspended in organ bath containing Kreb's solution maintained at 37±1^{0}c and continuously aerated with carbogen (95% O_2 + 5% CO_2).One end of tracheal chain was attached to a tissue holder at the base of the organ bath and the other end to a frontal lever. The suspended tracheal chain was allowed to equilibrate for 45 minutes under a load of 400mg. The responses

were recorded on a slow moving polyrite. The dose response curve for histamine in plane Kreb's solution was recorded. Histamine concentration which produced 50% response was selected and kept constant for determining antagonism activity of *Bytteneria herbacea*. The 50% dose response curve for histamine in the presence of aqueous and ethanolic extracts of *Bytteneria herbaceae* root were recorded on a polyrite.

Statistical analysis

All the values are expressed as mean ± SEM. The data was analyzed by one way ANOVA, followed by Tukey's multiple comparison test. $P<0.05$ was considered as significant.

RESULTS AND DISCUSSION:

In the present study aqueous and ethanolic extract of *Bytteneria herbaceae* was evaluated for antiasthmatic activity in rats.

Table 1: Phytochemical Investigation of Root Extracts of *Bytteneria Herbaceae*

Active constituents	Aqueous extract	Ethanolic extract
Alkaloids	+	+
Saponins	+	+
Flavonoids	+	+
Steroids	+	+
Carbohydrates	+	+
Tannins	+	+
Proteins	+	+
Aminoacids	+	+
Glycosides	+	+
Phenolic compounds	+	+
Anthraquinones	-	-

+: Presence of active constituents, - : absence of active constituents

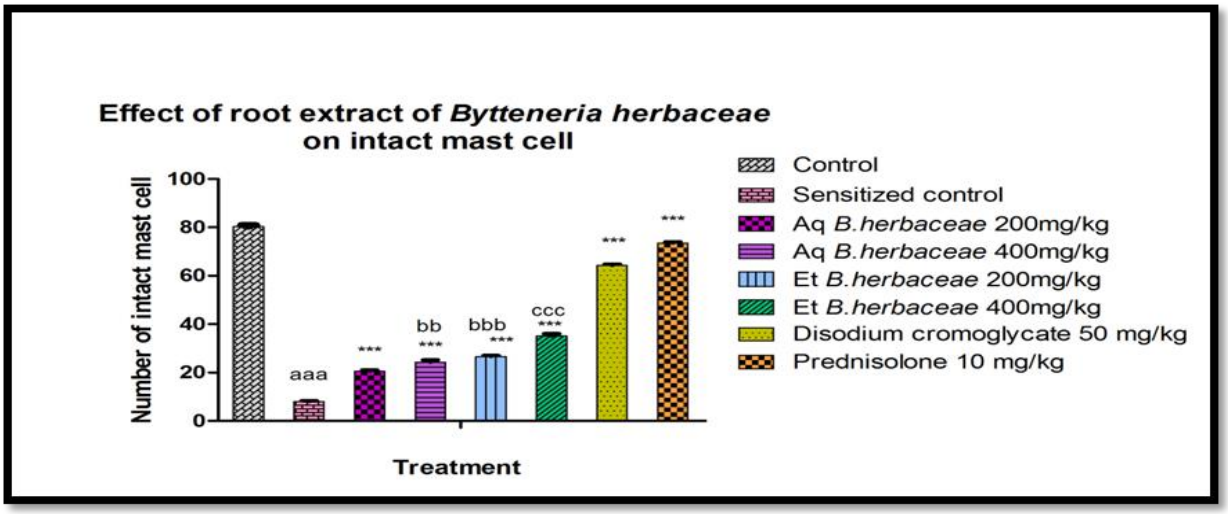

Fig 1: Effect of Aqueous and Ethanolic Extracts of *Bytteneria Herbaceae* Root on Intact Mast Cell.

n=6 Values are expressed as mean ± SEM, one way ANOVA, followed by Tuckey's multiple comparison test, $^{aaa}P<0.001$compared to control, *** $P<0.001$ compared to sensitized control, $^{bb}P<0.01$,$^{bbb}P<0.001$ compared to Aq 200 mg/kg *B.herbaceae*, ^{ccc} $P<0.001$ compared to Et 200 mg/kg and Aq 400 mg/kg *B.herbaceae*, Aq = Aqueous extract, Et= Ethanolic extract, ***B.herbaceae*** = *Bytteneria herbaceae*.

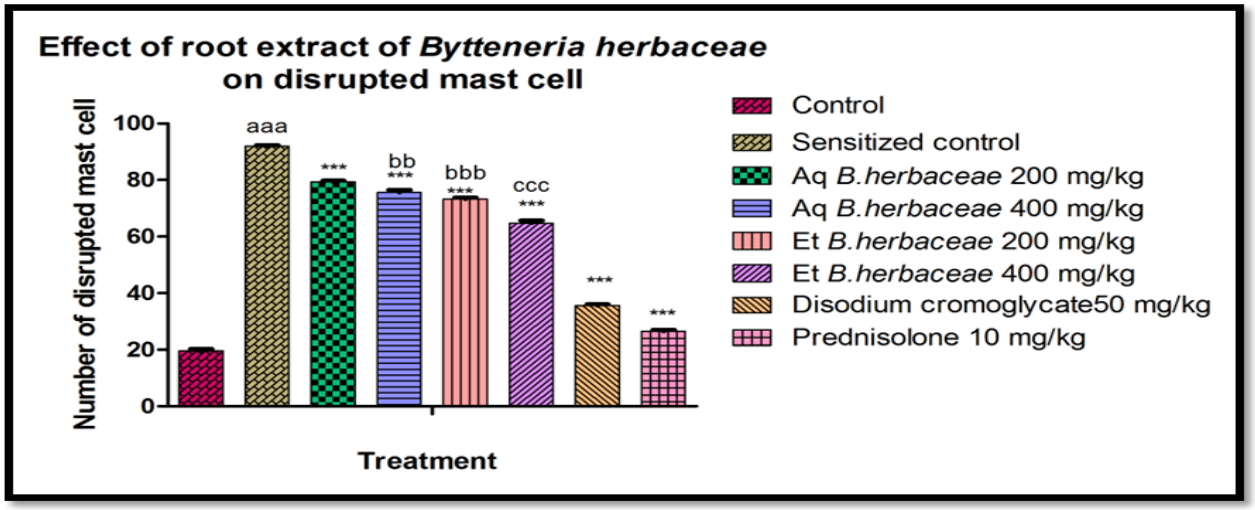

Fig 2: Effect of Aqueous and Ethanolic Extracts of *Bytteneria Herbaceae* Root on Disrupted Mast Cell.

n=6 Values are expressed as mean ± SEM, one way ANOVA, followed by Tuckey's multiple comparison test, $^{aaa}P<0.001$compared to control, ******* $P<0.001$ compared to sensitized control, $^{bb}P<0.01$, $^{bbb}P<0.001$ compared to Aq 200 mg/kg *B.herbaceae,* ^{ccc} $P<0.001$ compared to Et 200 mg/kg and Aq 400 mg/kg *B.herbaceae,* Aq = Aqueous extract, Et= Ethanolic extract, ***B.herbaceae*** = *Bytteneria herbaceae*

Phytochemical investigation of *Bytteneria herbaceae* root extracts indicated the presence of alkaloids, flavonoids, tannins, proteins, steroids, aminoacids, glycoprotein, glycosides and phenolic compounds as shown in table 1 also supported by other study [6].

Asthma is a chronic respiratory disease resulting in increased mucous production and airway hyper responsiveness. Hypersensitivity is an exaggerated immune response that develops after more than one exposed to a given antigen (allergen). Mast cell degranulation is important in the initiation of immediate responses following exposure to allergens.[4] In this study rats were sensitized by 0.5 ml of sheep serum and 0.5 ml triple antigen. Sensitization disrupted the mast cells. The disruption of mast cells is an important feature of anaphylaxis. Increase in the intact mast cells ($P<0.001$) (Fig 1) and When sensitized animals were treated with *Bytteneria herbaceae* (200 and 400 mg/kg, b.w. *p.o.*,) there was a significant reduction in the mast cell disruption (Fig 2) and. Mast cells stabilization of *Bytteneria herbaceae* was dose dependent and ethanolic extract was significantly ($P<0.001$) more potent than aqueous extract. It has been assumed that the process leading to histamine secretion may be mediated by calcium release from an intracellular store of mast cells resulting in disruption. Efficacies of standards were more than extracts at doses used.

Mast cell stabilization was reported in plant *Elephantopus scaber* L, containing chemical constituents such as flavonoids, steroids, carbohydrates, phenolic compounds and tannins [7]. Phytochemical screening of *Bytteneria herbaceae* showed presence of flavonoids, steroids, carbohydrates, tannins and phenolic compounds as shown in table 1. These chemical constituents may be responsible of membrane stabilizing potential of mast cell.

Histamine is an autacoid having profound physiological effect in the body. The contraction of tracheal or bronchial smooth muscle *invitro* has often been utilized for the study of contraction /dilation responses of agonists as well as antagonists. The goat tracheal chain preparation is suitable for screening the activity of extracts on respiratory smooth muscles.

The goat tracheal muscle has H_1, M_3 and β_2 receptors. The stimulation of H_1 receptor causes contraction of bronchial smooth muscle. It is reported that activation of α- adrenergic and H_1 histaminergic receptors causes activation of VIP (Vasoactive Intestinal Polypeptide) in cerebral cortex, which is responsible for release of histamine from sensory neurons [8]. Goat tracheal smooth muscles are contracted by histamine through the H_1 - receptor stimulation. This leads to activation of IP_3 and DAG pathway. This increased IP_3 is responsible for releasing the microsomal calcium, leads to phosphorylation of actin-myosin fibers of goat trachea causing the contraction.

Table 2: Effect of Aqueous Extracts of *Bytteneria Herbaceae* on Histamine Induced Contraction in Isolated Goat Tracheal Chain Preparation

Treatment	Height of contraction in mm	% Response
Histamine 5 µg	6.66 ± 0.2108	51.28 ± 0.8095
Histamine 10 µg	9.33 ± 0.2108	71.88 ± 1.064
Histamine 20 µg	13 ± 0.3651	100 ± 0.00
Histamine 40 µg	13 ± 0.3651	100 ± 0.00
100 µg *B.herbaceae* + 5 µg Histamine	5.33 ± 0.2108***	41.17 ± 1.912***
200 µg *B.herbaceae* + 5 µg Histamine	2.66 ± 0.210***	22.99 ± 0.5774***
400 µg *B.herbaceae* +5 µg Histamine	1.83 ± 0.1667***	15.44 ± 0.4349***
800 µg *B.herbaceae* +5 µg Histamine	0	0

n=6 Values are expressed as mean ± SEM, one way ANOVA, followed by Tuckey's multiple comparison test,$^{***}P<0.001$ compared to 5 µg Histamine, ***B.herbaceae*** = *Bytteneria herbaceae*.

Table 3: Effect of Ethanolic Extracts of *Bytteneria Herbaceae* on Histamine Induced Contraction Activity in Isolated Goat Tracheal Chain Preparation

Treatment	Height of contraction in mm	% Response
Histamine 10 µg	6.66 ± 0.2108	48.89 ± 2.037
Histamine 20 µg	8.66 ± 0.2108	63.55 ± 2.219
Histamine 40 µg	11 ± 0.3651	80.58 ± 2.904
Histamine 80 µg	13.66 ± 0.2108	100 ± 0.00
Histamine 120 µg	13.66 ± 0.2108	100 ± 0.00
100 µg *B.herbaceae* +10 µg Histamine	4.66 ± 0.2108***	34.22 ± 1.876***
200 µg *B.herbaceae* +10 µg Histamine	2.33 ± 0.2108***	17.02 ± 1.404***
400 µg *B.herbaceae* +10 µg Histamine	0	0

n=6 Values are expressed as mean ± SEM, one way ANOVA, followed by Tuckey's multiple comparison test,$^{***}P<0.001$ compared to 10 µg Histamine, ***B.herbaceae*** = *Bytteneria herbaceae*.

In the isolated goat tracheal chain preparation, both aqueous and ethanolic extracts of *Bytteneria herbaceae* antagonized the histamine induced contraction irreversibly (P<0.001) and it was dose dependent. Ethanolic extract was more potent than aqueous extract. Antihistaminic activity of *Pistacia Integerrima* galls was due to the presence of alkaloids, flavonoids, saponins and phenolic compounds. Therefore bronchodilator activity of *Bytteneria herbaceae* may be attributed to its chemical constituents.

CONCLUSION:

Outcome of the study concludes that Bytteneria herbaceae is used for the treatment of bronchial asthma due to its antihistaminic and mast cell stabilizing properties. The data from the present study confirmed that aqueous and ethanolic extract of Bytteneria herbaceae root at doses 200 and 400 mg/kg b.w.p.o., exhibits significant antiasthmatic activity. Treatment with extracts reduced mast cell degranulation in sensitized rats by mast cell stabilization and antagonized the histamine induced contraction in isolated goat tracheal chain preparation. Antiasthmatic activity of Bytteneria herbaceae root is due to the presence of rich content of alkaloids, flavonoids, phenolic compounds, tannins and saponins. Ethanolic extract was more potent than aqueous extract due to increased concentration of active constituents. Further studies are needed to isolate and confirm the individual chemical constituents responsible for the respective activity. Results of the study provide scientific evidence substantiating the traditional use of Bytteneria herbaceae in asthma.

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