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Cardioprotective potential of *Ocimum sanctum* in isoproterenol induced myocardial infarction in rats

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Abstract

Myocardial infarction (MI) was produced in rats with 85, 200 and 300 mg/kg of isoproterenol (ISO) administered subcutaneously (sc) twice at an interval of 24 h. Shift in antioxidant parameters, lactate dehydrogenase (LDH) together with morphological and histopathological changes were investigated. Two hundred mg/kg ISO dose was selected for the present study as this dose offered significant alteration in biochemical parameters along with moderate necrosis in heart. Effect of pre- and co-treatment of hydroalcoholic extract of *Ocimum sanctum* (Os) at different doses (25, 50, 75, 100, 200 and 400 mg/kg) was investigated against ISO (200 mg/kg) induced myocardial infarction in rats. Modulation of various biochemical parameters and membrane integrity was studied. Os at the dose of 25, 50, 75 and 100 mg/kg reduced significantly glutathione (GSH), superoxide dismutase (SOD) and LDH levels. It also inhibited the lipid peroxidation as observed by the reduced thiobarbituric acid reactive substances (TBARS) levels. In the present study Os at the dose of 50 mg/kg was found to demonstrate maximum cardioprotective effect. Above results were further confirmed by histopathological findings. Thus from the present study it is concluded that Os may be of therapeutic and prophylactic value in the treatment of MI. (Mol Cell Biochem **225**: 75–83, 2001)

Key words: isoproterenol, myocardial infarction, *Ocimum sanctum*, antioxidant enzymes

Introduction

Free radical reactions have been implicated in the pathology of many human diseases including atherosclerosis, ischemic heart disease, the aging process, inflammation, diabetes, immunodepression, the neurodegenerative diseases and other disease states [1]. Radicals and other reactive oxygen species are formed constantly in the human body and are removed by the enzymic and non-enzymic antioxidant defense system. Oxidative stress occurring when antioxidant defenses are inadequate can damage lipids, proteins, carbohydrates and DNA [2]. It is now well recognized that isoproterenol (ISO) a synthetic catecholamine in large doses produces myocardial infarction [3]. Amongst various mechanisms proposed to explain ISO induced cardiac damage, generation of highly cytotoxic free radicals through autooxidation of catecholamines has been implicated as one of the important causative factor. Free radical mediated peroxidation of membrane phospholipids and consequent changes in membrane permeability appears to be the primary target responsible for cardiotoxicity induced by ISO [4]. Proven cardioprotective and membrane stabilizing activity of few antioxidants in animal models of myocardial necrosis, further strengthen this theory [5]. Recently, several plants of Indian origin have been found to possess antioxidant properties and their beneficial effects in pathological conditions like atherosclerosis, ischemia, cancer, cataract, and liver dysfunction have been related to their antioxidant properties. Drugs with multiple mechanisms of protective action, including antioxidant properties, may be one step forward in human disease.

Despite the fact that *Ocimum sanctum* (Os) is an important medicinal plant having antioxidant properties, its cardioprotective activity against ISO induced myocardial infarction has not been studied. Os is known to have hypoglycemic and

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hypolipidemic [6, 7], adaptogenic [8], immunomodulatory [9], antioxidant [10], radioprotective [11] and anti-inflammatory properties [12].

In view of this, the present study was designed to investigate if oral administration of hydroalcoholic Os leaf extract has any protective action against ISO induced myocardial injury. Histopathological and biochemical changes induced by ISO have been monitored and their modulation with different doses of Os was evaluated.

Materials and methods

Isoproterenol HCl, dihydro-diphosphopyridine nucleotide (DPNH), phenazine methosulphate (PMS), nitroblue tetrazolium (NBT), 5,5-dithiobis (2-nitro benzoic acid) (DTNB), bovine serum albumin (BSA), 1,1,3,3-tetra methoxy propane, reduced glutathione (GSH), lactate dehydrogenase (LDH), superoxide dismutase (SOD) and triphenyl tetrazolium chloride (TTC) were purchased from Sigma Chemical Co., USA. Dinitrophenylhydrazine solution (DNPH), sodium pyrophosphate (SPP), sodium dodecyl sulphate (SDS), thiobarbituric acid (TBA), and trichloroacetic acid (TCA) were obtained from Sisco Research Laboratories, Bombay, India. All other chemicals were of analytical grade. Hydroalcoholic leaf extract of Os was a generous gift from Dabur Pharmaceuticals, India.

Hydroalcoholic leaf extract of Os was prepared by powdering approved dried leaves. Hydroalcoholic solution in the ratio of 20:80 (water:alcohol) prepared separately. A weighed quantity of the coarsely powdered leaves was then mixed with 8 times of the hydroalcoholic solution and kept under agitation for 4 h with mild heating (about 40–45°C). The solution then filtered to keep the filtrate. The residue again was extracted with 6 times of hydroalcoholic solution for about 4 h. This was filtered and both filtrates are to be mixed together. This extract was then concentrated and spray dried to obtain the solid extract, which complied with the specifications.

Wistar Albino rats of either sex, weighing 150–200 g, were used in the study, which was conducted according to protocols approved by the All India Institute of Medical Sciences Committee on Animal Care and Use. Rats were obtained from the Central Animal House facility of Hamdard University, New Delhi, India. They were kept in standard laboratory conditions under natural light and dark cycle. The rats were fed normal diet (Gulmohar feed, Delhi) and water *ad libitum*.

ISO dose determination

A pilot study was carried out by inducing myocardial infarction in three different groups of rats (n = 8) by administering 85, 200 and 300 mg/kg body wt ISO subcutaneously (sc) twice at an interval of 24 h. Myocardial infarction and shifts in biochemical parameters were determined as per standard protocols.

Treatment protocol

The animals were divided into three main groups. The first group consisted of control rats (saline treated); the second group was administered ISO and third group was administered Os.

The third group was administered in a single dose hydroalcoholic leaf extract of Os on the body wt base (mg/kg). This group was further sub-grouped into six groups according to the dose of Os administered (25–400 mg). Group 1 – saline treated control; group 2 – ISO treated control; group 3 – Os treated groups; group 3a – 25 mg; group 3b – 50 mg; group 3c – 75 mg; group 3d – 100 mg; group 3e – 200 mg; group 3f – 400 mg.

ISO was administered subcutaneously (200 mg/kg) twice at an interval of 24 h. Os (lyophilized hydroalcoholic leaf extract) dissolved in distilled water was given to each animal orally for 12 days and ISO administered sc on 11th and 12th day. The animals were sacrificed 24 h after the second dose of ISO, under chloroform anesthesia. Hearts were removed and processed immediately for morphological and histopathological studies. For performing biochemical estimations hearts were immediately stored in liquid nitrogen till further analysis.

Determination of myocardial necrosis by direct staining

Using triphenyl tetrazolium chloride (TTC) dye described by Lie *et al*. [13].

Myocardium of rat was frozen immediately after removal. When the tissue was firm, the heart was sliced into 1 mm segments and incubated at 37°C for 20 min in 1% TTC. The formazan precipitate resulting from the reaction of lactate dehydrogenase in normal and ischemic regions delineated the area at risk from the infarcted tissue.

Biochemical estimations

Hearts removed from liquid nitrogen were weighed. Ten percent homogenate was prepared in 0.1 M Tris-buffer, pH 7.4 and used for all the assays.

Estimation of LDH

Lactate dehydrogenase was estimated by the method described by Cabaud and Wrobleuski [14]. The tissue homoge-

nate was centrifuged at 8,000 rpm for 15 min. Supernatant was collected and to 0.1 ml of the supernatant, 0.9 ml of double distilled water was added. To this 0.1 ml of 1% DPNH solution was added and placed at 37°C in a water bath along with a separate tube containing pyruvic acid-buffered substrate (0.02% in 0.05 M phosphate buffer) for 15 min. One ml of pre-incubated pyruvic acid-buffered substrate was then added to each tube containing the sample mixture. Exactly 30 min after adding the substrate, 1 ml of DNPH (0.02% in 1 N HCl) was added and were removed from water bath, mixed by swirling, and tubes were allowed to stand for 20 min at room temperature. Ten ml of 0.4 N NaOH was then added and vortexed. Percent transmittance was read at 440 nm on a Beckmen's spectrophotometer within 10 min. At the same time a standard graph with different amount of LDH was obtained for determining the amount of LDH in the samples. One unit of LDH has been defined as the amount of protein that will reduce 1.0 µmole of pyruvate to L-lactate per min at pH 7.5 at 37°C.

Estimation of GSH

Glutathione was estimated by the method described by Ellman *et al*. [15]. Protein free supernatant was obtained by addition of equal volume of 10% TCA to the tissue homogenate and centrifuged at 5000 rpm for 10 min. To 0.5 ml of the supernatant, 2 ml of K_2HPO_4 buffer at pH 8.4, 0.5 ml of DTNB prepared in 1% tri-sodium citrate was added and vortexed. The absorbance of the resulting yellow colour was recorded within 10 min at 412 nm on a Beckmen's spectrophotometer. Parallel blank and standards were run for determining the amount of GSH in the samples.

Estimation of SOD

Superoxide dismutase was estimated by the method described by McCord and Fridovich [16] with slight modifications. The tissue homogenate was centrifuged at 10,000 rpm for 15 min at 4°C under cold conditions to obtain cytosol fraction. The supernatant was collected and to 50 µl of sample, 950 µl of double distilled water was added. Assay mixture contained 1.2 ml of sodium pyrophosphate buffer (0.052 M, pH 8.3), 0.1 ml 186 µM PMS, 0.3 ml 300 µM NBT, 0.2 ml DPNH (780 µM), appropriately dilute enzyme preparation and water in a total volume of 3 ml. Reaction was started by addition of DPNH at 30°C and stopped after 90 sec by the addition of 1 ml glacial acetic acid. The chromogen formed was extracted by 4 ml of n-butanol. The mixture was allowed to stand for 10 min, centrifuged and butanol layer was taken out. Colour intensity of the chromogen in the butanol layer was measured at 560 nm in Beckmen's spectrophotometer against a butanol blank. Parallel blank and standards were run for determining the amount of GSH in the samples. One unit of enzyme activity has been defined as enzyme concentration required to inhibit the optical density at 560 nm of chromogen production by 50% in 1 min under the assay conditions and expressed as specific activity in milliunits/mg protein.

Estimation of TBARS

Thiobarbituric reactive substances were estimated by the method described by Okhawa *et al*. [17]. Briefly, to 0.2 ml of tissue homogenate, 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% TBA were added in succession. Volume was made up to 4 ml with double distilled water. The mixture was incubated for 60 min at 95°C in a temperature controlled water bath. After cooling, the pink colored complex was extracted with 5 ml of butanol:pyridine (15:1) mixture. Organic layer was separated and absorbance was observed at 532 nm in the Beckmen's spectrophotometer. Blank and standards were run simultaneously.

Estimation of protein

Protein estimation in the tissue sample was done by the method of Lowry [18]. To appropriately diluted samples, 1 ml of alkaline copper solution mixture (50 ml 2% Na_2CO_3 in 0.1 N NaOH + 1 ml 0.5% $CuSO_4.5H_2O$ in 1% sodium potassium tartarate) was added; tubes were vortexed and kept for 10 min. To the above solution 0.1 ml of Folin-ciocalteau phenol reagent (1:5 in 0.1 N HCl) was then added, vortexed and kept for 30 min. Finally absorbance was read at 740 nm. BSA was used as standard to calculate the protein content of the samples.

Histopathological studies

Myocardial tissue after removal was immediately fixed in 10% buffered neutral formalin solution. After fixation was complete, tissues were embedded in paraffin and serial sections were cut. Each section was stained with hematoxylin and eosin. The sections were examined under light microscope and photomicrographs were taken.

Statistical analysis

Descriptive statistics such as mean and standard deviation has been calculated for each and every variable for each group. One-way analysis of variance (ANOVA) has been applied for statistical analysis with post-hoc analysis (Bonferroni Mul-

tiple Range Test) and a value of $p < 0.05$ has been considered as statistical significance level.

Results

Detection of myocardial necrosis by direct staining using TTC in phosphate buffer saline showed that in all ISO groups there was a significant leakage of LDH as compared to saline control (Fig. 1). In the control group, viable myocardial tissue was stained brick red as evident by the formation of red formazan with LDH of the myocardial tissue. However, in all ISO administered groups scattered patches of necrotic tissue were clearly visible as the unstained regions (from where the LDH has leaked out of the myocardial membrane due to oxidative stress). Necrotic patches were present more in ISO 300 mg/kg administered group as compared to the groups administered 200 mg/kg ISO (Fig. 2) and 85 mg/kg ISO respectively. Taken together these data suggest that the level of necrosis by ISO is dose-dependent.

Further, biochemical estimations also revealed a significant fall in the levels of LDH and antioxidant parameters in all ISO treated groups as compared to control. There was a 32.99, 40.69 and 46.01% fall in LDH levels and 40.47, 49.46 and 56.62% fall in GSH levels (Fig. 3) respectively at $p < 0.05$ for the above doses of ISO as compared to control. Antioxidant enzyme levels of SOD demonstrated statistically significant fall of 43.70, 50.88 and 42.02% at $p < 0.05$ in 85, 200 and 300 mg/kg of ISO administered groups respectively (Fig. 3). Lipid peroxidation (LP) marker TBARS was observed to be elevated by 93.73, 121.13 and 135.67% at $p < 0.05$ for 85, 200 and 300 mg/kg of ISO administered groups respectively (Fig. 3). A significant fall in LDH, GSH and SOD levels with elevated TBARS levels in heart tissue samples together with morphological changes confirmed the development of ischemia with the three tested doses of ISO in the present study. A careful examination and comparison of biochemical parameters for the above three doses of ISO in the pilot study revealed that ISO showed a dose dependent effect. Our results for ISO 85 mg/kg (Fig. 4) on histopathological examination showed only slight visible alteration in the membrane damage as against clear visible damage of myocardial membrane integrity for ISO 200 mg/kg (Fig. 5) which progresses to severe damage to the membrane with ISO 300 mg/kg (Fig. 6), when compared to saline control (Fig. 7). Based on the results of pilot study 200 mg/kg ISO, which produced moderate myocardial damage, was selected to evaluate the cardioprotective potential of pre- and co-treatment of Os on ISO induced myocardial infarction.

A statistically significant restoration in LDH and GSH levels were observed when rats were pre and co-treated with 25, 50, 75 and 100 mg/kg of Os as compared to ISO 200 mg/kg group (Fig. 8). Os at the above doses elucidated significant restoration in SOD and TBARS towards normal levels. However further increment of Os at the dose of 200 and 400 mg/kg did not show any significant cardioprotective effect on these biochemical parameters. The minimum dose of Os offering maximum and statistically significant cardioprotection was found to be 50 mg/kg. Percent improvements were 62.52, 64.12, 54.90 and 58.31% in SOD levels and

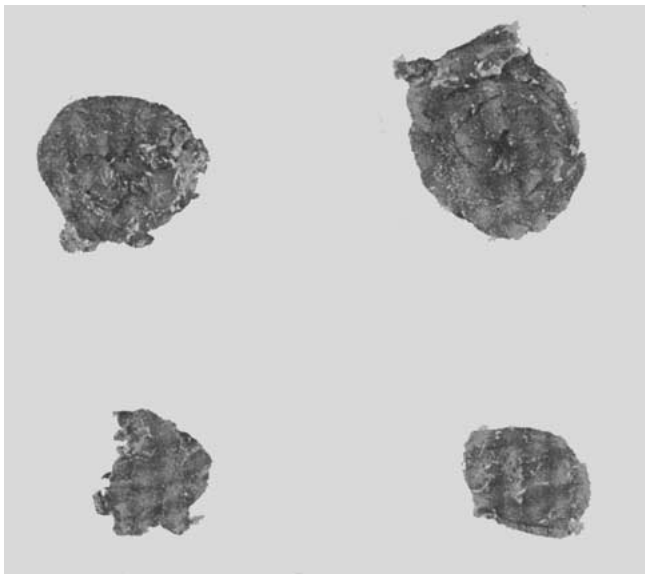

Fig. 1. Transverse sections of heart from a rat of the control group. The tissue are stained brick red (dark region) with 1% triphenyl tetrazolium chloride (TTC) solution.

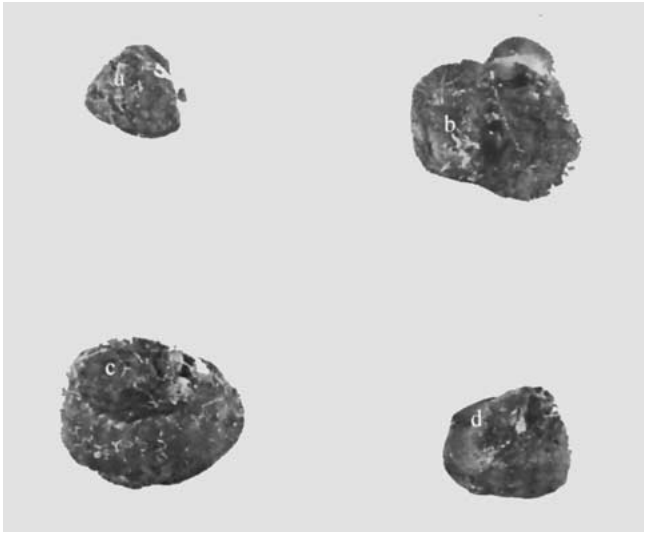

Fig. 2. Transverse sections of heart from a rat of the pilot study group administered isoproterenol (ISO) 200 mg/kg showing the areas of focal necrosis, marked as a, b, c, d (unstained white regions).

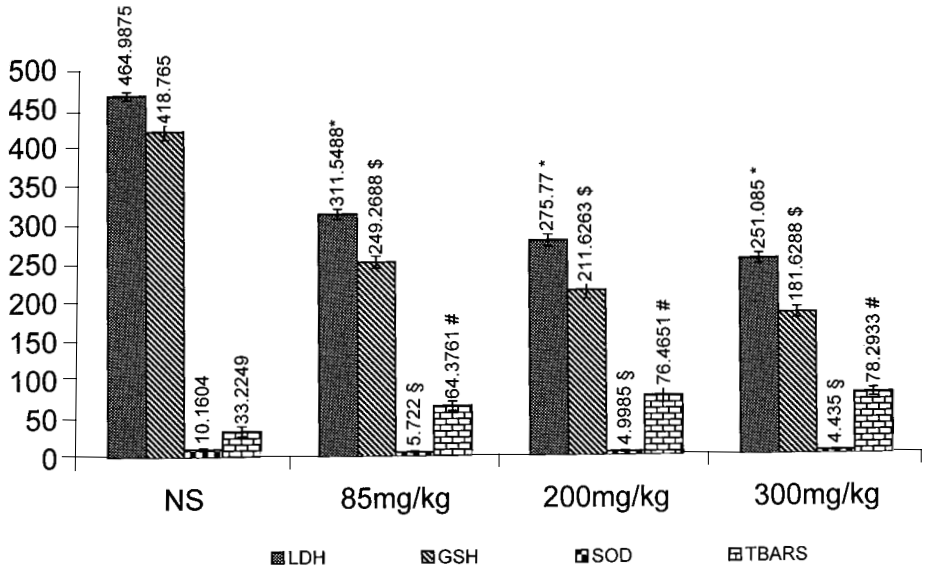

Fig. 3. Depicts the changes in lactate dehydrogenase (U/g wet wt.); glutathione (μg/g wet wt.); superoxide dismutase (U/mg protein) and lipid peroxide (nmol/g wet wt.) levels in the rat heart on administration of Isoproterenol (ISO) in a dose of 85, 200 and 300 mg/kg. Values are mean ± S.D. of 8 experiments. *$p < 0.05$; $^{\$}p < 0.05$; $^{\S}p < 0.05$; $^{\#}p < 0.05$ as compared to control group (normal saline).

20.13, 38.37, 34.01 and 30.32% in TBARS (Fig. 9) for 25, 50, 75 and 100 mg/kg of Os respectively as compared to ISO 200 mg/kg administered group.

The above morphological and biochemical findings were further confirmed by histopathological studies. Histopathological examination of myocardial tissue of saline control depicted clear integrity of myocardial cell membrane (Fig. 7). Endocardium and pericardium were seen within the normal limits. No inflammatory cells infiltration was seen in the saline administered rat heart. In 85 mg/kg ISO administered (Fig. 4) group, focal myonecrosis with myophagocytosis and lymphocytic infiltration (myocarditis) was observed. In 200 mg/kg ISO administered (Fig. 5) group, clear myonecrotic areas were observed which were more as compared to 85 mg/kg ISO administered group. There was complete myonecrosis with fibroblastic proliferation and presence of chronic inflammatory cells. Marked edema and vacuolar changes along with subendocardial myonecrotic patches were clearly visible in

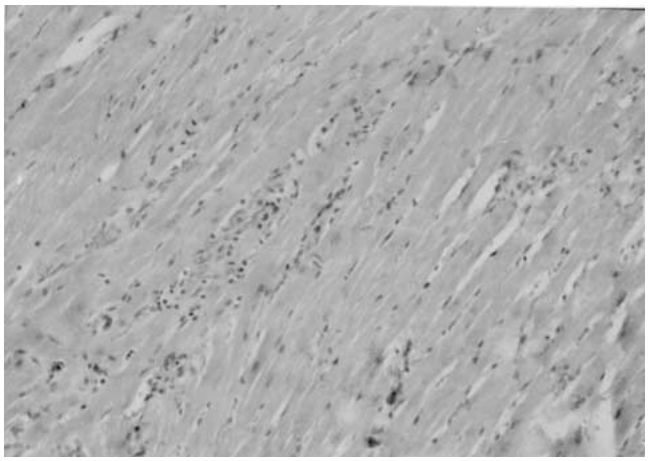

Fig. 4. Photomicrograph of rat heart of the pilot study group administered isoproterenol 85 mg/kg showing focal myonecrosis with myophagocytosis and lymphocytic infiltration (myocarditis). (H & N ×100).

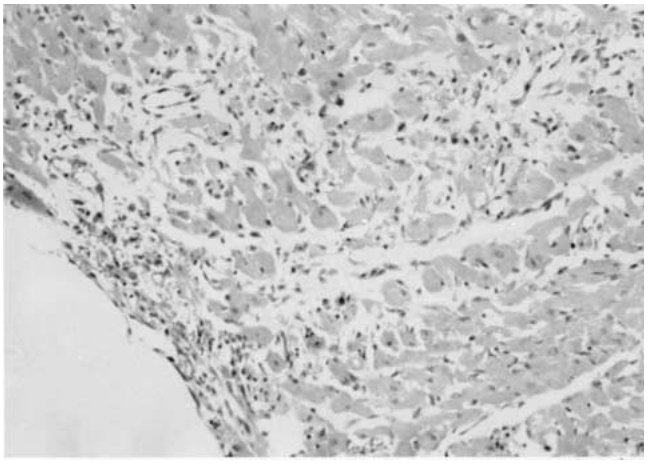

Fig. 5. Photomicrograph of rat heart of the pilot study group administered isoproterenol 200 mg/kg showing more and complete areas of focal myonecrosis with fibroblastic proliferation. In subendocardium vacuolar changes and prominent edema along with chronic inflammatory cells are clearly visible. (H & N ×100).

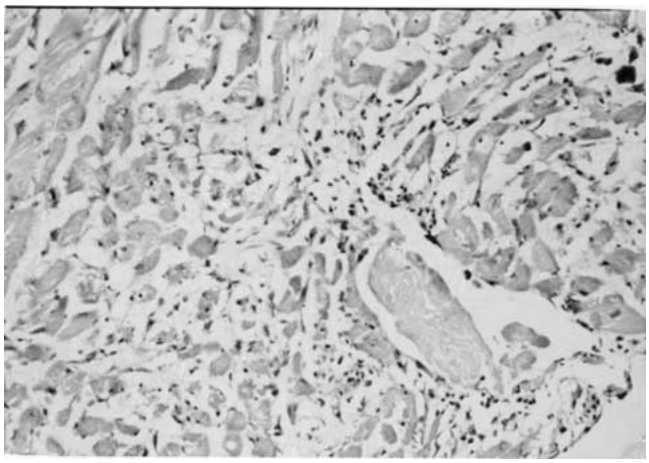

Fig. 6. Photomicrograph of rat heart of the pilot study group administered isoproterenol 300 mg/kg showing confluent areas of focal myonecrosis. In subendocardium, massive necrosis of muscle fibres and inflammatory cells seen. Vacuolar changes and prominent edema is present. (H & N ×100).

200 mg/kg ISO group. In 300 mg/kg ISO (Fig. 6) group, confluent areas of myonecrosis and subendocardial edema with chronic inflammatory cells were observed. Gradation of myocardial damage in this group was more than the 200 mg/kg ISO group. In the 50 mg/kg Os (Fig. 10) treated group no confluent areas of multiple subendocardial damage was seen. Inflammatory cells in reduced density were observed. Vacuolar changes and edema were present to the lesser extent as compared to ISO 200 mg/kg administered group. However, there was no inter-dose significant level of difference in the improvement attained histopathologically with Os at the doses of 25, 50, 75 and 100 mg/kg.

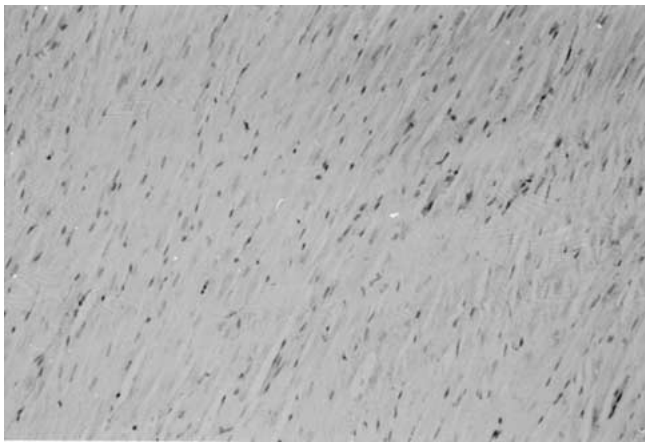

Fig. 7. Photomicrograph showing normal architecture of rat heart of saline injected control group. Endocardium and pericardium are seen within normal limits with no inflammatory cells. (H & N ×100).

Discussion

Animals develop 'infarct like' lesions when injected with ISO, a potent synthetic catecholamine. These lesions are morphologically similar to those of 'coagulative myocytolysis' (COAM) or myofibrillar degeneration, one of the findings described in acute myocardial infarction (AMI) and sudden death in man [19].

Though the pathogenesis of AMI has not yet been completely understood, the studies on ISO induced cardiotoxicity clearly demonstrate the involvement of oxidative stress in this pathology [4, 20]. Therapeutic intervention that could improve impaired antioxidant defense mechanisms or diminish free radical production in the ischemic myocardium has been of great interest [4]. Recently there has been an upsurge of interest to explore the cardioprotective potential of natural products [5].

A number of investigations have suggested that catecholamines in large doses produce myocardial necrosis [3, 4, 21]. Mechanisms proposed to explain this catecholamine-induced necrosis include an increase in cAMP levels [22, 23], intracellular calcium overload, and exhaustion of high-energy phosphates [24]. Since catecholamines readily undergo oxidation, it has been suggested that the oxidation products of catecholamines, rather than catecholamines per se, are responsible for myocardial changes observed following the administration of the parent compounds [25]. There is strong evidence that adrenochrome and other oxidation metabolites of catecholamines can cause cell necrosis and contractile failure in the rat heart [26, 27]. It is also known that autoxidation of catecholamines results in the generation of highly cytotoxic free radicals [28]. Free radicals can initiate the formation of alkyl, alkoxy and hydroperoxy radicals plus hydroperoxides from polyunsaturated fatty acids. The localization of highly unsaturated fatty acids in membrane makes the latter vulnerable to free radical induced LP [29]. These studies strongly suggests that free radicals play an important role in catecholamine-induced cardiotoxicity by causing peroxidation of membrane phospholipids, which can result in permeability changes in the membrane as well as intracellular calcium overload.

Free radical scavenging enzymes such as catalase, superoxide dismutase, glutathione peroxidase are the first line cellular defense against oxidative injury, decomposing O_2 and H_2O_2 before their interaction to form the more reactive hydroxyl radical (OH·). The equilibrium between these enzymes is an important process for the effective removal of oxygen stress in intracellular organelles. The second line of defense consists of the non- enzymatic scavenger's viz., ascorbic acid, α tocopherol, ceruloplasmin and sulphydryl containing compounds, which scavenge residual free radicals escaping decomposition by the antioxidant enzymes.

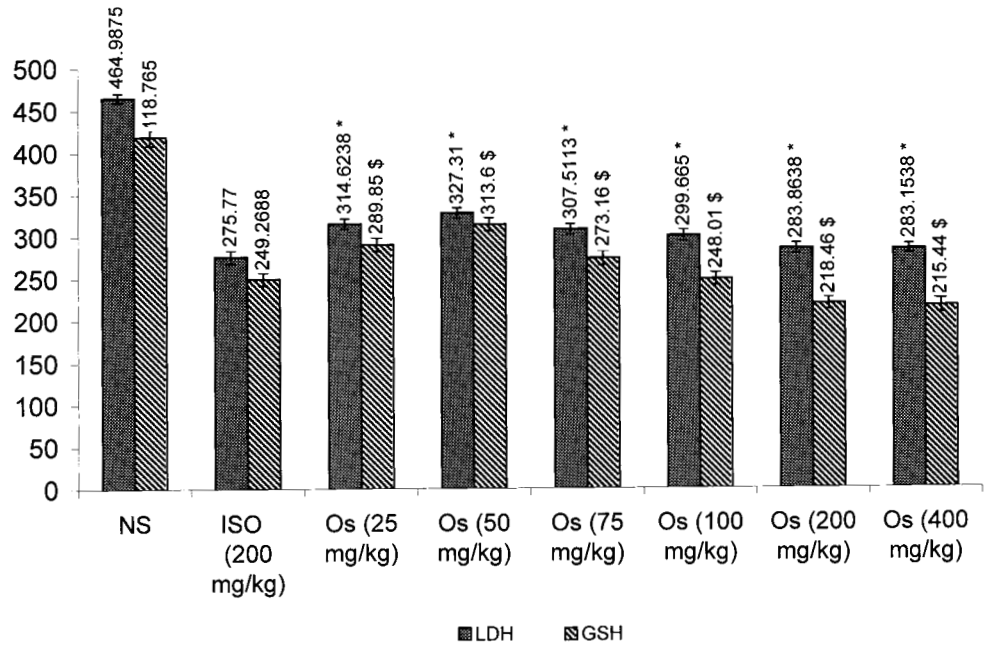

Fig. 8. Depicts the changes in lactate dehydrogenase (U/g wet wt.); glutathione (μ/g wet wt.) levels in isoproterenol (ISO) 200 mg/kg induced myocardial infarction after *Ocimum sanctum* (Os) treatment in a dose of 25–400 mg/kg in the rat heart. Values are mean ± S.D. of 8 experiments. *$p < 0.05$; $^{\$}p < 0.05$ as compared to control group.

Besides, antioxidant enzymes and physiological antioxidants, alteration in LDH has been considered as one of the most important marker of myocardial infarction. Furthermore, Wexler and Kittinger [30] demonstrated that there was a dramatic rise and fall in serum CPK and LDH following ISO induced MI in rats, and the degree of rise and fall in serum enzyme activities were commensurate with the extent of the myocardium infarcted. Various studies have shown an

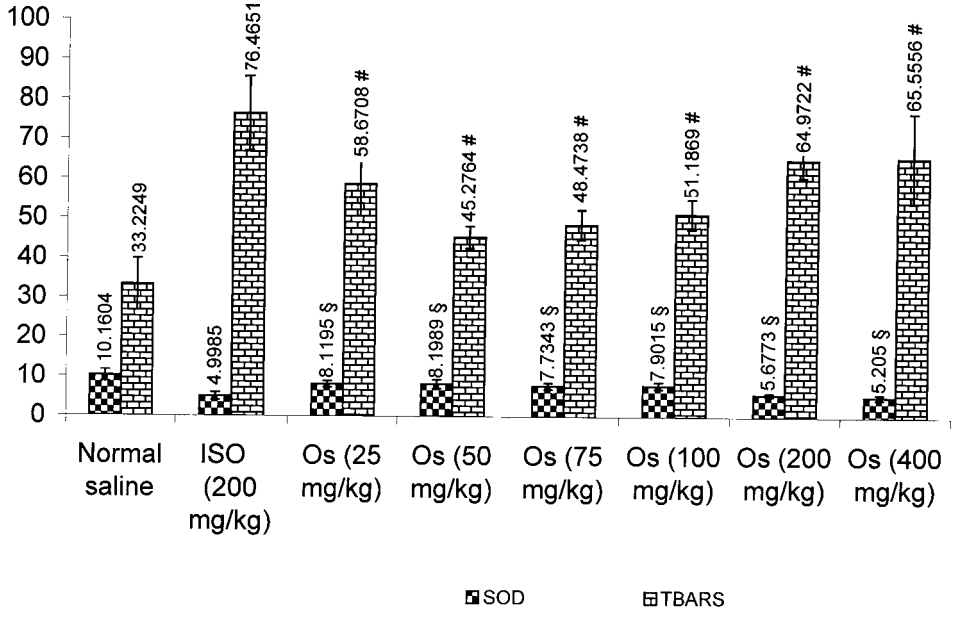

Fig. 9. Depicts the changes in superoxide dismutase (U/mg protein) and lipid peroxide (nmol/g wet wt.) levels in isoproterenol (ISO) 200 mg/kg induced myocardial infarction after *Ocimum sanctum* (Os) treatment in a dose of 25–400 mg/kg in the rat heart. Values are mean ± S.D. of 8 experiments. $^{\$}p < 0.05$; $^{\#}p < 0.05$ as compared to control group.

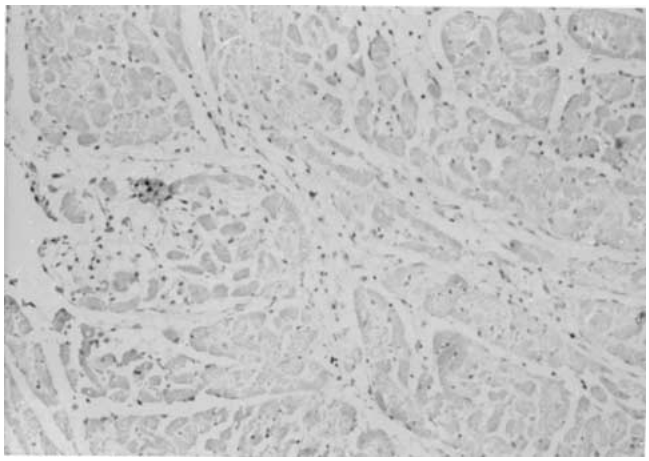

Fig. 10. Photomicrograph of rat heart of the *Ocimum sanctum* pre- and co-treated group administered 50 mg/kg showing decreased degree of necrosis and less infiltration of inflammatory cells. Occasional areas of focal myonecrosis are visible. (H & N ×100).

increase in plasma LDH activity with the consecutive injections of ISO and appearance of degenerative changes in myocardial cell membranes [31]. In the present study, LDH was estimated in heart tissue and a significant ($p < 0.05$) fall in the levels was observed in ISO treated rats to those of control. This observation is in conformity with previous reports and can be attributed to the fact that LDH, being the myocardial enzyme, leaked out from the tissue to plasma on development of degenerative changes in myocardial cell membranes. Detection of myocardial necrosis by direct staining using TTC dye, which forms a red formazan precipitate with LDH of the viable myocardial tissue also confirmed that in all ISO administered groups there was a significant leakage of LDH as compared to control.

In the present investigation, the pilot study demonstrated dose dependent development of severity of myocardial necrotic tissue which was confirmed morphologically, biochemically and histopathologically. Our results are in confirmation with earlier studies, which also have shown that the severity and extent of myocardial damage varies directly with the dose and rate of ISO administration. In this study, it was observed that 200 mg/kg dose of ISO, induced marked lesions in myocardium and significantly altered various biochemical parameters. Therefore, further studies related to evaluation of cardioprotective effect of the Os was conducted using 200 mg/kg of ISO.

With this background information the present study was designed to systematically evaluate the hydroalcoholic extracts of Os leaves for its potential as a cardioprotective agent. For this, effect of Os on morphology, biochemistry and histopathology of heart was studied against ISO induced myocardial infarction.

In the present study pre- and co-treatment of Os exhibited significant protection against ISO induced histopathological and biochemical changes. The cadioprotective mechanism(s) appear to be through modulation of various antioxidant parameters thereby improving the overall antioxidant defense of the myocardial tissue. Present data on GSH, SOD, and TBARS demonstrated that antioxidant status of the myocardial cell in ISO treated group is significantly hampered. Significant fall in GSH levels, impaired SOD activity together with increased LP appears to be the initial insult to the tissue making it more susceptible to oxidative damage. Increased ·OH production in such a compromised situation may be responsible for the observed membrane damage as evidenced by the elevated LP in terms of TBARS.

Glutathione is implicated in the removal of free oxygen species such as H_2O_2, superoxide radicals, alkoxy radicals, and maintenance of membrane protein thiols and as a substrate for glutathione peroxidase (GPX) and glutathione-s-transferase (GST). Enhanced levels of GSH could either be because of its increased synthesis or due to improved glutathione reductase activity in presence of Os. Experiments performed in the present study demonstrates inhibition of the LP, enhancement of SOD activity and improvement in GSH levels in Os administered groups, which further implies that the cardioprotective effect of Os may be by virtue of its antioxidant properties. Antioxidant properties of Os could be attributed to its constituents like eugenol, flavonols, flavones and anthocyanins etc. Pre- and co-treatment of animals with Os (25, 50, 75 and 100 mg/kg) offered a significant at $p < 0.05$ protection against ISO induced MI. However Os at doses 200 and 400 mg/kg failed to exhibit any significant cardioprotection in the present study, which might be due to the prooxidant activity at higher doses.

The fall in SOD levels may be due to the involvement of superoxide free radical in myocardial cell damage. A decrease in activity of SOD can result in the decreased removal of superoxide ion, which can be harmful to the myocardium [33]. It is possible that in presence of Os either generation of free radical itself is impaired or enhanced SOD activity could effectively scavenge the first free radical superoxide from the system.

Serum lactate dehydrogenase has been reported to elevate markedly in ISO induced MI due to its leakage from heart tissue as a consequence of ISO induced LP and membrane damage [31]. In the present study, the level of LDH falls significantly ($p < 0.05$) in heart tissue following membrane damage. A significant protection in heart LDH levels is indicative of the fact that Os have cardioprotective action and maintain membrane integrity of myocytes.

On histopathological examination, ISO 200 mg/kg group, (Fig. 5) demonstrates focal myonecrosis and chronic infiltration of inflammatory cells. Marked vacuolar changes and

edema were seen. Pre- and co-treatment with Os (50 mg/kg) demonstrated reversal of myonecrosis and lymphocytic infiltration (myocarditis) seen with ISO treated group. Inflammatory cells were seen with reduced density in the Os treated groups as compared to ISO 200 mg/kg group confirming further the cardioprotective activity exerted by the hydroalcoholic leaf extract of Os in the present study. Our data indicate that Os may provide potential therapeutic value in the treatment of MI. If the beneficial effects of Os can be reproduced in human beings, these findings may represent a novel prophylactic therapy for MI.

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