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EFFECTS OF *NIGELLA SATIVA* SUPPLEMENTATION FOR ONE MONTH ON CARDIAC RESERVE IN RATS

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Abstract : *Nigella sativa* (*N. sativa*) has a long history of use in folk medicine**.** In a current study performed in this laboratory, two-month dietary supplementation with *N. sativa* extract to normal rats has shown a homogenous cardiac hypertrophy and enhanced cardiac contractility at baseline conditions. In the present study, shorter (one-month) duration of oral *N. sativa* administration was adopted to detect possible earlier cardiac responses. In addition, *in vitro* cardiac stress by the beta adrenoceptor agonist isoproterenol was used to assess the intrinsic cardiac reserve mechanisms.

The hearts of *Nigella*-treated rats developed a moderate but significant hypertrophy that was evident by an increase in the heart weight to body weight ratio. The observed *Nigella*-induced cardiac hypertrophy was associated with an increase in the baseline cardiac inotropic properties as well as the maximal peak tension generation upon progressive cardiac stress by isoproterenol infusion. The demonstrated selective enhancement of the inotropic reserve favours the physiological nature of *Nigella*-induced cardiac hypertrophy, similar to that provoked by exercise training.

Key words : *Nigella sativa* cardiac adrenergic reserve cardiac trophic reserve cardiac inotropic reserve cardiac hypertrophy inotropy chronotropy

INTRODUCTION

The cardiac reserve concept describes the ability of the heart to adjust to demands placed upon it. Cardiac reserve mechanisms are numerous and include those that enhance cardiac output components; heart rate, stroke volume and contractility, as well as, those that modulate myocardial energetics and vascular capacity.

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The importance of the cardiac adrenergic reserve is evident as much of the cardiovascular response to stress is mediated by the sympathetic nervous system. Furthermore, adrenergic responsiveness has been shown by all the intrinsic properties of the cardiac muscle. In fact the cardiac adrenergic reserve constitutes an essential mechanism to increase the cardiac performance in most of the situations characterized by augmented circulatory demands (1). In addition, alteration in adrenergic receptor/signal pathways has been implicated in models of reduced cardiac function, such as hypertension (2, 3) coronary heart disease and heart failure (4), and obesity (5).

Likewise, the experimental changes in mechanical performance of the isolated hearts in response to progressively increasing doses of isoproterenol (ISO) infusion, the well known β-adrenergic stimulant, reflect the intrinsic ability of the hearts to enhance it performance to manage stressful conditions. Multiple functional data could be extruded from the study of the ISO dose-response curves. An increase in the maximal response and the calculated delta change from the pre-infusion level of a given cardiac trait, reflect an increase in cardiac reserve for this property. Moreover, shifts in the dose response curve, reflect changes in myocardial adrenergic responsiveness. While, an earlier response to lower ISO doses (left-shift) reflects increased adrenergic responsiveness, a maintained response to higher doses (right-shift) indicates better tolerance to adrenergic stress.

The black seed, *Nigella sativa* (*N. sativa*) **is** a member of the family of Ranunculaceae, and commonly grows in the Middle East, Western Asia and Europe. In the Arab countries, it is called “Al Habbah Al-Sawda”, or “Habbet- elbaraka” and in Asian countries it is known as “Klonji”. It has been used for ages for various ailments in folk medicine and a number of pharmaco-therapeutic effects of *N. sativa* and its active ingredient, thymoquinone, have been demonstrated (6).

In a current study, performed in this laboratory, long-term (2 months) dietary supplementation with *N. sativa* extract has shown a favorable advantage on some intrinsic properties of the heart. In addition to induction of a homogenous cardiac hypertrophy, the baseline mechanical activity of the isolated perfused hearts of these rats demonstrated an enhanced cardiac inotropy suggesting that the cardiac trophic response to *N. sativa* was a physiological type of hypertrophy (7). In the present study, one-month duration of *N. sativa* administration was adopted to investigate the possibility of earlier cardiac responses with shorter courses of *Nigella* supplementation. In addition, not only the baseline cardiac performance of the isolated hearts was assessed but also the chronotropic and inotropic reserve mechanisms in response to *in vitro* cardiac stress by the beta adrenoceptor agonist isoproterenol (ISO).

MATERIAL AND METHODS

Animal model

Thirty normal adult Wistar albino male rats were obtained from the animal house of the College of Medicine, King Faisal University. Their weights ranged from 150–250 gm. The animals were housed at

controlled room temperature of 22°C and allowed free access to water and laboratory chow. Body weight determination was done weekly for each rat, to adjust the oral dose of *N. sativa*. The study was performed according to the institutional rules of King Faisal University considering animal experiments.

Rats were divided equally into experimental and control rats. Nigella-treated rats received a daily oral dose of 800 mg/kg of *N. sativa*. This dose has been chosen because it corresponds to the submaximal dose of thymoquinone producing hypotensive effect in rats (8). The seeds (10 g) were grounded and the powder was added to distilled water (100 ml) at room temperature to prepare a crude suspension of 100 mg *N. sativa*/ml water, a few minutes before each feeding. The volume of the suspension needed to supply the required dose of *N. sativa* was given daily for the experimental group through an orogastric feeding. An equivalent volume of water was administered by an orogastric tube to the control rats.

Isolated perfused heart preparation

After one month of *N. sativa* oral supplementation each rat was weighed and injected intraperitoneally with 5000 IU heparin sodium. Rats were anaesthetized with intraperitoneal phenobarbital (40 mg/kg, body weight). Through a thoracic incision the heart was excised and mounted on Langendorff preparation. The heart was then perfused in a Langendorff preparation as described before (9). After initiating retrograde heart perfusion, the heart was allowed to stabilize for approximately 20 minutes. The perfusion fluid was a modified Krebs-Henseleit bicarbonate buffer of pH 7.4 equilibrated with O_2:CO_2 (95:5) at 37°C and containing (in mM): NaCl: 118, KCl: 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, Na_2EDTA 0.5, and Dextrose 11.

Isolated perfused heart preparation was allowed to stabilize for 15 minutes prior to recording the baseline values of heart rate (HR) developed peak tension (PT), maximum rate of tension development (dp/dt_{max}), time to peak tension (TPT), and half relaxation time (HRT). TPT was measured as the time from the onset of contractile response to the peak of developed tension and HRT was measured as the time required for tension to fall from its peak to 50% of the response.

Isoproterenol dose-response curves

Isoproterenol (ISO) was diluted, by the same buffer used to perfuse the heart, and infused just above the aortic cannula by means of a Minipuls 3 peristaltic pump (Gilson). The original dose of isoproterenol was 0.00005 mg/ml in the container of the pump. The pump speed was increased sequentially to reach the following concentrations in the preparation: (0.7, 1.4, 2.6, 4, 5.4) $\times 10^{-4}$ mg/ml. The total duration of infusion for each concentration was 3 minutes. Reported values were the average measurements taken during the final 20 seconds of each 3 minute period when the function had stabilized. Dose response relations were constructed by assessing the chronotropic and contractile responses to incremental concentrations of ISO.

Heart weights

The heart was cleaned from fat and fibrous tissue, and dried with filter paper before determining the weight of the whole heart and the left ventricle. Cardiac indices were calculated as ratio of whole heart weight/body weight (HW/BW), and ratio of left ventricular weight/whole heart weight (LV/HW).

Data analysis and statistics

For the statistical evaluation of the results, we used Statistical Package for Social Sciences (SPSS) version 10 for windows. All data were expressed as mean±SEM. Unpaired 't' tests were done to determine differences between experimental group and the control group. Paired sample 't' tests were used to determine the difference in respective preinfusion values and values at different doses for different variables for both the experimental and control groups. A P-value of <0.05 was considered statistically significant.

RESULTS

The baseline values, the maximal response to isoproterenol (ISO) and the delta change, difference between the maximal and the basal values, for heart rate (HR) showed no significant difference between the hearts of *N. sativa* treated rats and their matching controls (Table I). The ISO-dose response curves (Fig. 1) showed a significant decline in HR at higher ISO doses in the control group but the hearts of *N. sativa* treated rats maintained their HR even at the highest dose of ISO.

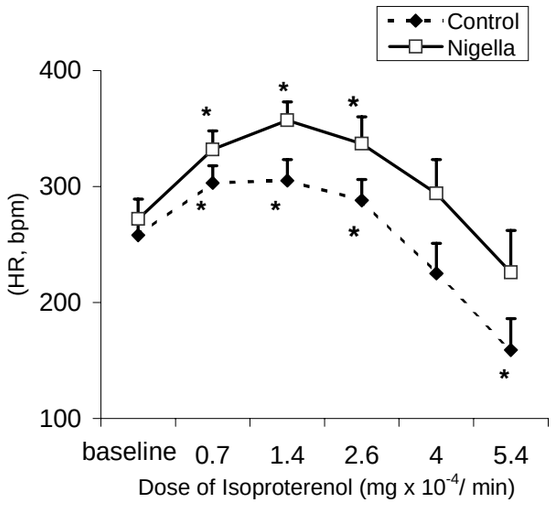

Fig. 1: **Dose response curve:** Effect of graded isoproterenol infusion on heart rate (HR) of hearts isolated from one month *Nigella*-treated rats (n=13)compared to their controls (n=13).

*P<0.05, compared to preinfusion value.

TABLE I: Effect of graded isoproterenol infusion on hearts isolated from one month *N. sativa* treated rats compared to their controls on Langendorff preparation.

	Baseline activity		*Maximum*		*Delta change*	
	Control	*N. sativa*	*Control*	*N. sativa*	*Control*	*N. sativa*
HR (bpm)	258±14	272±17	327±17	372±17	80±18	95±12
PT (gms)	0.46±0.07	1.4±0.21*	1.07±0.12	2.55±0.36*	0.61±0.13	1.16±0.19*
dp/dt_{max} (gm/sec)	9.15±1.05	31.4±5.4*	14.2±1.96	52.8±10.4*	5.27±1.62	23.7±8.12*
TPT (msec)	101±7	74±6*	79±7	59±6*	28±9	14±6
HRT (msec)	61±3	55±4	46±4	41±3	16±3	14±3

Heart rate (HR), peak tension (PT) developed, maximum rate of tension development (dp/dt_{max}), time to peak tension (TPT) and half relaxation time (HRT). Control rats: n=13, Nigella-treated rats: n=13. Data are presented as mean±SEM; *P<0.05.

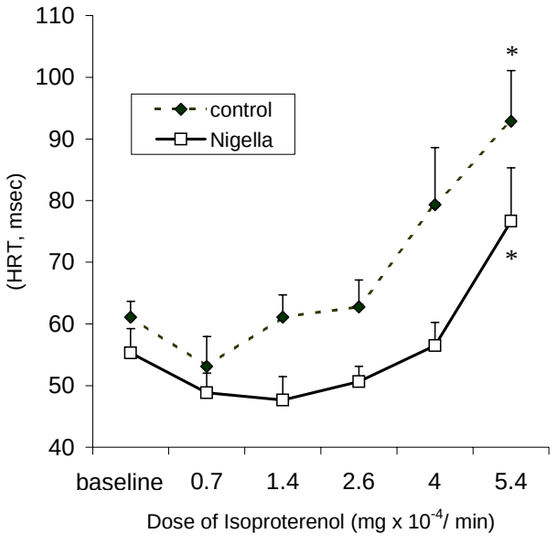

Fig. 5: **Dose response curve:** Effect of graded isoproterenol infusion on half relaxation time (HRT) of hearts isolated from one month *Nigella*-treated rats (n=13) compared to their controls (n=13).

*P<0.05, compared to preinfusion value.

TABLE II: Effect of *N. sativa* oral supplementation for one month on body weight (BW) and heart weight (HW), left ventricular weight (LV) and cardiac weight indices.

	Control (n=13)	*N. sativa (n=13)*
BW (gm)	250±6	253±5
HW (mg)	919±35	1077±44*
HW/BW (mg/gm)	3.7±0.15	4.26±0.16*
LV (mg)	667±23	756±26*
LV/HW (mg/mg)	0.73±0.02	0.71±0.02

Data are presented as mean±SEM, *P<0.05.

change in body weight of studied rats compared to their controls. The whole heart (HW) and the left ventricular (LV) weights as well as the HW/BW ratio were significantly increased in *N. sativa* treated rats. This increase in heart weight had involved all cardiac chambers as evident by the non-significant changes in LV/HW ratio.

DISCUSSION

The baseline contractile activity of the hearts isolated from *Nigella*-treated rats was significantly higher than their matched control rats. This was manifested by significant increases in the developed peak tension (PT) and the maximal rate of tension generation per unit time (dp/dt_{max}), which is a better index of myocardial contractility (10). When these hearts were perfused with progressively increasing doses of ISO, they showed significantly higher maximal inotropic response and reserve. The results of the dose response curves of the hearts of *Nigella*-treated rats showed a left shift indicating an enhanced PT and dp/dt_{max} responsiveness to lower ISO doses.

While the time to peak tension (TPT), an index for the duration of the cardiac contraction phase, was significantly enhanced in hearts of *Nigella*-treated rats, the half relaxation time (HRT), which indicates the duration of the cardiac relaxation phase, was not significantly changed. The selective enhanced inotropic effects of *N. sativa* administration without a parallel lusitropic effects could be explained by the independent mechanisms of the ventricular systolic and diastolic functions. Some investigators explained the dissociation of inotropy and lusitropy by specific phosphorylation of different regulatory proteins that drive the contractile machinery (11).

In contrast to the increased contractile performance of the isolated heart of the *Nigella*-treated rats, neither their baseline heart rates nor their heart rate responses to ISO were enhanced. These results denote the selective action of *N. sativa* on the inotropic properties of the heart and exclude the possibility of recruitment of force-frequency relationship as in explanation for

the enhanced inotropic reserve.

The dissociation between the inotropic and chronotropic effects *Nigella* reflect dissociation of the mechanical and the electric properties of the cardiac muscle and could be explained by a selective effect of *Nigella* on the contractile (working) cells rather than the pace-maker cells of the heart.

The functional dissociation in the latency, direction, and magnitude of changes in the intrinsic cardiac properties has been repeatedly demonstrated in experimental and clinical studies by various pharmacological and pathophysiological interventions. El-Bahai et al (12) reported a selective decrease in peak tension development in the hearts isolated from envenomentated rats despite non-significant changes in chronotropy and myocardial flow rate. Furthermore, Ayobe et al (13) reported an enhanced cardiac inotropy following exercise program in rats which was associated with reduced cardiac chronotropy. Upon de-training the hearts were de-conditioned with earlier loss of the resting bradycardia but with preservation of the exercise-induced enhanced inotropy. Similarly, reduced heart rate responses to ISO, despite enhancement of other indices of ventricular systolic performance were observed after exercise training in human subjects (14) and experimental animals (15).

The dissociation of inotropic effects of *N. sativa* from the lusitropic effects, on one hand, and the inotropic from the chronotropic effect, on the other hand, may be attributed to different cardiac adrenoceptors subtypes, properties, distribution and regulation (16, 17). While B1 adrenergic stimulation enhances both inotropic and lusitropic properties of the ventricle B2 adrenergic stimulation was associated with less inotropic and not lusitropic effects (18). Xiao et al (19) suggested that B2 adrenergic receptor stimulation activates an additional phosphate that offset the protein kinase A activity and subsequent phosphorylation of the cellular regulatory proteins.

The hearts of *Nigella*-treated rats developed a moderate but significant hypertrophy that was evident by an increase in the heart weight to body weight ratio (HW/BW).

Cardiac hypertrophy is the major reserve mechanism by which the heart can augment its output in the face of increased physiological or pathological hemodynamic demands. To execute this response, the myocardium is equipped with a large number of neurohumoral and intracellular reactive cascade systems (20). Most of hypertrophic stimuli bind to membrane receptors that activate various intracellular cascades of protein kinases. Differential activation of these pathways leads to distinctive cardiac phenotypes (21), while physiological hypertrophy signaling pathways promote protein synthesis, myocytes size and enhanced systolic function with no evidence of histopathology (22). In contrast, pathological hypertrophy signaling pathways depress contractility and the response to beta-adrenergic stimulation (23, 24).

In conclusion, the observed *Nigella*-induced cardiac hypertrophy in this study was associated with increase in cardiac inotropic properties at the baseline conditions as well as upon cardiac stress by beta-adrenergic stimulation; which favors the suggestion that it is a physiological hypertrophy similar to that induced by exercise training. Further research to probe this assumption and to explore the sustainability of these effects after withdrawal of *Nigella* supplementation is suggested.

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