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Effect of the Volatile Oil of *Nigella sativa* Seeds and Its Components on Body Temperature of Mice: Elucidation of the Mechanisms of Action

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Abstract – The effect(s) of the volatile oil (VO) of *Nigella sativa* and its two components, α-pinene and ρ-cymene on body temperature of male and female conscious mice were studied. Further investigations to delineate the mechanism(s) of action of the observed effect(s) by using various blockers involved in the central regulation of body temperature were made. VO and α-pinene caused significant reductions in rectal body temperature at is and 30 minute after treatment. ρ-cymene had negligible effect on body temperature of mice. Cyproheptadine inhibited VO and α-pinene-induced hypothermia significantly. Nalbuphine inhibited α-pinene-induced hypothermia significantly but did not affect VO-induced hypothermia. Droperidol potentiated VO and α-pinene-induced hypothermia to a non-significant level; whereas atropine potentiated VO-induced hypothermia non-significantly. The study confirms further the role of serotoninergic receptors in the mechanism(s) of the observed pharmacological effects of the VO of *Nigella sativa*. It also indicated a possible role of opioid receptors in α-pinene-induced hypothermia.
Keywords – *Nigella sativa*, α-pinene, ρ-cymene, receptor blockers, serotoninergic receptors

Introduction

Nigella sativa (Black Seed) is an annual herb that belongs to the family Ranunculaceae (Linnaeus, 1753). The seeds of *N. sativa* are used as a flavoring agent in certain foods. It has also been used in folk medicine for many medicinal uses originating mainly from Islamic literature. The basis of these uses go back to the saying of Prophet Mohammad (peace be upon him) "Black seed contains cure for every illness except death" (Al-Bukhari, 1976). This has strongly pushed a lot of scientific work to reveal secrets of this plant in the cure of various illnesses. The volatile oil of *N. sativa* was studied extensively (El Tahir, *et al.*, 1993a, 1993b; Shaheen, 1996) where many pharmacological actions were documented as well as their possible mechanism(s). *N. sativa* volatile oil contains about 24% w/w as thymoquinone (Canonoica *et al.*, 1963). It also contains monoterpenes (46%) which are composed mainly of ρ-cymene (31.7%) and α-pinene (9.3%) (Aboutabl *et al.*, 1986). Since one of the traditional uses of *N. sativa* involves its use as a remedy for fever (Al-Gawzia, 1957) it was of value to test its effect on fever and/or body temperature. Al-Naggar *et al.* (2003) found that the aqueous and methanolic extracts of defatted *N. sativa* produced a significant decrease in rectal temperature in mice 30 min. after administration. Al-Ghamdi (2001) found no effect of the aqueous extract of *N. sativa* seeds on yeast-induced pyrexia. Since it was noticed that different fractions of *N. sativa* may produce different pharmacological effects it was thought of interest to study the effect of the volatile oil of *N. sativa* on body temperature of mice and to compare the obtained effect with that of its two main components: ρ-cymene and α-pinene.

Furthermore, attempts were made to elucidate the mechanisms of action of the oil and its components. The previous work of El Tahir, *et al.* (1993a, 1993b) has shown involvement of acetylcholine and serotonin transmitters in both respiratory and cardiovascular effects of the VO of *N. sativa.* Various receptor blockers were used to help in the explanation of the mechanism of action of the induced changes on body temperature of mice.

Experimental

VO extraction – Ground sun-dried *N. sativa* Linn. Seeds, variety Hispidula (product of the Sudan) "identified by Dr. Abdulrahman" VO was extracted by direct steam

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distillation of ground *N. sativa* seeds as described by Gad *et al.*, (1963). The distillate was extracted with diethylether which yielded about 1.5 ml of VO in each Kg of seeds.

Animals – Male and Female mice (WSR strain, Animal House of The Central Lab. For Drug and Food Analysis) (20 - 30 gm in weight) were divided into 24 groups (6 - 24 animal/group).

Methods – The effect of VO and its components (ρ-cymene and α-pinene) on the body temperature of conscious mice was investigated according to the methods of Little *et al.*, (1986) and Gray *et al.*, (1987). The test substances were administered i.p. at various doses and changes in rectal body temperature were followed using a digital thermometer, Apelex (France). For this purpose the probe of an Apelex thermometer was inserted to a depth of 2.5 cm into the mouse rectum at an ambient temperature of 25 °C. To investigate the effect of VO or its components the rectal temperature was recorded at the following time intervals: one min before administration of any drug, 5, 15, 30, 60, 120, and 180 min after administration of the test substance. Some animals received no treatment where the spontaneous changes in body temperature were measured and were used as control.

To investigate the influence of various receptor blockers, the sub-maximal doses of VO or its components were selected (200 µl/kg) and the blocker was given at time 0. The VO or its component was given 15 - 30 min later. The changes in body temperature were recorded before injection and exactly 30 min after. The absolute change in body temperature in °C was calculated. This was compared to the effect of VO or its components at 30 min following their sole injection. Some groups of mice received the blocker only where the effects on body temperature were recorded. The effect of the blocker was always subtracted from the test group.

The following blockers were used: cyproheptadine (MSD, USA) (2 mg/kg), atropine (E. Merck, Germany) (1 mg/kg), droperidol (Johnson & Johnson) (1 mg/kg), and nalbuphine (DuPont Merck, Germany) (10 mg/kg). All blockers were injected via i.p. route. All blockers were solubilized/diluted in water or saline. VO doses were calculated and drawn in Hamilton micolitre syringe (50 µl) (Germany) then injected i.p.using 1 ml syringe after mixing with 0.2 ml isotonic saline (as emulsion). Pure ρ-cymene (Riedel-Dehaen, Germany) and α-pinene (Hopkin's & William, UK) were used as VO components.

Statistical analysis – All values reported were mean ± SE mean with N equals the number of animals used in each group. Statistical analyses were performed using student non-paired 't' test.

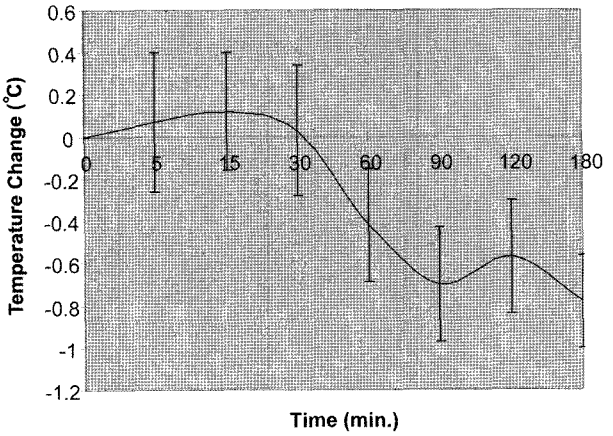

Fig. 1. Changes in body temperature in control mice. Animals received no treatment where spontaneous changes in body temperature over 3 hours recorded. Max increase in body temperature at 15 min (less than 0.2 °C) Maximum decrease in body temperature at 90 min. (less than 0.8 °C).

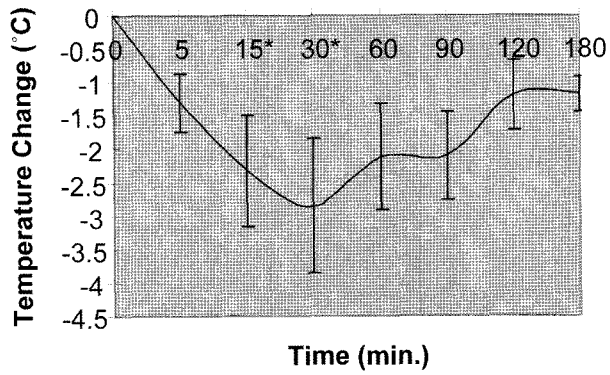

Fig. 2. Effect of VO (200 µl/kg) on mice body temperature. Intraperitoneal administration of VO (200 µl/kg) into mice produced time-dependent decreases in body temperature. Significant decreases were observed 15 and 30 min. after administration ($P < 0.02$, $N = 6$).

Results and Discussion

In control mice, there was no change in body temperature during the first 30 minutes but there was less than 1 °C decrease during the next 150 min (Fig. 1). Administration of the VO in the dose range 100 - 400 µl/kg (i.p.) induced dose dependent decreases in body temperature. The maximum decrease was observed 30 min. following administration of each dose e.g. 4 °C following the administration of 400 µl/kg i.p. The effect of a submaximal dose (200 µl/kg i.p.) is shown in Fig. 2. The maximum reduction was observed 30 min following administration of the oil. After that reversal of the hypothermia started but there was no full recovery till 3 hours following injection.

Table 1. Effect of cyproheptadine (2 mg/kg i.p.) for 30 min on VO (200 μl/kg)-induced hypothermia in mice

	VO (N = 34)	cyproheptadine followed by VO (N = 6)
average change in Temp. in °C (± SEM)	−2.85 (± 1.00)	−1.00[a] (± 0.60)

[a] The difference was significant compared to control (P < 0.01).

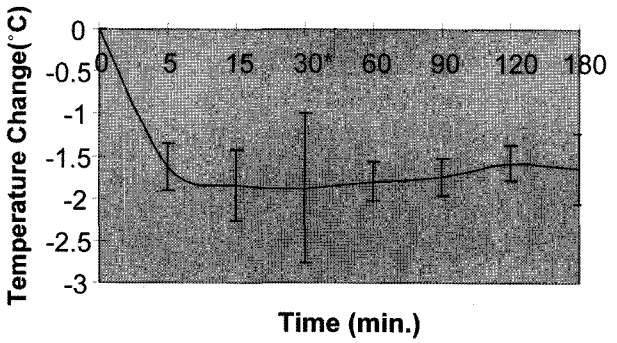

Fig. 3. Effect of α-pinene (100 μl/kg) on mice body temperature. Intraperitoneal administration of α-pinene (100 μl/kg) into mice produced time-dependent decreases in body temperature. Significant decreases observed at 15 and 30 min (P < 0.02, N = 12).

The reduction in body temperature was statistically significant at 15 and 30 min compared to control (P < 0.02, N = 6). The 30-min point was therefore selected as the critical point where the effects of various blockers were tested.

Effect of receptor blockers – Treatment of mice with the serotonin receptor blocker, cyproeptadine (2 mg/kg) for 30 min (i.p.) significantly blocked the effect of VO on body temperature as it induced an average reduction of 1 °C compared to 2.85 °C with VO alone (Table 1). The difference was significant compared to control (P < 0.01, N = 6)).

Treatment of the animals with atropine or droperidol (1 mg/kg i.p.) for 30 minutes before the VO induced non-significant enhancement of hypothermia in the range of 40 - 50% (N = 6). Treatment with nalbuphine (1 mg/kg i.p.) did not affect VO-induced hypothermia.

Effect of α-pinene – Treatment of mice with α-pinene in the dose range of 50 to 200 μl/kg induced dose dependent decreases in body temperature. Fig. 3 show the effect of submaximal dose of 100 μl/kg i.p. Here again as in case of the VO, the maximum decrease was noted 30 min following the injection. The effect was sustained for 3 hours. The reduction in body temperature was statistically significant compared to control at 30 min. only (P < 0.02, N = 12).

Effect of receptor blockers – Treatment of mice with cyproheptadine (2 mg/kg i.p.) significantly blocked the effect of α-pinene on body temperature as reflected by an average reduction of 0.93 °C compared to 1.88 °C with α-pinene alone (Table 2).

The difference was significant compared to control (P < 0.02, N = 6).

Similarly, nalbuphine (10 mg/kg i.p.for 15 min) blocked the action of α-pinene on body temperature as reflected by an average reduction of 0.125 ± 0.11 °C compared with the control 1.88 ± 0.28 °C (P < 0.001, N = 12)

On the other hand, treatment of mice with atropine (1 mg/.kg i.p.) for 30 min didn't inhibit α-pinene induced hypothermia significantly. It only antagonized it by 11%.

Droperidol potentiated α-pinene-induced hypothermia non-significantly as noticed by 38% enhancement of hypothermia.

Effect of ρ-cymene – Treatment of mice with ρ-cymene in the dose range of 100 μl/kg to 300 μl/kg i.p. did not affect body temperature.

It is well known that the hypothalamus is the major site for the regulation of body temperature. The role of various neurotransmitters has been learned from microinjection technique into the pre-optic area and the anterior region of hypothalamus (PO/AH). (Blatteis, 1981).

There are many papers in the literature discussing the role of various neurotransmitters in the control of mammalian body temperature. Microinjection of ACh into (PO/AH) of rat lead to heat loss and hypothermia. (Beckman and Carlisle, 1969). Similarly, 5-HT has been shown to produce a decrease in rectal temperature in many species (Feldberg *et al.*, 1967) except cat where 5-HT lead to increase in body temperature from which it is concluded that 5-HT may have a role in central regulation of body

Table 2. Effect of cyproheptadine 2 mg/kg i.p for 30 min and Nalbuphine 10 mg/kg i.p. for 15 min on α-pinene (100 μl/kg i.p.)-induced hypothermia in mice

	α-pinene (N = 12)	cyproheptadine followed by α-pinene (N = 10)	nalbuphine followed by α-pinene (N = 12)
average change in °C (± SEM)	−1.88 (± 28)	−0.93[a] (± 0.09)	−0.125[b] (± 0.11)

[a] Difference was significant compared to control (P < 0.02).
[b] Difference was significant compared to control (P < 0.001).

temperature (Feldberg and Myers, 1963). Opioid system is more complex in the sense that β-endorphin injection in rodents produces hyperthermia at lower doses (Haidobro-Toro and Way, 1980) and hypothermia at higher doses (Bloom and Tseng, 1981) . Goldstein and Lowery (1975) reported significant hypothermia in male Wistar rats given naloxone 10 mg/kg (s.c.). Blockade of opioid receptors by naloxone leading to hypothemia indicated that opioid receptors may be operating under normal conditions leading to higher temperature set point. The response of most species to the administration of dopamine is hypothermia (Clark and Lipton, 1985).

In case of VO, atropine which is a non selective muscarinic blocker (Barnes, 1990) caused a trend towards almost enhancement of hypothemia in a non significant manner and didn't inhibit α-pinene-induced hypothermia suggesting dis-involvement of cholinergic mechanisms in the observed hypothermia.

However, cyproheptadine which is a serotonin receptor non-specific blocker (Niemegeers *et al.*, 1982) inhibited VO and α-pinene-induced hypothermia to a significant level indicating that 5-HT has probably a big role in the mechanism of VO and its component, α-pinene-induced hypothermia. Unfortunately there are no reports in the literature regarding effect α-pinene on serotoninergic receptors. Thus, VO-induced hypothermia was probably due to activation of serotoninergic receptors resulting from either a direct effect of the VO components on these receptors or due to release of 5-HT.

Nalbuphine is a mixed opioid agonist/antagonist (Roth *et al.*, 1988). Taking in consideration the agonistic effect of nalbuphine, it can be suggested that α-pinene-induced hypothermia ma be due to blockade of endogenous opioid receptors as their activation induced hyperthermia (Goldstein and Lowery, 1975). On the other hand taking in consideration the antagonistic opioid action of nalbuphine it can be suggested that α-pinene-induced hypothermia was due to activation of endogenous β-endorphin receptors, activation of which induced hypothermia similar to higher opioid doses (Bloom and Tseng, 1981). These results point to the antagonistic effect of α-pinene on opioid receptors which can be resolved via use of selective opioid receptor blockers.

Administration of the dopamine antagonist, droperidol (Oh, 1978) did not affect VO- or α-pinene-induced hypothermia suggesting that dopamine receptors have no role in the mechanism of VO-induced or α-pinene-induced hypothermia.

The results of this study indicate that *Nigella sativa* and its component α-pinene but not ρ-cymene have the potential of treating fevers and/or malignant hyperthermias. This needs to be further tested in humans.

It is concluded that VO and its component, α-pinene have a hypothermic action in mice. The hypothermic effect was blocked by cyproheptadine which drew the attention to involvement of serotonin receptors in *Nigella sativa* induced hypothermic effect as has been shown in the cardiovascular effects of the VO (El Tahir *et al.*, 1993a). The opioid receptors have a possible role in the mechanism of action of α-pinene. More studies are needed to confirm these findings. Understanding the mechanism of action of VO or its components can help in management of toxic cases of the oil and/or its components overdose.

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