Research Paper

Cardioprotective Activity of Garlic (Allium sativum) in Isoproterenol-Induced Rat Myocardial Necrosis: A Biochemical and Histoarchitectural Evaluation

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ABSTRACT: The current study dealt with the protective role of garlic oil on isoproterenol (ISO)-induced myocardial infarction (MI) in rats. Subcutaneous injection of ISO (20 mg/kg body weight in 1ml saline) to rats for 2 consecutive days offered significant alteration in biochemical parameters and moderate necrosis in heart. Effect of garlic oil oral treatment for 60 days (75 mg/kg body weight) was evaluated against ISO (20 mg/kg, sc)-induced cardiac necrosis. Levels of marker enzymes (AST, ALT, LDH and CPK) were assessed in serum, antioxidant parameters viz., reduced glutathione (GSH), and lipid peroxides were assayed in serum and heart homogenate. Significant myocardial necrosis, depletion of endogenous antioxidants and increase in serum levels of marker enzymes were observed in ISO-treated animals when compared with the normal animals. Garlic oil elicited a significant cardio protective activity by lowering the levels of serum marker enzymes and lipid peroxidation and elevated the levels of GSH. The present findings have demonstrated that the cardioprotective effects of garlic oil in ISO-induced oxidative damage may be due to an augmentation of the endogenous antioxidants and inhibition of lipid peroxidation of membrane.

KEYWORDS: Antioxidant activity, Cardioprotective, Cardiac necrosis, Garlic, Isoproterenol.

Introduction

Cardiovascular diseases (CVDs) are the most prevalent cause of death and disability worldwide. CVD, a group of disorders of the heart and the vasculature, includes high blood pressure, coronary heart disease, congestive heart failure, stroke and congenital heart defects. The World Health Organization (WHO) estimates that 17 million people die of cardiovascular disease annually (MacKay and Mensah, 2004). WHO predicts that deaths due to circulatory system diseases are projected to double by 2015 (Reddy, 1993). Myocardial infarction (MI) is the rapid development of myocardial necrosis caused by critical imbalance between the oxygen supply and the demand of the myocardium. Oxidative stress resulting from increased production of free radicals associated with decreased levels of antioxidants in the myocardium plays a major role in CVD such as ischemic heart disease, atherosclerosis, congestive heart failure, cardiomyopathy and arrhythmias (Das and Maulik, 1995).

Isoproterenol (ISO), a catecholamine, was administered in the present study, as it serves as a standard model to study the beneficial effect of many drugs on cardiac function. ISO is a synthetic β-adrenergic agonist that causes severe stress in the myocardium and cause necrosis in the heart muscle. ISO-induced myocardial necrosis showed membrane permeability alterations, which bring about the loss of function and integrity of myocardial membranes (Todd et al., 1980 and McCord, 1988). The pathophysiological changes following ISO administration are comparable to those taking place in human myocardial alterations (Wexler, 1978).

Many dietary antioxidants and some non-nutrient based antioxidants from plants such as sulphur containing compounds in garlic, phyto-oestrogens in soy, green tea, anthocyanins in red berries, lycopene in tomatoes, red and white wines from grape seeds are increasingly being
recognized as potential health promoters in reducing the risk of cardiovascular disease (CVD) and atherosclerosis (Walker, 1996). The prophylactic and therapeutic effects of many plant foods and extracts in reducing CVD have been reviewed (Ames et al., 1993).

Garlic (Allium sativum) is very effective on the basis of its antidiabetic, antithrombotic and anticancer effects (Augusti, 1996). Garlic and its products have been reported to effectively prevent high lipid levels in experimental animals and humans and also to inhibit oxidation of low density lipoprotein (Jain et al., 1993) and also have antioxidant activity (Banerjee et al., 2002). The high antioxidant potential of garlic may be a result of its high content of sulfur compounds (Prasad et al., 1995). The present study has been designed to evaluate the cardioprotective activity of garlic oil in ISO-induced cardiac damage in rats and attempts to understand the mechanism of its therapeutic effect with reference to biochemical markers and lipid peroxidation.

MATERIALS AND METHODS

Animals: Male Wistar albino rats (weighing 170–200 g) were maintained at the Animal house, Nantha College of pharmacy, Erode, India, and maintained under standard environmental conditions (12 h light/dark cycles at 25–28°C, 60–80% relative humidity). They were fed with a standard diet (Hindustan Lever, India) and water ad libitum and allowed to acclimatize for 14 days before the procedure. All the studies were conducted in accordance with the norms approved by Ministry of Social Justice and Empowerment, Government of India and Institutional Animal Ethics’ Committee guidelines/Approval No.688/02/CPCSEA).

Drugs and chemicals: Isoproterenol and Vitamin E were obtained from Ranbaxy laboratories, Mumbai, India. All other chemicals used were of analytical grade.

Garlic oil preparation: Commercial garlic pearls (containing 0.25% garlic oil) were crushed and thoroughly mixed with diethyl ether (B.P., 40–60°C) in a separating funnel. The etherial fraction was then separated and ether was allowed to evaporate. The remaining oily material was used as garlic oil (Jain and Konar, 1993).

Experimental design: The experimental animals were divided into four groups of six rats each.

Group I: Normal animals were served as control.

Group II: Normal animals were administered isoproterenol (20 mg/kg b.w., subcutaneously twice at an interval of 24 h) in physiological saline (Saravanan and Prakash, 2004).

Group III: Animals were orally administered with garlic oil (75 mg / kg b.w ) for 60 days and isoproterenol (20 mg/kg b.w) was administered subcutaneously twice at an interval of 24 h (Saravanan and Prakash, 2004).

Group IV: Animals were orally administered with vitamin E (50 mg/kg b.w for a period of 60 days) and isoproterenol (20 mg/kg b.w) was administered subcutaneously twice at an interval of 24 h.

Biochemical assays: After 60 days treatment, the fasted rats of various groups were sacrificed by cervical decapitation and blood was collected. A portion of the heart tissue was dissected, washed with ice cold saline and homogenized in 0.1M Tris HC1 buffer, pH 7.4. The super-natant was used for the assay of enzyme activity.

The activities of Aspartate amino transferase (AST) and Alanine amino transferase (ALT) in serum were determined spectrophotometrically by the method of Mohur and Cook (Mohur and Cook, 1975). The lactate dehydrogenase (LDH) activity in serum was assayed according to the method of King (1965). The creatine phosphokinase (CPK) activity in serum was determined by the method of Okinaka et al. (1961). Thiobarbituric acid reactive substance (TBARS) in tissue was estimated by the method of Okhawa et al. (1979). Glutathione (GSH) content was estimated by the method of Patterson and Lazaro (1955).

Histological studies: Animals were sacrificed on the day of withdrawal of blood, hearts were removed, washed immediately with saline and then fixed in 10% buffered formalin. The hearts stored in 10% buffered formalin, were embedded in paraffin, sections cut at 5 mm and stained with hematoxylin and eosin. These sections were then examined under a light microscope for histarchitectural changes.

Statistical analysis: All the grouped data were statistically evaluated with SPSS 10.0 software. Hypothesis testing methods included one way analysis of variance(ANOVA) followed by least significant difference(LSD) test; p value of less than 0.05 were considered to indicate statistical significance. All the results were expressed as the mean ± S.D. for six animals in each group.

Results

Table 1 shows the activities of marker enzymes such as LDH, CPK, AST and ALT in the serum of control and experimental groups of rats. Marked elevation (p < 0.05) in the activities of these enzymes were observed in group 2, IPL intoxicated rats when compared with group 1, control rats. Activities of these enzymes in serum were maintained
at near normal ($p < 0.05$) levels in the group 3 and 4 rats, pre-co-treated with garlic oil and Vit E respectively.

Levels of GSH and lipid peroxides in heart and serum are shown in Table 2. There was a significant increase in lipid peroxidation ($P < 0.05$) with significant decrease in glutathione content in group II rats when their values are compared to those of control rats. The activities of glutathione and lipid peroxide level in serum and heart were maintained at near normal ($p < 0.05$) levels in the group 3 and 4 rats, pre-co-treated with garlic oil and Vit E respectively.

Normal architecture of the myocardium was observed with no evidence of microscopic changes in the normal control group (Figure 1). The cardiac sections of the ISO-treated animals revealed degenerative changes in the muscle fiber, showing a coagulative necrosis (Figure 2). Pretreatment with SMCS and Vitamin E exerted a protective effect as evident from the normal myofibrillar structures with striations (Figure 3 and 4).

Table 1 Activities of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) in serum of control and experimental animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>CK (µkat/litre)</th>
<th>LDH (µkat/litre)</th>
<th>AST (µkat/litre)</th>
<th>ALT (µkat/litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (Group I)</td>
<td>179.14±7.67</td>
<td>371.69±6.47</td>
<td>159.17±6.47</td>
<td>365.13±13.16</td>
</tr>
<tr>
<td>Isoproterenol (Group II)</td>
<td>364.37±8.59</td>
<td>640.51±12.56</td>
<td>221.63±8.74</td>
<td>654.21±14.21</td>
</tr>
<tr>
<td>Isoproterenol + garlic oil (Group III)</td>
<td>191.17±7.19</td>
<td>395.73±3.17</td>
<td>172.35±7.84</td>
<td>390.10±10.33</td>
</tr>
<tr>
<td>Isoproterenol + Vitamin E (Group IV)</td>
<td>187.36±6.95</td>
<td>389.43±10.31</td>
<td>169.77±6.26</td>
<td>382.94±11.61</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.D for 6 animals in each group. The level of CK, LDH, AST and ALT in serum are expressed as µkat/litre. * P < 0.05

Group II compared with Group I
Group III compared with Group II
Group IV compared with Group II

Table 2 Levels of TBARS and glutathione in serum and heart of control and experimental groups of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS Serum</th>
<th>TBARS Heart</th>
<th>Glutathione Serum</th>
<th>Glutathione Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (Group I)</td>
<td>3.85 ± 0.35</td>
<td>3.63 ± 0.39</td>
<td>31.14 ± 2.31</td>
<td>4.36 ± 0.41</td>
</tr>
<tr>
<td>Isoproterenol (Group II)</td>
<td>7.34 ± 0.93</td>
<td>5.78 ± 0.60</td>
<td>20.53 ± 1.64</td>
<td>2.13 ± 0.23</td>
</tr>
<tr>
<td>Isoproterenol + garlic oil (Group III)</td>
<td>4.12 ± 0.57</td>
<td>3.94 ± 0.51</td>
<td>28.68 ± 2.44</td>
<td>4.12 ± 0.24</td>
</tr>
<tr>
<td>Isoproterenol + Vitamin E (Group IV)</td>
<td>3.91 ± 0.43</td>
<td>3.82 ± 0.48</td>
<td>29.51 ± 2.37</td>
<td>4.27 ± 0.46</td>
</tr>
</tbody>
</table>

Values are given ± S.D for groups of six animals in each group. Values are statistically significant at * p<0.05.

Units: Thiobarbituric acid reactive substances (nm) of TBA reactants/mg of protein; glutathione (nm) of GSH/g of tissue.

Group II compared with Group I
Group III compared with Group II
Group IV compared with Group II
Discussion

Myocardium contains an abundant concentration of diagnostic marker enzymes of MI and once metabolically damaged, it releases its contents into the extra cellular fluid (Suchalata and Shyamala Devi, 2004). Hence, in ISO myocardial infarcted rats, there was a decrease in activities of the marker enzymes AST, ALT, LDH and CPK in the heart homogenate, followed by an increase in their levels in serum. These findings confirm the onset of myocardial necrosis and leaking out of the marker enzymes from heart to blood (Sabeena Farvin et al. 2004). Furthermore, the amount of enzymes appearing in serum is proportional to the number of necrotic cells (Geetha et al., 1990). Hearn (1979) had reported that, of all the macromolecules that leak from the damaged tissue, enzymes because of their tissue specificity and catalytic activity are the best markers of tissue damage. The release of cellular enzymes reflects a non-specific alteration in the plasma membrane integrity and/or permeability as a response to β-adrenergic stimulation. When myocardial cells, containing CK, LDH, AST and ALT are damaged or destroyed due to deficient oxygen supply or glucose, the cell membrane becomes permeable or may rupture, which results in the leakage of these enzymes. This accounts for the decreased activities of CK, LDH, AST and ALT in heart tissue of ISO-induced rats. This might be due to the damage caused by the β-agonist that has rendered it leaky (Mathew et al., 1985). Garlic oil and Vit E pretreated isoproterenol administered rats maintained the level of lipid peroxides to near normal when compared to control. This could be due to the protective effect of garlic on the myocardium, reducing the myocardial damage thereby restricting the leakage of these enzymes.
Isoproterenol generates free radicals and stimulate lipid peroxidation, which may be a factor for causing damage to the myocardial membrane (Kakreja and Hess, 1992). Lipid peroxide is an important pathogenic event in myocardial infarction and the accumulated lipid peroxides reflects the various stages of the disease and its complications (Grylewski, 1980). It is reported that enhanced lipid peroxidation in serum and heart of isoproterenol-induced rats injure blood vessels, causing increasing adherence and aggregation of platelets to the injured sites (Grylewski, 1980). Garlic oil and Vit E pretreated isoproterenol administered rats maintained the level of lipid peroxides to near normal when compared to control. Decreased lipid peroxidation may be due to the sulfoxides present in the garlic which can directly trap electrons from other systems and thus prevent to a certain extent superoxide formation and scavenge many free radicals including OH (Klanns-Dietor, 1983).

Isoproterenol-intoxicated rats showed a significant decrease in glutathione levels in heart and serum. Decreased glutathione levels in isoproterenol administration may be due to its increased utilization in protecting SH containing proteins from lipid peroxides (Saravanan and Prakash 2004). GSH functions as a free radical scavenger in the repair of radical-induced cellular damage. Low level of GSH was observed during increase in cardiac stress caused by ISO administration (Saravanan and Prakash 2004). This observation supports our findings where we too have observed a decline in GSH level in isoproterenol intoxicated rats. Oral administration of garlic oil and Vit E along with isoproterenol intoxicated rats maintained the concentration of GSH at near normal levels.

Summary the study demonstrated that garlic oil (75 mg/kg) significantly alleviated ISO-induced myocardial necrosis. Histopathological examination of the myocardium further confirmed its cardio protective effects. Decreased myocardial necrosis (as evidenced by reduced AST, LDH and CPK release and histoarchitectural changes) and augmentation of endogenous antioxidants, all contribute to its cardio protective effect. These observations highlight that garlic is one of the promising herbal drug for improving defense mechanisms in the physiological systems against oxidative stress caused by ISO administration.

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References


