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Research Article

EVALUATION OF ANTI-ASTHMATIC ACTIVITY OF ETHANOLIC EXTRACT OF *EPHEDRA GERARDIANA* WALL IN MICE BY OVALBUMIN INDUCED METHOD

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ABSTRACT

Ephedra gerardiana Wall. have anti-inflammatory properties and in some instances smooth muscles relaxing effects. Since inflammation and airway smooth muscle contraction are two major components of asthma, the present study is going to investigate the effects of *Ephedra gerardiana* Wall. on airway inflammation and airway resistance in a mouse model of asthma. Mice were sensitized and challenged with ovalbumin (OVA) and treated intraperitoneally with Ephedra *gerardiana* Wall. The effect of *Ephedra gerardiana* Wall. was tested on airway inflammation, air way resistance and the increase of intracellular calcium in bronchial smooth muscle cells. In the present study we are going to evaluate the extent of lung inflammation according to Helsinki convention,OVA-specific immunoglobulin-E (IgE) titre by ELISA and histopathological studies. Ephedra gerardiana wall at 100 and 200 mg·kg^{-1} significantly reduced the total number of cells ($p < 0.001$; $n = 6$), eosinophils (*** $p < 0.001$) in BAL compared with the untreated group of OVA sensitised mice.

Keywords: Asthma, Inflammation, *Ephedra gerardiana,* Ovalbumin induced mice model, Immunoglobulin E.

INTRODUCTION

Asthma is a chronic inflammatory lung disease that can cause repeated episodes of cough, wheezing and breathing difficulty. Asthma is one of the most common chronic diseases of childhood, affecting more than 6 million children. Bronchial asthma is a chronic respiratory disorder affecting a large proportion of population throughout the world. The currently used drugs for the treatment of this disease in modern medicine are far from satisfactory as they provide only symptomatic relief, produce several adverse effects and may lose effectiveness on continued use. Asthma is characterized by a predisposition to chronic inflammation of the lungs in which the airways are reversibly narrowed. It occurs in 3 to 5% in all the people during their life span [1,2]. Asthma affects 7% of the population of the United States, [1] [2] 6.5% of British people and a total of 300 million worldwide. India has an estimated 15-20 million asthmatics. Asthma is a respiratory disorder caused by allergic hypersensitivity reactions. It is a disease that does not respect the boundaries of age, race, gender and 5000 deaths occurring annually. It is classified in to different types[3,4]. That

Are, **Extrinsic Asthma** occurs children and young adults who have atopic hyper sensitivity to foreign particles. An “Allergen" or an "antigen" is a foreign particle which enters in to the body.

Extrinsic asthma is caused by this type of immune system response to inhaled allergens such as pollen, animal dander or dust mite particles .It stimulate the production of IgE antibodies that binds to the surface of mast cells and basophills round the bronchial blood vessels.

Intrinsic Asthma is not allergy-related, in fact it is caused by anything except an allergy. E.g. Inhalation of chemicals such as cigarette smoke or cleaning agents,It is associated with chronic inflammation of Upper respiratory tract (URT), Aspirin trigger asthma,Stress, laughter, exercise, cold air, food preservatives or a myriad of other factors.**Mixed Asthma** name suggests, mixed asthma is a mixture of intrinsic and extrinsic asthma[5,6].

The present study was designed to investigate the Anti-asthmatic effect of the ethanolic of the dried whole plant of *Ephedra gerardiana* wall on ovalbumin induced Asthma in mice.

MATERIALS AND METHODS

Plant materials and animals

The plant material was Collected from local market and authentified by Professor Dr. P.Jayaraman ph.D, Director-Plant Anatomy Research Centre (PARC), Sakthi Nagar, W.Tambaram, Tamilnadu, India and the Authentification no is PARC /2009 /455.

Swiss Albino mice either sex weighing between 18-25g were selected for Anti asthmatic studies,respectively.The exp erimental method followed was as per the protocal of the institutional Animal Ethics Committee which duly approved the animal studies (IAEC 1505/PO/a/11 CPCSEA 2011)’The animals were acclimatised under standared conditions of temperature (23±1 ^{0}C),relative humidity (55±10%). Six animals were kept together as one group[7,8].

Extraction

The dried whole plant (250g) was powdered and passing through a 60 mesh and than extracted with 95% ethanol using a soxhlet apparatus. The extract was filtered through cotton wool plug and dried in vacuum rotary evaporator at 40-50 ^{0}C under vacuum.Complete dryness was achieved in a calcium chloride decicator and the dry extract was used for all experimental studies.

Drug and extract standardisation

The extract was suspended in distilled water containing 1% Tween 80 to produce a concentration of 80mg/ml.Ovalbumin I.P(SRL,India) was used to induce Asthma in mice while Dexamethsone(Himedia,India) was used as the standard for anti-asthmatic activity.

Asthma induction and assessment of lung inflammation

This Experiment were conducted according to the Helsinki convention. Balb/c and IACE protocols on either sex mice, 18–25 g, were sensitised with 50 µg chicken ovalbumin (OVA) conjugated to 2% aluminium hydroxide in 100 µL saline were given intraperitoneally (*i.p.*). Control mice were injected with saline. For the challenges, mice were anaesthetised with 3% isoflurane, intranasally (*i.n.*) challenged with 50 µl of 1.5% OVA or saline.

Description of the timing for the treatments is presentes. In protocol number 1, mice were treated I.P with *Ephedra gerardiana wall* Ethanolic extract at dose 100 mg·kg^{-1} during the OVA challenge period. In protocol number 2, mice were treated I.P with 200 mg·kg^{-1} of *Ephedra gerardiana wall Ehanolic* extract during the OVA challenge period. Mice were treated with 2.0 mg.kg^{-1} I.P DEXA on days 21, 22, and 23 only. Appropriate positive and negative controls were carried out in parallel with the treated groups. The induction with Ovalbumin was done on 1,7,14 and 21 days.

On day 24, mice were sacrificed by over-exposure to isoflurane, tracheotomised, and broncheoalveolar lavage (BAL) was performed [11-14].

Analysis of bronchoalveolar lavage fluid (BALF) and serum

The mice were bled 12 hours after the last OVA exposure by retroorbital puncture using heparin capillary tubes. Blood samples were centrifuged (10 minutes, 4°C, 1000 × *g*), and plasma was stored at −70°C until use. The lungs were washed three times with 0.5 ml saline to collect BALF. The BALF was centrifuged (10 minutes, 4°C, 1000 × *g*), and the total number of inflammatory cells in BALF was counted with a hemocytometer. Differential cell counts were conducted using cytospin techniques and Wright's staining by counting at least 200 cells. The levels of IL-4 (SRM medical College, Chennai) and IL-13 (R&D Systems, Minneapolis, USA) in BALF and total serum IgE (SRM medical College, Chennai) were determined by ELISA according to the manufacturer's protocol [15,16].

OVA - specific Immunoglobulin E titre by ELISA

On the day of the sacrifice, blood from mice were collected *via* the orbital sinus (after anaesthesia with 3% isoflurane). Serum OVA-specific immunoglobulin (Ig) E were measured as previously described , but with minor modifications; plaques were coated with anti-mouse IgE (SRM Medical college,Chennai) and levels of OVA specific IgE were expressed in arbitrary units of optical density.[17]

Histopathology studies

Lung sections from *Ephedra gerardiana wall* i.p. treated mice were fixed in Formulin solution, embedded in paraffin, cut in 0.5-µm sections, and stained with haematoxylin-eosin. Inflammatory parameters in lung tissue (peribronchial, perivascular and parenchymal infiltration of inflammatory cells) were evaluated blindly by a senior lung pathologist. Total histology score was calculated[18] and graded from 0–4, where

0 = normal lung and 4 = diffuse maximal inflammation.

Statistics:
Statistical analyses were made using an ANOVA table followed by a Fisher's post-hoc test to determine statistical significance between groups (significant result $p < 0.0001$).

RESULTS

Effect of Ephedra gerardiana wall on Ovalbumin induced eosinophilic airway inflammation:

Ephedra gerardiana wall at 100 and 200 mg·kg^{-1} significantly reduced the total number of cells ($p < 0.001$; $n = 6$), eosinophils (*** $p < 0.001$) in BAL compared with the untreated group of OVA sensitised mice. Ephedra gerardiana wall treatment during the challenges (days 21–23), sensitisation (days 1–20) significantly reduced the number of total cells in the BAL. It was as effective as 2.0 mg·kg^{-1} DEXA. Similar results were obtained for peripheral blood count and eosinophil number. These results were find out by using Leishman's stain.

Table1: Effect of Ephedra gerardiana wall on Ovalbumin induced eosinophilic airway inflammation

S.no	Group treatment	Total no.of leucocytic count (Per cu mm) (Mean ± SEM)	Diff. in eosinophil count (Per cu mm) (Mean±SED)
1	Normal	***884±2.92	***21.2±2.05
2	Control (OVA)	***4502 ± 16.4	***150.4±7.09
3	OVA+DEXA(2mg/kg)	1652±14.51	67.2±3.62
4	OVA+EG (100mg/kg)	2820±34.84	128.4±3.95
5	OVA+EG(200mg/kg)	***2104±12.81	***105.4±3.70

Values were given as mean ± S.D. for six mice in each group. Asthmatic Control group was compared with normal group. Valuesare statistically significant at

***$p < 0.0001$ As compared with Asthmatic Control

***$p<0.0001$ As compared with Normal

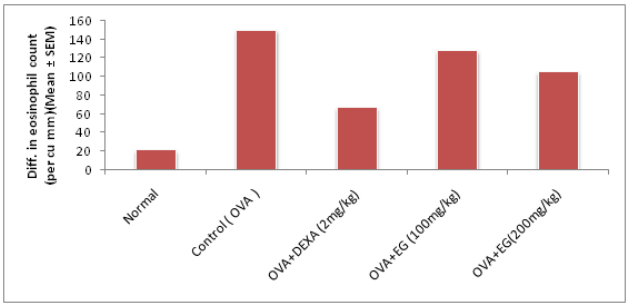


Figure 1: Differential eosinophilic count

Differential cell counts in the bronchoalveolar lavage (BAL) of intranasally treated *E. gerardiana wall* and dexamethasone (DEXA) treated mice, 2.0 mg·kg^{-1}. Administration of either *E. gerardiana wall* or DEXA intranasally significantly reduced the total cell and eosinophil counts in the BAL. *E. gerardiana* also reduced lymphocyte accumulation in BAL, while DEXA had effect only upon eosinophils:(*** $p< 0.0001$)

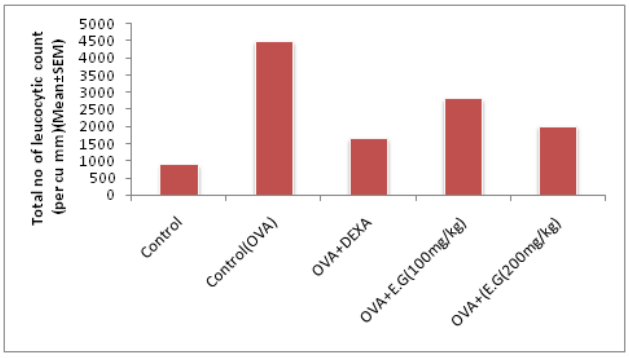


Figure 2: Total Leucocytic count

***Ephedra gerardiana wall* decreased levels of Th2 cytokines in BALF and total serum IgE:**

The levels of Th2 cytokines IL-4 and IL-13 in the BALF and total serum IgE were increased significantly by airway challenge with OVA when compared with those of the control group. The administration *of Ephedra gerardiana* reduced the concentrations of Th2 cytokines and total serum IgE.

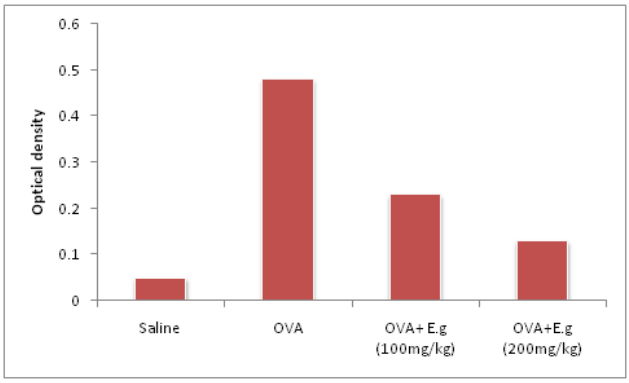
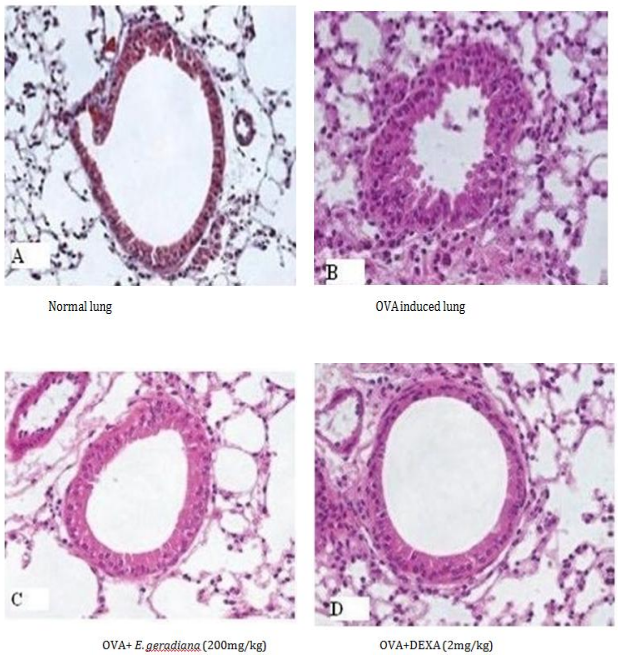


Figure 3:

Effect of different dosage of intraperitoneal *E. gerardiana* wall administration on ovalbumin (OVA)-specific immunoglobulin (Ig) E levels. Results are expressed as arbitrary optical density levels. Compared with nontreated mice, *E. gerardiana* reduced the serum levels of OVA-specific IgE in mice treated during the challenges (days 1–20; p = 0.01).

Histopathology

Histopathology shows a marked peribroncheal , perivascular and parenchymal inflammation of inflammatory cells in the OVA group compared with control mice. Mice treated with 200mg/kg *Ephedra gerardiana wall* showed decreased tissue inflammation by mononuclear cells and eosinophils compared with nontreated mice. DEXA showed a tendency to decrease tissue inflammation by inflammatory cells, but results were not significantly different from those of nontreated mice. These results were confirmed by a reduction of the total histological score in *Ephedra gerardiana wall* treated mice compared with nontreated mice. (P< 0.0001,n=6 mice per group)



Effect of *Ephedra gerardiana wal l* on the histology of lung tissue. (A) Control group , (B) egg albumin sensitized group , (C) egg albumin sensitized +*E. gerardiana* treated (200 mg/kg, I.P.) group , (D) egg albumin sensitized + DEXA treated (2 mg/kg, .I.P.) group .

LUNG HISTOLOGY

Histological analysis of the lungs from non-sensitized i.e. group I showed normal lung histology (Figure A). In contrast, similar to the BALF study, histological sections of lung tissue from group II mice exhibited airway inflammation, infiltration of eosinophils, lymphocytes and sub mucosal edema of the lungs, bronchoconstriction shown as lumen plugging by mucus and cells (Figure B). Treatment with *E.gerardiana* i.e. group III and group IV DEXA treated prevented the tissue edema, epithelial cell hypertrophy, infiltration of inflammatory cell and airway lumen plugging thereby decreasing inflammation and broncoconstriction which leads to normal lumen size (Figure C,& D).

DISCUSSION

In the present study, the ethanolic extract of the *Ephedra gerardiana* Wall was tested for Anti asthmatic property at doses of 100 and 200mg/kg body weight. Asthma is a common respiratory disease. The results from our earlier clinical study on *E.gerardiana* Wall suggest that, there was appreciable decrease in severity of symptoms of asthma and there also exists a simultaneous improvement in lung function parameters. The syndrome of bronchial asthma is characterized by wide spread narrowing of the bronchial tree due to the contraction of the smooth muscle in response to multiple stimuli resulting in the release of chemical mediators such as histamine .

The present study was carried out to verify if *E. gerardiana wall* could have a protective effect on the development of airway inflammation and responsiveness in asthma, and to evaluate possible mechanisms of action. Its dried twigs yield alkaloids (ephedrine and pseudo-ephedrine) which are used in drugs to treat hay fever and rashes of allergic origin. Tincture of *Ephedra* is used for cardiac and circulatory stimulation.

In addition to bronchodilating activity, a significant number of therapeutic approaches for bronchial asthma have been designed based on the antagonism of specific mediators released from mast cells. Mast cell degranulation is important in the initiation of immediate responses following exposure to allergens. Degranulated cells liberate mediators of inflammation such as histamine, leukotrienes, platelet activating factors and chemotactic factors for eosinophils,neutrophils etc. from mast cells . They play a significant role in airway inflammatory response such as airway eosinophilia, late asthmatic response and airway hyperresponsiveness as well as in immediate hypersensitivity reaction like bronchial contraction. Degranulation of mast cells has been taken as the criteria of positive anaphylaxis. Ketotifen fumarate, a well-known mast cell stabilizer, reduces synthesis of prostaglandins E2, thromboxane A2, leukotriene C4 and B4. It also inhibits release of histamine, serotonin and other inflammatory mediators from mast cells. Simultaneously it blocks H1 receptors. Khellin is a compound isolated from Ammi visnaga and its structural analogue furanochromone khellin. Cromolyn sodium, which is developed from the structural modification of Khellin (Cox et al., 1970) is the mast cell stabilizer used in the treatment of mild to moderate asthma. Adhatoda vasica, Albizzia lebbeck, Coleus forskohlii, Tylophora asthmatica etc. are several well known drugs from indigenous plant sources used in asthma and have been reported to have mast cell stabilizing activity.

Further, airway inflammation has been demonstrated in all forms of asthma. Even in mild asthma, there is an inflammatory response involving infiltration, particularly with activated eosinophils and lymphocytes, with neutrophils and mast cells. The degree of bronchial hyperresponsiveness and airway obstruction is closely linked to the extent of inflammation. Anti-inflammatory drugs suppress the inflammatory response by inhibiting infiltration and activation of inflammatory cells as well as their synthesis, or release and the effects of inflammatory mediators. Ethanolic extract of *E. gerardiana wall* possess potent anti-inflammatory activity, which was comparable to that of standard Dexamethasone. Since, serotonin, histamine and prostaglandins are the common mediators of both bronchial asthma and inflammation, the beneficial effect of Ethanolic extract of *E. gerardiana wall* could be due to inhibition of

their release possibly due to inhibition of the enzyme cycloxygenase leading to inhibition of prostaglandin synthesis.

Mice lung is used for screening of antihistaminic activity. Since it can besensitised with miner doses as it has well developed immune system. In the present study, *E.gerardiana Wall* (100 mg/kg, 200mg/kg) significantly inhibited the Ovalbumin induced contraction of mice lung preparation indicating its H1 receptor antagonistic activity and supports the anti asthmatic property of the plant.

The results support the hypothesis that these agonists have a protective effect on airway inflammation and responsiveness and that this effect may be related to antigen sensitisation and calcium metabolism. Taken together, these results confirm other published data by the current authors and others.

CONCLUSION

Thus, it can be concluded from the results obtained in the present investigation that *Ephedra gerardiana* wall possess significant antiasthmatic activity. At 100 & 200mg/kg the anti-asthmatic activity of *Ephedra gerardiana* wall can be attributed to its bronchodilating, antihistaminic (H1-antagonist) and anti-inflammatory property, suggestive of its potential in treatment and prophylaxis of asthma. Further detailed study needs to be Progressed to evaluate the clinical efficacy in the treatment of asthmatic patients.

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