

Pharmacological Evaluation of Hydroalcoholic Extract of *Clerodendrum Serratum* Leaves with Special Reference to Asthma on Guinea Pigs

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ABSTRACT

In the present study, hydroalcoholic extract of *Clerodendrum serratum* leaves was investigated for its potential to reverse some features of bronchial asthma in ovalbumin-induced murine model of asthma. *Clerodendrum serratum* commonly called bharangi, (family Solanaceae) is a well-known anti-allergic drug in Asian folk system of medicines. In the present work, pharmacological studies are done to provide scientific evidence for therapeutic potential of plant in allergic asthma. Asthma was induced in experimental rats with allergen suspension of ovalbumin and aluminum hydroxide followed by treatment with dexamethasone (2.5 mg/kg, po) or *C. serratum* leaves extract (100 and 200 mg/kg, b. w., po). Biomarkers of inflammatory response including cell counts, immunoglobulin E, cytokines such as interleukin (IL) -4, -5, -1 β , tumor necrosis factor- α (TNF- α), leukotriene (LTD-4), and nitrite concentration in blood as well as bronchial (BAL) fluid were tested. Lung functions in asthmatic and treated animals were evaluated as breathing rate and tidal volume. Treatment with *C. serratum* extract markedly ($p < 0.001$, $p < 0.01$, and $p < 0.05$) diminished infiltration of inflammatory cells, IgE, cytokines, and nitrites in blood serum and bronchial fluid. Improvement in lung functions ($p < 0.05$) of asthmatic animals after CSE treatment also supports our findings. Results of the study suggest therapeutic potential of *C. serratum* in allergic asthma that can be related to ability of plant to attenuate response of inflammatory cells and thereby, production of inflammatory and proinflammatory cytokines in airways.

Keywords: *Clerodendrum serratum* extract, asthma, ovalbumin, cytokines, phytochemicals

INTRODUCTION

Bronchial asthma is a very common noncommunicable lung disorder characterized by chronic inflammation, excessive mucus formation, reversible constriction, and hyperresponsiveness of airways usually triggered by allergens [1]. Another pathological feature of asthma is airway remodeling, described as structural and functional changes in lungs attributed to persistent inflammatory response in airways [2–5]. Annual increase in asthma prevalence by approximately 3.6% makes the disease a global issue of public health [6]. The classical dogma of asthma is central to CD4⁺T mediated T-helper type 2 (T_H2) cells production and IgE driven airway hyperresponsiveness. T_H2-induced immune response is advocated to production of large array of cytokines, mainly, IL-4, IL-5, and IL-13 and manifests in clinical pathology as high IgE antibodies and eosinophil levels in asthmatic individuals [7]. Together with T_H2 cells, innate lymphoid group 2 cells (ILC2) also play a key role in augmenting T_H2 response in the airways. These latter immune cells express transcription factor GATA3 and govern production type T_H2 related cytokine, IL-5 [8].

Glucocorticoids, widely accepted as main stay of asthma therapy, act by modulating. The response and nonspecific antiinflammatory response, and suppress innate immune response in individuals. Effects of corticosteroids on the cellular and humoral immunity are mediated via T_H1 cells. Treatment with other antiinflammatory drugs can provide benefit only to some extent in chronic patients. Despite several medical treatments available in the form of short-term and long-term relievers, there occurs an urgent need to seek more clinically effective targeted therapies in bronchial asthma. For decades, herbal drugs have been utilized as a source of new therapeutically active molecules, scaffolds, pharmacophores, and chemotypes. Among numerous drugs of natural origin, the genus *Clerodendrum* has been used ethnomedicinally for treatment of many diseases such as asthma, rheumatism, leucoderma in many countries of Asian continent. The biological effects of various species of *Clerodendrum* including *C. serratum* has been related to numerous category of bioactive principles including, terpenoids (serratum A, serratoside A and B, oleanolic acid, queretaroic acid; flavonoids and their glycosides (7, 40-trihydroxy-30-methoxyflavone, 6,7-trihydroxy-40-

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methoxyflavone; 7-glucopyranoside, scutellarein, apigenin-7-O-glucuronide; steroids (α -spinasterol, stigmasterol, campesterol, β -sitosterol) and others as cinnamic acid, iscosahydropicenic acid, ursolic acid, squalene, methyl palmitate, hexadecenoic acid [9–16]. These phytochemicals have exhibited considerable pharmacological activities including antifertility, anticancer, antioxidant, hepatoprotective, immunomodulator, anti-inflammatory, wound healing, and spermicidal in preclinical studies.

The plant forms an important constituent of several traditional antiasthmatic herbal formulations. However, the role of the plant in context to modulation of inflammatory markers in allergic asthma has never been investigated. In our study, we have investigated the potential of *C. serratum* standardized leaves extract (CSE) in ovalbumin induced airway inflammation in asthmatic animals.

MATERIALS AND METHODS

The hydroalcoholic extract was prepared for evaluation on animal rats. Male guinea pigs (*Cavia porcellus*) shall be utilized in this research since they are physiologically most related to human airways especially pertaining to histamine and allergen-induced bronchoconstriction.

Group Allocation

Animals will be randomly divided into the following groups:

1. Control group: Receiving no treatment.
2. Disease control group: in this group the disease will be induced by ovalbumin sensitization method as described in below.
3. Standard group: Montelukast 70mg/kg as a standard will be given to animals.
4. Treatment-1: *Clerodendrum serratum* 100mg/kg body weight.
5. Treatment-2: *Clerodendrum serratum* 200mg/kg body weight.

INDUCTION OF DISEASE:

Asthma will be induced using ovalbumin Sensitization Method. Ovalbumin is a glycoprotein derived from egg white, commonly used as an experimental allergen to induce asthma in animal models. When administered to guinea pigs along with an adjuvant such as aluminium hydroxide, it stimulates the immune system to produce antigen-specific IgE antibodies.

Upon subsequent exposure to aerosolized ovalbumin, a hypersensitivity reaction is triggered, leading to activation of Th2-type immune responses, release of inflammatory cytokines, and recruitment of eosinophils and mast cells to the airways.

This results in characteristic features of asthma, including airway inflammation, mucus hypersecretion, and bronchial hyperresponsiveness, closely mimicking the pathophysiology of human allergic asthma. The evaluation parameters are:

Sr. no.	Evaluation parameter
1.	bronchoalveolar lavage fluid (BALF): total cell count (lymphocytes, neutrophils, eosinophils, basophils and monocytes).
2.	Anti-oxidant parameter- MDA, MPO estimation
3.	Histopathological analysis of lungs

RESULTS

The practical yield of the plant extract was found to be 4.1 gm and %yield was found to be 8.5%w/w.

Table 1: Phytochemical screening of the plant:

S.R.N O.	CONSTITUENTS	TEST	INFERENCE
1.	Alkaloids	Dragendorff's test	Present
2.	Flavonoids	Shinoda test	Present
3.	Steroid	Salkowski steroid test	Present
4.	Phenols	Coumarin test	Present
5.	Cardiac glycosides	Keller Killiani test	Present
6.	Tannins	Lead acetate test	Present

The preliminary phytochemical analysis of the plant extract revealed the presence of several bioactive constituents. Alkaloids were detected using Dragendorff's test, while flavonoids were confirmed by the Shinoda test. The presence of steroids was indicated by the Salkowski test, and phenolic compounds were identified using the coumarin test. Cardiac glycosides tested positive in the Keller-Killiani test, and tannins were confirmed through the lead acetate test. The presence of these phytoconstituents suggests that the extract is rich in compounds with potential therapeutic properties.

Table 2: Estimation Of Total Flavonoid Content:

Concentration	Absorbance
20 μ g/ml	0.107
40 μ g/ml	0.242
60 μ g/ml	0.308
80 μ g/ml	0.451
100 μ g/ml	0.574
Hydroalcoholic extract of <i>C. serratum</i> : 50 μ g/ml	0.215

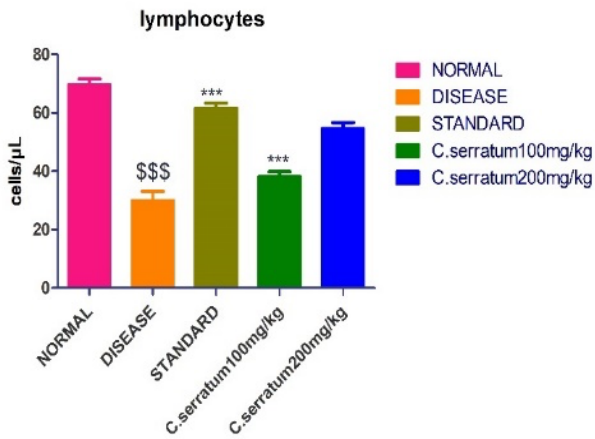


Figure 1: Effect Of *C. Serratum* On Lymphocyte Level Of Animal

Figure 3: Effect Of *C. Serratum* On Eosinophils Level Of Animals

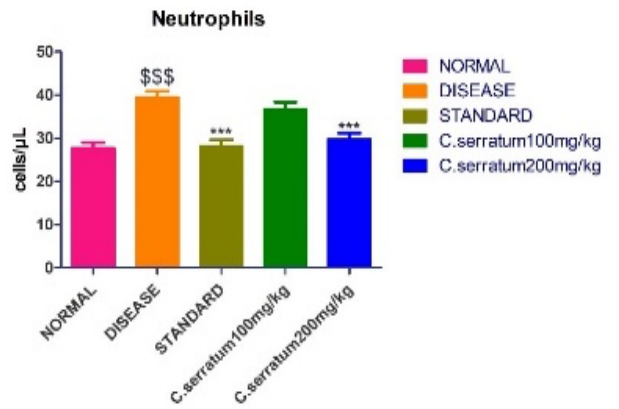


Figure 4: Effect Of *C. Serratum* On Neutrophils Level Of Animals:

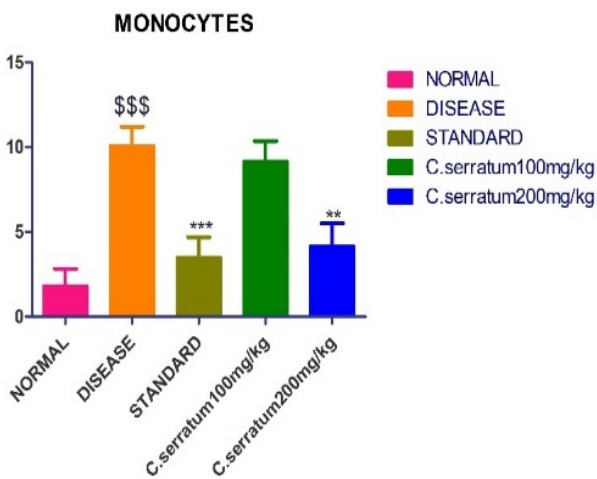


Figure 2: Effect Of *C. Serratum* On Monocytes Level Of Animals:

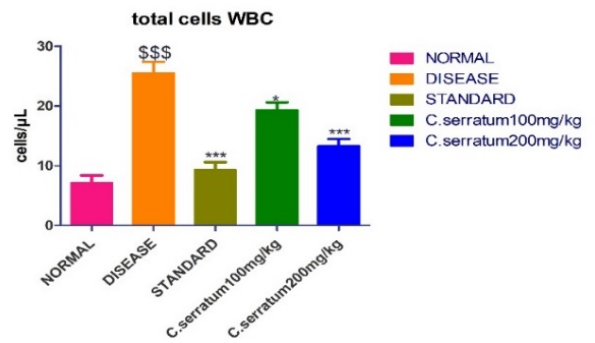


Figure 5: Effect Of *C. Serratum* On Total Cell Wbc Level Of Animals:

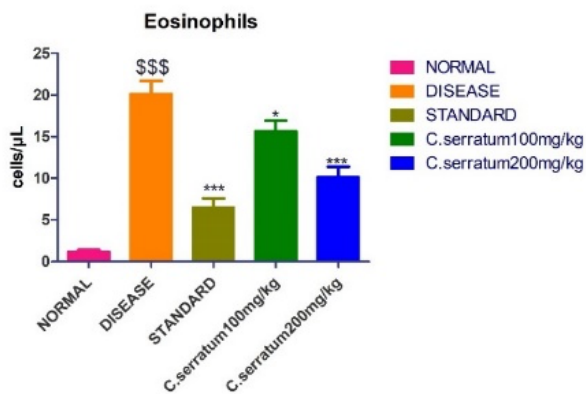


Figure 6: Effect Of *C.serratum* on Total Lung Protein:

CONCLUSION

The results of our study statistically proves and suggests the potential role of *C. serratum* root extract in treatment and management of allergic asthma by attenuating ongoing

inflammatory processes mediated through inhibiting the release of inflammatory and proinflammatory mediators and subsequent infiltration of eosinophils, lymphocytes, neutrophils into lungs airways. Further studies on bioactives present in *C. serratum* may be advised to investigate its mechanism of action at molecular and cellular levels.

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