Suppression of NF-κB-*p*65 and STAT3 by *Melicope pteleifolia* Extract Mitigates Ovalbumin-Driven Allergic Rhinitis in Mice

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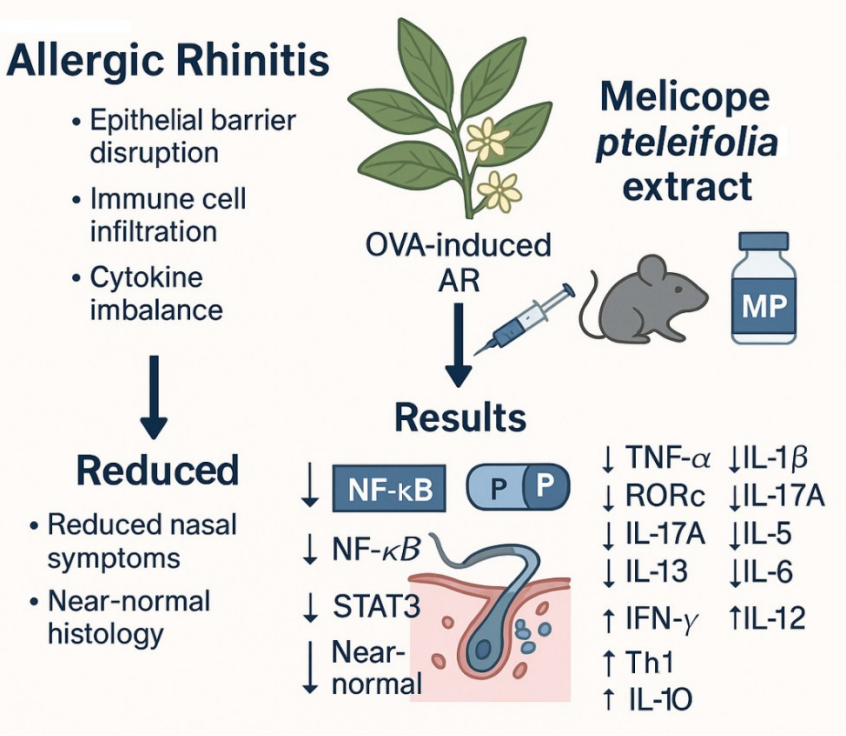
GRAPHICAL ABSTRACT



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Allergic rhinitis (AR) is an IgE-mediated inflammatory disorder of the nasal mucosa, characterized by epithelial barrier disruption, immune cell infiltration, and cytokine imbalance. This study evaluated the bioactivity of *Melicope pteleifolia* ethanolic extract (MP) in an ovalbumin (OVA)-induced mouse model of AR. Mice sensitized and challenged with OVA were treated orally with MP (50, 100, or 150 mg/kg, b.w.) or dexamethasone (2 mg/kg, b.w.). MP significantly and dose-dependently alleviated nasal symptoms, with the highest dose achieving effects comparable to dexamethasone. Nasal lavage fluid analysis revealed reductions in eosinophils, neutrophils, macrophages, and epithelial cells, while histological examination showed restoration of nasal-associated lymphoid tissue and septal mucosa. On the molecular level, MP suppressed NF-κB-p65 and IκBα phosphorylation, inhibited STAT3 signaling, downregulated Th17/Th2-associated markers (RORc, IL-17A, IL-5, IL-13, IL-6), and enhanced anti-inflammatory and Th1 cytokines (IL-10, IFN-γ, IL-12). Collectively, these results demonstrate the broad anti-inflammatory and immune-modulating potential of MP, highlighting its value as a promising non-steroidal candidate for AR therapy. While the present work primarily establishes pharmacological bioactivity, these insights may also provide a scientific foundation for exploring *Melicope pteleifolia* in future biomaterial-based biomedical applications.

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Keywords: Ovalbumin; Cytokine; Eosinophils; NF-κB-p65; IκBα

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INTRODUCTION

Allergic rhinitis (AR) is an immunoglobulin E–mediated inflammation of the nasal mucosa triggered by innocuous airborne allergens—pollens, dust mites, animal dander, molds and cockroach proteins—leading to sneezing, rhinorrhea, congestion, and itching, often accompanied by ocular symptoms (Incorvaia *et al.* 2018). It affects 10 to 30% of adults and up to 40% of children in Western countries, and an estimated 400 million people worldwide, causing impaired sleep, diminished work and school performance, and reduced quality of life (Small *et al.* 2018). Annual direct costs for doctor visits, medications and immunotherapy, together with indirect costs from absenteeism and "presenteeism," reach billions of dollars in high-income nations, yet AR remains under-recognized and

undertreated.

First-line pharmacotherapy combines second-generation oral antihistamines—which block histamine's effects without sedation—and intranasal corticosteroids, which suppress mucosal inflammation (Wheatley and Togias 2015). Leukotriene receptor antagonists, mast-cell stabilizers (*e.g.*, cromolyn sodium), and combination intranasal sprays (*e.g.*, azelastine + fluticasone) serve as adjuncts for persistent or complex cases (Sur and Plesa 2015). Allergen-specific immunotherapy (subcutaneous or sublingual) is the only disease-modifying option, offering long-term remission and reducing progression to asthma; however, it requires years of treatment, suffers from variable adherence, carries risk of systemic reactions, and lacks standardized dosing (Seidman *et al.* 2015).

These therapies often fail to achieve full symptom control and entail side effects: oral antihistamines can still cause mild sedation or anticholinergic effects; intranasal steroids may irritate mucosa or cause nosebleeds; and prolonged systemic corticosteroids risk metabolic disturbances and bone loss (Al Suleimani and Walker 2007; Hoyte and Katial 2011). Thus, there is a clear need for new drugs that combine rapid, sustained relief with improved safety and that target the immune mechanisms driving AR rather than merely masking symptoms (Nathan 2007).

Emerging biologics—monoclonal antibodies such as anti-immunoglobulin E (anti-IgE) (omalizumab) and agents against key cytokines—have shown promise in early trials by interrupting the allergic cascade at its origin, though high cost, limited access and unknown long-term safety limit widespread use (Tajiri *et al.* 2014). Small-molecule approaches, including phosphodiesterase 4 inhibitors, aim to modulate intracellular inflammatory signaling with fewer systemic effects (Janosova *et al.* 2020).

Plants offer another reservoir of potential therapeutics. Phytochemicals—flavonoids, alkaloids, terpenoids and phenolic acids—exhibit antihistaminic, anti-inflammatory, antioxidant and immunomodulatory properties (De Smet 1997). Butterbur (*Petasites hybridus*) sesquiterpenes inhibit leukotriene synthesis and mast-cell degranulation, reducing sneezing and congestion comparably to antihistamines but without sedation (Jackson *et al.* 2004). Quercetin (*e.g.* in onion and *Ginkgo biloba*) stabilizes mast cells and scavenges reactive oxygen species (Min *et al.* 2007). Stinging nettle (*Urtica dioica*) extracts block Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling, lowering Interleukin (IL)-4, IL-13 and Tumor Necrosis Factor alpha (TNF-α) (Johnson *et al.* 2013). Curcumin from turmeric (*Curcuma longa*) suppresses eosinophil infiltration and downregulates Cyclooxygenase-2 (COX-2) and 5-lipoxygenase (Chung *et al.* 2012; Thakare *et al.* 2013). *Perilla frutescens'* rosmarinic acid inhibits complement activation and eosinophil chemotaxis (Takano *et al.* 2004), and licorice (*Glycyrrhiza glabra*) glycyrrhizin exerts corticosteroid-like effects (Wahab *et al.* 2021). Traditional Chinese formulas (*e.g.*, Xin Yi San, Yu Ping Feng San) combine herbs for synergistic immunomodulation (Yang *et al.* 2010; Luo *et al.* 2017).

Melicope pteleifolia (Rutaceae), "Tenggek burung" or "Ge Ji," is a Southeast Asian shrub whose leaves, stems and bark yield alkaloids (dictamnine, skimmianine), flavonoids (quercetin, kaempferol derivatives), coumarins, and essential oils (β-caryophyllene, α-humulene, limonene) (Yao *et al.* 2020). Its alkaloid fractions disrupt microbial membranes and inhibit nucleic acid synthesis, showing antibacterial and antifungal activity (Yao *et al.* 2024). Flavonoids and coumarins scavenge reactive oxygen species and inhibit NF-κB–mediated TNF-α, IL-1β and COX-2 expression (Pietta 2000; Panche *et al.* 2016; Dias *et al.* 2021). It has been shown that *M. pteleifolia* extracts markedly reduce

inflammatory mediators (TNF-α, IL-6, nitric oxide), improving survival in septic (Lipopolysaccharide/cecal ligation and puncture) (Yao *et al.* 2024). They also scavenge free radicals *in vitro* (DPPH/ABTS assays), reflecting strong antioxidant activity (Binh *et al.* 2025). The cited authors also showed that leaf extracts inhibit xanthine oxidase and reduce uric acid formation *in vitro*, indicating potential benefit in hyperuricemia/gout. Given its potent anti-inflammatory and immunomodulatory constituents, *M. pteleifolia* merits investigation as a novel treatment for AR.

In summary, the high prevalence, substantial burden and limitations of current AR therapies underscore the urgent need for new drugs. The plant-derived phytochemicals offer pathways toward treatments that are more effective, safer and potentially disease-modifying. Therefore, this study evaluated *M. pteleifolia*'s pharmacological effects on AR and elucidate its underlying mechanisms.

EXPERIMENTAL

Plant Collection

The fresh plant of *Melicope pteleifolia* was collected from the gardens of the Hangzhou Traditional Chinese Medicine Hospital affiliated to Zhejiang Chinese Medical University, Zhejiang, China, and was identified and characterized by the botanist of the Institute. The harvested whole plant was allowed to dry in shade for 15 days in open atmosphere in hygienic condition. The air-dried whole plant was then later pulverized to afford the raw crude powder of *Melicope pteleifolia* (MP).

Ethanol Extract of *Melicope pteleifolia*

A total of 500 g of crude powder of *Melicope pteleifolia* was extracted with 10 L of 75% ethanol for three times. The resulting extracts were combined, filtered, and concentrated *in-vacuo* to furnish dried ethanolic extract of *Melicope pteleifolia* (MP).

Animals

The Male BALB/c mice (18 to 22 g, 6 to 8 weeks old) was obtained from the facility of Institutional animal house and were housed under standard conditions of 21 ± 2 °C and a 12 h light/dark cycle with access to food and water *ad libitum*. Animals received humane care in compliance with the guidelines of the National Institutes of Health. The animal study is approved by the animal ethical committee of The Second Affiliated Hospital of Xi 'an Jiaotong University, China (Approval No. 20240012).

Induction of Ovalbumin (OVA) induced AR in Mice

On days 1, 8, and 15, OVA-induced AR animals were made sensitive by injecting 200 µL of saline containing 50 µg of OVA (Sigma, USA) anchored to 1 mg of aluminum hydroxide (Thermo Scientific, USA). After that, for 13 days, 1 h prior to the intranasal exposure of OVA, mice were given 200 µL of MP in the corresponding dose or dexamethasone (Dex) orally once daily. The OVA group of mice were administered 200 µL of saline. The mice were intranasally challenged with 1 mg/mL OVA, 20 µL into each nostril daily, for one week following the last sensitization, from days 22 to 28. Mice were killed 24 h following the final OVA challenge.

Groups

The animals were randomly divided in to six groups (n=6 each group) as follows,

1. Control: Received saline only, without OVA sensitization or challenge.
2. OVA (Disease Control): Sensitized and challenged with OVA, but received no treatment.
3. MP-50: OVA-sensitized mice treated with MP extract at 50 mg/kg b.w.
4. MP-100: OVA-sensitized mice treated with MP extract at 100 mg/kg b.w.
5. MP-150: OVA-sensitized mice treated with MP extract at 150 mg/kg b.w.
6. Dex (Standard Control): OVA-sensitized mice treated with dexamethasone (2.5 mg/kg b.w.)."

Evaluation of Allergic Reactions and Symptoms

Following the administration of 20 µL of OVA at a concentration of 1 mg/mL per into both nasal cavities, the mice were subsequently placed in an observation cage. For a period of 15 min, immediately following the end of the most recent OVA intranasal test, the patterns of nasal rubbing and sneezing behavior were recorded.

Serum and Nasal Lavage Fluid Collection and Cell Count

At 24 h following the last challenge, all mice were slaughtered, and the blood was collected by heart puncture. The blood was centrifuged to obtain the serum. Approximately 1 mL of nasal lavage fluid (NALF) was collected using an 18-gauge catheter from 3 mice in each group. After performing a partial resection of the trachea, the catheter was put into the tracheal in the direction of the upper airway into the nasopharynx. The nasal passages were gently perfused with 1 mL of cold phosphate-buffered saline (PBS). The NALF was subjected to centrifugation at 10,000×g for 10 min at 4 °C to collect the supernatant. The serum and NALF were kept at a temperature of -70 °C. The cells that had been lavaged were resuspended in the same volume of cold PBS so that a hemocytometer could be used to count the total number of cells. A total of 150 µL of NALF was spun onto slides using a cytospin apparatus (Centrifuge 5403, Eppendorf, Germany) at a speed of 1000 revolutions per minute for ten minutes at a temperature of 4 °C. The Diff-Quik Staining reagent (1-5-1 Wakinohama-Kaigandori, Chuo-Ku, Kobe, Japan) was used to stain the cell in accordance with the methods provided by the manufacturer.

Histological Examination

For nasal histological analysis, the heads were fixed in 10% neutral buffered formalin for a period of three days at a temperature of 23 ± 2 °C. Subsequently, they were decalcified in an ethylenediaminetetraacetic acid (EDTA) decalcifying solution for a period of five days at a temperature of 23 ± 2 °C. The samples were first embedded in paraffin, and then they were dehydrated using a succession of ethyl alcohol and xylene reactions. For the purpose of evaluating the general morphology, the sections were cut at a thickness of 4 µM and stained with hematoxylin and eosin (H&E) provided by Sigma, USA. Histological alterations were recoded, and results are presented quantitatively as bar graphs; representative images were not included in this study to avoid potential subjectivity in interpretation and to ensure objective, reproducible assessment.

Quantification of the Cytokines

Levels of cytokines in IL-1β, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17A, Interferon gamma (IFN-γ), TNF-α, retinoid-related orphan receptor gamma t (RORc), Nuclear Factor kappa B p65 subunit (NFκBp65), inhibitor of nuclear factor kappa-B (IκB), phosphorylation of NFκBp65 (p-NFκBp65), IκB (p-IκB), Signal transducer and activator of transcription 3 (STAT3), and phosphorylation of STAT3 (p-YSTAT3) were determined using Cytokine quantitation kits (BD Biosciences, USA) with the manufacturer protocols.

Statistical Analysis

Software developed by Graph Pad, La Jolla, CA, USA, was used to evaluate the results. After doing a one-way ANOVA, we used Student's test to see whether the differences between the groups were statistically significant. The results of separate studies were shown as the averages plus or minus the standard error of mean (S.E.M.). At a 95% confidence level ($P < 0.05$), significance was deemed to exist.

RESULTS AND DISCUSSION

MP Alleviated the Allergic Nasal Symptoms Induced by OVA

Allergic rhinitis (AR) is a common IgE-mediated hypersensitivity disorder characterized by symptoms such as sneezing, nasal rubbing, rhinorrhea, and nasal congestion (Tran *et al.* 2011). Among these, nasal rubbing and sneezing are hallmark behavioral indicators of nasal hypersensitivity in murine models and are widely used to assess the severity of allergic inflammation and the efficacy of anti-allergic agents. Therefore, reduction in these parameters is considered a reliable measure of therapeutic potential in AR studies (Al Suleimani and Walker 2007).

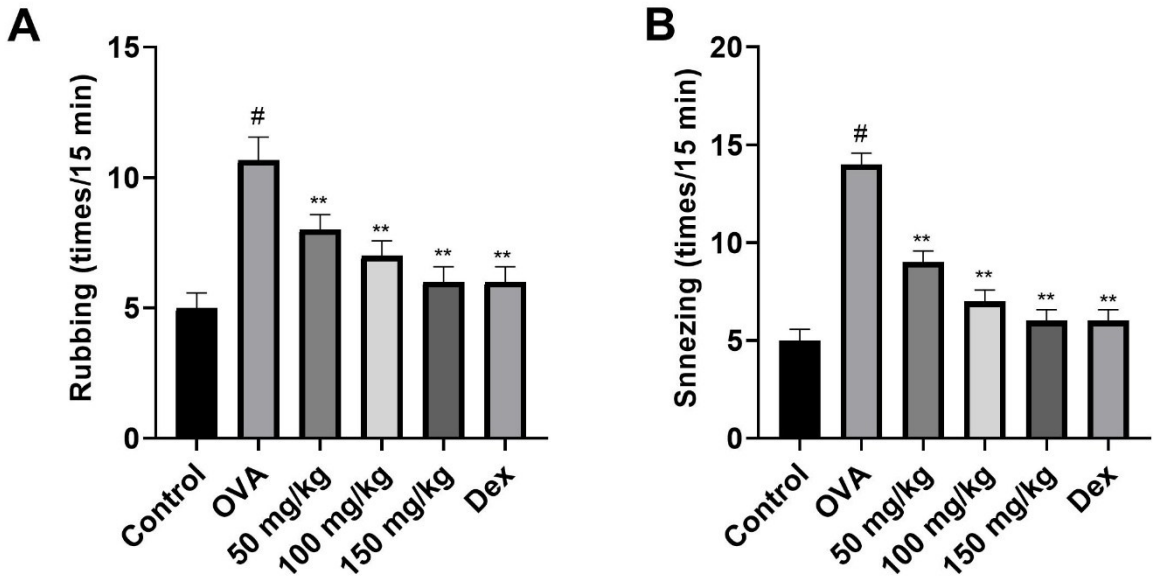

Fig. 1. Effect of MP on OVA-induced nasal symptoms in mice. Mice were sensitized and challenged with ovalbumin (OVA) to induce allergic rhinitis. Treatment with MP at 50, 100, and 150 mg/kg significantly reduced the frequency of nasal rubbing and sneezing in a dose-dependent manner. The 150 mg/kg dose showed comparable efficacy to the standard treatment, dexamethasone (2 mg/kg). #p<0.05 vs. control and **p<0.01 vs. OVA group. Mean ± S.E.M.

In the present study, the anti-allergic potential of MP was evaluated in an ovalbumin (OVA)-induced murine model of allergic rhinitis (Fig. 1). OVA sensitization and subsequent intranasal challenge led to a significant increase in nasal rubbing and sneezing episodes, reflecting successful induction of allergic nasal inflammation. However, treatment with MP significantly attenuated these allergic symptoms in a dose-dependent manner. Mice treated with 50, 100, and 150 mg/kg of MP showed progressive reductions in the frequency of nasal rubbing and sneezing compared to the OVA-induced AR group. The highest dose (150 mg/kg) demonstrated the most pronounced effect, showing a significant decrease in both parameters, with results comparable to the standard anti-inflammatory drug dexamethasone (2 mg/kg). These findings suggest that MP possesses potent anti-allergic activity.

MP Inhibited the Infiltration of Diverse Inflammatory Cell Types in NALF

AR is marked not only by the classic nasal symptoms but also by a characteristic influx of inflammatory cells into the nasal mucosa and lumen (Azman *et al.* 2021).

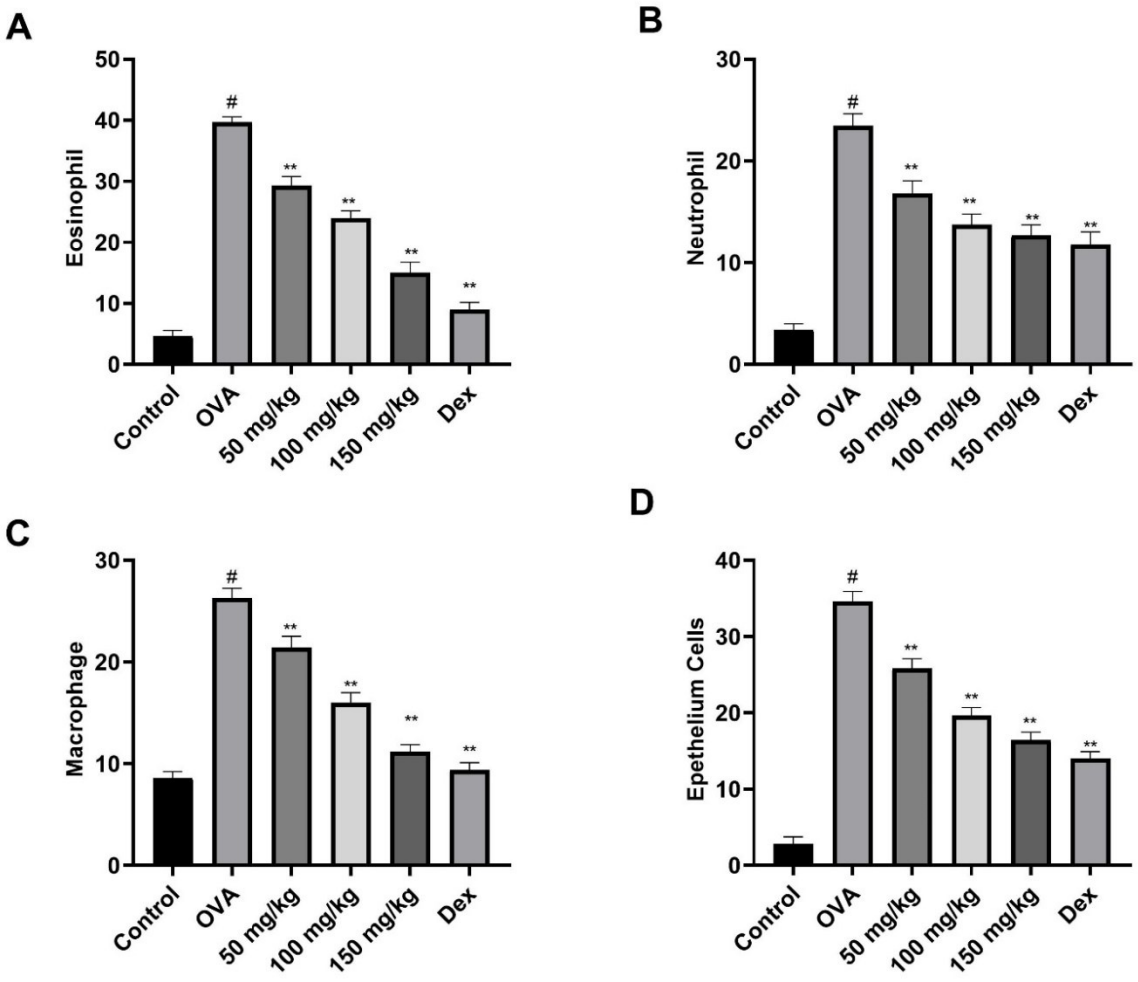

Fig. 2. MP inhibits OVA-induced inflammatory cell infiltration in nasal airway lavage fluid (NALF). NALF was collected 24 hours after the final ovalbumin (OVA) challenge and total and differential cell counts were performed. (A) Eosinophil counts, (B) Neutrophil counts, (C) Macrophage counts, (D) Shed epithelial cell counts. $^{\#}p<0.05$ *vs.* control and $^{**}p<0.01$ *vs.* OVA group. Mean ± S.E.M.

Eosinophils are central effectors of late-phase allergic inflammation: they release cytotoxic granule proteins (*e.g.*, major basic protein) and generate lipid mediators that perpetuate tissue damage and mucus hypersecretion (O'Sullivan and Bochner 2018). Neutrophils, although more typically associated with acute infection, contribute to AR pathophysiology by producing reactive oxygen species and proteases that exacerbate epithelial injury (Rosales 2018). Macrophages orchestrate both the initiation and resolution of inflammation through antigen presentation, phagocytosis of debris, and secretion of cytokines (*e.g.*, IL-1β, TNF-α) (Zhou *et al.* 2022; Wo *et al.* 2023). Finally, sloughed epithelial cells in nasal airway lavage fluid (NALF) reflect barrier disruption and are themselves active sources of chemokines (*e.g.*, eotaxin, RANTES) that recruit additional leukocytes (Williams *et al.* 2015). Quantifying the numbers of these cell types in NALF thus provides a robust readout of disease severity and therapeutic efficacy.

In OVA-sensitized and -challenged mice, total NALF cell counts were dramatically elevated compared to non-sensitized controls, with marked increases in eosinophils, neutrophils, macrophages, and epithelial cells (Fig. 2) (Chan *et al.* 2024). Administration of MP at 50, 100, and 150 mg/kg led to dose-dependent reductions in each cell population. These inhibitory effects were comparable to those observed with dexamethasone (2 mg/kg). The profound reduction of eosinophils in NALF by MP underscores its potential to attenuate the hallmark effector phase of AR. By curbing eosinophil influx, MP likely limits the release of toxic granule contents and downstream tissue remodeling (Fettrelet *et al.* 2021). The concomitant decrease in neutrophils suggests that MP also moderates acute innate inflammatory responses, which may prevent collateral epithelial damage and further antigen penetration (Silvestre-Roig *et el.* 2019). Suppression of macrophage numbers indicates an impact on both the amplification of local cytokine networks and antigen-driven adaptive responses, potentially shifting the nasal mucosal milieu toward resolution rather than perpetuation of inflammation (Guan *et al.* 2025). Finally, diminished epithelial cell shedding reflects preservation of barrier integrity, which is critical for preventing allergen re-exposure and breaking the cycle of chronic inflammation (Zeyneloglu *et al.* 2025).

MP Ameliorated the Nasal Mucosa Disorder and Thickness

The nasal-associated lymphoid tissue (NALT) epithelium and the underlying septal mucosa play central roles in the pathogenesis of allergic rhinitis (AR) (Hiroi *et al.* 1998; Debertin *et al.* 2003; Padayachee *et al.* 2021). NALT serves as an inductive site for antigen uptake and presentation, initiating local IgE class switching and recruitment of effector cells. Hyperplasia and hypertrophy of the NALT epithelium reflect heightened immune activation, whereas mucosal thickening of the nasal septum indicates edema, inflammatory cell infiltration, and goblet cell hyperplasia—features that underlie nasal congestion and obstruction in AR patients (Takaki *et al.* 2018). Quantifying changes in epithelial architecture and mucosal thickness by hematoxylin & eosin (H&E) staining thus provides crucial morphological evidence of disease severity and therapeutic efficacy.

The results revealed marked epithelial hyperplasia in NALT areas and significant mucosal thickening of the septum in OVA-sensitized/challenged mice (Fig. 3A) due to intense local immune activation and edema. Oral administration of MP at escalating doses (50, 100, 150 mg/kg) produced progressive normalization of these parameters, where MP at the highest tested dose of 150 mg/kg significantly reduced both epithelial and mucosal dimensions, effects that were statistically indistinguishable from those of dexamethasone (2 mg/kg). The reversal of NALT epithelial hyperplasia by MP suggests suppression of antigen-driven lymphoid activation at the mucosal interface, potentially *via* inhibition of

dendritic cell maturation or down-regulation of local T helper 2 cell (Th2) cytokines (*e.g.*, IL-4, IL-13). The concurrent reduction in septal mucosal thickness indicates diminished vascular permeability, edema, and inflammatory cell recruitment—key drivers of nasal obstruction. Together, these histological improvements underpin the symptomatic relief observed (*e.g.*, decreased sneezing and rubbing) and mirror the cellular suppressive effects seen in NALF.

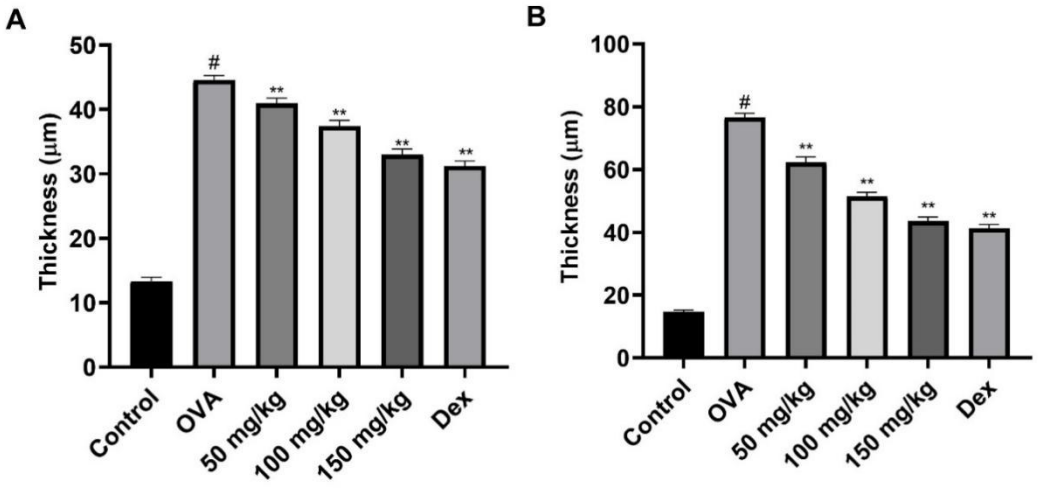

Fig. 3. Effect of MP on the nasal-associated lymphoid tissue (NALT) region. (A) NALT epithelium, and (B) Septum mucosa. #p<0.05 *vs.* control and **p<0.01 *vs.* OVA group. Mean ± S.E.M.

Mechanistically, the protective effects on mucosal architecture may stem from MP's capacity to stabilize mast cells and inhibit mediator release (*e.g.*, histamine, leukotrienes), thereby preventing the cascade of endothelial activation and plasma exudation. Additionally, MP could modulate epithelial-derived alarmins (*e.g.*, TSLP, IL-33), curtailing the feedback loop that perpetuates mucosal inflammation and remodeling.

In sum, MP effectively ameliorates both immunological (NALT epithelial hyperplasia) and structural (septal mucosal thickening) hallmarks of AR, paralleling the efficacy of dexamethasone. These findings reinforce MP's promise as a multi-targeted therapeutic agent that addresses not only symptoms but also underlying mucosal pathology in allergic rhinitis.

MP Inhibited the NF-κB-p65/IκBα Signalling and Related Cytokines

The transcription factor NF-κB plays a central role in the pathogenesis of allergic rhinitis by driving the expression of pro-inflammatory cytokines and adhesion molecules that perpetuate mucosal inflammation and leukocyte recruitment (Wee *et al.* 2017). In resting cells, NF-κB dimers (primarily p65/p50) are sequestered in the cytoplasm by the inhibitory protein IκBα. Allergen exposure activates upstream kinases (*e.g.*, IKK), leading to phosphorylation of IκBα, its ubiquitination and degradation, and consequent release and nuclear translocation of NF-κB-p65 (Wang *et al.* 2013). Once inside the nucleus, both non-phosphorylated and phosphorylated isoforms of p65 bind promoter regions of genes encoding TNF-α and IL-1β, among others, amplifying vascular permeability, mucus secretion, and inflammatory cell influx (Rahman and Fazal 2011). Thus, the levels and phosphorylation status of NF-κB-p65 and IκBα, together with concentrations of TNF-α and IL-1β, serve as critical biomarkers of both NF-κB pathway activation and disease

severity in allergic rhinitis (Wang *et al.* 2013; Qiao and Chen 2021; Tian *et al.* 2023).

As shown in Fig. 4, in OVA-sensitized and challenged mice, nasal mucosal extracts revealed pronounced increases in phosphorylated NF-κB-p65 (p-p65) and phosphorylated IκBα (p-IκBα), accompanied by elevated total p65 and a marked reduction of total IκBα compared with non-sensitized controls. Oral administration of MP at 50, 100, and 150 mg/kg resulted in dose-dependent suppression of both p-p65 and p-IκBα levels; at the highest dose, MP also restored non-phosphorylated IκBα expression and significantly reduced total NF-κB-p65 to near baseline levels, mirroring the effects of dexamethasone (2 mg/kg). In parallel, ELISA measurements of nasal lavage fluid showed that OVA challenge elevated TNF-α and IL-1β concentrations by roughly three- to four-fold. MP treatment produced a dose-dependent decline in these cytokines, again closely matching dexamethasone's profile. Therefore, MP exerts its anti-inflammatory effects in allergic rhinitis by dual modulation of the NF-κB pathway: it stabilizes IκBα (preventing its phosphorylation and degradation) and it inhibits the phosphorylation of NF-κB-p65 itself, thereby blocking both the release and the transcriptional activity of NF-κB. The consequent down-regulation of TNF-α and IL-1β disrupts the autocrine and paracrine feedback loops that fuel mucosal edema, cellular infiltration, and mucus hypersecretion. By targeting both non-phosphorylated and phosphorylated isoforms of key signaling proteins, MP offers a multifaceted way to dampen NF-κB-mediated inflammation, positioning it as a promising non-steroidal alternative to corticosteroids for the management of allergic rhinitis.

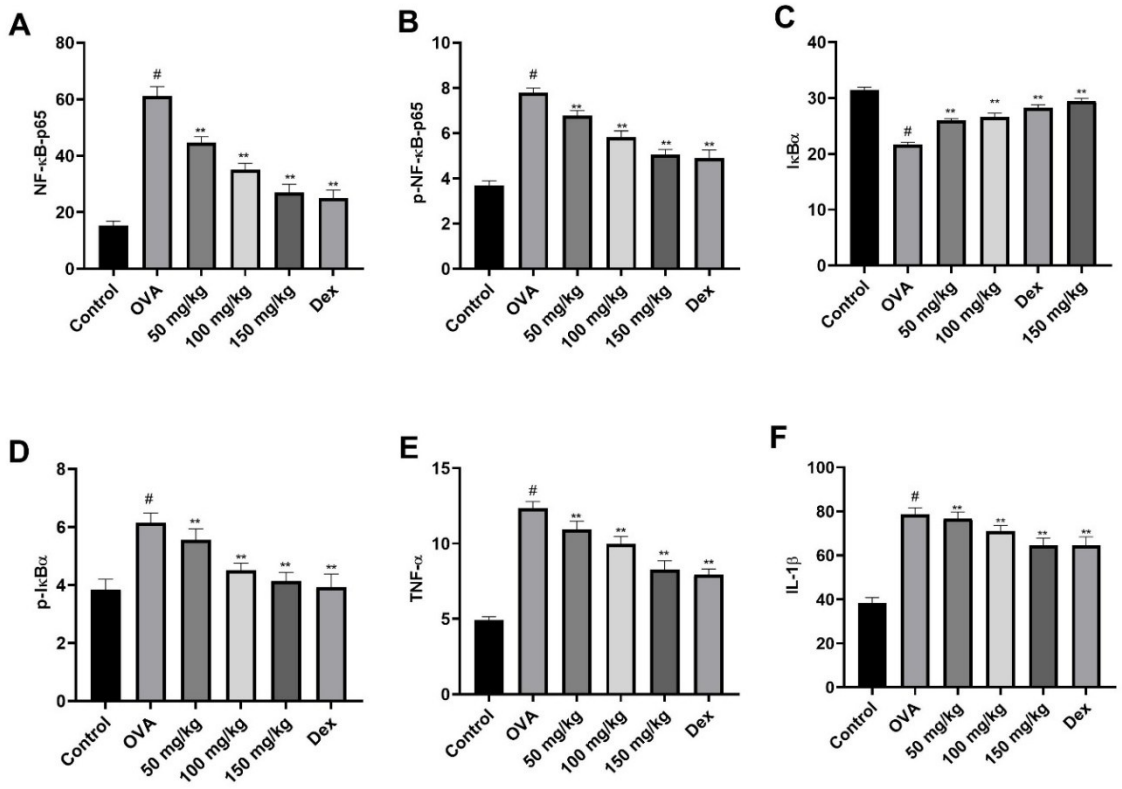

Fig. 4. MP attenuates NF-κB-p65/IκBα signaling and pro-inflammatory cytokine production in OVA-induced allergic rhinitis. (A) total NF-κB-p65, (B) phosphorylated NF-κB-p65 (p-p65), (C) total IκBα, (D) phosphorylated IκBα (p-IκBα), (E) TNF-α, and (F) IL-1β. #p<0.05 *vs.* control and **p<0.01 *vs.* OVA group. Mean ± S.E.M.

The balance between pro-inflammatory and regulatory T-cell responses is a critical determinant of allergic rhinitis (AR) severity and chronicity (Erkan *et al.* 2020). Signal transducer and activator of transcription 3 (STAT3) is a key transcription factor that, once phosphorylated on tyrosine 705 (p-YSTAT3), drives differentiation of naïve $CD4^+$ T cells into Th17 and Th2 subsets and promotes production of cytokines such as IL-17A, IL-5, IL-13, and IL-6, all of which contribute to eosinophilic inflammation, mucus hypersecretion, and tissue remodeling in AR (Wang *et al.* 2019, 2022; Van Nguyen *et al.* 2020; Phan Van *et al.* 2024).

Conversely, STAT3 inhibition favors the development of regulatory T cells (Tregs), which secrete IL-10, and skews toward Th1 responses characterized by IFN-γ and IL-12 production, thereby counteracting allergic inflammation (Goodman *et al.* 2011; Aqel *et al.* 2021; He *et al.* 2024). As shown in Fig. 5, In OVA-sensitized and challenged mice, analysis of nasal mucosal cytoplasmic extracts showed robust up-regulation of total STAT3 and p-YSTAT3, paralleled by increases in RORc (the Th17 lineage-defining transcription factor), IL-17A, IL-5, IL-13, and IL-6, whereas levels of IL-10, IFN-γ, and IL-12 were markedly suppressed. Oral treatment with MP at 50, 100, and 150 mg/kg led to a dose-dependent reduction in both STAT3 and p-YSTAT3 protein levels, with the highest dose restoring these signaling molecules nearly to baseline. These findings indicate that MP suppresses the STAT3-mediated Th17/Th2 axis and restores immunoregulatory and Th1-mediated counter-regulation, thereby rebalancing T-cell responses to ameliorate allergic inflammation in AR.

The data suggest that MP contains bioactive compounds capable of (1) mast cell stabilization, (2) direct kinase inhibition (*e.g.*, IKK, JAK2/STAT3), and (3) modulation of transcription factors. The synergistic interplay between NF-κB and STAT3 pathways—both of which converge on cytokine gene promoters—likely underlies MP's robust efficacy.

Future fractionation and phytochemical profiling will be critical to isolate the principal active constituents and to determine pharmacokinetic properties. Given its efficacy comparable to dexamethasone but with a potentially improved safety profile, MP could be developed as an adjunct or alternative therapy for AR, particularly in patients seeking non-steroidal options.

However, limitations of this study include the use of a single allergen model and lack of long-term safety data. Additionally, precise identification of MP's molecular targets and potential off-target effects will require in vitro kinase assays and toxicological evaluations.

The present study offers straightforward, green-chemistry–friendly extraction process and the prospect of cultivating *M. pteleifolia* as a standardized crop offering potential to minimize reliance on wild harvesting and promote sustainable sourcing, while repurposing extraction byproducts supports a circular-economy approach. Importantly, by providing a plant-based alternative to long-term steroid use, this research could expand affordable AR management options—especially in biodiverse, resource-limited regions—highlighting the broader promise of biodiversity conservation to fuel drug discovery and socioeconomic development.

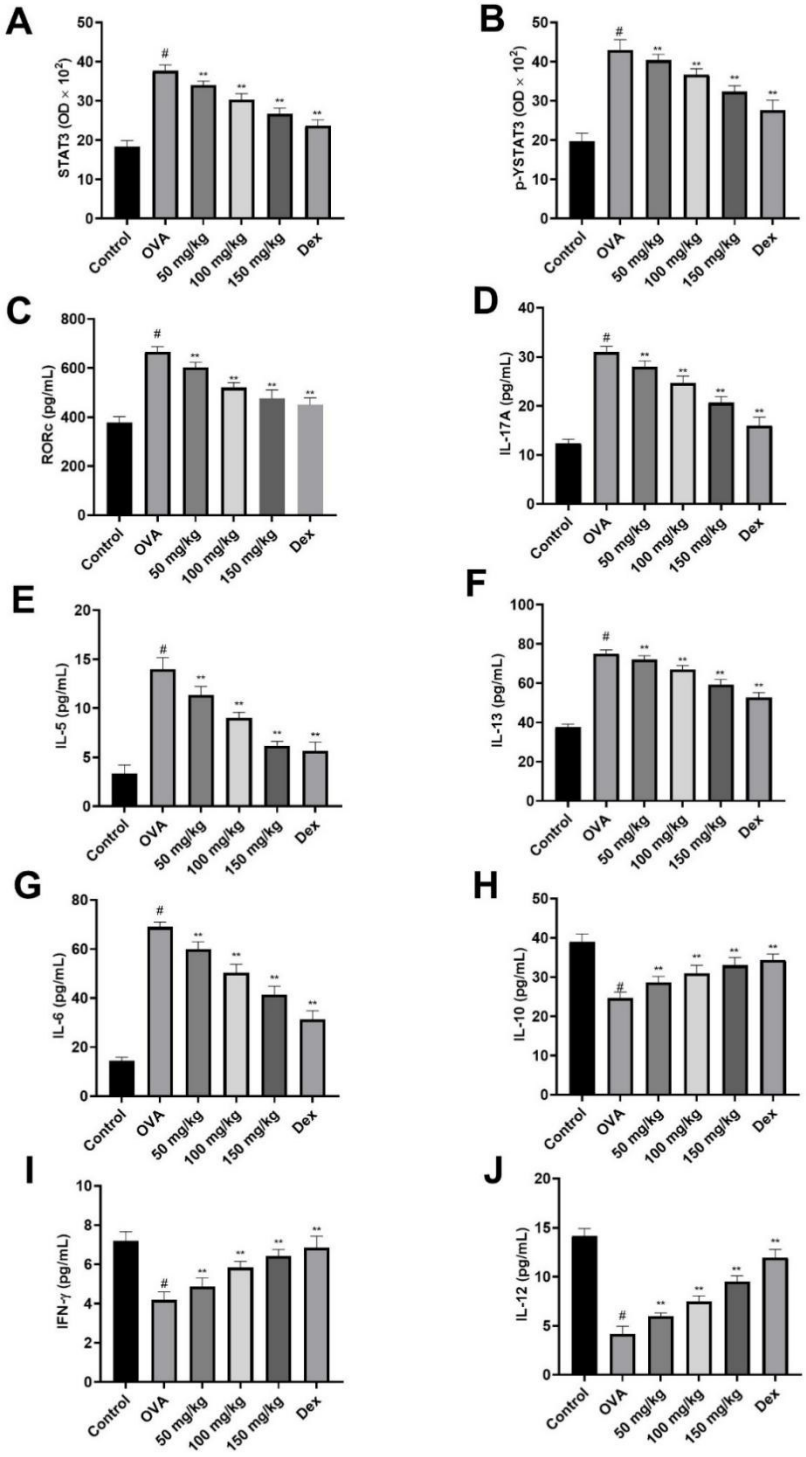

Fig. 5. MP modulates STAT3 signaling and T-cell cytokine profiles in OVA-induced allergic rhinitis. (A) total STAT3 and (B) tyrosine-phosphorylated STAT3 (p-YSTAT3), (C) RORc, (D) IL-17A, (E) IL-5, (F) IL-13, (G) IL-6, (H) IL-10, (I) IFN-γ, (J) IL-12. $^{\#}p<0.05$ *vs.* control and $^{**}p<0.01$ *vs.* OVA group. Mean ± S.E.M.

CONCLUSIONS

1. *Symptom Relief*: *Melicope pteleifolia* (MP) significantly reduced ovalbumin (OVA)-induced nasal rubbing and sneezing in a dose-dependent manner, with the 150 mg/kg dose matching dexamethasone's efficacy.
2. *Anti-inflammatory Effects*: MP inhibited infiltration of eosinophils, neutrophils, macrophages, and epithelial cells in nasal lavage fluid (NALF), and preserved nasal-associated lymphoid tissue (NALT) epithelial integrity and septal mucosal thickness.
3. *Signal Pathway Modulation*: MP suppressed both total and phosphorylated NF-κB-p65/IκBα and STAT3/p-YSTAT3 signaling, leading to marked reductions in TNF-α, IL-1β, IL-17A, IL-5, IL-13, and IL-6.
4. *Immune Rebalancing*: Treatment with MP elevated Treg-associated IL-10 and Th1 cytokines (IFN-γ, IL-12), shifting the immune response away from pathogenic Th2/Th17 profiles.
5. *Therapeutic Promise*: With multi-targeted anti-allergic actions comparable to corticosteroids but potentially fewer side effects, MP is a strong candidate for further development as a non-steroidal therapy for allergic rhinitis.

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ERRATUM: January 23, 2026; A minor labelling error occurred in Fig. 4. The Y-axis labels for *TNF-α* (Fig. 4E) and *IL-6* (Fig. 4F) were mistakenly shown as *p-IκBα*. This was a typographical oversight and does not affect the underlying data, analyses, or conclusions of the study.