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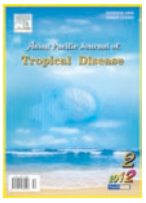

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Cardioprotective effect of *Nelumbo nucifera* on mitochondrial lipid peroxides, enzymes and electrolytes against isoproterenol induced cardiotoxicity in Wistar rats.

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ABSTRACT

Objective: To explore the protective effect of Nelumbo nucifera leaf extract (NNE) on the levels of mitochondrial lipid peroxides, activities of TCA cycle and respiratory chain enzymes and electrolytes in isoproterenol (ISO) induced myocardial infarction (MI) in rats. **Methods:** Oral pretreatment with NNE to ISO-induced rats for a period of 21 days, then heart mitochondria were isolated for the estimation of various biochemical parameters. **Results:** Rats induced with ISO showed significant increase in mitochondrial lipid peroxides with subsequent decrease in TCA cycle enzymes such as isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH) and α-ketoglutarate dehydrogenase (α-KGDH) and respiratory chain enzymes like NADH-dehydrogenase and cytochrome-c-oxidase. In addition, the levels of sodium and calcium increased and potassium decreased. Oral pretreatment with NNE to ISO-induced rats for a period of 21 days significantly reverted these biochemical parameters to near normal status. **Conclusions:** Thus, our study suggests that NNE treatment restore energy status of the mitochondria, thereby maintaining near normal function.

1. Introduction

Catecholamines cause deleterious effect on heart, which is associated with structural, functional and biochemical alterations. Isoproterenol (ISO), a synthetic catecholamine and α-adrenergic agonist, causes necrosis of rat heart muscle. ISO-induced myocardial infarction (MI) serves as a well standardized model to study the beneficial property of numerous drugs and cardiac function. Cardiac function depends on adequate delivery of oxygen and oxidizable substrate to generate sufficient amount of ATP to meet energy demand. Myocardial ischemia results in alterations of cardiac function and ultrastructure, which leads to interruption of the mitochondria beside with the inactivation of the enzymes concerned with the energy metabolism of myocardium[1].

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, hepatotoxicity, arrhythmia and hyperlipidemia. Traditionally, leaves are used to treat diarrhea fever and inflammatory skin conditions. Young leaves used to treat rectal prolapsed, raktapitta, or bleeding disorders; alleviate thirst and inflammations and to promote strength, virility, and intellect [2].

In our previous communication, we have reported that *Nelumbo nucifera* leaf extract (NNE) possess cardioprotective effect by maintaining the activities of cardiac marker enzymes and other biochemical parameters, also reported that NNE posses free radical scavenging and antioxidant properties in ISO-induced rats[3,4]. Based on the previous reports, this report communicates the preventive role of NNE on mitochondrial lipid peroxides, TCA cycle and respiratory chain enzymes and mitochondrial electrolytes in ISO-induced MI in rats.

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2. Materials and methods

2.1. Experimental Animals

All the experiments were carried out with Adult male albino rats of Wistar rats 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 (IAEC NO : P.Cog-11/06). They were housed in polypropylene cages (47cmx34cmx20cm) lined with husk, renewed every 24 h under a 12:12 h light/dark cycle at around 22℃ and had free access to tap water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Maharashtra, India).

2.2. Drugs and Extract Preparation

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). The extract preparation was described earlier[4].

2.3. Chemicals

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

2.4. 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[4].

2.5. Experimental design

A tot al number of 24 rats were used in the experiment, 6 rats of each group.

Group I: Normal control rats; Group II: Normal rats + NNE (400 mg/kg) ;

Group III: ISO control rats; Group IV: NNE (400 mg/kg) + ISO.

Nelumbo nucifera leaf extract (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. The heart tissue was dissected out immediately and washed in ice-cold saline.

2.6. Isolation of Heart Mitochondrial Fractions

Heart mitochondria were isolated by the method of Takasawa *et al*[5]. The heart tissue was put into ice-cold 50 mmol/L Tris-HCl (pH 7.4) containing 0.25 mol/L sucrose and homogenized. The homogenates were centrifuged at 700 r/min for 20 min and the supernatant obtained was again centrifuged at 9 000 r/min for 15 min. The pellets were then washed with 10 mM Tris-HCl (pH 7.8) containing 0.25 M sucrose and finally resuspended in the same buffer and used for the estimation of various biochemical parameters

2.7. Biochemical estimations

The levels of mitochondrial thiobarbituric acid reactive substances (TBARS), and the activities of malate dehydrogenase (MDH), isocitrate dehydrogenase (ICDH), α-ketoglutarate dehydrogenase (α-KGDH), succinate dehydrogenase (SDH) were assayed. The activities of NADH-dehydrogeanse and cytochrome-C-oxidase were assayed according to the methods described earlier[1]. According to the method of Ballentine and Burford[6], the sample was digested and used for electrolyte estimations. The levels of sodium and potassium were measured flame photometrically, and the calcium level in mitochondria was determined atomic emission spectrometrically.

2.8. 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 ± SD from 6 rats in each group. P values <0.05 were considered as significant.

3. Results

The levels of heart mitochondrial TBARS in normal and ISO-induced rats are shown in Figure 1. The levels of heart mitochondrial TBARS significantly increased in ISO-induced rats. Oral pretreatment with NNE for a period of 21 days to ISO-induced rats significantly decreased the levels of mitochondrial TBARS in ISO-induced rats.

Activities of the heart mitochondrial TCA cycle enzymes such as ICDH, SDH, MDH and -KGDH and respiratory chain enzymes like NADH-dehydrogenase and cytochrome -c-oxidase in normal and ISO-induced rats are shown in Table 1. The activities of these enzymes were declined significantly in ISO-induced rats when compared with normal control rats. Oral pretreatment with NNE to ISO-induced rats significantly increased the activities of these enzymes in ISO-induced rats.

Table 1.
Effect of Nelumbo nucifera leaf extract on the activities of the heart mitochondrial ICDH, succinate dehydrogenase, malate dehydrogenase and ▮ -ketoglutarate dehydrogenase, cytochrome-c-oxidase and NADH-dehydrogenase.

Groups	Group I	Group II	Group III	Group IV
Isocitrate dehydrogenase	580.4± 26.1a	585.7 ± 22.4a	392.5 ± 19.7b	551.3 ± 27.4c
Succinate dehydrogenase	216.2± 17.5a	219.8 ± 13.5a	118.0 ± 13.6b	192.0 ± 12.8c
Malate dehydrogenase	291.5±17.3a	294.5 ± 20.1a	177.3 ± 12.0b	252.3 ± 18.1c
▮ -ketoglutarate dehydrogenase	125.5 ± 8.2a	127.2 ± 10.2a	80.2 ± 7.2 b	112.0 ± 6.4c
Cytochrome-c-oxidase	0.4 ± 0.0a	0.42 ± 0.0a	0.3 ± 0.0b	0.4 ± 0.0c
NADH-dehydrogenase	152.3 ± 12.1a	154.2 ± 10.8a	92.5 ± 4.7b	136.3 ± 9.2c

Activity is expressed as nmoles of NADH oxidized/h/mg protein for ICDH; nmoles of succinate oxidized/min/mg protein for SDH; nmol of NADH oxidized/min/mg protein for MDH; nmol of ferrocyanide formed/h/mg protein for ▮ -KGDH; nmol of NADH oxidized/min/mg protein for NADH-dehydrogenase; nmol/min/mg protein for cytochrome-c-oxidase. Each value is mean ± SD for 6 rats in each group. Values not sharing a common superscript differ significantly with each other (P<0.05, DMRT).

Table 2.
Effect of Nelumbo nucifera leaf extract on heart mitochondrial electrolytes levels in control and experimental groups of rats.

Groups	Sodium (nmol/mg protein)	Potassium(nmol/mg protein)	Calcium(nmol/mg protein)
Group I	3.85 ± 0.14a	6.43 ± 0.41a	8.41 ± 0.47a
Group II	3.82 ± 0.31a	6.36 ± 0.31a	8.30 ± 0.25a
Group III	6.64 ± 0.41b	4.21 ± 0.24b	12.46 ± 1.00b
Group IV	4.32 ± 0.33d	5.84 ± 0.50c	9.56 ± 0.69c

Values are expressed as mean ± SD for 6 animals in each group Values not sharing a common superscript differ significantly with each other (P<0.05, DMRT).

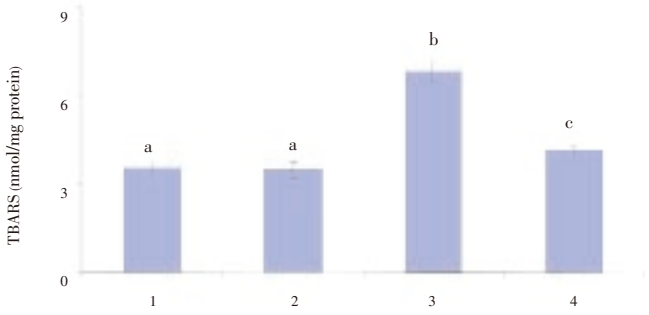

Figure 1. Effect of Nelumbo nucifera leaf extract on the heart mitochondrial thiobarbituric acid reactive substances.
Each value is mean ± SD for 6 rats in each group. Values not sharing a common superscript differ significantly with each other (P<0.05).

The levels of cardiac mitochondrial sodium, potassium, and calcium in normal and ISO-induced rats are shown in Table 2. The levels of sodium and calcium were significantly increased with subsequent decrease in potassium levels in ISO-induced rats. On administration of NNE to ISO-induced rats significantly minimized the alterations in the levels of these mitochondrial trace elements in ISO-induced rats.
For all the parameters studied, NNE treatment to normal rats didn't show any significant effect.

4. Discussion

ISO mediated its toxicity through α-1 and α-2-adrenoceptors, which are responsible for the positive inotropic and chronotropic effects[7]. Prolonged oxidative stress in failing myocardium results in damage to mitochondrial DNA, ROS generation and consequent cellular injury leading to functional decline. Thus, mitochondria serve as a source and target of a ROS mediated injury in heart. Increased levels of mitochondrial free radical production observed under pathological conditions such as ischemia is associated with impairment of mitochondrial structure and function. In this study, we have observed increased levels of cardiac mitochondrial TBARS in ISO-induced rats, which indicate the increased lipid peroxidation process, which also could be accredited to insufficiency of antioxidant system[8]. Pretreatment with NNE to ISO-induced rats for 21 days significantly decreased the heart mitochondrial TBARS. This could be due to free radical scavenging, antioxidant and anti lipoperoxidative properties of NNE, thus indirectly responsible for the decreased levels of mitochondrial TBARS in ISO-induced rats.

We have observed a decrease in the activities of TCA cycle enzymes in the heart mitochondria in ISO-induced rats, which are located in the outer membrane of mitochondria and easily affected by excessive production of free radicals by ISO. During hypoxia and the subsequent generation of lipid peroxides and hydrogen peroxide leads to initiation of chain reactions, which could responsible for mitochondrial damage. Burton et al[9] have reported that ROS produced during ischemia, damages a variety of macromolecules including enzymes. Pretreatment with NNE to ISO-induced rats significantly increased the activities of these enzymes, which could be due to its ability to prevent free radical formation and free radical scavenging properties of NNE.

NADH- dehydrogenase and Cytochrome-c-oxidase are located in the inner mitochondrial membrane and are concerned in the production of ATP. These enzymes have an absolute requirement of cardiolipin for its activity. In this study, the activities of these enzymes were decreased in the heart mitochondria of ISO-induced rats. This could be due

to enhanced PL degradation resulting in the non-availability of cardiolipin for their functional activity. NNE pretreatment increased the activities of NADH-dehydrogenase and cytochrome-c-oxidase in ISO-induced rats[10]. This could be due to the inhibition of PL degradation in the biological membranes and maintain the required quantity of cardiolipin in the membrane PL for the activities of these enzymes.

The levels of mitochondrial electrolytes play an important role in the normal function of the myocardium. Significantly elevated Na+ and Ca^{2+} and decreased K+ observed in ISO-induced rats in this study. Increased in Ca^{2+} could be due to increased Ca^{2+} uptake following ISO-administration. The increase in Ca^{2+} cycling across the mitochondrial membrane provoked by the combined Na^{+} and Ca^{2+} overload of cardiac myocytes leads to the depletion of cellular ATP[11]. Ischaemia leads to increased sodium Na+ and Ca^{2+} and decreased K+ levels. Rats treated with NNE significantly minimized these alterations in ISO-induced rats.

ISO-administration to rats leads to various biochemical alterations in the sub cellular organelles like mitochondria. In this study, an increased levels of mitochondrial TBARS and decreased the activities of TCA cycle and respiratory chain enzymes and altered electrolytes were observed in ISO-induced rats. Pretreatment with NNE significantly decreased the levels of mitochondrial TBARS and increased the activities of TCA cycle and respiratory chain enzymes and also maintained the levels of mitochondrial electrolytes in rats.

Conflict of interest statement

We declare that we have no conflict of interest.

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