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EVALUATION OF CARDIOPROTECTIVE EFFICACY OF *NELUMBO NUCIFERA* LEAF EXTRACT ON ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION IN WISTAR RATS

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ABSTRACT

Intake of diets rich in antioxidant from medicinal plants have been reported to be associated with reduced risk of cardiovascular diseases. The present study was aimed to appraise the protective role of *Nelumbo nucifera* leaf extract in isoproterenol-induced myocardial infarction in rats. Subcutaneous injection of isoproterenol (85 mg/kg) to male albino Wistar rats, exhibited a significant raise in the levels/activities of cardiac marker such as cardiac troponin T, creatine kinase-MB, creatine kinase, lactate dehydrogenase, asparate transaminase, alanine transaminase in serum with subsequent decrease in the heart. Isoproterenol-induced rats also showed a significant increase in the levels of blood glucose, serum urea, uric acid, creatinine and homocysteine and decrease in plasma protein. Pretreatment with *Nelumbo nucifera* (100, 200 and 400 mg/kg) daily for a period of 21 days positively altered the activities of cardiac markers and other biochemical parameters to isoproterenol-induced rats. Thus, the results of our study demonstrate that *Nelumbo nucifera* possess cardioprotective role against experimentally induced cardiac toxicity.

KEYWORDS

Myocardial infarction, Isoproterenol, *Nulumbo nucifera*, Marker enzymes, Medicinal plants.

INTRODUCTION

Myocardial infarction (MI) occurs due to the interruption of blood supply to part of the heart, leading to destruction of cardiac cells. This is due to blockage of coronary arteries following the rupture of an atherosclerotic plaque in the wall of arteries. Catecholamines cause deleterious effect on heart, which is associated with structural, functional and biochemical alterations[1]. Isoproterenol (ISO), a synthetic catecholamine and β-adrenergic agonist, causes necrosis of rat heart muscle[2]. ISO-induced MI serves as a well standardized model to study the beneficial effects of many drugs and cardiac function.

Nowadays research has been focused on medicinal plants and food products derived from medicinal plants that have been found to have certain preventive measures in the treatment of cardiovascular disease (CVD). *Nelumbo nucifera* has been reported to treat obesity[3], hepatotoxicity, arrhythmia[4] and hyperlipidemia. *Nelumbo nucifera* seeds are commonly used as folk remedy in the treatment of tissue inflammation, cancer, antiemetic, and given to children as diuretic and refrigerant[5]. It is also used as a cooling medicine for skin diseases, leprosy and considered as antidote to poison[6]. Traditionally, leaves are used to treat diarrhea fever and inflammatory skin conditions. Young leaves are taken with sugar to treat rectal prolapse; useful in many varieties of raktapitta, or bleeding disorders. It alleviates thirst and inflammations and to promote strength, virility, and intellect[7-9].

This study was undertaken to assess the efficacy of *Nelumbo nucifera* leaves extract (NNE) on cardiac troponin T (cTnT), cardiac marker enzymes such as creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), aspratate transaminase (AST) and alanine transaminase (ALT), glucose, urea, uric acid, creatinine and protein in normal and ISO-induced MI in rats.

MATERIALS AND METHODS

Experimental animals

Adult male albino rats of Wistar strain weighing 150-200 g were purchased from Venkateswara Enterprises, Bangalore, India. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethical Committee of Vinayaka Missions University (IAEC NO : P.Cog-1/06). They were housed in polypropylene cages (47x34x20 cm) lined with husk, renewed every 24 h under a 12:12 h light/dark cycle at around 22˚C and had free access to tap water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Maharashtra, India). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fibre, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% nitrogen free extract (carbohydrates). The diet provided metabolisable energy of 3, 600 kcal.

Drugs

Leaves of *Nelumbo nucifera* were purchased from local market, Chennai, Tamilnadu, India, and were authenticated by National Institute of Herbal Science Plant Anatomy Research Centre, West Tambaram, Chennai, Tamilnadu, India (Authentication No: PARC/2010/596).

Extract preparation

Dried leaves of *Nelumbo nucifera* were coarsely powdered and 1 kg of this powdered plant material was extracted with the help of the soxhlet apparatus using methanol as a solvent. The solvent from the methanolic extract was removed under vacuum distillation; dried material (brown colored, yield 11.25% w/w with respect to dry starting material) was kept in a desiccators. This methanolic extract was dissolved in distilled water for further experiments.

Chemicals

Isoproterenol hydrochloride, methanol, pyruvate, alpha-ketogultarate, aspartate were purchased from Sigma Chemical Company, St. Louis, MO, USA. All other biochemicals and chemicals used in the study were of analytical grade.

Induction of experimental myocardial infarction

Isoproternol (85 mg/kg) was dissolved in normal saline and injected subcutaneously to rats at an interval of 24 hours for 2 days[10].

Experimental design

A total number of 36 rats were used in the experiment, 6 rats of 6 groups.

Group 1	Normal control rats
Group 2	Normal rats + NNE (400 mg/kg)
Group 3	ISO control rats
Group 4	NNE (100 mg/kg) + ISO
Group 5	NNE (200 mg/kg) + ISO
Group 6	NNE (400 mg/kg) + ISO

NNE was dissolved in distilled water and administered to rats orally for a period of 21 days. At the end of the experimental period, after 12 h of second ISO-injection, all the rats were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and sacrificed by cervical decapitation. Blood was collected and plasma & serum were separated and used for various biochemical estimations. The heart tissue was excised immediately from the animals, washed off blood with ice-chilled physiological saline and used for further biochemical estimations. A known weight of the heart tissue was homogenized in appropriate buffer solution. The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

Biochemical assays

The level of cTnT in serum was estimated using standard kit by electro chemiluminescence immunoassay (Roche Diagnostics, Switzerland). Creatine kinase-MB activity was assayed using a commercial kit obtained from Agappe Diagnostics, Kerala, India. Activities of cardiac marker enzyme CK was assayed[11]. The reaction mixture contained 0.05 ml of serum, 0.1 ml of substrate, 0.1 ml of ATP solution and 0.1 ml of cysteine–hydrocholoride solution. The final volume was made up to 2.0 ml and incubated at 37°C for 30 minutes. The reaction was arrested by the addition of 1.0 ml of TCA and the contents subjected to centrifugation. 0.1 ml of the supernatant was made upto 4.3 ml with water. 1.0 ml of ammonium molybdate reagent was added incubated at room temperature for 10 minutes. 0.4 ml of ANSA was added and the color developed was read at 640 nm after 20 minutes.

Activity of Lactate dehydrogease (LDH) was assayed by Nieland method[12]. To a set of tubes, 1 ml of the buffered substrate and 0.1 ml of sample was added and the tubes were

incubated at 37°C for 15 min. After adding 0.2 ml of NAD solution, the incubation was continued for another 15 min. The reaction was then arrested by adding 1ml of DNPH reagent and the tubes were incubated for further period of 15 min at 37°C. 0.1 ml of serum was added to blank tubes after arresting the reaction with DNPH. 7 ml of sodium hydroxide solution was added and the color developed was measured at 420 nm. Activities of AST and ALT were assayed[13]. In different tubes, 1 ml of the buffered substrate was added to 0.1 ml of sample and incubated at 37°C for 30min. Then 1 ml of DNPH reagent was added to arrest the reaction. To the blank tubes, 0.1 ml of sample was added only after the addition of DNPH reagent. The tubes were kept aside for 15 min, and then 10 ml of sodium hydroxide was added and read at 520 nm.

The levels of blood glucose were determined[14]. 0.1 ml of blood was added to 1 ml of 0.05 M sodium hydroxide. Then 0.1 ml of 10% zinc sulphate was added and mixed well and centrifuged. To 0.2 ml of the supernatant, 4.0 ml of the enzyme-dye reagent was added. The tubes were placed in a water bath at 37°C for 45 min. The colour developed was read at 430 nm. The levels of urea were determined by the method of Geyer and Dabich[15]. 0.2 ml of sample was deproteinised with 2.8 ml of TCA. To 2 ml of the supernatant obtained by centrifugation, 1 ml of DAM-TSC reagent and 1.5 ml of acid ferric reagent were added and the solution was heated in a boiling water bath for 15 minutes. Aliquots of standard urea and blank containing 2 ml water were also treated in a similar manner. After cooling, the color developed was read at 520 nm. Creatinine was estimated by the method of Slot[16]. To 3 ml of deproteinized supernatant (0.1 ml of serum + 3.9 ml 10% TCA), 2 ml of alkaline picrate solution was added. Blank containing 3 ml of water and aliquots of standard in 3 ml of water were also treated in a similar manner. After 30 minutes the colour was measured at 520 nm.

The levels of protein were estimated by Lowery et al[17]. 0.5 ml of tissue homogenate was precipitated with 0.5 ml of 10% TCA, centrifuged for 10 min and the precipitate was dissolved in 1.0 ml of 0.1 N NaOH. 0.1 ml of aliquot was taken and made upto 1.0 ml with distilled water. Then, 4.5 ml of alkaline copper reagent was added and allowed to stand at room temperature for 10 min. After incubation, 0.5 ml of Folin's-Ciocalteau reagent was added and the blue colour developed was read at 620 nm after 20 min. Uric acid in serum was estimated using a commercial kit (Qualigens Diagnostics, Mumbai, India). The axis homocysteine enzyme immunoassay was used for the quantitative determination of total L-homocysteine.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using SPSS software package 9.05. Results were expressed as mean ± S.D. from 6 rats in each group. *P* values <0.05 were considered as significant.

RESULTS

Tables 1-3 represent the effect of *Nulumbo nucifera* leaf extract (NNE) on the levels of cTnT, and the activities of serum CK-MB, CK, LDH, AST and ALT in serum and heart in normal and ISO-induced rats. Rats induced with ISO, showed a significant increase in the levels and the activities of these cardiac enzymes in serum and a significant decrease in the activities of these enzymes in the heart when compared to normal control rats. Pretreatment with NNE (100, 200 and 400 mg/kg) daily for a period of 21 days significantly minimized the alterations in the activities of these enzymes in ISO-induced rats when compared to ISO-alone induced rats.

Table 1

Effect of Nelumbo nucifera leaf extract (NNE) on the activities of creatine kinase-MB (CK-MB) and cardiac troponin T (cTnT) in serum of normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

Groups	CK-MB (IU/L)	cTnT (ng/ml)
Normal control rats	58.44 ± 3.88[a]	ND
Normal rats + NNE (400 mg/kg)	56.75 ± 4.26[a]	ND
ISO control rats	97.74 ± 7.21[b]	0.91 ± 0.04[a]
NNE (100 mg/kg) + ISO	74.26 ± 5.30[c]	0.83 ± 0.03[b]
NNE (200 mg/kg) + ISO	71.66 ± 2.78[c]	0.80 ± 0.06[b]
NNE (400 mg/kg) + ISO	63.21 ± 5.11[d]	0.64 ± 0.05[c]

ND-Not Detectable
Each value is mean ± S.D. for 8 rats in each group.
Values not sharing a common superscript (a, b, c, d and e) differ significantly with each other (P < 0.05, DMRT).

Table 2

Effect of Nelumbo nucifera leaf extract (NNE) on the activities of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) in serum of normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

Groups	CK (IU/L)	LDH (IU/L)	AST (IU/L)	ALT (IU/L)
Normal control rats	248.8 ± 15.8[a]	133.7 ± 7.8[a]	42.2 ± 2.03[a]	22.3 ± 1.16[a]
Normal rats + NNE (400 mg/kg)	234.7 ± 14.12[a]	128.1 ± 9.2[a]	40.4 ± 2.91[a]	21.1 ± 1.72[a]
ISO control rats	419.9 ± 26.6[b]	212.3 ± 18.3[b]	80.2 ± 3.33[b]	37.3 ± 2.66[b]
NNE (100 mg/kg) + ISO	342.2 ± 23.5[c]	172.6 ± 8.3[c]	63.2 ± 3.01[c]	31.1 ± 1.35[c]
NNE (200 mg/kg) + ISO	328.5 ± 27.2[c]	168.9 ± 7.7[c]	61.6 ± 3.84[c]	30.7 ± 2.10[c]
NNE (400 mg/kg) + ISO	288.6 ± 23.2[d]	151.2 ±10.6[d]	50.8 ± 4.35[d]	26.0 ± 1.60[d]

Each value is mean ± S.D. for 8 rats in each group.
Values not sharing a common superscript (a, b, c, d and e) differ significantly with each other (P < 0.05, DMRT).

Table 3

Effect of Nelumbo nucifera leaf extract (NNE) on the activities of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) in heart of normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

Groups	CK	LDH	AST	ALT
Normal control rats	15.6 ± 1.18[a]	103.7±7.52[a]	51.32±2.03[a]	20.31±1.26[a]
Normal rats + NNE (400 mg/kg)	15.7 ± 1.12[a]	107.1± .32[a]	50.20±3.91[a]	21.19±1.91[a]
ISO control rats	7.76 ± 0.61[b]	58.4 ± 4.30[b]	31.98±2.33[b]	8.05±0.55[b]
NNE (100 mg/kg) + ISO	9.70 ± 0.85[c]	77.9 ± 5.08[c]	40.85±2.01[c]	12.85± 1.05[c]
NNE (200 mg/kg) + ISO	10.11±1.02[c]	80.3 ±5.31[c]	41.36±3.80[c]	13.06±2.14[c]
NNE (400 mg/kg) + ISO	13.2 ± 1.02[d]	91.1 ± 6.30[d]	46.11±3.15[d]	18.10±1.85[d]

CK activity: µmol of phosphorus liberated/min/mg protein. LDH, AST and ALT activity: nmol of pyruvate liberated/min/mg protein.
Each value is mean ± S.D. for 8 rats in each group.
Values not sharing a common superscript (a, b, c, d and e) differ significantly with each other (P < 0.05, DMRT).

Tables 4 & 5 present the effect of NNE on the levels of blood glucose, serum urea, uric acid, creatinine and homocysteine and plasma protein in normal and ISO-induced rats. ISO-induced rats showed a significant increase in the levels of these parameters except protein, when compared to normal control rats. Administration of NNE to ISO-induced rats significantly minimized the alterations in the levels of these parameters in ISO-induced rats when compared to ISO-alone induced rats.

Table 4

Effect of Nelumbo nucifera leaf extract (NNE) on the levels of glucose, urea, uricacid and creatinine in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

Groups	Glucose (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Normal control rats	59.8 ± 5.08[a]	26.4 ± 2.41[a]	3.62 ± 0.30[a]	0.31±0.03[a]
Normal rats + NNE (400 mg/kg)	57.2 ± 4.12[a]	25.4 ± 1.32[a]	3.40 ± 0.14[a]	0.30±0.03[a]
ISO control rats	115.2±8.63[b]	41.3 ± 3.30[b]	5.96 ± 0.53[b]	0.87±0.07[b]

NNE (100 mg/kg) + ISO	80.2 ± 4.85[c]	34.0 ± 2.18[c]	4.31 ± 0.31[c]	0.61±0.03[c]
NNE (200 mg/kg) + ISO	74.5±27.2[c]	33.2 ± 2.47[c]	4.17 ± 0.26[c]	0.58±0.03[c]
NNE (400 mg/kg) + ISO	67.2 ± 3.12[d]	29.4 ± 2.64[d]	3.94 ± 0.45[d]	0.42±0.03[d]

Each value is mean ± S.D. for 8 rats in each group.
Values not sharing a common superscript (a, b, c, d and e) differ significantly with each other (P < 0.05, DMRT).

Table 5
Effect of Nelumbo nucifera leaf extract (NNE) on the levels of protein and homocysteine in serum in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

Groups	Protein (g/dl)	Homocysteine (μg/ml)
Normal control rats	8.12 ± 0.58[a]	7.52 ± 0.50[a]
Normal rats + NNE (400 mg/kg)	8.23 ± 0.61[a]	7.47 ± 0.55[a]
ISO control rats	5.32 ± 0.28[b]	20.56± 1.35[b]
NNE (100 mg/kg) + ISO	6.55 ± 0.36[c]	14.3 ± 0.86[c]
NNE (200 mg/kg) + ISO	6.73 ± 0.44[c]	13.5 ± 0.71[c]
NNE (400 mg/kg) + ISO	7.40 ± 0.60[d]	9.15 ±0.54[d]

Each value is mean ± S.D. for 8 rats in each group.
Values not sharing a common superscript (a, b, c, d and e) differ significantly with each other (P < 0.05, DMRT).

For all the parameters studied, oral administration of NNE (100, 200 and 400 mg/kg) daily for a period of 21 days to normal rats showed a minor effect, but it was not statistically significant. NNE at a dose of 400 mg/kg showed a better effect than 100 and 200 mg/kg in ISO-induced MI in rats.

DISCUSSION

Free radical generation and lipid peroxidation process are involved in ISO-induced cardiac damage. ISO-injection produces excessive production of free radicals resulting from oxidative metabolism of catecholamines[18]. These radicals cause the loss of membrane integrity with disintegration of polyunsaturated fatty acids (PUFA) and exert unfavorable influences on heart structure and function. Reactive oxygen species (ROS) may attack biomolecules like proteins, nucleic acids and carbohydrates, but their main target is PUFA, where the process of initiation of lipid peroxidation occurs[19].

Troponin T is a unique cardiac marker, which is released from infarcting myocardium. Troponin C, I and T are proteins that form thin filaments of muscle fibers and regulate the movement of contractile proteins in muscle tissue[20]. cTnT have consistently been shown

for identifying myocardial necrosis than traditional markers [21]. Several evidences suggest that measurement of cardiac cTnT is at least as sensitive and specific as CK-MB. Furthermore, monitoring serum troponin is has been successfully utilized for risk stratification of unstable angina patients with a higher rate of adverse cardiac events in patients with increase concentrations[22]. The observed elevation in the levels of serum cTnT predicts the risk of both cardiac death and subsequent infarction in ISO-induced rats. Treatment with the NNE results in reduced level of troponin-T which is recognized as a better myocardial injury marker. Rats pretreated with NNE for a period for 21 days significantly decreased the levels of cTnT in ISO-induced rats, indicates the cardioprotective effect and preserves myocardial membrane integrity.

The serum enzymes viz, CK-MB, CK, LDH, AST and ALT serve as sensitive indices to assess the severity of MI. In ISO-induced rats, increased activities of these marker enzymes in and their concomitant reduction in the heart homogenate, confirm the onset of myocardial necrosis[23]. This finding concurs with a previous reported study[24], which indicates that of all the macromolecules release from damaged heart, and these enzymes because of their tissue specificity, are considered as best markers of MI. The oral pretreatment with NNE significantly prevented the ISO-induced release of these enzymes from the myocardium into the passage and help to maintain these activities at near normal status, indicating the cardio protective action of NNE.

In this study, we have observed that, ISO-administration causes an increase in the levels of blood glucose, serum urea, uric acid, creatinine, homocysteine and decrease in plasma proteins. The increase in blood urea, creatinine, and homocysteine can accelerate the progression of MI. Elevated levels of glucose in ISO-induced rats may be due to the release of glucose from the liver and also due to the reduced glucose oxidation. The possible mechanism for the observed anti-hyperglycemic effect of NNE treatment may be due to increased ability of insulin to mediate tissue glucose uptake, and thus helpful to maintain glucose homeostasis[25]. Decreased levels of proteins are due to enormous production of free radicals by ISO[26]. Prior oral administration of NNE retained near normal levels of these parameters and thus reduces the risk of MI. This is due to the presence of phytoconstituents in the extract, which may possess glucose lowering, free radical scavenging, antioxidant, and membrane stabilizing properties. These properties of NNE offer protection against the ISO-induced oxidative stress.

The results obtained from our study indicate that NNE offers protection to rat myocardium by decreasing the levels of marker enzymes in serum and increasing in heart and maintaining the levels of biochemical parameters in ISO-induced rats.

This could be due to its glucose lowering, free radical scavenging, antioxidant, and membrane stabilizing properties. On the basis of these findings, we speculate that NNE possesses cardioprotective activity against ISO-induced MI in rats.

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