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PHARMACOLOGICAL EVALUATION OF USHNAVAYU MENI CHOORANAM (UMC) IN OVA ALBUMIN INDUCED ALLERGIC RESPONSE IN MICE

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ABSTRACT

The prevalence of inflammatory and allergic airway diseases such as asthma has significantly increased in recent decades. Considering the side effects of long-term use of current therapeutics, identifying a new therapeutic strategy from natural resources is essential. Herbal therapeutics have been used in healthcare since time immemorial. Studies have been carried out globally to verify their efficacy and some of the findings have led to the production of plant-based medicines. The global market value of medicinal plant products exceeds $100 billion per annum. As per the siddha literature the formulation Ushnavayu meni chooranam (UMC) comprises of two novel herbs Acalypa indica and Cuminum cyminum indicated for the treatment of airway inflammation and obstruction, hence the main objective of the proposed study is to evaluate the anti-allergic potential of the formulation UMC in ova albumin challenged mice model. From the result analysis of the present investigation it was evident that there was a significant increase in allergic and inflammatory responses on the ova albumin challenged mice which was evidenced with in bronchial secretion, constriction and also significant increase in the level of Eosinophil, Neutrophil, WBC and monocytes count in mice belongs to group II. Treatment with trial drug UMC at both the dose level has shown significant decrease in the Eosinophils, WBC, lymphocyte and monocyte level. Further Procalcitonin (PCT) which is an actual index of inflammation and allergy was found decreased in UMC treated animals. It was concluded from the data's that trial drug UMC possess promising anti-allergic and bronchodilator property in the tested model

KEY WORDS: ***Allergic airway, Asthma, Siddha, Herbal therapeutics, Ushnavayu meni chooranam, Ova albumin, Anti-allergic, Bronchodilator***

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1. Introduction

Allergic asthma is a chronic inflammatory disease that recognized by airway inflammation and obstruction, mucus hyper secretion and airway hyper responsiveness [1]. The prevalence of inflammatory and allergic airway diseases such as asthma has significantly increased in recent decades. The asthma is associated to T-helper (Th) type 2 cells response, immunoglobulin (Ig) E-mediated mast cell activation, and other inflammatory factors, including eosinophils, B cells, cytokines and chemokines [2]. In addition, Th2 lymphocytes play important role in initiation and progression of allergic diseases such as asthma through their ability to release interleukin (IL)-4 and IL-5 [3].

Initiation of allergic response occurs with allergen presentation by antigen-presenting cells to CD4+ T cells. Antigen-activated CD4+ T cells induce several characteristic features of asthma, including the secretion of Th2-type cytokines such as IL-4, IL-5, and IL-13, which are responsible for IgE production by B cells and eosinophil activation and recruitment [4,5]. IL-4 is the most important Th2-type cytokine in inducing isotype switching to IgE in B lymphocytes [6]. IL-5 plays a major role in the maturation and recruitment of eosinophils into the airway [7]. IL-13 induces many features of allergic lung disease, including goblet cell metaplasia and mucus hypersecretion, which all contribute to airway obstruction [8].

Currently, inhaled corticosteroids are the most effective available treatment for asthma and they are used as the first-line therapy for persistent asthma in adults and children in many countries. However, systemic absorption of inhaled corticosteroids may have deleterious effects over long term use [9]. Traditional treatments for asthma include inhaled corticosteroids (ICS), short-acting β2-agonist (SABA), leukotriene receptor antagonists (LTRAs), and long-acting β2-agonist (LABA) [10]. However, these treatments still failed in some nonresponsive patients. It is believed that the nonresponsiveness to these treatments is caused by some inflammatory processes that are not targeted by currently therapies [11].

Effective and safe alternative medication for this disease management has become part of the major interest in scientific field nowadays. Herbs based traditional medicine had been used for asthma treatment as well. For a long time, the treatment of asthma relies on application of glucocorticoids. However, besides the side effect of glucocorticoids, some patients were nonresponsive for glucocorticoids [12,13]. On the other hand, as human monoclonal antibodies targeting specific cytokine receptors based therapy are under clinical trials, the antigenicity for these antibodies in human needs to be considered. Therefore, new treatment based on herbs which came from traditional medicine provides a novel way for fighting asthma. Ushnavayu meni chooranam (UMC) comprises of two novel herbs Acalypa indica and Cuminum cyminum indicated for the treatment of airway inflammation and obstruction, hence the main objective of the proposed study is to evaluate the anti-allergic potential of the formulation UMC in ova albumin challenged mice model.

2. Materials and Methods

2.1. Experimental Animals

Healthy Balb-C mice weighing between 20-25 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit (AHU). A 12 light / dark cycle were maintained .Room temperature was maintained between 22 ± 2° C and relative humidity 60–65%. They were provided with food (Sai feeds, Bangalore, India) and water ad libitum. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama Institute of science and technology, Chennai, Tamil Nadu, India

2.2. Experimental Methodology

Animals were randomly divided in four group of 6 mice each (one normal control, second albumin challenged, three and four are drug treatment groups).Animal belongs to group I received normal saline 0.1ml. Group II mice received injection of ova albumin (1 mg/kg) along with aluminum hydroxide in intraperitoneal route from day 1 to 7. Animal belongs to group III received ova albumin (1 mg/kg) along with aluminum hydroxide in intraperitoneal and treated with 200mg/kg dose of Ushnavayu meni chooranam (UMC) (p.o) 1 hr before albumin injection for seven days. Animal belongs to group IV received

intraperitoneal injection of ova albumin (1 mg/kg) along with aluminum hydroxide in intraperitoneal and treated with 400mg/kg dose of Ushnavayu meni chooranam (UMC) (p.o) before albumin injection for seven days.

2.3. Induction of Leukocytosis [14-17]

Balb- C mice were used for this study is challenged with ova albumin (1 mg/kg) along with aluminum hydroxide in intraperitoneal route results in abnormal increase in Total WBC, Procalcitonin, eosinophil etc.

2.4. Blood collection [18,19]

At the end of the study after overnight fast all mice were anesthetized by intra muscular injection with pentobarbital sodium. Blood collected by ocular puncture for biochemical estimations of Total WBC, Procalcitonin, eosinophil count.

2.5.Histopathology

At the end of the study all animals were sacrificed and lung was harvested and stored in the fixative solution (10% formalin) and cut into 10 µm thickness. Staining was done by using hematoxylin and eosin

3.Results

3.1. Effect of UMC on haematology profile of mice challenged with ova albumin induced allergic response

Result analysis of the present investigation evident that there was a significant increase allergic and inflammatory responses on the ova albumin challenged mice which was evidenced with in bronchial secretion, constriction and also significant increase in the level of Eosinophil, Neutrophil, WBC and monocytes count in mice belongs to group II. Treatment with trial drug UMC at both the dose level has shown significant decrease in the Eosinophils, WBC, lymphocyte and monocyte level. Further Procalcitonin (PCT) which is an actual index of inflammation and allergy was found decreased in UMC treated animals. It was concluded from the data's that trial drug UMC possess promising anti-allergic and bronchodilator property. Data's were tabulated in Table 1.

Table 1: Effect of UMC on haematology profile of mice challenged with ova albumin induced allergic response

GROUP	WBC count ($\times 10^3$ µl)	Eosinophils (%)	Neutrophils $10^3/mm^3$	Lymph (%)	Mon (%)	PCT (%)	Lung Weight in Gms
GROUP I	3.55 ±0.17	1.35±0.10	2.13± 0.24	66.22 ±1.5	4.11± 0.15	0.93±0.06	0.21±0.01
GROUP II	18.7 ±0.26	5.81±0.40	6.18± 0.55	93.2± 0.93	8.58± 0.27	6.16±0.10	0.59±0.03
GROUP III	8.03 ±0.64	2.81±0.17	3.83± 0.30	75.08 ±1.02	6.78± 0.30	3.49±0.09	0.37±0.02
GROUP IV	5.81 ±0.14	2.06±0.13	3.45± 0.20	70.82 ±2.62	5.11± 0.15	2.2±0.09	0.31±0.02

Values are mean ± S.E (n = 6 per group)

3.2. Effect of UMC on Lung histology of Mice challenged with ova albumin

Light microscopic observation of mice lung sample reveals normal bronchial blood vessels and connective tissue with no signs of pulmonary oedema in control group mice. Further arrangement of epithelial and muscular appears normal in sample belongs to group I. Significant reduction in bronchial lumen with deposition of collagen and migration of inflammatory cells were observed in group II. Perfect network of simple squamous epithelium with occasional evidence of inflammation and degeneration were observed in group III. Bronchial opening appears regular with no signs of infiltration and inflammation. As shown in Figure 1.

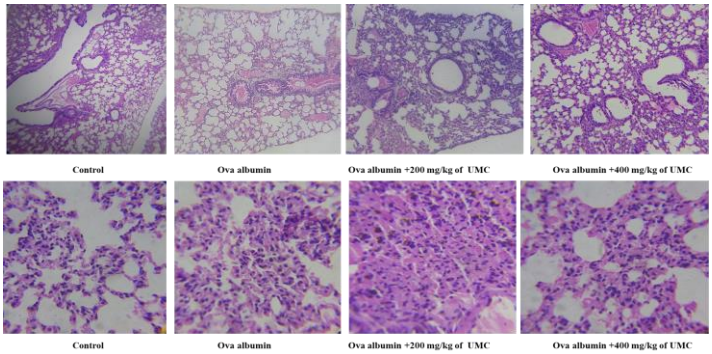

Figure 1: Lung histology of control and drug treated mice challenged with ova albumin

4. Discussion

Allergic asthma generally presents with symptoms of wheezing, coughing, breathlessness, and airway

inflammation. It is a chronic inflammatory disease of the airways, characterized by airway eosinophilia and goblet cell hyperplasia with mucus hypersecretion to inhaled allergens and nonspecific stimuli [20,21]. In particular, eosinophilic inflammation is considered the hallmark of airway inflammation in asthma [22]. The inflammatory process in allergic asthma is dominated by Th2 cells that produce IL-4, IL-5, and IL-13 [24], which activate eosinophils and induce the production of IgE by B cells [25].

Mast cells have long been associated with allergic asthma as these cells are the only resident cells in the airway that can form an interaction with allergen by means of Immunoglobulin E (IgE) bound to the high affinity IgE receptor (FcεRI)[26]. Mast cell degranulation was estimated by measuring the levels of mediators being released including beta hexosaminidase in BALF [27,28].

In susceptible individual, the inflammation causes the symptoms of wheezing, coughing, chest tightness and breathlessness [29]. The respiratory airways of an asthmatic patient show abnormalities such as epithelial denudation, goblet cell metaplasia, sub-epithelial thickening, increased airway smooth muscle mass, bronchial gland enlargement, angiogenesis and alterations in extracellular matrix components involving large and small airways [30].The incidence of asthma diseases has increased dramatically in the last decade. Approximately 300 million people worldwide suffer from asthma and it can be fatal if left untreated [31].

The emphasis on the use of medicinal plants had hitherto been placed on the treatment rather than prevention of diseases. However, there exists in the literature considerable report in recent times on research work on the use of medicinal plants and their constituents in disease prevention. A WHO expert group defined traditional medicine as the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental, or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing [32].

Animal studies are useful tools to investigate airway inflammatory responses, and in particular, the murine ovalbumin (OVA)-sensitization and challenge protocol is utilized routinely to study experimental allergic asthma. OVA-induced asthma has been recognized as a disease characterized by mucus overproduction, increased levels of inflammatory cells influx into the lungs, airway occlusion and thickness of the bronchial wall which are the hallmark of allergic asthma [33]. The changes in inflammatory cell influx and histopathological of the lungs of OVA-induced mice show that airways inflammation have been generated successfully in accordance with the previous studies [34,35]. From the result analysis of the present investigation it was evident that there was a significant increase allergic and inflammatory responses on the ova albumin challenged mice which was evidenced with in bronchial secretion, constriction and also significant increase in the level of Eosinophil, Neutrophil, WBC and monocytes count in mice belongs to group II. Treatment with trial drug UMC at both the dose level has shown significant decrease in the Eosinophils, WBC, lymphocyte and monocyte level. Further Procalcitonin (PCT) which is an actual index of inflammation and allergy was found decreased in UMC treated animals. It was concluded from the datas that trial drug UMC possess promising anti-allergic and bronchodilator property.

Mucus overproductions are the prominent histopathological feature in allergic asthma and this can be observed in OVA-induced mice. Additionally, inflammation has always being related to reactive oxygen species (ROS) where overproduction of ROS can lead to the compromised cellular functioning and increased inflammation by damaging nucleic acids, lipids, proteins, and mitochondria [36]. In the case of allergic asthma, inflammatory cells recruited to asthmatic airways are capable of generating reactive oxygen species (ROS) thus contributed to tissue injury and inflammatory reactions in the airways [37].

Light microscopic observation of mice lung sample reveals normal bronchial blood vessels and connective tissue with no signs of pulmonary oedema in control group mice. Further arrangement of epithelial and muscular appears normal in sample belongs to group I. Significant reduction in bronchial lumen with deposition of collagen and migration of inflammatory cells were observed in group II. Perfect network of simple squamous epithelium with occasional evidence of inflammation and degeneration were observed in group III. Bronchial opening appears regular with no signs of infiltration and inflammation.

5. Conclusion

In conclusion, we have witnessed that the siddha formulation Ushnavayu meni chooranam has the potential to mitigate OVA-induced allergic asthma of mice by alleviating asthma-related histopathological changes in the airway. The ability of formulation to halt the mucus overproduction, epithelium thickness, reduction in eosinophils, neutrophils and lymphocytes suggested that the formulation Ushnavayu meni chooranam may prove to be a useful therapeutic approach for the treatment of allergic asthma.

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