Original Article

Effects of two-months Nigella sativa supplementation on cardiac hemodynamics and adrenergic responsiveness

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Abstract

Objective: To study the effects of two months Nigella sativa (N. sativa) oral supplementation to normal rats on cardiac haemodynamics in vivo, the ionotropic and chronotropic properties of the isolated hearts in vitro, and the cardiac responsiveness to progressive adrenergic stimulation by isoproterenol.

Methods: The cardiac workload, after 2 months of N. sativa oral supplementation to normal adult rats, was assessed in vivo by measuring the cardiac pressure-rate product (mean arterial blood pressure multiplied by heart rate). Cardiac performance in vitro was evaluated by calculating the tension-rate product (developed peak tension multiplied by heart rate). The cardiac inotropic and chronotropic adrenergic responsiveness of N. sativa supplemented rats were evaluated in a Langendorff heart model upon graded infusion of the beta-adrenergic agonist isoproterenol.

Results: The isolated hearts of Nigella-treated rats maintained their normal cardiac adrenergic responsiveness, with a selective enhancement of both the tension-rate product and the inotropic reserve. In contrast, the in vivo cardiac pressure-rate product in N. sativa supplemented rats was not significantly different from the control group.

Conclusion: The demonstrated favourable results of N. sativa supplementation on the intrinsic cardiac contractile properties without evidence of an increased cardiac work load or energy consumption in vivo makes N. sativa an attractive inotropic agent with an economic haemodynamic profile. Further research is recommended to explore the usefulness of N. sativa in cardiovascular disorders associated with systolic dysfunction (JPMA 59:363; 2009).

Introduction

Cardiac hypertrophy represents an adaptation process to enhance cardiac performance by adjusting its ventricular mass.[1] Such a cardiac trophic response is shown to be induced by interaction of several mechanical, neurohormonal and local cardiac growth factors.[2] Molecular interplay between these factors and the intracellular reactive cascade systems is under investigation.[3] Whereas pathological cardiac hypertrophy is associated with deterioration of cardiac function, the physiological hypertrophy, observed in athletes, is associated with improved myocardial inotropic and chronotropic properties

Another major pathway to enhance cardiac performance is the increase in adrenergic responsiveness. Beta adrenergic receptors are of particular importance in the mammalian heart, where they regulate rate and force of cardiac contraction and relaxation. Blunting of cardiac adrenergic responsiveness has been implicated in experimental models of hypertension, coronary heart disease and heart failure.[4]

Identification of changes in intrinsic cardiac properties in the intact animals, are limited because of the systemic haemodynamic and neurohormonal factors in vivo. In contrast, the use of the isolated Langendorff heart perfusion model, in vitro, eliminates the influences of cardiac preload and afterload as well as the neurohormonal influences. Thus, it reflects the intrinsic responsiveness of the heart and allows one to study the local cardiac regulatory processes independent of the systemic haemodynamic and neurohormonal alterations.

Prevalence of different forms of cardiovascular diseases with their associated complications such as heart failure is increasing, but the treatment options are limited due to unwanted side effects and high costs.[5] Agents that can enhance cardiac responsiveness and cardiac reserve without compromising the myocardial oxygen utilization efficiency would economize cardiac work and may prove to be beneficial for disorders associated with decreased cardiac nutrition and/or perfusion.

The black seed, Nigella sativa (N. sativa), a member of the family of ranunculaceae, is commonly used as a natural food additive. In Arab world it is known as "Habat-ul- Sauda" whereas in subcontinent it is called "Kalonji".[6] It has been shown to have many beneficial effects on various systems including smooth muscle relaxation[6] and lowering of blood pressure.[7]

Though researchers have investigated the effects of acute administration of extracts and components of N. sativa on blood pressure[7] and heart activity,[8] there are hardly any reports dealing with cardiac effects of long term oral supplementation of N. sativa. In a recent study in this laboratory, cardiac hypertrophic and inotropic effects were

reported after two months N. sativa oral supplementation to normal rats.[9] In continuation, the present study was carried out to 1) determine the in vivo cardiac work load represented by heart rate and mean arterial blood pressure and their algebraic product, after two months of N. sativa oral supplementation to Wistar albino rats and 2) evaluate the cardiac inotropic and chronotropic properties and their adrenergic responsiveness to infusion of progressively increasing doses of the beta-adrenergic agonist isoproterenol (ISO) in a Langendorff heart perfusion model in vitro.

Methodology

Animal model: The study was performed in 2006 at King Faisal University, Dammam, Saudi Arabia, after approval of Ethical committee of the institution, considering animal experiments. Fifty (50) normal adult Wistar albino male rats, weighing 150-250 gm, acquired from a closed colony of King Faisal University, were divided equally into experimental and control groups. Only male rats were used in the study to avoid the heterogeneity in hormonal responses and effects in two sexes. The rats were kept at controlled room temperature of 22ºC 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.

Nigella-treated rats received a daily oral dose of 800 mg/kg body weight of N. sativa. This dose was chosen because it corresponds to the submaximal dose of thymoquinone (the active ingredient of N. sativa) producing hypotensive effect in rats.[7] Oral supplementation was used so that the components of seeds may be absorbed normally in the body and exert a physiological effect. The seeds (10 g) were ground 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 tube. An equivalent volume of water was administered by orogastric feeding to the control rats.

Cardiac haemodynamic profile: Each rat was weighed and injected intraperitoneally with 5000 IU heparin sodium. Rats were anaesthetized with intraperitoneal phenobarbital (40mg/kg body weight). Aorta was identified through a midline thoracic incision, cannulated and connected to a pressure transducer (MLT 844; AD Instruments, Australia). The signal from the transducer was filtered and amplified and sent to an analog-to-digital converter (Power Lab data acquisition and analysis system: ADInstruments, Australia) attached to a personal computer. The signal was recorded for 15-30 seconds and saved for later analysis. Mean arterial blood pressure and heart rate were calculated from this record with the help of Chart 5 (ADInstruments) software. The in vivo cardiac pressure rate product (PRP) was calculated by algebraically multiplying the heart rate and the mean blood pressure.

Isolated Perfused Heart Preparation: The heart was excised and mounted on Langendorff preparation. The aortic stump was slipped about 3 mm on the grooved perfusion cannula and secured by a silk ligature. A simple gravity system was used for perfusion of the coronary arteries via the ostium. Retrograde perfusion was started from a reservoir placed 75 cm above the level of the heart. The perfusion fluid was a modified Krebs-Henseleit bicarbonate buffer of pH 7.4 equilibrated with O2:CO2 (95:5) at 37°C and containing (in mM): NaCl: 118, KCl: 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25, Na2EDTA 0.5, and Dextrose 11.

A weight was attached to the heart apex and left to hang freely exerting resting tension of one gram. After initiating perfusion the isolated hearts preparations were allowed to stabilize for 15 minutes prior to recording the baseline values of heart rate (HR), myocardial flow rate (MFR), developed peak tension (PT), and maximum rate of tension development (dp/dtmax). Tension developed by the heart was measured by a light weight isometric transducer (Myograph Narcobio system) that was connected through a bridge amplifier to the PowerLab data recording system. With the help of Chart 5 program (ADInstruments) PT and (dp/dtmax) were automatically calculated from the tracing in user defined areas. Heart rate was determined by counting the number of peaks in a fixed interval of time. MFR was determined by a timed measurement of the effluent from the isolated perfused heart. The in vitro cardiac tension-rate product (TRP) was calculated by algebraically multiplying the heart rate and the developed peak tension.

Adrenergic responses of the isolated hearts (HR, MFR, PT, dp/dtmax): Isoproterenol (ISO) was delivered by the same buffer saline 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 ISO 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, 6.5) ×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 (HR, MFR) and contractile (PT, dp/dtmax) responses to incremental concentrations of ISO.

Hearts were excluded from analysis if initially a regular rhythm was not established after 20 minutes stabilization period or if during the experimental procedure they developed arrhythmia. If such exclusion criteria were met all data from the affected heart were omitted from the analysis.

Data Analysis and Statistics: Data are expressed as mean ± SEM. Using the statistical package for social sciences (SPSS:

version 10), 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 mentioned doses for different variables for both the experimental and control groups. A p-value of <0.05 was considered statistically significant.

Results

Mechanical performance of the isolated hearts: Figure 1A and 1B show that the baseline and maximum heart rate (HR) and myocardial flow rate (MFR) achieved in response to ISO infusion were significantly higher in Nigella-treated rats ($p < 0.05$). However the delta changes (the difference between the maximal and the baseline) of these values were not significantly different compared to hearts of the control rats. The baseline and maximum peak tension (PT) and dp/dtmax achieved in response to ISO infusion were significantly higher in the hearts of Nigella-treated rats ($p < 0.05$) with a significantly higher delta change from the baseline to maximum for both the PT and dp/dtmax, reflecting significantly enhanced inotropic reserve (Figure 1C and 1D).

ISO dose -response relationships: Generally the ISO dose response curves of studied cardiac function parameters showed similar pattern in all the hearts with higher levels in the hearts of Nigella-treated rats compared to their matched controls. Figure 2A shows that infusion of ISO caused a significant increase in the HR in response to first, third and fourth doses in the control group compared to their pre-infusion levels. Similarly, the hearts of Nigella-treated rats showed a significant increase in the HR up to fourth dose of ISO infusion compared to their respective pre-infusion levels. As regards the MFR, the hearts isolated from the control group as well as hearts of the Nigella group rats showed non significant changes in response to the first three doses of ISO infusion. Both groups showed a significant decrease in MFR to the later higher three doses compared to their pre-infusion levels (Figure 2B).

Figure 2C and 2D show that upon ISO infusion, the hearts of the control rats developed a significant increase in their PT and dp/dtmax in response to all doses of ISO infusion except the last dose. The hearts of Nigella-treated rats showed a more extended responsiveness with significant increase in all doses, and the response was significantly higher up to the last dose compared to their respective basal values.

In vitro haemodynamics: Table shows that the in vitro cardiac tension-rate-product (TRP) was significantly higher in Nigella-treated rats as compared to the control.

In vivo hemodynamics: Table shows that the rats treated with N. sativa for two months showed no significant changes in their heart rate (HR) and mean arterial blood pressure (MAP) compared to their matched control rats. Consequently the cardiac pressure-rate-product (PRP) did not show any significant difference between the two groups.

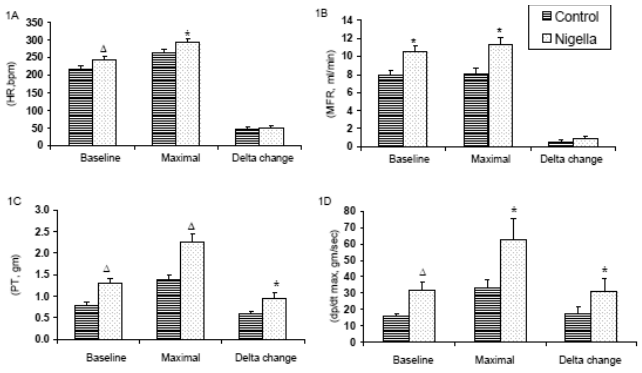

Figure 1: Cumulative results of baseline, maximal response to ISO infusion and delta change in different parameters of heart performance in nigella-treated (n=24) and control (n=24) rats. A: heart rate (HR); B: myocardial flow rate (MFR); C: peak tension (PT); D: maximum rate of tension generation (dp/dtmax).
*Data are means ± SEM. Significance of differences between experimental group and control group calculated by unpaired 't' tests; * $p<0.05$; Δ $p<0.01$.*

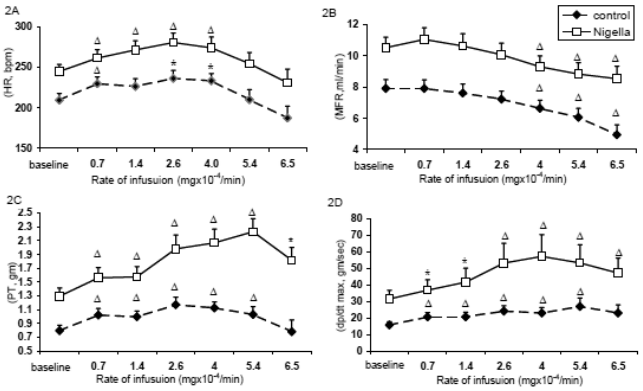

Figure 2: Dose response curves of isoproterenol infusion for different parameters of heart performance in nigella-treated (n=24) and control (n=24) rats. A: heart rate (HR); B: myocardial flow rate (MFR); C: peak tension (PT); D: maximum rate of tension generation (dp/dtmax).
*Data are means ± SEM. Significance of differences from the respective preinfusion value calculated by paired 't' tests; * $p<0.05$; Δ $p<0.01$*

Discussion

Effects of N. sativa on adrenergic responsiveness of the isolated hearts

The present study shows an overall increase in the baseline intrinsic activities of the isolated heart preparation after 2 months of N. sativa supplementation. Furthermore, when the isolated hearts were perfused with progressively increasing doses of ISO, these hearts showed a significantly higher maximal heart rate, myocardial flow rate and tension generation compared to their matched control hearts. However, only the calculated delta change for peak tension generation, representing the inotropic reserve, was significantly different from the matched control hearts. These results denote the selective action of N. sativa on the inotropic properties of the

heart which may be attributed to differences in cardiac adrenoceptors subtypes, their properties, or their distribution and regulation.[10,11]

The partial dissociation of the effects of N. sativa on cardiac chronotropic and inotropic activities has been reported in exercise-induced cardiac hypertrophy with lower resting heart rate in presence of increased contractility. Selective down regulation of beta adrenoceptors of the sino-atrial node in addition to increased vagal tone were proposed to explain sinus bradycardia in human athletes and experimental animals following chronic dynamic exercise.[12,13]

Table: Effect of N. sativa oral supplementation for two months on heart rate (HR), mean arterial blood pressure (MAP) and the cardiac pressure rate product (PRP) in vivo and the heart rate (HR), peak tension developed (PT) and the cardiac tension rate product (TRP) in vitro.

	Control (n= 24)	N. sativa (n= 24)
in vivo		
HR (beats/ min)	359 ± 10	347 ± 8
MAP (mm Hg)	86 ± 2	90 ± 3
PRP (HR x MAP)	31016 ± 234	31327 ± 198
in vitro		
HR) (beats/ min)	209±8	245±9**
PT (gm)	0.8 ±0.08	1.3 ±0.12**
TRP (HR x PT)	163±16	335±34***

Data are presented as mean ± SEM
* p<0.05 ** p<0.01 *** p<0.001

Effects of N. sativa on the cardiac haemodynamic profile

N. sativa supplementation produced a significant increase in the cardiac tension-rate product of the isolated hearts, calculated as the product of developed peak tension and the heart rate. In contrast, the in vivo cardiac pressure-rate product, calculated as the mean arterial blood pressure multiplied by the heart rate of the intact rats, was not significantly changed compared to the normal control rats.

Taking in consideration that the principal determinants of cardiac output are the stroke volume and heart rate, and those of the mean arterial pressure are the cardiac output and peripheral resistance, the absence of significant changes in heart rate and mean arterial pressure with N. sativa supplementation, despite the significant increase in myocardial contractility, may indicate that the cardiac output and blood pressure in these rats were maintained by higher stroke volume and lower total peripheral resistance, respectively.

The non-significant change in heart rate of Nigella-treated rats compared to the increased chronotropy of their isolated hearts may be explained by a decrease in the systemic or cardiac sympathetic activity and/or increased vagal tone in the intact animals. However, the maintained adrenergic responsiveness of the isolated hearts in vitro and the associated maintenance of the blood pressure in vivo are in favour of a decrease in the systemic sympathetic activity with maintained cardiac sympathetic activity. In fact, a systemic decrease in sympathetic tone of the resistance vessels with vasodilation effect might have prevented the expected increase in blood pressure with the Nigella-induced inotropic effect on the ventricular stroke volume. In support of this proposal of decreased sympathetic drive in vivo, is the observed increase in heart rate and tension-rate-product when the hearts were perfused outside the body independent of systemic neurohormonal factors.

The observed non-significant changes in cardiac haemodynamics with N. sativa supplementation differ from some other studies, where a decrease in heart rate with administration of the N. sativa volatile oil to normal rats[7] or alloxan-induced diabetic rabbits[14] was observed. In another study, an oral dose of N. sativa extract (0.6 mL/kg/day) was associated with significant diuresis and decreased arterial pressure after 15 days of treatment.[15] The dissimilarity of these results could be explained by the difference in the preparation of N. sativa used, its dose and duration of administration and the experimental animal species. Some investigators found a reduction in the heart rate and contractility in isolated guinea pig hearts infused by extract of N. sativa.[8] However their experiments involved the in vitro acute effects of infusion of N. sativa extracts on the isolated hearts which is quite different from long term in vivo supplementation for two months.

The increase in intrinsic cardiac contractility independent of systemic sympathetic activity may comprise many physiological advantages. First, the high levels of catecholamines have been shown to be lethal to cardiac myocytes. It is proposed that exposure to norepinephrine exert a direct toxic effect on cardiac myocytes in cell culture, an action that could be mediated by increase in both cAMP and calcium influx.[16] Second, myocytes death by apoptosis was observed in rat ventricular myocytes exposed in culture to norepinephrine for 24 hours.[17] Third, catecholamines are known to stimulate formation of harmful reactive oxygen (O2) free radicals.[18,19]

The increased cardiac contractility and contractile reserves with N. sativa supplementation without an associated increase in blood pressure and heart rate makes N. sativa an attractive inotropic agent in cardiac diseases associated with systolic dysfunction. In contrast to the deleterious effects of many pharmacological inotropic agents, augmentation of cardiac contractility induced by N. sativa would not overdrive the heart with maintained haemodynamic profile. It is well documented that the cardiac work, represented by the

pressure-rate product, is the chief determinant of myocardial O_2 demand. Consequently it may be suggested that the enhanced intrinsic cardiac properties induced by N. sativa supplementation were not associated with proportionate increase in myocyte O_2 consumption. This enhanced mechanical efficiency of the myocardial contractile machinery after N.sativa supplementation is in sharp contrast to effects of beta-adrenergic stimulants. One major drawback of beta-adrenergic stimulants is that they produce a disproportionate increase in myocardial O_2 requirements relative to the increase in mechanical function, thereby lowering myocardial O_2 utilization efficiency and depleting myocardial energy reserves.[20-22] Heart failure, regardless of its diverse etiopathogenesis, is characterized by loss of energy-dependent cellular functions.[23]

Clinical extension of these data to human condition is evidently a promising application. Besides the favourable metabolic properties of N. sativa, its inotropic economic potential makes it a promising alternative or an adjuvant to other adrenergic inotropic agents in treatment of patients with inadequate cardiac performance. In fact, modulation of myocardial growth without adversely affecting contractile function is increasingly recognized as a potentially promising approach in prevention and treatment of heart failure.[24]

In conclusion, long-term dietary supplementation with N. sativa extract has shown a favourable advantage on the intrinsic contractile properties of the heart. Moreover a physiologic cardiac hypertrophy develops with a shift in cardiac performance to more economic haemodynamic profile, where the enhanced inotropy was not associated with increased cardiac workload or oxygen demands, resulting in more efficient energy utilization.

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