ORIGINAL ARTICLE

THE AMELIORATIVE EFFECT OF FOENICULUM VULGARE (FENNEL/SAUNF) ON BLOOD OVALBUMIN-INDUCED ALLERGIC AIRWAY INFLAMMATION

MUHAMMAD UMAR TALHA[1], NEELOFAR YOUSAF WARRAICH[2], AYELA EMAN ZIA[3], RABIA RAFIQUE[4], HANNAH PIRZADA[5], BINISH ANWAR[6]

[1] Assistant Prof. of Pharmacology, Narowal Medical College, Narowal.[2]Associate Prof. of Pharmacology, Akhtar Saeed Medical and Dental College, Lahore.[3] Associate Prof. of Pharmacology, CMH Lahore Medical College and Institute of Dentistry, Lahore.[4]Assistant Prof, of Pharmacology, CMH Lahore Medical and Dental College, Lahore, [5]Assistant Prof of Pharmacology, Nishtar Medical University, Multan. [6]Assistant Prof of Pharmacology, Al-Aleem Medical College, Lahore

ABSTRACT

Background: Allergic asthma is defined by airway hyper-responsiveness, inflammation, and remodeling. It is characterized by recurring episodes of wheezing, dyspnea, chest constriction, and coughing. Foeniculum vulgare (Fennel/Saunf) is a biennial medicinal and aromatic plant in the Apiaceae family. It exhibits remarkable medicinal attributes, including anti-inflammatory, analgesic, antioxidant, antibacterial, anticancer, anti-stress, and cytotoxic effects.

Objectives: This study evaluates the anti-inflammatory effect of *Foeniculum vulgare* methanolic extract (FME) of two different doses in blood in an ovalbumin (OVA)-induced allergic airway inflammation using a mouse model.

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

Results: FME significantly reduced TLC and eosinophil when compared to the disease group ($p<0.001$).

Conclusion: FME has shown ameliorative activity which is comparable to dexamethasone anti-inflammatory activity, in OVA-induced allergic airway inflammation, suggesting it a beneficial natural remedy for management of asthma.

Key Words: Foeniculum vulgare, Asthma, Blood, Inflammation, Mice

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Correspondence to: *Neelofar Yousaf Warraich*
Associate Prof of Pharmacology, Akhtar Saeed Medical and Dental College, Lahore, Pakistan

Email: *neelofar.warraich3705@gmail.com*

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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] Classical signs and symptoms are 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 manifested by Th2 mediated cytokines, such as IL-4, 5, 6 and 13.[6]

Different pharmacological modalities used to treat asthma are, controller medication include corticosteroids, long-acting bronchodilator, leukotriene modifiers, phosphodiesterase inhibitors and monoclonal 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.[7]

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. [8]

Foeniculum vulgare(Fennel/Saunf) is a biennial medicinal and aromatic plant belonging to the family *Apiaceae.*[9] Studies have shown that it possesses extraordinary medical properties like anti-inflammatory, analgesic, antioxidant, anti-bacterial, anti-cancer, anti-stress and cytotoxic activity.[10] In addition it is also used as carminative, digestive, diuretic and also in the treatment of many respiratory and gastrointestinal ailments.[11] Its seeds(fruit)[11] 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.[12]

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.[13] Considering the anti-inflammatory and ant oxidant effect of ***Foeniculum vulgare*****; t**his study was conducted to see the Ameliorative Effect of Foeniculum Vulgare (Fennel/Saunf) on Blood; Ovalbumin-Induced Allergic Airway Inflammation.

METHODS

This study was carried out in the department of Pharmacology and Shahbaz Research Centre of KEMU. Experimental Research Laboratory of PGMI was also used for animal care and procedures. Forty healthy BALB/c mice (6–8 weeks old) were obtained from the animal house of PGMI. The study was approved by the Institutional Review Committee; Letter no. 123/RC/KEMU. September 2017 to April 2018.

Inclusion criteria: Healthy, Male BALB/c mice, of 6-8 week were included in the study.

Exclusion criteria: Mice with any kind of disease.

Preparation of Experimental Animals: Healthy, male BALB/c strain of mice were bought from UVAS Lahore and randomly divided into five groups with 8 mice in each group from A to E. They were housed in clean cages, provided rodent chow and water ad libitum. Mice were kept at room temperature with natural day and night cycle. Animal handling and care was conducted strictly in accordance with the rules and regulations of ethical committee. A period of one week was given for acclimatization before the start of research study.

Plant material: Fennel seeds were purchased from local market and the proper identification of the herb was done by the Head of Botany Department of GC University; Lahore vides no. GC. Herb. Bot 35202 dated 03-05-2018.

Preparation of methanolic extract of *Foeniculum Vulgare* seeds: The authenticated seeds of *Foeniculum vulgare* (1000g) were washed and air dried in sunlight. Methanolic extract of seeds was prepared in PCSIR Lahore. Dried seeds were ground into fine powder by electric grinder (C & N laboratory mill). Seeds powder was extracted with 80% methanol by using orbital shaker (orbital shaker Lab line) for 8hr at room temperature. The solid material was separated from extract by using Whatman filter paper no.1. The residue was re-extracted twice and then this extract was pooled. Vacuum drier (Mitchell Dryers, Manchester UK) was used to evaporate the solvent at 45^{o}C. Dark brown colored gelatinous material of 34.11g that was soluble in distill water was obtained. Percentage yield was 3.41%. It was kept in glass bottle that was caped securely, protected from sunlight and stored at -4^{o}C for further use. (14)

Drugs and dosage:

1. Ovalbumin 20µg emulsified in 2mg $Al(OH)_3$ in 0.1ml of PBS.(15)
2. Dexamethasone 1mg/Kg. (16)
3. Methanolic extract of *Foeniculum vulgare* 200 mg/kg (17) and 300mg/kg.

Induction of Allergic airway inflammation: Asthma was induced by intra peritoneal sensitization and subsequent intra nasal challenge with ovalbumin.

By intraperitoneal sensitization and intra nasal Challenge: Induction of allergic airway inflammation was done by intra peritoneal sensitization and subsequently airway challenge of OVA through nasal instillation. Mice in all experimental groups were intra-peritoneally sensitized with 20µg of ovalbumin which was emulsified in 2 mg of Al $(OH)_3$ (used as adjuvant) in 0.1 ml volume of PBS at day 0 and 14 except the normal control group (group A), that was sensitized with only PBS. After 1 week of sensitization, mice were given intranasal challenge with ovalbumin (1 mg) dissolved in 1ml of PBS and it was given once daily for seven consecutive days through nasal instillation from 21st to day 27th. Mice in control group were intra-nasally challenged with PBS only.[18]

Treatment protocol: One week after 2nd sensitization that was 21st day, experimental group C and D received methanolic extract of fennel seeds by oral route with 1cc disposable syringe with 7Fr nasogastric tube attached to it, at 200mg/kg and 300/kg of body weight respectively for one week (from day 21 to day 27) one hour before OVA challenge. Group E was given dexamethasone 1mg/kg of the body weight, orally daily for seven consecutive days 60 minutes before OVA challenge. Group B was disease group. Mice from normal control group A were sensitized and challenged with PBS only.

Preparation of dose of methanolic extract of *Foeniculum Vulgare:* For group C, 200mg of the fennel extract was completely dissolved in 5ml water so that:

For 1000g mouse required dose = present in 5ml which contain 200mg of the extract

For (X) g of mouse = 5/1000*(X) or 0.005*(X)

X = weight of mouse in grams

For group D, 300mg of the extract was completely dissolved in 5ml water and dose was calculated by the above given formula.

Preparation of dose of dexamethasone: Dexamethasone was given at dose of 1mg/kg and it was calculated by 5mg of dexamethasone dissolved in 25ml of distill water so that there was 1mg dexamethasone in 5ml of distill water

For 1000g mouse required dose = present in 5ml of distill water

For (X) g of mouse = 5/1000* (X) or 0.005*(X)

X = weight of mouse in grams

Grouping Of Animals: Animals were placed randomly into five groups having eight mice in a group. Each group was kept in separate cages.

Group A (Control group): Mice in this group were sham sensitized with PBS intra-peritoneally on day 0 and 14 and challenged intra-nasally with PBS solution from day 21 to 27.[18]

Group B (Positive control group): Mice in this group were sensitized with OVA on day 0 and day 14 then challenged intra nasally with OVA from day 21 to 27. [18]

Group C (Methanolic extract treatment group 200mg/kg): Mice in this group were sensitized intra-peritoneally with OVA on day 0 and day14, challenged intra-nasally with OVA from day 21 to 27 and treated with methanolic extract 200mg/kg orally one hour before each intranasal challenge.[18]

Group D (Methanolic extract treatment group 300mg/kg): Mice were sensitized intra-peritoneally with OVA on day 0 and 14, challenged intra-nasally with OVA from day 21 to 27 and treated with methanolic extract 300mg/kg orally one hour before each intranasal challenge.[18]

Group E (Dexamethasone treatment group): Mice in this group were sensitized intra-peritoneally with OVA on day 0 and 14, challenged intra nasally with OVA from day 21 to 27 and treated with the 1mg/kg of dexamethasone orally one hour before each intranasal challenge.[18]

DATA COLLECTION PROCEDURE:

Euthanization: One day after the last challenge and treatment that was the 28th day, mice in all the groups were sacrificed under light chloroform vapors anaesthesia.[18]

Inflammatory Cells of Blood: Blood was taken by intra cardiac puncture in EDTA containing tubes. Total and differential leukocyte count was determined by automated hematological analyzer Sysmex XT 1800i.[19]

Effect of fennel extract on total leukocyte count (TLC) in blood: There was a significant increment in total leucocyte count of group B (disease group) as compared to control Group A (10.33±1.69 vs 6.75±0.78) that showed ovalbumin had induced the allergic airway inflammation in diseased group and resulted in recruitment of leukocytes. Treatment with low dose methanolic extract of fennel (group C) showed significant curtailment in TLC count as compared to group B (7.52±0.94 vs 10.33±1.69). Treatment with high dose methanolic extract of fennel (Group D) also showed reduction in TLC count as compared to disease group (7.00±1.16 vs 10.33±1.69). Similarly, dexamethasone treatment (Group E) also significantly reduce leukocytes count when compared to Group B (6.90±1.15 vs 10.33±1.69). When ANOVA was applied, difference was significant among groups with p-value of <.001.

Post-hoc analysis by Tukey's test was used for pair wise comparison of groups. There was an increase in TLC count in group B as compared to group A with a significant p-value of <.001, whereas a significant decrease in TLC count of groups C, D and E when compared with that of group B with p- value of <.001, <.001, <.001 respectively. However, TLC count of group C, D and E was insignificant in comparison to group A.

DISCUSSION

This study was to find out the role of *Foeniculum vulgare* on allergic airway inflammation in mouse model. This research study was carried out in the Pharmacology Department and Advance Research Center for Biomedical Sciences of KEMU and PGMI Lahore. Five groups each containing 8 mice were included in the study. 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 followed by treatment with dexamethasone. TLC and DLC of blood were measured to see the inflammatory effect in mouse model.

In our study there was a significant increase in TLC in group B as compared to normal control group with p-value of <.001. Mice in groups which were treated with low and high dose of *Foeniculum vulgare* seed extract, reduced the count significantly when compared to group B with p-value of <.001 and <.001 respectively. This result was in accordance with a previous study in which effect of Hydro-Alcoholic

Extract of *Foeniculum vulgare* Mill on Leukocytes and Hematological Tests in Male Rats was seen and *Foeniculum vulgare* increased red and white blood cells probably due to the presence of polyphenols and antioxidant activity of *Foeniculum vulgare* and reduced negative effects of free radicals on blood cells.[20]

Similarly, dexamethasone treatment in mice of group E showed significant decrease in the TLC with <.001 in comparison to group B which was in consistent with a previous study showing successful ameliorative effect of dexamethasone. (21) There was no significant difference of TLC between group C, D and E and also their difference with control group A. There was no significant difference of TLC between group C, D and E and also their difference with group A. This result was in accordance with a previous study in which effect of Hydro-Alcoholic Extract of *Foeniculum vulgare* Mill on Leukocytes and Hematological Tests in Male Rats was seen and *Foeniculum vulgare* increased red and white blood cells probably due to the presence of polyphenols and antioxidant activity of *Foeniculum vulgare* and reduced negative effects of free radicals on blood cells.[20]

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

Parameter (Blood)	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	6.75±0.78	10.33±1.69 [a]	7.52±0.94 [b]	7.00±1.16 [b]	6.90±1.15 [b]	<.001
Lymphocytes%	65.98±2.32	71.13±3.08 [a]	67.39±2.75 [b]	66.38±2.53 [b]	66.49±1.90 [b]	.002
Neutrophils %	22.46±1.28	20.08±4.14	21.80±3.86	22.19±1.14	22.50±1.84	.394
Eosinophils %	1.87±0.66	6.62±2.37 [a]	4.52±1.50 [b]	3.13±0.96 [b]	2.61±0.99 [b]	<.001

[a] show 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,
Group D =high dose FME, Group E = Dexa treated

In differential leukocytes count, Lymphocytes% and Eosinophils% were significantly higher in group B as compared to normal control group with p-value of .002 and <.001 respectively. [22] Low dose fennel treatment group showed reduction in Lymphocytes% and Eosinophils% count significantly when compared to that of group B with p-value of 0.04 and 0.02 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 0.006 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 0.007 and <.001 respectively. [23] There was no significant difference of Lymphocytes% and Eosinophils% count between group C, D and E and also their difference with group A. [20]

Lee et al. demonstrated that fennel inhibits NF-κB activation and lowers IL-6/TNF-α levels in lung tissue [24] and other work shows F. vulgare extracts block 5-lipoxygenase–mediated leukotriene synthesis.[25] Our results are congruent with these reports: the reduction in eosinophils and lymphocytes likely reflects suppression of Th2 cytokines (IL-4, IL-5, IL-13) and mediators that drive allergic inflammation, an effect that has not been previously demonstrated in asthma.

There were no significant changes in percentage of neutrophils in blood of all groups. This is expected in a classic allergic (eosinophilic) asthma model, where neutrophils play a minor role. The lack of neutrophil response suggests FME primarily modulates the Th2-eosinophil axis rather than neutrophilic pathways. Indeed, volatile fennel oil has been reported to inhibit neutrophil activation in [26], but neutrophil involvement was not prominent in our model.

Previous studies have documented fennel's anti-inflammatory effects in many other aspects [27], but none have examined its impact on allergic airway leukocyte profiles. Our results showed that treatment with *Foeniculum vulgare* seed extract and dexamethasone in ovalbumin exposed mice reduced the inflammatory cells count of blood toward normal.

CONCLUSION

In conclusion, FME exhibits potent anti-inflammatory and immunomodulatory activity in OVA-induced asthma, essentially normalizing leukocyte counts similarly to dexamethasone.

Future studies should delineate the molecular mechanisms involved: for example, profiling Th2/Th1 cytokines (IL-4, IL-5, IL-13, IFN-γ) and signaling pathways (e.g. NF-κB, MAPKs) in lung tissue will clarify how FME exerts its effects. Assessing airway histopathology, remodeling markers, and cytokine expression will further validate fennel's anti-asthmatic potential and help in developing it as a complementary therapy.

ETHICAL APPROVAL

Ethical approval of article was granted by the Institutional Review Board of King Edward Medical University vide reference No 123/RC/KEMU dated 08 February, 2018.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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AUTHOR'S CONTRIBUTIONS

MUT: Concept, design, Manuscript writing
NYW, AEZ: Manuscript writing, Critical Review
RR: Data collection, data analysis
HP: Manuscript writing
BA: Critical Review, final approval
All Authors: Approval of the final version of the manuscript to be published

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