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RESEARCH ARTICLE

CARDIOPROTECTIVE EFFECT OF B-SITOSTEROL ON LIPID PEROXIDES AND ANTIOXIDANT IN ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION IN RATS: A HISTOPATHOLOGICAL STUDY

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ABSTRACT

This study aimed to evaluate the cardioprotective effect of β-sitosterol on lipid peroxides, enzymatic and non-enzymatic antioxidants and histopathological findings in isoproterenol-induced myocardial infarction in rats. Subcutaneous injection of isoproterenol (85 mg/kg) at an interval of 24 h for 2 days to male Wistar rats showed a significant increase in the levels of thiobarbituric acid reactive substances and hydroperoxide, whereas the activities of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and non-enzymatic antioxidants like reduced glutathione, vitamin E & C were significantly decreased. Oral pretreatment with β-sitosterol (10, 20 and 40 mg/kg, respectively) to isoproterenol-induced rats daily for a period of 21 days showed a significant reduction in the levels of lipid peroxidative products and improved the antioxidant status. This could be due to free radical scavenging and antioxidant property of β-sitosterol. Thus, our finding demonstrates that β-sitosterol prevents the alterations in lipid peroxidation and antioxidants status during isoproterenol-induced myocardial infarction in rats. Histopathological alterations due to isoproterenol administration markedly reduced by β-sitosterol treatment. Thus, our study shows that β-sitosterol possess cardioprotective effect in experimentally induced myocardial infarction.

INTRODUCTION

Isoproterenol (ISO), a synthetic catecholamine and β-adrenergic agonist, is reported to induce myocardial infarction (MI) as a result disturbance in physiological balance between production of free radicals and antioxidative defense system (Rathore *et al*., 1998; Srivastava *et al*., 2007. ISO, upon oxidation leads to increases lipid peroxidation through enhanced free radical formation and causes severe stress in the cardiac tissue resulting in infarct like necrosis (Chattopadhyay *et al*., 2003; Rajadurai and Stanely Mainzen Prince 2007). Experimental induction of MI by ISO in animal is a well established model to evaluate the protective role of various cardioprotective agents. MI is an acute state of necrosis of the myocardium that occurs as a result of critical imbalance between coronary blood supply and myocardial demand (Gilski 2005). MI affects a high proportion of the population throughout the world. MI continues as a major public health problem, in developing countries and makes significant contribution to the mortality statistics (Wang *et al*., 2009).

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β-sitosterol is a naturally occurring plant sterol, one of the most prevalent vegetable-derived phytosterols, which is found in numerous plants including rice, wheat, corn, nut, peanut, etc. It is structurally related to cholesterol (Fig. 1) (Plat and Mensink 2005). Plant sterols (phytosterols) have attracted much attention because of their capability to reduce cholesterol absorption in the intestine and, hence, to protect against cardiovascular diseases (CVD) (Pegel 1997). The major natural sources of phytosterols and phytostanols in the human diet are plant oils, such as corn, cereal grains, grape seed, and soybean, sunflower or olive oil. β-sitosterol has been reported to exhibit a multitude of bioactivities, which include, antioxidant (Yoshida and Niki 2003), anticancer, anti-inflammatory (Backhouse *et al*., 2008), angiogenic (Choi *et al*., 2002), chemopreventive (Imanaka *et al*., 2002) and immunomodulatory activities (Bouic 2001). β-sitosterol is reported to poisers hypercholesterolemia effect (Malini and Vanithakumari 1990). Moreover, β-sitosterol also has antiseptic, antineoplastic and antipyretic effects. The aim of the present study is to evaluate the cardioprotective effect of β-sitosterol on lipid peroxides, antioxidants and histopathological findings in normal and ISO-induced MI in male albino Wistar rats.

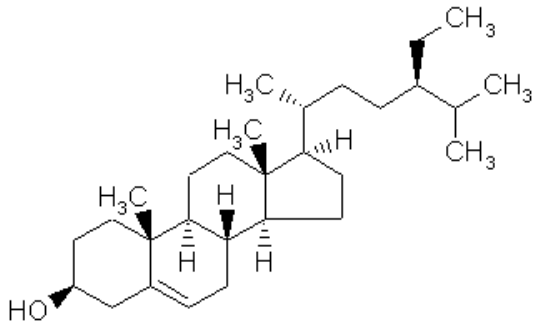

Fig. 1. Structure of β-sitosterol

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 at around 22ºC 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 & Science, Rasipuram. (MCAS/Ph.D.,/01/2012-2013).

Drugs and chemicals

Isoproterenol hydrochloride, β-sitosterol, reduced nicotinamide adenine dinucleotide (NADH), and phenazine methosulphate (PMS) were purchased from Sigma Chemical Company, St. Louis, MO, USA. Thiobarbituric acid (TBA), 1, 1' ,3 , 3' tetramethoxy propane, butylated hydroxy toluene (BHT), xylenol orange, dithionitro bis benzoic acid (DTNB), ascorbic acid, 2, 2' dipyridyl, p-phenylene diamine and sodium azide were obtained from Himedia laboratory, Mumbai, India. All other chemicals used in the 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 two days (Rajadurai and Stanely Mainzen Prince 2006).

Experimental design

A total 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 days.

Group1: Normal control rats
Group2: Normal rats + β-sitosterol (40 mg/kg)
Group 3: ISO control rats (85 mg/kg)
Group 4: ISO + β-sitosterol (10 mg/kg)
Group5: ISO + β-sitosterol (20 mg/kg)
Group6: 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. Blood was collected and plasma separated by centrifugation. Heart tissue was excised immediately and rinsed in ice-chilled normal physiological saline. A known weight of the heart tissue was homogenized in appropriate solution. The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

Biochemical assays

Plasma thiobarbituric acid reactive substances (TBARS) were estimated by the method of Yagi (1987). TBARS were quantitated by their reactivity with thiobarbituric acid (TBA) in acidic conditions to generate a pink coloured chromophore, which was read at 530 nm. TBARS in the heart was estimated by the method of Fraga *et al*. (1988). In this method, malondialdehyde and other TBARS were measured by their reactivity with TBA in acidic conditions to generate a pink coloured chromophore, which was read at 535 nm. Estimation of plasma and cardiac tissue lipid hydroperoxides (HP) was done by the method of Jiang *et al*. (1992). In this method, oxidation of ferrous ion (Fe^{2+}) under acidic conditions in the presence of xylenol orange led to the formation of a chromophore, which was read at 560 nm. Superoxide dismutase (SOD) activity in the myocardium was assayed by the method of Kakkar *et al*. (1984).

Superoxide radicals react with nitroblue tetrazolium in the presence of reduced nicotinamide adenine dinucleotide and produce formazon blue. SOD removes the superoxide radicals and inhibits the formation of formazon blue. The intensity of the colour is inversely proportional to the activity of the enzyme and read at 560 nm. The activity of catalase in myocardium was assayed by the method of Sinha (1972). In this method, dichromate in acetic acid is converted to perchromic acid and then to chromic acetate when heated in the presence of hydrogen peroxide. The chromic acetate formed was measured at 620 nm. Estimation of GSH in plasma and the heart tissue was done by the method of Ellman (1959). This method is based on the development of yellow colour, when dithionitro benzoic acid is added to compounds containing sulfhydryl groups. The colour developed was read at 412 nm. GPx activity was assayed by the method of Rotruck *et al* (1973). A known amount of enzyme preparation was allowed to react with hydrogen peroxide and GSH for a specified time period. The GSH content remaining after the reaction was measured by Ellman's reaction. The activity of GST was assayed in the cardiac tissue following the increase in the absorbance at 340 nm using 1-chloro-2,4- dinitro benzene as

substrate by the method of Habig and Jakoby (1981). Vitamin C in plasma and the heart tissue was estimated by the method of Omaye *et al*. (1979). The ascorbic acid is converted into dehydro ascorbic acid in the presence of thiourea, a mild reducing agent and then coupled with 2,4-dinitrophenyl hydrazine (DNPH). The coupled DNPH is converted into a red coloured complex when treated with sulphuric acid, which was read at 530 nm. The levels of Vitamin E in plasma and the concentration in cardiac tissue were estimated by the method of Baker *et al*. (1980). This method involves the reduction of ferric ion to ferrous ion by α-tocopherol and the formation of red coloured complex with 2, 2'-dipyridyl. The absorbance of the chromophore was measured at 520 nm.

Histopathological examination

After the sacrifice of the normal and experimental rats, the heart was rapidly dissected and washed immediately with saline and then fixed in 10% buffered neutral formalin solution. After fixation, the heart tissue was processed by embedding it in paraffin. Then, the heart tissue was sectioned and stained with hematoxylin and eosin (H&E). The sections were examined under the light microscope for histopathological changes and photomicrographs were taken.

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

RESULTS

Table 1 shows the levels of TBARS and HP in plasma and the heart of normal and ISO-induced rats. Rats injected with ISO, showed a significant (P<0.05) increase in the levels of TBARS and HP in plasma and the heart.

Oral pretreatment with β-sitosterol (10, 20 and 40 mg/kg) to ISO-induced rats for a period of 21 days significantly (P<0.05) decreased the levels of TBARS and HP in plasma and the heart when compared with ISO-alone induced rats. The activities of SOD and catalase in the heart of normal and ISO-induced rats are shown in Table 2. Rats induced with ISO, exhibited a significant (P<0.05) decrease in the activities of these antioxidant enzymes, when compared with normal control rats. Pretreatment with β-sitosterol to ISO-induced rats significantly (P<0.05) increased the activities of these antioxidant enzymes.

Table 3 presents the protective role of β-sitosterol on the activities of myocardial GPx and GST in heart and the levels of GSH in plasma and the heart in normal and ISO-induced rats. ISO-induced rats showed a significant (P< 0.05) decrease in the activities of these antioxidant enzymes and the levels of GSH on comparison with normal control rats. Oral administration of β-sitosterol to ISO-induced rats significantly (P< 0.05) increased the activities/level of these antioxidants when compared with ISO-alone induced rats.

Table 4 exhibits the effect of β-sitosterol on the levels of plasma and heart vitamin C and E in normal and ISO-induced rats. Rats induced with ISO, exhibited a significant (P< 0.05) decrease in the levels of vitamin C and E in plasma and heart. Oral administration of β-sitosterol to ISO-induced rats significantly (P< 0.05) increased the levels of vitamin C and E in plasma and the heart.

Table 5 shows the protective effect of β-sitosterol on the degree of histopathological changes of myocardial tissues in normal and ISO-induced rats. ISO-alone induced myocardium (Fig. 2.c) showed separation of cardiac muscle fibres, with oedema and accumulation of inflammatory cells in the infracted zone. Pretreatment with β-sitosterol at a dose of 10 mg/kg (Fig. 2.d) showed mild infarction with coagulative necrosis, mild oedema and accumulation of inflammatory cells in the area of separated muscle fibres.

Table 1. Effect of β-sitosterol on the levels of TBARS and HP in plasma and the heart in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats

Groups	Plasma TBARS (nM/ml)	Plasma HP (values × 10^{-5} mM/dl)	Tissue TBARS (mM/100g wet tissue)	Tissue HP (mM/100g wet tissue)
Normal control	2.83 ± 0.12^{a}	10.09 ± 0.52^{a}	0.83 ± 0.05^{a}	20.7 ± 1.45^{a}
Normal + β-sitosterol (40 mg/kg)	2.79 ± 0.18^{a}	10.15 ± 0.66^{a}	0.81 ± 0.04^{a}	20.4 ± 1.26^{a}
ISO (85 mg/kg)	6.53 ± 0.28^{b}	19.14 ± 1.05^{b}	1.54 ± 0.07^{b}	43.6 ± 3.12^{b}
β-sitosterol (10 mg/kg) + ISO	4.37 ± 0.19^{c}	14.86 ± 0.81^{c}	1.24 ± 0.05^{c}	30.2 ± 1.87^{c}
β-sitosterol (20 mg/kg) + ISO	4.26 ± 0.19^{c}	14.21 ± 0.72^{c}	1.19 ± 0.07^{c}	29.7 ± 2.06^{c}
β-sitosterol (40 mg/kg) + ISO	3.39 ± 0.21^{d}	12.10 ± 0.57^{d}	0.96 ± 0.06^{d}	24.5 ± 1.54^{d}

Each value is mean±S.D. for six rats in each group.
Values not sharing a common superscripts (a, b, c and d) differ significantly at P < 0.05 (DMRT).

Table 2. Effect of β-sitosterol on the activities of SOD and catalase in the heart of normal and ISO-induced MI in rats

Groups	SOD (U/mg protein)	Catalase (μmoles of H_2O_2 consumed/min/mg protein)
Normal control	11.42 ± 0.56^{a}	8.24 ± 0.36^{a}
Normal + β-sitosterol (40 mg/kg)	11.55 ± 0.48^{a}	8.30 ± 0.35^{a}
ISO (85 mg/kg)	6.14 ± 0.30^{b}	3.59 ± 0.18^{b}
β-sitosterol (10 mg/kg) + ISO	7.89 ± 0.25^{c}	5.62 ± 0.13^{c}
β-sitosterol (20 mg/kg) + ISO	8.12 ± 0.26^{c}	5.81 ± 0.17^{c}
β-sitosterol (40 mg/kg) + ISO	10.16 ± 0.60^{d}	7.74 ± 0.22^{d}

Each value is mean±S.D. for six rats in each group.
Values not sharing a common superscripts (a, b, c and d) differ significantly at P < 0.05 (DMRT).

Table 3. Effect of β-sitosterol on the activities of myocardial GPx, GST and the levels of reduced GSH in plasma and the heart in normal and ISO-induced MI in rats

Groups	Heart GPx (µg of GSH consumed/min/mg protein)	Heart GST (nmoles of CDNB conjugated/min/mg protein)	Plasma GSH (mg/dL)	Heart GSH (mM/g wet tissue)
Normal control	5.12 ± 0.27[a]	750.2 ± 27.2[a]	20.87 ± 1.83[a]	9.23 ± 0.48[a]
Normal + β-sitosterol (40 mg/kg)	5.08 ± 0.21[a]	764.4 ± 30.9[a]	21.20 ± 1.56[a]	9.41 ± 0.37[a]
ISO (85 mg/kg)	1.89 ± 0.18[b]	460.7 ± 21.6[b]	11.80 ± 0.77[b]	4.76 ± 0.28[b]
β-sitosterol (10 mg/kg) + ISO	3.28 ± 0.13[c]	535.2 ± 28.5[c]	14.92 ± 0.96[c]	6.03 ± 0.25[c]
β-sitosterol (20 mg/kg) + ISO	3.40 ± 0.17[c]	551.0 ± 40.1[c]	15.07 ± 1.10[c]	6.14 ± 0.17[c]
β-sitosterol (40 mg/kg) + ISO	4.61 ± 0.22[d]	683.9 ± 32.4[d]	18.44 ± 1.05[d]	8.56 ± 0.50[d]

Each value is mean±S.D. for six rats in each group.
Values not sharing a common superscripts (a, b, c and d) differ significantly at P < 0.05 (DMRT).

Table 4. Effect of β-sitosterol on the levels of vitamin C and E in plasma and the heart and ceruloplasmin in plasma in normal and ISO-induced MI in rats

Groups	Plasma vitamin C (mg/dL)	Plasma vitamin E (mg/dL)	Heart vitamin C (µmol/mg protein)	Heart vitamin E (µmol/mg protein)
Normal control	1.83 ± 0.10[a]	1.76 ± 0.11[a]	1.05 ± 0.06[a]	0.84 ± 0.05[a]
Normal + β-sitosterol (40 mg/kg)	1.91 ± 0.12[a]	1.79 ± 0.12[a]	1.07 ± 0.04[a]	0.87 ± 0.06[a]
ISO (85 mg/kg)	0.89 ± 0.06[b]	0.92 ± 0.06[b]	0.46 ± 0.03[b]	0.38 ± 0.06[b]
β-sitosterol (10 mg/kg) + ISO	1.16 ± 0.03[c]	1.12 ± 0.05[c]	0.57 ± 0.04[c]	0.50 ± 0.03[c]
β-sitosterol (20 mg/kg) + ISO	1.45 ± 0.04[c]	1.41 ± 0.04[c]	0.72 ± 0.06[c]	0.62 ± 0.03[c]
β-sitosterol (40 mg/kg) + ISO	1.62 ± 0.07[d]	1.59 ± 0.09[d]	0.89 ± 0.05[d]	0.71 ± 0.04[d]

Each value is mean±S.D. for six rats in each group.
Values not sharing a common superscripts (a, b, c and d) differ significantly at P < 0.05 (DMRT).

Table 5. Effect of β-sitosterol on the degree of histopathological changes in myocardium in normal and ISO-induced MI in rats

Groups	Necrosis	Oedema	Inflammatory cells
Normal control	A	A	A
Normal + β-sitosterol (40 mg/kg)	A	A	A
ISO (85 mg/kg)	+++	++	+++
β-sitosterol (10 mg/kg) + ISO	++	+	++
β-sitosterol (20 mg/kg) + ISO	+	+	+
β-sitosterol (40 mg/kg) + ISO	A	+	A

Photomicrographs were used to evaluate the damage in the heart tissues: (A) no changes: (+) mild changes: (++) moderate changes: (+++) marked changes.

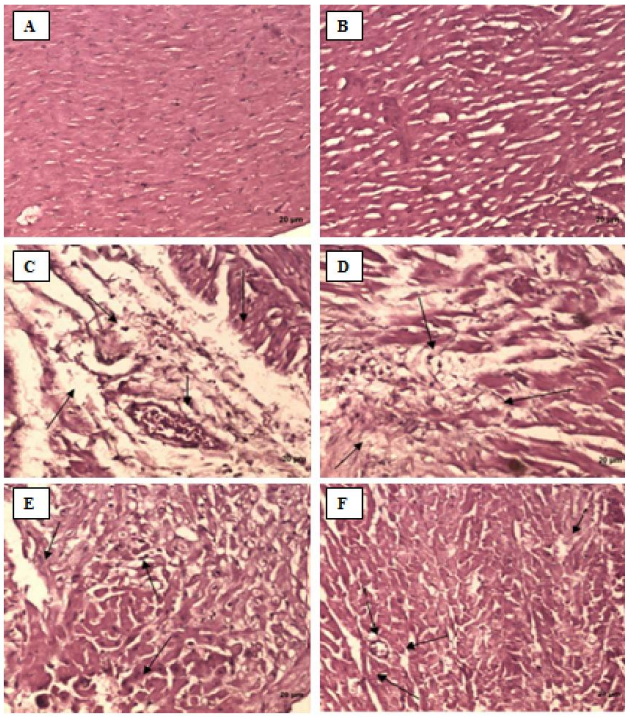

Fig. 2. Histopathological examination of the heart in normal and ISO-induced MI in rats. All the sections are in H&E 20X. (A) Normal untreated group showing normal cardiac fibers without any infarction oedema and inflammatory. (B) Normal + β-sitosterol (40 mg/kg) treated heart tissue showing normal cardiac fibers without any infarction, oedema and inflammatory cells. (C) ISO-control rats showing separation of cardiac muscle fibers, with oedema and accumulation of inflammatory cells in the infracted zone. (D) ISO + β-sitosterol (10 mg/kg) treated rats showing mild infarction with coagulative necrosis, mild oedema and accumulation of inflammatory cells in the area of separated muscle fibers. (E) ISO + β-sitosterol (20 mg/ kg) treated rats showing mild oedema and a few inflammatory cells with a trace amount of necrosis. (F) ISO + β-sitosterol (40 mg/ kg) treated rats showing mild oedema without necrosis and accumulation of inflammatory cells.

Pretreatment with β-sitosterol at a dose of 20 mg/kg (Fig.2.e) showed mild oedema and a few inflammatory cells with a trace amount of necrosis. Pretreatment with β-sitosterol at a dose of 40 mg/kg (Fig. 2.f) showed mild oedema without necrosis and accumulation of inflammatory cells. β-sitosterol (40 mg/kg) administration to normal rats did not have any histopathological changes in the myocardium (Fig. 2.b). Fig. 2.a shows the normal architecture of the rat myocardium. For all the parameters studied, oral pretreatment with β-sitosterol (40 mg/kg) to normal rats for a period of 21 days showed a minor effect, but it was not statistically significant ($P<0.05$). β-sitosterol at a dose of 40 mg/kg showed a better effect than the other two doses (10 and 20 mg/kg) in ISO-induced MI in rats.

DISCUSSION

ISO-administration to rats causes several physiological, functional, biochemical and pathological changes, resembling the symptoms of human MI (Padmanabhan and Prince 2006; Loh *et al*., 2007; Zhou *et al*., 2008). Autooxidation of catecholamines via β-receptor on the cardiac membrane leads to the formation of its metabolite quinine. Qunine reported to produce free radicals, which damages the myocardial membrane. Myocardial injury related symptoms include cardiac dysfunction, elevated lipid peroxidation, failure of antioxidant mechanisms and cellular necrosis in heart (Yates *et al*., 1981; Karthick and Stanely Mainzen Prince 2006; Thompson and Hess 1986). Lipid peroxidation, a type of oxidative deterioration of polyunsaturated fatty acids (PUFA) has linked with altered membrane structure and enzyme inactivation. A significant increase in the levels of lipid peroxidation products such as TBARS and hydroperoxides in plasma and the heart clearly indicates that increased oxidative stress in ISO-induced rats. Alterations in the metabolism of lipid peroxides are closely associated with myocardial damage due to free radicals produced by ISO (Sroka and Cisowski 2003). Oral pretreatment with β-sitosterol (10, 20 and 40 mg/kg), for a period of 21 days significantly decreased the levels of TBARS and HP in ISO-induced rats. The free radical inhibitory action of β-sitosterol is attributed to its antioxidant property, which effectively scavenges the ROS and decreases lipid peroxidation products (Comporti 1987).

Superoxide radicals plays an important role in the formation of ROS such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which induce oxidative damage in lipids, proteins and deoxyribonucleic acid (Guarnieri *et al*., 1980). Hydroxyl radical is responsible for lipid peroxidation, which impairs the normal function of cell membranes, motility and permeability. A significant decrease in the activities of antioxidative enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX) were observed in ISO-treated rats. It is also reported that ISO-induced cardiotoxicity is partly due to the formation of oxygen free radicals (OFR) and sulfhydril reactivity through a variety of oxidation products and decrease in SOD activity may result in the decreased removal of superoxide anions, which can cause myocardial damage (Meister 1988). Therefore, the observed decrease in the activities of SOD and CAT could be due to the increased generation of ROS, such as superoxide and H_2O_2, which in turn led to the inhibition of these enzymes activities. Rats pretreated with β-sitosterol showed increased activities of these enzymes, which suggest that β-sitosterol may have the ability to prevent the deleterious effects induced by free radicals in ISO-induced rats. Glutathione (GSH), a tripeptide, which is used as an electron donor for the enzyme GPx during the process of reduction of peroxides and it can also react directly with oxygen radical as a free radical scavenger (Dickinson and Forman 2002). It is also reported that in ISO-induced rats, GSH has a direct antioxidant function by reacting with superoxide radicals, peroxy radicals and singlet oxygen followed by the formation of oxidized GSH and other disulfides. It forms an important substrate for GPx, GST and several other enzymes, which are involved in free radical scavenging action. Decreased GSH levels in ISO-induced rats may be due to its enhanced utilization for augmenting the activities of GPx and GST (Lil *et al*., 1988). GSH levels depleted by ISO was significantly restored due to the administration of β-sitosterol.

Vitamin E is the major lipid-soluble antioxidant present in cell membranes and lipoproteins that protects against oxidative modification of lipids. Vitamin C can directly scavenge singlet oxygen, superoxide and hydroxyl radicals. It reduces the risk of CVD by reducing blood pressure, blood cholesterol and the formation of oxidized LDL cholesterol (Ondrejickova *et al* 1991). In our study, decreased concentration of vitamin C and E in plasma and heart were observed in ISO-induced rats. Pretreatment with β-sitosterol to ISO-induced rats significantly increased the levels of vitamin C and E in both plasma and heart. Kuiper *et al*. (1998), have stated that the estrogenic potency of several phytoestrogens, showed, these compounds bind both estrogen receptor subtypes, as described for β-sitosterol (Gutendorf and Westendorf 2001). Wang *et al*. (2003) also suggested that estrogens exert stong antioxidant activities. The effects of β-sitosterol on the enzymes involved in antioxidant action is seems to be mediated by estrogen receptor activation. The chemical structure of β-sitosterol, resembles that of cholesterol except for the presence of an ethyl group at the 24 carbon position of the side chain (Ling and Jones 1995). It's well known that β-sitosterol influences cholesterol absorption due to its structural similarity, which may be one of the reasons for the decreased levels of lipid peroxidative products. The histopathological findings of the ISO-induced myocardium showed separation of cardiac muscle fibres, with oedema and accumulation of inflammatory cells and infracted zone. On treatment with β-sitosterol (20 and 10 mg/kg) the pathological changes viz, separation of cardiac muscle fibres, oedema and accumulation of inflammatory cells are drastically diminished. There were mild oedema without necrosis and accumulation of inflammatory cells at the dose of β-sitosterol (40 mg/kg) pretreated rat myocardium. This indicates that administration β-sitosterol to ISO-induced rats posses cardioprotection by reducing the cardiac tissue damage, which is due to indirect action of reducing the event of lipid peroxidation and elevating the levels of antioxidants on the cardiac tissue in ISO-induced rats.

Conclusion

In our experiment, we found that β-sitosterol protected myocardium from ISO-induced myocardial functional and structural injury via favourably improved biochemical and histopathological and suggesting its cardioprotective action. In

conclusion, the present study provided experimental evidence that β-sitosterol maintained the antioxidant enzyme levels and improved cardiac performance in ISO-induced rats. This finding might be rational to understand the beneficial effects of β-sitosterol on cardio protection against myocardial injury, in which oxidative stress was long known to contribute to the pathogenesis, and interpretation.

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