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Protective Role of Phytic Acid on Cardiac Mitochondrial Enzymes during Isoproterenol-Induced Myocardial Infarction in Rats

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Abstract

The present study is to evaluate the preventive role of phytic acid on mitochondrial enzymes in isoproterenol (ISO)- induced myocardial infarction in male albino Wistar rats. Rats subcutaneously injected with ISO (85 mg/kg) at an interval of 24 h for 2 days, resulting in significant ($p < 0.05$) increase in the levels of mitochondrial lipid peroxides. ISO-induction also showed significant ($p < 0.05$) decrease in the activities of mitochondrial TCA cycle enzymes such as isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, and α-ketoglutarate dehydrogenase and respiratory chain enzymes such as NADH dehydrogenase and cytochrome c ox idase. Oral pretreatment with phytic acid (25 and 50 mg/kg) to ISO-induced rats daily for a period of 56 days significantly ($p < 0.05$) reduced the alterations in all the biochemical parameters and regulated the normal mitochondrial function. Transmission electron microscopic (TEM) study also correlated with these biochemical findings. Thus, the findings demonstrate that phytic acid prevents alterations in ISO-induced MI in rats.

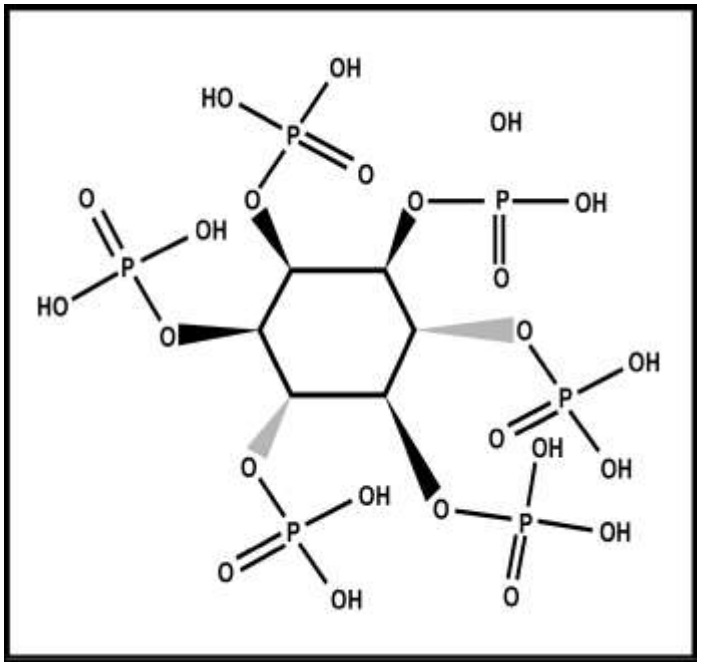
Keywords: Myocardial Infarction; Isoproterenol; Phytic acid; Mitochondria; Transmission Electron Microscopy

INTRODUCTION

Cardiovascular disease is long established as the leading cause of death in developed countries. Acute MI is usually characterized by varying degrees of chest pain, discomfort, sweating, weakness, nausea, vomiting, arrhythmias and sometimes causing loss of consciousness. Chest pain is the most common symptom of MI and is often described as pressure or squeezing sensation. Chest pain is more likely caused by MI [1,2]. In recent times, it has emerged as the leading cause of ill health in economically developing countries [3]. A large proportion of the world's population lives in developing countries and both the current and projected impact of CVD in these countries are enormous. Already more than four fifths of all cardiovascular deaths occur in developing countries and the great majority of the growth in global CVD burden over the next 20 years will be in these [4] The WHO has predicted that the deaths attributable to CVD have increased in parallel to the expanding population in India and CVD now accounts for a large proportion.

Isoproterenol (1-[3,4 dihydroxyphenyl]-2-isopropyl amino ethanol hydrochloride) is a synthetic catecholamine and β-adrenergic agonist, which has been found to cause severe stress in the myocardium resulting in infarct like necrosis of heart muscles [5]. During this reaction, highly toxic oxygen derived free radicals are generated, which are deterimental to extracellular and intracellular enzymes and proteins. Furthermore, free radicals could initiate the peroxidation of membrane bound PUFAs, leading to both functional and structural myocardial injury [5]. ISO-induced myocardial necrosis is a multifunctional incidence, which includes the role of hypoxia, coronary microcirculatory effects, membrane permeability alterations and the excessive formation of free radicals [7].

FIGURE 1: Structure of phytic acid



Dietary factors play a vital role in the development of various human diseases including CVD. Recently, there has been an upsurge of interest to explore the cardioprotective potential of natural products [8]. Natural products have lesser side effects than synthetic drugs. Phenolic acids have received much attention because of their role in the prevention of many human diseases, particularly atherosclerosis and cancer due to their antioxidant properties [9]. These compounds possess one or more aromatic rings bearing hydroxyl substituents act as antioxidants as a result of the reactivity of the phenolic moiety.

Phytic acid, myo-inositol hexaphosphate, is a plant component existing in most grain, legumes, wheat and rice bran and virtually every kind of mammalian cell [10,11]. It is considered to be the most abundant storage form of phosphorus present in food grains. It is usually a mixture of calcium/magnesium/ potassium salts of inositol hexaphosphoric acid and is the primary source of phosphorus soy and corn is shown to adversely impact mineral bioavailability and protein solubility when present in animal feeds [12].

MATERIALS AND METHODS

Experimental Animals

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 of Bharathidasan University (Approval no.BDU/IAEC/2011/31/29.03.2011). All the experiments were carried out with male albino Wistar rats weighing 140–160 g, obtained from the Central Animal House, Rajah Muthiah Institute of Health Sciences, Annamalai University, Annamalai Nagar, Tamil Nadu, India. They were housed in polypropylene cages (47 × 34 × 20 cm) lined with husk, renewed every 24 h under 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, Pune, India). The pellet diet consists of 21% protein, 5% lipids, 4% crude fiber, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamins and 55% nitrogen free extract. The diet provides metabolizable energy of 3,600 kcal.

Drugs and Chemicals

Isoproterenol hydrochloride, phytic acid, cytochrome c, *N*-phenyl *p*-phenylene diamine, oxaloacetate, sodium succinate, potassium ferricyanide, and α- ketoglutarate were purchased from Sigma Chemical Company (St. Louis, MO). All other chemicals used in the study were of analytical grade.

Induction of Experimental Myocardial Infarction

Isoproterenol (85 mg/kg) was dissolved in normal saline and injected subcutaneously into rats at an interval of 24 h for 2 days [13].

Experimental Design

The rats were grouped as 10 rats in each group. Two rats from each group were used for TEM studies. Group 1: Normal control rats; Groups 2; normal rats treated with phytic acid (25 and 50 mg/kg); Group 3; ISO (85 mg/kg) control rats; Groups 4 rats pretreated with phytic acid (25 and 50 mg/kg) and then subcutaneously injected with ISO. Phytic acid was dissolved in distilled water and administered to rats orally using an intragastric tube daily for a period of 56 days [13].

At the end of the experimental period, after 12 h of second ISO injection, all the rats were anesthetized with sodium pentobarbital and sacrificed by cervical decapitation. The heart tissue was excised immediately from the animals and washed off blood with ice-chilled physiological saline.

Isolation of Heart Mitochondrial Fractions

Heart mitochondria were isolated by the method of Takasawa et al. (1993) The heart tissue was put into ice-cold 50 mM Tris–HCl (pH 7.4), containing 0.25 M sucrose, and homogenized. The homogenates were centrifuged at 700×*g* for 20 min, and then the supernatant obtained were centrifuged at 9000×*g* 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.

Biochemical Estimations

The concentration of mitochondrial thiobarbituric acid reactive substances (TBARS) was estimated (Fraga et al., 1998). Activities of isocitrate dehydrogenase (ICDH), suc- cinate dehydrogenase (SDH), malate dehydrogenase (MDH), α-ketoglutarate dehydrogenase (α-KGDH), NADH dehydrogenase, and cytochrome c oxidase were assayed[17]. Protein content in the mitochon- drial fraction was estimated by the method of Lowry et al. (1951).

Transmission Electron Microscopic Studies

Small pieces of heart were taken and rinsed in 0.1 M phosphate buffer (pH 7.2). Approximately, 1-mm heart pieces were trimmed and immediately fixed into 3% ice-cold glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) and kept at 4° C for 12 h. Then, tissues processing for TEM studies were carried out. The grids containing sections were stained with 2% uranyl acetate and 0.2% lead acetate. Then, the sections were examined under a transmission electron microscope.

Statistical Analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using Statistical Package for the Social Sciences

(SPSS) software package version 9.05. Results were expressed as mean ± S.D. for eight rats in each group. *p* values < 0.05 were considered significant.

RESULTS

Isoproterenol-induced myocardial infarction was confirmed by elevated levels of TBARS in rats. Table 1 shows levels of mitochondrial TBARS in the heart of normal and experimental rats. Rats induced with ISO showed a significant ($p < 0.05$) increase in the levels of mitochondrial TBARS when compared with normal control rats. Oral pretreatment with phytic acid (25 and 50 mg/kg) to ISO-induced rats daily for a period of 56 days significantly ($p < 0.05$) decreased the levels of mitochondrial TBARS in the heart when compared with ISO-alone induced rats.

Table 1. Effect of Phytic acid on the levels of heart mitochondrial TBARS in normal and isoproterenol (ISO) – induced myocardial infarcted rats.

Groups	Mitochondrial TBARS (nmoles/mg ptn)
Normal control	3.16 ± 0.27 [a]
Normal + phytic acid (25 mg/kg)	3.09 ± 0.23 [a]
Normal + phytic acid 50 mg/kg)	3.14 ± 0.20 [a]
ISO (85 mg/kg) control	8.35 ± 0.47 [b]
Phytic acid (25 mg/kg) + ISO	5.33 ± 0.40[c]
Phytic acid (50 mg/kg) + ISO	4.72 ± 0.31[d]

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

Table 2: Effect of phytic acid on the activities of the heart mitochondrial isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH) and α-ketoglutarate dehydrogenase (α-KGDH) in normal and isoproterenol (ISO)-induced myocardial infracted rats.

Groups	ICDH	SDH	MDH	α-KGDH
Normal control	648.4±37.2[a]	222.3±17.1[a]	315. ±17.2 [a]	104.2±5.3[a]
Normal + phytic acid (25 mg/kg)	652.2±43.5[a]	224.2±15.2[a]	316.3±21.1[a]	104.5±6.7[a]
Normal + phytic acid 50 mg/kg)	653.1±32.3[a]	224.5±13.2[a]	317.4±19.8[a]	104.2±7.4[a]

ISO (85 mg/kg) control	412.6±23.5[b]	121.1±10.3[b]	190.4±10.2[b]	69.2±4.1[b]
Phytic acid (25 mg/kg) + ISO	543.7±41.3[c]	181.3±14.2[c]	263.2±17.2[c]	84.2±4.3[c]
Phytic acid (50 mg/kg) + ISO	612.1±35.2[d]	208.2±16.8[d]	296.4±16.2[d]	92.3±4.2[d]

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

Activities of the heart mitochondrial TCA cycle enzymes such as ICDH, SDH, MDH and α-KGDH were decreased significantly in ISO-induced rats when compared with normal control rats. Oral pretreatment with phytic acid (25 and 50 mg/kg) to ISO-induced rats significantly increased the activities of these enzymes when compared with ISO- alone induced rats (Table 2).

Table 3: Effect of phytic acid on the activities of the heart mitochondrial NADH-dehydrogenase and cytochrome-c-oxidase in normal and isoproterenol (ISO)-induced myocardial infarcted rats.

Groups	**NADH-dehydrogenase**	**Cytochrome c-oxidase**
Normal control	161.5±10.0[a]	0.451±0.01[a]
Normal + phytic acid (25 mg/kg)	163.1±12.3[a]	0.453±0.01[a]
Normal + phytic acid 50 mg/kg)	165.5±13.9[a]	0.454±0.02[a]
ISO (85 mg/kg) control	84.7±4.8[b]	0.287±0.02[b]
Phytic acid (25 mg/kg) + ISO	120.7±7.8[c]	0.358±0.01[c]
Phytic acid (50 mg/kg) + ISO	146.4±7.8[d]	0.411±0.01[d]

Activity is expressed as nmoles of NADH oxidized/min/mg protein for NADH-dehydrogenase; nmoles/min/mg protein for cytochrome-c-oxidase. Each value is mean ± S.D. for 6 rats in each group. Values not sharing a common superscript (a,b,c,d) differ significantly with each other $P < 0.05$ (DMRT).

The activities of the heart mitochondrial respiratory chain enzymes such as NADH-dehydrogenase and cytochrome-c-oxidase in normal and ISO-induced rats are shown in

Table 3. The activities of these enzymes were significantly decreased in ISO-induced rats when compared with normal control rats. Oral pretreatment with phytic acid (25 and 50 mg/kg) to ISO-induced rats significantly increased the activities of these enzymes when compared with ISO-alone induced rats.

Effect of phytic acid on heart mitochondria-TEM study

The TEM images of the heart mitochondria are shown in figure (a-d). Normal control rats showed the normal architecture of the heart mitochondria (Figure 2- a). Phytic acid (50 mg/kg) treated normal rats heart mitochondria showed no pathological changes (Figure 2-b). ISO-induced rats showed swelling of mitochondria and loss of cristae with vacuolation (Figure 2-c). Rats pretreated with phytic acid (50 mg/kg) to ISO-induced rats showed mild dissociation of cristae without swelling and vacuolation figure (2-d).

Fig 2 (a-d): Effect of phytic acid on the heart mitochondria (TEM –study)

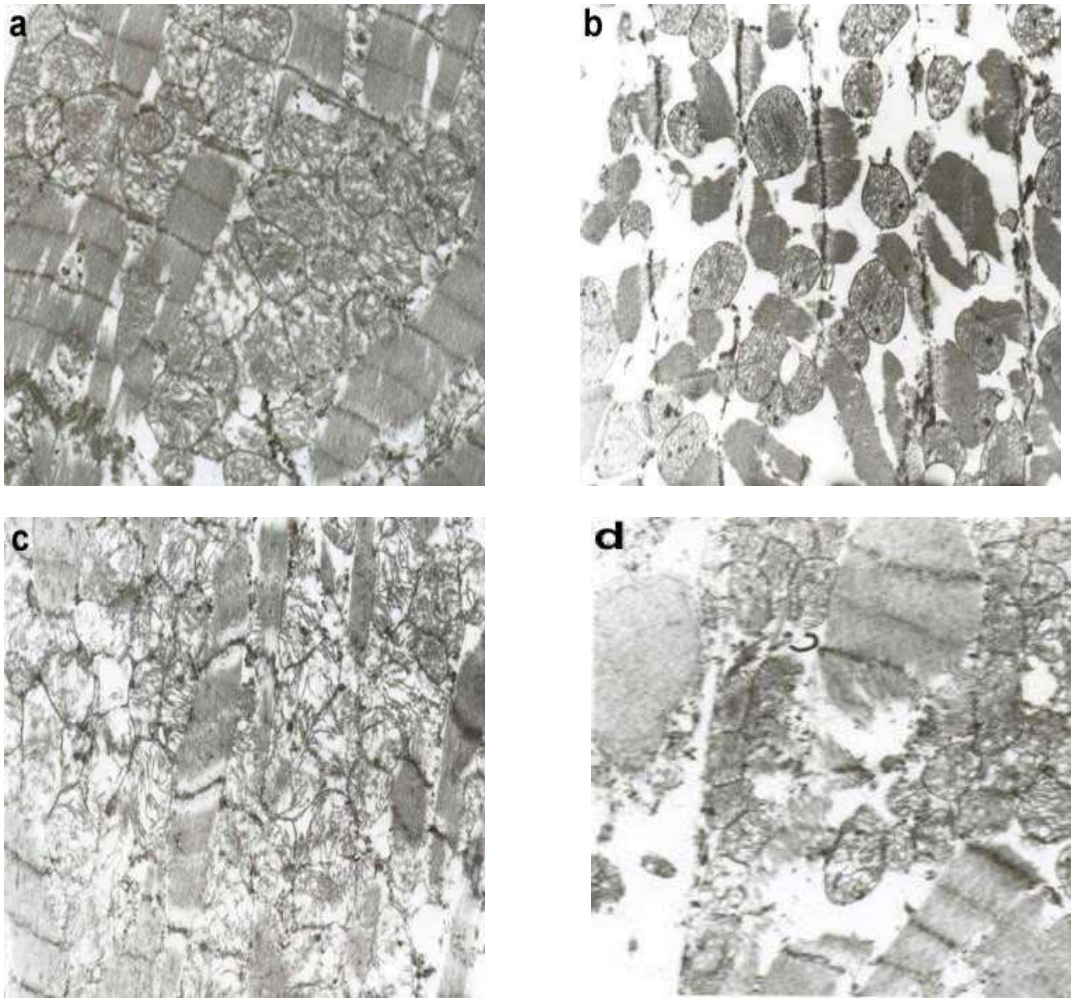


FIGURE 2

Normal group showing normal architecture of the heart mitochondria; (b) normal and phytic acid (25 mg/kg) treated rats heart showing no pathological changes; (c) ISO-induced (85 mg/kg) cardiac mitochondria showing swelling of mitochondria and loss of cristae with vacuolation; (d) phytic acid (50 mg/kg) and ISO-induced heart mitochondria showing mild dissociation of cristae without swelling and vacuolation.

DISCUSSION

Thus, ISO produces relative ischemia or hypoxia due to myocardial hyperactivity and coronary hypotension [18] and induce myocardial ischemia due to cytosolic Ca^{2+} overload [19]. Grimm et al. (1998) have reported that a toxic dosage of ISO caused characteristic myocardial damage that subsequently resulted in heart failure. ISO-administration causes ischemic necrosis in rats, which closely resembles histological damage seen in human MI. Free radicals could initiate the peroxidation of membrane bound polyunsaturated fatty acids (PUFA), leading to both functional and structural myocardial injury [21]. The effects of isoproterenol (ISO) on heart are mediated through β_1 and β_2 adrenoceptors. Both β_1 and β_2 adrenoceptors mediate the positive inotropic and chronotropic effects to β adrenoceptor agonists [22].

Increased levels of mitochondrial free radical production experiential under pathological conditions, such as ischemia, are associated with impairment of mitochondrial structure and function. We observed increased levels of heart mitochondrial TBARS in ISO- induced rats indicating increased lipid peroxidation, which could be credited to deficiency of antioxidant system [23]. Pretreatment with phytic acid to ISO- induced rats daily for a period of 56 days decreased the levels of heart mitochondrial TBARS. Flavonoid activities mainly depend on their antioxidant and chelating properties, which are responsible for the inhibitory effect of flavonoids on lipid peroxidation. In this context, Jagetia and Reddy have reported that phytic acid treatment reduced the levels of TBARS in radiation induced lipid peroxidation [24].

We have observed a decrease in the activities of tricarboxylic acid cycle enzymes, such as ICDH, SDH, MDH, and α-KGDH, in the heart mitochondria in ISO- induced rats. These enzymes are located on the outer membrane of mitochondria and could be pretentioused by excessive production of free radicals by ISO. Our results are in agreement with previous reports [25,26]. Pretreatment with phytic acid to ISO-induced rats significantly increased the activities of tricarboxylic acid cycle enzymes. This could be due to its ability to prevent free radical formation and free radical scavenging proper- ties of phytic acid.

Biological membranes and subcellular organelles rich in PUFA are the major sites for the free radical mediated damage. Activation of lipid peroxidation in mitochondria

corresponds with changes in lipid composition, which includes a decrease in the levels of total and readily oxidizable lipid, cardiolipin. Cytochrome c oxidase and NADH dehydrogenase are present in the inner mitochondrial membrane and are involved in the synthesis of high-energy compound ATP. These enzymes have an absolute requirement of cardiolipin. The activities of these enzymes were decreased in the heart mitochondria of ISO-induced rats. This could be due to enhanced phospholipids degradation resulting in the nonavailability of cardiolipin for their functional activity. Previous studies also reported diminished ac- tivities of cytochrome c oxidase and NADH dehydro- genase in rats induced with ISO [25,26].

Pretreatment with phytic acid increased the activities of NADH dehydrogenase and cytochrome c oxidase in ISO-induced rats. Our results indicate that pretreatment with phytic acid substantially prevented the excessive impairment of these enzyme activities. Furthermore, our study suggests that phytic acid may restore energy status of the mitochondria, thereby maintaining membrane integrity. This could be due to the inhibition of PLs degradation in the biological membranes and maintain the levels of cardiolipin in the membrane PLs.

The transmission electron micrograph of the mitochondria of the heart in ISO-induced rats showed swelling morphology of mitochondria and loss of cristae with vacuolation. The swollen morphology is typical for mitochondria that have been subjected to ischemic and hypoxic conditions [27], which could be due to the accumulation of lipid peroxide products as a result of GSH depletion [28]. Rats pretreated with phytic acid (25 mg/kg) in ISO-induced rats showed mild swelling with dissociation of cristae and rats pretreated with phytic acid (50 mg/kg) in ISO-induced rats showed mild dissociation of cristae without swelling and vacuolation. phytic acid (25 and 50 mg/kg) treated normal rats heart mitochondria showed no pathological changes, which indicates that phytic acid does not possess any adverse effects under normal conditions. The anti-lipid peroxidative and antioxidant property of phytic acid could have reduced the mitochondrial lipid peroxidation and maintain the normal functioning of mitochondria in the myocardium.

CONCLUSION

In conclusion, the results obtained from our study specified that phytic acid offers protection to the cardiac mitochondria by decreasing the levels of lipid peroxides and maintaining the activities of mitochondrial enzymes in ISO-induced rats. This could be due to its antioxidant as well as membrane-stabilizing effects. TEM study also supports these biochemical findings in ISO-induced rats. Restoration of cellular normalcy accredits the cytoprotective role of phytic acid, as phytic acid possessed protective effect on mitochondria, which is a crucial element involved in both triggering and mediating the cardioprotective responses in myocardial cells. Thus, this study may have a significant impact on the clinical treatment of myocardial diseases. On the basis of the previous and

present findings, we speculate that phytic acid could be an effective chemopreventive agent against ISO-induced myocardial infarction and associated oxidative stress.

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