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Cardioprotective Effect of *Bacopa monneira* Against Isoproterenol-Induced Myocardial Necrosis in Rats

[1]Mukesh Nandave, [1]Shreesh K. Ojha, [1]Sujata Joshi, [2]Santosh Kumari and [1]Dharamvir S. Arya
[1]Department of Pharmacology, All India Institute of Medical Sciences,
New Delhi-110029, India
[2]Division of Plant Physiology, Indian Agriculture Research Institute,
Pusa, New Delhi-110012, India

Abstract: The effects of standardized hydro-alcoholic lyophilized extract of *Bacopa monneira* (Bm) in isoproterenol (ISP)-induced myocardial necrosis were studied. Wistar albino male rats were randomly divided to sham, ISP control and *Bacopa monneira* treated groups. *Bacopa monneira* was administered in doses of 50, 100, 150 or 200 mg kg^{-1} orally for 30 days to *Bacopa monneira* treated groups while sham and ISP control groups received saline orally for the same duration. On day 29 and 30, ISP (85 mg kg^{-1}) was administered subcutaneously at an interval of 24 h to ISP control and *Bacopa monneira* treated groups. On day 31, hemodynamic parameters were recorded before all rats were sacrificed. Hearts were excised and processed for biochemical, histopathological and ultrastructural assessment. Significant cardiac dysfunction, decline in endogenous antioxidant defence [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx) and reduced glutathione (GSH)], myocyte specific injury markers [myocardial lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) isoenzyme] as well as increase in lipid peroxidation marker [malonaldialdehyde (MDA)] were observed in ISP control group as compared to sham control. Of the different doses studied, *Bacopa monneira* (150 mg kg^{-1}) produced maximum cardioprotection as evidenced by significant restoration of endogenous antioxidants, myocardial LDH and CK-MB isoenzyme activities and decrease in MDA. Histopathological and ultrastructural findings also reconfirmed the cardioprotective effect of the extract. The significance of these results is discussed in relation to cardioprotective effects of *Bacopa monneira* against ISP-induced cardiotoxicity.

Key words: Isoproterenol, cardiotoxicity, *Bacopa monneira*, antioxidants

INTRODUCTION

Myocardial Infarction (MI) is a disease with major health and socio-economic implications for both the individual patient and society. The current therapy for MI includes Angiotensin-Converting Enzyme (ACE) inhibitors, beta-adrenergic receptor antagonists and antiplatelet agents. However, the presently available options for the pharmacotherapy of MI are still inadequate in reducing the high mortality and thus novel and effective therapeutic modalities are needed for the treatment of MI.

The deleterious role of Free Radicals (FRs) in myocardial damage induced by ischemia is well established. A growing body of evidence demonstrates increased rate of production of FRs in ischemic heart disease (Dhalla *et al.*, 2000). Under normal physiological circumstances, formation of FRs is limited by endogenous antioxidant defense system. In ischemic heart disease, the production of FRs is increased at a rate that overwhelms the capacity of endogenous antioxidant defense system for detoxification, thereby resulting in FR mediated oxidative damage (Cuzzocrea *et al.*, 2001). Herbal medicines possessing antioxidant and free radical scavenging activities may, therefore, have protective role in cardiovascular diseases and provide viable alternatives. There is growing trend towards use of herbal medicines worldwide to treat a wide range of pathological conditions including cardiovascular diseases. This may be due to their relative safety, lack of significant side effects and generally lower cost compared to conventional medicine (Kamboj, 2000). *Bacopa monniera* (Family: Scrophulariaceae), commonly known as Brahmi, has been widely used in Ayurveda, the Indian System of Medicine. It is a glabrous somewhat succulent, creeping herb, rooting at the nodes, with numerous prostrate branches. The leaf and flower bearing stems are 10-30 cm long and arise from creeping stems that form roots at the nodes.

Corresponding Author: Dr. D.S. Arya, Department of Pharmacology, All India Institute of Medical Sciences, New Delhi-110029, India Tel: +91-11-26588500/26588700 Fax: +91-11-265882663/+91-11-26588641

The growth habit of Bacopa therefore resembles that of peppermint. Medicinal properties and uses of *Bacopa monniera* have been well documented in Charak Samhita (100 AD), an ayurvedic treatise, in which its role in the management of anxiety, poor cognition and lack of concentration has been mentioned. In India and Pakistan, the plant has been used as a cardiac tonic, gastro-protective and diuretic. These beneficial effects are thought to stem from its antioxidant, adaptogenic and anti-inflammatory properties (Rai *et al.*, 2003; Pawar *et al.*, 2001; Sairam *et al.*, 2001; Bhattacharya *et al.*, 2000; Tripathi *et al.*, 1996). However, no study has investigated its cardioprotective potential against exogenous stress in the heart.

The present study was conducted to test whether *Bacopa monniera* extract offers myocardial protection in a model of ISP-induced myocardial necrosis in rats. The present investigation also aimed to understand the physiological and biochemical mechanism of its therapeutic effects by examining hemodynamic, endogenous antioxidants and lipid peroxidation markers. Results were further confirmed by histopathological and ultrastructural examination.

MATERIALS AND METHODS

Plant material and composition of extract: Standardized lyophilized hydro-alcoholic whole plant extract of *Bacopa monniera* was procured from Sanat Products Ltd. (New Delhi, India). The extractive value of 1 g sample in water was 58.14% and in 50% v/v methanol was 52.04%. The fractions were analyzed by high performance liquid chromatography (HPLC, Waters, USA). The extract obtained was of the highest purity with 28.26% w/w of bacosides A and B on dried weight basis. A specimen (No. CVS-1415) has been kept at the Cardiovascular Laboratory, Department of Pharmacology, All India Institute of Medical Sciences (AIIMS), New Delhi for further reference.

Chemicals: Isoproterenol was procured from Sigma Chemicals, (St Louis, USA) and all other chemicals used in the study were of analytical grade.

Animals: Wistar albino male rats, weighing 150 to 200 g, were used in the study. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee and conformed to the Indian National Science Academy Guidelines for the Use and Care of Experimental Animals in research. This study was conducted in Department of Pharmacology, AIIMS from August 2006 to January 2007. Animals were obtained from the Central

Animal House Facility of AIIMS, New Delhi, India and were housed in polyacrylic cages ($38\times23\times10$ cm) with not more than four animals per cage. They were housed under standard laboratory conditions with 12 h light/12 h dark cycles and maintained at humidity of $50\pm5\%$ and an ambient temperature of $24\pm2°C$. All experiments were performed between 9:00 and 16:00 h. The animals were allowed free access to standard pellet diet (Ashirwad; India) and tap water *ad libitum*. The commercial pellet diet contained 55% carbohydrates, 24% protein, 5% fat, 0.6% calcium, 0.3% phosphorous, 4% fiber, 10% moisture and 9% ash w/w. The animals were allowed to acclimatize for one week before the experiments.

Treatment protocol: The animals were randomly allocated into three main groups. Sham and ISP control rats were administered 0.9% normal saline orally once daily for one month. *Bacopa monniera* treated rats were further divided into four subgroups and received standardized lyophilized extract of *Bacopa monniera* at doses of 50, 100, 150 or 200 mg kg^{-1} once daily for 1 month. The animals in ISP control and *Bacopa monniera* treated groups were administered ISP (85 mg kg^{-1}, subcutaneously) on day 29 and 30 at an interval of 24 h (Diaz-Munoz *et al.*, 2006; Li *et al.*, 2006; Chauhan and Naik, 2005; Rona, 1985).

On day 31, all rats were anesthetized intraperitoneally with pentobarbitone sodium (60 mg kg^{-1}). Atropine (4 mg kg^{-1}) was administered along with the anesthetic to maintain the heart rate, especially during surgery and to reduce tracheobronchial secretions. Body temperature was monitored and maintained at 37°C during the experimental protocol. The neck was opened with a ventral midline incision to perform tracheostomy. The rats were ventilated with room air from a positive pressure ventilator (Inco, Ambala, India) using compressed air at a rate of 90 strokes min^{-1} and a tidal volume of 10 mL kg^{-1}. Ventilator setting and PO$_2$ were adjusted as needed to maintain the arterial blood gas parameters within the physiological range. The left jugular vein was cannulated with polyethylene tube for continuous infusion of normal saline solution. The right carotid artery was cannulated with a cannula filled with heparinized saline and connected to CARDIOSYS CO-101 (Experimentria, Hungary) using a pressure transducer for the measurement of MAP and HR. After the recording of hemodynamic parameters, all rats were sacrificed with an overdose of anesthetic (sodium pentobarbitone 100 mg kg^{-1}, intravenously). Hearts were excised and immediately processed for histopathological and ultrastructural studies. For biochemical analysis, the hearts were snapped frozen in liquid nitrogen until analysis. Out of 16 rats in each group, 8 rats were used for hemodynamic and biochemical studies while the remaining 8 rats were used for histopathological (4 rats)

and ultrastructural (4 rats) studies. The standardized isoproterenol dose (85 mg kg⁻¹, subcutaneously) was selected according to the previous studies (Sharma *et al.*, 2001).

Biochemical studies: The frozen hearts were thawed and weighed. A 10% homogenate of myocardial tissue was prepared in 50 mM phosphate buffer, pH 7.4 and an aliquot was used for the assay of malonaldialdehyde according to the method described by Ohkawa *et al.* (1979). The homogenate was centrifuged at 7000 rpm for 15 min and the supernatant was used for estimation of antioxidant parameters: superoxide dismutase (Misra and Fridovich, 1976), catalase (Aebi, 1974), glutathione peroxidase (Paglia and Valentine, 1967) and reduced glutathione (Maron *et al.*, 1979). Lactate dehydrogenase (Cabaud and Wrobleuski, 1958) and creatine kinase-MB isoenzyme (Lamprecht *et al.*, 1974), myocyte injury marker enzymes were also estimated in the same supernatant. Protein was estimated by method of Lowry *et al.* (1951).

Histopathological studies: At the end of the experiment, hearts were excised and immediately fixed in 10% buffered neutral formalin solution. The fixed tissues were embedded in paraffin and serial sections (5 μm thick) were cut. Each section was stained with hematoxylin and eosin (H and E). The sections were examined under the light microscope (Nikon. Tokyo, Japan) and photomicrographs were taken. Representative area images were captured in an image analysis system. The Image Analyzer consisted of BX-50 Research Microscope (Olympus, Japan), Coolsnap 10 bit Digital Camera (Media Cyberneticus, USA) and Pentium 4 computer (Compaq, India) with an image analysis software Image Plus Pro (Media Cyberneticus, USA).

Ultrastructural study (transmission electron microscopy): At the end of experiment, small pieces of myocardial tissue (approximately 1-2 mm in thickness) were immediately fixed in ice-cold Karnovsky's fixative. The tissues were then washed in phosphate buffer (0.1M, pH 7.4) and postfixed for 2 h in 1% osmium tetroxide in the same buffer at 4°C. The specimens were then washed in phosphate buffer, dehydrated with graded acetone and then embedded in araldite CY 212 to make

tissue blocks. The semithin as well as ultrathin sections (80-100 nm) were cut by an ultramicrotome (Ultracut E, Reichert, Austria). The sections were stained with uranyl acetate and lead acetate and examined under transmission electron microscope (Morgagni 268 D, Fei Co., The Netherlands) operated at 60 KV. At least four hearts from each group were examined for ultrastructural changes.

Statistical analysis: Summary statistics, mean and standard deviation, were calculated for all variables for each group. One-way Analysis of Variance (ANOVA) was applied for statistical analysis and Student's t-test was used for biochemical analysis. The $p<0.05$ was considered as the statistical significance level.

RESULTS

Effect of pre- and co-treatment with *Bacopa monniera* on ISP-induced hemodynamic changes: Table 1 shows mean±SD values of Mean Arterial Pressure (MAP) and Heart Rate (HR) from sham, ISP control and Bm treated groups. In ISP control group, both the hemodynamic variables that are MAP and HR remained depressed throughout the experimental duration as compared to sham baseline values. *Bacopa monniera* treatment significantly restored the MAP at a dose of 150 and 200 mg kg⁻¹ day⁻¹ ($p<0.05$) but failed to restore MAP at the dose of 50 and 100 mg kg⁻¹ day⁻¹ as compared to ISP control. However, brahmi treatment significantly restored the HR only at a dose of 150 mg kg⁻¹ day⁻¹ ($p<0.05$) and failed to restore the HR at doses of 50, 100 and 200 mg kg⁻¹ day⁻¹.

Effect of *Bacopa monniera* on endogenous antioxidant enzymes and lipid peroxidation marker: In the present study, ISP administration resulted in significant depletion of myocardial antioxidant enzymes: SOD, CAT and GSHPx ($p<0.05$, Table 2) compared to sham control group. In *Bacopa monniera* 150 and 200 mg kg⁻¹ treated groups, a significant increase in the activity of SOD ($p<0.05$, Table 2) was observed as compared to ISP control group. *Bacopa monneira* at doses of 100, 150 and 200 mg kg⁻¹ significantly restored the activities of CAT ($p<0.05$, Table 2) and GSHPx ($p<0.05$, Table 2) as compared to ISP control. Isoproterenol administration also resulted in significant decline in myocardial GSH content

Table 1: Effect of *Bacopa monniera* (Bm) treatment on hemodynamic parameters in different experimental groups

Groups	Sham	ISP control	ISP + Bm-50	ISP + Bm-100	ISP + Bm-150	ISP + Bm-200
MAP (mmHg)	135±10.0	095±9.9#	105.00±7.69	102.09±8.9	127±10.0*	110.90±7.91*
HR (Beats/min)	385±25.5	311±30.9#	278.01±33.09	280.09±34.19	365±19.9*	301.25±52.03

MAP: Mean Arterial Pressure; HR: Heart Rate. #: $p<0.05$ vs Sham; *: $p<0.05$ vs ISP control. The values are mean±SD of eight experiments
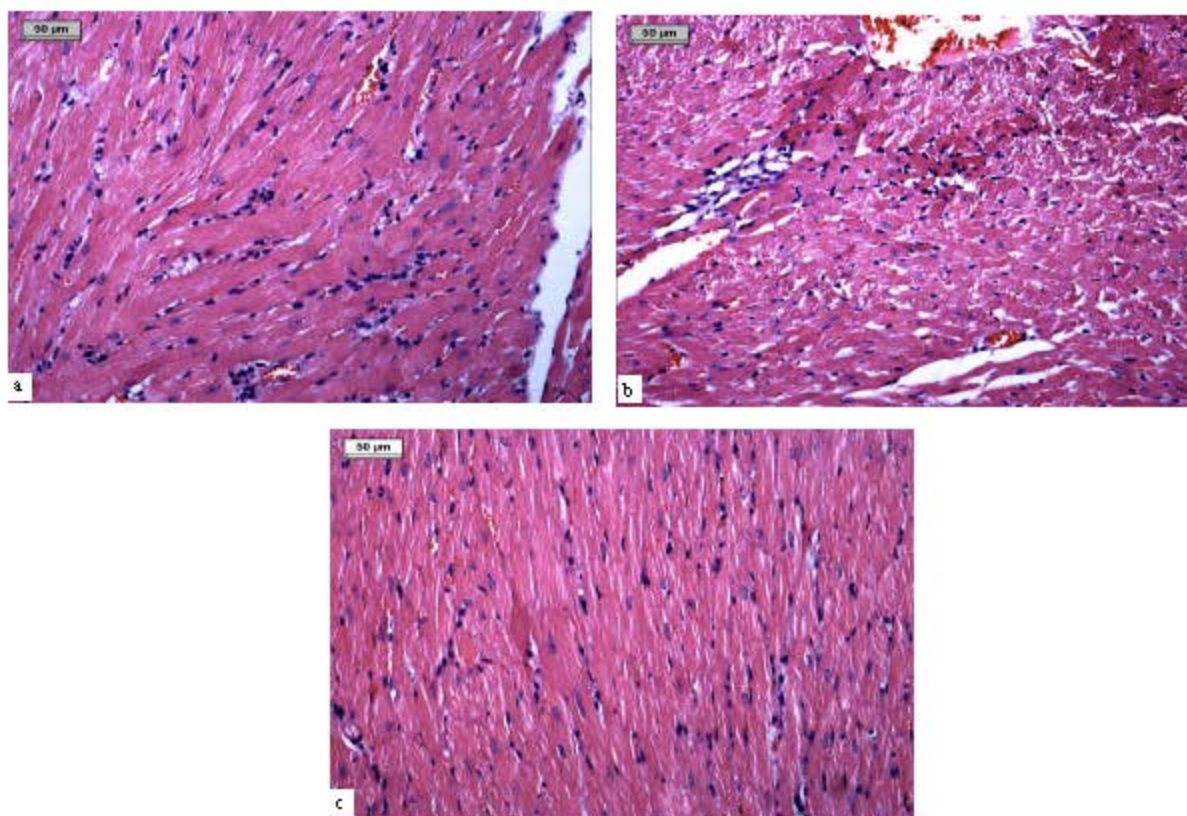
Fig. 1: a) Photomicrograph showing normal architecture of rat heart of sham group. Endocardium and pericardium are seen within normal limits with no infiltration of inflammatory cells (H and E X100). (b) Photomicrograph of rat heart subjected to isoproterenol-induced focal myonecrosis with myophagocytosis and lymphocytic infiltration. In subendocardium vacuolar changes and prominent oedema along with chronic inflammatory cells are clearly visible (H and E X100) and (c) Photomicrograph of rat heart of the *Bacopa monneira* treated group administered 150 mg kg^{-1} showing decreased degree of myonecrosis and lesser infiltration of inflammatory cells (H and E X100)

(p<0.05, Table 2) as compared to sham. However, significant restoration in GSH levels (p<0.05, Table 2) was observed when rats were pre- and co-treated with *Bacopa monneira* at 150 and 200 mg kg^{-1} doses as compared to ISP control group.

It is also observed that myocardial lipid peroxidation marker, MDA, (p<0.05, Table 3) was significantly elevated in the ISP control group in comparison to sham. However, *Bacopa monneira* (100, 150 and 200 mg kg^{-1}) treatment significantly (p<0.05) prevented the rise in myocardial content of MDA following ISP induced myocardial injury.

Effect of *Bacopa monniera* on myocyte injury markers: MDA levels generally correlated inversely with myocardial creatine kinase-MB (CK-MB) isoenzyme and lactate dehydrogenase (LDH) activity. The levels of myocardial LDH and CK-MB isoenzyme were depleted significantly (p<0.05, Table 3) in the ISP control group. Chronic treatment with *Bacopa monneira* at the doses of 100, 150 and 200 mg kg^{-1} demonstrated significant

restoration of CK-MB isoenzyme activities (p<0.05, Table 3) as compared to ISP control. However, the myocardial LDH activity was significantly restored by *Bacopa monniera* only at doses of 150 and 200 mg kg^{-1}.

Effect of *Bacopa monniera* on histoarchitectural changes: As compared to sham control group (Fig. 1a), hearts from ISP control group showed significant myonecrosis, vacuolar changes and edema along with fibroblastic proliferation and infiltration of chronic inflammatory cells (Fig. 1b). However, hearts from *Bacopa monniera* 150 mg kg^{-1} (Fig. 1c) treated rats showed significant improvement in the degree of myonecrosis, infiltration of inflammatory cells and lesser vacuolar changes as well as edema compared to the ISP control.

Effect of pre and co-treatment with Bm on ISP-induced ultrastructural changes: The ultrastructural changes observed in different experimental groups are presented in (Fig. 2 a-d). The ultrastructure of the myocardium from

Table 2: Effect of *Bacopa monniera* (Bm) on myocardial antioxidants in different experimental groups

Groups	Sham	ISP control	ISP + Bm-50	ISP + Bm-100	ISP + Bm-150	ISP + Bm-200
SOD (Units mg^{-1} protein)	7.94±2.90	3.40±1.40*	3.90±0.74	5.10±1.19	6.78±1.35*	5.43±1.28*
CAT (Units mg^{-1} protein)	21.10±3.10	11.90±2.00*	16.99±6.34	20.51±8.75*	23.70±6.710*	19.67±6.09*
GSH (µmol g^{-1} tissue)	1.86±0.69	1.22±0.50*	1.30±0.15	1.25±0.09	1.59±0.31*	14.5±0.40*
GSHPx (Units mg^{-1} protein)	0.33±0.12	0.19±0.08*	0.19±0.03	0.36±0.09*	0.44±0.12*	0.37±0.04*

SOD: Superoxide dismutase; CAT: Catalase; GSH: Reduced Glutathione; GSHPx: Glutathione peroxidase. The values are mean±SD of eight experiments. *: $p<0.05$ vs sham; *: $p<0.05$ vs ISP control. One unit of SOD inhibits the rate of auto-oxidation of adrenaline by 50% at pH 7 at 25°C. One unit of CAT activity represents amount of enzyme required to decompose 1 µmol of H_2O_2/min. One unit of GSHPx activity is defined as amount of enzyme required to utilize 1 mmol of NADPH/min at 25°C

Table 3: Effect of *Bacopa monniera* (Bm) on lipid peroxidation and myocyte injury markers in different experimental groups

Groups	Sham	ISP control	ISP + Bm-50	ISP + Bm-100	ISP + Bm-150	ISP + Bm-200
MDA (nmol g^{-1} tissue)	66.5±13.9	99.9±9.9*	84.40±19.8	81.3±17.8*	82.00±9.7*	83.00±15.9*
CK-MB (IU mg^{-1} protein)	162.4±27.3	59.4±21.3*	87.90±30.01	97.4±29.6*	115.09±20.09*	115.12±29.01*
LDH (IU mg^{-1} protein)	210.3±36.9	113.7±16.9*	145.01±39.0	150.2±37.2	178.09±37.12*	186.50±34.02*

MDA: Malonaldialdehyde; CK-MB: Creatine phosphokinase-MB isoenzyme; LDH: Lactate dehydrogenase. The values are mean±SD of eight experiments. *: $p<0.05$ vs sham; *: $p<0.05$ vs ISP control. One unit of CK-MB isoenzyme is defined as the amount of enzyme that will transfer 1 µmol of phosphate from phosphocreatine to ADP per min at pH 7.4 and a temp of 30°C. One unit of LDH is defined as the amount of enzyme required to reduce 1 µmol of pyruvate to D-lactate per min at pH 7 at a temperature of 25°C
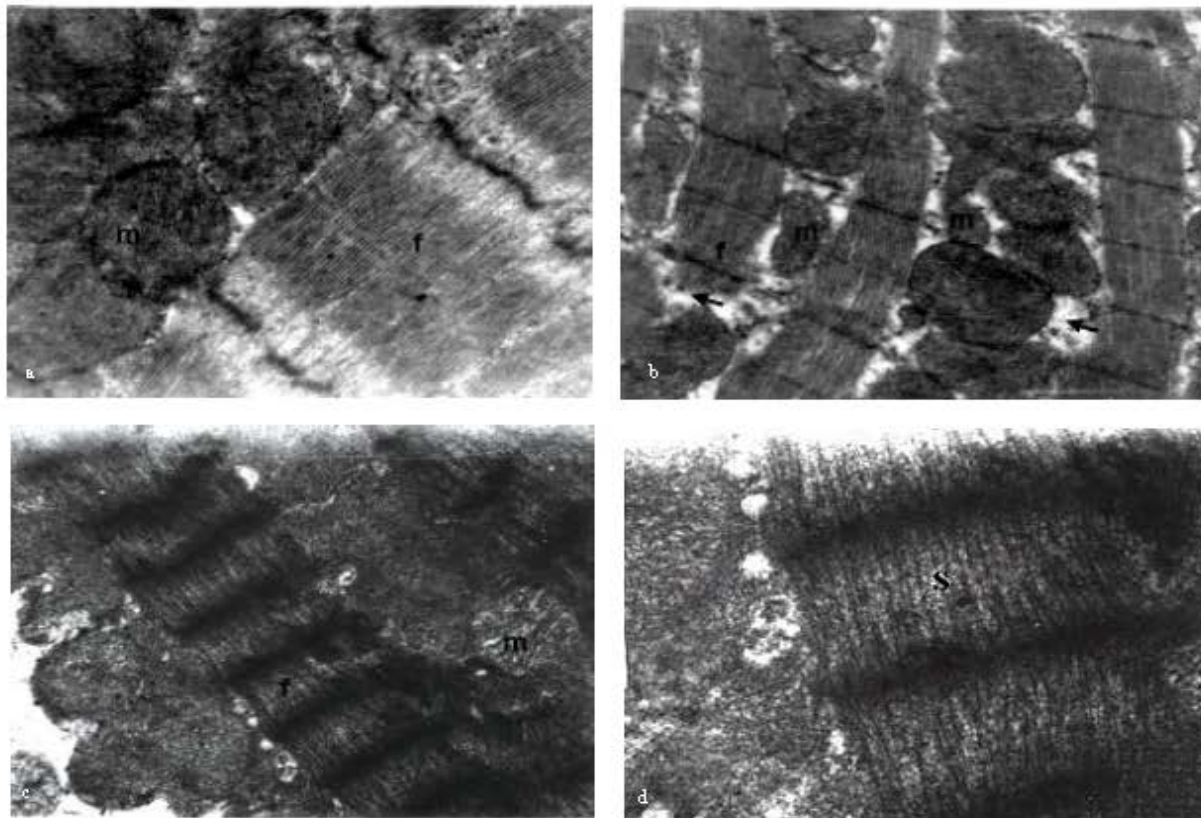

Fig. 2: a) Representative transmission electron micrographs showing normal ultrastructure of rat myocardium of sham group with normal architecture of myofibrils (f) and mitochondria (m) (1000X). (b) Electron micrographs of rat myocardium of isoproterenol control group showing extensive muscle necrosis (arrow) with significant disruption of myofibrils (f) (2800 x) and (c, d) Electron micrographs of rat myocardium treated with Bm 150 mg kg^{-1} day^{-1} for 30 days in addition to challenged with isoproterenol showing myofibrils without disruption and less intracytoplasmic vacuoles (8000 x) and (2800 x)

sham control rats was normal in appearance as evidenced by normal architecture of myofibrils (f) and mitochondria (m) (Fig. 2a). In ISP control group, the myocardial damage was marked by significant disruption of myofibrils (f), sarcomere (s) with several intracytoplasmic vacuoles (Fig. 2b). However, chronic pre and co-treatment

of animals with Bm at dose of 150 mg kg^{-1}, showed structural protection of the myocardium from these ISP-induced ultrastructural changes as evidenced by myofibrils without disruption and rare intracytoplasmic vacuoles (Fig. 2c, d).

DISCUSSION

The present study demonstrated the cardioprotective effect of *Bacopa monniera* in ISP-induced model of myocardial necrosis by improving endogenous antioxidant defense system. Additionally, *Bacopa monniera* ameliorated the cardiac dysfunction induced by isoproterenol. Isoproterenol, a synthetic beta-adrenergic receptor agonist, has been shown to produce myocardial necrosis in experimental animals. The infarct-like lesions produced by ISP are particularly marked in subendocardial regions of left ventricle and interventricular septum and resembles those produced by myocardial ischemia in humans (Diaz-Munoz *et al.*, 2006; Li *et al.*, 2006; Chauhan and Naik, 2005; Rona, 1985). The pathogenesis of ISP-induced myocardial necrosis is not completely understood. However, a number of studies support the role of oxidative stress as major determinant of necrotic damage to myocardium following ISP administration (Diaz-Munoz *et al.*, 2006; Trivedi *et al.*, 2006).

It has been suggested that during stressful situations like myocardial ischemia, the excessive rise in catecholamines overwhelms the capacity of such enzymatic metabolism and thus diverts the catecholamines to production of oxidative products like adrenochrome via enzymatic or spontaneous oxidation reactions (Kalyanaraman *et al.*, 1984). Such oxidative products of catecholamines, rather than catecholamines, *per se*, have been suggested to produce the characteristic myocardial damage, as adrenochrome and other oxidative metabolites induce cell necrosis, contractile failure, ventricular arrhythmias and subcellular changes in myocardium (Srivastava *et al.*, 2007; Hanna *et al.*, 2004; Tappia *et al.*, 2001). Further superoxide radicals have been proposed to play an important role in catecholamine-induced cardiotoxicity (Rajadurai and Prince, 2007; Rump *et al.*, 2001).

Due to cardioprotection offered by various antioxidant compounds like vitamin E, ascorbic acid against ISP-induced myocardial necrosis in experimental animals (Yogeeta *et al.*, 2006; Rajadurai and Prince, 2005), it has been proposed that free radicals generated during oxidation of catecholamines lead to peroxidation of membrane lipids, thus altering the permeability of cellular and subcellular membranes and oxidation of-SH groups of enzymatic proteins, like Ca^{2+}ATPase and Na^{+}-K^{+} ATPase.

Change in membrane permeability and inactivation of enzymatic proteins leads to excessive influx and diminished efflux of calcium resultant into intracellular calcium overload which in turn leads to depletion of high-energy phosphate stores and impairment of mitochondrial function (Nakayama *et al.*, 2007; Diaz-Munoz *et al.*, 2006; Dhalla *et al.*, 1996) and ultimately the transition from reversible to irreversible injury of the myocardial fibres during ISP-induced myocardial necrosis.

The present study clearly demonstrated the ISP-induced myocardial oxidative stress, evidenced by a significant fall in endogenous antioxidant enzymes (SOD, CAT and GSHPx) and GSH content along with a concomitant rise in MDA levels. The depletion in activity of GSHPx in the ISP control group might be correlated to decreased availability of its substrate, GSH. Hence, the imbalance between enzymatic as well as non-enzymatic endogenous antioxidant defence and generation of free radicals may have caused myocardial necrosis through induction of oxidative and associated enhanced lipid peroxidation. *Bacopa monniera* treatment, in doses of 100, 150 and 200 mg kg^{-1}, not only increased the levels of SOD, CAT, GSHPx and GSH but also attenuated increase in lipid peroxidation product, MDA, in comparison with ISP control group. Such attenuation may be due to the blunting of free radical mediated lipid peroxidation of the membrane lipids.

Keeping in view the above findings, it is tempting to speculate that cardioprotection which was observed in ISP induced myocardial necrosis is attributed to the presence of potent antioxidant components i.e. bacosides A and B. The antioxidant effect of *Bacopa monniera* as seen in the present study is consistent with previous studies (Rai *et al.*, 2003; Pawar *et al.*, 2001; Sairam *et al.*, 2001; Bhattacharya *et al.*, 2000; Tripathi *et al.*, 1996).

Moreover, LDH and CK-MB isoenzyme are cytosolic enzymes and are sensitive markers of ischemic myocyte injury (Deodato *et al.*, 1999). Depletion in myocardial LDH and CK-MB isoenzyme levels during ISP-induced myocardial necrosis indicates altered membrane permeability and leakage of these soluble enzymes (Khalid and Ashraf, 1993). This significant depletion in myocardial LDH and CK-MB isoenzyme activities observed in the study is in agreement with similar findings in other studies (Mohanty *et al.*, 2004). It is observed that, chronic *Bacopa monniera* administration restored the myocardial LDH and CK-MB isoenzyme activities, which indicate protection of the myocardium against ISP-induced exogenous stress. Therefore, it is very likely that *Bacopa monniera* provides cardioprotection due to its antioxidant and anti-peroxidative properties. The results were further

confirmed by histopathological and ultrastructural assessment. This is the first demonstration of cardioprotective effect of *Bacopa monniera* against ISP-induced myocardial necrosis in rats.

In conclusion, the lyophilized hydro-alcoholic extract obtained from *Bacopa monniera*, in doses of 100, 150 and 200 mg kg^{-1}, provides significant cardioprotection against ISP-induced exogenous stress in rats. Among the various doses evaluated in the present study, a dose of 150 mg kg^{-1} exhibited maximum cardioprotective activity. The exact mechanism for such cardioprotection by *Bacopa monniera* extract is unclear, but may involve its antioxidant and anti-peroxidative actions. Considering its efficacy and traditional acceptability, further research is warranted to establish its role in the pharmacotherapy of ischemic heart disease.

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