



## Phytic acid Ameliorates Mitochondrial Lipid Peroxides, Antioxidants and Lipids in Isoproterenol-Induced Myocardial Infarction in Wistar Rats

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### Article info

### Abstract

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Aim of the present study is to evaluate the preventive role of phytic acid on mitochondrial lipid peroxides, antioxidants and lipids in isoproterenol (ISO)-induced myocardial infarction (MI) in male Wistar rats. Rats subcutaneously injected with ISO (85 mg/kg) at an interval of 24 h for 2 days resulted in a significant increase in the levels of mitochondrial lipid peroxides with a significant decrease in the activities of mitochondrial antioxidants (SOD, catalase, glutathione peroxidase, glutathione-S-transferase and reduced glutathione). Induction of ISO also showed a significant increase in the levels of mitochondrial cholesterol, triglycerides and free fatty acids with a subsequent decrease in the levels of phospholipids. Oral pretreatment with phytic acid (25 and 50 mg/kg) to ISO-induced rats daily for a period of 56 days significantly decreased the levels of mitochondrial lipid peroxides with a significant increase in the activities of mitochondrial antioxidants and significantly minimized the alterations in the mitochondrial lipid levels in ISO-induced rats. Thus, the findings demonstrate that phytic acid prevents alterations in mitochondrial lipid peroxides, antioxidants and lipids in ISO-induced MI in rats.

**Keywords:** phytic acid; isoproterenol; myocardial infarction; mitochondria.

## INTRODUCTION

Cardiovascular disease is the principal cause of death worldwide. According to the World Health Organization (WHO), CVD was estimated to have caused over 15 million deaths comprising more than a quarter of all deaths in that year [1]. About 7.2 million of these deaths were due to ischemic heart disease (IHD) and 5.5 million were due to cerebrovascular disease (stroke). By the year 2020, it is estimated that 37% of all deaths worldwide will be from CVD. The great majority of these deaths will be due to stroke and heart attack. No other diseases currently cause this number of deaths or have such a large projected increase in the total number of deaths over this time period. The effects of isoproterenol (ISO) on heart are mediated through  $\beta_1$  and  $\beta_2$  adrenoceptors. Both  $\beta_1$  and  $\beta_2$  adrenoceptors mediate the positive inotropic and chronotropic effects to  $\beta$  adrenoceptor agonists [2]. Thus, ISO produces relative ischemia or hypoxia due to myocardial hyperactivity and coronary hypotension [3] and induce myocardial ischemia due to cytosolic  $\text{Ca}^{2+}$  overload [4]. 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.

Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs [6]. Traditional medicinal plants are often cheaper, locally available and can be easily consumable as raw or simple medicinal preparations. Nowadays, traditional medicinal practices form an integral part of complementary or alternative medicine. Although their efficacy and mechanism of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents [7]. The Rasayanas are prepared from several plant extracts, which contain strong antioxidants and are used as rejuvenators or nutritional supplements [8].

Phytopharmaceuticals are gaining importance in allopathic as well as traditional medicine owing to their non-addictive and non-toxic in nature. Novel antioxidants may offer an effective and safe means of counteracting some of the problems and bolstering the body's defense against free radicals and CVD [9]. In the past decade, substantial progress has been made concerning knowledge of bioactive components in plant foods and their links to health. Phytochemicals with antioxidant properties tend to be brightly colored because they contain chromophores, ie, a series of alternating single-bonded and double-bonded carbons.

Phytic acid, myo-inositol hexaphosphate (1,2,3,4,5,6- hexakis dihydrogen phosphate), 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 (accounts for 65-85% of seed total phosphorus [12]) 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 are shown to adversely impact mineral bioavailability and protein solubility when present in animal feeds [13]. The accumulation of phosphorus in soils and the threat to surface water quality that may result from phosphorus losses to water ways due to runoff or leaching are major challenges facing animal agriculture today. It possesses a broad range of bioactivities. It has been long used as safe natural antioxidant by removing active oxygen in the body and suppressing lipid peroxide production. It absorbs excess iron ions, enhancing immunity and has an effect on heart disease, liver dysfunction, dermatitis and anticancer actions. It is known as further to prevent kidney stone formation and cholesterol deposition. The remarkable affinity of IP6 for iron totally inhibits this metal's ability to catalyze the formation of hydroxyl radicals which cause oxidation and cancer.

Phytic acid was shown to inhibit OH formation and decrease lipid peroxidation catalyzed by iron and ascorbic acid in human erythrocytes [14]. The protective effect of IP6 was suggested based on its antioxidant effect or its ability to alter cell signaling pathways or antioxidant enzyme that detoxify the ROS [15].

## **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.

**Induction of experimental myocardial infarction.** Isoproterenol (85 mg/kg) was dissolved in normal saline and injected subcutaneously to rats at an interval of 24 h for two days [16]. ISO-induced myocardial infarction was confirmed by elevated activities of serum creatine kinase (CK), CK-MB and lactate dehydrogenase.

### 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; Group 2 ; normal rats treated with phytic acid (25 and 50 mg/kg); Group3; 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.

At the end of the experimental period, after 12 h of the second ISO-injection, all the rats were anesthetized with sodium pentobarbital and killed by cervical de-capitation. The heart tissue was excised immediately from the animals, and blood washed off 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  $\mu$  g for 20 min and then the supernatants obtained were centri-fuged at 9000  $\mu$  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 assays:** The concentration of thiobarbituric acid reactive substances (TBARS) was estimated by the method of Fraga *et al.* (1988). The activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione-S-transferase (GST) were assayed by the methods of Kakkar *et al.* (1984), Sinha (1972), Rotruck *et al.* (1973) and Habig and Jackoby (1981), respectively. Glutathione (GSH) levels were estimated by the method of Ellman (1959). Lipids were extracted by the method of Folch *et al.* (1957). The levels of total cholesterol and triglycerides (TGs) were estimated by the method of Zlatkis *et al.* (1953) and Foser and Dunn (1973). Free fatty acids (FFAs) and phospholipids (PLs) levels were estimated by the methods of Falholt *et al.* (1973) and Zilversmit and Davis (1950). Protein content of the mitochondria was estimated by the method of Lowry *et al.* (1951).

### 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  $\mu$  SD for eight rats in each group. Values of  $p < 0.05$  were considered significant.

## RESULTS

### Effect of phytic acid on mitochondrial antioxidants

Table 1 shows the levels of mitochondrial SOD and catalase in the heart of normal and ISO-induced rats. Rats induced with ISO showed a significant decrease in the activities of these antioxidant enzymes in the heart when compared to normal control rats. 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 1. Effect of phytic acid on the activities of heart mitochondrial superoxide dismutase (SOD) and catalase in of normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.**

Groups	SOD (U/100mg protein)	Catalase (units/mg protein)
Normal control	8.64±0.67 <sup>a</sup>	6.46 ±0.37 <sup>a</sup>
Normal + phytic acid (25 mg/kg)	8.71 ±0.82 <sup>a</sup>	6.51 ±0.46 <sup>a</sup>
Normal + phytic acid 50 mg/kg)	8.78±0.70 <sup>a</sup>	6.56 ±0.38 <sup>a</sup>
ISO (85 mg/kg) control	5.40 ±0.30 <sup>b</sup>	2.05 ±0.16 <sup>b</sup>
Phytic acid (25 mg/kg) + ISO	6.24± 0.57 <sup>c</sup>	4.15 ±0.27 <sup>c</sup>
Phytic acid (50 mg/kg) + ISO	7.23± 0.68 <sup>d</sup>	5.11 ± 0.35 <sup>d</sup>

SOD units: 1U is defined as the enzyme concentration required to inhibit the OD at 560 nm of chromogen production by 50% in 1 min. Each value is mean±S.D. for eight rats in each group. Values not sharing a common superscripts (a, b, c, and d) differ significantly at  $P < 0.05$  (DMRT).

Table 2 depicts the activities of mitochondrial GPx and GST and the levels of GSH in normal and ISO-induced rats. Rats induced with ISO showed a significant decrease in the activities of mitochondrial GPx and GST and the levels of GSH in the heart when compared to normal control rats. Pretreatment with phytic acid (25 and 50 mg/kg) to ISO-induced rats significantly increased the activities of these enzymes and the levels of GSH in the heart mitochondrial when compared with ISO-alone induced rats.

**Table 2. Effect of phytic acid on the activities of the heart mitochondrial glutathione peroxidase (GP<sub>x</sub>) and glutathione-S-transferase (GST) and the levels of reduced glutathione (GSH) in normal and isoproterenol (ISO)-induced myocardial infarcted rats.**

Groups	GP <sub>x</sub> (Units/100 mg protein)	GST (Units/100 mg protein)	GSH (Units/100 mg protein)
Normal control	1.76±0.08 <sup>a</sup>	53.4±3.67 <sup>a</sup>	5.12±0.27 <sup>a</sup>
Normal + phytic acid (40mg/kg)	1.80±0.08 <sup>a</sup>	53.1±2.76 <sup>a</sup>	5.17±0.28 <sup>a</sup>
Normal + phytic acid 50 mg/kg)	1.81±0.010 <sup>a</sup>	54.1±4.28 <sup>a</sup>	5.20±0.39 <sup>a</sup>
ISO (85 mg/kg) control	0.95±0.07 <sup>b</sup>	34.7±2.39 <sup>b</sup>	3.02±0.12 <sup>b</sup>
Phytic acid (40 mg/kg) + ISO	1.41±0.12 <sup>c</sup>	42.4±2.35 <sup>c</sup>	4.02±0.28 <sup>c</sup>
Phytic acid (50 mg/kg) + ISO	1.63±0.10 <sup>d</sup>	49.1±4.56 <sup>d</sup>	4.64±0.21 <sup>d</sup>

GPX unit-nmoles of GSH consumed/min/100 mg protein; GST unit – nmoles of CDNB conjugated/min/100 mg protein. CDNB – 1- chloro 2,4-dinitro benzene. 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).

**Table 3. Effect of phytic acid on the levels of the heart mitochondrial total cholesterol, triglycerides (TGS), free fatty acids (FFAs) and phospholipids (PLs) in normal and isoproterenol (ISO)-induced myocardial infarcted rats.**

Groups	Total cholesterol (nmoles/mg protein)	TGs (nmoles/mg protein)	FFAs (nmoles/mg protein)	PLs (nmoles/mg protein)
Normal control	32.1±2.1 <sup>a</sup>	19.8±1.5 <sup>a</sup>	9.98±0.45 <sup>a</sup>	482.1±28.64 <sup>a</sup>
Normal + phytic acid (25 mg/kg)	31.4±1.8 <sup>a</sup>	19.3±1.3 <sup>a</sup>	9.92±0.23 <sup>a</sup>	479.2±26.2 <sup>a</sup>
Normal + phytic acid 50 mg/kg)	31.2±1.9 <sup>a</sup>	19.1±1.2 <sup>a</sup>	9.89±0.67 <sup>a</sup>	479.3±23.2 <sup>a</sup>
ISO (85 mg/kg) control	50.2±3.8 <sup>b</sup>	36.2±1.8 <sup>b</sup>	16.5±1.12 <sup>b</sup>	331.3±23.5 <sup>b</sup>
Phytic acid (25 mg/kg) + ISO	41.0±2.88 <sup>c</sup>	27.4±2.48 <sup>c</sup>	13.9±1.018 <sup>c</sup>	405.3±27.38 <sup>c</sup>
Phytic acid (50mg/kg) + ISO	36.2±2.5 <sup>d</sup>	23.5±1.8 <sup>d</sup>	11.1±0.87 <sup>d</sup>	442.3±19.2 <sup>d</sup>

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)).

Table 3 illustrates the levels of the heart mitochondrial cholesterol, TGs, FFAs, and PLs in normal and ISO-induced rats. Rats induced with ISO showed a significant increase in the levels of the heart mitochondrial cholesterol, TGs and FFAs with subsequent decrease in the levels of PLs when compared to normal control rats. Pretreatment with phytic acid (25 and 50 mg/kg) to ISO-induced rats significantly decreased levels of the heart mitochondrial cholesterol, TGs and FFAs and increased the levels of PLs when compared with ISO-alone induced rats.

## **DISCUSSION**

Damage to the myocardial cells arises due to the generation of toxic reactive oxygen species (ROS) such as superoxide radicals, H<sub>2</sub>O<sub>2</sub> and hydroxyl radical [30]. It is well known that cardiovascular disease (CVD) is directly or indirectly related to oxidative damage that shares a common mechanism of molecular and cellular damage. Auto-oxidation of catecholamines results in the generation of highly cytotoxic free radicals [31]. Free radicals could initiate the peroxidation of membrane bound polyunsaturated fatty acids (PUFA), leading to both functional and structural myocardial injury [32]. The effects of isoproterenol (ISO) on heart are mediated through  $\beta_1$  and  $\beta_2$  adrenoceptors. Both  $\beta_1$  and  $\beta_2$  adrenoceptors mediate the positive inotropic and chronotropic effects to  $\beta$  adrenoceptor agonists [33]. Thus, ISO produces relative ischemia or hypoxia due to myocardial hyperactivity and coronary hypotension [34] and induce myocardial ischemia due to cytosolic Ca<sup>2+</sup> overload [35]. 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.

Oxidative stress in cells or tissues results in the enhanced generation of ROS and/or depletion of the antioxidants in the defense system, thereby causing an imbalance between the prooxidants and antioxidants. The ROS generations in tissues are efficiently scavenged by the enzymatic and non-enzymatic antioxidant systems. The decrease in the activities of antioxidant enzymes is in close relationship with the induction of lipid peroxidation [37]. Enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione-S-transferase (GST) are the primary defense system against oxidative stress.

In our study, ISO-induced rats exhibited a decrease in the activities of SOD and catalase in the heart. SOD plays an important role in protecting the cells from oxidative damage by converting superoxide radicals into H<sub>2</sub>O<sub>2</sub>, which is further metabolized by catalase to molecular oxygen and water. The decrease in the activities of these antioxidant enzymes might be due to myocardial cell damage. Superoxide radicals generated at the site of

damage modulates SOD and catalase resulting in decreased activities of these enzymes and accumulation of superoxide anion, which also damages the myocardium.

Increased levels of mitochondrial free radical production observed under pathological conditions such as ischemia is 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 attributed to deficiency of antioxidant system[38]. Pretreatment with phytic acid to ISO-induced rats decreased the levels of heart mitochondrial TBARS.

Aerobic organisms are endowed with antioxidant enzymes and non-enzymic antioxidants. However, when ROS generation exceeds the antioxidant capacity of cells, oxidative stress develops, potentially causing tissue damage [39]. The observed decrease in the activities of mitochondrial SOD and catalase may be due to increased generation of ROS, such as superoxide and hydrogen peroxide, which in turn leads to the inhibition of these enzyme activities.

Studies have shown that GSH depletion and accumulation of its oxidized form (GSSG), occur in heart muscles within minutes of oxidative stress and precede abnormalities in electrical and contractile functions. GSH is utilized for the inactivation of lipid peroxides through the activity of GPx, which generates GSSG as byproducts. GSH is also required for the conjugating activity of GST, which detoxifies reactive byproducts of lipid peroxidation and other xenobiotic molecules [40]. The observed decrease in the concentration of GSH and the activities of GPx and GST in ISO-induced rats in our study was supported by previous report [41]. The decrease in the activities of GPx and GST in ISO-induced rats could be due to reduced availability of its substrate, GSH [42]. A decrease in the activity of GPx makes mitochondria more susceptible to oxidative damage, which leads to mitochondrial dysfunction [43].

Pretreatment with phytic acid significantly increased the activities of mitochondrial SOD, catalase, GPx and GST as well as increased the levels of mitochondrial GSH in the heart of ISO-induced rats.

Rats induced with ISO showed a significant increase in the levels of mitochondrial cholesterol and TGs. A significant increase in mitochondrial cholesterol is well associated with myocardial ischemia [44]. Altered cholesterol levels in the mitochondrial membrane affects the fluidity, permeability of the ions and the activities of membrane bound enzymes. Increased levels of the mitochondrial cholesterol suggest the redistribution of cholesterol. We also observed a significant increase in the levels of FFAs and decrease in the levels of PLs in mitochondria of the heart in ISO-induced rats. This could be due to accelerated degradation of membrane PLs by phospholipase. Accelerated degradation of

membrane PLs by phospholipase and lysophospholipase has been related to membrane dysfunction and ischemic injury [45]. Accumulation of FFAs is a consequence of changes in the metabolism. These changes in the metabolism of subcellular fractions may lead to damage of the mitochondrial membranes in cardiac myocytes, which may lead to the disorders of contractile properties of the myocardium. Similar results have been reported by other workers in ISO-induced rats [46].

Pretreatment with phytic acid significantly decreased the levels of mitochondrial cholesterol, TGs and FFAs and increased PLs in ISO-induced rats. It has been reported that supplementation of phytic acid decreased the levels of lipids in high fat and high cholesterol fed rats [47]. The carbonyl and hydroxyl groups of phytic acid may scavenge the free radicals and indirectly helped to decrease the levels of lipids by reducing or inhibiting the lipid peroxidation process.

### **CONCLUSION**

This study shows that phytic acid exhibits cardioprotective effect in ISO-induced MI in rats by decreasing the levels of mitochondrial lipid peroxides, increasing antioxidants status and minimizing the alterations in the levels of lipids in ISO-induced rats. This could be due to the prevention or inhibition of lipid peroxidative system by its antioxidant effect and lipid lowering effect of phytic acid. This study also further strengthens the cardioprotective effect of phytic acid.

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