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Research Article

Pharmacological Evaluation of Anti-Histaminic activity of *Boerhaavia diffusa*

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ABSTRACT

The present investigation was undertaken to evaluate the anti-histaminic activity of ethanolic extract of *Boerhaavia diffsa* Linn roots (BD) in experimental animals. BD was evaluated for anti-histaminic activity using isolated goat tracheal chain preparation and histamine induced Bronchoconstriction in Guinea pig. BD significantly inhibited dose dependent contraction of goat tracheal chain produced by histamine and also showed significant protection by prolonging Preconvulsion dyspnoea time (PCD) in guinea pigs. Thus, BD showed anti-histaminic and bronchodilating activity against histamine and hence possesses potential role in the treatment of asthma.

Keywords: Anti-histaminic, *Boerhaavia diffusa*, Bronchoconstriction, Chlorphenaramine Maleate.

I. INTRODUCTION

Asthma is a chronic inflammatory lung disease that can cause repeated episodes of cough, wheezing and breathing difficulty. During an acute asthma episode, the airway lining in the lungs becomes inflamed and swollen and excess mucus production occurs in the airway.[1] The prevalence of allergy and asthma has risen in the recent years despite an improvement in the general health of the population. Approximately 300 million people worldwide currently have asthma, with estimates suggesting that asthma prevalence increases globally by 50% every decade. With the projected increase in the proportion of the world‘s urban population from 45% to 59% in 2025, there is likely to be a marked increase in the number of asthmatics worldwide over the next two decades.[2] Although the fundamental causes of asthma are not completely understood, the strongest risk factors for developing asthma are inhaled asthma triggers such as pollens and moulds, tobacco smoke; and Chemical irritants in the workplace.

Large numbers of drugs are used for in the treatment of asthma. However none of them seems to be an ideal drug. The currently used drugs for the treatment of asthma in modern medicine are far from satisfactory as they provide only symptomatic relief, produce several adverse effects and may lose effectiveness on continued use. The search for new drug is still the need of the day. *Boerhaavia diffusa* (Linn.) belonging to the family Nyctaginaceae, contains a large number of such compounds as flavonoids, alkaloids, steroids, triterpenoid, boeravinone A-F.,[3] flavonoids are having anti-inflammatory and antioxidant activity. [4] Alkaloids are having anti-inflammatory and antioxidant activity.[5] Traditionally, the root juice of *Boerhaavia diffusa* is used in treating asthma, scanty urine, and internal inflammation disorders. Therefore, by considering by considering the traditional claim, reported pharmacological activity and chemical constituents present in plant, the need was felt to investigate the antiasthmatic activity of *Boerhaavia diffusa* (Linn.) in laboratory animals.

II. MATERIALS AND METHODS

Procurement of Plant

The roots of *Boerhavia diffusa* Linn. (Family: Nyctaginaceae) were collected from local market

of Pune. The *Boerhavia diffusa* root sample was authenticated and certified by Authentication Service at Agharkar Research Institute, Pune.

Preparation of extract [6]

The extraction of course powdered roots of *Boerhaavia diffusa* (Linn.) was carried by Soxhlation method using 95% ethanol. The extract was then filtered and concentrated. The % yield of ethanolic extract of *Boerhaavia diffsa* Linn roots (BD) was 5.8 gm. (5.8% w/w).

Procurement of Animals

Dunkin-Hartley Guinea pigs weighing 350-400 gm of both sexes were brought from Serum institute, hadapsar, Pune-411028. They were housed under standard laboratory conditions of temperature (25 ± 20C) and 12/12 hr light/dark cycle at least 1 week before experimentation on animals. Animals had free access to standard pellet diet (Amrut laboratory animal feed, Sangli-Maharashtra.) and water ad libitum. Laboratory animal handling and experimental procedures were performed in accordance with the guidelines of CPCSEA. (Approval No: 198/99). Goat trachea was obtained from a slaughter house and kept in Krebs's solution.

Acute Toxicity Study [7, 8]

Albino rats of either sex weighing 200-250 gm were used in the study. Acute oral toxicity was performed as per Organization for Economic Co-operation and Development (OECD)-423 guidelines. There was no behavioral abnormality and zero mortality was recorded till 48 h post treatment with the dose 2000mg/kg, Therefore 1/10th of the dose 2000mg/kg of BD was selected i.e., 200mg/kg as middle dose. Three different doses (100, 200 and 400 mg/kg, p.o) of BD were later chosen for this study based on the acute toxicity testing.

METHODS

Isolated Goat tracheal chain preparation [9, 10]

The goat tracheal tissue was obtained immediately after slaughter of animals. Pieces of trachea were collected in freshly prepared ice-cold oxygenated Kreb's solution (Composition (mM): NaCl, 115; KCl, 4.7; CaCl2, 2; NaHCO3, 25; KH2PO4, 1.2; MgCl2, 1.2; glucose, 11.5). Goat trachea was then cut into individual rings and tied together in series to form a chain. It was suspended in bath containing Kreb's solution and maintained at 37 ± 1ºC, a stream of air was bubbled through the organ tube (1 bubble/sec). One end of the tracheal muscle was attached to S-shaped aerator and the other attached to isotonic frontal writing lever to a drum. The tissue was allowed to equilibrate for 45 min under a load of 400 mg. A dose response curve for histamine was recorded at variant molar concentrations by maintaining 15 min time cycle. After obtaining dose response curve of histamine (30ug/ml) on trachea, the BD (100 µg/ml) was added to reservoir and same doses of histamine were repeated. Graph of percentage of maximum contractile response on ordinate and negative log of molar concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and in presence of BD and standard drug Chlorpheniramine maleate (1 µg/ml)[15&16.]

Histamine induced Bronchoconstriction in Guinea pig[11]

Overnight fasted guinea pigs were randomly divided into five groups (n=5). Prior to drug treatment, each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The Preconvulsive dyspnea time (PCD) was noted for each animal. The Preconvulsive dyspnoea time is the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion. As soon as preconvulsive dyspnea commenced, animals were removed from the chamber and placed in fresh air to recover from dyspnea for 24 hours. This time for preconvulsive dyspnea was recorded as basal value. After 24 hours, animals belonging to group I served as control and were administered with phosphate buffer (1ml/kg, p.o.); Animals belonging to group II were administered with Chlorpheniramine maleate (2 mg/kg, i.p.) while group III to V were received respective doses of BD. These animals were again subjected to histamine aerosol later at an interval of 1 hr, 4 hr and 24 hr and to determine Preconvulsive dyspnea time (PCD). The protection offered by the treatment was calculated by using the following formula:

$$\% \text{ protection} = (1 - T1/T2) \times 100$$

T1 =the mean of PCT before administration of test drugs.
T2 = the mean of PCT after administration of test drugs at 1 hr, 4 hr and 24 hrs.

Statistical Analysis

The results of various studies were expressed as mean ± SEM and analyzed statistically using one way ANOVA followed by Student's t-Test to find out the level of significance. Data were considered statistically significant at minimum level of $p < 0.05$.

III. RESULTS AND DISCUSSION

Effect of Boerhaavia diffusa extract on histamine induced contraction of isolated goat tracheal chain preparation

In the present study, histamine (30µg/ml) produced dose dependent contraction of goat tracheal chain preparation maximum percentage of contractile

response versus negative log molar concentration of histamine.

The modified physiological salt solution containing Chlorpheniramine maleate (1 µg/ml) significantly inhibited (p<0.01) the contractile effect of histamine. The modified physiological salt solution containing ethanolic extract of *Boerhaavia diffusa* (100 µg/ml) significantly inhibited (p<0.01) the contractile effect of histamine. Hence Chlorpheniramine maleate and ethanolic extract of *Boerhaavia diffusa* (100 µg/ml) shifted the DRC of Histamine towards the right side indicating that there was competitive antagonism between histamine and both the drugs for histaminergic receptors. (Table 1).

Effect of ethanolic extract of Boerhaavia diffusa on histamine induced Bronchoconstriction in guinea pigs

The guinea pigs when exposed to 0.2 % w/v histamine aerosol showed signs of progressive dyspnoea leading to convulsions. Chlorpheniramine maleate (2 mg/kg, i.p) significantly prolonged (p<0.01) the preconvulsive dyspnoea in 1st, 4th and 24th hr as compared to control and the percent % protection observed was 93.97, 78.37 & 65.60 respectively. The ethanolic extract of roots of *Boerhaavia diffusa* at doses of 100 mg/kg (p<0.05) and at the dose of 200 and 400 mg/kg p.o (p<0.01) significantly prolonged the preconvulsive dyspnoea at 1st, 4th hr and 24 hr as compared to control. Thus showed more protection against preconvulsive dyspnoea as compared to control, following exposure to histamine aerosol. The percent protection observed for BD at the dose of 100 mg/kg was 48.51, 59.94 & 41.55 at 1st, 4th and 24th hr respectively. The percent protection observed for ER at the dose of 200 mg/kg was 70.25, 66.63 & 65.74 in 1st, 4th and 24th hr respectively. The percent protection observed for ER at the dose of 400 mg/kg was 73.08, 69.02& 68.82 in 1st, 4th and 24th hr respectively. (Table 2).

DISCUSSION

Asthma is a chronic inflammatory disorder of airways. In allergic asthma, the body initiates an immune response to an allergen such as pet dander, dust-mites, mold or pollen that results in IgE antibodies. Mediators include histamine, prostaglandins and leukotrienes. Additionally, the reaction can be enhanced by the recruitment of eosinophils. Ultimately the mediators promote vascular permeability, smooth-muscle contraction and mucus production, which cause symptoms of asthma including airway constriction, coughing, shortness of breath and wheezing.

Histamine contracts the trachea-bronchial muscle of guinea pig, goat, horse, dog and man.[13] Goat tracheal chain is easier to handle and also much more sensitive than guinea pig tracheal chain. Spasmogens such as histamine (0.1-102.4 mg), acetylcholine (0.1-12.8µg) and barium chloride (0.1-51.2 mcg) show dose dependent contraction on goat tracheal chain preparation.[9] Histamine antagonism is modulated by using the relaxing factors and may be due to the suppression of histamine H_1-receptors. 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. In the present study, the potential of ethanolic extract of *Boerhaavia diffusa* has antagonized the histamine induced contractions on goat tracheal chain preparation which have shown a significant relaxation indicated by right shift of DRC of histamine.

Histamine is one of the major inflammatory mediators in the immediate phase of asthma, causing airway hyper responsiveness and bronchial airway inflammation. The study regarding involvement of H_1 and H_2 receptors has been done in guinea pigs using respiratory smooth muscle and it was confirmed that there is prominent involvement of H_1 receptors as compared to H_2 receptors which when stimulated worsening the condition of asthma. Histamine when inhaled has been shown to induce bronchoconstriction by direct H_1-receptor activation[14, 15] The guinea pigs exposed to histamine aerosol showed signs of progressive dyspnoea i.e. difficulty in breathing, leading to convulsions. In the present study, treatment with ethanolic extract of *Boerhaavia diffusa*, showed significant prolongation in preconvulsive dyspnoea time but prolongation was found to be less as compared to standard Chlorpheniramine maleate. The effect may be due to its H_1- receptor blocking or bronchodilating activity and thus may contribute in the management of asthma.

IV. CONCLUSION

It can be concluded that ethanolic extract of roots of *Boerhaavia diffusa* may possesses antihistaminic activity which may be due to H_1- receptor blocking or bronchodilating activity. Thus *Boerhaavia diffusa* roots may be used in the management of asthma.

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Table 1: Effect of *Boerhaavia diffusa* extract on histamine induced contraction of isolated goat tracheal chain preparation

S. No	Dose of Histamine (30µg /ml) (ml)	- ve Log molar concentration of Histamine	% Maximum Contraction		
			Control	Test	Standard
1	0.1	6.38	21.24 ± 0.89	16.52 ± 1.02	9.64 ± 0.69
2	0.2	5.91	48.14 ± 1.75	36.91±1.32**	20.80 ± 1.04**
3	0.4	5.54	61.34 ± 2.03	49.33±2.94**	33.78±1.12**
4	0.8	5.21	76.49 ± 1.24	65.66±1.58**	41.99±1.19**
5	1.6	4.89	89.69 ± 2.39	75.48±0.94**	54.25±1.33**
6	3.2	4.59	100 ± 0.00	79.26±2.10**	58.55±2.03**

Control = D.R.C. of histamine (30µg /ml) in absence of *Boerhaavia diffusa* (100 µg/ml) extract; **Test** = D.R.C. of histamine (30µg /ml) in presence of *Boerhaavia diffusa* (100 µg/ml) extract; **Standard** = D.R.C. of histamine (30µg /ml) in presence of Chlorpheniramine maleate (1 µg/ml)

Table 2: Effect of ethanolic extract of *Boerhaavia diffusa* on histamine induced Bronchoconstriction in guinea pigs

Groups (n = 5)	Preconvulsive dyspnea (in Sec) (Mean ± SEM) at				Percent protection (%)		
	Before treatment	After treatment					
		1 hr	4hr	24hr	1 hr	4hr	24hr
I (Control)	49.75± 1.57	50.40± 1.58	46.34± 0.85	53.10± 1.22	---	----	---
II (Std)	48.29± 1.89	800.83± 36.80**	223.32± 5.59**	140.38± 10.72**	93.97	78.37	65.60
III (BD100)	49.04± 0.89	95.24± 5.48*	122.41± 2.33**	83.90± 6.13*	48.51	59.94	41.55
IV (BD200)	45.55± 1.08	153.09± 10.26**	136.51± 4.29**	132.94± 7.36**	70.25	66.63	65.74
V (BD400)	50.08± 0.92	186.04± 12.29**	161.68± 6.43**	160.60± 4.23**	73.08	69.02	68.82

Group- I (Control) = Aerosolized Histamine (0.2 % w/v); **Group-II (Std)** = Aerosolized Histamine (0.2 % w/v) + Chlorpheniramine maleate (2 mg/kg, i.p.); **Group-III (BD-100)** = Aerosolized Histamine (0.2 % w/v) + Ethanolic extract of *Boerhaavia diffusa* (100 mg/kg, p.o.); **Group-IV (BD-200**) = Aerosolized Histamine (0.2 % w/v) + Ethanolic extract of *Boerhaavia diffusa* (200 mg/kg, p.o.); **Group-V (BD-400)** = Aerosolized Histamine (0.2 % w/v) + Ethanolic extract of *Boerhaavia diffusa* (400 mg/kg, p.o.)

REFERENCES

1. Maddox L and Schwartz DA. The Pathophysiology of Asthma. Annu Rev Med. 2002; 53:477-498.
2. Masoli M, Fabian D and Holt S. Global Initiative for Asthma (GINA) program: the global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy. 2004;59:469-478.
3. Masoli M, Fabian D and Holt S. Global Initiative for Asthma (GINA) program: the global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy. 2004;59:469-478.
4. Raj Narayana K, Sripal Reddy M and Chaluvadi MR. Bioflavonoids classification, pharmacological, biochemical Effects and therapeutic potential. Indian J of Pharmacol. 2001;33:2-16.
5. Maiza-Benabdesselam F, Khentache S and Bougoffa K. Antioxidant activities of alkaloid extracts of two Algerian species of Fumaria : Fumaria capreolata and Fumaria bastardii Rec. Nat Prod. 2007;(1)2-3:28-35.
6. Mehrotra S, Singh VK and Maurya R. Antilymphoproliferative Activity of Ethanolic Extract of Boerhaavia diffusa Roots. Experimental and Molecular Pathology. 2002;72:236–242
7. OECD Guideline For The Testing of Chemicals: Acute oral toxicity-Acute Toxic Class Method; 2001; 423.
8. Ghosh MN. Toxicity studies. In: Fundamentals of experimental pharmacology. 2nd ed. scientific book agency; 1984;153-55.
9. Kulshreshtha S, Misra SS and Sharma AL. Response of the goat trachea to some autonomic drugs. Indian J Pharmacol. 1983;15:107-110.

10. Nag Chaudhari AK and Lahiri SC. Use of goat trachea isolated tracheal chain preparation. Indian J Pharmacol. 1974; 6:149-151.
11. Pandit P, Singh A and Bafna AR. Evaluation of Antiasthmatic activity of curculigo orhioides gaerth. Rhizomes. Indian journal of Pharmaceutical sciences. 2008;440-444.
12. Mitra R and Gupta RC. Punarnava – An Ayurvedic drug of repute. Economic Botany Information Service, National Botanical Research Institute, Lucknow, Uttar Pradesh, India. Appl Bot Abstr. 1997;17(3):209-227.
13. Ehright T and Chua S. Pulmonary eosinophilic syndromes. Ann allergy. 1989;62:277-283.
14. Sheth UK, Dadkar NK and Kamath NG. Selected topics in experimental pharmacology. Bombay: Kothari Book Depot, India. 1972;5:63.
15. Thosar A, Mayee R and Kondapure A. Evalution of antiasthmatic activity of calotropis gigantea roots. Asian J Pharm Clin Res. 2011;4(2):3335.