

Potential Adjuvant Effects of *Nigella sativa* Seeds to Improve Specific Immunotherapy in Allergic Rhinitis Patients

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Key Words

Nigella sativa · Phagocytosis · Intracellular killing activity · Allergic rhinitis · Active immunotherapy

Abstract

Objective: To investigate the effects of *Nigella sativa* seed supplementation on symptom levels, polymorphonuclear leukocyte (PMN) functions, lymphocyte subsets and hematological parameters of allergic rhinitis. **Subjects and Methods:** Twenty-four patients randomly selected from an experimental group of 31 (mean age 34 years) sensitive to house dust mites with allergic rhinitis and a control group of 8 healthy volunteers (mean age 23 years) were treated with allergen-specific immunotherapy in conventional doses for 30 days. After a month of immunotherapy, 12 of the 24 patients and the 8 healthy volunteers were given *N. sativa* seed supplementation (2 g/day orally) for 30 days. The remaining 12 patients continued only on immunotherapy during the same period. The other 7 patients were given 0.1 ml saline solution subcutaneously once a week as a placebo. The symptom scores, PMN functions, lymphocyte subsets and other hematological parameters were evaluated before and after all treatment periods. **Results:** There was a statistically significant increase in the phagocytic and intracellular killing ac-

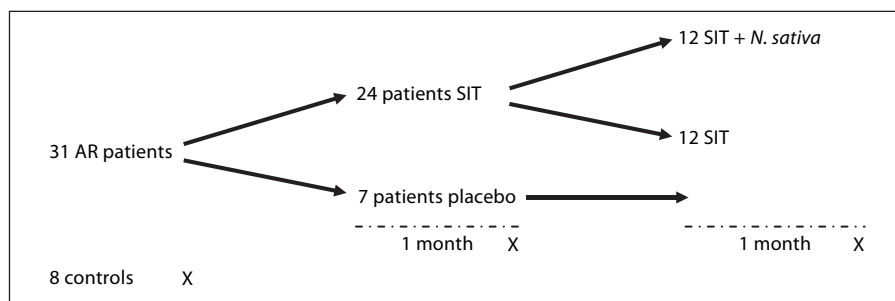
tivities of PMNs of patients receiving specific immunotherapy, especially after the addition of *N. sativa* seed. The CD8 counts of patients receiving specific immunotherapy plus *N. sativa* seed supplementation significantly increased compared to patients receiving only specific immunotherapy. PMN functions of healthy volunteers significantly increased after *N. sativa* seed supplementation compared to baseline. **Conclusion:** *N. sativa* seed supplementation during specific immunotherapy of allergic rhinitis may be considered a potential adjuvant therapy.

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Introduction

Linn. *Nigella sativa*, also known as black seed or black cumin, is a member of the Ranunculaceae family, an annual herbaceous plant that grows in countries bordering the Mediterranean sea, Pakistan and India [1]. It has been used as a natural remedy in many Middle Eastern countries for over 2,000 years. It is a traditional folk medicine used for a wide range of illnesses, such as bronchial asthma, headache, dysentery, infections, obesity, back pain, hypertension, dermatological and gastrointestinal problems [2, 3].

Fig. 1. Diagram of the study design. Symptom scores (X), PMN functions, lymphocyte subsets and other hematological parameters were evaluated. AR = Allergic rhinitis.



N. sativa seed is commonly eaten alone or in combination with honey and in many food preparations [4]. Black cummin seeds are of importance as a carminative and spice, as they are often used as a condiment in bread and other dishes [5]. Black seed contains more than 100 valuable nutrients, approximately including protein: 21%, carbohydrates: 38% and plant fats and oils (fixed oil and volatile oil): 35%. The active ingredients of black seed are thymoquinone, nigellone and fixed oils. Other ingredients include linoleic acid, oleic acid, calcium, potassium, iron, zinc, magnesium, selenium, vitamin A, vitamin B, vitamin B₂, niacin, vitamin C and essential oil, and monosaccharides in the form of glucose, rhamnose, xylose and arabinose [2, 4, 5].

Several studies have focused on an explanation of the mechanism of the beneficial effects of *N. sativa*. In relatively low concentrations, nigellone, the carbonyl polymer of thymoquinone, has been found to be effective in inhibiting histamine release from mast cells in vitro [4]. *N. sativa* oil has been demonstrated to modify leukotriene synthesis and inhibit histamine release [3]. It has been shown that *N. sativa* increased the proliferative response of spleen cells to allogenic cells and their IL-3 production [6].

N. sativa seed constituents possess potential immunomodulatory effects and are reported to have antibacterial, antifungal, antihelminthic and antiviral properties. For instance, *N. sativa* seed extracts and thymoquinone have shown potential protective effects against *Schistosoma mansoni* infections, and antibacterial activity against several bacterial strains including *Escherichia coli*, *Bacillus subtilis*, *Streptococcus faecalis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* as well as against the pathogenic yeast *Candida albicans* and fungus [4].

The mechanism of action of *N. sativa* on allergic diseases is still unclear. Büyüköztürk et al. [7] demonstrated that *N. sativa* oil had no effects on Th1 and Th2 cytokine production of splenic mononuclear cells in mice. How-

ever, other studies have shown that it affects Th2 cytokine production in the lung [8–10]. The main active constituent, thymoquinone, has been found to attenuate allergic inflammation by inhibiting Th2 cytokines and eosinophil infiltration in the airways. On the other hand, there is no sufficient study related to the clinical benefits of black seed on the symptoms of allergic patients. Recently, Kalus et al. [11] have shown that the oil of *N. sativa* was effective in relieving symptoms of allergic diseases. In the present study, we have assessed the effects of *N. sativa* seed supplementation on symptom levels, peripheral blood polymorphonuclear leukocyte (PMN) functions (phagocytic and intracellular killing activity) and lymphocyte subsets in patients with allergic rhinitis.

Subjects and Methods

Subjects

Thirty-one patients (mean age 34 years) with allergic rhinitis sensitive only to house dust mites were enrolled in the experimental group. Exclusion criteria were the existence of an infectious, rheumatic, malignant or any other systemic disease and detection of a seasonal allergen sensitivity. The control group consisted of 8 healthy volunteers (mean age 23 years). The diagnosis of allergic rhinitis was based on history, physical examination and skin prick tests.

Treatment with Specific Immunotherapy and *N. sativa* Seed Supplementation

After diagnosis of house dust mite sensitivity, 24 patients from the experimental group were randomly selected to begin treatment with subcutaneous allergen-specific immunotherapy (SIT, Alutard D. *pteronysinus* + *D. farinea*, ALK-Abello, Denmark) using the conventional doses [12] (fig. 1). After a month of immunotherapy, 12 of them and all the healthy volunteers were given *N. sativa* seed supplementation (2 g/day orally) for 30 days. The remaining 12 patients continued to take immunotherapy only during seed supplementation. A third group of patients (n = 7) were given 0.1 ml saline solution subcutaneously once a week as a placebo for 2 months (fig. 1). *N. sativa* (L.) (MARE 9025) was authenticated by Dr. Ertan Tuzlacı, Department of Pharmaceutical

Table 1. Comparison of lymphocyte subsets in different stages of the study

Therapy	Lymphocyte subsets				
	CD3	CD19	CD4	CD8	CD16/56 (NK)
Before SIT	70.64 ± 8.82	12.18 ± 4.85	40.45 ± 10.96	31.36 ± 6.93	20.00 ± 11.65
After 1st month of SIT	68.45 ± 10.49	11.09 ± 5.64	42.64 ± 6.48	26.73 ± 6.3*	13.91 ± 5.82
After SIT + <i>N. sativa</i> seed supplementation (30 days)	70.36 ± 9.43	11.18 ± 7.06	40.91 ± 7.14	30.55 ± 5.6*	13.00 ± 5.36
After 2nd month of SIT	76.00 ± 3.36	7.42 ± 2.50	43.71 ± 7.8	31.00 ± 6.87	9.85 ± 2.79

Results are expressed as mean ± SD. ANOVA and Student-Newman-Keuls test were performed on the results (repeated measures of ANOVA and Student-Newman-Keuls Multiple comparison test). * p < 0.05.

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Measurement of Allergic Rhinitis Symptoms

Patients were asked to rate their symptom levels on a visual analog scale (VAS, 0 = no symptoms, 10 = worst symptoms) at the first visit after the first month of SIT and after the SIT + *N. sativa* supplementation placebo or at the end of the second month of SIT.

Isolation of PMNs

Peripheral blood samples (10 ml) from patients with allergic rhinitis and healthy volunteers were drawn with ethylenediaminetetraacetic acid (EDTA). PMNs from venous blood with EDTA (1×10^7 cells/ml) were isolated by the Ficoll-Hypaque gradient centrifugation method previously described [13].

Measurement of PMN Activities

Phagocytosis and intracellular killing activity were assayed by modifying the method of Alexander et al. [14]. In the modified method, Ficoll was used instead of dextran and PMNs were counted by microscope instead of using the standard pour plate technique. PMN viability was assayed as 98% by trypan blue staining. PMNs were suspended in Hanks' buffered salt solution (HBSS) and cell density was adjusted by dilution (1×10^7 cell/ml) [15–17].

A clinical strain of *C. albicans* was used in order to determine the phagocytic and intracellular killing activities of PMNs. *C. albicans* viability was assayed as greater than 98% by methylene blue staining. PMNs were suspended in HBSS and incubated at 37°C for 30 min in a shaking incubator. In a separate tube, *C. albicans* was suspended in HBSS and then an aliquot of sterile human serum (1:4) was added to induce opsonization and the mixture was incubated at 37°C for 30 min. Subsequently, opsonized yeast cells were added to the PMN tube. The final mixture contained 5×10^6 PMNs/ml and 5×10^6 yeasts/ml. Dead yeast cells were determined by adding 0.01% methylene blue stain (1:1 ratio) in the last 5 min of the incubation. The phagocytic activity was determined by the percentage of PMNs that had phagocytosed yeast cells. Intracellular killing activity was determined by the percentage of PMNs that included killed yeast cells [15, 17, 18].

Immunomonitoring by Flow Cytometry

Patient immune profiles were determined by analyzing blood samples, whole blood lysis on flow cytometry (Becton Dickinson FACSCalibur) following heparinization and treating with monoclonal antibodies (CD3, CD4, CD8, CD19, CD16/56-NK). Ten thousand cells were counted in the appropriate lymphocyte gate for each tube's sample in flow cytometry and then the percentage values of all markers were determined by using CellQuest software program.

Statistics

The data were analyzed using ANOVA and paired Student-Newman-Keuls multiple comparison test.

Results

There were no significant differences between study groups in terms of lymphocyte subsets, except the CD8 counts that showed a significant decrease when analyzed after the first month of SIT (table 1, p < 0.05). The number of CD8 T cells after SIT recovered when SIT patients received a 1-month supplementation with *N. sativa* seeds. The PMN functions for different treatment modalities and periods are given in table 2. Phagocytic and intracellular killing activities of patients who received SIT for 1 month and SIT plus *N. sativa* seed supplementation for 30 days showed significant increases compared to phagocytic and intracellular killing activities before SIT (p < 0.001, p < 0.01). Phagocytic and intracellular killing activities of PMNs of the same patients who received SIT plus *N. sativa* seed supplementation for 30 days also showed significant increases compared to those after 1 month of SIT (table 2, p = 0.002, p = 0.05). However, PMN activities of those patients who were given only SIT were not different at the end of the second month compared to the results obtained after the first month of SIT (p > 0.05).

Table 2. Comparison of PMN functions in different periods of the study

Therapy periods	PMN functions	
	phagocytosis, %	intracellular killing activity, %
Before SIT (n = 31)	40.17 ± 9.80 ^a	1.75 ± 1.54 ^b
After 1st month of SIT (n = 24)	57.67 ± 15.13 ^c	3.00 ± 2.00 ^d
After SIT + <i>N. sativa</i> seed supplementation (30 days) (n = 12)	70.50 ± 8.17 ^e	4.84 ± 2.27 ^f
After 2nd month of SIT only (n = 12)	53.28 ± 4.38 ^g	1.71 ± 1.88 ^h
Placebo (saline solution) (n = 7)	36.85 ± 11.58 ⁱ	1.00 ± 0.81 ^j

Results are expressed as means ± SD. ANOVA and Student-Newman-Keuls test were performed on the results (repeated measures of ANOVA and Student-Newman-Keuls multiple comparisons test). * p < 0.05, p^(c vs. a) < 0.0001, p^(d vs. b) = 0.01, p^(e vs. c) = 0.002, p^(f vs. d) = 0.05, p^(e vs. g) = 0.002, p^(f vs. h) < 0.001, p^(e vs. i) < 0.0001, p^(f vs. j) = 0.0003.

Phagocytic and intracellular killing activities of PMNs of the same patients who received SIT plus *N. sativa* seed supplementation for 30 days also showed significant increases compared to those after 2 months of SIT (table 2, p = 0.002, p < 0.001). PMN phagocytic and intracellular killing activities of patients receiving placebo were significantly lower compared to patients who received either SIT or SIT + *N. sativa* seed supplementation (p < 0.0001, p = 0.0003). PMN functions of healthy young volunteers significantly increased after *N. sativa* seed supplementation compared to those before supplementation (table 3, p < 0.001, p < 0.01).

According to the one-way ANOVA test, power analysis test was done and power was found as (1 - β) = 0.9298 for phagocytosis and (1 - β) = 0.8426 for intracellular killing activity when α = 0.05.

Discussion

This study demonstrated that *N. sativa* supplementation has beneficial effects on the symptoms of patients with allergic rhinitis. These results confirmed the findings obtained by Kalus et al. [11], who showed the efficacy of orally given *N. sativa* oil in allergic rhinitis, bronchial asthma and atopic dermatitis. From this point of view, *N. sativa* may be considered an appropriate 'adjuvant' for the therapy of allergic diseases. It is well known

Table 3. PMN functions of healthy young volunteers before and after *N. sativa* seed supplementation (n = 8)

<i>N. sativa</i> seed supplementation (2 g/day for 30 days orally)	PMN functions	
	phagocytosis, %	intracellular killing activity, %
Before supplementation	43.88 ± 10.41	1.25 ± 1.38
After supplementation	69.13 ± 8.91 ^{**}	3.62 ± 2.20 [*]

Results are expressed as means ± SD. Paired t test was performed on the results. * p < 0.01, ** p < 0.001.

that SIT, which depends on the administration of increasing doses of allergen extracts, is the only specific and curative approach for the treatment of IgE-mediated allergy. In recent years, adjuvant therapies have been investigated in order to increase the efficacy of SIT in the therapy of allergic diseases. *Mycobacterium vaccae*, lipopolysaccharide, *Lactobacillus* spp. and oral bacterial extracts have been examined for their adjuvant effects [19].

The beneficial effects of *N. sativa* seed supplementation on the symptoms of allergic rhinitis may be due to its antihistaminic properties. Chakravarty [3] demonstrated that when rat macrophages were incubated with nigellone thymoquinone (carbon polymer) obtained from *N. sativa* seed, nigellone inhibited histamine release from macrophages, intracellular calcium release, protein kinase C activation and oxidative energy metabolism [3]. Preclinical [1, 6] and clinical [2] studies have also shown antihistaminic effects of *N. sativa* seed using a gastric ulcer model induced by oral administration of ethanol, which caused a significant increase in mucosal histamine content. In rats pretreated with *N. sativa* oil before ulcer induction, gastric mucosal histamine content significantly decreased compared with the nonpretreated group [1]. Another study showed that *N. sativa* oil inhibits the COX and 5-lipoxygenase pathways of arachidonic acid metabolism and decreases the synthesis of thromboxane and leukotrienes [16]. Since leukotrienes are potent mediators of asthma and histamine plays an important role in immediate hypersensitivity reactions, the above findings might explain the mechanism mediating the efficacy of *N. sativa* on allergic diseases and asthma. When nigellone was administered to children and adults during the treatment of bronchial asthma, *N. sativa* oil decreased the IgE level, eosinophil count

and endogenous cortisol in plasma and urine [2], indicating the effectiveness of *N. sativa* oil and seeds as an adjuvant for the treatment of allergic diseases. Also no sign of toxicity was observed.

In our study the PMN functions of patients who received SIT plus *N. sativa* seed supplementation increased compared to those taking only SIT or placebo. As is seen in the Results section, the power analysis is in accordance with the p values that have been determined by repeated measures of ANOVA and Student-Newman-Keuls multiple comparisons test shown in table 2.

Currently, only one study has explored the effects of *N. sativa* on the leukocyte phagocytic activity of PMNs in vitro. In that study, no effect of *N. sativa* or its fractions was noticed on the phagocytic or killing activities of PMNs in the presence of *S. aureus* bacteria. On the other hand, *N. sativa* seed supplementation with specific immunotherapy increased CD8 cell count, which was decreased after the 1st month of specific immunotherapy. The mechanism of *N. sativa* stimulation of the increase in PMN functions and CD8 cells is unclear. Haq et al. [6] demonstrated that *N. sativa* seeds activated T lymphocytes to secrete IL-3 and to cause enhanced IL-1 β production. In a later study, the same authors had fractionated *N. sativa* proteins by ion exchange chromatography and showed that some proteins have suppressive and other stimulatory properties in lymphocyte cultures [20]. It may be speculated that an important number of cytokines induced by *N. sativa* produce immunomodulatory changes.

The augmented PMN function by *N. sativa* seed supplementation can explain the beneficial effect of this sup-

plementation on allergic rhinitis patients. The results of this study showed that *N. sativa* also increased the PMN functions in healthy controls, indicating that this effect is not specific to allergic patients. Although there is no confirmed evidence that allergic rhinitis patients are more susceptible to upper respiratory tract infections than normal, it has been observed that such infections lead to symptom aggravation in rhinitis patients, last longer and tend to become chronic. From this point of view, it can be speculated that augmented PMN functions stimulated by *N. sativa* supplementation may improve the response to microorganisms in rhinitis patients.

Conclusion

Our findings suggest that *N. sativa* seed supplementation may have beneficial immunomodulatory and anti-allergic effects on allergic patients and healthy subjects as well. Our results show that *N. sativa* seed supplementation together with SIT seem to achieve better clinical recovery of the suppressed immune system of allergic patients. The potential role of *N. sativa* as an adjuvant therapy in patients treated with SIT should be further studied and clarified.

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