Gen. Pharmac. Vol. 24, No. 5, pp. 1123–1131, 1993
Printed in Great Britain.

0306-3623/93 $6.00 + 0.00

THE CARDIOVASCULAR ACTIONS OF THE VOLATILE OIL OF THE BLACK SEED (*NIGELLA SATIVA*) IN RATS: ELUCIDATION OF THE MECHANISM OF ACTION

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(Received 19 February 1993)

Abstract—1. The effects of the volatile oil (V.O.) of the black seed (*Nigella sativa*) on the arterial blood pressure and heart of urethane-anaesthetized rats were investigated and the effects were compared with those of its constituent thymoquinone (T.Q.).

2. Intravenous administration of V.O. in the dose range (4–32 μl kg.$^{-1}$) or T.Q. (0.2–1.6 mg kg^{-1}) to rats decreased the arterial blood pressure and the heart rate in a dose-dependent manner.

3. The effects of V.O. were significantly antagonized by treatment of the animals with cyproheptadine, hexamethonium atropine and by spinal pithing.

4. Treatment of the animals with reserpine (5 mg kg^{-} 1 day^{-1} for 2 days) significantly antagonized the cardiovascular depressant effects induced by 4 and 8 μl of V.O. kg^{-1} but not those induced by the larger doses.

5. T.Q.-induced cardiovascular depressant effects were significantly antagonized by atropine and cyproheptadine but not by reserpine.

6. The results suggested that V.O.-induced cardiovascular depressant effects were mediated mainly centrally via indirect and direct mechanisms that involved both 5-hydroxytryptaminergic and muscarinic mechanisms. The direct mechanisms may be due to the presence of T.Q. in the V.O. The V.O. seemed to possess the potential of being a potent centrally acting antihypertensive agent.

1. INTRODUCTION

The black seed, *Nigella Sativa* Linn., family Ranunculaceae has been shown to contain >30% of a fixed oil and 0.4–0.45% w/w of a volatile oil (V.O.) (Hashim and El-Kiey, 1962; El-Alfy *et al.*, 1975). The V.O. has been shown to contain 18.4–24% thymoquinone (T.Q.) and many monoterpenes such as *P*-cymene and α-pinene (Canonica *et al.*, 1963; El-Dakhakhny, 1963; Aboutabl *et al.*, 1986). Pharmacological investigations revealed the ability of the V.O. to exert antibacterial and antifungal properties (Rathee *et al.*, 1982), anthelmintic activity against tape worms (Agarwal *et al.*, 1979) and chloretic effects in dogs (Mahfouz *et al.*, 1962). In Arabian folk medicine the whole seeds alone or in incombination with honey or garlic are promoted for treatment of hypertension. It was thus thought of interest to examine the influence of the V.O. on the arterial blood pressure and heart rate of rats and to compare its actions with those of its constituent T.Q.

2. METHODS

2.1. Extraction of the V.O.

Sun-dried *Nigella sativa* Linn. seeds, variety hispidula (product of the Sudan) were crushed and their V.O. was extracted using steam-distillation as described by Gad *et al.* (1963). The oil was separated from the water using diethylether. The latter was completely removed from the oil via distillation under reduced pressure (400 m bar at 40°C). The oil was then stored at 25°C until required.

2.2. Preparation of rats for measurement of systemic arterial blood pressure and heart rate

Male Wistar rats (250 g) were anaesthetized with urethane 1.25 g kg^{-1} i.p. and prepared for measurement of systemic arterial blood pressure and heart rate as described previously (El-Tahir *et al.*, 1991a). The right external jugular vein was cannulated for admininistration of drugs. The arterial cannula was connected to an ITT Cannon blood pressure transducer fitted to a physiograph recorder (MK-1-V-P-Narco Bio-systems).

2.3. Effect of receptor blockers and synthesis inhibitors

The doses and pretreatment times of the various receptor blockers or synthesis inhibitors used were similar to those previously found adequate to antagonize the effects of their respective agonists (El-Tahir *et al.*, 1991a, b). In addition, some experiments were performed to confirm the effectiveness of the used blockers on the effects produced by their specific agonists.

To investigate the influence of various drugs on V.O.- or T.Q.-induced cardiovascular changes dose–response curves for V.O. and T.Q. were first obtained. The submaximal dose of each substance was then selected. The animals were then pretreated with the receptor blocker or synthesis inhibitor for a specific time which ranged from 5–60 min depending upon the drug under test (see Results) followed by re-administration of the submaximal dose of either V.O. or T.Q. Changes in arterial blood pressure induced by each treatment were then quantified in mmHg using the calibration

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system built-in the physiograph. The changes in the heart rate were quantified as percentage change compared with that before treatment. The percentage changes exerted by each blocker or synthesis inhibitor on the effects induced by the submaximal doses of V.O. or T.Q. were then calculated.

2.4. *Effect of reserpine*

To investigate the effect of treatment of the rats with reserpine on V.O.- or T.Q.-induced cardiovascular changes, the animals were treated with reserpine (5 mg kg^{-1} day^{-1} i.p. for 2 consecutive days) and used 18 hr after the last dose.

2.5. *Effect of spinal pithing on V.O.-induced cardiovascular changes*

Male Wistar rats (250 g) were anaesthetized with urethane and prepared for measurement of arterial blood pressure and heart rate as described above. The trachea was cannulated and connected to an air pump rodent ventilator (Scientific and Research Institutes Ltd., U.K.) for artificial respiration using room air and a tidal volume of 6 ml of air kg^{-1} body wt. The temperature of the animal was maintained by using an overhead tungsten lamp. A dose–response curve of the V.O. was obtained and a submaximal dose was selected. Then the animal was pithed using a modification of the method reported by Gillespie *et al.* (1970). A stainless steel rod 2 mm in diameter and 15 cm long was inserted through the left eye orbit and directed downwards towards the spinal cord. The rod was pushed into the spinal cord down to the level of the third lumbar segment. Thereafter, the animal was allowed to stabilize for 30 min. Then the submaximal dose of V.O. was re-administered and its cardiovascular effects were then calculated. The obtained responses were then compared with those obtained prior to pithing. After each experiment the position of the rod inside the spinal cord was checked by opening the thorax and abdomen and verifying the exact location of the pithing rod inside the spinal cord.

2.6. *Drugs used*

The drugs used were: T.Q. (Sigma, U.S.A.), atropine sulphate (E. Merck, Germany), mepyramine maleate (Drug Pharmaceutical Trading, B.U., Holland), ranitidine hydrochloride (Glaxo, U.K.), hexamethonium bromide (Koch-Light Laboratories Ltd., U.K.), indomethacin sodium (Dumex Ltd., Denmark), mepacrine hydrochloride (Sigma), nordihydroguaiaretic acid (Sigma), hydrocortisone (BDH Ltd., U.K.), methylene blue (E. Merck), $CaCl_2$ (BDH), diethylether (BDH), reserpine (Fluka A.G., Germany) and urethane (BDH).

2.7. *Solubilization of the drugs used*

V.O. was solubilized in platelet poor plasma (PPP) obtained from the same animal under test. The ratio of V.O.

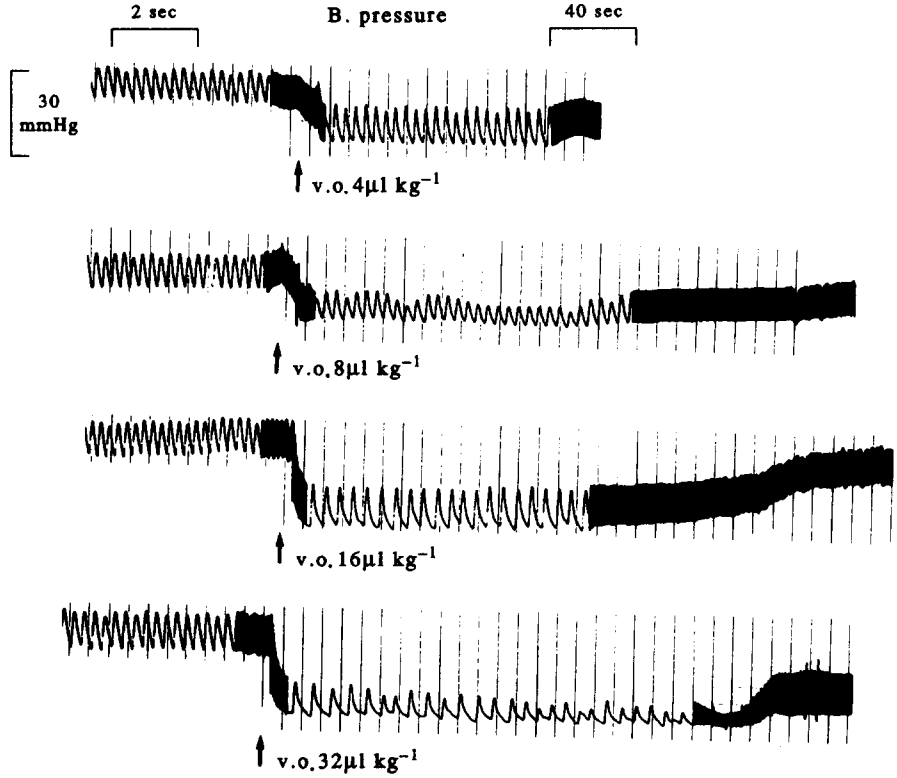

Fig. 1. Effect of the V.O. of the black seed on the arterial blood pressure and heart rate of a urethane-anaesthetized rat. Administration of V.O. (4–32 μl kg^{-1} i.v.) decreased the arterial blood pressure by 12–26 mmHg and the heart rate by 10–35%. In this figure and the subsequent ones, the 40-sec scale represents the normal speed of the recording system whereas the 2-sec scale represents the recording speed when it was increased to enable counting of the heart rate before administration of the test drug and at the time of maximum decrease in the arterial blood pressure. The vertical scale to the left of the figure represents the scale used to quantify the changes in the arterial blood pressure in mmHg following administration of the oil.

Table 1. Effect of the V.O. of the black seed *Nigella sativa* on the arterial blood pressure and heart rate of urethane-anaesthetized rats

Dose of V.O. (μl kg^{-1})	(mmHg) Mean ± SEM	Decrease in blood pressure Decrease in the heart rate (%)
4	6.7 ± 1.3*	5.1 ± 1.7
8	13.1 ± 3.4*	12.2 ± 1.9*
16	18.6 ± 4.6**	27.6 ± 5.9**
32	31.2 ± 3.9**	29.2 ± 8.1**

*$P < 0.05$; **$P < 0.1$; $n = 12$ compared with control.

to PPP was 1:4 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 × 5 cm glass tubes just before administration of the different doses. Hydrocortisone was solubilized in PPP (50 mg ml^{-1}), T.Q. in ethanol (0.4 g ml^{-1}), nordihydroguaiaretic acid in ethanol (100 mg ml^{-1}), 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) (2.5 mg ml^{-1}). All other drugs were dissolved in saline.

2.8. 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 V.O. on the systemic arterial blood pressure and heart rate of rats

Intravenous administration of V.O. in the dose range (4–32 μl kg^{-1}) to urethane-anaesthetized rats induced dose-dependent decreases in the systemic arterial pressure and heart rate. Figure 1 shows one of these experiments. In this experiment the normal heart rate was 255 beats min^{-1}. Administration of V.O. in doses of 4, 8, 16 and 32 μl kg^{-1} decreased the arterial blood pressure by 12, 15, 25 and 26 mmHg, respectively. The corresponding decreases in the heart rate were 10, 11, 33 and 35%, respectively. Both blood pressure and heart rate returned to pretreatment levels within 5 min. The cumulative findings mean ± SE mean from 12 such experiments are shown in Table 1.

3.2 Effect of receptor blockers and synthesis inhibitors

Treatment of rats with cyproheptadine (1 mg kg^{-1} for 5 min) significantly antagonized the cardiovascular depressant effects induced by the V.O. (16 μl kg^{-1}) (Fig. 2). The cumulative percentage antagonism from 4 such experiments was 54.2 ± 4.1%

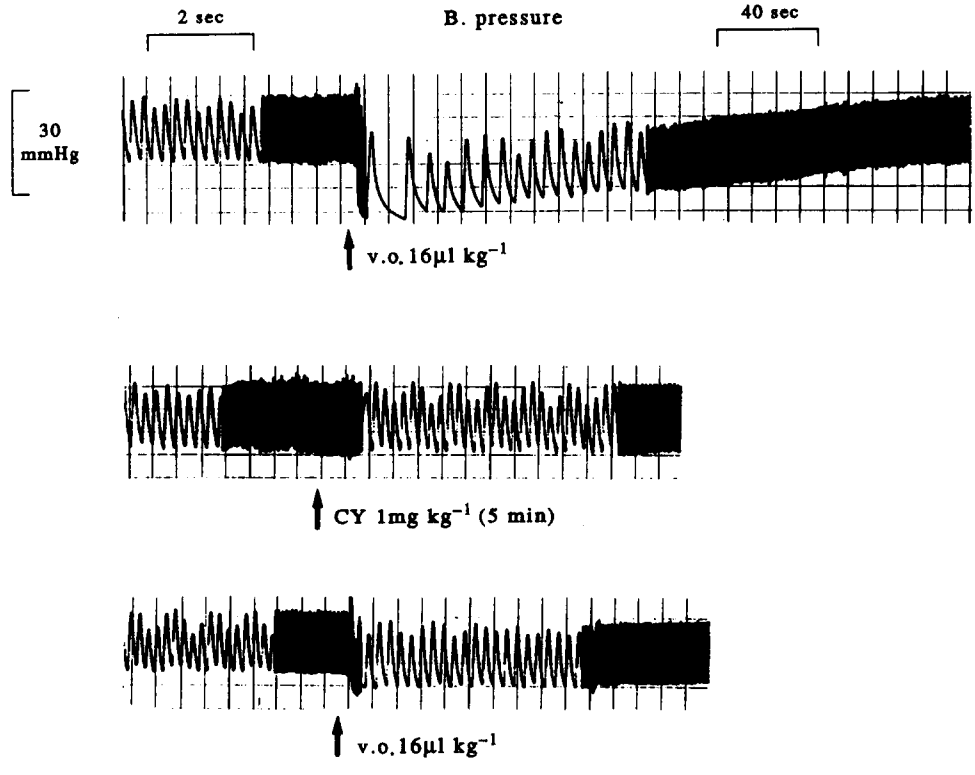

Fig. 2. Effect of cyproheptadine on V.O.-induced cardiovascular depressant effects in the rat. Administration of the V.O. (16 μl kg^{-1}) to the urethane-anaesthetized rat decreased the arterial blood pressure by 18 mmHg and the heart rate by 36.3%. Pretreatment of the animal with cyproheptadine (CY, 1 mg kg^{-1} for 5 min) antagonized the V.O.-induced decrease in blood pressure by 67% and the heart rate by 46%.

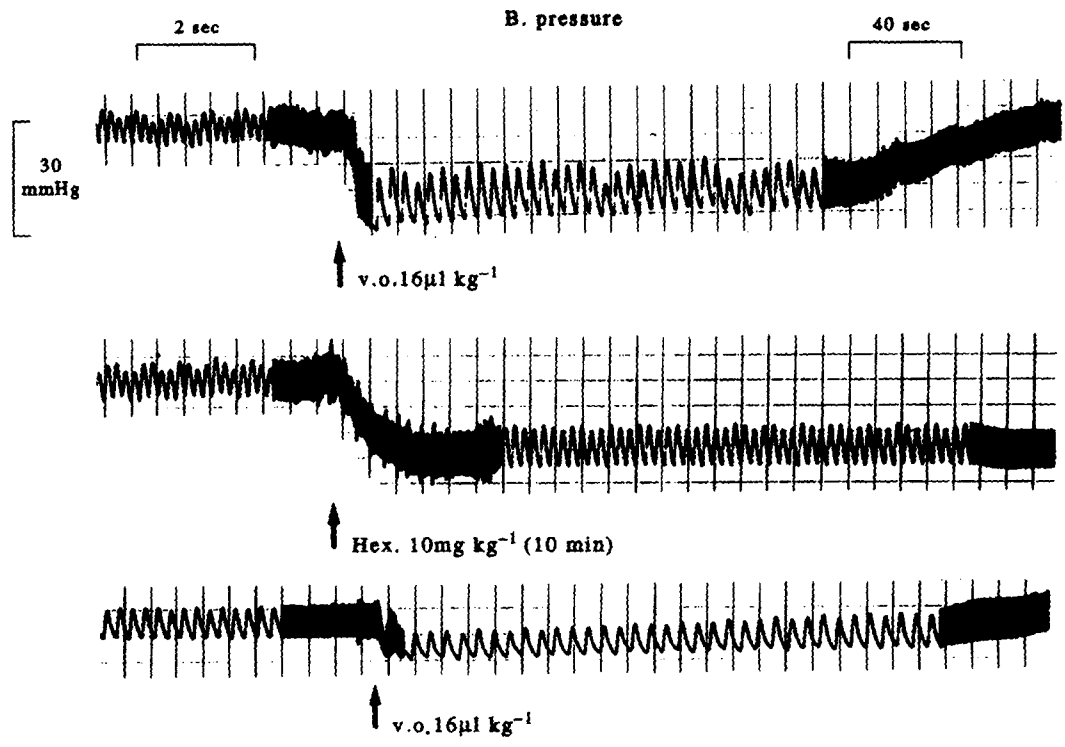

Fig. 3. Effect of hexamethonium on V.O.-induced cardiovascular depressant effects in the rat. Administration of the V.O. (16 μl kg^{-1}) to a urethane-anaesthetized rat decreased the arterial blood pressure by 23 mmHg and the heart rate by 27%. After pretreatment of the animal with hexamethonium (Hex, 10 mg kg^{-1} for 10 min), the same dose of V.O. decreased the arterial blood pressure by 7.5 mmHg and the heart rate by 12.6%.

(with regard to arterial pressure) and 59.8 ± 8.5% (with regard to the heart rate) ($P < 0.01$, $n = 4$). Similarly, treatment of the animals with atropine (1 mg kg^{-1} for 5 min) antagonized V.O.-induced decreases in the blood pressure and heart rate by 41.4 ± 6.6 and 79.4 ± 26.5%, respectively ($P < 0.01$, $n = 3$). Furthermore, treatment of the animals with hexamethonium (10 mg kg^{-1} for 10 min) significantly antagonized V.O.-induced decreases in the arterial pressure and heart rate by 60.3 ± 6.3 and 53.1 ± 5.5%, respectively ($P < 0.01$, n = 4) (Fig. 3).

Treatment of the animals with mepyramine (10 mg kg^{-1} for 5 min), ranitidine (10 mg kg^{-1} for 5 min), $CaCl_2$ (240 mg kg^{-1} for 3 min), methylene blue (20 mg kg^{-1} for 30 min), mepacrine (4 mg kg^{-1} for 30 min), nordihydroguaiaretic acid (20 mg kg^{-1} for 30 min), indomethacin (10 mg kg^{-1} for 30 min) or hydrocortisone (10 mg kg^{-1} for 60 min) did not affect V.O.-induced cardiovascular depressant effects.

3.3. *Effect of reserpine*

Treatment of rats with reserpine (5 mg kg^{-1} i.p. day^{-1} for 2 days) significantly decreased the cardiovascular depressant effects induced by small doses (4 and 8 μl kg^{-1}) of V.O. ($P < 0.01$, $n = 4$) but did not significantly affect the depressant effects induced by the larger doses (10 and 32 μl kg^{-1}). Table 2 depicts the cardiovascular depressant effects of V.O. in control and reserpine-treated animals.

3.4. *Effect of spinal pithing*

Figure 4 shows the cardiovascular effects of V.O. (16 μl kg^{-1}) in a rat before and after spinal pithing. In this experiment administration of V.O. to the intact rat decreased the arterial blood pressure by 25 mmHg and the heart rate by 28%, however, the corresponding decreases after spinal pithing were 2 mmHg and 12.5%, respectively. In 4 similar experiments spinal pithing decreased V.O.-induced decreases in arterial blood pressure and heart rate by

Table 2. Effect of the V.O. of the black seed *Nigella sativa* on the arterial blood pressure and heart rate of reserpinized rats

Dose of V.O. (μl kg^{-1})	Decrease in blood pressure (mmHg)		Decrease in the heart rate (%)	
	Control	Treated	Control	Treated
4	6.0 ± 1.4	0.00*	5.2 ± 1.4	0.3 ± 1.1*
8	11.95 ± 3.1	5.2 ± 1.7*	13.5 ± 1.7	4.7 ± 3.1*
16	19.1 ± 3.9	12.8 ± 4.2	28.1 ± 5.1	16.9 ± 5.2
32	29.6 ± 4.1	19.7 ± 5.2	31.1 ± 5.6	30.6 ± 5.4

*$P < 0.01$, $n = 4$ compared with the vehicle-treated animals.

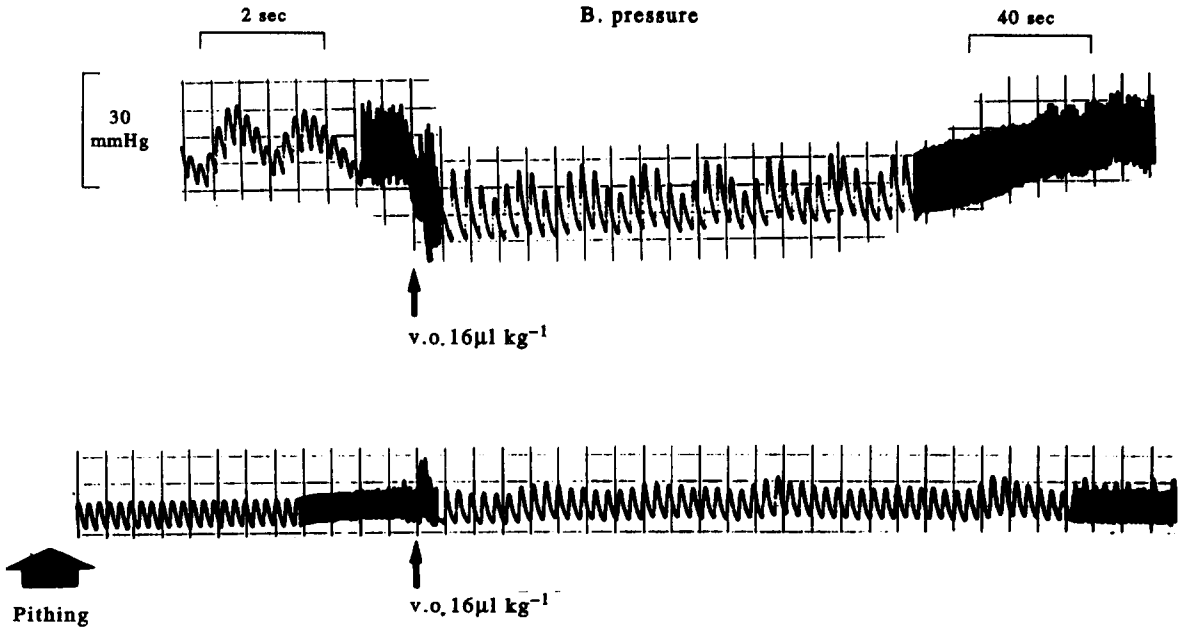

Fig. 4. Effect of spinal pithing on V.O.-induced cardiovascular depressant effects in the rat. Administration of the V.O. (16 μl kg^{-1}) to a urethane-anaesthetized rat decreased the arterial blood pressure by 25 mmHg and the heart rate by 28%. Administration of the same dose after pithing decreased the blood pressure by 2 mmHg and the heart rate by 12.5%.

86.7 ± 4.4 and 56.7 ± 8.7%, respectively ($P < 0.01$, $n = 4$).

3.5. Effect of T.Q.

Intravenous administration of T.Q. to urethane-anaesthetized rats in the dose range (0.2–1.6 mg kg^{-1}) induced dose-dependent decreases in both the arterial blood pressure and the heart rate. Figure 5 shows one of these experiments. In this experiment administration of T.Q. in doses of 0.4, 0.8 and 1.6 mg kg^{-1} decreased the arterial blood pressure by 3, 15 and 18 mmHg and the heart rate by 0, 29 and 53%, respectively. The cumulative findings from 8 such experiments are shown in Table 3.

3.6. Effect of atropine and cyproheptadine

Treatment of rats with atropine (1 mg kg^{-1} for 5 min) significantly antagonized the T.Q.-induced decrease in the blood pressure and heart by 73.8 ± 3.6 and 65.3 ± 4.5%, respectively ($P < 0.01$, $n = 4$). Figure 6 shows one of these experiments. In this experiment administration of T.Q. (0.8 mg kg^{-1}) decreased the arterial blood pressure by 15 mmHg and the heart rate by 29.6%. The corresponding decreases following pretreatment with atropine were 4.5 mmHg and 8.3%, respectively. Similarly, treatment of rats with cyproheptadine (1 mg kg^{-1} for 5 min) antagonized T.Q.-induced decreases in blood pressure and heart rate by 92.2 ± 3.6 and 74.3 ± 4.8%, respectively ($P < 0.01$, $n = 4$).

3.7. Effect of reserpine

Treatment of rats with reserpine (5 mg kg^{-1} i.p. day^{-1} for 2 days) did not significantly affect T.Q.-induced cardiovascular depressant effects compared with control animals ($P > 0.05$, $n = 4$). Table 4 depicts the cardiovascular depressant effects of T.Q. (0.4–1.6 mg kg^{-1}) in control and reserpine-treated animals.

4. DISCUSSION

The results of this study clearly demonstrated that treatment of rats with the V.O. of the black seed decreased both the arterial blood pressure and heart rate. Both effects were antagonized by the ganglionic blocker, hexamethonium (Paton and Zaimis, 1952), the non-selective muscarinic receptor blocker atropine (Barnes, 1990) and the 5-hydroxytryptaminergic (5-HT) and histamine H_1 receptor blocker, cyproheptadine (Niemegeers *et al.*, 1982). The effects were not antagonized by the histamine H_1 receptor blocker, mepyramine (Niemegeers *et al.*, 1982) the H_2 receptor blocker, ranitidine (Daly *et al.*, 1979) the phospholipase A_2 inhibitors mepacrine and hydrocortisone (Moncada and Vane, 1978), the lipoxygenase inhibitor nordihydroguairetic acid (Borgeat and

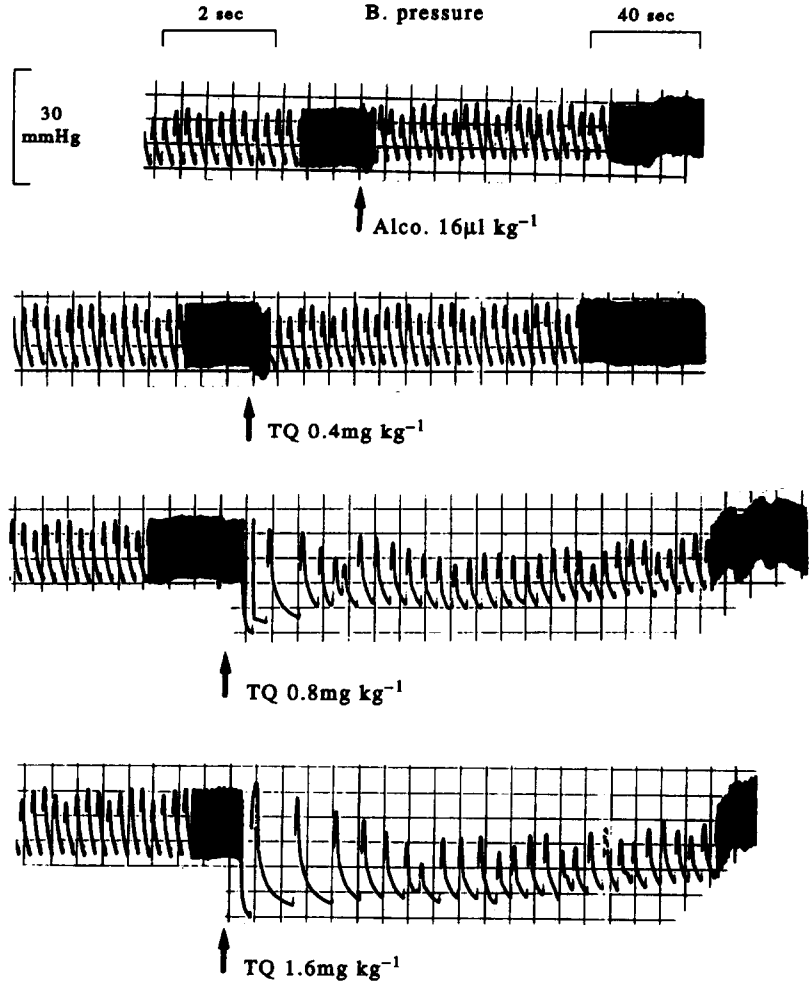

Fig. 5. Effect of T.Q. on the arterial blood pressure and heart rate of a urethane-anaesthetized rat. Administration of alcohol—the solvent of T.Q.—in a dose of 16 μl kg^{-1} (Alco., 16 μl kg^{-1}) to the rat did not affect the arterial blood pressure or the heart rate. However, administration of T.Q. 0.4, 0.8 and 1.6 mg kg^{-1} in the same volume of alcohol decreased the arterial blood pressure by 3, 15 and 18 mmHg, respectively and the heart rate by 0, 29 and 53%, respectively.

Samuelsson, 1979), the prostaglandin cyclo-oxygenase inhibitor indomethacin (Moncada and Vane, 1978), $CaCl_2$ or the cyclic GMP inhibitor, methylene blue (Ignarrow *et al.*, 1986). Furthermore, pretreatment of the animals with reserpine which depletes neuronal (Sharif *et al.*, 1989) and cellular (Ridzwan *et al.*, 1988) monoamines significantly antagonized the effects induced by small but not large doses of V.O. In addition, spinal pithing that destroyed the neuronal connections between the medullary adrenergic neurones and the spinal preganglionic sympathetic neurones (Ruggiero *et al.*, 1985) completely blunted V.O.-induced decreases in the arterial pressure and significantly antagonized the induced bradycardia. Collectively, these findings suggest the following conclusions:

Table 3. Effect of T.Q. on the arterial blood pressure and heart rate of urethane-anaesthetized rats (Mean ± SEM)

Dose of T.Q. (mg kg^{-1})	Decrease in blood pressure (mmHg)	Decrease in the heart rate (p%)
0.2	1.2 ± 1.2	3.5 ± 2.1
0.4	9.6 ± 1.6*	8.5 ± 3.3
0.8	15.1 ± 1.4*	26.1 ± 4.3*
1.6	17.5 ± 3.0*	36.4 ± 6.6*

*$P < 0.05$, $n = 8$ compared with the control.

(a) V.O. seemed to act both directly and indirectly to activate both 5-HT and cholinergic mechanisms.

(b) V.O.-induced decrease in arterial blood pressure was mainly centrally mediated in the brain and/or the spinal cord whereas the induced bradycardia may have involved both central and peripheral mechanisms.

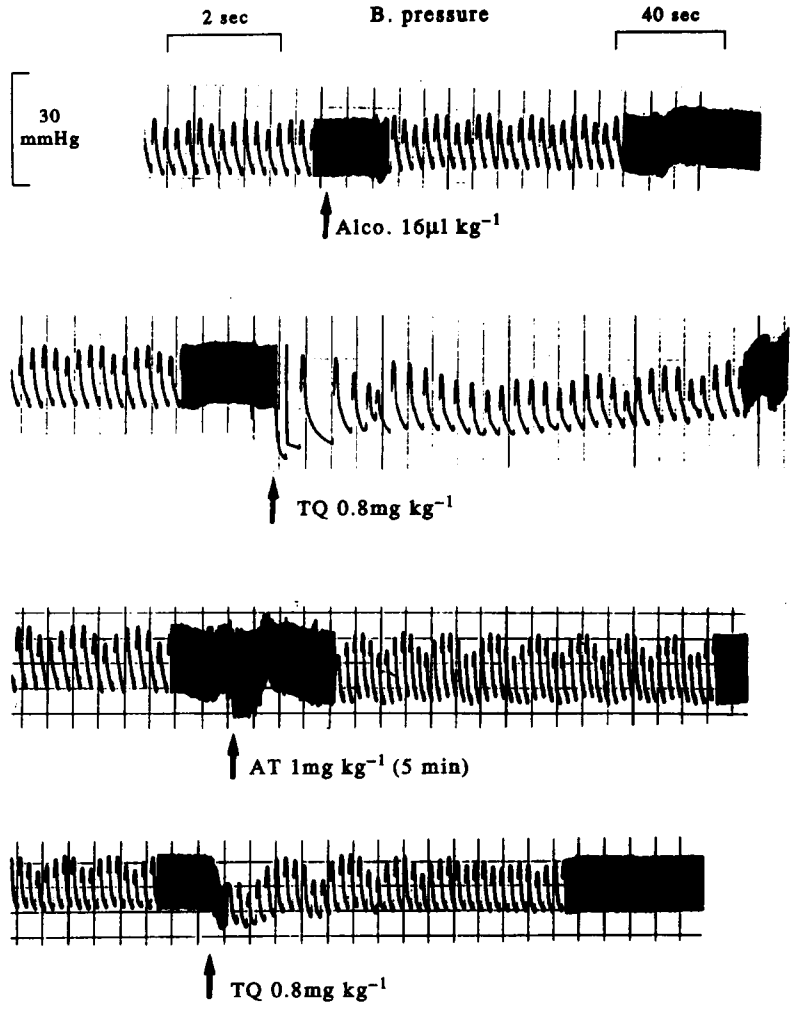

Fig. 6. Effect of atropine on T.Q.-induced cardiovascular depressant effects in the rat. Administration of alcohol—the solvent of T.Q.—in a dose of 16 μl kg^{-1} (Alco., 16 μl kg^{-1}) did not affect the arterial blood pressure or the heart rate of the rat. Administration of T.Q. in a dose of 0.8 mg kg^{-1} (T.Q., 0.8 mg kg^{-1}) decreased the arterial pressure by 15 mmHg and the heart rate by 30%. The corresponding decreases after pretreatment of the animal with atropine (AT, 1 mg kg^{-1} for 5 min) were 4.5 mmHg and 8.3%, respectively.

(c) V.O.-induced effects did not involve release of eicosanoids, PAF, EDRF, histamine or blockade of calcium channels.

The V.O. direct mechanisms may involve direct activation of both 5-HT and muscarinic receptors by some V.O. constituents whereas the indirect mechanisms may involve stimulation of 5-HT release from brain 5-HT neurones, circulating platelets and/or mast cells located on the perivascular sites of the cranial blood vessels. This suggestion is based on the observations that both ascending and descending 5-HT neurones have been identified in the brain stem of various mammals (Strack *et al.*, 1989) and that both rodent's platelets and mast cells contain 5-HT (Jansson, 1970; Kibby, 1970).

Table 4. Effect of T.Q. on the arterial blood pressure and heart rate of reserpinized rats.

Dose of T.Q. (mg kg^{-1})	Decrease in blood pressure (mmHg)		Decrease in the heart rate (%)	
	Control	Treated	Control	Treated
0.4	9.8 ± 1.4	11.2 ± 4.4	9.5 ± 4.3	15.7 ± 4.7
0.8	15.6 ± 1.6	16.1 ± 4.7	27.4 ± 4.3	34.0 ± 5.3
1.6	17.9 ± 3.2	16.8 ± 4.4	36.9 ± 6.1	35.2 ± 4.5

Previous studies on 5-HT-induced cardiovascular effects revealed that 5-HT-induced cardiovascular depressant effects are usually associated with 5-HT$_1$ and 5-HT$_3$ receptors (Saxena and Lawang, 1985). Cyproheptadine is an old 5-HT receptor blocker known to interact with both 5-HT$_1$ and

5-HT_2 receptors (Williams and Martin, 1982; Cocks and Angus, 1983; Hiner *et al.*, 1986; Leff *et al.*, 1987; Hoyer *et al.*, 1989). Similarly, the non-selective muscarinic receptor blocker atropine has also been shown to antagonize the cardiovascular depressant effects induced via activation of 5-HT_{1A} receptors in the rat (Fozard *et al.*, 1987). Thus, V.O.-induced cardiovascular depressant effects mediated via 5-HT mechanisms seemed to be more likely a result of activation of 5-HT_1 receptors. On the other hand, the muscarinic component of the V.O.-induced cardiovascular depressant effects seemed to involve the cardiac muscarinic receptors. Atropine is known to block these receptors (Barnes, 1990).

The most likely site within the brain that contains 5-HT_1 receptors and that was probably affected by direct and indirect mechanisms activated by V.O. seemed to be the nuclei tractus solitarii/vagal nuclei complex (NTS/VN complex) within the medulla oblongata. Indeed this area is shown to contain 5-HT_{1A} receptors (Sporton *et al.*, 1989). Injection of selective 5-HT_{1A} agonists in this complex induced cardiovascular depressant effects in rats (Sporton *et al.*, 1989).

Thus, the most likely explanation for V.O.-induced cardiovascular depressant effects may be as follows: V.O. may act to stimulate release of 5-HT from its brain stem neurones, platelets and/or cranial perivascular mast cells. Both the released 5-HT and some V.O. constituents may act to activate 5-HT_{1A} receptors located on the NTS/VN complex resulting in ultimate inhibition of the vasomotor centre (Brown and Guynet, 1984) and activation of efferent vagal fibres reaching the heart resulting in release of acetylcholine and activation of cardiac muscarinic receptors (Spyer, 1982). All these actions will result in decreases in the arterial blood pressure and the heart rate.

Administration of the V.O. constituent T.Q. mimicked the actions of the V.O. except that its effects seemed to be mediated directly since they were not significantly affected by depletion of 5-HT with reserpine. Thus, the direct component of the cardiovascular depressant effects induced by V.O. seemed to be due to the presence of T.Q.

In conclusion, this study clearly demonstrated the cardiovascular depressant effects of the V.O. of the black seed which seemed to be mediated via central mechanisms that involved direct and indirect activation of 5-HT_{1A} located in the NTS/VN complex within the medullary area in the brain stem and muscarinic receptors located in the heart. Thus, the V.O. seemed to possess the potential of a centrally acting antihypertensive agent.

5. SUMMARY

The V.O. of the black seed (*Nigella sativa*) was extracted using steam distillation and diethyl ether. Intravenous administration of the V.O. in the dose range (4–32 μl kg^{-1}) decreased the systemic arterial pressure of the urethane-anaesthetized rats by 6–31 mmHg and the heart rate by 5–29%.

Pretreatment of the animals with cyproheptadine or atropine (1 mg kg^{-1} for 5 min) significantly antagonized V.O.-induced cardiovascular depressant effects by 41–79%. Furthermore, the effects were significantly decreased by spinal pithing of the animals.

Pretreatment of the animals with reserpine (5 mg kg^{-1} day^{-1} i.p. for 2 days) significantly decreased the effects induced by 4 and 8 μl of V.O. kg^{-1} but not those induced by larger doses.

The V.O. cardiovascular depressant effects were not affected by pretreatment of the animals with mepyramine, ranitidine, mepacrine, hydrocortisone, nordihydroguaiaretic acid, indomethacin, $CaCl_2$ or methylene blue.

Intravenous administration of the V.O. constituent T.Q. in the dose-range (0.2–1.6 mg kg^{-1}) induced similar cardiovascular effects that were significantly antagonized by pretreatment of the animals with cyproheptadine or atropine (1 mg kg^{-1} for 5 min) but not with reserpine.

It was concluded that V.O.-induced cardiovascular depressant effects may be due to indirect and direct mechanisms mediated centrally in the brain stem with involvement of both 5-HT and cholinergic muscarinic mechanisms. The direct actions may be due to the presence of T.Q. in the V.O.

Acknowledgements—We sincerely thank the Phytochemical Unit of the Centre of Medicinal, Aromatic and Poisonous Plants, College of Pharmacy, King Saud University for the kind help and assistance in extraction of the V.O. We also thank Mr Barkatullah Javed for his patience 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. In *Progress in Essential Oil Research, Proceedings of the International Symposium on Essential Oils* (Edited by Brunke E.-J.), pp. 44–55. De Gruyter, Berlin, Germany.

Agarwal R., Kharya M. D. and Shrivastava R. (1979) Antimicrobial and anthelmintic activities of the essential oil of *Nigella Sativa* Linn. *Indian J. Exp. Biol.* **17**, 1264–1265.

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

Bill D. J., Hughes I. E. and Stephens R. J. (1989) The thermogenic action of α-2-adrenoreceptor agonists in reserpinized mice are mediated via a central postsynaptic α-2-adrenoreceptor mechanisms. *Br. J. Pharmac.* **96**, 133–143.

Borgeat P. and Samuelsson B. (1979) Metabolism of arachidonic acid in polymorphonuclear leukocytes. Structural analysis of novel hydroxylated compounds. *J. Biol. Chem.* **254,** 7865–7869.

Brown D. L. and Guynet P. G. (1984) Cardiovascular neurons of brain stem with projections to spinal cord. *Am. J. Physiol.* **247,** R1009–R1016.

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

Cocks T. M. and Angus J. A. (1983) Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. *Nature* **305,** 627–630.

Daly M. J., Humphray J. M. and Stables R. (1979) Antagonizm of vasodepressor and gastric secretory responses to histamine by ranitidine and cimetidine in the anaesthetized dog. *Br. J. Pharmac.* **67,** 414P.

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,** 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 diethylcarbamazine and mefloquine on PGI_2 synthesis by the rat thoracic aorta and myometrial tissues. *Gen. Pharmac.* **22,** 837–846.

Fozard J. R., Mir A. K. and Middlemiss N. N. (1987) Cardiovascular response to 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) in the rat: site of action and pharmacological analysis. *J. Cardiovasc. Pharmac.* **9,** 328–347.

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 J. S., McLaren A. and Pollock D. (1970) A method of stimulating different segments of the autonomic outflow from the spinal column to various organs in the pithed cat and rat. *Br. J. Pharmac.* **40,** 257–267.

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

Hiner B. C., Roth J. H. L. and Peroutka S. J. (1986) Antimigraine drug interactions with 5-hydroxytryptamine 1A receptors. *Ann. Neurol.* **19,** 511–513.

Hoyer D., Waeber C., Schoeffter P., Palacios J. M. and David A. (1989) 5-HT_{1C} receptor mediated stimulation of insitol phosphate production in pig choroid plexus. A pharmacological characterization. *Naunyn-Schmiedeberg's Archs Pharmac.* **339,** 252–258.

Ignarrow L. L., Harbison R. G., Wood K. S. and Kadowitz P. J. (1986) Dissimilarities between methylene blue and cyanide on relaxation and cyclic GMP formation in endothelium-intact intrapulmonary artery caused by nitrogen oxide containing vasodilators and acetylcholine. *J. Pharmac. Exp. Ther.* **236,** 30–36.

Jansson S. E. (1970) Cytoplasmic distribution of endogenous and exogenous 5-HT in rat peritoneal mast cells. *Acta Physiol. Scand.* **80,** 345–352.

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

Leff P., Martin G. R. and Morse J. M. (1987) Differential classification of vascular smooth muscle and endothelial cell 5-HT receptors by use of tryptamine analogues. *Br. J. Pharmac.* **91,** 321–331.

Mahfouz M., Dakhakhany M., Gemel A. and Moussa H. (1962) Chloretic action of *Nigella sativa* seed oil. *Egyptian Pharmac. Bull.* **44,** 225–229.

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 D. J. E., Awouters F. H. L. and Janssen P. A. J. (1982) The *in vivo* pharmacological profile of histamine (H_1) antagonists in rat. *Drug Dev. Res.* **2,** 559–566.

Paton W. D. M. and Zaimis E. J. (1952) The methonium compounds. *Pharmac. Rev.* **4,** 219–253.

Rathee P. S., Mishra S. H. and Kaushal R. (1982) Antimicrobial activity of essential oil, fixed oil and unsaponifiable matter of *Nigella sativa* L. *Indian J. Pharmac. Sci.* **44,** 8–10.

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.

Ruggiero D. A., Ross C. A., Anwar M., Park D. H., Joh T. H. and Reis D. J. (1985) Distribution of neurons containing phenylethanoloamine *N*-methyltransferase in medulla and hypothalamus of rat. *J. Comp. Neurol.* **239,** 127–154.

Saxena P. R. and Lawang A. (1985) A comparison of cardiovascular and smooth muscle effects of 5-HT and 5-carboxamidotryptamine, a selective agonist of 5-HT_1 receptors. *Archs Int. Pharmacodyn. Ther.* **277,** 235–252.

Sharif N. A., Towle A. G., Burth 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.

Sporton S. C. E., Shepheard S. L., Jordan D. and Ramage A. G. (1989) Evidence for the involvement of 5-HT_{1A} receptors in the control of cardiac vagal motoneurones in the anaesthetized rat. *Br. J. Pharmac.* **97,** 409P.

Spyer K. M. (1982) Central nervous integration of cardiovascular control. *J. Exp. Biol.* **100,** 109–128.

Strack A. M., Sawyer W. B., Pratt K. B. and Loewy A. D. (1989) DNS cell groups regulating the sympathetic outflow to adrenal gland as revealed by transneuronal cell body labelling with pseudorabies virus. *Brain Res.* **491,** 274–296.

Williams M. and Martin G. E. (1982) Selectivity of cyproheptadine as assessed by radioligand binding. *J. Pharm. Pharmac.* **34,** 58–59.