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# Efficacy of *Zanthoxylum armatum* fruit on isoproterenol induced myocardial infarction in rats

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**ABSTRACT:** The present study was elucidating the cardioprotective activity of hydroethanoic extract of *Zanthoxylum armatum* (*Z. armatum)* fruit on serum cardiac markers, lipid profile and the level of antioxidants in experimentally induced myocardial infarction (MI). The hydroethanolic extract of *Z. armatum* fruit was administered at a dose of 200 and 400 mg/kg body weight for 30 days to male Wistar albino rats. On 28$^{th}$ and 29$^{th}$ day, isoproterenol (ISO) (8.5mg/100g body weight) was administered to induce MI. Animals were sacrificed; blood and heart tissues were removed and the biochemical parameters were carried out. Serum markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB) and troponin –T were significantly ($p<0.05$) increased after ISO treatment. Altered lipid profile and significant changes in enzymic and non- enzymic antioxidants were also observed in MI. The altered levels were brought back to near normal by the administration of hydroethanoic extract of *Z. armatum* fruit which might be due to the active phytoconstituents present in it..

**Keywords:** Antioxidant enzymes, cardiac markers, isoproterenol, lipid profile, *Z. armatum*.

## 1. Introduction

Cardiovascular disease (CVD) is the first leading cause of death worldwide, claiming about 17.1 million lives a year. In India particularly in urban areas 31.7 % of deaths occur due to MI [1]. It is mainly due to the changes in lifestyle. In 1970 the incident of CVD was only about 7% and was increased up to 39% in 2017. MI is a key component of the trouble of CVD which is associated with complications and mortality [2]. The chronic development of atherosclerotic lesions in MI is due to elevated serum levels of myocardial markers such as CK-MB, AST and LDH including troponin – T [3,4]. High levels of serum total cholesterol (TC), low density lipoprotein (LDL), triglycerides (TG) and decreased levels of high density lipoprotein (HDL) are correlated positively in the coronary atherosclerosis [5].

Pharmacological induction of MI by subcutaneous administration of ISO in rats is a well standardized and frequent representation to study the defensive property of many drugs and cardiac function [6]. It is a synthetic catecholamine and β-adrenergic agonist induces severe stress in the myocardium resulting in the necrosis of the heart muscle. It has been proposed by various mechanisms and one of it includes the production of toxic free radicals through the auto oxidation of catecholamine [7].

Many modern drugs are effective in the treatment of CVD, but their application is limited because of their side effects. Recently, there has been an upsurge of interest to search the cardioprotective potential of natural foodstuffs [8]. At present, natural products and medicinal plants have gained much attention as possible source of antioxidants due to their excellent efficiency in opposition to reactive oxygen species (ROS) stimulate pathologies [9]. *Z. armatum* is an important medicinal plant extensively used locally for curing several diseases. Though ancient literature states the use of this extract for CVD, there is lack of scientific information regarding the effect of *Z. armatum* fruit. In our previous study, *Z. armatum* fruit was characterized for its phytoconstituents and antioxidants. However, the pharmacological exploitation of this fruit has been rarely reported and hence the fruits of *Z. armatum* were evaluated on the cardiac changes associated with ISO induced MI for its cardioprotective activity.

## 2. Materials and Methods

### Animals

Male Wistar albino rats (Rattus norvegicus) weighing 150–180g were obtained from animal house of PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India and used for this study. They were housed in polypropylene cages (47×34×20 cm) lined with husk, renewed every 24 h under a 12:12 h light and dark cycle at around 22 °C. They were fed on a standard pellet diet (AVM Cattle and Poultry Feeds, Coimbatore) and water *ad libitum*. The clearance of the ethical committee for experimentation on animals was obtained before the start of the experiment (Proposal No: 158/PO/bc/99/CPCSEA). The experiment was carried out according to the guidelines approved by the Animal Ethical Committee of PSG Institute of Medical Sciences and Research.

### Materials

*Z. armatum* fruit was identified by ABS Botanical Garden, Tamilnadu, India (AUT/ECP/101). Extract was prepared by the maceration procedure from dried fruits using 50% ethanol for 5 days. Isoproterenol hydrochloride was purchased from Sigma Chemical Co., St. Louis, MO, USA. All the other chemicals and reagents used were of analytical grade.

### Induction of MI

ISO hydrochloride was used to induce MI in rats. Animals were injected subcutaneously with freshly prepared ISO hydrochloride in sterile normal saline at a dose of 8.5mg/100g body weight.

### Experimental design

Animals were divided into six groups of six rats in each group. The Group I: The rats received only standard rat pellet for 30 days. These animals serve as healthy controls. Group II: Normal rats were orally treated with hydroethanolic extract of *Z. armatum* fruit using an intragastric tube (400mg/kg body weight for 30 days). Group III: Rats were injected with ISO (8.5mg/100g body weight) subcutaneously twice at an interval of 24 h dissolved in normal saline). Group IV: Rats were orally pretreated with hydroethanolic extract of *Z. armatum* fruit (200mg/kg body weight for 30 days) and then injected with ISO (8.5mg/100g body weight) subcutaneously twice at an interval of 24 h on 28$^{th}$ and 29$^{th}$ day. Group V: Rats were orally pretreated with hydroethanolic extract of *Z.armatum* fruit (400mg/kg body weight for 30 days) and then injected with ISO (8.5mg/100g body weight) subcutaneously twice at an interval of 24 h. Group VI: Rats were orally pretreated with standard drug verapamil (1mg/ kg body weight for 30 days) and then injected with ISO subcutaneously twice at an interval of 24 h. At the end of the experimental period i.e., 12 h after the second dose of ISO injection, all the rats were scarified by cervical dislocation under mild chloroform anesthesia. Blood was collected and serum was separated after centrifugation at 2500rpm. The heart tissue was excised immediately and thoroughly washed with ice-cold physiological saline. Serum was used for various biochemical estimations.

### Biochemical estimations in serum

The activities of AST, ALT and LDH were estimated by the standard methods [10,11]. CK-MB was assayed using commercial kit obtained from Agappe diagnostics, Kerala, India. The level of troponin-T was estimated by using chemiluminescence immunoassay (ECLIA) with Elycsys Tro-T High sensitive immunoassay (Roche Diagnostics limited, Switzerland). The level of TC, TG and HDL were determined by using standard diagnostic kits (Reckon Diagnostic Ltd). Low density

lipoprotein- cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) were determined by using Fridwald's formula [12].

### Preparation of tissue homogenate

One gram of heart tissue was taken and homogenized with 0.1M cold tris buffer (pH 7.4) in a potter homogenizer fitted with Teflon plunger at 600 revolutions per minute for 3 minutes. The homogenate was taken for the analysis of various biochemical parameters.

### Estimation antioxidants in the heart tissue

Tissue lipid peroxidation level was determined as thiobarbituric acid reactive substances (TBARS) by the method of Nichans & Samuleson, 1968 [13]. The enzymic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione –S-transferase (GST) [14-17] and non enzymic antioxidants vitamin – C (Vit-C), vitamin – E (Vit-E) and glutathione were assayed by using standard methods [18-20].

### Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using SPSS Software Package version 12.00. Results were expressed as mean ± SD (standard deviation) from six rats in each group. $P<0.05$ were considered to be significant.

## 3. Results and Discussion

In the present study, table 1 shows the activities of cardiac markers such as AST, ALT, LDH, CK-MB and troponin-T in control and experimental rats. There was a significant ($p<0.05$) increase in enzyme activities in the serum of ISO treated rats when compared to the control rats. It is due to the leakage from the damaged heart tissue into the blood stream as a result of necrosis induced by ISO in rats. Administration of hydroethanolic extract of *Z. armatum* fruit (200 and 400mg/kg body weight) significantly reverted the ISO induced elevation in the activities of AST, ALT, LDH, CK-MB and Troponin-T in serum. There was no significant difference between standard drug verapamil treated rats, control rats and *Z. armatum* alone treated rats.

ISO induced myocardial injury which brings about the loss of functions and integrity of myocardial membranes and also altered the membrane permeability [21]. The primary disturbances of induced MI have been reported to develop adenylate cyclase activity, resulting in increased formation of cAMP, which in turn would lead to the elevated lipid accumulation in the myocardium [22]. Biochemical alterations in ISO induced cardiomyopathy include changes in cardiac marker enzymes, lipid metabolizing enzymes, lipid profile, antioxidant levels and electrolyte levels in the blood as well as in the myocardial tissue [23,24].

**Table – 1** Effect of hydroethanolic extract of *Z. armatum* fruit on cardiac markers

| | AST (IU/L) | ALT (IU/L) | LDH (IU/L) | CK-MB (IU/L) | Troponin-T (ng/ml) |
|---|---|---|---|---|---|
| Group-I | 79.43± 2.04 | 69.52± 1.01 | 176.12± 2.73 | 151.13± 3.4 | 0.57± 0.02 |
| Group-II | 77.32± 6.86* | 70.53± 2.74* | 173.4± 3.07* | 146.12± 3.9* | 0.54± 0.02* |
| Group-III | 316.13± 2.34a* | 298.12± 3.18a* | 356.13± 3.06a* | 64.24± 2.12a* | 1.72± 0.14a* |
| Group-IV | 114.61± 3.09abc* | 132.21± 2.76abc* | 206.24± 4.9abc* | 99.78± 2.78abc* | 0.96± 0.06abc* |
| Group-V | 89.37± 2.04abc* | 106.3± 2.49abc* | 169.38± 3.1abc* | 117.12± 2.4abc* | 0.74± 0.05abc* |
| Group-VI | 80.68± 2.91ab* | 72.48± 2.39ab* | 169.14± 2.99ab* | 136.2±3.95ab* | 0.63± 0.01ab* |

Values are mean ± SD of six samples in each group. *- significant at 5% level ($p<0.05$)
Group comparison: a- GI vs GII, GIII, GIV, GV, GVI.
b- GIII vs GIV, GV, GVI.
c- GVI vs GIV, GV.

Furthermore, the level of diagnostic markers CK-MB, LDH and troponin T were increased in ISO-treated rats that indicates myocardial damage and our finding was in consonance with an earlier report. Troponin –T has been shown to be highly sensitive and specific marker of myocardial injury. Increased serum marker activities might be due to enhanced susceptibility of myocardial membrane to the ISO mediated peroxidative damage resulting in increased release of these enzymes into the blood stream [25]. Administration of hydroethanolic extract of *Z. armatum* fruit significantly prevented the ISO induced elevation in the activities of diagnostic marker enzymes in serum indicating the cardioprotective activity of *Z. armatum* fruit. This reduction in enzyme levels could be due to its action on maintaining membrane integrity restricting the leakage of these enzymes.

Table –2 reveals a significantly ($p<0.05$) increased levels of TG, TC, LDL, VLDL-c and decreased level of HDL-c in ISO treated rats when compared to control rats. Oral pretreatment with two different concentrations of hydroethanolic extract of *Z.armatum* fruit significantly ($p<0.05$) decreased the levels of TG, TC, LDL-c, VLDL-c and increased the level of HDL-c in ISO induced rats. The lipid profile was found to be normal when standard drug verapamil was administered. Extract alone treated rats have shown to have a normal lipid picture as that of control rats.

Chagoya de Sanchez et al., (1998) [26] have reported that ISO causes increased levels of circulatory and myocardial lipids such as TC, TG, free fatty acids and phospholipids.

**Table – 2** Effect of hydroethanolic extract of *Z. armatum* fruit on lipid profile

| Groups | Serum (mg/dl) | | | | |
|---|---|---|---|---|---|
| | TC | VLDL-c | LDL-c | HDL-c | TG |
| Group-I | 84.56± 1.18 | 20.76± 0.03 | 59.7± 0.8 | 26.52± 1.7 | 74.46± 3.2 |
| Group-II | 77.24± 1.09* | 22.47± 0.44** | 61.78± 0.7** | 24.27± 3.21** | 78.92± 3.09** |
| Group-III | 217.73±3.54a* | 72.78± 0.43a** | 117.12± 1.31a** | 10.78± 0.65a** | 187.24± 1.96a** |
| Group-IV | 146.42± 2.7abc* | 49.48± 1.02abc** | 83.81± 0.41abc** | 17.27± 1.2abc** | 127.2± 3.7abc** |
| Group-V | 117.7± 2.14abc** | 36.97± 0.19abc** | 67.24± 0.12abc** | 22.47± 1.07abc** | 98.24 ± 2.78abc** |
| Group-VI | 103.21± 1.98ab** | 24.29± 0.32ab** | 65.78± 0.72ab** | 29.28± 2.13ab** | 87.28± 3.1ab** |

Values are mean ± SD of six samples in each group. *- significant at 5% level ($p<0.05$)

**Table-3:** Effect of hydroethanolic extract of *Z. armatum* fruit on Lipid peroxidation in control and experimental rats.

| Groups | TBARS (nmoles /mg protein) |
|---|---|
| Group-I | 1.98± 0.12 |
| Group-II | 2.01± 0.09* |
| Group-III | 5.94±0.32a** |
| Group-IV | 3.84±0.17abc** |
| Group-V | 2.99± 0.24abc** |
| Group-VI | 2.64± 0.21ab** |

Values are mean ± SD of six samples in each group. *- significant at 5% level ($p<0.05$)

It also lowers the HDL-C and enhances the LDL-C levels in the circulation that results in the arterial deposition. ISO further promotes the degradation of lipids [27].

Table – 3 indicates an ISO- induced myocardial infracted rats showed a significantly ($p<0.05$) increased level of thiobarbituric acid reactive substances (TBARS) in the heart tissue homogenate compared to control rats. Pretreatment with hydroethanolic extract of *Z. armatum* fruit protected the heart mitochondrial membrane against lipid peroxidative damage by reducing the ISO-treated lipid peroxidation. The ROS in heart tissue were controlled in standard drug treated rats and hydroethanolic extract of *Z. armatum* fruit alone treated rats. MDA, a cytotoxic production of lipid peroxidation is a biomarker for oxidative stress and indicate free radical production and consequent tissue damage [28]. The MI induced oxidative stress depresses the calcium transport and result in intracellular calcium overload and dysfunctioning of ventricles [29].

hydroethanolic extract of *Z. armatum* fruit significantly ($p<0.05$) improved the activities of these enzymes.

Cells are protected against oxidative stress by antioxidant enzymes such as SOD, CAT and GPx. SOD is an enzyme that catalyzes dismutation of superoxide anion into $O_2$ and $H_2O_2$ [31]. The biochemical role of GPx is to reduce lipid peroxides to their corresponding alcohols and to reduce free $H_2O_2$ to water. SOD and GPx play an important role in preventing tissue damage through oxidation.

In the present study, a significant reduction in the activities of glutathione dependent antioxidants enzymes (GPx and GST) and antiperoxidative enzymes such as SOD and CAT with a concominant deciline in the level of reduced glutathione was observed in the heart tissue of experimental and control rats. Decreased activities of these enzymes lead to the accumulation of $H_2O_2$ and lipid peroxides and make myocardial cell membrane more susceptible to oxidative damage.

**Table-4:** Effect of hydroethanolic extract of *Z. armatum* fruit on enzymic antioxidants in control and experimental rats.

| Groups | SOD (U/mg protein) | CAT (µmoles of $H_2O_2$ utilized/min/ mg protein) | GPx (µmoles of GSH utilized/min/mg protein) | GST (nmoles of NADPH utilized/ min / mg protein) |
|---|---|---|---|---|
| Group-I | 2.61± 0.21 | 1.76± 0.2 | 5.06± 0.4 | 0.74± 0.01 |
| Group-II | 2.42± 0.2* | 1.45± 0.3* | 4.96± 0.3* | 0.70± 0.04* |
| Group-III | 0.97±0.01a** | 0.36±0.02a** | 2.68±0.31a** | 0.21±0.01a** |
| Group-IV | 1.44±0.03abc** | 1.01±0.1abc** | 3.05±0.2abc** | 0.48±0.02abc** |
| Group-V | 1.94± 0.03abc** | 1.41± 0.02abc** | 3.98± 0.03abc** | 0.56± 0.01abc** |
| Group-VI | 2.32± 0.01ab** | 1.62± 0.08ab** | 4.7± 0.04ab** | 0.62± 0.03ab** |

Values are mean ± SD of six samples in each group. *- significant at 5% level ($p<0.05$)
1 unit (U) of SOD = amount of enzyme causing 50% inhibition of NBT reduction /min/mg/protein.

Table-4 illustrate the activities of SOD, CAT, GPx and GST in heart tissue of normal and experimental group of rats. Significantly ($p<0.05$) decreased enzyme activities in the heart tissue were observed in ISO induced rats when compared to control rats. The observed decline in the activities of enzymic antioxidants in ISO induced cardiac tissue might be due to radical generated at the site of damage which modulates these enzymes resulting in decreased activities [30]. Pretreatment with

Reduction noticed in the activities of the antiperoxidative enzymes in ISO induced MI might be due to the increased generation of reactive oxygen radical which in turn lead to the inactivation of these enzymes [32, 33]. Pretreatment with hydroethanolic extract of *Z. armatum* fruit significantly improved the activities of SOD, CAT, GPx and GST. Thus *Z. armatum* fruit reduces myocardial damage caused by free radicals in cardiac tissue in ISO induced rats.

**Table-5:** Effect of hydroethanolic extract of Z. armatum fruit on the levels of non-enzymatic antioxidants in control and experimental rats

| Groups | Heart tissue (mg/g) | | |
|---|---|---|---|
| | Vit-C | Vit-E | Glutathione |
| Group-I | 1.52± 0.02 | 1.97± 0.01 | 5.41± 0.04 |
| Group-II | 1.47± 0.06* | 1.84± 0.03* | 5.02± 0.03* |
| Group-III | 0.65± 0.01a** | 0.76± 0.01a** | 2.41± 0.05a** |
| Group-IV | 0.98± 0.03abc** | 1.02± 0.04abc** | 3.81± 0.06abc** |
| Group-V | 1.12± 0.01abc** | 1.62± 0.01abc** | 4.35± 0.03abc** |
| Group-VI | 1.37± 0.01ab** | 1.78± 0.02ab** | 4.86± 0.06ab** |

Values are mean ± SD of six samples in each group. *- significant at 5% level ($p<0.05$)

Table-5 demonstrate a significant ($p<0.05$) fall in the levels of Vit-C, Vit- E and glutathione is ISO induced rats when compared to control rats. But there was no significant difference between control, standard drug verapamil and hydroethanolic extract of *Z. armatum* fruit alone treated animals. Oral administration of the extract substantially enhanced the levels of non-enzymatic antioxidants in the myocardial rats.

Non enzymatic antioxidants Vit-C and Vit- E are easily inactivated by lipid peroxides or reactive oxygen species, which results in decreased levels of these in cardio toxicity induced rats. The major antioxidant is Vit-C, which acts as the first line of defense during oxidative stress. It is also important to maintain Vit-E level by reducing the Vit-E radical to its oxidized form. Thus antioxidants are important not only because they react with free radicals directly but also they act synergistically with one another [34]. GSH is an intracellular thiol that participates directly in the destruction of $H_2O_2$. Depletion of mitochondrial GSH is a major mechanism for inducing an imbalance in the mitochondrial function. ISO reduce glutathione levels, leading to the loss of membrane integrity, myocytotoxicity and finally producing myocardial necrosis. The decreased activity of GPx observed in ISO induced rats is due to the reduced availability of the substrate GSH [35].

## 4. Conclusion

In conclusion, the hydroethanolic extract of *Z. armatum* fruit can be considered as a good protector against ISO induced toxicity in rats which prevent the myocardium of rat heart from oxidative damage due to its non-toxicity profile, acceptable route of administration (oral) and boosting the antioxidant capacity. Further characterization and purification of the bioactive compounds would be necessary to determine the exact mechanism of action of *Z. armatum* fruit.

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## Conflict of interest:

There are no conflicts of interest.

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## Competing Interests:

The authors declare that they have no competing interests.