Research Article

Protective role of black cumin (*Nigella sativa*) on isoproterenol induced myocardial infarction in rats

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ABSTRACT

Background: For many years, serologic markers have been used to assist cardiologists in the diagnosis and management of patients with myocardial infarction (MI). Once the serologic markers such as cardiac marker enzymes come to normal level upon a treatment, it clearly shows the treatment drug has the role in the management of MI.

Objectives: The present study is designed to evaluate the effect of *Nigella sativa* seeds called black cumin on isoproterenol induced myocardial infarction in experimental rats.

Materials and methods: The rats were randomly divided into four groups of 6 rats each. Group 1 rats received 1.0 ml of 0.5% carboxymethyl cellulose (CMC) throughout the experimental period and served as the control. Group 2 rats received CMC as in group 1 and isoproterenol (85 mg/kg body weight) intraperitoneally twice at an interval of 24 hours on the 14th and 15th days. The rats in group 3 received black cumin (150 mg/kg body weight) intragastrically for a period of 15 days. Group 4 rats received black cumin and isoproterenol as said above and the experiment was terminated on 16th day and animals were sacrificed by cervical decapitation after an overnight fast. Blood was collected for the estimation of biochemical parameters and heart dissected out for biochemical estimation and histopathological examination.

Results: Along with VLDL, TG, cholesterol, free fatty acids, the levels of marker enzymes in serum such as AST, ALT, LDH, CK, and tissue lipid profile of TG, cholesterol, free fatty acids were significantly decreased ($p < 0.05$), whereas the levels of CK-MB and HDL, LDL in serum and tissue lipid profile of phospholipids were significantly increased ($p < 0.05$) in rats pretreated with black cumin compared to that of the group which received isoproterenol alone.

Conclusion: Pretreatment with black cumin offered a protective effect against isoproterenol induced myocardial infarction in rats as evidenced by cardiac markers and lipid profile of the heart tissue.

Key words: Myocardial Infarction, lipid peroxidation, black cumin, cardiac markers

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INTRODUCTION

Myocardial infarction (MI) is a therapeutic enigma which is the principal cause of death in both developed and developing countries as a result of cardiovascular diseases, and has been the object of intense investigation by clinicians and basic medical scientists.[1] Although clinical care is improved, public awareness is raised and health innovations are widely used, myocardial infarction still remains the leading cause of death worldwide.[2] In India, the number of patients being hospitalized for MI, commonly known as heart attack, is increasing over the past 35 years and male patients have shown a more striking increase.[3] MI results from the prolonged myocardial ischemia with necrosis of myocytes due to interruption of blood supply to an area of heart.[4]

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Experimental models for myocardial infarction greatly help to identify interventions that probe the pathogenesis underlying myocardial infarction. Each experimental model, with its own inherent advantages and disadvantages, helps the investigator to select the most appropriate study design. The pathophysiological changes occurred in heart following isoproterenol administration in rats are comparable to those taking place in human myocardial infarction.[5] Isoproterenol, a synthetic catecholamine and β-adrenergic agonist, causes severe stress in the myocardium resulting in infarction like necrosis of the heart muscle.[6] It is very well established that isoproterenol induced MI in rats is accompanied with an increase in cardiac marker enzymes and altered lipid profile. Isoproterenol induced MI involves membrane permeability alterations that bring about loss of function and integrity of myocardial membrane. It also has been reported to show many metabolic and morphologic aberrations in the heart tissue of experimental animals.[7] It also increases the levels of low-density lipoprotein (LDL) and, cholesterol in the blood, which inturn leads to the formation of atherosclerosis in the arteries thus favoring coronary heart disease.[8] Lipids and lipoproteins play an important role in the pathology of myocardial infarction. To achieve the greatest possible reduction in MI risk, treatment strategies should be aimed at reducing the increased levels of circulatory lipids and maintaining the normal levels of lipoproteins.[9]

Many modern drugs are effective in preventing cardiovascular diseases, but their use is often limited because of their adverse side effects.[10] Dietary factors play a key role in the prevention of various human diseases including cardiovascular diseases. Epidemiological studies have shown that diets rich in fruits, herbs, and spices are associated with low risk of cardiovascular diseases.[11] *Nigella sativa* L., a dicotyledonous of Ranunculaceae family, is an amazing herb with a rich historical and religious background and has been employed for thousands of years as a spice and food preservative, as well as a protective and curative remedy for numerous disorders.[12] The seeds have long been used in the middle and far East as a traditional medicine for a wide range of illness including bronchial asthma, headache, dysentery, infections, obesity, back pain, hypertension and gastrointestinal problems. Its use in skin condition such as eczema has also been recognized worldwide.[13] The seeds of *Nigella sativa,* commonly known as black cumin are the source of the active ingredients of this plant. Various therapeutic effects, such as antioxidant[14], anti-inflammatory[15], anticancer[16], antihistaminic[17], antibacterial effects[18] have been described for *Nigella sativa.* Thymoquinone, the active constituent of *Nigella sativa* seeds, is a pharmacologically active quinone, prevents oxidative injury in various *in vitro* and *in vivo* studies in rats[19, 20] and also has been suggested that thymoquinone may quench oxidant radicals and prevents membrane lipid peroxidation in tissues.[21] We had previously reported that black cumin could be protective against the isoproterenol induced myocardial damage as it significantly increased the levels of antioxidant enzymes and decreased the oxidative stress produced by isoproterenol.[22] Therefore, the present study has been designed to elucidate the effect of black cumin (*Nigella sativa*) on isoproterenol induced cardiac damage with reference to marker enzymes and lipid profile.

MATERIALS AND METHODS

Animals

The Wistar strain male albino rats, weighing 110-150 g were selected for the study. They were housed in plastic cages with filter tops under controlled conditions of 12 hr light/12 hr dark cycles 50% humidity at 28°C. The animals were allowed to feed a commercial pellet diet comprised of 20% crude protein, 5% fat, 4% crude fiber, 8% ash, 2% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamins and 55% nitrogen free extract that obtained from Venkateshwara enterprises, Bangalore, India and water *ad libitum*. The animals were treated strictly according to the CPCSEA guidelines and the study was conducted after obtaining permission from Institutional Animal Ethics Committee (IAEC) of the institution.

Chemicals

Isoproterenol was obtained from the Sigma Chemical Company, St. Louis, MO, USA. All the other chemicals and reagents were purchased from Himedia Laboratories, Mumbai, India.

Formulation and administration of black cumin

The black cumin seeds of *Nigella sativa* were purchased from the local market in Salem, Tamilnadu, India. The seeds were grinded with a grinder into powder and dissolved in freshly prepared carboxymethyl cellulose and each animal belonging to two different groups received black cumin at a dose of 150 mg/kg body weight[22] everyday by intragastric intubation.

Induction of myocardial infarction

Myocardial infarction was induced by intraperitoneal (i.p.) injection of isoproterenol hydrochloride 85 mg/kg body weight, dissolved in physiological saline, for two consecutive days (14^{th} and 15^{th} day).[23]

Study design

The rats were randomly divided into four groups of 6 rats each. Group 1 rats received 1.0 ml of 0.5% carboxymethyl cellulose (CMC) throughout the experimental period of 15 days and served as the untreated control. Group 2 rats received CMC as in group 1 and given isoproterenol (85 mg/kg body weight) intraperitoneally twice at an interval of 24 hours on the 14^{th} and 15^{th} day. The rats in group 3 received black cumin via intragastric intubation at a daily dose of 50 mg/kg body weight for a period of 15 days. Group 4 rats received black cumin as in group 3 for 15 days and they also received isoproterenol (85 mg/kg body weight) injections intraperitoneally twice at an interval of 24 hours. The experiment was terminated on 16^{th} day and all the animals were sacrificed by cervical decapitation after an overnight fast.

Preparation of hemolysate

Blood was collected in heparinized tubes and plasma separated by centrifugation at 2000 x g for 10 min. Serum was collected and used for the determination of cardiac marker enzymes, lipoproteins and lipid metabolites like triglycerides, phospholipids, free fatty acids and cholesterol. Hemolysate was prepared by lysing a known volume of erythrocytes with hypotonic phosphate buffer pH 7.4. The contents were then centrifuged at 3000 x g for 10 min at 2˚C to separate the hemolysate.

Preparation of tissue homogenate

Heart tissue were removed immediately after sacrificing and washed with ice-cold saline and homogenized by using tris buffered pH 7.8 in a tissue homogenizer.

Biochemical estimation

Lipids from the heart tissues were extracted by the method of Folch.[24] Total cholesterol was determined from serum and heart tissue by the method of Zlatkis.[25] Lipid extract was treated with ferric chloride acetic acid reagent to precipitate the proteins. The protein free supernatant was treated with concentrated H_2SO_4. A reddish purple color formed was read in a Spectronic 20 colorimeter at 560 nm. The protein values are expressed as mg/100g tissue or mg/dl. Phospholipids were determined from serum and heart tissue by the method of Zilversmit.[26] The formation of stable blue color, which was read in a colorimeter at 680 nm. The amounts of phospholipids are expressed as mg/100 g tissue or mg/dl. Triglycerides were determined from serum and heart tissue by the method of Foster.[27] The absorbance of yellow colored compound was read in a Spectronic 20 colorimeter at 405 nm. The triglyceride content is expressed as mg/100 g tissue or mg/dl. Free fatty acids were determined from serum and heart tissue by the method of Falholt.[28] The amounts of free fatty acids are expressed as mg/100 g tissue or mg/dl.

Activities of aspartate and alanine transaminase (AST and ALT respectively) from serum were assayed by the method of Reitman.[29] The liberated oxaloacetate and pyruvate reacts with 2, 4-dinitrophenyl hydrazine to form 2, 4-dinitrophenyl hydrazone, which was read at 540 nm. Activities of AST and ALT were expressed as IU/L. The activity of lactate dehydrogenase (LDH) from serum was assayed in serum using a commercial kit (Product No. 72351) purchased from Qualigens Diagnostics, Mumbai, India. The enzyme activity was expressed as IU/L for serum. Creatine kinase (CK) activity from serum was assayed by the method of Okinada.[30] The enzyme activity was expressed as IU/L for serum. Creatine kinase-MB (CK-MB) activity

from serum was assayed using a commercial kit (Product No. 11207001) obtained from Agappe Diagnostics, Kerala, India. The enzyme activity in serum was expressed as IU/L.

Statistical analysis

The data presented as mean ± standard deviation. Results were analyzed using one-way analysis of variance [ANOVA] and the group means were compared by Duncan's Multiple Range Test [DMRT] using SPSS version 12 for windows. A p value of <0.05 was considered as statistically significant.

RESULTS

Changes in the levels of serum marker enzymes

Table 1 shows the effect of black cumin on the circulating levels of marker enzymes (AST, ALT, CK and LDH) of control and experimental animals. Circulating levels of (AST, ALT, CK and LDH) status were significantly increased except CK-MB showed decreased levels in isoproterenol (group 2) treated rats compared to control rats (group 1), whereas the activity of the marker enzymes (AST, ALT, CK and LDH) were significantly decreased except CK-MB showed increased levels in black cumin + isoproterenol treated rats (group 4) compared to untreated isoproterenol treated rats (group 2). The activities of marker enzymes (AST, ALT, CK and LDH) were near to control rats treated with black cumin (group 3) as compared to untreated control rats (group 1).

Changes in the levels of serum lipoproteins

Table 2 shows the effect of black cumin on lipoproteins (VLDL, LDL and HDL) of control and experimental animals. Circulating levels of lipoprotein VLDL was significantly increased except HDL and LDL showed decreased levels in isoproterenol treated rats (group 2) as compared to the control rats (group 1), whereas the activity of lipoprotein VLDL was significantly decreased except HDL and LDL showed increased levels in black cumin + isoproterenol treated rats (group 4) as compared to untreated isoproterenol treated rats (group 2). The activities of lipoproteins (VLDL, LDL and HDL) were near to control rats treated with black cumin (group 3) as compared to untreated control rats (group 1).

Changes in the levels of serum lipid profile

Table 3 shows the effect of black cumin on serum lipid metabolism (triglyceride, cholesterol, free fatty acids and phospholipids) of control and experimental animals. Circulating levels of triglyceride, cholesterol, free fatty acids and phospholipids were significantly increased in isoproterenol treated rats (group 2) as compared to the control rats (group 1), whereas the activity of triglyceride, cholesterol, free fatty acids and phospholipids) were significantly decreased in black cumin + isoproterenol treated rats (group 4) as compared to untreated isoproterenol treated rats (group 2). The activities of triglyceride, cholesterol, free fatty acids and phospholipids were near to control rats treated with black cumin (group 3) as compared to untreated control rats (group 1).

Changes in the levels of heart tissue lipid profile

Table 4 shows the effect of black cumin on tissue lipid metabolism (triglyceride, cholesterol, free fatty acids and phospholipids) of control and experimental animals. Tissue levels of triglyceride, cholesterol and free fatty acids were significantly increased except phospholipids showed decreased levels in isoproterenol treated rats (group 2) as compared to the control rats (group 1), whereas the activity of triglyceride, cholesterol and free fatty acids were significantly decreased except phospholipids showed increased levels in black cumin + isoproterenol treated rats (group 4) as compared to untreated isoproterenol treated rats (group 2). The activities of tissue levels of triglyceride, cholesterol, free fatty acids and phospholipids were near to control rats treated with black cumin (group 3) as compared to untreated control rats (group 1).

DISCUSSION

Cardiovascular disease is a major global health problem reaching epidemic proportions in the Indian subcontinent[31] and low and middle income countries, accounting for 78% of all deaths.[32] Myocardial cell protection and prevention of cell ischemia or necrosis have been therapeutic targets for a long time. New therapies are needed to treat myocardial ischemia because current treatment has only a limited impact on survival and annual costs.[33]

Table 1: Effect of black cumin on serum marker enzymes of control and experimental rats

Groups	AST (UL^{-1})	ALT (UL^{-1})	LDH (UL^{-1})	CK (UL^{-1})	CK-MB (UL^{-1})
Control	25.55 ± 0.29	13.32 ± 0.48	83.18 ± 0.51	135.33 ± 0.53	162.31 ± 0.56
Isoproterenol (85 mg/kg)	53.63 ± 0.72*	24.88 ± 0.41*	158.35 ± 0.60*	189.27 ± 0.58*	80.24 ± 0.47*
Black cumin (150 mg/kg)	20.61 ± 0.25	11.85 ± 0.53	60.3 ± 0.56	120.58 ± 0.48	165.43 ± 0.39
Isoproterenol + black cumin	39.93 ± 0.44†	17.56 ± 0.25†	118.15 ± 0.66†	150.55 ± 0.29†	145.57 ± 0.69†

The results are expressed as mean ± SD for 6 animals in each group.
AST - aspartate transaminase,
ALT - alanine transaminase
LDH - lactate dehydrogenase
CK - Creatine kinase
CK-MB - Creatine kinase-MB
*p < 0.05 vs. control
†p < 0.05 vs. isoproterenol

Table 2: Effect of black cumin on serum lipoproteins of control and experimental rats

Groups	HDL (UL^{-1})	LDL (UL^{-1})	VLDL (UL^{-1})
Control	50.37± 0.26	68.2 ± 0.46	20.51 ± 0.29
Isoproterenol (85 mg/kg)	30.45 ± 0.26*	40.52 ± 0.29*	28.37 ± 0.35*
Black cumin (150 mg/kg)	55.43 ± 0.29	52.46 ± 0.30	16.52 ± 0.27
Isoproterenol + black cumin	45.45 ± 0.26†	59.28 ± 0.50†	22.54 ± 0.23†

The results are expressed as mean ± SD for 6 animals in each group.
HDL - High density lipoprotein
LDL - Low density lipoprotein
VLDL - Very low density lipoprotein
*p < 0.05 vs. control
†p < 0.05 vs. isoproterenol

Table 3: Effect of black cumin on serum lipid products of control and experimental rats

Groups	Triglycerides	Cholesterol	Free Fatty acids	Phospholipids
Control	132.02 ± 0.69	76.25 ± 1.27	24.61 ± 0.73	80.5 ± 0.67
Isoproterenol (85 mg/kg)	181.59 ± 1.04*	115.19 ± 1.10*	37.26 ± 0.71*	61.49 ± 0.64*
Black cumin (150 mg/kg)	119.06 ± 0.44	56.06 ± 0.94	20.67 ± 0.72	82.43 ± 0.34
Isoproterenol + black cumin	143.34 ± 0.43†	85.37 ± 0.73†	32.74 ± 0.39†	76.51 ± 0.74†

The results are expressed as mean ± SD for 6 animals in each group.
*p < 0.05 vs. normal
†p < 0.05 vs. isoproterenol

Table 4: Effect of black cumin on tissue lipid metabolism of control and experimental rats

Groups	Triglycerides	Cholesterol	Free Fatty acids	Phospholipids
Control	3.37 ± 0.37	2.51 ± 0.25	4.29 ± 0.21	13.48 ± 0.23
Isoproterenol (85 mg/kg)	5.43 ± 0.39*	4.65 ± 0.23*	5.64 ± 0.24*	7.35 ± 0.25*
Black cumin (150 mg/kg)	2.33 ± 0.26	1.35 ± 0.19	2.35 ± 0.199	14.56 ± 0.28
Isoproterenol + black cumin	2.96 ± 0.38†	2.86 ± 0.23†	4.57 ± 0.26†	12.49 ± 0.24†

The results are expressed as mean ± SD for 6 animals in each group.
*$p < 0.05$ vs. normal
†$p < 0.05$ vs. isoproterenol

The present investigation is aimed to evaluate and explore the cardioprotective effect of black cumin, seeds of *Nigella sativa* on isoproterenol induced myocardial infarction in rats. Myocardium contains an abundant concentration of diagnostic marker enzymes of myocardial infarction viz., circulating lipids, CK, LDH, trans-aminases and once metabolically damaged, release of its content into the extra cellular fluid serves as the diagnostic markers of myocardial tissue damage.[34]

Isoproterenol is well known cardiotoxic agent due to its ability to destruct myocardial cells. Its administration brought about a significant decrease in the activities of cardiac marker enzymes, which leads to subsequent increase in the activities of these enzymes in the serum. This might be due to the damage in the heart tissue, rendering the leakage of enzymes into the serum.[35] The release of cellular enzymes reflects the alterations in plasma membrane integrity and/or permeability as a response to β-adrenergic stimulation. Acute β-adrenergic receptor stimulation not only rapidly generates reactive oxygen species, but also down regulates copper-zinc superoxide dismutase enzyme activity, protein and mRNA and reduces glutathione level, leading to the loss of membrane integrity, inducing heart contractile dysfunction and myocyte toxicity finally producing myocardial necrosis.[36] The enzymes such as AST, ALT, CK and LDH serve as sensitive indices to assess the severity of myocardial infarction.[37]

In our study we observed significant increase in the level of marker enzymes (AST, ALT, CK and LDH) in serum of isoproterenol treated rats, which is in line with previous report.[38] It is noticeable that isoproterenol induced rats showed increased levels of AST and ALT when compared to control rats. This finding could be a consequence of a reduction in the number of viable myocytes due to enhanced cell death in heart, as these animals showed the highest levels of AST, ALT, LDH and CK.[39] Treatment with black cumin (150 mg/kg) decreased the levels of these marker enzymes clearly pointed out that black cumin could be highly cardio-protective against the MI. Our previous findings also supported that black cumin have the cardioprotective activity against isoproterenol induced MI due to its strong antioxidant property.[22]

We also observed a marked decline activity of CK-MB in serum of isoproterenol treated rats. CK-MB is mainly found in the heart muscle, where more than 20% of the total CK activity is present as CK-MB, and nearly 80% as CK-MM. CK-MB leaks out from myocardium due to disintegration of the contractile apparatus & increased sarcoplas-mic permeability leads to increased concentration in the serum and always detectable in patients with recent infarcts.[41] Previous reports showed that in isoproterenol induced rats there is marked rise in lipid peroxidation with concomitant fall in myocardial CK-MB activity.[42]

The decreased level of CK-MB in myocardium also confirms the injured state of myocardium peroxidation with concomitant fall in myocardial CK-MB activity.[42] The decreased level of CK-MB in myocardium also confirms the injured state of myocardium. Treatment with black cumin (150 mg/kg) showed decreased activity of CK-MB isoenzyme in serum suggesting the prevention of myocardial injury.

Lipid metabolism plays an important role in myocardial necrosis produced by ischemia.[43] In our study we observed increased levels of total CH and LDL, decreased levels of HDL in isoproterenol treated rats. Our results are in line with previous findings.[44] An increase in concentration of total cholesterol and LDL cholesterol, and a decrease in HDL cholesterol are associated with raised risk of myocardial infarction.[45] There is a growing body of evidence from epidemiologic, clinical and laboratory data indicating that elevated triglyceride levels are an independent risk factor for cardiovascular disease.[46] Hyperglyceridemic patients at a risk for cardiovascular disease often develop a lipoprotein profile characterized by elevated triglyceride, dense LDL, and low HDL cholesterol which causes myocardial membrane damage.[47] Treatment with black cumin (150 mg/kg) prevented the elevation of triglycerides, cholesterol and LDL in serum, signifying that the myocardial membrane is intact and not damaged. Our previous study also supported that black cumin have strong antioxidant activity against isoproterenol induced MI thereby restore the normal levels of antioxidant enzymes and decreased the reactive oxygen species.[22]

The treatment of black cumin (150 mg/ kg body weight) showed significant reduction in isoproterenol induced elevated serum marker enzymes. This is probably due to the protective effect of the black cumin on the myocardium; this reduced the extent of myocardial damage and thereby restricted the leakage of these enzymes from the myocardium. In our study, we found that black cumin protected myocardium from isoproterenol induced myocardial functional and structural injury via near normalization levels of diagnostic marker enzymes. The observed myocardial protective effect of black cumin could be due to its protective activity in the presence of phytoconstituents such as thymoquinone, dithymoquinone, thymohydroquinone, thymol, carvacrol, tanethole and 4-terpineol.[41]

In conclusion, the results of the present study indicated that the prior administration of the black cumin attenuates isoproterenol induced MI. The cardioprotective effect of the black cumin is probably related to its ability to strengthen the myocardial membrane by its membrane stabilizing action.[48] Further research is needed to purify and identify the specific bioactive compounds that are responsible for the cardioprotective action of black cumin seeds.

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