

Antinociceptive effect of watery suspension of *Nigella sativa* Linn. seeds in mice

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Abstract

The present study was designed to investigate the antinociceptive activity of the watery suspension of powdered *Nigella sativa* seeds in mice. The antinociceptive effect of the watery suspension of *Nigella sativa* seeds compared with that of Naproxen was evaluated by formalin test in which the stimulus is standardized and measures potency and duration of response. In this test, however, *Nigella sativa* seeds watery suspension significantly ($P<0.05$) reduced nociceptive reaction both at early and late phases whereas, Naproxen attenuated the nociceptive response in late phase of test only. Pre-treatment with Naloxone (1 mg / kg B.W.IM.) significantly ($P<0.05$) reversed the analgesic effect of *Nigella* seeds at late phase only. This may implicate an opioid activity of *Nigella* seeds constituents (particularly thymoquinone) participating in its analgesic activity which characterized by inhibitory effect on nociceptive system and / or inflammatory mediators.

Keywords: Analgesic, *Nigella sativa*, Watery suspension, Mice.

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التأثير المضاد للألم للعالق المائي لبذور الحبة السوداء *Nigella sativa* Linn. في الفئران

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الخلاصة

صممت هذه الدراسة لتقصي التأثير المضاد للألم للعالق المائي لبذور الحبة السوداء المطحونة في الفئران. تم تقييم التأثير المضاد للألم لعالق الحبة السوداء ومقارنته مع تأثير دواء النابروكسين باستخدام اختبار الفورمالين، حيث يتميز هذا الاختبار بالتوحيد القياسي لحافز الألم كما ويقاس قوة ومدة الإيعاز المحدث للألم. سبب العالق المائي للحبة السوداء انخفاضا معنويا ($P<0.05$) في الإحساس بالألم مقاسا بطريقة اختبار الفورمالين في كل من المرحلتين المبكرة والمتأخرة في حين خفض دواء النابروكسين الإحساس بالألم في المرحلة المتأخرة من الاختبار فقط. من جهة أخرى أدى إعطاء النالكسون (1ملغم/كغم في العضل) إلى إبطال الفعل التسكينى لعالق الحبة السوداء بشكل معنوي ($P<0.05$) في المرحلة المتأخرة للاختبار فقط. من خلال هذه الدراسة يمكن الاستنتاج ضمنا إن الفعل التسكينى لبعض مكونات الحبة السوداء (خاصة النايموكوينون) مشابه لفعل الأفيونات إذ تتميز بفعالها المثبط لمواقع السيطرة على الألم أو النواقل الالتهابية أو كلاهما معا.

Introduction

There is an increasing interest towards medicinal plants and their active ingredient since 1980. One of the most important motives was the synthetic drugs which show off their dangerous side effect by time, whereas medicinal

plants have generally centuries-long use and little unknown side effects (1).

One of the most famous herbs which has used in the treatment of diseases was *Nigella sativa* which was substantial historical tradition in medicine, it is the black seeds referred to by the prophet Muhammad (PBUH) as

having healing powers, the prophet (PBUH) said, "Hold onto the use of the black seed for it has a remedy for every illness except death". This prophetic reference in describing black seed not to be exaggerated as recent research has provided evidence, which indicate that black seed contain ability to significantly boost the human immune system – if taken over the time, and it act on the principle of assisting the body's own natural healing process in over coming illness or maintaining health.

Black seed is also identified as the curative black cumin in the Holy Bible, and is described as the magic remedy of Hippocrates and Disocoredes.

In spite of large number of pharmacological studies carried out world wide on *Nigella sativa* seed, recent references like (2) explained that *Nigella sativa* is truly an amazing combination, but there are still many components that haven't been yet identified, but research is going on around world.

Scrutiny of published articles showed that there is a need for more studies to investigate many medicinal and pharmacological activities of the seeds including its anti-nociceptive activity.

It is well known that traditionally and publically plants in general and *Nigella* seeds in particular are taken or prescribed for patients in the form of a kind of tea i.e. the powder of the seeds is macerated or simply boiled to prepare a remedy. It has then been resumed that a need to perform an investigation as an aim of the present study focusing on the anti- nociceptive activity of watery suspension of *Nigella sativa* seeds.

Materials and methods

Animals

Twenty albino Swiss mice of either sex weighing 24-32 g with an average (28±2) were obtained from the animal house of Al-kindy company for production of veterinary vaccines.

Mice were housed in groups of (4) per plastic cage (30 × 10×10) cm in insulated room until the beginning of experiments. Standard rodent diet (Commercial Feed Pellets) and tap water were freely available.

Plant Material

Dry *Nigella sativa* Linn.seeds were purchased from a local market in Baghdad, and were identified in the National herbarium at Abu Graib.

Preparation of Seeds Suspension

To (15) gm of *Nigella* seeds, (100) ml of distilled water were added, mixed and ground by a blender, this was made for 5-6 intervals, 60 seconds each time, 60 second apart, at room temperature until complete grinding and mixing.

A stock of a crude suspension a few minutes before each experiment was prepared by taking 10 ml from stock solution contain 150 ml, then a volume of 0.1 ml / 10 gm B.W with a maximum of 0.3 ml.

Formaline Test

Twenty albino Swiss mice were used in this experiment divided randomly into (4) groups, five animals for each group. At least 30 minutes before performing formalin test, animals of all groups were pretreated as follows: Group (1): Treated orally with distilled water. Group (2): Treated orally with watery suspension of *Nigella sativa* seeds at a dose of (1000) mg / kg B.W. based on our previous study (3). Group (3): Treated orally with Naproxen at a dose of (500) mg / kg B.W (4). Group (4): Injected with naloxone (1 mg / kg B.W S/C) (5), and fifteen minutes later treated with watery suspension of *Nigella sativa* (1000 mg / kg B.W).

The nociceptive response was measured by formaline test, as a model of persistent somatic pain first described by Dubuisson and Dennis (6), by a method modified by Hunskaar and Hole (3) and Ok Lee *et al.* (7). Each mouse was placed in a transport plastic cage and left for five minutes before formalin injection to allow adaptation to the new environment.

Ten microliters of 2% formalin injected S / C into the planter region of the right hind paw of mice in each group. The injection of diluted formalin S/C produces two pain-related behavioral components that can be measured in terms of nociceptive response, namely flinching (is one of the pain-related behaviors of the formalin model and is characterized by spontaneous, rapid and brief shaking or lifting of the paw) and licking of the injected paw.

Measurement of nociceptive response was carried out immediately after formalin injection and continued for (60) minutes.

A comparison of the antinociceptive activity was made between group (2) (pretreated with watery suspension of *Nigella sativa* seeds), group (3) (pretreated with Naproxen) and control group.

We further investigated the probable modulatory properties of opioid receptors on the antinociceptive effect of the suspension, by applying naloxone as an opioid receptors antagonist. The antinociceptive activity of watery suspension of *Nigella sativa* in this group, (group 4) was assessed and compared with that in animals of group (2) (pretreated only with watery suspension of *Nigella sativa*).

Data were analyzed statistically by using one way analysis of variance (ANOVA). Analyses were performed, probability value less than 0.05 was considered statistically significant.

Results

Injection of diluted formalin solution into the paw of hind foot of mice induced a biphasic nociceptive response (Licking and flinching of the injected paw), an early phase during the first five minutes following injection of formalin and a late phase starting (15) minutes after injection and lasting for (45) minutes. The gap between the two phases (early and late) show diminution of nociceptive response. The number of licking and flinching was (88 ± 1.41) and (95 ± 1.61) at the early and late phase respectively.

Oral administration of *Nigella sativa* attenuated the nociceptive response in both the early and late phase of the formalin test (figure 1). In contrast, anti-nociceptive effect of Naproxen was found only during the late phase. Subcutaneous injection of Naloxone (1mg/kg B.W) significantly antagonized the effect of *Nigella sativa* on the early phase response, but it failed to reverse *Nigella sativa* – induced antinociception in the late phase (figure 1 and table 1).

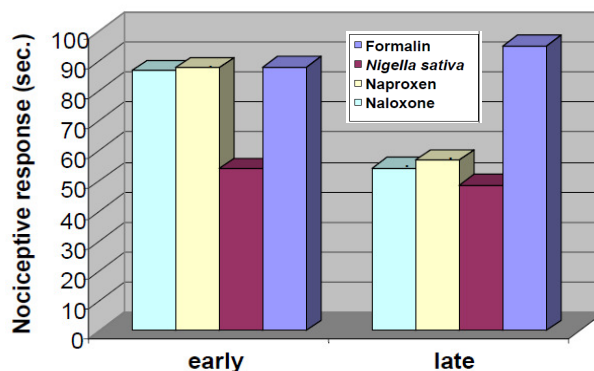


Figure (1): Formalin test, nociceptive response (seconds) for mice treated with *Nigella sativa* seeds watery suspension.

Table (1): Nociceptive response in mice treated orally with watery suspension of *Nigella* seeds, Naproxen, distilled water and mice injected with Naloxone before oral treatment with *N.s.* (formalin test).

Groups of mice	Nociceptive response (number of licking and flinching)	
	Early phase (0-5) minute after injection	Late phase (15-45) minute after injection
Treated orally with distilled water before injection of formalin (control)	88 ± 1.41 A	95 ± 1.61 A
Treated orally with <i>N.s</i> watery suspension before injection of formalin	54 ± 1.22 B	48.2 ± 2.22 B
Treated orally with Naproxen before injection of formalin	87.6 ± 1.80 A	56.8 ± 1.77 B
Pretreated by injection of Naloxone (sc) then treated orally with <i>N.s</i> watery suspension before injection of formalin	86.8 ± 1.59 A	54.2 ± 5.30 B

Figures represent mean ± standard error. Different large letters represent significant difference between groups vertically at (P < 0.05). Similar letters represent insignificant difference between groups.

Discussion

It has, however, been difficult to separate the influence of the anti-inflammatory activity from pure analgesia in standard animals tested by using classical analgesic tests, so the formalin test is used for this purpose and for shed a light on mechanism of analgesic activity of watery suspension of *Nigella Sativa*. This test is a statistically valid technique having two advantages over other pain tests (as hot plate test). Little or no restraint is necessary, permitting unhindered observation of the complete range of behavioral responses, and pain stimulus is continuous rather than transient, thus bearing greater resemblance to most clinical pain (6).

When formalin test was performed by injection of 10 microliter, 2% diluted formalin at the planter region, a biphasic response to formalin was observed (licking and flinching) with an early phase starting immediately after injection lasting for (5) minutes and a late phase 15-60 minutes after injection. An interphase depression to pain response was noticed where a decrease in pain behavior occurred between (5) and (15) minutes after injection. Virtually all mice went through a period during which display less pain behavior.

The early phase seemed to be caused predominantly by C-fiber activation due to the peripheral stimulus, while the late phase appeared to be dependent on the combination of an inflammatory reaction in the peripheral tissue and functional changes in the dorsal horn of the spinal cord.

These functional changes seemed to be initiated by the C-fiber barrage during the early phase (4 and 8). The diminution of pain response that occurred after the first phase of the formalin test (interphase period) may be due to active inhibitory processes (auto analgesia) triggered by the initial pain (9 and 7).

We performed the formalin test for testing the probable analgesic activity of the watery suspension of *Nigella sativa* seeds, it is interesting to note that administration of *Nigella sativa* watery suspension showed an antinociceptive effect at both the early and late phase of formalin responses, as do centrally acting antinociceptive drugs (10). These findings may indicate that watery suspension of *Nigella sativa* can exert an antinociceptive activity via a central mechanism.

The antagonistic interaction between *Nigella sativa* suspension and naloxone in the early phase of formalin response makes it conceivable that the supraspinal opioid receptors are implicated in *Nigella sativa* - induced antinociception on the early phase response to formalin.

The difference in naloxone sensitivity to *Nigella sativa* antinociception between the early and late phase may be suggestive for the possibility that antinociceptive action of *Nigella sativa* in the formalin test is mediated by mechanism (S) other than direct stimulation of opioid receptors located in the central nervous system.

Although watery suspension of *Nigella sativa* attenuated the late phase response to formaline, we can not exclude the possibility that *Nigella sativa* - induced antinociception in the late phase is partly mediated by peripheral mechanisms. A previous study (11) revealed that thymoquinone as well as *Nigella sativa* oil inhibited the generation of eicosanoids such as thromboxane B2 and Leukotriene B4. Drugs, which suppress these inflammatory mediators like naproxen, which is used in this experiment and showed attenuation of the late phase nociceptive responses in the formalin test and that came in agreement with that reported by Hunskaar and Hole (4) that the non-steroidal anti-inflammatory drugs (indomethacin and Naproxen) and the steroids dexamethasone and hydrocortisone inhibited only the late phase.

One might infer that the anti-inflammatory effect of watery suspension of *Nigella sativa* seeds is implicated in antinociceptive effect in the late phase.

Nigella sativa significantly inhibited the behavioral changes caused by acute nociceptive stimuli (hot plate, early phase and late phase of the formalin test), these results suggest that watery suspension of *Nigella sativa* seeds induced antinociceptive is due to an inhibitory effect of this seeds on the nociceptive systems and / or inflammatory mediators.

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