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ORIGINAL ARTICLE

The possible prophylactic effect of *Nigella sativa* seed extract in asthmatic patients

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ABSTRACT

In previous studies, the relaxant, anticholinergic (functional antagonism) antihistaminic, effects of *Nigella sativa* have been demonstrated on guinea-pig tracheal chains. In the present study, the prophylactic effect of boiled extract of *N. sativa* on asthmatic disease was examined. Twenty-nine asthmatic adults were randomly divided into control group (14 patients) and study group (15 patients), and they were studied for 3 months. In the study group 15 mL/kg of 0.1 g% boiled extract and in the control group a placebo solution was administrated daily throughout the study. Asthma symptom score, asthma severity, frequency of symptoms/week and wheezing were recorded in the beginning (first visit), 45 days after treatment (second visit), and at the end of the study (third visit). Pulmonary function tests (PFTs) were also measured, and the drug regimen of the patients was evaluated at three different visits. All asthma symptoms, frequency of asthma symptoms/week, chest wheezing, and PFT values in the study group significantly improved in the second and third visits compared with the first visit ($P < 0.05$ to $P < 0.001$). In addition, further improvement of chest wheezing and severity of disease on the third visit were observed compared with the second visit in this group ($P < 0.05$ for both cases). In the third visit all symptoms in the study group were significantly different from those of the control group ($P < 0.01$ to $P < 0.001$). However, in the control group, there were only small improvements in some parameters in just the second visit. The usage of inhaler and oral β-agonists, oral corticosteroid, oral theophylline and even inhaler corticosteroid in the study group decreased at the end of the study while there were no obvious changes in usage of the drugs in control subjects. The results of phase I study generally suggest a prophylactic effect of *N. sativa* on asthma disease and warrant further research regarding this effect.

INTRODUCTION

Nigella sativa is a grassy plant with green to blue flowers and small black seeds, which grows in temperate and cold climate areas. The seeds of *N. sativa* contain thymoquinone, monoterpenes such as *p*-cymene and α-pinene [1], the alkaloids nigellidine and nigellimine [2,3] and a saponin [4]. All chemical composition of the plant was summarized in a recent review [5].

Several therapeutic effects, including anti-asthma and dyspnoea, have been described for the seeds of *N. sativa* in ancient Iranian medical books [6]. In Arabian folk medicine also, the whole black seeds alone or in combination with honey are prompted for treatment of bronchial asthma. There is evidence of relaxant effects of the volatile oil from this plant on different smooth muscle preparations including rabbit aorta [7], rabbit jejunum [8], and guinea-pig isolated tracheal muscle [9]. Mahfouz and El-Dakhakhny [10] reported that the volatile oil from *N. sativa* protected guinea-pigs against histamine-induced bronchospasm, but it did not affect histamine H_1 receptors in isolated tissues. However, in an in vivo study, increasing respiratory rate and intratracheal pressure of guinea-pigs due to

i.v. administration of volatile oil from *N. sativa* has been demonstrated [11].

The results of our studies have also shown differing pharmacological effects of *N. sativa* on guinea-pig tracheal chains, including: relaxant and functional antagonistic effects on muscarinic receptors [12], inhibitory effect on histamine (H_1) receptors [13], inhibitory effect on calcium channels [14], opening effect on potassium channels [15] and stimulatory effect on β-adronceptors [16]. The antitussive effect of this plant on the guinea-pig [17] was also demonstrated. Both systemic and local administrations of essential oil from this plant are showed to have anti-inflammatory activity [18]. The therapeutic effect of *N. sativa* oil on patients with allergic diseases (allergic rhinitis, bronchial asthma, atopic eczema) was also demonstrated [19]. In addition, Labib Salem in a recent review summarized the immunomodulatory and therapeutic properties of the *N. sativa* L. seed and emphasized the potent immunomodulatory effects of this plant [5].

Therefore, in the present study, the prophylactic effect of boiled extract from *N. sativa* on asthmatic airways was examined.

MATERIALS AND METHODS

Plant, extract and drugs

Nigella sativa was collected from Torbat Heydarieh (northeast Iran), and its seeds were dried at room temperature in the absence of sunlight. The plant was identified by botanists in the herbarium of Ferdowsi University of Mashhad; and the specimen number of the plant is 293-0303-1. The boiled extract of the seeds of the plant was prepared as follows: 10 g of the chopped, dried plant was boiled with 100 mL distilled water for 15 min and allowed to cool at room temperature. The extract was then filtered with a clean cotton cloth, and the volume of extract was adjusted to 100 mL by evaporation. Therefore, the final extract concentration was 0.1 g%. The constituents of the essential oil of *N. sativa* was assessed using HPLC (Schimadzue (Tokyo, Japan), SSPD-10AVP) by a phytochemsit with ODS column [20].

Patients

Twenty-nine asthmatic patients were recruited from the Asthma Clinic, Ghaem Medical Centre, Mashhad University of Medical Sciences, and divided to control group (14 patients, 2 male, 12 female, aged 48.20 ± 11.91 years, height 157.80 ± 7.81 cm) and study group (15 patients, 4 male, 11 female, aged 35.87 ± 12.79 years, height 161.50 ± 9.38 cm) in random order. The researcher was unaware of the allocation of patients in the two groups. All patients had the following criteria: (1) previously diagnosed asthma by a physician and having two or more of the following symptoms: recurrent wheeze, recurrent cough or tightness at rest; wheeze, cough or tightness during the night or early morning; wheeze or cough during exercise, (2) having forced expiratory volume in 1 s (FEV_1) and peak expiratory flow (PEF) less than 80% of predicted values, (3) had no history or symptoms of cardiovascular or other respiratory diseases that required treatment (excluding the common cold). The studied patients had moderate to severe asthma according to GINA guidelines [21]. The protocol was approved by the Ethics Committee of our institution, and each subject gave informed consent. The study was carried out during spring and summer 2005.

Treatment duration and administered drugs

Each patient was treated for a period of 3 months and was visited and controlled three times during treatment duration. The treatment regimen of all studied patients included inhaled corticosteroid, mostly beclomethasone dipropionate (400–1400 μg depending on the severity of the disease) and in some cases fluticasone dipropionate (500 μg), inhaler and oral β-agonists, oral corticosteroid and oral theophylline. In addition, the patients of the study group were given 15 mL/kg of 0.1 g% boiled extract (containing 10 g% glucose) daily; and those of the control group were given a semi-roasted glucose solution (10 g% in saline) as a placebo for *N. sativa* extract throughout the study. The placebo solution did not contain any chemical that could affect asthma disease. The study was performed in the double-blind manner.

Protocol

Medical examination was performed and asthma symptoms were taken in all patients at the beginning, in the middle (45 days after starting the study on each patient), and at the end of the study. Asthma symptom score was counted according to *Table I* [22]. The degree of wheezing was considered between 0 and 3 as follows: no wheezing = 0, barely heard wheezing = 1, moderate wheezing = 2, and loud wheezing = 3. Pulmonary function tests were also measured in the beginning and at the end of the study using a spirometer with a pneumotachograph sensor (Model ST90; Sangyo Co., Ltd, Fukuda, Japan). Prior to the pulmonary function testing, the required manoeuvre was demonstrated by

Table I The criteria for asthma severity score.

Symptom	Frequency	Score
Night wheezing	None	0
	Sleeping well with a little wheezing	1
	Waking once at night	2
	Waking most of night	3
Night cough	None	0
	Sleeping well with a little cough	1
	Waking once at night	2
	Waking most of night	3
Exercise cough and wheezing	Non-existent during strong exercise	0
	Existence only during strong exercise	1
	Existence during climbing stairs	2
	Existence during ordinary activity	3
Morning cough, tightness and wheezing	None	0
	Existence in case of exertion	1
	Mild symptoms without exertion	2
	Waking in the morning due to symptoms	3
Day time cough, tightness and wheezing	None	0
	Once a day	1
	Two or more times a day	2
	Affecting day-time activity	3
Total score		16

the operator, and subjects were encouraged and supervised throughout test performance. Pulmonary function testing was performed using the acceptable standards outlined by the American Thoracic Society with subjects in a standing position and wearing nose clips [23]. All tests were carried out between 10:00 and 17:00 h. Lung function tests were performed three times in each subject by an acceptable technique [22]. The highest level for forced vital capacity (FVC), FEV_1, PEF, maximal expiratory flow at 75%, 50%, and 25% of the FVC (MEF_{75}, MEF_{50}, and MEF_{25} respectively) was taken independently from the three curves.

Data analysis

The data of asthma symptom score, chest wheeze, frequency of occurrence of symptoms/week and PFT values were expressed as mean ± SEM because the variability of these data among asthmatic subject were considerable; but those of height and age were expressed as mean ± SD because there were small differences in these data between subjects. The percentage change in the asthma symptom score, chest wheeze, frequency of occurrence of symptoms/week and PFT values in the middle (second visit) and at the end of the study (third visit) were calculated as follows: [data of the second or third visit − data of the first visit (baseline values)]/data of the first visit × 100.

All data were compared between the beginning, the middle and the end of the study (three visits) using one-way analysis of variance (ANOVA) with Tukey–Kramer multiple post hoc test. The data of control and study groups were compared using unpaired '*t*' test. The difference of percentage of patients using each type of drug between each two visits was tested by chi-square testing on 2×2 contingency tables. Significance was accepted at $P < 0.05$.

RESULTS

Constituents of the essential oil of *N. sativa*

The main constituents of the essential oil of *N. sativa* included thujene α (8.2%), pinene α (2%), pinene β (2.9%), terpinene α (1.8%), cymenen P (41.7%), limonene (3%), terpinene G (12.8%), campholenel α (9.7%), carvacrol (2.2%) and thymoqunone (2%) [20].

Asthma symptoms

All symptom scores, according to GINA guidelines, improved after even 45 days treatment of asthmatic patients in the study group. In the control group only night wheezing, exercise wheeze and cough, and morning wheeze and cough in the second visit were significantly lower than in the first visit ($P < 0.05$ to $P < 0.01$). However, there was no significant improve-

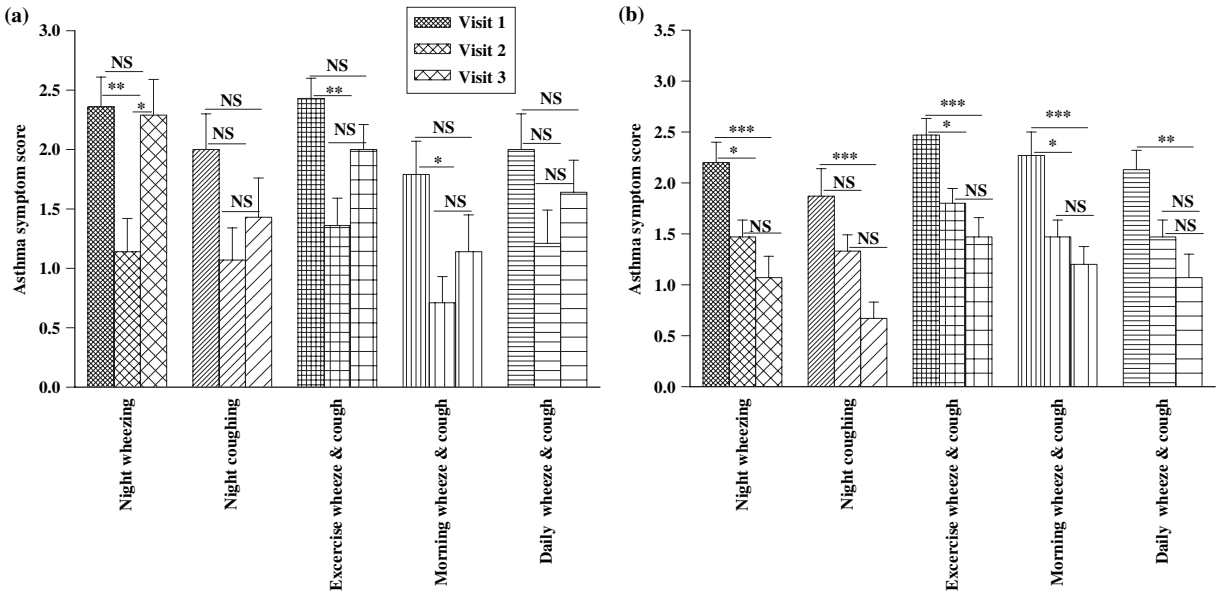

Figure 1 Comparison of symptom score of control (a) and study (b) groups of asthmatic patients at the beginning (fine filled bars), middle (medium filled bars) and at the end of 2-month study (coarse filled bars). Statistical difference in different parameter between three visits: NS, non-significant difference, $^{*}P < 0.05$, $^{**}P < 0.002$, $^{***}P < 0.001$.

Table II Asthma symptoms and severity in control and study groups of patients in the beginning, and their percentage decrease in the middle and at the end of the study.

	Beginning		Middle		End	
Symptoms	Control group	Study group	Control group	Study group	Control group	Study group
Night wheezing	2.36 ± .25	2.20 ± 0.20 NS	55.94 ± 10.13	34.44 ± 5.97 NS	5.99 ± 8.50	65.56 ± 0.12***
Night coughing	2.00 ± 0.30	1.87 ± 0.27 NS	41.67 ± 11.15	33.32 ± 6.90 NS	33.33 ± 11.59	74.45 ± 7.78**
Exercising W and C	2.43 ± 0.17	2.47 ± 0.163 NS	40.47 ± 9.03	28.87 ± 5.00 NS	15.46 ± 8.97	53.32 ± 7.49**
Morning W and C	1.79 ± 0.28	2.27 ± 0.23 NS	32.14 ± 10.26	32.21 ± 4.73 NS	14.29 ± 8.35	51.11 ± 7.54**
Daily W and C	2.00 ± 0.3	2.13 ± 0.19 NS	41.74 ± 14.48	35.55 ± 5.11 NS	7.14 ± 9.48	60.00 ± 10.26*
Weekly W and C	6.07 ± 0.74	4.47 ± 0.45 NS	24.31 ± 4.74	40.06 ± 4.36*	10.48 ± 9.97	58.45 ± 5.25***
Chest wheezing	2.57 ± 0.17	2.6 ± 0.13 NS	26.17 ± 4.85	23.32 ± 4.54 NS	22.61 ± 7.74	44.43 ± 3.12*
Asthma severity	3.20 ± 0.19	3.27 ± 0.18 NS	29.20 ± 5.25	23.32 ± 3.27 NS	5.36 ± 3.87	49.45 ± 3.50***

W, wheezing; C, coughing. All values were quoted as mean ± SEM. Statistical difference in different parameter between control and study group: NS, non-significant difference, $^{*}P < 0.01$, $^{**}P < 0.005$, $^{***}P < 0.001$.

ment in symptoms between the third (end of 3-month study) and the first visits (*Figure 1a*). In the study group, all asthma symptoms were improved significantly in the second ($P < 0.05$ to $P < 0.001$) and third visits ($P < 0.001$ for all symptoms) than in the first visit (baseline values) except night cough in the second visit (*Figure 1b*). In addition, there was no significant difference in symptoms between the third and second visit in the study group. While at the beginning of the study there were no significant differences in asthma symptoms between control and study groups; in the third visit, there was significantly more reduction in all symptoms in the study subjects compared with the control group ($P < 0.01$ to $P < 0.001$) (*Table II*).

Severity of asthma and wheezing

Asthma severity score, frequency of occurrence of asthma symptoms/week, and chest wheezing were also improved at the end of the study (after 3-month treatment) in study groups. In the control group only chest wheezing and severity of asthma in the second visit were significantly lower than the first visit ($P < 0.05$ for

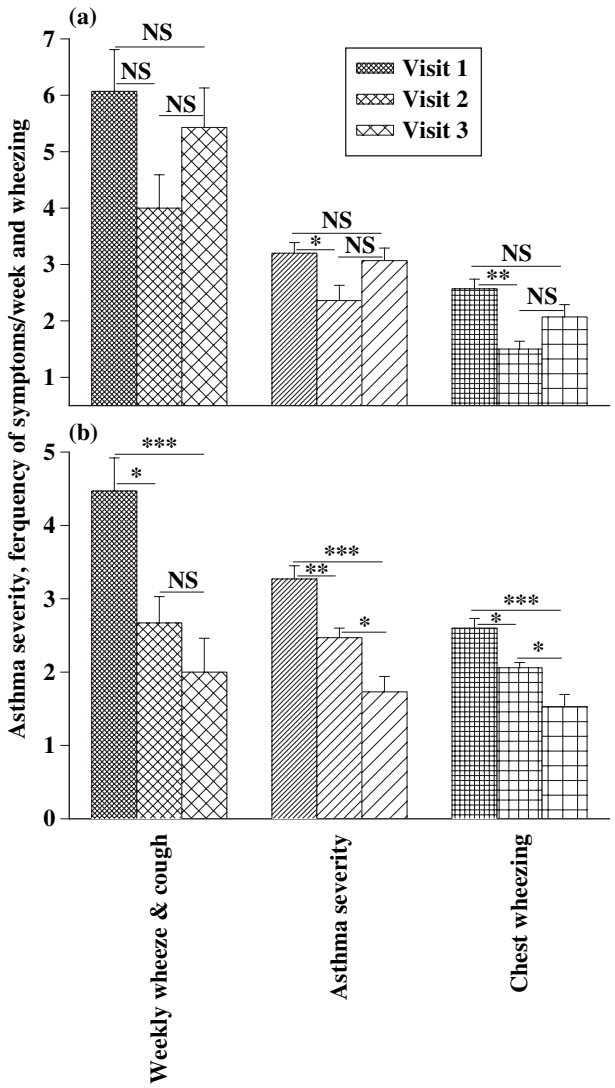

Figure 2 Comparison of severity of asthma according GINA guideline, frequency of asthma symptoms/week and chest wheeze of control (a) and study (b) groups of asthmatic patients at the beginning (fine filled bars), middle (medium filled bars) and at the end of 2-month study (coarse filled bars). Statistical difference in different parameter between three visits: NS, non-significant difference, $^{*}P < 0.05$, $^{**}P < 0.002$, $^{***}P < 0.001$.

both cases). However, there was no significant difference in the above parameters between the third and first visits (*Figure 2a*). In the study group, the asthma severity score, frequency of asthma symptoms/week, and chest wheezing were significantly lower in the second and third visits than in the first visit ($P < 0.05$ and $P < 0.001$ for all cases in second and third visits respectively), (*Figure 2b*). In addition, there was significant improvement in the asthma severity score and chest wheezing in the third visit compared with the second visit in the study group ($P < 0.05$), (*Figure 2b*). While at the beginning of the study there was no significant difference in the asthma severity score, frequency of asthma symptoms/week, and chest wheezing between control and study groups, in the third visit, there was significantly more reduction in all symptoms in study subjects compared with the control group ($P < 0.05$ to $P < 0.001$) (*Table II*).

Pulmonary function tests

All PFT variables were abnormally low in both control and studied asthmatic patients at the beginning of the study (33.00 ± 4.85% to 62.27 ± 4.50%). PFT variables were improved after 45 days of treatment in study group; and there was further improvement in PFTs at the end of the study. In the control group, there were only significant increases in FEV_1 and MMEF in visit 2 compared to visits 1 and 3 ($P < 0.05$ to $P < 0.001$) (*Figure 3a*). In the study group, in the second visit most PFT values (except MEF_{75} and MEF_{50}) and the third visits all PFT values were significantly improved compared with first visits ($P < 0.001$ for all cases) (*Figure 3b*). The values of FVC and MMEF also significantly increased in the third visit compared with the second visit in the study group ($P < 0.01$ for both cases). Although at the beginning of the study PFF in the control group was lower than in the study group ($P < 0.05$), in the second and third visits all PFT variables in the study group increased; and in the third visit, there was significantly more increase in all PFT values (except MEF_{25}) in study subjects compared with the control group, and they were more significantly different than in the control group ($P < 0.05$ to $P < 0.001$) (*Table III*).

Treatment regiment and inhaler using technique

The usage of inhaler and oral β-agonists, oral corticosteroid, oral theophylline and even inhaler corticosteroid of the study group was decreased at the end of the study while there were no obvious changes in usage of these types of drugs in control subjects ($P < 0.01$ to $P < 0.001$) (*Table IV*).

DISCUSSION

The results of the present study showed improvement in symptom score, asthma symptom/week, chest wheeze, and especially in PFT values in patients receiving extract of *N. sativa* compared with the control group. Although

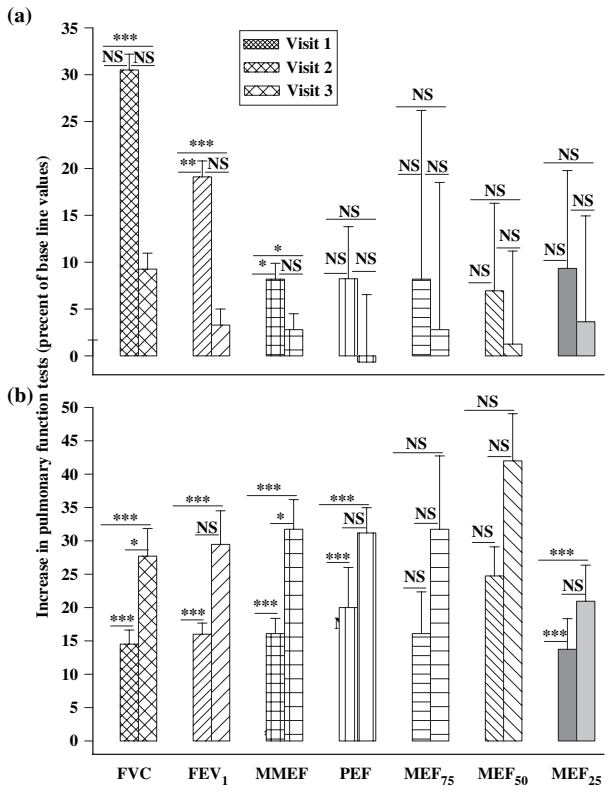

Figure 3 The percentage increase in pulmonary function tests in proportion to the baseline values of control (a) and study (b) groups of asthmatic patients at the middle (fine filled bars) and the end of 3-month study (medium filled bars). FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; PEF: peak expiratory flow; MEF_{75}, MEF_{50} and MEF_{25}: maximal expiratory flow at 75%, 50% and 25% of the FVC, respectively. All values of PFTs were quoted as percentage predicted. Statistical difference in different parameter between three visits: NS, non-significant difference, $^{*}P < 0.05$, $^{**}P < 0.002$, $^{***}P < 0.001$.

in the present study asthmatic patients were treated for a short period of time, there were significant improvements in PFT values in the studied group. The PFT values in the studied group were increased more than 20% at the end of the study period (3 months) and became close to normal values. The asthma symptom scores were also improved in the study group, and patients were almost symptom-free at the end of the study. The asthma severity was also improved from moderate persistent to severe persistent and achieved intermittent to mild persistent according to the GINA guideline [21]. The chest wheeze of patients in the study group was significantly reduced after 3 months of treatment. The amount and types of drugs in the treatment regimen of this group of patients were also decreased due to improvement of asthma severity. All patients in the study group were able to do almost normal activity at the end of the study. However, there were minimal changes in symptom score, asthma symptom/week, chest wheeze and PFT values in the control group.

The PFT values in the control group were lower than in the study group; and their symptoms score, asthma severity and chest wheeze were non-significantly greater. These differences indicated more severe disease and expectation of more pronounced response to treatment, but the response to the same treatment regiment without the extract of *N. sativa* was less than that of the study group. In fact, in the second visit (after 45 days of treatment) there were some improvements in the different parameter of the control group which support their treatment response. However, their treatment response was much lower than in the study group and returned to baseline values at the third visit. Although the patients employed in the study used different types of corticosteroid, they were divided randomly into two groups. Therefore, the treatment regiment of the patients does not influence the outcome of the therapy.

The smaller effect of the extract from *N. sativa* on some PFTs, especially on MEF_{25}, may indicate that this plant has little effect on small airways. In fact, this finding is supported by our previous study indicating that this plant has a minimum effect in this value of PFT. The results of this study confirm those of previous studies indicating a relaxant effect of this plant on airway smooth muscle [12].

The main pathological feature of asthmatic patients is airway inflammation, and all prophylactic drugs used in treatment of this disease are aimed to reduce this phenomenon. Therefore, the mechanism of prophylactic effect of this plant on asthma is perhaps due to its suppressing effect on airway inflammation. In fact, the inhibitory effects of the essential oil of *N. sativa* and thymoquinone have been shown on both cyclooxygenase and 5-lipooxygenase pathways of arachidonic acid metabolism and also on membrane lipid peroxidation [24]. In addition, the inhibitory effect of this plant on histamine (H_1) receptor seen in our previous study [13] can contribute to its anti-inflammatory effect. The antitussive effect of *N. sativa* has been shown in our previous study [17]. Furthermore, it was shown that both systemic and local administrations of the essential oil from this plant have an anti-inflammatory activity [18]. The therapeutic effect of *N. sativa* oil on patients with allergic diseases (allergic rhinitis, bronchial asthma, atopic eczema) was also demonstrated [19]. In

Table III Pulmonary function tests (PFTs) in control and study groups of asthmatic patients at the beginning and their percentage increase in middle and the end of the study.

	Beginning		Middle		End	
PFTs	Control group	Study group	Control group	Study group	Control group	Study group
FVC	54.21 ± 3.30	62.27 ± 4.50 NS	30.5 ± 10.25	14.53 ± 2.10 NS	9.26 ± 5.47	27.71 ± 4.15*
FEV_1 (L)	52.1 ± 4.50	58.8 ± 5.00 NS	19.1 ± 8.37	16.00 ± 1.66 NS	3.30 ± 6.50	29.47 ± 5.04**
PEF (L/s)	38.8 ± 3.70	53 ± 5.21*	8.23 ± 5.56	20.00 ± 6.01 NS	−0.66 ± 7.20	31.18 ± 3.80***
MMEF	37.7 ± 5.30	42.13 ± 5.70 NS	8.19 ± 8.70	16.10 ± 2.27 NS	2.80 ± 10.23	31.74 ± 4.44**
MEF_{75} (L/s)	33 ± 4.85	50 ± 7.40 NS	21.60 ± 7.98	16.10 ± 6.25 NS	7.71 ± 8.52	31.74 ± 11.00***
MEF_{50} (L/s)	37.71 ± 5.18	41.67 ± 5.45 NS	6.95 ± 9.34	24.74 ± 4.36 NS	1.26 ± 9.93	42.00 ± 7.06***
MEF_{25} (L/s)	45.9 ± 7.45	49.87 ± 5.70 NS	9.34 ± 10.44	13.75 ± 4.58 NS	3.64 ± 11.30	20.95 ± 5.40 NS

FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; PEF: peak expiratory flow; MEF_{75}, MEF_{50} and MEF_{25}: maximal expiratory flow at 75%, 50% and 25% of the FVC, respectively. All values of PFTs were quoted as mean ± SEM of percentage predicted. Statistical difference in different parameter between control and study groups: NS, non-significant difference, $*P < 0.05$, $**P < 0.005$, $***P < 0.001$.

Table IV Different type of drugs in treatment regimen in control and study groups of asthmatic patients at the beginning, middle and the end of the study (percentage of total patients in each group).

	Beginning		Middle		End	
Type of drugs	Control group	Study group	Control group	Study group	Control group	Study group
Inhaler salbotamol	80	73	86.6 NS	70 NS	93.3* ns	68 NS ns
Oral salbotamol	26.5	15	13.3*	12.5 NS	26.6 NS†	0***,††
Salmetrol inhaler	0	0	20***	0 NS	6.6*†	0 NS ns
Inhaler corticosteroids	26.5	32	80***	21 NS	53.3***,††	15** ns
Oral corticosteroid	26.6	30	26.6 NS	14**	20 NS ns	6.25*** ns
Oral theophylline	53.3	68.5	86.6***	47.5**	46.6 NS††	23***,††
Antihistamine	6.6	0	33.3***	0 NS	6.6 NS††	0 NS ns

Statistical difference in the percentage of patients using each type of drug between beginning with middle and the end of the study: NS, non-significant difference, $*P < 0.05$, $**P < 0.002$, $***P < 0.001$. Statistical difference in the percentage of patients using each type of drug between middle and the end of the study: ns, non-significant difference, $†P < 0.05$, $††P < 0.001$. Inhaler corticosteroids included beclomethasone dipropionate and fluticasone dipropionate. Oral corticosteroid drug of the treatment regimen of patients was prednisolone. Antihistamine drugs were included in treatment regimen of allergic asthmatic patients to prevent allergic symptoms.

addition, Labib Salem in a recent review summarized the immunomodulatory and therapeutic properties of the *N. sativa* L. seed and emphasized on potent immunomodulatory effects of this plant [5]. In addition Ali and Blunden also summarized different pharmacological effect of *N. sativa* including effect on asthma disease, inflammation and immune system and indicated its different constituents [25]. Therefore, as indicated in ancient Iranian medical books this plant could have therapeutic effects on respiratory diseases. However, more studies are required revealing the different therapeutic effect, effective substance(s) and mechanism(s) of action of *N. sativa*.

Regarding the safety of this remedy, many in vivo studies including the study of Kalus et al. [19] were done on this plant and there is no any report on adverse reaction of *N. sativa*. In addition, hepato-protective effect of this plant also was shown [26,27]. Furthermore, in a comprehensive review, the safety properties of the *N. sativa* L. seed was emphasized [5].

In conclusion, the results of phase I study generally suggest a prophylactic effect of *N. sativa* on asthma disease and warrant further research regarding this effect.

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