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Nigella sativa protects against isoproterenol-induced myocardial infarction by alleviating oxidative stress, biochemical alterations and histological damage

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ABSTRACT

Objective: To evaluate the cardioprotective effect of *Nigella sativa* L. (*N. sativa*) in isoproterenol-induced myocardial infarction (MI).

Methods: Groups were treated with different doses of ethanol extract of *N. sativa* (EENS) and *N. sativa* oil alone and along with enalapril for 28 days. MI was induced by subcutaneous administration of isoproterenol (85 mg/kg) in two consecutive doses. Levels of cardiac biomarkers and antioxidant enzymes such as creatine kinase–*N*-acetyl-L-cysteine, lactate dehydrogenase, aspartate aminotransferase, malondialdehyde, superoxide dismutase, reduced glutathione and catalase were evaluated along with gross histopathological examination.

Results: Isoproterenol (85 mg/kg) induced MI by causing the significant ($P < 0.01$) reduction in the activity of cardiac biomarkers (creatine kinase–*N*-acetyl-L-cysteine, lactate dehydrogenase, aspartate aminotransferase) and antioxidant markers (superoxide dismutase, catalase, glutathione) along with significant ($P < 0.01$) increase in the level of malondialdehyde. Furthermore, histopathological evaluation also confirmed the isoproterenol-induced MI. Pretreatment with EENS (800 mg/kg) and combination of EENS (800 mg/kg) with enalapril (1 mg/kg) significantly ($P < 0.01$) prevented the development of these alteration and restored activity of cardiac biomarkers as well as antioxidant markers almost near to normal levels. Histopathological evaluation of cardiac tissue further confirmed the restoration of biochemical activity.

Conclusions: Experimental findings thus indicate that EENS (800 mg/kg) demonstrated cardioprotective effect against isoproterenol-induced MI by restoring cardiac biomarkers and antioxidant status.

1. Introduction

Oxidative stress causes an imbalance between the production of reactive oxygen species and the efficacy of the cellular antioxidant defense system, leading to an altered redox status which contributes to endothelial dysfunction and apoptosis [1–3]. Earlier studies have demonstrated that during ischemic injury, oxidative stress produced by the generation of reactive oxygen species plays a critical role in the development of myocardial infarction (MI) [4,5]. Several researchers have reported the correlation of free radicals with endothelial injury, dysfunction and cardiovascular disease progression [6,7].

Isoproterenol is a chemically synthesized catecholamine and β-adrenergic agonist, which causes severe stress to the myocardium leading to infarct-like necrosis [8,9]. Catecholamine generates free radicals that induce cardiotoxicity [10]. Compromised antioxidant resistance leads to metabolic and contractile impairments, and alteration in the membrane permeability consequent to lipid peroxidation and irreversible damage to the myocardial membrane [11–13].

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All experimental schedules involving animals were conducted in accordance to Committee for the purpose of Control and Supervision on Experiments on Animals (CPCSEA) and approved by the Institutional Animal Ethics Committee (IAEC) (project no#570), Jamia Hamdard (Hamdard University), New Delhi.

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The seeds of *Nigella sativa* L. (Ranunculaceae) (*N. sativa*) commonly known as black seed or black cumin, are used in herbal medicine for the treatment and prevention of a number of diseases like bronchitis, asthma, diarrhea, rheumatism and skin disorders and to support immune system. The seeds contain oils (fixed and essential), proteins, alkaloids and saponins. Biological activities of *N. sativa* are due to thymoquinone, a major constituent of essential oils, which is also present in the fixed oils. *N. sativa* seeds have been reported to show anticancer, antioxidant, gastroprotective, analgesic and anti-inflammatory effects [14–17]. A recent study has shown that the *N. sativa*-treated rats developed a moderate but significant cardiomyocyte hypertrophy due to increase in heart weight to body weight ratio. This cardiomyocyte hypertrophy was associated with an increase in the baseline cardiac inotropic properties [18]. *N. sativa* oil has also been reported to reduce the cyclosporine-A injury in rat's myocardium, which is demonstrated by normal cardiac histopathology, decreased lipid peroxidation, improved antioxidant enzyme status and cellular protein oxidation [19]. Enalapril, an orally active angiotensin-converting enzyme inhibitor, is an ester prodrug. Since, it significantly decreases blood pressure and also improves heart failure symptoms [20,21] and has antioxidant activity [22], it is selected as a standard drug for comparison in this study. The present study was designed to evaluate the effects of different doses of ethanol extract of *N. sativa* (EENS) in isoproterenol-induced MI in Wistar rats.

2. Materials and methods

2.1. Plant extract

N. sativa seeds were purchased from Khari Baoli (a street in Delhi, India, known for its Asia's largest wholesale spice market selling all kinds of herbal drugs). *N. sativa* seeds were crushed into a moderately coarse powder using pestle and mortar. Powdered seeds were (500 g) extracted with ethanol (80%) in a Soxhlet apparatus for 72 h. A semisolid extract was obtained after complete removal of alcohol under reduced pressure, using vacuum rotary evaporator at 40 °C. The yield value of extract was found to be 26% (w/w). The extract was stored in refrigerator, until used. The extract was suspended in 5% carboxymethyl cellulose in normal saline just before oral administration.

2.2. Animals

All experimental schedule involving animals was conducted in accordance to Committee for the purpose of Control and Supervision on Experiments on Animals (CPCSEA) and approved by the Institutional Animal Ethics Committee (IAEC) (project no#570), Jamia Hamdard (Hamdard University), New Delhi. Albino rats (Wistar strain) of either sex, weighing 200–250 g were procured from the Central Animal House Facility, Jamia Hamdard, New Delhi. The animals were kept in polypropylene cages (6 in each cage) under standard laboratory conditions (12 h light and 12 h dark cycle) and had a free access to commercial pellet diet (Amrut Rat Feed, Nav Maharashtra Chakan Oil Mills Ltd., New Delhi, India) and drinking water *ad libitum*. The animal house temperature was maintained at (25 ± 2) °C.

2.3. Chemicals

Oil of *N. sativa* was purchased from Mohammedia products (Andhra Pradesh). Isoproterenol and enalapril were purchased from Sigma Chemicals, St. Louis, Missouri, USA. All other chemicals were of analytical grade obtained from SD Fine Chemicals, India. Double distilled water was used for all procedures.

2.4. Induction of experimental MI

Weighed amount of isoproterenol was freshly prepared in distilled water at the time of induction of MI. Isoproterenol (85 mg/kg) was injected via *s.c.* route in rats for two consecutive days *i.e.* on the 27th and 28th days respectively with 24 h interval to induce MI [9].

2.5. Experimental protocol

A total of 48 animals were used for this study. Rats were randomly divided into eight groups with six rats in each group. Group I (normal control rats) received physiological saline solution with 0.5% carboxy methylcellulose (1 mL/day) orally for 28 days and on the 27th and 28th days, 0.1 mL physiological saline was given *s.c.* at 24 h interval. Group II served as toxic group; rats received physiological saline solution with 0.5% carboxy methylcellulose (1 mL/day) orally for 28 days and on the 27th and 28th days, isoproterenol (85 mg/kg) was given *s.c.* at 24 h interval. Rats in Groups III and IV received EENS (400 and 800 mg/kg/day) orally for 28 days and on the 27th and 28th days, isoproterenol (85 mg/kg) was given *s.c.* at 24 h interval. Rats in Group V received *N. sativa* oil (2.5 mL/kg/day) orally for 28 days and on the 27th and 28th days, isoproterenol (85 mg/kg) was given *s.c.* at 24 h interval. Group VI (standard control rats) received enalapril (1 mg/kg/day) orally for 28 days and on the 27th and 28th days, isoproterenol (85 mg/kg) was given *s.c.* at 24 h interval. Rats in Group VII received EENS (800 mg/kg/day) in combination with enalapril (1 mg/kg/day) orally for 28 days and on the 27th and 28th days, isoproterenol (85 mg/kg) was given *s.c.* at 24 h interval. Group VIII served as *per se* group; rats received only EENS (800 mg/kg/day) orally for 28 days.

2.6. Biochemical parameters

Twenty-four hours after the last dose, blood samples were collected from the tail vein, and serum was separated. Then, all the animals were sacrificed under light ether anesthesia and hearts were dissected out for the estimation of following parameters: lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and creatine kinase–*N*-acetyl-L-cysteine (CK–NAC) which were estimated as biomarkers of myocardial damage. These biomarkers were estimated as leaflet instruction supplied by manufactured company. Cardiac malondialdehyde (MDA) levels were estimated as a marker of lipid peroxidation by the method of Okhawa *et al.* [23] and antioxidant enzymes were measured by evaluating the levels of catalase (CAT) [24], superoxide dismutase (SOD) [25] and reduced glutathione (GSH) [26]. Protein levels were estimated by the method of Lowry *et al.*, by using bovine serum albumin as a standard [27].

2.7. Histopathological studies

Myocardial tissues were fixed in 10% formalin, routinely processed and embedded in paraffin wax. Paraffin section (5 μm) was cut on glass slides and stained with hematoxylin and

eosin (H&E), and examined under a light microscope by pathologist, blinded to the study groups.

2.8. Statistical analysis

Statistical analysis was carried out using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA). Data were expressed as mean ± SEM for six rats per group. Groups of data were compared with the ANOVA followed by Dunnett's *t*-test. Values were considered statistically significant when $P < 0.05$.

3. Results

3.1. Effects of EENS on MDA level

Figure 1 shows that the levels of MDA significantly increased in toxic group as compared to the normal control group. Pretreatment with EENS (800 mg/kg) significantly ($P < 0.05$) decreased the levels of MDA in cardiac tissues to (0.197 ± 0.038) nmol MDA/mg protein as compared to the toxic group. Combination treatment with EENS (800 mg/kg) and enalapril (1 mg/kg) in isoproterenol-induced MI showed more significant ($P < 0.01$) reduction in the elevated level of MDA. Pretreatment with EENS (400 mg/kg) did not produce any significant ($P > 0.05$) effect on MDA levels as compared to the toxic group. Pretreatment with EENS (800 mg/kg) significantly ($P > 0.05$) increased MDA level as compared to EENS (400 mg/kg). There was no significant difference between the effects of EENS (800 mg/kg) and enalapril (1 mg/kg) treatment.

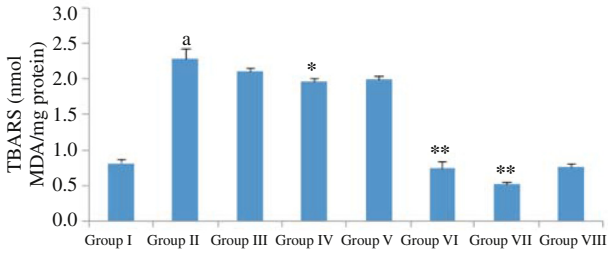

Figure 1. Effect of EENS on the levels of MDA in the cardiac tissues. Values are expressed as mean ± SEM from a group of six animals. [a]: $P < 0.05$ when compared with control; [*]: $P < 0.05$, [**]: $P < 0.01$ when compared with toxic group.

3.2. Effects of EENS on GSH level

Figure 2 shows that isoproterenol (85 mg/kg) treatment significantly ($P < 0.01$) decreased GSH levels in myocardial tissues to (3.010 ± 0.116) μmol/g of wet tissue compared to the normal control group. Pretreatment with EENS (800 mg/kg) significantly ($P < 0.01$) increased the levels of GSH to (6.620 ± 0.077) μmol/g of wet tissue as compared to isoproterenol group. Also, the combination treatment of EENS (800 mg/kg) with enalapril (1 mg/kg) significantly ($P < 0.01$) increased the levels of GSH to near normal levels. Pretreatment with EENS (400 mg/kg) non-significantly ($P > 0.01$) increased the levels of GSH as compared to the toxic group. EENS (800 mg/kg) significantly ($P < 0.01$) increased the levels of GSH as compared to EENS (400 mg/kg). Pretreatment with *N. sativa* oil non-significantly ($P > 0.01$) changed the level of GSH as compared to the toxic group. *Per se* group showed no significant changes in terms of GSH level as compared to the normal control group.

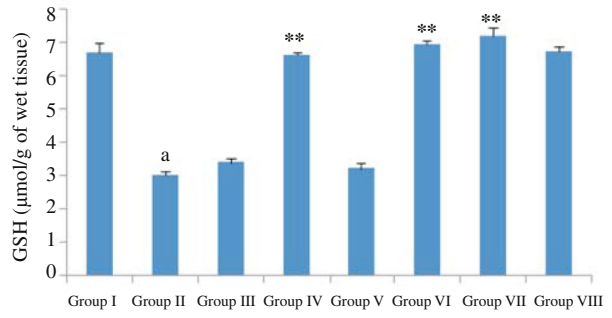

Figure 2. Effect of EENS on the levels of GSH in cardiac tissues. Values are expressed as mean ± SEM from a group of six animals. [a]: $P < 0.05$ when compared with control; [**]: $P < 0.01$ when compared with toxic group.

3.3. Effects of EENS on SOD activity

Isoproterenol (85 mg/kg) treatment group significantly ($P < 0.01$) decreased the SOD activity in heart tissues to (4.29 ± 0.24) μmol/g of wet tissue compared to the control group (Figure 3). Pretreatment with EENS (800 mg/kg) significantly ($P < 0.05$) increased the activity of SOD as compared to the isoproterenol control group. Pretreatment with EENS (400 mg/kg) non-significantly ($P > 0.05$) changed the activity of SOD as compared to the isoproterenol control group. EENS (800 mg/kg) significantly ($P < 0.05$) increased the activity of SOD as compared the EENS (400 mg/kg). Pretreatment with combination of dose of EENS (800 mg/kg) and enalapril (1 mg/kg) significantly ($P < 0.01$) increased the activity of SOD in cardiac tissue to (7.17 ± 0.35) μmol/g of wet tissue as compared to the isoproterenol control group. *Per se* group showed no significant changes in the activity of SOD as compared to the normal control group.

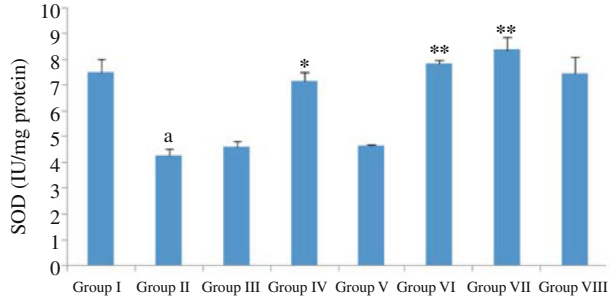

Figure 3. Effect of EENS on the activity of SOD in cardiac tissues. Values are expressed as mean ± SEM from a group of six animals. [a]: $P < 0.05$ when compared with control; [*]: $P < 0.05$, [**]: $P < 0.01$ when compared with toxic group.

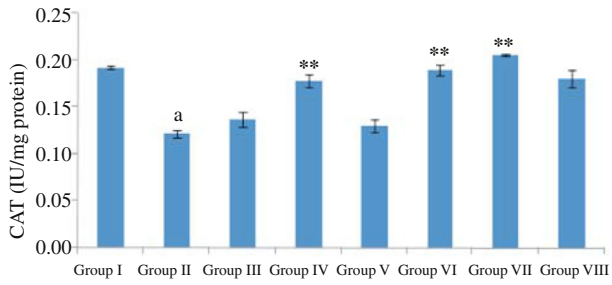

Figure 4. Effect of EENS on the activity of CAT in cardiac tissues. Values are expressed as mean ± SEM from a group of six animals. [a]: $P < 0.05$ when compared with control; [**]: $P < 0.01$ when compared with toxic group.

3.4. Effects of EENS on CAT activity

Figure 4 shows that the activity of CAT in cardiac tissues of normal control rats was found to be (0.192 ± 0.002) IU/mg of protein. Isoproterenol (85 mg/kg) treatment significantly ($P < 0.01$) reduced the level of CAT in cardiac tissue to (0.121 ± 0.004) IU/mg protein as compared to the normal control group. Pretreatment with EENS (800 mg/kg) and combined treatment with EENS (800 mg/kg) and enalapril (1 mg/kg) significantly ($P < 0.01$) increased the levels of CAT in cardiac tissue to (0.178 ± 0.007) IU/mg protein as compared to toxic group. Pretreatment with EENS (400 mg/kg) non-significantly ($P > 0.05$) changed the activity of CAT in cardiac tissue as compared the toxic group. Pretreatment with EENS (800 mg/kg) non-significantly ($P > 0.05$) changed the activity of CAT in cardiac tissue as compared to group treated with combination of dose of *N. sativa* and enalapril (800 mg/kg + 1 mg/kg) and enalapril (1 mg/kg) respectively. *Per se* group showed no significant changes in the activity of CAT as compared to the normal control group.

3.5. Effects of EENS on CK–NAC, LDH and AST activity

Figure 5 shows the significant ($P < 0.01$) elevation of the levels of CK–NAC, LDH and AST in toxic control group as compared to the normal control group. Pretreatment with EENS (800 mg/kg)

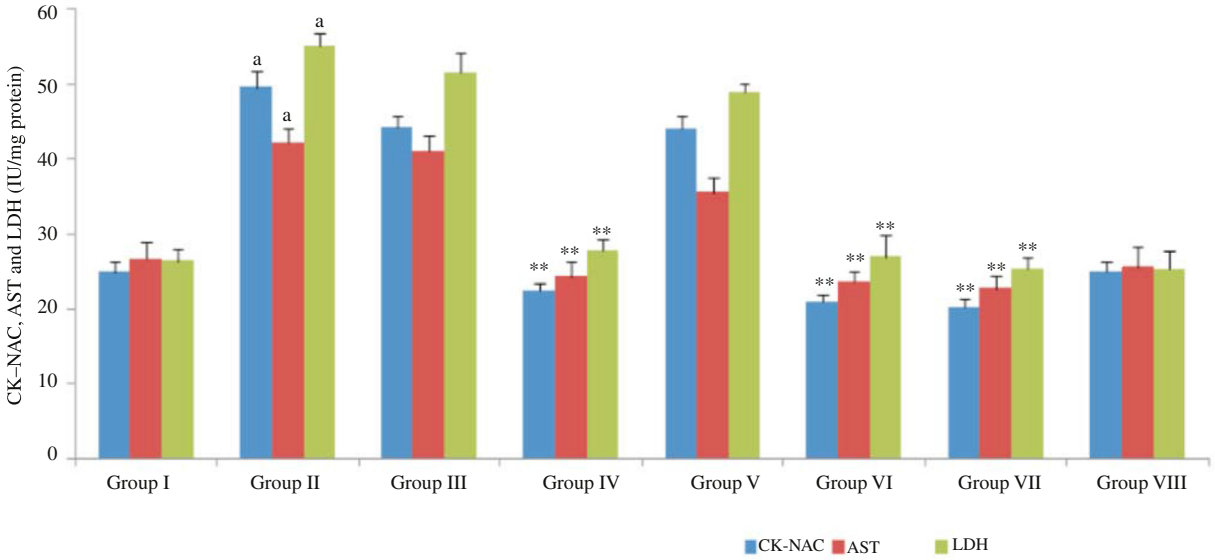

Figure 5. Effect of EENS on the activities of CK–NAC, AST and LDH in serum.
Values are expressed as mean ± SEM from a group of six animals. [a]: $P < 0.05$ when compared with control; [**]: $P < 0.01$ when compared with toxic group.

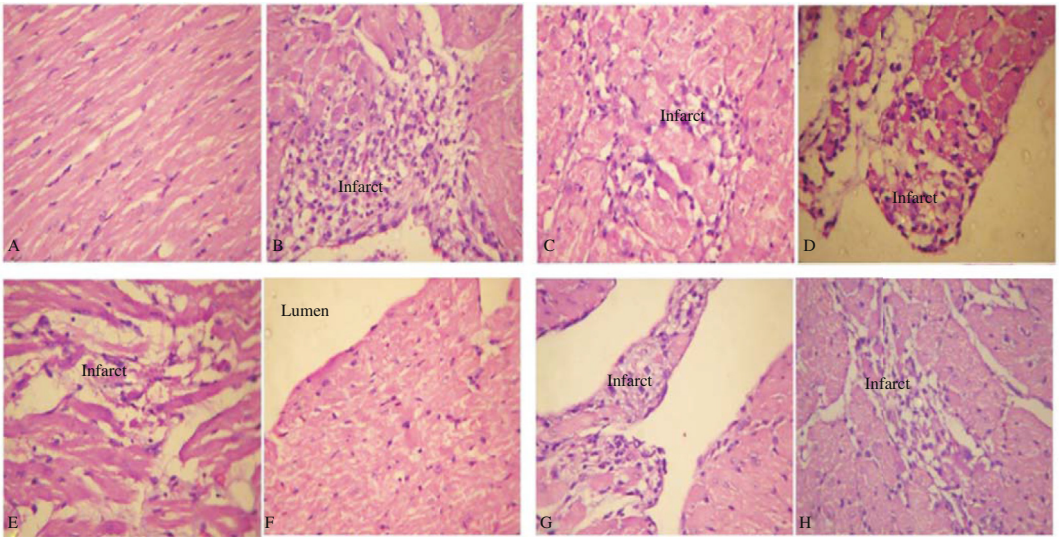

Figure 6. High power photomicrograph (H&E, 400×) of ventricle wall's of rat.
A: Normal control group (normal cardiac muscle fibers with striation in cytoplasm and elongated nuclei in animal of control group); B: Toxic control (extensive loss of muscle fibers, several inflammatory cells and undamaged muscle fibers in the surrounding area in animal that received isoproterenol) (85 mg/kg, *s.c.*); C: Loss of muscle fibers and scattered inflammatory cells in the infracted area in animal that received *N. sativa* extract (400 mg/kg, *p.o.*); D: Loss of muscle fibers and scattered inflammatory cells in animal that received *N. sativa* (800 mg/kg, *p.o.*); E: Loss of muscle fibers and scattered inflammatory cell in animal that received enalapril (1 mg/kg, *p.o.*); F: Muscle fibers with striation in cytoplasm and elongated nuclei in *per se* group; G: Loss of muscle fibers, scattered inflammatory cells and isolated residual muscle fibers in animal that received *N. sativa* oil (2.5 mL/kg, *p.o.*); H: Loss of muscle fibers and few inflammatory cells in animal that received *N. sativa* extract (800 mg/kg, *p.o.*) + enalapril (1 mg/kg, *p.o.*).

and co-administration of EENS (800 mg/kg) with enalapril (1 mg/kg) significantly restored the level of these cardiac markers as compared to the toxic group. Pretreatment with enalapril (1 mg/kg) significantly ($P < 0.01$) increased the level of these cardiac markers as compared to the toxic control group. Pretreatment with *N. sativa* oil non-significantly ($P > 0.05$) changed the level of these cardiac markers as compared to the toxic group. Pretreatment with EENS (800 mg/kg) non-significantly ($P > 0.05$) increased the level of these cardiac markers as compared to the EENS (400 mg/kg). There were no significant changes in the level of these cardiac markers in *per se* group compared to the normal control group.

3.6. Histopathological study

The histopathological evaluation of H&E stained sections of myocardial tissue (Figure 6) from toxic group showed the inflammatory changes, edema and necrosis indicating the isoproterenol induced damage to the cardiac tissue. Table 1 shows myocardial score of experimental group. Amongst the pretreatment groups with EENS (400 and 800 mg/kg) and *N. sativa* oil (2.5 mL/kg), the best results were achieved in the EENS (800 mg/kg) group. Histopathological analysis showed that best protection against isoproterenol-induced (85 mg/kg) MI was achieved by enalapril (1 mg/kg), and combination with EENS (800 mg/kg) and enalapril (1 mg/kg).

Table 1
Effect of EENS on histopathological change in experimental group.

Group	Inflammatory cells	Edema	Necrosis
Group I	–	–	–
Group II	+++	++	++
Group III	++	++	++
Group IV	–	–	–
Group V	++	++	++
Group VI	–	–	–
Group VII	–	–	–
Group VIII	–	–	–

–: No changes; ++: Moderate changes; +++: Marked changes.

4. Discussion

Seeds of *N. sativa* have long been used as a traditional medicine for the treatment and prevention of a number of ailments. Thymoquinone is the active constituent of *N. sativa*, which possesses strong antioxidant action. This property of thymoquinone has been reported in several studies [28,29]. Enalapril also possess free radical scavenger and antioxidant properties [22,30]. Enalapril has been shown to have potential effects on the cardiovascular system through reduction in mean arterial pressure, preload, and afterload which contribute towards preservation of cardiac contractility [31]. Because of the above properties of enalapril, it has been used as standard drug in this study. In this study, isoproterenol caused MI through the abnormalities in the myocardial contraction and generation of free radical. Enalapril might have produced their effect by downregulation of free radical generation in isoproterenol-induced MI. *N. sativa* has been reported to possess excellent antioxidant activity by reducing the elevated MDA concentrations, increasing the lowered GSH and SOD content in heart cell [15,32] and the effect of *N. sativa* on isoproterenol-induced MI in rat has not been yet reported in literature. The present study was designed to evaluate the cardioprotective effects of EENS on isoproterenol-induced MI in rats.

Thiobarbituric acid reactive substances are breakdown product of lipids; also MDA is a measure of lipid peroxidation in the tissues. Increase in the levels of MDA in heart tissue of rats treated with isoproterenol indicated increased lipid peroxidation. The inhibition of elevation in the levels of MDA in heart tissue of rats pretreated with *N. sativa* (800 mg/kg) and enalapril suggests an inhibitory effect of the drugs on lipid peroxidation.

GSH is one of the important non-enzymatic defenses, essential for maintaining cell integrity because of its reducing properties [33]. Its key function is to serve as the reductant of toxic peroxides and it also helps in keeping the enzymes in an active state, by preventing the conversion of sulfhydryl group to disulfide group. Deficiency or depletion of this enzyme causes damage to the macromolecules or membranes. The deficiency of GSH caused by isoproterenol indicates its interaction with biomembrane and subsequent peroxidizing action. The inhibitory effect on depletion of GSH levels by the pretreatment of *N. sativa* (800 mg/kg) and enalapril (1 mg/kg) in rats revealed the protective effect of these drugs on isoproterenol-induced myocardial necrosis and this effect may be due to the antioxidant property of these drugs.

SOD and CAT are important enzymes for maintaining cell integrity because of their reducing properties. Reduction in myocardial SOD and CAT activities strongly suggests overwhelming superoxide radical generation and hydrogen peroxide formation following isoproterenol administration [34]. The inhibitory effect on depletion of SOD and CAT levels by pretreatment of rats with EENS (800 mg/kg) and enalapril (1 mg/kg) revealed the protective effect of these drugs on isoproterenol-induced MI.

Injury to the myocardium causes release of cardiac enzyme such as CK–NAC, AST and LDH from the injured heart muscle cell into the blood stream. In this study, elevated levels of CK–NAC, LDH and AST in isoproterenol-treated rats were found, which was also confirmed by the previous study [9]. Pretreatment of rats with EENS (800 mg/kg) and enalapril significantly ($P < 0.01$) reduced the elevated level of CK–NAC, AST and LDH, indicating the possible cardioprotective effect of drugs.

Isoproterenol-induced MI is also manifested by altered histopathological features including marked interstitial edema, necrosis, focal cytoplasmic vacuolation, focal myocardial fibrosis, disorganization of myocardium of myofibrillar loss. The normal control and *per se* (800 mg/kg) group showed normal cardiac tissue. The samples from the groups pretreated with *N. sativa* (800 mg/kg) and combination with the *N. sativa* (800 mg/kg) and enalapril (1 mg/kg) showed a normal myocardium and no evidence of focal cytoplasmic vacuolation and necrosis.

The results indicated a protective effect of the EENS in 800 mg/kg doses similar to that seen in the enalapril (1 mg/kg) group, but more protection against isoproterenol-induced (85 mg/kg) myocardial necrosis was achieved by the enalapril (1 mg/kg) and EENS combinations. Amongst the groups treated with isoproterenol (85 mg/kg) and *N. sativa* extract or *N. sativa* oil, the best results were achieved in the EENS (800 mg/kg) group. The normal control and *per se* (800 mg/kg) groups showed normal heart muscle while the toxic control group showed a large infarction of the heart muscle. Thus, our findings indicate that EENS (800 mg/kg) has cardioprotective effect against isoproterenol-induced MI model in rats.

Our study suggests that EENS (800 mg/kg, *p.o.*) significantly protected rat against isoproterenol-induced MI. The mechanism of prohibition might have restored lipid peroxidation levels, myocardial endogenous antioxidants and cardiac biomarker enzymes. Furthermore, EENS (800 mg/kg, *p.o.*) also preserved the cardiac tissue which was established by histopathological study.

Conflict of interest statement

We declare that we have no conflict of interest.

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