Effect of β-Sitosterol on Cardiac Troponins, Marker Enzymes and Biochemical Parameters in Isoproterenol-Induced Myocardial Infarction

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Abstract

Increasing trend of cardiovascular disease (CVD) is not completely controlled by allopathic medicine. However, usage of plant based medicine have a great impact on CVD. This study evaluated the preventive role of β-sitosterol on heart weight, cardiac markers and other biochemical parameters in isoproterenol (ISO)-induced myocardial infarction (MI) in Wistar rats. β-sitosterol pretreatment period of 21 d after ISO (85 mg/kg) induced experimental rats for 2 d were carried out. Rats injected with ISO (85 mg/kg) showed significantly increased levels of heart weight, elevated activities of cardiac marker enzymes such as creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) in serum with consequent depletion in their activities in heart. The levels of cardiac troponin T (cTnT), troponin I (cTnI), glucose, uric acid, serum iron were significantly increased with succeeding decrease in the levels of total proteins and A/G ratio. Wistar rats were pretreated with β-sitosterol (10, 20 and 40 mg/kg) for a period of 21 d to ISO-induced rats showed a significant decrease in heart weight and minimized the alterations in the activity levels of cardiac marker enzymes and other biochemical parameters. This could be due to the minimization of cardiac damage to ISO-induced rats. Thus, the results of our study show that β-sitosterol possess cardioprotective role in ISO-induced MI in rats.

Keywords: β-sitosterol, isoproterenol, myocardial infarction, cardiac troponin T, cardioprotective role.

Introduction

Catecholamines are produced under stress conditions and are also administered in circumstances of cardiac stress to sustain blood pressure and cardiac function in patients. It is an important regulator of myocardial contractility and metabolism. However, excess amounts of catecholamines are responsible for cellular damage, observed in clinical conditions like angina, transient myocardial hypoxia, acute coronary insufficiency and subendocardial infarct. Due to the generation of reactive oxygen species (ROS), catecholamines contribute to oxidative stress (Yogeeta et al., 2006). Isoproterenol [1-(3,4-dihydroxy phenyl)-2-isopropyl amino ethanol hydro chloride] (ISO) is a synthetic catecholamine and β-adrenergic agonist, cause severe stress in the myocardium resulting in infarct like necrosis of the heart muscles (Wexler, 1978). The advantages of ISO-induced myocardial infarction (MI) can be comparable to physical occlusion of the coronary artery, as a less invasion accomplished without the complicating factors of general anesthesia and lack of foreign body remaining in the heart. The physiological, pathological, biochemical and histological changes that take place in rat’s heart following MI induced by ISO are analogous to those changes taking place in MI in humans (Geng et al., 2004).

Ischemic heart disease (IHD) is a leading cause of death in India, with an estimated 3 million deaths per year accounting 25% of all transience (WHO, 2004). IHD is a condition, in which unevenness between myocardial oxygen supplies and demand to the myocardium via the coronary circulation results in hypoxia and accumulation of waste metabolites. There is substantial evidence that ischemic tissue generates oxygen-derived free radicals, which are involved in the further damage of the myocardium, finally leads to MI (Jeremias et al., 2009). MI, the most dreaded sequel among IHD, is invariably followed by several biochemical alterations such as cardiac markers, lipid peroxidation, hyperglycemia, hyperlipidaemia and other biochemical alterations, leading to qualitative and quantitative alterations of myocardium (Bhandari et al., 2008). Though the clinical concern is enhanced, communal understanding is increased; still MI remains the leading cause of death (Aronow, 2006). Obviously, search for an effective drug that can ameliorate CVD is imperative. As plant based drugs are considered to be safe and economic, several research scientists have tried from time to time with phytochemicals to regulate CVD (Wiffen et al., 2002).
β-Sitosterol (24-ethyl-5-cholestan-3-ol), is widely distributed in plant kingdom and found in *Nigella sativa*, *Serenoa repens* (Saw palmetto), *Cucurbita pepo* (pumpkin seed), *Pygeum africanum*, black cumin seed, pecans, cashew fruit, avocados, rice bran, wheat germ, corn oils, soybeans, sea-buckthorn and wolfberries (Moreau et al., 2002). β-sitosterol, a well-known plant sterol has been reported to reduce serum cholesterol and prevent cardiovascular events mainly by inhibition of cholesterol absorption in the intestines (Miettinen et al., 1995). β-sitosterol posses various pharmacological properties and known to regulate key molecules involved in inflammation, the immune response, anti-cancer defenses and apoptosis (Bouric and Lamprecht, 1997; Bouic, 2002). As of our knowledge, there is no previous study on the cardioprotective role of β-sitosterol on ISO-induced MI in rats. Hence, to evaluate the cardioprotective effect of β-sitosterol, the following parameters were studied viz., cardiac troponin-T and I (cTnT and cTnI), cardiac marker enzymes (creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT), glucose, uric acid, serum iron, plasma total protein and A/G ratio in normal and ISO-induced MI in Wistar rats.

**Materials and methods**

**Experimental animals:** All the experiments were carried out with male albino Wistar rats weighing 140-160 g, obtained from Venkateswara Enterprises, Bangalore, 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 dark cycle 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). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fibre, 7.5% ash, 1.38% and 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 3,600 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 Institutional Animal Ethics Committee (IAEC) of Muthaymmal College of Arts and Science, Rasipuram, TN, India (Approval number: MCAS/Ph.D./01/2012-2013).

**Drugs and chemicals:** Isoproterenol hydrochloride, β-sitosterol, trichloroacetic acid (TCA), 2,2′ dipyrydyl, sodium sulphite reagent were purchased from Sigma Chemical Company, St. Louis, MO, USA. Creatine kinase (CK), lactate dehydrogenase (LDH), alanine transaminase (ALT), aspartate amino transaminase (AST), glucose, uric acid, total protein and A/G ratio all the kits were purchased from Agappe Diagnostics, kerala India. All other chemicals used in this study were of analytical grade.

**Induction of experimental myocardial infarction:** Isoproterenol (85 mg/kg) was dissolved in normal saline and injected subcutaneously to rats at an interval of 24 h for 2 d (Rajadurai and Prince, 2006).

**Experimental design:** A total number of 36 rats were used in the experiment. The rats were randomly divided into 6 groups of 6 rats each. β-sitosterol was suspended in carboxy methyl cellulose (CMC) and administered to rats orally using an intragastric tube daily for a period of 21 d.

- **Group 1:** Normal control rats
- **Group 2:** Normal rats + β-sitosterol (40 mg/kg)
- **Group 3:** ISO control rats (85 mg/kg)
- **Group 4:** ISO + β-sitosterol (10 mg/kg)
- **Group 5:** ISO + β-sitosterol (20 mg/kg)
- **Group 6:** ISO + β-sitosterol (40 mg/kg)

At the end of the treatment period, all the rats were anaesthetized with pentobarbital sodium (35 mg/kg, i.p.) and sacrificed by cervical decapitation and blood was collected in two tubes, i.e., one with anticoagulant (ethylene diamine tetra acetic acid) for plasma separation and another without anticoagulant for serum separation. Both the plasma and serum were separated from each sample and used for the biochemical analysis.

Immediately after sacrifice, heart tissues were excised in ice cold saline condition. They were blotted free of blood and tissue fluids. A known weight of the heart tissue was homogenized in buffer solution. The homogenate was centrifuged and the supernatant was used for the estimation of biochemical parameters.

**Assays of cardiac markers:** The levels of cardiac Troponin T and I in serum were estimated using a standard kit by electro chemiluminescence immunoassay (Catalogue no. 12017423, Roche Diagnostics, Switzerland). Assay of cardiac marker enzymes: Activity of serum and heat creatine kinase activity was estimated by Witt and Trendelenburg (1982) method using a commercial kit (Product No. 11404002), Lactate dehydrogenase activity was estimated by Weisshaar et al. (1975) method using a commercial kit (Product No. 11407002), Aspartate transaminase activity was estimated by Climb (1976) method using a commercial kit (Product No. 11408001), Alanine amino transferase activity was estimated by Thefeld et al. (1974) method using a commercial kit (Product No. 11409001).

**Biochemical assays:** Albumin levels were estimated by Doumas et al. (1971) using a commercial kit (Product No. 11002001), Total proteins levels were estimated by Doumas (1971) using a commercial kit (Product No. 11002001), Glucose levels were estimated by Trinder, (1969) method using a commercial kit (Product No. 11406001), Serum uric acids levels were estimated by Fossati et al. (1980) method using a commercial kit (Product No. 11413001). Serum iron content was estimated by the method of Ramsay (1969).
Table 1. Effect of β-sitosterol on the activities of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) in serum in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>CK(IU/L)</th>
<th>LDH(IU/L)</th>
<th>AST(IU/L)</th>
<th>ALT(IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>208.2 ± 12.6a</td>
<td>106.7 ± 6.1a</td>
<td>29.6 ± 1.7a</td>
<td>22.1 ± 1.1a</td>
</tr>
<tr>
<td>Normal + β-sitosterol (40 mg/kg)</td>
<td>205.3 ± 10.6a</td>
<td>104.3 ± 5.7a</td>
<td>29.2 ± 1.5a</td>
<td>21.6 ± 1.3a</td>
</tr>
<tr>
<td>ISO control</td>
<td>422.2 ± 31.2c</td>
<td>192.6 ± 12.2b</td>
<td>65.6 ± 4.2c</td>
<td>37.2 ± 2.6c</td>
</tr>
<tr>
<td>ISO + β-sitosterol (10 mg/kg)</td>
<td>325.5 ± 18.8c</td>
<td>142.1 ± 10.4c</td>
<td>45.8 ± 3.6c</td>
<td>31.2 ± 2.0c</td>
</tr>
<tr>
<td>ISO + β-sitosterol (20 mg/kg)</td>
<td>291.3 ± 20.7c</td>
<td>133.8 ± 11.2c</td>
<td>42.5 ± 3.2c</td>
<td>28.4 ± 1.7c</td>
</tr>
<tr>
<td>ISO + β-sitosterol (40 mg/kg)</td>
<td>265.6 ± 10.9d</td>
<td>118.5 ± 8.3d</td>
<td>36.4 ± 1.8d</td>
<td>25.9 ± 1.5d</td>
</tr>
</tbody>
</table>

*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 2. Effect of β-sitosterol on the activities of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) heart in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>CK(IU/L)</th>
<th>LDH(IU/L)</th>
<th>AST(IU/L)</th>
<th>ALT(IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>20.7 ± 0.8a</td>
<td>87.2 ± 4.2a</td>
<td>41.6 ± 2.4a</td>
<td>21.3 ± 0.74</td>
</tr>
<tr>
<td>Normal + β-sitosterol (40 mg/kg)</td>
<td>20.5 ± 1.2a</td>
<td>86.5 ± 3.8a</td>
<td>41.2 ± 2.7a</td>
<td>28.0 ± 0.9a</td>
</tr>
<tr>
<td>ISO control</td>
<td>9.8 ± 0.7b</td>
<td>58.1 ± 2.6b</td>
<td>20.3 ± 0.9b</td>
<td>10.4 ± 0.4b</td>
</tr>
<tr>
<td>ISO + β-sitosterol (10 mg/kg)</td>
<td>15.5 ± 0.9c</td>
<td>66.2 ± 2.9c</td>
<td>33.6 ± 1.8c</td>
<td>16.2 ± 0.8c</td>
</tr>
<tr>
<td>ISO + β-sitosterol (20 mg/kg)</td>
<td>17.0 ± 1.3c</td>
<td>68.5 ± 3.4c</td>
<td>34.3 ± 1.3c</td>
<td>16.5 ± 1.1c</td>
</tr>
<tr>
<td>ISO + β-sitosterol (40 mg/kg)</td>
<td>18.6 ± 1.4d</td>
<td>77.3 ± 4.1d</td>
<td>37.7 ± 1.6d</td>
<td>18.6 ± 1.2d</td>
</tr>
</tbody>
</table>

*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).*

**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 9.00. Results were expressed as mean ± S.D. from six rats in each group. P values <0.05 were considered as significant.

**Results**

Figure 1 and 2 showed the effects of β-sitosterol on heart weight, the level of cardiac Troponins T and I in normal and ISO-induced MI rats. Rats induced with ISO showed significant (P<0.05) increase in the heart weight and the level of cTnT and cTnl. Oral pretreatment with β-sitosterol (10, 20, 40 mg/kg) for a period of 21 d significantly decreased the levels of heart weight and cTnT and cTnl in ISO-induced MI rats. Table 1 and 2 represent the effect of β-sitosterol on the activities of serum and heart CK, LDH, AST and ALT in normal and ISO-induced rats. Rats induced with ISO showed a significant (P<0.05) increase in the activities of these cardiac marker enzymes in serum with subsequent decrease in the activities of these enzymes in the heart when compared to normal control rats. Pretreatment with β-sitosterol significantly (P<0.05) minimized the alterations in the activities of these enzymes in ISO-induced rats when compared to ISO-alone induced rats. Figure 3 showed the effects of β-sitosterol on the level of glucose in normal and ISO-induced MI rats. ISO administered rats showed a significant (P<0.05) increase in the levels of plasma glucose on comparison with normal control rats. Pretreatment with β-sitosterol to ISO-induced rats significantly decreased the level of plasma glucose in ISO-induced MI rats. Table 3 depicts the levels of serum uric acid, serum iron, plasma total proteins and A/G ratio in normal and ISO-induced rats. In ISO-induced group of rats, the levels of serum uric acid and serum iron were increased significantly (P<0.05) with decrease in the levels of plasma total protein and A/G ratio when compared with normal control rats. Oral administration of β-sitosterol to ISO-induced rats significantly minimized the alterations in the levels of these parameters in ISO-induced rats when compared with ISO-alone induced rats.
For all the parameters studied, oral administration of β-sitosterol (10, 20 and 40 mg/kg respectively) to normal rats for a period of 21 d showed a minor effect but it was not statistically significant. Pretreatment with β-sitosterol (10, 20 and 40 mg/kg respectively) to ISO-induced rats significantly minimized the alterations in all the parameters studied in a dose dependent manner. β-sitosterol at a dose of 40 mg/kg showed a better effect than the other two doses (10 and 20 mg/kg respectively) in ISO-induced rats.

**Discussion**

Pharmacological induction of MI by subcutaneous administration of ISO in rats is convenient due to its smaller size of coronary arteries (Rona et al., 1959). ISO-induced cardiac lesions are morphologically similar to those of ‘coagulative myocytolysis’ or myofibrillar degeneration, which is one of the important finding described in acute MI and sudden death in man (Baroldi, 1974). Administration of ISO acts on β1 and β2 adrenoceptors, stimulation of these receptors leading to positive inotropic and chronotropic effects. Various mechanism have been proposed to explain the toxic role of ISO on myocardium, including increased cAMP (Bhagat et al., 176), increased intracellular Ca++ overload (Bloom and Davis, 1972), depletion of high energy phosphate and oxidative stress (Singal et al., 1982). Excessive generation of cellular toxic free radicals, due to the auto-oxidation metabolic products (ISO undergo auto-oxidation produces quinones, which react with oxygen to produce superoxide anion (O2−) and H2O2) (Singal et al., 1982). In ISO-induced rats, a significant increase in the levels of heart weight has been observed.

Enhancement in the heart weight could be due to excessive accumulation of water content, oedematous intramuscular space moreover widespread necrosis of cardiac muscle fibres followed by the invasion of damaged tissues by the inflammatory cells (Rajadurai and Prince, 2007). Oral pretreatment with β-sitosterol for a period of 21 d significantly decreased the heart weight in ISO-induced rats.

Cardiac Troponins T and I are the cardiac specific proteins and highly sensitive, precise and released when myocardial cell damage occurs. Hence, Troponins are the ideal biomarkers for MI, which are present at high concentration in the myocardium, whereas absent in non-cardiac tissues. The release of cardiac troponin has been associated with the severity of infarction (Bertinchant et al., 2000). In this study, we have observed elevated levels of cTnT and cTnl in ISO-induced rats. This could be due to action of quinine, which produces free radicals. It is well accepted that when ISO undergo biotransformation, it produces quinine as metabolite, which is involved is cardiac damage. Pretreatment with β-sitosterol for a period of 21 d significantly decreased these troponins levels in ISO-induced rats. The decline in the troponin levels indicates the reduction in cardiac injury. Myocardium contains plentiful concentrations of diagnostic markers of MI and once metabolically damaged, it releases its contents into the extracellular fluid (Upaganlawar et al., 2009). Enzymes, the macromolecules that leak from the damaged tissue, because of their tissue specificity and catalytic activity, are the best markers of tissue damage. Hence, in ISO-induced myocardial infarcted rats, there was a decrease in activities of the marker enzymes CK, LDH, AST and ALT in the heart homogenate, followed by an increase in their levels in serum (Panda and Naik, 2008). These findings confirm the onset of myocardial necrosis and leaking out of the marker enzymes from heart to blood (Farvin et al., 2004). The amount of marker enzymes is directly proportional to ISO-induced necrotic lesions present in the myocardium. In the present study, β-sitosterol pretreatment to MI-induced rats reduce the cardiac damage and restrict the leakage of enzymes as evident from a significant reduction in the activities of cardiac marker enzymes in serum. An increased level of plasma uric acid, which is one of the markers of risk for CVD, has been observed in ISO-induced MI.

**Table 3. Effect of β-sitosterol on the levels uric acid, serum iron, plasma total proteins and albumin/globulin (A/G) ratio in normal and ISO-induced MI in rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Uric acid (mg/dL)</th>
<th>Serum iron (µg/dL)</th>
<th>Plasma total protein (g/dL)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>2.12 ± 0.15a</td>
<td>38.7 ± 2.6a</td>
<td>6.62 ± 0.31a</td>
<td>1.40 ± 0.06a</td>
</tr>
<tr>
<td>Normal + β-sitosterol (40 mg/kg)</td>
<td>2.11 ± 0.12b</td>
<td>38.2 ± 2.4a</td>
<td>6.58 ± 0.28a</td>
<td>1.42 ± 0.07a</td>
</tr>
<tr>
<td>ISO control</td>
<td>4.75 ± 0.27c</td>
<td>84.8 ± 5.2a</td>
<td>5.20 ± 0.19d</td>
<td>0.71 ± 0.04d</td>
</tr>
<tr>
<td>β-sitosterol (10 mg/kg) + ISO</td>
<td>3.30 ± 0.21c</td>
<td>63.4 ± 4.2c</td>
<td>5.65 ± 0.23c</td>
<td>0.99 ± 0.07c</td>
</tr>
<tr>
<td>β-sitosterol (20 mg/kg) + ISO</td>
<td>3.13 ± 0.20c</td>
<td>61.4 ± 3.8c</td>
<td>5.72 ± 0.21c</td>
<td>1.02 ± 0.09c</td>
</tr>
<tr>
<td>β-sitosterol (40 mg/kg) + ISO</td>
<td>2.53 ± 0.18d</td>
<td>50.2 ± 3.5c</td>
<td>6.13 ± 0.36d</td>
<td>1.21 ± 0.06d</td>
</tr>
</tbody>
</table>

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).
During ischemia, there is build-up of purine metabolites from ATP and ADP, which provide the required substrate, hypoxanthine and the electron acceptor, oxygen leading to the formation of uric acid and more importantly to the generation of \( O_2^{-} \) and hydrogen peroxide (H\(_2\)O\(_2\)) (Ashraf and Samra, 1993). Pre-treatment with \( \beta \)-sitosterol reduced the plasma uric acid levels in ISO-induced rats. Thus, \( \beta \)-sitosterol prevented the increase in the levels of uric acid, thereby decreasing the generation of \( O_2^{-} \) and H\(_2\)O\(_2\) in ISO-induced rats by their free radical scavenging effects. Decreased levels of serum iron were observed in ISO-induced rats. During ischemia, free iron is released from heme-dependent proteins like hemoglobin and myoglobin thus, increases prostaglandin metabolism and \textit{in vivo} lipid peroxidation (Halliwell and Gutteridge, 1989). Administration of \( \beta \)-sitosterol to ISO-induced rats decreased the levels of serum iron. This effect might be due to the free radical scavenging and antioxidant property of \( \beta \)-sitosterol. A decrease in the levels of plasma total proteins and A/G ratio observed by us in ISO-induced rats could be due to increased free radical production by ISO. Pretreatment with \( \beta \)-sitosterol significantly increased the levels of plasma total proteins and A/G ratio, this, could be due to the ability of \( \beta \)-sitosterol to scavenge free radicals and to inhibit lipid peroxidation. We observed increased levels of blood glucose in ISO-control rats. ISO injection is associated with pronounced metabolic abnormalities in blood glucose levels. The observed increased glucose in ISO-induced rats might be due to the enhanced glyconeogenesis and less utilization by the peripheral tissues in MI in rats. Pretreatment with \( \beta \)-sitosterol significantly decreased the levels of blood glucose and uric acid in ISO-induced rats.

**Conclusion**

In conclusion, our study clearly demonstrates that \( \beta \)-sitosterol administration to ISO-induced rats for 21 d possess significant cardioprotection by decreasing the heart weight, minimizing the alterations in the activities of the marker enzymes and decreasing the levels of Troponins and reducing the changes in the biochemical parameters (glucose, serum iron, uric acid, protein and A/G ratio). These effects could be due to membrane protective action of \( \beta \)-sitosterol by scavenging the free radicals and its antioxidant action.

**References**


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