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Effects of aqueous and macerated extracts from *Nigella sativa* on guinea pig isolated heart activity

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Received October 8, 2004, accepted February 17, 2005

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Pharmazie 60: 943–948 (2005)

Several therapeutic effects including those on digestive and gynaecological disorders, against asthma and dyspnea have been described for the seeds of *Nigella sativa*. In the present study, the effects of aqueous and macerated extracts from *Nigella sativa* on heart rate and contractility of the isolated heart were examined. Isolated guinea-pig hearts were perfused through aorta in the Langendorff mode. Heart rate (HR) and contractility were determined on the presence of four concentrations of aqueous and macerated extract from *Nigella sativa* (0.5, 1.0, 2.0 and 5.0 mg%) and diltiazem, a calcium channel blocker (0.1, 1, 10 and 100 μM) in comparison with baseline values in two different groups of experiments as follows: 1) Perfused heart with ordinary Krebs solution (group 1 experiments, $n = 9$). 2) Perfused heart with calcium free Krebs solution (group 2 experiments, $n = 8$). In group 1 three higher concentrations of diltiazem (1, 10 and 100 μM) and both extracts (1.0, 2.0 and 5.0 mg%) showed a significant reduction in heart rate ($P < 0.001$). However, only two larger concentrations of diltiazem (10 and 100 μM) and macerated extract (2.0 and 5.0 mg%) and three concentrations of the aqueous extract (1.0, 2.0 and 5.0 mg%) caused a significant reduction in heart contractility in this group ($P < 0.001$). In group 2 only 100 μM diltiazem, caused significant reduction in heart contractility. However, two concentrations of macerated extract (2.0 and 5.0 mg%) and three higher concentrations of aqueous extract (1.0, 2.0 and 5.0 mg%) caused significant reductions in heart rate and contractility in this group ($p < 0.05$ to $p < 0.001$). There were significant negative correlations between concentrations of both extracts and diltiazem and their effect on heart rate and contractility in both groups ($p < 0.01$ to $p < 0.001$). These results showed a potent inhibitory effect of both extracts from *Nigella sativa* on both heart rate and contractility of guinea pig heart that was comparable and even higher than that of diltazem. The results of the present study may be due to calcium channel inhibitory or an opening effect for the plant on potassium channels of the isolated heart.

1. Introduction

Nigella sativa L. (Ranunculaceae) is a grassy plant with green to blue flowers and small black seeds, which grows in temperate and cold climate areas. The seeds of *Nigella sativa* contain thymoquinone, monotropens such as *p*-cymene and α-pinene (El-Dakhakhny 1963) Nigellidine (Atta and Malik 1995), Nigellimine (Atta and Malik 1985) and a saponin (Ansari and Sadiy 1989).

Several therapeutic effects have been described for the seeds of *Nigella sativa* in ancient Iranian medical books (Avesina 1990). *Nigella sativa* has long been known for its medical use as an antispasmodic, especially against gastrointestinal disorders or respiratory ailments, in many countries.

There is evidence of relaxant effects of the volatile oil from this plant on different smooth muscles including rabbit aorta (Aqel 1992a), rabbit jejunum (Aqel 1993), and isolated tracheal muscles of guinea pigs (Reiter and Brandt 1985). Mahfous and El-Dakhakhny (1960) reported that the volatile oil from *Nigella sativa* protected guinea pigs against histamine-induced bronchospasm but it did not affect histamine H_1 receptors in isolated tissues (Mahfouz and El-Dakhakhny 1960). However, in an *in vivo* study, increasing respiratory rate and intratracheal pressure of guinea pigs due to i.v. administration of volatile oil from *Nigella sativa* has been demonstrated (El-Tahir et al. 1993). In our recent studies, a relaxant effect on guinea tracheal chain for this plant was shown (Boskabady and Shahabi 1997). In addition the anticholinergic and histamine H_1 receptor blocking effects of this plant on isolated guinea pig tracheal chains were also demonstrated (Boskabady and Shahabi 1997, Boskabady and Shiravi 2000). In addition an inhibitory effect of this plant on calcium channels of guinea pig tracheal chains was also shown in another study (Boskabady and Shirmohammadi 2002). In the present study the inhibitory effects of aqueous and macerated extracts from this plant on heart rate and contractility of isolated guinea pig hearts were examined.

Table 1: Effect of four different concentrations of extracts from *Nigella sativa* and diltiazem on heart rate of isolated of guinea pig's hearts in two groups of experiments

Experimental design		Group 1	St.Dif vs. B.	Group 2	St.Dif vs. B.	St.Dif vs. G.1
Baseline		272.33 ± 12.16		150.85 ± 10.30		$p < 0.001$
	0.1 μM	245.33 ± 14.81	NS	145.71 ± 10.72	NS	$p < 0.001$
Diltiazem	1.0 μM	202.22 ± 16.46	$p < 0.01$	140.00 ± 13.18	NS	NS
	10 μM	143.77 ± 11.41	$p < 0.001$	122.85 ± 10.30	NS	NS
	100 μM	68.22 ± 5.62	$p < 0.001$	110.85 ± 9.94	NS	NS
Baseline		269.75 ± 12.25		158.66 ± 7.96		$p < 0.001$
	0.5 mg%	237.55 ± 16.1	NS	139.42 ± 11.40	NS	$p < 0.001$
Aqueous	1.0 mg%	192.66 ± 16.30	$p < 0.01$	113.14 ± 14.13	$p < 0.05$	$p < 0.01$
extract	2.0 mg%	119.55 ± 20.19	$p < 0.001$	80.00 ± 16.58	$p < 0.01$	NS
	5.0 mg%	37.33 ± 8.33	$p < 0.001$	41.14 ± 11.00	$p < 0.001$	NS
Baseline		238.66 ± 13.11		121.55 ± 9.29		$p < 0.001$
	0.5mg%	215.00 ± 14.17	NS	108.44 ± 10.79	NS	$p < 0.001$
Macerate	1.0mg%	198.00 ± 12.37	$p < 0.05$	94.88 ± 10.58	NS	$p < 0.001$
extract	2.0 mg%	179.33 ± 12.72	$p < 0.01$	75.77 ± 12.71	$p < 0.05$	$p < 0.001$
	5.0 mg%	143.66 ± 12.13	$p < 0.001$	47.33 ± 13.86	$p < 0.001$	$p < 0.001$

Values are presented as mean ± SEM. Group 1 (G.1): experiments on isolated guinea pig heart in the presence of ordinary Krebs solution (n = 9). Group 2: experiments on isolated guinea pig heart in the presence of calcium free Krebs solution (n = 8). St. Dif: Statistical difference, NS: non significant difference, B: baseline

2. Investigations, results and discussion

The present study investigated the effects of aqueous and macerated extracts from *Nigella sativa* on heart rate and heart contractility in the presence of ordinary and calcium free Krebs solution.

In group 1 experiments three concentrations of diltiazem (1, 10 and 100 μM) and both extracts (1.0, 2.0 and 5.0 mg%) significantly reduced heart rate of guinea pigs compared to baseline values ($p < 0.05$ to $p < 0.001$, Table 1). In group 2 experiments, only two concentration of macerated extract (2.0 and 5.0 mg%) and three higher concentration of aqueous extract (1.0, 2.0 and 5.0 mg%) significantly reduced heart rate of guinea pigs compared to baseline values ($p < 0.05$ to $p < 0.001$, Table 1).

In group 1 experiments, two concentrations of diltiazem (10 and 100 μM) and macerated extract (2.0 and 5.0 mg%) and three concentrations of aqueous extract (1.0, 2.0 and 5.0 mg%) significantly reduced heart contractility of guinea pigs compared to baseline values ($p < 0.05$ to $p < 0.001$, Table 2). In group 2 experiments only one concentration of diltiazem (100 μM), two higher concentrations of macerated extract (2.0 and 5.0 mg%) and three last concentrations of aqueous extracts (1.0, 2.0 and 5.0 mg%) significantly reduced heart contractility of guinea pigs compared to baseline values ($p < 0.05$ to $p < 0.001$, Table 2).

In group 1 there was no significant difference between the effect of diltiazem and aqueous extract on both heart rate and contractility. However, the effects of macerated extract (2.0 and 5.0 mg%) on both heart rate and contractility were significantly lower than those of diltiazem and aqueous extract ($p < 0.01$ to $p < 0.001$, Fig. a and c).

In group 2 the effects of both extracts on heart rate were significantly greater than that of diltiazem ($p < 0.05$ to $p < 0.001$, Fig. b). However, the effect of aqueous extract (5.0 mg%) on heart contractility was significantly greater than those of both diltiazem (100 μM) and macerated extract ($p < 0.05$ to $p < 0.001$, Fig. d).

The baseline heart rate prior to addition of diltiazem and both extracts in group 2 were significantly lower than those of group 1 ($p < 0.001$ for all cases, Table 1). In

Table 2: Effect of four different concentrations of extracts from *Nigella sativa* and diltiazem on contractility of isolated of guinea pig's hearts in two groups of experiments

Experimental design		Group 1	St.Dif vs. B.	Group 2	St.Dif vs. B.	St.Dif vs. G.1
Baseline		2.30 ± 0.18		1.18 ± 0.07		$p < 0.001$
	0.1 μM	2.06 ± 0.23	NS	1.11 ± 0.08	NS	$p < 0.01$
Diltiazem	1.0 μM	1.84 ± 0.22	NS	0.97 ± 0.091	NS	$p < 0.05$
	10 μM	0.95 ± 0.19	$p < 0.01$	0.91 ± 0.07	NS	NS
	100 μM	0.44 ± 0.14	$p < 0.001$	0.77 ± 0.068	$p < 0.05$	NS
Baseline		2.37 ± 0.20		1.21 ± 0.09		$p < 0.001$
	0.5 mg%	2.02 ± 0.21	NS	1.02 ± 0.10	NS	$p < 0.01$
Aqueous	1.0 mg%	1.62 ± 0.17	$p < 0.05$	0.80 ± 0.07	$p < 0.05$	$p < 0.01$
extract	2.0 mg%	1.31 ± 0.18	$p < 0.01$	0.65 ± 0.09	$p < 0.01$	NS
	5.0 mg%	0.28 ± 0.08	$p < 0.001$	0.34 ± 0.10	$p < 0.001$	NS
Baseline		2.21 ± 0.33		1.48 ± 0.11		$p < 0.001$
	0.5 mg%	2.08 ± 0.31	NS	1.42 ± 0.12	NS	NS
Macerate	1.0 mg%	1.85 ± 0.27	NS	1.2 ± 0.11	NS	NS
extract	2.0 mg%	1.60 ± 0.26	$p < 0.05$	1.00 ± 0.08	$p < 0.05$	NS
	5.0 mg%	1.13 ± 0.24	$p < 0.01$	0.80 ± 0.13	$p < 0.01$	NS

For abbreviations see Table 1

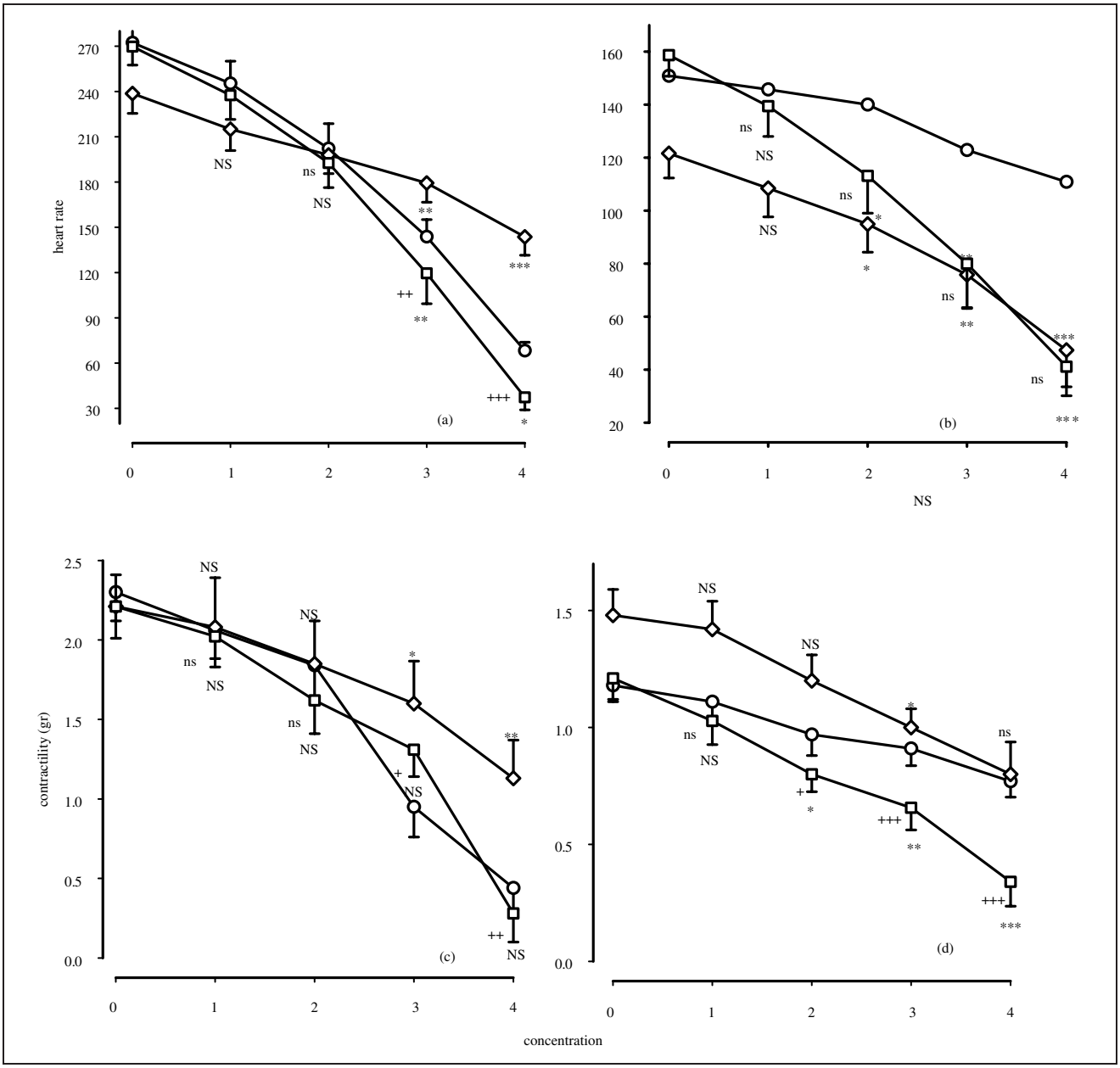

Fig.: Concentration response curves of aqueous (□) and macerated (◇) extracts from *Nigella sativa*, and diltiazem (○), on heart rate and contractility of isolated guinea pigs in group 1 (in the presence of ordinary Krebs solution, n = 9), (a and b) and group 2 experiments (in the presence of calcium free Krebs solution, n = 8), (c and d). Tested concentrations for extract were 0.5, 1, 2 and 5 mg% and for diltiazem 0.1, 1, 10 and 100 μM. Statistical differences between the effect of extracts with that of diltiazem; NS: non-significant difference, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical differences between the effect of two extracts; ns: non-significant difference, + $p < 0.05$; ++ $p < 0.01$; +++ $p < 0.001$

addition the heart rate obtained in the presence of only the lowest concentration of diltiazem (0.1 μM), two lower concentrations of the aqueous extract (0.5 and 1.0 mg%) and all concentrations of the macerated extract were significantly lower than those of group 1 ($p < 0.01$ to $p < 0.001$, Table 1). Baseline heart contractility obtained prior to addition of diltiazem and both extracts in group 2 were also significantly lower than those of group 1 ($p < 0.01$ for all cases, Table 2). The heart contractility in the presence of two lower concentrations of diltiazem (0.1 and 1.0 μM) and aqueous extract (0.5 and 1.0 mg%) in group 2 were also significantly lower than those of group 1 ($p < 0.05$ to 0.01, Table 2).

There were significant negative correlations between both heart rate and heart contractility with concentration of diltiazem and both extracts ($p < 0.01$ to $p < 0.001$, Table 3).

The results of the present study show a concentration dependent decrease in both heart rate and heart contractility due to aqueous and macerated extracts from *Nigella sativa* in the presence of ordinary Krebs solution (group 1 experiments). The results of group 1 indicated a potent and concentration dependent inhibitory effect of both extracts from *Nigella sativa* on heart rate and contractility. The effect of both extracts, especially those of aqueous extract on heart rate and contractility, were very similar to those of diltiazem. These similarities may suggest an inhibitory effect of the extracts from *Nigella sativa* on calcium channels of the isolated heart.

To explore the effect of extracts of this plant on calcium channels more precisely, their effects on heart rate and contractility of isolated heart were re-examined in the presence of calcium free Krebs solution in group 2 experi-

Table 3: Correlations between the effects of extracts from *Nigella sativa* on heart rate and contractility of isolated guinea pig heart with concentrations in two groups of experiments

Experimental Groups		Aqueous extract		Macerated extract		Diltiazem	
		r	p value	r	p value	r	p value
Group 1	HR	−0.829	$p < 0.001$	−0.557	$p < 0.001$	−0.703	$p < 0.001$
	Cont	−0.478	$p < 0.001$	−0.352	$p < 0.001$	−0.365	$p < 0.001$
Group 2	HR	−0.738	$p < 0.001$	−0.545	$p < 0.001$	−0.236	$p < 0.01$
	Cont	−0.555	$p < 0.001$	−0.314	$p < 0.001$	−0.362	$p < 0.001$

ments. In this group of experiments, the effects of two extracts on heart contractility were similar to those on heart rate in group 1; but higher concentrations of diltiazem also significantly inhibited heart contractility.

The effect of macerated extract in group 2 seems to be more similar to that of diltiazem because it has less inhibitory effect on both heart rate and contractility. However, higher concentrations of aqueous extract in group 1 reduced both heart rate and contractility to the same level as those of group 2 suggesting a complete inhibition of the calcium channel. Although the higher concentrations of macerated extract in group 2 also reduced heart contractility to a level similar to group 1, even the highest concentrations of this extract on group 1 did not reduce heart rate to the level of group 2. These findings suggest that extracts of *Nigella sativa* and especially the aqueous extract almost completely inhibited calcium channels of isolated guinea pig heart. The significant negative correlation between concentrations of different solutions and their effect on heart rate and heart contractility strongly supported concentration dependent effects of both extracts and diltiazem.

There are three groups of calcium channel blocker drugs including 1) dihidropyridins such as nifidipine and nicardipine which act mainly on smooth muscle, 2) fenylalkylamins such as verapamil which mainly act on heart muscle, 3) benzothiazepins such as diltiazem that act on smooth muscles (Hurwitz et al. 1991). The results of the present study may indicate that the effect of *Nigella sativa* extracts is more similar to the fenylalkylamin group. In fact the results of the present study i.e. the depressant effect of diltiazem on heart rate is supported by a study by Gerhard et al. (1995). The diltiazem concentrate used on the present study was also very similar to that of the earlier study (Gerhard et al. 1995), however the minute concentrations of extracts from *Nigella sativa* caused an effect similar to that of the concentration of diltiazem used in the present study. These findings may indicate that the extracts of *Nigella sativa* have a very potent inhibitory effect on calcium channels and/or an opening effect on potassium channels of the isolated guinea pig heart.

A comparison of the effect of the two extracts and diltiazem also showed that the effect of aqueous extract in group 1 on both heart rate and contractility were very similar to those of diltiazem. However, the effects of the two higher concentrations of macerated extracts in group 1 on both heart rate and contractility were significantly less than those of aqueous extract and diltiazem. These findings suggest that the aqueous extract inhibited calcium channels of the isolated heart in a very similar manner as diltiazem. In group 2, the effects of both extracts on heart rate were similar but more pronounced than that of diltiazem. However, the effect of last concentrations of only aqueous extract on contractility in group 2 was higher than that of diltiazem. The slopes of concentration response curves in the presence of extract were different from those obtained in the presence of diltiazem for both heart rate and contractility. The greater effect of both extracts from *Nigella sativa* on heart rate in group 2 and differences in the slope of concentration response curves between extracts and diltiazem may indicate an additional effect for extracts and especially aqueous extracts other than calcium channel inhibitory effect. This additional effect could be a potassium channel opening effect for extracts because a positive inotropic effect of potassium channel blocker has been demonstrated (Kocis et al. 2003). In fact, in our previous study a potassium channel opening effect for extracts of *Nigella sativa* has been suggested (Boskabady et al. 2004). Although there is no report regarding the active constituents of this plant on calcium channels different studies showed a calcium channel blocking effect for different extracts of the plant on smooth muscle (Aqel 1992b; Gilani et al. 2001; Boskabady and Shirmohammadi 2002). In addition it was observed that one of the main constituents of the plant, thymoquinon, has a calcium channel inhibitory effect.

The other mechanisms such as cholinergic stimulatory and/or adrenergic inhibitory effects of the plant could be responsible for the observed effect of the aqueous extract on the isolated heart. Therefore, the most probable mechanisms of action of *Nigella sativa* is its inhibitory effect on calcium channels of the guinea pig heart. However, our previous studies showed anticholinergic (Boskabady and Shahabi 1997) and β-adrenergic stimulatory (Boskabady et al. 2003) effects. Therefore, the exact mechanism of this plant on heart rate and contractility should be clarified in further studies.

The difference between the effects of two extracts may be due to a variation in extract composition due to the method of extraction. Although both aqueous and macerated extracts are aqueous in nature, the methods of extraction for the two extracts are different. For preparing aqueous extracts the seeds should be exposed to water steam (100 °C) for 18–24 h. But for macerated extract it should be macerated with water at room temperature for 48 h while shaking intermittently. Therefore the ingredients of the two extracts may be different. In aqueous extract more substances may be extracted, but some ingredients are destroyed due to the high temperature. However, the effect of the two extracts was not statistically different.

In conclusion, the results of the present study show inhibitory effects of macerated and aqueous extracts from *Nigella sativa* on rate and contractility of the isolated guinea pig heart and suggests a possible potent inhibitory effect of the plant on calcium channels of isolated guinea pig heart. However, the mechanisms of action of extract and especially those of aqueous extract may partially differ from that of diltiazem.

3. Experimental

3.1. Plant and extracts

Nigella sativa was collected from Torbat Heydarieh (north-east Iran) in the spring of 2002, and dried at room temperature in the absence of sunlight. Botanists in the herbarium of Ferdowsi University of Mashhad identified the plant; and the specimen number of the plant is 293-0303-1.

The aqueous extract was prepared as follows: Fifty grams of the chopped and dried plant was extracted with 300 ml of distilled water by a Soxhlet apparatus. Distilled water (300 ml) was added to a glass balloon in the lower part of suxhelat apparatus which was placed over a heater. The heater was set to boil distilled water continuously. The water steam flew through the middle part of the apparatus containing plant powder. The steam was then cooled and converted to liquid as it passed through a tube in the upper portion of the Soxhlet with a helix tube inside through which tap water flowed by means of a side tube, then added to the balloon in the lower part. This procedure was continued for 18–24 h until the liquid water returning to the balloon through the side tube became colourless. For the macerated extract, the same amount of plant was macerated with 300 ml distilled water (on a shaker) for 48 h. The solvent of both extracts was then removed under reduced pressure until the extract volumes reached 20 ml. Plant ingredient concentration in the final extracts was 10 g% in both extracts. The amount of thymoquinone, thymohydroquinone and thymol in the essential oil of *Nigella sativa* is 0.53, 0.77 and 0.91% respectively (Ghoshe et al. 1999). However, there is no information regarding the amount of main constituents in aqueous and macerated extracts of this plant but their amount in extracts seems to be lower than in the essential oil.

3.2. Tissue preparation

Dunkin Hartley guinea pigs of either sex, with a body weight of 400–500 g, were provided by the Razi Institute, Mashhad, Iran. Guinea pigs were killed by a blow on the neck, the hearts were rapidly isolated, and were washed in ice-cold saline. The coronary circulation was perfused through aorta on a modified Langendorff apparatus at a constant perfusion pressure of 70 mm Hg (Chopicki et al. 2002). The hearts were perfused with K-H buffer solution (37 °C, pH 7.4, saturated with 95% O_2 and 5% CO_2). The K-H buffer solution contained the following (in mmol/L): NaCl 118, $NaHCO_3$ 25.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, and glucose 11.0.

All the hearts were first perfused with K-H solution for 20–30 min for stabilization in a Langendorff apparatus and then the effects of extracts from *Nigella sativa* and diltiazem were studied.

3.3. Protocols

The effects of four different concentrations of aqueous and macerated extracts from *Nigella sativa* (0.5, 1.0, 2.0 and 5.0 mg% from each extract) and diltiazem (0.1, 1.0, 10 and 100 μM) on heart rate and contractility were examined and compared to baseline values. For studying the influences of different concentrations of each solution, each concentration of the solutions was given a one-minute intracoronary infusion and the heart rate and contractility was recorded in last 30 s. During the experiments each heart served as its own control before injection of each solution. In the absence of pharmacological interventions, both heart rate and contractility were reproducible (Chopicki et al. 2003). The effects of different solutions were tested with two different experimental designs as follows:

1. Perfused heart with ordinary Krebs solution (group 1 experiments, $n = 9$).
2. Perfused heart with calcium free Krebs solution (group 2 experiments, $n = 8$).

The effects of extracts and diltiazem in two groups of experiments were examined in two different series of animal hearts. The effects of two extracts and diltiazem in each heart were performed randomly with a 30-min resting period of the heart between examining the effect of each two solutions while the heart was perfused with ordinary Krebs. In all experiments, the heart rate (HR) and contractility were recorded on a kymograph (ET8 G-Boulitt, Paris) and were measured after fixation. The local Animal Research Committee of Mashhad University of Medical Sciences approved the experimental procedures used in the present study.

3.4. Statistical analysis

The data of the heart rate and contractility were expressed as mean ± SEM. The data of heart rate and contractility obtained in the presence of different concentrations of extracts, diltiazem, and baseline were compared using ANOVA test in each group. The effect of each concentration of extracts and diltiazem between two groups was compared using unpaired t-test. The effect of two extracts and diltiazem were related to the concentrations of the solutions using least square regression. Significance was accepted at $p < 0.05$.

Acknowledgement: This study was financially supported by the Vice Presidency of Research of Mashhad University of Medical Sciences.

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