JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/34453
RESEARCH ARTICLE

Inhalation Therapy with *Clerodendrum serratum* Linn. Extract: An Experimental Study on Ovalbumin-induced Asthma in Rats

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Abstract

Bronchial asthma is a chronic inflammatory condition marked by airway constriction, inflammation, and structural changes known as airway remodeling. *Clerodendrum serratum* Linn., a plant traditionally used in *Ayurvedic* medicine, has shown anti-inflammatory and bronchodilatory effects. This research work aims to study the potential anti-asthmatic properties of an inhalable formulation prepared from *C. serratum* extract in an ovalbumin-induced asthma rat model. The formulation was evaluated for its effects on serum leukocytes and eosinophils, mast cells, total tissue protein, airway inflammation, and histopathological changes in the lungs. The results showed that the inhalable formulation significantly reduced the symptoms of asthma in the rat model. The formulation led to a significant reduction in the levels of serum leukocytes and eosinophils, key indicators of asthma inflammation. It effectively stabilized mast cells, preventing their degranulation, and reducing airway reactivity. A decrease in total tissue protein levels was observed, suggesting reduced tissue damage associated with asthma. The formulation also demonstrated a substantial reduction in airway inflammation, as evidenced by histopathological changes in the lungs and reduced Th2 cytokines namely Tumour necrosis factor α and Interleukin 4, in bronchoalveolar lavage fluid. These effects may be attributed to the anti-inflammatory, mast cell stabilizing, and immunomodulatory properties of the formulation. The study provides scientific evidence supporting the traditional use of *C. serratum* in the treatment of asthma and demonstrates its potential as a therapeutic alternative for the treatment of respiratory diseases.

Keywords: Asthma, Anti-inflammatory, *Bharangi*, *Clerodendrum serratum*, Herbal, Reduced Serum Leukocyte and Eosinophil Counts, Th2 Cytokines

1. Introduction

Bronchial asthma is a long-term inflammatory condition of the airways characterized by bronchoconstriction, airway edema, airway hypersensitiveness, eosinophil infiltration, and a predominance of Type II helper T cells (Th2). The repeated damage and regeneration caused by chronic inflammation may result in structural changes in the airway, known as airway remodeling. Airway remodeling is distinguished by thickening of the bronchial wall, subepithelial fibrosis, raised smooth muscle mass, angiogenesis, and growing mucous glands. This remodeling is hypothesized to contribute to hyperresponsive airways and irreversible airflow

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Article Received on: 18.07.2023 Revised on: 25.10.2023 Accepted on: 29.10.2023

limitation[1]. It is caused by genetic and environmental variables and can be induced by allergies, exercise, and stress[2]. People of any age can develop asthma, and the symptoms can range from mild to very serious. Some of the symptoms are wheezing, coughing, tightness in the chest, and shortness of breath. Severe asthma can be lethal and requires immediate medical attention[3]. In recent years, the prevalence of asthma has increased, particularly in wealthy nations. Although the specific origin of this increase is unknown, variables such as air pollution, dietary and lifestyle changes, and greater exposure to allergens are thought to have played a role[4].

The recent consensus statements on asthma recommend intensive airway inflammation treatment. Glucocorticoids (GCs), particularly inhaled GCs, remain the cornerstone of asthma treatment due to their significant reduction of inflammation, endurance, and early onset of action. Inhaled GC administration at low doses for an extended period is typically regarded as safe. However, it is important to note that prolonged and high-dose use of GCs can lead to adverse effects such as osteoporosis, cataracts, and adrenal suppression[1]. Therefore, it is crucial for healthcare providers to monitor the dosage and duration of GC use in asthma patients.

Clerodendrum serratum Linn., traditionally known as *Bharangi*, belongs to the family Verbenaceae and has been used since the ancient period to alleviate various ailments. The Sanskrit word *Bharangi* literally means that which is glorious[5]. *C. serratum* is a shrub with various activities, including anti-inflammatory and bronchodilatory effects[6]. The use of this plant in "*tamaka swasa*" is reported in "*Arka prakasha of Lankapati Ravana*"[7]. *Tamaka swasa* is a type of respiratory disorder characterized by sudden attacks of breathlessness and wheezing. The reference to the use of *C. serratum* in ancient *Ayurvedic* texts highlights its potential therapeutic benefits for such respiratory conditions. Recently, Arora P *et al.* studied the effects of *Clerodendrum serratum* root extract on asthma in animal models. Results showed that the extract reduced airway inflammation, bronchoconstriction, and oxidative stress, suggesting its potential use as a therapeutic option[8]. Further research is needed to develop a novel formulation, test different doses, and conduct clinical trials to establish the appropriate dosage and duration of treatment.

So, based on the ethnobotanical claims of the plant's therapeutic capabilities, the current study explores the possible anti-asthmatic activities of an inhalable formulation derived from the extract of *C. serratum* in an ovalbumin-induced asthma rat model. The study aims to evaluate the efficacy of the inhalation formulation in improving respiratory function and reducing airway inflammation, thus providing scientific evidence to support the traditional use of *Clerodendrum serratum* Linn. as an herbal remedy for asthma. The study evaluated the said effects with important parameters like serum leukocyte and eosinophil counts, mast cell stabilization, total tissue protein levels, and alleviation of airway inflammation and inflammatory mediators.

2. Methods

2.1 Materials and Experimental Procedures

Plants of *C. serratum* were procured from hilly regions of Waghai District – Gujarat, India, in the month of December 2020 and authenticated by Department of Botany, Maharaja Sayajirao University of Baroda, Vadodara (specimen number LP001) (Figure 1).

The novel formulation was prepared by mixing a specific proportion of dried hydroalcoholic extract of *Clerodendrm serratum* roots with lactose carrier obtained from Carry Excipients (DFE Pharma) Germany, MEGGLE Pharma, Germany.

For sensitization, a 2 mg/ml suspension of Ovalbumin (OVA) (SRL Pvt. Ltd., India) in Phosphate-Buffered Saline (PBS) was prepared. The precipitation of this suspension was aided by the addition of aluminum hydroxide gel at a 1:1 ratio.

To perform the airway allergen challenge, Ovalbumin (OVA) was mixed with Phosphate-Buffered Saline (PBS) to make a 1% weight/volume suspension. The animals were then sensitized by subjecting them to an aerosol of ovalbumin using a commercially available nebulizer (Medequip Healthcare Solutions, Karnataka, India) with an output rate of 0.2 ml/min. The animals were sensitized using a histamine chamber (Vijay Scientific, Nadiad, India) as part of the experimental protocol.

2.2 Animals

Male Wistar albino rats weighing 180–250 g were purchased from Sun Pharma Advanced Research Company (SPARC), Vadodara, India, for the

Figure 1. Picture of *C. serratum* plant (Source: Picture captured at the time of collection).

experiment. The protocol (PIPH 08/21) for the experiment was approved by the Institutional Animal Ethical Committee (IAEC), and all the experimental studies were performed according to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA), Animal Welfare Division, New Delhi, India. All the rats were housed in cages, where they had free access to a standard pellet diet and water. A constant temperature of 22 ± 1 °C, and relative humidity of 55 ± 5 % were maintained in housing with a 12/12-hour light/dark cycle.

2.3 Study Design

In this study, rats were randomly assigned to one of the following six groups (n = 6) (Figure 2).

Group 1 (NC): Rats were sensitized and challenged with 0.03 ml/g PBS.

Group 2 (DC): Rats were sensitized and challenged with OVA.

Group 3 (SC): Rats were sensitized, challenged with OVA, and treated with Budesonide 5 µg/g.

Group 4 (NF 1): Rats were sensitized and challenged with OVA and treated with Novel Formulation Dose 1 (1.28 µg/g)

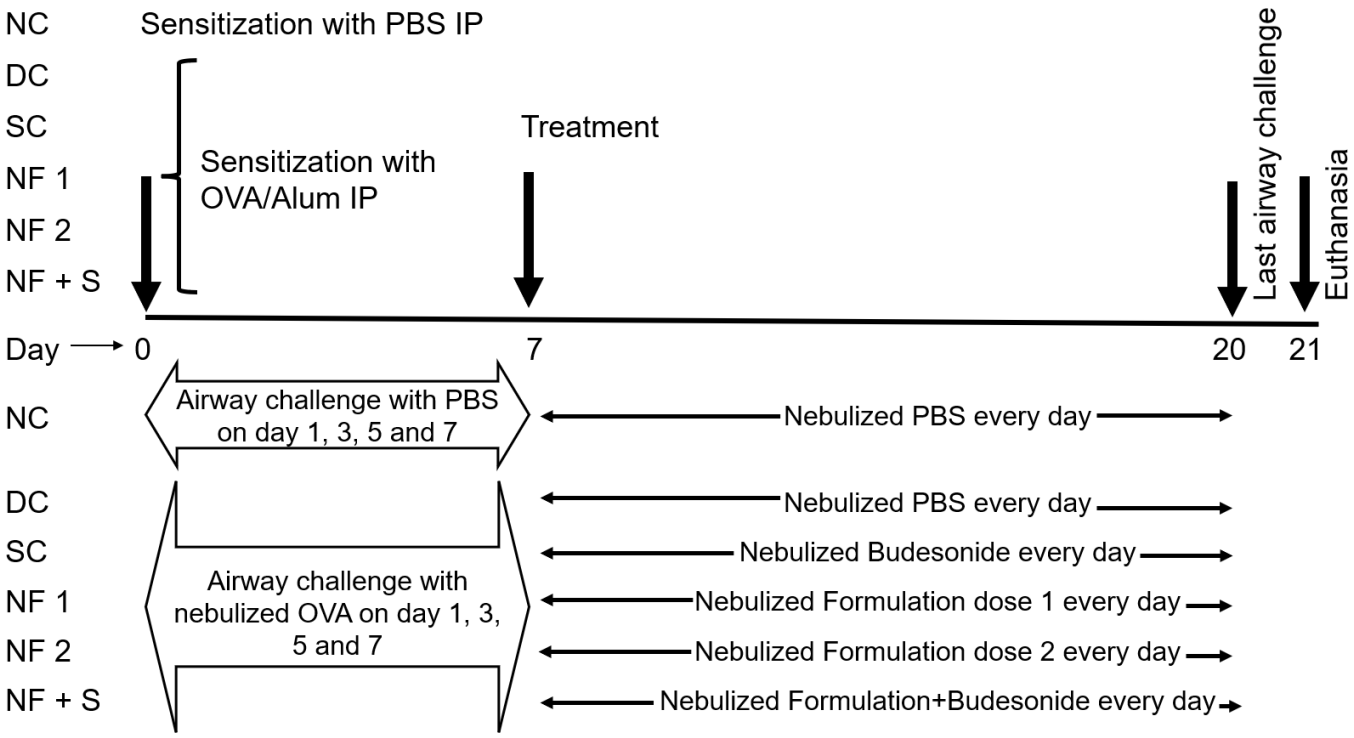

Figure 2. Timeline of the experimental protocol.

Group-5 (NF 2): Rats were sensitized and challenged with OVA and treated with Novel Formulation Dose 2 (2.55 µg/g)

Group-6 (S+NF): Rats were sensitized, challenged with OVA, and treated with Novel Formulation Dose 1, 1.28 µg/g + Budesonide 5 µg/g.

2.4 Study Protocol

The studies were conducted using an OVA-induced asthma model in rats, as reported previously[1,9,10]. As depicted in Figure 2, rats were sensitized intraperitoneally with Ovalbumin (OVA) precipitated with aluminum hydroxide gel in PBS on day 0 for the brief (21 day) study group. After the sensitization, an airway challenge was performed on the first, third, fifth, and seventh days of the study. A last airway challenge was performed on the animals 18 hours before their euthanasia. The rats in the Normal Control (NC) group received an intraperitoneal injection of PBS to sensitize them, and then they were given an aerosolized PBS dose to act as a challenge.

On the 21st day, Serum leukocyte and eosinophil count, Tissue total protein (lungs), Mast stabilization, inflammatory cytokines (Tumor Necrosis Factor α and Interleukin 4) in Bronchiolar Lavage Fluid (BALF), and histology of lungs were investigated.

2.4.1 Serum Leukocyte and Eosinophil Count

Blood samples were collected immediately after euthanasia and were collected in an EDTA collection tube. Total leukocyte count and Absolute Eosinophil count were obtained using flow cytometry.

2.4.2 Mast Stabilization

Bronchiolar lavage was done with normal saline. 2ml of normal saline was flushed into the bronchi by using Ryle's tube (number 5). Mast cells were collected by centrifuging collected BALF at 5000rpm for 10 minutes at 4°C. The cells in the generated pellet were resuspended in 0.5ml saline and were stained with Toluidine blue stain and observed under a microscope.

2.4.3 Tissue Total Protein (Lungs)

After euthanasia, lungs were removed, rinsed, and cleansed with normal saline, and 0.1g of lung tissue was homogenized in phosphate-buffered saline buffer solution (2 mL) at a ratio of 1:2 (w/v; 1 g tissue with 2 mL PBS, pH 7.4). Homogenates were centrifuged at 5000 rpm for 15 min at 4°C in a temperature-controlled centrifuge. The supernatants were used for the Estimation of protein by Lowry's method using a UV 1800 Spectrophotometer, in Kyoto, Japan.

2.4.4 Tumour Necrosis Factor α (TNF α)

Bronchiolar lavage was done with normal saline. 2ml of normal saline was flushed into the bronchi by using Ryle's tube (number 5). The clear supernatant was collected by centrifuging collected BALF at 5000 rpm for 10 min at 4°C and was analyzed for TNF α by Enzyme-Linked Immunosorbent Assay (ELISA) with Rat TNF α kit obtained from Krishgen Biosystems, Mumbai, in duplicate. All the plates were analyzed on an automated ELISA plate reader (iMark, Bio-Rad, USA).

2.4.5 Interleukin 4 (IL 4)

Bronchiolar lavage was done with the normal saline. 2 ml of normal saline was flushed into the bronchi by using Ryle's tube (number 5). The clear supernatant was collected by centrifuging collected BALF at 5000rpm for 10 min at 4°C and was analyzed for IL 4 by enzyme-linked immunosorbent assay (ELISA) with Rat IL 4 kit obtained from Krishgen Biosystems, Mumbai, in duplicate. All the plates were analyzed on an automated ELISA plate reader (iMark, Bio-Rad, USA).

2.4.6 Histopathology

At the completion of the research project, rats from each of the groups were euthanized and their lungs were removed, and washed with saline solution before being stored in 10% neutral formalin. These tissues were trimmed and routinely processed. Using a rotary microtome, tissue blocks that had been fixed in paraffin wax were cut into slices of 4–5 m thickness. The Haematoxylin and Eosin-stained slides were studied under a microscope and photomicrographs to study histopathology were taken at 10X using CX23 Biological Microscope (Olympus, Kyoto, Japan).

2.5 Statistical Analysis

All the data collected during the experiments were mentioned as mean ± Standard Deviation (SD). Statistical analysis of obtained responses was performed by one-way analysis of variance (ANOVA) for assessing

differences amongst multiple groups, using Excel software (Office 365, Microsoft Corporation, United States). P<0.05 was considered statistically significant.

3. Results

The study involves extracting bioactive compounds from *C. serratum* using hydroalcoholic solvent, followed by formulating the extract into a formulation using a lactose carrier to ensure stability and optimal aerosolization properties. The prepared formulation was then evaluated in an animal model of ovalbumin-induced asthma to assess its potential therapeutic effects on Serum leukocytes and Eosinophils, Mast cells, total tissue protein, airway inflammation, and histopathological changes in the lungs.

3.1 Inhaled Formulation Reduces Serum Leukocyte and Eosinophil Count in OVA-Induced Asthmatic Rat

When compared to the NC group, OVA exposure to rats resulted in a significant increase in serum leukocytes and eosinophils (P<0.05). OVA-induced serum leukocyte and eosinophil counts were considerably reduced by nebulized inhaled formulation (P<0.05) (Figure 3).

3.2 Inhaled Formulation Stabilises Degranulation of Mast Cells in BALF in OVA-induced Asthmatic Rat

Upon allergic response, the Degranulation of Mast cells in BALF leads to an increase in inflammatory cytokines like IL-4, IL-5, and TNF α. When compared to the NC group, OVA exposure resulted in a significant increase in Degranulated Mast cells (P<0.05). OVA-induced degranulation of Mast cells in BALF was considerably reduced by nebulized inhaled formulation (P<0.05) (Figure 4).

3.3 Inhaled Formulation Reduces Total Tissue Protein in OVA-induced Asthmatic Rat

Proteins were significantly increased in lung tissue in asthmatic animals, in comparison to the NC group, OVA

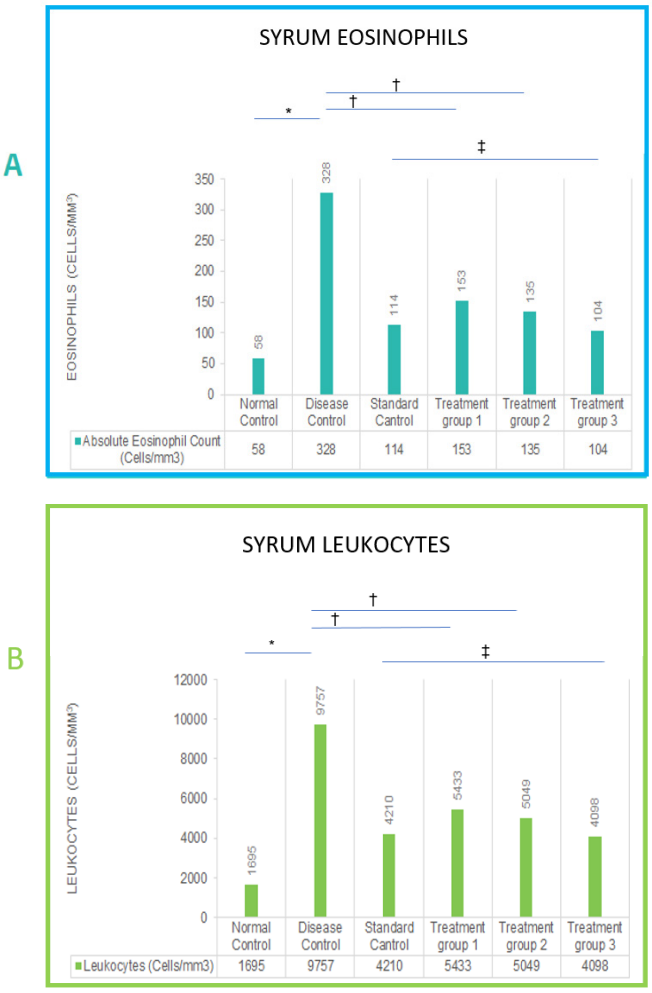

Figure 3. Nebulized inhaled formulation reduces serum leukocyte and eosinophil. **(A).** Serum Eosinophil count. **(B).** Serum leukocyte count. Data are expressed as mean ± SD (n = 6) and analyzed by one-way ANOVA. *P<0.01 compared to NC group; †P<0.01 compared to DC group; ‡P>0.05 compared to SC group.

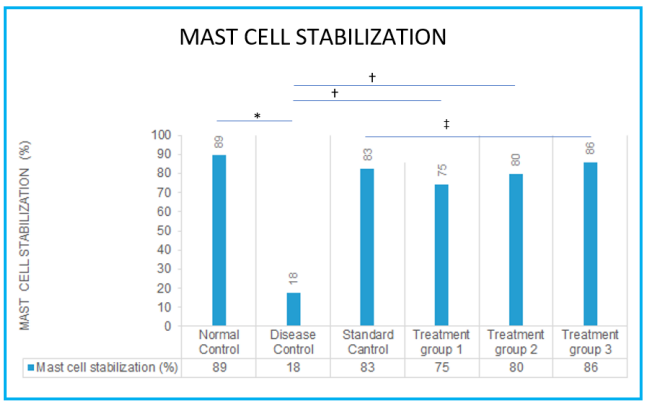

Figure 4. Nebulized inhaled formulation stabilises degranulation of mast cells in BALF Data are expressed as mean ± SD (n = 6) and analysed by one-way ANOVA. *P<0.01 compared to NC group; †P<0.01 compared to DC group; ‡P>0.05 compared to SC group.

exposure resulted in a significant increase in Total tissue protein (P<0.05). Total tissue protein was considerably lower in treatment groups (P<0.05) (Figure 5).

3.4 Inhaled Formulation Alleviates the OVA-induced Airway Inflammation

The inflammatory changes in H and E-stained lung tissues of rats were observed using light microscopy. There were no significant differences in the inflammation and bronchial contraction between the Treatment and NC groups (Figure 6). Moreover, severe inflammation bronchial contraction around the bronchi, and massive goblet cell hyperplasia in the bronchial lumen were observed in the DC group. The airway lumen was less obstructed in the treatment group as compared to the DC group (Figure 6).

3.5 Inhaled Formulation Reduces the OVA-Induced Th2 Cytokines in BALF

OVA-induced allergic asthma is characterized by an imbalance of Th1/Th2 cytokines. Therefore, we measured the concentrations of TNF α and IL-4 in BALF. Compared to the NC group, the DC group had higher concentrations of TNF α and IL-4 (P<0.05). However, the treatment groups had lower concentrations of TNF α and IL-4, in comparison to the DC group (Figure 7).

4. Discussion

The inhaled formulation used in the study appears to have multiple pharmacological pathways to alleviate the symptoms of OVA-induced asthma. The main mechanisms of action are as follows:

4.1 Anti-inflammatory Activity

The inhaled formulation reduces airway inflammation by suppressing the release of pro-inflammatory cytokines like TNF-α and IL-4 in Bronchoalveolar Lavage Fluid (BALF). This action may be attributed to the presence of anti-inflammatory compounds in the formulation, such as flavonoids, phenolics, and

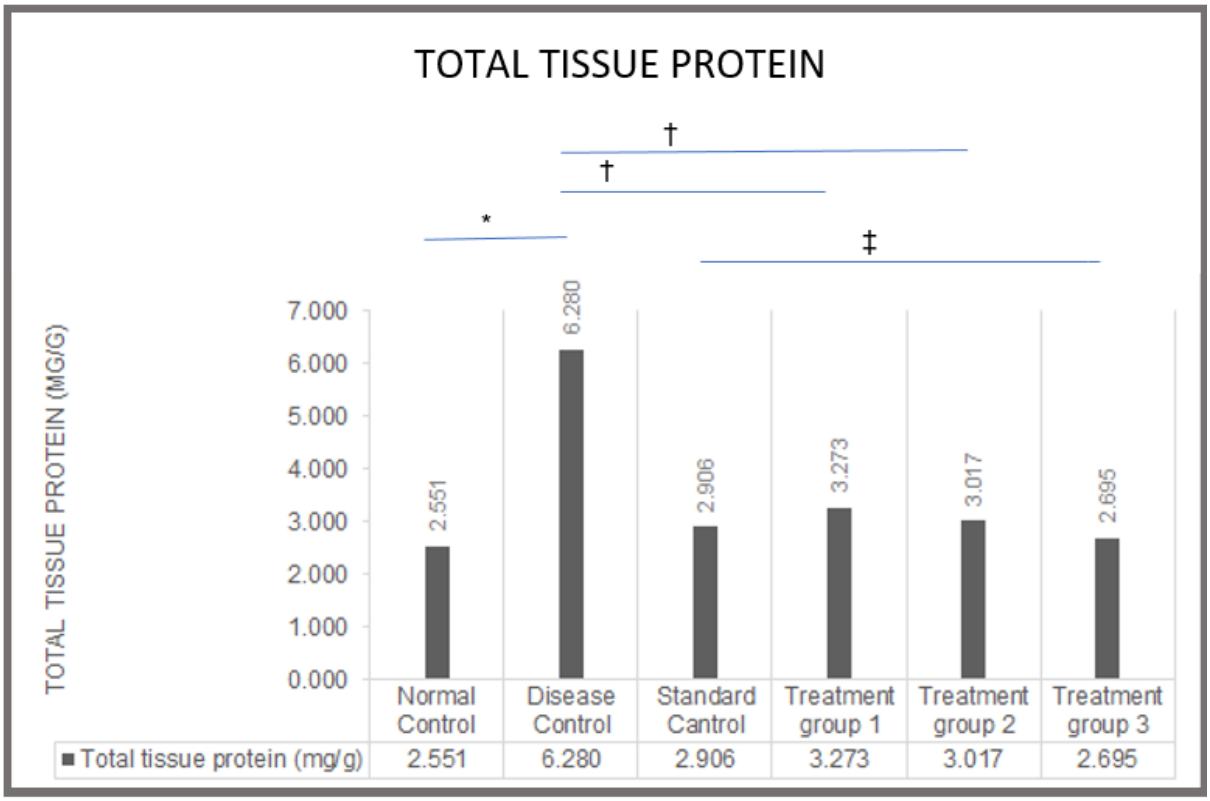

Figure 5. Nebulized inhaled formulation reduces Total tissue protein. Data are expressed as mean ± SD (n = 6) and analyzed by one-way ANOVA. *P<0.01 compared to NC group; †P<0.01 compared to DC group; ‡P>0.05 compared to SC group.

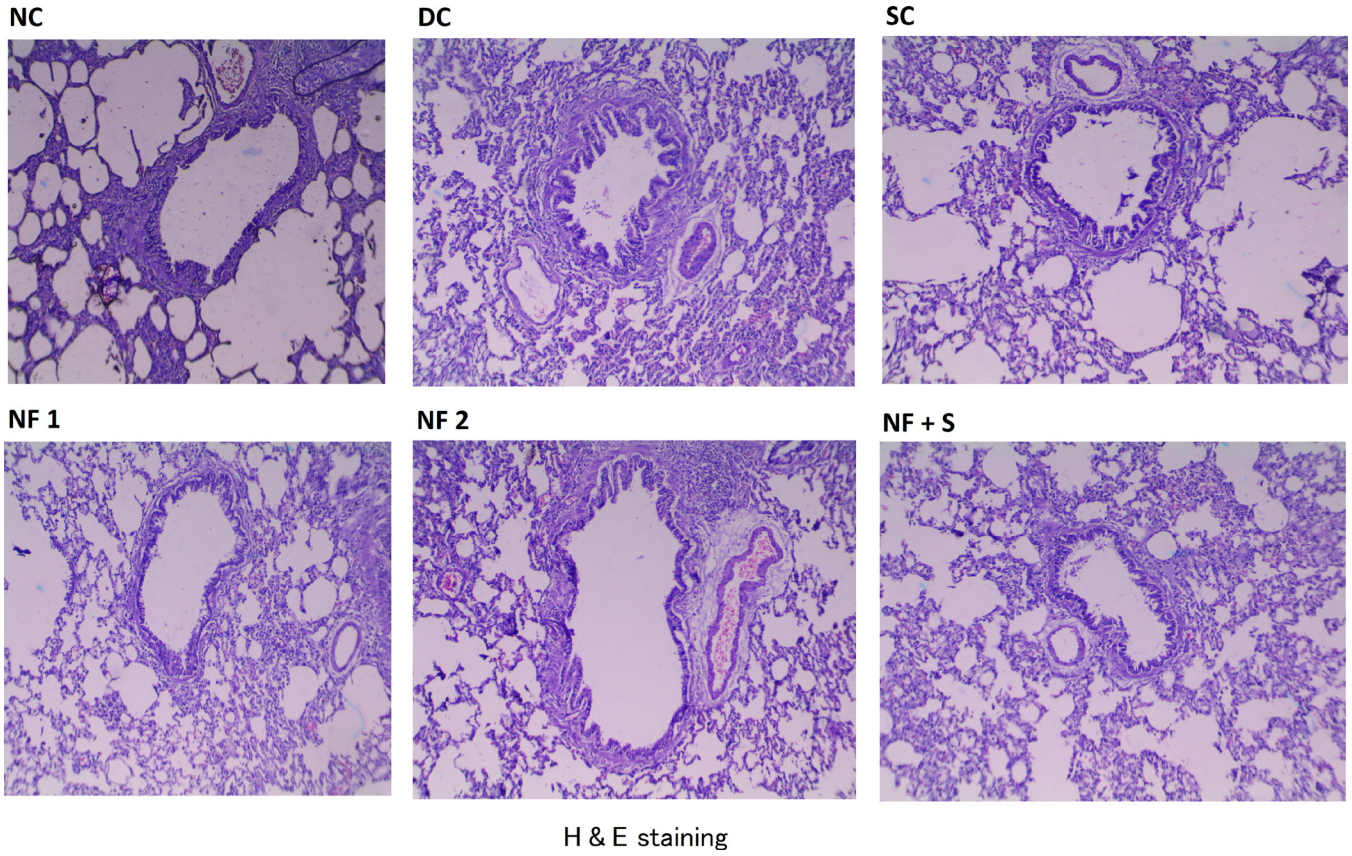

Figure 6. Inhaled formulation alleviates the OVA-induced airway inflammation as indicated in histological results of lung tissues using hematoxylin and eosin (H and E) staining (magnification: × 10).

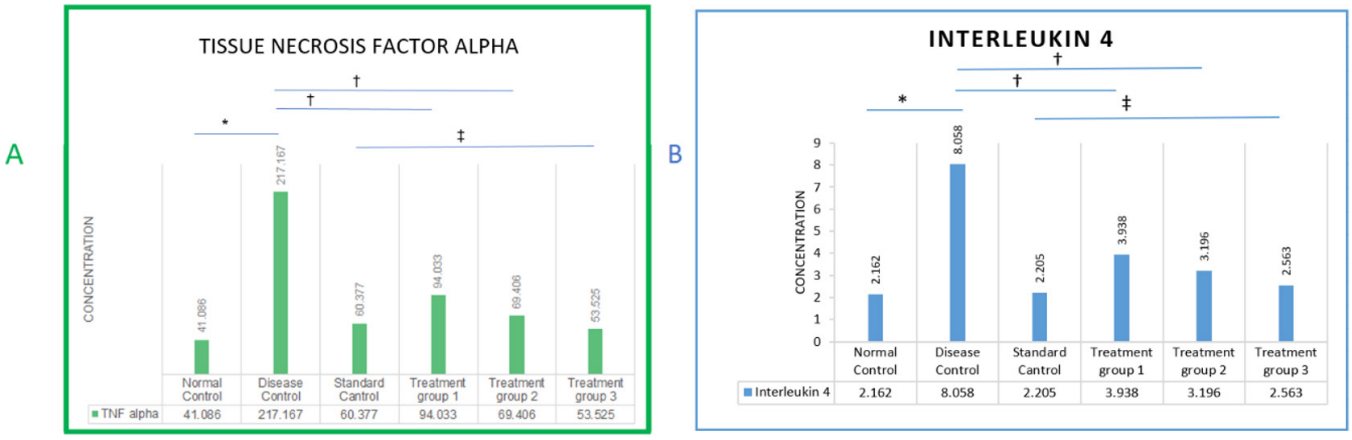

Figure 7. Inhaled formulation reduces the OVA-induced TNF α and IL-4 in BALF. Data are expressed as mean ± SD (n = 6) and analyzed by one-way ANOVA. *P<0.01 compared to NC group; †P<0.01 compared to DC group; ‡P>0.05 compared to SC group.

terpenoids. These compounds have been reported to inhibit the activation of inflammatory cells, particularly mast cells, and eosinophils, by modulating the activity of signaling pathways such as the NF-κB pathway[11,12].

4.2 Mast Cell Stabilizing Activity

The formulation reduces the degranulation of mast cells in BALF. Mast cells perform a crucial role in the pathogenesis of asthma by releasing various inflammatory mediators such as histamine,

leukotrienes, and cytokines upon activation. The stabilization of mast cells by the formulation may be affected by the presence of flavonoids and phenolics, which have been reported to inhibit mast cell degranulation and thereby reduce airway inflammation[13].

4.3 Reduction in Eosinophil and Leukocyte Count

The inhaled formulation reduces serum eosinophil and leukocyte count in OVA-induced asthmatic rats. Eosinophilic inflammation is a characteristic feature of allergic asthma, and the reduction in eosinophil and leukocyte count by the formulation may be attributed to the inhibition of the activity of eosinophils and other inflammatory cells[11-13].

4.4 Reduction of Th2 Cytokines in BALF

The study has shown to modulate the Th1/Th2 balance by decreasing Th2 cytokines such as IL-4, and IL-13. This effect is thought to be mediated by the active compounds in *C. serratum* extracts, such as oleanolic acid, and flavonoids[11,12].

4.5 Reduction in Total Tissue Protein

The administration of *Clerodendrum serratum* has shown promising effects in reducing tissue total protein levels, which gets elevated in oxidative stress associated with asthma[14]. Studies have demonstrated that the extract of *Clerodendrum serratum* possesses bioactive compounds that exhibit protein-reducing properties.

5. Conclusion

The inhaled formulation of *Clerodendrum serratum* Linn. used in the study appears to have multiple pharmacological pathways that contribute to its efficacy in alleviating the symptoms of OVA-induced asthma. The formulation exerts its beneficial effects through its anti-inflammatory, antioxidant, mast cell stabilizing, and immunomodulatory activities. These properties of *Clerodendrum serratum* Linn. help to reduce airway inflammation, oxidative stress, and mast cell activation, which are key factors in the development and progression of asthma. Additionally, the immunomodulatory effects of the preparation may regulate immune responses and prevent excessive immune reactions that can worsen asthma symptoms. Furthermore, the formulation has shown promising results in preclinical studies and may have potential as a therapeutic option for other respiratory diseases beyond OVA-induced asthma. However, further clinical trials are needed to confirm its safety and efficacy in humans.

6. Acknowledgement

The authors are thankful to Carry Excipients (DFE Pharma) Germany and Meggle Pharma, Germany for providing needed materials for formulation development.

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