**Bacopa monniera** augments endogenous antioxidants and attenuates myocardial injury

Ipseeta Ray Mohanty, Ujjwala Maheshwari, Daniel Joseph, Vijay Moghe

1 Department of Pharmacology, MGM Medical College, Navi Mumbai, India
2 Department of Pathology, MGM Medical College, Navi Mumbai, India

**Abstract**

*Bacopa monniera* (Bm), commonly known as brahmi, is widely used in the Indian System of Medicine as a nootropic agent. The present study was designed to investigate the effects of oral administration of Bm (25, 75 and 150 mg/kg) for 21 days on: (i) myocardial antioxidant system and (ii) oxidative stress induced by isoproterenol (ISP) in the rat heart. Oral administration of Bm (75 and 150 mg/kg) per se to healthy experimental rats for 21 days significantly augmented endogenous antioxidants and reduced basal lipid peroxidation (p<0.05). Hearts were also subjected to ISP (85 mg/kg), subcutaneously on 20th and 21st day. Significant myocyte injury, decline in antioxidant status and elevation in thiobarbituric acid reactive substances (TBARS) levels was observed in this group. Interestingly, ISP induced biochemical and histopathological perturbations were significantly prevented by Bm (75 mg/kg) pre-treatment. The results indicate that Bm (75 mg) administration causes myocardial adaptation and protects rat hearts from oxidative stress associated with ISP.

**Keywords:** oxidative stress, myocardial infarction, medicinal herbs, *Bacopa monniera*, antioxidants.

**INTRODUCTION**

Although the last two decades have been exciting times for ischemic heart disease research, current treatments are inadequate in reducing the high mortality. This suggests that novel and effective therapeutic targets for the treatment of myocardial infarction (MI) are needed (Rastogi et al., 2004; Reddy et al., 1993; Destefano et al., 1993). Considerable efforts have been made in the exploration of the potential for exogenous antioxidants and free radical scavengers to supplement endogenous antioxidant system and limit free radical injury, with mixed success and failure (Brekhan et al., 1969). One reason why exogenous antioxidants have limited success in the prevention of ischemia and reperfusion injury may be due to the inaccessibility of large molecules to the key intracellular sites of oxidative damage. Under such circumstances, other options need to be explored which will help in circumventing this problem, that is, whether, by any means, it is possible to stimulate or augment the endogenous antioxidant defense system of the heart (Das et al., 1993).

Hence, a concept is now emerging of ‘adaptogenic drugs’-drugs that increase non-specific resistance of the users to a variety of stresses. Myocardial adaptation appears to be a highly promising approach to reduce cellular injury due to ischemia and reperfusion. It has been shown that myocardial adaptation could prevent ischemic damage, myocardial stress and cardiac arrhythmias in both animals and humans (Das et al., 1993). Such adaptation also restricts the impairment of cardiac electric stability and contractility in MI. The current literature emphasizes that cellular adaptation to oxidative stress depends primarily on specific antioxidants and DNA repair enzymes systems that are coordinated at a molecular level (Rajak et al., 2004). Indeed most studies have demonstrated that myocardial adaptation is associated with maximization of antioxidant defenses, evoking induction of the expression of new genes and proteins (Marczin et al., 2003). These observations lead to the emergence of a new discipline of ‘adaptive medicine’ which offers for new therapeutic strategies in both experimental and clinical disease development. Although the exact mechanism of such an adaptation is not known, it has been speculated that it works through the oxidative...
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stress response because a number of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx), and antioxidants such as Glutathione (GSH) have been found to be stimulated following myocardial adaptation. The adaptogenic property of various herbs like Ocimum sanctum, Bacopa monniera and Withania somnifera has been reported in various experimental studies. These herbs allow one to adapt to a variety of heightened stressful circumstances (Das et al., 1993; Marczin et al., 2003).

Therefore, with the point of view that it might be interesting and possibly fruitful to study the effect of herbal extract of *Bacopa monniera* in the setting of ISP induced myocardial injury, the present investigation was planned to unravel the cardioprotective potential of this time tested herbal drug Bm, commonly known as brahmi has gained world wide recognition as a memory booster and used for the treatment for epilepsy and bronchial asthma. The whole plant is used and the active ingredients bacosides are mainly responsible for it’s antioxidant, immuno-modulatory and adaptogenic properties (Russo and Borrelli, 2005). The effect of the herbal extracts on myocardial antioxidant system and oxidative stress induced by ISP in rat heart was investigated. Indices of oxidant-antioxidant balance: lipid peroxidation product: TBARS, endogenous antioxidant: GSH, antioxidant enzymes {SOD, CAT, GSHPx} were incorporated in the study design to evaluate the antioxidant and adaptogenic effects of Bm. In addition, myocardial enzyme creatine phosphokinase (CPK) and histopathological assessment of injury were undertaken to evaluate the cardioprotective effects of Bm.

**MATERIALS AND METHODS**

**Experimental animals**

Adult male Wistar rats, 10 to 12 weeks old, weighing 150 to 200g 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. Animals were obtained from the Animal Facility of Mahatma Gandhi Mission Medical College, Navi Mumbai, India. 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 under natural light and dark cycles (approximately 14 h light/10 h dark) and maintained at humidity 60±5% and an ambient temperature of 25±2°C. All experiments were performed between 9.0 and 16.0 h. The animals were allowed free excess to standard diet (Ashirwad; Chandigarh) and tap water *ad libitum* and allowed to acclimatize for one week before the experiments. Commercial pellet diet contained 24% protein, 5% fat, 4% fiber, 55% carbohydrates, 0.6% calcium, 0.3% phosphorous, 10% moisture and 9% ash w/w.

**Chemicals**

All Chemicals were of analytical grade, purchased from Sigma Chemical Co., St Louis, USA. Hydro-alcoholic lyophilized extracts of *Bacopa Monniera* was procured from Dabur Research Foundation, India. Double distilled water was used in all biochemical assays.

**Experimental groups and treatment protocol**

The animals were assigned to the following experimental groups.

**BASELINE EVALUATION PROTOCOL**

In this group healthy experimental animals were used to evaluate baseline values of various parameters investigated in this study i.e rats without any pathologic challenge to the heart.

**Group 1 – Saline control group (Sham):**

Rats were administered 0.9% normal saline per orally using a feeding cannula for 21 days and then sacrificed on the 22nd day. There were nine animals in this group.

**Group 2 –Bacopa monniera (Bm) control group:**

This group was divided into three subgroups comprising of nine rats each.

Total rats in this group were twenty seven: Group 4a-
Cardioprotective effects of *B. monniera* ISP+25mg/kg (IBm-25), Group 4b - ISP+75 mg/kg (IBm-75), Group 4c - ISP+150mg/kg (IBm-150).

**Experimental parameters studied**

**Biochemical studies**

A ten-percent homogenate of myocardial tissue was prepared in 50mM phosphate buffer, pH 7.4 and an aliquot was used for the assay of Thio-barbituric acid reactive substances according to the method described by Ohkawa (Ohkawa et al., 1979). The homogenate was centrifuged at 7000 rpm for 15 minutes and the supernatant was used for the estimation of the glutathione (Maron et al., 1979), glutathione peroxidase (Paglia and Valentine, 1967), superoxide dismutase (Misra et al., 1976), Catalase (Aebi, 1974) and protein (Lowry et al., 1951). Creatinine phosphokinase was estimated spectrophotometrically using a kit from Randox Laboratories, USA (Lamprecht et al., 1974).

**Histopathological studies**

At the end of the experiment, myocardial tissue was immediately fixed in 10% buffered neutral formalin solution. The tissues were carefully embedded in molten paraffin with the help of metallic blocks, covered with flexible plastic moulds and kept under freezing plates to allow the paraffin to solidify. Cross sections (5 μm thick) of the fixed myocardial tissues were cut. These sections were stained with hematoxylin and eosin (H&E) and visualized under light microscope to study the light microscopic architecture of the myocardium. The degree of necrosis was graded and scored.

**Assessment of body weight gain and mortality in different experimental groups**

Body weight of rats in all the groups was recorded every week and the change in body weight was calculated after 21 days feeding. At the same time mortality if any during the 21 days of oral administration of the respective drugs was also recorded in all the experimental groups.

**Statistical analysis**

All numerical data in text, figures and tables are expressed as the mean ± SD. Statistical analysis was performed by one-way analysis of variance (ANOVA) or repeated measures ANOVA when data were Compared within and between study groups, followed by the Bonferroni post hoc test. Differences were considered statistically significant at p<0.05.

**RESULTS**

**Biochemical parameters**

without ISP- induced myocardial necrosis

On chronic feeding of Bm for 21 days, no significant change in basal GSH (Table 1 [Supplementary data]) was observed in the Bm (25, 75 & 150 mg/kg) control groups as compared to the sham. A significant augmentation of endogenous antioxidant enzymes CAT (p<0.05) and SOD (p<0.01) in Bm (75 & 150 mg/kg) control group was seen as compared to sham. In addition, Bm only at 75 mg/kg dose significantly (p<0.05) augmented basal GSHPx activity and reduced baseline TBARS levels in comparison to sham (Table 1). No significant change in basal myocardial SOD, CAT and GSHPx activity and GSH levels was observed in the Bm-25 control group (Table 1). Also,
none of the doses of Bm studied produced any change in basal CPK activity in comparison to sham.

**Following ISP induced myocardial injury**

A significant decrease in GSH level (p<0.05, Fig. 1) and activities of SOD, CAT (p<0.05, Table 2) as well as an increase in TBARS level (p<0.01, Fig. 2) were observed in the ISP control group as compared to sham group. In addition, in the ISP control group, myocardial injury as indicated by significant leakage of myocardial CPK levels (p<0.01, Fig. 3) in comparison to sham was observed.

A significant restoration of endogenous antioxidant enzymes SOD in Bm (75, 150 mg/kg, p<0.05) and GSHPx in Bm (75 mg/kg, p<0.05) treated groups were seen as compared to ISP control (Table 2). However, Bm treatment failed to significantly protect the animals against ISP induced alterations in the antioxidant enzyme activity of CAT (Table 2).

In addition, Bm (75, 150 mg/kg) treatment significantly prevented alterations in myocardial contents of TBARS (Fig. 2) following ISP induced myocardial injury Bm (75 mg/kg), prevented decrease in myocardial GSH contents (Fig. 1) as compared to ISP control group. In this context, it is important to note that that Bm at a dose of 25 mg/kg did not exhibit any antioxidant effects. Bm treatment at the doses (75 mg/kg) elucidated a significant restoration (p<0.05) in CPK activity as compared to ISP control (Fig. 3).

**Histopathological studies**

Microscopic histology revealed that the non-infarcted myocardium in the sham group is characterized by an organised pattern and shows normal architecture of the myocardium (Fig. 4). However, marked morphological and pathological degeneration of myocardium was seen in the heart sections of the rat from the ISP control group (Fig. 5). The section showed myonecrosis with fibroblastic proliferation, infiltration of inflammatory cells, marked intramyocellular edema, besides vacuolar degeneration and rounded nuclei as compared to sham group (Fig. 5).

Bm(75 mg/kg) treatment prevented myonecrosis, infiltration of inflammatory cells, edema and vacuolar changes as compared to the ISP control. In the IBm-75 group and there was only occasional loss of myofiber and edema as well as inflammation was minimal compared to other groups (Fig. 6). In the IBm-150 treated group patchy areas of necrosis of muscle fiber with mild edema and inflammation was observed as compared to ISP control. However, in the IBm-25 treated group of the study protocol the degree of edema and necrosis was nearly comparable to that of ISP control group with similar morphological changes.

**Assessment of body weight gain and mortality in different experimental groups**

No mortality was observed in the sham-operated group (Sham) comprising of nine rats. In the saline-treated control group (ISP Control), of the nine rats subjected to ISP induced myocardial injury, 1 rat died during the ischemic period. In the IBm-150 group, 1 rat each died on 18th day of oral feeding. There was no mortality in any of the other Bm treated groups. There was no statistically significant change in the weight gain and mortality pattern in any of the groups studied.

**DISCUSSION**

Supramaximal doses of isoproterenol induces subendocardial myocardial ischemia, hypoxia, necrosis, and finally fibroblastic hyperplasia with decreased myocardial compliance and inhibition of systolic and diastolic function, is widely used as a model of evaluating cardioprotective drugs and studying myocardial consequences of ischemic disorders (Rona et al., 1963). The oxidation of hydroxyl groups in catecholamines leading to the conversion into quinolones and the subsequent
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formation of adreochromes most probably account for the hazardous effects of isoproterenol. During this reaction, highly toxic oxygen derived free radicals are generated which are detrimental to extracellular and intracellular enzymes and proteins. Furthermore free radicals could initiate the peroxidation of membrane bound polyunsaturated fatty acids, leading to both functional and structural myocardial injury (Rona et al., 1963; Karthikeyen et al., 2007).

While direct evidence of ROS-induced cardiac injury during hypoxia or ischemia and reperfusion in humans is lacking (due to inadequate methodology), many studies have shown increase in biomarkers of oxidant production and/or decrease in antioxidant capacity during myocardial infarction (Marczin, 2003). In the present study biochemical indicators of oxidative damage, viz the lipid peroxidation product, TBARS, endogenous antioxidant: GSH, antioxidant enzymes: SOD, CAT, GSHPx and myocardial enzyme: CPK has been evaluated. CPK is an intracellular enzyme, responsible for the transfer of phosphate to ADP from ATP (Boehm et al., 2007). Leakage of this enzyme is a strong evidence for loss of sarcolemmal integrity due to ischemia and reperfusion induced injury to the myocardial cells. The study was designed to evaluate the effects of oral administration of Bm on a) myocardial antioxidant system and b) oxidative stress induced by ISP in rat heart.

Increase in TBARS as a marker of lipid peroxidation in conditions of myocardial ischemia and reperfusion is well documented both in clinical and experimental studies (Ferrari et al., 2004; Priscilla and Prince, 2009). The results of the present study concur with earlier findings. In the present study, along with significant myocardial lipid peroxidation, myocardial GSH content, SOD and CAT activities were also depleted significantly following ischemia and reperfusion induced injury. However, the activity of the antioxidant enzyme, GSHPx was not significantly reduced. Superoxides are the major and the first formed OFRs and SOD is the enzyme, which dismutates superoxides to form H₂O₂ and O₂. The function of SOD has often been termed as primary defense against OFR, because this enzyme prevents further generation of free radicals (Yu, 1994). Catalase is also a major primary antioxidant enzyme that catalyses this function through the GSH system (Chang et al., 2003). Furthermore, cellular defense mechanisms rely on autolysis as well as inactivation of H₂O₂ by CAT to produce water and oxygen.

It appears that, in the present study the major burden of neutralizing the isoproterenol induced oxidative stress, was borne out of GSH, SOD and CAT and to a lesser extent by GSHPx. This is reflected by the extent of depletion of the respective antioxidants. There are several studies, which have documented the evidence of depletion of different antioxidant compounds along with the increase of TBARS in different in vitro and in vivo models (Yim et al., 1990; Khaper et al., 1997). Thus, the increased TBARS production and the reduced levels of endogenous antioxidants provide strong evidence for the occurrence of oxidative stress during ISP induced myocardial injury.

‘Adaptogenic’ property of various herbs like Ocimum sanctum, Bacopa monniera and Withania somnifera, first time reported by Brekhman and associates in Eleuthrococcus and Panax ginseng has already been reported in various experimental studies (Wang et al., 1998; Bhattacharya, 2000a; Bhattacharya et al., 2003b; Rege et al., 1999). These herbs allow one to adapt to a variety of heightened stressful circumstances. Although the exact mechanism of such adaptation is presently not known, it has been proposed that these drugs may act by inducing a number of antioxidant enzymes such as SOD, CAT, GSHPx and antioxidants such as GSH, proteins like heat shock protein (HSP) in the heart (Wang et al., 1998; Das et al., 1993; Rajak et al., 2004).

The present study demonstrated the adaptogenic property of Bm. Oral administration of Bm per se to healthy experimental animals (rats without any pathologic challenge to the heart); resulted in a significant increase in myocardial GSHPx, CAT along with SOD activity as compared to sham group. Increase in antioxidant levels following chronic Bm treatment might considerably improve the myocardium’s defense against oxidative stress and account for the cardioprotective effect of Bm. Any increase in SOD activity is beneficial in the event of increased free radical generation (Chang et al., 2003; Wang et al., 1998). However, it has been reported that an augmented SOD activity, without a concomitant rise in the activity of CAT and/or GSHPx might be detrimental, since SOD activity, generated hydrogen peroxide as a metabolite, which is more cytotoxic than oxygen radicals and must be scavenged by CAT or GSHPx. A simultaneous increase in CAT and/or GSHPx activity is essential for an overall beneficial effect of an increased SOD activity (Yim et al., 1990; Baker et al., 2004). Thus, simultaneous increase in myocardial SOD, GSHPx and CAT activities observed in the present study with Bm (75 mg/kg) underscores the distinct importance of enhanced beneficial effects of this herbal extract.

In addition, subsequent to ISP induced myocardial injury, Bm treatment demonstrated significant antioxidant activity. It decreased the level of TBARS compared to ISP Control group, which could be imparted due to reduced formation of TBARS from fatty acids. Furthermore, protection against ISP induced oxidative stress in Bm treated rat hearts was evidenced by preservation of endogenous antioxidants enzyme SOD and GSHPx.

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It is well established that myocardial injury leads to loss of structural integrity and increased permeability. As described earlier, a good correlation between the histopathological evidence of myocardial necrosis and the enzymatic activity of enzyme CPK elucidates that the degree of CPK leakage from myocardium corresponds well with the myocardial injury (Ferrari et al., 2004). In the present study, Bm(75 mg/kg) treatment significantly prevented leakage of myocardial enzyme CPK and preserved the myofiber architecture as compared to the ISP control group.

Nature has been a source of medicinal treatments for thousands of years and plant-derived products continue to play an essential role in the primary health care of about 80-85% of the world’s population. Hence, these herbal extracts traditionally used have been evaluated scientifically in the present study with an aim to define the role of these agents in limiting the deleterious affects of ISP induced myocardial injury by providing scientific data to validate their use as prophylactic approaches or as an adjunct to standard treatment (Synthetic compounds employed in conventional treatment protocols) of ischemic heart disease.

Development of indigenous herbal products may be a boon in developing countries like India and South East Asian Nations as the exogenous antioxidants are costly and therefore patients belonging to weaker sections of the society may be non-complaint in therapy on long term basis. In summary, the present study for the first time provided experimental evidence that Bm maintained the antioxidative enzymes and membrane integrity following ISP administration. Bm causes myocardial adaptation by augmenting endogenous antioxidants and protects rat heart from oxidative stress associated myocardial injury. This study will also provide a lead for further studies in the field of cardiovascular pharmacology.

CONCLUSION

The present study, demonstrates that Bm offered significant protection against ISP induced myocardial necrosis through a unique property of enhancement of endogenous antioxidants without producing any cytotoxic effects.

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