Comparison the analgesic activity of watery suspension of *Nigella sativa* Linn. seeds with Naproxen in mice

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Abstract

The present study was designed to investigated the antinociceptive effect of the watery suspension of *Nigella sativa* seeds compared with that of Naproxen by using hot plate test, which mainly measures the threshold of stimulus required to elicit a response , in mice at 30, 60, 90, 120 minutes after administration of the test agents. Twenty four albino Swiss mice of either sex were divided in four groups with (6) animals each, the first group was treated orally with 1000 mg/kg BW of watery suspension of Nigella seeds, the second group was treated orally with naproxen at dose of 500 mg/kg BW ,the third group drenched with 500mg/kg BW of watery suspension of Nigella seeds and 250 mg/kg BW of naproxen ,whereas the last group serve as control. *Nigella sativa* seeds suspension significantly ($p<0.05$) prolonged the latency of response at all post-treatment observation times (30, 60, 90 and 120 minutes) , the analgesic action of naproxen was diminished with time compared with Nigella seeds. Combination of equal amounts of Nigella seeds and Naproxen (half the originally used doses) caused significant increment ($p<0.05$) of analgesic reaction time longer than shown by each agent when given alone indicating an obvious synergistic effect between watery suspension of Nigella seeds and Naproxen.

Introduction

Since prehistoric times, humans have attempted to alleviate ailments or injuries with the aid of plant parts or herbal preparations. Ancient civilizations have recorded various prescriptions of this kind. In the herbal formularies of medieval times numerous plants were promoted as remedies. In modern medicine, where each drug is required to satisfy objective criteria of efficacy, hundreds of reputedly curative plants species have survived as drugs with documented effectiveness(1). One of these herbs was *Nigella sativa* which has substantial historical tradition in medicine. In spite of large number of pharmacological studies carried out world wide on *Nigella sativa* seed, recent references like (3) 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. Pain is unpleasant sensory and emotional experience associated with actual

or potential tissue damage ,or described in terms of such damage. It is a common symptom and important because it both signals “disease”(in the broadest sense) and aids diagnosis.However, by definition ,it is a wretched sensation ,and its relief is one of the most duties of a doctors(4) .*Nigella sativa* oil was used traditionally as a topical treatment for pain and stiffness in joint (5).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 analgesic activity of watery suspension of *Nigella sativa* seeds, and extending to compare with other drug “naproxen” in mice by using hot plate test at different post –treatment time intervals .

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 ($30 \times 10 \times 10$) 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:

Based on our previous study(15), to (10) 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 100mg, then a volume of 0.1 ml / 10 gm B.W with a maximum of 0.3 ml.

Oral administration of *Nigella* seed suspension showed significantly lower reaction time (analgesia) than treatment with Naproxen at 30 and 60 minutes. Reaction time of both treatments, however, approached each other with time with no significant difference at 90 and 120 minutes. Reaction times of both treatments were significantly higher than those of control values along the course time of treatment. This proved presence of analgesic effect of *Nigella* seeds suspension. Results indicate

Analgesic effect of *Nigella* seed suspension By Hot Plate Test:

Twenty four albino Swiss mice were used in this experiment and divided randomly into (4) groups (6 each)

Group (1): Treated orally with 1000 mg / kg B.W of watery suspension of *Nigella sativa* seeds only based on our previous study(6).

Group (2): Treated orally with 500 mg / kg B.W of Naproxen (7).

Group (3): Treated orally with both (500) mg / kg B.W watery suspension of *Nigella sativa* and (250) mg / kg B.W of Naproxen.

Group (4): Treated orally with distilled water only and served as control group.

Thirty minutes after administration, each animal in all groups was dropped gently on a plate maintained at 55 ± 1 C°. This constant temperature produces two behavioral components in mice that can be measured in terms of their reaction times, namely licking and flicking of the paw and jumping as described by (8 and 9). Measurement of reaction time were carried out also after 30, 60, 90, 120 minutes after administration of the test agents in order to gain more information about the analgesic activity of watery suspension of *Nigella sativa* seeds and its possible interaction with Naproxen.

Results

longer analgesic action of *Nigella* seed suspension than Naproxen, hence, the analgesic action of the later appeared to diminish with time. Combination of equal amounts of *Nigella* seeds suspension and Naproxen (half the originally used doses) resulted in prolongation of analgesic reaction time, which was rather longer than that shown by each agent when given alone referring an obvious synergistic effect (table,1) and (figure,1).

Table (1): Antinociceptive (reaction time) in mice treated orally with watery suspension of *Nigella* seeds, Naproxen or both together (hot plate test).

Groups of mice	Time after administration (minutes)			
	30	60	90	120
Treated orally with watery suspension of <i>N.s</i>	19.16 ± 0.79 A	20.00 ± 0.85 A	24.00 ± 0.63 A	22.33 ± 0.80 A
Treated orally with Naproxen	26.00 ± 0.52 B	24.50 ± 0.76 B	23.50 ± 0.76 A	21.83 ± 0.90 A
Treated orally with watery suspension of <i>N.s</i> then with Naproxen	29.00 ± 0.96 C	29.33 ± 0.95 C	30.00 ± 0.85 B	28.16 ± 1.32 B
Control treated orally with distilled water only	15.66 ± 0.49 D	14.66 ± 0.49 D	15.16 ± 0.60 C	14.50 ± 0.42 C

groups Figures represent mean ± standard error.

Different large letters represent significant difference between groups vertically ($P < 0.05$).

Similar large letters represent insignificant difference between vertically.

Figure(1):Antinociceptive reaction in mice treated orally with watery suspension of *N.s*,Naproxen or both together.(hot plate test)

Discussion

The present study demonstrated that the oral administration of watery suspension of *Nigella sativa* seeds produced a suppressive effect on nociceptive responses caused by thermal nociceptive stimuli in mice, as compared with animals in control group which were exposed to the same condition and with animals treated with naproxen. This antinociceptive effect of *Nigella sativa* is partly attributable to its component, thymoquinone which have been reported to produce antinociceptive effect

through either indirect activation of supraspinal opioid receptors (10) or by inhibiting the cyclooxygenase and lipooxygenase pathways of arachidonic acid metabolism as reported by (11) who proposed that the inhibition of eicosanoid generation of the fixed oil of *Nigella sativa* is greater than is expected from its content of thymoquinone, so it could be concluded that there are substances other than thymoquinone responsible for analgesic activity such as p-cymene and pinene proved

to have analgesic effects (3). Flavonoid, Rutine which present in *Nigella sativa* (12) and belongs to Quercetin group has an inhibitory effect on 5-lipoxygenase pathway (the main pathway for production of chemical mediators important in pain and inflammatory processes). (13). Some alkaloids, as reported in many situations in the literature (14), have important role in relieving pain. Chemical analysis of *Nigella sativa* seeds revealed the presence of alkaloids (15,16,17), which may share other substance like flavonoids, and probably other substances, in relieving pain through elevation of pain threshold or diminishing inflammatory symptoms by inhibiting some chemical mediators important in initiating pain mechanism such as prostaglandins, leukotrienes and histamine discussed in a previous section of this study. Other mechanisms suggestive for analgesia as an activity of *Nigella sativa* seed suspension, are either depressing the ability of nociceptors to bind to chemical mediators or

decreasing the conducting rate of nerve impulses along nerve fibers responsible for propagation and transport of impulses. The duration of analgesic action of orally administered watery suspension of *Nigella sativa* seeds, varied with variable latency periods, it was significantly greater than the tolerance period of control animals 30 and 60 minutes after administration, but shorter than that of naproxen, *Nigella* and naproxen reaction periods approached each other as tested 90 minutes after treatment. The duration of reaction time due to *Nigella sativa* suspension, however, was greater than that of naproxen tested at 120 minutes after treatment. In addition, when *Nigella sativa* seeds suspension and naproxen were given simultaneously, the duration of their action, at all latency times, was significantly longer as compared to duration of reaction time due to *Nigella* suspension or naproxen each alone, this may suggest or support the possibility of different sites on which they act.

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مقارنة التأثير المسكن للعالق المائي لبذور الحبة السوداء *Nigella sativa*

Linn مع دواء النابروكسين (Naproxen) في الفئران

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الخلاصة

صممت هذه الدراسة لتحري التأثير المضاد للألم للعالق المائي لبذور الحبة السوداء في الفئران ومقارنتها مع دواء النابروكسين (Naproxen) باستخدام طريقة الصفيحة الساخنة، والتي تقيس عتبة المحفز اللازمة لإظهار الاستجابة للألم، تمت مراقبة الحيوانات وذلك بعد فترات (30، 60، 90، 120 دقيقة) بعد الإعطاء. تم استخدام أربع وعشرون من الفئران البيضاء من كلا الجنسين وتم تقسيمها إلى أربع مجاميع وبواقع (6) حيوانات لكل مجموعة. تمت معالجة المجموعة الأولى فمويًا بجرعة (1000) ملغم/ كغم من وزن الجسم، وجرعت المجموعة الثانية فمويًا بدواء النابروكسين بجرعة (500) ملغم/ كغم من وزن الجسم، وفي حين جرعت المجموعة الثالثة بمقدار (500) ملغم/ كغم من وزن الجسم من العالق المائي للحبة السوداء و (250) ملغم/ كغم من وزن الجسم بدواء النابروكسين واعتبرت المجموعة الأخيرة كمجموعة سيطرة. أظهر عالق الحبة السوداء زيادة معنوية وعلى مستوى احتمال ($p < 0.05$) في فترة عم الاستجابة في جميع فترات المشاهدة بعد التجريع (30، 60، 90، 120 دقيقة)، كما لوحظ انخفاض التأثير المسكن لدواء النابروكسين بمرور الوقت عند مقارنته بالعالق المائي لبذور الحبة السوداء. إن استخدام مزيج مكون من كميات متساوية من الحبة السوداء دواء النابروكسين (نصف الجرعة المستخدمة أصلاً) سبب زيادة معنوية وعلى مستوى احتمال ($p < 0.05$) في وقت التسكين عند مقارنتها مع كل مادة إذا ما أعطيت بصورة منفردة مؤشراً على وجود تأثير تآزري واضح بين العالق المائي للحبة السوداء و دواء النابروكسين.

*البحث مستل من أطروحة الماجستير للباحث الأول.