J Physiol Sci (2015) 65:285–292
DOI 10.1007/s12576-015-0367-6

ORIGINAL PAPER

The protective effect of α-hederin, the active constituent of *Nigella sativa*, on tracheal responsiveness and lung inflammation in ovalbumin-sensitized guinea pigs

Saeideh Saadat · Mostafa Mohammadi · Maryam Fallahi · Rana keyhanmanesh · Mohammad Reza Aslani

Received: 28 November 2014 / Accepted: 13 February 2015 / Published online: 10 March 2015

Abstract Many investigations have demonstrated the prophylactic effect of *Nigella sativa* on asthma disease. One of its active constituents is α-hederin. In the present study, the preventive effect of two different concentrations of α-hederin on tracheal responsiveness and lung inflammation in ovalbumin-sensitized guinea pigs was examined. Forty male adult Dunkin-Hartley guinea pigs were randomly divided into the control (C), sensitized (S) and sensitized pretreated groups with thymoquinone (3 mg/kg i.p., S + TQ), low-dose α-hederin (0.3 mg/kg i.p., S + LAH) and high-dose α-hederin (3 mg/kg i.p., S + HAH). The responsiveness of tracheal smooth muscle (TR) to methacholine, histamine and ovalbumin was assessed. Moreover, total and differential white blood cell counts in lung lavage fluid were examined. Compared with the S group, the mean EC50 value in the S + LAH group increased significantly ($p < 0.05$). The mean EC50 value of histamine contraction in the S + LAH and S + HAH groups was significantly higher than in the S group ($p < 0.05$). In all pretreated groups, the TR to ovalbumin decreased in comparison to the S group ($p < 0.001$). Both the S + HAH and S + LAH groups showed significantly decreased TR compared to the S + TQ group ($p < 0.01$–$p < 0.01$). Total WBC and eosinophil counts in all pretreated groups decreased significantly in comparison with the S group (0.001–0.01). There was a significant increase in neutrophil, lymphocyte and monocyte counts in the pretreated groups compared to the S group ($p < 0.001$–$p < 0.05$). The basophil count in the S + TQ and S + HAH groups was significantly lower than in the S group ($p < 0.01$–$p < 0.05$). This study suggested that α-hederin has anti-inflammatory and bronchodilatory effects like thymoquinone.

Keywords Asthma · α-hederin · Tracheal responsiveness · Lung inflammation · Ovalbumin

S. Saadat · M. Mohammadi · M. Fallahi · M. R. Aslani
Department of Physiology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

S. Saadat
Department of Physiology, Faculty of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

R. keyhanmanesh (✉)
Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
e-mail: keyhanmaneshr@tbzmed.ac.ir; rkeyhanmanesh@gmail.com

Introduction

Allergic asthma is a severe inflammatory disorder of the lungs with bronchial hyperreactivity, recruitment of eosinophils, mast cells and lymphocytes, and hyperplasia of the smooth muscle and goblet cells, often related to increased serum IgE concentrations [1]. The inflammatory response of asthma is considered to be increased by T helper 2 type cells through multiple cytokines [2].

Nigella sativa (black seed) is an important medicinal herb. In many countries, for example, the Arabian, Asian and African countries, black seed oil is used as a natural cure for many diseases, including different allergies [3]. However, the exact mechanism of action has not been identified yet.

Much research carried out in vitro and studies on laboratory animals and humans in vivo have been carried out to examine its different pharmacological features [4].

The potential prophylactic effect of this plant has been demonstrated in asthmatic patients. Moreover, the prophylactic and bronchodilatory effects of the boiled extract

from *N. sativa* on asthmatic patients have been shown [5, 6]. In addition, the beneficial role of *N. sativa* in reducing the wheezing associated with lower respiratory tract illness in children [7] and adult asthmatic attacks has been reported [8]. The antitussive effect of *N. sativa* in guinea pigs has also been documented [9, 10]. Moreover, the preventive effect of the hydroethanolic extract of *N. sativa* in ovalbumin-sensitized guinea pigs and rats, an animal model of asthma, was also shown [11–13]. Recently, the preventive effect of the hydroethanolic extract of this plant was proven in the tracheal responsiveness of cigarette smoke-exposed guinea pigs [14].

Many active components have been separated from *N. sativa*, including thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellimine-N-oxide, nigellicine, nigellidine and α-hederin. α-Hederin was also separated from *Hedera helix* (*Hedera helix* L.), a famous plant known as ivy or English ivy and a member of the Araliaceae family. α-Hederin has been shown to have an anti-inflammatory effect [15].

In the present study, the preventive effect of two different concentrations of α-hederin on tracheal responsiveness and lung inflammation in ovalbumin-sensitized guinea pigs was examined.

Materials and methods

Forty male adult Dunkin-Hartley guinea pigs (400–700 g) were selected for the study. They were accommodated in individual cages kept at 22 ± 2 °C on a 12-h light/dark cycle. After 10 days, the animals were randomly divided into five groups: the control group (C); sensitized group (S); sensitized groups pretreated with thymoquinone (Sigma Chemical Ltd., UK), 3 mg/kg [13] (S + TQ); low-dose α-hederin (Extrasynthese Co., France), 0.3 mg/kg (S + LAH); and high-dose α-hederin, 3 mg/kg (S + HAH). These were injected intraperitoneally on the 10th day of the induction protocol.

This study was approved by the ethics committee of Tabriz University of Medical Sciences.

Animal sensitization

Sensitization of the animals to ovalbumin was done using a previously described method [16]. Briefly, guinea pigs were sensitized to ovalbumin (Sigma Chemical Ltd., UK) by injecting 100 mg i.p. and 100 mg s.c. on the 1st day and a further 10 mg i.p. on the 8th day. From the 14th day, sensitized animals were exposed to an aerosol of 4 % ovalbumin for 18 ± 1 days for 4 min daily. The aerosol was administered in a 30 × 20 × 20-cm closed chamber using a nebulizer (Omron Healthcare, Inc., USA). Control animals were treated similarly, but saline was used instead of the ovalbumin solution.

Tissue preparations

Tracheas were detached from guinea pigs killed by a hit on their neck. Each trachea was separated into ten rings (each containing 2–3 cartilaginous rings). Afterwards, all the rings were cut open opposite the tracheal muscle and sutured together to form a tracheal chain [16]. Tissue was then suspended in a 20-ml organ bath (Schuler Organ Bath type 809, March-Hugstetten, Germany) containing Krebs-Henseleit solution of the following composition (mM): NaCl 120, $NaHCO_3$ 25, $MgSO_4$ 0.5, KH_2PO_4 1.2, KCl 4.72, $CaCl_2$ 2.5 and dextrose 11. The Krebs solution was kept at 37 °C, pH 7.4, and gassed with 95 % O_2 and 5 % CO_2. Tissue was suspended under an isotonic tension of 1 g and allowed to balance for at least 1 h while being washed with Krebs solution every 15 min [17].

An isometric transducer (AD Instruments, Spain) with a sensitivity range of 0–25 g was used to measure the responses. These responses were kept on a four-channel PowerLab recorder (ML-750; March-Hugstetten, Germany) after amplification with an ML/118 quadri-bridge amplifier (March-Hugstetten, Germany).

Assessment of the tracheal response to methacholine

The concentration-response curve of the tracheal chain was recorded in each experiment. Successive concentrations of methacholine hydrochloride (Sigma Chemical Ltd., UK) including 10^{-7}–10^{-2} M dissolved in saline were added every 3 min. The contraction caused by each concentration and the effect attaining a plateau in all experiments were documented at the end of 3 min. Then the percentage of the tracheal smooth muscle contraction due to each concentration of methacholine in proportion to the maximal contraction obtained by its final concentration was plotted against the log concentration of methacholine. A concentration-response curve of methacholine was obtained for the tracheal chain of each animal studied. The useful concentration of methacholine causing 50 % maximum response (EC50) was calculated from the methacholine response curve in each experiment by using 50 % of the maximum response in the *Y* axis and measuring the dose of methacholine causing this response in the *X* axis. The contractility response to 10 μM methacholine as the intensity of the contraction was also measured [17].

Assessment of the tracheal response to histamine

Like tracheal responsiveness to methacholine, successive concentrations of histamine (Sigma Chemical Ltd., UK)

including 10^{-7}–10^{-2} M dissolved in saline were added every 3 min, and the contraction caused by each concentration was documented at the end of these intervals [18]. Then the percentage of the tracheal smooth muscle contraction due to each concentration of histamine in proportion to the maximal contraction obtained by its final concentration was plotted against the log concentration of histamine. The useful concentration of histamine causing 50 % maximum response (EC50) was calculated.

Measurement of the tracheal response to ovalbumin

The tracheal response of all animals to a 0.1 % solution of ovalbumin was evaluated in each selected animal as follows: 0.5 ml of 4 % ovalbumin solution (dissolved in saline) was added to the 20-ml organ bath, and the contraction of tracheal chain was recorded after 10 min, shown as a proportion (in percentage) of the contraction obtained with 10 μM methacholine [17]. Measurements of the tracheal response to methacholine and OA were done in random order.

Lung lavage and white blood cell count

Compatible with preparing the tracheal chain, a cannula was placed into the remaining trachea, and the lungs were washed with 5 ml of saline four times (total 20 ml). One milliliter of lung lavage fluid (LLF) was stained with Turk solution and counted in duplicate in a hemocytometer (in a Burker chamber). The Turk solution consisted of 1 ml glacial acetic acid, 1 ml gentian violet solution 1 % and 100 ml pure water. The remaining LLF was centrifuged at 2,500×g at 4 °C for 10 min. Then the supernatant was removed, and a smear was prepared from the cells and stained with Wright-Giemsa. Differential cell examination was done under a light microscope by counting 100 cells twice, and the percentage of each cell type was measured according to staining and morphological criteria [17].

Statistical analysis

All results were considered as mean ± SEM. The data of four sensitized groups were compared with controls using one-way analysis of variance (ANOVA) with the Tukey-Kramer post-test. Furthermore, the data of the three treated groups were compared with sensitized guinea pigs using one-way analysis of variance (ANOVA) with Tukey-Kramer or LSD post tests. The data of the α-hederin-treated groups were compared with the S + TQ group using the unpaired t test. In addition, the data of the α-hederin-treated groups were compared using the unpaired t test.

Results

Tracheal response to methacholine

The responsiveness of tracheal chains to different concentrations of methacholine is shown as concentration-response curves in Fig. 1. Concentration-response curves in all groups (except the S + LAH group) showed leftward shifts compared to the control group. In comparison to the sensitized group, all groups shifted to the right. The highest tracheal response was observed in the S group and the least in the S + LAH group (Fig. 1).

The mean EC50 value in the sensitized and S + TQ groups was significantly decreased compared with the control group ($p < 0.01$ for both). Compared with the S group, the mean EC50 value increased significantly ($p < 0.05$) only in the S + LAH group. The mean EC50 value in the S + LAH group was significantly higher than in the S + TQ group, but there were no significant differences among the α-hederin-treated groups (Table 1).

The mean value of contractility in all groups (except S + TQ) increased significantly in comparison with the control group ($p < 0.001$ for the S group, $p < 0.05$ for the S + LAH and S + HAH groups). All pretreated groups showed significantly decreased contractility compared with the S group ($p < 0.05$ for the S + TQ group, $p < 0.001$ for the S + LAH group, $p < 0.01$ for the S + HAH group). There were no significant differences among the pretreated groups (Table 1).

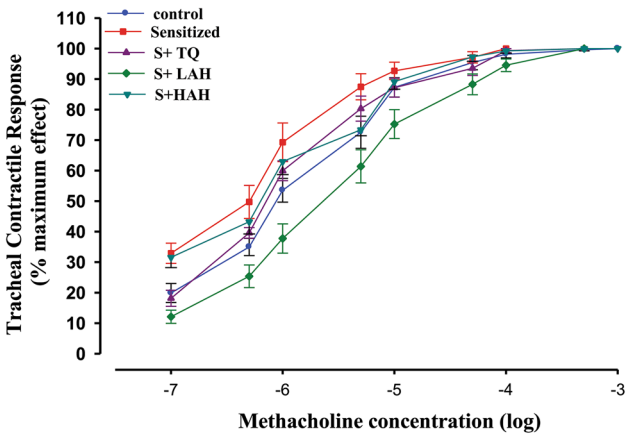

Fig. 1 Cumulative log concentration-response curves of methacholine-induced contraction of isolated trachea in control (C), sensitized (S), sensitized pretreated with thymoquinone (S + TQ), sensitized pretreated with low-dose α-hederin (S + LAH) and with high-dose α-hederin (S + HAH) guinea pigs in the organ bath (for each group, $n = 7$)

Table 1 Values of tracheal response to methacholine (EC50-Met) and histamine (EC50-His), response to ovalbumin (OA), contractility and total white blood cells (WBCs) of LLF in control (C), sensitized (S), S treated with thymoquinone (S + TQ), S treated with low-dose (S + LAH) and high-dose α-hederin (S + HAH) guinea pigs (for each group, $n = 6$)

	C	S	S + TQ	S + LAH	S + HAH
EC50-Met (μM)	3.95 ± 0.85	0.56 ± 0.22	0.77 ± 0.04	2.87 ± 0.87	2.22 ± 0.72
		++	++ ns	NS *	NS ns
				#	# ns
Contractility	0.60 ± 0.05	1.68 ± 0.08	1.03 ± 0.24	0.93 ± 0.12	0.98 ± 0.15
		+++	NS *	+ ***	+ *
				ns	**ns** ns
EC50-His (μM)	9.92 ± 1.14	1.54 ± 0.70	5.23 ± 1.67	4.94 ± 1.09	5.73 ± 1.44
		+++	+ ns	+ *	+ *
				ns	**ns** ns
OA	1.9 ± 1.15	72.88 ± 9.28	15.01 ± 2.37	7.78 ± 1.01	3.5 ± 1.18
		+++	+++ ***	++ ***	NS ***
				#	## !
Total WBC count	333.17 ± 31.11	878.33 ± 96.11	479.17 ± 50.62	368.33 ± 53.00	407.33 ± 62.47
		+++	+ **	NS ***	NS **
				ns	**ns** ns

Values are presented as mean ± SEM. The WBC data are their counts in 1 ml of LLF. Statistical differences between the data of control vs. other groups: *NS* nonsignificant difference; +, $p < 0.05$; ++, $p < 0.01$; +++, $p < 0.001$. Statistical differences between the data of sensitized vs. pretreated groups: *ns* nonsignificant difference; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Statistical differences between the S + TQ group vs. the S + LAH and S + HAH groups: ***ns*** nonsignificant difference; #, $p < 0.05$; ##, $p < 0.01$. Statistical differences between the S + LAH and S + HAH groups: ns nonsignificant difference; !, $p < 0.05$

Tracheal response to histamine

Concentration-response curves in all groups were shifted to the left compared to the control group, and all cases were shifted to the right with respect to the sensitized group. The S + TQ group was the closest to the control group but did not reach the control level. The curve of the S + LAH group was very close to that of the S + TQ group. The least tracheal responsiveness was seen in the C group (Fig. 2).

The mean EC50 value in all groups decreased significantly in comparison to the control group ($p < 0.001$ for the S group, $p < 0.05$ for all pretreated groups). The mean EC50 value in all pretreated groups was significantly higher than in the S group ($p < 0.05$ for both). There was no significant difference among the pretreated groups (Table 1).

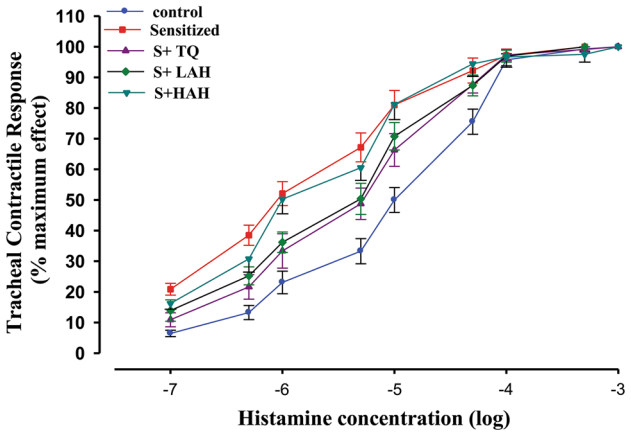

Fig. 2 Cumulative log concentration-response curves of histamine-induced contraction of isolated trachea in the control (C), sensitized (S), sensitized pretreated with thymoquinone (S + TQ), sensitized pretreated with low-dose α-hederin (S + LAH) and high-dose α-hederin (S + HAH) guinea pigs in the organ bath (for each group, $n = 7$)

Tracheal response to ovalbumin

Tracheal responsiveness to ovalbumin in all groups (except the S + HAH group) was significantly higher than in the controls ($p < 0.001$ for the S and S + TQ groups, $p < 0.01$ for the S + LAH group). In all pretreated groups, the tracheal response to ovalbumin decreased in comparison to the S group ($p < 0.001$ for all pretreated groups). Both the S + HAH and S + LAH groups showed significantly decreased tracheal response compared to the S + TQ group ($p < 0.05$ for the S + LAH group, $p < 0.01$ for the S + HAH group). Moreover, there was a significant difference between the S + LAH and S + HAH groups ($p < 0.05$, Table 1).

Total WBC count in lung lavage fluid

The amount of WBCs in the S and S + TQ groups was significantly higher than in the control group ($p < 0.001$ for the S group, $p < 0.05$ for the S + TQ group). The WBC count in all pretreated groups decreased significantly in comparison with the sensitized group ($p < 0.01$ for the S + TQ group, $p < 0.001$ for the S + LAH group, $p < 0.01$ for the S + HAH group). There was no significant difference among the pretreated groups (Table 1).

Differential count of WBCs in lung lavage fluid

There was a significant increase in the eosinophil count of all groups (except the S + HAH group) compared with the control group ($p < 0.001$ for the S group, $p < 0.01$ for the S + TQ group, $p < 0.05$ for the S + LAH group). The mean value of the eosinophil count in all pretreated groups decreased significantly in comparison to the S group ($p < 0.001$ for all pretreated groups). There was a significant difference between the α-hederin-treated groups compared with the S + TQ group ($p < 0.05$ for the S + LAH group, $p < 0.001$ for the S + HAH group). The eosinophil count of the S + HAH group was significantly lower than that of the S + LAH group ($p < 0.01$, Fig. 3a).

Compared to controls, the neutrophil count only decreased significantly in the S group ($p < 0.01$). There was a significant increase in all pretreated groups compared to the S group ($p < 0.001$ for the S + TQ group; $p < 0.01$ for the S + LAH group; $p < 0.05$ for the S + HAH group). There was no significant difference between the pretreated groups (Fig. 3b).

The lymphocyte count declined significantly in all groups compared to the control group ($p < 0.001$ for the S and S + TQ groups, $p < 0.01$ for the S + LAH group, $p < 0.05$ for the S + HAH group). There was a significantly increased lymphocyte count in the pretreated groups compared with the S group ($p < 0.01$ for the S + TQ group, $p < 0.001$ for the S + LAH and S + HAH groups). There was a significant difference between the α-hederin-treated groups compared to the S + TQ group ($p < 0.05$ for the S + LAH group, $p < 0.01$ for the S + HAH group). There was a significant difference between the S + LAH and S + HAH groups, too ($p < 0.05$, Fig. 3c).

Compared to the control group, there was a significant decrease in the monocyte count in the S group ($p < 0.05$), but it showed significant increments in the S + LAH and S + HAH groups ($p < 0.01$ for the S + LAH group, $p < 0.05$ for the S + HAH group). There was no significant difference between the S + TQ and control groups. The number of monocytes was significantly elevated in all pretreated groups compared to the S group ($p < 0.05$ for the S + TQ group, $p < 0.001$ for the S + LAH group, $p < 0.01$ for the S + HAH group). The S + HAH and S + LAH groups showed a significant increase compared to the S + TQ group ($p < 0.05$ for the S + LAH group, $p < 0.01$ for the S + HAH group). There was no significant difference between the S + LAH and S + HAH groups (Fig. 3d).

In comparison to the basophil count of the control group, there was a significant increase in the S group ($p < 0.05$) and significant decrease in the S + TQ group ($p < 0.05$). There was no significant difference in the basophil count between the α-hederin-treated groups and controls. The basophil count in the S + TQ and S + HAH groups was significantly lower than that in the S group ($p < 0.01$ for S + TQ group, $p < 0.05$ for the S + HAH group). The S + LAH group showed a significantly elevated basophil count compared to the S + TQ group ($p < 0.05$), but there was no significant difference between the S + HAH and S + TQ group. Moreover, there was no significant difference between the S + HAH and S + LAH groups (Fig. 3e).

Discussion

In the present study, the effects of α-hederin on tracheal responsiveness to methacholine, histamine and OA as well as the total and differential cell count in lung lavage fluid of ovalbumin-sensitized guinea pigs were examined.

The results showed significantly increased tracheal responsiveness to methacholine, histamine and OA as well as an increased contractility response, increment of the LLF total WBC count, eosinophil and basophil number, but decreased neutrophil, lymphocyte and monocyte counts in the sensitized group compared to control animals. These results were similar to the results of previous studies [12, 16]. Moreover, tracheal responsiveness to methacholin in all groups was higher than in histamine-induced contractions. This might be due to the direct effect on smooth muscle or indirect effect of histamine via nerves or mediators.

The drugs used for preventing asthma should be able to reduce airway inflammation, which is the main characteristic of this disease. In the present study, most of above changes were improved by peritoneal injection of thymoquinone. The anti-inflammatory and antitussive effect of thymoquinone on different animal models of asthma has been shown in previous studies [10, 13, 16, 19]. Previously, the main effect of thymoquinone was shown to be on LLF total WBC and eosinophil counts [20], which our current study confirmed.

In this investigation, the injection of two different doses of α-hederin could reduce the tracheal responsiveness to

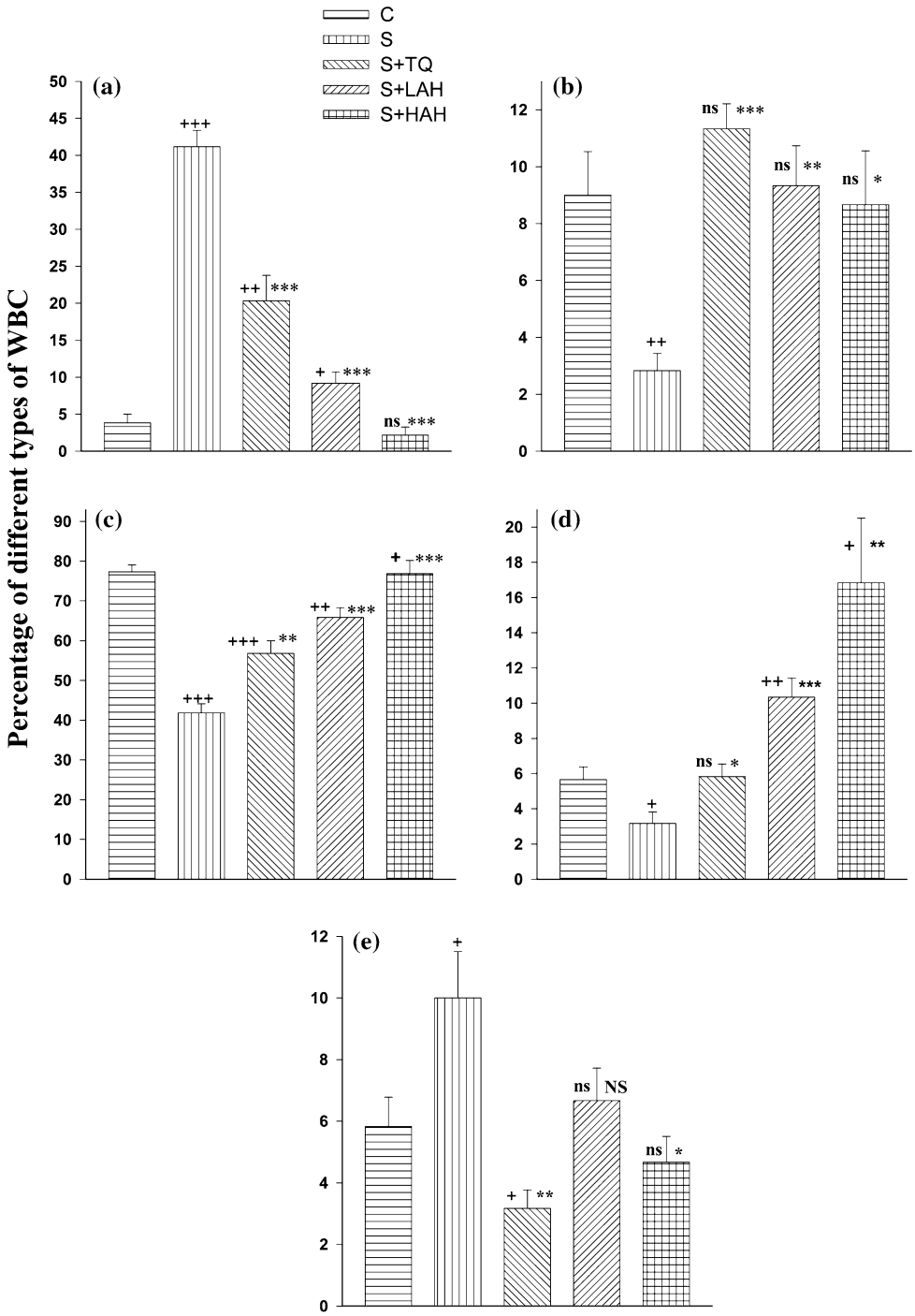

Fig. 3 The percentages of eosinophils (**a**), neutrophils (**b**), lymphocytes (**c**), monocytes (**d**) and basophils (**e**) in lung lavage fluid in the control, sensitized (S), sensitized pretreated with thymoquinone (S + TQ), sensitized pretreated with low-dose α-hederin (S + LAH) and high-dose α-hederin (S + HAH) guinea pigs (for each group, $n = 15$). Statistical differences between the control and different groups: *ns*, nonsignificant difference; +, $p < 0.05$; ++, $p < 0.01$; +++, $p < 0.001$. Statistical differences between pretreated groups vs. the sensitized group: *NS*, nonsignificant difference; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

methacholine, histamine and ovalbumin as well as the contractility and EC50 increment compared to the sensitized group. Even low-dose α-hederin can decrease the tracheal responsiveness to methacholine to lower levels compared to the control level.

α-Hederin is a triterpenoid saponin. Triterpenoids have a variety of biological effects, e.g., anti-inflammatory [21]. Preclinical studies analyzing living cells and the regulation of β_2-adrenoceptors recently identified α-hederin as the main active constituent among saponins isolated from ivy [22]. Gepdiremen described the acute anti-inflammatory activity of α-hederin in carrageenan-induced rat paw edema [15]. In clinical studies, treatment of acute and chronic bronchial inflammatory diseases with a dry extract of ivy leaves improved symptoms such as coughing and viscous mucus secretion as well as lung function parameters such as increased airway resistance in patients with obstructive airway diseases [23].

The exact mechanisms of action of α-hederin are not clear yet, but a previous study reported an elevated β_2-adrenergic responsiveness in α-hederin pretreated human airway smooth muscle cells after stimulation with terbutaline and forskolin [22]. Another study suggested that α-hederin elevated β_2-adrenergic responsiveness by increasing cAMP levels [23]. Elevated cAMP levels lead to an increased phosphorylation of myosin light-chain kinase (MLCK) by protein kinase A (PKA), which caused a reduced sensitivity of MLCK to calcium [24]. Moreover, cAMP inhibited calcium ion release from intracellular pools and decreased calcium entry into cells [25]. α-Hederin also inhibited the internalization of β_2-adrenergic receptors in stimulated airway smooth muscle cells [22]. Hegener and coworkers have suggested that the secretolytic and bronchodilating properties found in *Hedera helix* extract are due to its saponin content, particularly α-hederin as an inhibitor of B2 receptor endocytosis, establishing an indirect B2 sympathomimetic action [26].

Also an antioxidant effect was suggested for α-hederin. Airway inflammatory cells could produce active types of oxygen such as superoxide and peroxide. These active types of oxygen directly caused bronchial contraction, stimulated histamine release from mast cells and also excreted mucus by epithelial airway cells [27]. On the other hand, oxidative stress played an important role in airway inflammation in asthma disease [28], and a decrease in different components of the antioxidant defense system (enzymatic or nonenzymatic) in patients with asthma was observed [29].

In this study, the prescription of α-hederin at two different doses decreased the total WBC count, eosinophil and basophil numbers, and increased the number of neutrophils, monocytes and lymphocytes in LLF compared to the sensitized group. These changes indicated that the injection of α-hederin before the induction of asthma in ovalbumin-sensitized animals had a great impact on the total WBC count, eosinophil and lymphocyte numbers. These results showed that α-hederin had more impact on eosinophilic inflammation in animals sensitized with ovalbumin.

This study showed that the peritoneal injection of both doses of α-hederin could decrease the tracheal responsiveness to ovalbumin. This amount of reduction was more than the effect of thymoquinone at the studied dose. Regarding the changes in the lungs and airways of the studied animals, the results showed that the effects of α-hederin on the eosinophil and lymphocyte counts of LLF were greater than those of thymoquinone. In addition, the results of this study indicated that α-hederin had dilatory and antiasthmatic effects similar to those of thymoquinone. Furthermore, a high dose of α-hederin had a greater decreasing effect on tracheal responsiveness to ovalbumin and the LLF eosinophil count compared to a low dose. Even the LLF eosinophil count in the pretreated group with high-dose α-hederin came down to the control limit. In conclusion, α-hederin probably has anti-inflammatory and dilatory effects like thymoquinone.

Acknowledgments This is a report of a database from a thesis entitled "Effect of α-hederin on tracheal responsiveness, changes in inflammatory cells of bronchoalveolar lavage and blood cytokines in asthmatic guinea pigs" registered at the Drug Applied Research Center of Tabriz University of Medical Sciences.

Conflict of interest None declared.

References

1. Wills-Karp M (1999) Immunologic basis of antigen-induced airway hyper responsiveness. Annu Rev Immunol 17(1):255–281
2. Ray A et al (2010) Regulatory T cells in many flavors control asthma. Mucosal Immunol 3(3):216–229
3. Kalus U et al (2003) Effect of *Nigella sativa* (black seed) on subjective feeling in patients with allergic diseases. Phytother Res 17(10):1209–1214
4. Randhawa M, Al-Ghamdi M (2002) A review of the pharmacotherapeutic effects of *Nigella sativa*. Pak J Med Res 41(2):1–10
5. Boskabady MH et al (2007) The possible prophylactic effect of *Nigella sativa* seed extract in asthmatic patients. Fundam Clin Pharmacol 21(5):559–566
6. Boskabady MH, Mohsenpoor N, Takaloo L (2010) Antiasthmatic effect of *Nigella sativa* in airways of asthmatic patients. Phytomedicine 17(10):707–713
7. Ahmad J, Khan RA, Malik MA (2010) A study of *Nigella sativa* oil in the management of wheeze associated lower respiratory tract illness in children. Afr J Pharm Pharmacol 4(7):436–439
8. Al-Jawad FH et al (2012) Broncho-relaxant activity of *Nigella sativa* versus anthemis nobilis in chronic bronchial asthma; a comparative study of efficacy. IOSR J Pharm 2:81–83
9. Boskabady M, Kiani S, Jandaghi P (2004) Antitussive effect of *Nigella sativa* in guinea pigs. Pak J Med Sci 20:224–228
10. Hosseinzadeh H, Eskandari M, Ziaee T (2008) Antitussive effect of thymoquinone a constituent of *Nigella sativa* Seeds in guinea pigs. Pharmacologyonline 2:480–484
11. Boskabady MH et al (2011) The effect of *Nigella sativa* extract on tracheal responsiveness and lung inflammation in ovalbumin-sensitized guinea pigs. Clinics 66(5):879–887
12. Boskabady M-H et al (2011) Potential immunomodulation effect of the extract of *Nigella sativa* on ovalbumin sensitized guinea pigs. J Zhejiang Univ Sci B 12(3):201–209
13. Aziz AEA, El Sayed NS, Mahran LG (2011) Anti-asthmatic and anti-allergic effects of thymoquinone on airway-induced hypersensitivity in experimental animals. J Appl Pharm Sci 1(8):109–117
14. Keyhanmanesh R et al (2014) *Nigella sativa* pretreatment in guinea pigs exposed to cigarette smoke modulates in vitro tracheal responsiveness. Iranian Red Crescent Med J 16(7):e10421
15. Gepdiremen A et al (2005) Acute anti-inflammatory activity of four saponins isolated from ivy: alpha-hederin, hederasaponin-C, hederacolchiside-E and hederacolchiside-F in carrageenan-induced rat paw edema. Phytomedicine 12(6):440–444
16. Keyhanmanesh R et al (2010) Effect of thymoquinone on the lung pathology and cytokine levels of ovalbumin-sensitized guinea pigs. Pharmacol Rep 62(5):910–916
17. Pejman L et al (2014) The effect of adenosine A2A and A2B antagonists on tracheal responsiveness, serum levels of cytokines and lung inflammation in guinea pig model of asthma. Adv Pharm Bull 4(2):131

18. Boskabady MH, Harati M (1998) Increased histamine H1 receptor blockade by chlorpheniramine in tracheal chains of asthmatic guinea pigs. Med J Islam Repub Iran 12(3):265–271
19. Kalemci S et al (2012) Effectiveness of thymoquinone in the treatment of experimental asthma. La Clinica terapeutica 164(3):e155–e158
20. Keyhanmanesh R et al (2009) The effect of thymoquinone, the main constituent of *Nigella sativa* on tracheal responsiveness and white blood cell count in lung lavage of sensitized guinea pigs. Planta Med 75:1–9
21. Jeong HG, Lee SS (1999) Suppressive effects of α-Hederin on 2,3,7,8-tetrachlorodibenzo-p-dioxin-mediated murine Cyp1a-1 expression in the mouse hepatoma Hepa-1c1c7 cells. Cancer Lett 138(1):131–137
22. Sieben A et al (2009) α-Hederin, but not hederacoside c and hederagenin from hedera helix, affects the binding behavior, dynamics, and regulation of β2-adrenergic receptors†. Biochemistry 48(15):3477–3482
23. Wolf A et al (2011) Pre-treatment with α-hederin increases β-adrenoceptor mediated relaxation of airway smooth muscle. Phytomedicine 18(2):214–218
24. Giembycz M, Newton R (2006) Beyond the dogma: novel β2-adrenoceptor signalling in the airways. Eur Respir J 27(6):1286–1306
25. Johnson M (2001) Beta2-adrenoceptors: mechanisms of action of beta2-agonists. Paediatr Respir Rev 2(1):57–62
26. Hegener O et al (2004) Dynamics of β2-adrenergic receptor-ligand complexes on living cells. Biochemistry 43(20):6190–6199
27. Cluzel M et al (1987) Enhanced alveolar cell luminol-dependent chemiluminescence in asthma. J Allerg Clin Immunol 80(2):195–201
28. Talati M et al (2006) Oxidant stress modulates murine allergic airway responses. Free Radic Biol Med 40(7):1210–1219
29. Sackesen C et al (2008) A comprehensive evaluation of the enzymatic and nonenzymatic antioxidant systems in childhood asthma. J Allerg Clin Immunol 122(1):78–85