



The Effect of Aqueous Extract of *Nigella sativa* Seeds on Smooth Muscle of Isolated Trachea in Sheep

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Abstract:

The present study was designed to determine the effect of the aqueous extract of powdered *Nigella sativa* seeds on pre-contracted smooth muscle isolated from the trachea in sheep. Parts of the trachea were taken from freshly slaughtered sheep and were kept viable in Krebs–Henseleit solution at (37 C°), then the smooth muscles were isolated from the trachea and proceeded for studying its contraction in physiograph. Aqueous extract of *Nigella sativa* seeds was prepared and six concentrations of the aqueous extract were used (50, 100, 150, 200, 250, and 300 mg / 10 ml). The smooth muscle was stimulated for contraction with different drugs (acetylcholine or histamine). Next, one milliliter of the prepared six serially increasing concentrations of the *Nigella sativa* aqueous extract was added separately to pre-contracted isolated tracheal smooth muscle to determine any probable inhibitory effect of the seeds extract. Aqueous extract of *Nigella sativa* seeds caused relaxation of tracheal smooth muscle pre-contracted with acetylcholine or histamine which may have taken place through stimulation of the non-adrenergic – non-cholinergic system.

Keywords: *Nigella sativa*, isolated trachea, sheep, smooth muscle.

Introduction:

Although many countries, including the United States, England, France, Germany, and others, have advanced in the manufacture of synthetic drugs, these countries have advanced in the study of medicinal plants, as evidenced by the number of publications on medicinal plants and their attempts to make medicine available in their countries [1]. One of the well-known medicinal plant is *Nigella sativa* which is also known as black seeds. It is a natural cure for a wide range of disease conditions, including, inflammation, cough, eczema, and flu-like symptoms [2]. *Black seeds* have been used traditionally to relief of asthma, also its effect on the isolated smooth muscle of the trachea that has been studied experimentally. Medicinal plants in general, and *Nigella sativa* seeds in particular, are well known for being consumed or prescribed for people in the form of a type of tea, in which the powdered seeds are macerated or simply boiled to prepare a cure.

It has been recorded that thymoquinone-induced relaxation of guinea pig isolated trachea [3]. The crude extract of *Nigella sativa* seeds was studied *in vitro* and it has been concluded that causes relaxation of carbachol, histamine and K⁺ induced contraction of isolated guinea pig trachea [4 and 5]. Both aqueous and macerated extracts of *Nigella sativa* showed inhibitory effect on pre-contracted tracheal chain of guinea pig in the presence of krebs solution [6]. To the best of our knowledge, scrutiny of published articles showed that there is no previous study focused on studying the effect of watery extract of black seeds on the isolated tracheal smooth muscle specifically in sheep. Therefore, the goal of the current study is to determine the effect of the aqueous extract of *Nigella sativa* seeds on the contracted smooth muscle of isolated trachea from sheep.



Materials and Methods:

Aqueous Extract Preparation:

Dry *Nigella sativa* Linn. seeds were purchased from a local market in Baghdad, and were identified in the National herbarium at Abu Graib. Fifty grams of *Nigella sativa* seeds were macerated in (300) ml of distilled water, completely ground and mixed by an electric blender, then the suspension was transferred into glass container, tightly stoppered and shaken intermittently at room temperature with an electric shaker for (48) hours then filtered first through filter sac, the clear filtrate was concentrated with aid of a rotary evaporator at a temperature of 50 C° and dried in an incubator[7]. The yielded concentrated extract was weighed and kept frozen at (-20C°) until usage.

Drugs Used and Tissue Preparation:

In this experiment the following drugs were used:

- a) Synthetic acetylcholine iodide (Muscarinic receptor stimulant) 50 µg / ml.
- b) Propranolol (β-adrenoceptor blocker) 9 µg / ml.
- c) Atropine sulphate (muscarinic receptor blocker) 0.6 µg / ml.
- d) Synthetic histamine 0.05 mg / ml.

Concentrations of 50, 100, 150, 200, 250 and 300 mg / 10 ml of *Nigella sativa* seed extract were prepared in distilled water. Portion of trachea was taken from freshly slaughtered sheep, preserved and kept viable in Krebs–Henseleit solution at 20-25 C° in tightly closed container until usage. The tracheal part then transferred into a Petri dish that contained Krebs–Henseleit solution at (37 C°) and the surrounding connective tissue was removed, then one cartilaginous ring from the lower part of trachea was taken and cut so that a small piece of the ring was kept attached to each end of the muscle. This was suspended into the organ bath which contains (60) ml of Krebs–Henseleit solution at (37

C°) supported with oxygen through an aerator. Suspension of the isolated tissue was made by ligation one of its ends to the aerator and the other end to the transducer's arm.

Protocol:

1-The tension of the transducer's arm was equilibrated so that the pen of the physiograph runs on the baseline before addition of test substances and also after washing with Krebs–Henseleit solution following each addition.

2-Maximal contraction of the isolated muscle was induced by addition of acetylcholine. One milliliter of six serially increasing concentrations of the aqueous extract of *Nigella sativa* seeds (previously prepared) was added separately to examine their probable inhibitory effect reflected as markings on the physiogram.

3-To make sure of blockade of muscarinic receptors, atropine was added after careful wash of the tissue followed by acetylcholine.

4-The tissue was washed for several times to get rid of any remnant of atropine, then watery extract of *Nigella sativa* seed was added followed by acetylcholine to see whether the extract imitates the inhibitory effect of atropine.

5-After wash, maximal contraction of the isolated muscle was induced by acetylcholine followed with propranolol (non-selective β adrenoceptor blocker). Aqueous extract of the seeds was added without wash to examine any probable adrenergic activity of the extract.

6-Histamine was added to the suspended tissue to obtain maximal contraction. One milliliter of six serially increasing concentrations of the *Nigella sativa* watery extract (previously prepared) were then added each separately to determine any probable inhibitory effect of the plant extract.

Results

Nigella sativa seeds were extracted by maceration and sieved to get rid of debris and

fibers. Extraction yielded 18% of the dry matter. The prepared aqueous extract of



Nigella seed caused relaxation of smooth muscle of sheep trachea suspended in organ bath after a maximal contraction induced by acetylcholine or histamine. Concentrations of the aqueous extract beyond 5 mg/ ml gave immediate and concentration- dependent relaxant effect to tracheal smooth muscle. Relaxation was demonstrated by a decline of pen tracing beneath the base line of physiogram. Relaxant effect of all concentrations continued until washing (table, 1 and figure, 1). Several washings of the isolated tissue allowed restoration of its normal condition. Addition of atropine to the isolated trachea prevented its contraction by subsequently added acetylcholine. Addition of the Nigella seed extract, however, didn't clearly prevent contraction of the tracheal smooth muscle by acetylcholine. To precontracted tracheal smooth muscle, propranolol (β -adrenoceptor blocker) was added. Successive addition of Nigella seeds aqueous extract caused relaxation of the muscle. Likewise, concentrations of 50, 100, 150, 200, 250 and 300 mg/10ml of the aqueous extract of Nigella seeds induced immediate and concentration – dependent relaxation of smooth muscle isolated sheep trachea in which maximal contraction was induced by histamine. Relaxation continued after each concentration until washing (table, 2 and figure, 2)

Table (1): The inhibitory effect of aqueous Nigella seeds extract on isolated tracheal muscle of sheep contracted with acetylcholine.

Concentration mg/ 10 ml	Degree of decline from baseline of physiogram (mm)
50	3 ± 0.48
100	4 ± 0.48
150	6 ± 0.61
200	8 ± 0.59
250	9 ± 0.48
300	9 ± 0.56

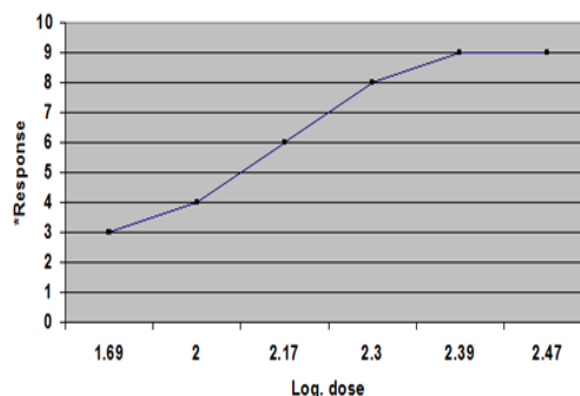


Figure (1): Degree of relaxation of isolated tracheal muscle of sheep by N. seeds extract pre-contracted by acetylcholine.

Figures represent mean \pm standard error. Number of animals = 6. Relaxation effect of all concentrations was immediate and continued until washing.

* Degree of decline (mm) from base line of physiogram.

Table (2): The inhibitory effect of aqueous extract of Nigella seeds on isolated tracheal muscle of sheep contracted with histamine.

Concentration mg/ ml	Degree of decline from baseline of physiogram (mm)
50	2 ± 0.42
100	4 ± 0.48
150	5 ± 0.48
200	7 ± 0.54
250	8 ± 0.48
300	8 ± 0.57

Figures represent mean \pm standard error. Number of animals = 6. Relaxation effect of all concentrations was immediate and continued until washing.

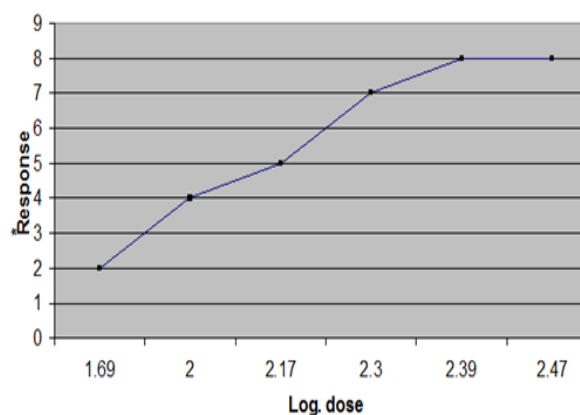


Figure (2): Degree of relaxation of isolated tracheal muscle of sheep by N. seeds extract pre-contracted by histamine.

* Degree of decline (mm) from base line of physiogram.



Discussion:

The response of tracheal smooth muscle is different for the same kind and same concentration of relaxant. This response is different even among individuals within the same species depending upon health condition [8]. The tracheal smooth muscle isolated from patients died after chronic asthma had maximal response to acetylcholine greater than isolated trachea from non-asthmatic patients [9]. Since tracheal smooth muscle is relaxed in normal conditions, any relaxant agent causes no obvious relaxation, whereas the smooth muscle of an isolated asthmatic patient may show clear response in this attitude. Aqueous extract of *Nigella sativa* seeds, however, is not expected to cause detectable relaxation of tracheal smooth muscle unless contraction is previously induced by an excitatory agent like acetylcholine or histamine. Extent of relaxation of the tracheal smooth muscle was obviously proportional to different concentrations of aqueous extract of *Nigella sativa* seeds used in this study. Low concentrations, however, caused slight relaxant action demonstrated by a decline below the base line on the physiogram. This weak response may be due to weak contractility of tracheal muscle and disability to perform spontaneous movement at least after low concentrations of acetylcholine and histamine. Moreover, the tracheal smooth muscle had a rather simple contraction ability and not a continuous rhythmic contraction and relaxation [8], so the relaxation of tracheal muscle caused a merely slight decline below the base line on the physiogram. Higher concentration of *Nigella sativa* extract, however, caused pronounced relaxation of tracheal smooth muscle. A concentration of (50) mg / 10 ml displayed a decline of (3) mm and (2) mm on the physiogram following contraction induced by acetylcholine and histamine respectively. Maximal relaxation showed a decline of (9) mm and (8) mm following addition of a concentration of (300) mg / 10 ml of the

extract proceeded by contraction induced by acetylcholine and histamine respectively. Regardless of concentration of *Nigella* extract added, tracheal smooth muscle responded immediately with gradual decline beneath baseline, which continued until subsequent wash. Intensive relaxation response might probably be due to large number of receptors occupied. Successive addition of different concentrations of *Nigella sativa* extract to immersed isolated trachea and when no inter-concentration wash was practiced, the organ continued to relax and displayed continuous decline on the physiogram, this may be attributed to cumulative and partially irreversible effect. In another study, the isolated epithelial tissue from trachea of a dog was sensitized to acetylcholine and histamine; they suggested that contraction and relaxation of smooth muscle of isolated trachea are governed by multiple factors and complex systems including the presence of contraction inhibitor factor, and neuronal control [10]. Contraction of tracheal smooth muscle induced by acetylcholine was prevented in this study by addition of atropine through blockade of muscarinic receptors. This contraction, however, could not be reversed by addition of *Nigella sativa* extract, this may be attributed to functional antagonism exerted by the extract to acetylcholine rather than specific blockade of muscarinic cholinergic receptors. Contraction of tracheal muscle induced by acetylcholine followed by propranolol (β -blocker) was reversed by addition of *Nigella sativa* seeds extract. This excludes the possibility of relaxation being caused by the action of *Nigella sativa* extract through its effect on β -receptors. Several suggestions for the mechanism of the relaxant action of *Nigella sativa* seed extract may be proposed. First is the stimulation of inhibitory Non-adrenergic, non-cholinergic nervous system (NANC). Secondly, a competitive effect on histamine receptor (H1)



suggested by Boskabady and Sheiravi [11] after their work on chains of guinea pig trachea. And thirdly through calcium antagonistic action suggested by Boskabady

et al. [12] where this antagonistic effect of *Nigella sativa* extract to calcium may contribute to the relaxant effect of the plant.

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