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RESEARCH ARTICLE

CARDIO PROTECTIVE EFFECT OF NELUMBO NUCIFERA FLOWER EXTRACT AGAINST ISOPROTERENOL INDUCED OXIDATIVE STRESS IN MALE SWISS ALBINO RATS

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ABSTRACT

Nelumbo nucifera Gaertn (Family- Nymphaeaceae) is a well-known plant in ancient medical sciences. The leaf of Nelumbo nucifera Gaertn (family Nymphaeaceae) has been used for summer heat syndrome as home remedy in Japan and China, and it has recently been used to treat obesity in China. So the present study investigate the cardioprotective effect of Nelumbo nucifera flowers in isoproterenol induced rats. Cardiomyopathy was induced by a subcutaneous administration of isoproterenol. The positive hypertrophy response of isoproterenol caused a severe oxidative stress in the myocardium through increased lipid per oxidation. Nelumbo nucifera was administered intraperitoneally at a dose of 200 mg/kg for a period of 30 days. On 29th and 30th days the rats were induced with isoproterenol (20 mg/100g subcutaneously twice at an interval of 24 hours). At the end of experimental period, on day 29 th, 48 hour after injection of ISO administration, the animals were sacrificed under an overdose of anaesthesia. Blood was collected and was used for the determination of diagnostic cardiac marker enzyme and the heart was excised immediately, cleaned free of extraneous material and perfused with ice cold saline which are used for enzymic and non enzymic antioxidant estimations and also stored in 10% formalin, which are used for Histopathological studies

INTRODUCTION

The role of traditional medicines in the solution of health problems is invaluable on a global level. Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine, Krentz *et al*., 2005. Modern treatment methods can have many side effects. In addition, using medication continuously may involve economic burden on the user,Chatterjea*et al*., 2002. Some plant extracts have diagnostic markers ofmyocardial infarction such as creatine phosphokinase (CK), and lactate dehydrogenase (LDH). These enzymes are tightly bound to the contractile apparatus of the cardiac muscle tissue and any serious insult to the heart muscle will evoke the release of these enzymes into the serum,Nayak*et al* ., 2011.Aging is associated with complex and diversified changes of cardiovascular structure and function. Advanced age may induce a decline in bodily functions and overall cardiovascular performance even in the absence of overt disease. Coronary artery disease (CAD) is the single most important disease entity in terms of both mortality and morbidity in the entire world population Ferrari *et al*., 2003. Isoproterenol (ISO), a synthetic catecholamine and β-adrenergic agonist that causes severe stress in myocardium and infarct-like necrosis of the heart muscles. ISP induced myocardial injury involves membrane permeability alterations, which brings about the loss of functions and integrity of myocardial membranes. ISO induced myocardial necrosis is a well-known standard model to study the beneficial effect of many drugs on cardiac dysfunction,Suchalatha*et al*., 2004.*Nelumbo nucifera* Gaertn. (Family: Nymphaeaceae), an aquatic herb with stout creeping rhizome found throughout India up to an altitude of 1,800m. *N. nucifera* is commonly found growing in ponds, tanks and jheels; it is often cultivated for its elegant sweet flowers. All parts of this plant are employed medicinally in theindigenous systems of medicine, Jung *et al*., 2003. The white flower is considered to be nutritive and a good tonic in general. So the present study was carried out to find the cardioprotective effect of methanolic extract of *Nelumbo nucifera* in isoproterenol induced swiss albino rats.

MATERIALS AND METHODS

Plant materials

N.nucifera flowers were collected in Nagarkovil district, Tamil Nadu, The plant got authentication (BSI/SRC/5/23/2012-13/Tech 1700) from botanical survey of India, Coimbatore.

Preparation of methanolic extract of N.nucifera (MeNnF)

The whole N.nucifera flower were dried under the shade. Dried flower were coarsely powdered and extracted with a mixture of methanol: water (7:3, v/v) by a Soxhlet apparatus at 50°C. The solvent was completely removed and obtained dried crude extract which was used for investigation.

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Drugs usage

Isoproterenol and Metacord drug were used for the study, all drugsolutions were freshly prepared in saline beforeeach experiment.

Induction of myocardial infarction

Cardiomyopathy was induced by Intraperitoneal (i.p.) injection of isoproterenol hydrochloride (85 mg/kg body weight, dissolved in saline, for two consecutive days (29th and 30th day).

Experimental animals

Twenty five male swiss albino rats were weighing 110-150 g were selected for the study. They were bought from a central animal breeding station, Kerala. The rats were maintained under standard laboratory conditions (temperature 25±2ºC) with dark/light cycle (14/10h). The animals were kept in neat cages, bottomed with husk and fed standard pellet diet and water ad libitum. The rats were acclimatized to laboratory conditions for 15 days before the commencement of the experiments. All procedures described were reviewed and approved by the Institutional Animals Ethical Committee (Reg no: 623/02/b/CPCSEA).

Experimental design

The groups were divided in to five groups, each group having five rats.

Group I: served as a control

Group II: Rats were administered with isoproterenol (85 mg/kg body weight for the period of 24 hrs interval for two days).

Group III: Rats were treated with standard drug metacord (200 mg/kg body weight) intraperitoneally for a period of 30 days.

Group IV: Rats were treated with MeNnF (150 mg/kg body weight) intraperitoneally for a period of 30 days.

Group V: Rats were treated with MeNnF (200 mg/kg body weight)intraperitoneally for a period of 30 days.

At the end of experimental period, on day 29 th and 30th, 48 hour after injection of isoproterenol administration or saline, the animals were sacrificed under an overdose of anaesthesia. Blood was collected and was used for the determination of diagnostic cardiac marker enzyme and the heart was excised and prepared for heart tissue homogenate for biochemical estimations and Histopathological studies.

From Serum

At the end of the experimental period, i.e. 48 hour after the last injection of isoproterenol, the experimental animals were sacrificed; blood was collected from heart and the serum separated was used for the determination of diagnostic marker enzymes.

From Homogenate

The heart tissue was excised immediately and washed with chilled isotonic saline. The heart tissue homogenates prepared in ice-cold 0.1 M Tris-HCl buffer, pH 7.2 were used for the determination of lipid peroxides (LPO), SOD, catalase activity The heart was excised and some portion were placed in formalin for histology.

Biochemical Estimations

The Serum total cholesterol, triglycerides, HDL- C, LDL- C and the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), CK-MB and troponin T were estimated using commercially available kits. Antioxidant enzymes activity such as Catalase (CAT), super oxide dismutase (SOD), reduced glutathione (GSH), lipid peroxidase (LPO) in heart homogenate were analysed.

Statistical analysis

Results are expressed as the mean ± SD. Statistical significance was evaluated by one way analysis of variance (ANOVA) using SPSS version. (17.0) and the individual comparisons were obtained by the Duncan's multiple range test (DMRT) (Duncan, 1957). A value of $p<0.05$ was considered to indicate a significant difference between groups. For biochemical parameters comparison was made using student "t" test ($p < 0.05$).

RESULTS

Biochemical estimations revealed a significant fall in the levels of antioxidant parameters in ISO treated groups (II) as compared to control. In group II showed an increased levels of lipoproteins and cholesterol than control. At the same time Group IV and V showed a reduction in lipo proteins and cholesterol than standard drug treated group (III). Cardiac marker enzyme such as AST, ALT and troponin were increased significantly in group II, when compared to all experimental groups decreased CK-MB level was noted in group II.

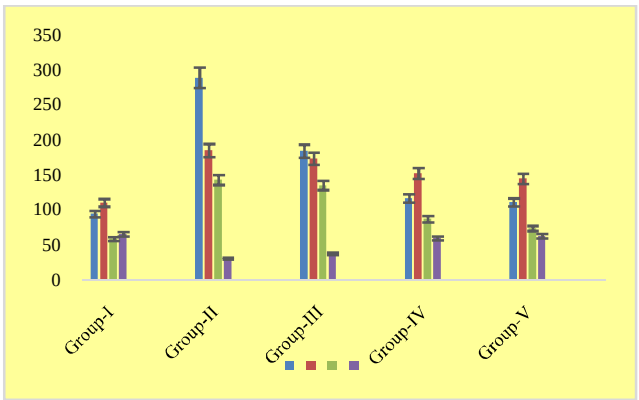

Figure 1 Levels of Total cholesterol and Triglycerides in experimental groups

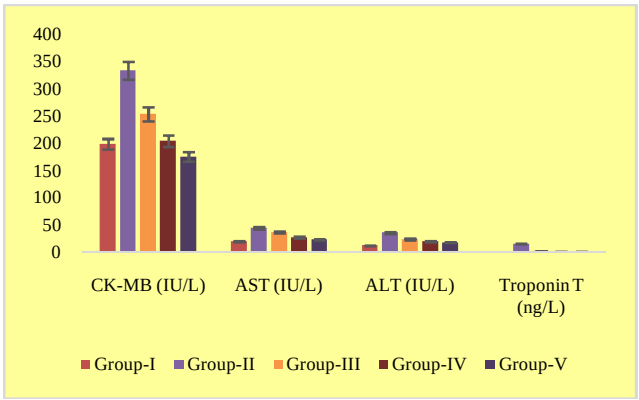

Figure 2 Serum cardiac enzyme level in Different experimental group of Rats

The levels of antioxidant CAT, SOD, GSH showed a high range and LPO level was significantly decreased it was noted in group V, High levels of LPO were observed in group II and Group III. The reduced levels of antioxidant were noted in group III. Histopathological finding shows,in normal rats the myocardium membrane showed adequate cellularity and normal morphology. Myocyte were healthy and there was no evidence of myocyte necrosis, vascular proliferation, macrophage activity and muscle hypertrophy in control group.In the ISO-control group (group II) there were morphological changes that were strongly suggestive of isoproterenol-induced myocardial injury. Large areas of coagulative necrosis were seen with neutrophil infiltrate, diffused interstitial edema and pale myocytes with fading nuclei and decreased striations.

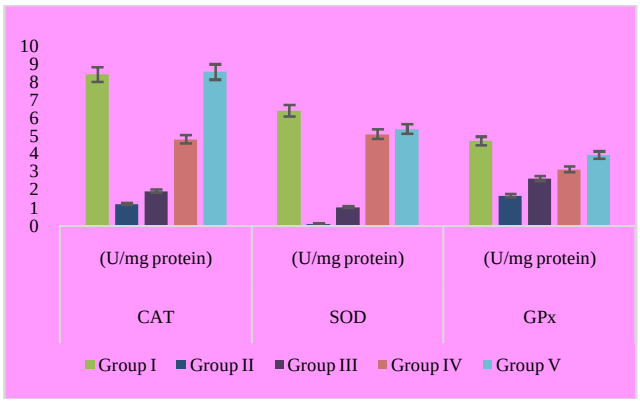

Figure 3 Levels of catalase,superoxide dismutase and glutathione peroxidase in the heart homogenate

In ISO groups treated with MeNnF (group V), many areas of myocyte debris were disintegrated with the presence of macrophages, suggesting that the myocytes were removed by macrophage activity. Macrophage activity was prominent in areas of injury and it increased with increasing doses of MeNnF. This was also true for areas of vascular proliferation which were both present in numerous areas throughout the myocardium and increased with increasing doses of MeNnF.

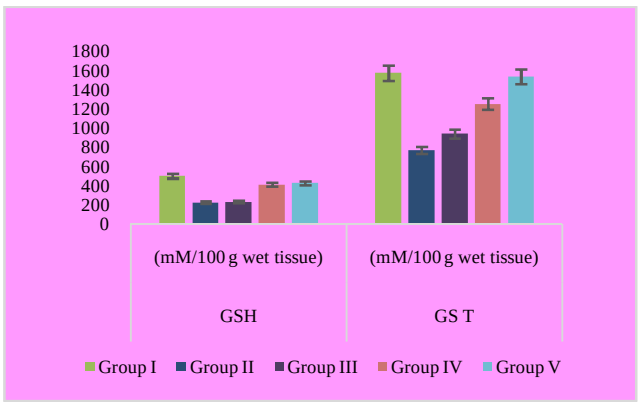

Figure 4 Levels of Reduced glutathione and Glutathione S transferase in the heart tissue

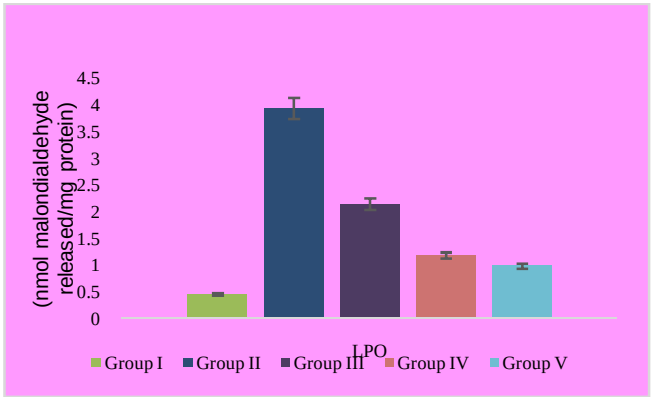

Table 5 Levels of lipid peroxidase in heart homogenate

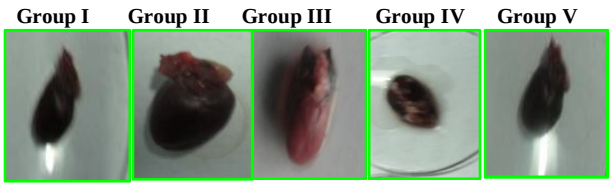

Plate1 Dissected heart from Different experimental groups

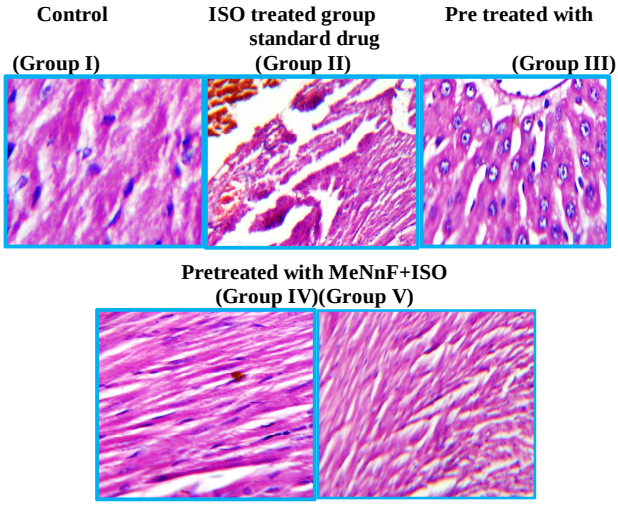

Plate 2 Histopathological changes in experimental groups

DISCUSSION

Isoproterenol [1-(3,4-dihydroxyphenyl)-2-isopropyl amino ethanol hydrochloride] is a synthetic catecholamine and beta-adrenergic agonist that induces severe stress in the cardiac muscle leading to development of cardiomyopathy. The Cardiomyopathy is produced due to its action on the cardiac β1-receptors. ISO-induced myocardial necrosis showed membrane permeability alterations, which bring about the loss of function and integrity of myocardial membrane (Nirmala *et al*., 1994). ALT, AST, troponin and CK – MB were present in cardiac muscle, injury to these tissues results in the release of the enzyme of the blood stream. Increased levels are found in cardiomyopathy. Increased levels of ALT, AST, troponin and CK-MB in serum 'the diagnostic markers', were due to the leakage of these enzymes as a result of necrosis induced byISOin rats (Ramadoss et al., 2012).In this study, ISO treated group showed the increased level of cardiac enzyme ,its due to the leakage of enzyme in to heart muscle. In group IV and V showed the normal level of cardiac enzyme, it may be due to the presence of antioxidant in the plant extract.Lipid profile is

generally considered as a reflection of the tissue metabolism and the permeability of cell membrane to various ions, which in turn depends on lipid composition. Lipids play an important role in the pathogenesis of various diseases, Radika *et al*., 2011. In the present study, the level of total cholesterol, triglycerides and LDL-C was increased in isoproterenol induced group (II) as compared with control rats. Chylomicrons and VLDL transport the cholesterol in the circulation. We have noted a significant decrease in LPO, GSH and troponinT level (figure II and IV) where as in homogenate the levels were vice-versa in both normal and pre-treated with plant extract of*N.nucifera.* Lipid peroxide is an important pathogenic event in myocardial injury and the accumulated lipid peroxides reflects the various stages of the disease and its complications Rai *et al*., 2006.

Free radical scavenging enzymes such as catalase, superoxide dismutase, glutathione peroxidase and glutathione-S transferase are the first line cellular defence against oxidative injury, decomposing O2 and H2O2 before interacting to form the more reactive hydroxyl radical (OH). The equilibrium between these enzymes is an important process for the effective removal of oxygen stress in intracellular organelles. Glutathione plays an important role in the regulation of variety of cell function and in cell protection from oxidative injury, Nigam 2007. In this study, significant reduction in the activities of glutathione-dependent antioxidant enzymes (GPX and GST) and antiperoxidative enzymes (SOD and CAT) with a concomitant decline in the level of reduced glutathione was observed in the heart tissue of Group II rats as compared to Group I normal control animals. Depletion of GSH results in enhanced lipid peroxidation and excessive lipid peroxidation can cause increased GSH consumption as observed in the present study.

Pre-treatment with intraperitoneal administered of *N.nucifera* to the retention of near normal activities of the clinicalmarker enzymes in the serum and cardiac tissue. Pre-treatment with *N.nucifera* was associated with a decreased release of enzymes from the cardiac cell fractions, which could be due to the membrane stabilizing effect of *N.nucifera* on the cardiac cell membrane. The antioxidant property is due to methanolic extract of *N.nucifera* scavenging for oxygen free radicals, resulting in the preservation of cellular viability serving, secondarily to preserve cardiac cell and thereby, retaining near normal functioning of the cardiac cell thus preventing myocardial necrosis.

Histopathological and biochemical findings of this study indicates that the methanolic extract of *N.nucifera* flower possess antioxidant properties in and protects myocardium against isoproterenol-induced oxidative stress. The most important protective mechanism offered by MeNnF is through its ability to decrease lipid hydro peroxides and to increase the superoxide dismutase and glutathione level. Thus the methanolic extract of *N.nucifera* flower has been shown to possess cardioprotective effect against isoproterenol-induced cardiomyopathy in rats.

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