RESEARCH ARTICLE

Preventive effect of *Zanthoxylum armatum* fruit on mitochondrial lipids alteration in isoproterenol-induced myocardial infarcted rats

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Abstract

Background: Mitochondrial lipids are essential for the mitochondrial architecture, the activity of respiratory proteins, and the transport of proteins into the mitochondria. **Aim:** In this study, the preventive role of *Zanthoxylum armatum* fruit on mitochondrial lipids alteration induced by isoproterenol hydrochloride (ISO) was evaluated in myocardial infarcted (MI) rats. **Methods:** Oral pre-treatment with hydroethanolic extract of *Z. armatum* fruit to ISO-induced rats was done for 30 days followed by the isolation of mitochondria from the heart tissue. The lipids such as total cholesterol (TC), phospholipids (PL), triglycerides (TG), and free fatty acids (FFA) in the heart mitochondria and lipid hydroperoxides and thiobarbituric acid reactive substances (TBARS) in the heart tissue were evaluated. **Results:** ISO-induced MI rats showed a significantly ($P < 0.05$) increased mitochondrial TC, TG, and FFA with a subsequent decrease in PL. Significantly increased levels of lipid hydroperoxides and TBARS were observed in the heart tissue of ISO-treated rats. Oral pre-treatment with hydroethanolic extract of *Z. armatum* fruit to ISO-induced rats significantly ($P < 0.05$) reverted these alterations near to normal status. **Conclusion:** Pre-treatment with *Z. armatum* fruit favorably restored the biochemical alterations demonstrating that *Z. armatum* fruit has a significant protective effect on cardiac mitochondrial function against ISO-induced MI in rats.

Key words: Isoproterenol hydrochloride, mitochondrial lipids, oxidative stress, thiobarbituric acid reactive substances, *Zanthoxylum armatum* fruit

INTRODUCTION

Mitochondria are vital subcellular organelles and are called the powerhouse of the cell. They are not only involved in energy generation and electron transport chain, but they are the primary source of reactive oxygen species (ROS) in the cell.[1] Adenosine triphosphate (ATP) synthesis and electron transport chain begin in the mitochondria, which is required for cardiovascular compression and unwinding.[2] The decline in oxygen supply among myocardial damage debilitates energy generation by mitochondria.[3,4] Ischemia happening during myocardial damage is a potential reason for expanded free radicals that may harm the cell membrane and inactivate the components of tricarboxylic acid cycle and unsaturated fat oxidation.[5] Myocardial mitochondrial damage is thought to be an essential trigger for the pathogenesis of coronary illness and is, especially, vulnerable to oxidative anxiety. Delayed oxidative stress in fizzling myocardium brings about damage to mitochondrial ROS generation and ensuing cell damage in failing heart. Imperfect mitochondrial capacity is a major characteristic for the failing heart.[6]

Isoproterenol hydrochloride (ISO) initiated myocardial ischemia is considered as a standout among the most broadly utilized experimental model. ISO incited cardiotoxicity has

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been appeared to be intervened through various systems, including membrane lipid peroxidation (LPO), free radical development, mitochondrial damage, and diminished movement of Na^+–K^+ ATP action.[7] Furthermore, ISO causes myocardial ischemia due to exorbitant creation of free radicals coming about because of oxidative digestion of catecholamines.[8] Colossal generation of free radicals may bring about the loss of capacity and integrity of myocardial membranes. Therefore, ISO produces relative ischemia or hypoxia due to myocardial hyperactivity and coronary hypotension.[9]

Along these lines, the suitability of the myocardial cell depends generally on the uprightness of a few membrane systems. Mitochondrial damage assumes a crucial part in the pathology of myocardial infarcted (MI). In our past research, we have found that the defensive impact of hydroethanolic extract of *Zanthoxylum armatum* fruit on serum marker enzymes, lipid lowering action, and cell reinforcement properties in ISO prompted MI rats. The current study is a piece of our finding that logically reveals that the security of heart mitochondria is controlled by mitochondrial lipids which rummage the free radicals in the mitochondrial membrane. The consequences of the research could fill in as a stage for the improvement of a system based therapeutic approach for the administration of MI.

METHODS

Collection of Plant Material and Preparation of Extract

The plant *Z. armatum* fruit was collected from Kolli hills, India. The taxonomic identity of the plant was confirmed from the ABS Botanical Conservation, Research and Training Centre, Salem, Tamil Nadu, India. (Voucher Specimen No: AUT/ECP/101). The fruits were dried at room temperature and extracted with 50% hydro ethanol. Then, it was filtered, dried in rotary vacuum evaporator, and used for analysis.

Animals

Male Wistar albino rats (Rattus norvegicus) weighing 100–120 g were obtained from animal house of PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India. They were housed in polypropylene cages under a 12:12 h light and dark cycle at around 37°C. The rats had free access to tap water and food. They were fed on a standard pellet diet (AVM Cattle and Poultry Feeds, Coimbatore) and water *ad libitum*. The Ethical Committee Clearance for experimentation on animals were obtained before the start of the experiment (Proposal No: 158/PO/bc/99/CPCSEA). The experiment was carried out according to the guidelines of the committee approved by the Animal Ethical Committee of PSG Institute of Medical Sciences and Research, Coimbatore.

Induction of MI

ISO was used to induce MI in rats. Animals were injected subcutaneously with freshly prepared ISO in sterile normal saline at a dose of 20 mg/100 g body weight.

Experimental Design

Animals were divided into six groups of six rats in each group.

1. Group 1: The rats received only standard rat pellet for 30 days. These animals serve as healthy controls.
2. Group 2: Rats were orally treated with hydroethanolic extract of *Z. armatum* fruit using an intragastric tube (400 mg/kg body weight for 30 days).
3. Group 3: Rats were injected with ISO (20 mg/100 g body weight) subcutaneously twice at an interval of 24 h on 28th and 29th day.
4. Group 4: Rats were orally pretreated with hydroethanolic extract of *Z. armatum* fruit (200 mg/kg body weight for 30 days) and then injected with ISO (20 mg/100 g body weight) subcutaneously twice at an interval of 24 h on 28th and 29th day.
5. Group 5: Rats were orally pretreated with hydroethanolic extract of *Z. armatum* fruit (400 mg/kg body weight for 30 days) and then injected with ISO (20 mg/100 g body weight) subcutaneously twice at an interval of 24 h on 28th and 29th day.
6. Group 6: Rats were orally pretreated with standard drug verapamil (1 mg/kg body weight for 30 days) and then injected with ISO subcutaneously twice at an interval of 24 h on 28th and 29th day.

At the end of the experimental period, i.e., 12 h after the second dose of ISO injection, all the rats were sacrificed by cervical dislocation under mild chloroform anesthesia. The heart tissue was excised immediately and thoroughly washed with ice-cold physiological saline and homogenized in 5.0 ml of 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged, and the supernatant was used for various biochemical estimations.

Separation of Heart Mitochondrial Fractions

Heart mitochondria were isolated by the method of Takasawa with slight modifications.[10] The heart tissue was put into ice-cold medium containing 250 mM sucrose, 0.5 mM ethylenediaminetetraacetic acid, 50 mM Tris HCl (pH 7.4), and homogenized. The minced blood-free tissue was then resuspended in 20 mL of isolation medium containing 0.1% (w/v) defatted bovine serum albumin and transferred to a 50 mL glass homogenizer. The suspension was incubated for 1 min (4°C) and then rehomogenized. The homogenate was subjected to differential centrifugation at 4°C to isolate mitochondria. Mitochondrial fraction was finally resuspended in the same buffer (final concentration 0.2% v/v) in ice for

15 min. To determine LPO, the mitochondrial pellet was dissolved in a buffer consisting of 175 mM KCl and 10 mM Tris (pH 7.4) so as to remove sucrose.

Estimation of Thiobarbituric Acid Reactive Substances (TBARS), Lipid Hydroperoxides, and Lipids

Heart TBARS and lipid hydroperoxides were estimated by the methods of Niehaus and Samuelsson[11] and Jiang *et al.*[12] The level of lipids such as total cholesterol (TC) and triglycerides (TG) were determined using standard diagnostic kits (Reckon Diagnostic Ltd.) and phospholipids (PL) and free fatty acids (FFA) were assayed by the method of Rouser *et al.*,[13] and Hron and Menahan[14] in the heart mitochondria.

Statistical Analysis

The results were articulated as a mean ± standard deviation. Statistical analysis was carried between the experimental groups using one-way analysis of variance (ANOVA) employing statistical package for social science (SPSS version 16.0). *Post hoc* testing was performed for intergroup comparisons using Fisher's least significant difference tests. The level of significance was set as $P < 0.05$.

RESULTS AND DISCUSSION

Figures 1 and 2 show the levels of LPO products such as TBARS and lipid hydroperoxides in the heart and heart mitochondria of normal and experimental rats. ISO-induced MI rats (Group-3) showed a significantly increased level of TBARS and lipid hydroperoxides in the heart tissue homogenate and heart mitochondria compared to control rats (Group-1). Pre-treatment with hydroethanolic extract of *Z. armatum* fruit (Groups 4 and 5) protected the heart and heart mitochondrial membrane against LPO damage by reducing LPO induced by ISO. The ROS in heart tissue was controlled in standard drug-treated rats (Group 6). There was no significant difference between plant extract treated rats (Group 2) and normal rats (Group 1).

In recent years, research is focused on the function of mitochondria in MI, as the energy for contraction comes mainly from the oxidative metabolism of mitochondria in the myocytes. Lipid peroxidation is a major mechanism of free radical-mediated cell injury.[15] Malondialdehyde is the product of LPO derived from polyunsaturated fats which is measured as TBARS and LPO. Elevated levels of TBARS and lipid hydroperoxides in the heart and heart mitochondria decreased the membrane fluidity and altered membrane permeability which uncouples oxidative phosphorylation.[16] ISO-treated cardiotoxic rodent (Group 3) demonstrated expanded levels of LPO in the heart which could be credited to the amassing of ROS in the heart and irreversible harm to the myocardial

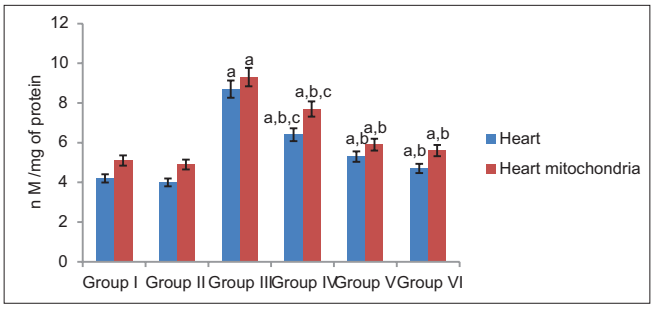

Figure 1: Level of thiobarbituric acid reactive substances in the heart and heart mitochondria. Values are mean ± standard deviation of six samples in each group. *Significant at 5% level ($P < 0.05$). Group comparison: (a) G1 versus G2, G3, G4, G5, G6. (b) G3 versus G4, G5, G6. (c) G6 versus G4, G5

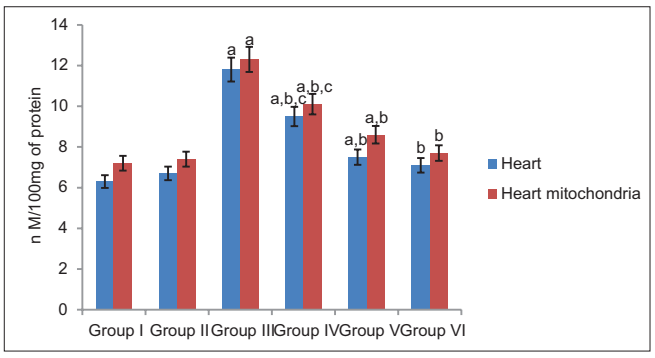

Figure 2: Level of lipid hydroperoxides in the heart and heart mitochondria. Values are mean ± standard deviation of six samples in each group. *Significant at 5% level ($P < 0.05$)

membrane.[17] Activated LPO is an essential pathogenic occasion in MI, and the levels of lipid peroxide indicate the extent of infection. Pre-treatment with hydroethanolic extract of *Z. armatum* fruit reduced the levels of TBARS in ISO-induced rats. Our results confirmed that the *Z. armatum* fruit has an anti-lipid peroxidative action against ISO-induced mitochondrial damage which may be due to inhibition of lipid peroxide production and blocking the ROS generation.

The heart mitochondrial fraction of MI rats revealed altered levels of mitochondrial lipids which indicate altered cardiac function. Increased levels of TC, FFA, and TG were observed in the heart mitochondrial fraction of ISO-intoxicated rats. The level of PL was significantly decreased in ISO-induced rats (Group 3) as compared to the control rats (Group 1). Administration of different concentrations of hydroethanolic extract of *Z. armatum* fruit on ISO-induced rats (Groups 4 and 5) significantly ($P < 0.05$) decreased the level of TC, FFA, and TG, whereas the levels of PL were significantly increased when compared to the ISO-induced rats (Group 3). The lipid metabolism was found to be normal when standard drug verapamil was administered (Group 6). Extract alone treated rats (Group 2) shown to have normal lipid profile like control rats [Table 1].

Administration of ISO enhanced the levels of mitochondrial lipids, which is a clear evidence for altered cardiac function and

Table 1: Effect of hydroethanolic extract of *Z. armatum* fruit on cardiac mitochondrial lipids

	Heart mitochondria (nmoles/ mg protein)			
	TC	PL	FFA	TG
Group-I	84.56+1.18	20.76+0.03	59.7+0.8	74.46+3.2
Group-II	79.24+1.09	22.47+0.44	61.78+0.7	78.92+3.09
Group-III	217.73+3.54[a]	12.78+0.43[a]	117.12+1.31[a]	187.24+1.96[a]
Group-IV	146.42+2.7[abc]	16.97+1.02[abc]	83.81+0.41[abc]	127.2+3.7[abc]
Group-V	117.7+2.14[ab]	19.48+0.19[ab]	67.24+0.12[ab]	98.24 +2.78[ab]
Group-VI	103.21+1.98[ab]	21.08+0.32[b]	65.78+0.72[ab]	87.28+3.1[ab]

Values are mean±standard deviation of six samples in each group. [a, b, c]Significant at 5% level (P<0.05). Group comparison: (a) G1 versus G2, G3, G4, G5, and G6. (b) G3 versus G4, G5, and G6. (c) G6 versus G4 and G5. *Z. armatum: Zanthoxylum armatum*, TC: Total cholesterol, PL: Phospholipids, FFA: Free fatty acids, TG: Triglycerides

ultrastructure. Taegtmeyer reported that there is an accumulation of FFA in MI as the oxidation of FFA is decreased due to lack of oxygen supply in MI.[18] FFA elevation in ischemia also inhibits respiratory activities and diminishes cardiac function.[19] A significant increase in mitochondrial cholesterol is well associated with myocardial ischemia. Changes in membrane cholesterol are associated with MI as it affects membrane permeability of ions and fluidity.[20] Hypercholesterolemia actuates oxidative stress by the generation of free radicals and inhibits the enzymatic antioxidant activities.[21] Pre-treatment with *Z. armatum* fruit significantly decreased the levels of mitochondrial cholesterol, TGs, and FFA in ISO-induced rats. The phytoconstituents of *Z. armatum* fruit scavenge the free radicals and indirectly helped to decrease the levels of lipids.

Mitochondrial membrane is rich in PL which is highly vulnerable to attack free radical, an important deterioration in the biological membranes.[22] Decreased levels of mitochondrial PL were observed in ISO-induced MI rats indicating increased free radical formation, which could be attributed to a deficiency of the antioxidant system. This may be the reason for decreased levels of PL in mitochondrial fractions of the heart of ISO-induced rats. Pre-treatment with hydroethanolic extract of *Z. armatum* fruit significantly increased the levels of PL and it might be due to the ability to scavenge free radical and protect PL in mitochondria.

CONCLUSION

The present study shows that Z. *armatum* fruit exerts its cardioprotective effect by bringing down the levels of LPO products and mitochondrial lipids in ISO-treated rats. Thus, *Z. armatum* fruit shielded the heart from myocardial damage by scavenging free radicals and hindering the peroxidation of lipids in the mitochondria.

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