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ORIGINAL ARTICLE

Cardiovascular Effects of Nigella Sativa on Isolated Rabbit Heart and its Impact on Cardiac Remodeling

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ABSTRACT

Background: Black Cumin/Nigella sativa (NS) which belongs to the botanical family of Ranunculaceae commonly grows in Eastern Europe, the Middle East, and Western Asia. Its prolonged use can produce physiological changes with or without affecting the architecture of different organs like the heart (cardiac remodeling). The data for the cardiovascular benefits of black cumin are not well established scientifically.
Objectives: To determine the direct cardiovascular effects of Nigella Sativa extract on heart rate, cardiac contractility (apical force), ECG and coronary flow on normal heart with and without cardiac remodeling.
Methods: This experimental study was conducted on forty-two (42) rabbits. These rabbits were divided into seven groups, each comprising of six animals (Group I-VI without cardiac remodeling and Group VII with cardiac remodeling). NS was given to these groups in different doses i.e., Group I (NS=10ug), Group II (NS=30ug), Group III (NS=100ug), Group IV (NS=300ug), Group V (NS=3000ug), Group VI (NS=10000ug) and VII (NS=300ug). Radnoti working heart system was used to determine the effects of NS on heart rate, cardiac contractility (apical Force), ECG and coronary flow on normal heart with and without cardiac remodeling. The results were analyzed using SPSS version 28.
Results: Results of this study revealed negative chronotropic and positive inotropic effects without ECG changes in the normal heart and with ECG changes in remodeled heart.
Conclusions: Prolonged use of Nigella sativa can lead to disturbed ECG by affecting the conducting tissue.
Key Words: Cardiac Remodeling, Apical force, Ischemia and Reperfusion

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INTRODUCTION

Alternative medicine has become popular worldwide over the past 20 years.[1] Thus, Nigella sativa (NS) probably had thirty five important roles in the practices of the ancient Egyptians. The Hippocrates and the Dioscorides referred them as Melanthion.[2,3]

Cardiac remodeling is defined as genome expression resulting in molecular, cellular, and interstitial changes and manifested as changes in size, shape and function of the heart resulting from cardiac load or injury.

Cardiac remodeling is influenced by hemodynamic load and the neuro-hormonal factors.[4,5] There are several beneficial pharmacological effects attributed to various crude or purified components of these seeds including antihistaminic, antihypertensive, hypoglycemic, anti-inflammatory, and the immunity booster effects of NS seeds along with significant antineoplastic activities.[6-8]

Seeds of NS are frequently used in folk medicine in the Middle East and some Asian countries. These studies collectively provide early indication that further development of agents derived from NS seeds could be useful in modern medicine. Clinical use of NS has inotropic potential as an alternative to other treatment options in patients with decreased cardiac performance.[9] Cardiac hypertrophy is defined as the enlargement of the cardiomyocytes.[10]

There are two forms of cardiac hypertrophy: physiological and pathological, the later is the result of adaptive response to the pathological stimuli and usually progresses to the heart failure. It may be precipitated by pathological insults such as stress overload secondary to hypertension, valvular heart disease or injury of the cardiac muscle due to ischemia and infarction.[11]

METHODS

This experimental study was carried out in the Pharmacology Department of Shalamar Medical and Dental College, Lahore after the approval of the project from Institutional Review Board (SMDC/IRB/03-01/144) and the Guide for the care and use of laboratory animals published by the US National Institutes of Health (85-23, revised 1996) was followed.

Study population included forty-two male rabbits, 1.5-2 Kg in weight. These experimental animals were divided into seven groups and each group had 6 animals. The six groups were given different doses of NS for 8 weeks to remodel the heart. Experimental Group I (NS=10ug), Group II (NS=30ug), Group III (NS=100ug), Group IV (NS=300ug), Group V (NS=3000ug), and Group VI (NS=10000ug) were given NS. Group IV (NS=300ug) showed optimal effects, so it was selected and renamed as Group VII. The baseline recording of all the parameters (such as heart rate, apical force of cardiac contractility, coronary flow and ECG) was done and then the effects of ischemia and reperfusion produced by ligation and unligation of coronary vessel of isolated beating heart were observed.

Preparation of Nigella Sativa Extract:

70% methanol extract of NS was prepared by extracting the contents from 200gm of nigella powder dissolved in 500ml of distilled water and then in 500ml of methanol. Various dilutions were prepared from standard solution of 3mg/ml.

Preparation of Heart for Perfusion:

Forty-two (42) male rabbits (weighing between 1.5-2 kg) were injected 5000IU (1ml) heparin through the marginal ear vein one hour before slaughtering. Hearts were removed from the chest after midsternal incision and immediately placed in an ice cold Krebs-Henseleit solution. Each heart was mounted on Radnoti student heart system (AD Instrument) via aorta. It was allowed to stabilize for 30 mins in the perfusate.

Ninety five percent O_2, 5% CO_2, and Krebs-Henseleit solution with pH of 7.35 were used at 37˚C. The isometric transducer was connected through a bridge amplifier (FE221) and BP Transducer (MLT 1199) to the Power Lab data recording system (AD Instrument - 8/35 Model PL 3508) to record the contractility. Three ECG leads (Bioamplifier FE 136-0368) were used to record heart rate (HR) and ECG changes.

Coronary flow (CF) was determined by a timed measurement of the effluent as well as electronic drop recorder (Drop Counter

MLT 226). Ischemia was produced by ligating and unligating LAD (left anterior descending branch of left coronary artery.[12]

The cardiac remodeling was produced by daily feeding dose of 800mg/kg body weight of NS for 4 weeks. This dose was chosen because it corresponds to the submaximal dose of thymoquinone (the active ingredient of N. sativa) producing hypotensive effect in rats.[13]

Statistical Analysis

Graph Pad prism was used for statistical analysis and wherever necessary paired or unpaired "t" test was applied.

RESULTS

The selected samples of 42 rabbits, control and experimental, remained intact and healthy during the research period of 1.5 months. In the dose response, the effects of NS were observed on the heart rate (Fig:1a) and the cardiac contractility (Fig:1b). There was dose dependent negative effect on heart rate and positive effect on cardiac contractility in dose range of 10-300µg of NS (Fig:2a,2b). Effects of the lowest affective dose i.e., 300ug of NS were compared in the remodeled hearts during ischemia induction and after reperfusion. Negative chronotropic effect and positive inotropic effect during ischemia and reperfusion were recorded (Fig:2a,2b). The effects of highest doses of NS were observed on the ECG and coronary flow. There was no significant change in T wave amplitude, ST height, R wave amplitude and QT interval. (Table 1) There was significant decrease in coronary flow with NS (10000ug) which may be due to significant increased contractility (Table 1). Mechanical factors HR, AF and CF and ECG parameters were compared in normal and remodeled hearts. After remodeling there was no significant effect on heart rate, cardiac contractility and coronary flow. However, there were significant negative changes in T wave amplitude, ST height, R wave amplitude and QT interval manifesting quite significant effect of the NS on the conducting tissue (Table 2).

Table 1: Effects of Nigella Sativa (NS) on Normal Heart

Dose of NS µg	ECG Changes				Coronary Flow
	QT Interval (sec)	R wave Amplitude (µV)	ST Height (µV)	T wave Amplitude (µV)	(ml/min)
	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD
Baseline	17±4.0	232±40	133±26.4	125.7±2.5	6.5±2.03
300	16±4.0	238±40	68±15.7	168.4±20.5	6.5±1.18
p value	0.3534	0.6131	0.221	0.2377	0.6782
Baseline	17±4.0	216±33	130±20.9	165.3±25.5	6.4±1.75
3000	14±3.0	102±19	40±8.92	92.15±9.2	6.3±1.81
p value	0.4848	0.2836	0.1741	0.3852	0.4848
Baseline	11±2.0	101±24	21±5.12	31.19±7	6±2.52
10000	15±3.0	150±1.6	90±6.61	131±0.07	3.6±2.52
p value	0.3627	0.3683	0.3934	0.4041	0.0182*

Paired t-test was applied; p<0.05considered statistically significant

Table 2: Effect on Cardiovascular Parameters before and after Remodeling with Nigella Sativa

	Before Remodeling	After Remodeling	p value
	mean±SD	mean±SD	
Heart Rate (beats/min)	181±15	157±23.9	0.5183
Apical Force of Cardiac Contractility (grams)	8.6±1.2	10.6±3.3	0.2466
Coronary Flow (ml/min)	8±0.8	10±0.9	0.5686
QT interval (sec)	17±4.0	0.17±0.02	< 0.0001*
R wave Amplitude (µV)	170±57	294±1.1	< 0.0001*
ST Height (µV)	82±22	144±2.3	< 0.0001*
T wave Amplitude (µV)	125.2±27	80±0.4	< 0.0001*

*Paired t-test was applied; *p<0.05considered statistically significant*

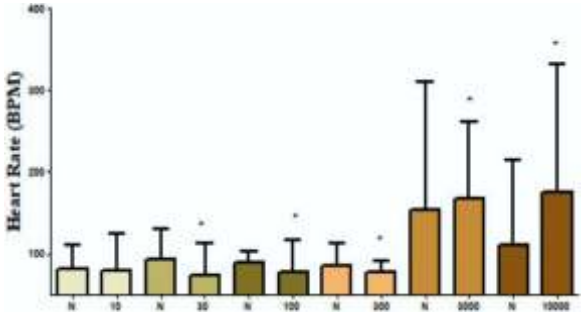

Fig 1a: Effects of increasing doses of Nigella Sativa on Normal Heart

p*value <0.05 statistically significant

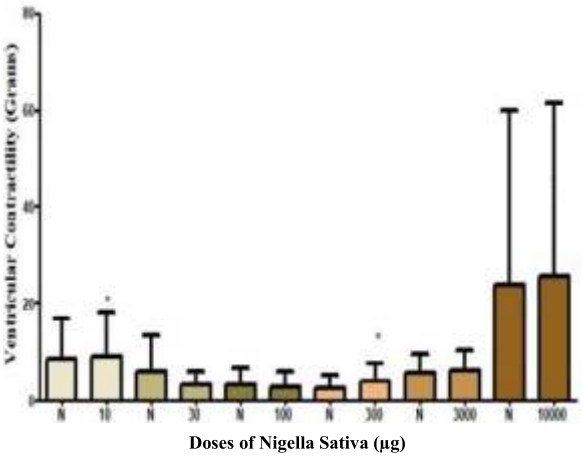

Fig 1b: Effects of increasing doses of Nigella Sativa on ventricular contractility

p*value <0.05 statistically significant

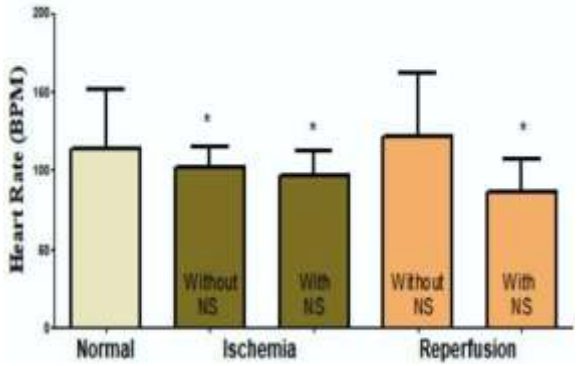

Fig 2a: Effects of 300µg Nigella Sativa on remodeled heart (Heart Rate) during ischemia and reperfusion

p*value <0.05 statistically significant

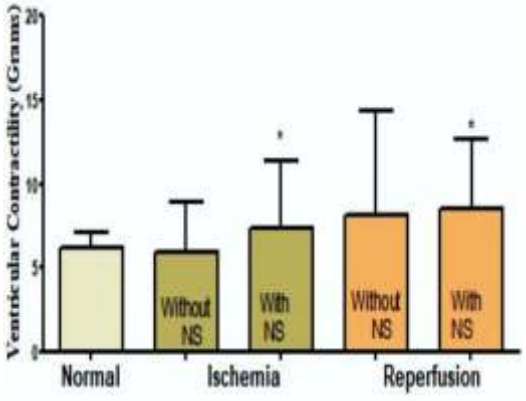

Fig 2b: Effects of 300µg Nigella Sativa on remodeled heart (Ventricular Contractility) during ischemia and reperfusion

p*value <0.05 statistically significant

DISCUSSION

Cardiac remodeling is defined as genome expression resulting in molecular, cellular and interstitial changes manifested as changes in size, shape and function of the heart resulting from cardiac load or injury. Remodeling is adaptive process followed by a maladaptive (late) phase of myocardium – the myocyte, the interstitial cells, the vascular endothelium, and the immune cells Transforming growth factor β (TGF-β) is involved in the cardiac hypertrophy and fibrosis of the left ventricle.

The 'black seed is a considered universal healer. Nigella sativa is gastro-protective and is also used for asthma. The effects of Nigella sativa (Ns) including n-hexane, dichloromethane, methanol, and aqueous fractions, on tracheal smooth muscle of guinea pigs were also examined.[14] The most potent relaxant effect was seen with methanol and dichloromethane fractions.[15,16]

In our experimental study setup, the isolated re-perfused heart is lacking neuro-humoral control of the body. There is no cholinergic or adrenergic innervation and high doses of NS have produced increased coronary flow as result of coronary vessel relaxant effect. The role of electrolytes potassium and calcium becomes important. The effect of NS (2, 4, 6, 8, 10, and 14 mg/ml) on rat aortic smooth muscle contracted by both KCl and phenylephrine had been observed. The decreased heart rate in normal perfused heart in present study may be due to inhibition of calcium channels (Fig:2a,2b). The effects of NS on heart rate and contractility of isolated heart in the presence of calcium free Krebs solution have been observed in the past.[17]

The isolated perfused hearts of Nigella treated rats showed the physiological cardiac

hypertrophy in rats induced by long term NS supplementation, baseline peak tension and the maximum rate of tension development.[18] The bradycardia at low dose and tachycardia at high dose are related with the calcium channel blocking effects of NS as confirmed by various other studies.[19-21] Positive inotropic effects were observed in our study. Sequence of molecular, biochemical and mechanical events can lead to heart failure, myocyte hypertrophy. Clinically there are changes in size, shape and function of the heart resulting from cardiac load or injury. Hemodynamic load and neuro-hormonal activation may be responsible for increased contractility.[22,23] The effects of lowest affective dose (300ug) of NS were observed and compared with the remodeled hearts during ischemia and reperfusion. Negative chronotropic effect and positive inotropic effect during ischemia and reperfusion were recorded. The remodeling in present study i.e., regular feeding of NS (800mg/kg) ameliorates all the mechanical dysfunctions (Table 2). The significant changes in the amplitude of T wave, ST segment, R wave and QT interval are as a result of cellular potassium and calcium homeostasis. In another study NS supplementation induced moderate global (homogenous) cardiac hypertrophy evident by enhanced levels of baseline peak tension, maximum rate of tension development, altered heart rate and myocardial flow rate.[24]

CONCLUSION

Ventricle remodeling was produced in the rabbits. On the left ventricular stress was observed through ischemia. The changes are measureable from mechanical outcomes or ECG. In an intact animal local and systemic factors play a role. In the isolated heart observed effects are either enhancement or amelioration of specified parameters. Results of this study revealed decreased heart rate (negative chronotropic) and increased cardiac contractility (positive inotropic effects) without ECG changes in the normal heart and with ECG changes with remodeled hearts (Table1&2). There is scope to observe the drug interactions of the NS with antihypertensive, cardiotonic and antianginal drugs.

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Conflict of interest:

All authors declared no conflict of interest.

Contributors:

ASAB: Central idea, data analysis, data interpretation, critical review of final draft.
AS: Data analysis, write up of the draft and critical review.
MKB: Statistical analysis, critical review of the manuscript for final approval.
MAB: Data analysis, drafting of work.
MU: Contribution to data collection and data analysis.
MAK: Drafting, Investigations & supervision of the project.
All authors approved the final version and signed the agreement to be accountable for all aspects of work.

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Data sharing statement:

The data is available from the corresponding author upon request.

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