Global Journal of Pharmacology 5 (1): 40-49, 2011
ISSN 1992-0075

Evaluation of the Effect of *Onosma bracteatum*, Wall (Boraginaceae) Using Experimental Allergic and Inflammatory Models

Kalpana G. Patel, Jaysukh R. Detroja, Tanmay A. Shah, Kirti V. Patel and Tejal R. Gandhi

Anand Pharmacy College, Shri Ram Krishna Seva Mandal Campus,
Near Townhall, Anand, Gujarat, India

Abstract: Allergic disease such as allergic asthma, is a hypersensitivity reaction initiated by immunological mechanism, the primary pathophysiological abnormality being bronchial wall inflammation leading to airway narrowing. *Onosma bracteatum* (*O. bracteatum*) is known traditionally in Ayurveda to possess anti-asthmatic activity. Hence, the present investigation was undertaken to evaluate the potential effect of dried ethanolic extract of aerial parts of *O. bracteatum* on experimental allergic reactions and inflammation. Experimental models studied were vascular permeability induced by acetic acid, carrageenan induced hind paw edema, passive paw anaphylaxis, passive cutaneous anaphylaxis and allergic pleurisy. Pretreatment with ETOB (59.77 and 68.72% inhibition at 5 and 10 mg/kg doses) significantly ($P<0.05$) inhibited vascular leakage induced by acetic acid. Oral pre-treatment with ethanolic extract of *O. bracteatum* also inhibited carrageenan induced paw edema (52.79 and 74.79% at 5 h., $P<0.05$) and allergen induced edema (65.95 and 73.04% at 1 h., $P<0.05$) at doses of 5 and 10 mg/kg. Pretreatment with ETOB (5 and 10 mg/kg, p.o.) as compared to control group significantly ($P<0.05$) inhibited PCA by 21.99 and 48.49% and also significantly inhibited the eosinophil accumulation (57.75 and 77.75%, $P<0.05$) in the pleural cavity, respectively. These findings demonstrated that the dried ethanolic extract from the aerial parts of *O. bracteatum* possess significant antiallergic and anti-inflammatory activities. This activity may be mediated by inhibiting plasma exudation, reducing the release of mediators such as histamine, inhibition of mast cell degranulation and inhibition of eosinophil accumulation thereby preventing the release of cytokines and chemokines.

Key words: *Onosma bracteatum* · Allergy · Inflammation · Vascular permeability · Mast cell · IgE

INTRODUCTION

Allergic asthma, recognized as immediate-type hypersensitivity reaction characterized by increased responsiveness of the tracheobronchial tree to a variety of stimuli, which may be spontaneous, allergen-related or drug-induced and the primary pathophysiological abnormality being bronchial wall inflammation leading to airway narrowing [1, 2]. Studies using bronchoalveolar lavage (BAL) and bronchial biopsies have demonstrated that a variety of cells and mediators are involved, with IgE and mast cells implicated in the acute response, eosinophils and eosinophil granule proteins in the late response and with T cells, particularly Th2 cells, orchestrating these responses through their production of cytokines such as IL-4, IL-5, IL-9 and IL-13 [3, 4].

The prevalence and severity of allergic asthma has been steadily increasing over past 20 years together with an estimated 300 million individuals affected across the globe [5 - 7]. The disease statistics clearly necessitates the increasing need for drugs targeting the mechanisms involved in eosinophil and neutrophil activation and accumulation, anti-IgE therapy for the management of asthma. Additionally, currently available inhaled bronchodilators and antiinflammatory drugs are effective in most asthmatics, but this palliative therapy requires long term daily administration and is associated with major limitations owing to low efficacy, associated adverse events and compliance issues [8]. As a result there is high prevalence of usage of herbal medicine for treatment of this disease [9].

Corresponding Author: Kalpana Patel, Anand Pharmacy College, Shri Ram Krishna Seva Mandal Campus,
Near Townhall, Anand, Gujarat, India, Fax: 02692-250020, E-mail: kalpana_jpatel@yahoo.com.

Onosma bracteatum, Wall (Family Boraginaceae, commonly known as Gaozaban, Gojihva) is one such medicinal plant known traditionally in Ayurveda for the treatment of asthma and bronchitis [10, 11]. Aerial parts of *Onosma bracteatum* (*O. bracteatum*) are prescribed by Ayurvedic practitioners in bronchial asthmatic patients. Preliminary studies performed at our laboratory has established the efficacy of ethanolic extract of aerial parts of *O. bracteatum* in decreasing bronchial hyperresponsiveness and bronchoconstriction through systematic studies on bronchoalveolar lavage, acetyl choline and histamine induced bronchospasm, histamine estimation in lungs and lung cytology in guinea pigs [12]. The pathophysiology of asthma involves multiple factors leading to hyperresponsiveness and bronchoconstriction, such as mast cell degranulation [13], neurogenic dysfunction [14], involvement of T-lymphocytes and eosinophils [15, 16], altered immunosuppressive macrophages [17, 18], overproduction of proinflammatory cytokines [19, 20] and immunoglobulins (IgE, IgG & IgD) [21], plasma protein exudation [22]. Therefore, the present work aimed to prove the antiallergic and anti-inflammatory activity based on studies focusing upon inflammation of the airway and the immune responses at cellular and molecular levels.

MATERIALS AND METHODS

Plant: Aerial parts of *O. bracteatum* were obtained from commercial supplier of Ahmedabad. The plant was identified and authenticated at the Department of Bioscience, Veer Narmad South Gujarat University, Surat, Gujarat.

Preparation of the Plant Extract: The aerial parts reduced to coarse powder were macerated with ethanol for 48 hrs and filtered. Combined extracts from the three consecutive extractions were evaporated under reduced pressure to obtain dry extract. The extract was stored in cool and dry place and used for pharmacological evaluation. This ethanolic extract of aerial parts of *O. bracteatum* will be referred as ETOB in the following text (extractive value 2% w/w) (ethanolic extractive value 1 %w/w).

Reagents: Egg albumin and aluminum hydroxide hydrate gel (alum) were purchased from S D Fine-Chem Limited, India. Freund's adjuvant emulsion, carrageenan and evans blue were purchased from HiMedia, India. All other chemicals used were of analytical grade.

Animal Protocol: Swiss albino mice and wistar albino rats of either sex housed in standard conditions of temperature (22 ± 2°C), relative humidity (55 ± 5 %) and light (12 hrs light/dark cycles) were used. Animal studies were approved by the Institutional Animal Ethics Committee (protocol no 6012 and 8004), as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Vascular Permeability Induced by Acetic Acid [23, 24]: Swiss albino mice were randomly allocated to four groups each containing six animals. Group I (model control), Group II (standard), Group III (ETOB-5) and Group IV (ETOB-10) received saline, Indomethacin (20 mg/kg), ETOB (5 mg/kg) and ETOB (10 mg/kg) orally, respectively. Thirty minutes later, mice received an intravenous injection of 0.5% Evans blue saline solution (0.1 ml/10 g body weight) and an intraperitoneal injection of 0.6% acetic acid (10 ml/kg). After 20 min. the dye that leaked into the peritoneal cavity was collected by lavaging with 10 ml distilled water and was transferred to 10 ml volumetric flask through glass wool. To each flask, 0.1 ml of 0.1 N sodium hydroxide solution was added and volume made upto the mark with distilled water followed by measurement of absorbance at 610 nm (Shimadzu Double beam Spectrophotometer 1650 PC).

Carrageenan-Induced Paw Edema: Wistar albino rats were randomly allocated to four groups each containing six animals. Group I (model control), Group II (standard), Group III (ETOB-5) and Group IV (ETOB-10) received saline, diclofenac sodium (20 mg/kg), ETOB (5 mg/kg) and ETOB (10 mg/kg) orally, respectively. After 30 min. of administration, edema was induced in the right hind paw of rats by subplantar injection of 0.1 ml/rat of 1.0% carrageenan in saline. The paw volume was measured using mercury column plethysmometer before carrageenan and 1, 3, 5 and 24 h after carrageenan injection [25]. Paw edema was expressed as percentage increase in paw volume and the anti-inflammatory effect was expressed as percentage inhibition in comparison to the model control group [26].

Passive Paw Anaphylaxis

Preparation of Antiserum: For preparation of antiserum, wistar albino rats of either sex selected randomly were injected intraperitoneally with 0.2 ml of 10 % w/v egg albumin and 0.2 ml of *Bordetella pertussis* vaccine on day 1, 3 and 5. Twenty one days after the first immunization, blood was collected from retro-orbital plexus under light

ether anesthesia. The collected blood was allowed to clot and serum was separated by centrifugation at 1500 rpm. The separated serum was stored at -20°C until it was used for further experiment.

Sensitization and Hypersensitivity: Wistar albino rats were randomly allocated to four groups each containing six animals. Group I (model control), Group II (standard), Group III (ETOB-5) and Group IV (ETOB-10) received saline, ketotifen (1 mg/kg), ETOB (5 mg/kg) and ETOB (10 mg/kg) orally once daily for seven days, respectively. Two hours after the last dose of administration on seventh day, rats were passively sensitized into the left hind paw with 0.1 ml of undiluted serum. Twenty four hours after sensitization, the rats were challenged in the left hind paw with 10µg of egg albumin in 0.1 ml saline. The hind paw volume was measured after 30 minutes by volume displacement method using mercury column plethysmometer [27]. The difference in the reading prior to and after challenge represents the edema volume and the anti-anaphylactic effect was expressed as the percentage inhibition.

Passive Cutaneous Anaphylaxis

Preparation of Antiserum: Rat antiserum containing IgE was prepared according to method described earlier [28]. In brief, rats were immunized with 0.5 ml of suspension containing 1 mg of egg albumin and 10 mg of aluminum hydroxide gel (s.c.) and 1 ml of inactive bacterial suspension of *Bordetella pertussis* (i.p.), simultaneously. Seven days later, they were immunized again following the same procedure. Fourteen days later, rats were anesthetized with ketamine and diazepam and blood was withdrawn from the carotid artery; followed by separation of rat antiserum.

Sensitization and Hypersensitivity: Wistar albino rats were randomly allocated to four groups each containing six animals. The antiserum diluted 2-fold with saline (50 µl) was intradermally injected into 2 sites on the shaved dorsal skin of rats. After 48 h, rats were challenged with 0.5 ml of saline containing 2 mg egg albumin and 5 mg Evans blue via tail vein. Group I (model control), Group III (ETOB-5) and Group IV (ETOB-10) received saline, ETOB (5 mg/kg) and ETOB (10 mg/kg) orally 1 h. before the challenge of egg albumin respectively. Disodium cromoglycate (2 mg/kg) was intravenously injected to Group II animals just before the challenge of egg albumin. After 30 min. the animals were sacrificed and the skin surrounding spots were removed. The amount of dye was then determined colorimetrically after extraction with 0.5 ml of 1 M KOH and 4.5 ml of a mixture of acetone and phosphoric acid (in a ratio of 13:5 v/v) at 620 nm (Shimadzu Double beam Spectrophotometer 1650-PC) [29].

Allergic pleurisy: Swiss albino mice were randomly allocated to five groups each containing six animals: Group I (normal control), Group II (model control), Group III (standard), Group IV (ETOB-5) and Group V (ETOB-10). Active sensitization of Swiss albino mice was achieved with a subcutaneous injection of Freund's complete adjuvant emulsion (100 µL) containing egg albumin (100 µg). Fourteen days later, mice were challenged with an intrathoracic injection of egg albumin (50 µL, 12.5µg/cavity) [30, 31]. Briefly, an adapted needle was inserted into the right side of the thoracic cavity of egg albumin-sensitized animals to permit the intrapleural administration of egg albumin diluted in sterile pyrogen-free saline (50 µL). At 24 hr after the stimulus, mice were anaesthetized and their thoracic cavities were rinsed with 1 mL phosphate buffer saline containing 10 mM EDTA, pH 7.4. Total leukocyte counts were made with an automated cell counter (Cell Dyn 3200SL). Differential cell counts were made by light microscopy stained with Leishman's stain. Group IV and V animals fasted overnight, received ETOB (5 mg/kg) and ETOB (10 mg/kg) orally 1h before stimulation. Group II were similarly treated with vehicle alone. In Group III, dexamethasone was given intraperitoneally (2 mg/kg) 24 and 1 hr before stimulation.

Statistical Analysis: The results of various studies were expressed as mean ± SEM and analyzed statistically using one way ANOVA with Dunnett post hoc test to find out the level of significance. Data were considered statistically significant at $p < 0.05$.

RESULTS

Effect of ETOB on Vascular Permeability Induced by Acetic Acid in Mice: After 20 minutes of intraperitoneal administration of acetic acid, the content of Evans blue dye that leaked in the peritoneal fluid was measured by colorimetry. The amount of dye measured was found to be higher in model control animals. Pretreatment with ETOB (5 mg/kg and 10 mg/kg) significantly ($P<0.05$) inhibited extravasation of dye induced by acetic acid resulting in 59.77 and 68.72% inhibition, respectively compared to control group thus indicating decrease in vascular permeability. Standard, indomethacin (20 mg/kg) also produced significant inhibition of the exudation (70.69%, $P<0.05$) as compared to control group (Table 1).

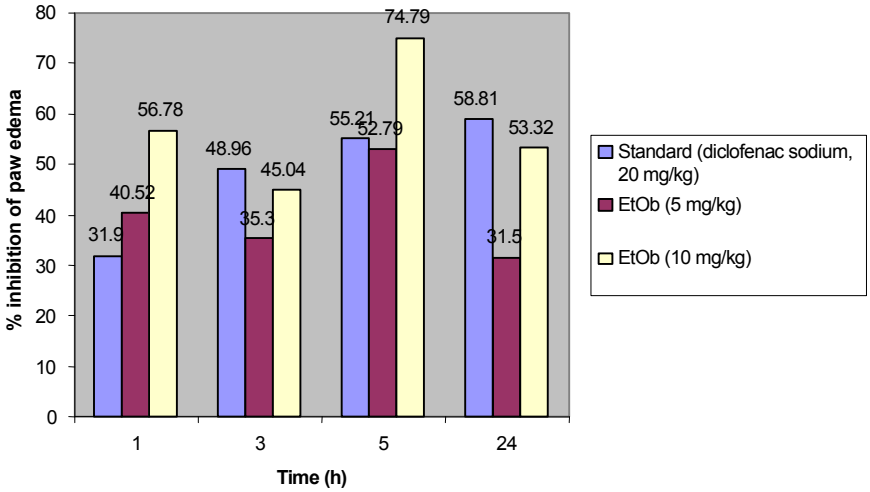

Fig. 1: Percent inhibition of carrageenan induced paw edema

Table 1: Effect of ETOB on vascular permeability induced by acetic acid

Group	Evans blue concentration(µg/ml)	% inhibition
Control	1.474±0.121	-
Standard (Indomethacin, 20 mg/kg)	0.432±0.14*	70.69
ETOB (5 mg/kg)	0.593±0.161*	59.77
ETOB (10 mg/kg)	0.461±0.111*	68.72

Statistical Analysis by one way ANOVA with Dunnett post hoc test
Values are mean ± SEM, n=6 in each group
Significantly different from control group *P<0.05

Table 2: Effect of ETOB on carrageenan induced inflammation in rats

Group	% edema observed after 60 minutes	% edema observed after 180 minutes	% edema observed after 300 minutes	% edema observed after 24 hrs
Control	22.95±1.57	36.66±2.95	43.51±2.97	11.46±1.17
Standard (diclofenac sodium, 20 mg/kg)	15.63±2.46	18.71±2.16	19.49±2.05*	4.72±0.99
ETOB (5 mg/kg)	13.65±1.40	23.72±4.55	20.54±2.79*	7.85±0.74
ETOB (10 mg/kg)	9.92±1.58	14.99±2.05	10.97±1.18*	5.35±0.60

Statistical Analysis by one way ANOVA with Dunnett post hoc test
Values are mean ± SEM, n=6 in each group
Significantly different from control group *P<0.05

Effect of ETOB on Carrageenan Induced Paw Edema: The intraplantar injection of carrageenan to the footpad in rats induced an increase in the paw thickness. This edema had a rapid onset and reached a peak at 5 hour after the challenge, followed by a gradual decrease thereafter (Table 2).

Pretreatment with ETOB (5 mg/kg and 10 mg/kg, p.o.) resulted in 40.52 and 56.78% inhibition at 1 h, 52.79 and 74.79% inhibition at 5 h and 31.5 and 53.32% inhibition at 24 h as compared to control. Both doses of ETOB (5 mg/kg and 10 mg/kg, p.o.) on pretreatment showed significant (P<0.05) inhibition of percentage of edema as compared to control (maximal inhibition of 52.79 and 74.79% at 5 mg/kg and 10 mg/kg at 5 h) (Table 2 and Figure 1). Similarly, pre-treatment with diclofenac sodium (20 mg/kg; p.o.) was able to inhibit edema by 58.81% upto 24 h (Table 2).

Effect of ETOB on Passive Paw Anaphylaxis: A marked inflammatory response in the form of paw edema was developed after 30 minutes on intraplantar administration of egg albumin into the hind paw of rats passively sensitized with serum containing IgE and these edema volumes measured plethysmometrically were significantly higher in model control animals (Table 3).

Table 3: Effect of ETOB on allergen induced passive paw anaphylaxis

Group	% edema observed after 60 minutes	% inhibition of edema at 60 minutes
Control	67.66±3.86	-
Standard (ketotifen,1 mg/kg)	21.38±1.42*	68.40
ETOB (5 mg/kg)	23.04±1.97*	65.95
ETOB (10 mg/kg)	18.24±1.55*	73.04

Statistical Analysis by one way ANOVA with Dunnett post hoc test

Values are mean ± SEM, n=6 in each group

Significantly different from control group *P<0.05

Table 4: Effect of ETOB on passive cutaneous anaphylaxis reaction

Group	Concentration of Evans blue (µg/ml)
Control	3.32±0.19
Standard (disodium cromoglycate, 2 mg/kg)	1.65±0.15*
ETOB (5 mg/kg)	2.59±0.19*
ETOB (10 mg/kg)	1.71±0.13*

Statistical Analysis by one way ANOVA with Dunnett post hoc test

Values are mean ± SEM, n=6 in each group

Significantly different from control group *P<0.05

Table 5: Effect of ETOB on cell counts in allergic pleurisy

GROUP	Total leukocyte	Neutrophil	Lymphocyte	Eosinophil	Monocyte
Control	3575±411	1857.75±291.39	1567.25±169.71	65.5±14.06	83.25±17.95
Sensitized	5225±383.78*	3072.75±264.34*	2002±108.44	145±27.54*	111.75±5.63*
Standard (dexamethasone, 2 mg/kg)	2550±413.32@	1273.5±202.37@	1182.25±187.97@	32.75±9.3@	61.5±20.36
ETOB (5 mg/kg)	4675±925.9	2762.75±625.45	1757.75±291.07	61.25±10.43@	89.5±23.05
ETOB (10 mg/kg)	2231.25±257.67@	1113.5±120.22@	1024±141.91@	32.25±8.05@	52.25±9.69

Values are mean ± SEM, n=6 in each group

Significantly different from control group *P<0.05 (one way ANOVA)

Significantly different from sensitized group @P<0.05 (one way ANOVA with Dunnett post hoc test)

A significant (P<0.05) inhibition of paw edema was observed on pretreatment with standard drug, ketotifen (1 mg/kg, 68.40% inhibition) after 30 min. Pretreatment with two doses of 5 mg/kg and 10 mg/kg of ETOB resulted in significant inhibition of edema (65.95 and 73.04%, p<0.05 respectively) as compared to control group at 30 min (Table 3).

Effect of ETOB on Passive Cutaneous Anaphylaxis: 48 hours after local injection of serum containing IgE into the dorsal skin of rats followed by an intravenous antigenic challenge i.e. egg albumin and Evans blue resulted in passive cutaneous anaphylaxis (PCA) reaction. This cutaneous anaphylactic reaction was best visualized by the extravasation of the dye that was estimated by colorimetric procedure after extraction from skin. As shown in Table 4, egg albumin induced the extravasation of dye in model control animals as seen by higher concentration of dye measured by colorimetric estimation, eventually revealing increase in PCA reaction.

Pretreatment with ETOB (5 and 10 mg/kg, p.o.) and disodium cromoglycate (2 mg/kg, i.v.) showed a significant (P<0.05) inhibition of egg albumin induced extravasation of Evans blue dye as revealed from decreased dye concentration as compared to control group. The percent inhibition of PCA by ETOB (5 and 10 mg/kg, p.o.) and disodium cromoglycate (2 mg/kg, i.v.) was 21.99, 48.49 and 50.3%, respectively (Table 4).

Effect of ETOB on Allergic Pleurisy: Twenty-four hours after the intrathoracic injection of egg albumin, an intense accumulation of total and differential cells was observed in sensitized group as compared to control group (Table 5). Dexamethasone pre-treatment (2 mg/kg, i.p.) significantly (P<0.05) inhibited the influx of total and differential leukocytes as compared to sensitized group (Table 5). The oral pretreatment with ETOB (5 and 10 mg/kg, p.o.) significantly (P<0.05) inhibited the eosinophil accumulation by 57.75 and 77.75%, respectively as compared to sensitized group in

the pleural cavity (Table 5). Additionally, ETOB (10 mg/kg, p.o., $P<0.05$) significantly prevented the rise in the number of total leukocytes (57.3%), lymphocyte (48.85%), monocytes (53.24%) and neutrophils (63.76%). Conversely, the number of total leukocytes (10.53%), lymphocyte (12.2%), monocytes (19.91%) and neutrophils (10.08%) was not affected significantly on oral pre-treatment with ETOB (5 mg/kg, p.o.) (Table 5).

DISCUSSION

Inflammation associated with asthma, has received increased attention over the past decades [32]. Bronchial asthma is now no longer simply viewed as reversible airway obstruction or irritable airways. Rather it is now viewed primarily as an inflammatory illness that as a result has bronchial hyperreactivity and bronchospasm. Inflammation characterized by redness, warmth, edema and pain is elicited experimentally by numerous stimuli, e.g. infectious agents, ischemia, antigen-antibody interactions, chemical, thermal or mechanical injury [33]. In the present study, the effect of ETOB was evaluated on acute and sub-acute inflammatory models like vascular permeability and carrageenan induced rat paw edema.

Phlogistic substance like acetic acid leads to dilatation of arterioles and venules and to an increased vascular permeability [33]. As a consequence, fluid and plasma proteins are extravasated and edemas are formed. Increase in vascular permeability also releases histamine, prostaglandins and leukotrienes [34]. It is also possible that plasma exudates may readily pass the inflamed mucosa and reach the airway lumen through leaky epithelium, thus compromising epithelial integrity and reducing ciliary function and mucus clearance [35]. Thus, plasma protein leakage has been implicated to play a role in the induction of a thickened, engorged and edematous airway wall, resulting in the airway luminal narrowing correlating with bronchial hyper-reactivity and airway inflammation [36]. These effects of increased vascular permeability are counteracted by H_1-antihistaminics, inhibitors of arachidonic acid metabolism and by leukotriene receptor antagonists. It is also reported that increased vascular permeability is associated with bronchial inflammation and airway hyper-responsiveness in a murine model of asthma [37 - 39]. Consistent with these observations, it was found in the present study that amounts of plasma extravasation measured in terms of Evans blue dye concentration were greatly enhanced in model control. Interestingly, pretreatment with ETOB significantly inhibited vascular leakage induced by acetic acid and was comparable to indomethacin, thereby exhibiting its inhibitory effect on increased vascular permeability.

Secondly, the ability to inhibit the edema produced in the hind paw of the rat after injection of phlogistic agent like carrageenan, egg albumin can also serve as a proof of anti-inflammatory activity of drugs. The development of carrageenan induced edema is biphasic; the first phase occurs within 1 hr of carrageenan administration and is attributed to the release of cytoplasmic enzymes, histamine and serotonin from the mast cells [40]. This mediator release from mast cells is capable of increasing vascular permeability, of dilating capillaries and of producing the contraction of non-vascular smooth muscles [41]. The second phase (>1h) is mediated by an increased release of prostaglandins in the inflammatory loci and continuity between the two phases is provided by kinins. Additionally, it is also reported that carrageenan injection induces the production of cytokine, TNF-α [42]. In context to this, the present findings showed that oral pretreatment with ETOB produced a pronounced inhibitory effect on carrageenan induced inflammation. Thus, in the above study, the effectiveness of ETOB in acetic acid induced vascular permeability and carrageenan induced paw edema suggest their anti-inflammatory action.

The airway inflammation is now also considered to be an immunologically initiated, mediator driven event. Airway inflammation in asthma thus is a direct response of the immune system to a trigger resulting in a cascade of immunologic events that includes inflammatory cells and mediators [43]. The immune system thus includes three main components; *antibodies, inflammatory cells* and *inflammatory mediators*. Thus, experimental models were further selected to target two components i.e. antibodies and inflammatory cells; as inhibition of inflammatory mediator release has already been established in our previous pharmacological studies.

The antibody involved in allergic asthma, is IgE [43]. Thus, primary immune response to exposed allergen generates unique IgE antibodies that bind to the surface of mast cells. These mast cells live within various tissues of the body, including the bronchi. If the human is later re-exposed to an allergen by inhalation, the allergen binds to the surface bound IgE on mast cells in the bronchi. Binding of at least two IgE molecules, bridged by a single allergen molecule, is termed cross-linking. This cross-linking of IgE by allergen on the surface of the mast cell is the initial biologic event of an allergic reaction referred as an immediate hypersensitivity reaction (IHR),

thereby inducing release of biologically active mediators; the preformed mediators stored in the cytoplasmic granules (histamine and β-hexosaminidase) and the newly synthesized mediators (leukotrienes and cytokines-TNF-α, IL-6) within 30 min [44 - 46]. These mediators ultimately cause various symptoms of allergic asthma. In line with this notion, the reaction was replicated in experimental passive paw anaphylaxis model whereby passive immunization with serum containing IgE antibodies and then exposure to second antigen i.e. egg albumin caused an immediate hypersensitivity reaction in the form of paw edema [47]. ETOB produced prominent inhibitory effect at two different dose levels suggesting its action against inflammation driven immunologically. These results were found comparable to ketotifen, a known antihistaminic and anti-inflammatory agent.

Secondly, another experimental animal model with a mechanistic similarity to passive paw anaphylaxis is passive cutaneous anaphylaxis where anti-IgE antibody induces anaphylaxis with mast cells playing an important role [48]. Passive cutaneous anaphylaxis involves extravasation of the dye from rat skin that serves as a useful site for studying anaphylactic reaction, type I hypersensitivity reaction [49, 50]. It is worth noting that ETOB and standard drug, disodium cromoglycate probably through interference with the degranulation system inhibited the immediate type allergic reactions. This suggests that ETOB possess antiallergic activity.

Inflammatory cells are activated as a result of immunologic cascade. The concomitant presence of infiltrating cells, eosinophils, neutrophils and T lymphocytes in the bronchi of asthmatics plays a major role in the development of airway inflammation and the accompanying bronchial hyperreactivity [51, 52]. In the present study, the therapeutic potential of ETOB was evaluated by estimation of total and differential cell count in pleural fluid in allergic pleurisy in mice.

Among the inflammatory cells measured, eosinophil is the inflammatory cell most closely associated with asthma. The triggering and regulation of eosinophil accumulation in allergic inflammation depends on the release of cytokines and chemokines such as IL-4, IL-5 and CCL11/eotaxin in response to an antigen challenge [53].

First of all, to participate in the allergic inflammatory response, eosinophil must migrate from the circulation to the airway [54]. Circulating eosinophils migrate to the airways by phenomenon of cell rolling, through interaction with P-selectin and eventually adhere to the endothelium through the binding of integrins to adhesion proteins, VCAM 1 and ICAM 1. As eosinophils enter the matrix of the membrane, their survival is prolonged by IL-5. Upon reaching the airways, eosinophils can release a variety of cationic proteins contained in its cytoplasmic granules, such as eosinophil peroxidase (EPO) and major basic protein (MBP) that are responsible for injury and shedding of airway epithelium [55, 56]. Disruption of the epithelium leads to the exposure of the underlying mucosal structures and sensory nerve endings to allergen and irritants, contributing to the developing of nonspecific bronchial hyperreactivity [57].

Moreover, it is also well-established that there are increased eosinophil numbers in the airways of patients with ongoing asthma, even those with mild disease [58], whilst autopsy and biopsy studies have revealed a characteristic eosinophil infiltration of the airway mucosa [59, 60]. Similar replication was observed experimentally in the present study, where antigen challenge resulted in an increase in the number of eosinophils in pleural fluid. ETOB produced a pronounced inhibition of eosinophil accumulation in pleural fluid in mice that was comparable with standard drug, dexamethasone. These results suggest the protective role of ETOB which may be mediated by inhibition of eosinophil accumulation and release of eosinophil products.

Based on above results, our study demonstrates the effectiveness of ETOB in decreasing bronchial hyper responsiveness by possessing significant antiallergic and anti-inflammatory activity. These effects can be attributed to inhibition of histamine release by mast cell stabilization, modulation of eosinophil accumulation and decreasing vascular permeability and edema.

Moreover, the results of our preliminary phytochemical studies, credit the therapeutic action of *O. bracteatum* majorly to phenolic compounds and additionally also to saponins and flavonoid type of compounds.

Taken together, the present results suggest the potential of *Onosma bracteatum* as a herbal-based therapy for the treatment of bronchial asthma.

REFERENCES

1. Pride, N.B., 1992. Definition and clinical spectrum, Asthma. In: P.J. Barnes, (eds). Br. Med. Bull., 48: 1-9.
2. Jeffery, P.K., 1992. Pathology of asthma, Asthma. In: P.J. Barnes, ed. Br. Med. Bull., 48: 23-39.
3. Wills-Karp, M., J. Luyimbazi, X. Xu, B. Schofield, T.Y. Neben, C.L. Karp and D.D. Donaldson, 1998. Interleukin-13: central mediator of allergic asthma. Sci., 282: 2258-2260.

4. Nicolaides, N.C., K.J. Holroyd, S.L. Ewart, S.M. Eleff, M.B. Kiser, C.R. Dragwa, C.D. Sullivan, L. Grasso, L.Y. Zhang, C.J. Messler, T. Zhou, S.R. Kleeberger, K.H. Buetow and R.C. Levitt, 1997. Interleukin 9: a candidate gene for asthma. Proc. Natl. Acad. Sci. U.S.A., 94: 13175-13180.
5. Safwat, T., 2007. Towards a deep understanding of bronchial asthma. Egyptian J. Bronchol., 1(1): 120-124.
6. O' Byrne, P.M. and D.S. Postma, 1999. The many faces of airway inflammation - Asthma and Chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med., 159: S41-S66.
7. Giembycz, M.A., 2000. Phosphodiesterase 4 inhibitors and the treatment of asthma- where are we now and where do we go from here. Drugs, 59: 193-212.
8. Barnes, P.J. and S. Pederson, 1993. Efficacy and safety of inhaled corticosteroids in asthma. Report of a workshop held in Eze, France, October 1992. Am. Rev. Respir. Dis., 148: S1-S26.
9. Salib, R.J., A. Drake Lee and P.H. Howarth, 2003. Allergic rhinitis: past, present and the future. Clin. Otolaryngol., 28: 291-303.
10. Kirtikar, K.R. and B.D. Basu, 1999. Indian Medicinal Plants, second edition, Volume III, International Book Distributors, pp: 1699.
11. Nadkarni's, K.M., 2002. Indian Materia Medica, 3rd revised edition, Volume I, Popular Prakashan Pvt. Ltd., Bombay, India, pp: 871.
12. Patel, K.G., K.V. Patel and T.R. Gandhi, 2008. Evaluation of the effect of Onosma bracteatum Wall (Boraginaceae) on bronchial hyperreactivity in sensitized guinea pigs. Iran. J. Pharmacol. and Thera, 7(1): 35-41.
13. Chapel, H., M. Haeney, J. Mishbah and N. Snowden, 1999. Essentials of Clinical Immunology, pp 10-14, 4th edition, Oxford, Blackwell Science.
14. Busse, W.W., W.F. Calhoun and J.D. Sedgwick, 1993. Mechanism of airway inflammation in asthma. Am. Rev. Respir. Dis., 147: 520-524.
15. Corrigan, C.J. and A.B. Kay, 1992. T-cell and eosinophils in the pathogenesis of asthma. Immunol. Today, 13(2): 501-7.
16. Kline, J.N. and G.W. Hunninghake, 1994. T-lymphocyte dysregulation in asthma. Proc. Soc. Exp. Biol. Med., 207(3): 243-253.
17. Poulter, L.W., D.G. Janossy and C. Power, 1994. Immunological/ Physiological relationships in asthma: Potential regulation by lung macrophages. Immunol. Today, 15(6): 258-261.
18. Sipteri, M.A. and L.W. Poulter, 1991. Characterization of immune inducer and suppressor macrophage from normal human lung. Clin. Exp. Immunol., 83: 157-162.
19. Barnes, P.J. and F.V. Liew, 1991. Nitric oxide and asthmatic inflammation. Immunol. Today, 16(3): 128-130.
20. Najam, F.I.E., A.S.M. Giasuddin and A.H. Shembesh, 2001. Tumour necrosis factors in childhood Asthma. Indian J. Pediatr., 68(3): 217-222.
21. Najam, F.I.E., A.S.M. Giasuddin and A.H. Shembesh, 1999. Immunoglobulin isotypes in childhood asthma. Indian J. Pediatr., 66(3): 337-344.
22. Lee, K.S., S.R. Kim, H.S. Park, G.Y. Jin and Y.C. Lee, 2004. Cysteinyl leukotriene receptor antagonist regulates vascular permeability by reducing vascular endothelial growth factor expression. J. Allergy Clin. Immunol., 114: 1093-9.
23. Whittle, B.A., 1964. The use of changes in capillary permeability in mice to distinguish between narcotic and nonnarcotic analgesics Brit. J. Pharmacol., 22: 246-53.
24. Yesilada, E. and E. Kupeli, 2002. Berberis crataegina DC. root exhibits potent anti-inflammatory, analgesic and febrifuge effects in mice and rats J. Ethnopharmacol., 79: 237-48.
25. Harris, J.M. and P.S.J. Spencer, 1962. A modified plethysmographic apparatus for recording volume changes in rat paw. J. Pharm. Pharmacol., 14: 464-466.
26. Winter, C.A., E.A. Risley and W. Nuss, 1962. Carrageenan induced edema in hind paw of rats as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med., 111: 544-547.
27. Martel, R.R., J. Klicius and J. Pinski, 1978. Inhibition of passive anaphylaxis induced in the rat hind paw and peritoneal cavity by N-(2-oxo-3,5,7-cycloheptatrien-1-yl)-aminooxoacetic acid ethyl ester (AY-25,674): Can. J. Physiol. Pharmacol., 56(6): 1005-10.
28. Matsuda, H., Y. Dai, Y. Ido, M. Yoshikawa and M. Kubo, 1997. Studies on Kochiae Fructus IV. Anti-Allergic Effects of 70% Ethanol Extract and Its Component, Momordin Ic from Dried Fruits of Kochia scoparia L. Biol. Pharm. Bull., 20: 1165-1170.
29. Katayama, S., H. Shionoya and S. Ohtake, 1978. A new method for extraction of extravasated dye in the skin and the influence of fasting stress on passive cutaneous allergy in guinea pigs and rats. Microbiol. Immunol., 22: 89-101.
30. Sampaio, A.L., G.A. Rae and M.M. Henriques, 2000. Role of endothelins on lymphocyte accumulation in allergic pleurisy. J. Leukoc. Biol., 67: 189-195.

31. Henriques, M.G., V.B. Weg, M.A. Martins, P.M. Silva, P.D. Fernandes and R.S. Cordeiro, 1990. Differential inhibition by two hetrazepine PAF antagonists of acute inflammation in the mouse. Br. J. Pharmacol., 99: 164-168.
32. Hamano, KJ., G.J. Gleich, R.H. Gundel and S.R. White, 1991. In: The Airway Epithelium Physiology, Pathophysiology and Pharmacology, Farmer, S.G. and Hay, D.W.P., pp: 255-318.
33. Vogel, W.H., B.A. Scholkens, J. Sandow, G. Muller and W.F. Vogel, 2002. Drug discovery and evaluation, Pharmacological essays, Springer Verlag Berlin Heidelberg, New York, Second edition, pp: 725-771.
34. Miles, A.A. and E.M. Miles, 1952. Vascular reactions to histamine, histamine liberator and leukotaxine in the skin of guinea pigs. J. Physiol., 118: 228-257.
35. Persson, C.G., 1996. Epithelial cells: barrier functions and shedding restitution mechanisms. Am. J. Respir. Crit. Care Med., 153: S9-10.
36. Thurston, G., J.S. Rudge, E. Ioffe, H. Zhou, L. Ross, S.D. Croll, N. Glazer, J. Holash, D.M. McDonald and G.D. Yancopoulos, 2000. Angiopoietin-1 protects the adult vasculature against plasma leakage. Nat. Med., 6: 460-3.
37. Lee, K.S., S.R. Kim, H.S. Park, G.Y. Jin and Y.C. Lee, 2004. Cysteinyl leukotriene receptor antagonist regulates vascular permeability by reducing vascular endothelial growth factor expression. J. Allergy Clin. Immunol., 114: 1093-9.
38. Lee, Y.C., Y.G. Kwak and C.H. Song, 2002. Contribution of vascular endothelial growth factor to airway hyperresponsiveness and inflammation in a murine model of toluene diisocyanate-induced asthma. J. Immunol., 168: 3595-600.
39. Dai, Y., Y.P. Chan, L.M. Chu and P.P.H. But, 2002. Antiallergic and Anti-inflammatory Properties of the Ethanolic Extract from *Gleditsia sinensis*. Biol. Pharm. Bull., 25(9): 1179-1182.
40. Vinegar, R., W. Schreiber and R. Hugo, 1969. Biphasic development of carrageenan edema in rats. J. Pharmacol. Exp. Therapeutics, 166: 96-103.
41. Kim, D.Y. and M. Camilleri, 2000. Serotonin: a mediator of the brain-gut connection. Am. J. Gastroenterol., 95: 2698-2709.
42. Cunha, T.M., W.A. Verri, J.S. Silva, S. Poole, F.Q. Cunha and S.H. Ferreira, 2005. A cascade of cytokines mediated mechanical inflammatory hypernociception in mice. Proc. Natl. Acad. Sci. U.S.A., 105(5): 1755-1760.
43. Murray, J.F. and J.A. Nadel, 2000. Textbook of Respiratory Medicine, Volume I, Third edition, Philadelphia, Pa.: W.B. Saunders.
44. Galli, S.J., J. Kalesnikoff, M.A. Grimbaldeston, A.M. Piliponsky, C.M. Williams and M. Tsai, 2005a. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. Annu. Rev. Immunol., 23: 749-786.
45. Galli, S.J., S. Nakae and M. Tsai, 2005b. Mast cells in the development of adaptive immune responses. Nat. Immunol., 6: 135-142.
46. Kim, H.M., E.H. Lee and H.H. Cho, 1998. Inhibitory effect of mast cell-mediated immediate-type allergic reactions in rats by spirulina. Biochem. Pharmacol., 55: 1071-1076.
47. Mahajan, S.G., R.G. Mali and A.A. Mehta, 2006. Antianaphylactic and anti-inflammatory activity of hydroalcoholic extract of leaves of *Moringa Oleifera.* Planta Indica, 2(3): 9-12.
48. Jeong, H.J., P.D. Moon, J.Y. Um, J. Park, K.H. Leem, C.J. Kim, H.M. Kim and S.H. Hong, 2007. Effect of Gamiseunggal-Tang on immediate type allergic reaction in mice. Indian J. Med. Res., 125: 542-549.
49. Moon, P.D., H.Y. Shin, H.J. Na, H.J. Jeong, S.J. Kim, J.Y. Um, R.K. Park, H.M. Kim and S.H. Hong, 2007. Gamijeonssibaekchulsan regulates mast cell-mediated anaphylactic reaction. Acta Biochimica Polonica, 54(2): 349-355.
50. Horiuchi, M. and Y. Seyama, 2008. Improvement of the anti-inflammatory and antiallergic activity of *Bidens pilosa* L. var. *radiate* SCHERFF treated with enzyme (Cellulosine). J. Health Sci., 54(3): 294-301.
51. Azzawi, M., B. Bradley, P.K. Jeffrey, A.J. Frew, A.J. Wardlaw, G. Knowles, B. Assouf, J.V. Collins, S. Durham and A.B. Kay, 1990. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthmatics. Am. Rev. Respir. Dis., 142: 1407.
52. Bentley, A.M., Q. Meng, D.S. Robinson, Q. Humid, A.B. Kay and S.R. Durham, 1993. Increases in activated T-lymphocytes, eosinophils and cytokine mRNA expression for interleukin-5 and granulocyte/macrophage colony-stimulating factor in bronchial biopsies after inhalation challenge in atopic asthmatics. Am. J. Respir. Cell. Mol. Biol., 8: 835.
53. Brito, F.A., L.A. Lima, M.F.S. Ramos, M.J. Nakamura, S.C. Cavalher-Machado, A.C. Siani, M.G.M.O. Henriques and A.L.F. Sampaio, 2007. Pharmacological study of anti-allergic activity of *Syzygium cumini* (L.) Skeels. Braz. J. Med. Biol. Res., 40(1): 105-115.

54. Wardlaw, A.J., 1999. Molecular basis for selective eosinophil trafficking in asthma: a multistep paradigm. J. Allergy Clin. Immunol., 104: 917-926.
55. Motojima, S., E. Frigas, D.A. Loegering and G.J. Gleich, 1989. Toxicity of eosinophil cationic proteins for guinea-pig tracheal epithelium in vitro. Am. Rev. Respir. Dis., 139: 801.
56. Pretolani, M., C. ILuffi, E. Lapa J.R. Silva, D. Joseph, R.R. Lobb and G.B.B. Vargaftig, 1994. Antibody to Very Late Activation Antigen 4 Prevents Antigen-induced Bronchial Hyperreactivity and Cellular Infiltration in the Guinea Pig Airways. J. Exp. Med., 180: 795-805.
57. Underwood, S., M. Foster, D. Raeburn, S. Bottoms and J.A. Karlsson, 1995. Time course of antigen induced airway inflammation in the guinea pig and its relationship to airway hyperresponsiveness. Eur. Respir. J., pp: 2104-2113.
58. Beasley, R., R.W. Roche, J.A. Roberts and S.T. Holgate, 1989. Cellular events in the bronchi in mild asthma and after bronchial provocation. Am. Rev. Respir. Dis., 139: 806-817.
59. Djukanovic, R., W.R. Roche and J.W. Wilson, 1990. Mucosal inflammation in asthma. Am. Rev. Respir. Dis., 142: 434-457.
60. Underwood, S., M. Foster, D. Raeburn, S. Bottoms and J.A. Karlsson, 1995. Time course of antigen induced airway inflammation in the guinea pig and its relationship to airway hyperresponsiveness. Eur. Respir. J., pp: 2104-2113.