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# The relaxant effect of *Nigella sativa* on smooth muscles, its possible mechanisms and clinical applications

Rana Keyhanmanesh [1], Zahra Gholamnezhad [2], Mohammad Hossien Boskabady [2*]

[1] Tuberculosis and Lung Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2] Neurogenic Inflammation Research Centre and Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

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ABSTRACT

*Nigella sativa* (*N. sativa*) is a spice plant which has been traditionally used for culinary and medicinal purposes. Different therapeutic properties including the beneficial effects on asthma and dyspnea, digestive and gynecology disorders have been described for the seeds of *N. sativa*. There is evidence of the relaxant effects of this plant and some of its constituents on different types of smooth muscle including rabbit aorta, rabbit jejunum and trachea. The relaxant effect of *N. sativa* could be of therapeutic importance such as bronchodilation in asthma, vasodilation in hypertension and therapeutic effect on digestive or urogenital disorders. Therefore in the present article, the relaxant effects of *N. sativa* and its constituents on smooth muscles and its possible mechanisms as well as clinical application of this effect were reviewed.

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## Introduction

*Nigella sativa* (*N. sativa*) is a spice plant belonging to the family from Ranunculaceae (1). Different local names of the plant are; in Farsi, it's commonly known as Siah-Daneh or Fitch in south Asia, it is called Kalonji, its name in Arabic is Habat-ul-Sauda and its English, black cumin or black seeds. It is an annual grassy plant with green-to blue-colored flowers and black seeds.

Traditionally, there is a common Islamic belief that the 'black seed', is a panacea (universal healer) that is a remedy for all ailments, but cannot prevent aging or death (2-3). *N. sativa* has been traditionally used in Iran (4), the Indian subcontinent, Arabian countries (5) and Europe for culinary and medicinal purposes as a natural remedy for a number of illnesses and conditions (6-8). The fixed oil from seeds of *N. sativa* is useful in skin eruptions, paralysis, hemiplegia, back pain, rheumatoid and related inflammatory diseases (9-11). In view of the folkloric, the plant mixture extracts have been used for treatment of diabetes in the Middle East.

The extract of *N. sativa* was effective in reducing blood glucose due to inhibition of hepatic gluconeogenesis and its possible insulinotropic properties (12-18). In addition, different extracts of *N. sativa* and its constituents could lower blood pressure through blockade of calcium channels (19-24) and showed a potent inhibitory effect on both heart rate and contractility of isolated guinea pig heart which was comparable and even higher than that of diltiazem (25-26). *N. sativa* seeds and its constituent, thymoquinone were also found to reduce the levels of serum cholesterol, triglyceride and glucose as well as leukocyte and platelet counts (16, 23, 27-31). It has also inhibitory effects on arachidonic acid-induced platelet aggregation and blood coagulation (32). In the healthy female volunteers, it was shown that *N. sativa* crushed seeds and its total oil decreased glucose, prolactins, triglycerides and cholesterol level (33).

Moreover, *N. sativa* oil and its constituent, thymoquinone, were found to possess gastroprotective effect against gastric lesions and reduced the volume of gastric acid secretion, free acidity, total acidity and ulcer index (34, 35) which may be related to the conservation of the gastric mucosal redox state (36). Significant increase in mucin content, glutathione level and a significant decrease in mucosal histamine content in the stomach were also shown for the plant seed and thymoquinone (15, 37).

*N. sativa* and its constituents have been traditionally used to alleviate respiratory disorders such as asthma, bronchospasm and chest congestion (38,

*Corresponding author: Mohammad Hossien Boskabady, Neurogenic Inflammation Research Centre and Department of Physiology, School of Medicine, Mashhad, Post Code 9177948564, Iran. Tel : 098 511 8828565; Fax 0098 511 8828564; email: boskabadymh@mums.ac.ir, mhboskabady@hotmail.com

39). Nigellone, a carbonyl polymer of thymoquinone, was shown to be an effective prophylactic agent in asthma and bronchitis with higher efficacy in children than in adults (40, 41). Chakravarty (42) concluded that the nigellone inhibit effectively the histamine release from the mast cells, thus showing the basis for its traditional use in asthma. It was also suggested that *N. sativa* volatile oil might be a potentially useful respiratory stimulant (43). The antihistaminic effect of the plant was also reported (44, 45).

The prophylactic effect of boiled extract of *N. sativa* on asthmatic patients as improvement in the symptom score, asthma symptom/week, chest wheeze and pulmonary function test (PFT) values in a three months treatment period was also shown (46). In addition, the beneficial role of *N. sativa* in reducing wheeze associated with lower respiratory tract illness in children (47) and adult asthmatic attacks has been reported (48). The bronchodilatory effect of boiled extract of *N. sativa* on asthmatic patients was also documented by increasing pulmonary function tests and specific airway conductance (sGaw) of the patients after administration of the extract (49).

The antitussive effect of *N. sativa* and its constituent, thymoquinone in guinea pigs has been shown (50,51). Moreover, the preventive effect of hydroethanolic extract of *N. sativa* and its main constituent, thymoquinone, in ovalbumin sensitized guinea pigs and rats, as animal models of asthma was documented (52-56). In a current study, the preventive effect of the hydroethanolic extract of this plant was proved on the tracheal responsiveness of cigarette smoke-exposed guinea pigs (57). In another study, the preventive effect of long term administration of three different concentrations of carvacrol, another constituent of the plant has been shown in asthmatic guinea pigs (58).

With regard to various physiologic effects of smooth muscle relaxant effect such as bronchodilation in obstructive pulmonary diseases, reducing high blood pressure by vasodilation and due to several studies examining the relaxant effect of *N. sativa* and its constituents on smooth muscles, this property of the plant and its possible mechanism(s) as well as clinical application of this effect was reviewed in the present article.

## Methods

Online literature searches were performed using Medline, Pubmed, Iran medex, Scopus, and Google Scholar websites backed to 1959 to identify researches about *N. sativa*, its constituents and relaxant effect.

### *Constituents*

The seed oil of *N. sativa* was found to be rich in polyphenols and tocopherols (59-60). The plant's seeds contain a yellowish volatile oil; 0.5-1.6% (61) or 0.4–2.5% (62), a fixed oil; 35.6-41.6%, proteins; 22.7% (61), different amino acids (such as arginine, glutamic acid, leucine, lysine, methionine, tyrosine, proline and threonine), carbohydrates; 33.9% and fiber; 5.5% (63). The other compositions of the plant include; fats, crude fibers, minerals; e.g. Fe, Na, Cu, Zn, P, Ca and many vitamins. *N. sativa* seeds yield esters of fatty acids (61), free sterols, steryl esters, steryl glucosides and acylated steryl glucosides (64). Fatty acids were identified in the extract of the plant, which represented about 99.5% of the total fatty acid composition. The fixed oil is composed mainly of fatty acids consisted of four saturated fatty acids (17.0%) and four unsaturated fatty acids (82.5%). The major unsaturated fatty acids were linoleic acid (C18:2, 55.6%) and oleic acid (C18:1, 23.4%), while the main saturated fatty acids was palmitic acid (C16:0, 12.5%) and stearic (C18:0) acids (62, 65,66). β-sitosterol was the dominant sterol (69%); while campesterol and stigmasterol constituted 12% and 19%, of the total sterols respectively (62).

Pharmacologically active constituents of the volatile oil of the plant are thymoquinone; 27.8-57% (61) or 30–48% (67), dithymoquinone, thymohydroqunone and thymol. The content of thymoquinone is highest (57-78%) when water has withheld for 12 days (61). Nigellone is the carbonyl polymer of thymoquinone, isolates from *N. sativa* seeds. This polymer is far less toxic but retains much of the pharmacologic properties of thymoquinone, which is its active principle (42). Other components isolated from the essential oil of the plant included; alpha-pinene (13.75 %), ρ-cymene (7.1-15.5 %), carvacrol (5.8-11.6%), trans-anethole (0.25-2.3% or 38.3%), 4-terpineol (2.0-6.6%), longifoline (1.0-8.0%), limonene (4.3%) and carvone (4.0%), (39, 65, 68-69). Four alkaloids have been reported as constituents of *N. sativa* including; nigellicine (67, 70), nigellidine which contains an indazole nucleus (71-72), nigellimine (73) and isoquinolines (74).

Briefly, thirty-two compounds, constituting 86.7% of the volatile oil, were identified. The oil consisted of six phenyl propanoid compounds (46.1%), nine monoterpenoid hydrocarbons (26.9%), four monoterpenoid ketones (6.0%), eight nonterpenoid hydrocarbons (4.0%), three monoterpenoid alcohols (2.7%) and two sesquiterpenoid hydrocarbons (1.0%). So, the oil of *N. sativa* is characterized by a large amount of phenyl propanoids (65). Table 1 summa-rized the different chemical composition of the plant.

### *Relaxant effects on various types of smooth muscles*

The relaxant effect of various preparations of *N. sativa* and its constituents on different types of smooth muscles were demonstrated which reviewed in the following sections.

#### *Tracheal smooth muscle*

Reiter and Brandt examined the relaxant effects of 22 plants extracts from 11 different families including *N. sativa* on tracheal and ileal smooth

**Table 1.** Chemical composition of *N. sativa* based on the type of plant preparation

| Plant preparation | Constituents | Reference |
|---|---|---|
| Seed oil | Polyphenols and tocopherols | (59-60) |
| | Volatile oil | (61-62) |
| | Fixed oil | (61) |
| | Proteins | (61) |
| | Aminoacids (Arg, Glu, Leu, Lys, Met, Tyr, Pro, Thr) | (63) |
| | Carbohydrates | (63) |
| | Fibers | (63) |
| | Minerals ( Fe, Na, Cu, Zn, P, Ca) | (63) |
| | Vitamins | (63) |
| | Fat: esters of fatty acids, free sterols, steryl esters, steryl glucosides, acylated steryl glucosides | (61, 64) |
| | Alkaloids: Nigellicine | (67, 70) |
| | Nigellidine | (71-72) |
| | Nigellimine | (73) |
| | Isoquinolines | (74) |
| Volatile oil | Thymoquinone, Dithymoquinone, Thymohydroqunone or Thymol | (61, 67) |
| | Nigellone | (42) |
| | ρ-cymene, Carvacrol, t-anethole, 4-terpineol, limonene, carvone and longifoline | (65, 68-69) |

muscles of the guinea pig in comparison with the relaxant effects of catecholamines and phosphor-diesterase inhibitors. All of the oils had relaxant effects on tracheal smooth muscle. However, the extract of *N. sativa* had a higher relaxant effect on tracheal muscle than ileal smooth muscles (75). A previous study also showed that the volatile oil of *N.* sativa could protect guinea pigs against histamine-induced bronchospasm but it did not affect histamine $H_1$ receptors in isolated tissues (41).

Many studies demonstrated the relaxant effects of a crude extract including aqueous and macerated extracts of *N. sativa* seeds on isolated guinea pig tracheal preparation (76-78). In one study, the effect of three concentrations of aqueous extract of *N. sativa* (0.25, 0.5 and 1 g%) have been evaluated on calcium induced contraction of guinea pig tracheal smooth muscle. The results elucidated the rightward shift in calcium concentration response curves indicating the relaxant effect of this extract on isolated guinea pig tracheal smooth muscle compared to saline (79).

The relaxant effects of hydroethanolic, macerated, aqueous and lipid free macerated aqueous extract of *N. sativa* on tracheal smooth muscle of guinea pigs were also examined. The results showed that mainly water soluble substances of the plant were responsible for the relaxant effect of the plant on airway smooth muscle (80). The relaxant effects of four fractions of *N. sativa* including; n-hexane, dichloromethane, methanol and aqueous fractions, on tracheal smooth muscle of guinea pigs were also examined. These results showed a potent relaxant effect of most fractions of the plant on airway smooth muscle. The most potent relaxant effect was seen for methanol and dichloromethane fractions (81). In a recent study, in order to identify main constituents of the methanolic extract, the relaxant effects of five different methanolic fractions (20%, 40%, 60%, 80%, and 100%) of *N. sativa* on tracheal smooth muscle of guinea pigs were examined. The results of this study showed a potent relaxant effect of 20% methanolic fractions of this plant (82). Four constituents of 20%-methanolic fraction, consisting of two flavonoids (20-20% and 21-20% fractions) and two polysaccharides (1-20% and 2-20% fractions), were purified by analytical and preparative HPLC and their relaxant effects were examined on methacholine-precontracted guinea pig tracheal smooth muscle. The findings revealed that two flavonoids of 20%-methanolic fraction of *N. sativa* were its main relaxant constituent (83).

The effect of thymoquinone, the main constituent of the volatile oil of *N. sativa*, on the guinea pig isolated tracheal preparation showed that thymoquinone caused a concentration-dependent decrease in the tension of the tracheal smooth muscle (84). However, the results of this study was not in agreement to the findings of other study in which three cumulative concentrations (40, 80, and 120 μM) of thymoquinone did not show any relaxant effect on methacholine or KCl precontracted tracheal smooth muscle of guinea pigs (85).

In addition, the preventive effects of two concentrations of hydroethanolic extract of *N. sativa* on the tracheal responsiveness of ovalbumin sensitized guinea pigs, an animal model of asthma, were examined. The tracheal responses of treated groups with both concentrations of the plant to methacholine and ovalbumin were significantly decreased compared to those of sensitized group. Incubation of the tracheal smooth muscle with the *N. sativa* extract also caused a further decrease in the tracheal responsiveness to methacholine and ovalbumin and a decrease in the contractility response. These results confirmed the relaxant effect of the plant on airway smooth muscle (52). Moreover, the prophylactic effect of short term (56) and long term (54) administration of two doses of thymoquinone, the main constituent of *N. sativa* has also been shown similar results on tracheal smooth muscle. The potent relaxant effect of carvacrol, another constituent of *N. sativa* on guinea pig tracheal smooth muscle was also demonstrated (86-88).

**Table 2.** The relaxant effect of *Nigella sativa* and its constituents on different types of smooth muscle. In most studies, Smooth muscle was contracted, the extract was added and the effect was measured

| Type of smooth muscle (SM) | Effect | Reference |
|---|---|---|
| Trachea | Relaxant effect | (75-84) |
| | Protected against histamine-induced bronchospasm | (41) |
| | Preventive effects on the tracheal responsiveness | (52, 54) |
| Vascular | Depressant action on heart | (89) |
| | Vasorelaxation | (21, 90) |
| Gastrointestinal | Spasmolytic effect | (75, 91-93) |
| Urogenital | Inhibition of spontaneous movements | (1) |

*Vascular smooth muscle*

The essential oil from the seeds of *N. sativa* exhibited a depressant effect on the frog heart and a relaxant property on isolated vascular smooth muscles of rat (89). The vasorelaxation effect of cumulative concentration of the hydroethanolic extract of *N. sativa* (2, 4, 6, 8, 10, and 14 mg/ml) on rat aortic smooth muscle contracted by both KCl and phenylephrine was shown (21). In other study, the effect of thymoquinone on smooth muscle of pulmonary artery was assessed. The finding showed that thymoquinone caused a concentration-dependent decrease in the tension of the pulmonary arterial smooth muscles precontracted by phenylephrine (90).

*Gastro-intestinal and urogenital smooth muscle*

The oils of 16 plants including *N. sativa* inhibited the phasic contractions of the ileal myenteric plexus-longitudinal muscle preparation (75). The volatile oil and ethanol extract of *N. sativa* inhibited spontaneous movements of the rabbit jejunum and prevented contractions induced by high potassium ($K^+$) solution or acetylcholine. This inhibition was dose-dependent, reversible and was not affected by the addition of calcium to the organ bath. These data suggest that the plant has an antispasmodic effect, possibly due to a calcium antagonistic activity (91).

In another study, the inhibitory effect of different doses (10, 20, 40, 80, 100 mM) of thymoquinone on the contractility of guinea pig isolated ileum was evaluated. The findings indicated that the major constituent of *N. sativa*, thymoquinone, inhibit guinea pig isolated ileum contractility, and this effect may be responsible for the smooth muscle relaxant activity of this plant (92). For assessing the spasmolytic activity of thymoquinone, it was added cumulatively after using high $K^+$ concentration to depolarize the rabbit jejunum and guinea pig ileum smooth muscles in organ bath. In this investigation, sustained contractions were obtained with high $K^+$ which involves entry of $Ca^{2+}$ into the cells through voltage-operated $Ca^{2+}$ channels. Thymoquinone showed a concentration-dependent relaxant activity on both intestinal smooth muscle preparations. According to the results of this study, it has been concluded that thymoquinone-induced smooth muscle relaxation occurred via blockade of voltage-operated $Ca^{2+}$ channels in intestinal jejunal and ileal tissues (93). The volatile oil of *N. sativa* inhibited the spontaneous movements of rat and guinea pig uterine smooth muscle and also the contractions induced by oxytocin, showing its antioxytocic potential (1).

The relaxant effect of the plant on different types of smooth muscles was summarized in Table 2.

***Possible mechanisms of the relaxant effect of N. sativa on smooth muscles***

The relaxant effects seen for *N. sativa* on smooth muscles in various studies might be produced due to several different mechanisms which are described in this part of article.

*Calcium channel blocking effect*

Gilani and his colleagues studied the effects of extract of *N. sativa* seeds on isolated tracheal and ileal smooth muscle and showed that the aqueous-methanolic extract of this plant had spasmolytic effect mediated through calcium channel blocking activity. This study showed that the extract antagonized the contractions induced by histamine, carbachol and KCl. The relaxant effect of the plant on smooth muscles was suggested to be mediated via calcium channels. The above pharmacological activities of the petroleum ether fraction of the extract were about 10 times higher than those of the crude extract (76). The calcium-antagonistic effects of three increment concentrations (0.25, 0.5 and 1 g%) of the aqueous extract of *N. sativa* were also tested by measuring the cumulative concentration response curves of $CaCl_2$-induced contractions of isolated guinea pig tracheal smooth muscle in the presence of diltiazem, a calcium channel blocker and the extract. The results showed rightward shift in $CaCl_2$-concentration response curve in the presence of the extract similar to the effect of diltiazem which suggest a calcium antagonistic effect of *N. sativa* in isolated tracheal smooth muscle (79). However, the absence of inhibitory effects of the extracts on KCl induced contraction of tracheal smooth muscle suggested that the calcium channel blocking effect of this plant did not contribute to the relaxant effect of this plant on the tracheal smooth muscle of guinea pigs (78).

The effect of *N. sativa* aqueous extract on calcium channels was also examined more precisely by its effects on heart rate and contractility of isolated heart in the presence of calcium free Krebs solution. The findings indicated that the extract of this plant almost completely inhibited calcium channels of

isolated guinea pig heart (26). Previous experiment implied that *N. sativa* seed is able to relax the aorta and by inhibition of inositol triphosphate ($IP_3$) and/or ryanodine receptor-dependent release of intracellular $Ca^{2+}$(21). It was also shown that the voltage-dependent $Ca^{2+}$ channels are involved in KCl-induced contraction. Therefore, voltage-dependent $Ca^{2+}$ channels blockade is the suggested mechanism of vasorelaxant substances which affect KCl induced contractions (21, 94). The dose-dependent inhibitory effect of volatile oil of *N. sativa* on contractions of the rabbit jejunum induced by high potassium (K+) solution or acetylcholine was also shown which was not affected by the addition of calcium to the organ bath. These results also suggest that the antispasmodic effect of the plant is possibly due to a calcium antagonistic activity (91). The calcium channel blocking effect of *N. sativa* was also confirmed by various other studies (20, 22-24)

In other studies, the relaxant activity of thymoquinone, a bioactive component of *N. sativa* was evaluated on rabbit and guinea pig cardiac, intestinal and tracheal smooth muscles. The data of these studies suggested that its relaxant effect on smooth and cardiac muscles partially mediated via blockade of voltage-operated $Ca^{2+}$ channels (92-93).

The inhibited spontaneous movements of the rabbit jejunum and the preventive effect of volatile oil and ethanol extract of *N. sativa* on contractions induced by high potassium ($K^+$) solution or acetylcholine which was not affected by the addition of calcium to the organ bath also suggested a calcium antagonistic activity of the plant (91). Concentration-dependent relaxant effect of thymoquinone on intestinal smooth muscle preparations contracted with high $K^+$ which involves entry of $Ca^{2+}$ into the cells through voltage-operated $Ca^{2+}$ channels also showed involvement of voltage-operated $Ca^{2+}$ channels blockade in this effect (93).

*Potassium channel opening effect*

The inhibitory effect of aqueous and macerated extracts of *N. sativa* on contraction of tracheal smooth muscle in the presence of both ordinary and calcium free Krebs solutions have been examined previously (78). The extracts of *N. sativa* did not show any relaxant effect on KCl induced tracheal smooth muscle contraction, but they had a relaxant effect when the muscle was contracted by methacholine. These results may indicate an opening effect of the plant on potassium channels because, if a compound had a potassium channel opening effect, it would not have relaxant effects on smooth muscle contracted by KCl, while it could show a relaxant effect on methacholine induced muscle contraction. In addition, the bronchodilatory effect of potassium channel openers has been demonstrated previously (95) which support this suggestion.

On the other hand, membrane hyperpolarization is the result of an efflux of $K^+$ rises due to the opening of the $K^+$ channels in the vascular smooth muscle cells. This effect is followed by the closure of voltage-dependent $Ca^{2+}$ channels, leading to the reduction in $Ca^{2+}$ entry and vasodilation (96). Vascular smooth muscle cells express both $K_{ATP}$ and nonselective $K^+$ channel (97-98). A previous study indicated the nonselective blockade of the $K_{ATP}$ channel by *N. sativa* extract in rat aorta (21). The relaxant effect of different concentrations (50, 100, 200, 500 and 1000 μM) of thymoquinone has also shown in the isolated rat pulmonary artery. In this study, blocking of ATP-sensitive $K^+$ channel by glibenclamide significantly reduced thymoquinone-induced relaxation of pulmonary arterial smooth muscle. These findings also suggested that relaxation of pulmonary arterial smooth muscle by thymoquinone could be through $K^+$ channel activation (90).

***The anticholinergic and muscarinic receptor inhibitory effects of the plant***

The anticholinergic effect of aqueous and macerated extracts of *N. sativa* has been demonstrated by performing concentration response curve to methacholine, a muscarinic agonist in the presence of the extracts and atropine (77). The extracts of *N. sativa* and atropine showed rightward shifts in methacoline concentration response curve but the maximum response to methacholine was not achieved in the presence of macerated extract. These results indicated a competitive antagonistic effect of aqueous extract and a non-competitive antagonistic effect of macerated extract of *N. sativa* on muscarinic receptors (77). In addition, the existence of α-pinene in essential oil of this plant was demonstrated which showed anticholinergic activity (99).

The contribution of muscarinic receptors blocking effect on the relaxant effect of extracts of *N. sativa* was examined on incubated tracheal preparations with atropine. The results indicated a functional antagonism effect of aqueous extract from this plant at muscarinic receptors (78).

The inhibitory effect of carvacrol, a constituent of *N. sativa* on muscarinic receptors of tracheal smooth muscle as one possible mechanism responsible for its relaxant effect was also had been shown (87). The slopes of the Schild plot in the experimental groups were supported the notion of competitive antagonism of carvacrol on muscarinic receptors of guinea pig tracheal smooth muscle. These findings indicated a competitive antagonistic effect of carvacrol at muscarinic receptors of tracheal smooth muscle (87).

***Histaminic (H1 receptor) antagonistic activity***

Mahfous and EL-Dakhakhny reported that the volatile oil from *N. sativa* protected guinea pigs against bronchospasm induced by histamine, but in

**Table 3.** Possible mechanisms of the relaxant effect of *Nigella sativa* and its constituents

| Possible mechanisms | Type of muscle | Reference |
|---|---|---|
| Calcium antagonistic effect | Trachea | (76, 78-79, 92-93) |
| | Vascular | (21, 26, 92-94) |
| | Gastrointestinal | (92-93) |
| Potassium channel opening effect | Trachea | (95) |
| | Vascular | (21, 90) |
| Anticholinergic and anti-muscarinic effect | Trachea | (77-78, 87-88, 99) |
| Histaminic antagonistic activity | Trachea | (41, 77, 101-102) |
| Stimulatory effect on β2-adrenoceptors | Trachea | (103-105) |

isolated tissues, it did not affect histamine $H_1$ receptors (41). However, in an *in vivo* study, increasing respiratory rate and intra-tracheal pressure of guinea pigs due to intravenous administration of volatile oil from *N. sativa* has been demonstrated (100). The reason(s) of this discrepancy is unclear to us.

The concentration response curves of histamine induced contraction of smooth muscle were performed in the presence of saline, the plant extracts and chlorpheniramine. The results showed a parallel rightward shift in the presence of macerated extract compared to the curve obtained in the presence of saline similar to that of chlorphrniramine and the maximum response to histamine was also achieved. The values of concentration response - 1 (CR - 1) produced by the macerated extract was also significantly higher than that of chlorpheniramine. These results indicated a competitive antagonistic effect of macerated extract from *N. sativa* on histamine $H_1$ receptors of tracheal smooth muscles which was greater than that of chlorpheniramine at concentrations used (101). In fact, in a previous study, the relaxant effect of $H_1$ receptors antagonist, chlorpheniramine on smooth muscle was shown which supported the contribution of $H_1$ receptors inhibition in the relaxant effect of *N. sativa* on smooth muscles (102).

The inhibitory effect of carvacrol, a constituent of *N. sativa*, on histamine $H_1$ receptors was also investigated and the results showed parallel and concentration dependent rightward shifts in histamine concentration response curve and maximum response to histamine was achieved in the presence of carvacrol. This finding showed a competitive antagonistic effect for carvacrol on $H_1$ receptors of tracheal smooth muscles (103).

### *Stimulatory effect on β2- adrenoceptors*

The stimulatory effect of aqueous and macerated extracts from *N. sativa* on β2- adrenoceptors was examined by performing isoprenaline concentration response curves inducing relaxation of precontracted tracheal smooth muscle in the presence of saline, extracts and propranolol (103, 104). While propranolol caused a parallel rightward shift as expected, both extract lead to parallel leftward shift of the curves. These results may suggest a stimulatory effect of both extract from *N. sativa* on β2- adrenoceptors. The stimulatory effect of carvacrol, the constituent of the plant on β-adrenergic receptors was also evaluated similarly and the results showed concentration dependent leftward shift in isoprenaline concentration response curve obtained in the presence of carvacrol which suggested stimulatory effect of this compound on β-adrenergic receptors (105).

The vasorelaxation effect of cumulative concentration of the hydroethanolic extract of *N. sativa* on rat aortic smooth muscle contracted by both KCl and phenylephrine, a selective $\alpha_1$-adrenergic receptor agonist (21) and the relaxant effect of thymoquinone on smooth muscle of pulmonary artery precontracted by phenylephrine (90) indicated the involvement of $\alpha_1$-adrenergic receptor blockade in this effect.

### *Other possible mechanisms of the relaxant effect of N. sativa on smooth muscles*

The other possible mechanisms responsible for relaxant effect of *N. sativa* and its constituents included; stimulation of inhibitory non-adrenergic non-cholinergic nervous system (NANC) or inhibition of stimulatory NANC (106), methylxanthine activity of the plant (107), inhibition of phosphodiesterase (108), nitric oxide production because it was shown that nitric oxide induces airway smooth muscle cell relaxation (109). However, the extract of *N. sativa* caused a dose-dependent decrease in NO production (110). These results indicated that this mechanism cannot contribute in the relaxant effect of the plant on smooth muscles. Non-competitive blocking of serotonin, $\alpha_1$ and endothelin receptors (90), inhibition of eicosanoid generation, namely thromboxane $B_2$ and leukotriene $B_4$ (111), stabilizing of mast-cells and inhibiting the release of histamine (61), inhibition of $PGD_2$ synthesis and $Th_2$-driven immune response (112) also could contribute in the relaxant effect of the plant on smooth muscle. However, the contribution of these mechanisms in the relaxant effect of *N. sativa* should be examined in further studies. Table 3 summarized the possible mechanisms of *N. sativa* and its constituents.

### *Clinical applications*

The relaxant effect of *N. sativa* on tracheal smooth muscle could be of therapeutic value in treatment of airway obstruction in obstructive pulmonary diseases such as asthma and chronic pulmonary

**Table 4.** Possible clinical applications of the relaxant effect of *Nigella sativa* and its constituents on different types of smooth muscle

| Method | Possible effect | Reference |
|---|---|---|
| Respiratory system | Bronchodilatory effects | (46, 49, 84, 113) |
| | Elevation in FEV1and FVC values in asthmatic patients | (48, 49) |
| | Antitussive activity | (50, 51) |
| Cardiovascular system | Vasodilatory effect | (22-23) |
| | Lowering blood pressure and improving of lipid profile | (23) |
| Gastrointestinal tract | Spasmolytic effect | (75-76, 92, 114) |
| | Gastroprotective effect | (35-36) |
| Urogenital tracts | | (115) |

diseases (COPD). In fact, the bronchodilatory effects of the boiled extract of the plant on asthmatic patients (49) as well as its effect on airway of these patients (46) and chemical war victims who had airway obstruction (113) were demonstrated. In one of these studies (49), the boiled extract of *N. sativa* was administered orally and values of pulmonary function tests were measured repeatedly in 30 min interval until 180 min. The results showed increase in all pulmonary function tests values which was similar to the effect of oral theophylline and inhaled salbutamol both from the magnitude and duration of point of view (49). The comparative study also showed that the plant significantly increased the values of forced expiratory volume in one second (FEV1%) and forced vital capacity (FVC) with marked reduction in asthmatic attacks (48). Antitussive activity of this plant and its constituents has also shown in guinea pigs (50, 51). These studies further support the traditional use of black seeds either alone or in combination with honey to treat bronchial asthma (84).

Relaxant effect of black seed on vascular smooth muscle leads to vasodilatory effect which could indicate the therapeutic application of the plant in hypertensive patients (20, 22, 23). In fact, administration of *N. sativa* seed extract for 8 weeks reduced systolic and diastolic blood pressure in a dose-dependent manner and lowered the levels of total and LDL cholesterol in patients with mild hypertension (23).

The relaxant effect of the plant on gastrointestinal smooth muscle was also had been shown (75, 91-93). The aqueous and methanolic extract of *N. sativa* seeds and its constituent, thymoquinone, was showed to have spasmolytic effect which provides a scientific basis for its traditional use in diarrhea (76) and colitis (114). Moreover, both *N. sativa* and thymoquinone can partially protect gastric mucosa from acute alcohol-induced mucosal injury (35) and ischaemia/reperfusion induced gastric lesion (36).

With regard to the relaxant effect of *N. sativa* on urogenital smooth muscle, the plant also may have therapeutic values in the disorders of these systems associated with the obstruction of their tubes. In fact the protective effect of *N. sativa* on the kidney function in rats has been shown (115). Table 4 summarized the possible clinical application of *N. sativa*.

## Conclusion

In conclusion the relaxant effects of *N. sativa* and some of its constituents on trachea, vascular, gastrointestinal and urogenital smooth muscles were discussed in the present review article. The possible mechanisms of the relaxant effect of the plant and its constituents on smooth muscle as well as the clinical applications of this effect were also indicated. However, further clinical studies were needed to demonstrate responsible constituents of the plant, the exact mechanism of action and the clinical applications of this effect more clearly.

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