Original Article

Investigation of the effect of probiotic yogurt and fenugreek on allergic asthma

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Abstract

Background: Asthma as the variable degrees of airway obstruction is reversible and allergic asthma is a common form of asthma. Yogurt is coagulated milk and fenugreek as a plant is used in traditional medicine. Oral administration of yogurt (as food) can provide probiotic agents that have the main effects on immune responses. In this study, the effect of yogurt and fenugreek on asthma was studied. **Materials and Methods:** After producing of asthma model in BALB/c mice in 4 groups, groups were treated with yogurt, yogurt-probiotics, and yogurt-fenugreek. At least, total IgE in serum, IL-4, 5, 13 in BAL (Broncho-Alveolar lavage) fluid was measured. Histopathological sections were prepared and eosinophilic infiltration and mucus-secreting were investigated. **Results:** Eosinophilic infiltration and mucus hyper-secretion were decreased in treated groups. Total IgE in serum was decreased in asthma-yogurt-probiotic and asthma-yogurt-fenugreek groups in comparison with asthmatic and asthma-yogurt groups. The amount of IL-4 in BAL of the asthma-yogurt-fenugreek group was decreased. The amount of IL-5 in BAL of the asthma-yogurt-probiotic and asthma-yogurt-fenugreek groups was decreased. The amount of IL-13 was decreased significantly in three treated groups.**Conclusion:** This study showed that yogurt with fenugreek and probiotic has a strong effect on suppression of progression of airway inflammation and asthma pathophysiology.

Keywords: Herbal Medicine, Probiotic, Allergy, food, Cow's milk yogurt

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Introduction

Asthma is variable degrees of airway obstruction and this obstruction is reversible. In the last years, the prevalence and incidence of asthma were increased and estimated that almost 300 million people suffer from asthma [1]. Allergic asthma is a common form of asthma that involves the respiratory system and causes eosinophilia in bronchi which have the main role in airway inflammation [2].

Th2 cytokines [especially interleukin (IL) 4, 5, 13] play an important role in allergic asthma. IL-4 is involved in immunoglobulin (Ig) E production, IL-5 activates eosinophilia, and IL-13 leads to mucus hypersecretion [3]. An asthma attack can be managed with control of eosinophilic inflammation, bronchial muscle spasm, and mucus hypersecretion [4].

Yogurt is coagulated milk, it's obtained by lactic acid fermentation due to the presence of lactobacillus (LAB) bulgaricus and streptococcus thermophiles in milk. Traditional Persian medicine

(TPM) is one of the ancient traditional medicines and one of these prescripts is based on herbal medicine and another component is yogurt. According to the code of federal regulation of the FDA, yogurt is defined as the food produced or more of the optional dairy [5, 6].

The immune system is an important contributor to all of these diseases and the stimulatory effect of yogurt on the immune system has been investigated by animal models and human subjects. Some results support yogurt has the immune-stimulatory effects, but this hypothesis needs to be substantiated by several in vivo and in vitro tested [7]. Oral administration of yogurt and LAB can stimulate the immune system and LAB is essential for yogurt to exert immune-stimulatory effects. The investigations showed that the LAB cell wall contains the main immune-modulatory component [8]. Probiotics have been advocated as a novel therapeutic approach to respiratory disease. Oral administration of probiotic influences the respiratory macrobiotic is needed and yogurt has a large number of probiotic microorganisms [9].

Trigonella foenum-graecum (fenugreek) is one of the important plants for asthma treatment. [10]. Fenugreek is used as an appetite stimulant lung tonic and chest wall analgesia and enhances breathing and lung secretion[11]. This plant has anti-inflammatory and anti-oxidant effects [12, 13]. We aimed to investigate the effect of yogurt with and without probiotic as well as the effect of yogurt containing fenugreek extract on allergic asthma.

Methods

Animals. Female BALB/c mice with 6 weeks of age were purchased from the Pasteur Institute of Iran and kept in free access to food and water with standard conditions (18-24c temperature, 30-60% relative humidity, 12 hours light/dark cycle). It should be noted that all experiments were carried out to a high ethical standard and all ethical quid lines were applied under method approved by the animal ethics committee.

Antigen sensitization and treatment. Immunization was done with two methods; intraperitoneal injection and inhalation aerosolized. The mice were divided into five groups (n=7 mice in each group) as follows:

1) 1st group: allergic inflammatory asthma models were sensitized by intraperitoneal injection of 20µ of ovalbumin with 50µ of aluminum hydroxide (alum adjuvant) and 50µ of PBS on day 1 and this was repeated at 14 days. The mice were challenged with inhaled 1% ovalbumin solution aerosolized by an ultrasonic nebulizer for 30 min per day on days 24, 26, 28, and 30.

2) 2nd group: allergic asthma model with acute inoculation condition (similar to the first group) which received yogurt of cow's milk with 4% fat in an oral form on the 25, 27, 29 days.

3) 3rd group: allergic asthma model (similar to the first group) which on the 25, 27, 29 days received yogurt of cow's milk with 4% fat contains probiotics.

4) 4th group: allergic asthma model (similar to the first group) which received yogurt of cow's milk with 4% fat contains fenugreek extract (1%) on the 25, 27, 29 days.

5) 5th group: healthy mice have received only normal saline and not received OVA.

In all groups, 24h after the last inhalation was inoculated, sampling (blood, BALF, and lung tissue) were done.

Histopathology. Histopathological sections were prepared and stained with hematoxylin and eosin (H & E). Eosinophilic infiltration and mucus-secreting were investigated. The mucus stain in the histopathology sections was scored and attributed 5 score as follows; Score 1: 0≤ bronchial obstruction < 5%, Score 2: 5% ≤ bronchial obstruction < 25%, Score 3: 25% ≤ bronchial obstruction < 50%, Score 4: 50% ≤ bronchial obstruction < 75% and Score 5: 75% ≤ bronchial obstruction ≤ 100%.

Measurement of total IgE in serum. Total IgE was measured with ELISA by a specific ELISA kit.

Measurement of cytokines in BALF. The amount of IL-4, 5, 13 in BAL was measured with specific mice ELISA kit.

Statistical analysis. The Kolmogorov-Smirnov test for normal distribution, one-way analysis of variance (ANOVA) and t-test (comparing results from different groups) and the Mann-Whitney U test (comparing differences between positive and negative groups) were used. A p-value of less than 0.05 was considered to be significant.

Results

According to the histopathology study, eosinophilic infiltration in the asthmatic group is significantly increased in comparison with controls. The inflammation was decreased in asthma-yogurt and asthma-yogurt-probiotic groups, but the decrease of inflammation in asthma-yogurt-fenugreek was obvious (Fig.1 and 2). Mucus hypersecretion was decreased in asthma-yogurt and asthma-yogurt-probiotic groups but this effect was not statistically significant in comparison with the asthmatic group. Mucus hypersecretion was decreased significantly in the asthma-yogurt-fenugreek group as compared with the asthmatic group (Fig.1 and 2).

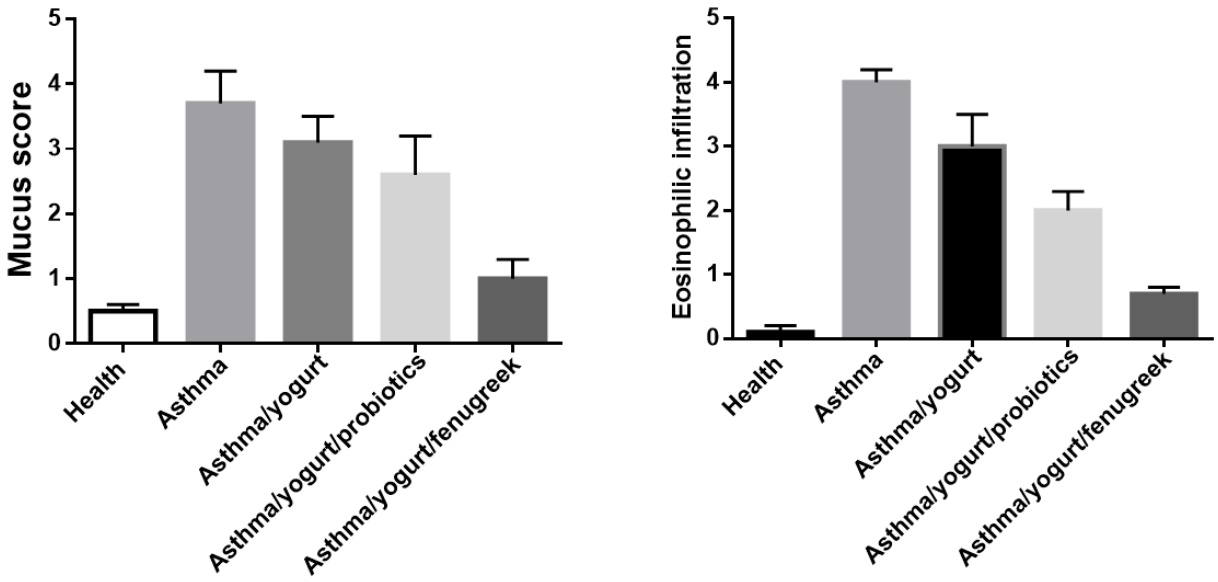

Fig.1. Eosinophilic infiltration and Mucus hyper-secretion in all studied groups.

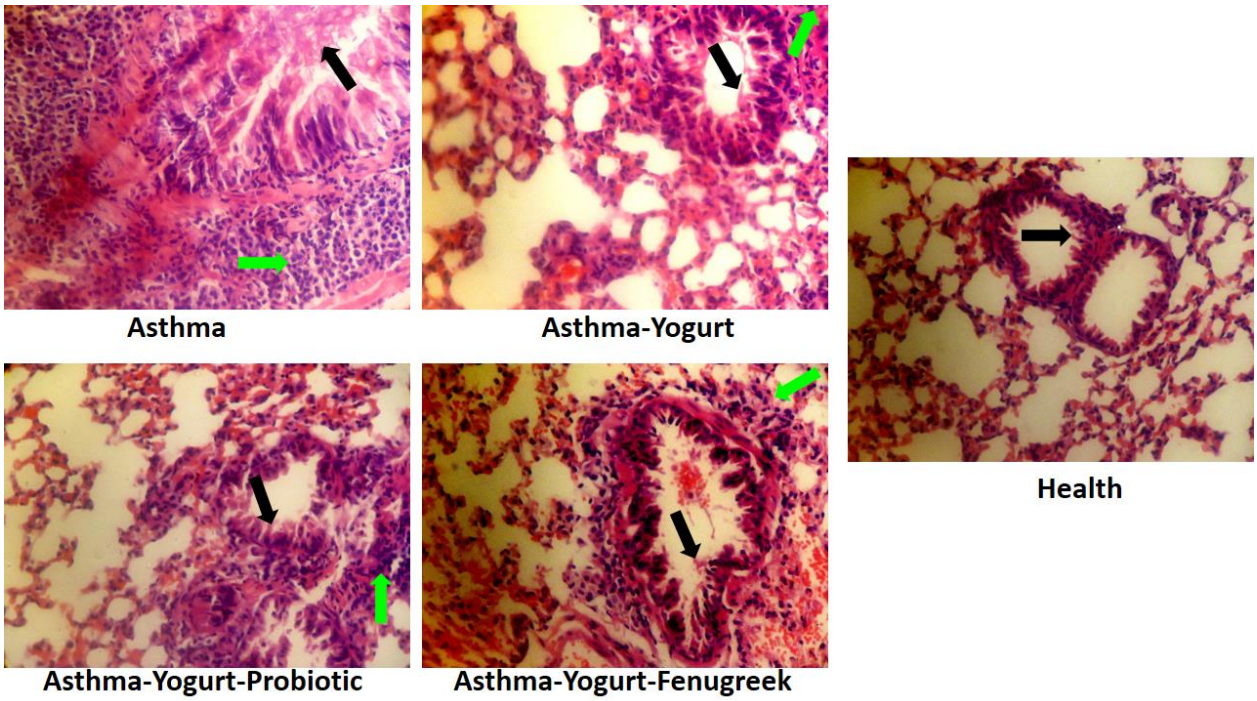

Fig.2. Eosinophilic infiltration (green arrow) and mucus hyper-secretion (black arrow) in histopathological sections of all studied groups.

Total IgE in serum was decreased in asthma-yogurt-probiotic and asthma-yogurt-fenugreek groups in comparison with asthmatic and asthma-yogurt groups. Decreasing of total IgE in the serum asthma-yogurt-fenugreek group was significant in comparison with asthma-yogurt-probiotic (Fig.3).

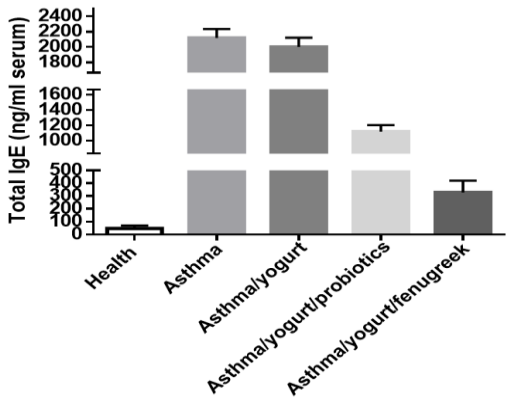

Fig.3. Serum level of total IgE in all studied groups (ng/ml)

The concentration of IL-4 in BAL of the asthma-yogurt-fenugreek group was decreased significantly as compared with the asthma group. The concentration of IL-5 in BAL of the asthma-yogurt-probiotic and asthma-yogurt-fenugreek groups was decreased significantly in comparison with the asthma group. The level of IL-13 was decreased significantly in three treated groups in comparison with the asthma group (Fig.4).

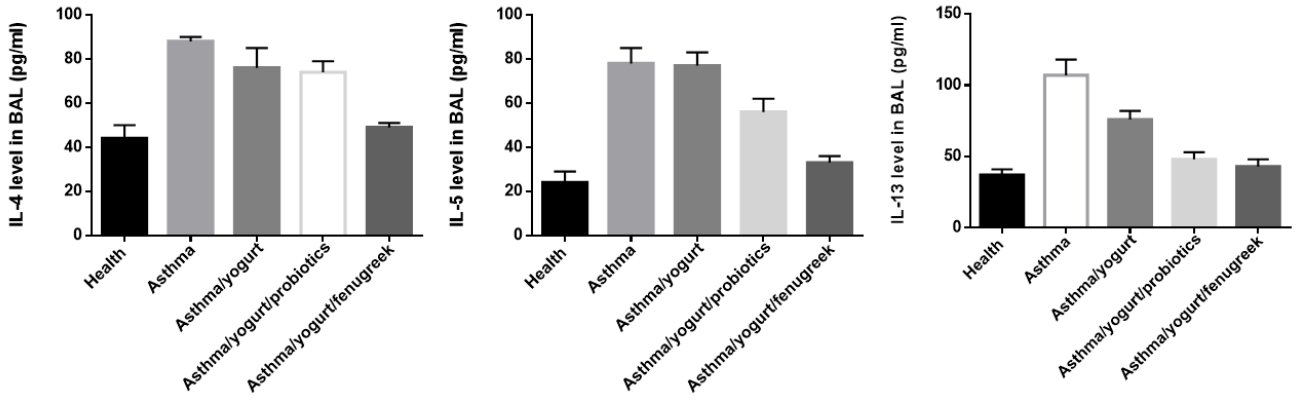

Fig.4. BAL fluid level of IL-4, 5 and 13 in all studied groups (pg/ml)

Discussion

In this study, different animal groups with allergic asthma were treated with yogurt and yogurt with probiotics and yogurt with Trigonella foenum-graecum. Cytokines are produced by immune cells and are involved in inflammation and immunity. The main underlying mechanism in allergic asthma is increased IL-4, 5, and 13 expressions in the lung which plays a main role in the severity of asthma. IL-4 increases allergic response in bronchial and induces the proliferation of Th2 cells that leads to the production of IgE. IL-5 leads to eosinophil activation and migration in bronchial and IL-13 is an important trigger for mucus secretion [14-17]. Thus, modulation of cytokine in the lung could decrease airway inflammation.

A wide range of herbal therapy is used in the treatment of asthma in Iranian traditional medicine [18].

The immune-stimulatory effects of yogurt are due to yogurts' bacterial components, but the mechanism of these effects has not been determined [19-21]. Commensal bacteria are the main immune-stimulatory factor of yogurt. LAB are gram-positive bacteria which to have immune-stimulatory properties [22]. Nonbacterial milk components also contribute to the immune stimulatory activity of yogurt peptides. Moreover, free fatty acids, calcium, and certain vitamin are shown to enhance the immune response [22].

The fenugreek as an annual plant is composed of more than 32 species which mostly found in central regions of Iran [23]. In Iranian traditional medicine, fenugreek was used as an appetite stimulant, lung tonic and enhances breathing and lung secretion [10, 24, 25]. Nowadays, anti-inflammatory and antioxidant effects of this plant have been approved [11, 12, 26]. This study showed improvement in all studied groups treated with yogurt, decreased level of eosinophilic attack, and downregulation of Th2 cytokines gene expression in the treated asthmatic groups. However, improvement by yogurt with probiotic is considerable than the only yogurt. A similar pattern was seen in the group treated with yogurt and Trigonella foenum-graecum too.

Probiotics have great potential to use as preventive and therapeutically approaches to immunity-related disease management [27]. There is evidence that yogurt has the potential ability to inhibit cytokine-induced allergy in bronchial and reduce mucus production.

The probiotics are cultures of beneficial bacteria that positively affect hosts by enhancing the

microbial balance and reduce the generation of pro-inflammatory cytokine in allergic inflammation [28-30].

To assess the anti-inflammatory effect of yogurt and Trigonella foenum-graecum and probiotics, histopathology studies were performed and the infiltrated inflammatory cells (especially eosinophils) were studied. Yogurt significantly reduced eosinophil infiltration. Yogurt with Trigonella foenum-graecum significantly reduced eosinophil infiltration compared with only yogurt group and yogurt with the probiotic group. Mucus production was reduced in yogurt treated mice compared with the control group. This effect was also augmented with Trigonella foenum-graecum and probiotics.

Goblet cells produce mucus and asthmatic airways mucus has hyper-secretion form [30-33]. The reduction in mucus production in yogurt-treated lung tissue attributed to the drop in Th2 cytokines levels.

Conclusion

These findings suggest that yogurt with Trigonella foenum-graecum and probiotics have beneficial effects on suppression of airway inflammation progression and remodeling in the lung. Additional studies are necessary to clarify the underlying mechanism in which yogurt and Trigonella foenum-graecum and probiotic affect the airway inflammation.

Conflicts of Interest

There is no conflict of interest.

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