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RESEARCH ARTICLE

Preventive effect of Phytic acid on lipids and lipoproteins in isoproterenol-induced Myocardial infarction in Wistar rats

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Abstract

This study was designed to evaluate the preventive effect of phytic acid in isoproterenol (ISO)-induced myocardial infarction (MI) in rats. Rats were pretreated with phytic acid (25 and 50 mg/kg body weight) orally for a period of 56 d. After the treatment period, ISO (85 mg/kg body weight) was administered subcutaneously to rats at an interval of 24 h for 2 d. It was noted that there was a significant increase in the levels of total, ester, and free cholesterol, triglycerides (TG), and free fatty acids (FFA) in serum and heart and decrease in heart phospholipids (PL) in ISO-induced rats. Altered levels of lipoproteins in plasma were also observed in ISO-induced rats. Pretreatment with phytic acid (25 and 50 mg/kg) for a period of 56 d significantly decreased the levels of total, ester, and free cholesterol, TG, FFA in serum and heart and increased PL in heart. It also minimized the alterations in serum lipoproteins in ISO-induced rats.

Keywords: Phytic acid, isoproterenol, myocardial infarction, cholesterol, triglycerides, free fatty acids.

Introduction

Myocardial infarction is one of the main causes of death from cardiovascular diseases. Myocardial ischemia occurs while myocardial oxygen demand exceeds oxygen supply causing cell injury known as myocardial infarction, which is one of the most fatal manifestations of cardiovascular diseases (Mohanty *et al*., 2004). An increased risk of coronary heart disease (CHD) is associated with high levels of serum total cholesterol (Grundy, 1986) and low density lipoprotein (LDL) (Brown and Goldstein, 1986) and decreased levels of high density lipoprotein (HDL) (Castelli *et al*., 1986). Isoproterenol (ISO), a synthetic catecholamine and β-adrenergic agonist has been found to cause severe stress in the myocardium resulting in necrosis of the heart muscles (Wexler and Greenberg, 1978). ISO-induced myocardial necrosis showed membrane permeability and alterations which fetch about the loss of function and veracity of myocardial membranes (Todd *et al.,* 1980). Alterations in lipid metabolism were also characteristic of ISO-induction in rats (Prince and Rajadurai, 2005). A number of substances have been identified for their ability to protect against experimental MI induced by ISO. Any substance which can prevent an attack or accelerate the process of recovery will have considerable clinical applications. Phytic acid is a plant component existing in most grains and legumes is usually regarded as an anti-nutritive factor and much attention has been focused on the biologic effects of phytic acid in human beings and animals (Muraoka and Miura, 2004; Coulibaly *et al*., 2011).

Over the last decade potential beneficial effects of phytic acid have also been recognized. *In vitro* studies indicate that phytic acid acts as an antioxidant through its iron chelating properties and considered to be the most abundant storage form of phosphorus present in food grains. The phytic acid accounts for 65-85% of seed total phosphorus (Abdul jabbar khan *et al*., 2007). In this study, cardioprotective action of phytic acid was investigated by studying the levels of lipids and lipoproteins in ISO-induced MI in male albino Wistar rats.

Materials and methods

Drugs and chemicals: Phytic acid, isoproterenol and digitonin were purchased from Sigma Chemicals (USA). Ammonium molybdate, aminonapthol sulfonic acid, hydroxylamine hydrochloride, dextran sulfate and glycerol trioleate were purchased from S.D. Fine Chemicals (Mumbai, India). All other chemicals used in the study are of analytical grade.

Experimental animals: 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, TN, India. They were housed in polypropylene cages (47 cm × 34 cm × 20 cm) lined with husk, renewed every 24 h under a 12:12 h light ark 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, India).

Table 1. Effect of phytic acid on total, ester and free cholesterol in serum and heart of normal and ISO- induced myocardial infarcted rats.

Groups	Normal Control	Normal + phytic acid (25 mg/kg)	Normal + phytic acid (50 mg/kg)	ISO (85 mg/kg) Control	Phytic acid (25 mg/kg) + ISO	Phytic acid (50 mg/kg) + ISO
Serum (mg/dL)						
Total cholesterol	88.12±4.87^{a}	87.01±4.12^{a}	85.73±5.23^{a}	140.13±7.87^{b}	112.23±7.24^{c}	96.75±6.01^{d}
Ester cholesterol	56.12±2.79^{a}	55.34±3.20^{a}	54.76±3.32^{a}	81.36±4.23^{b}	68.54±5.01^{c}	62.03±3.65^{d}
Free cholesterol	32.33±1.98^{a}	32.03±1.87^{a}	31.45±2.13^{a}	56.94±3.46^{b}	44.42±3.16^{c}	37.65±2.31^{d}
Heart (mg/g wet tissue)						
Total cholesterol	7.14±0.24^{a}	7.01±0.32^{a}	7.04±0.36^{a}	12.54±0.64^{b}	10.02±0.56^{c}	8.76±0.54^{d}
Ester cholesterol	4.27±0.1^{a}	4.42±0.23^{a}	4.42±0.26^{a}	7.54±0.43^{b}	6.12±0.36^{c}	5.41±0.28^{d}
Free cholesterol	2.76±0.13^{a}	2.87±0.18^{a}	2.76±0.16^{a}	5.12±0.28^{b}	4.02±0.24^{c}	3.45±0.21^{d}

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

The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.25% 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 3000 kcal. 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).

Induction of experimental myocardial infarction: Isoproterenol (85 mg/kg) dissolved in normal saline was injected subcutaneously to rats at an interval of 24 h for 2 d to induce experimental MI (Gupta *et al.,* 2004).

Experimental design: The animals were grouped as 6 rats in each group.
Group I: Normal control rats;
Groups II and III: Normal rats treated with phytic acid (25 and 50 mg/kg);
Group IV: ISO (85 mg/kg) control rats;
Groups V and VI: Rats pretreated with phytic acid (25 and 50 mg/kg) and then subcutaneously injected with ISO.
Phytic acid was dissolved in water and administered to rats orally using an intragastric tube daily for a period of 56 d. After the last treatment, all the rats were sacrificed by cervical decapitation after an overnight fast. Blood was collected and plasma was separated by centrifugation. Heart tissue was excised immediately and rinsed in ice-chilled normal saline. A known weight of the heart tissue was homogenized in 5 mL of 0.1 M Tris–HCl buffer (pH 7.4) solution. The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

Biochemical parameters: Lipids were extracted by the method of Folch *et al.* (1957). The levels of total, ester, and free cholesterol, triglycerides (TG), free fatty acids (FFA) and phospholipids (PL) were estimated (Zilversmit and Davis, 1950; Zlatkis *et al*., 1953; Foster and Dunn, 1973; Falholt *et al*., 1973; Varley *et al*., 1991). Phosphorus content was estimated by the method of Fiske and Subbarow (1925).

High-density lipoprotein (HDL) cholesterol was estimated using a commercial kit purchased from Qualigens Diagnostics (72201) (Mumbai, India). Very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) fractions were calculated as VLDL = Triglycerides/5 and LDL = Total cholesterol − (HDL cholesterol + VLDL cholesterol).

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. *p* values <0.05 were considered as significant. All the results were expressed as mean ± SD for 6 rats in each group.

Results

Total, free, and ester cholesterol levels in serum and heart in normal and experimental rats are shown in Table 1. Rats treated with ISO showed a significant (p<0.05) increase in total, free, and ester cholesterol levels when compared to normal rats. Pretreatment with phytic acid (25 and 50 mg/kg) for a period of 56 d to ISO-induced rats showed a significant (p<0.05) decrease in the levels of total, free, and ester cholesterol. Table 2 shows the levels of HDL, LDL, and VLDL in serum in normal and experimental rats. Rats treated with ISO showed a significant (p<0.05) increase in LDL and VLDL levels, with a significant (p<0.05) decrease in HDL levels when compared to normal rats. Pretreatment with phytic acid (25 and 50 mg/kg) to ISO-induced rats showed a significant (p<0.05) decrease in the levels of LDL and VLDL with subsequent increase in the levels of HDL cholesterol.

Table 3 and 4 shows the levels of TG, FFA and PL in serum and heart in normal and experimental rats. ISO-induced rats showed a significant (p<0.05) increase in the levels of TG, FFA in serum and heart and PL in serum with a significant decrease in PL in heart when compared to normal rats. Pretreatment with phytic acid (25 and 50 mg/kg) to ISO-induced rats significantly (p<0.05) decreased the levels of TG, FFA and significantly increased the concentration of PL in heart. Table 5 shows the activities of HMG-CoA reductase, LCAT and LPL in plasma and HMG-CoA reductase in liver in normal and experimental rats.

Table 2. Effect on phytic acid on serum LDL, VLDL and HDL in normal and ISO induced myocardial infracted rats.

Groups	LDL (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)
Normal control	56.43±3.43[a]	21.67±1.26[a]	8.74± 0.47[a]
Normal + phytic acid (25 mg/kg)	56.12±3.23[a]	22.16±1.46[a]	8.54±0.45[a]
Normal + phytic acid (50 mg/kg)	55.12±3.44[a]	22.32±1.37[a]	8.21±0.46[a]
ISO (85 mg/kg) control	112.17±5.06[b]	14.31±0.76[b]	13.72±0.76[b]
Phytic acid (25 mg/kg) + ISO	81.69±5.93[c]	18.54±1.28[c]	11.34±0.72[c]
Phytic acid (50 mg/kg) + ISO	67.23±4.21[d]	19.76±1.16[d]	9.65±0.54[d]

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

Table 3. Effect on phytic acid on TGs, FFAs and PLs in normal and ISO induced myocardial infracted rats.

Groups	Triglycerides (mg/dL)	Free Fatty Acids (mg/dL)	Phospholipids (mg/dL)
Normal control	3.76±0.34[a]	0.287±0.07[a]	28.87±1.36[a]
Normal + phytic acid (25 mg/kg)	3.62±0.06[a]	0.267±0.04[a]	29.76±2.74[a]
Normal + phytic acid (50 mg/kg)	3.56±0.42[a]	0.256±0.05[a]	29.26±1.61[a]
ISO (85 mg/kg) control	5.02±4.19[b]	0.507±0.04[b]	16.06±1.78[b]
Phytic acid (25 mg/kg) + ISO	4.74±4.32[c]	0.401±0.05[c]	21.76±1.23[c]
Phytic acid (50 mg/kg) + ISO	4.34±3.10[d]	0.343±0.07[d]	25.29±1.01[d]

Table 4. Effect on phytic acid on TGs, FFAs and PLs in normal and ISO induced myocardial infracted rats.

Groups	Triglycerides (mg/dL)	Free Fatty Acids (mg/dL)	Phospholipids (mg/dL)
Normal control	41.75 ±2.56[a]	26.22 ±1.60[a]	75.82 ±4.68[a]
Normal + phytic acid (25 mg/kg)	40.76 ±2.06[a]	25.27 ±1.82[a]	74.78 ±5.44[a]
Normal + phytic acid (50 mg/kg)	40.16 ±1.60[a]	25.74 ±1.59[a]	74.11 ±3.65[a]
ISO (85 mg/kg) control	68.06 ±3.29[b]	44.74 ±2.75[b]	93.16 ±5.75[b]
Phytic acid (25 mg/kg) + ISO	56.66 ±4.12[c]	36.03 ±1.70[c]	85.83 ±5.24[c]
Phytic acid (50 mg/kg) + ISO	48.17 ±2.10[d]	30.25 ±1.78[d]	79.80 ±4.11[d]

Table 5. Effect on phytic acid on the activities of lipid metabolizing enzymes in heart of control and experimental groups of rats.

Groups	Plasma HMG CoA reductase/Mevalonate Ratio	Liver (HMG CoA reductase /Mevalonate ratio)	LCAT (µmol of cholesterol esterified/h/mL plasma)
Normal control	1.83 ± 0.07[a]	1.14 ± 0.05[a]	32.5 ± 2.14[a]
Normal + phytic acid (25 mg/kg)	1.85 ± 0.08[a]	1.16 ± 0.08 [a]	32.9 ± 3.01[a]
Normal + phytic acid (50 mg/kg)	1.82 ± 0.07[a]	1.09 ± 0.03 [a]	33.1 ± 2.59[a]
ISO (85 mg/kg) control	1.21 ± 0.06[b]	0.65 ± 0.03 [b]	18.4 ± 1.26[b]
Phytic acid (25 mg/kg) + ISO	1.45 ± 0.09[c]	0.85 ± 0.07[c]	23.2 ± 1.76[c]
Phytic acid (50 mg/kg) + ISO	1.62 ± 0.12[d]	0.97 ± 0.09[d]	29.1 ± 2.05[d]

Rats treated with ISO showed a significant (p<0.05) increase in the activities of HMG-CoA reductase in liver when compared to normal rats. Lower ratio indicates higher enzyme activity and vice versa. Pretreatment with phytic acid (25 and 50 mg/kg) to ISO-induced rats significantly (p<0.05) decreased the activity of HMG-CoA reductase and the activities of LCAT and LPL in plasma of normal and ISO-induced rats. Rats treated with ISO showed a significant (p<0.05) decrease in these enzyme activities when compared to normal rats. Pretreatment with phytic acid (25 and 50 mg/kg) to ISO-induced rats significantly (p<0.05) increased the activities of these enzymes. In all the parameters studied, oral administration of phytic acid (25 and 50 mg/kg) to normal rats for a period of 56 d showed minor effects but none were statistically significant. Phytic acid at a dose of 50 mg/kg showed a better effect than the other dose (25 mg/kg) in ISO-induced rats.

Discussion

Administration of ISO causes ischaemic necrosis, which closely resemble the histological damage seen in human MI (Wexler, 1978). Free radical production and lipid peroxidation are concerned in ISO-induced cardiac damage. The cytotoxic free radicals source the loss of membrane integrity with disintegration of polyunsaturated fatty acids in the membrane bilayer and exert unfavorable influences on heart structure and function. The biochemical changes on ISO-administration in particular, changed lipid metabolism are comparable to those taking place in human (Rajadurai and Prince, 2005). Hypercholesterolaemia is a risk factor for the development of CVD, including atherogenesis MI, heart attack, and cerebral paralysis (Wald and Law, 1995). Cellular cholesterol homeostasis is an important factor in the prevention of CVD.

The concentration of cholesterol can be regulated by cholesterol biosynthesis, removal of cholesterol from circulation, absorption of dietary cholesterol, and excretion via bile and feces. The unregulated accumulation of cholesterol causes atherogenesis. ISO-induced rats showed a significant increase in total, ester, and free cholesterol levels in serum and heart. Increased levels of cholesterol lead to increase the membrane fluidity, regulate membrane permeability; alter internal viscosity, and also the internal chemical composition (Yeagle, 1986). Hypertriglyceridaemia is one of the risk factors for MI. The significant increase in serum and heart TG in ISO-induced rats might be due to a decrease in the activity of LPL resulting in decreased uptake of TG from the circulation (Sathish *et al.,* 2003). Pretreatment with phytic acid for a period of 56 d to ISO-induced rats showed a significant reduction in the levels of cholesterol and TG in serum and heart. Jeon *et al.* (2004) reported that phytic acid possesses a strong lipid lowering property in hyperlipidemic rabbits. Lipoprotein is an independent risk factor for the development of atherosclerotic disease. An increase in serum LDL and VLDL fractions along with a decrease in HDL was observed in ISO-induced rats. LDL formation occurs primarily in the catabolism of VLDL. LDL is capable of carrying the highest concentration of cholesterol and PL. It has also been suggested that oxidatively modified LDL rather than unmodified LDL is responsible for atherogenesis (Morimoto, 2000). Oxidized LDL promotes the production of several cytokines, immune cell chemo-attractant proteins, and growth factors. In addition, they increase platelet aggregation, which aggravates the lesion and causes arterial wall thickening (Zhao and Xu, 2000). An increased level of LDL shows a positive correlation with CAD, whereas HDL shows a negative correlation. HDL inhibits the uptake of LDL from arterial wall and facilitates the transport of cholesterol from peripheral tissues to the liver, where it is catabolized and excreted from the body (Sheela, 2001).

Pretreatment with phytic acid to ISO-induced rats minimized the alterations in serum lipoprotein levels by increasing HDL and decreasing LDL and VLDL cholesterol levels. Similar findings were reported earlier in ISO-induced rats by Todd (1980). Phytic acid and its derivative phytate possess anti-atherogenic effect in high-cholesterol diet-fed rats (Sheela, 2001). There is a report showing that phytic acid changes its structure to an active form (phytase) in the gastrointestinal tract during digestion or transfer to other target tissues (Lee *et al*., 2007). In general, the mechanisms of action of lipolytic hormones, such as catecholamines, on fat cells are believed to be mediated by the cAMP cascade: lipolytic hormones activate adenylate cyclase, thereby increasing cAMP formation (Cowieson, 1980). Then cAMP promotes lipolytic activity by activating cAMP-dependent protein kinase, which phosphorylates hormone sensitive lipase (HSL) resulting in the hydrolysis of stored triacylglycerol by HSL (Fain, 1980).

Oxygen free radicals are implicated as mediators of tissue injury in cardiovascular pathology. ISO administration produces cytotoxic free radicals, causes myocardial cell necrosis. Cytotoxic effects of reactive oxygen species are related in their reaction with membrane lipids (Morimoto *et al*., 2000). In this study, we have observed increased levels of FFA in serum and heart and PL in serum with subsequent decrease in PL in heart. The higher levels of FFA in serum and heart in ISO-induced rats might be due to increased lipolysis (Wexler 1978). Cell membranes are rich sources of PL and degradation of PL results in membrane dysfunction, which leads to cell injury. The altered levels of PL might be due to enhanced membrane degradation. The increased peroxidation of membrane PL releases FFA by the action of phospholipase A2 (Vimal and Devaki, 2004). Ca^{2+} has been reported to be one of the inducers of phospholipase A2. Therefore, increased levels of FFA in ISO-induced rats might be due to the indirect effect of Ca^{2+}. Phytic acid pretreatment significantly decreased the levels of FFA in serum and heart and PL in serum and significantly increased the concentration of PL in heart in ISO-induced rats. Phytic acid, a potent antioxidant, scavenged free radicals, inhibited lipid peroxidation and indirectly helped to maintain the levels of lipids in the serum, heart and heart mitochondria and serum lipoproteins in isoproterenol treated cardiotoxic rats. A decrease in cholesterol levels by phytic acid treatment might be correlated to the decreased activity of HMG-CoA reductase. Jeon *et al*. (2004) had reported that phytic acid possesses HMG-CoA reductase inhibitory activity in high cholesterol diet fed rabbits. The lipid lowering activity of phytic acid could also be due to its antioxidant property. Phytic acid pretreatment significantly increased the activities of LCAT and LPL in plasma of ISO-induced rats. The increased activities of LCAT and LPL may contribute in the regulation of circulatory lipid levels.

Conclusion

Preventive effect of phytic acid in isoproterenol induced myocardial infarction in rats was evaluated. It was noted that there was a significant increase in the levels of total, ester, and free cholesterol, triglycerides, and free fatty acids in serum and heart and decrease in heart phospholipids in ISO-induced rats. Pretreatment with phytic acid for a period of 56 d significantly decreased the levels of total, ester, and free cholesterol, TG, FFA in serum and heart and increased PL in heart. It also minimized the alterations in serum lipoproteins in ISO-induced rats.

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