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Effect of *Boerhaavia diffusa* on Experimental Induced Model of Asthma in Mice

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Jay H. Shah[1*], Dr. I. S. Anand[1], Dr. K. K. Shah[1]

1 Faculty of Pharmacy, Hemchandracharya North Gujarat University, Patan, Gujarat, India

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ABSTRACT

Asthma is a chronic inflammatory disorder characterized by airway inflammation and airway hyper-responsiveness to inhaled stimuli. The production and release of proinflammatory cytokines (TNF-α, IL-1β and IL-6) from monocytes/macrophages play critical role in the pathogenesis of chronic inflammatory diseases and immune response. *Boerhaavia diffusa* is a well-known medicinal plant in the Indian traditional system with immunomodulatory action. In present study, the anti-asthmatic activity of herbal immune modulator *Boerhaavia diffusa* was studied. Trypsin and egg albumin induced chronic model of asthma was used to evaluate the anti-asthmatic action of *Boerhaavia diffusa* in experimentally induced asthma in mice. Various parameters such as pO2, serum bicarbonate level, tidal volume, respiratory rate, eosinophil count in bronchoalveolar lavage fluid etc. were measured after the treatment with methanolic extract of *Boerhaavia diffusa* compared to control. The results of present study were in line with expected responses observed with standard anti-asthmatic agents. Thus, the present study has shown that *Boerhaavia diffusa* shows a beneficial effect in trypsin and egg albumin induced experimental model of asthma in mice and thereby suggesting its possible use in the treatment of asthma.

INTRODUCTION

Asthma is a chronic inflammatory disorder characterized by airway inflammation and airway hyper-responsiveness to inhaled stimuli. It is a very common disease with immense social impact which afflicts 300 million individuals worldwide [1]. The physiologic and clinical features of asthma derive from an interaction among the resident and infiltrating inflammatory cells in the airway surface epithelium, inflammatory mediators, and cytokines [2]. Cytokines are biologically highly potent peptides endogenously synthesized upon stimulation and they direct and modify the inflammatory response in asthma and likely determine its severity. Key cytokines include IL-1β (Interleukin-1β) and TNF-α (Tumor necrotic factor α), which amplify the inflammatory response [1].

Boerhaavia diffusa, belonging to the family of the Nyctaginaceae, is mainly a diffused perennial herbaceous creeping weed of India (known also under its traditional name as Punarnava). The root, leaves, aerial parts or the whole plant of *Boerhaavia diffusa* have been employed for the treatment of various disorders in the Ayurvedic herbal medicine. It is scientifically documented for its immunomodulatory [3] and immunosuppressive [4, 5], Anti-hepatotoxic and hepato-protective [6], anti-inflammatory [7], diuretic [8], anti- convulsions [9] activities. It is also reported to relieve muscular pain [10].

Animal models of asthma have been extensively used to examine mechanisms of disease, the activity of a variety of genes and cellular pathways, and to predict the safety of new drugs or chemicals before being used in clinical studies [11]. Asthma involves complex physiological process and as animals are biologically similar to human, animal usage is well established [12].

We undertook a study to evaluate the anti-asthmatic potential of methanolic extract of *Boerhaavia diffusa* (MEBD) in trypsin and egg albumin induced experimental model of asthma in mice.

MATERIALS AND METHODS

Animals: Healthy mice of either sex, weighing 25-30 gms were used for the animal experiment. The animals were housed at 25^0C; 12 hours light-dark cycles, in polypropylene cages with free access to food and water ad libitum during the course of experiment. The protocol was approved by IAEC (Institutional Animal Ethics Committee), K. B. Institute of

Pharmaceutical Education and Research, under the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guideline before carried out the project.

Study design: A combination of trypsin and egg albumin was used to induce asthmatic status in mice [13]. The animals were divided into 4 groups (n = 6). All animals (except group I) were exposed to trypsin aerosol (1 mg/ml, 1 ml/min) once daily for 5 min, followed by a rest of 2 hrs and then exposed to egg albumin aerosol (1% m/V, 1 ml/min) for 3 min. This procedure was repeated for 10 days and later egg albumin aerosol was discontinued whereas trypsin exposure was continued until the 21st day. The animals of group I were exposed to aerosol of saline for 5 minutes daily. Group II animals were exposed to trypsin and egg albumin but did not receive any drug treatment and they served as asthmatic control. After animals developed asthma, animals of group-III received Dexamethasone (5 mg/kg, *per oral*), animals of group-IV received MEBD (400 mg/kg, *per oral*) treatment from day 22 to day 35. On day 35, after 2 hrs of the last dose of treatment, only egg albumin challenge was given.

On day 1 (baseline) before any exposure, on day 21 (after trypsin exposure) and on day 35 (after treatment) the following parameters were measured for each animal: pO_2, Tidal volume, Respiratory rate, serum bicarbonate. On day 35, in addition to above parameters, Eosinophil count in BAL (Broncheo alveolar lavage) fluid and histopathology of lung tissue was done.

Measurement of pO2: The measurement of arterial O_2 tension (pO_2) was done with the help of pulse oxymeter [14, 15].

Measurement of serum bicarbonate level: About 1 to 2 ml of blood was collected from each animal under anesthesia after 1 hr of the exposure to egg albumin. The serum was separated from blood. For bicarbonate level measurement, 10 ml of 1.0 gm/dl saline was pipetted out in 100 ml beaker. To this, 0.1 ml of the serum and 2 drops of phenol red indicator was added and mixed well. In above mixture, 0.01 N NaOH was added dropwise till the endpoint was achieved (7.35 pH or color changed from yellow to pink). The volume of NaOH required was noted down and considered as control reading (X ml). In another set, 9.0 ml of 1 gm/dl saline was pipetted out in another 100 ml beaker, 0.01 N 1 ml HCl was added and mixed well. The above procedure was repeated and volume of NaOH required was noted (Y ml). Serum bicarbonate level (m. Eq/ltr) was measured based on the readings [16].

Measurement of tidal volume and respiratory rate: Tidal volume and respiratory rate were measured with the help of 'Respiratory volume transducer' that was used with 'strain gage coupler' and student physiograph.

Eosinophil count in BAL fluid: On 35th day of the study, the tracheobronchial tree was lavaged with 1 ml of saline 3 to 4 times. The fluid was collected and centrifuged at 2000 rpm for 5 min. The supernatant was discarded and the pellet was re-suspended in 0.5 ml saline. A thin film of 0.5 ml suspended saline was made on a clean grease-free slide using a smooth edged spreader. The film was fixed with methyl alcohol for 3 to 5 min and dried. A few drops of Geimsa stain in phosphate buffered saline (pH 6.8) were added and kept for 15 mins. The number of each type of leukocytes was determined under the microscope at 450x magnification [17].

Histopathology of lungs: On 35th day, the animals were sacrificed and lungs were dissected out. The procedure used for histopathological study was fixation of the tissue with formalin, embedding in paraffin blocks, sectioning with microtome (0.7 µ thickness) and finally stained by haematoxylin and eosin stain technique [18].

Statistical analysis: Experimental results were expressed as mean + SEM (Standard error of mean) for 6 animals. The results were computed statistically. Statistical significance of the difference in parameters amongst groups was determined by one-way ANOVA (Analysis of variance) followed by post hoc test. Paired t-test was also performed.

RESULTS AND DISCUSSION

In vivo study was conducted to establish the efficacy of MEBD in experimental model of asthma in mice. The findings of trypsin and egg albumin induced experimental mice model of asthma measuring tidal volume, serum pO2 level, respiratory rate, serum bicarbonate level, eosinophil count as well as observations from histopathological studies were in line with expected responses observed with standard anti-asthmatic agents.

Tidal volume is the volume of air inspired or expired per breath. As asthma is an obstructive disease, there is a difficulty in expiration resulting in reduction of the volume of air expired. In addition, there is shallow and rapid breathing thus decreasing the tidal volume and simultaneously increasing the respiratory rate. The lungs do not provide adequate respiratory exchange due to constricted air flow volume and the levels of oxygen in the blood begin to

fall. In the present study, a lower tidal volume was observed in asthmatic group compared to normal control group after trypsin exposure from baseline. There was a significant ($p<0.05$) increase in tidal volume in the animals subjected to dexamethasone and MEBD (figure 1).

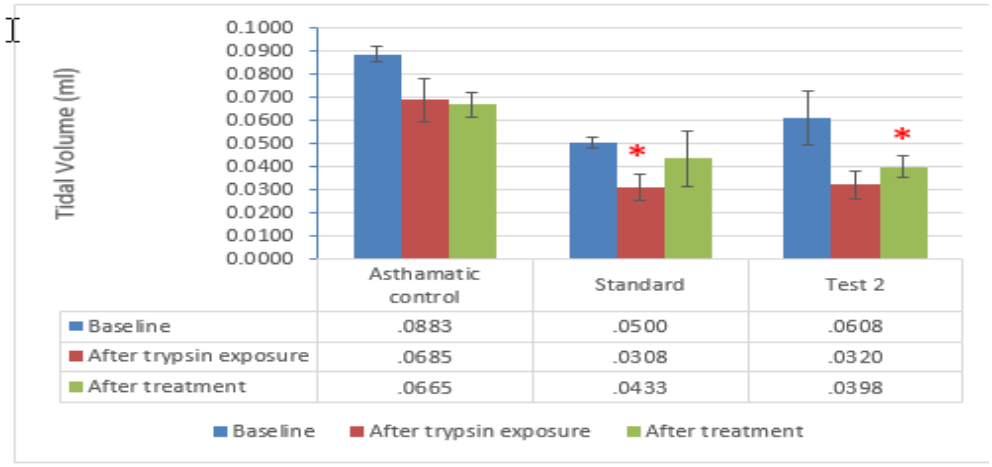

Figure 1: Effect of Dexamethasone and MEBD on tidal volume

Standard = dexamethasone, test 2= Methanolic extract of *Boerhaavia diffusa (MEBD)*

*Significant difference from 21st day ($p<0.05$) (paired t-test)

Similarly, significant ($p<0.05$) higher respiratory rate was observed in asthmatic group compared to normal control group after trypsin exposure from baseline. Significantly ($p<0.05$) lower respiratory rate was observed in dexamethasone and MEBD treated groups compared to asthmatic control group (figure 2).

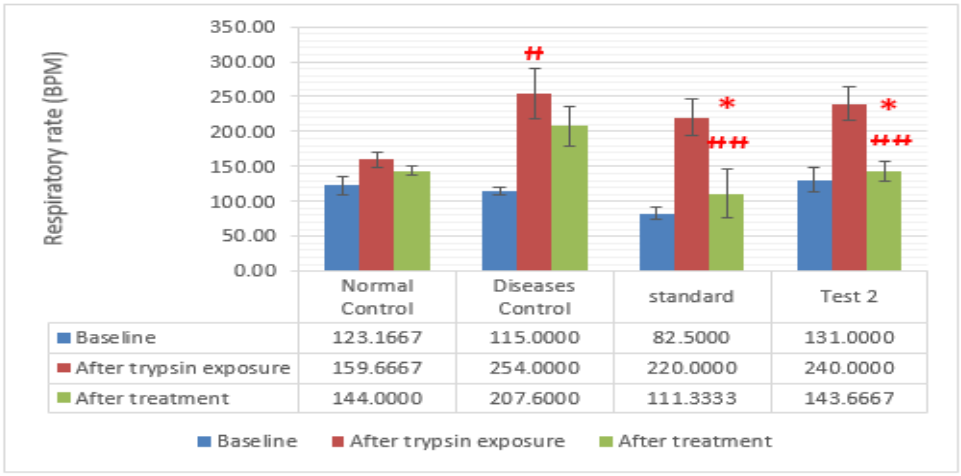

Figure 2: Effect of Dexamethasone and MEBD on Respiratory rate

Standard = Dexamethasone, test 2= *M*ethanolic extract *of Boerhaavia diffusa* (MEBD)

#Significant difference from baseline ($p<0.05$) (paired t-test)

*Significant difference from 21st day ($p<0.05$) (paired t-test)

##Significant difference from asthmatic control group ($p<0.05$) (One way ANOVA followed by post hoc test)

Further, in patients with asthma, analysis of blood gases reveals a severe hypoxemia with arterial oxygen (pO_2) lower than 60 mmHg [19]. In the present study, the sensitized animal when challenged with trypsin and egg albumin, showed a significant ($p<0.001$) lower serum pO_2 level compared to normal control animals similar to that of observed in patients suffering from asthma. pO2 level was significantly higher ($p<0.001$) in the animals subjected to dexamethasone and MEBD compared to asthmatic control group (figure 3) indicating improvement in condition.

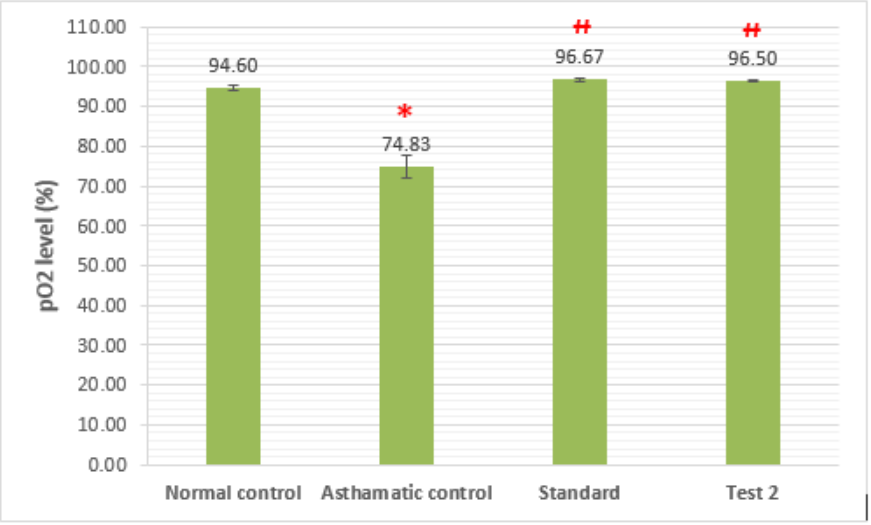

Figure 3: Effect of Dexamethasone and MEBD on pO2 level

Standard = Dexamethasone, test 2= *M*ethanolic extract of *Boerhaavia diffusa* (MEBD)

* Significant difference from normal control group ($p<0.001$)

Significant difference from asthmatic control group ($p<0.001$)

(One way ANOVA followed by post hoc test)

Additionally, among patients with asthma, as severity of airflow obstruction increases, pCO_2 first normalizes and subsequently increases. Increased pCO_2 level in serum will eventually result in increased bicarbonate levels because carbon dioxide in blood is transported as bicarbonates [20]. In present study, challenging animals with trypsin and egg albumin showed a significant higher serum bicarbonate level ($p<0.05$) in asthmatic control group as compared to normal control group. Serum bicarbonate level was significantly lower ($p<0.05$) in the animals subjected to Dexamethasone and MEBD (figure 4).

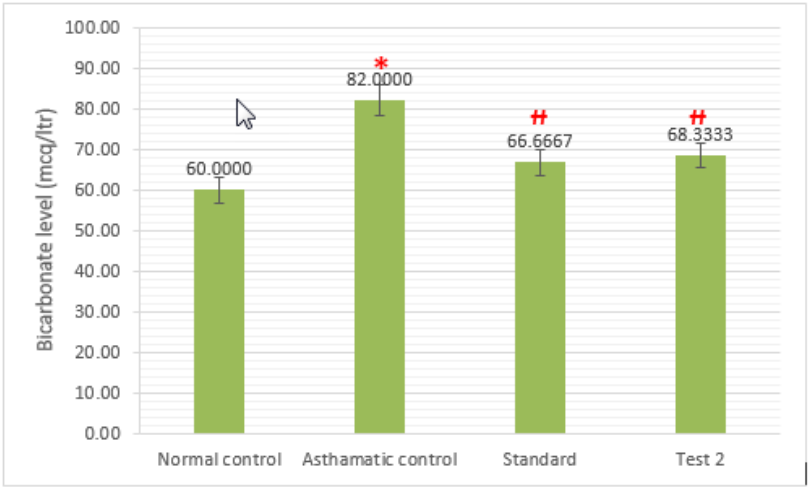

Figure 4: Effect of Dexamethasone and MEBD on serum bicarbonate level

Standard = Dexamethasone, test 2= *M*ethanolic extract of *Boerhaavia diffusa* (MEBD)

*Significant difference from normal control group ($p<0.05$)

#Significant difference from asthmatic control group ($p<0.05$)

(One way ANOVA followed by post hoc test)

Eosinophils are bone marrow derived granulocytes that play a central role in asthma. Increased numbers of eosinophils exist in the airways of most persons who have asthma [21]. An allergic reaction in the airways, caused by natural exposure to allergens, has been shown to lead to an increase in eosinophils in bronchoalveolar lavage [22]. Increases in eosinophils often correlate with greater asthma severity. In present study, challenging animals with trypsin and egg albumin showed a significant higher eosinophil count ($p<0.05$) in asthmatic control group as compared to normal control group. Eosinophil count was significantly lower ($p<0.05$) in the animals subjected to Dexamethasone and MEBD (figure 5).

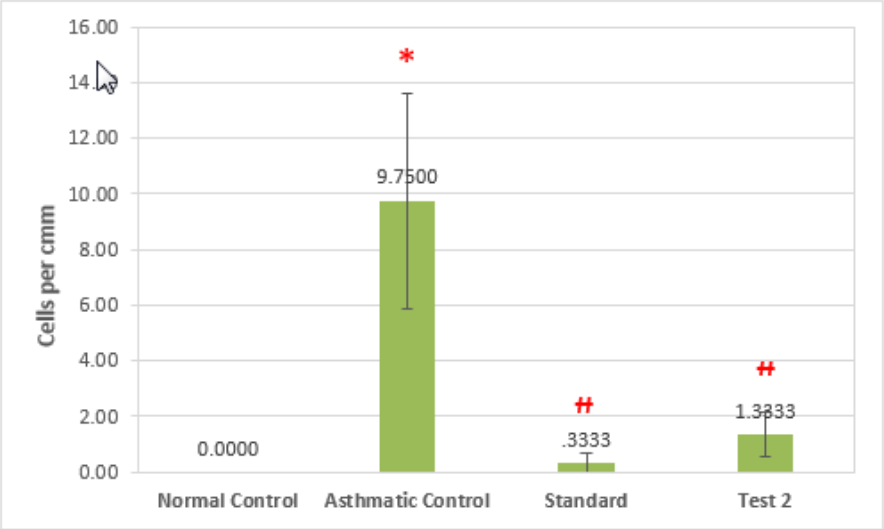

Figure 5: Effect of Dexamethasone and MEBD on eosinophil count

Standard = Dexamethasone, test 2= *M*ethanolic extract of *Boerhaavia diffusa* (MEBD)

*Significant difference from normal control group ($p<0.05$)

#Significant difference from asthmatic control group ($p<0.05$)

(One way ANOVA followed by post hoc test)

In asthma, the major physiological event leading to clinical symptoms is airway narrowing. Bronchial smooth muscle contraction (bronchoconstriction) occurs to narrow the airways in response to exposure to a variety of stimuli including allergens or irritants [23] which also cause decrease in the lumen size of the bronchiole. In present study, histopathological studies revealed an intact bronchial structure in normal control group (figure 6.a), whereas trypsin and egg albumin-sensitized animals showed Small (constricted) bronchus with inflammation (figure 6.b). In dexamethasone and MEBD treated animals, it showed near normal bronchus and alveoli with minimal inflammation with near normal cellular architecture (figure 6.c and figure 6.d).

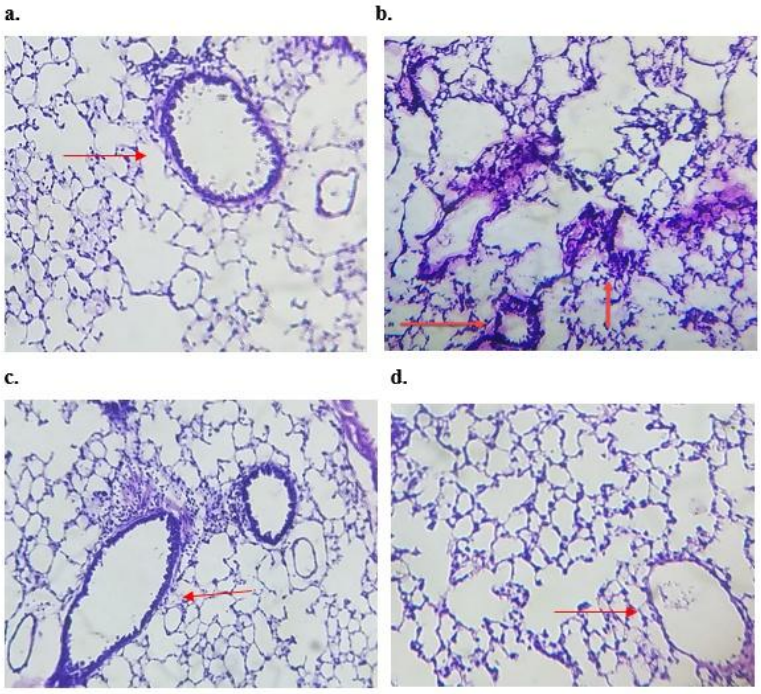

Figure 6: Photographs of tissue sections of lung isolated from mice (100x)

a = Normal control group, b = Asthmatic control group, c = Dexamethasone treated group, d = *M*ethanolic extract of *Boerhaavia diffusa* (MEBD) treated group

CONCLUSION

The results obtained in the present study have shown that MEBD shows a beneficial effect in trypsin and egg albumin induced experimental model of asthma in mice and thereby suggesting its possible use in inflammatory diseases such as Asthma. However, further detailed studies are required to establish its clinical relevance/therapeutic potential.

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