

ANTI-NOCICEPTIVE EFFECT OF *NIGELLA SATIVA* OIL, APPLE VINEGAR, HONEY AND THEIR COMBINATIONS IN LABORATORY MOUSE

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(Received 11 October 2006, Accepted 29 November 2006)

Keywords: Pain, Anti-nociceptive, *Nigella sativa*, Apple, honey.

ABSTRACT

An attempt was made to study the analgesic effect of oil extract of *Nigella sativa* seed, apple vinegar, honey and their combination. These materials were administered orally to male mice and were tested using a model of animal of pain (hot plate test).

The result showed that *N. sativa* oil had a good analgesic effect, increasing hot plate latencies by 57.7%. Similar analgesic effect was produced by apple vinegar. Honey was much less effective; it produced only 52% increase in hot plate time. The effect of apple vinegar and honey was dose dependent. Combination of *N. sativa* with vinegar or honey did not result in enhancement of effect of *N. sativa* oil when measured, one hour after administration. However, potentiation effect was found between *N. sativa* oil and apple vinegar when the analgesic effect was measured at 4 and 6 hours after oral administration.

INTRODUCTION

Pain affects hundreds of millions of people throughout the world (1). Moreover, it is one of the most common reasons why patients seek medical care and become so desperate for relief of their pain (2). A search for effective safe and cheap agents from natural sources such as plants is, therefore, worthwhile.

Nigella sativa was known to have analgesic and anti-inflammatory but not antipyretic effects in mice (3). Apple vinegar has also been shown to have anti-inflammatory activity in mice (4) and used as inhibitory agent against food – borne bacteria (5). Honey is used in traditional medicine for a wide –range of diseases including arthritis, anemia, bad breathe, bed wetting and bladder control, bladder infections, abscesses, bronchitis, burns, cough and several others (6). Vinegar and honey are commonly mixed by traditional therapists with different types of herbs and other materials. The present study is, therefore, an attempt to investigate the analgesic effects of *N. sativa* oil, apple vinegar and honey; and their combination in an animal model of pain (hot plate test).

MATERIALS AND METHODS

The oil extract of *N. sativa* and the natural honey were donated from Dr. Abdul-Basit Khalid Ahmed, College of Education / University of Basrah.

Preparation of apple vinegar: The apples (*Pyrus malus*) were cleaned by good washing and left to dry. The apples were cut to small pieces and put in a glass container which was filled and closed by a piece of cloth and left at room temperature for nine weeks (Apple vinegar was prepared by the assistant pharmacist: Abdul-Khaliq Hussein).

Animals: Balb/C Albino mice were bought from AL-Kindi Vaccine Company/ Baghdad. The animals were housed and breed as described by Yousif (7). Briefly, the mice were housed in a separate, light controlled room (white fluorescent light in from 6.00-18.00 hr

and darkness for the rest of the day), and temperature $25\pm C^{\circ}$). Food was prepared in the laboratory by mixing crude protein (15%), Soya bean (6%), wheat flour (50%), wheat bran (25%) vegetable oil(2%), milk powder (2%) and minerals & vitamin (1 g/kg) of the mixture. These materials were mixed with water, suitable form were prepared (as pollute) and put in oven at $40C^{\circ}$ to dry.

Experiment (1): Effect of *N. sativa* oil, apple vinegar, honey and the combination of *N. sativa* oil with vinegar or honey on animal models of pain (Hot plate test): Six groups of (18) male mice were used in this experiment. Each group of animals was tested by hot plate test. Each animal was placed on a metal plate (Lasso Company, India) maintained at ($55c^{\circ}$) and the latency of nociceptive responses such as licking, flicking of the hind limb or jumping was measured in seconds. Mice that showed the nociceptive response within 18 seconds were used as experimental animals. The latency of nociceptive response in this animal was expressed as hot plate latency which was measured before and 60 minutes after oral administration of the tested agent (8, 9).

Animal groups received the tested material as follows: group (1) received 0.2 ml distilled water; group (2) received 0.1ml *N. sativa* oil with 0.1ml distilled water, group (3) received 0.1ml apple vinegar with 0.1ml distilled water; group (4) 0.1ml honey with 0.1ml distilled water, group (5) 0.1ml *N. sativa* oil mixed with 0.1ml vinegar and group (6) received 0.1ml *N. sativa* oil mixed with 0.1 honey (mixing was made in the syringe of administration because of lack of miscibility). The final volume of each administration was 0.2 ml orally.

Experiment (2): Duration of analgesic effects of *N. sativa* oil, vinegar or their combination before and 1, 2, 4,6,24 hours, after oral administration: In this experiment, four groups of mice were used. Each group consisted of six mice, and tested by hot plate test [see Experiment (1)]. Group (1) received distilled water, group (2) *N. sativa* oil, group (3) apple vinegar and group (4) received *N. sativa* oil mixed with vinegar in doses as described in Experiment (1). Tests were performed before and 1, 2, 4, 6, and 24 hours after oral administration.

Experiment (3). Dose response relationship of apple vinegar and honey:

A-Dose response relationship of apple vinegar on painful stimuli in mice.

Four groups were used in this experiment; each group consisted of six mice. Each group of animals were tested by hot plate test. The mice were given graduated doses of vinegar; group (1) was given distilled water; group (2) undiluted vinegar; group (3) 1:2 dilution and group (4) was given 1:4 dilution of vinegar in distilled water. The doses were as described in Experiment (1). Hot plate test was performed one hour after oral administration.

B-Dose response relationship of honey diluted 1:1, 1:2 and 1:3 in distilled water: Three groups were used in this experiment; each group consisted of six mice. Each group of animal was tested by hot plate. The mice were given graduated doses of honey diluted in distilled water. Group (1) was given 1:1 dilution, group (2) was given 1:2 dilution, and group (3) was given 1:3 dilution. The doses were as described in Experiment (1). Hot plate test were performed one hour after oral administration.

Statistical analysis: Data were statistically evaluated using paired and unpaired t-tests. P values less than 0.05 were taken as statistically significant.

RESULTS

Experiment (1): Effect of *N. sativa* oil, apple vinegar, honey and the combination of *N. sativa* oil with vinegar or honey on Hot plate test: Oral administration of *N. sativa* oil showed in an increase in hot plate time by 57.7% compared to pre-administration measurement ($P<0.05$, Table1). Similar finding was found when vinegar was used alone (an increase of 73.7%), while treatment with honey alone caused a smaller increase

(38.1%). Although *N. sativa* oil when mixed with vinegar resulted in a slightly more increase in hot plate time compared to vinegar or *N. sativa* oil given alone, this difference is not statistically significant. On the other hand, honey mixed with *N. sativa* oil did not significantly enhance the analgesic effect of *N. sativa* oil (Table 1).

Experiment(2): Duration of analgesic effects of *N. sativa* oil ,vinegar or their combination before and 1,2,4,6,24 hours after oral administration, using hot plate test: Oral administration of *N. sativa* oil resulted in an increase in hot plate time by 68.7%, 88.6%, 98.7%, 116.6% and 5.6% at 1,2,4,6 and 24 hours after oral administration (Table 2). However, oral administration of vinegar resulted in higher increase in hot plate latencies in this experiment by 110.2%, 110.2%, 114.5%, 107.9% and 11.3% at 1, 2, 4, 6, 24 hours, after oral administration of vinegar (Table 2).

Oral administration of a mixture of *N. sativa* oil and vinegar produced analgesic effect higher than vinegar given alone in the first 6 hours after administration. This was evident at 4 and 6 hours rather than one hour after administration (Table.2).

Experiment (3): Dose –response relationship of apple vinegar and honey in pain models in mice

A- Dose –response relationship of apple vinegar in pain models in mice: Oral administration of 0.2ml of undiluted vinegar or its 1:2 and 1:4 dilutions in distilled water resulted in an increase in hot plate latencies by 92.3%, 85.8% and 20.9% with respect to pre-administration times respectively (Table 3).

B- Dose response relationship of honey diluted 1:1, 1:2 and 1:3 in distilled water in pain models in mice-Hot plate test: 1:1 dilution of honey in distilled water produced a mild analgesic effect (an increase of 38.7% in hot plate latency) with no significant effects of greater dilutions (1:2 and 1:3) with respect to pre-administration time (Table 4).

Table (1) Effect of *N. sativa* oil, vinegar, honey and combination of *N. sativa* oil with vinegar or honey on animal models of pain using hot plate test (n=18).

	Control (0.2ml dist. Water)	0.1ml <i>N.</i> <i>sativa</i> oil +0.1ml dist.water	0.1ml Apple vinegar +0.1ml dist.water	0.1ml Honey +0.1ml dist. water	0.1ml <i>N.</i> <i>sativa</i> oil + 0.1ml vinegar	0.1ml <i>N.</i> <i>sativa</i> oil + 0.1ml honey
Before oral administration	5± 0.9	5.2± 0.8	6.1± 0.5	6.3± 0.8	4.7± 0.8	4.6± 0.6
One hour after administration	5.2± 0.9	8.2±* 2.3	10.6±* 2.8	8.7±* 2.3	8.8±* 1.9	8.3±* 2.6
%change with respect to pre- administration time	↑4.3%	↑57.7%	73.7%	↑38.1%	↑87.3%	↑80.5%

Data are expressed as mean ±SD of hot plate time (in seconds).

Significant difference with respect to per-administration time: *p<0.05.

Table (2) Duration of analgesic effects of *N. sativa* oil ,vinegar or their combination before and 1,2,4,6,24 hours, after oral administration using hot- plate test (n=6)

	Control %	<i>N. sativa</i> %	Vinegar %	<i>N. sativa</i> + Vinegar %
1 hour	20	68.7*	110.2*	109.8*
2 hour	20.4	88.6*	110.2*	129.9
4hour	18.6	98.7*	114.5*	162.2*
6 hour	31.1	116.6*	107.9*	143.5*
24 hour	13.92	5.6	11.3	2.7

Data are presented as percent change with respect to pre-administration measurements.
Significant difference with respect to pre-administration time (p<0.001).

Table (3) Dose -response relationship of apple vinegar in hot plate test (n=6)

	Control	Undiluted Vinegar	1:2 dilution	1:4 dilution
Before oral administration	4.5± 0.4	3.9± 0.6	4.2± 0.7	4.3± 0.6
One hour after administration	4.1± 0.5	7.5±* 1.2	7.8±* 1.5	5.2± 0.8
%change with respect to pre-administration time	↓8.8%	↑92.3%	↑85.8%	↑20.9%

Data are expressed as mean ±SD of hot plate time (in seconds).
Significant difference with respect to pre-administration time: * p<0.01.

Table (4) Dose response relationship of honey diluted 1:1, 1:2, 1:3 in distilled water, using hot plate test (n=6).

	1:1	1:2	1:3
Before oral administration	4.9± 1.2	5.9± 1.3	4.9± 0.3
One hour after administration	6.8±* 0.9	6.7± 1.2	5.5± 0.5
% change with respect to pre-administration time	↑38.7%	↑13.6%	↑12.2%

Data are expressed as mean ±SD of hot plate time (in seconds).Significant difference with respect to pre-administration time: *p<0.01.

DISCUSSION

It is our intention to investigate the oil of *N. sativa* rather than its aqueous extract since the latter had been found to have both analgesic and anti-inflammatory activities in previous studies (3).

Nigella sativa oil was found in the present study to have a significant analgesic activity in animal model of pain. It increased hot plate latencies by 67% with respect to pre-administration measurement.

Abdel-fattah (10) also used *N. sativa* oil but the oil was suspended in propylene glycol. In our study, we used *N. sativa* oil alone or mixed with vinegar or honey in the same syringe of administration. *Nigella sativa* has been shown to have several other actions such as anti-platelet effect (11) and antioxidant effect (12). In addition, it contains a polymer (nigellone) that inhibits histamine release (13). The above-cited actions might contribute to its analgesic and anti-inflammatory effects. Thus, both aqueous extract of *N. sativa* (3) and its oil dissolved in propylene glycol (10), and its oil alone in the present study were found to have significant analgesic activities.

Although mixing vinegar with *N. sativa* oil did not enhance the analgesic activity of the latter measured one hour after oral administration, apple vinegar on its own resulted in a significant analgesic activity, which is comparable to that of *N. sativa* oil. However, the analgesic effect of mixing vinegar with *N. sativa* becomes higher at 4 and 6 hours after oral administration when compared with individual agents used alone.

Apple vinegar is not just diluted acetic acid but contains nutrients, minerals, vitamins, essential amino acids and several enzymes. It was shown to have anti-inflammatory activity in human with arthritis (14) and in mice (4).

Honey, on the other hand, produced an analgesic effect but it is much less than that produced by apple vinegar or *N. sativa* used individually. Honey when mixed with *N. sativa* did not enhance its analgesic effect measured one hour after oral administration.

Up to our knowledge, no published report was found in the literature showing honey to have analgesic effect. While it was shown to have antioxidant activity (15) and can accelerate healing of cutaneous wounds (16). It can, therefore, be concluded that *N. sativa* oil had a good analgesic effect in tail flick and hot plate tests in mice. Similar analgesic effect was produced by apple vinegar in both tests. Honey was much less effective. No synergistic effect was found between the three agents one hour after administration, but potentiation effect between *N. sativa* and apple vinegar was found 4 and 6 hours after oral administration.

التأثير المضاد للألم لزيوت الحبة السوداء وخل التفاح والعسل ومزجهم في الفئران المختبرية
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الخلاصة

أجريت محاولة دراسة التأثير المسكن لزيوت الحبة السوداء وخل التفاح والعسل ومزجهم. هذه المواد أعطيت عن طريق الفم إلى ذكور الفئران وفحصت باستخدام النموذج الحيواني للألم (فحص الصفيفة الحارة). أظهرت النتائج بأن لزيوت الحبة السوداء تأثير مسكن جيد وذلك بزيادة فترة الصفيفة الحارة بنسبة 57.7%. ونفس التأثير وجد بواسطة خل التفاح. أما العسل فكان أقل تأثيراً فقد سبب زيادة بنسبة 52% في فترة الصفيفة الحارة. وقد لوحظ بأن تأثير خل التفاح والعسل كان معتمداً على الجرعة. أما مزج الحبة السوداء مع الخل أو العسل فلم يحسن تأثير الحبة السوداء، مع هذا فقد وجد تأثير مقوي بين الحبة السوداء وخل التفاح عند قياس التأثير المسكن بعد 4 و 6 ساعة من الزرق الفموي.

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