Gen. Pharmac. Vol. 24, No. 5, pp. 1115–1122, 1993
Printed in Great Britain.

0306-3623/93 $6.00 + 0.00

THE RESPIRATORY EFFECTS OF THE VOLATILE OIL OF THE BLACK SEED (*NIGELLA SATIVA*) IN GUINEA-PIGS: ELUCIDATION OF THE MECHANISM(S) OF ACTION

KAMAL E. H. EL TAHIR*, MOHAMMAD M. S. ASHOUR and MOHAMMAD M. AL-HARBI
Department of Pharmacology, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

(Received 26 February 1993)

Abstract—1. The effect of the volatile oil (VO) of the black seed (*Nigella sativa*) on the respiratory system of the urethane-anaesthetized guinea-pig was investigated and compared with those of its constituent thymoquinone (TQ).

2. Intravenous administration of VO in the dose range (4–32 μl kg^{-1}) induced dose-dependent increases in the respiratory rate and the intratracheal pressure.

3. The effects of VO were significantly antagonized by treatment of the animals with mepyramine, atropine and reserpine. They were not antagonized by indomethacin, diethyl carbamazine or hydrocortisone.

4. Intravenous administration of TQ in the dose range (1.6–6.4 mg kg^{-1}) induced significant increases in the intratracheal pressure without any effect in the respiratory rate.

5. The results suggested that VO-induced respiratory effects were mediated via release of histamine with direct involvement of histaminergic mechanisms and indirect activation of muscarinic cholinergic mechanisms.

6. Removal of TQ from VO may provide a potential centrally acting respiratory stimulant.

1. INTRODUCTION

The black seed of *Nigella sativa* Linn., family Ranunculaceae contains >30% of a fixed oil and 0.4–0.45% of a volatile oil (VO) w/w (Hashim and El-Kiey, 1962; El-Alfy *et al.*, 1975). Chemical analysis of the VO revealed its content of thymoquinone (TQ) 18.4–24% (w/w) and 46% of monoterpenes such as *p*-cymene and α-pinene (Canonica *et al.*, 1963; El-Dakhakhny, 1963; Aboutabl *et al.*, 1975). During the 1960s Mahfouz and El-Dakhakhny (1960a) reported that i.m. or i.p. administration of the VO in a dose of (0.2 ml kg^{-1}) to guinea-pigs protected the animals against histamine-induced bronchospasm. However, VO did not affect histamine H_1 receptors in isolated tissues (Mahfouz and El-Dakhakhny, 1960b) but its constituent TQ was observed to increase histamine binding to plasma proteins (Mahfouz *et al.*, 1965). In Arabian folk medicine, the whole black seeds alone or in combination with honey are promoted for treatment of bronchial asthma. It was thus thought of interest to investigate the effects of VO and its constituent TQ on the respiratory system of the guinea-pig.

*To whom all correspondence should be addressed.

2. METHODS

2.1. Extraction of the volatile oil of the black seed

The VO of sun-dried black seeds of *Nigella sativa* Linn., variety hispidula (product of the Sudan) was obtained via steam distillation as described by Gad *et al.* (1963). The VO was isolated from the aqueous medium using diethyl ether. The latter was then completely removed via distillation under reduced pressure (400 mbar at 40°C). The VO was then stored at 25°C until required.

2.2. Preparation of guinea-pigs for measurement of normal respiration

Male guinea-pigs (500 g, from Experimental Animal Care Centre, King Saud University), were anaesthetized with aqueous urethane (1.5 g kg^{-1} i.p.). The right jugular vein was exposed, cannulated and heparin (1000 i.u. kg^{-1}) was then injected. The trachea was cannulated and connected to an ITT Cannon pressure transducer attached to a Physiograph recorder (MK-1-V-P-Narco Bio-Systems). The recording speed was usually set at 0.1 cm sec^{-1} but was occasionally increased to 1 cm sec^{-1} to enable counting the respiratory rate. All drugs were administered i.v. in a maximum volume of (0.4 ml kg^{-1}). Each dose was flushed using 0.2 ml of saline. The effect of each dose of VO or TQ was then examined at 0.5, 1 and 3 min following administration. The percentage change in the rate of respiration was then calculated. All doses were administered at intervals of 15 min. The effect of any solvent used was initially tested and its effect was then subtracted from that produced by the solubilized drug.

2.3. Effect of receptor blockers and synthesis inhibitors

To investigate the influence of receptor blockers and synthesis inhibitors on VO or TQ-induced respiratory changes the following procedure was used. Initially, a dose–response curve of VO or TQ was obtained and the

submaximal dose was selected and used to test the influence of various blockers. The percentage effectiveness of each blocker to antagonize the VO- or TQ-induced effects was then calculated. The doses and pretreatment times of receptor blockers and synthesis inhibitors used were similar to those used by previous workers or in our own laboratory (El Tahir *et al.*, 1991b; Buchan and Adcock, 1992). In addition some experiments were initially performed to confirm the effectiveness of the used blockers on the effects produced by their specific agonists.

2.4. *Effect of reserpine*

Guinea-pigs were initially treated with reserpine (0.5–5 mg kg^{-1} day^{-1} i.p.) for 2 consecutive days and the maximum tolerated dose was then selected. The animals were then treated with this dose which was (1 mg kg^{-1} day^{-1} i.p. for 2 days) and used 18 hr after the last dose.

2.5. *Preparation of the guinea-pigs for measurement of the intratracheal pressure (or pulmonary inflation pressure)*

Male guinea-pigs (500 g) were anaesthetized with urethane, and the jugular vein and trachea cannulated as outlined above. One limb of the T-shaped tracheal cannula was connected to an ITT pressure transducer attached to a Physiograph recorder (Narco Bio-systems) and the other limb was connected via plastic tubings to an air pump rodent ventilator (Scientific and Research Instruments Ltd, U.K.) for artificial respiration using room air and a tidal volume of 6 ml air kg^{-1} as reported by Tocker *et al.* (1990) and Buchan and Adcock (1992). The tracheal pressure was then displayed on the recording system. The effect of each dose on the intratracheal pressure was quantified in mmHg by the aid of the pressure calibration system built into the physiograph coupler. At the beginning of each experiment the sensitivity of the animal and the recording system was ascertained by injection of histamine (10–20 μg kg^{-1} i.v.).

2.6. *Preparation of the tracheal rings*

Male guinea-pigs (500 g) were killed, the neck opened, and the trachea located and freed from all connective tissues, and transferred to a petri dish containing Tyrode's solution. The trachea was then divided into individual rings that were tied together to form a tracheal chain and suspended in an oxygenated organ bath containing Tyrode's solution at 37°C to measure isometric contractions in the usual manner, using a force displacement transducer fitted to a Narco Physiograph.

2.7. *Drugs used*

The drugs used were thymoquinone (Sigma, U.S.A); hexamethonium bromide (Koch-Ligh Laboratories Ltd, U.K.); histamine diphosphate (Fluka, Germany); indomethacin sodium (Dumex Ltd, Denmark); mepacrine hydrochloride (Sigma, U.S.A); mepyramine maleate (Drug Pharmaceutical Trading B.U. Holland); nordihydroguaiaretic acid (Sigma, U.S.A.); reserpine (Fluka, Germany); theophylline (E. Merck, Germany); atropine sulphate (E. Merck, Germany); cyproheptadine hydrochloride (MSD, U.S.A); diethyl carbamazine citrate (Sigma, U.S.A); urethane and hydrocortisone (BDH, U.K.).

2.8. *Solubilization of drugs*

VO was solubilized in platelet-poor plasma (PPP) obtained from the same animal under test. The ratio of VO to PPP was 1:3 and injected into the animal using a Hamilton glass microlitre syringe. PPP was obtained by centrifuging 1.5 ml of the heparinized blood at 2000 rpm for 20 min using a MSE centrifuge. The solubilization of the oil in PPP was facilitated by vortexing for 1 min in (0.4 × 4 cm) glass tubes just before administration of the different doses. Thymoquinone was solubilized in ethanol; reserpine in a mixture of Tween 80 20%, benzyl alcohol 4% and citric acid 0.5% in water as described by Bill *et al.* (1989), nordihydroguaiaretic acid in alcohol, hydrocortisone in PPP and all other drugs in saline.

2.9. *Statistical analysis*

Significant differences between control and treated groups were calculated using Student's t-test for paired or non-paired samples as appropriate. All results were presented as mean ± SE mean with n = number of experiments performed.

3. RESULTS

3.1. *Effect of VO on the respiratory rate*

Intravenous administration of VO into urethane-anaesthetized guinea-pigs in the dose range (4–16 μl kg^{-1}) induced dose-dependent increases in the respiratory rate. The maximum increase was noted 0.5 min following administration of the oil. The rate returned to pretreatment value 3–5 min later. The increase in the rate was always preceded by a histamine-like bronchoconstriction. Figure 1 depicts one of these experiments. In this experiment the normal respiratory rate was 40 min^{-1} Administration of VO (8 μl kg^{-1}) increased the respiratory rate to 192, 152 and 40 min^{-1} at 0.5, 1 and 3 min following administration of the oil, respectively. The corresponding percentage increases in the frequency of respiration were 380, 280 and zero, respectively. Similarly, administration of (16 μl VO kg^{-1}) increased the frequency of respiration by 445, 165 and 27% at 0.5, 1 and 3 min, respectively. Administration of PPP in doses upto (32 μl kg^{-1}) did not affect respiration. The cumulative findings from 3–24 such experiments are shown in Table 1.

3.2. *Effect of receptor blockers and synthesis inhibitors on VO-induced increase in the respiratory rate*

Treatment of guinea-pigs with mepyramine (10 mg kg^{-1} for 5 min) significantly antagonized VO induced increase in the respiratory rate by 83.9 ± 2.6% ($P < 0.01$, $n = 3$). Figure 2 shows one of these experiments. Treatment of the animal with VO (8 μl kg^{-1}) increased the respiratory rate by 280%0.5 min after administration. However, pretreatment of the animal with mepyramine antagonized VO-induced increase in rate by 82% and prevented the induced bronchoconstriction. Similarly, treatment of the animals with atropine (1 mg kg^{-1} for 5 min), nordihydroguaiaretic acid (20 mg kg^{-1} for 30 min), mepacrine (4 mg kg^{-1} for 30 min) and theophylline (10 mg kg^{-1} for 5 min), significantly antagonized the VO-induced increase in respiratory rate. Table 2 shows the percentage antagonisms exerted by these drugs. However, pretreatment of the animals with diethylcarbamazine (12.5 mg kg^{-1} for 30 min), hydrocortisone (10 mg kg^{-1} for 60 min) and indomethacin (10 mg kg^{-1} for 30 min) did not antagonize the VO-induced increase in respiratory rate.

3.3. *Effect of reserpine*

Treatment of guinea-pigs with reserpine (1 mg kg^{-1} day^{-1} i.p. for 2 days) induced significant decreases in the VO-induced increase in respiratory rate at all doses tested ($P < 0.01$, $n = 6$). Table 3 shows the percentage increases in the respiratory rate 0.5 min after administration of VO to reserpine-treated and the control animals.

3.4. *Effect of thymoquinone (TQ) on the respiratory rate*

Administration of TQ in the dose range (1.6–6.4 mg kg^{-1}) did not significantly affect the respiratory rate ($P > 0.05$, $n = 6$) but induced dose-dependent bronchoconstriction.

3.5. *Effect of VO on the intratracheal pressure*

Administration of VO in the dose range (4–32 μl kg^{-1}) to guinea-pigs increased the intratracheal pressure in a dose-dependent manner. Figure 3 shows one of these experiments. In this experiment administration of VO in doses of (8, 16 and 32 μl kg^{-1}) increased the intratracheal pressure by 3, 3.6 and 3.9 mmHg. The maximum duration of the increase was < 1 min. Administration of the PPP in doses up to (200 μl kg^{-1}) did not affect the intratracheal pressure. The cumulative findings from 4–24 experiments are shown in Table 4.

3.6. *Effect of receptor blockers and synthesis inhibitors*

Treatment of the animals with mepyramine (10 mg kg^{-1} for 5 min) or with atropine (4 mg kg^{-1} for 5 min) antagonized VO (16 μl kg^{-1})-induced increase in the intratracheal pressure by 64.0 ± 3.7 and 70.6 ± 6.9%, respectively ($P < 0.01$, $n = 4$). Figure 4 depicts the effect of mepyramine on VO-induced increase in the intratracheal pressure. In this experiment pretreatment of the animal with mepyramine antagonized VO-induced increase in the intratracheal pressure by 87%.

Treatment of the animals with cyproheptadine (1 mg kg^{-1} for 5 min), mepacrine (4 mg kg^{-1} for 30 min), diethylcarbamazine (12.5 mg kg^{-1} for 30 min) or hydrocortisone (10 mg kg^{-1} for 60 min)

Table 1. Effect of VO of the black seed on the respiratory rate of guinea-pigs

Dose of VO (μl kg^{-1})	Percentage increase in the respiratory rate (Mean ± SEM)		
	Time (in min) after administration		
	0.5	1	3
4	85.2 ± 17.5	85.7 ± 27.5	18.1 ± 5.7
8	163.2 ± 19.6**	148.9 ± 21.3	39.1 ± 19.1
16	240.45 ± 23.2**	163.6 ± 23.4	96.5 ± 27.2*

*$P < 0.05$; **$P < 0.01$, $n = 3$–24.

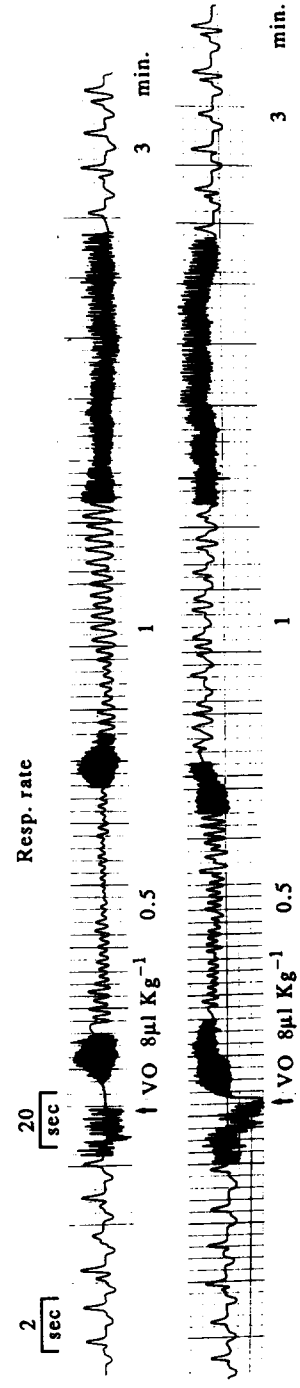

Fig. 1. Effect of black seed VO on the respiratory rate of guinea-pigs. Administration of VO (8 and 16 μl kg^{-1}) induced initial bronchoconstriction followed by a dose-dependent increase in the respiratory rate. The maximum increase in rate was observed 0.5 min after administration of VO. In this and the subsequent figures, the 20-sec scale represents the normal recording speed of the physiograph. The 2-sec scale represents the speed of the recording when it was increased to enable counting the respiratory rate. Times are in min (0.5, 1 and 3); the value shown below each record represents the time following administration of the oil.

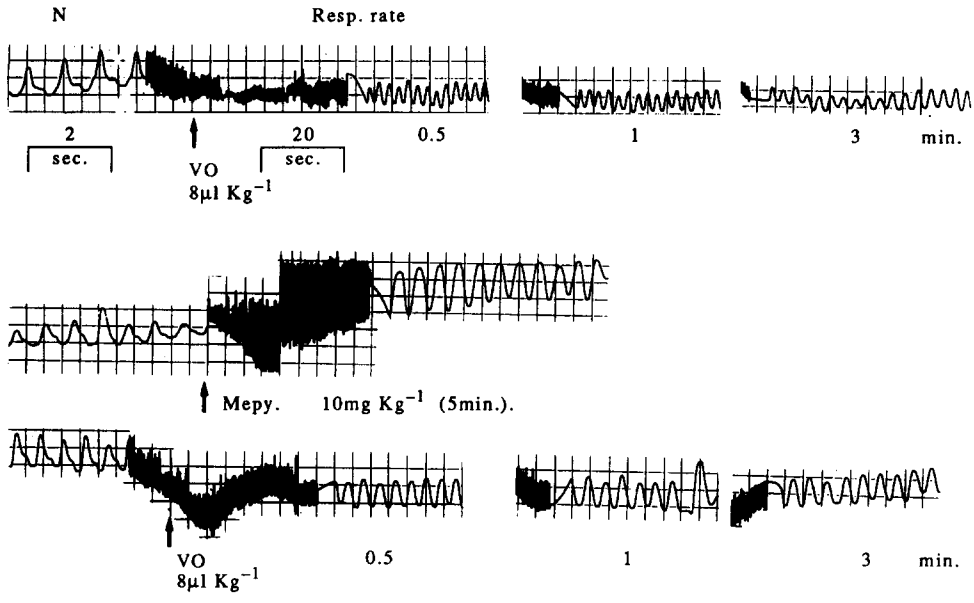

Fig. 2. Effect of mepyramine on the VO-induced increase in respiratory rate of the guinea-pig. Administration of VO (8 μl kg^{-1}) to the animal increased the respiratory rate by 280% 0.5 min after administration. After treatment of the animal with mepyramine (Mepy. 10 mg kg^{-1} for 5 min) the percentage increase in rate was 40%. Mepyramine antagonized VO effect by 82%. Mepyramine also prevented VO-induced initial bronchoconstriction.

did not antagonize VO-induced increase in the intratracheal pressure.

3.7. *Effect of reserpine*

Treatment of guinea-pigs with reserpine (1 mg kg^{-1} day^{-1} i.p. for 2 days) significantly antagonized VO-induced increases in intratracheal pressure ($P < 0.01$, $n = 6$). Figure 5 shows the effect of VO (4–32 μl kg^{-1}) on the intratracheal pressure of a reserpine-treated guinea-pig. In this experiment administration of VO in doses of (4, 8 and 16 μl kg^{-1}) failed to increase the intratracheal pressure but at a dose of (32 μl kg^{-1}) increased the pressure by 2.8 mmHg. The cumulative findings from 6 similar experiments are shown in Table 5.

3.8. *Effect of VO on the isolated trachea*

Addition of V.O. in the dose range (1–8 μl ml^{-1}) did not contract the tissue.

3.9. *Effect of thymoquinone (TQ)*

Administration of TQ in the dose range (1.6–6.4 mg kg^{-1}) to guinea-pigs increased intratracheal pressure in a dose-dependent manner. Figure 6 shows one of these experiments. In this experiment administration of TQ in doses of (1.6, 3.2 and 6.4 mg kg^{-1}) increased intratracheal pressure by 1.2, 1.9 and 3.2 mmHg, respectively. The maximum duration was < 1 min. In 5 similar experiments, the cumulative increases were 1.3 ± 0.5, 1.9 ± 0.9 and 3.1 ± 1.2 mmHg, respectively ($P < 0.05$, $n = 5$).

4. DISCUSSION

The results of this study demonstrated the ability of black seed volatile oil (VO) to induce dose-dependent increases in the respiratory rate and intratracheal pressure of urethane-anaesthetized guinea-pigs. The effects were significantly antagonized by pretreatment of the animals with reserpine, which depletes

Table 2. Effect of some receptor blockers, synthesis inhibitors and theophylline on VO-induced increase in respiratory rate 0.5 min after administration of VO

Drug	Dose (mg kg^{-1})	Percentage antagonism (mean ± SEM)
Mepyramine	10	83.9 ± 2.6**
Atropine	1	78.7 ± 8.1**
Mepacrine	4	42.5 ± 7.4*
Nordihydorguaiaretic acid	20	46.9 ± 7.7*
Diethylcarbamazine	12.5	6.1 ± 3.6
Theophylline	10	50.1 ± 9.3*

*$P < 0.05$; **$P < 0.01$, $n = 3$–5.

Table 3. Effect of black seed VO on the respiratory rate of reserpinized guinea-pigs

VO (μl kg^{-1})	Percentage increase in the respiratory rate 0.5 min after administration of VO (Mean ± SEM)	
	Control	Treated
4	83.5 ± 9.2	19.4 ± 4.2*
8	159.6 ± 6.2	40.3 ± 7.9*
16	233.7 ± 18.2	46.8 ± 9.1*

*$P < 0.05$, $n = 4$–24.

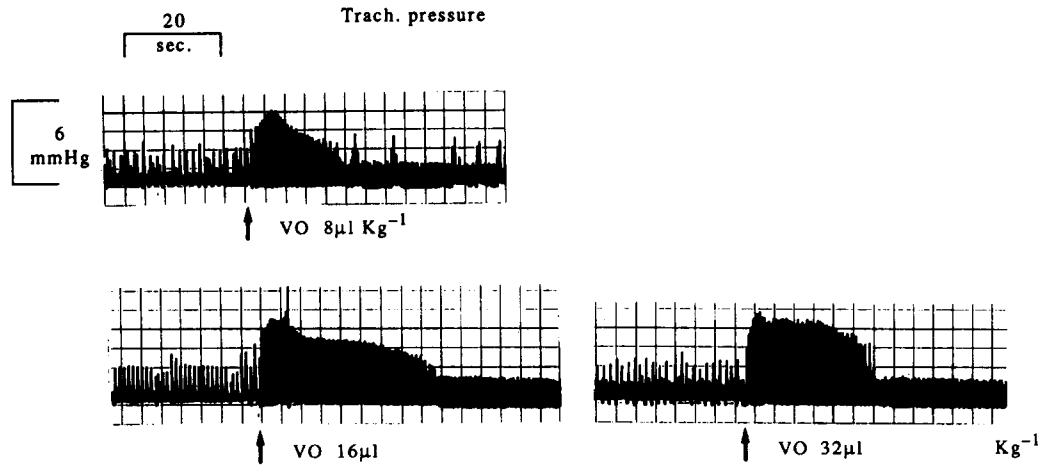

Fig. 3. Effect of black seed VO on the intratracheal pressure of guinea-pigs. Administration of the VO in doses of (8, 16 and 32 μl kg^{-1}) to the animal increased the tracheal pressure by 3, 3.6 and 3.9 mmHg respectively. The mmHg scale represents the scale used to quantify the increase in the intratracheal pressure in mmHg.

neuronal and cellular stores of monoamines (histamine, 5-HT and NA) (Kibby, 1970; Ridzwan *et al.*, 1988; Sharif *et al.*, 1989), mepyramine which blocks histamine H_1 receptors (Niemegeers *et al.*, 1982) and the non-selective muscarinic receptor blocker atropine (Barnes, 1990). The effects were not antagonized by the phospholipase A_2 inhibitor hydrocortisone, the prostaglandin cyclooxygenase inhibitor, indomethacin or the dual prostaglandin and leukotriene synthesis inhibitor, diethyl carbamazine (Moncada and Vane, 1978; El Tahir *et al.*, 1991a). Furthermore, VO failed to contract the isolated guinea-pig treachea.

Collectively, these findings suggest that VO-induced respiratory changes may be due to release of histamine and activation of both H_1 receptors and muscarinic M_3 receptors within the brain and peripheral structures involved in the regulation of respiration. Furthermore, the effects seemed not to involve release of any eicosanoid (prostaglandins or leukotrienes) or PAF.

The source of the released histamine may be brain histaminergic neurones (Watanabe *et al.*, 1984), peripheral mast cells (Gillespie *et al.*, 1968) and basophils (Joad and Casale, 1988). The muscarinic mechanisms seemed to be activated indirectly via histaminergic mechanisms. This is based on the inability of reserpine to deplete neuronal ACh stores (Mahotra, 1967). Thus, VO-induced respiratory effects may involve central and peripheral mechanisms. The most likely central sites to be involved in these effects are the medullary respiratory centre located in the nuclei tractus solitarii (NTS), the nucleus retroambigualis (Wayman, 1977; Kalia *et al.*, 1979) and the vagal nucleus (VN)—the nucleus ambiguus (Von Euler, 1973a, b). This NTS/VS complex is known to receive afferent sensory fibres arising from the peripheral pulmonary C-fibre sensory afferents located within the airway epithelium (Roberts *et al.*, 1981; Sant' Ambrogio, 1982) and sensory afferent fibres arising from the peripheral irritant receptors (or the rapidly adapting stretch receptors) located below the epithelium of the lungs (Sampson and Vidruk, 1975; Sant' Ambrogio, 1982).

Table 4. Effect of black seed VO on the intratracheal pressure of guinea-pigs

VO (μl kg^{-1})	Increase in tracheal pressure (mmHg)	Duration of effect (sec)
4	0.4 ± 0.2	6.5 ± 3.6
8	1.8 ± 0.2*	30.4 ± 4.8*
16	2.9 ± 0.2*	57.4 ± 6.9*
32	3.6 ± 0.8*	51.0 ± 22.8*

*$P < 0.05$, $n = 4$–24.

Thus, the most likely mechanisms which may explain at least in part the VO-induced respiratory changes may include:

(a) VO may act to stimulate histamine release from the medulary histaminergic neurones with the concomitant activation of H_1 receptors located in the NTS/VN complex. Such activation will lead to an increase in the respiratory rate via an increased activity of the descending medullary and spinal respiratory neurones and to an increase in the intratracheal pressure resulting from stimulation of the efferent vagal activity terminating in the pulmonary airways.

(b) VO may act to stimulate histamine release from pulmonary mast cells and the circulating basophils. The released amine may then activate

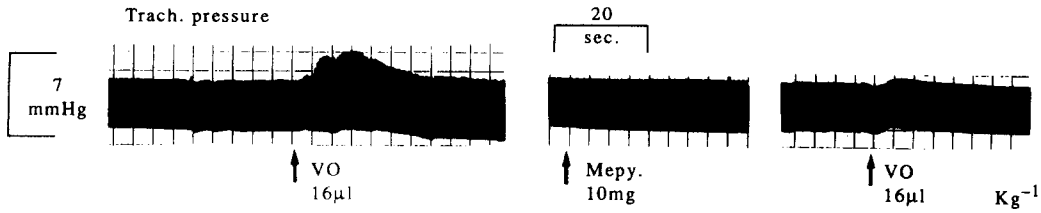

Fig. 4. Effect of mepyramine on the VO-induced increase in intratracheal pressure of guinea-pigs. Administration of the VO to the animal increased the intratracheal pressure by 2.6 mmHg. After treatment of the animal with mepyramine (Mepy. 10 mg kg^{-1} for 5 min), the same dose increased the pressure by 0.35 mmHg. Mepy. antagonized the effect of VO by 87%.

H_1 receptors located on the pulmonary C-fibre sensory afferents. The activity of these fibres will be transmitted to the NTS/VN complex via the pulmonary afferent vagal fibres with a consequent increase in the respiratory rate and bronchoconstriction. Indeed these C-fibres contain H_1 receptors (Coleridge and Coleridge, 1984) and are activated by many chemicals including histamine (Roberts *et al.*, 1981).

(c) VO may act to release histamine from pulmonary mast cells and circulating basophils. The released amine may then activate H_1 receptors located on the pulmonary irritant receptors. Such activity will be transmitted to the NTS/VN complex via the pulmonary afferent vagal fibres with the consequent activation of the respiratory centre and the efferent vagal activity reaching the pulmonary airways (Sant' Ambrogio, 1982). Indeed these irritant receptors contain H_1 receptors that are activated by histamine (Sampson and Vidruk, 1975; Bergen *et al.*, 1985).

(d) VO may act to stimulate histamine release from pulmonary mast cells or basophils. The released histamine may act locally to activate H_1 receptors in the pulmonary airways. However, this local action is unlikely because VO failed to contract the isolated trachea.

The significant blockade of VO-induced increase in the respiratory rate by both mepacrine (Mep) and nordihydroguaiaretic acid (NDG) is unlikely to be due to their inhibitory effects on eicosanoids synthesis, because other eicosanoid synthesis inhibitors such as hydrocortisone, indomethacin and diethylcarbamazine failed to block the VO-induced effect. Mep and NDG may depress the activity of the descending respiratory neurones reaching the spinal cord, or may act to inhibit release of the neurotransmitter, substance P released from the terminals of the sensory afferents impinging on the NTS/VN complex (Lundberg *et al.*, 1984).

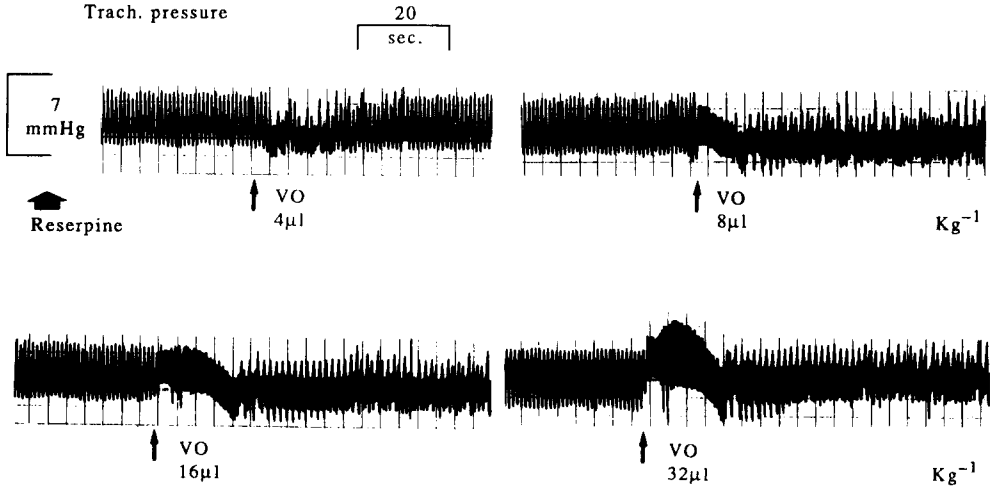

Fig. 5. Effect of black seed VO on the intratracheal pressure of reserpinized guinea-pigs. Pretreatment of the animal with reserpine (1 mg kg^{-1} day^{-1} for 2 days) completely suppressed the increase in intratracheal pressure usually induced by 4–16 μl VO kg^{-1} Administration of VO (32 μl kg^{-1}) increased the pressure by 2.8 mmHg.

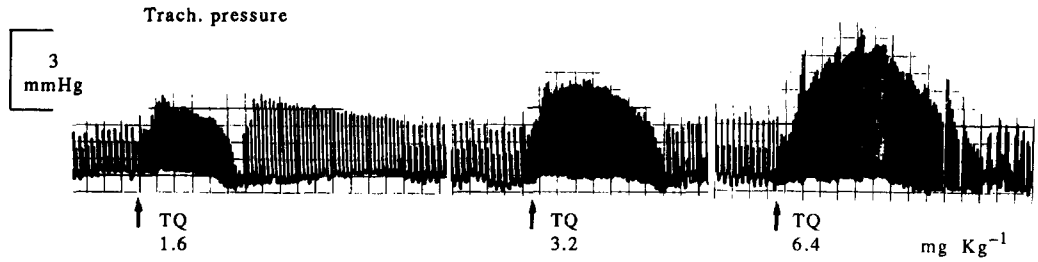

Fig. 6. Effect of thymoquinone (TQ) on the intratracheal pressure of guinea-pigs. Administration of TQ in doses of (1.6, 3.2 and 6.4 mg kg^{-1}) to the animal increased the intratracheal pressure by 1.25, 1.95 and 3.25 mmHg, respectively.

Theophylline-induced antagonism to the VO-induced increase in respiratory rate may be due to its ability to stabilize membranes of mast cells and basophils with consequent inhibition of their mediators' release (Peters *et al.*, 1982).

VO contains both thymoquinone (TQ) and monoterpenes (Canonica *et al.*, 1963; El-Dakhakhny, 1963; Aboutabl *et al.*, 1975). However, studies with TQ revealed its inability to affect the respiratory rate but it seemed to be a potent bronchoconstrictor. Thus, TQ seemed to be the VO component responsible for VO-induced bronchoconstriction, whereas the monoterpenes e.g. *p*-cymene and α-pinene may be responsible for the induced increase in respiratory rate. Thus, removal of TQ from VO may provide a potential respiratory stimulant for use in cases of central respiratory depression. Furthermore, the reported effectiveness of VO in the suppression of asthma needs to be re-investigated.

5. SUMMARY

The volatile oil (VO) of the black seed (*Nigella sativa*) was extracted using steam distillation and diethyl ether.

Intravenous administration of VO in the dose-range (4–16 μl kg^{-1}) to urethane-anaesthetized guinea-pigs increased the respiratory rate by 85–204%, 0.5 min after administration with a return to the normal rate 3–5 min later. It also increased the intratracheal pressure by 0.4–3.6 mmHg for a duration of < 1 min.

Table 5. Effect of black seed VO on the intratracheal pressure of reserpinized guinea-pigs

VO (μl kg^{-1})	Increase in the intratracheal pressure (mmHg)	
	Control	Treated
4	0.5 ± 0.15	0.17 ± 0.17*
8	1.8 ± 0.10	0*
16	2.8 ± 0.3	0.14 ± 0.06*
32	3.8 ± 1.1	1.6 ± 0.5*

$P < 0.01$, $n = 6$ compared with the control.

Treatment of the animals with mepyramine (10 mg kg^{-1} for 5 min), atropine (1 mg kg^{-1} for 5 min) or reserpine (1 mg kg^{-1} day^{-1} i.p. for 2 days) significantly antagonized VO-induced respiratory changes. The effects were not antagonized by treatment of the animals with indomethacin (10 mg/kg^{-1} for 30 min), diethylcarbamazine (12.5 mg kg^{-1} for 30 min) or hydrocortisone (10 mg kg^{-1} for 60 min).

Intravenous administration of thymoquinone (TQ) in the dose range (1.6–6.4 mg kg^{-1}) did not affect the respiratory rate of the guinea-pigs but induced dose-dependent increases in the intratracheal pressure by 1.3–3.1 mmHg.

It was concluded that a VO-induced increase in respiratory rate was due to constituents other than TQ, e.g. monoterpenes, whereas TQ was responsible for the induced increase in intratracheal pressure. Furthermore, the VO-induced respiratory changes were mainly mediated via release of histamine with direct involvement of histaminergic mechanisms and indirect activation of muscarinic cholinergic mechanisms. Thus, removal of TQ from VO may provide a potential centrally acting respiratory stimulant.

Acknowledgements—We sincerely thank Professor M. A. Al-Yahya, Chairman of the Centre of Medicinal, Aromatic and Poisonous Plants, College of Pharmacy, King Saud University for his kind permission and help in extraction of the volatile oil in the Phytochemical Unit of the Centre. We also thank Mr Barkatullah Javed for his patient and kindness in typing this manuscript.

REFERENCES

Aboutabl E. A., El-Azzouny A. A. and Hammerschmidt F. J. (1986). Aroma volatiles of *Nigella sativa* L. seeds. *Prog. Essent, Oil Res., Proc. Int. Symp. Essent. Oils* (Edited by Brunke E.-J.), pp. 49–55. De Gruyter, Berlin.

Barnes P. J. (1990) Muscarinic receptors in airways. Recent development. *J. Appl. Physiol.* **68,** 1777–1785.

Bergen D. R., Myers D. L. and Mohrman M. (1985) Activity of rapidly adapting receptors to histamine and antigen challenge before and after sodium cromoglycate. *Archs Int. Pharmacodyn. Ther.* **273,** 88–98.

Bill D. J., Hughes I. E. and Stephens R. J. (1989) The thermogenic actions of α-2-adrenoceptor agonists in reserpinized mice are mediated via a central postsynaptic

α-2-andrenoceptor mechanism. *Br. J. Pharmac.* **96,** 133–143.

Buchan P. and Adcock J. J. (1992) Capsaicin-induced bronchoconstriction in the guinea-pig: Contribution of vagal cholinergic reflexes, local axon reflexes and their modulation by BW 443C81. *Br. J. Pharmac.* **105,** 448–452.

Canonica L., Jommi G., Scolastico C. and Bonati A. (1963) The pharmacologically active principle in *Nigella sativa*. *Gazz. Chim. Ital.* **93**(11), 1404–1407.

Coleridge J. C. G. and Coleridge H. M. (1984) Afferent vagal C-fibre innervation of the lung and airways and its functional significance *Rev. Physiol. Biochem. Pharmac.* **99,** 1–110.

El-Alfy T. S., El-Fatatry H. M. and Toama M. A. (1975) Isolation and structure assignment of an antimicrobial principle from the volatile oil of *Nigella sativa* L. seeds. *Pharmazie* **30**(2), 109–111.

El-Dakhakhny M. (1963) Studies on the chemical constitution of Egyptian *Nigella sativa* L. seeds. II. The essential oil. *Planta Med.* **11,** 465–470.

El Tahir K. E. H., El Nasser M. A. S., Ageel A. M., El-Obeid H. A. and Al-Rashood K. A. (1991a) Cardiovascular depressant effects of *N*-Methyl- and *N*-isobutyl-1,2-diphenyl ethanolamines: Elucidation of the mechanisms of action. *Archs Int. Pharmacodyn. Ther.* **309,** 88–102.

El Tahir K. E. H., Al-Kharji A. M. and Ageel A. M. (1991b) Influence of diethylcarbamzine and mefloquine on PGI_2 synthesis by the rat thoracic aorta and myometrial tissues. *Gen. Pharmac.* **22,** 837–846.

Gad A. M., El-Dakhakhny M. and Hassan M. M. (1963) Studies on the chemical constitution of Egyptian *Nigella sativa* L. oil. *Planta Med.* **11,** 134–138.

Gillespie E. Levine R. J. and Malawista S. E. (1968) Histamine release from rat peritoneal mast cells: Inhibition by colchicine and potentiation by deuterium oxide. *J. Pharmac. Exp. Ther.* **164,** 158–165.

Hashim F. M. and El-Kiey M. A. (1962) *Nigella sativa* seeds of Egypt. *J. Pharm. Sci. United Arab Rep.* **3,** 121–133.

Joad J. and Casale T. B. (1988) Histamine and airway caliber. *Ann. Allergy* **61,** 1–7.

Kalia M. J., Feldman J. L. and Cohen M. I. (1979) Afferent projections to the inspiratory neuronal regions of the ventrolateral nucleus of the tractus solitarius in the cat. *Brain Res.* **171,** 135–141.

Kibby M. R. (1970) Kinetics of 5-HT exchange in rabbit blood platelets and its analysis by digital computer. *Biochem. J.* **119,** 39P.

Lundberg J. M., Hokfelt T., Martling C-R., Saria A. and Cuello C. (1984) Substance P immunoreactive sensory nerves in the lower respiratory tract of various mammals including man. *Cell Tissue Res.* **235,** 251–261.

Mahfouz M. and El-Dakhakhny M. (1960a) The isolation of a crystalline active principle from *Nigella sativa* seeds. *J. Pharm. Sci. United Arab Rep.* **1,** 9–19.

Mahfouz M. and El-Dakhakhny M. (1960b) Chemical and pharmacological properties of the new anti-asthmatic drug, nigellone. *Egypt Pharm. Bull.* **42,** 411–424.

Mahfouz M., Abdel-Maguid R. and El-Dakhakhny M. (1965) The effects of "Nigellone-Therapy" on the histaminopexic power of the blood sera of asthmatic patients. *Arzneimittel-Forsch. (Drug Res.)* **15,** 1230–1231.

Mahotra C. L. (1967). Central cholinergic transmission. *Indian J. Physiol. Pharmac.* **11,** 69–71.

Moncada S. and Vane J. R. (1978) Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2 and prostacyclin. *Pharmac. Rev.* **30,** 293–331.

Niemegeers C. J. E., Awouters F. H. L. and Janssen P. A. J. (1982) The *in vivo* pharmacological profile of histamine$_1$ antagonists in rat. *Drug Dev. Res.* **2,** 559–566.

Peters S. P., Schulman E. S., Schleimer R. P., Macglashan D. W., Newball H. H. and Lichtenstein L. M. (1982) Dispersed human lung mast cells: Pharmacologic aspects and comparison with human lung tissue fragments. *Am. Rev. Respir. Dis.* **126,** 1034–1039.

Ridzwan B. H., Mat J. A. M. and Waton N. G. (1988) The depletion effects of chlorpromazine, reserpine and ascorbic acid on tissue histamine of guinea-pigs. *Gen. Pharmac.* **19,** 631–636.

Roberts A. M., Kaufman M. P., Baker D. G., Brown J. K., Coleridge H. M. and Coleridge J. C. G. (1981) Reflex tracheal contraction induced by stimulation of bronchial C-fibres in dogs. *J. Appl. Physiol.* **51,** 485–493.

Sampson S. R. and Vidruk E. J. I. (1975) Properties of irritant receptors in canine lung. *Resp. Physiol.* **25,** 9–22.

Sant' Ambrogio G. (1982) Information arising from the trachea bronchial tree of mammals. *Physiol. Rev.* **62,** 531–569.

Sharif N. A., Towle A. G., Burt D. R., Mueller R. A. and Breese G. R. (1989) Co-transmitters: Differential effects of serotonin (5-HT)-depleting drugs on levels of 5-HT & TRH and their receptors in rat brain and spinal cord. *Brain Res.* **490,** 365–371.

Tocker J. E., Durham S. K., Welton A. F. and Selig W. M. (1990) Phospholipase A_2-induced pulmonary and hemodynamic responses in the guinea-pig. Effects of enzyme inhibitors and mediator antagonists. *Am. Rev. Respir. Dis.* **142,** 1193–1199.

Von Euler C., Hayward J. N., Martilla I. and Wyman R. J. (1973a) Respiratory neurones of the ventrolateral nucleus of the solitary tract of the cat: Vagal input, spinal connections and morphological identification. *Brain Res.* **61,** 1–22.

Von Euler C., Hayward J. N., Martilla I. and Wayman R. J. (1973b) The spinal connections of the inspiratory neurones of the ventrolateral nucleus of the tractus solitarius of the cat. *Brain Res.* **61,** 23–33.

Watanabe T., Taguchi V., Shiosaba S., Tanaka J., Kubota H., Terano Y., Tokyama M. and Wada H. (1984) Distribution of the histaminergic neurone system in the central nervous system of the rat: A flourescent immunohistochemical analysis with histidine decarboxylase as a marker. *Brain Res.* **295,** 13–25.

Wayman R. J. (1977) Neural generation of the breathing rhythm. *Ann. Rev. Physiol.* **39,** 417–448.