

Full Length Research Paper

Preventive effect of *Rhododendron arboreum* on cardiac markers, lipid peroxides and antioxidants in normal and isoproterenol-induced myocardial necrosis in rats

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This study was designed to investigate the preventive effect of ethanolic extract of *Rhododendron arboreum* (ERA) against isoproterenol-induced myocardial ischemia in rat myocardium. Wister rats were pretreated with ERA (100, 250, 500 mg kg⁻¹) orally for 42 days and then intoxicated with isoproterenol (ISO, 150mg/kg body weight, s.c. for 2 days). MI induced in rats by subcutaneous injection of ISO-(150 mg/kg body weight) at interval of 24 h for 2 days showed significant elevation in serum cardiac marker enzymes like lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT). Lipid peroxidative product like lipid hydroperoxidase was elevated in tissue and serum, decline in enzymatic antioxidant status (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic reduced glutathione antioxidants. Pretreatment with ERA to ISO-treated rats caused a significant protective effect. Administration of ERA in normal rats did not have any significant effect on cardiac markers, lipid peroxidation and antioxidant levels. The results of our study show that ERA possesses protective effect result from suppression of oxidative stress in ISO-induced myocardial infarcted rats. Histopathological examination further confirmed the cardioprotective effect of ERA against ischemic insult.

Key words: Myocardial infarction, isoproterenol, antioxidants, *Rhododendron arboreum*, oxidative stress.

INTRODUCTION

Cardiovascular disease will be the most important cause of mortality in India by the year 2015 (Gilski and Borkenhagen, 2005). The cardiovascular system is susceptible to many chronic diseases such as myocardial infarction (MI). MI is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand (De Bono and Boon, 1992). Damage to the myocardial cell arises due to the generation of toxic reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radical (Vaage and Valen, 1993). It is well

known that CVD are directly or indirectly related to oxidative damage that shares a common mechanism of molecular and cellular damage.

The model of isoproterenol-induced myocardial ischemia is considered as one of the most widely used experimental model to study the beneficial effects of many drugs and cardiac function (Wexler, 1978). Myocardial infarction induced by isoproterenol, a β -adrenergic agonist has been reported to show many metabolic and morphological aberrations in the heart tissue of the experimental animals similar to those observed in human myocardial infarction (Nirmala and Puvanakrishnan, 1990). The primary disturbance of isoproterenol-induced myocardial infarction has been reported to enhance adenylate cyclase activity resulting in increased cAMP formation, which in turn would have

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lead to higher lipid accumulation in myocardium (Subhash et al., 1978). It is also well known that ISO generate free radicals and stimulate lipid peroxidation, which cause irreversible damage to the myocardial membrane (Sathish et al., 2006). Increase in formation of ROS during ischemia/reperfusion and the adverse effects of oxygen radicals on myocardium have been well established by both direct and indirect measurements. Thus, increased production of ROS may be a unifying mechanism in ischemic injury progression; anti-oxidants may be the therapeutic value in this setting.

Epidemiological studies have shown that diets rich in fruits, herbs and spices are associated with a low risk of CVD (Banerjee and Maulik, 2002). Flavonoids, plant-derived antioxidants, are defined as non-nutritive dietary components that are abundant in foods (Boyle et al., 2000). Consumption of flavonoids containing food and beverages has been proposed as a useful practice to limit oxidative damage in the body (Cherubini et al., 1999). The protective role of flavonoids involves several mechanisms of action: a direct antioxidant effect, inhibition of enzymes of the oxygen-reduction pathway, sequestration of transient metal cations (Robak and Gryglewski, 1996; Cotelle, 2001; Rice-Evans, 2001). Recently, attention has been focused on non-nutrient phytochemicals and polyphenols such as the flavonoids, alkaloids and xanthenes derived from different plant species as potent therapeutic agents in the prevention and management of CVD due to their antioxidant nature (Pauletti et al., 2003).

The leaves of *Rhododendron arboreum* were reported to contain Quercetin 3-O- beta -D-glucopyranosyl [1- >6]-O- alpha -L-rhamnopyranoside, pectolarigenin 7-Orutinoside, 7,2'-dimethoxy-4',5-methylene dioxyflavanone (Kamil and Shafiullah, 1995). Flavonoids, isolated from the leaves of *R. arboreum* were found to have potent antioxidant property (Dhan et al., 2007) the plant *R. arboreum* have been reported for anti-inflammatory (Shyam and Kalpana, 1988). In the absence of reliable cardioprotective drugs in modern medicine, there are numbers of medicinal preparations in the Ayurvedic system of Indian medicine recommended for the treatment of cardiac disorders. Their usage is in vogue since centuries are quite often claimed to offer significant relief. However, no scientific information is available regarding the cardioprotective effect of *R. arboreum*. This study addresses the preventive effect of ethanolic extract of *R. arboreum* (ERA) in noninvasive myocardial infarction rat model against oxidative damage, focus and correlate functional, biochemical and histopathological changes.

MATERIALS AND METHODS

Plant materials

Leaves of the plant *R. arboreum* were collected (in the month of October) from the surrounding fields of Meghalaya. The identification of plant was made by Department of Botany, K. N. G.

College, Jowai, India. The voucher specimen (Ref No: 08/P.colog/2006-2007) of the plant material has been deposited in the Department of Pharmacology.

Plant extract

Freshly collected *R. arboreum* L. whole plant was dried under shade and the dried material was milled to obtain a coarse powder. The ethanolic extract of powder was prepared by the process of continuous extraction (Soxhlation), extract was stored in desiccator for further study.

Acute toxicity studies

Acute oral toxicity studies carried out for ethanolic extract of *R. arboreum* using acute toxic class method, according to OECD guidelines No. 423. Based on toxicity study three doses 100, 250, 500 mg kg⁻¹ were selected (OECD, 1996).

EXPERIMENTAL

Animals

All the experiments were carried out with adult male albino Wistar rats, 200 to 250 g (Bionees, Tumkur, Bangalore). Rats were housed in polyacrylic cages (38X23X10 cm) with not more than four animals per cage. They were housed in an air conditioned room and were kept in standard laboratory conditions under natural light dark cycle (approximately 14 h light/ 10 h dark) maintained humidity 60±5% an ambient temperature of 25±2°C. All animals were allowed free access to standard diet (Amrut rat feed, Bangalore) and tap water *ad libitum*. Allowed to acclimatize for 1 week before the experiments. Commercial pellet diet contained 22% protein, 4% fat, 4% fiber, 36% carbohydrates and 10% ash w/w. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control Supervision of Experiments on Animals (CPCSEA), New Delhi, India. Approved by the Institutional Animal Ethical Committee of Acharya and B. M. Reddy College of Pharmacy, Bangalore (Approval No. IAEC /PP //03/2007-2008).

Chemicals

Isoproterenol was procured from the Sigma- Aldrich chemicals Ltd, St. Louis, USA. 5, 5'-Dithiobis(2-nitrobenzoic acid) (DTNB), reduced glutathione were obtained from Himedia Laboratories, Mumbai. All the other chemicals procured from Merck laboratories, Nice chemicals, Loba chemie, Sd. fine chemicals and were of analytical grade.

Induction of myocardial ischemia

Myocardial ischemia was induced by subcutaneous injection of isoproterenol hydrochloride (ISO, 150 mg kg⁻¹ dissolved in saline, once a day for 2 days (Rone et al., 1959).

Experimental protocols

The rats were divided into 9 groups of 6 rats as follows: group 1, maintained as normal control group received 0.2 ml saline orally to each animal for 42 days; group 2, maintained as vehicle control group received 0.2 ml 80% DMSO orally to each animal for 42

days; group 3, maintained as ISO control without any drug treatment (positive control) given 150 mg kg⁻¹ subcutaneously once a day for 2 days; groups 4, 5 and 6 received ERA orally 100, 250, 500 mg kg⁻¹ respectively for 42 days; groups 7, 8 and 9, received ERA orally 100, 250, 500 mg kg⁻¹ respectively for 42 days and then subcutaneously injected with isoproterenol (150 mg kg⁻¹ dissolved in saline, once a day for 2 days at interval of 24 h).

Twenty-four hours after the second dose of isoproterenol, all the rats were anaesthetized with thiopental sodium (30 mg kg⁻¹, intraperitoneally). Blood samples were collected from carotid artery and allowed to clot for 30 min at room temperature. The serum separated by centrifugation at 2500 rpm at 30°C for 15 min and used for estimation of marker enzymes namely, AST, LDH, ALT and malondialdehyde (MDA). The heart was dissected out, washed immediately in ice-chilled physiological saline, blotted and weighed. A known weight of the heart tissue was homogenized in 0.1 M Tris-HCl (pH 7.4) buffer solution to produce 10% w/v homogenate. An aliquot was used for estimation of malondialdehyde (MDA). The homogenate was centrifuged at 3000 rpm, 4°C for 5 min, supernatant were used for assays of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH). Similarly, hearts were also fixed in 10% buffered neutral formalin for histological studies.

Marker enzyme assays

The marker enzymes LDH, AST, and ALT were assayed in serum using standard kits supplied from Swemed diagnostics, Bangalore, India. The results were expressed as IU/L for LDH, AST, and ALT.

Assay of lipid peroxidase

Tissue and serum lipid peroxidation was evaluated by measurement of thiobarbituric acid reactive substance (Ohkawa et al., 1975; Yagi, 1976). Malondialdehyde has been identified as the product of lipid peroxidation, which reacts with thiobarbituric acid resulting into chromogen, this chromogen extracted from organic solvent and absorbency of organic phase is determined at 532 nm. The level of lipid peroxides in heart tissue is expressed as n.mol of MDA/g tissue weight and serum expressed as n.mol of MDA/ml.

Assay of superoxide dismutase (SOD)

The assay for estimation of SOD is based on the principle of inhibitory effects of SOD on reduction of nitro blue tetrazolium dye by superoxide anions generated by the photo-oxidation of hydroxylamine hydrochloride (Kono, 1978).

Assay of catalase

The reaction mixture contained 3 ml of 0.66 M phosphate buffer (pH 7.0), 30% w/v H₂O₂ in the sample cuvette (Luck, 1963). The reference cuvette contained 3 ml of 0.66 M phosphate buffer (pH 7.0). The reaction was started by adding heart tissue homogenates to the sample and reference cuvettes. The rate of elimination of hydrogen peroxide by catalase was measured by recording the time (in second) required for 0.05 decline of absorbance at 240 nm. Catalase activity (in international units) was calculated by following formula was expressed in terms of units/ml.

$$\frac{\text{Units in assay mixture} \times \text{Dilution}}{\text{Volume of sample}} = \text{Units/ml wet tissue}$$

Assay of glutathione peroxidase (GPx)

To 0.2 ml of tissue homogenate, add 0.2 ml of 0.8 mM EDTA, 0.1 ml of sodium azide, 0.1 ml of 4 mM GSH, 0.1 ml H₂O₂ solution, 0.4 ml of 0.4 M phosphate buffer (pH 7.0) (Rotruck et al., 1973). Incubate at 37°C for 10 min; to this add 0.5 ml of 10% TCA centrifuged at 2000 rpm for 10 min, supernatant add 0.1 ml of 0.04% DTNB solution, optical density read at 420 against blank (without homogenate) results were expressed as heart tissue GPx (% inhibition).

Assay of reduced glutathione (GSH)

To 0.1 ml tissue homogenate/serum add equal volume of 10% TCA the tissue homogenate centrifuged at 5000 rpm for 10 min. To 0.1 ml of supernatant, 2.0 ml of 0.6 mM DTNB reagent 1.9 ml of 0.2 M phosphate buffer (pH 9.0) were added vortexed (Ellman, 1959). The absorbance was measured at 412 nm against a blank containing TCA instead of supernatant. A series of std. treated in a similar way also run to determine the glutathione content and results were expressed as heart tissue GSH (mg g⁻¹ wet tissue) and serum GSH (mg ml⁻¹).

Histopathological examination

Heart tissue was fixed in 10% buffered neutral formalin solution. The tissue were embedded in paraffin, sectioned at 5 µm stained with hemato-xilin and eosin (H&E). The sections were examined under light microscope to study the light microscopic architecture of the myocardium, and then photomicrographs were taken.

Statistical analysis

All data were expressed as mean ± S.D from n=6 rats in each groups, statistical analysis was performed using one-way ANOVA followed by Dunnet's test. P < 0.01 was accepted as statistically significant.

RESULTS

Histopathology slides

Table 1 shows the effect of ERA on serum cardiac markers in normal and isoproterenol-treated rats. Isoproterenol-treated rats showed a significant (P<0.01) increase in the activity of LDH, AST, ALT in serum when compared with normal rats. Pretreatment with ERA (100, 250, 500 mg kg⁻¹) for 42 days significantly (P<0.01) dose dependently decreased the activity of these enzymes in the serum of isoproterenol-treated rats. Table 2 illustrates the effect of ERA on heart tissue and serum malondialdehyde (MDA) in normal and isoproterenol-treated rats. Isoproterenol-treated rats showed a significant increase (P<0.01) in MDA in serum and heart tissue when compared with normal rats. Pretreatment with ERA (100, 250, 500 mg kg⁻¹) to isoproterenol treated rats for 42 days significantly (P<0.01) dose dependently decreased the levels of MDA in both serum and heart tissue.

Table 3 represents the effect of ERA on myocardial

Table 1. Effect of ERA on serum lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT) in normal and ISO-induced myocardial infarcted rats.

Groups	LDH (IU L ⁻¹)	AST (IU L ⁻¹)	ALT (IU L ⁻¹)
Control	186.57 ±15.60	116.93±10.60	87.02 ±5.27
80% DMSO	174.69 ±3.60	115.09±7.80	88.74±11.20
ISO 150 mg	702.08 ±58.61 ^{a**}	242.27±30.49 ^{a**}	207.01±12.94 ^{a**}
ERA 100 mg	183.10 ±46.30 ^{b**}	111.40±23.15 ^{b**}	96.48±18.95 ^{b**}
ERA 250 mg	197.55 ±11.74 ^{b**}	106.01±10.39 ^{b**}	91.76±11.05 ^{b**}
ERA 500 mg	197.65 ±11.65 ^{b**}	114.28±6.49 ^{b**}	90.88±9.93 ^{b**}
ERA 100 mg+ISO	498.56 ±14.14 ^{a**b**}	193.52±23.84 ^{a**b**}	181.38±20.88 ^{a**b**}
ERA 250 mg+ISO	410.86 ±21.56 ^{a**b**}	156.13±15.81 ^{a**b**}	157.46±15.74 ^{a**b**}
ERA 500 mg+ISO	284.81 ±13.16 ^{a**b**}	123.06±25.32 ^{b**}	104.82±10.75 ^{b**}

Values are expressed as mean ± SD; n=6; a=Compared with control group: ** p<0.01, * p<0.05. b=Compared with ISO group: ** p<0.01, * p<0.05.

Table 2. Effect of ERA on serum and tissue malondialdehyde (MDA) in normal and ISO-induced myocardial infarcted rats.

Groups	MDA (n. mol ml ⁻¹)	MDA (n. mol g ⁻¹ wet tissue)
Control	46.54±4.69	71.72±5.37
80% DMSO	47.19±4.35	71.14±6.88
ISO 150 mg	263.98±16.83 ^{a**}	186.18±10.95 ^{a**}
ERA 100 mg	48.82±2.47 ^{b**}	72.31±4.27 ^{b**}
ERA 250 mg	48.17±5.74 ^{b**}	70.22±1.62 ^{b**}
ERA 500 mg	48.49±4.17 ^{b**}	69.7±1.18 ^{b**}
ERA 100 mg+ISO	188.80±6.37 ^{a**b**}	141.11±9.82 ^{a**}
ERA 250 mg+ISO	123.69±5.87 ^{a**b**}	125.44±9.11 ^{b**}
ERA 500 mg+ISO	87.87±7.31 ^{a**b**}	93.15±2.61 ^{b**}

Values are expressed as mean ± SD; n=6; a=Compared with control group: ** p<0.01, b=Compared with ISO group: ** p<0.01.

Table 3. Effect of ERA on superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) in the heart of normal and ISO-induced myocardial infarcted rats.

Groups	SOD (% inhibition)	GPx (% inhibition)	Catalase (units/ml)
Control	0±0	0±0	2.29±0.08
80% DMSO	4.13±1.47	3.74±1.59	2.28±0.08
ISO 150 mg	100±0 ^{a**}	100±0 ^{a**}	1.07±0.07 ^{a**}
ERA 100 mg	5.95±1.84 ^{b**}	3.90±1.45 ^{b**}	2.23±0.08 ^{b**}
ERA 250 mg	5.35±1.95 ^{b**}	4.05±1.28 ^{b**}	2.33±0.07 ^{b**}
ERA 500 mg	7.14±2.25 ^{b**}	4.21±0.88 ^{b**}	2.27±0.07 ^{b**}
ERA 100 mg+ISO	73.19±9.78 ^{a**b**}	70.66±3.24 ^{a**b**}	1.64±0.09 ^{a**b**}
ERA 250 mg+ISO	48.19±7.40 ^{a**b**}	56.16±2.70 ^{a**b**}	1.97±0.10 ^{b**}
ERA 500 mg+ISO	20.32±3.11 ^{a**b**}	30.88±3.33 ^{a**b**}	2.00±0.10 ^{b**}

Values are expressed as mean ± SD; n=6; a=Compared with control group: ** p<0.01, b=Compared with ISO group: ** p<0.01.

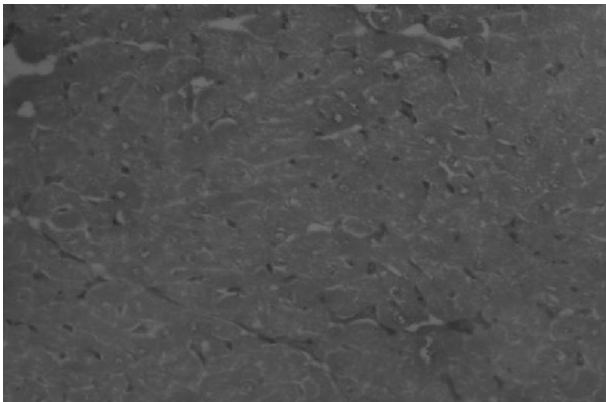
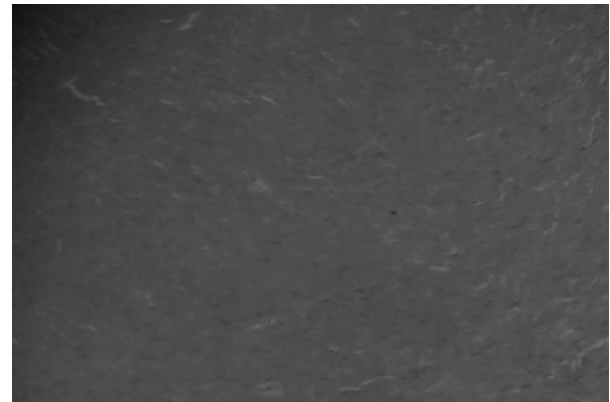
antioxidants, such as SOD, catalase and GPx in normal and isoproterenol-induced rats. Rats treated with

isoproterenol showed a significant (P<0.01) decrease in the activity of these enzymic antioxidants in the heart as

Table 4. Effect of ERA on serum heart reduced glutathione (GSH) in normal and ISO-induced myocardial infarcted rats.

Groups	GSH (mg ml ⁻¹)	GSH (mg g ⁻¹ wet tissue)
Control	3.28±0.09	2.32±0.05
80% DMSO	3.28±0.05	2.34±0.05
ISO 150 mg	0.77±0.04 ^{a**}	0.68±0.07 ^{a**}
ERA 100 mg	3.21±0.15 ^{b**}	2.28±0.08 ^{b**}
ERA 250 mg	3.22±0.10 ^{b**}	2.29±0.05 ^{b**}
ERA 500 mg	3.19±0.09 ^{b**}	2.28±0.06 ^{b**}
ERA 100 mg+ISO	1.28±0.06 ^{a**b**}	1.27±0.07 ^{a**b**}
ERA 250 mg+ISO	1.80±0.03 ^{a**b**}	1.55±0.06 ^{a**b**}
ERA 500 mg+ISO	2.58±0.09 ^{a**b**}	1.86±0.09 ^{a**b**}

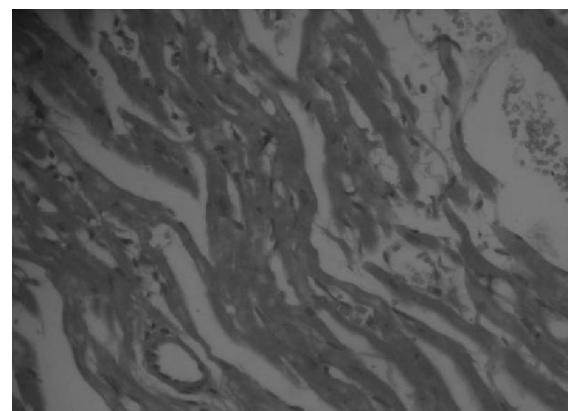
Values are expressed as mean±SD; n=6; a=Compared with control group: ** p<0.01, b=Compared with ISO group: ** p<0.01.

**Figure 1.** Control group: Heart sample showing very mild vacuolation (could be spontaneous).**Figure 2.** 80% DMSO Group: No abnormality detected.

compared with normal rats. Pretreatment with ERA (100, 250, 500 mg kg⁻¹) to isoproterenol-treated rats for 42 days exerted a significant (P<0.01) dose dependent increase in the activity of these enzymes. Table 4 shows the effect of ERA on serum and heart GSH in normal and isoproterenol-induced rats. Rats treated with isoproterenol, showed a significant decrease in the concentration GSH in serum and heart as compared with normal rats. Administration of ERA (100, 250, 500 mg kg⁻¹) to isoproterenol treated rats for 42 days significantly (P<0.01) dose dependently increased the concentration of these antioxidants. Treatment with ERA at doses of 100, 250, 500 mg kg⁻¹ to normal rats did not have any significant effect.

Histopathological examination of heart tissues

On histopathological examination, heart tissue from isoproterenol-treated rats showed moderate degree of myocardial degeneration, vacuolation and inflammatory

**Figure 3.** ISO Group: Section of heart showing moderate degree of myocardial degeneration, vacuolation and inflammatory cell infiltration.

cell infiltration (Figure 3) as compared to control group (Figure 1) and vehicle control group (80% DMSO) (Figure 2). Pretreatment with ERA (100, 250, 500 mg kg⁻¹

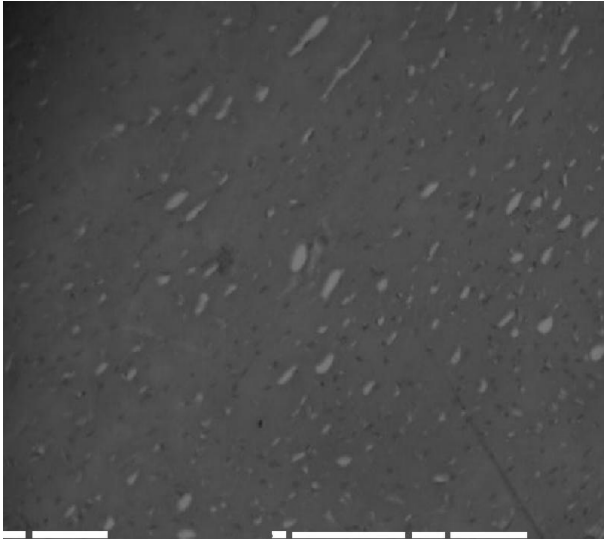


Figure 4. ERA 100 mg/kg: Section of heart showing moderate degree of myocardial vacuolation and degeneration.

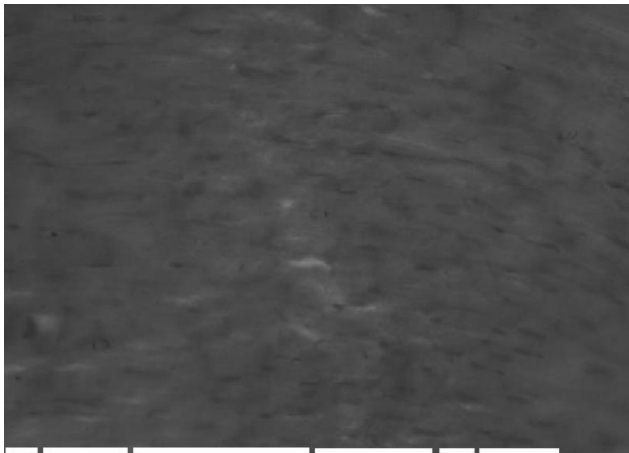


Figure 5. ERA 250 mg/kg: Section of heart showing moderate degree of myocardial degeneration and mild degree of hemorrhage.

respectively) demonstrated marked improvement in isoproterenol-induced myocardial degeneration, vacuolation and inflammatory cell infiltration (Figures 7, 8 and 9). Heart tissue from treatment with sham control, vehicle control, ERA at doses of (100, 250, 500 mg kg⁻¹ respectively) to normal rats did not have any histopathological changes (Figures 4, 5 and 6).

DISCUSSION

Oxygen-derived free radicals are known to play a vital role in the genesis of various cardiovascular disorders (Marx, 1987; Mayers, 1985). Myocardial ischemia occurs

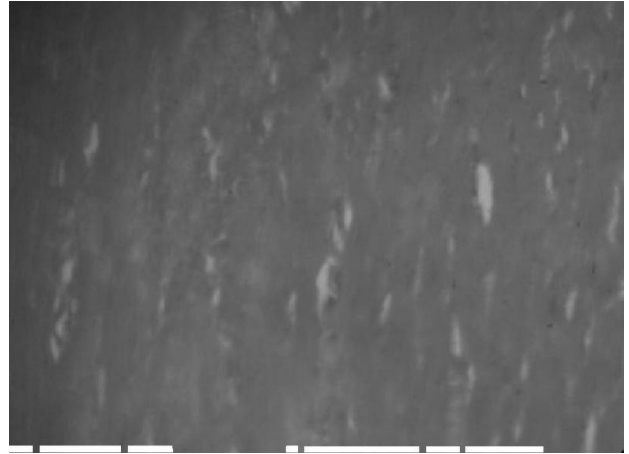


Figure 6. ERA 500 mg/kg: Section of heart showing mild degree of myocardial degeneration and mild degree of edema.

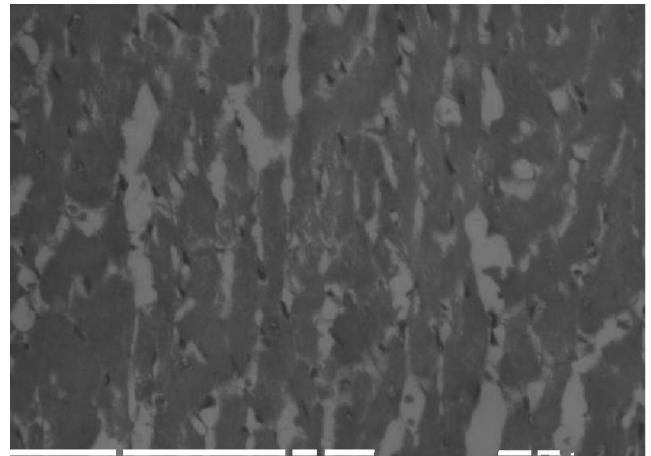


Figure 7. ERA 100 mg/kg + ISO: Section of heart showing mild degree of myocardial degeneration and mild degree of edema.

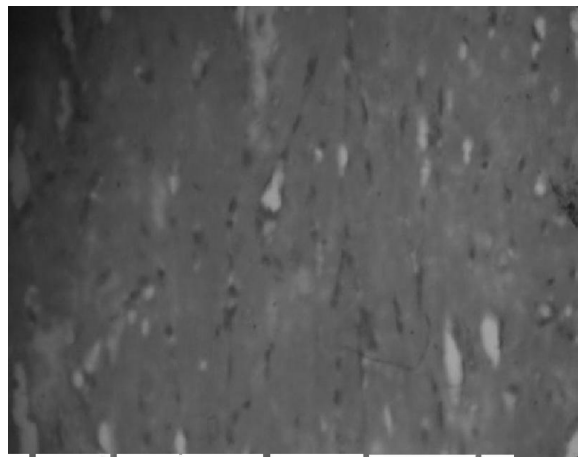


Figure 8. ERA 250 mg/kg +ISO: Section of heart showing mild degree of myocardial degeneration and mild degree of edema.

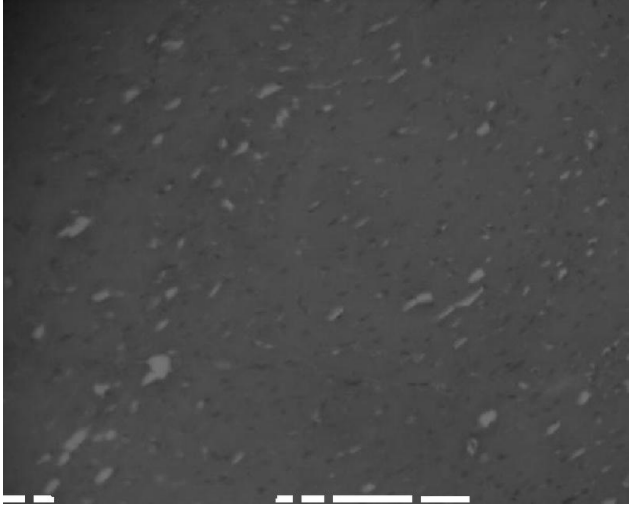


Figure 9. ERA 500 mg/kg + ISO: No abnormality detected.

when myocardial oxygen demand exceeds the oxygen supply. If this condition is not reversed, myocardial infarction precipitates. Reperfusion of the ischemic myocardium can restore oxygen supply which causes a burst of oxygen consumption with the consequent generation of free radicals, resulting in imbalance of antioxidative processes. This process may result in a loss of contractile function of the heart and lead to severe myocardial cell damage, termed as reperfusion injury (Jacobson, 1974).

Recently, there has been an increasing interest in the protective function of dietary antioxidants, for extending life span. Several antioxidants such as vitamins A and C, β -carotene, uric acid, ubiquinol and flavonoids, have been found to play an important role in the non-enzymatic protection against oxidative stress (Okada et al., 2001). Oxidative stress can damage many biological molecules, proteins and DNA, they are more significant targets of injury than the lipids, with lipid peroxidation often occurring late in the injury process (Halliwell, 1993). Oxygen derived free radicals also play a significant role in a large variety of CVD, including atherosclerosis and ischemic heart disease (Marx, 1987). Our study shows that ERA treatment in myocardial infarcted animals prevented the raise in infarct size, lipid peroxidation, serum marker enzymes and decrease in antioxidants.

Effect on cardiac marker enzymes

The cardiac marker enzymes of MI are LDH, AST and ALT. ISO-treated rats showed an increase in the activity of these enzymes in serum. The increase in the activity of marker enzymes in serum could be due to leakage of these enzymes from the heart, as a result of MI injury; but in treatment group with ERA, there was a significant

effect on these marker enzymes in serum of pretreated MI rats.

Effect on lipid peroxidation

Lipid peroxides play an important role in myocardial cell damage. Enormous amounts of ROS, like superoxide, hydrogen peroxide and hydroxyl radicals, are produced during MI. Significant elevation in the n. mol concentration of MDA was observed in ISO-treated rats. Lipid peroxidation is an important pathogenic event in MI and accumulation of lipid peroxides reflects the various stages of this disease and its complications (Neely et al., 1973).

It is known that isoproterenol produces free radicals and these free radicals are involved in membrane damage, leading to increase in levels of MDA. On treatment with ERA, the n. mol concentrations of MDA were significantly decreased in ISO-treated rats. The free radical inhibitory activity of ERA is attributed to its antioxidant property which effectively scavenges the ROS and decreases lipid peroxidation end products.

Effect on antioxidants

Effect on enzymic antioxidants

In-vivo and *in-vitro* studies, as well as epidemiological studies, suggest an inverse correlation between the severity of oxidative-stress-induced diseases and levels of antioxidants (Oka et al., 1999). Free radical scavenging enzymes such as SOD, catalase and GPx are the first line of cellular defense against oxidative injury. The equilibrium between these enzymes is an important factor for the effective removal of ROS in intracellular organelles (Milei et al., 1978). ISO-treated rats showed decreased activity of SOD and catalase in heart.

A decrease in the activity of these antioxidant enzymes can lead to formation of oxygen hydrogen peroxide, which in turn can form the toxic hydroxyl radical (OH). The decrease in the activity of SOD and catalase may be due to myocardial cell damage. The increased activity of the myocardial catalase and SOD is associated with decreased levels of lipid peroxidation in ERA (100, 250, 500 mg kg⁻¹) treated ISO groups.

This can result in decreased formation of toxic intermediates. The enzyme GPx is a well-known first line of defense against oxidative stress, which in turn requires glutathione as co-factor. GPx catalyses the oxidation of GSH to GSSG at the expense of H₂O₂. The observed decreased activity of GPx in the study might be due to the reduced concentration of GSH in ISO-treated rats. Oral pre-treatment with ERA increases the activity of GPx in ISO-treated rats.

Effect on non-enzymatic antioxidant

The second line of defense consists of the non-enzymatic scavengers, namely reduced glutathione and ascorbic acid containing compounds, which scavenge residual free radicals escaping decomposition by the antioxidant enzymes (Kloner et al., 1974). Reduced glutathione GSH one of the major constituent of erythrocytes plays an important role in providing protection against oxidative damage. It has been proposed that antioxidants, which maintain the concentration of GSH, may restore the cellular defense mechanism, block lipid peroxidation and protect the tissue against oxidative damage (Chugh et al., 1999).

A decrease in the concentration of GSH in ISO-treated rats might be due to its utilization by the glutathione-dependent antioxidant process. During ISO-induced myocardial necrosis, the level of enzymatic and non-enzymatic antioxidants decreases significantly leading to increased free radical formation. These radicals cascade a number of reactions that could be harmful to the myocardium (Samuleson, 1997). The significant increase in the activity of GSH in ISO-induced rats treated with ERA (100, 250, 500mg kg⁻¹) could prevent free radical formation during myocardial necrosis. Histopathological examination further confirmed the protective effect of ERA on the MI heart.

Conclusion

In the present investigation it was observed that pretreatment of ERA (100, 250, 500mg kg⁻¹) offer dose dependent protection from myocardial injury in ISO-induced MI. Pretreated with ERA groups were prevented against the increase in serum and tissue lipid peroxidation, serum cardiac marker enzyme LDH, AST, ALT and the decrease in both enzymatic and non-enzymatic antioxidants in ISO treated rats. Antioxidant effect could be attributed to the presence of flavonoids in ERA. This protective effect of ERA was further confirmed by histopathological report.

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