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Trigonella foenum-graecum alleviates airway inflammation of allergic asthma in ovalbumin-induced mouse model

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ABSTRACT

Trigonella foenum-graecum, a member oldest medicinal plant in the fabaceae (legumes) family, is used as a herb, spice, and vegetable, and known for its olfactory, laxative, and galactagogue effects. However, the inhibitory effect of *Trigonella foenum-graecum* on allergic inflammatory response remains unclear, therefore, we investigated the precise role of *Trigonella foenum-graecum* in the allergic asthma and revealed the effects of *Trigonella foenum-graecum* in regulating airway inflammation and its possible mechanism. Allergic asthma was initiated in BALB/c mice by sensitized with OVA emulsified in aluminum on days 1 and 14, then aerosol challenged with OVA on days 27, 28 and 29. Some mice were administered *Trigonella foenum-graecum* by oral gavage before challenge. Then mice were evaluated for the presence of airway inflammation, production of allergen-specific cytokine response and lung pathology. *Trigonella foenum-graecum* significantly ameliorated the number of inflammatory cells in BALF and alleviated lung inflammation. It also reduced the collagen deposition and goblet cells. Meanwhile, *Trigonella foenum-graecum* treatment evidently decreased the high expression of Th2 cytokines and increased the Th1 cytokines in BALF and lung homogenates. *Trigonella foenum-graecum* showed a significant inhibition of serum IgE and anti-OVA IgG₁. In this study, our data suggest that *Trigonella foenum-graecum* has a significant anti-inflammatory effect and it may prove to be an efficacious therapeutic reagent on allergic asthma.

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1. Introduction

Allergic asthma is a serious allergic disease that is characterized by airway inflammation [1]. The pathological changes in asthma include airway obstruction, mucus hyperproduction, and increased infiltration of inflammatory cells, including eosinophils, mast cells, and lymphocytes [2]. As we all known, asthma is a multifactorial and complex disease in which the repetition of allergen challenges leads to airway remodeling eventually. The resulting airway inflammation is thought to be caused by a breakdown of immune tolerance toward environmental antigens and leads to a T helper

type 2 (Th2)- biased immune response [3]. Th2 cytokines can also lead to the production of allergen-specific immunoglobulin (Ig)E antibodies by B cells [4]. Activated mast cells release inflammatory mediators, such as interleukin (IL)-1 β and tumor necrosis factor (TNF)- α . The structural changes of the airway wall include epithelial denudation, subepithelial fibrosis, mucus gland hypertrophy, myofibroblast and smooth muscle proliferation, and angiogenesis [2,3]. *Trigonella foenum-graecum* (Fenugreek) is a legume crop that is used as a spice in cooking and in small quantities is categorized as “Generally Recognized as Safe” by the U.S. Food and Drug Administration [5]. Historically, *Trigonella foenum-graecum* is one of the oldest medicinal plants, known for its olfactory, laxative, and galactagogue effects. More recently, immunostimulatory, anti-diabetic, anti-hypertensive and cholesterol-lowering activities have been demonstrated [6]. Due to its amazing therapeutic and medical properties it is one of the oldest medicinal plants known

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and has long been recognized as a traditional medicine in different parts of the world and in Egypt as well [5].

Traditional treatments for asthma include inhaled corticosteroids (ICS), short-acting β_2 -agonist (SABA), leukotriene receptor antagonists (LTRAs), and long-acting β_2 -agonist (LABA) [7,8]. However, asthma attacks and exacerbations can still occur, as the treatment does not alter the underlying pathology [9]. Unfortunately, prolonged use of drug therapy can cause local and systemic side effects, including oral candidiasis, dysphonia, growth failure, accelerated loss of bone mass, elevated intraocular pressure, and mild tachyphylaxis [10]. Therefore, need to develop the novel treatment for asthma with fewer side effects, and the *Trigonella foenum-graecum* which is natural products is a goal for new therapies. However, its effect on allergic asthma has been still rarely studied, and need to be verified.

In the present study, we aimed to investigate the effects of *Trigonella foenum-graecum* on airway inflammation of allergic asthma in OVA-induced mouse model and to reveal its mechanism. *Trigonella foenum-graecum* treatment of group attenuated airway inflammation by decreased the inflammatory cell numbers and Th2 cytokines. Furthermore, *Trigonella foenum-graecum* ameliorates the OVA-induced allergic asthma in mice.

2. Materials and methods

2.1. Animals and experimental protocol

Specific pathogen-free male 5-week-old BALB/c mice, weighing approximately 20 g, were purchased from Damool Science (Daejeon, Korea). They were housed 5 per cage in a laminar air-flow cabinet maintained at $23 \pm 2^\circ\text{C}$ at a relative humidity of $55 \pm 10\%$ throughout the study. All animal experiments were performed in compliance with NIH guideline for the care and use of laboratory animals and were approved by the Animal Research Committee of Chonbuk National University.

In this study, the mice were divided randomly into four groups ($n = 5$ per group), namely control group, ovalbumin (OVA) group, *Trigonella foenum-graecum* (Fenugreek) group and dexamethasone (Dex) group. First sensitization was performed on day 1 by intraperitoneal injection of $50\ \mu\text{g}$ of OVA (Grade V, Sigma, St. Louis, MO, USA) emulsified in 1 mg of alum (Imject Alum; Pierce, Rockford, IL, USA) in a total volume of $200\ \mu\text{L}$. Control group received saline only (NACL 0.9%; B. Braun Medical BV, Oss, The Netherlands). On day 14, second sensitization was performed by intraperitoneal injection of $50\ \mu\text{g}$ of OVA in saline. From days 15–26, Mice of Fenugreek group and Dex group were received *Trigonella foenum-graecum* (20 mg/mL) and Dex (2.5 mg/mL) by oral gavage. Mice of control group and OVA group were sham received saline. On days 27, 28 and 29, the OVA group, Dex group and *Trigonella foenum-graecum* group were challenged with inhalation of 5% OVA solution in saline, for 20 min by ultrasonic nebulization. Mice of control group were sham challenged with saline. Mice were sacrificed 24 h after the last OVA exposure for airway inflammation (Fig. 1).

2.2. Bronchoalveolar lavage fluid (BALF) leukocytes count

After sacrifice, 24 h after the last OVA challenge, blood was taken from the eye of the mice. Then serum samples were collected after centrifugation ($1000 \times g$, 10 min, 4°C) and stored at -80°C until analysis. The airway lumina were washed through a tracheal cannula with 1 mL volumes of PBS. This was repeated 2 times with 1 mL PBS only. The (BALF) was centrifuged and the supernatants of BALF were (stored at -80°C) subjected to ELISA assay with kits. The cell pellets were resuspended in 1 mL PBS. The total BALF cells were counted double-blindly using a hemocytometer. Total cell numbers

were conducted using trypan blue staining and standard with light microscopy. Cytospin cell preparations were made by BALF onto clean glass slides for 10 min ($150\ \mu\text{L}$, 4°C), and staining with Diff-Quik (1-5-1 Wakonohama-Kaigandori, Chuo-Ku, Kobe Japan). Differential cell numbers of eosinophils, macrophages, neutrophils, and lymphocytes were counted by light microscopy.

2.3. Lung histology analysis

After the final OVA challenge, the mice were sacrificed, then, performing BAL fluid and the lung lobes were removed for histological examination, lungs were fixed in 10% formalin, and embedded in paraffin, sections of $5\ \mu\text{m}$ thickness were deparaffinized and hydrated, and stained with hematoxylin and eosin (H&E), congo red, periodic acid Schiff (PAS), and Masson's Trichrome staining.

2.4. Cytokine levels

Quantitative assessments of IL-5, IL-6, IL-10, IL-1 β , TNF- α and INF- γ in BAL fluid and lung tissue homogenization were conducted enzyme-linked immunosorbent assay kits (R&D Systems Inc. USA) according to the manufacturer's protocol. Lung tissues were homogenized in a saline to concentration of 100 mg/mL with a complete Mini, EDTA-Free Protease Inhibitor (Roche Applied Science), and debris-free supernatant was used for cytokine measurement.

2.5. Measurement of total IgE and anti-OVA IgG1

After the final challenge, the blood was collected and centrifuged. The serum was then separated and concentrations of total IgE and anti-OVA IgG1 levels were measured in each group using ELISA kits.

2.6. Statistical analysis

Each experiment was repeated three times with $n = 5$ animals per group. Data are expressed as mean $\pm$ SEMs. Statistical comparisons were performed using one-way ANOVA, followed by the Fisher test. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Effect of *Trigonella foenum-graecum* on airway inflammation in BALF

To examine the effects of *Trigonella foenum-graecum* on asthma model of mice, total cells and differential cells, as well as numbers of macrophages, lymphocytes, neutrophils and eosinophils in BALF were counted. OVA challenged group induced significantly increased total cell numbers and eosinophils levels than control group (Fig. 2B). However, treatment with *Trigonella foenum-graecum* (Fenugreek) group markedly depressed the inflammatory cells in BAL fluid exerted similar suppressive effects with positive group of DEX (Fig. 2B). As showed in cytospin cell preparations, total cells and inflammation cells number were markedly increased in OVA group than control group. However, *Trigonella foenum-graecum* (Fenugreek) group and Dex group showed little cells were determined and the cells were mostly macrophages (Fig. 2A).

3.2. Effect of *Trigonella foenum-graecum* on airway inflammation in lung tissues

To evaluate the histological changes after *Trigonella foenum-graecum* treatment for OVA-induced asthma model, the level of

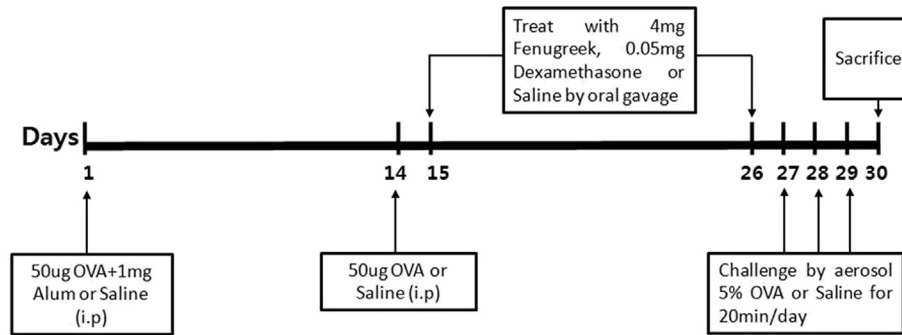


Fig. 1. Experimental protocol for allergic asthma mouse model. Mice were sensitized intraperitoneally (i.p) on days 1 and 14. *Trigonella foenum-graecum* (Fenugreek) and Dex were administrated by oral gavage on days 15–26. From days 27–29, mice were challenged by aerosol with OVA. Mice of control group were sham treated with saline. Mice were sacrificed 24 h after the last challenge for further analysis.

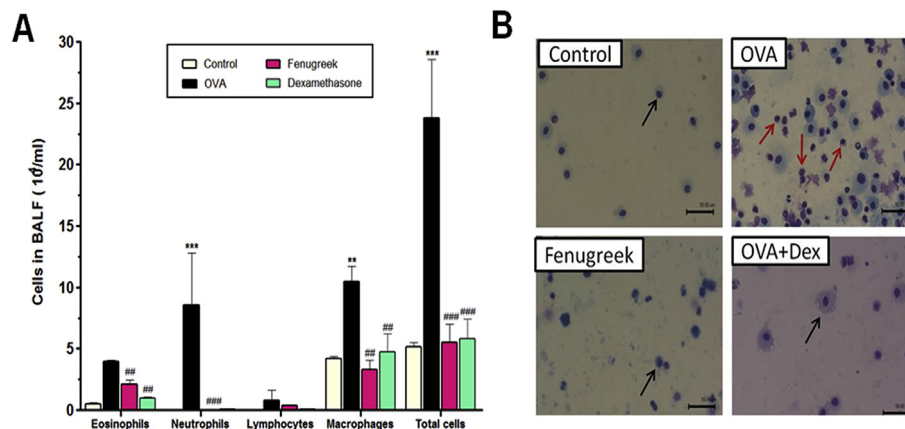


Fig. 2. Effect of *Trigonella foenum-graecum* (Fenugreek) on (A) total and differential cell numbers and (B) infiltrations of inflammatory cells in BALF. (A) Total and differential cell numbers within a hemocytometer were counted using a light microscope. Each bar represents the mean $\pm$ SEM of 5 mice. *** $p < 0.001$, ** $p < 0.01$, significantly different from the value of control group. ### $p < 0.001$, ## $p < 0.01$, significantly different from the value of OVA group. (B) Cytospin cell preparations were made by BALF onto clean glass slides and staining with Diff-Quik ($\times 200$ original magnification). The increased eosinophils (red arrows) and macrophages (black arrows) were observed. Scale bar = 100 μ m. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

general pathological alteration, eosinophils infiltration, mucus production, goblet cell hyperplasia, and bronchial subepithelial fibrosis were examined in lung tissue. We observed severe infiltration of inflammatory cells around the respiratory tract and blood vessel in OVA group (Fig. 3). Mice of *Trigonella foenum-graecum* (Fenugreek) group and Dex group showed no or little inflammation cells around the airway. Mucus of overproduction and goblet cell hyperplasia were observed in the airway wall (bronchi) and eosinophils infiltration was increased in peribronchial in OVA group compared with the control group. However, *Trigonella foenum-graecum* (Fenugreek) treatment group showed significant reduction of goblet cell and eosinophil numbers (Fig. 3B and C). Masson's-Trichrome staining revealed prominent fibrotic deposit under bronchial epithelia and around the blood vessel in the OVA group. In contrast, treatment with *Trigonella foenum-graecum* and Dex mice displayed reduction of collagen deposition (Fig. 3D).

3.3. Effects of *Trigonella foenum-graecum* on the production of cytokines in BALF and homogenized lung tissues

Following sensitization and challenges, the levels of IL-5, TNF- α , INF- γ , IL-1 β , IL-6 and IL-10 in lung homogenates and secreted TNF- α , INF- γ and IL-10 in BALF were quantified from OVA-induced allergic mice were determined by ELISA. The levels of Th2 cytokines, including IL-5 and IL-6, inflammatory cytokines IL-1 β and

TNF- α were highly augmented in OVA group. However, the administration of *Trigonella foenum-graecum* (Fenugreek) group reduced the levels of all 4 cytokines. The levels of INF- γ and IL-10 both in BALF and lung homogenates were up-regulated by *Trigonella foenum-graecum* treatment (Fig. 4). Therefore, the results showed that *Trigonella foenum-graecum* suppressed the airway inflammation by down-regulated the inflammatory cytokines.

3.4. Effect of *Trigonella foenum-graecum* with IgE and anti-ova-IgG1 levels in serum

In order to examine whether the *Trigonella foenum-graecum* effected the IgE and anti-OVA IgG1 production in allergic mouse model, the serum concentrations of IgE and anti-OVA IgG1 were measured in each group using ELISA (Fig. 4). *Trigonella foenum-graecum* (Fenugreek) group reduced the both of serum IgE and anti-OVA IgG1.

4. Discussion

In the present study, we investigate the effect of *Trigonella foenum-graecum* (Fenugreek) in OVA-induced allergic asthma mouse model. Our results demonstrated that *Trigonella foenum-graecum* has anti-inflammatory effects in allergic asthma resulting from the down regulating the inflammatory cells, adjustment the balance of

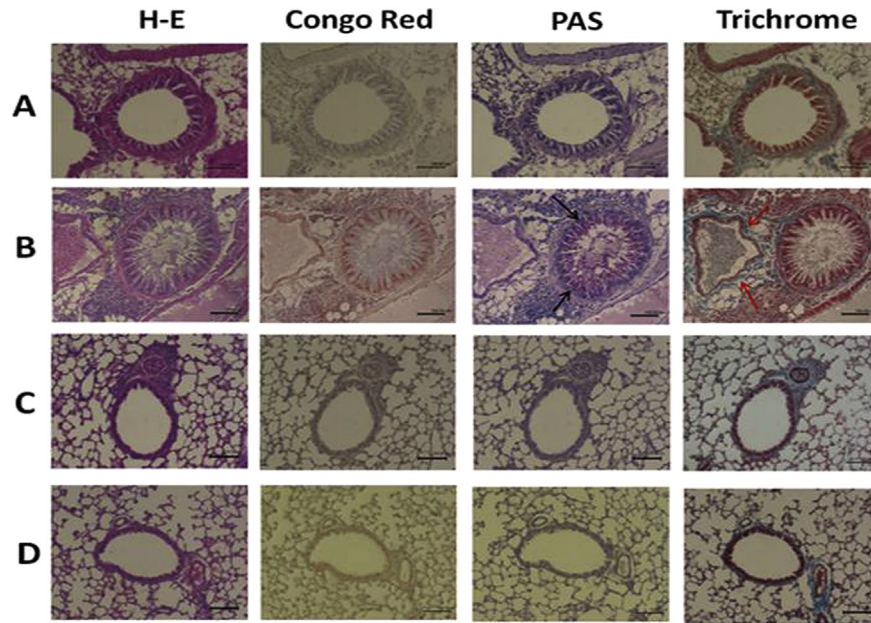


Fig. 3. Effect of *Trigonella foenum-graecum* (Fenugreek) on allergic airway inflammation in lung tissues. Lung tissues were stained with hematoxylin and eosin (H–E), periodic acid Schiff (PAS), congo red and Masson's-Trichrome staining ($\times 200$ original magnification). Overproduction of mucus and goblet cell hyperplasia (black arrows) and prominent fibrotic deposit (red arrows) were revealed. (A) Control group, (B) OVA group, (C) Fenugreek group, (D) Dex group. Scale bar = 100 μm . (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

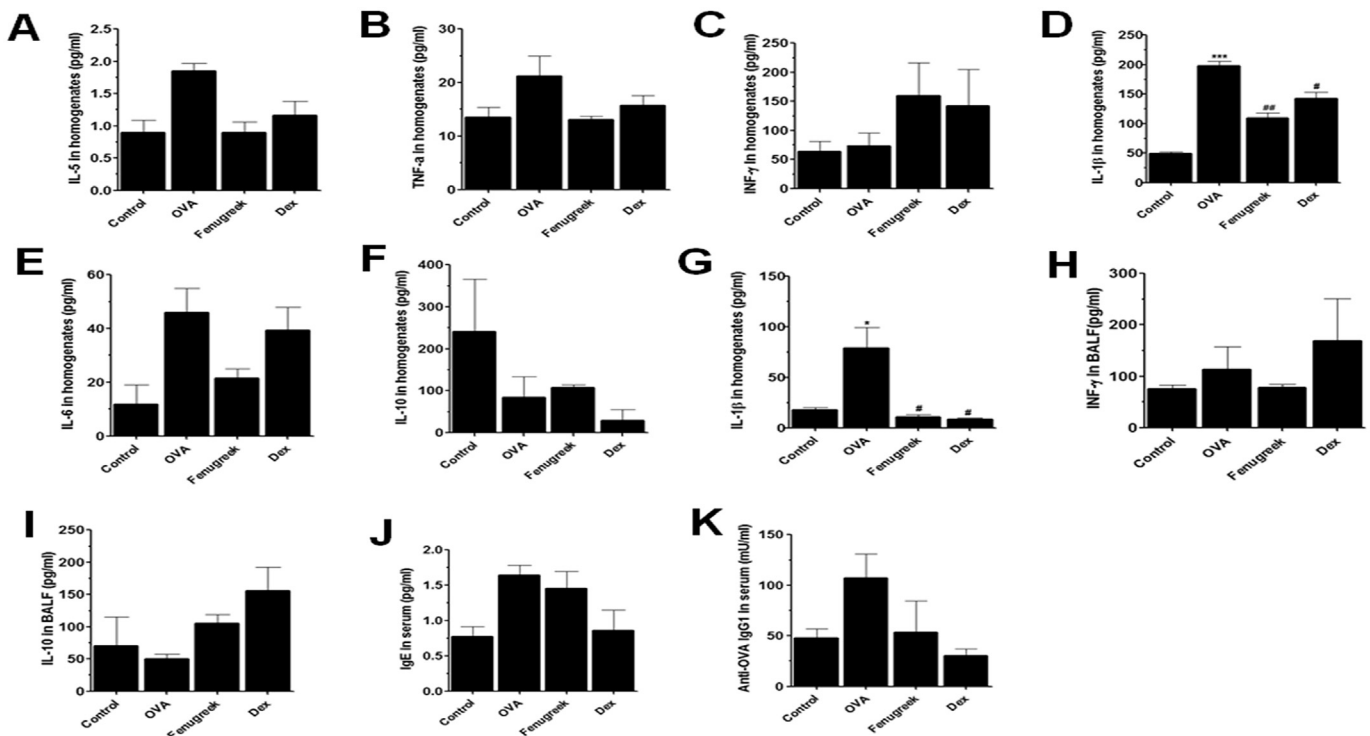


Fig. 4. Production of cytokines in BALF and lung homogenates. The levels of (A) IL-5, (B) TNF- α , (C) INF- γ , (D) IL-1 β , (E) IL-6 and (F) IL-10 were determined in lung homogenates. Secreted (G) TNF- α , (H) INF- γ and (I) IL-10 in BALF were quantified from OVA-induced allergic mice. The measurement of IgE (J) and anti-OVA IgG1 (K) in serum. Each bar represents the mean $\pm$ SEM of 5 mice. * $p < 0.05$, significantly different from the value of control group. ** $p < 0.01$, # $p < 0.05$, significantly different from the value of OVA group.

the Th1/Th2 cytokines and decreased the serum concentration of IgE and anti-OVA IgG₁.

As we all known, asthma is one of the most common chronic diseases worldwide, characteristic of airway inflammation and

airway remodeling [11]. The airway inflammation in this model is characterized by excessive airway eosinophil and other inflammatory cell infiltration, goblet cell hyperplasia with excessive airway mucus and elevated IgE and Th2 cytokines [12–14].

A central mediator of asthma is the IgE antibody, which is produced by sensitized allergen-specific B cells [15]. Allergens increase IgE levels in the serum of susceptible subjects subsequent to stimulation [15]. IgE antibodies then bind to the high-affinity IgE receptor, Fc epsilon receptor 1 (FcεRI), present in mast cells, eosinophils, and basophils, thereby sensitizing these cells to allergen exposure [15]. In our study, *Trigonella foenum-graecum* treatment significantly blocked production of IgE and anti-OVA-IgG1 in serum (Fig. 3).

Mast cells are widely distributed, especially in connective tissue and mucosal surfaces, and provide a useful and powerful tool for studying both stimulus-secretion coupling and allergic disorders [16]. Mast cells are responsible for a variety of allergic disorders including allergic rhinitis, dermatitis, asthma, and food allergies, as well as catastrophic anaphylactic reactions to insect stings and some drugs [16]. Compound 48/80 induces signal transduction pathways, which leads to exocytosis, and degranulation of mast cells causing histamine release [17].

In recent years, Th2 cells have been known as the dominant modulators in regulated downstream cell differentiation and proliferation including B cells class switch to secreting IgE, IgG1 and eosinophils, and attract a large number of inflammatory cell infiltrations in the lungs [18]. There is a close relationship between the IgE-mediated immune response and the elevated Th2 cytokines in allergic asthma [19]. To demonstrate the mechanism of effect of *Trigonella foenum-graecum* on the inflammation we examine the cytokines levels and inflammatory cells in allergic asthma model. In this study we found to OVA significantly enhanced the level of Th2 cytokines, including IL-5, IL-6, IL-1β and TNF-α (Fig. 4), and increased inflammatory cell infiltration such as eosinophils and macrophages (Figs. 2,3). *Trigonella foenum-graecum* treatment evidently lowered Th2 cytokines and inflammatory cells especially eosinophils in BALF and lung homogenates (Fig. 3). Therefore *Trigonella foenum-graecum* is effective as Dex in inhibiting the allergic inflammation. Several studies have shown that modulation of Th1/Th2 cytokines can inhibit airway inflammation in OVA-induced mice [20,21]. Our data demonstrate that *Trigonella foenum-graecum* group up-regulated the Th1 cytokines, including INF-γ and IL-10 (Fig. 3). These findings effectively proved that the *Trigonella foenum-graecum* regulated the balance of Th cells. We guess the *Trigonella foenum-graecum* attenuated the expression of Th2 cytokines may lead to anti-inflammatory effect.

Prominent characteristics of allergic asthma are overproduction of antigen-specific IgE, extreme immunological responses, and consequent inflammation and clinical symptoms include excessive mucus secretion and bronchospasm [19]. Treatment with *Trigonella foenum-graecum* significantly suppressed the deposition of collagen within the epithelium of lung tissue and reduced the mucus producing goblet cells and eosinophils around the airway (Fig. 3). So *Trigonella foenum-graecum* has inhibitory effect on airway inflammation.

Natural products contribute new strategy to novel drug development efforts and have therefore played a significant role in drug discovery and development for treatment of some inflammatory diseases [19].

In conclusion, our data have provide evidence that treatment with *Trigonella foenum-graecum* has a significant anti-inflammatory effect in allergic asthma and may prove to be an efficacious therapeutic reagent on allergic asthma by suppressing proinflammatory cytokines and inflammatory cell infiltration.

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