

Ajwain oil attenuates allergic response of ovalbumin-induced allergic rhinitis via alteration of inflammatory, oxidative stress, and Th1/Th2 responses

Jingjing Li¹ | Prashant Raghav² | Chunyan Hu³ 

¹Department of Otolaryngology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xibei Hospital, Xi'an, China

²Department of Pharmacy, Dharamveer Singh Rajput Memorial College of Pharmacy, Moradabad, India

³Department of Otolaryngology-Head and Neck Surgery, Children's Hospital Affiliated to Xi'an Jiaotong University, Xi'an, China

Correspondence

Chunyan Hu, Department of Otolaryngology-Head and Neck Surgery, Xi'an Children's Hospital, Xi'an 710003, China.
Email: ertonghu@sina.com

Abstract

Background: Allergic rhinitis (AR) is an immune inflammatory-related disorder that affects the nasal mucosa. Free radicals play a crucial role in the expansion of allergic reaction and the researcher used the antioxidant therapy to treat the disease. *Trachyspermum ammi* L. (Ajwain oil) is popular traditional medicine. It has been proved their potential effect on various diseases. Ajwain oil showed anti-tumor, antioxidant, antidiabetic, anti-inflammatory, and anti-bacterial properties. Yet, the anti-allergic effect of Ajwain oil is still not explored. In this experimental study, an ovalbumin (OVX)-induced AR model was used to scrutinize the anti-allergic, antioxidant and anti-inflammatory effects of Ajwain oil. **Materials and Methods:** OVX was used to establish the AR model (sensitization days 1, 8, and 15) and given the oral treatment of Ajwain oil and Montelukast for 13 days. The spleen, lungs, and body weight were estimated. Sneezing, nasal discharge and rubbing are also estimated. Immunoglobulin-E (IgE), histamine, malondialdehyde (MDA), superoxide dismutase (SOD), heme oxygenase-1 (HO-1), nuclear factor erythroid 2-related factor 2 (Nrf2), and inflammatory cytokines were scrutinized. **Results:** Ajwain oil significantly ($p < .001$) suppressed sneezing, nasal discharge and nasal rubbing along with increasing the spleen, lung and body weight. Ajwain oil significantly ($p < .001$) decreased the level of IgE, histamine, MDA, Nrf2, HO-1, and increased the level of SOD. Ajwain oil significantly ($p < .001$) suppressed the number of eosinophils, neutrophils, macrophages, and epithelial cells. Ajwain oil significantly prevented the activation of the NF- κ Bp65 and STAT3 signaling pathways that led to enhancing the synthesis of anti-inflammatory cytokines and reducing the inflammatory, allergen-specific type 2T helper cells (Th2), Th17 cytokines. **Conclusion:** The obtained data suggests that Ajwain oil has a promising anti-allergic against allergic rhinitis in mice via anti-allergic, antioxidant, and anti-inflammatory effects.

Practical applications

Allergic rhinitis is a serious life-threatening disease. Inflammatory reaction plays an important role in the expansion of AR diseases. Ajwain oil considerably increased

Abbreviations: AR, allergic rhinitis; ELISA, enzyme-linked immunosorbent assay; HO-1, heme oxygenase-1; IgE, immunoglobulin-E; IL-4, interleukin-4; IL-5, interleukin-5; MDA, malondialdehyde; Nrf2, nuclear factor erythroid 2-related factor 2; OVX, ovalbumin; SOD, superoxide dismutase; STAT-2, signal transducer and activator of transcription 2; STAT-3, signal transducer and activator of transcription 3; Treg, regulatory T; W/D, wet/dry.

the spleen weight and reduced lung weight. Ajwain oil suppressed the nasal rubbing, sneezing, and nasal discharge. Ajwain oil considerably suppressed the immunoglobulin and inflammatory cytokines. The result suggests that Ajwain oil having the potential effect against the allergic rhinitis.

KEYWORDS

Ajwain oil, allergic rhinitis, inflammation, oxidative stress, Th1/Th2 response

1 | INTRODUCTION

AR is a type of nasal inflammatory reaction that occurs due to immune system overreacts to allergens breathed and it is a common and serious public health concern (Li et al., 2020). AR is considered chronic and immune inflammatory disease, which affects the inner lining of nasal membrane that resultant induces various characteristic symptoms such as sneezing, congestion, nasal rubbing, rhinorrhea, congestion, and lacrimation (Aswar et al., 2015; Zhang et al., 2015). AR affected around 400–500 million people work productivity and quality throughout the world. AR is an immunoglobulin-E (IgE) attributed inflammatory disease observed in the nasal mucosa, categorized via the activation and infiltration of inflammatory cells viz., eosinophils and mast cells (Hemmati et al., 2019; Kim et al., 2018; Li et al., 2018). For treating AR, anti-histamine and anti-allergic drugs are commonly used. These treatments can reduce the inflammatory response system. Although AR is not a life-threatening condition, clinical symptoms such as itching, rhinorrhea, sneezing, and nasal congestion make life more difficult and add to the financial burden (Wang et al., 2020; Yang et al., 2021; Yuan et al., 2019). AR affected the various organs via induction and alteration of edema. In the later phase of the AR reaction, when leukocytes are released into the afflicted organs, the mast cell secretes chemokines and cytokines. Asthma's clinical symptoms include increased mucus production, airway edema, and chronic bronchospasm followed by protracted inflammation, resulting in anaphylactic shock (Kim et al., 2018; Li et al., 2020; Zhang et al., 2015).

Exposure to environmental allergens activates the Th2 which were boosted the production of Th2 cytokines such as antigen-specific (IgE), interleukin-4 (IL-4), and IL-5 and finally causing the hyper-responsiveness in nasal mucosa (Bui et al., 2020; Bui, Piao, Hyeon, Fan, Van Nguyen, et al., 2019). During the induction of allergic response, activates the Th1 and Th2 cells appear to be mutually antagonistic (Bing et al., 2019; Zhang et al., 2019). During the physiological conditions, the population of Th1 and Th2 exist in balance and cross-regulate each other. The Th1/Th2 equilibrium necessary for balancing the immune homeostasis (Zhang et al., 2019).

Previous study already confirmed the relationship between oxidative stress and chronic inflammation (Fu et al., 2018; Kaya et al., 2019). Oxidative stress causes an imbalance between endogenous antioxidant protection and oxidative power, favoring oxidative harm, which has been linked to the spread of asthma and AR (Bui et al., 2020; Bui, Piao, Hyeon, Fan, Van Nguyen, et al., 2019).

In addition, the development of innovative therapeutic drugs that target pathophysiological dysfunction is critical for the treatment of AR. Until now, scientists have discovered a plethora of natural compounds with anti-inflammatory and antioxidant properties.

GATA-3 is a transcription factor that has been shown to be expressed particularly in Th2 cells, whereas T-box protein present in T-cells (T-bet) is a transcription factor that is specifically induced in Th1 cells (Piao et al., 2020). The gene expression of T-bet and GATA-3 boosted during the differentiation of T-cell, that takes plays an important role in the expansion of Th1 and Th2 lineages. Furthermore, apart from an abnormal Th2 response, the underlying mechanism of allergic rhinitis is crucial and involves additional pathways such as antioxidant and anti-inflammatory (Piao et al., 2020). According to a recent study, regulatory T (Treg) cells play a crucial role in immunological homeostasis. Treg cells limit proliferation and activate the T cell effector, either directly or through antigen-presenting cells, in a cell contact-dependent way. Foxp3 (forkhead family transcription factor) plays a key role in Treg cells in lowering tissue inflammation and regulating the body's immune system stability (Eifan et al., 2012). Th17 cells had the opposite effect as Treg cells, despite the fact that ROR γ t is a splice variant of ROR γ , which is a key element in Th17 cellular development. ROR γ t induction has also been shown to be dependent on signal transducer and activator of transcription 3 (STAT-3) activity. The transcriptional profile of Th17 cells may be regulated by Signal transducer and activator of transcription 2 (STAT-2) and ROR γ t (Eifan et al., 2012; Piao et al., 2020; Zhang et al., 2019). In the early stages of an allergic reaction, the interaction of IgE with the high-affinity receptor of the Fc region (Fc ϵ RI) of mast cells-induced histamine release, vasodilation, and increased vascular permeability of the cell (Eifan et al., 2012; Piao et al., 2020).

The use of the species has been a long history. Species use as enhancers and preserve the food due to the presence of various antioxidant phytochemicals. In recent years, spices having the incredible effects due to the present of various potential bioactive compounds. Currently, spices/medicinal plants to reduces the effect of synthetic drugs and its adverse effects. Spices/medicinal plants having higher demands due to various potential and beneficial effects (Bui et al., 2020; Bui, Piao, Hyeon, Fan, Van Nguyen, et al., 2019; Kim et al., 2018; Yuan et al., 2019).

Trachyspermum ammi L. (family: Apiaceae), also known as Ajwain oil are the common species, having higher aromatic flavor and nature and commonly used in the Chinese system of medicine (Chahal et al., 2017; Rajput et al., 2013). *T. ammi* L. cultivated in China, India,

Afghanistan, Pakistan, Iran, and Iraq. It is having various pharmacological effects such as antimicrobial, antioxidant, estrogenic, hepatoprotective, antidiabetic, acaricidal, anti-inflammatory, antithrombotic, and antihirsutism (Chahal et al., 2017; Moein et al., 2015; Rajput et al., 2013; Zarshenas et al., 2014). The anti-allergic rhinitis effect of Ajwain oil (100%) has been currently unclear. The current investigation was designed to investigate the protective effect of Ajwain oil against the OVA-induced allergic rhinitis mouse model.

2 | MATERIALS AND METHODS

2.1 | Chemical

OVA were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dexamethasone was purchased from the Innovative Research of America (Toledo, OH, USA) and aluminum hydroxide was purchased from Thermo Scientific (Rockford, IL, USA).

2.2 | Experimental rodent

BALB/c (sex- male; 20 ± 5 g) was procured from the animal house for the current experimental protocol. The mice were kept in the air-conditioned room with standard condition such as $20 \pm 5^\circ\text{C}$ temperature; 60%-5% relative humidity with 12-hr light and 12-hr dark cycle. The whole experimental study was conducted as per the guidelines of Care and Use of Experimental Animals and whole experimental study was approved by the institutional animal ethics committee.

2.3 | Experimental protocol

For inducing AR, OVA-induced AR model was used (Bui, Piao, Hyeon, Fan, Van Nguyen, et al., 2019). The rats were grouped into five groups and each group contains the six mice in each group.

Group I: normal control group (mice without challenge and sensitization; 16–28 days, mice were received the oral administration of saline, once a day),

Group II: OVA control mice were inoculated at regular intervals with OVA (50 μg) and 1 mg alum (200 μl) through intraperitoneal injection (days 0, 7, and 14). The mice were then given OVA (10 mg/ml, 20 μl /nostril) through intranasal instillation for 5 days (days 21–28) to begin the sensitization period. From days 16 to 28, the mice were given one dose of saline orally once a day.

Group III: AO-treated group, mice were challenged and immunized following the same protocol as OVA control. From days 16 to 28, the mice received the oral administration of AO (5 mg/kg) for consecutive days (13 days). From days 21 to 28 AO was orally administered 1 hr before the OVA challenge,

Group IV: AO-treated group, mice was challenged and immunized following the same protocol as OVA control. From days 16 to

28, the mice received the oral administration of AO (10 mg/kg) for consecutive days (13 days). From days 21 to 28 AO was orally administered 1 hr before the OVA challenge,

Group V: AO-treated group, mice were challenged and immunized following the same protocol as OVA control. From days 16 to 28, the mice received the oral administration of AO (20 mg/kg) for consecutive days (13 days). From days 21 to 28 AO was orally administered 1 hr before the OVA challenge and

Group VI: ML-treated group, mice were challenged and immunized following the same protocol as OVA control. From days 16 to 28, the mice received oral administration of ML (10 mg/kg) for consecutive days (13 days). From days 21 to 28 AO was orally administered 1 hr before the OVA challenge, respectively. All the data treatment carried in this experimental study is presented in Figure 1.

After completing the experimental protocol (28 days), the mice were sacrificed and lung tissue (along with the nasal lavage fluid), and nasal were collected for further analysis.

2.4 | Spleen/hepatic index

The body weight of all group rats was estimated weekly using the electronic weight scale. At the end of the study, the rats were anesthetized using the ether and finally sacrificed to remove the liver and spleen tissue (Aswar et al., 2015). The hepatic and spleen index were estimated using the following formula

$$\text{Spleen/liver index} = \frac{\text{Spleen} \times \text{body weight}}{\text{liver weight}} \times 10$$

2.5 | Nasal symptom scores

For estimating the nasal symptoms, the frequency of sneezing behaviors and nasal rubbing were counted. After intranasal administration of OVA from days 22 to 28, the frequency of sneezing behavior

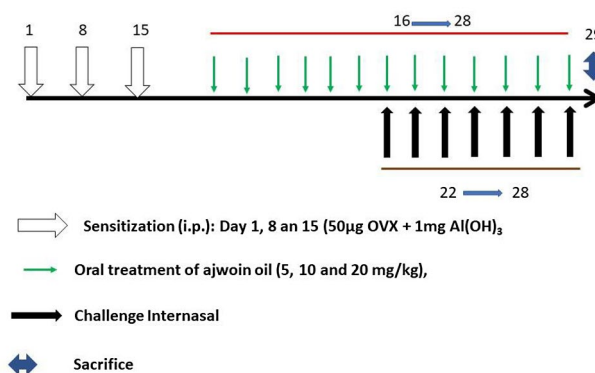


FIGURE 1 The establishment of an allergic rhinitis rodent model and given treatment (Ajwain oil). In this experimental study, the mice were sensitized on days 1, 8, and 15 and ovalbumin challenged on days 22–28. The mice received the oral administration of Ajwain oil (5, 10, and 20 mg) and Montelukast (10 mg/kg) for 13 days, respectively

and nasal rubbing was counted after 15 min and also counted the experimental conditions (blinded observation) (Şahin et al., 2021; Xu et al., 2021; Zhang & Kang, 2020).

2.6 | Lung wet/dry ratio

Lung tissue wet/dry (W/D) ratio was scrutinized for estimating lung edema. Briefly, right lung tissue of all group rats was excised and immediately recorded the wet weight. After that, the lungs were kept in an incubator for 24 hr at 80°C, and then calculated the W/D weight.

2.7 | Enzyme-linked immunosorbent assay

The rats were sacrificed at the end of the experimental protocol and blood was obtained by puncturing the orbital sinus. To separate the serum, the blood samples were kept at room temperature for 30 min before being centrifuged at 5,000×g for 15 min.

2.8 | Estimation of Th1/Th2/Th17, cytokines, and related transcription factor

Anti-inflammatory cytokines such as IL-12 and IFN- γ were measured using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems) according to the manufacturer's instructions.

Inflammatory cytokines such as IL-4, IL-13, and IL-5 were measured in the Th2 response using ELISA kits that were used according to the manufacturer's instructions (R&D Systems).

TNF- α , IL-6, and IL-1 were measured using ELISA kits that were used according to the manufacturer's instructions (R&D Systems).

2.9 | Malondialdehyde, superoxide dismutase, Nrf2/HO-1, and NF- κ B pathway

The supernatant was used for the determination of lipid peroxidation by estimating the level of malondialdehyde (MDA). The previously reported method was used for estimating superoxide dismutase (SOD) with minor modification (Aswar et al., 2015; Piao et al., 2020). The level of HO-1 and Nrf2 was estimated using the ELISA kits following the manufacture protocol (R&D Systems, Minneapolis, MN, USA). Furthermore, the supernatant was also used for estimating the I κ B, NF- κ B, P-I κ B, and P-NF- κ B in the NALF.

2.10 | Statistical analysis

The results are analyzed using GraphPad Prism Software 7 (La Jolla, CA, USA) and presented as mean $\pm$ SEMs. Statistical analysis was carried out using the Student's *t*-test and ANOVA with Dunnett's test. Where *p* < .05 was considered as the significant.

3 | RESULT

3.1 | Spleen weight, lung weight, and body weight

OVX-induced AR mice showed decreased spleen weight (Figure 2a), lung weight (Figure 2b), and body weight (Figure 2c). Ajwain

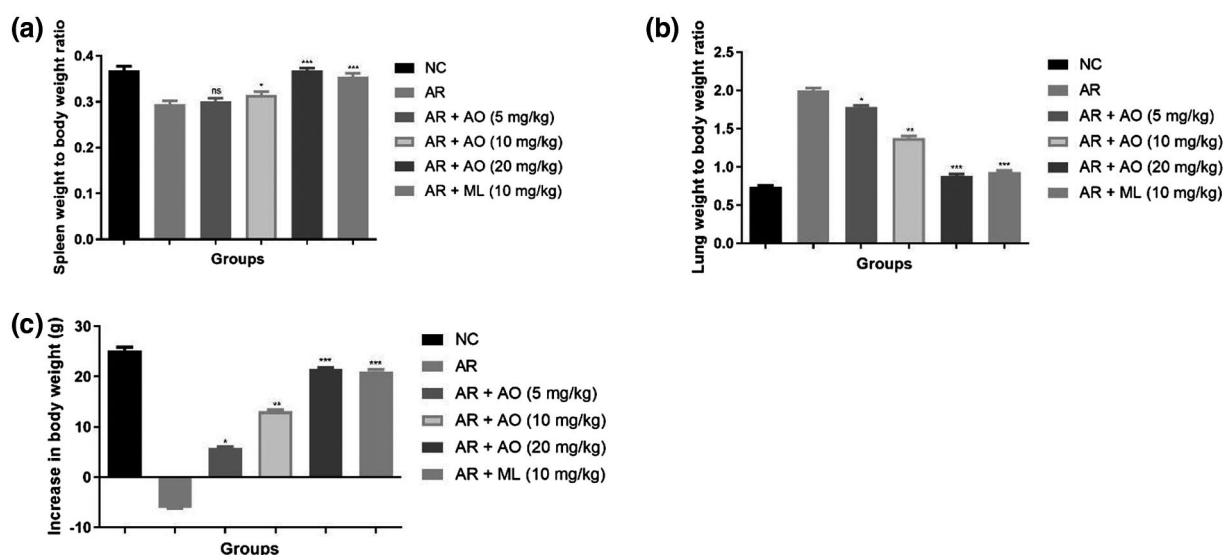


FIGURE 2 The effect of Ajwain oil and Montelukast on the spleen weight, lung weight, and body weight of OVX-induced allergic rhinitis (AR) in mice. (a) Spleen weight to body weight ratio, (b) lung weight to body weight ratio, and (c) effect in body weight. Data are shown as mean $\pm$ SEM. All data were compared using Dunnett's test. **p* < .05; ***p* < .01; and ****p* < .001 compared to AR mice

oil-treated mice significantly ($p < .001$) increased the spleen weight, lung weight, and body weight. ML (10 mg/kg)-treated mice significantly increased the spleen, lung, and body weight.

3.2 | Sneezing, nasal rubbing and discharge

During the allergic reaction, enhanced the sneezing reaction, nasal rubbing, and discharge from the nasal. OVX-induced mice exhibited increased sneezing (Figure 3a), nasal rubbing (Figure 3b), and nasal discharge (Figure 3c) and Ajwain oil-treated mice suppressed sneezing, nasal rubbing, and nasal discharge. ML-treated mice showed similar effects such as reduced reaction of sneezing, nasal rubbing, and nasal discharge.

3.3 | IgE and histamine

IgE and histamine are important biomarker for allergic rhinitis. OVX-induced mice demonstrated increased levels of IgE (Figure 4a) and histamine (Figure 4b); Ajwain oil treatment significantly ($p < .001$) suppressed the level of IgE and histamine.

3.4 | Epithelial cells and different inflammatory cells

OVX-induced mice showed the increased epithelial cells and different inflammatory cells (eosinophil, neutrophil, and macrophage). OVX-induced mice showed increased levels of eosinophils (Figure 5a), neutrophils (Figure 5b), macrophages (Figure 5c) and epithelial cells (Figure 5d). OVX-induced mice treated with Ajwain oil and ML significantly declined epithelial cells and different inflammatory cells.

3.5 | MDA and SOD

During increasing the oxidative stress, enhanced the MDA level and declined the SOD level. OVX-induced mice showed the boosted level of MDA (Figure 6a) and reduced level of SOD (Figure 6b) and Ajwain oil treatment significantly ($p < .001$) suppressed the MDA level and boosted the SOD levels.

3.6 | Nrf2/HO-1

The Nrf2 (Figure 7a) and HO-1 (Figure 7b) level was boosted in the OVX-induced mice and Ajwain oil-treated mice significantly ($p < .001$) suppressed the level of Nrf2 and HO-1.

3.7 | Inflammatory cytokines

OVX-induced mice showed the altered levels of TNF- α (Figure 8a), IL-4 (Figure 8b), IL-5 (Figure 8c), IL-6 (Figure 8d), IL-10 (Figure 8e), IL-12 (Figure 8f), IL-13 (Figure 8g), IL-17 (Figure 8h), and IFN- γ (Figure 8i) compared to other group mice. Ajwain oil- and ML-treated rats significantly ($p < .001$) modulated the level of inflammatory cytokines.

3.8 | T-bet, GATA-3 and RoR- γ

OVX-induced mice showed an increased level of T-bet (Figure 9a) and reduced level of GATA-3 (Figure 9b), RoR- γ (Figure 9c). Ajwain oil- and ML-treated mice significantly ($p < .001$) suppressed the level of T-bet and boosted the level of GATA-3 and RoR- γ .

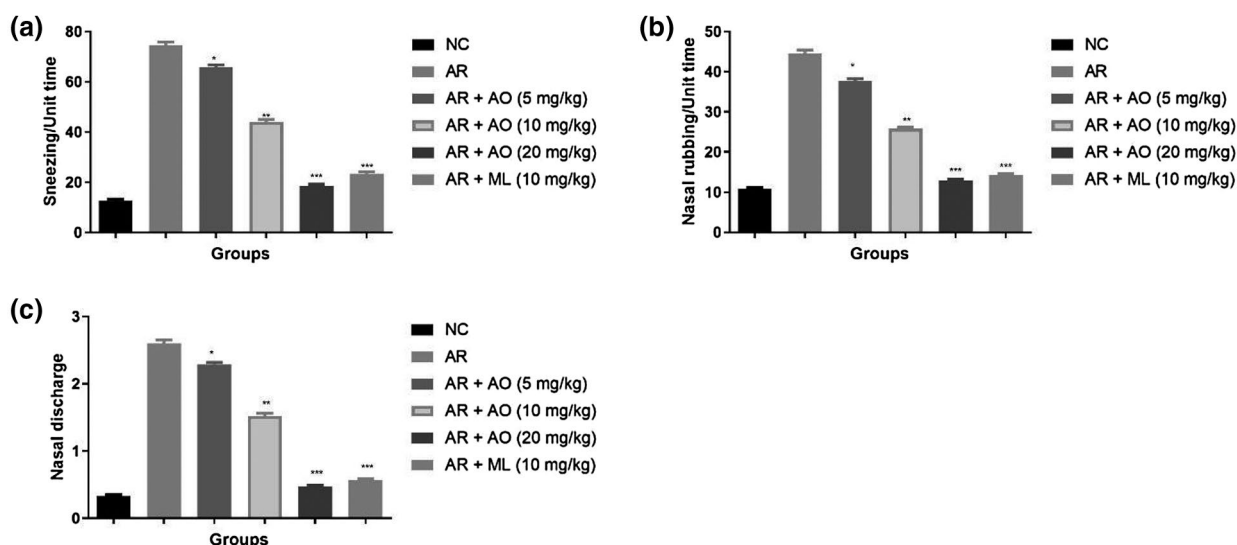


FIGURE 3 The effect of Ajwain oil and Montelukast on sneezing, nasal rubbing and nasal discharge of OVX-induced allergic rhinitis (AR) in mice. (a) Sneezing, (b) nasal rubbing, and (c) nasal discharge. Data are shown as mean $\pm$ SEM. All data were compared using Dunnett's test. * $p < .05$; ** $p < .01$; and *** $p < .001$ compared to AR mice

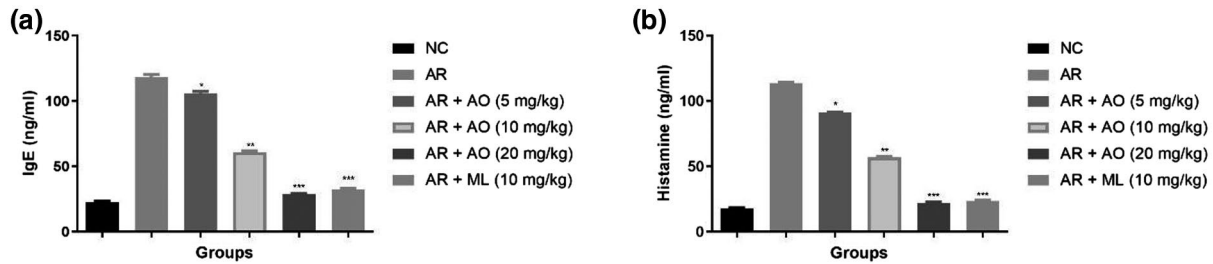


FIGURE 4 The effect of Ajwain oil and Montelukast on the immunoglobulin-E (IgE) and histamine of OVX-induced allergic rhinitis (AR) in mice. (a) IgE and (b) histamine. Data are shown as mean $\pm$ SEM. All data were compared using Dunnett's test. * $p < .05$; ** $p < .01$; and *** $p < .001$ compared to AR mice

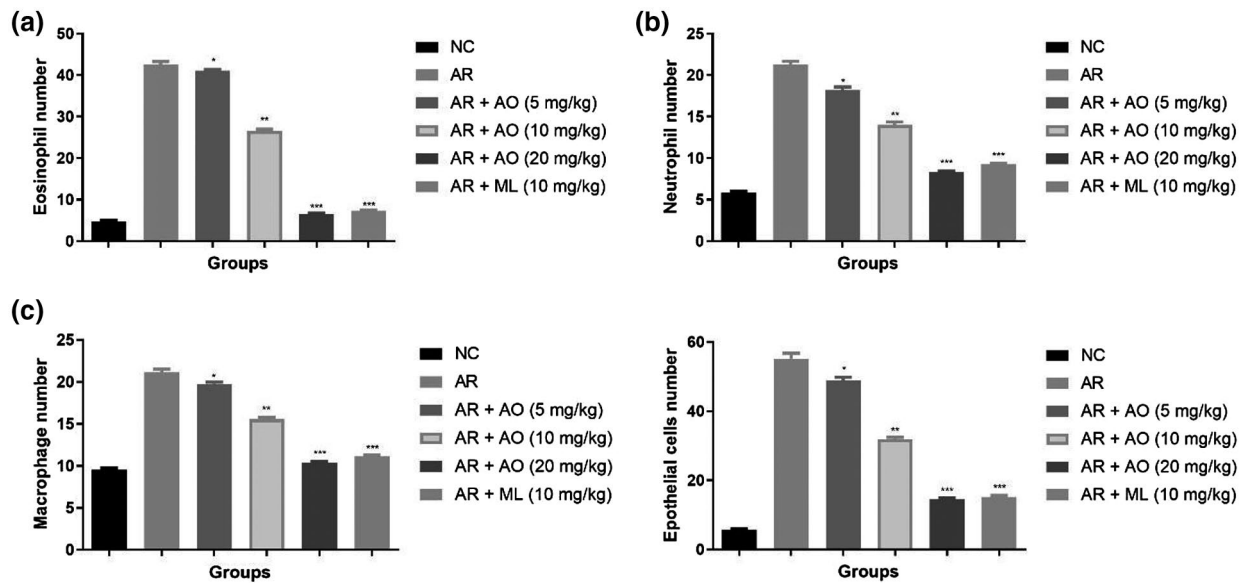


FIGURE 5 The effect of Ajwain oil and Montelukast on epithelial cells and different inflammatory cells of OVX-induced allergic rhinitis (AR) in mice. (a) Eosinophil number, (b) neutrophil, (c) macrophage, and (d) epithelial cells. Data are shown as mean $\pm$ SEM. All data were compared using Dunnett's test. * $p < .05$; ** $p < .01$; and *** $p < .001$ compared to AR mice

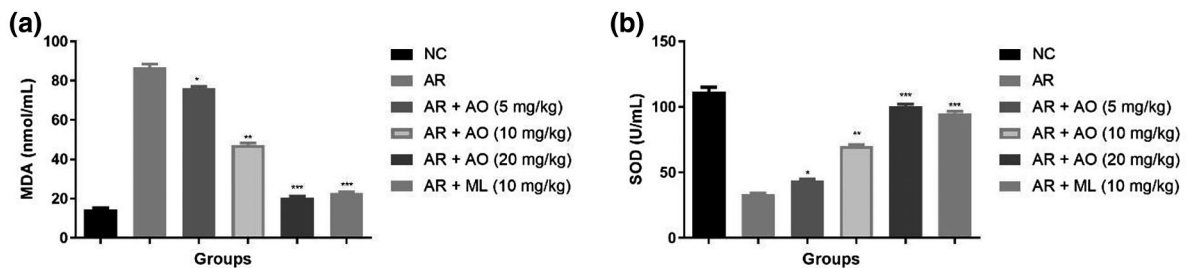


FIGURE 6 The effect of Ajwain oil and Montelukast on MDA and SOD level of OVX-induced allergic rhinitis (AR) in mice. (a) MDA and (b) SOD. Data are shown as mean $\pm$ SEM. All data were compared using Dunnett's test. * $p < .05$; ** $p < .01$; and *** $p < .001$ compared to AR mice

3.9 | NF κ Bp65/I κ B α signaling

For estimating the anti-inflammatory effect of Ajwain oil, phosphorylation of I κ B α and NF κ B65 were estimated. OVX-induced mice showed

enhanced expression of NF κ B65 (Figure 10a) and P-NF κ B65 phosphorylation (Figure 10b). The reduced expression of I κ B (Figure 10c) and increased the P-I κ B level (Figure 10d). Ajwain oil significantly ($p < .001$) altered the expression of NF κ B65, P-NF κ B65, I κ B, and P-I κ B.

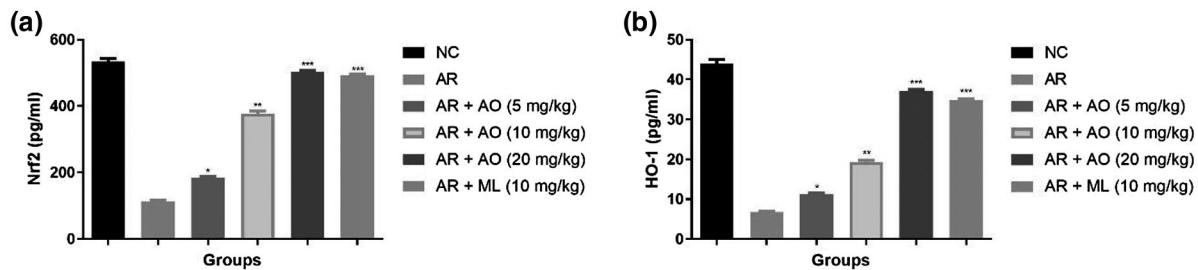


FIGURE 7 The effect of Ajwain oil and Montelukast on the Nrf2 and HO-1 level of OVX-induced allergic rhinitis (AR) in mice. (a) Nrf2 and (b) HO-1. Data are shown as mean $\pm$ SEM. All data were compared using Dunnett's test. * $p < .05$; ** $p < .01$; and *** $p < .001$ compared to AR mice

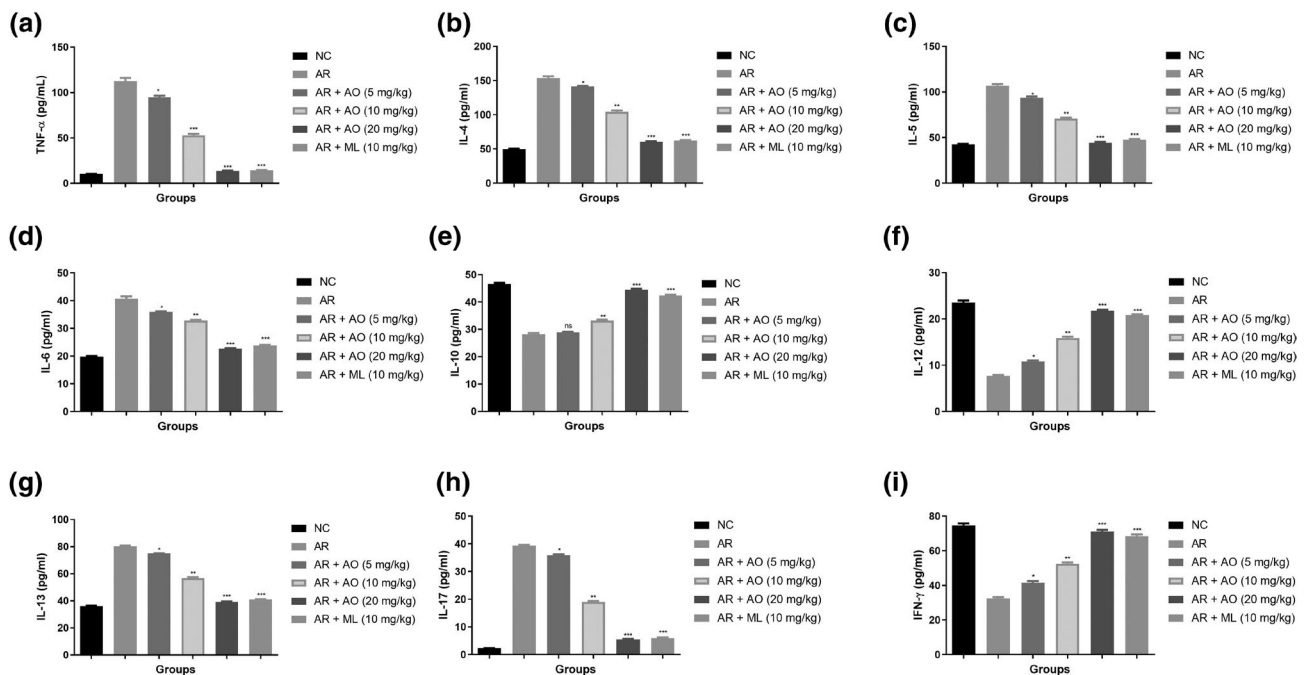


FIGURE 8 The effect of Ajwain oil and Montelukast on the levels of cytokines of OVX-induced allergic rhinitis (AR) in mice. (a) TNF- α , (b) IL-4, (c) IL-5, (d) IL-6, (e) IL-10, (f) IL-12, (g) IL-13, (h) IL-17, and (i) INF- γ . Data are shown as mean $\pm$ SEM. All data were compared using Dunnett's test. * $p < .05$; ** $p < .01$; and *** $p < .001$ compared to AR mice

3.10 | STAT3 signaling

STAT3 level and p-STAT3 (tyrosine phosphorylated STAT3) in the cytoplasm of OVX-induced AR mice were estimated. The level of STAT3 (Figure 11a) and p-STAT3 (Figure 11b) significantly higher in the OVX group mice and Ajwain oil and ML-treated mice significantly ($p < .001$) suppressed the level.

4 | DISCUSSION

Allergic rhinitis is a common inflammation-related disease and it became global threat to public health. Therefore, a lot of diagnosis and protective treatment has been made to cure AR disease, but still lot of patients fail or sometimes they are unsatisfied with the

treatment (Piao et al., 2020; Wang et al., 2021). It is well documented that the progression of AR is connected with the inflammatory response (type 1) and during the progression of AR, inflammatory cytokines are secreted into the nasal mucosa. The invading allergen is recognized by antigen-presenting cells in the nasal mucosa, which then present it to T lymphocytes. Meanwhile, IL-4 (inflammatory cytokines) generated by antigen-presenting cells promotes the development of naive CD4 T cells into Th2 CD4 cells. Moreover, various inflammatory cytokines are released from polarized Th2 CD4 T cells and promote the progression of AR (Bui, Piao, Hyeon, Fan, Choi, et al., 2019; Piao et al., 2020; Shao et al., 2017). Furthermore, reducing or inhibiting inflammatory cytokine release throughout the course of AR would be critical for AR treatment (Gu et al., 2020; Piao et al., 2020). Ajwain oil has showed the antioxidant, anticancer, antibacterial, anti-inflammatory, anti-diabetic, and neurological effects.

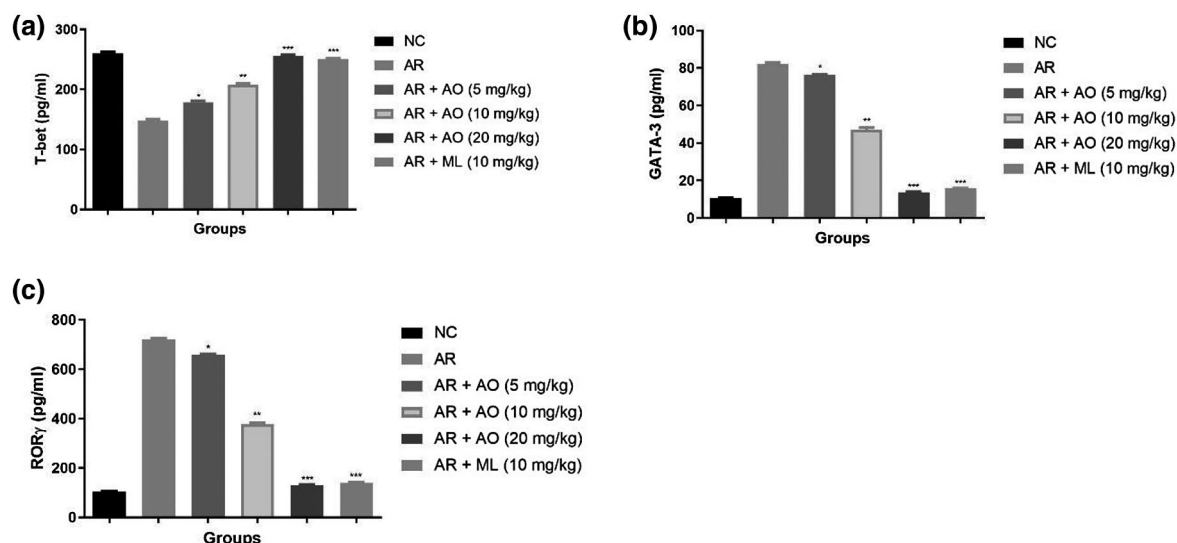


FIGURE 9 The effect of Ajwain oil and Montelukast on the levels of T-bet, GATA, and RoR-γ of OVX-induced allergic rhinitis (AR) in mice. (a) T-bet, (b) GATA-3, and (c) RoR-γ. Data are shown as mean $\pm$ SEM. All data were compared using Dunnett's test. * $p < .05$; ** $p < .01$; and *** $p < .001$ compared to AR mice

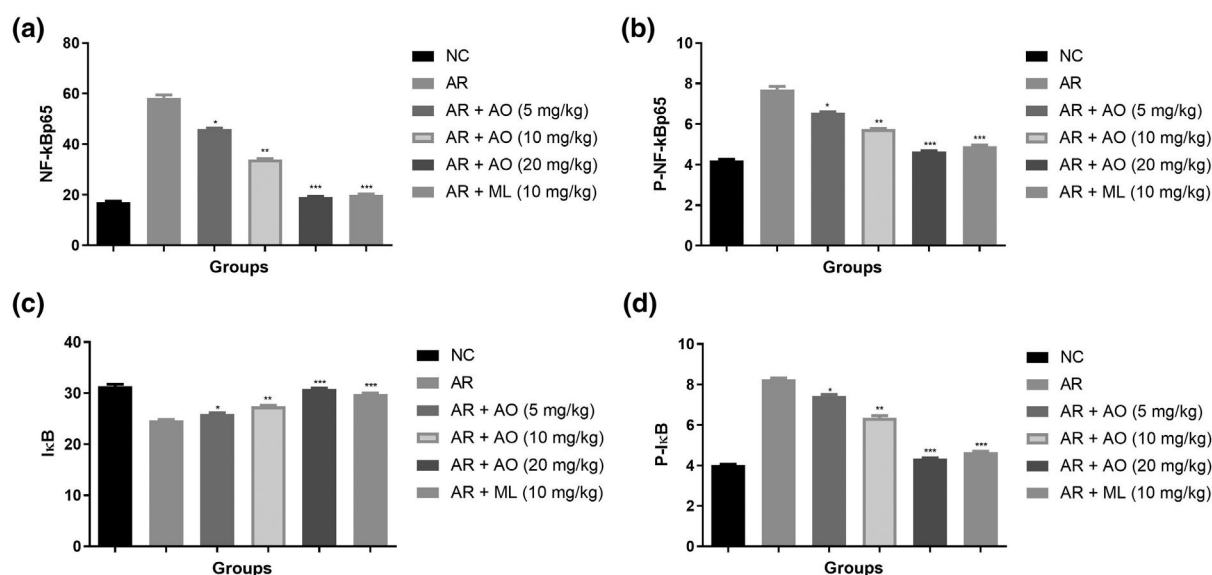


FIGURE 10 Figure showed the effect of ajwain oil and montelukast on the level of NFκB, P-NFκB, IκB and P-IκB of OVX induced allergic rhinitis (AR) in mice. (a) NFκB, (b) P-NFκB, (c) IκB and (d) P-IκB. mean $\pm$ SEM. All the data compared using the Dunnett's test. * $p < .05$; ** $p < .01$; and *** $p < .001$ compared to AR mice

In this experimental study, we scrutinized the protective effect of Ajwain oil on AR.

Nasal itching, nasal mucous, and sneezing are common typical symptoms of AR. In an animal model, nasal behavior is an excellent indicator of pharmacodynamic assessment (Kandhare et al., 2013). In the current investigation, we estimated the efficacy of Ajwain oil against OVX-induced AR in rats. Ajwain oil significantly suppressed the incidence of nose scratching and sneezing, especially the higher dose of Ajwain oil exhibited the beneficial effect. Compared with the standard group, the Ajwain oil group rats had a significant beneficial

effect (after 28 days of administration), which corresponds to the previous research (Chen, He, et al., 2020). The result suggests that the Ajwain oil having protective effect against AR by reducing the nasal-allergic symptoms in rats.

Mast cells and eosinophils are the significant effector cells of AR. Both are the high-affinity IgE binding sites of mast cell surface and huge number of vasoactive mediators and pro-inflammatory factors are stored in the cytoplasm (Zhao et al., 2019). Nasal secretion and peripheral blood (eosinophilic granulocytes) are enhanced through the secretion of mediators of mast cell degranulation. Eosinophils

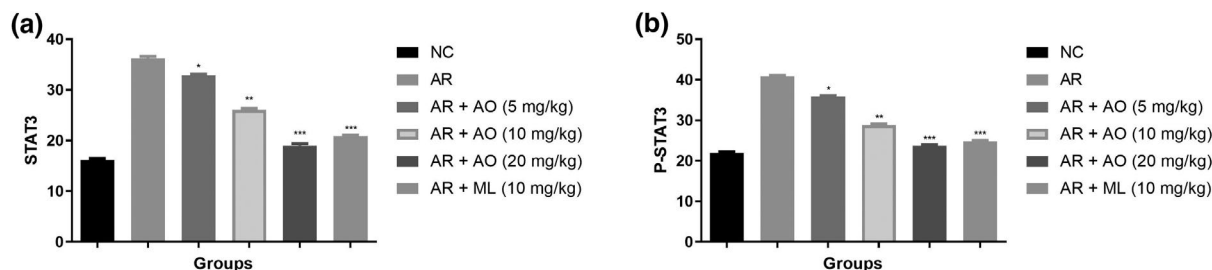


FIGURE 11 Figure showed the effect of ajwain oil and montelukast on the level of STAT-3 and P-STAT-3 of OVX induced allergic rhinitis (AR) in mice. (a) STAT-3 and (b) P-STAT-3. mean $\pm$ SEM. All the data compared using the Dunnett's test. * $p < .05$; ** $p < .01$; and *** $p < .001$ compared to AR mice

also damage the cilia and epithelium of the respiratory tract, various mediators in mast cells and free histamine, thus encouraging the expansion of allergy reaction (Aswar et al., 2015). The current investigation observed that Ajwain oil can suppress the eosinophils and number of mast cells in the nasal mucosa, improve the eosinophil infiltration in the nasal dry membranes and also maintain the expansion of AR.

It is well documented that IgE is a significant mediator inducing the hypersensitivity. Allergen stimulated the body and start the generation of IgE antibody (Aswar et al., 2015; Kim et al., 2018; Li et al., 2020). IgE is the significant biomarker of allergic diseases including allergic asthma and AR. IgE can bind with the high affinity IgE receptor on the surface of mast cells and basophils to sensitize them and start the secretion of inflammatory mediators such as leukotriene and histamine when exposed to the same allergen again (Kim et al., 2018; Li et al., 2018; Wang et al., 2020). IgE is a significant biomarker to scrutinize the efficiency of anti-allergic drugs and scrutinize the possible mechanism. The result showed that Ajwain oil can suppress the IgE level in the nasal lavage fluid and serum of rats.

Histamine is a significant marker of nasal allergy. The secretion of histamine starts after the contact of allergens with basophils and mast cells (Li et al., 2020). During the allergy reaction, histamine activates the H1 receptor on vascular endothelial cells, which further induce the enhancement in C-GMP in cells, consequently producing pro-inflammatory effects, resulting in dilation in small vessels, enhanced gland secretion and microvascular permeability (Bing et al., 2019; Hemmati et al., 2019; Li et al., 2020). Mostly oral anti-histamine is considered the first-line drug for the treatment of AR. Our experimental result suggests that the Ajwain oil having the anti-histamine effect and also enhanced the nasal symptoms in the AR model.

The research suggests that AR pathogenesis in modern medicine depends on the Th1/Th2 theory. IL-4, IFN- γ , and IL-5 are the Th1/Th2-related cytokines and IL-17 is the Th17-related cytokines (Fu et al., 2017; Hemmati et al., 2019). IL-4 boost maturation and cell differentiation co-operate with the IL-13 to increase the transformation to B-cell into the plasma cell synthesis and build the immune memory and quickly synthesized the more specific IgE allergen (Bing et al., 2019; Hemmati et al., 2019; Li et al., 2020). Moreover, IL-4, a significant cytokine of up-regulating IgE, IFN- γ is an anti-allergic

factor of respiratory tract and biological antagonist of IL-4 synthesis. In the development and activation of eosinophils, IL-5 plays a critical function, increased survival time and is a significant marker in the delayed phase of hypersensitivity (Chen, Chan, et al., 2020; Piao et al., 2020; Wang et al., 2020). Airway hyper responsiveness requires IL-5-mediated eosinophil infiltration in the trachea (Chen, Chan, et al., 2020; Piao et al., 2019). The results of the current experimental study showed that IL-5 and IL-4 were significantly reduced by Ajwain oil and increased the IFN- γ level. Th17 cells have a key function in the spread of allergy disorders caused by Th2. IL-17 may increase the aggregation of neutrophils by inducing the activation of mast cells and IL-8. AR group rodents exhibited the increased IL-17 levels and Ajwain oil-treated rodent showed the decrease level of IL-17.

It is well known that NF κ B/I κ B signaling pathway is involved in the expansion of the inflammatory reaction. Previous research suggests that the enlargement of the cell with IL-1 β and TNF- α increased the level of NF κ B (Li et al., 2017). TNF- α -induced the activation of NF κ B and phosphorylation of I κ B in the endothelial cell (Piao et al., 2020). We have found that the increased level of IL-1 β , TNF- α , and Ajwain oil significantly reduced the level of IL-1 β and TNF- α . Moreover, a recent investigation suggests that STAT3 in T cells is necessary for the expansion of allergic inflammation (Eifan et al., 2012). During the differentiation of Th2 cells, activate the STAT3, and the presence of STAT3 increases the production of Th2 cytokine (Piao et al., 2020). In the normal condition, the STAT3 live in the dormant condition in the cytoplasm, once it is activated, STAT3 translocates to the nucleus and increases the IL-6 expression (Eifan et al., 2012). Similarly, ROR γ and STAT3 are required for the Th17 cell differentiation (Piao et al., 2020). We estimated the expression of p-STAT3 and STAT3 in the cytoplasm. In this experimental study, Ajwain oil significantly suppressed the expression of STAT3 and blocked the p-STAT3 step.

Previous research suggests that the interaction between the NF κ Bp65 and STAT3 in the human breast cancer cells (Eifan et al., 2012). It is well known that the phosphorylation process of STAT3 was blocked, the STAT3 could not translocate into the nucleus and not interact with the NF- κ B (Piao et al., 2020). The interaction and activation between the NF- κ B and STAT3 play an important role in maintaining the inflammatory cells (Eifan et al., 2012). In this study, Ajwain oil significantly reduced the level of STAT3 and NF κ Bp65 in the cytoplasm by blocking the phosphorylation process.

5 | CONCLUSION

On the basis of the most recent findings, we can say that Ajwain oil has a preventive and protective effect on the regulation of allergic reaction through suppressing the accumulation of inflammatory cells, activation of STAT3 signaling as well as inflammatory-related cytokines. Ajwain oil control the expansion of AR through regulation of T-cell immune response, suppression of eosinophil infiltration, IgE and histamine level. The result suggests that Ajwain oil provides a promising strategy for immunotherapy in the allergic rhinitis. Still, a more molecular level investigation needs to identify the possible mechanism.

AUTHOR CONTRIBUTIONS

Jingjing Li: Investigation; Methodology; Project administration; Writing-original draft; Writing-review & editing. **Prashant Raghav:** Conceptualization; Data curation; Writing-original draft; Writing-review & editing. **Chunyan Hu:** Conceptualization; Data curation; Resources; Software; Supervision; Validation; Visualization; Writing-original draft; Writing-review & editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Chunyan Hu  <https://orcid.org/0000-0002-7912-1061>

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