

Protective effect of aqueous extract of *Moringa oleifera* Lam. stem bark on serum lipids, marker enzymes and heart antioxidants parameters in isoproterenol-induced cardiotoxicity in Wistar rats

Mahendra A Gunjal, Abhishek S Shah, Alok S Wakade, Archana R Juvekar*

Department of Pharmaceutical Sciences and Technology
Mumbai University Institute of Chemical Technology, Nathalal Parikh Marg, Matunga, Mumbai-400 019, India

Received 12 October 2009; Accepted 20 September 2010

Cardio protective potential of aqueous extract of *Moringa oleifera* Lam. (Family-Moringaceae) stem bark on serum lipids profiles, myocardial marker enzymes in serum and heart homogenate, antioxidant parameters in heart homogenate and histopathological findings in isoproterenol (ISO)-induced cardiotoxicity in rats was evaluated. Subcutaneous injection of ISO (85 mg/kg) to Wistar rats showed a significant ($P<0.05$) increase in the levels of thiobarbituric acid reactive substances along with a significant decrease in the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase and reduced glutathione in the heart. It also significantly increased the level of cholesterol, triglycerides, VLDL and LDL with significant decrease in the level of HDL, increased the level of myocardial marker enzymes CK, LDH, ALT and AST in serum and concomitantly decreased in heart. Oral administration of aqueous extract of *M. oleifera* (250 and 500 mg/kg) to ISO-induced rats daily for a period of 21 days showed a significant improvement in lipid profile along with marker enzymes in serum and heart homogenate. It also decreased the levels of thiobarbituric acid reactive substances and improved antioxidant status by increasing the activities of antioxidant enzymes. Histopathological findings of the myocardial tissue showed the protective role of *M. oleifera* in ISO-induced cardiotoxicity in rats. The effect at 500 mg/kg dose was more pronounced.

Keywords: *Moringa oleifera*, Drumstick tree, Isoproterenol, Marker enzymes, Antioxidant enzymes, Cardioprotection, Hypolipidemic.

IPC code; Int. cl.⁸—A61K 36/00, A61P 9/00, A61P 17/18

Introduction

Ischemic heart disease has emerged as a major health problem and is predicted that by the year 2020 this disease will persist as the major and the most common threat to human life¹. The pathogenic mechanism of myocardial ischemic damage is still not completely understood, but the role of oxygen-derived free radicals (OFRs) in myocardial ischemia is established, although not completely characterized.

Oxidative stress resulting from increased production of free radicals is associated with decreased levels of antioxidants in the myocardium and plays a major role in cardiovascular diseases². Damage to the myocardial cells arises due to the generation of toxic reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide and hydroxyl radical³. ISO, a synthetic catecholamine and

a β -adrenergic agonist causes severe oxidative stress in the myocardium, resulting in infarct like necrosis of the heart muscles⁴. Catecholamines rapidly undergo auto-oxidation and has been suggested that the oxidative products of catecholamines are responsible for the changes in the myocardium⁵. Exposure of the heart to high concentrations of catecholamines had been reported to result in the development of necrotic lesions in the myocardium of experimental animals⁶. By studying the biochemical alterations that take place in an animal model, it is possible to gain more insight into the mechanisms leading to the altered metabolic process in human myocardial infarction (MI).

Phytopharmaceuticals are gaining importance in allopathic as well as traditional medicine owing to their non-addictive and less toxic nature. Novel antioxidants may offer an effective and safe means of counteracting some of the problems and bolstering the body's defense against free radicals and cardiovascular diseases. *Moringa oleifera* Lam

*Correspondent author

E-mail: arj04@rediffmail.com, abhishekshah@yahoo.com

Tel: 022-24145616 Ext. 428; Fax: 91-22-24145614

(Family-Moringaceae), commonly known as Drum stick tree, grows largely in Asia, tropical Africa and tropical America. Various parts of this tree have been studied for several pharmacological actions. It is reported to be useful as antispasmodic, anti-inflammatory and diuretics⁷. The leaves are reported to have antihypertensive, hypocholesterolaemic, anti-ulcer and wound healing properties⁸⁻¹¹. The hypolipidemic activity of its fruits was also reported by Mehta *et al*¹². The bark extract has been shown to possess anti-fungal and anti-tubercular activity¹³. Moreover, hydro-alcoholic extract of Drumsticks was reported to possess significant antioxidant potential by increasing the activities of catalase, glutathione peroxidase (GPx), glutathione reductase (GR) with decreasing the hepatic MDA level¹⁴. The plant also showed protective effect against antitubercular drugs by decreasing liver lipid peroxidation and by enhancing antioxidant activity¹⁵. Hence, the present study was undertaken to evaluate cardio-protective activity of aqueous extract of *M. oleifera* stem bark (MOA).

Materials and Methods

Plant material and extraction

The fresh bark of *M. oleifera* was collected from the hilly areas of Rahuri, District Ahmednagar, Maharashtra. It was shade dried at room temperature and coarsely pulverized to powdered form. The dried powdered bark was then extracted with distilled water (100 g/l) using maceration technique. The aqueous extract was then dried at 40°C using a vacuum evaporator and investigated for cardioprotective activity. The yield of aqueous extract was found to be 10.2% w/w of dried bark powder.

Experimental animals

Male Wistar rats (150-200 g) were used for cardioprotective activity. The rats were obtained from Haffkine Biopharmaceuticals, Parel, Mumbai. The animals were housed under good hygienic conditions in the departmental animal house. Standard conditions of temperature (22°C ± 2°C), 12 h/12 h light and dark cycle was provided to animals and they were fed with standard pellet diet (Amrut India Ltd., Pune) and had access to water, *ad libitum*. All the experiments were performed in accordance with the Institutional Animal Ethics Committee (IAEC) constituted as per directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals

(CPCSEA), under the Ministry of Animal Welfare Division, Government of India, New Delhi.

Chemicals

Isoproterenol hydrochloride (Sigma, USA) was used to induce myocardial infarction in rats. The biochemical kits used for biochemical analysis were obtained from Merck India Limited, Mumbai. All other chemicals were procured from standard local sources.

Acute toxicity study

Oral acute toxicity study in Wistar rats was carried out for MOA in accordance with OECD (Organization for Economic Co-operation and Development) guideline no. 423. Wistar rats (3 Male + 3 Female) weighing between 150-200 g were used for evaluation of acute toxicity test. Animals were kept on fasting overnight prior to MOA administration. After the period of fasting, animals were weighed and MOA was administered at the dose of 2000 mg/kg by gavage. Following administration of MOA, food was held for further 3-4 h and observations were made at 30 min, 1 h, 2 h, 3 h, 4 h, 24 h and once a day till the day 14. Rats were examined for various observations like weight change, tremors, convulsion, salivation, diarrhoea, lethargy, sleep, coma and death. Along with that the cage side observations like changes in the skin, fur, eyes and behavioral pattern were studied. As the extract did not show any toxic effect at the dose of 2000 mg/kg and was required to dose for 21 days, the toxicity was further studied at the dose of 5000 mg/kg in the similar manner. The extract was found to be safe at the dose of 5000 mg/kg.

Grouping of animals

Animals were grouped into following four groups, each group consisting of six animals.

Control: Administered water as vehicle only.

ISO 85 mg/kg (Negative control): Administered with water as vehicle for 21 days and on 22nd and 23rd day. Isoproterenol (ISO) (85 mg/kg body weight in 0.9% saline, s.c.), was administered at an interval of 24 h¹⁶.

MOA 250 mg/kg: Rats treated with MOA (250 mg/kg, p.o.) for 21 days and on 22nd and 23rd day. Isoproterenol (85 mg/kg body weight in 0.9% saline, s.c.), was administered at an interval of 24 h.

MOA 500 mg/kg: Rats treated with MOA (500 mg/kg, p.o.) for 21 days and on 22nd and 23rd day

Isoproterenol (85 mg/kg body weight in 0.9% saline, s.c.), was administered at an interval of 24 h.

Collection of serum and preparation of tissue

After the experimental period, on 24th day, the blood was collected and serum was separated after centrifugation for 30 min at 3500 rpm at 4°C. The heart tissues were dissected out immediately and washed in ice-cold saline. Heart tissue (1 g) was weighed accurately and homogenized in 10 ml of 0.1 M Tris-HCl buffer (pH 7.4) in ice cold condition. The homogenate was centrifuged at 2500 × g and the clear supernatant solution was taken for the estimation of biochemical parameters. The remaining portion of heart was stored in 10% buffered neutral formalin for histopathological evaluation.

Biochemical assay

Serum triglycerides, total cholesterol and high density cholesterol (HDL) levels were determined enzymatically by colorimetric specific kits (Merck India Ltd.). Low density cholesterol (LDL) and very low density cholesterol (VLDL) were calculated by the method of Varley *et al*¹⁷. Serum marker enzymes, such as alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK) were assayed by using biochemical kits (Merck India Ltd.).

Tissue lipid peroxide level was determined as thiobarbituric acid reactive substances (TBARS) by the method of Okhawa *et al*¹⁸. Reduced glutathione (GSH) was determined by the method of Moron *et al*¹⁹. Glutathione-S-transferase (GST) activity was determined by the method of Mannervik and Guthenberg²⁰ while glutathione reductase (GR) activity was assayed by the method of Shaedle and Bassham²¹. The activity of Glutathione peroxidase (Gpox) and catalase was assayed by the method of Chance and Maehly²². Superoxide dismutase (SOD)

activity was determined according to the method of Kakkar *et al*²³.

Histopathological examination

The heart tissue obtained from all experimental groups were washed immediately with saline and then fixed in 10% buffered neutral formalin solution. After fixation, the heart tissue was processed embedding in paraffin. Then, the heart tissue was sectioned and stained with hematoxylin and eosin and examined under high power microscope and photomicrographs were taken.

Statistical analysis

Results were expressed as Mean ± S.D. from six rats in each group. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnet's test for multiple comparisons with the level of significance chosen at $P < 0.05$.

Results

The levels of serum triglycerides and cholesterol of control and treated animals are given in Table 1. Rats induced with ISO, showed a significant ($P < 0.05$) increase in serum levels of triglycerides, total cholesterol, LDL, VLDL with significant decrease ($P < 0.05$) in level of HDL called good cholesterol when compared to control rats. Oral pretreatment with MOA (250 and 500 mg/kg) to ISO treated rats significantly ($P < 0.05$) decrease the levels of cholesterol, triglycerides, VLDL, LDL and significantly ($P < 0.05$) increase HDL level at 500 mg/kg when compared with ISO alone induced rats.

The effects of MOA on marker enzymes (AST, ALT, CK, LDH) in serum and heart homogenate of ISO treated animals are shown in Tables 2 and 3, respectively. ISO-treated rats showed a significant ($P < 0.05$) increase in serum marker enzymes when compared with control group. Oral pretreatment with

Table 1: Effect of aqueous extract of *Moringa oleifera* stem bark (MOA) pretreatment on Isoproterenol (ISO) induced changes in the serum TGL, TC, LDL, VLDL and HDL

Treatment	TGL (mg/dl)	TC (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)
Control	46.91 ± 3.08	66.13 ± 3.0	18.27 ± 2.81	9.38 ± 0.61	38.48 ± 0.87
ISO (85 mg/kg)	93.82 ± 5.1#	96 ± 3.3#	60.18 ± 3.6#	18.76 ± 1#	17.04 ± 0.6#
MOA (250 mg/kg)	82.72 ± 3.7*	85.6 ± 5.1*	51.62 ± 4.7*	16.54 ± 0.7*	17.5 ± 0.6
MOA (500 mg/kg)	57.38 ± 2.9*	66.7 ± 2.1*	31.02 ± 1.4*	11.4 ± 0.6*	24.19 ± 0.7*

Values are expressed in Mean ± S.D. (n=6), significantly different ($P < 0.05$) from control group (#), ISO group (*). TGL: Triglycerides, TC: Total cholesterol, LDL: Low density cholesterol, VLDL: Very low density cholesterol, HDL: High density cholesterol.

Table 2: Effect of aqueous extract of *Moringa oleifera* stem bark (MOA) pretreatment on Isoproterenol (ISO) induced changes in the activities of serum AST, ALT, LDH and CK

Treatment	AST(U/L)	ALT(U/L)	LDH(U/L)	CK(U/L)
Control	95.68 ± 2.58	31.66 ± 1.77	36.15 ± 4.9	83.24 ± 3.8
ISO (85 mg/kg)	151.2 ± 3.37#	61.69 ± 1.84#	82.56 ± 6.21#	141.4 ± 4.56#
MOA (250 mg/kg)	148.28 ± 4.7	40.5 ± 2.62*	50.47 ± 3.81*	138.1 ± 6.1
MOA (500 mg/kg)	133.17 ± 5.6*	36.43 ± 1.2*	39.68 ± 6.32*	123.4 ± 4.8*

Values are expressed in Mean ± S.D. (n=6), significantly different ($P < 0.05$) from vehicle control group (#), ISO group (*). AST: Aspartate transaminase, ALT: Alanine transaminase, LDH: Lactate dehydrogenase, CK: Creatine Kinase, U/L: Unit per litre

Table 3: Effect of aqueous extract of *Moringa oleifera* stem bark (MOA) pretreatment on Isoproterenol (ISO) induced changes in the activities of heart AST, ALT, LDH and CK

Treatment	AST(U/L)	ALT(U/L)	LDH(U/L)	CK(U/L)
Control	53.19 ± 3.96	109.64 ± 2.34	68.25 ± 2.46	43.24 ± 3.8
ISO (85 mg/kg)	17.11 ± 1.57#	74.26 ± 2.8#	38.11 ± 4.03#	27.34 ± 2.56#
MOA (250 mg/kg)	14.89 ± 0.88	78.33 ± 1.91	41.52 ± 3.68*	28.16 ± 2.1
MOA (500 mg/kg)	23.28 ± 3.79*	112.55 ± 7.53*	53.27 ± 3.9*	38.43 ± 2.8*

Values are expressed in Mean ± S.D. (n=6), significantly different ($P < 0.05$) from control group (#), ISO group (*). AST: Aspartate transaminase, ALT: Alanine transaminase, LDH: Lactate dehydrogenase, CK: Creatine kinase, U/L: Unit per litre.

Table 4: Effect of aqueous extract of *Moringa oleifera* stem bark (MOA) pretreatment on Isoproterenol(ISO) induced changes in the activities of heart antioxidant enzymes and lipid peroxidation

Treatment	Control	ISO (85 mg/kg)	MOA (250 mg/kg)	MOA (500 mg/kg)
SOD	8.6 ± 1.2	4.4 ± 0.9#	4.8 ± 1.1	7.0 ± 0.7*
CAT	21.52 ± 2.8	4.6 ± 1.2#	5.48 ± 1.6	15.24 ± 1.2*
GSH	2.43 ± 0.09	0.77 ± 0.03#	1.14 ± 0.04*	1.53 ± 0.07*
GR	48.1 ± 6.9	20.8 ± 2.0#	27.4 ± 1.8	41.1 ± 2.8*
GST	492 ± 17	170 ± 10#	142 ± 23	206 ± 19*
GPOX	11.92 ± 1.46	2.6 ± 0.54#	3.66 ± 0.7	6.92 ± 1.34*
TBARS	4.35 ± 0.4	6.48 ± 0.48#	5.94 ± 0.3*	4.84 ± 0.3*

Values are expressed in Mean ± S.D. (n=6), significantly different ($P < 0.05$) from control group (#), ISO group (*) [SOD: IU (mg protein)⁻¹; CAT: μ moles of H₂O₂ decomposed (min)⁻¹ (mg protein)⁻¹; Reduced glutathione (GSH): μ g (mg protein)⁻¹; Glutathione reductase(GR): μ mol NADH oxidized min⁻¹ (mg protein)⁻¹; Glutathione-S-transferase (GST): nmoles CDNB conjugate formed min⁻¹ mg protein⁻¹; Glutathione peroxidase (Gpox): μ mol of tetraguaiacol formed min⁻¹mg protein⁻¹]

MOA 250 mg/kg to ISO treated rats significantly ($P < 0.05$) decrease the levels of LDH and ALT in serum while MOA 500 mg/kg decrease the levels of AST, ALT, CK and LDH in serum. In heart homogenate, ISO-treated group showed a significant ($P < 0.05$) decrease in marker enzymes when compared with control, which return to normal by pretreatment with MOA.

Table 4 illustrates the effect of MOA on activities of antioxidant enzyme (SOD, catalase, GR, GST, G_{POX}, GSH and TBARS) in heart homogenate of ISO treated animals. The activities of SOD, catalase, GR,

GST, G_{POX} and GSH were lowered significantly ($P < 0.05$) and the level of lipid peroxidation (TBARS) was increased significantly ($P < 0.05$) owing to the myocardial infarction in ISO-induced rats when compared with control group. Oral pretreatment with MOA at 500 mg/kg successfully ($P < 0.05$) prevented the decrease in the activity of these enzymes and resulted in decreased TBARS formation. This effect was probably owing to removal of excess free radicals generated by isoproterenol.

Plate 1 shows the effect of MOA on the degree of histopathological changes in myocardial tissues of the

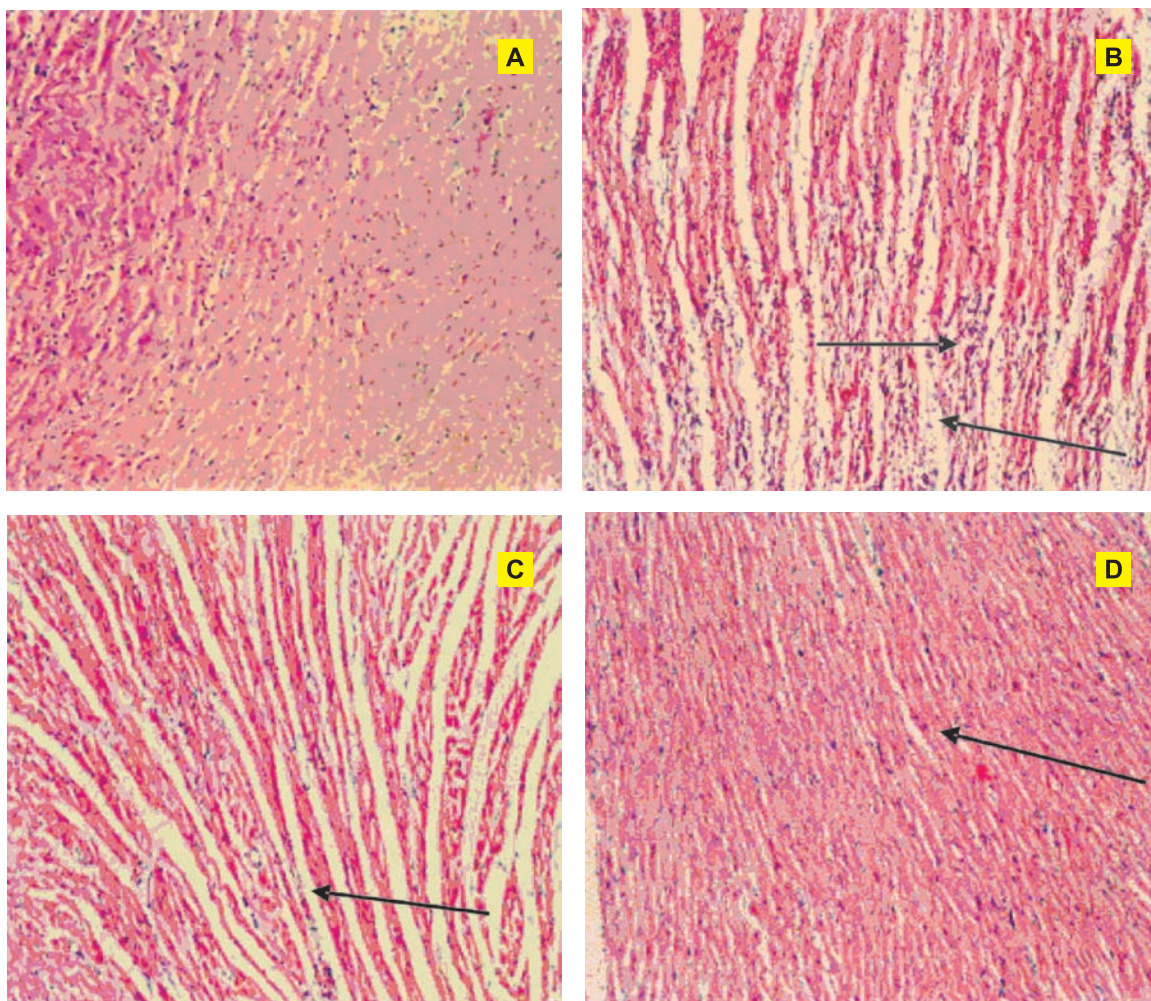


Plate 1: Effect of treatment on histopathology of heart (10x); A. Control, untreated group shows normal cardiac fibers without any fraying of infarction; B. ISO (85 mg/kg) alone treated group shows infarcted zone with oedema and inflammatory cells; C. MOA (250 mg/kg + ISO 85 mg/kg) treated group shows moderate oedema and inflammatory cells with decreased area of coagulative necrosis of myocardial fibers; D. MOA (500 mg/kg + ISO 85 mg/kg) treated group shows mild oedema but no infarction and inflammatory cells.

ISO-induced rats. Histopathological findings of the ISO induced myocardium showed infarcted zone with oedema and inflammatory cells and separation of cardiac muscle fibers. Oral pretreatment with MOA 250 mg/kg showed myocardium with decreased area of infarction with coagulative necrosis and inflammatory cells with moderate oedema. Oral pretreatment with MOA 500 mg/kg showed myocardium with mild oedema but no infarction and inflammatory cells and the cardiac fibers were within the normal limits. For all the parameters studied, oral pretreatment of MOA (250 and 500 mg/kg) to ISO induced rats for a period of 21 days showed a significant ($P < 0.05$) improvement in the myocardial infarction and indicates the prophylactic cardio protective effect of MOA.

Discussion

The effects of ISO on heart are mediated through β_1 and β_2 adrenoceptors. Both β_1 and β_2 adrenoceptors mediate the positive inotropic and chronotropic effects to β adrenoceptor agonists²⁴. Thus, ISO produces relative ischemia or hypoxia due to myocardial hyperactivity and coronary hypotension²⁵. Additionally, ISO causes myocardial ischemia due to excessive production of free radicals resulting from oxidative metabolism of catecholamines²⁶. Grimm *et al*²⁷ have reported that a toxic dosage of ISO caused characteristic myocardial damage that subsequently resulted in heart failure.

ISO administration has been reported to stimulate adenylate cyclase activity resulting in enhanced cAMP formation which enhances lipid biosynthesis

resulting in hyperlipidemia²⁸. In the present investigation ISO administration increased TG, TC, LDL and VLDL levels with a significant decrease in HDL. The levels of TG and cholesterol were significantly reduced by pretreatment with MOA, which could be due to the increased activity of extrahepatic lipoprotein lipase²⁹ which is responsible for the circulating lipoprotein in a non-atherogenic direction by efficient lipolysis of triglyceride rich lipoprotein in heart, skeletal muscle and adipose tissue³⁰⁻³². Present study also showed the significant decrease in HDL in ISO treated animals, which was prevented by pretreatment with MOA at 500 mg/kg. HDL is known to be involved in the transport of cholesterol from tissues to the liver for excretion into the bile and thus called "good cholesterol". Thus the cholesterol lowering activity of MOA could be mediated through increasing the activity of extrahepatic lipoprotein lipase which increased hydrolysis of triglycerides that result in the transfer of lipids and apolipoproteins to HDL and thereby facilitate their excretion.

The free radicals generated by ISO challenge, initiate lipid peroxidation of membrane bound polyunsaturated fatty acids, leading to an impairment of structural and functional integrity of myocardial membrane³³. The need for assessing the size of experimental infarction arises while evaluating the drugs for the beneficial effect against myocardial infarction. The serum enzymes serve as sensitive indices to assess the severity of myocardial infarction.

Myocardium contains an abundant concentration of many enzymes, viz. AST, CK and LDH and once metabolically damaged releases its content into extracellular fluid³⁴. Hence, in ISO-induced rats, the increased activities of serum ALT, AST, LDH and CK accompanied by their concomitant reduction in heart homogenate confirm the onset of myocardial necrosis³⁵. Pretreatment with MOA showed the normalization of the activity of diagnostic marker enzymes when compared with isoproterenol treated rats, indicating the antioxidant potential of MOA, which protects heart from lipid peroxidative damage.

Pharmacological augmentation of endogenous myocardial antioxidants has been identified as a promising therapeutic approach in diseases associated with increased oxidative stress. In present study, ISO-induced rats exhibited decrease activities of SOD and catalase in the heart. SOD plays an important role in protecting the cells from oxidative damage by

converting superoxide radicals into hydrogen peroxide, which is further metabolized by catalase to molecular oxygen and water. The decrease in the activities of these antioxidant enzymes might be due to myocardial cell damage. Superoxide radicals generated at the site of damage modulates SOD and catalase resulting in the decreased activities of these enzymes and accumulation of superoxide anion, which also damages the myocardium³⁶. Pretreatment with MOA significantly increased the activities of SOD and catalase in the heart of ISO-induced rats, which demonstrated an important role of MOA in regulating antioxidative capacity.

Decreased concentration of GSH in the heart and decreased activities of glutathione dependent enzymes such as Gpx, GR and GST in the heart of ISO-induced rats were observed in present study. GSH is an abundant and ubiquitous antioxidant, a tripeptide and essential biofactor synthesized in all living cells. It functions mainly as an effective intracellular reductant³⁷ and protects the cells from free radical mediated damage caused by drugs and ionizing radiation. It forms an important substrate for Gpx, GR, GST and several other enzymes, which is involved in the free radical scavenging action³⁸. In the heart, Gpx is a major enzymatic mechanism for the disposal of peroxides, a prolonged depression in the activity of this enzyme may lead to the intracellular peroxide accumulation. Gpx catalyzes the reduction of hydroperoxides and hence increasing the levels of GSSG (glutathion disulfide). On the other hand, GR is primarily responsible for the reduction of GSSG and for maintenance of the GSH/GSSG ratio in cells and thus the level of GSH³⁹. GST acts like peroxidase and removes the stable peroxides from the system, resulting in the reduction of peroxide-induced damage³⁸. Decreased GSH levels might be due to increased utilization in protecting 'SH' containing proteins from lipid peroxides. The unavailability of GSH may decrease the activities of Gpx, GR and GST in ISO-induced rats. Pretreatment with MOA significantly increased the concentration of GSH in the heart and the activities of Gpx, GR and GST in the heart of ISO-induced rats.

The histopathological findings of the ISO-induced myocardium showed infarcted zone with oedema and inflammatory cells and separation of cardiac muscle fibers. Pretreatment of MOA (250 mg/kg) showed decreased area of infarction with coagulative necrosis and inflammatory cells with moderate oedema in

myocardium. There was mild oedema but no infarction and inflammatory cells and the cardiac fibers were within the normal limits in myocardium of rats pretreated with MOA (500 mg/kg). The protection might have been mediated through MOA induced increase in basal myocardial antioxidant enzyme activities.

Conclusion

The prophylactic cardioprotective use of stem bark of the plant *M. oleifera* has been confirmed, as the extract displayed hypolipidemic and antioxidant effect in ISO-induced cardiotoxicity. Further, it would be interesting to isolate the possible constituents that are responsible for cardioprotective activity and establish the possible mechanism(s) of action. We are working on isolation of cardioprotective compounds from this extract, and this will be reported at a later date.

Acknowledgement

The authors are highly thankful to University Grant Commission for the generous financial support. Thanks are due to Dr. Ganesh Iyer, Botanist at Ramnarayan Ruia College, Mumbai-400019 for authentication of the fresh bark of *M. oleifera*. For histopathological studies, assistance of Dr. D. P. Chaudhari, Animal house Incharge, Haffkine Biopharmaceuticals, Parel, Mumbai is gratefully acknowledged.

References

- 1 Anonymous, From what will die in 2020? *Lancet*, 1997, **349**, 1263-1263.
- 2 Das DK and Maulik N, Protection against free radical injury in the heart and cardiac performance, *In: Sen CK, Packer L, Hanninen O, eds., Exercise and Oxygen Toxicity*, Elsevier Science, Amsterdam 1995, 359-388.
- 3 Vaage J and Valen G, Pathophysiology and mediators of ischemiareperfusion injury with special reference to cardiac surgery, *Scand J Thorac Cardiovasc Surg*, 1993, **41**, 1-18.
- 4 Wexler BC, Myocardial infarction in young vs. old male rats; pathophysiologic changes, *Amer Heart J*, 1978, **96**, 70-80.
- 5 Yates JC and Dhala NS, Induction of necrosis and failure in the isolated perfused rat heart with oxidized isoproterenol, *J Mol Cell Cardiol*, 1975, **7**, 807-816.
- 6 Knuffman NM, Van Der Laarse A, Vliegen HW and Brinkman CJ, Quantification of myocardial necrosis and cardiac hypertrophy in isoproterenol-treated rats, *Res Commun Chem Pathol Pharmacol*, 1987, **57**, 15-32.
- 7 Caceres A, Saravia A, Rizzo S, Zabala L, De Leon E and Nave F, Pharmacologic properties of *Moringa oleifera* II: Screening for antispasmodic, antiinflammatory and diuretic activity, *J Ethnopharmacol*, 1992, **36**, 233-237.
- 8 Dangi SY, Jolly CI and Narayanan S, Antihypertensive activity of the total alkaloids from the leaves of *Moringa oleifera*, *Pharm Biol*, 2002, **40**, 144-148.
- 9 Ghasi S, Nwobodo E and Ofili JO, Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam. in high-fat diet fed Wistar rats, *J Ethnopharmacol*, 2000, **69**, 21-25.
- 10 Pal SK, Mukherjee PK and Saha BP, Studies on the antiulcer activity of *Moringa oleifera* leaf extract on gastric ulcer models in rats, *Phytother Res*, 2006, **9**, 463-465.
- 11 Rathi BS, Bodhankar SL and Baheti AM, Evaluation of aqueous leaves extract of *Moringa oleifera* Linn for wound healing in albino rats, *Indian J Exp Biol*, 2006, **44**, 898-901.
- 12 Mehta K, Balaraman R, Amin AH, Bafna PA and Gulati OD, Effect of fruits of *Moringa oleifera* on the lipid profile of normal and hypercholesterolaemic rabbits, *J Ethnopharmacol*, 2003, **86**, 191-195.
- 13 Bhatnagar SS, Santapau H, Desai JDH, Yellore S and Rao TNS, Biological activity of Indian medicinal plants. Part I. Antibacterial, antitubercular and antifungal action, *Indian J Med Res*, 1961, **49**, 799-805.
- 14 Bharali R, Tabassum J and Azad MR, Chemomodulatory effect of *Moringa oleifera* Lam, on hepatic carcinogen metabolising enzymes, antioxidant parameters and skin papillomagenesis in mice, *Asian Pac J Cancer Prev*, 2003, **4**, 131-139.
- 15 Ashok Kumar N and Pari L, Antioxidant action of *Moringa oleifera* Lam. (Drumstick) against antitubercular drugs induced lipid peroxidation in rats, *J Med Food*, 2003, **6**, 255-259.
- 16 Prabhu S, Jainu M, Sabitha KE and Shyamala Devi CS, A study on the beneficial effect of mangiferin on isoproterenol induced myocardial infarction in rats-research article, *Pharmacol Mag*, 2005, **1**, 92-96.
- 17 Varley H, Gowenlock AH and Bell M, Practical Clinical Biochemistry, 5th ed. Heinemann Medical Book, London, 1983, 625-685.
- 18 Okhawa H, Ohishi N and Yagi K, Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction, *Anal. Biochem*, 1979, **95**, 351-358.
- 19 Moron MS, Depierre JW and Manrerirk B, Levels of glutathione, glutathione reductase and glutathione peroxidase in rat lung and liver, *Biochem Biophys Acta*, 1979, **582**, 67-78.
- 20 Mannervik B and Guthenberg C, Glutathione transferase (Human placenta), *Meth Enzymol*, 1981, **77**, 231-235.
- 21 Shaedle M and Bassham JA, Chloroplast glutathione reductase, *Plant Physiol*, 1977, **59**, 1011-1012.
- 22 Chance B and Maehly AC, Assay of catalase and peroxidases, *Meth Enzymol*, 1955, **2**, 764-755.
- 23 Kakkar P, Das B and Vishwanathan PN, A modified spectrophotometric assay of superoxide dismutase, *Indian J Biochem Biophys*, 1984, **21**, 130-132.
- 24 Brodde OE, β_1 and β_2 adrenoceptors in the human heart: properties, function, and alteration in chronic heart failure, *Pharmacol Rev*, 1991, **43**, 203-242.
- 25 Yeager JC and Iams SG, The haemodynamics of isoproterenol- induced cardiac failure in rats, *Circ Shock*, 1981, **81**, 151-163.
- 26 Singal PK, Beamich RE and Dhalla NS, Potential oxidative pathways of catecholamines in the formation of lipid peroxides and genesis of heart disease, *Adv Exp Med Biol*, 1983, **161**, 391-440

- 27 Grimm D, Elsner D, Schunkert H, Pfeifer M, Griese D, Bruckschlegel G, Muders F, Riegger GAJ and Kromer EP, Development of heart failure following isoproterenol administration in the rat: role of rennin-angiotensin system, *Cardiovasc Res*, 1998, **37**, 91-100.
- 28 Subhash D, Narinder KK and Nityanand S, Effect of isoprenaline on lipid profile and cardiac enzymes in rats, *Indian J Exp Biol*, 1978, **16**, 376-378.
- 29 Ithayarasi AP and Shyamala Devi CS, Effect of α -tocopherol on isoproterenol induced changes in lipid and lipoprotein profile in rats, *Indian J Pharmacol*, 1997, **29**, 399-404.
- 30 Shimada M, Ishibashi S, Inaba T, Yagyu H, Harada K, Osuga JI, Ohashi K, Yazaki Y and Yamada N, Suppression of diet-induced atherosclerosis in low density lipoprotein receptor knockout mice over expressing lipoprotein lipase, *Proc Natl Acad Sci, USA* 1996, **93**, 7242-7246.
- 31 Zsigmond E, Kobayashi K, Tzung KW, Li L, Fuke Y and Chan L, Adenovirus-mediated gene transfer of human lipoprotein lipase ameliorates the hyper-lipidemias associated with apolipoprotein E and LDL receptor deficiencies in mice, *Hum Gene Ther*, 1977, **8**, 1921-1933.
- 32 Yagyu H, Ishibashi S, Chen Z, Osuga JI, Okazaki M, Perrey S, Kitamine T, Shimada M, Ohashi K, Harada K, Shionoiri F, Yahagi N, Gotoda T, Yazaki Y and Yamada N, Over expressed lipoprotein lipase protects against atherosclerosis in apolipoprotein E knockout mice, *J Lipid Res*, 1999, **40**, 1677-1685.
- 33 Suchalata S and Shyamala Devi CS, Protective effects of *Terminalia chebula* against experimental myocardial injury induced by isoproterenol, *Indian J Exp Biol*, 2004, **42**, 174-178.
- 34 Sharma M, Kishore K, Gupta SK, Joshi S and Arya DS, Cardioprotective potential of *Ocimum sanctum* in isoproterenol induced myocardial infarction in rats, *Mol Cell Biochem*, 2001, **225**, 75-79.
- 35 Saxena KK, Gupta B and Srivastava VK, Creatine kinase and aspartate transaminase in experimental model to predict size of cardiac infarct, *Indian J Exp Biol*, 1998, **26**, 235-236.
- 36 Rajadurai M and Stanely Mainzen Prince P, Preventive effect of naringin on lipid peroxides and antioxidants in isoproterenol-induced cardiotoxicity in Wistar rats, Biochemical and histopathological evidences, *Toxicology*, 2006, **228**, 259-268
- 37 Rahman I and MacNee W, Lung glutathione and oxidative stress: Implications in cigarette smoking-induced airway disease, *Amer J Physiol*, 1999, **277**, 1067-1088.
- 38 Jagetia GC, Reddy TK and Kedlaya R, Influence of naringin on ferric iron induced oxidative damage *in vitro*, *Clin Chim Acta*, 2004, **347**, 189-197.
- 39 Jamieson DJ, Oxidative stress responses of the yeast *Saccharomyces cerevisiae*, *Yeast*, 1998, **14**, 1511-1527.