

ORIGINAL ARTICLE

Survey the effect of Herbal Medicine on pathogenesis of Eosinophilic Chemotactic Factors in experimental Allergic Asthma

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ABSTRACT

Herbal Medicine is the oldest and safest route for treatment of many types of acute and chronic diseases. *Althaea officinalis*, *Malva sylvestris*, *Mentha longifolia*, *Tribulus terrestris* are traditionally that used for cure and strengthening. The aim of this study was to investigate effect of these Plants on migration of Eosinophils to the airways in the experiential allergic asthma. In this experiment; 64 guinea pigs were divided into 4 groups. In group 1 after sensitization by Ovalbumin they were challenged by Ovalbumin and buffer, then they received these Plants; group 2 was sensitized and challenged by Ovalbumin without receiving any Plants. The third group was challenged only by buffer, and the last group was challenged without sensitization. The comparison of statistical analysis among groups showed that the number of Eosinophils in groups which received these Plants, significantly decrease in comparison with control group ($P \leq 0.05$). The comparison of the second and third groups shows that the second group has a significant increase in the number of Eosinophils in comparison with the third group. The data showed that these Plants had an important effect on preventing the migration of Eosinophils to the airways in comparison with control group (and group one?). The Histopathologic sections of Lung and Bronchoalveolar area also, was implicated these results.

Key Words: Allergic Asthma, Eosinophilic chemotaxy, Herbal Medicine

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INTRODUCTION

Allergic diseases such as asthma, food borne allergies, atopic dermatitis, and allergic rhinitis are common diseases which their incidence rate has increased during the last 30 years. Asthma is a complicated multi-factor pulmonary disease which is diagnosed through reversible blockage, the increase in external bronchial responses and chronic inflammation of bronchial system [1-3]. Asthma is one of the most common chronic diseases of childhood, causing substantial morbidity [4, 5].

Asthma is defined as a chronic inflammatory disorder with intermittent symptoms of cough, dyspnea, wheezing and chest pain. Allergic asthma represents an important public health issue with significant growth over the years and affects individuals of all ages from infants to the elderly prevailing as the major cause of illness and consequent hospitalization in children of developed countries altering both patients and families' normal activities and therefore impairing life quality. Allergic asthma is also associated with high direct and indirect health costs, especially related with diagnosis and treatment [6-8].

So many Environmental and genetically factors are effective in this disease. Allergy is one of the immunological responses which are categorized under the hyper sensitivity reactions [9, 10]. There is a high genetic potential for evolution the allergy immediate hypersensitivity reaction. There are factors

which increase the allergy that in its most severe systematic form which is anaphylaxis, causes the cells to constrict the airways until they cause asphyxia and heart strokes to death [11-13].

Asthma is defined with reversible and periodic contractions of bronchi as a result of different stimulants. Asthma is divided into two groups which is based on the being or lack of a factor in immune disorders. 1) External asthma: starts with hyper sensitivity reactions type which is caused by an external antigen. Three forms of external asthma have been diagnosed so far. 2) Internal asthma: This deals with non-immune beginner mechanisms [12, 14-16].

The presence of asthma allergy is one kind of the asthma, asthma with external origin that is called allergic asthma or atopic asthma which is the most common type of asthma. The diagnosis of allergic asthma is mainly based on the presence of IgE (type I hyper sensitivity). The allergic asthma like type I hyper sensitivity making the TCD⁴⁺ Th₂ cells sensitive which release cytokines like interleukin 4 and 5 (IL-4 and IL-5) which support the making of IgE, the increase of growth of mast cells (IL4) and the activation of Eosinophil (IL5) which have an important role in allergic asthma [17, 18].

The gathered Eosinophils take effects through chemotactic factors of the cells PAF, IL-5 and Eotaxin and produce the basic protein and cationic protein which are poison for the epithelial cell. Therefore the Eosinophils can make the allergic response stable and severe [13, 19]. Eosinophils produce lipid mediators and cytokines which cause the increase in the allergic response. Eosinophils play a main role in the bronchial asthma.

Two mechanisms are involved in the Eosinophils activation: one: The Th₂ cells and mast cells by producing cytokines like IL-5 and a chemokine called Eotaxin. Two: Eosinophils by the Eotaxins and mast cells productions like Histamine and product of breaking it like acid imidiazon acetic, leukoterian B₄ and Baf reabsorbed to the location of mast cells degranulation [12, 19, 20].

Eosinophils and their poisonous products can cause the increase in the airways response. The levels of Eotaxin in epithelium and submucosal are related to the numbers of the Eosinophils and in the plasma of asthmatic individuals the increasing of Eotaxin are related directly to the severity of the disease. The main cell origins of the Eotaxin are the epithelial and the endothelial cells but smooth muscle, mast cells, fibroblast and Eosinophils can produce Eotaxin. Allergic diseases of airways have been known as an inflammatory disease [21-23].

Althaea officinalis, *Malva sylvestris*, *Mentha longifolia*, *Tribulus terrestris* are Herbal Medicine that used for cure and strengthening of patients. These have very useful effects and are safe and old cure for many diseases. In this study we want to research the anti-asthma effect of these Plants when mixed of them are used together.

MATERIALS AND METHOD

In this study we diluted 10 ml of pure sterile Ovalbumin in 50 ml sterile saline buffer (in 0.5ml of this solution, there is 100 µg of Ovalbumin that is used in injection as 100 µg dose) and we sterile the solution with Heater-shaker and magnet and then we kept it in the refrigerator and in each injection into peritoneal 0.5 ml of it was injected to guinea pigs. *Althaea officinalis*, *Malva sylvestris*, *Mentha longifolia*, *Tribulus terrestris* (24-27) four Plants that were used for this research. Powder of these Plants (*Mentha* 40%, *Malva* 30%, *Althaea* 15%, *Tribulus* 15%) were mixed and then this powder was boiled in the Distilled water for 15 min then this fluid was filtered and kept in sterile conditions.

Discharges and Bronchoalveolar fluid were taken from the guinea pig which was challenged and it was sensitive to Ovalbumin, it should be placed in a hanks buffer. Environment for the analysis of the results, comparison, providing the slide and staining were worked, so that no changes accrue in them.

64 male guinea pigs were divided in to four groups and then one of these groups was chosen as the normal control and this process was done for the next 3 groups.

For the first group, after sensitization by 0.5ml Ovalbumin injection in peritoneal space with dose of (20 mg/ml) in the next step challenged through the nose and bronchi, and then prescribed the fluid that is provided through oral with 0.5ml per day for each animal for a week and then anesthetized animals, Bronchoalveolar fluid and mucosal secretion were taken through bronchus and were put into Hank's buffer and then provided slide and stained, then Eosinophils were counted and finally results were analyzed and differences with the control groups were surveyed. Slide of Blood samples were provided too and Eosinophils were counted.

About the second group, like the first group at first, sensitization was done in 14 days and then guinea pigs were challenged through the nose and bronchi with Ovalbumin like the first group, then bronchoalveolar fluid was received and counting was done.

About the third group as the next control group, this group was sensitize with Ovalbumin like the first and the second group, then was challenged with buffer and was sampled like the rest.

About the fourth group as normal control group was challenged only by buffer, slide was prepared and stained then Eosinophils were counted. In the end of this process, animals were euthanatized then from lung and bronchoalvoular of them Histopathological sections were provided.

RESULTS

About the samples of bronch, the effect of these Plants in decreasing of Eosinophils in groups that was received the drug had significantly meaningful difference with control ($p \leq 0.05$). The number of Eosinophils in the first group which received the drug was 11.8% and was decreased compared with the control group that was 36.5% (shown in Tab 1). About the comparison of other groups, the statistical analysis interpretation with Donkan test showed that averages had significant differences ($p \leq 0.05$). The data gathered from the blood samples was compared with Donkan test (Tab 1) that in this case also, like bronchoalvoular fluid samples, significant difference was observed between group 1 and group 3 (control) ($p \leq 0.05$) (Pic 1).

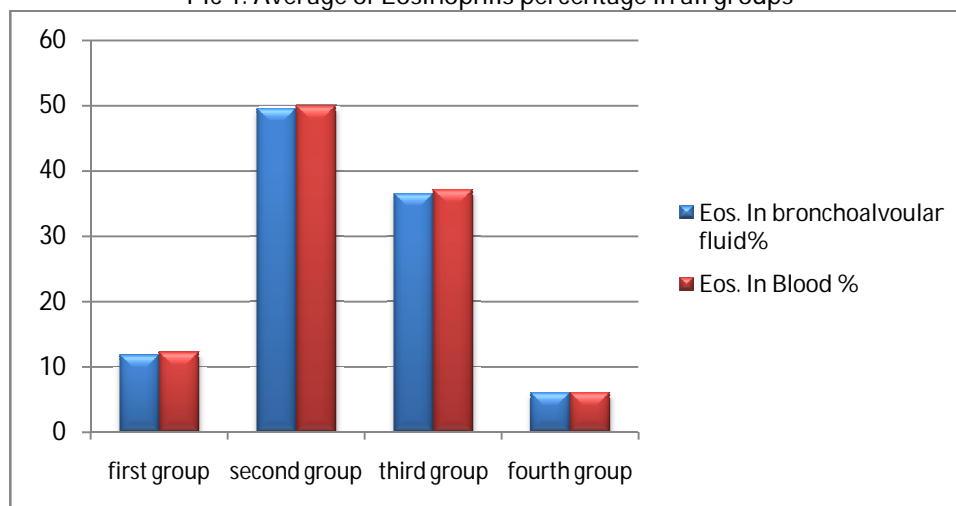
Tab 1: percentage of Eosiniphils in bronchoalvoular fluid and Blood samples

	Eos % in samples of bronchoalvoular fluid	Eos % in samples of Blood
First group	11.8	12.2
Second group	49.5	49.9
Third group	36.5	37.1
Fourth group	6	6

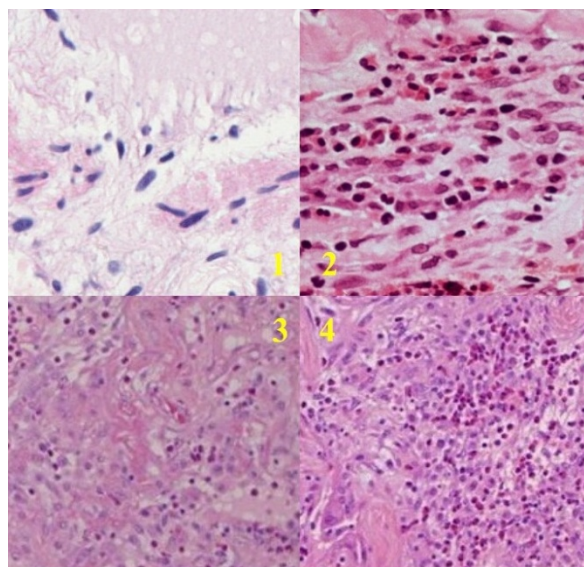
In the Histopathological sections of Lung Eosinophils were decreasing in the interstitial tissue in the group that received drug and edema wasn't observed in the alveolar ducts and interstitial tissue on the treatment group (Pic 2).

In the sections of bronchoalvoular on the treatment group, inflammation of Risesen muscles was very little. Edema, Eosinophils infiltration, erythema and hyperemia were decreased. These results showed that this drug (four plants) had very useful effect on Allergic in Respiratory system.

Pic 1. Average of Eosinophils percentage in all groups



Pic 1. Histopathological sections of Lung Eosinophils. First group section is number 1 that was compared with second group in number 2 and show increasing of Eos in second group. Fourth group section is number 3 that was compared with third group in number 4 and show increasing of Eos in third group.



DISCUSSION AND CONCLUSION

In the current study, the results showed that these Herbal medicines had Anti-allergy effect through decreasing the number of Eosinophils in Blood and mucosa in spite of this fact that its presentation accrued in a short time. the number of Eosinophils in bronchoalvoular fluid in the group that took the drug was significantly decreased in Comparison with control group, and comparison of the samples were obtained from bronchoalvoular fluid and blood samples confirms this effect of these Herbal medicine.

In stuy of Massanari et al role of Th2 and Eosinophils were important in Asthma. With regards to pathophysiology, asthma is recognized by T-helper 2 (Th2)-cell- driven chronic inflammation, and a variety of inflammatory mediators (such as cytokines, chemokines, signaling molecules, adhesion molecules and growth factors) from immune cells and structural cells in the airways are involved in various stages of asthma. Suppressive effect of verproside isolated from *Pseudolysimachion longifolium* on airway inflammation in a mouse model of allergic asthma [28].

Wang and et al findings indicate that the SNPs rs969129 (in intron 2) and rs1494555 (in exon 4) in the IL7R gene may be associated with mite-sensitive allergic asthma in Taiwanese children [29].

Asthma is similarly associated with allergic and non-allergic rhinitis, suggesting a link between upper and lower airways beyond allergy associated inflammation. Only children with allergic rhinitis had increased bronchial responsiveness and elevated FeNO, suggesting different endotypes of asthma symptoms in young children with allergic and nonallergic rhinitis [30].

The Histopathological sections support this results and showed that the inflammation and Eosinophils infiltration were decreased in the group that was given herbal medicine and this decreasing was meaningful from others groups.

Fukunoa and et al After being challenged by an aerosol of OVA, the total number of cells in the BAL fluid was 57.6_12.3_104/ml and 5.9_3.8_104/ml in OVA sensitized and non-sensitized mice, respectively. The increase in OVA-sensitized mice was statistically significant. The increase of cell number was mainly due to an increase of eosinophils. The number of eosinophils in BAL fluids from OVA-sensitized mice was 37.3_14.3_104/ml, and was significantly larger than that of the non-sensitized group, 1.6_1.5_104/ml [31].

Ketotifen is an effective prophylaxis for asthma in children and adult (32, 33). Ketotifen significantly inhibited the chemotaxis of eosinophils to fMLP, IL-5 and eotaxin. The production of reactive oxygen species induced by eotaxin and sIgA was decreased by ketotifen, showing a more pronounced effect when cells were activated by eotaxin [34] but there was no difference in the mean decreases in lung function from pre-exercise baseline values after three doses of ketotifen than with placebo [35].

This is a suitable drug for allergic asthma without any side effects and over dose of this has no poisoning effect. Short time of using this drug has useful effect and using of this is easy for ever patients. Results of these research shows that this drug is very useful and effective drug for reduction of Eosinophils migration and activation so that reduce of the effects of these cells to beginning of asthma. Therefor using of this is important for patients in economic, safety, hygienic, treatment, side effect, benefit and using this drug is very benefit from other Chemical and Herbal drug.

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