Evaluation of cardioprotective activity of *Pandanus odoratissimus* leaves against isoproterenol induced myocardial infarction in albino rats

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**INTRODUCTION**

Cardiovascular disorder and its resulting complications is the major cause for the mortality and morbidity in the developing countries. Among the total mortality rate of CVD the Coronary Artery Disease plays a prominent role which includes Ischemic Heart Disease like Atherosclerosis and Myocardial Infarction. Most of the CVD occurs due to the Cardiotoxicity by Chemotherapy treatment and its complications [1]. Myocardial Infarction [MI] pathologically denotes the death of cardiac myocytes due to extended ischemia, which may be caused by an increase in perfusion demand or a decrease in blood flow. It happens when blood stops flowing properly to part of the heart and the heart muscle is injured due to not receiving enough oxygen. Usually this is because one of the coronary arteries that supplies blood to the heart develops a blockage due to an unbalanced buildup of white blood cells, cholesterol and fat deposition [2]. Coronary arteries are blood vessels that supply the heart muscle with blood and oxygen. Blockage of a coronary artery deprives the heart muscle of blood and oxygen, causing injury to the heart muscle [3].

**Epidemiology**

Worldwide, the burden of chronic diseases such as cardiovascular diseases, cancer, diabetes and obesity is increasing rapidly. In 2005, chronic diseases contributed approximately 69% of the 56.5 million total reported deaths in the world and 46% of the global burden of disease [4]. Cardiovascular diseases (CVD) is the name for the cluster of disorders afflicting the heart and blood vessels, including hypertension (high blood pressure), coronary heart disease (heart attack), cerebrovascular disease (stroke), heart failure and peripheral vascular disease [5]. In 2001, CVD alone contributed to a third of global deaths and by 2020 it would become the leading cause of death in
developing countries. Developing countries like India are also struggling to manage the impact of CVD along with the growing burden of several disorders. By the year 2020, it will account for one third of the deaths [6]. Current projections suggest that by the year 2020, India will have the largest CVD burden in the world. The prevalence of these diseases is more in urban than in rural areas due to their modified lifestyle [7].

MATERIALS AND METHOD
Plant Material
The plant of *Pandanus odoratissimus* leaves were collected from palamaner located in chittoor district of Andhra Pradesh in the month of February and they were identified and authenticated by the taxonomist Dr. Jayaraman, Director of Plant Anatomy Research Centre (PARC), Chennai. The leaves were shade dried at room temperature and made to powdered as in coarse form by pulverization. The powdered material was passed through a no. 60 mesh sieve.

Preparation of extract
The powdered plant material. The powdered material was soaked in Pet-ether for overnight for defatification and followed by Soxhalation with Hydro-alcoholic solvent [8]. The extracts were concentrated by using a water bath temperature not exceeding 40°C and subjected to drying in a lyophilizer till dry extract was obtained.

Phytochemical screening
The extract was subjected to preliminary phytochemical screening for the detection of various phytochemical constituents like tannins, alkaloids, saponins, glycosides, terpenes, phenolics, flavonoids, carbohydrates, proteins and steroids, by using simple and standard qualitative methods described by Kokate [9].

PHARMACOLOGICAL STUDY
Animals
Healthy albino rats of either sex weighing about 150-180 g were used during the study. The animals were procured from SreeVidyaniKethan College of Pharmacy, Tirupati, India. Before initiation of experiment, the rats were acclimatized for a period of 7 days. Standard environmental conditions such as temperature ranging from 18 to 32°C, relative humidity (70%) and 12 hrs dark/light cycle were maintained in the quarantine. All the animals were fed with rodent pellet diet and water under strict hygienic conditions. All procedures were performed in accordance to CPCSEA guidelines after approval from SreeVidyaniKethan College of Pharmacy, Tirupati, India[Approval no. SVCP/IAEC/1-013/2013-2014, dated on 14 march 2014].

Acute oral toxicity study
The acute toxicity of ethanolic extract of *Pandanus odoratissimus* was carried out as per OECD-423 guidelines for safe dose administration to animals. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose level 2000mg/kg body weight and observed for 14 days. The results showed no sign of toxicity till 14 days at a starting dose of 2000 mg/kg body weight. Hence, 1/10th were considered as test dose for the study [10].

Induction of Myocardial Infarction
Isoproterenol was obtained from A to Z Pharma in Chennai. Myocardial infarction was induced by 85mg/kg body weight of isoproterenol, dissolved in normal saline and given through subcutaneous route injection for two consecutive at an interval of 24hrs and on days 14 and 15, for the rats in the isoproterenol control.

Experimental design
Then rats are divided into 5 groups, each group having 6 animals. Group I: received normal saline (10ml/kg/day/p.o)Group II: received Isoproterenol (85mg/kg/s.c), Group III: received standard drug Atenolol(10mg/kg/i.p) + Isoproterenol (85mg/kg/s.c), Group IV: received hydroalcoholic extract of *Pandanus odoratissimus*(200mg/kg/p.o) + Isoproterenol (85mg/kg/s.c), Group V: received hydroalcoholic extract of *Pandanus odoratissimus* (400mg/kg/p.o) + Isoproterenol (85mg/kg/s.c), The extract, standard were given for 15 days and Isoproterenol given for 14 & 15 day.

Sample collection
After the experimental period of 15 days, the rats was sacrified under mild anesthsia. The heart was dissected out, immediately washed in ice-cold saline and a homogenate is prepared in 0.1M Tris HCl buffer (pH 6.4). Homogenate is centrifuged and supernatant was used for the assay of glutathione and lipid peroxides in serum and heart homogenate. The collected samples (Serum & homogenate) were used for analysis of different
biochemical parameters and assay of marker enzymes.

Biochemical parameter Estimation
The parameters and serum marker enzymes analyzed are, LPO (Lipid Peroxidation), GSH (Glutathione), CK (Creatine Kinase), LDH (Lactate Dehydrogenase), SOD (Superoxide dismutase), CAT (Catalase).

Lipid Peroxidation
The quantitative estimation of lipid peroxidation was determined by the concentration of thiobarbituric acid reactive substances in heart using the method of Ohkawa and Yagi. The amount of malondialdehyde (MDA) formed was quantified by reaction with TBA and used as an index of lipid peroxidation. The results was expressed as µ mole of MDA/g of wet tissue using molar extinction coefficient of the chromophore and 1,1,3,3,tetraethoxypropane. The homogenizate with 1.15% KCl (10% weight/volume). The assay mixture consisting of 0.1 mL of tissue homogenate, 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 mol of 20% acetic acid (adjusted to pH 3.5 with NaOH) and 1.5 mL of 0.8% aqueous solution of thiobarbituric acid is heated for 60 min at 95°C. Thereafter, the mixture is cooled and extracted with 5 mL of mixture of n-butanol and pyridine (15:1 volume/volume). After centrifugation at 4000 rpm for 10 min, the organic phase is assayed spectrophotometrically at 532 nm. Tetraethoxypropane (in amounts of 2 nmol, 4 nmol, 6 nmol and 8 nmol) served as an external standard. Lipid peroxide levels in myocardium were expressed as µmol/gm of wet tissue [12].

Glutathione (GSH) was assayed by the method of Habig. To 0.1 mL of homogenate, 1.0 mL of 0.3 M phosphate buffer (pH 6.5), 1.7 mL of water and 0.1 mL of 30 mM CDNB were added. After incubation at 36.8°C for 15 min, 0.1 mL of GSH is added and change in optical density at 340 nm for 3 min at intervals of 3 s. Reaction mixture without the enzyme is used as a blank. The GSH activity was expressed as µ mole/gm wet tissue [13].

Superoxide Dismutase
SOD level will be estimated by the method of Kakkar. The assay mixture contained 0.1 mL of sample, 1.2 mL of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 mL phenazinemethosulphate (186 µM), 0.3 mL of 300 µM nitrobluetetrazolium and 0.2 mL of NADH (750 µM). The reaction is started by the addition of NADH. After incubation at 30°C for 90 s, the reaction is stopped by the addition of 0.1 mL glacial acetic acid. The reaction mixture is stirred vigorously with 4.0 mL of n-butanol, and the mixture is allowed to stand for 10 min. After centrifuging the mixture, the butanol layer is separated. Colour intensity of the chromogen in the butanol is measured at 560 nm spectrophotometrically, and the concentration of SOD is expressed as unit/mg protein. Where as the levels of Creatine kinase myocardial isoenzyme, Lactate dehydrogenase and catalase was estimated by using the standard kits obtained from Bioreactors, Triupathi.

Histopathological Studies
Histological examination of isolated rat’s heart section confirmed myocardial injury with isoproterenol to determine the myocardial necrosis by direct staining the myocardium of rat was frozen immediately after removal. Then the tissue is firm, the heart is sliced into 3 - 5 mm thick slice from the apex toward the atrioventricular groove and incubated in 1% solution of 2, 3,5-triphenyltetrazolium chloride (TTC) in phosphate buffer saline with pH 6.4 at 37°C for 20 min. The sections was examined under light and photographs was taken to determine the intensity of tissue damage in the myocardium.

RESULTS
Phytochemical screening
Phytochemical screening of ethanolic extract were showed the presence of flavonoids , tannins, saponins, glycosides, phenols, carbohydrates, proteins, aminoacids and alkaloids was present. Where as fats, oils, gum and mucilage, triterpenoids were absent.
Table 1. Effect of HAE of *P. odoratissimus* LPO and GSH

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups</th>
<th>Treatment</th>
<th>LPO µmole/gm wet tissue</th>
<th>GSH µmole/gm wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I</td>
<td>Normal</td>
<td>35.67±1.856</td>
<td>3.452±0.1438</td>
</tr>
<tr>
<td>2</td>
<td>Group II</td>
<td>ISO induced</td>
<td>85.50±2.320**</td>
<td>1.237±0.05858***</td>
</tr>
<tr>
<td>3</td>
<td>Group III</td>
<td>Atenolol and ISO</td>
<td>44.00±1.592***ab</td>
<td>2.833±0.05136***bc</td>
</tr>
<tr>
<td>4</td>
<td>Group IV</td>
<td>HAE of P.o(200mg/kg)and ISO</td>
<td>52.12±1.844***ab</td>
<td>1.770±0.08112**b</td>
</tr>
<tr>
<td>5</td>
<td>Group V</td>
<td>HAE of P.o(400mg/kg)and ISO</td>
<td>46.83±1.4***b</td>
<td>2.580±0.06105**b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6 rats), *P* values: ***< 0.001, ** < 0.01, * < 0.05 NS: Non-Significant. Comparisons are made between (a) - compared to normal Group I Vs Group II, (b) - compared to negative control Group II Vs Group III, Group IV, Group V. LPO - Lipid Peroxidation, GSH – Glutathione.

Effect of Lactate dehydrogenase and Creatine kinase -myocardial isoenzyme

Table 2. Effect of HAE of *P. odoratissimus* LDH and CK-MB

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups</th>
<th>Treatment</th>
<th>LDH (IU/L)</th>
<th>CK-MB (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I</td>
<td>Normal</td>
<td>34.50±1.478</td>
<td>71.97±1.801</td>
</tr>
<tr>
<td>2</td>
<td>Group II</td>
<td>ISO induced</td>
<td>62.17±1.195***a</td>
<td>167.5±2.680**a</td>
</tr>
<tr>
<td>3</td>
<td>Group III</td>
<td>Atenolol and ISO</td>
<td>42.83±1.701**b</td>
<td>97.67±2.985***b</td>
</tr>
<tr>
<td>4</td>
<td>Group IV</td>
<td>HAE of P.o(200mg/kg)and ISO</td>
<td>55.83±2.212**b</td>
<td>129.8±1.869***b</td>
</tr>
<tr>
<td>5</td>
<td>Group V</td>
<td>HAE of P.o(400mg/kg)and ISO</td>
<td>47.83±1.833**b</td>
<td>117.8±2.120* b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6 rats), *P* values: ***< 0.001, ** < 0.01, * < 0.05 NS: Non-Significant. Comparisons are made between (a) - compared to normal Group I Vs Group II, (b) - compared to negative control Group II Vs Group III, Group IV, Group V. LDH – Lactate Dehydrogenase, CK-MB – Creatine Kinase in Myocardial Isoenzyme.

Effect of Superoxide dismutase and Catalase

Table 3. Effect of HAE of *P. odoratissimus* SOD and CAT

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups</th>
<th>Treatment</th>
<th>SOD Unit/mg Protein</th>
<th>CAT Unit/mg Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I</td>
<td>Normal</td>
<td>10.80±0.07442</td>
<td>24.67±1.358</td>
</tr>
<tr>
<td>2</td>
<td>Group II</td>
<td>ISO induced</td>
<td>4.837±0.04507***a</td>
<td>11.50±0.7638***a</td>
</tr>
<tr>
<td>3</td>
<td>Group III</td>
<td>Atenolol and ISO</td>
<td>8.317±0.04287***ab</td>
<td>20.52±0.9916**ab</td>
</tr>
<tr>
<td>4</td>
<td>Group IV</td>
<td>HAE of P.o(200mg/kg)and ISO</td>
<td>6.942±0.03736**b</td>
<td>17.33±1.256**b</td>
</tr>
<tr>
<td>5</td>
<td>Group V</td>
<td>HAE of P.o(400mg/kg)and ISO</td>
<td>7.908±0.04012***ab</td>
<td>20.35±0.6325***ab</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6 rats), *P* values: ***< 0.001, ** < 0.01, * < 0.05 NS: Non-Significant. Comparisons are made between (a) - compared to normal Group I Vs Group II, (b) - compared to negative control Group II Vs Group III, Group IV, Group V. SOD – Superoxide Dismutase, CAT – Catalase.

Fig 1. Effect of HAE of *P. odoratissimus* LPO

A significant increase in LPO level in heart was observed in Cardiotoxicity induced animals when compared with control group. Whereas administration of HAE of *P. odoratissimus* (200mg/kg and 400mg/kg) decreased the LPO level in comparison to Isoproterenol treated group. As shown in Tab-1, Fig -1
Cardioprotective effect of HAE of *P. odoratissimus* was supported by increased antioxidant level. Decreased level of GSH were observed in tissue homogenate of disease control group, a significant increase in GSH levels was observed in HAE of *P. odoratissimus*(200mg/kg, 400mg/kg) treated groups. This increase in GSH level is possibly required to overcome excessive oxidative stress. As shown in Tab-1, Fig-2.

A significant increase in LDH level in heart was observed in Cardiotoxicity induced animals when compared with control group. Whereas administration of HAE of *P. odoratissimus*(200mg/kg and 400mg/kg) decreased the LDH level in comparison to Isoproterenol treated group. As shown in Tab-2, Fig-4.

Cardioprotective effect of HAE of *P. odoratissimus* was supported by increased antioxidant level. Decreased level of SOD were observed in tissue homogenate of disease control group, a significant increase in SOD levels was observed in HAE of *P. odoratissimus*(200mg/kg, 400mg/kg) treated groups. This increase in SOD level is possibly required to overcome excessive oxidative stress. As shown in Tab-3, Fig-5.
A significant decrease in CAT level in heart was observed in Cardiotoxicity induced animals when compared with control group. Where as administration of HAE of *P. odoratissimus* (200mg/kg and 400mg/kg) increased the CAT level in comparison to Isoproterenol treated group. As shown in Tab-3, Fig -6.

Histopathological studies

**Fig 7. Histopathological examination of the Myocardium of Heart**

*Group I*  
*Group II*  
*Group III*  
*Group IV*  
*Group V*
Group I - Myoctes with normal cell structure without any deviation.

Group II - Myocytes with severe damaged cell Necrosis.

Group III - Myoctes with mild multifocal cell Necrosis.

Group IV - Myoctes with marked cell Necrosis.

Group V - Myoctes with mild to moderate cell Necrosis.

DISCUSSION

The oxygen radical hypothesis is importance because of its potential mechanisms in the myocardial injury. It has been demonstrated that exogenous free radicals cause cellular calcium loading with inhibition of the sarcoplasmic reticulum calcium ATPase and inhibition of the sodium potassium ATPase leading to sodium mediated calcium gain [14]. Oxygen radicals cause lipid peroxidation that can result in cell membrane break down causing cell apoptosis and cell necrosis [15]. Reactive oxygen species (ROS) are formed at an accelerated rate in ISO-treated myocardium. Cardiacmyocytes, endothelial cells, and infiltrating neutrophils contribute to this ROS production and can lead to cellular dysfunction and necrosis. Infarct-like lesions are produced in the myocardium when injected with ISO. myocardial necrosis induced by ISO is probably due to a primary action on the sarcoplasmic membrane, followed by stimulation of adenylate cyclase, activation of Ca2+ and Na+ channels, exaggerated calcium inflow and excess of excitation-contraction coupling mechanism leading to energy consumption and cellular death. Free radicals generated by ISO, initiate lipid peroxidation of the membrane bound polyunsaturated fatty acids, leading to impairment of membrane structural and functional integrity [16]. The metabolic damage of myocardium results in increase in the concentration of the marker enzymes like lipid peroxidation, glutathione, lactate dehydrogenase, creatine kinase myocardial isoenzyme, catalase and superoxide dismutase. Oxygen, although essential for tissue survival, can be injurious in myocardial infarction. In the present study the ability of oxygen radical scavengers to improve mechanical, sarcoplasmic reticulum, and mitochondrial function suggested that oxygen free radicals participated in isoproterenol induced myocardial infarction.

The present study revealed the cardioprotective effect of hydroalcoholic extract of Pandanus odoratissimus isoproterenol induced myocardial infarction in rats. Administration of isoproterenol leads to formation of reactive oxygen species in irreversible manner which precipitate in hypoxia cellular dysfunction and necrosis [17]. All this reaction leads the biological system in to oxidative stress and resulting apoptosis.

The level of glutathione, Catalase and superoxide dismutase is significantly reduced in disease control when compared with that of normal control group.Upon treatment with HAE of P. odoratissimus dose 200mg/kg and 400mg/kg b.w (p.o) significantly increased the decreased levels of glutathione, Catalase and superoxide dismutase in a dose dependant manner (p < 0.01). The level of lipid peroxidation, lactate dehydrogenase and creatine kinase myocardial isoenzyme is significantly increased in disease control when compared with that of normal control group. Upon treatment with HAE of P. odoratissimus dose 200mg/kg and 400mg/kg b.w (p.o) significantly reduced the increased levels of lipid peroxidation, lactate dehydrogenase, and creatine kinase myocardial isoenzyme in a dose dependant manner (p < 0.01). Histopathological studies of heart tissue showed structural damages like myocyte cell damage in myocardial layer. Cells were completely damaged in negative control. The drug treated groups showed regenerative changes when compared with negative control.

CONCLUSION

Present study concluded that the Hydroalcoholic extract Pandanus odoratissimus showed an significant effect on cardioprotective activity at an dose of 400 mg/kg body weight and showed an significant values of biochemical parameters in serum levels, antioxidant and heart. Regenerative changes in histopathological study, presence of flavonoids may contribute for the significant cardioprotective effect.

REFERENCES


