SOME CARDIOVASCULAR EFFECTS OF THE DETHYMOQUINONATED *NIGELLA SATIVA* VOLATILE OIL AND ITS MAJOR COMPONENTS α-PINENE AND p-CYMENE IN RATS.

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تم بحث تأثير الزيت الطيار لحبة البركة (الحبة السوداء) الذي تم نزع الثايموكينون منه وكذلك تأثير إثنين من مكوناته الرئيسية، وهما ألفا باينين وباراسايمين وذلك على الجهاز القلبي الوعائي للجرذان المبنجة باليوريثان. وقد تسبب إعطاء الزيت الطيار وألفا باينين وباراسايمين وريدياً بجرعة تتراوح بين 2 – 16 ميكرولتر/كغ في خفض كل من ضغط الدم الشرياني ومعدل ضربات القلب. وهذه التأثيرات تم تضادها معنوياً بمعالجة الجرذان بالهكساميثونيوم وبقطع الحبل الشوكي. أما بطء القلب المستحث فقد تم صده بالأتروبين. لذلك يبدو أن التأثيرات القلبية الوعائية المهبطة تتوسطها آليات مركزية من المحتمل أنها تشمل المركز الوعائي الحركي في الغمد النخاعي مع انخفاض ناتج في التدفق السمبتاوي المركزي الذي يصل إلى القلب والأوعية الدموية. إضافة إلى ذلك تسببت معالجة الصفيحات الدموية للجرذان بالزيت الطيار المنزوع منه الثايموكينون، وبالألفا باينين أ والباراسايمين بجرعات 5- 20 ميكرولتر/مل في تثبيط غير معنوي لتكدس الصفيحات المستحث بالأدينوزين ثنائي الفوسفات بشكل غير معتمد على الجرعة. ويمكننا أن نستنتج أن إخلاء الزيت العطري الطيار من محتواه من مادة الثايموكينون التي يعرف عنها أنها تضيق الشعب الهوائية وتسبب التقرح المعدي ، لم يقض على التأثيرات القلبية الوعائية المهبطة للزيت.

The influence of the dethymoquinonated volatile oil (DE-TQ) of *Nigella sativa* (the black seed) and its two major components α-pinene and *p*-cymene was examined on the cardiovascular system of urethane-anaesthetized rats. Intravenous administration of the DE-TQ, α-pinene or *p*-cymene in the dose range (2-16 μl kg^{-1}) decreased both the arterial blood pressure and the heart rate. These effects were significantly antagonized by treatment of the animals with hexamethonium and by spinal pithing of the rats. The induced bradycardias were also blocked by atropine. Thus, the induced cardiovascular depressant actions seemed to be mediated via central mechanisms that probably involved the vasomotor centre in the medulla with the resultant decrease in the central sympathetic outflow reaching the heart and blood vessels. Furthermore treatment of rat platelets *in vitro* with DE-TQ, α-pinene or *p*-cymene in doses of (5-20 μl ml^{-1}) produced non-significant and non-dose-dependent inhibitions of adenosine diphosphate-induced aggregation. It can be concluded that deprivation of the whole volatile oil of its constituent thymoquinone which is known for its bronchoconstricting and gastric ulcerogenic actions did not abolish the cardiovascular depressant actions of the oil.

Introduction

Studies about the pharmacological actions of the volatile oil of the black seed *Nigella sativa* in both, rats and guinea-pigs revealed its ability in doses of (4-32 μl kg^{-1}) (i.v.) to decrease the arterial blood pressure and to induce bradycardia in a dose-dependent manner (1,2). Analysis of the mechanisms underlying these observed actions suggested the involvement of central mechanisms and partial peripheral mechanisms that involved serotoninergic and M_2 muscarinic mechanisms, respectively (1,2). Furthermore, studies on the respiratory system of the guine-pig revealed the ability of the volatile oil in doses of (4-32 μl Kg^{-1}) (i.v.) to induce both bronchoconstriction and tachypnea (3). Detailed analysis of the mechanisms revealed the ability of

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the oil to release histamine from the respiratory mast cells with the concomitant direct activation of histaminergic receptors and indirect activation of muscarinic-cholinergic mechanisms via mechanisms that involved sensory C filbres (carried in the afferent vagus nerve) and the efferent vagal nerves (3). Furthermore, administration of thymoquinone - the major component of the volatile oil – in doses of (1.6 – 6.4 mg kg^{-1}) (i.v.) into guinea-pigs induced only bronchoconstriction and no tachypnea (3) whereas its administration in smaller doses (0.2-1.6 mg Kg^{-1}) (i.v.) into rats decreased both the arterial blood pressure and the heart rate (1).

The volatile oil of *N sativa* is known to contain thymoquinone, *p*-cymene and α-pinene in concentrations of 18-24, 31.7 and 9.3%, respectively (4,5,6). The striking similarity between the actions of thymoquinone and the volatile oil raised the question: do the actions of the volatile oil depend upon its content of thymoquinone or do other constituents such as *p*-cymene and α-pinene contribute to the observed actions? It should be noted that removal of thymoquinone may remove both bronchconstriction (3) and gastric ulcers induced by the oil (7).

Thus, this study is a partial attempt to answer the above question. For this purpose the oil was deprived of its thymoquinone and the effects of the de-thymoquinonated oil together with those of pure *p*-cymene and α-pinene were investigated on the arterial blood pressure, the heart rate and platelets aggregation of the rat.

Methods

A. Phytochemical studies:

A.1. Source of the seeds of Nigella sativa:

The seeds used in this study were of Ethiopian origin bought in Riyadh market, Saudi Arabia. They were correctly identified and authenticated by Dr. Sultanul Abedin, the taxonomist at the Research Centre at the College of Pharmacy, King Saud University. A sample of the seeds is retained in the Herbarium of the Centre.

A.2 Extraction of volatile oil of Nigella sativa:

The volatile oil of *Nigella sativa* seeds was extracted using direct steam distillation using VWR-Volatile oil distillation apparatus (VWR Co., U.S.A.) as described before (1,3).

A.3 Identification of the major components of the volatile oil:

The presence of the major components of the volatile oil namely thymoquinone, *p*-cymene and α-pinene in the extracted oil was confirmed using thin layer chromatography. For this purpose silica gel plates (0.2 mm thickness) with fluorescent indicator 254 nm (Riedel-de-Haen, Germany) were used together with the solvent system n-hexane : ethyl acetate (80 : 20 v/v) as described by Canonica *et al*. (4). The oil together with authentic thymoquinone, *p*-cymene and α-pinene were applied to the plate. After development, the plate was examined under UV light to locate the various spots.

A.4 Separation of thymoquinone from the oil:

To dethymoquinonate the oil, a slight modification of Canonica *etal*. (4) method was used. Thirty ml of the volatile oil were placed in a 200-ml glass beaker and 150 ml of n-hexane were added and mixed. The beaker was then placed in a freezer set at –69°C and left overnight. In the morning the whole solution above the slightly yellowish crystalline precipitate was aspirated and the remainder was filtered rapidly and the crystals were collected. The n-hexane in the aspirated fluid was then distilled off at 40°C under reduced pressure (400 mbar) using a Rota-Evaporator (Buchi HB. 140, Germany). The oil obtained after distilling off n-hexane was collected and dried in a vacuum oven for seven hours to remove all traces of hexane. The obtained oil was then subjected to thin layer chromatography as described above to confirm the absence of thymoquinone. Similarly, the collected precipitated crystals were subjected to thin layer chromatography to confirm their identity as thymoquinone.

B. Pharmacological studies.

B.1 Animals used:

In this study male Wistar rats (200-250g body weight) provided by the Experimental Animals Care Centre, King Saud University, Riyadh were used. The animals were kept in rooms with 12h dark and 12h light cycle. Water and food were provided *adlib*. All animals were anaesthetized using 25% (w/v) aqueous urethane in a dose of (1.25g kg^{-1} i.p.).

B.2 Preparation of rats for cardiovascular measurement:

The method used to prepare Wistar male rats for

the measurement of the arterial blood pressure and heart rate was as described before (1). Before administration of any drug, the mean arterial blood pressure of each animal was measured using a mercury manometer. The effect of the test substance on the arterial blood pressure were quantified by the aid of the built-in calibration system of Narco Physiograph Coupler No. 7179 (MK-1-Narco-Bio Systems, U.S.A.). The influence of the different treatments on the heart rate was quantified by increasing the speed of recording from the normal of 0.05 cm/sec to 1 cm/sec for 5-10 seconds and then counting the rate per unit time. The changes in the heart rate were reported as percentage change from the pre-drug administration rate.

B.3 Spinal pithing of rats:

The connections between the brain medullary cardiovascular centers and the spinal preganglionic sympathetic fibres throughout the thoracolumbar outflow were severed by spinal pithing of the rats using a modification of the method described by Gillespie *et al*. (8). In brief, male Wistar rats (275 g) were anaesthetized with urethane (l.25 g kg^{-1}) (i.p.). The trachea was then cannulated and connected to a rodent ventilator pump (Scientific and Research Institute Ltd., U.K.) to initiate an artificial respiration at a tidal volume of 10 ml kg^{-1} body weight. A stainless steel rod (diameter 2 mm, and length 15 cm) was inserted through one eye orbit and directed downwards to enter the spinal cord and pushed further downwards to reach the third lumbar vertebrum. Thereafter, the rat was prepared for measurement of the arterial blood pressure. A submaximal dose of any tested substance was then administered (i.v.) and its effect on the arterial blood pressure and heart rate was quantified as described above. The mean effects obtained were then compared with the effects observed in non-pithed rats. After each experiment, the successfulness of each pithing process was checked by opening the abdomen and the thorax of the animal and tracing the position of the pithing rod within the lumen of the spinal cord. Only results from successfully pithed rats were included in the results.

B.4 Studies using receptor blockers and enzyme inhibitors:

To investigate the mechanism(s) of action of the de-thymoquinonated oil and its constituents on the cardiovascular parameters in rats, various drugs that are known to block specific receptors or to inhibit synthesis of various autacoids known to be involved in the normal regulation or modulation of the arterial blood pressure were used.

The doses used in this study for each of these drugs and the pretreatment times used were consistently shown in our laboratory and by others to effectively block or inhibit their respective agonists (1, 2, 9, 10).

B.5 Effect of test substances on adenosine diphosphate (ADP)-induced platelets aggregation:

Rat's platelet-rich plasma (PRP) was prepared for induction of ADP-induced platelet aggregation as described before (11). Rat blood was obtained using cardiac puncture and mixed with trisodium citrate 3.6% aqueous solution in a ratio of 1 : 9 (citrate : blood). The blood was centrifuged at 1000 rpm for 10 min and the PRP was then aspirated, pooled and used for induction of ADP-induced aggregation using an aggregometer (Chrono-Log, U.S.A.). The dose of ADP used to induce control aggregation was the smallest dose that produced full irreversible aggregation. This was found to be (30 μM final concentration in the cuvettes). PRP was divided into 1-ml aliquots and each sample was used once either for control or to test the effect of any substance under test. Any test substance was incubated with PRP for 2 min at 37°C before addition of ADP. The percentage change in the ADP-induced aggregation in the treated platelets was then calculated with reference to the control effect of ADP.

B.9 Solubilization of the test substances:

All test substasnces used in this study namely the de-thymoquinonated volatile oil, α-pinene and *p*-cymene were solubilized in platelets-poor plasma obtained from the same animal under test. For this purpose 1 ml of blood was collected in citrate solution from each animal under test via the carotid artery. The blood was centrifuged at 2500 rpm (MSE centrifuge) for 20 minutes. The platelet-poor plasma (PPP) was then aspirated. Test substances were solubilized in the PPP in a ratio of 1:4 (substance : PPP). All injections were made (i.v) using Hamilton glass microlitre syringes (50 and 100 μl). In all experiments performed the influence of the solvent (PPP) in the maximum dose injected was initially tested on the parameter under test. The

injected solvent did not influence any of the measured parameters to any significant effect.

B.7 Solubilization of the receptor blockers and enzyme inhibitors:

All the receptor blockers and enzyme inhibitors were dissolved in water. Hydrocortisone was dissolved in ethanol and diluted with water as required. Indomethacin was used as its sodium salt.

B.8 Statistical analysis:

Significant differences between the various treatments were calculated using Student's 't' test (for paired and non-paired samples as appropriate). All results were presented as mean ± s.e. mean with N = number of experiments performed.

B.9 Sources of Chemicals used:

ADP and thymoquinone (Sigma Chemical Co., U.S.A.); *p*-cymene (Riedel De Haen, Germany); α-pinene (Hopkins and Williams, U.K.); methylene blue (E. Merck, Germany). All other chemicals (BDH, U.K.).

Results

3.1 Major Components of Nigella sativa volatile oil:

Exposure of the Ethiopean *Nigella sativa* volatile oil to TLC as described in the methods section revealed the presence of α-pinene (R_f = 0.39), thymoquinone (R_f = 0.52) and P-cymene (R_f = 0.7). Other three spots with R_f values 0.788, 0.83 and 0.97 were also observed but not identified.

2 Effectiveness of de-thymoquinonation

Dilution of *Nigella sativa* volatile oil with n-hexane and storage at -69°C overnight completely precipitated thymoquinone. Indeed, the oil obtained after filtration and evaporation of n-hexane under vacuum revealed complete absence of thymoquinone as tested by TLC. Furthermore, the precipitated crystals also proved to be thymoquinone as checked by TLC and authentic thymoquinone. Thus the obtained oil can be labeled as dethymoquinonated oil (DE-TQ.V.O.).

3.3 Basal values of the mean systemic arterial blood pressure and heart rate of the urethane anaesthetized rats:

Using a mercury mannometer (B. Braun, Melsungen, type 864665) for the determination of the basal mean arterial blood pressure of the anaesthetized rats revealed that the mean value was 113.5 ± 3.7 mm Hg. The mean heart rate of the same animals was 291 ± 20 beats min^{-1}.

4. Effect of DE TQ.V.O., α-pinene and p-cymene on the arterial blood pressure and heart rate of urethane anaesthetized rats.

Intravenous administration of DE TQ.V.O. in the dose range of (2-16 μl kg^{-1}), α-pinene (1-4 μl kg^{-1}) or *p*-cymene (2-16 μl kg^{-1}) into the anaesthetized rats decreased the arterial blood pressure and the heart rate in a dose-dependent manner. The cumulative findings from 5-8 separate experiments are shown in Tables 1, 2 and 3). The effects of α-pinene (2 & 4 μl kg^{-1}) , (DE-TQ.V.O.) and *p*-cymene of doses of (4, 8 and 16 μl kg^{-1}) were significant ($P < 0.05$, N = 5-6). Most of the decreases in the arterial blood pressure were significant ($P < 0.05$, N = 5-6).

Table 1. Effect of de-thymoquinonated volatile oil (DeTQ) on the arterial blood pressure and the heart rate of urethane-anaesthetized rats.

DeTQ (μl/kg)	Decrease in arterial blood pressure (mmHg) mean ± s.e.mean	%Decrease in heart rate mean ± s.e.m.
2	10.3 ± 4.0	7.8 ± 5.0
4	26.4 ± 0.7*	31.7 ± 8.0*
8	33.5 ± 8.3**	34.1 ± 9.8*
16	39.5 ± 9.0***	53.6 ± 3.0***

***P < 0.005, **P < 0.01 & *P < 0.05 compared with pretreatment level. N = 5-6 (t-test).

Table 2. Effect of α-pinene on the arterial blood pressure and the heart rate of urethane-anaesthetized rats.

α-pinene (μl/kg)	Decrease in arterial blood pressure (mmHg) mean ± s.e.mean	%Decrease in heart rate mean ± s.e.mean
1	16.9 ± 4.4**	0.00 ± 3.4
2	21.4 ± 8.0*	30.6 ± 7.8*
4	23.5 ± 7.8*	33.8 ± 7.4*

**P < 0.005 & *P < 0.05 compared with pretreatment level. N = 5-8 (t-test).

Table 3. Effect of *p*-cymene on the arterial blood pressure and the heart rate of urethane-anaesthetized rats.

p-cymene (µl/kg)	Decrease in arterial blood pressure (mmHg) mean ± s.e.mean	%Decrease in heart rate mean ± s.e.mean
2	17.0 ± 6.6	5.3 ± 2.1
4	26.9 ± 7.9*	16.1 ± 3.1**
8	32.7 ± 4.3**	25.3 ± 10.7*
16	42.7 ± 7.5**	34.4 ± 6.3**
32	29.1 ± 4.7	33.7 ± 11.7**

***P < 0.005,**P < 0.05 compared with pretreatment level. N = 4-5 (t-test).

3.5 Effect of Receptor blockers and enzyme inhibitors:

Intravenous treatment of rats with indomethacin (10 mg kg^{-1} for 30 min), mepacrine (10 mg kg^{-1} for 30 min), hydrocortisone (20 mg kg^{-1} for 60 min), methylene blue (20 mg kg^{-1} for 20 min), mepyramine (10 mg kg^{-1} for 5 min) and/or ranitidine (10 mg kg^{-1} for 5 min) did not block any of the cardiovascular changes observed following intravenous administration of (DE T.Q. V.O.), α-pinene or *p*-cymene.

However, treatment of rats with hexamethonium (10 mg kg^{-1} for 5 min) significantly blocked all of the cardiovascular changes observed following (i.v.) administration of submaximal doses of DE. T.Q. V.O., α-pinene and *p*-cymene (P< 0.05, N = 4 each). The cumulative findings are shown in Table 4.

Table 4: Effect of hexamethonium (10 mg/kg) on *p*-cymene, α-pinene and de-thymoquinonated *Nigella sativa* volatile oil (DeTQ)-induced cardiovascular depressant effects in rats.

Treatment (µl/kg)	% Effectiveness in antagonizing the decrease in	
	Blood Pressure	Heart Rate
DeTQ(8)	61.7 ± 10.5*	81.5 ± 9.2*
α-pinene (4)	79.2 ± 10.0*	93.1 ± 6.9*
p-cymene (8)	86.1 ± 8.3*	81.2 ± 1.3*

*P < 0.05 compared with control effect before blocker (t-test). (N = 4).

Treatment of animals with atropine or cyproheptadine (1 mg kg^{-1} for 5 min) did not affect DE. T.Q. V.O., α-pinene or *p*-cymene-induced hypotension but induced significant antagonisms in the induced bradycardias. For instance atropine antagonized the induced bradycardias by 100% in all cases (P < 0.01, N = 4 each). Whereas cyproheptadine antagonized the induced bradycardias by 78.1 ± 2.3, 90.8 ± 9.3 and 49.9 ± 7.1%, respectively for the above three substances(P < 0.05, N = 4).

3.6 Effect of spinal pithing:

Destruction of the connection between the medullary cardiovascular centres and the pre-ganglionic sympathetic fibres in the spinal cord resulted in sharp falls in both the arterial blood pressure and the heart rate and completely prevented DE. T.Q. V.O., α-pinene or *p*-cymene-induced cardiovascular changes (N = 3 each).

3.7 Effect on ADP-induced platelet aggregation:

Exposure of rat platelets to ADP in concentrations of 2.5 – 30 µM (final concentration) induced dose-dependent aggregations. The minimum dose that produced irreversible aggregation was 30 µM. Treatment of rat platelets with DE. T.Q. V.O., *p*-cymene or α-pinene in doses of 5 – 20 µl ml^{-1} for 2 min at 37°C inhibited ADP-induced aggregation by 13 – 22 % but all inhibitions were insignificant (P > 0.05, N = 4 for each). The effect of the smaller doses were similar to those of the larger doses i.e., the observed effects were not dose dependent.

Discussion

The results of this study clearly demonstrated the ability of the de-thymoquinonated volatile oil (DETQ) of *Nigella sativa* and its two components α-pinene and *p*-cymene to decrease both the arterial blood pressure and heart rate. These effects are qualitatively similar to those of the whole non-de-thymoquinonated volatile oil observed previously (1). Volume-to-volume the three substances seemed to be equipotent with regard to their ability to decrease the arterial blood pressure. However, when the substances were tested at doses of 4 µl kg^{-1} both DE-TQ and α-pinene seemed to produce more bradycardia than *p*-cymene which was not statistically significant.

The cardiovascular depressant effects of the three test substances were not blocked by the

prostaglandin cyclooxygenase inhibitor inodomethacin, the phospholipase A_2 inhibitors mepacrine and hydrocortisone, the histamine H_1 receptor blocker mepyramine and the H_2 receptor blocker ranitidine. These findings suggest the disinvolvement of prostaglandins, leukotrienes, histamine and the platelet activating factor (PAF) in the observed cardiovascular depressant actions. Furthermore, the failure of methylene blue-which is known for its antagonism to nitric oxide-induced actions – suggests the disinvolvement of vascular [NO] in the induced actions (1). However, the significant blockade of the induced effects by the ganglionic blocker hexamethonium and spinal pithing coupled with the successful antagonism of the induced bradycardia by the muscarinic receptor blocker-atropine and the tripple muscarininc/5-hydroxytryptaminergic/histaminergic blocker-cyproheptadine point to the involvement of central cardiovascular regulatory mechanisms in the observed actions. Such mechanisms are known to involve the nucleus tractus solitarius (NTS), the vasomotor centre (VMC) and the vagal nuclei (the nucleus ambiguous and the dorsal vagal motor nucleus) (12, 13, 14). The most likely central mechanism seemed to involve inhibition of VMC with the consequent decrease in the sympathetic outflow reaching the peripheral blood vessels and the heart resulting in decreases in both the arterial blood pressure and the heart rate. It should be noted that absence of the test substances induced bradycardia following spinal pithing excluded the involvement of the central vagal nuclei and the peripheral cardiac parathympathetic ganglia and neurones in the observed bradycardia.

Addition of ADP to rat platelets induced dose-dependent aggregation. This effect is believed to result from interaction of ADP with its specific platelet membrane receptors such as the G_q protein coupled-receptor (P_2Y_1) and the G_i protein coupled-receptor ($P_2\ Y_{cyc}$) (15, 16). Activation of these receptors induces depolarization of the platelets membranes with the consequent opening of the membrane Ca^{2+} channels and the concomitant influx of extracellular Ca^{2+} and release of intracellular Ca^{2+} (17). Since the antiaggregatory effect of DE-TQ, α-pinene and p-cymene was non-dose dependent the observed inhibitions did not seem to result from interaction with ADP specific receptors. It is more likely that the observed effects were due to non-specific interactions of the substances with the platelets membranes' phospholipids.

In conclusion, the results of this study revealed that deprivation of the whole volatile oil of *Nigella sativa* of its contained thymoquinone did not abolish the cardiovascular depressant actions of the oil. This seemed to be due to the ability of its other two major components α-pinene and *p*-cymene to possess inherent cardiovascular depressant effects. Thus, the results of this study point to the potential inherent hypotensive effect and bradycardiac action of the DE TQ without any fear from thymoquinone - induced bronchocontriction (3) and gastric ulceration (7) that are liable to accompany the use of the whole volatile oil for the same purpose.

References

1. El Tahir KEH, Ashour MM and Al-Harbi MM. The cardiovascular actions of the volatile oil of the black seed (*Nigella sativa)* in rats: Elucidation of the mechanism of action. Gen Pharmacol 1993; 24: 1123-1131.
2. El Tahir KEH and Ageel AM. Effect of the volatile oil of *Nigella sativa* on the arterial blood pressure and heart rate of the guinea-pig. Saudi Pharm J 1994; 2: 163-168.
3. El Tahir KEH, Ashour MM and Al Harbi MM. The respiratory effects of the volatile oil of the black seed (*Nigella sativa)* in guinea-pigs: Elucidation of the mechanism of action. Gen Pharmacol 1993; 24: 1115-1122.
4. Canonica L, Jommi G, Scolastico C and Vonati A. The pharmacologically active principle in *Nigella sativa.* Gazz Chim. Ital 1963; 93: 1404-1407.
5. Aboutabl EA, El-Azzouny AA and Hammerschmidt FJ. Aroma volatile oil of *Nigella sativa* seeds. Prog Essent Oil Res 1986; 16: 49-55.
6 Houghton PJ, Zaka R, Hera PD and Hoult JRS. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Medica 1995; 61: 33-36.
7. Marozzi FJ Jr. Kocialski AB and Malane MH. Studies on the antihistaminic effects of thymoquinone and quercetin. Drug Res 1970; 10: 1574-1577.
8. Gillespie JS, McLaren A and Pollonk D. A method of stimulating different segments of the autonomic outflow from the spinal column to various organs in pithed cat and rat. Br J Pharmac 1970; 40: 257-267.
9. El Tahir KEH, El-Naser MAS, Ageel AM, El-Obaid HA and Al-Rashood KA. Cardiovascular depressant effects of N-methyl and N-isobutyl-1,2-diphenyl ethanolamines: Elucidation of the mechanism of action. Archiv Int Pharmacodyn Ther 1991; 309: 88-102.
10. Buchan P and Adcock JJ. Capsaicin-induced bronchoconstriction in the guinea-pig: contribution of vagal cholinergic reflexes, local axon reflexes and their modulation by BW44 3C81. Br J Pharmac 1992; 105: 448-452.
11. El Tahir KEH. Influence of niridazole and chloroquine on

arterial prostacyclin synthesis. Br J Pharmac 1987; 92: 567-576.
12. Spyer KM. Central nervous integration of Cardiovascular control. J Exp Biol 1982; 100: 109-128.
13. Barman SM and Gebber GL. Axonal projection patterns of ventrolateral medulla-spinal sympathoexcitatory neurons. Neurophysiology 1985; 53: 1551-1566.
14. Brown DL and Guynet PG. Cardiovascular neurons of brain stem with projections to spinal cord. Am J Physiol 1984; 247: R 1009-R1016.
15. Hechler B, Lean C, Vial C, Vigne P, Frelin C, CazenaveJP and Gachet C. The P_2 Y receptor is necessary for ADP-induced platelet aggregation Blood 1998; 92: 152-159.
16. Geiger J, Honing-Lie P, Schanzenbacher P and Walter U. Ligand specificity and ticlopidine effects distinguish 3 human platelet ADP receptors. Eur J Pharmacol 1998; 351: 235-246.
17. Hourani SM and Hall DA. Receptors for ADP on human blood platelets. Trends Pharmacol Sci 1994; 15: 103-108.