Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 7, 2014

Original Article

CARDIOPROTECTIVE EFFICACY OF *NELUMBO NUCIFERA* LEAF EXTRACT ON GLYCOPROTEIN, MEMBRANE BOUND ATPASE AND LYSOSOMAL ENZYMES AGAINST ISOPROTERENOL INDUCED CARDIOTOXICITY IN WISTAR RATS

***R. SUBASHINI[1]**

[1]Department of Biochemistry and Biotechnology, Annamalai University, Annamalainagar 608002, Tamil Nadu, India.
Email: dr.r.subashini@gmail.com

Received: 24 May 2014 Revised and Accepted: 07 Jul 2014

ABSTRACT

Objective: *Nelumbo nucifera*, an Indian Ayurvedic herbal medicine, which provides significant protection against myocardial infarction and hypertension. However, there is lack of information regarding the effect of NNE on the cardiac changes associated with isoproterenol (ISO)-induced myocardial infarction (MI) This study aimed to evaluate the combined preventive effects of NNE on glycoprotein, membrane bound ATPases and lysosomal enzymes in isoproterenol induced myocardial infarcted rats.

Methods: Male rats were pretreated with *Nelumbo nucifera* leaf extract (NNE) (400 mg/kg) orally daily for 21 days. After pretreatment, rats were induced myocardial infarction by isoproterenol (100 mg/kg) at an interval of 24 h for 2 days to induce myocardial infarction.

Results: Isoproterenol treated rats showed decreased levels of heart creatine kinase and lactate dehydrogenase. The activity of sodium potassium adenosine triphosphatase was decreased and the activities of magnesium adenosine triphosphatase and calcium adenosine triphosphatase were increased in isoproterenol treated rats. Also, the activities of β-glucuronidase, β-N-acetylglucosaminidase, β-galactosidase, cathepsin-B and D were increased (serum and heart), but the activities of β-glucuronidase and cathepsin-D were decreased in lysosomal fraction and increased in cytosolic fraction of the heart in isoproterenol treated rats. Pretreatment with NNE to isoproterenol treated rats normalized all the biochemical parameters studied.

Conclusion: The observed effects are due to their membrane stabilizing property and this property might be due to decreased lipid peroxidation..

Keywords: Isoproterenol,Lysosome, Myocardial infarction, *Nelumbo nucifera.*

INTRODUCTION

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

Isoproterenol, a synthetic catecholamine and β-adrenergic agonist, induced rats have used as a model for several cardiac dysfunctions [2].The deleterious effects of ISO on heart is well known, and also associated not only with functional alterations, but also with numerous morphological and biochemical changes [3]. Ideally, animal models of human pathological conditions are mimic the cellular and physiological processes responsible for the pathological conditions in man [4] reported that the administration of ISO to rats produces "infarct-like" myocardial necrosis in the absence of significant coronary artery lesions. This observation led to the "relative hypoxia" was responsible for the observed cardiac necrosis [5]. Increased cardiac inotropy and chronotropy after adrenergic stimulation caused a relative imbalance between myocardial oxygen demand and blood flow, such that demand and supply [6].

Myocardial infarction is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand. Particularly the lack of blood supply is caused by closure of the artery (coronary artery) that supplies that particular part of the heart muscle with blood. The artery is closed or narrowed by a plaque that usually occurs in a coronary artery by atherosclerosis. Serum cholesterol and low density lipoprotein (LDL) cholesterol has consistently been shown to be a significant risk factor for CHD and other major CVD [7]. A high level of blood cholesterol is a major risk factor for CHD and also a secondary risk factor for stroke. Higher levels of LDL can slowly build up in the inner walls of the arteries that feed the heart and brain and narrow those arteries. An unhealthy cholesterol balance can lead to deposition of fat in the arteries called plaque. Plaque narrows the arteries and decreases blood flow to the heart, which can cause a heart attack [8].

Biologically active plant chemicals termed as phytochemicals and these traditional nutrients possess beneficial effects on human health [9]. Phytochemicals are naturally occurring and it appears to work alone or combination and perhaps in conjunction, with vitamins and other nutrients in food to prevent, halt or lessen disease. These non-nutritive chemicals scavenges free radicals, ROS and subsequent oxidative stress have been correlated to many human disorders including those of the kidney, eye, lung, liver, nervous system, heart and cardiovascular system [10]. Therefore, make sure to consume wide variety of fruits and herbs to get health benefits. Consequently, foods in our diet that can aid in prevention of these diseases are major interest to both the scientific community and the general public.

Nowadays research has been focused on food and medicinal plants that have been found to have certain preventive measures with fewer side effects in the treatment. *Nelumbo nucifera* has been reported to treat obesity, hepatotoxicity, arrhythmia and hyperlipidemia. Traditionally, leaves are used to treat diarrhea, fever and skin inflammations. Young leaves used to treat rectal prolapsed, raktapitta, or bleeding disorders; alleviate thirst, to promote strength and virility [11]. It used in folk remedy to treat tissue inflammation, leprosy, cancer diseases and also used as a cooling medicine, antiemetic and antidote for the poisons.

In our previous communication, we have reported that *Nelumbo nucifera* leaf extract (NNE) possess cardio protective 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[12][13]. Based on the previous reports, communicates the preventive role of NNE on mitochondrial lipid peroxides, TCA cycle and antioxidant enzymes. NNE significantly reduces lipid peroxidation production and improves cardiac sustainability in myocardial infarcted rats. Hence, this study was undertaken to assess the efficacy of NNE in the treatment of MI.

MATERIALS AND METHODS

Plant Material

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

Preparation of Alcoholic Extract (NNE)

Alcoholic extract of the dried leaves of *Nelumbo nucifera* was prepared and coarsely powdered 1 kg of plant material was extracted in 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 was kept in a desiccators [12]. This methanolic extract was dissolved in distilled water for further experiments.

Chemicals

(±) Isoproterenol hydrochloride, reduced nicotinamide adenine dinucleotide (NADH), oxaloacetate, bovine serum albumin (BSA), N-phenyl-p-phenylenediamine, p-nitrophenyl-β-D-glucuronide, β-nitrophenyl-β-D-N-acetyl glucosaminide, sodium dodecyl sulphate (SDS) and α-N-benzoyl-DL-arginine-p-nitroanilide hydrochloride (BAPNA), and phosphate buffered saline (PBS) were purchased from Sigma Chemical Company, St. Louis, MO, USA. Methanol was purchased from Anilax chemicals, USA. All other chemicals used for the experiment is of analytical grade.

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 Animal Ethical Committee (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 22C 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.

Induction of experimental myocardial infarction

Isoproterenol (85 mg/kg) was dissolved in normal saline and injected subcutaneously to rats at an interval of 24 hours for 2 days [12].

Experimental Design

In this experiment, a total of 32 male albino Wistar rats were randomly divided into four groups of eight rats of each. Group I: Normal control rats, Group II: Rats were orally treated with NNE 400 mg/kg alone daily for 21 days using an intra gastric tube, Group III: Rats were subcutaneously injected with ISO alone (85 mg/kg) at an interval of 24 h for 2 days (on 20th and 21st days), Group IV: Rats were pretreated with NNE 400mg/kg daily for 21 days and then subcutaneously injected with ISO (85 mg/kg) for 2 days. At the end of the experimental period, after 12 h of second ISO injection, (i.e. on 16th day) all the rats were anesthetized and then sacrificed by cervical decapitation. Blood was collected and subsequently plasma and serum were separated by centrifugation. The heart tissue was excised immediately from the animals, washed off blood with ice-chilled physiological saline and stored for further biochemical estimations. A known weight of the heart tissue was homogenized in 5 ml of 0.1 M Tris–HCl (pH-7.4) buffer solution. The homogenate was centrifuged at 3000 rpm for 5 min and the supernatant was used for the estimation of various biochemical parameters.

Biochemical Estimations

Glycoprotein components (Hexose, hexosamine, sialic acid and fucose) were estimated in plasma and heart. The heart samples were defatted prior to estimation according to the method of Folch et al. (1957) [14]. Protein-bound hexose was estimated by the method of Dubois and Gilles (1956) [15]. Hexosamine was estimated by the method of Wagner (1979) [16]. Fucose was estimated by the method of Dische and Shettles (1948) [17]. Sialic acid was estimated by the method of Warren (1959) [18]. The activity of Na^+/K^+-ATPase was assayed according to the procedure of Bonting (1970) [19]. The activity of Ca^{2+}-ATPase was assayed according to the method of Hjerten and Pan (1983) [20]. The activity of Mg^{2+}-ATPase was assayed by the method of Ohnishi et al. (1982) [21]. The lysosomal fraction of the heart tissue was isolated by the method of Wattiaux (1977) [22]. The activity of β-glucuronidase was assayed by the method of Kawai and Anno (1971) [23]. The activity of β -N-acetyl glucosaminidase was assayed by the method of Moore and Morris (1982) [24]. The activity of β -galactosidase was assayed by the method of Conchie et al. (1967) [25]. The activity of cathepsin-D was assayed by the method of Sapolsky et al. (1973) [26]. Protein in the tissue extract was estimated by the method of Lowry et al. (1951) [27].

Statistical Analysis

Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). Using SPSS software package version 9.05. The results were expressed as + SD from eight rats in each group, and mean P values <0.05 were considered as significant.

RESULTS

Effect of NNE on glycoproteins

The level of glycoprotein (hexose, hexosamine, fucose and sialic acid) in serum and heart of normal and ISO-induced rats is shown in Tables 1&2. Significantly increased levels of glycoproteins were observed in serum and heart of ISO-induced rats when compared with normal control rats. Pretreatment with NNE significantly decreased the levels of glycoprotein in serum and heart of ISO-induced rats when compared with ISO-alone induced rats.

Table 1:

Groups	Hexose (mg/dL)	Hexosamine (mg/dL)	Fucose (mg/dL)	Sialic acid (mg/dL)
Normal control rats	122.8 ± 6.12[a]	19.3 ± 1.50[a]	23.6 ± 1.25[a]	33.5 ± 1.98[a]
Normal rats + NNE (400 mg/kg)	120.5 ± 9.81[a]	18.7 ± 1.11[a]	23.2 ± 1.33[a]	33.9 ± 1.65[a]
ISO control rats	223.6 ± 14.4[b]	33.6 ± 2.09[b]	44.1 ± 2.18[b]	47.5 ± 2.77[b]
NNE (400 mg/kg) + ISO	139.6 ± 10.1[c]	23.7 ± 1.70[c]	27.4 ± 1.85[c]	36.5 ± 2.15[c]

Each value is mean ± S.D. for 6 rats in each group. Values not sharing a common superscript (a, b and c) differ significantly with each other (P<0.05, DMRT)

Table 2:

Groups	Hexose (mg/g defatted tissue)	Hexosamine (mg/g defatted tissue	Fucose (mg/g defatted tissue)	Sialic acid (mg/g defatted tissue)
Normal control rats	125.1 ± 6.14[a]	6.43 ± 0.41[a]	20.2 ± 1.47[a]	32.5 ± 2.03[a]
Normal rats + NNE (400 mg/kg)	123.6 ± 7.31[a]	6.36 ± 0.31[a]	20.8 ± 1.25[a]	32.2 ± 2.57[a]
ISO control rats	190.3 ± 10.1[b]	10.09 ± 0.74[b]	44.9 ± 2.52[b]	57.9 ± 4.11[b]
NNE (400 mg/kg) + ISO	141.2 ± 8.33[c]	7.61 ± 0.50[c]	24.0 ± 1.69[c]	39.3 ± 2.77[c]

Each value is mean ± S.D. for 6 rats in each group. Values not sharing a common superscript (a, b and c) differ significantly with each other (P<0.05, DMRT)

Effect of NNE on the activities of membrane bound enzymes

Table 3 illustrate the effect of NNE on the activities of sodium/potassium dependent adenosine tri phosphatase (Na^+/K^+-ATPase), calcium and magnesium dependent adenosine tri phosphatase Ca^{2+} and Mg^{2+}-ATPases in normal and ISO-induced rats. The activity of Na^+/K^+-ATPase decreased significantly and the activities of Ca^{2+} and Mg^{2+}-ATPases increased significantly in the heart of ISO-induced rats when compared to normal control rats.

NNE pretreatment to ISO-induced rats significantly increased the activity of Na^+/K^+-ATPase and decreased the activities of Ca^{2+} and Mg^{2+}-ATPases in the heart when compared to ISO-alone induced rats.

Table 3:

Groups	Na^+K^+ATPase (µmoles of Pi liberated/min/mg protein)	Ca^{2+}ATPase (µmoles of Pi liberated/min/mg protein)	Mg^{2+}ATPase (µmoles of Pi liberated/min/mg protein)
Normal control rats	0.61 ± 0.04[a]	1.23 ± 0.07[a]	6.21 ± 0.15[a]
Normal rats + NNE (400 mg/kg)	0.63 ± 0.05[a]	1.31 ± 0.05[a]	6.30 ± 0.23[a]
ISO control rats	0.26 ± 0.01[b]	3.25 ± 0.14[b]	9.40 ± 0.52[b]
NNE (400 mg/kg) + ISO	0.50 ± 0.04[c]	1.84 ± 0.06[c]	7.44 ± 0.69[c]

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

Effect of NNE on the activities of lysosomal enzymes

Tables 4 & 5 show the activities of serum and the heart lysosomal hydrolases (β-glucuronidase, β-N-acetyl glucosaminidase and β-galactosidase) in normal and ISO-induced rats. ISO-induced rats showed a significant increase in the activities of these enzymes when compared to normal control rats. Oral pretreatment with NNE to ISO-induced rats significantly decreased the activities of these enzymes in serum and the heart when compared with ISO-alone induced rats.

Table 4:

Groups	β-glucuronidase	β-N-acetyl glucosaminidase	β-galactosidase
Normal control rats	12.4 ± 1.12[a]	26.2 ± 1.72[a]	18.2 ± 0.92[a]
Normal rats + NNE (400 mg/kg)	12.6 ± 0.80[a]	26.6 ± 1.24[a]	18.7 ± 1.05[a]
ISO control rats	27.9 ± 1.34[b]	44.2 ± 2.63[b]	38.5 ± 2.45[b]
NNE (400 mg/kg) + ISO	15.8 ± 1.06[c]	30.1 ± 2.02[c]	22.4 ± 1.08[c]

Units: µmoles of p-nitro phenol liberated/hr/100 mg protein for β-glucuronidase, β-N-acetyl glucosaminidase and β-galactosidase. Each value is mean ± S.D. for 8 rats in each group. Values not sharing a common superscript (a, b and c) differ significantly with each other (*P*<0.05, DMRT)

Table 5:

Groups	β-glucuronidase	β-N-acetyl glucosaminidase	β-galactosidase
Normal control rats	24.1 ± 1.52[a]	47.2 ± 2.17[a]	40.1 ± 3.05[a]
Normal rats + NNE (400 mg/kg)	24.4 ± 1.18[a]	48.0 ± 3.10[a]	39.8 ± 3.45[a]
ISO control rats	46.6 ± 3.34[b]	78.2 ± 4.15[b]	71.3 ± 5.13[b]
NNE (400 mg/kg) + ISO	29.5 ± 1.78[c]	55.7 ± 3.51[c]	46.4 ± 3.70[c]

Units: µmoles of p-nitro phenol liberated/hr/100 mg protein for β-glucuronidase, β-N-acetyl glucosaminidase and β-galactosidase. Each value is mean ± S.D. for 8 rats in each group. Values not sharing a common superscript (a, b, and c) differ significantly with each other (P<0.05, DMRT)

DISCUSSION

Effect of NNE on glycoproteins

Hexose, hexosamine, fucose and sialic acid are the basic components of glycoproteins. The levels of glycoproteins are reported to be significantly increased in CVD [28]. An increase in glycoprotein components has been reported to relate to the duration, severity and existence of degenerative vascular diseases [29]. Glycoproteins are important components of intracellular matrix, cell membrane and membranes of the sub cellular organelles [30] [31]. Suggested that glycoproteins are involved in the myocardial necrosis and repair. The functions of glycoprotein in stabilizing the tissue may be involved in maintaining the structural stability of collagen fibrils. Our study evidently shows an increase in the glycoprotein components namely hexoses, hexosamine, fucose, sialic acid in ISO-induced rats. The elevation in the levels of serum glycoprotein components might be due to secretion from cell membrane glycoconjugates into the circulation [32]. Increase in glycoprotein

level could also be due to increased synthesis to repair the damaged membrane structure by peroxidation. The NNE pretreated rats showed a significant decrease in these glycoprotein levels compared to ISO- administered rats.

Maintenance of ambient levels of heart glycoprotein of NNE treated rats could be due to the scavenging of free radicals levels and decrease the process of lipid peroxidation by their antioxidant property.

Table 6:

Groups	Serum		Heart	
	Cathepsin-B	Cathepsin-D	Cathepsin-B	Cathepsin-D
Normal control rats	12.83 ± 0.89^{a}	19.1 ± 1.18^{a}	21.7 ± 1.45^{a}	29.5 ± 2.09^{a}
Normal rats + NNE (400 mg/kg)	12.53 ± 0.92^{a}	19.4 ± 1.05^{a}	21.5 ± 1.70^{a}	28.7 ± 1.92^{a}
ISO control rats	19.65 ± 1.21^{b}	32.3 ± 2.32^{b}	35.1 ± 2.80^{b}	42.7 ± 3.15^{b}
NNE (400 mg/kg) + ISO	14.68 ± 1.08^{c}	23.7 ± 1.57^{c}	26.5 ± 2.05^{c}	33.6 ± 2.41^{c}

Each value is mean ± S.D. for 8 rats in each group. Values not sharing a common superscript (a, b and c) differ significantly with each other ($P < 0.05$, DMRT). Cathepsin-B activity: µmoles p-nitro phenol liberated/hr/100 mg protein for serum and the heart. Cathepsin-D activity: µmoles of tyrosine liberated/hr/100 mg protein for serum and the heart.

Table 6 shows the activities of cathepsin-B and cathepsin-D in serum and the heart of normal and ISO-induced rats. The activities of cathepsin-B and cathepsin-D were increased significantly in ISO-induced rats when compared with normal control rats. Oral pretreatment with NNE to ISO-induced rats significantly decreased the activities of these enzymes in serum and the heart when compared with ISO-alone induced rats.

Effect of NNE on membrane bound enzymes

The activity of many membrane bound enzymes and transport system is dependent on the physical state of the membrane lipid microenvironment. ATPases are intimately associated with the plasma membrane and participates in the energy requiring translocation of sodium, potassium, calcium and magnesium [33]. Determination of membrane associated enzyme activities like ATPases indicate the alterations in membrane under pathological conditions. The abnormalities in sodium/potassium dependent adenosine tri phosphatase (Na^+/K^+-ATPase) and calcium dependent adenosine tri phosphatase (Ca^{2+}-ATPase) activities with accompanied increase in base line sodium and calcium concentration are well documented in cardiac dysfunction.

In this study, we observed decreased activities of Na^+/K^+-ATPase and increased activities of Ca^{2+} ATPase and Mg^{2+}-ATPase in ISO-induced rats. Na^+/K^+-ATPase, located in the cardiac sarcolemma is considered to be involved in the maintenance of intracellular Na^+ and K^+ concentrations in the myocardium. Inactivation of Na^+/K^+-ATPase occurs due to enhanced lipid peroxidation by ISO. The inhibition of Na^+/K^+-ATPase activates the Na^+ and Ca^{2+} exchange mechanism, which play a role in regulating the cellular calcium levels [34]. Membrane Ca^{2+}-ATPase is responsible for fine tuning of intracellular calcium as well as the contractility and excitability properties of muscles. Ca^{2+}-ATPase is the major active calcium transport protein responsible for the maintenance of normal intracellular calcium levels.

Pretreatment with NNE increased the activity of Na^+/K^+-ATPase and decreased the activities of Ca^{2+} and Mg^{2+}-ATPases in ISO-induced rats. Increased Na^+/K^+ ATPase activity due to pretreatment of NNE could regulate the intracellular Ca^{2+} levels, thereby protecting the myocardium from excess damage by maintaining the membrane integrity. Elevation of Na^+ concentration operates to depress Ca^{2+} effect and augment Ca^{2+} influx. These effects show membrane stabilizing property of NNE. The polyphenols in NNE by preventing the oxidative stress, protein glycation and hyperlipidemia may have reduced the factors responsible for disturbed fluidity and stability of the membranes and membrane bound ATPases.

Effect of NNE on Lysosomal enzymes

Lysosomal enzymes are significant intermediaries of acute and chronic inflammatory diseases, which cause harmful effect to connective tissues. Altered activities of these lysosomal enzymes were observed in patients with MI and in experimental animal models. Therefore, considerable attention has been focused on alteration in the lysosomal enzyme activities that may related to ischemic or hypoxic myocellular damage. During MI, when myocardial cell death and degeneration occurs, proteolysis of necrotic myocardium also occurs with a concomitant influx of inflammatory cells at the infarct margins [35].

Lysosomes are membrane bound structures that play a major role in the secretion and transport processes. Lysosomal hydrolytic enzymes are involved in the degradation of most of the cellular constituents. The intracellular release of lysosomal enzymes and their subsequent extralysosomal activity may play a crucial role in the progressive modifications that lead from reversible myocardial ischemia to irreversible MI [36]. Lytic action of these enzymes, damage of the lysosomal membrane and alterations in the fragility of lysosomes may be among the earliest structural alterations that occur during the development of ischemic injury [37].

We have observed increased activities of lysosomal enzymes such as β-glucuronidase, β-N-acetyl glucosaminidase, β-galactosidase, cathepsin-B and cathepsin-D in serum and the heart of ISO-induced rats. The release of β-glucuronidase is used as an index of lysosomal membrane integrity [38]. The release of lysosomal enzymes into the cytoplasm stimulates inflammatory mediators (eg. oxygen radicals and prostaglandin), which then stimulate tissue disruption [39].

Pretreatment with orally administered NNE led to the retention of near normal activities of the lysosomal enzymes in the serum and heart of ISO-induced rats. The anti-oxidant property is due to NNE, scavenging the oxygen free radicals, resulting in the preservation of cellular viability serving, secondarily, to preserve lysosomes and thereby, retaining near normal functioning of the lysosomes.

This might be due to inhibiting the release of lysosomal enzymes by decreasing membrane damage, thereby enhancing the stability of lysosomes. Like other various medicinal plants NNE has antioxidant and free radical scavenging properties, these properties of NNE could be responsible for preventing and reducing the membrane damage in ISO-induced rats.

CONCLUSION

The protective effect of NNE in preventing free radical mediated myocardial damage and thereby eliminating the acute fatal complications by protecting the membrane damage against ISO-induced infarction. NNE pretreatment also shows the inhibition of necrosis and reduced inflammation in ISO induced rats. The free radical scavenging, antioxidant, lipid lowering and membrane stabilizing properties of NNE could responsible for these effects on histology of the myocardium. Epidemiological studies suggest that diet rich in herbs and medicinal plants are protective against cardiovascular disease (CVD). It could be concluded that regular consumption of medicinal plants like NNE could offers protection to the heart. Further clinical trials are warranted before NNE could be developed as a drug for the cardiovascular disorders.

CONFLICT OF INTEREST

The authors do not have any conflict of interest to declare.

REFERENCES

1. Subashini R, Sumathi P. Cardioprotective effect of Nelumbo nucifera on mitochondrial lipid peroxides, enzymes and electrolytes against isoproterenol induced cardiotoxicity in Wistar rats. Asian Pacific Journal of Tropical Disease 2;2012:588-91.
2. Wexler BC, Kittinger D. Isoprenaline-induced myocardial infarction in spontaneously hypertensive rats. Eur Soc Cardiol 1963;13:450-58.
3. Yogeeta SK, Gnanapragasam A, Kumar SS, Subhashini R, Sathivel A, Devaki T. Synergistic interactions of ferulic acid with ascorbic acid:its cardioprotective role during isoproterenol induced myocardial infarction in rats. Mol Cell Biochem 2006;283(1-2):139-46.
4. Heezen J, Menzies C, Heart J. Rona l, Drug discovery and evaluation:pharmacological assays. Eur 1959;25:250-54.
5. Somani KJ, Williams MJ, Restieaux NJ, Low CJ. Myocardial infarction in young people with normal coronary arteries. Heart, 1970:79;191-94.
6. Rona G. Catecholamine cardiotoxicity. J Mol Cell Cardiol 1985;17(4):291-306.
7. Simon R, J. Smoking educational status and health inequity in India. Ind Res 2000;124:15-22.
8. Agostino RB, Heart J. Primary and subsequent coronary risk appraisal:new results from the Framingham study. Am 2000;139:272-81.
9. Duthie SJ, Dobson VL. Dietary flavonoids protect human colonocyte DNA from oxidative attack in vitro. Eur J Nutr 1999;38(1):28-34.
10. Ahmed RS, Sharma SB. Biochemical studies on combined effects of garlic (Allium sativum Linn) and ginger (Zingiber officinale Rosc) in albino rats. Indian J Exp Biol 1997;35(8):841-3.
11. Cutler G, Perry KM, Tjian R. Adf-1 is a nonmodular transcription factor that contains a TAF-binding Myb-like motif. Mol Cell Biol 1998;18(4):2252-61.
12. Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases part I:General considerations, the epidemiologic transition, risk factors, and impact of urbanization. Circulation 2000;104:2746-753.
13. Raj CD, Shabi MM, Jipnomon J, Dhevi R, Gayathri K, Subashini U, et al. Terminalia arjuna's antioxidant effect in isolated perfused kidney. Res Pharm Sci 2012;7(3):181-8.
14. Subashini R, Rajadurai M, J. Evaluation of cardioprotective efficacy of Nelumbo nucifera leaf extract on isoproterenol-induced myocardial infarction in Wistar rats. Int Biosci 2011;2:285-94.
15. Lowry OH, Rosebrough MJ, Farr AL, Randall RJ, J. Protein measurement with Folins-phenol reagent. Chem 1951;193:265-75.
16. Falholt K, Lund B, Falholt W. An easy colorimetric micromethod for routine determination of free fatty acids in plasma. Clinica chimica acta;international journal of clinical chemistry 1973;46(2):105-11.
17. Dubois M, Gilles KA, Academic NY, New P. Methods in Enzymology1956.
18. Wagner WD. A more sensitive assay discriminating galactosamine and glucosamine in mixtures. Anal Biochem 1979;94(2):394-6.
19. Dische L, Shettles LB, J. Specific colour reactions of methyl pentoses and spectrophotometric micromethod for their determination. Chem 1948;175:595-604.
20. Warren L, J. Thiobarbituric acid assay of sialic acid. Chem 1959;234:1974-5.
21. Bonting SL. In:Membrane and ion transport. Wiley Interscience London 1970;257.
22. Hjerten S, Pan H. Purification and characterization of two forms of low affinity Ca2+ ATPase from erythrocyte membrane. Biochem Biophys Acta 1983;728:281-88.
23. Wattiaux R, Jamieson DM. In:Mammalian cell membranes1977.
24. Apel K, Hirt H. Reactive oxygen species:metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 2004;55:373-99.
25. Mourelle M, Franco MT. Erythrocyte defects proceed the onset of CCl4 induced liver cirrhosis. Protection by silymarin. Life Sci, 1991:48;1083-90.
26. Conchie J, Gelman AL, Levvy GA. Inhibition of glycosidases by aldonolactones of corresponding configuration. The C-4-and C-6-specificity of beta-glucosidase and beta-galactosidase. The Biochemical journal 1967;103(3):609-15.
27. Sapolsky AI, Altman RD, Howell DS. Cathepsin D activity in normal and osteoarthritic human cartilage. Fed Proc 1973;32(4):1489-93.
28. Lowry OH, Rosebrough MJ, Farr AL, J. R, J. Randall measurement with Folins-phenol reagent. Chem 1951;193:265-75.
29. Lindberg G, Eklund GA, Gullberg B, Råstam L. Serum sialic acid concentration and cardiovascular mortality. BMJ (Clinical research ed) 1991;302(6769):143-6.
30. Mathew S, Menon PV, Kurup PA. Changes in glycoproteins in isoproterenol-induced myocardial infarction in rats. Indian J Biochem Biophys 1982;19(1):41-3.
31. Zachariah B, Basu D. Surface carbohydrates in cell-sociology. Indian J Biochem Biophys 1993;30(6):422-5.
32. Judd JT, Wexler BC. Myocardial connective tissue metabolism in response to injury. II. Investigation of the mucopolysaccharides involved in isoproterenol-induced necrosis and repair in rat hearts. Circ Res 1970;26(1):101-9.
33. Crook MA, Earle K, Morocutti A, Yip J, Viberti G, Pickup JC. Serum sialic acid, a risk factor for cardiovascular disease, is increased in IDDM patients with microalbuminuria and clinical proteinuria. Diabetes Care 1994;17(4):305-10.
34. Mourelle M, Franco MT. Erythrocyte defects precede the onset of CCl4-induced liver cirrhosis. Protection by silymarin. Life Sci 1991;48(11):1083-90.
35. Trump BF, Berezesky IK, Sato T, Laiho KU, Phelps PC, DeClaris N. Cell calcium, cell injury and cell death. Environ Health Perspect 1984;57:281-7.
36. Sathish V, Ebenezar KK, Devaki T. Synergistic effect of Nicorandil and Amlodipine on tissue defense system during experimental myocardial infarction in rats. Mol Cell Biochem 2003;243(1-2):133-8.
37. Sudharsan PT, Mythili Y, Selvakumar E, Varalakshmi P. Lupeol and its ester ameliorate the cyclophosphamide provoked cardiac lysosomal damage studied in rat. Mol Cell Biochem 2006;282(1-2):23-9.
38. Ravichandran LV, Puvanakrishnan R, Joseph KT. Alterations in the heart lysosomal stability in isoproterenol induced myocardial infarction in rats. Biochem Int 1990;22(2):387-96.
39. Paritha IA, Devi CS, J. Effect of α-tocopherol on isoproterenol induced myocardial infarction in rats. Ind Pharmacol 1997;40:297-302.