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Antiasthmatic Activity of Alcoholic Extract of *Clerodendrum serratum* Induced by Ovalbumin

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ABSTRACT

Anti-asthmatic activity of the alcoholic extract of *Clerodendrum serratum* roots in ovalbumin induced experimental mice model. The roots were extracted with ethanol and the anti-asthmatic activity of the extract in ovalbumin-induced asthma in albino mice was evaluated. The parameters assessed were assessment of lung inflammation, OVA-specific immunoglobulin E titre by ELISA and histopathology of lung. The extract (100 and 200 mg/kg I.p) inhibited ovalbumin induced asthma by decreasing releasing of inflammatory mediators. *Clerodendrum serratum* roots extract has potent anti-asthmatic activity. Its anti-asthmatic property probably acts via a reduction in inflammatory mediator's release. The present study indicates that *Clerodendrum serratum* has significant anti-asthmatic property.

Keywords: Asthma, Inflammation, Airway hyper-responsiveness and Ovalbumin Ig E.

1. INTRODUCTION

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. Asthma is a chronic inflammatory lung disease. That can cause repeated episodes of cough, wheezing and breathing difficulty [1]. 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. Asthma affects 7% of the population of the United States, 6.5% of British people and a total of 300 million worldwide [2]. 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 [3-5].

The present study was designed to evaluatethe Anti-asthmatic effect of the alcoholic of the dried roots of *Clerodendrum serratum*on ovalbumin induced Asthma in mice.

2. MATERIALS AND METHODS

2.1. Animals

Swiss Albino mice either sex weighing between 18-25g were selected for Anti asthmatic studies,respectively.The animals were acclimatised under standared conditions.

2.2. Extraction

The dried roots(300g) was powdered and passing through a 80 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 40-60^{0}C under vacuum and complete dryness.

2.3. Drug and Extract Standardisation

The extract was suspended in distilled water containing 2% Tween 80 to produce a concentration of 80mg/ml. Ovalbumin I.P(SRL,India) was used to induce Asthma in mice.

2.4. Asthma Induction and Assessment of Lung Inflammation

Mice, 18–25 g, were sensitized with 50µg chicken ovalbumin (OVA) conjugated to 2% aluminium hydroxide in 100µL saline was given intraperitoneally (*i.p.*). Control mice were injected with saline. For the challenges, mice were anaesthetised with 5% isoflurane, intranasally (*i.n.*) challenged with 50µl of 1.5% OVA or saline. Mice were treated I.P with *Clerodendrum serratum* alcoholic extract at dose 100 mg/kg during the OVA challenge period. Mice were treated i.p with 200 mg/kg of *Clerodendrum serratum* alcoholic extract during the OVA challenge period.

Appropriate positive and negative controls were carried out in parallel with the treated groups. The induction with Ovalbumin was done on day 1 to day 23 but for every 7days for three weeks. On day 24, mice were sacrificed, broncheoalveolar lavage (BAL) was performed.

2.5. Analysis of Broncho-Alveolar Lavage Fluid (BALF) and Serum

Blood samples were retro-orbital 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. Total number of inflammatory cells in BALF was counted with a hemocytometer. The levels of IL-4 and IL-13 in BALF and total serum Ig E were determined by using ELISA [6].

2.6. OVA - Specific Immunoglobulin **E** Titre by ELISA

After sacrificing the animal collected blood from mice retro-orbital puncture. Serum OVA – specific immunoglobulin (Ig) E were measured in the units of optical density.

2.7. Histopathology Studies

Lung sections from *Clerodendrum serratum* i.p treated mice were fixed in Formalin solution, embedded in paraffin. Cut in 0.5-µm sections, and stained with haematoxylin-eosin. Inflammatory parameters in lung tissue (inflammatory cells) were evaluated. Total histology score was calculated and graded from 0–4.

Where, 0 = normal lung and 4 = diffuse maximal inflammation.

2.8. Statistics

Statistical analyses were made using an ANOVA followed by Dunnet;s t- test. Statistical significance was determined at $p < 0.0001$.

3. RESULTS AND DISCUSSION

*Clerodendrum serratum*at 100mg/kg and 200 mg/kg significantly reduced the total number of cells ($p < 0.001$) eosinophils (** $p < 0.001$) in BAL compared with the untreated group of OVA sensitised mice. *Clerodendrum serratum* treatment during the challenges significantly reduced the number of total cells in the BAL. Similar results were obtained for peripheral blood count and eosinophil number.

Table1: Effect of *Clerodendrum serratum* on Ovalbumin induced eosinophilic airway inflammation

S.no	Group treatment	Total no.of leucocytic count/mm	Diff. in eosinophil count/mm
1	Normal	**884±2.92	**24.2±2.05
2	Control (OVA)	**4512 ± 16.4	**160.4±7.09
3	OVA+CS (100mg/kg)	2828±34.84	128.4±3.95
4	OVA+CS (200mg/kg)	**2104±12.81	**105.4±3.70

All values are shown as Mean ± S.D and n=6

**p < 0.0001 indicate when compared with Controlgroup

**p<0.0001 indicate when compared with Normal group

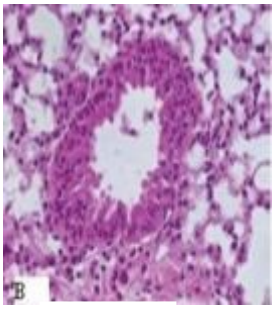
3.1. Differential Eosinophilic Count

Differential cell counts in the bronchoalveolar lavage (BAL) of intranasally treated *Cleodendrum serratum* Administration *Cleodendrum serratum* significantly reduced the total cell, eosinophil and also lymphocyte accumulation (** p< 0.0001).

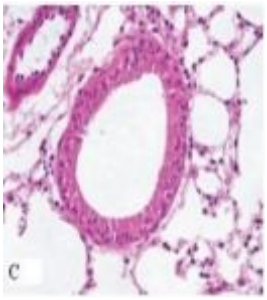
3.2. **Total** Leucocytic Count

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 the control group. The administration *of Clerodendrum serratum* reduced the concentrations of Th2 cytokines and total serum IgE.

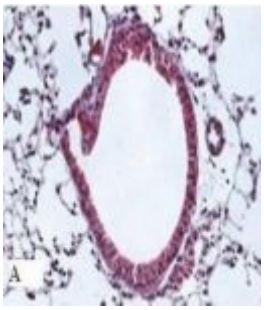
3.3. Lung Histology



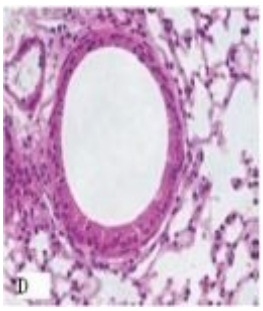
Normal



OVA induced lung



OVA+ *CS*(100mg/kg)



OVA+ CS(200mg/kg)

Histological analysis of the lungs from non-sensitized i.e. group-I showed normal lung histology. 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, broncho-constriction shown as lumen plugging by mucus and cells Treatment with *Cleodendrumserratum* i.e. group-III and group-IV infiltration of inflammatory cell and airway lumen plugging thereby decreasing inflammation and broncho-constriction which leads to normal lumen size.

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 [7]. Asthma is a common respiratory disease. The results from our earlier clinical study on *Clerodendrum serratum* suggest that, there was appreciable decrease in severity of symptoms of asthma and there also exists a simultaneous improvement in lung function parameters.

The present study was carried out to verify if *Clerodendrum serratum* could have broncho-dilating 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 de-granulation is important in the initiation of immediate responses following exposure to allergens. They play a significant role in airway inflammatory response such as airway eosinophilia. Degranulation of mast cells has been taken as the criteria of positive anaphylaxis.

The degree of bronchial hyper-responsiveness 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 [8]. Alcoholic extract of *Clerodendrum serratum* possess potent anti-inflammatory activity. Since, serotonin, histamine and prostaglandins are the common mediators of both bronchial asthma and inflammation, the beneficial effect of Alcoholic extract of *Clerodendrum serratum* could be due to inhibition of their release possibly due to COX inhibition leading to inhibition of prostaglandin synthesis [9].

Mice lung is used for screening of antihistaminic activity. Since it can be sensitized with minor doses as it has well developed immune system. In the present study, *Cleodendrum serratum* (100 mg/kg, 200mg/kg) significantly inhibited the Ovalbumin induced contraction of mice lung preparation supports the anti asthmatic activity.

4. CONCLUSION

In the present study *Clerodendrum serratum* possess significant *anti-asthmatic* activity. Anti-asthmatic activity of *Clerodendrum serratum* can be attributed to its bronchodilating and anti-inflammatory property.

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