



Disponible en ligne sur

ScienceDirect
www.sciencedirect.com

Elsevier Masson France

EM|consulte
www.em-consulte.com



Original article

Effects of oral administration of *Ocimum basilicum* on goblet cell hyperplasia and upstream cytokine gene expression in allergic asthma

Effets de l'administration orale d'*Ocimum basilicum* sur l'hyperplasie des cellules caliciformes et de l'expression en amont des gènes des cytokines dans l'asthme allergique

E. Mehrabi Nasaba^a, S.M. Athari^b, B. Motlagh^c, S.S. Athari^{d,*}

^a Cardiologist, Tehran Heart Center, Tehran University of Medical Sciences, Tehran, Iran

^b Department of Biology, Faculty of Basic Sciences, Maragheh University, Maragheh, Iran

^c Department of Biochemistry and Nutrition, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

^d Department of Immunology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

ARTICLE INFO

Article history:

Received 29 November 2018

Accepted 18 February 2019

Available online xxx

Keywords:

Herbal medicine
Allergy
Asthma
Gene
Cytokine

ABSTRACT

Background. – *Ocimum basilicum* (OB) possess antioxidant and anti-inflammatory properties. The components of this plant have been used to treat respiratory disorders such as asthma.

Aim. – This study examined the effects of OB seeds on upstream cytokine gene expression and bronchial goblet cell hyperplasia in a murine asthma model.

Materials and methods. – The anti-inflammatory and immunomodulatory effects of daily consumption of OB seeds were investigated by gene expression of interleukins IL-8, 9, 11, 25 and 33 and eotaxin production. Two groups of 6-week-old female BALB/c mice were sensitized by intraperitoneal injection of ovalbumin (OVA) plus alum on days 1 and 14. On days 24, 26, 28 and 30, the mice were exposed to OVA in saline for 30 minutes using a nebulizer. One of the asthmatic groups received OB seeds orally on days 25, 27 and 29. Identical tests were conducted on mice receiving saline as negative controls.

Results. – OB significantly reduced mRNA expression for IL-8, 9, 11, 25, 33 and eotaxin in bronchoalveolar lavage fluid (BALF) from the mice ($P < 0.05$). Further, goblet cell hyperplasia was significantly decreased in OB-treated mice ($P < 0.05$).

Conclusion. – Our results show that OB may inhibit expression of Th2 upstream cytokines and goblet cell hyperplasia in OVA-induced asthma and thus alleviate allergic asthma in the mouse model.

© 2019 Elsevier Masson SAS. All rights reserved.

R É S U M É

Ocimum basilicum (OB) a des propriétés antioxydantes et anti-inflammatoires. Les composants de cette plante (NDLR: le basilic) ont été utilisés pour traiter des pathologies respiratoires telles que l'asthme.

But de l'étude. – Cette étude vise à déterminer les effets des graines d'OB sur l'expression en amont des gènes des cytokines et l'hyperplasie des cellules caliciformes bronchiques sur un modèle d'asthme murin.

Matériel et méthodes. – Les propriétés anti-inflammatoires et immunomodulatrices de la consommation quotidienne de graines d'OB ont été abordées par l'action sur l'expression des gènes assurant la production d'IL 8,9,11,25 et 33 et de l'éotaxine. Deux groupes de souris femelles BALB/c âgées de 6 semaines ont été sensibilisés par l'injection intrapéritonéale d'ovalbumine (OVA) et d'alun aux jours 1 et 14. Les jours 24, 26, 28 et 30 les souris ont été exposées à des nébulisations de 30 minutes d'OVA dans du sérum salé. Un des groupes de souris asthmatiques a reçu par voie orale des graines d'OB aux jours 25, 27 et 29. Des tests identiques ont été réalisés sur un groupe contrôle de souris recevant du sérum salé seul.

* Corresponding author.

E-mail addresses: ss.athari@gmail.com, ss.athari@zums.ac.ir (S.S. Athari).

Résultats. – L'administration d'OB a réduit de façon significative l'expression d'IL 8,9,11,25,33 et d'éotaxine dans le liquide de lavage bronchoalvéolaire (BALF) ($p < 0,05$). De plus l'hyperplasie des cellules caliciformes était significativement réduite chez les souris traitées par OB ($p < 0,05$).

Conclusion. – Nos résultats montrent que l'OB peut inhiber en amont l'expression des gènes codant pour les cytokines Th2 et l'hyperplasie des cellules caliciformes dans l'asthme induit par l'OVA et ainsi améliorer l'asthme allergique dans un modèle murin.

© 2019 Elsevier Masson SAS. Tous droits réservés.

1. Introduction

Allergic asthma is currently a significant public health problem with around 20% of the world's population affected. Due to the chronic nature of the disease, it is associated with morbidity and mortality [1]. Airway inflammation leads to epithelial injury, subepithelial thickening, and goblet cell and airway hyperplasia. Activated T-helper (Th)2 lymphocytes at the injury site cause inappropriate allergic responses [asthma, (3)]. Excessive production of Th2 cytokines such as IL-4, IL-5, IL-9 and IL-13 in allergic airway inflammation induce activation and infiltration of inflammatory cells such as eosinophils and induce intense allergic responses [2].

Ocimum basilicum Linn. (sweet basil) is a popular culinary herb belonging to the *Lamiaceae* family. It includes different species and varieties distributed in the tropical regions of Asia, Africa and Central and South America [3]. This plant possesses anti-inflammatory [4–6], antimicrobial [7], antidiabetic [8] and antioxidant properties [9,10]. In addition to these health-promoting properties, several studies have evaluated and confirmed the therapeutic effects of this plant on asthma and other respiratory disorders [4,11]. There is a lack of studies about the effects of OB seeds on the expression of cytokines involved in the pathogenesis of allergic asthma, and the aim of this study was thus to assess the anti-asthmatic effect of OB seeds in the mouse model.

2. Methods

2.1. Animals

Six-week-old female BALB/c mice were obtained from the Pasteur Institute of Iran (Tehran, Iran). The animals were given food and water ad libitum in an allergen-free environment at a temperature of between 18 to 24 °C and with a 12 h light/dark cycle.

2.2. Antigen sensitization, challenge and treatment

The mice were immunized using two methods: intraperitoneal injection and inhalation of OVA in an aerosol. The animals were divided into the following groups: group 1 was actively sensitized by intraperitoneal injection of 20 µg OVA (Sigma Aldrich, USA) with 1 mg of alum on days 1 and 14. The mice then inhaled a 1% OVA solution (in sterile physiological saline) aerosolized by an ultrasonic nebulizer (NE-U07, Omrom, Japan) on days 24, 26, 28, and 30 for 30 minutes per day (positive controls); group 2 included allergic asthma models (similar to group 1) receiving OB in feed on days 25, 27 and 29; group 3 comprised healthy mice receiving normal saline (negative controls).

OB seeds were administrated orally in food. The seeds were ground and 5 g of OB seeds were mixed with 95 g of standard mouse feed (5% concentration). The mice were then given all feed thus produced by oral gavage on days 25, 27 and 29. On the other days, they had free access to normal food and water.

After day 31, exams were performed, and the groups were compared using statistical methods.

Table 1

Primers used in Real-time PCR.

Amplified cytokine	Forward & Reverse	Sequence (5'-3')
GAPDH	Forward Reverse	TGTTCTACCCCAATGTGT GGTCTCAGTGTAGCCCAAG
IL-8	Forward Reverse	CACCTCAAGAACATCCAGAGCT CAAGCAGAACTGAATACCATCG
IL-9	Forward Reverse	CATCAGTGTCTCTCCGTCCCACTGATG GATTTCTGTGTGGCATTGGTCAG
IL-11	Forward Reverse	AAGCTGTGTTTTCGCTGGT AAGCTGCAAAGATCCCAATG
IL-25	Forward Reverse	CGGAGGAGTGGCTGAAGTGGAG ATGGGTACCTTCTCGCCATG
IL-33	Forward Reverse	TGAGACTCCGTTCTGGCCTC CTCTCATGCTTGGTACCCGAT
Eotaxin	Forward Reverse	CTGCTCACGGTCACTTCCTT GGGTCAGCACAGATCTCTT

2.3. RNA extraction from bronchoalveolar lavage fluid (BALF)

BAL fluid was collected from animals, centrifuged in sterile centrifuge tubes for 5 minutes at 500 rpm at 4 °C, and then washed twice with PBS. The cell pellet was suspended in PBS. Total RNA was extracted from BALF using TRIzol reagent according to the manufacturer's specifications. The RNA concentration was quantified by measuring the optical density (OD) at 260 nm (Eppendorf, Homburg, Germany). The extracted RNA was stored at – 70 °C pending cDNA synthesis.

2.4. Construction of cDNA from total RNA in BALF

Total RNA samples were converted to single-stranded cDNA using the Maxima First Strand cDNA Synthesis Kit according to the manufacturer's instructions (RevertAid, Fermentas Life Sciences, International INC, Canada). The product of the first strand cDNA synthesis was used directly in qPCR or stored at – 20 °C for up to one week, and at – 70 °C for longer storage periods.

2.5. Real-time quantitative PCR

In the present study, real-time PCR was carried out using SYBR Green I as a fluorescent dye. PCR amplification was performed using a Rotor-Gene SYBR Green PCR kit from QIAGEN and a Rotor-Gene Q thermocycler (QIAGEN, Hilden, Germany). The sequences of primers used in the PCR are listed in Table 1. After an initial activation step at 95 °C for 5 min (hot start DNA), reactions were run under the following conditions: 40 cycles of denaturation at 95 °C for 5 s, and combined annealing/extension at 60 °C for 10 s at which fluorescence was acquired. Amplification specificity was checked using the melting curve generated by heating the PCR product slowly at a rate of 1 °C s⁻¹ from 60 °C to 95 °C. Relative gene expression levels were normalized to GAPDH mRNA levels. To avoid an impressing effect of inter-run variations on reference gene stability, the gene expression of all reference genes was quantified for each sample in triplicate within the same qPCR plate. The PCR efficiency of the target genes, IL-8, 9, 11, 25, 33, eotaxin and endogenous control

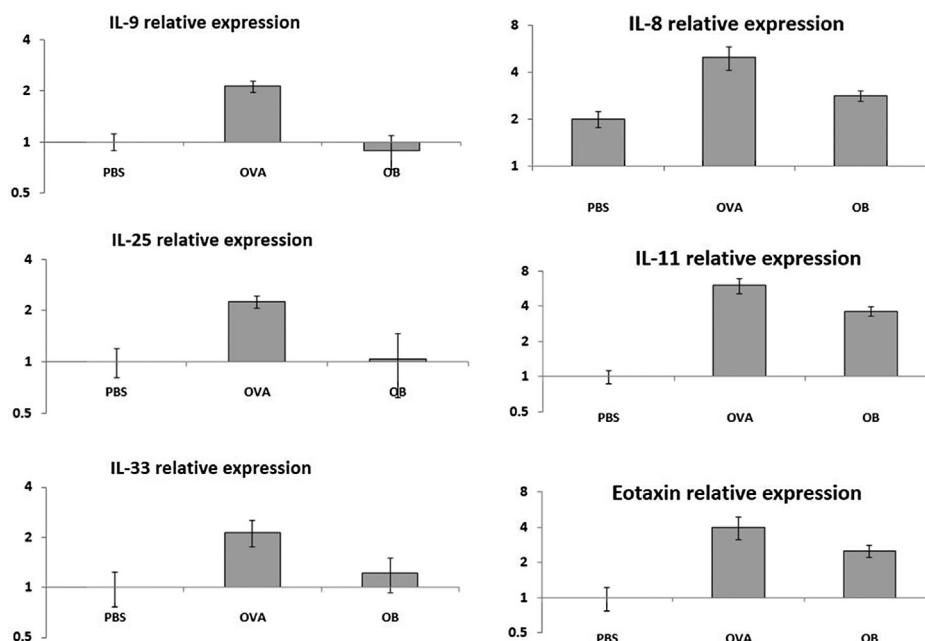


Fig. 1. Effects of OB on mRNA expression for IL-8, IL-9, IL-11, IL-25, IL-33 and eotaxin. Data is shown as mean $\pm$ SD for 14 BALB/c mice in each group. A significant increase in mRNA expression for all cytokines was noted in OVA-sensitized mice (OVA-group) compared to PBS-treated controls. OB significantly reduced mRNA expression for IL-8, 9, 11, 25 and 33 and eotaxin in mice ($P < 0.05$). PBS: PBS-exposed group; OVA: non-treated bronchial asthma group; OB: bronchial asthma group treated with OB.

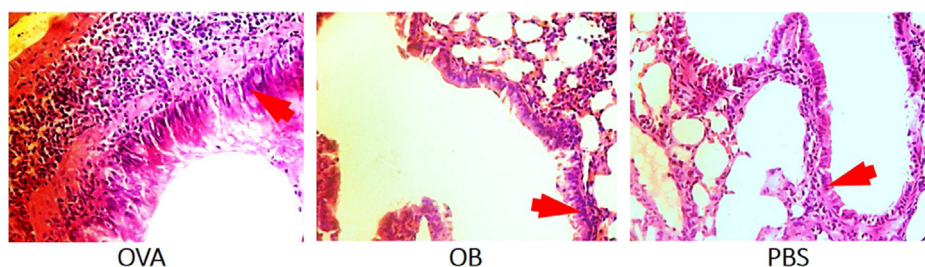


Fig. 2. Goblet cell hyperplasia in lung bronchi (black arrow). Goblet cell hyperplasia was increased in the OVA group vs. the PBS group. In the OB-treated group, goblet cell hyperplasia was reduced vs. the OVA group.

glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were calculated from the slopes of the standard curves (primer sequences are listed in Table 1).

2.6. Histopathology

Histopathological sections were prepared and stained with hematoxylin and eosin (HE) to detect goblet cell hyperplasia.

2.7. Statistical analysis

Data were analyzed with one-way ANOVA followed by Tukey's test using GraphPad Prism software (GraphPad, San Diego, CA). The values were expressed as mean $\pm$ Standard Error on the Mean (SEM), and differences with $P < 0.05$ were considered significant.

3. Results

3.1. Effect of OB on cytokine and chemokine gene expression

Cytokines, including IL-8, 9, 11, 25, 33 and eotaxin as chemokine, were measured by real-time PCR assay of their mRNA expression in BALF. Fig. 1 shows that OVA-treated mice exhibited higher mRNA expression of these cytokines. The level of cytokine expression was significantly reduced in mice receiving OB.

3.2. Goblet cell hyperplasia

To confirm the allergic airway inflammation model, the positive and negative controls were compared in terms of differences in increased incidence of goblet cell hyperplasia in the airways (histopathology sections) (Fig. 2). A Mann-Whitney U test was used to compare the positive and negative control groups. There were significant differences between the two groups in terms of goblet cell hyperplasia ($P < 0.05$) (Fig. 3). Treatment with OB in OVA-sensitized mice significantly reduced goblet cell hyperplasia more than 2-fold ($P < 0.05$). OB had a significant effect on goblet cell hyperplasia of the airways vs. the asthmatic group.

4. Discussion

We evaluated the effects of OB seeds on allergic asthma responses characterized by differential release of cytokines and goblet cell hyperplasia in an experimental model of OVA-sensitized mice. The results of the present study show that treatment of OVA-sensitized mice with OB seed could alleviate inflammatory cytokines and goblet cell hyperplasia in the airways.

Inflammation plays a key role in the pathophysiology of asthma. The interaction of numerous cell types and multiple mediators with the airways ultimately results in bronchial inflammation. Thus, inhibiting or eliminating Th2 cells is a beneficial strategy in

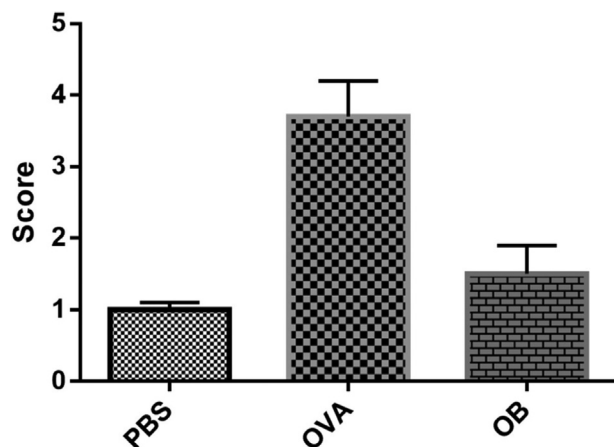


Fig. 3. Pulmonary goblet cell hyperplasia scores in all three groups.

asthma treatment [12]. While corticosteroids are widely used as anti-inflammatory drugs to control this problem, long-term steroid use has numerous undesirable side effects [13,14].

Adjunctive treatments such as medicinal plants with few side effects may replace chemical drugs. Several studies have indicated the anti-inflammatory and anti-asthmatic effects of OB [4–6,11] and the results of the present study are consistent with these findings. OB oil may inhibit the lipoxygenase pathway and reduce inflammation [5]. The effect of OB on suppression of the cytokines involved in allergic asthma was investigated for the first time in the present study. The results of one study showed a potent relaxant effect of OB comparable with theophylline on the tracheal chains of guinea pigs [15], although this effect was less marked than that of theophylline. Several studies have shown that interventions targeting Th2 cytokines may improve asthma symptoms to some extent. Inhibition of two or all of the cytokines secreted simultaneously from Th2 cells proved effective in clinical trials [16–18]. In the present study, OB exhibited an inhibitory effect on cytokines.

Further, according to certain papers, enzymes like matrix metalloproteinases (MMPs) 2, 7, 9 and 12 are involved in airways hyperactivity and inflammation [19–21]. Activation of MMP-7 leads to inflammatory activity in asthmatic lungs through IL-25 and inhibition of the development of regulatory T cells [21]. Sands et al. [22] previously showed that targeting MMPs could have a beneficial effect in asthma treatment. The results of the present study show that OB seeds dramatically reduce gene expression of IL-25 and IL-33 in OVA-sensitized asthmatic mice. Recently, experimental studies have demonstrated undesirable effects of IL-25 and IL-33 on eosinophilia generation, overexpression of type 2 cytokines, and mucus hypersecretion [23–25]. Suppression of IL-25 and IL-33 leads to reduction of inflammation in pulmonary tissue and consequently helps to ease asthma [26]. Several clinical studies of allergic asthma have reported overexpression of these two interleukins in serum and bronchial mucosa [27,28]. According to several studies, levels of IL-25 and IL-33 correlate with the severity of allergic diseases [29]. IL-25 is required to up-regulate levels of IL-13, which can in turn activate smooth muscle response [30]. Lei et al. [31] showed that vaccination against IL-33 could reduce levels of IL-17A, IL-25 and IL-33 in lung tissue of mice models of asthma. Indeed a recent investigation demonstrated that IL-33 has an important role in the rapid induction of airway contraction by stimulating prompt expansion of IL-13–producing type-2 innate lymphoid cells, whereas IL-25-induced responses are slower and less potent [32,33]. A reduction of IL-13 in the bronchi thus leads to decreased mucus production and goblet cell hyperplasia.

OB extract contains a mixture of phytochemicals, but these are not the sole components and OB seeds were therefore used in

this study to treat allergic asthma. *Ocimum basilicum* seed contains mucilage that can reduce the quantity of mucus (produced by goblet cells) in the airways [34]. Essential oil of OB has an anti-inflammatory effect on the respiratory system [35]. It also has relaxant effects, and, with its other effects, it can be used as an anti-allergic and anti-asthma treatment. OB can open obstructed airways and improve breathing flow [36]. Eftekhari et al. (2018) reported that OB extract and its chemical compounds, especially rosmarinic acid (one of the main phenolic compounds), have antioxidant properties and can control asthma, which is associated with oxidant stress. In addition, rosmarinic acid has an anti-inflammatory effect and can help control inflammatory diseases such as allergic asthma. In addition, the relaxant effects of OB on tracheal smooth muscle can decrease bronchospasm and improve the quality of air flow [37]. At the very least, OB has excellent potential for use as an anti-asthma drug and may be used in asthma patients to achieve pronounced effects with few side effects and low costs.

5. Conclusion

We investigated the anti-inflammatory and immunomodulatory effects of OB in a mouse model of allergic asthma based on Th2 cytokine expression and goblet cell hyperplasia in the airways of lung tissue. The results demonstrated the efficacy of daily consumption of OB seeds in inhibiting the key cytokine production and goblet cell hyperplasia involved in OVA-induced asthma in mice.

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgment

This research is a continuation of the published manuscript entitled: Study effect of *Ocimum basilicum* seeds on mucus production and cytokine gene expression in allergic asthma mice model. S. Masoume Athari, E. Mehrabi Nasab, S. Shamsadin Athari. Revue Française d'Allergologie, Volume 58, Issue 7, November 2018, Pages 489–493.

References

- [1] Bousquet J, Mantzouranis E, Cruz AA, Ait-Khaled N, Baena-Cagnani CE, Bleecker ER, et al. Uniform definition of asthma severity, control, and exacerbations: document presented for the World Health Organization Consultation on Severe Asthma. *Journal of Allergy and Clinical Immunology* 2010;126(5):926–38.
- [2] Lee S-Y, Chang Y-S, Cho S-H. Allergic diseases and air pollution. *Asia Pacific Allergy* 2013;3(3):145–54.
- [3] Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular aspects of Medicine* 2006;27(1):1–93.
- [4] Singh S, Agrawal SS. Anti-asthmatic and anti-inflammatory activity of *Ocimum sanctum*. *International Journal of pharmacognosy* 1991;29(4):306–10.
- [5] Singh S. Mechanism of action of anti-inflammatory effect of fixed oil of *Ocimum basilicum* Linn. *Indian Journal of Experimental Biology* 1999;37(3):248–52.
- [6] Rakha P, Nagpal M, Sharma S, Parle M. Study of Anti-Inflammatory potential of Ethanolic Fraction of Seeds of *Ocimum basilicum* Linn. *Research Journal of Pharmacology and Pharmacodynamics* 2009;1(2):79–81.
- [7] Suppakul P, Miltz J, Sonneveld K, Bigger SW. Antimicrobial properties of basil and its possible application in food packaging. *Journal of agricultural and food chemistry* 2003;51(11):3197–207.
- [8] Narendhirakannan R, Subramanian S, Kandaswamy M. Biochemical evaluation of antidiabetic properties of some commonly used Indian plants on streptozotocin-induced diabetes in experimental rats. *Clinical and Experimental Pharmacology and Physiology* 2006;33(12):1150–7.
- [9] Lee K-g, Shibamoto T. Determination of antioxidant potential of volatile extracts isolated from various herbs and spices. *Journal of Agricultural and Food Chemistry* 2002;50(17):4947–52.
- [10] Jayasinghe C, Gotoh N, Aoki T, Wada S. Phenolics composition and antioxidant activity of sweet basil (*Ocimum basilicum* L.). *Journal of Agricultural and Food Chemistry* 2003;51(15):4442–9.

- [11] Joshi RK. Phytoconstituents, traditional, medicinal and bioactive uses of Tulsi (*Ocimum sanctum* Linn.): A review. *Journal of Pharmacognosy and Phytochemistry* 2017;6(2):261–4.
- [12] Broide DH. Molecular and cellular mechanisms of allergic disease. *Journal of Allergy and Clinical Immunology* 2001;108(2):S65–71.
- [13] Bosnjak B, Stelzmueller B, Erb KJ, Epstein MM. Treatment of allergic asthma: modulation of Th2 cells and their responses. *Respiratory research* 2011;12(1):114.
- [14] Bateman ED, Hurd S, Barnes P, Bousquet J, Drazen J, FitzGerald M, et al. Global strategy for asthma management and prevention: GINA executive summary. *European Respiratory Journal* 2008;31(1):143–78.
- [15] Boskabady MH, Kiani S, Haghir B. Relaxant effects of *Ocimum basilicum* on guinea pig tracheal chains and its possible mechanism (s). *DARU Journal of Pharmaceutical Sciences* 2005;13(1):28–33.
- [16] Webb DC, Cai Y, Matthaie KI, Foster PS. Comparative roles of IL-4, IL-13, and IL-4R α in dendritic cell maturation and CD4⁺ Th2 cell function. *The Journal of Immunology* 2007;178(1):219–27.
- [17] Leckie MJ. Anti-Interleukin-5 Monoclonal Antibodies. *American Journal of Respiratory Medicine* 2003;2(3):245–59.
- [18] Brightling C, Saha S, Hollins F. Interleukin-13: prospects for new treatments. *Clinical & Experimental Allergy* 2010;40(1):42–9.
- [19] Page K, Ledford JR, Zhou P, Wills-Karp M. A TLR2 agonist in German cockroach frass activates MMP-9 release and is protective against allergic inflammation in mice. *The Journal of Immunology* 2009;183(5):3400–8.
- [20] Warner RL, Lukacs NW, Shapiro SD, Bhagarvathula N, Nerusu KC, Varani J, et al. Role of metalloelastase in a model of allergic lung responses induced by cockroach allergen. *The American journal of pathology* 2004;165(6):1921–30.
- [21] Goswami S, Angkasekwinai P, Shan M, Greenlee KJ, Barranco WT, Polikepahad S, et al. Divergent functions for airway epithelial matrix metalloproteinase 7 and retinoic acid in experimental asthma. *Nature immunology* 2009;10(5):496.
- [22] Sands MF, Ohtake PJ, Mahajan SD, Takyar SS, Aalinkel R, Fang YV, et al. Tissue inhibitor of metalloproteinase-1 modulates allergic lung inflammation in murine asthma. *Clinical Immunology* 2009;130(2):186–98.
- [23] Kurowska-Stolarska M, Kewin P, Murphy G, Russo RC, Stolarski B, Garcia CC, et al. IL-33 induces antigen-specific IL-5⁺ T cells and promotes allergic-induced airway inflammation independent of IL-4. *The Journal of Immunology* 2008;181(7):4780–90.
- [24] Kim MR, Manoukian R, Yeh R, Silbiger SM, Danilenko DM, Scully S, et al. Transgenic overexpression of human IL-17E results in eosinophilia, B-lymphocyte hyperplasia, and altered antibody production. *Blood* 2002;100(7):2330–40.
- [25] Hurst SD, Muchamuel T, Gorman DM, Gilbert JM, Clifford T, Kwan S, et al. New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *The Journal of Immunology* 2002;169(1):443–53.
- [26] Ballantyne SJ, Barlow JL, Jolin HE, Nath P, Williams AS, Chung KF, et al. Blocking IL-25 prevents airway hyperresponsiveness in allergic asthma. *Journal of Allergy and Clinical Immunology* 2007;120(6):1324–31.
- [27] Raeiszadeh Jahromi S, Mahesh P, Jayaraj B, Madhunapantula SRV, Holla AD, Vishweswaraiah S, et al. Serum levels of IL-10, IL-17F and IL-33 in patients with asthma: a case-control study. *Journal of Asthma* 2014;51(10):1004–13.
- [28] Stolarski B, Kurowska-Stolarska M, Kewin P, Xu D, Liew FY. IL-33 exacerbates eosinophil-mediated airway inflammation. *The Journal of Immunology* 2010;185(6):3472–80.
- [29] Bønnelykke K, Sleiman P, Nielsen K, Kreiner-Møller E, Mercader JM, Belgrave D, et al. A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbations. *Nature genetics* 2014;46(1):51.
- [30] Préfontaine D, Lajoie-Kadoch S, Foley S, Audusseau S, Olivenstein R, Halayko AJ, et al. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. *The Journal of Immunology* 2009;183(8):5094–103.
- [31] Lei Y, Boinapally V, Zoltowska A, Adner M, Hellman L, Nilsson G. Vaccination against IL-33 inhibits airway hyperresponsiveness and inflammation in a house dust mite model of asthma. *PloS one* 2015;10(7):e0133774.
- [32] Barlow JL, Peel S, Fox J, Panova V, Hardman CS, Camelo A, et al. IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction. *Journal of allergy and clinical immunology* 2013;132(4):933–41.
- [33] Masoume Athari S, Mehrabi Nasab E, Shamsadin S, Athari. Study effect of *Ocimum basilicum* seeds on mucus production and cytokine gene expression in allergic asthma mice model. *Revue Française d'Allergologie* 2018;58(7):489–93.
- [34] Sayyad FJ, Sakhare Sfurti S. Isolation, Characterization and Evaluation of *Ocimum basilicum* Seed Mucilage for Tableting Performance. *Indian J Pharm Sci* 2018;80(2):282–90.
- [35] Filip S. Basil (*Ocimum basilicum* L.) a Source of Valuable Phytonutrients. *Int J Clin Nutr Diet* 2017;3:118.
- [36] Boskabady MH, Kiani S, Haghir B. Relaxant effects of *Ocimum basilicum* on guinea pig tracheal chains and its possible mechanism(s). *Daru* 2005;3(1):28–33.
- [37] Eftekhari N, Moghimi A, Boskabady MH. The Effects of *Ocimum basilicum* Extract and Its Constituent, Rosmarinic Acid on Total and Differential Blood WBC, Serum Levels of NO, MDA, Thiol SOD, and CAT in Ovalbumin Sensitized Rats. *Iran J Pharm Res* 2018;17(4):1371–85.