

# Oral Administration of *Zataria multiflora* Extract Decreases IL-17 Expression in Perennial Allergic Rhinitis

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## Abstract

**Background:** Rhinitis, which occurs most commonly as allergic rhinitis and affects 20% of the world's population, is a major health care burden causing significant morbidity. Considering the high prevalence of allergic rhinitis and anti-inflammatory effects of thyme, a favorite condiment, we performed a randomized clinical trial to determine whether thyme can relieve allergic rhinitis symptoms and affect the expression of TH17- and T-regulatory cell- (Treg) related cytokines IL-17, TGF- $\beta$ , FOXP3, and IL-10.

**Methods:** Thirty patients with allergic rhinitis symptoms and positive skin prick test for common aero allergens were randomly assigned to experimental or control groups. The experimental group received thyme or *Zataria multiflora* (ZM) extracts and the control group received placebo for two months. Expression of IL-17, TGF- $\beta$ , FOXP3, and IL-10 was evaluated in all subjects by real-time PCR before and after intervention.

**Results:** After treatment IL-17 expression was significantly less in the ZM group than in controls ( $p < 0.05$ ), while TGF- $\beta$ , FOXP3, and IL-10, expression were not significantly changed.

**Conclusions:** Given the significant effect of thyme in reducing symptoms of allergic rhinitis and decrease IL-17 gene expression and because allergic rhinitis is a multifactorial disease, the administration of thyme extract along with conventional treatments may benefit allergic rhinitis sufferers.

**Keywords:** Allergic rhinitis, IL-17, Herbal product, Thyme, *Zataria multiflora*.

## Introduction

Allergic rhinitis is a common inflammatory disorder that affects 400 million individuals worldwide of all ages, particularly in developed countries (1). Due to the increasing rate of atopic disorders in this century, a significant proportion of these patients suffer from allergic rhinitis (2). The illness is characterized by sneezing, clear rhinorrhea, nasal congestion, and pruritus (3). In addition to these clinical symptoms the condition can also affect wellbeing in other ways including fatigue, sleep disorders, irritability, difficulties at school or work, and even social

relationships (4). Allergic rhinitis is mediated by early- and late-phase hypersensitivity responses (5). Antihistamines, decongestants, and corticosteroids are the main therapeutic treatments (6).

*Zataria multiflora* (ZM) is an edible thyme-like plant widely recognized as thyme and Avishan Shirazi in Iran (7). It grows in Iran, Pakistan, and Afghanistan and is a common food condiment (8). The main components of ZM extract are thymol, carvacrol, (8) and *p*-cymene (9). *Zataria multiflora* is not only a popular condiment but also widely used in

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traditional medicine for analgesia, diarrhea, infectious diseases, and gastrointestinal problems (10). It has also been shown to have anti-inflammatory and anti-nociceptive effects due to the wide range of biological properties (8). Due to the increase in allergic conditions and recent developments in the field of herbal remedies (11) we performed a double-blind randomized control study to evaluate the effects of ZM syrup on subjects with allergic rhinitis with focus on possible role of T helper 17 and T regulatory related cytokines including IL-17, TGF- $\beta$ , FOXP3, and IL-10 in allergic rhinitis.

## Materials and Methods

### Study population

Forty-three individuals with histories of seasonal allergic rhinitis for at least two years and at least one positive skin prick test for common aeroallergens of the region (12) were eligible for the study. All the patients were from the clinic of allergy, Ghaem hospital, Mashhad, Iran. Patients diagnosed with allergic rhinitis from Nov 2012 to Jan 2013 were enrolled to avoid the effects of seasonal allergy in the spring and summer. All the patients were examined by two allergists and all fulfilled the criteria of allergy according to GLORIA (Global Resources in Allergy) (13). The investigation protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences (code: 910801) and the Iranian Registry of Clinical Trials (code: IRCT2016121823235N6). All patients signed written informed consent agreements.

To focus on thyme effects patients taking other herbal products were excluded from the study. Patients with systemic disease, sinusitis, or pregnancy, or those receiving antioxidant or anti-inflammatory treatments were excluded. Few of the enrolled patients experienced side effects of thyme, which included nausea, diarrhea, and vomiting. Although the side effects were generally mild, these patients were excluded from study. In addition, some patients withdrew for other reasons. Finally, of the 30 patients enrolled, 15 received ZM syrup in addition to their conventional treatments and 15 received the placebo. All the patients received Cetirizine tablets and nasal spray. Neither the patients nor physicians were aware of the ingredients in the syrup. Throughout the study the

general health and allergic rhinitis of the subjects were assessed via SNOT22 questionnaire (14).

### Preparation of ZM syrup

First, a 20% ZM hydro alcoholic extract was purchased from Giah Essence (Gorgan, Iran). The ingredients of the extract are shown in Table 1. The extract was diluted with National Formulary (NF), which is used as the basis of syrups in Iran pharmacies, to achieve the final concentrations of thymol and carvacrol of 20.5 and 2.85 mg/100 ml, respectively. The placebo was NF syrup with thyme artificial flavor. The syrup was prepared in 120 ml bottles and patients drank 10 ml of the extract three times/day for two months.

**Table 1.** ZM extract ingredients

Ingredients	Amount/100 mL
Thymol	55.4 mg
Carvacrol	7.7 mg
Flavonoids	63.2 mg
Dried Substance	1.6 g
Methyl Paraben	0.2%*
Propyl Paraben	0.05%*

\*Preservatives

### Gene expression

Total RNA was isolated from peripheral blood applying TriPure Isolation Reagent (Roche, Germany) according to the manufacturer's instructions. The mRNA was reverse transcribed using a RevertAid<sup>TM</sup> H Minus First Strand cDNA Synthesis kit (Fermentas, Germany). FOXP3, IL-10, IL-17, and TGF- $\beta$  expression was evaluated before and after treatment via SYBER green real-time PCR.

### Statistical analysis

Data was analyzed with SPSS software (version 16, USA). We used a t-test to compare the case and control groups. To analyze the normal distribution in the two groups, the Kolmogorov-Smirnov test was applied. Due to the normal distribution of data in two groups, we used the Pair T test to determine the differences. McNemar's and chi-squared ( $\chi^2$ ) tests were applied for qualitative parameters. Differences were considered to be significant when  $p < 0.05$ .

## Results

Of the 30 allergic rhinitis patients in this study, 17 (56.7%) were female and 13 (43.3%) were male. The patients' mean, minimum, and maximum ages were 33, 11, and 67 years, respectively. No significant differences were found between the experimental and control groups for sex or age distributions.

Based on the SNOT22 questionnaires, the ZM syrup had significantly greater alleviatory effects than the placebo ( $P<0.01$ ). Data about men and SD can be vividly seen in Table 2.

**Table 2.** Grades of SNOT22 questioner before and after treatment with ZM syrup and placebo.

	Groups	Mean $\pm$ SD
Before treatment	A (Placebo)	47.5 $\pm$ 2
	B (ZM)	32 $\pm$ 9.7
After treatment	A (Placebo)	37.7 $\pm$ 22
	B (ZM)	9.4 $\pm$ 6

**Table 3.** Cytokine mRNA expression before and at the end of the experiment.

Cytokine		Groups Control	Case	P Value
IL-10	Before treatment	0.2 $\pm$ 0.8	0.32 $\pm$ 0.63	0.08
	After treatment	0.3 $\pm$ 0.9	0.18 $\pm$ 0.6	
IL-17	Before treatment	0.12 $\pm$ 0.35	0.48 $\pm$ 1.05	0.04*
	After treatment	0.19 $\pm$ 0.34	0.15 $\pm$ 0.45	
FOXP3	Before treatment	0.21 $\pm$ 0.64	0.82 $\pm$ 2.06	0.13
	After treatment	0.29 $\pm$ 0.53	0.16 $\pm$ 0.44	
TGF- $\beta$	Before treatment	0.12 $\pm$ 0.4	0.46 $\pm$ 0.8	
	After treatment	0.33 $\pm$ 0.4	0.09 $\pm$ 0.13	

No significant differences in cytokine expression were found between the two groups before treatment. After treatment no significant differences were found for IL-10, TGF- $\beta$ , or FOXP3; however, IL-17 expression was significantly less in the ZM than in the control group ( $P<0.05$ ) (Table 3).

## Discussion

Based on the SNOT22 questionnaire the ZM syrup reduced symptoms in allergic rhinitis patients.

Boskabady analyzed the role of ZM in various inflammatory issues, including its relaxing effect on tracheal smooth muscle through competitive antagonism of histamine H1 receptors and demonstrated the stimulatory effect of the extract on  $\beta$ -adrenergic receptors and perhaps a blocking effect on muscarinic receptors (15). Another study showed that this plant had a preventive effect equal to that dexamethasone, phospholipase A2, histamine, and nitric oxide in sensitized guinea pigs (7).

Another investigation showed that carvacrol, the main constituent of ZM, inhibited lung inflammation and increased IgE, eosinophil peroxidase, and total and differential white blood cells in a guinea pig model (16). These data support the hypothesis that ZM modulates immunity by changing cytokine secretion profiles by upregulating IFN- $\gamma$  while downregulating IL-4 to interfere with the Th1/Th2 balance (17). Thus, ZM may be therapeutic for inflammatory, atopic, and some infectious diseases (15). In many of these conditions an appropriate Th1/Th2 balance is necessary to prevent undesirable allergic events. (17).

In our study IL-17 expression was less in patients who received ZM treatment than in those who had not. It has been shown in allergic diseases that T-regulatory (Treg) and Th17 immune cells have opposing immunomodulatory effects in the inflammatory process (18); therefore, we expected to see an increase in FOXP3 expression along with decreased IL-17. In our study FOXP3 decreased in allergic rhinitis

patients after ZM treatment. Our results differed from those of other studies that focused on the phenotype or functional analyses of Tregs and Th17 cells. In those studies IL-10 production by macrophages, B cells, and T cells was increased (19). Also TGF $\beta$  was increased and, together with IL-10, TGF $\beta$  might contribute to regulatory T-cell function and immunoglobulin class switching to IgA, IgG1, and IgG4 (20).

Limitations of this study included the small number of subjects and short period of ZM treatment. It is also possible that FOXP3, TGF $\beta$ , and IL-4 are not affected by ZM in two months and a longer period will be needed in future studies.

Our study demonstrated the therapeutic effect of ZM on allergic rhinitis patients. *Zataria multiflora*

also reduced IL-17, indicating that IL-17 may play a greater role in reducing allergic rhinitis symptoms than IL-10, FOXP3, or TGF $\beta$ . Because ZM is native to southwest Asia, and a well-known, inexpensive folk remedy it would likely be well-accepted by patients. Therefore, we suggest its use in addition to conventional treatment. Its use may also reduce the adverse side effects of corticosteroids and other therapeutic agents by reducing their effective dosages.

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**Conflict of interest:** None to be declared.

## References

- Greiner AN, Hellings PW, Rotiroti G, Scadding GK. Allergic rhinitis. *The Lancet*. 2012;378(9809):2112-22.
- De Marco R, Cappa V, Accordini S, Rava M, Antonicelli L, Bortolami O, et al. Trends in the prevalence of asthma and allergic rhinitis in Italy between 1991 and 2010. *European Respiratory Journal*. 2012;39(4):883-92.
- Wheatley LM, Togias A. Allergic rhinitis. *New England Journal of Medicine*. 2015;372(5):456-63.
- Bousquet PJ, Demoly P, Devillier P, Mesbah K, Bousquet J. Impact of allergic rhinitis symptoms on quality of life in primary care. *International archives of allergy and immunology*. 2012;160(4):393-400.
- Rondón C, Campo P, Togias A, Fokkens WJ, Durham SR, Powe DG, et al. Local allergic rhinitis: concept, pathophysiology, and management. *Journal of Allergy and Clinical Immunology*. 2012;129(6):1460-7.
- Braido F, Scichilone N, Lavorini F, Usmani O, Dubuske L, Boulet L, et al. Manifesto on small airway involvement and management in asthma and chronic obstructive pulmonary disease: an Interasma (Global Asthma Association-GAA) and World Allergy Organization (WAO) document endorsed by Allergic Rhinitis and its Impact on Asthma (ARIA) and Global Allergy and Asthma European Network (GA 2 LEN). *Asthma Research and Practice*. 2016;2(1):12.
- Boskabady MH, Jalali S, Farkhondeh T, Byrami G. The extract of *Zataria multiflora* affect tracheal responsiveness, serum levels of NO, nitrite, PLA2, TP and histamine in sensitized Guinea pigs. *Journal of ethnopharmacology*. 2014;156:301-8.
- Sajed H, Sahebkar A, Iranshahi M. *Zataria multiflora* Boiss.(Shirazi thyme)—an ancient condiment with modern pharmaceutical uses. *Journal of ethnopharmacology*. 2013;145(3):686-98.
- Saei-Dehkordi SS, Tajik H, Moradi M, Khalighi-Sigaroodi F. Chemical composition of essential oils in *Zataria multiflora* Boiss. from different parts of Iran and their radical scavenging and antimicrobial activity. *Food and Chemical Toxicology*. 2010;48(6):1562-7.
- Ghasemi Dehkordi N. Iranian herbal pharmacopoeia. Ministry of Health Pub Tehran. 2002;1:105.
- Ariaee-Nasab N, Vahedi Z, Vahedi F. Inhibitory effects of cinnamon-water extract on human tumor cell lines. *Asian Pacific Journal of Tropical Disease*. 2014;4:S975-S8.
- Oskouei YM, Hosseini RF, Ahanchian H, Jarahi L, Ariaee N, Azad FJ. Report of Common Aeroallergens among Allergic Patients in Northeastern Iran. *Iranian journal of otorhinolaryngology*. 2017;29(91):89.
- Van Cauwenberge P, De Belder T, Vermeiren J, Kaplan A. Global resources in allergy (GLORIA):

allergic rhinitis and allergic conjunctivitis. Clinical & Experimental Allergy Reviews. 2003;3(1):46-50.

14. Jalessi M, Farhadi M, Kamrava SK, Amintehran E, Asghari A, Hemami MR, et al. The reliability and validity of the persian version of sinonasal outcome test 22 (snot 22) questionnaires. Iranian Red Crescent Medical Journal. 2013;15(5):404.

15. Boskabady MH, Tabanfar H, Gholamnezhad Z, Sadeghnia HR. Inhibitory effect of *Zataria multiflora* Boiss and carvacrol on histamine (H1) receptors of guinea-pig tracheal chains. Fundamental & clinical pharmacology. 2012;26(5):609-20.

16. Boskabady MH, Tabatabaee A, Jalali S. Potential effect of the extract of *Zataria multiflora* and its constituent, carvacrol, on lung pathology, total and differential WBC, IgE and eosinophil peroxidase levels in sensitized guinea pigs. Journal of Functional Foods. 2014;11:49-61.

17. Boskabady MH, Mehrjardi SS, Rezaee A, Rafatpanah H, Jalali S. The impact of *Zataria multiflora* Boiss extract on in vitro and in vivo Th 1/Th 2 cytokine (IFN- $\gamma$ /IL4) balance. Journal of ethnopharmacology. 2013;150(3):1024-31.

18. Kleinewietfeld M, Hafler DA, editors. The plasticity of human Treg and Th17 cells and its role in autoimmunity. Seminars in immunology; 2013: Elsevier.

19. Petti FB, Liguori A, Ippoliti F. Study on cytokines IL-2, IL-6, IL-10 in patients of chronic allergic rhinitis treated with acupuncture. Journal of traditional Chinese medicine= Chung i tsa chih ying wen pan/sponsored by All-China Association of Traditional Chinese Medicine, Academy of Traditional Chinese Medicine. 2002;22(2):104-11.

20. Larché M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. Nature Reviews Immunology. 2006;6(10):761-71.