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ORIGINAL ARTICLE

The Anti-inflammatory Effect of *Foeniculum Vulgare* (Fennel/Saunf) on Bronchoalveolar Lavage Fluid (BALF) in Ovalbumin-Induced Allergic (OVA) Airway Inflammation in a Mouse Model

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ABSTRACT

Objective: This study evaluates the anti-inflammatory effect of *Foeniculum vulgare* methanolic extract (FME) of two different doses on bronchoalveolar lavage fluid (BALF) in an ovalbumin (OVA)-induced allergic airway inflammation mouse model.

Methodology: BALB/c female mice were divided into five groups (n=8): Normal control, disease control (OVA-sensitised), FME-treated (200 mg/kg), FME-treated (300 mg/kg), and standard dexamethasone-treated. Sensitization was done using intraperitoneal OVA and aluminium hydroxide followed by intranasal OVA challenge. Then, FME was administered orally for 7 days. Afterwards, BALF was collected, and analysis was carried out for total leukocyte count (TLC) and total differential leukocyte count (DLC).

Results: FME significantly reduced TLC and eosinophils when compared to the disease group ($p<0.001$). Similarly, lymphocytes and neutrophils were also notably reduced ($p<0.01$).

Conclusion: FME demonstrates anti-inflammatory activity, comparable to dexamethasone, in OVA-induced allergic airway inflammation and suggests a potential natural remedy for asthma management.

Keywords: Asthma, Bronchoalveolar Lavage Fluid, *Foeniculum vulgare*, Inflammation, Mice, Inbred BALB C, Phytotherapy.

Authors' Contribution:
[1,2]Conception; Literature research; manuscript design and drafting; [3,4]Critical analysis and manuscript review; [5,6]Data analysis; Manuscript Editing.

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Introduction

Allergic asthma is a complex disorder characterized by airway hyper-responsiveness, inflammation and remodeling.[1] It includes infiltration of inflammatory cells, goblet cell hyperplasia and thick mucus plugging in small airways, deposition of collagen under basement membrane, smooth muscle hypertrophy of bronchioles, edema of the airways and activation of mast cells.[2] It is manifested by recurrent episodes of wheezing, breathlessness, chest tightness and coughing associated with airflow

obstruction.[3] According to WHO about 300 million people of all ages are suffering from asthma and approximately 250000 people die from asthma every year.[4] Clinical and experimental findings established that asthma can be elicited by exposure to various stimuli like an allergen, tobacco smoke, perfume, pets, exercise, warm, cold air and emotional stress.[5]

Inflammatory mediators released during inflammation play central role in pathophysiology of allergic airway inflammation. Most of the features are manifested by Th2 mediated cytokines,such as IL-4, IL-5 and 13.[6,7] Elevated amount of Th2 cytokines IL-4, 5, 6 and 13 with increased number of eosinophils are observed in allergic asthmatic patients.[8] Among these cytokines, IL-4 is key factor in the differentiation of T cells into Th2 type which generates a Th2 dominant immune response(6,7), that leads to secrete further cytokines IL4, 5, 9 and 13.[9] IL5 is not involved only in bronchoconstriction and allergic airway inflammation but also play important role in differentiation, maturation and recruitment of eosinophils in airway.[10-11] IL-6 a cytokine produced by inflammatory cells is produced by primary lung epithelial cells in response to variety of different stimuli including allergens, respiratory virus and exercise.[12] Raised level of IL-6 in serum of asthmatic patients have been found.[13]

Different pharmacological modalities used to treat asthma are, controller medication include corticosteroids, long-acting bronchodilator, leukotriene modifiers, phosphodiesterase inhibitors and monocolonal antibodies. Quick relievers include short acting bronchodilator, anticholinergic, systemic corticosteroids and phosphodiesterase inhibitors.[2] Corticosteroids are commonly used for the treatment of allergic asthma as anti-inflammatory agents. However, their use is associated with various undesired adverse effects such as reduced bone metabolism, adrenal suppression and reduced growth in children.[14]

Keeping in view these side effects of corticosteroids, efforts are being made to develop more specific and safer therapies for asthma. Plant based medicines are increasingly used in developing countries to maintain health of people due to their safety, affordability and easy availability.[15] *Foeniculum vulgare (Fennel/Saunf)* is a biennial medicinal and aromatic plant belonging to the family *Apiaceae.* Generally considered indigenous to the shores of Mediterranean Sea but has become widely naturalised in many parts of the world especially on dry soils near the sea coast and on the river banks.[16] Studies have shown that it possesses extraordinary medical properties like anti-inflammatory, analgesic, antioxidant, anti-bacterial, anti-cancer, anti-stress and cytotoxic activity.[17] In addition it is also used as carminative, digestive, diuretic and also in the treatment of many respiratory and gastrointestinal ailments.[18] Its seeds (fruit) methanolic extract exhibit analgesic, anti-inflammatory and anti-allergic activity in it may be due to its immunosuppressive properties and inhibition of cyclooxygenase and lipoxygenase pathways.[19] Another study shows that its anti-inflammatory effect in lipopolysaccharide induced acute lung injury in mice is by regulating the production of pro inflammatory mediators, transcription factors and nitric oxide.[20]

This study was conducted to establish the anti-inflammatory effect of methanolic extract of fennel in ovalbumin induced allergic airway inflammation.

Methodology

This study was carried out in the Department of Pharmacology and Shahbaz Research Centre of King Edward Medical University. Experimental Research Laboratory of PGMI was also used for animal care and procedures. Forty healthy BALB/c mice (6–8 weeks old) were taken from the animal house of PGMI, Lahore. Mice were acclimatized for one week with standard diet and water ad libitum.

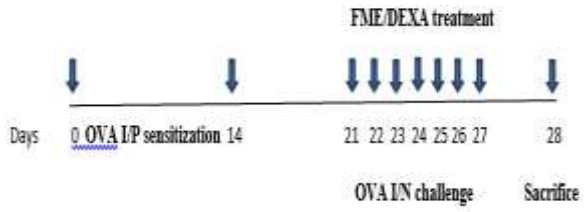
Animals: Female BALB/c mice (6–8 weeks, 20–25 g) were procured and housed under standard conditions.

Plant Material: Foeniculum vulgare (Fennel/Saunf) seeds was purchased from local market and proper identification of the seeds was carried out by the authorized personnal from the Botany Department of GC University, Lahore. After identification seeds were washed and dried under shade. 20g Grounded seed were extracted with 200ml of 80% methanol (80:20 methanol:water v/v) by using orbital shaker for 8hr at room temperature. The extract was separated from solid by using whatman no.1 filter paper. The remaining residue was re-extracted twice and extract was pooled. The solvent was removed under vacuum at 45oC by using rotatory vacuum evaporator and was be stored at -40C until used for further study.[21]

Study Design: Mice were divided into five groups (n=8 per group) randomly and named as:

1. Group A; Normal control (saline)
2. Group B; Disease control (OVA-sensitized)
3. Group C; FME-treated group (200 mg/kg)
4. Group D; FME-treated group (300 mg/kg)
5. Group D; Dexamethasone-treated group (2 mg/kg)

Induction of Allergic airway inflammation:



By intraperitoneal sensitization and intra nasal Challenge: Induction of allergic airway inflammation will be performed by intra-peritoneal sensitization and airway challenge of OVA through nasal inhalation 20 µg of OVA will be dissolved in 2 mg of aluminum hydroxide in a volume of 0.1 ml PBS. All the mice will be intraperitoneally sensitized with OVA at day 0 and day 14 except the control group (group A), which will be sham sensitized with PBS only. One week after the sensitization, the mice will be given intranasal challenge with OVA (1 mg/ml of PBS)once daily for seven consecutive days i.e from day 21 to day 27. The mice in the control group will be challenged intranasally with PBS only.[22]

Treatment: One week after 2nd sensitization i.e 21st day, group C and D will receive methanolic extract of fennel seeds at 200mg/kg (19) and 300/kg body weight respectively orally for 7 consecutive days (up to day 27) one hour before OVA challenge. Similarly group E will be given dexamethasone 1mg/kg body weight[23] orally daily for 7 consecutive days one hour before challenge. Group B is diseased group. Mice from control group A will be sensitized and challenged with PBS only and will also be given orally for treatment.[22]

Euthanization: Twenty-four hours after the last challenge and respective treatment, all the mice will be sacrificed.[22]

Inflammatory Cells in Broncho Alveolar Lavage Fluid: After taking trachea out with lungs intact, BALF was in 1.5 ml tubes by gradual instillation and withdrawal of 1 ml of ice cold PBS through trachea and lungs. A Neubauer hemocytometer
was used to determine TLC in BALF. DLC was be determined by cytocentrifugation of BALF on a glass slide. The BALF was subsequently fixed with methanol and Giemsa Wright staining was used to count on the basis of differential morphology.[22]

Statistical Analysis: Data was statistically analysis by using SPSS (version 21) and graph pad prism version 6. Quantitative variables were demonstrated as Mean ± S.D. One-way ANOVA was applied to see the statistical significance between mean of different groups followed Tukey's post hoc test for multiple comparison of groups

The study was approved by the Institutional Review Committee; Letter no. 123/RC/KEMU. September 2017 to April 2018

Results

Leukocyte Count in BALF: Disease control group showed significantly increased levels of TLC and

Eosinophils (p<0.001). FME significantly reduced Total Leucocyte Count compared to the OVA group (p<0.001) which was similar to dexamethasone. (Table 1, Figure 1)

Differential Count: Eosinophils and Lymphocytes were markedly reduced significantly in FME treated groups (p<0.01), indicating suppressed airway inflammation. There were no significant changes in percentage of neutrophils in BALF of all groups. (Table 1)

Table No.1: Comparison of mean of total leucocytes count and differential cell count in BALF of all groups by ANOVA.

Parameter (BALF)	Group -A Mean ±SD	Group -B Mean ±SD	Group -C Mean ±SD	Group -D Mean ±SD	Group -E Mean ±SD	P-value
TLC 10^3/uL	0.64± 0.09	2.95± 1.42 [a]	1.33± 0.38 [b]	1.17± 0.43 [b]	0.94± 0.29 [b]	<.001
Lymphocytes %	74.50± 1.77	80.63± 1.68 [a]	77.25± 2.25 [b]	75.50± 2.44 [b]	75.00± 2.26 [b]	<.001
Neutrophils %	14.75± 2.18	12.38± 3.02	14.13± 2.80	14.50± 1.77	15.13± 1.24	.165
Eosinophils %	2.25± 0.70	7.25± 2.49 [a]	4.25± 1.03 [b]	2.75± 1.16 [b]	2.37± 0.74 [b]	<.001

[*a] shows a significant difference with group A
[b] shows a significant difference with group B
Group A = normal control, Group B = disease control, Group C = low dose FME treated, Group D =high dose FME treated, Group E = Dexa treated

Discussion

This research was conducted to find out the role of *Foeniculum vulgare* on allergic airway inflammation in mouse model. This research was carried out in the Pharmacology department and Advance Research Center for Biomedical Sciences of KEMU and PGMI Lahore. There were 5 groups each containing 8 mice. One was of normal control group, second group was positive control, third group mice were sensitized and challenged with ovalbumin and then treated by low dose of fennel extract, fourth group mice were sensitized and challenged with ovalbumin and then treated by high dose of fennel extract, fifth group mice were sensitized and challenged with ovalbumin and then treated with dexamethasone. TLC and DLC of BALF was measured to see the inflammatory extent in mice.

TLC of BALF of group B was a significantly higher as compared to group A with p-value of <.001. In group C and group D, which were treated with low and high dose of *Foeniculum vulgare* seed extract respectively, reduced the count significantly when compared to group B with p-value of <.001 and <.001 respectively. Similarly, dexamethasone treatment in mice of group E showed significant decrease in the TLC with <.001 in comparison to group B. There was no significant difference in TLC between group C, D and E and their difference with group A.

In differential leukocytes count, Lymphocytes% and Eosinophils% in BALF were significantly raised in group B as compared to group A with p-value of <.001 and <.001 respectively. Low dose fennel treatment group showed reduction in Lymphocytes% and Eosinophils% count significantly when compared to that of group B with p-value of 0.02 and 0.001 respectively. High dose fennel treatment group showed reduction in Lymphocytes% and Eosinophils% count significantly when compared to that of group B with p-value of <.001 and <.001 respectively. Similarly, dexamethasone treatment group showed reduction in Lymphocytes% and Eosinophils% count significantly when compared to that of group B with p-value of <.001 and <.001 respectively. There was no significant difference of Lymphocytes% and Eosinophils% count between group C, D and E and their difference with group A.

There were no significant changes in percentage of neutrophils in BALF of all groups.

Total inflammatory cells count was raised in BALF after ova exposure similar to previous study in which same mode was used to produce inflammation. [24]

Results of our study showed that treatment with *Foeniculum vulgare* seed extract and dexamethasone in ovalbumin exposed mice reduced the inflammatory cells count of BALF towards normal. This result was comparable to another study in which *Trigonella foenum-graecum,* a member oldest medicinal plant in the fabaceae (legumes) family, TLC and DLC were reduced in ova exposed allergic asthmatic mice.[25] Reduction in inflammatory cells count in BALF is also evident by a study in which fennel essential oil decreased the count in acute lung injury induced by lipopolysaccharide.[26] No study at that time of conduction of study had shown the effect of *Foeniculum vulgare* on inflammatory cells count of BALF in ovalbumin induced airway inflammation, so it was dinstictive at that time distinctive is this respect.[27]

Conclusion

Results of our study showed that methanolic extract of *Foeniculum vulgare* possesses anti-inflammatory effect in ovalbumin exposed allergic airway inflammation in mice. It ameliorates the allergic airway inflammatory changes by lessening the inflammatory cells count in BALF. These findings of *Foeniculum vulgare* are comparable to dexamethasone. However, this study did not explore the underlying molecular mechanisms or cytokine modulation. Future studies on cytokine profiling and molecular pathway analysis may further strengthen its anti-inflammatory effect and may be beneficial.

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