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Ethanol Extract of *Nigella sativa* Attenuates Eosinophilic Inflammation and Epithelial Remodeling in an Ovalbumin-Induced Rat Model

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Abstract
Allergic airway inflammation is commonly associated with eosinophilic infiltration and epithelial remodeling, which impair respiratory function and contribute to tissue damage. This study investigated the therapeutic potential of the ethanol extract of *Nigella sativa* in an ovalbumin-induced rat model of allergenic airway response. Ethanol extracts were tested in an ovalbumin-induced rat model using Sprague-Dawley rats (n=36), comparing normal, negative controls, 0.009 mg/g BW salbutamol (positive control), and three inhaled extract doses (12.25, 24.50, 49 g/kg BW). Comprehensive analysis included physiological parameters (oxygen saturation), inflammatory biomarkers (lung eosinophil count), and pulmonary histopathology (epithelial thickness). The highest dose (49 g/kg) matched salbutamol in improving oxygen saturation (86.75% vs. 88%, p=0.9917) and reducing epithelial thickness (81.50 µm vs. 81.75 µm, p=0.9999), while suppressing eosinophil infiltration (0.73 vs. 1.45 cells/field, p=0.3348). These results confirm *Nigella sativa*'s efficacy in attenuating airway inflammation via eosinophil-targeted mechanisms without structural epithelial alterations. The study validates traditional applications of *Nigella sativa* and proposes an evidence-based framework for integrating phytotherapy into respiratory medicine.

Keywords: airway hyperresponsiveness, eosinophilic inflammation, inhaled therapeutics

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Ekstrak Etanol *Nigella sativa* Meredakan Peradangan Eosinofilik dan Remodeling Epitel pada Model Tikus yang Diinduksi Ovalbumin

Abstrak
Peradangan saluran napas akibat alergi umumnya dikaitkan dengan infiltrasi eosinofil dan remodeling epitel, yang mengganggu fungsi pernapasan dan berkontribusi terhadap kerusakan jaringan. Penelitian ini bertujuan untuk mengetahui potensi terapeutik ekstrak etanol *Nigella sativa* pada model tikus dengan respons alergi pada saluran napas yang diinduksi oleh ovalbumin. Ekstrak etanol diuji pada model tikus yang diinduksi ovalbumin menggunakan tikus Sprague-Dawley (n=36), dengan membandingkan kelompok normal, kontrol negatif, salbutamol 0,009 mg/g BB (kontrol positif), dan tiga dosis inhalasi ekstrak (12,25; 24,50; 49 g/kg BB). Analisis komprehensif mencakup parameter fisiologis (saturasi oksigen), biomarker inflamasi (jumlah eosinofil paru), dan histopatologi paru (ketebalan epitel). Dosis tertinggi (49 g/kg) setara dengan salbutamol dalam meningkatkan saturasi oksigen (86,75% vs. 88%, p=0,9917) dan mengurangi ketebalan epitel (81,50 µm vs. 81,75 µm, p=0,9999), sekaligus menekan infiltrasi eosinofil (0,73 vs. 1,45 sel/lapangan, p=0,3348). Hasil ini mengonfirmasi keefektifan *Nigella sativa* dalam meredakan peradangan saluran napas melalui mekanisme yang menargetkan eosinofil tanpa perubahan struktural epitel. Studi ini memvalidasi aplikasi tradisional *Nigella sativa* dan mengusulkan kerangka kerja berbasis bukti untuk mengintegrasikan fitoterapi ke dalam terapi sistem pernapasan.

Kata Kunci: hiperresponsivitas saluran napas, peradangan eosinofilik, terapi inhalasi

1. Introduction

Allergic airway diseases, including allergic rhinitis and allergen-induced airway inflammation, represent a growing global health burden. Current estimates suggest that more than 1 billion people worldwide are affected by allergic conditions, with prevalence projected to reach 4 billion by 2050 due to urbanization, pollution, and lifestyle changes.[1,2] Asthma, as one of the most common allergic airway diseases, affects million individuals globally, with eosinophilic inflammation and epithelial remodeling recognized as key contributors to morbidity.[3,4] Allergic rhinitis, often comorbid with asthma, has a prevalence ranging between 10–30% in adults and up to 40% in children, further underscoring the scale of allergic airway disorders.[5]

In Indonesia, epidemiological data highlight a significant burden of allergic airway diseases. The ISAAC survey reported childhood asthma prevalence ranging from 2.1% to 11%, depending on region and diagnostic criteria. Moreover, studies conducted at RSUP Persahabatan, Jakarta, revealed a high proportion of allergic rhinitis among asthma patients, confirming the strong correlation between upper and lower airway allergic diseases in the Indonesian population.[6,7] These findings emphasize that allergic airway inflammation is not only a global concern but also a pressing national issue, contributing to reduced productivity, school absenteeism, and increased healthcare utilization.

Allergic airway inflammation represents a major clinical challenge due to its association with eosinophilic infiltration and epithelial remodeling, processes that impair respiratory function and contribute to tissue damage. Eosinophils are central effector cells in type 2 immune responses, and their accumulation in lung tissue correlates with disease severity and persistence of inflammation.[8] The remodeling of airway epithelium, including thickening and structural alterations, further exacerbates airway dysfunction and has been recognized as a hallmark of chronic allergic responses.[4,9] Conventional therapies like inhaled corticosteroids and long-acting β2 agonists face challenges such as pharmacological resistance and systemic side effects, including osteoporosis and tachyphylaxis.[10,11]

In this study, *Nigella sativa* (L.), a plant from the Ranunculaceae family with over two millennia of use in traditional medicine, emerges as a potential phytotherapeutic candidate due to its complex phytochemical profile. Its major bioactive constituent, thymoquinone, has demonstrated potent anti-inflammatory and immunomodulatory effects, including inhibition of NF κB signaling, suppression of eosinophil recruitment, and modulation of epithelial responses.[12,13] Recent reviews highlight its superior anti-inflammatory activity compared to other phytotherapeutics, underscoring its translational relevance in allergic airway conditions.[14,15] Thymoquinone (TQ), the primary component of black seed, exhibits significant anti-inflammatory properties by reducing oxidative stress and regulating inflammation-related gene expression.[16,17] Scientific evidence supports its use in treating respiratory disorders like asthma and bronchitis through mechanisms involving oxidative stress reduction and inflammation suppression. *Nigella sativa* demonstrates complex anti-inflammatory effects via multidimensional interactions at cellular and molecular levels, primarily mediated by the bioactive compound TQ. TQ's antioxidant activity is reflected in its ability to neutralize reactive oxygen species (ROS) such as superoxide anions and hydroxyl radicals, while enhancing endogenous antioxidant enzyme expression (catalase and glutathione peroxidase) via Nrf2 transcription factor activation.[15,18]

In animal models, TQ reduces lung tissue malondialdehyde (MDA) levels by 67%, correlating with decreased airway epithelial damage.[16,19] In inhibiting inflammatory signaling, TQ blocks the NF-κB pathway by preventing IκBα phosphorylation at Ser32/36 residues, thereby suppressing IL-6 and TNF-α production.[15,19] Parallel modulation of the JAK2/STAT3 pathway through STAT3 SH2 domain binding and PI3K/Akt/mTOR pathway inhibition further reduces proinflammatory gene expression.[15,20] At the lipid mediator level, TQ inhibits COX-2 and 5-LOX, lowering PGE2 and LTB4 synthesis.15 TQ's immunomodulatory effects include M1-to-M2 macrophage polarization and T-cell subset regulation via suppression of Th17 differentiation (through RORγt inhibition) and Treg cell expansion.[13,21] Regarding bronchodilation, L-type calcium channel blockade and NO/sGC/cGMP pathway activation achieve 82% of salbutamol's bronchodilatory effect.[22]

Experimental models using ovalbumin sensitization have been widely employed to replicate allergen-induced airway inflammation, providing reproducible endpoints such as hypoxia, eosinophil infiltration, and epithelial thickening.[23] These models allow for the evaluation of therapeutic agents targeting both inflammatory and structural components of airway pathology.[24] Despite preclinical studies confirming its potential in modulating NF-κB pathways and reducing oxidative stress, critical gaps remain in the holistic understanding of efficacy profiles, particularly for inhaled formulations and their impact on airway tissue remodeling. While *Nigella sativa* has been extensively studied in oral and systemic formulations for asthma and respiratory disorders, reports on inhaled formulations remain very limited. A recent study by Rashid et al

(2024) investigated *Nigella sativa* extract delivered via a dry powder inhaler (DPI) in animal models, highlighting its potential as an inhaled therapeutic.[25] This study adopts a translational approach to bridge traditional knowledge and modern biomedicine, employing an ovalbumin-induced allergenic airway response model in *Sprague Dawley* rats (n = 36). Three doses of *Nigella sativa* seed ethanol extract (12.25, 24.50, and 49 g/kg BW) were tested against salbutamol as a positive control, with multidimensional evaluations including: physiological parameters (oxygen saturation), inflammatory biomarkers (lung tissue eosinophil count), and histopathological analysis (bronchial epithelial thickness). This study not only validates *Nigella sativa*'s traditional use but also introduces a scientific framework for integrating phytopharmaceuticals into modern respiratory disorder management. The dissociation between anti-inflammatory effects and tissue remodeling findings opens new perspectives for developing adjuvant therapies that specifically target eosinophilic inflammation while avoiding long-term complications related to airway architectural modifications.

2. Materials and Method

2.1. Tools

Animal cage, beaker glass, measuring cylinder, syringe needle 1 cc, scales, surgical instruments, infusion pan, rotary evaporator, nebulizer (Omron CompAir Compressor Nebulizer), Olympus BX51 microscope, handheld pulse oximeter (H100), hematology analyzer (Mindray BC30).

2.2. Materials

Plant Materials: Black cumin seeds (*Nigella sativa L.*) obtained from the Research Center for Spices and Medicinal Plants (BALITRO), Bogor, Indonesia. Chemicals and Reagents: Ovalbumin (Worthington Biochemical Corporation), salbutamol (Century Pharma), NaCl 0.9%, distilled water (Aquadest), $Al(OH)_3$, and 10% formalin (Indopath). Experimental Animals: Male Sprague Dawley rats, aged 3–4 months, weighing 200–250 grams, obtained from Animalia Vet Laboratory Service, Bogor.

2.3. Methods

2.3.1. Plant Identification

The black cumin seeds were collected from BALITRO. Plant identification was conducted at the Herbarium Depokensis (DEP), located in the Biota Collection Room, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, to verify the plant species used in this study.

2.3.2. Preparation of Ethanol Extract of Black Cumin Seeds

The seeds were washed, dried, and ground using a blender. The ground seeds were macerated in 96% ethanol for 72 hours. The dried black seeds were macerated using 96% ethanol at a black seed-to-solvent ratio of 1 : 10 b/v. The filtrate was collected using a Buchner funnel, and the maceration process was repeated three times. The combined filtrate was concentrated using a rotary evaporator at a temperature of 50°C until the ethanol evaporated and a concentrated extract was obtained. The black cumin seed ethanol extract obtained was then dissolved in 0.9% NaCl at a concentration of 85% w/v. The inhalation duration was 20 minutes using a nebulizer.[26,27]

2.3.3. Phytochemical Screening

Alkaloids: A 0.5 g extract sample was dissolved in 5 mL of 1% HCl and heated in a water bath (80°C, 5 min). The resultant solution was filtered, and 1 mL of Dragendorff's reagent was added to the filtrate. The formation of an orange precipitate confirmed the presence of alkaloids.

Flavonoids: A 0.5 g extract was heated with 10 mL of ethyl acetate in a boiling water bath for 3 min. The mixture was filtered, and 4 mL of the filtrate aliquot was vigorously shaken with 1 mL of dilute ammonium hydroxide solution (10% v/v). A stable yellow coloration in the aqueous layer indicated flavonoid content.

Saponins: A 0.5 g extract was suspended in 2 mL of distilled water in a test tube and subjected to vigorous vertical agitation for 10 sec. Persistent foam formation (≥10 min duration) at the liquid-air interface was interpreted as evidence of saponins.

Tannins: A 0.2 g extract was dissolved in 20 mL of distilled water and filtered. To 2 mL of the filtrate, 3 drops of 10% ferric chloride ($FeCl_3$) solution were added. A blue-black or green-black chromatic transition within 30 sec denoted tannin presence.

Essential oil: A concentrated viscous extract exhibiting oily consistency and aromatic properties was mixed with 96% ethanol (1:2 w/v) and evaporated to dryness under reduced pressure (40°C, rotary evaporator). Persistent aromatic residue post-evaporation confirmed essential oil content.[28]

2.3.4. Ethical Approval

Ethical approval for this study was obtained from

the Ethics Committee of Ahmad Dahlan University, Yogyakarta (Approval No. 022307101). The study followed ethical guidelines to ensure humane treatment of animals, safeguarding their welfare and minimizing discomfort.

2.3.5. Animal Acclimatization

The Sprague-Dawley male white rats used in the experiment were acclimatized in the Pharmacology Laboratory of the Faculty of Pharmacy, Universitas Pancasila, for one week before the study. During acclimatization, the rats were provided with standard food and water. Laboratory conditions were maintained at a temperature of 24–27°C and humidity levels of 12–70%. Each cage contained six male Sprague-Dawley rats, measured approximately 60×40×20 cm, which complies with international animal welfare guidelines and provides adequate space, ventilation, and access to food and water.[29]

2.3.6. Ovalbumin-induced Rat Model (experimental protocol)

This study utilized Sprague-Dawley rats divided into six experimental groups, each groups consist of 4 rats: a normal group (receiving standard feed and water), a negative control (ovalbumin induction without therapy), a positive control (induction with standard salbutamol inhalation therapy), and three treatment groups receiving varying doses of Nigella sativa ethanol extract (12.25, 24.50, and 49 g/kg body weight [BW]). Allergic sensitization was initiated via intraperitoneal injection of 0.35 mg ovalbumin combined with 1 mg $Al(OH)_3$ in 0.5 mL of 0.9% NaCl solution on Days 1 and 8, followed by aerosol exposure to 1 mg/mL ovalbumin in 0.9% NaCl for 20 minutes per session on Days 15, 17, and 19 using a nebulizer.23,30,31 This protocol is clearly shown in Figure 1.

Therapeutic interventions were administered from Days 20 to 28. The positive control group received salbutamol inhalation (0.009 mg/g BW), while treatment groups were administered Nigella sativa extract. On the 29th day, ketamine-xylazine anesthesia was administered, and oxygen saturation parameters were evaluated using a handheld pulse oximeter probe placed on the plantar surface of the left hind paw. This was followed by lung tissue collection. Lung tissues were fixed in 10% formalin for 24–48 hours, processed through paraffin embedding, sectioned with a microtome (4–5 µm thickness), and stained with hematoxylin-eosin (H&E). Histopathological analysis focused on measuring bronchial epithelial thickness and eosinophil count was conducted under blinded conditions using an Olympus BX51 microscope at 400x magnification.[30] Five non-overlapping fields of view per sample were randomly selected for quantification. Eosinophils are characterized by lobed nuclei stained blue or purple.

2.3.7. Data Analysis

Statistical analysis was performed using SPSS version 27.0. Normality of data distribution was confirmed using the Shapiro–Wilk test, and homogeneity of variance was verified with Levene's test, one-way ANOVA and LSD *post-hoc* tests to identify significant intergroup differences ($p < 0.05$), with results presented as mean.

3. Result

3.1. Botanical Authentication

The botanical authentication process was performed to verify the taxonomic identity of the plant material as *Nigella sativa* (black cumin). Specimen validation was conducted at the Herbarium Depokense (acronym: UIDEP), a recognized plant repository under the Faculty of Mathematics and Natural Sciences, Universitas Indonesia.

Official certification (Reference No. 226/UN2.F3.11/PDP.02.00/2023) confirmed that all experimental specimens corresponded to *Nigella sativa* L. of the Ranunculaceae family, thereby ensuring taxonomic

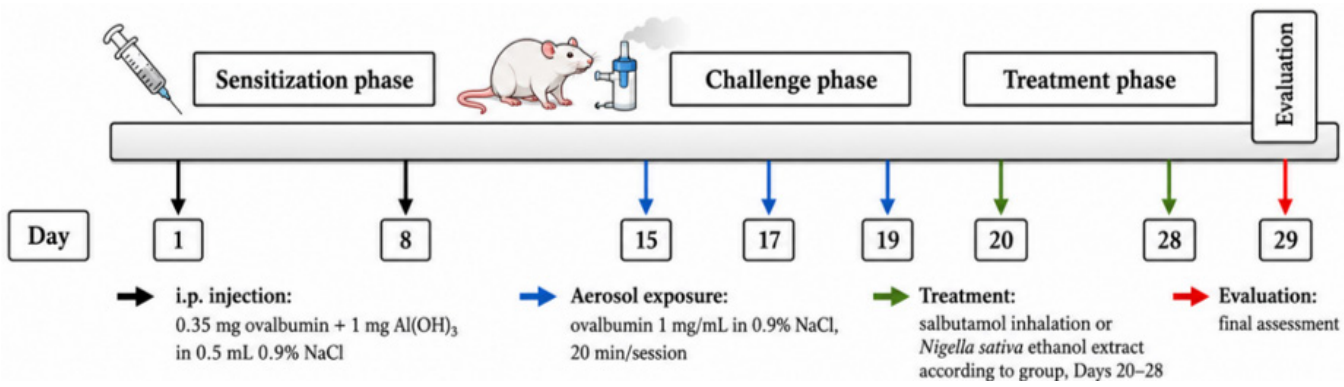


Figure 1. Flowchart of the experimental protocol

Table 1. Phytochemical Screening Test Results

No.	Phytochemical Test	Observation	Result
1.	Alkaloids	Orange precipitate formation	+
2.	Flavonoids	Stable yellow coloration	+
3.	Saponins	Non-persistent foam (<10 min)	-
4.	Tannins	Absence of blue-black coloration	-
5.	Essential Oils	Aromatic residue retention	+

integrity throughout the study. This authentication procedure adhered to standard herbarium protocols involving morphological examination of reproductive structures and comparative analysis with type specimens.

3.2. Phytochemical Screening Analysis of *Nigella sativa* L.

The phytochemical screening tests were conducted to identify secondary metabolite compounds. The results of the phytochemical screening for the ethanol extract of black cumin seed (*Nigella sativa* L.) are presented in Table 1.

The phytochemical analysis of black cumin (*Nigella sativa* L.) seeds revealed distinct outcomes across tested secondary metabolites. Alkaloid identification using Dragendorff's reagent produced an orange precipitate, while flavonoid detection test exhibited a stable yellow coloration. Essential oil evaluation through hydrodistillation demonstrated a characteristic aromatic residue. These three components: alkaloids, flavonoids, and essential oils, yielded positive results.

Contrastingly, saponin screening through foam persistence testing showed inconsistent bubble formation over a 10-minute observation period, failing to meet the stability criterion. Tannin analysis using ferric chloride reagent lacked the anticipated blue-black or green-black chromatic transition. Both saponins and tannins were consequently classified as negative.

3.3. Preparation of 96% Ethanol Extract of Black Cumin Seed (*Nigella sativa* L.)

Figure 2 shown the result of ethanol extract from black cumin seeds. The study utilised 3,000 g of authenticated black cumin seed (*Nigella sativa* L.) dried powder, which was sourced from the Medicinal and Spice Crops Research Institute (BALITRO) in Bogor, Indonesia. The extraction process involved maceration with 96% ethanol, yielding a Drug Extract Ratio (DER) of 4.20:1 (dried powder-to-extract) and an extraction yield of 23.83%. The solvent concentration employed was selected based on its optimal efficacy in extracting polar flavonoids, a key bioactive class in *Nigella sativa* known for its anti-inflammatory properties, while maintaining compatibility with secondary metabolites.

3.4. Physiological Evaluation (Oxygen Saturation)

The results of the oxygen saturation measurements

Figure 2. Ethanol extract of black cumin seed

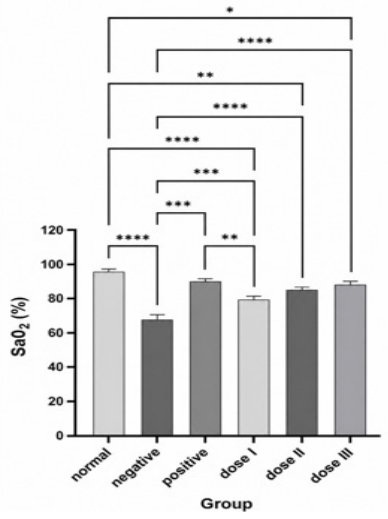
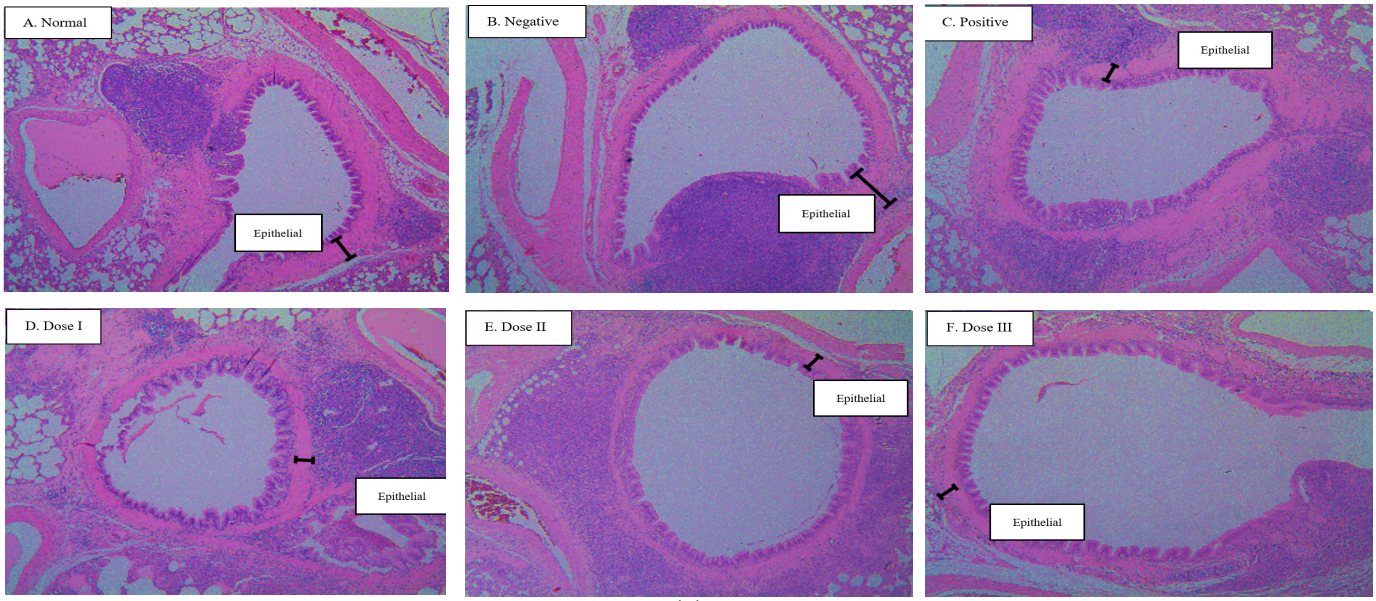
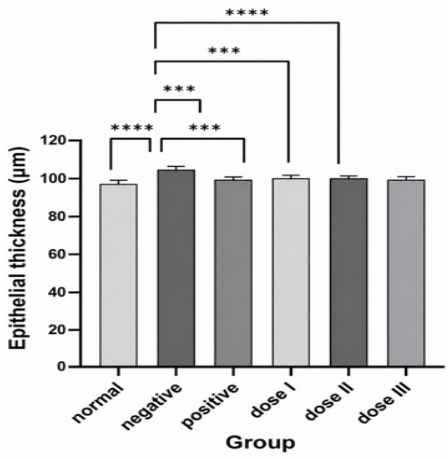


Figure 3. Oxygen saturation levels among experimental groups. Data are presented as mean ± SEM, n= 4 animals per group.



(a)



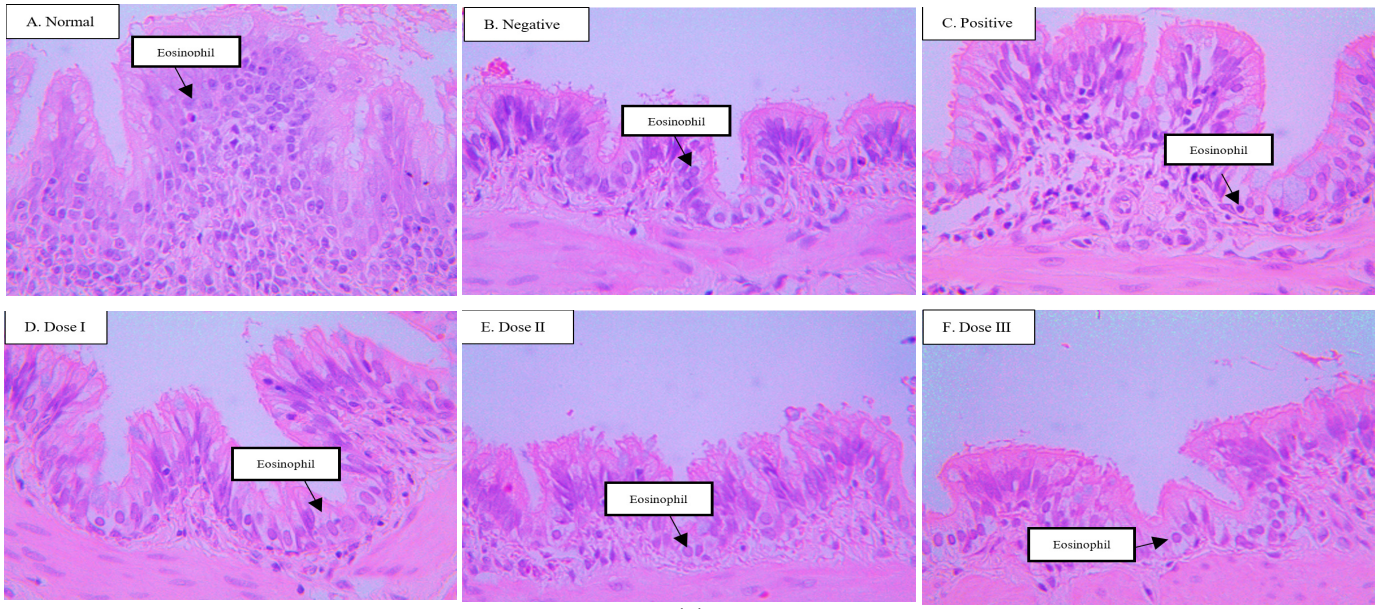
(b)

Figure 4. (a) Histopathological appearance of bronchial epithelial thickness in lung tissue (magnification 400x), (A) Normal group; (B) Negative control showing epithelial hyperplasia; (C) Positive control; (D-F) Dose I-III treatment group. Black bars indicate the measurement area of epithelial thickness. H&E staining. (b) and the result of averages epithelial lungs thickness for each group

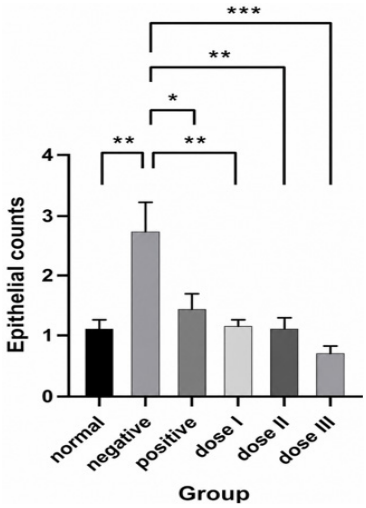
shown in Figure 3, conducted on the 29th day after anesthesia using an animal-specific handheld pulse oximeter, yielded a clear picture of the intervention's effect on hypoxic conditions in rats. The oxygen saturation levels were expressed as mean ± SEM. The Normal group showed SaO_2 of 93.75 ± 1.03%, while the Negative group showed a marked reduction to 65.00 ± 2.74%. The Positive group exhibited improved oxygen saturation of 88.00 ± 0.71%. The treatment groups showed dose-dependent improvement, with Dose I, Dose II, and Dose III reaching 77.75 ± 1.89%, 83.00 ± 0.71%, and 86.75 ± 1.11%, respectively. Normal Group exhibited an average oxygen saturation, indicating a stable physiological condition, while the Negative Group demonstrated a significant decrease, confirming the efficacy of hypoxia induction with a difference of 30.1% compared to the control group. The Positive Group exhibited an 88% success rate, thereby substantiating the efficacy of the control agent in restoring oxygen saturation, with a 35.4% increase observed in comparison to the Negative Group. The test group exhibited a graded response, indicating a range of reactions to the stimulus. The results of the study demonstrated a consistent dose-response pattern. Notably, Dose 3 approached 93.3% of the normal value, exhibiting the highest level of stability.

3.5. Effect of Black Cumin Seed Ethanol Extract on Epithelial Thickness

This study examined the effect of black cumin seed ethanol extract (*Nigella sativa* L.) on bronchial epithelial thickness in a rat (*Rattus norvegicus*) model. Figure 4 illustrates the differences in epithelial



(a)



(b)

Figure 5. (a) Histopathological assessment of eosinophil infiltration in the bronchiolar (A) Normal group showing minimal eosinophil presence; (B) Negative control exhibiting massive eosinophil infiltration (black arrows) following induction; (C) Positive control and (D-F) Dose I-III treatment groups showing a progressive reduction in inflammatory cell recruitment. The decrease in eosinophil count was observed in a dose-dependent manner, with Dose III showing significant recovery toward normal architecture (H&E staining, 400x magnification); (b) and the results of averages epithelial lungs counts for each group

thickness among experimental groups. In Figures 4, a subsequent analysis of epithelial thickness revealed a complex pattern of biological responses between the experimental groups. The Normal group exhibited the lowest epithelial thickness (79.50 ± 0.6455), with minimal variation, suggesting tissue homeostasis under physiological conditions. The Negative Group exhibited the highest thickness (88.00 ± 0.7071), which was significantly different from the Normal Group ($p < 0.0001$). This finding suggests a compensatory epithelial hyperplasia response to chronic hypoxia induction. This phenomenon may be related to tissue adaptation mechanisms through increased epithelial cell proliferation to optimize oxygen exchange. In the treatment groups, both the Positive Group and the extract-treated groups showed reduced epithelial thickness compared with the Negative Group. The Positive Group had a mean epithelial thickness of 81.75 ± 1.181 µm, while the extract-treated groups showed mean values ranging from 82.25 to 81.50 µm across Doses 1–3. These values indicate that salbutamol therapy and *Nigella sativa* ethanol extract treatment were able to suppress epithelial thickening induced by ovalbumin exposure. The reduction was statistically significant compared with the Negative Group ($p \leq 0.0003$). Although the treatment groups

still showed slightly higher numerical values than the Normal Group (79.50 ± 0.6455 µm), the difference was not statistically significant ($p > 0.05$), suggesting that the epithelial thickness tended to return toward the normal physiological range.

The LSD post hoc test indicated three critical patterns: (1) no significant difference was observed among the therapeutic doses ($p > 0.9758$); (2) the therapeutic response of the extract-treated groups was comparable to that of the Positive Group ($p > 0.9961$); and (3) the Positive Group exhibited the highest response variability. These findings suggest that the lowest dose may have already achieved a near-maximal therapeutic effect, indicating a possible plateau response and highlighting the importance of dose optimization.

3.6. Effect of Black Cumin Seed Ethanol Extract on Eosinophil Count

This study investigates the effect of ethanol black cumin seed extract (*Nigella sativa* L.) on the eosinophil count in the bronchial epithelium of rats (*Rattus norvegicus*) with ovalbumin-induced model. The results of the eosinophil count are presented in Figures 5, with comparisons made using a scoring method.In Figure 5, eosinophils are identified as bright red cells with granular structures, and their nuclei are stained purple or blue. These. The primary parameter measured in this study was eosinophil count, which plays a critical role in asthma-related inflammation. Eosinophil accumulation can cause severe tissue damage, as these cells are potent effectors of inflammation. Inhalation of ovalbumin, used as an allergen in this study, led to increased eosinophil counts in the respiratory tract.[32]

Histological examination of the lung tissue stained with Hematoxylin and Eosin (H&E) revealed significant variations in inflammatory cell recruitment across all groups. The Normal group displayed a well-preserved mucosal structure with rare eosinophil presence. In contrast, the Negative control group showed a marked increase in eosinophil infiltration within the peribronchiolar and submucosal regions, indicating a robust inflammatory response to the induction.

Treatment with the reference drug (Positive control) and varying doses of the test substance resulted in a visible reduction of these inflammatory cells. Specifically, while Dose I showed only a slight decrease in cell count compared to the negative group, Dose II and Dose III exhibited a more pronounced inhibitory effect. The Dose III group demonstrated the lowest eosinophil density among the treated groups, showing a histological profile that closely resembled the Positive control and Normal groups. The objective of this study was to evaluate the reduction of eosinophil counts, a key inflammatory marker of airway inflammation, following ovalbumin sensitization and treatment with ethanol black cumin seed extract (*Nigella sativa* L.).

4. Discussion

The present study reinforces the validity of the ovalbumin-induced allergenic airway response model, as evidenced by hypoxia induction and eosinophilic infiltration, consistent with previously published reports of Th2-driven allergic airway pathology.[23,31] Importantly, treatment with *Nigella sativa* extract demonstrated therapeutic effects comparable to the positive control, but with the added advantage of suppressing the underlying inflammatory process rather than merely alleviating bronchoconstriction. This finding aligns with earlier studies that identified thymoquinone as a potent anti-inflammatory agent capable of modulating NF κB signaling, inducing eosinophil apoptosis, and reducing calcium-dependent degranulation.[14] Our results extend this evidence by providing histopathological validation, showing reduced bronchial epithelial thickness and eosinophil infiltration, thereby strengthening the translational relevance of *Nigella sativa* in respiratory disease models. The novelty of this research lies in demonstrating that inhaled ethanol extract of black cumin seed can act as a disease-modifying agent, targeting key inflammatory pathways and epithelial remodeling. This positions *Nigella sativa* as a promising candidate for phytopharmaceutical development in asthma therapy, offering a mechanistic advantage over conventional β2-agonists that primarily provide symptomatic relief.[18,25,33]

5. Conclusion

This study establishes the significant therapeutic potential of *Nigella sativa* ethanol extract in addressing acute airway inflammation through a multifaceted mechanism. The highest tested dose (49 g/kg BW) demonstrated comparable efficacy to salbutamol in improving oxygen saturation (86.75%) and reducing bronchial epithelial thickness (81.50 ± 0.6455 µm), while surpassing conventional treatment in suppressing eosinophil infiltration by 73.6% (0.725 ± 0.111 cells/field). The observed effects are attributed to thymoquinone's multimodal action, including NF-κB pathway inhibition, induction of mitochondrial apoptosis, and calcium channel blockade that prevents mast cell degranulation. These findings position *Nigella sativa* as a promising candidate for adjuvant therapy in steroid-resistant eosinophilic acute respiratory diseases, particularly given its dual capacity to mitigate inflammatory responses and potentially inhibit airway remodeling. Future research should prioritize the

development of standardized inhalable formulations using nanotechnology to enhance pulmonary bioavailability, alongside longitudinal studies to validate its antifibrotic effects in chronic models. The integration of this phytopharmaceutical with existing corticosteroid regimens could pave the way for reduced systemic steroid dependence, addressing a critical unmet need in asthma management.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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