BASIC RESEARCH

The effect of *Nigella sativa* extract on tracheal responsiveness and lung inflammation in ovalbumin-sensitized guinea pigs

Mohammad Hossein Boskabady,[I,II] Rana Keyhanmanesh,[III,IV] Saeed Khamneh,[V] Mohammad Ali Ebrahimi[VI]

[I] Dept. of Physiology and Pharmaceutical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. [II] Pharmaceutical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. [III] Dept. of Physiology, Tabriz University of Medical Sciences, Tabriz, Iran. [IV] Drug applied research center Tabriz University of Medical Sciences, Tabriz, Iran. [V] Tuberclosis and lung research center, Tabriz University of Medical Sciences, Tabriz, Iran. [VI] Dept. of Basic Sciences, Islamic Azad University, Tabriz branch, Iran.

OBJECTIVE: To examine the preventive effect of a hydro-ethanolic extract of *Nigella sativa* on the tracheal responsiveness and white blood cell count in the lung lavage fluid of sensitized guinea pigs.

METHODS: Three groups of guinea pigs sensitized to intraperitoneally injected and inhaled ovalbumin were given drinking water alone (group S), drinking water containing a low concentration of *N. sativa* extract (group S+LNS) or drinking water containing a high concentration of *N. sativa* extract (group S+HNS). The tracheal responses of control animals (group C) and the three groups of sensitized guinea pigs ($n=7$ for all groups) to methacholine were measured by the assessment of the tracheal smooth muscle response to increasing concentrations of methacholine, and the effective concentration causing 50% of the maximum response (EC_{50}) was determined. Tracheal responses to 0.1% ovalbumin and white blood cell counts in the lung lavage fluid were also examined.

RESULTS: The tracheal response of the group S guinea pigs to both methacholine and ovalbumin was significantly higher than the response of the controls ($p<0.01$ for both cases). The tracheal responses of the S+LNS and S+HNS groups to both methacholine and ovalbumin were significantly decreased compared to those of the S group ($p<0.05$ to $p<0.01$). The total white blood cell and eosinophil counts in the lung lavage fluid of group S were significantly higher than those of group C ($p<0.01$). The white blood cell counts in both treated groups showed significant improvements ($p<0.01$ for both cases).

CONCLUSIONS: These results demonstrate the preventive effect of the *N. sativa* extract on the tracheal response and lung inflammation in sensitized guinea pigs.

KEYWORDS: *Nigella sativa*; Asthma; Tracheal responsiveness; Sensitization; Inflammation.

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E-mail: boskabadymh@mums.ac.ir

Tel.: 98 511 8413579

INTRODUCTION

Asthma is an inflammatory disorder of the airway[1] characterized by increased airway responsiveness (AHR) to many stimuli.[2] There is a close correlation between airway inflammation, AHR and asthma severity.[3] Many inflammatory cells are involved in the airway inflammation in asthma,[4] and these cells produce more reactive oxygen species (e.g., superoxides, peroxides and hypohalites) than cells obtained from normal subjects.[5] These reactive oxygen species directly induce the contraction of airway smooth muscle preparations and also appear to stimulate histamine release from mast cells and mucus secretion from airway epithelial cells.[6]

The anti-inflammatory activity of both the systemic and local administration of the essential oil of *Nigella sativa* has been shown.[7] The therapeutic effect of the oil of this plant on patients with allergic diseases (e.g., allergic rhinitis, bronchial asthma and atopic eczema) has also been demonstrated.[8] In addition, in a recent review, Labib Salem summarized the immunomodulatory and therapeutic properties of the *N. sativa* L. seed and emphasized the potent immunomodulatory effects of this plant.[9] Our previous works have demonstrated the different pharmacological effects of *N. sativa* on guinea pig tracheal chains, including relaxant and functionally antagonistic effects on muscarinic receptors,[10] an inhibitory effect on histamine (H_1) receptors,[11] an inhibitory effect on calcium channels,[12] an opening effect on potassium channels[13] and a stimulatory effect on β-adrenoceptors.[14] Furthermore, an antitussive effect of this plant in the guinea pig[15] has also

been demonstrated, and a possible prophylactic effect of this plant has been observed in asthmatic patients.[16]

In the present study, the protective effect of *N. sativa* on tracheal responsiveness and lung inflammation in sensitized guinea pigs was examined.

MATERIALS AND METHODS

Plant, extract and drugs

N. 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 hydro-ethanolic extract was prepared using a maceration method as follows: 500 g of chopped *Nigella sativa* seeds were mixed with 450 cc of 50% ethanol for 72 hours at 40℃. This process was repeated three times. The solutions were dried by rotary evaporation at 50℃.

Animal sensitization

The study was approved by the ethics committee of the Mashhad University of Medical Sciences. The sensitization of animals to OA was performed using the method described by McCaig.[17-18] Briefly, guinea pigs (weight; 587.85 ± 9.25 g, mean ± SD) were sensitized to OA (Sigma Chemical Ltd, UK) by injecting 100 mg i.p. (1 mL) and 100 mg s.c. (1 mL) on day one and a further 10 mg i.p. (1 mL) on day 8. From day 14, sensitized animals were exposed to an aerosol of 4% OA for 4 mins a day for 18 ± 1 days. The aerosol was administered in a closed, 30×20×20 cm–chamber. Control animals were treated similarly, but saline was used instead of the OA solution (Fig. 1).

Animal groups

The study was performed using control animals (group C), treated the same as the sensitized group but with normal saline (0.9%) instead of OA; these animals were given drinking water alone] and three different groups of sensitized animals, which were given drinking water containing different concentrations of the extract during the sensitization period as follows ($n = 7$ for each group; a total of 28 guinea pigs were studied, Fig. 1):

1) Drinking water alone (group S, an animal model of asthma),
2) Drinking water containing 0.125 mg/ml *N. sativa* extract (group S+LNS) and
3) Drinking water containing 0.25 omg/ml *N. sativa* extract (group S+HNS).

The total volume of drinking water consumed by the animals during the study protocol was 95.21 ± 3.24 ml (mean ± SD), which was very similar among the different groups.

Tissue preparations

Guinea pigs were euthanized by a blow on the neck, and the trachea was removed. Each trachea was cut into 10 rings, each containing 2–3 cartilaginous rings). All of the rings were then cut open opposite of the trachealis muscle and sutured together to form a tracheal chain.[19]

The tissue was then suspended in a 20 ml organ bath (Schuler organ bath type 809, March-Hugstetten, Germany) containing a Krebs-Henseliet solution (120 mM NaCl, 25 mm $NaHCO_3$, 0.5 mM $MgSO_4$, 1.2 mM KH_2PO_4, 4.72 mM KCl, 2.5 mM $CaCl_2$ and 11 mM dextrose). The solution was maintained at 37℃ and gassed with 95% O_2 and 5% CO_2.

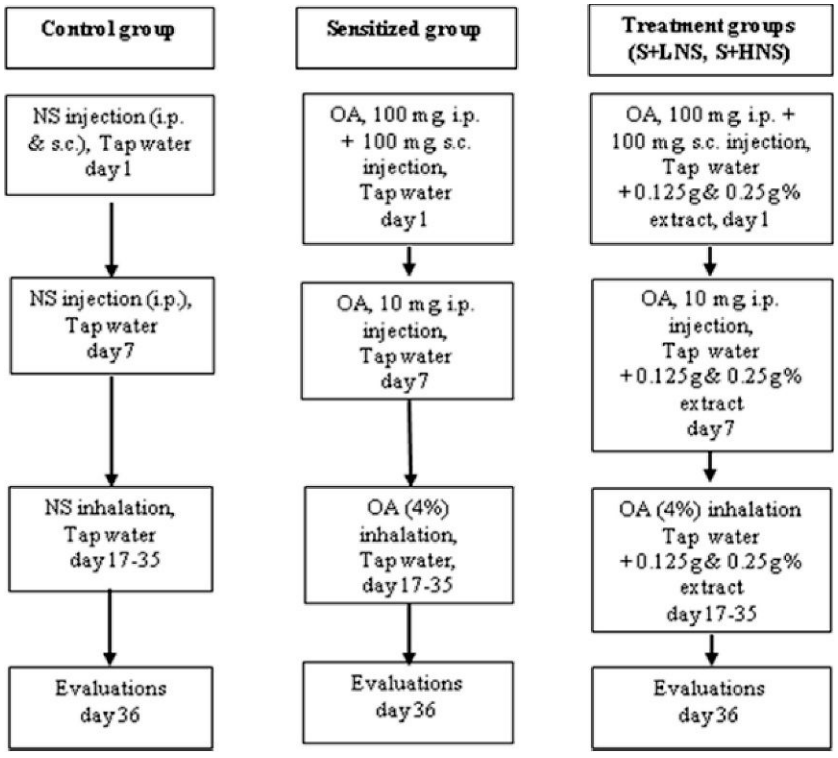

Figure 1 - Experimental timeline for the control, sensitized and treatment groups.

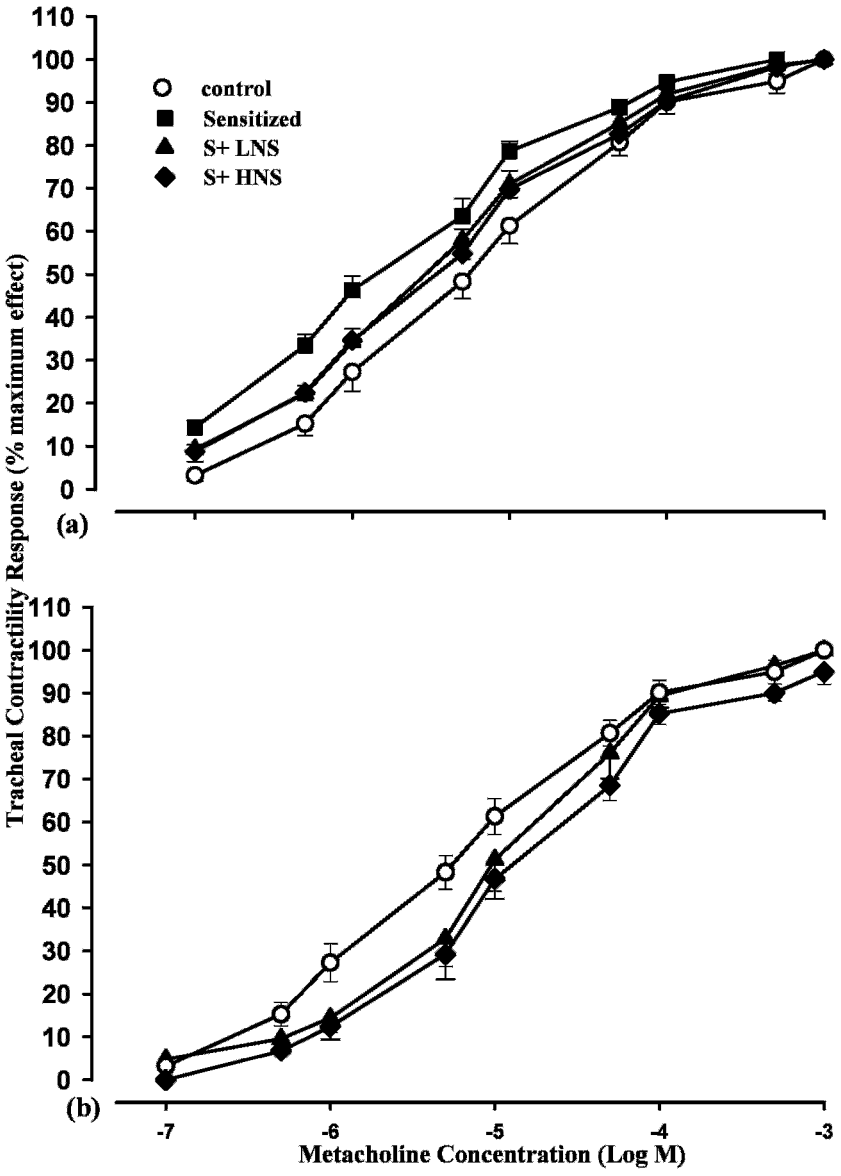

Figure 2 - Cumulative log concentration-response curves of methacholine-induced contraction of trachea isolated from control (C), sensitized (S), S treated with low dose of *N. sativa* extract (S+LNS) and S treated with high dose of *N. sativa* extract (S+HNS) guinea pigs in the absence (a) or presence of 0.25 g% *N. sativa* extract for 7 mins (b; for each group, $n = 7$).

The tissue was suspended under an isotonic tension (1 g) and allowed to equilibrate for at least 1 h, while it was washed with Krebs solution every 15 min.

Responses were measured using a vernier control type 850 N sensor with a sensitivity range of 0–20 g and resolution of 0.2 mm/turn (Hugo-Sachs Elektronik, Germany); these were amplified (ML/118 quadribridge amp, March- Hugstetten, Germany) and recorded on a powerlab recorder (ML-750, 4 channel recorder, March-Hugstetten, Germany).

Assessment of tracheal response to methacholine

In each experiment, a cumulative log concentration-response curve of methacholine hydrochloride (Sigma Chemical Ltd, U.K.)–induced contraction of the tracheal chain was obtained. Increasing concentrations (from 10^{-7} to 10^{-3} mM) were added every 3 min. The contraction due to each concentration was recorded at the end of 3 min, and the effect reached a plateau in all experiments. To generate the curve, the percentage of contraction of the tracheal smooth muscle due to each concentration of methacholine in proportion to the maximum contraction obtained by its final concentration was plotted against the log concentration of methacholine.

The effective concentration causing 50% of the maximum response (EC_{50}) using the methacholine concentration-response curve in each experiment was measured. In addition, the contractility response to 10 μM methacholine as the magnitude of contraction was also measured.

Measurement of tracheal response to ovalbumin

The tracheal response of all animals to a 0.1% OA solution was measured as follows: 0.5 ml of a 4% OA solution was added to the 20 ml organ bath, and the degree of tracheal chain contraction was recorded after 15 min; this was then

expressed as the proportion (in percentage) of the contraction in response to 10 μM methacholine. The measurement of the tracheal responsiveness to methacholine and OA were performed in a random order.

The measurements of tracheal responsiveness to methacholine and OA were also repeated in tissues incubated with 0.25 g% *N. sativa* extract for 7 min in the S+LNS and S+HNS groups ($n=7$ for each group).

Lung lavage and white blood cell counts

Coincident with preparing the tracheal chain, a cannula was inserted into the remaining trachea, and the lungs were lavaged four times with 5 mL of saline (total: 20 mL). Of this lung lavage fluid (LLF), 1 mL was stained with Turk solution, and the stained cells were counted in duplicate using a hemacytometer (in a Burker chamber). The Turk solution consisted of 1 mL glacial acetic acid, 1 mL of 1% Gentian Violet Solution and 100 mL of distilled water.[20]

The remaining LLF was centrifuged at 2500×*g* at 4℃ for 10 min, and the supernatant was removed. A smear was prepared from the cells and stained with Wright-Giemsa. Differential cell counts (based on staining and morphological criteria) were obtained using a light microscope by counting 400 cells, and the percentage of each cell type was calculated.[20]

Statistical analysis

The tracheal response to methacholine (EC_{50}), tracheal contractility response, tracheal response to OA, total WBC numbers and differential WBC counts are presented as the mean±SEM. As determined using the Kolmogorov Smirnov test, these data had a normal distribution. The data from the sensitized group were compared with data from the control guinea pigs and the two groups of animals treated with the extract using a one-way ANOVA with a post hoc Dunnett's test. The data obtained from measuring tissues incubated in the presence or absence of the *N. sativa* extract were compared using a paired *t*-test. A *p* value less than 0.05 was considered significant. The InStat software (GraphPad Software, Inc.) was used for the statistical analysis.

RESULTS AND DISCUSSION

Tracheal response to methacholine

The concentration-response curves of methacholine in non-incubated tissues with *N. sativa* extract showed a leftward shift in group S when compared to group C. However, the curves of the S+LNS and S+HNS groups were shifted to the right when compared to group S (Fig. 2a). In incubated trachea, the curves of the S+LNS and S+HNS groups shifted to the right when compared to group C (Fig. 2b).

In non-incubated tissues, the mean EC_{50} value of the group S tracheal chains (1.27±0.18 μM) was significantly lower than that of group C (5.31±0.71 μM, $p<0.01$, Fig. 3a). The mean EC50 value of the pre-treatment groups [S+LNS (3.21±0.55 μM, $p<0.05$) and S+HNS (3.21±0.25 μM, $p<0.01$)] tracheal chains was significantly improved when compared to that of group S (Fig. 3a). However, the mean EC_{50} value of the S+LNS and S+HNS tracheal chains was still significantly lower than that of group C ($p<0.05$ for both cases, Fig. 3a).

The mean EC_{50} value of the incubated tissues from the S+LNS (9.67±1.91 μM) and the S+HNS (12.33±2.27 μM) groups was significantly higher than that of group S ($p<0.01$ for both groups) and even group C ($p<0.01$ for only high concentrations, Fig. 3b).

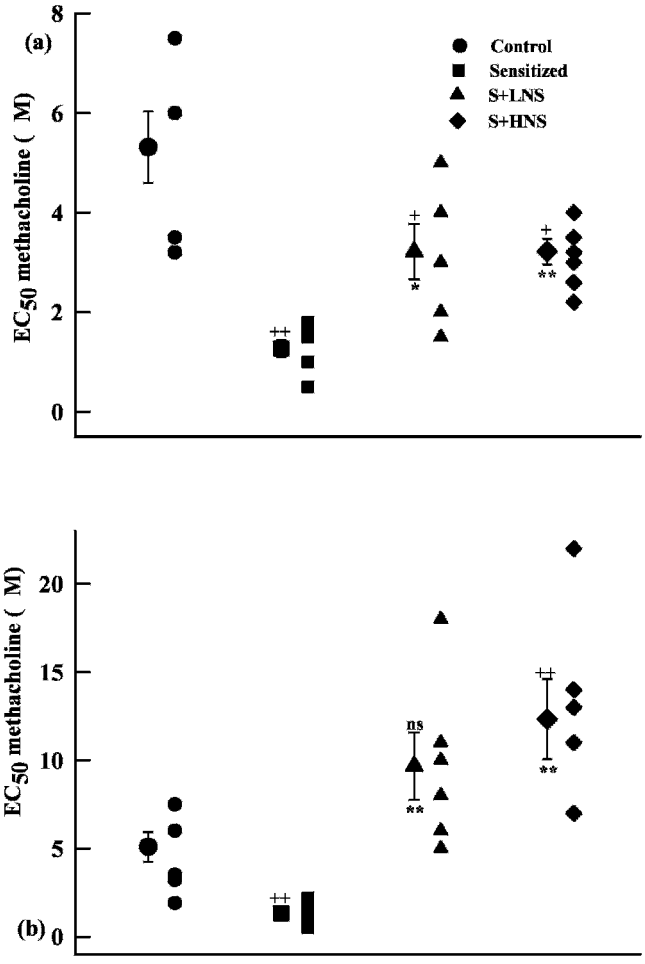

Figure 3 - Individual values and the mean±SEM (larger symbols with bars) of the tracheal response to methacholine (EC_{50}) in tissues either non incubated treated (a) or treated with 0.25 g% *N. sativa* extract (b; for each group, $n=7$). Statistical differences between the control and different groups: +; $p<0.05$, ++; $p<0.01$. Statistical differences between the S+LNS and S+HNS *vs.* sensitized group: **: $p<0.01$. There was no significant difference in the effects of the two different concentrations of the extract.

Tracheal response to ovalbumin

The tracheal response of the non-incubated group S tracheal chains (73.38±9.86%) to OA was significantly higher than the response of group C tracheal chains (7.65±3.85%, $p<0.01$, Fig. 4a). The tracheal response of the treatment groups [S+LNS (40.71±4.84%, $p<0.05$) and S+HNS (35.17±4.74%, $p<0.01$)] to OA was significantly improved when compared to the response of group S tracheal chains (Fig. 4a). However, the tracheal response of the treatment groups to OA was still significantly higher than the response of group C tracheal chains ($p<0.01$ for both groups; Fig. 4a).

The tracheal responses of the incubated tissues from groups S+LNS and S+HNS to OA were lower than the response of group S ($p<0.01$ for both groups) and were not statistically different from that of group C (Fig. 4b).

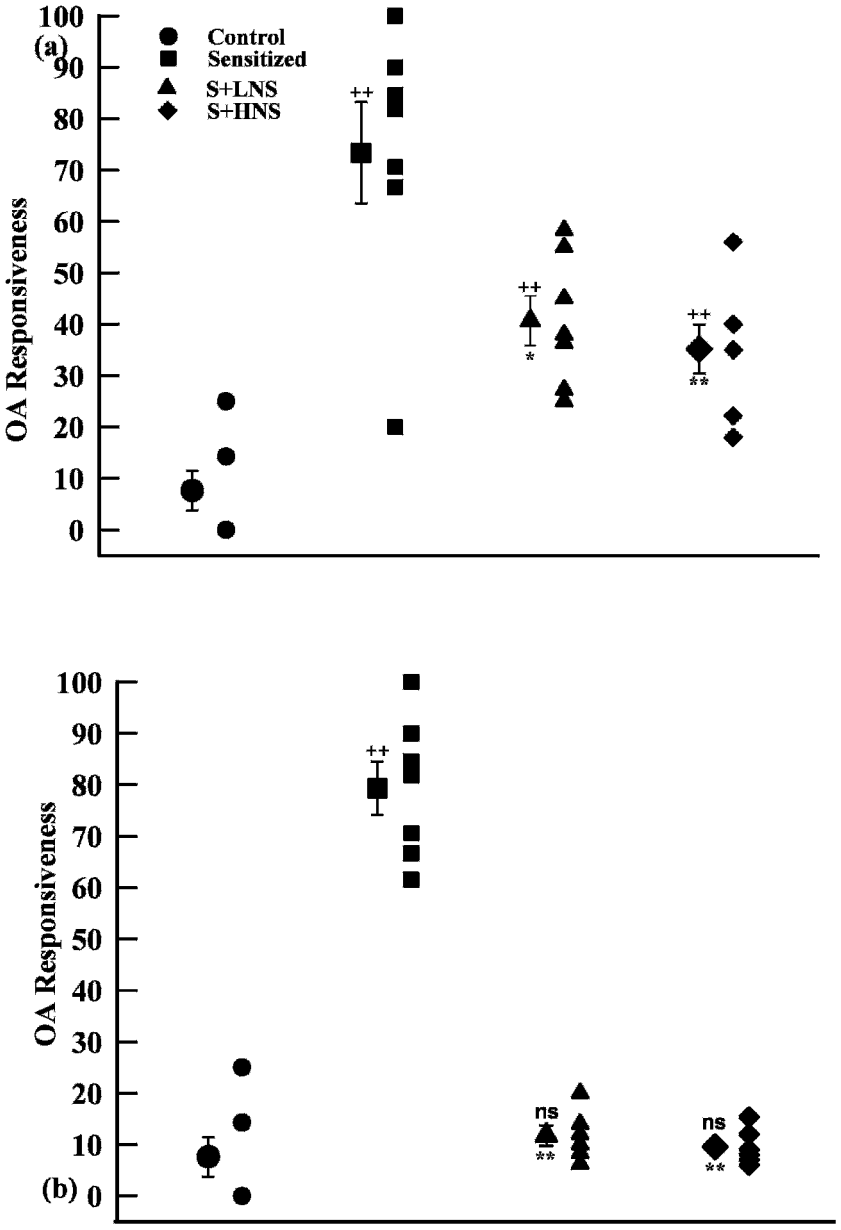

Figure 4 - Individual values and mean ± SEM (larger symbols with bars) of the tracheal response to ovalbumin in the absence (a) or presence of 0.25 g% *N. sativa* extract (b; for each group, $n = 7$). The data are presented as the percent contraction in proportion to the contraction obtained following treatment with 10 μM methacholine. Statistical differences between the control and different groups: ns; non-significant difference, ++; $p<0.01$. Statistical differences between S+LNS and S+HNS *vs.* sensitized group: *: $p<0.05$, **: $p<0.01$. There was no significant difference in the effects of the two different concentrations of the extract.

Contractility

Treatment with a high concentration of *N. sativa* extract caused a significant decrease in contractility when the treated group was compared with the S group ($p<0.05$). There was no significant difference in the contractility response of both pretreated groups when they were compared to group C (Fig. 5b). Furthermore, the contractility response of the incubated group S+HNS tracheal chains was lower than the response of group S ($p<0.01$).

Total white blood cell counts

The total white blood cell (WBC) count in the LLF from guinea pigs in group S (2127.22 ± 184.3 cells/μl) was significantly higher than that from group C (461.66 ± 60 cells/μl, $p<0.01$) (Fig. 5a). The WBC counts of both treated groups [S+LNS (936.25 ± 89.46 count/microl) and S+HNS (883.12 ± 58.82 cells/μl)] showed significant improvement when compared to that of group S ($p<0.01$ for both cases, Fig. 5a). However, the WBC count of the S+LNS group was still significantly higher than that of group C ($p<0.01$; Fig. 5a).

Differential WBC counts in lung lavage fluid

There was a significant decrease in the number of neutrophils, lymphocytes and monocytes, and a significant increase in the number of eosinophils, in the LLF from group S when compared to group C ($p<0.01$ for all subsets, Figs. 6a–d). The administration of either concentration of the extract to sensitized animals (group S+LNS and S+HNS)

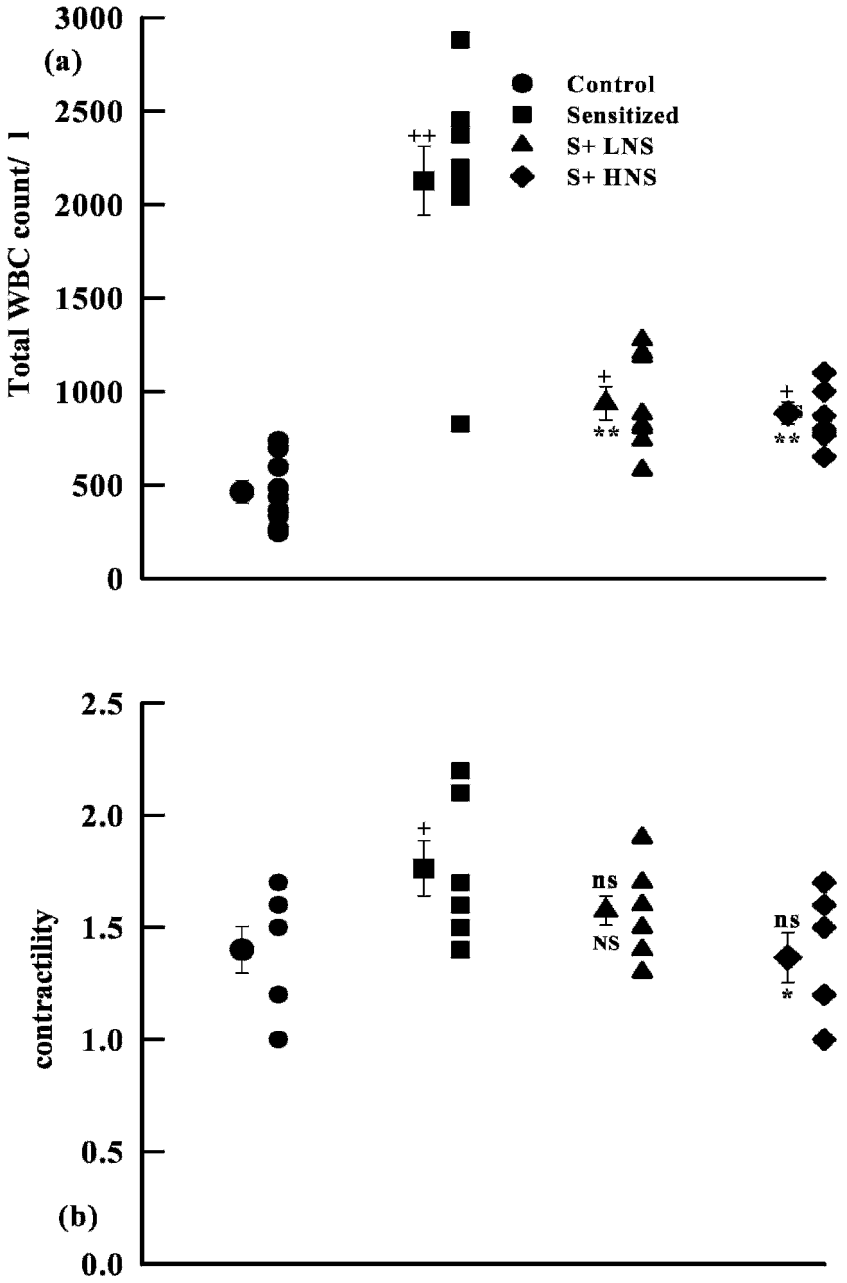

Figure 5 - Individual values and the mean ± SEM (larger symbols with bars) of the total WBC count (a) and tracheal contractility response to 10 μM methacholine (b; for each group, $n = 7$). Statistical differences between the control and different groups: ns; non-significant difference, +; $p<0.05$. Statistical differences between the S+LNS and S+HNS *vs.* sensitized group: NS; non-significant difference, *: $p<0.05$, **: $p<0.01$. There was no significant difference in the effects of the two different concentrations of the extract.

resulted in a significant improvement in the neutrophil and monocyte counts ($p<0.05$ to $p<0.01$) but did not affect the eosinophil and lymphocyte counts. There were still significant differences in the number of eosinophils, neutrophils, lymphocytes and monocytes between both treated groups and group C ($p<0.05$ to $p<0.01$, Figs. 6a–d).

Differences between two concentrations of the extract

The mean EC_{50} value for the tracheal chains of pre-treated group with lower concentrations of *N. sativa* extract (S+LNS), both in non-incubated and incubated tissues was not significantly lower than treated group with higher concentration, and both the tracheal response to OA and the contractility response were greater than the responses seen in the group treated with the higher concentration (Figs. 3, 4 and 5b).

The WBC counts and the counts of the different WBC subsets in animals treated with the lower concentration of *N. sativa* extract were not significantly greater than the counts of the animals treated with the higher concentration of the extract (Figs. 5a and 6).

Differences between the incubated and non-incubated tissues

Incubation of the tissues with *N. sativa* extract caused a significant increase in the EC_{50} and a significant decrease in

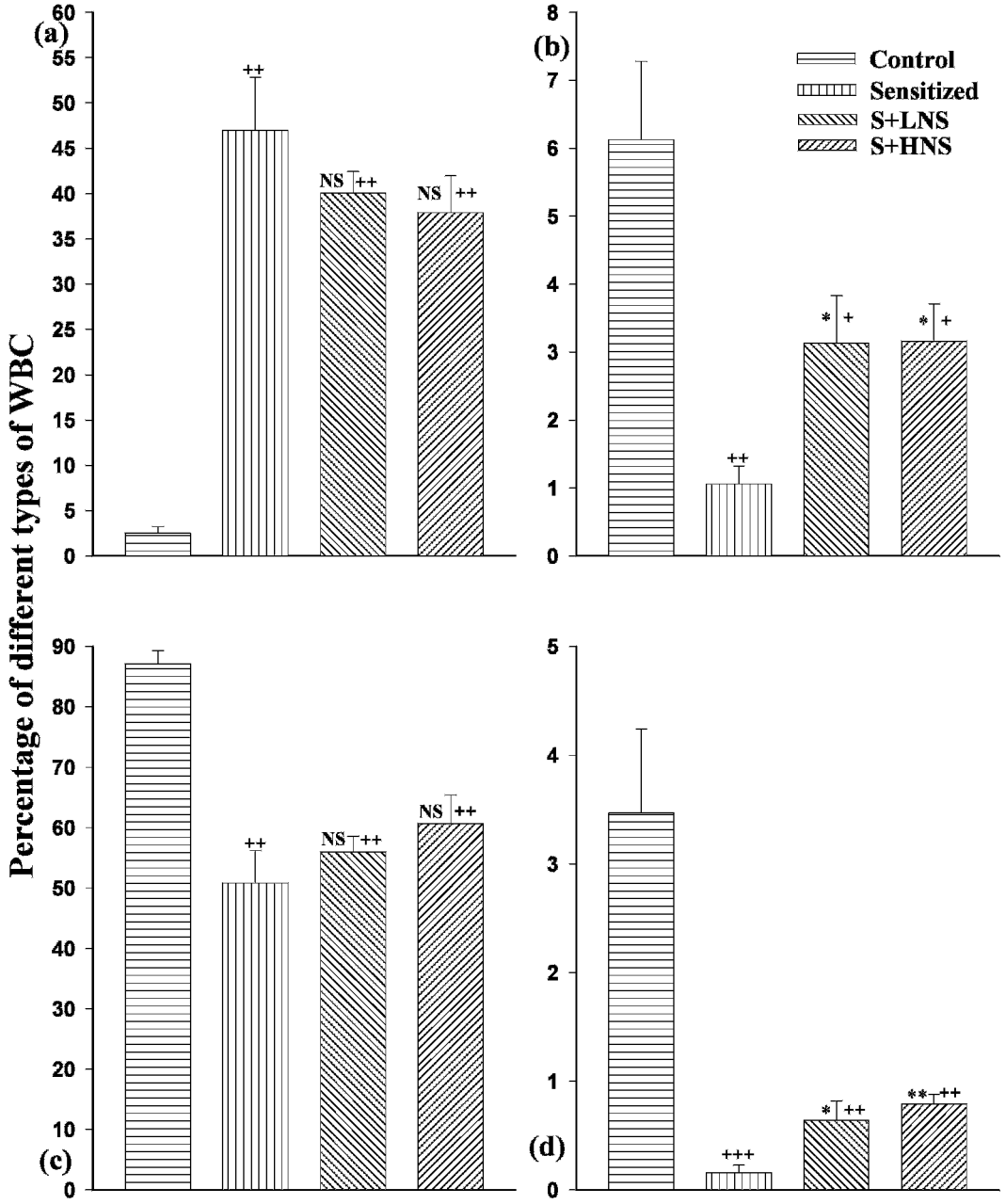

Figure 6 - The eosinophil (a), neutrophil (b), lymphocyte (c) and monocyte (d) fraction of the lung lavages of control, sensitized (S), S treated with low dose *N. sativa* extract (S+LNS) and S treated with high dose *N. sativa* extract (S+HNS) guinea pigs (for each group, $n = 8$). Statistical differences between the control and different groups: ns; non-significant difference, +; $p<0.05$, ++; $p<0.01$. Statistical differences between the S+LNS and S+HNS groups *vs.* the sensitized group: NS; non-significant difference,*: $p<0.05$, **: $p<0.01$. There was no significant difference in the effects of the two different concentrations of the extract.

the tracheal response to OA in the S+LNS and S+HNS groups when compared to the tissues not incubated with the extract ($p<0.001$ for all cases). Incubation of the tissues with the *N. sativa* extract also resulted in a non-significant decrease in the contractility response of the S+LNS and S+HNS groups when compared to the tissues not treated with the extract.

DISCUSSION

In the present study, we demonstrated the preventive effect of the long-term administration of a hydroethanolic *N. sativa* extract on the tracheal responsiveness of sensitized guinea pigs to methacholine and OA, the contractility response to methacholine, the increased WBC count and changes in differential WBC counts.

In addition, incubation of the tracheal chains from the S+LNS and S+HNS animals with the *N. sativa* extract (i.e., short-term administration of the extract) caused a further decrease in the tracheal responsiveness to methacholine and OA and a decrease in the contractility response, such that all of these values became very close to the control values.

The treatment of sensitized animals with *N. sativa* effectively restored the total WBC counts and the neutrophil

and monocyte changes. These findings demonstrate that *N. sativa* mainly affects the neutrophilic changes of sensitized animals. The results also indicate that *N. sativa* has a preventive effect on tracheal responsiveness—one of the main characteristic features of asthma.

The main pathological feature of asthmatic patients is airway inflammation, which causes the most characteristic feature of the disease, increased airway responsiveness. As such, all prophylactic drugs used in the treatment of asthma aim to reduce this inflammation. The preventive effect of the long-term administration of the *N. sativa* extract on the tracheal responsiveness of sensitized animals may be due to its ability to suppress airway inflammation. This hypothesis is supported by the preventive effect of *N. sativa* extract on the airway responsiveness of sensitized guinea pigs. In fact, an *N. sativa* essential oil has been shown to have inhibitory effects on both the cyclooxygenase and the 5-lipoxygenase pathways of arachidonic acid metabolism and on membrane lipid peroxidation.[21] In addition, both the systemic and local administration of the essential oil have an anti-inflammatory activity.[7] The inhibitory effect of this plant against the histamine (H_1) receptor, seen in our previous study,[11] can contribute to its anti-inflammatory effect. An antitussive effect of *N. sativa* has also been shown.[15] The therapeutic effect of *N. sativa* oil on patients with allergic diseases (e.g., allergic rhinitis, bronchial asthma and atopic eczema) has also been demonstrated.[8] In a recent review, Labib Salem summarized the immunomodulatory and therapeutic properties of the *N. sativa* L. seed and emphasized the potent immunomodulatory effects of this plant.[9] Furthermore, Ali and Blunden also summarized the different pharmacological effects of *N. sativa,* including its effect on asthma, inflammation and the immune system, and they indicated its different constituents.[22]

We observed a significant difference in the eosinophil and lymphocyte counts between the sensitized and treated groups and an increase in neutrophil recruitment in the treated groups when compared to the sensitized groups. However, there was a significant reduction in total WBC counts in the treated groups when compared to the sensitized animals (from approximately 2200 to 800). Therefore, if we consider the absolute differential WBC counts, there was a reduction of all cell types in the treated groups, which could be corrected.

The inhibitory effect of the short-term administration of the extract (incubation of the tissues with the extract) on tracheal responsiveness to methacholine and OA and the contractility response may be due to its relaxant effect on the tracheal chains, which has been previously reported.[10] The effect of *N. sativa* extract on the tracheal responsiveness of pre-treated animals suggests a synergistic effect of short- and long-term administration of the extract, which was shown in a previous study.[10]

The lack of a difference between the two different concentrations of the extract indicates that the maximum preventive effect of the plant extract was obtained at the lowest concentration used in this study.

As indicated in ancient Iranian medical books, this plant may have therapeutic effects on respiratory diseases, including asthma. However, more studies are required to elucidate the different therapeutic effects, effective substance(s) and mechanism(s) of action of *N. sativa.*

Regarding the safety of this remedy, several *in vivo* studies, including the study of Kalus et al.,[8] have shown no adverse reaction to *N. sativa*. Additionally, a hepatoprotective effect of this plant has also been reported,[23-24] and, in a comprehensive review, the safety of the *N. sativa* seed has been emphasized.[9]

CONCLUSIONS

The results of the present study illustrate the preventive effect of *N. sativa* on tracheal responsiveness, with a greater effect seen in response to methacholine than to OA. The results also suggest that *N. sativa* has both a relaxant (bronchodilatory) and a preventive effect on asthma.

ACKNOWLEDGMENTS

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