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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