

Research Article

Nigella Sativa Essential Oil Prevents Airway Inflammation in Ovalbumin Sensitized Guinea-pigs

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Abstract

Objectives: *Nigella sativa* has been investigated as an adjuvant and prophylactic agent for asthma. This study was planned to investigate the effect of *Nigella sativa* essential oil on guinea pig model of allergic airway inflammation.

Methods: In this study conducted at PGMI, Lahore, eighteen guinea pigs were divided into three groups. Disease Control (DC) and *Nigella sativa* (NS) group were sensitized with intraperitoneal injection of ovalbumin on day 0 and 14 and then challenged with inhalation of 1% ovalbumin on days 22, 23 and 24. Normal control (NC) was given equivalent treatment with phosphate buffer saline (PBS). NS group was given 5 mg/kg *Nigella sativa* oil orally before every intranasal challenge. At the end of study, blood and bronchoalveolar lavage (BAL) samples were collected for estimation of total leukocyte counts (TLC), differential leukocyte counts (DLC) and eosinophil percentage.

Results: Both sensitized groups had significantly raised TLC in both blood and BAL fluid. *Nigella sativa* treatment lowered TLC in blood insignificantly but highly significantly in BAL fluid. Similarly, eosinophil percentage was significantly high in both sensitized groups and *Nigella sativa* treatment was able to affect a highly significant reduction in both blood and BAL fluid.

Conclusion: *Nigella sativa* reduces airway inflammation when used before allergic airway challenge. It may be considered as a prophylactic agent to asthma therapy.

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Introduction

Chronic inflammatory airways disorder asthma is characterized by hyper responsiveness and limitation of respiratory tract control¹. Inflammation of bronchial tissues aggravates the bronchial hyper responsiveness and they become swollen and hypertrophied. Whenever exposed to triggers or allergens, the patients develop cough, dyspnea and chest tightness². Occurrence of asthma is rising worldwide due

to various types of allergens particularly in developed countries. Most of them are indoor allergens which include tobacco-smoke, dust-mite, fungus and cockroaches as well as outdoor pollution³.

Airways hyper responsiveness is defined as airway constriction in response to nonspecific stimuli. This hyper responsiveness is the result of inflammation involving certain cell types, which are characteristic of asthma. These cells include mast cells that liberate

prostaglandins and leukotrienes which are responsible for an immediate reaction. Eosinophils are found in bronchial walls and their secretions liberating interleukins and different other chemokines. This continuous inflammatory process causes remodelling of bronchial structure in the long run manifested as increased bronchial hyperresponsiveness⁴.

To reduce the risk of acute attack, it is advised to avoid known allergens particularly in children⁵. Use of therapeutic agents includes bronchodilators, anti-inflammatory drugs like corticosteroids, monoclonal antibodies and antibiotics. Generally, bronchodilators and corticosteroids are delivered as aerosol or powders directly into the lungs and airways consequently decrease the doses requirements and unwanted effects⁶. These agents, though useful, carry potentially severe adverse effects and are not always fully successful at controlling asthma. Search for relevant alternates is, therefore, need of the day.

Nigella sativa (NS) commonly known as Kalonji or black seed, is used for prophylaxis and treatment of asthma in traditional medicine. Its major constituent thymoquinone (TQ), which has been shown many biological properties including anti-inflammatory, antimicrobial, cytoprotective and antioxidant activities⁷. *Nigella sativa* decreases low density lipoprotein cholesterol level significantly thus prevents atherogenesis and increases high density lipoprotein cholesterol level⁸. This ingredient also has the ability to interact with a variety of proteins. TQ can also inhibit certain protein-protein interactions. It has also been found through various studies that *Nigella sativa* has no side effects on liver and kidney⁷.

Use of *Nigella sativa* in different forms improved wheezing, breathlessness, chest tightness and cough in 10% of asthmatic patients⁹. In a study on chemical war victims, *Nigella sativa* aqueous seed extract was used resulting in improvement in pulmonary function¹⁰. Preventive effect of *Nigella sativa* has been demonstrated on COPD model¹¹. In another randomized controlled trial, *Nigella sativa* seeds were given for a period of three months along with inhaled maintenance therapy, with minimal improvement in pulmonary function and inflammation in partly controlled asthma¹². The evidence for beneficial effect of *Nigella sativa* in bronchial asthma is scanty¹³ requiring further investigation. Objective of this

study was to observe the effect of *Nigella sativa* oil on airway inflammation in sensitized guinea pigs.

Methods

This animal experimental controlled study was conducted at Post Graduate Medical Institute; Lahore after approval from institutional ethical committee. Sample size was taken according to the number of animal in similar studies¹⁴. Eighteen guinea pigs of either gender 350 to 490 g body weight procured from Zoo Park Lahore were included and were kept in animal house of PGMI Lahore at 22°C – 24°C with natural light-dark cycle. They had free access to food and water. Eighteen animals were divided into 3 groups, normal control (NC), disease control (DC) and *Nigella sativa* treated (NS). They were sensitized and challenged with ovalbumin¹⁴ and treated as depicted in table 1. Ovalbumin was from Alfa aesar GmbH & co KG, alum from Biosector, Denmark, Phosphate Buffer Saline (PBS) from Sigma Aldrich, Germany and *Nigella sativa* oil was prepared at PCSIR Laboratories, Lahore.

Nigella sativa seeds (Kalonji) were purchased from local market. After being ground they were subjected to hydro distillation in completely dried reverse Dean-Stark apparatus (Sattar 1989) for six hours. The extracted essential oil was stored at 4°C. Yield of essential was 0.2%. Weight of one ml essential oil of *Nigella sativa* was one gram and it was diluted in 90% ethyl alcohol to make volume of individual dose easily measurable.

Blood was drawn at the end of study from anaesthetised animal through cardiac puncture. Afterwards, bronchoalveolar lavage (BAL) fluid sampling was done on euthanized animals by instilling and withdrawing 0.5 ml of ice cold PBS through trachea. Total leukocyte counts (TLC), differential leukocyte counts (DLC) and eosinophil percentage were estimated in blood and BAL by manual method.

SPSS 20 was used for statistical analysis. Normality of data was tested by Shapiro Wilk test. Homogeneity of variance was tested by Leven's statistics. Mean and standard deviation were calculated as descriptive measures. ANOVA followed by post hoc Tukey test were used to assess the effect of *Nigella sativa* oil. The level of significance was ≤ 0.05 .

Results

Blood TLC was significantly higher in DC group than NC (p-value 0.043). *Nigella sativa* treatment lowered blood TLC but difference was insignificant compared to both DC and NC (Fig 1). DLC of blood is given in table 2. Eosinophil percentage was highest in DC group. *Nigella sativa* treatment caused a significant fall in eosinophil percentage than DC (p-value 0.001) but it was still significantly higher than NC (p-value 0.001) (Fig 2).

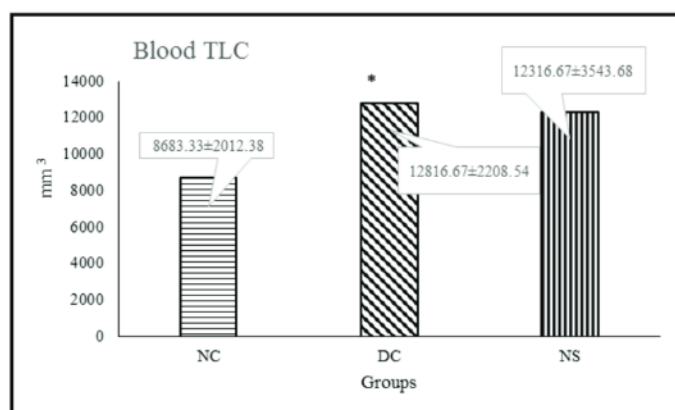


Fig. 1: Total Leukocyte Count (Mean±SD) of Blood (n=6).

* - p-value < 0.05 vs NC

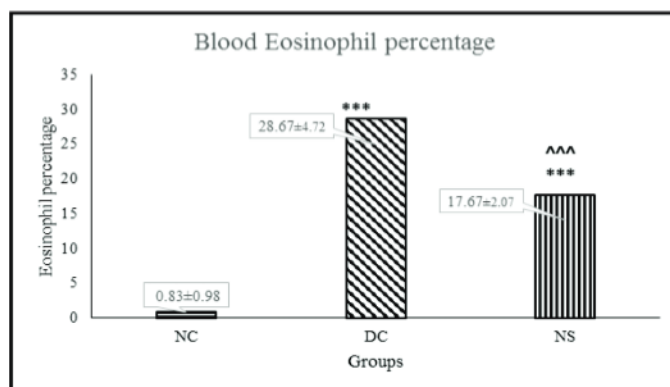


Fig. 2: Eosinophil Percentage (mean±SD) of Blood (n=6).

*** p-value < 0.001 vs NC; ^^ p-value < 0.001 vs DC

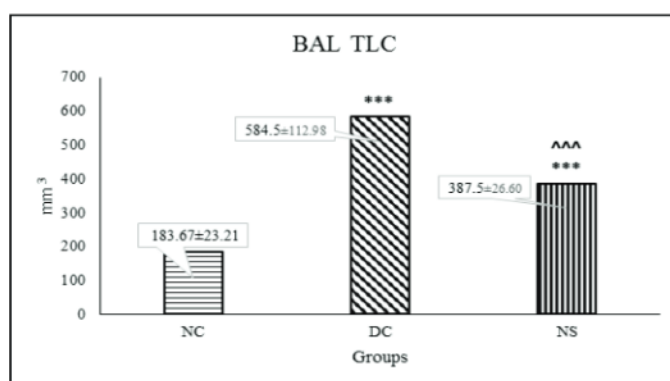


Fig. 3: Total Leukocyte Count (Mean±SD) of BAL Fluid (n=6).

*** - p-value < 0.001 vs NC; ^^ - p-value < 0.001 vs DC

Table 1: Animal Grouping and Experimental Interventions

Days	Normal Control (NC)	Disease Control (DC)	<i>Nigella sativa</i> (NS)
Sensitization Day 0 & 14	1.5 ml PBS intraperitoneally	100 µg ovalbumin and 200 mg alum in PBS intraperitoneally	100 µg ovalbumin and 200 mg alum in PBS intraperitoneally
Intranasal Challenge Day 22, 23, 24	Intranasal challenge with PBS	Intranasal challenge with 1% ovalbumin in PBS	Intranasal challenge with 1% ovalbumin in PBS
Treatment Day 22, 23, 24	0.5 ml/ kg ethyl alcohol orally one hour before each challenge	0.5 ml/ kg ethyl alcohol orally one hour before each challenge	5 mg/0.5 ml alcohol/ kg <i>Nigella sativa</i> oil orally one hour before each challenge

Table 2: Differential Leukocytes Count (Mean±SD) of Blood

Group n=6	Neutrophil %	Lymphocyte %	Eosinophil %	Monocyte %
Normal control	25.33±5.85	69.50±5.43	0.83±0.98	4.33±3.08
Disease control	25.33±10.11	48.67±10.71	28.67±4.72	2.17±1.60
<i>Nigella sativa</i>	22.50±25.33	57.50±6.44	17.67±2.07	2.33±1.63

Table 3: Differential Leukocytes Count (Mean±SD) of BAL Fluid

Group n=6	Neutrophil %	Lymphocyte %	Eosinophil %	Monocyte %
Normal control	23.33±23.33	62.00±8.39	9.33±3.72	5.33±3.72
Disease control	19.50±4.23	49.50±6.75	48.00±2.61	2.00±1.10
<i>Nigella sativa</i>	17.83±3.43	49.33±4.32	29.17±3.87	3.67±2.16

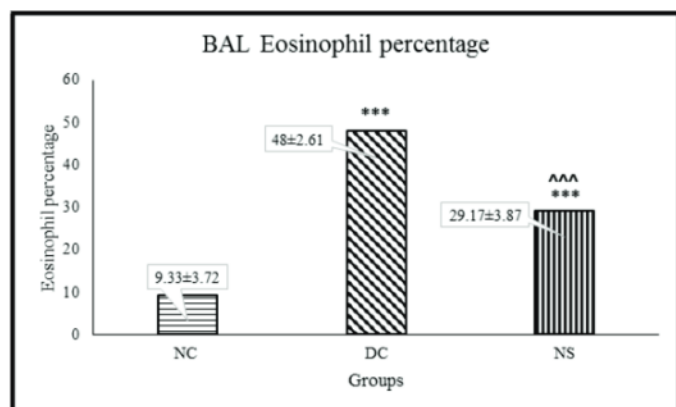


Fig. 4: Eosinophil Percentage (mean±SD) of BAL Fluid (n=6).

***p-value<0.001 vs NC; ^^^p-value<0.001 vs DC

BAL fluid TLC had highest value in DC group, lowest in NC group and significant fall (p-value <0.001 vs DC) in NS group (Fig 3). DLC of BAL fluid is given in table 3. Like blood eosinophil percentage was the highest in DC group. Significant lowering was observed with *Nigella sativa* treatment (p-value <0.001 vs DC) but it was still significantly higher than NC (p-value <0.001) (Fig 4).

Discussion

Medicinal plants are highly valued all over the world as rich sources of therapeutic agents for prevention of various medical conditions including asthma. They are widely used due to their safety, efficacy, cultural acceptability and lower number of side effects as compared to synthetic drugs. *Nigella sativa* is one of these medicinal herbs widely grown and used for anti-inflammatory activity.¹⁵

Present study was conducted on guinea pigs, which can be easily sensitized for asthma model.¹⁶ In this study, disease control group had markedly higher blood TLC as compared to normal control group. *Nigella sativa* group had lower TLC as compared to that of disease control group but significantly higher count as compared to normal control. Similar study by Keyhanmanesh et al demonstrated the preventive effect of thymoquinone; a main constituent of *Nigella sativa* on guinea pig asthma model.¹⁷ This study supports the results of present study. Allergic airway inflammation shows increased eosinophil percentage in blood as well as in bronchial secretions.¹⁸ In the present study disease control group had very high eosinophilic percentage in the blood. *Nigella sativa* treatment reduced this rise significantly with p value

0.001 but not upto the normal level. Supporting these results is a study on mice where *Nigella sativa* fixed oil treatment reduced eosinophil percentage significantly with p value 0.05 as compared to sensitized group¹⁹. Similarly effect of *Nigella sativa* on peripheral blood eosinophil count, immunoglobulin production and inflammatory cells in mouse model of allergic air way inflammation showed significantly reduced levels of all parameters²⁰.

When allergic airway inflammation is induced by ovalbumin, there is increase in inflammatory cell count of BAL fluid¹⁶ as observed in disease control group of this study. *Nigella sativa* treated guinea pigs had lower BAL fluid eosinophil percentage as compared to DC, but it was still significantly higher as compared to normal animals with p values < 0.001. Studies on mice asthmatic models treated with *Nigella sativa* oil²¹ and thymoquinone^{22,23} made similar observations. In a study using the same model of ovalbumin sensitized guinea pigs, different constituents of *Nigella sativa* were used. Total leukocyte and eosinophil counts in all treated groups decreased significantly in comparison with the disease model group.²⁴ A randomized double-blind placebo-controlled experiment was recently conducted in asthmatic patients.²⁵ *Nigella sativa* oil 500mg twice daily for one month was used as supplement, which significantly decreased the eosinophil count with p value 0.013.

In the present study lower TLC and eosinophil percentage in blood and BAL fluid after *Nigella sativa* treatment, in the beginning of paragraph suggests some anti-inflammatory role of this herbal product in allergic asthma. Some studies have explored the underlying mechanism of this beneficial effect, throwing light on various pathways. One of them is through nitric oxide pathway¹⁹, others consider inhibition of prostaglandin production²² and leukotriene biosynthesis²³ as major mechanisms involved. Thus, one can say that multiple mechanisms may be involved and depending upon dose used, one may dominate over other.

In this study standard methodology was adopted to validate the traditional uses of herbal compound *Nigella sativa* while unfortunately due to unavailability of facilities advanced and sophisticated tools were not used to determine the molecular

mechanisms.

Conclusion

Essential oil of *Nigella sativa* reduces airway inflammation in guinea pig model of allergic airway inflammation. Effect of ovalbumin sensitization was more marked on BAL fluid. Similarly effect of NS treatment was also more on BAL fluid as compared to blood parameters. Controlled trials may be conducted on humans to evaluate its role as adjuvant and prophylactic agent in asthma.

Ethical Approval: Given

Conflict of Interest: None

Funding Source: None

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