

RESEARCH

Open Access



Anti-allergic and anti-inflammatory effects of *Gastrodia elata* Blume extract in ovalbumin-induced asthma rat model

Jeong Su Park¹, Yeon Su Lee¹, Da Eun Jung¹, Ji Won Seo¹, Hyeon Jeong Na¹, Jin Woo Hong¹ and Jae-Ho Shin^{1*} 

Abstract

Background Allergic asthma is a chronic inflammatory disease in which bronchial inflammation causes narrowing of the bronchi when exposed to allergens, resulting in coughing, wheezing, and difficulty breathing. *Gastrodia elata* Blume (GEB) is a perennial Orchidaceae plant native to alpine areas and is known to be effective in anti-inflammatory and anticonvulsants. This study evaluated the anti-inflammatory and anti-allergic effects of GEB extract in a rat model of allergic asthma induced by ovalbumin. This study evaluated the anti-inflammatory and anti-allergic effects of GEB extract in a rat model of allergic asthma induced by ovalbumin. Twenty-four 6-week-old Wistar rats were divided into three groups: control group (CON), ovalbumin (OVA) -induced group, and GEB treatment group. Except for the CON group, the remaining groups were sensitized to OVA by intraperitoneal injection, and asthma was induced by OVA intranasal instillation. The CON and OVA groups were administered distilled water, and the GEB group was administered 7 g/kg of GEB extract for 11 days.

Results Serum total IgE levels were decreased in the GEB group compared to the OVA group. Also, lung IL-4, IL-5, and IL-13 levels were significantly lower in the GEB group than in the OVA group. Histopathological analysis using hematoxylin and eosin and periodic acid Schiff staining, the tracheal and alveolar walls of the OVA group were thickened, and there was increased infiltration of inflammatory cells in the bronchi, perivascular, and alveolar spaces. As for lung damage caused by OVA, GEB treatment reduced the infiltration of inflammatory cells into the bronchi and blood vessels, and the alveolar spaces were maintained, showing a structure similar to that of the CON group. Immunohistochemical analysis showed that IL-4, IL-5, CD206, and MPO expression levels were reduced in the GEB group compared to the OVA group.

Conclusions This suggests that GEB treatment has an anti-inflammatory and anti-allergic effect by reducing the levels of IgE and the cytokines IL-4, IL-5, and IL-13 and ameliorating histopathological changes in an asthma rat model.

Keywords *Gastrodia elata* Blume, Allergic asthma, Anti-inflammation, Anti-allergic, Ovalbumin, Rat

*Correspondence:

Jae-Ho Shin
shinh0157@gmail.com

¹Department of Biomedical Laboratory Science, Graduate School, Eulji University, Seongnam 13156, Korea



© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Background

Asthma affects approximately 300 million people worldwide, leading to numerous deaths each year. Despite advances in medical technology, the global impact of asthma remains high. Allergic asthma is a chronic inflammatory disease of the airways, typically triggered by inhalation of allergens and characterized by increased mast cells, eosinophils, macrophages, and neutrophils [1–3]. This disease causes recurrent symptoms of wheezing, breathlessness, chest tightness, and coughing at night or daybreak, in severe cases, lung function may decline, and breathing difficulties may even progress to respiratory failure [1, 4]. It occurs particularly frequently in childhood and is characterized by airway obstruction, airway hyperresponsiveness, and airway inflammation resulting from exposure to various irritants [5]. Type 1 allergy causes asthma through an immune response to allergens mediated by T helper 2 (Th2) cells [6–8]. To mimic the pathogenesis of human asthma and evaluate the safety and efficacy of treatment, asthma was induced in animal models such as mice, rats, and guinea pigs using various allergens such as ovalbumin (OVA), house dust mites, and molds [9, 10]. Among them, OVA is the primary protein found in chicken egg whites. It is known to be the most commonly used allergen in asthma experiments because it causes severe allergic lung inflammation with features similar to human asthma [10, 11]. When an allergen enters the body, it is processed by macrophages, natural killer (NK) cells, and dendritic cells and produces cytokines such as interleukin-4 (IL-4), IL-5, and IL-13 [12, 13]. The cytokines activate B cells to secrete immunoglobulins, induce class switching from IgM to IgE, and promote differentiation into M2 macrophages. Cytokines and IgE secreted in this way cause inflammation and lung damage [14–16].

Inhaled corticosteroids are used to treat allergic asthma, a chronic inflammatory disease, but they can cause side effects such as dysphonia and candidiasis [17, 18]. For this reason, it is necessary to find natural substances that can help prevent and improve asthma and have fewer side effects. Many natural products, including Kanakasaba [19], Anatabine [20], and *Olea europaea* L [21], have been studied for the treatment and prevention of respiratory diseases such as asthma. *Gastrodia elata* Blume (GEB) is a plant from the Orchidaceae plant and is distributed in mountainous regions of East Asia, including Korea, China, and Japan. It is currently used clinically to treat dizziness, headaches, epilepsy, convulsions, and high blood pressure [22, 23]. Additionally, recent studies have shown that GEB extract prepared with 70% ethanol has anti-inflammatory effects [24]. Phenolic compounds found in GEB extract, such as 4-hydroxybenzaldehyde, 4-hydroxy benzyl alcohol, benzyl alcohol, bis-(4-hydroxyphenyl)methane, 4-(4'-hydroxybenzyloxy)benzyl methyl

ether, 4-hydroxy-3-methoxybenzyl alcohol, 4-hydroxy-3-methoxybenzaldehyde, 4-hydroxy-3-methoxybenzoic acid, and 1,2-bis (4-(β -D-glucopyranosyloxy)benzyl) citrate (parishin), exhibit antiasthmatic effects in a guinea pig asthma model [25]. Like this, the main active ingredients of GEB have been reported to have various pharmacological activities such as neuroprotection, anti-inflammatory, and anti-asthma. However, the anti-inflammatory and anti-allergic actions of GEB extract in asthma rat models have not been identified.

To date, the anti-inflammatory and anti-allergic effects of GEB extract in asthma models have not been clearly elucidated. Given the growing interest in natural product-based health functional foods with fewer side effects, it is necessary to clarify the pharmacological effects of GEB on allergic asthma for its potential use as a functional food ingredient. Therefore, this study investigated the histopathological changes, immune cells, and cytokines in lung tissues to evaluate the potential protective effects of GEB in an OVA-induced allergic asthma model that is very similar to the pathophysiology of human asthma. Through this approach, we aimed to elucidate the potential of GEB as a novel natural anti-asthmatic agent and contribute to its utilization as a safer and more effective health functional food for allergic diseases.

Methods

Preparation of GEB extract

GEB was provided by the Rural Development Administration (Jeonju, Jeonbuk State, Korea). The GEB was dried using hot air at 70 °C. The dried GEB was coarse-ground. Distilled water (DW) 10 times the weight (g) of coarse-ground GEB was added, extracted four times at 96 °C, and the first to fourth extracts were mixed. DW was added to the extract, and the mixture was concentrated under reduced pressure and then lyophilized. The lyophilized GEB extract was sterilized through irradiation. To analyze the components of GEB, 1 mL of GEB extract and 4 mL of 50% methanol were added to the vortex for 10 s. The mixed samples were subjected to ultrasonic extraction for 30 min. The ultrasonically extracted sample was centrifuged at 10,000 rpm for 10 min, and the supernatant was separated and filtered through a 0.45 μ m syringe filter. Afterward, the GEB extract was analyzed using HPLC–DAD (High-performance liquid chromatography-diode array detector) (Table 1). GEB extract was freshly prepared by completely dissolving 7 g of GEB powder in 10 mL of DW at the time of the experiment.

Chemicals and reagents

Albumin from chicken egg white (OVA, A5378-1G) and aluminum hydroxide (Alum, 239,186-25G) were purchased from Sigma-Aldrich (St Louis, MO, USA). Rat interleukin-4 (IL-4, CSB-E04635r), interleukin-5 (IL-5,

Table 1 HPLC–DAD instrumental analysis conditions for the analysis of GEB extract

LC (Liquid chromatography)		Agilent 1100 series					
Column		Capcellpak C18 UG1200 (4.6×250 nm, 5 µm)					
Mobile phase		A: Water: ACN = 99:1 B: ACN					
Gradient mode	Time (min)	0	15	20	25	25.1	30
	B (%)	0	0	90	90	90	0
Flow		1.2 mL/min					
Injection volume		10 µL					
Detector	DAD detector	270 nm					

CSB-E07435r) and interleukin-13 (IL-13, CSB-E07454r), enzyme-linked immunosorbent assay (ELISA) kits were obtained from Cusabio (Wuhan, P.R. China), and rat total IgE ELISA kit (ab157736) was obtained from Abcam (Inc., Cambridge, MA, USA). To perform immunohistochemistry (IHC), anti-IL-4 antibody (ab9811, Abcam), anti-IL-5 antibody (LS-B14355, LSBio, Seattle, WA, USA), anti-CD206 antibody (ab64693, Abcam), anti-myeloperoxidase antibody (ab208670, Abcam), and ABC-horseradish peroxidase (HRP) kit (PK6101) was obtained from Vector Laboratories (Burlingame, CA, USA) and 3,3'-diaminobenzidine (DAB) Substrate Kit (34,002) was purchased from Thermo Fisher Scientific (Waltham, MA, USA).

Animals

Male Wistar rats (6 weeks old, 180–230 g) were purchased from Orient Bio (Seongnam, Gyeonggi-do, Korea). The animals were housed in cages measuring 260 mm (W)×420 mm (D)×180 mm (H) (2–3 rats per cage) with free access to food (24.52% protein, 12.41% fat, 63.07% carbohydrates) and tap water and were maintained under the following conditions: temperature, 22±2°C; Humidity, 50±10%; circadian cycle, 12-h light/dark cycle. The body weight (BW) and food and water intake were measured twice a week during the experimental period.

Experimental design

After 1 week of adaptation to standard housing conditions, animals were randomly divided into 3 groups with 8 rats per group: i) Control (CON) group, ii) OVA-induced asthma group, and iii) GEB-treated group (Supplementary Fig. 1).

On days 1, 2, 3, and 11, the OVA and GEB groups were sensitized by dissolving 200 µg of OVA in 1 mL of saline containing 10 mg of alum as an adjuvant, and the CON group received the same volume of saline with 10 mg of alum added. On days 20, 21, and 22, 100 µL of 1% OVA in saline was intranasally instilled in the OVA and GEB groups, while the CON group received the same amount of saline intranasally. GEB extract was administered orally once daily from day 11 to day 23 at the maximum

nontoxic dose of 7 g/kg. Twenty-four hours after the last administration, the rats were fasted overnight and sacrificed under anesthesia using isoflurane (Halocarbon, Georgia, USA). Blood was collected from the abdominal aorta. Blood samples were placed in a serum-separating tube (SST) and centrifuged at 3,000 rpm and 4 °C for 15 min to obtain the serum. The serum was stored at –80 °C for biochemical analysis. The left lung was stored in 10% neutral buffered formalin solution for histopathological and immunostaining analysis, and the right lung was stored at –80 °C for biochemical analysis.

Bronchoalveolar lavage fluid (BALF) collection and differential cell counts

To collect BALF, rats were slowly instilled with 3 mL of cold phosphate-buffered saline (PBS) three times after endotracheal intubation. This resulted in a collected average volume of 1.95±0.04 mL of BALF. The collected BALF was centrifuged at 2,000 rpm and 4 °C for 5 min. After centrifugation, all supernatants were collected and stored at –80 °C, and the cell pellets were suspended in 100 µL of PBS. Subsequently, 3×10⁴ cells were added to the slide in 200 µL of PBS and centrifuged using cyto-spin (Cellspine, Hanil, Gimpo, Korea) equipment. After drying, the slides were stained with Diff-Quik reagent (38,721, Sysmex, Kobe, Japan). To observe changes in the number of eosinophils and neutrophils in BALF, the total number of eosinophils and neutrophils was counted in four fields of view on Diff-Quik stained slides.

Measurement of total IgE in serum

Total IgE levels were measured according to the ELISA manufacturer's instructions using serum stored at –80 °C. Absorbance was measured at 450 nm using SkanIt™ software for a microplate reader (Thermo Fisher Scientific).

Measurement of cytokines in lung

To measure the cytokines IL-4, IL-5, and IL-13 in lung homogenates, in ice, 100 mg of lung tissue was homogenized in 1 mL of PBS and stored overnight at –20 °C. After subjecting the homogenate to two freeze–thaw cycles to break the cell membrane, it was centrifuged at 5,000 xg and 4 °C for 5 min. The obtained supernatant

Table 2 Scoring methods for histopathological observation

Grade	Diagnostic criteria
0	No bronchial and perivascular inflammatory cells
1	Few scattered bronchial and perivascular inflammatory cells
2	Focal inflammatory cells around the bronchi and blood vessels
3	One layer of inflammatory cells encircling the bronchi and blood vessels
4	Two or more layers of inflammatory cells encircling the bronchi and blood vessels

was used and measured according to the ELISA manufacturer's instructions. Absorbance was measured at 450 nm using SkanIt™ software for a microplate reader (Thermo Fisher Scientific).

Histopathological analysis

The fixed lung tissue was dehydrated with alcohol, washed with xylene, and embedded in paraffin. The blocks were then cut into 4 μm thick sections. The sections were stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS), mounted with Permount, and observed using a light microscope (Olympus, Tokyo, Japan). Lungs were observed on H&E-stained slides to evaluate peribronchial inflammatory cell infiltration, alveolar inflammatory cell infiltration, and alveolar wall changes. The lung inflammation score was performed in a blinded manner following the method described by Zeng Z et al. [26]. The degree of lung inflammation was evaluated using the following scoring system: 0 = no bronchial and perivascular inflammatory cells; 1 = few scattered bronchial and perivascular inflammatory cells; 2 = focal inflammatory cells around the bronchi and blood vessels; 3 = one layer of inflammatory cells encircling the bronchi and blood vessels; 4 = two or more layers of inflammatory cells encircling the bronchi and blood vessels (Table 2). PAS staining, which stains neutral mucus, was performed

to confirm mucus secretion caused by inflammation in the bronchi.

Immunohistochemical analysis

IL-4, IL-5, CD206, and MPO expression levels associated with inflammatory cells, such as eosinophils, neutrophils, and macrophages, were evaluated in lung tissues using immunohistochemistry. The lung tissue was sectioned at 4 μm thickness on silane-coated slides. After deparaffinization and dehydration, the sections were boiled in a microwave for 10 min using citrate buffer (pH 6.0) for antigen retrieval and then cooled at room temperature for 30 min. Anti-IL-4 antibody (1:500), anti-IL-5 antibody (1:1000), anti-myeloperoxidase antibody (1:1000), CD206 antibody (1:1000), and MPO antibody (1:1000) were incubated overnight at 4 °C as primary antibody. The samples were visualized using 3,3'-diaminobenzidine tetrahydrochloride (DAB), counterstained with Mayer's hematoxylin, and examined using a light microscope.

Statistical analysis

The significance of the differences in mean values among the experimental groups was determined using a one-way analysis of variance (ANOVA), followed by Tukey's HSD test. The level of statistical significance was set at $p < 0.05$. SPSS (version 28.0; SPSS Inc., Chicago, USA) calculated probability values. All the results in this study are expressed as mean ± standard deviation (SD).

Results

Active ingredient content of GEB extract using HPLC–DAD

GEB extract contains the active ingredients known as 4-hydroxybenzyl alcohol and gastrodin (Fig. 1).

Effects of GEB on inflammatory cell counts in BALF

The total cell count in BALF was significantly increased in the OVA group compared to the CON group and

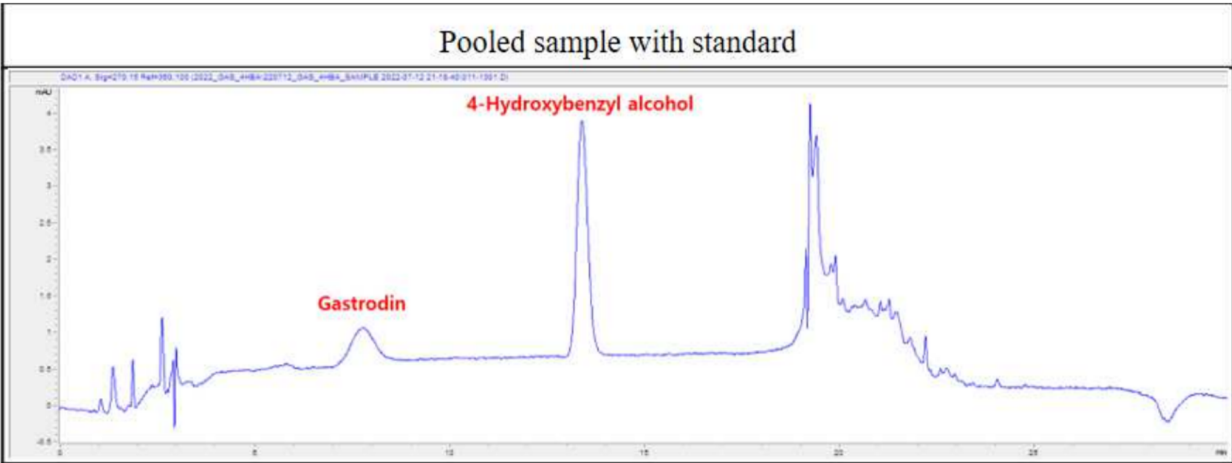


Fig. 1 Active ingredients of GEB extract

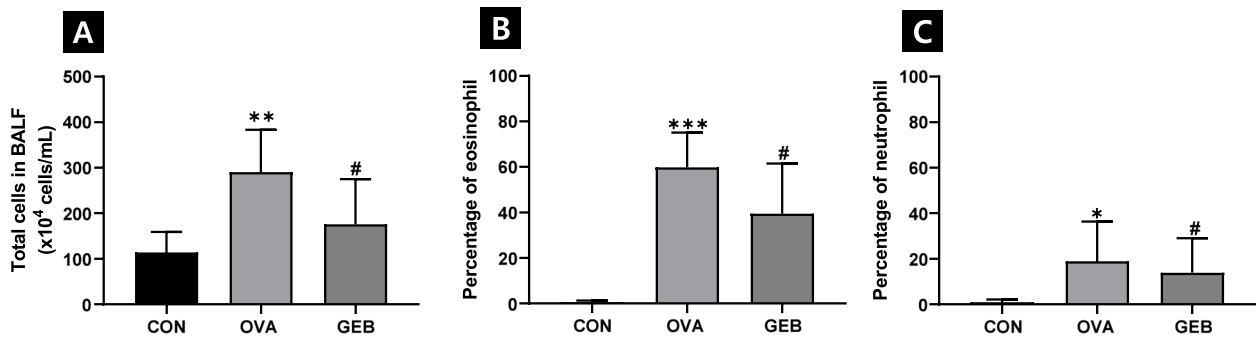


Fig. 2 Effect of GEB on inflammatory cells in asthmatic rats. Mean values of (A) total cell counts in BALF, (B) percentage of eosinophil, and (C) percentage of neutrophil. Values are expressed as mean $\pm$ SD of 8 rats per group. Compared with the CON group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; compared with the OVA group, # $p < 0.05$

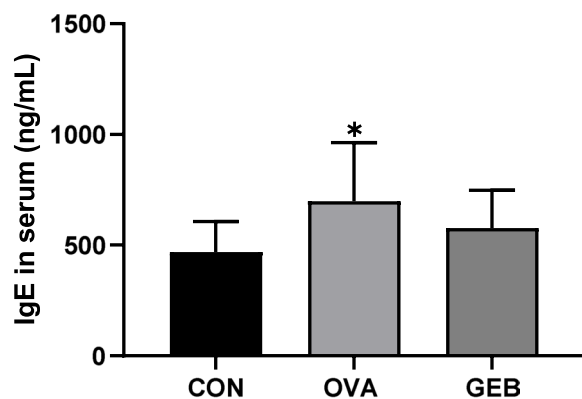


Fig. 3 Total IgE level in serum. Total IgE levels in serum were quantified using enzyme-linked immunosorbent assay (ELISA). Values were mean $\pm$ SD of 8 rats per group. Compared with the CON group, * $p < 0.05$

significantly decreased in the GEB group compared to the OVA group (Fig. 2A). Additionally, when checking the asthma status of rats after drug treatment for asthma caused by OVA administration in BALF, eosinophils, and neutrophils, which are generally increased in asthma diseases, were scarcely found in the CON group. Eosinophils and neutrophils were found in the OVA and GEB groups administered OVA, but the levels of eosinophils

and neutrophils were markedly reduced in the GEB group compared to the OVA group (Fig. 2B, C).

Effects of GEB on the total IgE in serum

The total IgE level in the serum of the OVA group was significantly higher than that of the CON group. The total IgE level in the GEB group tended to decrease compared to the OVA group. GEB treatment has been shown to reduce IgE production due to the inhibition of allergic responses (Fig. 3).

Effects of GEB on cytokines in the lung

To analyze type 2 cytokines induced by GEB in rats with induced asthma, we examined type 2 cytokine levels in the lungs of rats administered GEB. In the OVA group, the levels of IL-4 (Fig. 4A), IL-5 (Fig. 4B), and IL-13 (Fig. 4C) were dramatically increased in contrast to the CON group. Compared with the OVA group, the levels of IL-4, IL-5, and IL-13 in the GEB group were significantly downregulated.

Effects of GEB on airway inflammation and mucus secretion in lung

H&E staining showed normal structures of the bronchi and lungs in the CON group (Fig. 5A, D). The tracheal

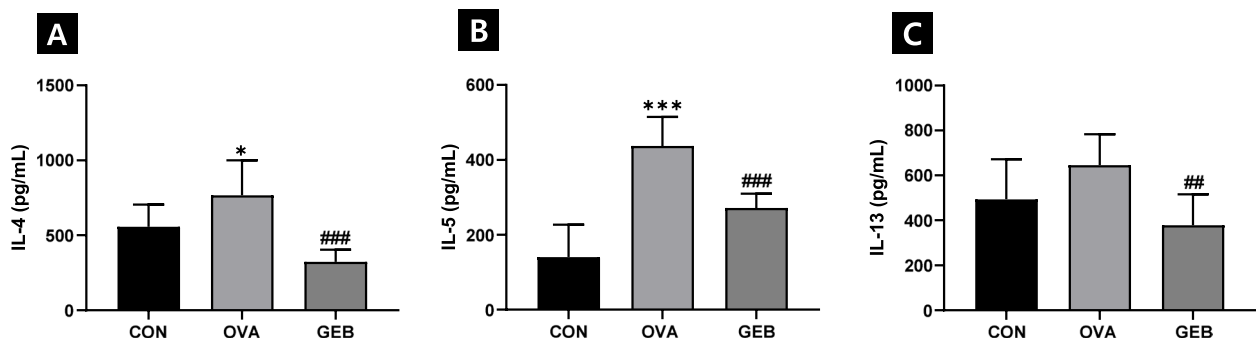


Fig. 4 Changes in cytokines in the lung. Levels of cytokines (A) IL-4, (B) IL-5, and (C) IL-13 were measured using ELISA in lung homogenates of OVA-induced asthmatic rats. Values were mean $\pm$ SD of 8 rats per group. Compared with the CON group, * $p < 0.05$, *** $p < 0.001$; compared with the OVA group, ## $p < 0.01$, ### $p < 0.001$

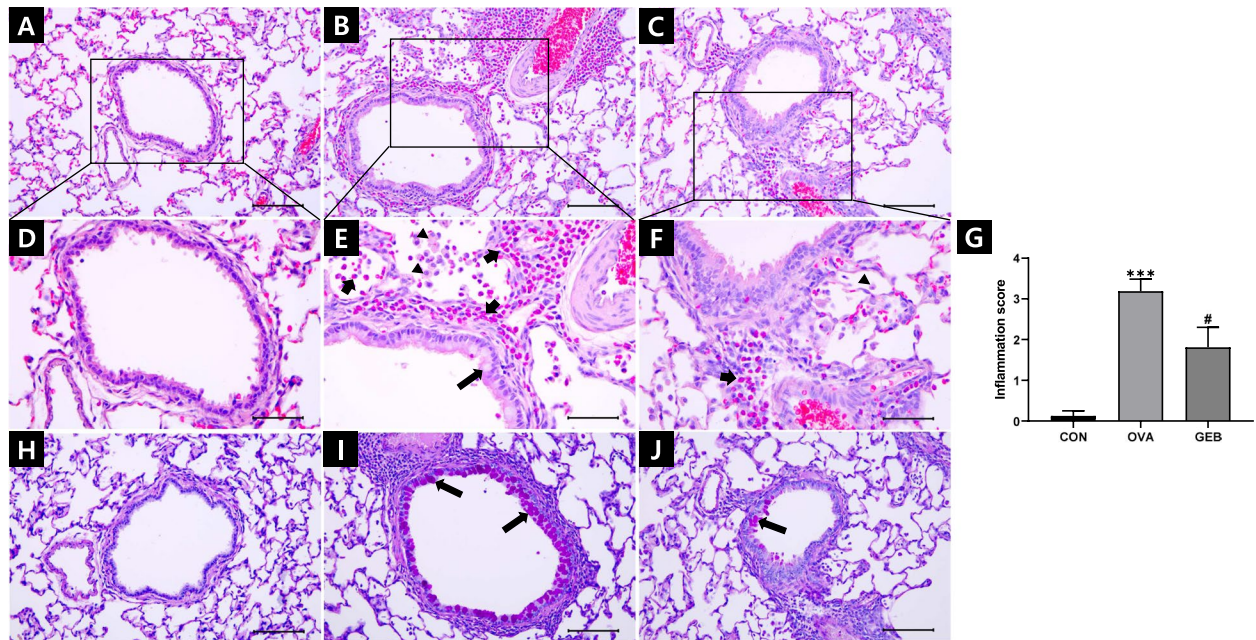


Fig. 5 Histopathological changes in H&E and PAS stained lung. Representative tissue sections of the lung were stained with H&E and PAS. (A–F) H&E, (H–J) PAS, (A, D, H) CON group, (B, E, I) OVA group, (C, F, J) GEB group, (G) Inflammatory cell scoring results. The infiltration of inflammatory cells such as eosinophils in the bronchial, perivascular, and alveolar spaces (arrowheads), goblet cells (long arrows), and alveolar macrophages (short arrows). PAS-positive cells (long arrows). Scale bars in A–C and H–J are 100 μ m, and in D–F, 50 μ m, each. Values were mean $\pm$ SD of 8 rats per group. Compared with the CON group, *** p < 0.001; compared with the OVA group, # p < 0.05

and alveolar walls of the OVA group were thickened, and there was increased infiltration of inflammatory cells in the bronchi, perivascular, and alveolar spaces (Fig. 5B, E). OVA-induced damage was ameliorated by GEB treatment. The GEB treatment reduced inflammatory cell infiltration into blood vessels, bronchioles, and alveolar spaces, and alveolar spaces were maintained. GEB treatment reduced inflammatory cells and structural changes in the alveolar space of the lung and tracheal tissue (Fig. 5C, F). The lung inflammation score was markedly higher in the OVA group compared with the CON group. However, the OVA group had dramatically lower scores than the GEB group (Fig. 5G). As a result of confirming histopathological changes in lung tissue stained with PAS, normal mucus production was not observed in the bronchioles of the CON group, but an increase in mucus production was observed in the bronchioles of the OVA group (Fig. 5H, I). In contrast, bronchioles in the GEB group showed decreased mucus production as inflammation decreased (Fig. 5J).

Effects of GEB on the expression of cytokines, CD206, and MPO in the lung

Immunohistochemical staining was performed to confirm the expression of cytokines and the M2 macrophage marker CD206 in the lung tissue of OVA-induced asthmatic rats. The expression of IL-4 in the OVA group was higher than in the CON group (Fig. 6B). Compared with

the OVA group, the expression in the GEB group was lower (Fig. 6C). IL-5, a key mediator of eosinophil activation, was upregulated in the OVA group compared to the CON group (Fig. 6E). The expression of IL-5 was lower in the GEB group (Fig. 6F). The expression of CD206, an M2 macrophage marker, was increased in the OVA group (Fig. 6H). On the other hand, the expression of CD206 was downregulated in the GEB group (Fig. 6I). The expression of MPO, an enzyme contributing to neutrophil formation, was increased in the OVA group compared to the CON group. This increase was reduced in the GEB group (Fig. 6K, L).

Discussion

Allergic asthma is a chronic respiratory disease characterized by the contraction of bronchial smooth muscles and an increase in inflammatory cells and cytokines due to chronic inflammation triggered by external allergen substances [27, 28]. Inflammation occurs in the body during tissue damage processes such as cell death, cancer, ischemia, and degeneration [29, 30]. It is known that when asthma is induced in rats, inflammatory cells, cytokines, and lung damage increase due to inflammation [7, 31–33]. This study aimed to determine if GEB treatment could reduce inflammatory cells and cytokines, as well as alleviate lung damage in OVA-induced rats.

Eosinophils are known to increase in parasitic infections, autoimmune diseases such as rheumatoid arthritis

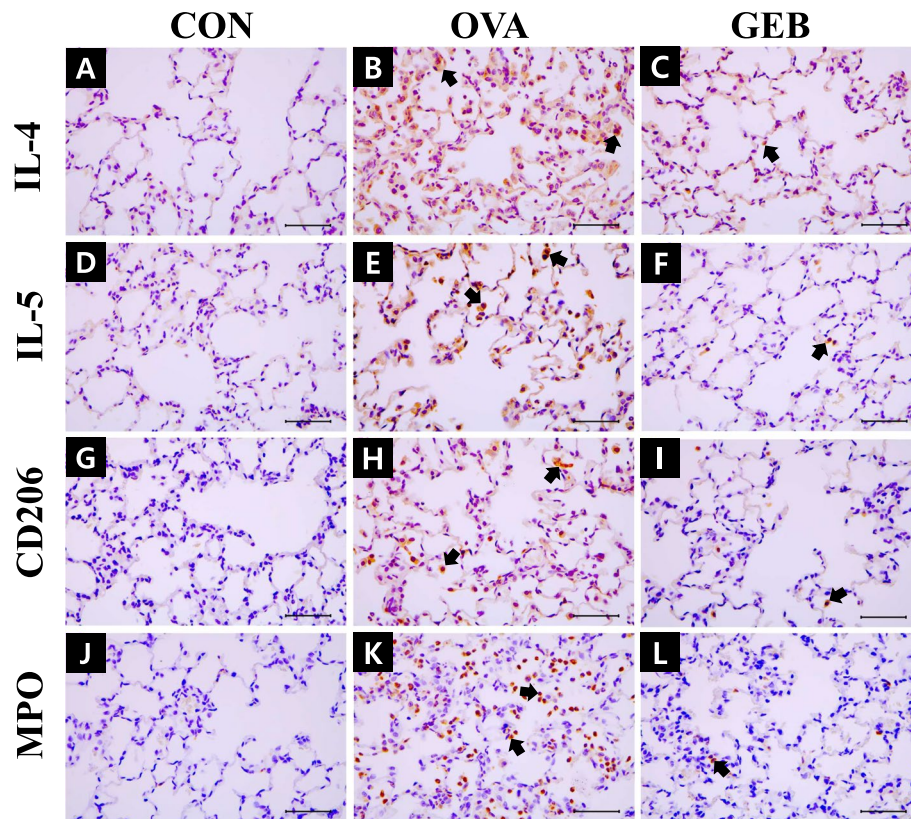


Fig. 6 IL-4, IL-5, CD206, and MPO expression in immunohistochemical staining of the lung. A Representative histopathological section of the lungs was stained by immunohistochemistry. (A–C) IL-4, (D–F) IL-5, (G–I) CD206, (J–L) MPO, (A, D, G, J) CON group, (B, E, H, K) OVA group, (C, F, I, L) GEB group. IL-4, IL-5, CD206, and MPO-positive cells (arrows). Scale bars in 50 μm

and systemic lupus erythematosus, and allergic diseases such as asthma, while neutrophils are known to increase due to allergies or immune reactions [34, 35]. In this experiment, we found dramatic increases in total cell counts, eosinophil counts, and neutrophil counts in the BALF of OVA-induced asthmatic rats. However, the inflammatory response to OVA was mitigated with GEB treatment. In addition, the cytological findings of BALF are consistent with the results of Chen YR et al. [36], who reported an increase in the total cell count, eosinophil count, and neutrophil count in the BALF of mice administered OVA and decreased after treatment. Activation of eosinophils triggers the release of histamine and type 2 cytokines from mast cells, and the released histamine can activate other immune cells, such as neutrophils and alveolar macrophages, promoting inflammation in asthma [37]. It is known that when asthma is induced by OVA, smooth muscle hypertrophy occurs in lung tissue, along with inflammatory cell infiltration around the bronchi, blood vessels, and alveoli [38–40]. In the lung structure of OVA-induced asthmatic rats, numerous inflammatory cells were observed in the bronchi, blood vessels, and alveolar spaces, and inflammatory cells infiltrated around the alveoli, narrowing the alveolar space

and increasing alveolar macrophages. GEB treatment maintained alveolar space by reducing inflammatory cells in the bronchi, blood vessels, and surrounding alveoli, as well as reduced alveolar macrophages. This was consistent with the results of BALF inflammatory cells. In the allergic asthma pathway, airway hyperresponsiveness is closely associated with elevated eosinophil levels in the lungs. The reduction of inflammatory cells in BALF and lung tissue indicates that they play an important role in the treatment of allergen-induced bronchial hyperresponsiveness [19].

A representative disease demonstrating a Th2 dominant response is allergic asthma. When naive T cells are stimulated by a specific allergen, they differentiate into Th2 cells and produce type 2 cytokines IL-4, IL-5, and IL-13, which promote airway eosinophilia, excessive mucus production, bronchial hyperresponsiveness, and IgE synthesis [41, 42].

The generated IgE binds to the high-affinity IgE receptor on the surface of mast cells and degranulates them, and the degranulated cells release histamine and cytokines to induce mucus production and bronchial remodeling [12]. We determined whether treatment with GEB reduces total IgE levels in the serum

of OVA-induced asthma rats. Total IgE levels in the serum of OVA-induced asthmatic rats increased, while GEB treatment decreased total IgE levels. A cytokine closely related to IgE is IL-4, a key cytokine in allergic diseases that mainly acts in the early stages of asthma and promotes the process of synthesizing IgE and replacing macrophages with M2 cells [43, 44]. In addition, studies have reported that blocking the IL-4 pathway can reduce serum total IgE levels in rats [45, 46]. IL-4 levels in lung from OVA-induced asthma rats were significantly increased compared to controls and markedly decreased by GEB treatment. This indicates that GEB suppresses the activation of B cells into plasma cells by reducing the level of IL-4, a type 2 cytokine, which was stimulated by OVA, thereby reducing IgE production and alleviating allergic reactions.

IL-5 is produced by Th2 cells, mast cells, airway smooth muscle, and epithelial cells, and it is associated with increased eosinophils during allergies or parasitic infections [47, 48]. The link between IL-5 and eosinophilic inflammation has been demonstrated in animal and human asthma experiments, where inhibition of IL-5 with monoclonal antibodies reduced eosinophils caused by allergens or chronic diseases [47]. Additionally, when OVA was induced, an IL-5-dependent eosinophil influx was observed in BALF and lung tissue [49]. This study found that treating OVA-infected rats with GEB could significantly reduce eosinophil counts in BALF and lungs by suppressing IL-5 levels. Consistent with our report, Ou G et al. [50] found that the levels of IL-5 and eosinophils were significantly reduced in OVA mice.

IL-13 is primarily involved in the later stages of allergic reactions and is a cytokine secreted by CD4 cells, NK T cells, mast cells, and eosinophils. IL-13 induces tissue remodeling, regulates barrier function, induces differentiation and proliferation of goblet cells, and promotes mucus production [51]. Goblet cell hyperplasia and excessive mucus production in the airway epithelium are characteristic of asthma and obstructive pulmonary disease, primarily due to an increase in eosinophils, which cause tissue damage and inflammation, leading the lungs to secrete mucus for protection [52, 53]. The association of IL-13 with goblet cell production and mucus secretion has already been demonstrated in animal models to influence monocyte infiltration, goblet cell proliferation, acid and neutral mucus overproduction, epithelial airway fibrosis, and crystal precipitation [54]. In chronic asthma, persistent airway inflammation caused by OVA leads to airway remodeling. Our results showed that GEB could significantly reduce the level of IL-13 in the lung. Bronchial epithelial cells serve as an important barrier against invading allergens. These cells secrete mucus, which becomes stuck to dust and is then expelled through the ciliary movement of the respiratory tract [55]. However,

allergens can stimulate an inflammatory response in bronchial epithelial cells, causing them to differentiate into goblet cells, which release large amounts of mucus that block the airways, causing breathing difficulties and even death from suffocation [56]. We confirmed through PAS staining that GEB treatment reduced mucus secretion in OVA-induced asthma rats. Our results showed that GEB could significantly reduce the levels of IL-13 and mucus secretion. This shows that GEB treatment has the potential to improve airway inflammation by regulating airway remodeling through inhibition of the IL-13 pathway.

Alveolar macrophages are divided into M1 cells and M2 cells based on the stimulator. M1 macrophages are predominantly activated in models of acute lung injury and are stimulated by interferon- γ (IFN- γ) and Lipopolysaccharide (LPS) to induce non-allergic immune responses [16, 57]. Meanwhile, the macrophages that are primarily activated in allergic asthma models are M2 macrophages [9]. Alveolar macrophages differentiate into M2 macrophages by IL-4 and IL-13, which induce allergic immune responses and are involved in apoptosis and removal of necrotic tissue [58]. CD206 is a mannose receptor expressed in M2 macrophages and serves as a marker of M2 macrophages [59]. Neutrophils, the first immune cells to respond when inflammation occurs, are a cell type found during inflammation and airway narrowing in asthma. When they accumulate in the lung inflammation site, monocytes from the blood are recruited and differentiate into macrophages [35, 60, 61]. MPO is a heme-containing peroxidase expressed primarily in neutrophils and less frequently in monocytes [62]. In asthma patients, IL-4 and IL-5 are secreted, which generate eosinophils and induce airway inflammation and airway remodeling [63, 64]. Our experiments showed that GEB treatment suppressed the expression of IL-4 and IL-5 and decreased the expression of CD206, a marker of M2 macrophages, and MPO, which is involved in neutrophil production. Therefore, we have confirmed that GEB can improve lung function by protecting against inflammation in asthmatic rats.

Asthma is a lung disease characterized by lung inflammation due to persistent stimulation by allergens. When allergens enter, naive T cells are stimulated to differentiate into Th2 cells and secrete type 2 cytokines. Type 2 cytokines stimulate the production of IgE, an important immunoglobulin in allergic diseases, and M2 macrophages and are involved in airway remodeling, mucus secretion, and inflammatory cell infiltration into the airways. In Our data, GEB reduced the levels of type 2 cytokines, decreased inflammatory cells in the airway and lung, reduced mucus secretion, and suppressed the production of IgE and M2 macrophages (Fig. 7). The main active ingredients in

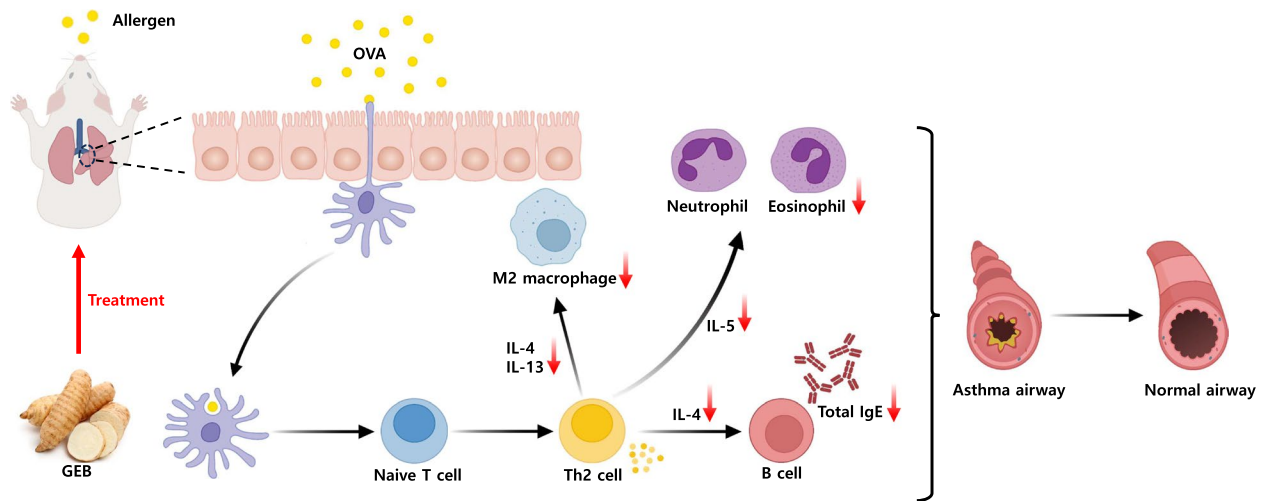


Fig. 7 The suggested mechanisms on the anti-allergic and anti-inflammatory effects of GEB extract

GEB extract are 4-hydroxybenzyl alcohol and gastrodin. Among them, 4-hydroxybenzyl alcohol has been proven to have an anti-allergic effect in a guinea pig asthma model [25], and gastrodin has been proven to have an anti-inflammatory effect in several studies [65–67]. In our study, the GEB extract containing these active ingredients demonstrated anti-allergic and anti-inflammatory effects in asthma. Therefore, GEB extract has been proven to be a natural product that can relieve allergic asthma. However, the current concentration of GEB extract is the maximum dose that can be administered without toxicity, but it is too high to be safely applied to humans. Future research is needed to reduce the total amount of this extract and make it more concentrated so that it can be applied to humans.

Conclusions

We demonstrated that treatment of OVA-induced rats with GEB has the potential to alleviate asthma symptoms, such as airway inflammation and mucus hypersecretion, by reducing type 2 cytokines, IgE, and inflammatory cells. In conclusion, this study shows that GEB is a promising candidate for improving asthma with anti-inflammatory and anti-allergic effects.

Abbreviations

CON	Control
OVA	Ovalbumin
GEB	Gastrodia elata Blume
NK	Natural killer
HPLC-DAD	High-performance liquid chromatography-diode array detector
Alum	Aluminum hydroxide
ELISA	Enzyme-linked immunosorbent assay
IHC	Immunohistochemistry
HRP	Horseradish peroxidase
BW	Body weight
SST	Serum separation tube
BALF	Bronchoalveolar lavage fluid

PBS	Phosphate-buffered saline
i.p.	Intraperitoneal injection
i.n.	Intranasal instillation
Th2	T helper 2
IL-4	Interleukin 4
IL-5	Interleukin 5
IL-13	Interleukin 13
H&E	Hematoxylin and eosin
PAS	Periodic acid-Schiff
DAB	3,3'-Diaminobenzidine tetrahydrochloride
SD	Standard deviation

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42826-025-00252-8>.

Supplementary Material 1.

Acknowledgements

None

Authors' contributions

JSP, YSL, and DEJ participated in the study investigation and data organization. JWS, HJN, and JWH helped with the original draft preparation and visualization. JHS managed this study conceptualization and supervision. All authors have read and agreed to the published version of the manuscript.

Funding

This project was supported by the Rural Development Administration business (Project No. RS-2022-RD009980).

Data availability

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

Declarations

Ethics approval and consent to participate

The experimental procedure was approved by the Institutional Animal Care and Use Committee of Eulji University (EUIACUC 22–11).

Consent for publication

All authors consent for publication.

Competing interests

The authors declare that they have no competing interests.

Received: 31 October 2024 / Revised: 24 June 2025 / Accepted: 7 September 2025

Published online: 16 September 2025

References

- Olin JT, Wechsler ME. Asthma: pathogenesis and novel drugs for treatment. *BMJ*. 2014;349:g5517.
- Zhang X, Xu Z, Wen X, Huang G, Nian S, Li L, et al. The onset, development and pathogenesis of severe neutrophilic asthma. *Immunol Cell Biol*. 2022;100(3):144–59.
- Ferguson JE, Patel SS, Lockey RF. Acute asthma, prognosis, and treatment. *J Allergy Clin Immunol*. 2017;139(2):438–47.
- Park HS, Kim SY, Kim SR, Lee YC. Targeting abnormal airway vascularity as a therapeutic strategy in asthma. *Respirology*. 2010;15(3):459–71.
- Mims JW. Asthma: definitions and pathophysiology. *Int Forum Allergy Rhinol*. 2015;5(Suppl 1):S2–6.
- Krusche J, Basse S, Schaub B. Role of early life immune regulation in asthma development. *Semin Immunopathol*. 2020;42(1):29–42.
- Akar-Ghbiril N, Casale T, Custovic A, Phipatanakul W. Allergic endotypes and phenotypes of asthma. *J Allergy Clin Immunol Pract*. 2020;8(2):429–40.
- Kobayashi T, Iijima K, Dent AL, Kita H. Follicular helper T cells mediate IgE antibody response to airborne allergens. *J Allergy Clin Immunol*. 2017;139(1):300–13 e7.
- Abdelaziz MH, Abdelwahab SF, Wan J, Cai W, Huixuan W, Jianjun C, et al. Alternatively activated macrophages; a double-edged sword in allergic asthma. *J Transl Med*. 2020;18(1):58.
- Aun MV, Bonamichi-Santos R, Arantes-Costa FM, Kalil J, Giavina-Bianchi P. Animal models of asthma: utility and limitations. *J Asthma Allergy*. 2017;10:293–301.
- Thakur VR, Khuman V, Beladiya JV, Chaudagar KK, Mehta AA. An experimental model of asthma in rats using ovalbumin and lipopolysaccharide allergens. *Heliyon*. 2019;5(11):e02864.
- Suraya R, Nagano T, Katsurada M, Sekiya R, Kobayashi K, Nishimura Y. Molecular mechanism of asthma and its novel molecular target therapeutic agent. *Respir Investig*. 2021;59(3):291–301.
- Habib N, Pasha MA, Tang DD. Current understanding of asthma pathogenesis and biomarkers. *Cells*. 2022. <https://doi.org/10.3390/cells11172764>.
- Gour N, Wills-Karp M. IL-4 and IL-13 signaling in allergic airway disease. *Cytokine*. 2015;75(1):68–78.
- Adachi T, Alam R. The mechanism of IL-5 signal transduction. *Am J Physiol*. 1998;275(3):C623–33.
- Jiang Z, Zhu L. Update on the role of alternatively activated macrophages in asthma. *J Asthma Allergy*. 2016;9:101–7.
- Bleecker ER, Menzies-Gow AN, Price DB, Bourdin A, Sweet S, Martin AL, et al. Systematic literature review of systemic corticosteroid use for asthma management. *Am J Respir Crit Care Med*. 2020;201(3):276–93.
- Heffler E, Madeira LNG, Ferrando M, Puggioni F, Racca F, Malvezzi L, et al. Inhaled corticosteroids safety and adverse effects in patients with asthma. *J Allergy Clin Immunol Pract*. 2018;6(3):776–81.
- Arora P, Ansari SH, Anjum V, Mathur R, Ahmad S. Investigation of anti-asthmatic potential of Kanakasa in ovalbumin-induced bronchial asthma and airway inflammation in rats. *J Ethnopharmacol*. 2017;197:242–9.
- Abdo W, Haziri I, Dmerdash M, Alnasser SM, Hakamy A, Ali E, et al. Anatabine attenuates ovalbumin-induced asthma via oxidative stress and inflammation mitigation and Nrf2/HO-1 signaling upregulation in rats. *Life Sci*. 2022;308:120954.
- Rouibah Z, Ben Mensour A, Rekik O, Boumendjel M, Taïbi F, Bouaziz M, et al. Chemical composition, antioxidant activities, in an allergic asthma model, of *Olea europaea* L. leaf extracts from Collo (Skikda, Algeria). *Drug Chem Toxicol*. 2022;45(1):197–208.
- Zhu H, Liu C, Hou J, Long H, Wang B, Guo D, et al. *Gastrodia elata* Blume polysaccharides: a review of their acquisition, analysis, modification, and pharmacological activities. *Molecules*. 2019. <https://doi.org/10.3390/molecules24132436>.
- Jang JH, Son Y, Kang SS, Bae CS, Kim JC, Kim SH, et al. Neuropharmacological Potential of *Gastrodia elata* Blume and Its Components. *Evid Based Complement Alternat Med*. 2015;2015:309261.
- Ahn EK, Jeon HJ, Lim EJ, Jung HJ, Park EH. Anti-inflammatory and anti-angiogenic activities of *Gastrodia elata* Blume. *J Ethnopharmacol*. 2007;110(3):476–82.
- Jang YW, Lee JY, Kim CJ. Anti-asthmatic activity of phenolic compounds from the roots of *Gastrodia elata* Bl. *Int Immunopharmacol*. 2010;10(2):147–54.
- Zeng Z, Lin X, Zheng R, Zhang H, Zhang W. Celastrol alleviates airway hyper-responsiveness and inhibits Th17 responses in obese asthmatic mice. *Front Pharmacol*. 2018;9:49.
- Gans MD, Gavrilova T. Understanding the immunology of asthma: pathophysiology, biomarkers, and treatments for asthma endotypes. *Paediatr Respir Rev*. 2020;36:118–27.
- Finotto S. Resolution of allergic asthma. *Semin Immunopathol*. 2019;41(6):665–74.
- Azab A, Nassar A, Azab AN. Anti-inflammatory activity of natural products. *Molecules*. 2016. <https://doi.org/10.3390/molecules21101321>.
- Arulselvan P, Fard MT, Tan WS, Gothai S, Fakurazi S, Norhaizan ME, et al. Role of Antioxidants and Natural Products in Inflammation. *Oxid Med Cell Longev*. 2016;2016:5276130.
- Wang CN, Lee YL, Lin YP, Chung WH, Tzeng YM, Lee CC. Ovatodiolide suppresses allergic airway inflammation and hyperresponsiveness in a murine model of asthma. *Eur J Pharmacol*. 2017;812:9–17.
- Schatz M, Rosenwasser L. The allergic asthma phenotype. *J Allergy Clin Immunol Pract*. 2014;2(6):645–8; quiz 9.
- Nguyen V, Zhang Q, Pan F, Jin Q, Sun M, Tangthianachana J, et al. Zi-Su-Zi decoction improves airway hyperresponsiveness in cough-variant asthma rat model through PI3K/AKT1/mTOR, JAK2/STAT3 and HIF-1 α /NF- κ B signaling pathways. *J Ethnopharmacol*. 2023;314:116637.
- Yamauchi K, Sasaki N, Niisato M, Kamataki A, Shikanai T, Nakamura Y, et al. Analysis of pulmonary allergic vasculitis with eosinophil infiltration in asthma model of mice. *Exp Lung Res*. 2010;36(4):227–36.
- Poto R, Shamji M, Marone G, Durham SR, Scadding GW, Varricchi G. Neutrophil extracellular traps in asthma: friends or foes? *Cells*. 2022. <https://doi.org/10.3390/cells11213521>.
- Chen YR, Xiang XD, Sun F, Xiao BW, Yan MY, Peng B, et al. Simvastatin Reduces NETosis to Attenuate Severe Asthma by Inhibiting PAD4 Expression. *Oxid Med Cell Longev*. 2023;2023:1493684.
- Van Hulst G, Batugedara HM, Jorssen J, Louis R, Bureau F, Desmet CJ. Eosinophil diversity in asthma. *Biochem Pharmacol*. 2020;179:113963.
- Liang S, Zhao Y, Chen G, Wang C. Isoorientin ameliorates OVA-induced asthma in a murine model of asthma. *Exp Biol Med (Maywood)*. 2022;247(16):1479–88.
- Qian J, Ma X, Xun Y, Pan L. Protective effect of forsythiaside A on OVA-induced asthma in mice. *Eur J Pharmacol*. 2017;812:250–5.
- Li Y, Yan B, Wu Y, Peng Q, Wei Y, Chen Y, et al. Ferroptosis participates in dibutyl phthalate-aggravated allergic asthma in ovalbumin-sensitized mice. *Ecotoxicol Environ Saf*. 2023;256:114848.
- Lambrechts BN, Hammad H, Fahy JV. The cytokines of asthma. *Immunity*. 2019;50(4):975–91.
- Barnes PJ. Targeting cytokines to treat asthma and chronic obstructive pulmonary disease. *Nat Rev Immunol*. 2018;18(7):454–66.
- Ling XJ, Wei JF, Zhu Y. Aiming to IgE: drug development in allergic diseases. *Int Immunopharmacol*. 2023;121:110495.
- Zhang N, Xu J, Jiang C, Lu S. Neuro-Immune Regulation in Inflammation and Airway Remodeling of Allergic Asthma. *Front Immunol*. 2022;13:894047.
- Massey O, Suphioglu C. Recent advances in the inhibition of the IL-4 cytokine pathway for the treatment of allergen-induced asthma. *Int J Mol Sci*. 2021. <https://doi.org/10.3390/ijms222413655>.
- Nur Husna SM, Md Shukri N, Mohd Ashari NS, Wong KK. IL-4/IL-13 axis as therapeutic targets in allergic rhinitis and asthma. *PeerJ*. 2022;10:e13444.
- Greenfeder S, Umland SP, Cuss FM, Chapman RW, Egan RW. Th2 cytokines and asthma. The role of interleukin-5 in allergic eosinophilic disease. *Respir Res*. 2001;2(2):71–9.
- Nagase H, Ueki S, Fujieda S. The roles of IL-5 and anti-IL-5 treatment in eosinophilic diseases: asthma, eosinophilic granulomatosis with polyangiitis, and eosinophilic chronic rhinosinusitis. *Allergol Int*. 2020;69(2):178–86.
- Kung TT, Jones H, Adams GK 3rd, Umland SP, Kreutner W, Egan RW, et al. Characterization of a murine model of allergic pulmonary inflammation. *Int Arch Allergy Immunol*. 1994;105(1):83–90.
- Ou G, Liu Q, Yu C, Chen X, Zhang W, Chen Y, et al. The Protective Effects of Maresin 1 in the OVA-Induced Asthma Mouse Model. *Mediators Inflamm*. 2021;2021:4131420.

51. Rael EL, Lockey RF. Interleukin-13 signaling and its role in asthma. *World Allergy Organ J.* 2011;4(3):54–64.
52. Fahy JV. Goblet cell and mucin gene abnormalities in asthma. *Chest.* 2002;122(6 Suppl):320S–S326.
53. Tan D, Lu M, Cai Y, Qi W, Wu F, Bao H, et al. Sumoylation of Rho-associated protein kinase 2 induces goblet cell metaplasia in allergic airways. *Nat Commun.* 2023;14(1):3887.
54. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest.* 1999;103(6):779–88.
55. Goleva E, Berdyshev E, Leung DY. Epithelial barrier repair and prevention of allergy. *J Clin Invest.* 2019;129(4):1463–74.
56. Barrett NA, Shalek AK. Revisiting airway epithelial remodeling in type 2 immunity: Beyond goblet cell metaplasia. *J Allergy Clin Immunol.* 2019;144(5):1158–60.
57. Kadamoto S, Izumi K, Mizokami A. Macrophage polarity and disease control. *Int J Mol Sci.* 2021. <https://doi.org/10.3390/ijms23010144>.
58. Saradna A, Do DC, Kumar S, Fu QL, Gao P. Macrophage polarization and allergic asthma. *Transl Res.* 2018;191:1–14.
59. Tsuchiya K, Suzuki Y, Yoshimura K, Yasui H, Karayama M, Hozumi H, et al. Macrophage mannose receptor CD206 predicts prognosis in community-acquired pneumonia. *Sci Rep.* 2019;9(1):18750.
60. Jia M, Fu H, Jiang X, Wang L, Xu J, Barnes PJ, et al. DEL-1, as an anti-neutrophil transepithelial migration molecule, inhibits airway neutrophilic inflammation in asthma. *Allergy.* 2024;79(5):1180–94.
61. Zhang B, Zeng M, Zhang Q, Wang R, Jia J, Cao B, et al. *Ephedrae herba* polysaccharides inhibit the inflammation of ovalbumin induced asthma by regulating Th1/Th2 and Th17/Treg cell immune imbalance. *Mol Immunol.* 2022;152:14–26.
62. Lin W, Chen H, Chen X, Guo C. The roles of neutrophil-derived myeloperoxidase (MPO) in diseases: the new progress. *Antioxidants.* 2024;13(1):132.
63. Boonpiyathad T, Sozener ZC, Satitsuksanoa P, Akdis CA. Immunologic mechanisms in asthma. *Semin Immunol.* 2019;46:101333.
64. Galvao J, Cavalcante-Silva LHA, de Almeida LE, Carvalho DCM, Alves AF, Mascarenhas SR. Ouabain modulates airway remodeling caused by Th2-high asthma in mice. *Int Immunopharmacol.* 2022;109:108808.
65. Hu Y, Li M, Chen XY, Huang CH, Cao HY, Wang GJ, et al. Gastrodin Ameliorates the Inflammation, Oxidative Stress, and Extracellular Matrix Degradation of IL-1beta-Mediated Fibroblast-Like Synoviocytes via Suppressing the Gremelin-1/NF-kappaB Pathway. *Discov Med.* 2024;36(186):1441–52.
66. Xue X, Li F, Xu M, Chen B, Zhao Y, Wang M, et al. Gastrodin ameliorates atherosclerosis by inhibiting foam cells formation and inflammation through down-regulating NF-kappaB pathway. *Nutr Metab (Lond).* 2023;20(1):9.
67. Qu LL, Yu B, Li Z, Jiang WX, Jiang JD, Kong WJ. Gastrodin ameliorates oxidative stress and proinflammatory response in nonalcoholic fatty liver disease through the AMPK/Nrf2 pathway. *Phytother Res.* 2016;30(3):402–11.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.