Original research article

Berbamine protects the heart from isoproterenol induced myocardial infarction by modulating eNOS and iNOS expressions in rats

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\textbf{A R T I C L E  I N F O}

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\textbf{A B S T R A C T}

\textbf{Aim:} The current study was designed to investigate the effect of berbamine (BBM) on isoproterenol (ISO) induced changes in cardiac marker enzymes, myocardial oxidative stress, lipid profile and expression of inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) in male Wistar rats. Rats were pretreated with BBM (25 mg/kg) through intraperitoneal injection for 7 days followed by induction of myocardial infarction (MI) by subcutaneous injection of ISO (85 mg/kg) for last two days. Key findings: In the present study, the histopathological findings of the heart tissue showed that BBM treatment significantly minimized the damage induced by ISO. BBM pretreatment showed a significant decrease in heart weight, serum marker enzymes, lipid peroxidation and significant increase in cardiac endogenous enzymatic and non-enzymatic antioxidants compared to the ISO-treated group. In addition, we observed significantly upregulated eNOS expression and downregulated iNOS expression in BBM pretreated group. Thus, BBM protected the rat’s heart from ISO-induced myocardial infarction by its antioxidant, and antilipidemic properties. Significance: The results of the present investigation suggested that BBM efficiently ameliorated the ISO-induced myocardial infarction in rats.

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\textbf{Introduction}

Cardiovascular diseases (CVD) are the major cause of mortality in both developed and developing countries (WHO, 2010). Myocardial infarction (MI) is an acute condition of myocardial necrosis that occurs as a result of decreased blood supply to a part of the myocardium, which causes more biochemical changes, inducing myocardial cell death, ultimately resulting in cardiac dysfunction (Ahsan et al., 2014). It has been reported that excessive generation of free radicals due to oxidative metabolism of catecholamines leads to qualitative and quantitative alteration of the myocardium (Sahu et al., 2014). The stress-induced cardiac dysfunction is mainly associated with adrenergic overstimulation as a result of catecholamine overproduction (Shao et al., 2012). Isoproterenol {4-[1-hydroxy-2-(propan-2-ylamino)-ethyl]benzene-1,2-diol, C\textsubscript{11}H\textsubscript{17}NO\textsubscript{3}} is a synthetic catecholamine and β-adrenoceptor agonist which induces myocardial necrosis (Shaik et al., 2012). The pathophysiological changes in ISO-induced MI in rats mimic those of human MI. Hence, ISO-induced MI is widely used in evaluation of the therapeutic efficacy of several phytoconstituents against catecholamine induced cardiac dysfunction (Kumar et al., 2017). ISO undergoes auto-oxidation, generates free radicals leading to elevated lipid peroxidation resulting in accumulation of intracellular calcium which in turn cause irreversible myocardial membrane damage (Huang et al., 2018).

Diverse allopathic medicines have been investigated earlier for the treatment of MI which includes beta-blockers and calcium antagonists and they caused several side effects like dysrhythmia and toxicity (Panda et al., 2017; Shirole et al., 2013). Very often alternative medicines, particularly plant based drugs are considered more effective and safe. So, there is an urgent need to find new alternatives for the successful treatment of MI.

Natural compounds serve as a rich source of antioxidants, which help in restoring and maintaining the altered biochemical parameters (Azoleia et al., 2013). They exert their biological activities through the presence of biologically active compounds like alkaloids, flavonoids, vitamins, and polyphenols (Abhilash et al., 2010). Berbamine (BBM; C\textsubscript{27}H\textsubscript{40}N\textsubscript{2}O\textsubscript{6}), a bis-benzylisoquinoline alkaloid, derived from roots, stem and bark of Barberry plants such as Berberis aristata (Berberidaceae) has been used in traditional medicine (Yang et al., 2014; Zhao et al., 2011). Commonly known as the Indian
Barberry tree turmeric, this shrub is found growing wild in the sub-Himalayan tract (Pai et al., 2012). BBM has several medicinal properties and has been widely used as anti-arrhythmia, anti-tumor, antipyretic and anti-inflammatory agent. Further, cardioprotective, anti-hypertensive and immunomodulatory properties of BBM have also been reported (Kupeli et al., 2002; Xu et al., 2004; Yang et al., 2014; Zheng et al., 2017). Previous reports suggest that BBM significantly reduced the blood pressure in various animals such as cat, dog, and rabbit and antagonize the arrhythmias induced by the adrenaline in the isolated working heart of Guinea Pig (Baofeng et al., 1991; Zhou et al., 1980). Zhang et al. (2012) have shown that BBM elicits protective effect against ischemia/reperfusion injury in isolated rabbit heart. Hence, the present study was designed to investigate the effect of BBM on cardiac marker enzymes, oxidative stress, antioxidant enzymes, lipid profile, and iNOS and eNOS expression in ISO-induced myocardial infarction in rats.

Materials and methods

Chemicals

Berberine dihydrochloride (Berberidaceae) is a natural compound derived from Berberis aristata and isoproterenol were obtained from Sigma-Aldrich, St. Louis, MO, USA. All other chemicals used were of analytical grade.

Animals

Wistar male rats were selected for the present study and were purchased from Kerala agricultural university, Mannuthy, weighing 200 ± 10 g each (40–45 days old) and were maintained at a temperature of 28 ± 2 °C, with a normal 12 h:12 h light:dark cycle. The animals were nourished with commercially available pelleted rat chow (Sai Durga private limited, Bangalore) and water ad libitum. Whole experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and regulations, and the research was permitted by the Institutional Animals Ethics Committee (IAEC), PSG Institute of Medical Sciences and Research (Ethical approval number: 209/2013/IAEC, India).

Induction of experimental myocardial infarction

The experimental myocardial infarction was induced in rats by administration of ISO (85 mg/kg body weight/day) subcutaneously for 2 days at the 24 h interval (Paul et al., 2017; Tasatargil et al., 2017).

Pilot study for dose fixation of test BBM

A pilot study was conducted with four different doses of BBM (15, 20, 25, and 30 mg/kg body weight/day) in ISO-treated rats. The optimum dose of BBM was determined by estimating the activity of serum creatine kinase (CK) and cardiac lipid peroxidation (LPO). There was no significant change in the activity of serum CK in BBM (15, 20, 25, and 30 mg/kg) alone treated rats compared to control rats whereas the LPO level was slightly increased in rats which received 30 mg/kg dose. BBM pretreatment resulted in a dose dependent decrease in the activity of serum CK and LPO level in ISO-treated rats till 25 mg/kg dose whereas an increase in the CK activity and LPO levels were observed in ratspretreated with 30 mg/kg of BBM followed by ISO administration. Hence, 25 mg/kg dose was selected as an optimum dose for further investigation (Fig. 1 and 2).

Experimental design

After a week of acclimatization, the experimental animals were grouped into four, comprising six rats each. Rats in group I (normal control) received standard diet and were given normal saline through intraperitoneal injection for a period of 7 days. Group II animals were injected with ISO (85 mg/kg body weight/day) dissolved in physiological saline subcutaneously (on 8th and 9th) for 2 days at the 24 h interval for the induction of myocardial infarction. In group III, animals were injected with BBM (25 mg/kg body weight/day) dissolved in physiological saline intraperitoneally for 7 days. In group IV, the animals were pretreated with BBM (25 mg/kg body weight/day) for 7 days and then injected with ISO (85 mg/kg body weight) for two days.

After 12 h from the last injection of ISO, all the animals were anesthetized by using diethyl ether and then sacrificed by cervical decapitation. Blood was collected and the serum was separated and used for biochemical assays. The heart tissue was dissected out, washed in ice-cold saline, patted dry, weighed, and stored at –80 °C for further analysis. From the remaining tissue, 100 mg was weighed and homogenized in 5.0 ml of chilled 0.1 M Tris-HCl buffer in Potter-Elvehjem Teflon homogenizer and the homogenate was used for biochemical investigation.

![Image]

Fig. 1. Effect of BBM on serum creatine kinase (CK) activity. Each bar represents mean ± SEM (n = 6). One-way ANOVA followed by Tukey’s multiple comparison test. Where, ISO – isoproterenol, BBM – berberine, and BBM + ISO – berberine pretreated ISO-induced rats. * P < 0.001 compared to the control group; † P < 0.001 compared to the isoproterenol group; # non-significant as compared to the control; $ Non-significant as compared to the isoproterenol group.

![Image]

Fig. 2. Effect of berberamine on thiobarbituric acid reactive substances (TBARS) level. Each bar represents mean ± SEM (n = 6). One-way ANOVA followed by Tukey’s multiple comparison test. Where, ISO – isoproterenol, BBM – berberine, and BBM + ISO – berberine pretreated ISO-induced rats. * P < 0.001 compared to the control group; † P < 0.001 compared to the isoproterenol group; # non-significant as compared to the control; $ Non-significant as compared to the isoproterenol group.
Heart weight/body weight ratio

The weight of the whole heart and the body weight were weighed. The ratio of heart weight to body weight (HW/ BW) was then calculated.

Histopathology

Heart was dissected out, washed thoroughly in 0.9% saline, and heart slice were fixed in 10% neutral buffered formalin and processed by standard procedure for paraffin embedding, and serial sections of about 5 μm size were cut. The heart tissue sections were stained with hematoxylin and eosin (H&E) dyes. The photomicrographs of heart sections were taken using light microscopy (Olympus, Japan).

TTC staining

The myocardial infarct size was measured using triphenyl tetrazolium chloride (TTC) dye described by Lie et al. (1975). Rat myocardium was frozen immediately after removal. The heart was sliced into 1 mm segments and incubated in 1% TTC solution prepared in phosphate buffer (pH 7.4) at 37° C for 20 min, following which they were fixed with 10% formalin. A camera with macrofons was used to take colour photographs of heart slices. Infarct size was analyzed using image processing software (Imagej, Version 1.44p, NIH, USA). The infarcted area of the individual slice was measured and divided by the total area of that slice to obtain the fraction of the affected area per slice.

Biochemical estimations in cardiac tissue

Markers of oxidative stress induction

2', 7'-Dichlorofluorescin diacetate (DCF-DA) dye was used to estimate the ROS generation in heart tissue (Shinomol and Muralidhara, 2007). Briefly, the homogenate was diluted 1:20 times with ice-cold Locke's buffer to obtain a concentration of 5 mg tissue/mL. The reaction mixture (1 ml) containing Locke’s buffer (pH 7.4), 0.2 ml tissue homogenate and 10 ml of DCF-DA (5 mM) was incubated for 15 min at room temperature. After 30 min of further incubation, the conversion of DCF-DA to the fluorescent product 2', 7'-Dichlorofluorescin (DCF) was measured in a multimode reader (Bio-Tek Instruments) with excitation at 484 nm and emission at 530 nm. The values were expressed as % relative fluorescence compared to the control.

Biochemical estimations in serum

Estimation of serum myocardial injury markers

The ISO-induced myocardial injury was studied through the estimation of serum cardiac marker enzymes creatine kinase (CK), lactate dehydrogenase (LDH), and aspartate transaminase (AST). Serum CK activity was determined by the method of (Okinaka et al., 1961); LDH activity was assayed according to the method of (King, 1965), and the activity of AST was determined according to the method of (Bergmeyer et al., 1974).

Effect of BBM on ISO-induced nitric oxide release

The NO released into the serum was estimated by the method of Stuehr and Marletta (1987). Briefly, 50 μl of serum was reacted with an equal volume of Griess reagent (0.1% naphthylethylenediamine, 1% sulfanilamide in 5% H3PO4) for 10 min at room temperature in the dark. The absorbance at 550 nm was determined.

Extraction and estimation of lipids in serum

Lipids were extracted according to the method of Folch et al. (1957) from heart tissue. The levels of total cholesterol, triglycerides, and phospholipids in homogenate of myocardial tissue were estimated using standard kits (Agappe Diagnostics Kit, India) and read in microplate reader (Bio-Tek Instruments).

Biochemical estimations in cardiac tissue

Markers of oxidative stress induction

2', 7'-Dichlorofluorescin diacetate (DCF-DA) dye was used to estimate the ROS generation in heart tissue (Shinomol and Muralidhara, 2007). Briefly, the homogenate was diluted 1:20 times with ice-cold Locke's buffer to obtain a concentration of 5 mg tissue/mL. The reaction mixture (1 ml) containing Locke’s buffer (pH 7.4), 0.2 ml tissue homogenate and 10 ml of DCF-DA (5 mM) was incubated for 15 min at room temperature. After 30 min of further incubation, the conversion of DCF-DA to the fluorescent product 2', 7'-Dichlorofluorescin (DCF) was measured in a multimode reader (Bio-Tek Instruments) with excitation at 484 nm and emission at 530 nm. The values were expressed as % relative fluorescence compared to the control.

The generation of oxidative stress was determined by estimating the level of thiobarbituric acid reactive substances (TBARS) in the heart tissue homogenate by the method of Ohkawa et al. (1979). Briefly, the reaction mixture contained 0.2 ml of homogenate, 1.5 ml of acetic acid (pH 3.5, 20%), 1.5 ml of 0.8% thiobarbituric acid (0.8% w/v) and 0.2 ml SDS (8% w/v). The mixture was heated to boiling for 45 min and TBARS adducts were extracted into 3 ml of 1-butanol and TBARS was measured in a microplate reader (Bio-Tek Instruments) at 532 nm and quantified as malondialdehyde (MDA) equivalents using 1, 1, 3, 3-tetramethoxypropane as the standard.

Myocardial antioxidant status

Tissue homogenate was used for the assay of endogenous anti-oxidant enzymes [superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione-S-transferase (GST), and glutathione peroxidase (GPx)] SOD was determined by the method of (Marklund and Marklund, 1974). CAT was estimated by the method given by (Takahara et al., 1960). The level of GSH was determined according to the method of (Moron et al., 1979). GST and GPx were estimated by the methods described by (Habig et al., 1974; Rotruck et al., 1973), respectively.

Extraction and estimation of lipids in heart tissue

Lipids were extracted according to the method of (Folch et al., 1957) from heart tissue. The levels of total cholesterol, triglycerides, and phospholipids in homogenate of myocardial tissue were estimated using standard kits (Agappe Diagnostics Kit, India) and read in microplate reader (Bio-Tek Instruments).

Real-time PCR analysis of eNOS and iNOS expressions

RNA isolation: Total genomic RNA was isolated from the heart using TRI reagent (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer’s protocol. Concentration of isolated total RNA was quantified by NanoDrop ND-1000 Spectrophotometer.

Synthesis of cDNA: 1 μg of total RNA was used for cDNA conversion. Synthesis of cDNA and PCR were performed as per the manufacturer’s instructions using the kit purchased from Thermo Scientific, India.

Quantitative RT-PCR analysis: Maxima SYBR Green/ROX ready mix real time PCR kit (Thermo Scientific) was utilized as per manufacturer’s instructions for reverse transcription and amplification. The following primers were used, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), (forward) 5'-TTC TTG TGC AGT GCC AGC CTC GTC-3' and (reverse) 5'-TAG GAA CAC GGA AGG CCA TGC CAG-3'; eNOS, (forward) 5'-CGA GAT ATC TTC AGT CCC AAG C-3' and (reverse) 5'-GTG CAT TTG CTG CTC TCT ACG-3'; iNOS, (forward) 5'-ATG GAA CAG TAT AAG GCA AAC ACC-3' and (reverse) 5'-GTG CTT CTG CGA TGT CAT GAG CAA AGG-3'. The PCR products were analyzed on a 2% agarose gel, and the relative quantitation of the gene expression was performed using the comparative C(T) method (Livak and Schmittgen, 2001). The data were normalized against internal control, GAPDH and expressed as relative expression (Fold change).
Statistical analysis

Data analyses were performed using SPSS 17. All data were expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance followed by Tukey’s multiple comparison tests. Differences between treatments were considered statistically significant at \( P < 0.001 \).

Results

Effect of BBM pretreatment on heart weight to body weight ratio in ISO-induced myocardial infarction in rats

Table 1 shows the effect of BBM pretreatment on heart weight and heart weight/body weight ratio in ISO-induced myocardial infarction in rats. In the present study, we observed increased heart weight and the heart weight to body weight ratio in the ISO-treated group, though there was no considerable difference in the body weight compared to control group. Heart weight and heart weight to body weight ratio was decreased in BBM pretreated group compared to ISO-induced group. BBM alone treated rats showed no significant change in heart weight compared to control.

Histopathological findings

Fig. 3A and B shows histopathological photographs of the heart tissues of experimental rats. Histopathological examination of the heart of control rats showed normal myofibrillar structure and architecture. The heart tissue of rats subjected to ISO-administration showed myofibrillar degeneration, necrosis and inflammatory cells with separated muscle fibers. The rats pretreated with BBM followed by ISO-administration showed lesser inflammation and necrosis compared to the ISO-alone treated rats. In rats treated with BBM alone no significant histological changes of myocardium were observed.

Effect of BBM on ISO-induced area of myocardial infarction

The myocardial infarct size was measured by TTC macroscopic enzyme mapping assay. Fig. 4A and B shows the heart sections of rats after TTC staining. Control group showed viable tissue without any infarction. In ISO-induced group unstained regions represent the infarcted tissue. BBM alone treated rat heart showed results similar to those of control rats. The heart slice of the BBM pretreated group exhibited a major portion stained positively an indication of viable tissue with reduced infarct size.

Effect of BBM on ISO-induced changes in cardiac injury markers

Fig. 5 shows the effect of BBM on the activities of CK, LDH, and AST in the serum of experimental rats. Significantly \( P < 0.001 \) increased activities of serum cardiac marker enzymes such as CK, LDH, and AST were observed in ISO-treated rats compared to control rats. The activities of serum marker enzymes were decreased significantly \( P < 0.001 \) in BBM pretreated rats than ISO-induced rats.

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Isoproterenol</th>
<th>Berbamine</th>
<th>Berbamine + Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight (g)</td>
<td>0.74 ± 0.06</td>
<td>0.98 ± 0.02*</td>
<td>0.72 ± 0.06*</td>
<td>0.77 ± 0.05**</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>255 ± 257</td>
<td>253 ± 1.96*</td>
<td>247 ± 4.6*</td>
<td>257 ± 2.36*</td>
</tr>
<tr>
<td>Heart weight to body weight ratio (g)</td>
<td>0.290 ± 0.003</td>
<td>0.387 ± 0.001*</td>
<td>0.291 ± 0.003*</td>
<td>0.299 ± 0.002**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n = 6 rats), One way ANOVA followed by Tukey’s multiple comparison test. * \( P < 0.001 \) compared to the isoproterenol group; ** non-significant as compared to the control.

BBM inhibited ISO-induced oxidative stress in the heart

To investigate whether ISO administration generates the ROS in the heart tissue, the ROS level was estimated using fluorescent probe DCF-DA. As shown in Fig. 6A, ISO administration significantly \( P < 0.001 \) increased the intracellular ROS level in tissue homogenate when compared to the control. The value of ROS was expressed as relative fluorescence compared to the control value which was taken as 100%. ISO-induced ROS generation in heart tissue was reduced significantly \( P < 0.001 \) by BBM pretreatment compared to ISO alone administered rats.

Treatment of rats with ISO resulted in a statistically significant \( P < 0.001 \) elevation of LPO in tissue homogenate compared to control rats. The level of LPO was significantly \( P < 0.001 \) reduced in BBM pretreated rats compared to ISO alone induced rats (Fig. 6B). It may be due to the free-radical scavenging action of BBM.

Effect of BBM on ISO-induced changes in myocardial antioxidants

The level of GSH and activities of antioxidant enzymes such as SOD, CAT, GPx, and GST were significantly \( P < 0.001 \) reduced in ISO alone treated rats compared to control rats. The activities of antioxidant enzymes and the level of GSH were increased significantly \( P < 0.001 \) in rats pretreated with BBM compared to ISO alone treated rats. BBM alone treated rats showed no significant changes in the antioxidant status (Table 2).

Effect of BBM on lipid profile

Table 3 shows the effect of ISO and BBM on total cholesterol, phospholipids, and triglycerides levels in both serum and tissue of the different experimental groups. ISO-administration significantly \( P < 0.001 \) increased the serum and tissue total cholesterol and triglyceride levels compared with control. In addition, ISO-treatment increased the level of serum phospholipids with a concomitant decrease in tissue phospholipid level. Whereas, the levels of serum and tissue total cholesterol and triglyceride were significantly \( P < 0.001 \) decreased in BBM pretreated group. The level of tissue phospholipids was increased considerably \( P < 0.001 \) with decreased serum phospholipid in BBM pretreated rats. There were no significant difference in total cholesterol, phospholipids, and triglycerides levels in the serum and heart tissues of BBM alone treated rats compared to control.

Administration of ISO showed a significantly \( P < 0.001 \) increased level of serum LDL-C and VLDL-C and decreased level of serum HDL-C compared to control rats. We found decreased serum LDL-C and VLDL-C level and increased HDL-C level in BBM pretreated rats compared to ISO alone treated rats. No significant difference was observed in BBM alone treated rats when compared to control (Table 4).

Effect of BBM on iNOS and eNOS mRNA expression

ISO-administration induced a significant \( P < 0.001 \) increase in iNOS mRNA expression levels compared to control group.
when normalized with GAPDH. The expression of iNOS mRNA levels was significantly ($P < 0.001$) reduced in BBM treated group compared to ISO alone induced group. ISO-treatment produced a significant decrease in eNOS mRNA expression levels compared with control group. The enhanced expression of eNOS was observed in BBM pretreated rats compared to ISO-treated rats (Fig. 7A and B).

**Effect of BBM on nitrite levels**

Consistent with increased iNOS mRNA expression, ISO-treatment significantly ($P < 0.001$) enhanced the level of serum nitrites than control. While BBM pretreatment considerably ($P < 0.001$) reduced the serum nitrite levels compared to ISO alone induced rats (Fig. 8).

**Discussion**

The pathophysiological changes induced in rats upon ISO-administration are similar to changes seen in human MI. Therefore, the cardioprotective efficacy of various drugs is evaluated using the ISO-induced MI as an experimental model (Othman et al., 2017). Plant based dietary agents have been reported to reduce the risk of cardiac diseases (Remya et al., 2013). BBM, a natural compound isolated from *Berberis aristata* has been used to treat various diseases (Zheng et al., 2017). The present study was designed to investigate the cardioprotective efficacy of BBM against ISO induced cardiotoxicity by analyzing the cardiac markers, antioxidant status, lipid profile and expressions of eNOS and iNOS.

In this study we observed an increase in the heart weight and heart weight to body weight ratio in ISO-administered rats.
Increased water content, edematous intramuscular space, increased protein content and infiltration of inflammatory cells to damaged areas in ISO treated rats was reported earlier by Moradi-Arzeloo et al. (2016). The present study shows that pretreatment with BBM significantly decreased heart weight as well as heart weight to body weight ratio as compared to ISO alone treated rats. Further, histopathological examination of the myocardium in ISO-induced rats showed a severe infarcted area with inflammatory cells which confirms the ISO-induced necrosis. Rats pretreated with BBM followed by ISO-administration showed minimal histological changes with reduced inflammation. The results of the present study indicate cardioprotective efficacy of BBM against ISO induced cardiac hypertrophy.

ISO-mediated oxidative stress results in myocardial damage which result in the leakage of cytosolic enzymes creatine kinase (CK), lactate dehydrogenase (LDH), and aspartate transaminase (AST), into the circulation (Alam et al., 2018; Patel et al., 2012). The results of the present investigation are in line with above reports. BBM pretreatment resulted in the decreased levels of these enzymes in serum compared to ISO alone treated rats due to the significantly reduced cardiac damage which could be attributed to the free radical scavenging and antioxidant nature of BBM.
Table 2
Effect of berbamine pretreatment on endogenous antioxidant levels, and the activities of antioxidant enzymes in isoproterenol-induced myocardial infarction in rats.

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Control</th>
<th>Isoproterenol</th>
<th>Berbamine</th>
<th>Berbamine + Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH</td>
<td>8.83 ± 0.160</td>
<td>4.74 ± 0.352*</td>
<td>8.81 ± 0.116*</td>
<td>7.36 ± 0.120**</td>
</tr>
<tr>
<td>SOD</td>
<td>162.98 ± 2.31</td>
<td>95.56 ± 4.11*</td>
<td>157.30 ± 2.64*</td>
<td>134.11 ± 2.40**</td>
</tr>
<tr>
<td>CAT</td>
<td>5.61 ± 0.06</td>
<td>2.24 ± 0.06</td>
<td>5.47 ± 0.06*</td>
<td>4.36 ± 0.07**</td>
</tr>
<tr>
<td>GPx</td>
<td>27.44 ± 1.65</td>
<td>14.9 ± 1.56*</td>
<td>27.15 ± 1.56*</td>
<td>20.33 ± 1.38**</td>
</tr>
<tr>
<td>GST</td>
<td>779.9 ± 13.3</td>
<td>562.8 ± 16.7*</td>
<td>764.4 ± 14.8*</td>
<td>669.5 ± 12.6**</td>
</tr>
</tbody>
</table>

Units are expressed as follows, GSH-nmoles of glutathione released/mg of protein; SOD-U/mg of protein; CAT-nm of H2O2 decomposed/min/mg of protein; GPx-µg of GSH utilized/min/mg of protein; GST-nm of CDNB conjugated/min/mg of protein. Results are shown as mean ± SEM (n = 6). One way ANOVA followed by Tukey’s multiple comparison test. * P < 0.001 compared to the control group; ** P < 0.001 compared to the isoproterenol group; * non-significant as compared to the control.

Table 3
Effect of berbamine pre-treatment on serum and tissue total cholesterol, triglycerides, and phospholipids in isoproterenol-induced myocardial infarction in rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Isoproterenol</th>
<th>Berbamine</th>
<th>Berbamine + Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (mg/dl)</td>
<td>82.8 ± 1.4</td>
<td>147.6 ± 2.2*</td>
<td>81.4 ± 1.6*</td>
<td>94.5 ± 1.8**</td>
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<tr>
<td>Total cholesterol</td>
<td>55.3 ± 1.9</td>
<td>89.8 ± 2.2*</td>
<td>56.5 ± 1.4*</td>
<td>61.6 ± 1.8**</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>61.5 ± 2.8</td>
<td>90.7 ± 3.2*</td>
<td>64.2 ± 2.7*</td>
<td>75.4 ± 2.4**</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>93.5 ± 2.8</td>
<td>101.7 ± 3.5*</td>
<td>89.7 ± 2.6*</td>
<td>104.5 ± 3.2**</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>Isoproterenol</th>
<th>Berbamine</th>
<th>Berbamine + Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue (mg/g of tissue)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total cholesterol</td>
<td>22.7 ± 0.8</td>
<td>47.7 ± 0.7*</td>
<td>23.6 ± 0.8*</td>
<td>29.6 ± 0.4**</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>8.9 ± 0.3</td>
<td>14.3 ± 0.6*</td>
<td>8.6 ± 0.3*</td>
<td>10.7 ± 0.9</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>29.6 ± 0.5</td>
<td>11.5 ± 0.8*</td>
<td>29.2 ± 0.4*</td>
<td>26.8 ± 0.5**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n = 6). One-way ANOVA followed by Tukey’s multiple comparison test. * P < 0.001 compared to the control group; ** P < 0.001 compared to the isoproterenol group; * non-significant as compared to the control.

Table 4
Effect of berbamine on Serum HDL-C, LDL-C, and VLDL-C in normal and isoproterenol-induced myocardial infarction in rats.

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>Isoproterenol</th>
<th>Berbamine</th>
<th>Berbamine + Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-C (mg/dl)</td>
<td>36.2 ± 1.2</td>
<td>19.6 ± 2.4*</td>
<td>36.7 ± 1.8*</td>
<td>31.7 ± 1.2**</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>45.7 ± 1.4</td>
<td>83.4 ± 18*</td>
<td>44.8 ± 13*</td>
<td>56.3 ± 1.7**</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>8.79 ± 0.9</td>
<td>15.3 ± 0.9*</td>
<td>8.62 ± 0.8*</td>
<td>10.6 ± 1.1**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n = 6). One-way ANOVA followed by Tukey’s multiple comparison test. * P < 0.001 compared to the control group; ** P < 0.001 compared to the isoproterenol group; * non-significant as compared to the control.

Fig. 7. A. Represents RT-PCR gel showing mRNA expression of eNOS and iNOS in control and experimental groups of rat hearts. GAPDH was used as an internal control. Values are expressed as mean ± SEM (n = 6). One-way ANOVA followed by Tukey’s multiple comparison test. Where, ISO – isoproterenol, BBM – berbamine, BBM + ISO – berbamine + isoproterenol, eNOS – endothelial nitric oxide synthase, GAPDH – glyceraldehyde-3-phosphate dehydrogenase, iNOS – induced nitric oxide synthase. * P < 0.001 compared to the control group; ** P < 0.001 compared to the isoproterenol group; * non-significant as compared to the control. B. Represents relative quantitation of the gene expression normalized with GAPDH mRNA expression. Values are expressed as mean ± SEM (n = 6). One-way ANOVA followed by Tukey’s multiple comparison test. Where, ISO – isoproterenol, BBM – berbamine, BBM + ISO – berbamine + isoproterenol, eNOS – endothelial nitric oxide synthase, GAPDH – glyceraldehyde-3-phosphate dehydrogenase, iNOS – induced nitric oxide synthase. * P < 0.001 compared to the control group; ** P < 0.001 compared to the isoproterenol group; * non-significant as compared to the control.
Excessive generation of ROS by ISO-administration leads to myocardial membrane damage which in turn causes an increased level of lipid peroxidation (Singh et al., 2013). Peroxidation of endogenous lipid might be a major factor involved in the cytotoxic nature of excessive dose of ISO and linked to pathogenic events such as myocardial necrosis and accumulation of lipid hydroperoxides indicating the damage of the cardiac muscles (Sahu et al., 2015). In the current study, an increased level of ROS and lipid peroxidation products were observed in ISO-treated rats which causes the cardiac tissues more prone to oxidative damage. Whereas, BBM pretreatment significantly reduced ROS and lipid peroxidation levels in ISO-induced rats. Thus, the results of the present study suggest that BBM protects the heart from ISO-induced free radical damages. The results of our study corroborate with an earlier study where pretreatment with Paeonol and Danshensu combination reduced ROS and LPO level in ISO administered rats and thus offered protection against ISO induced oxidative stress (Li et al., 2016).

Accumulating evidence has shown that excessive generation of ROS results in the depletion of antioxidants, thus causing an imbalance of cellular pro- and antioxidants. The enzymatic and non-enzymatic antioxidants constitute cellular defense which efficiently scavenge the intracellular ROS. Thus, cellular lipid peroxidation is inversely proportional to the antioxidant status (Jagetia et al., 2003). SOD plays an important role in protecting the cells from oxidative damage by converting superoxide radicals into hydrogen peroxide, which is further metabolized into molecular oxygen and water by catalase (Rajadurai and Stanely Mainzen Prince, 2006). GSH serves as a substrate for GPx, GST and several other enzymes, which is involved in the free radical scavenging action. GPx and GST enzymes act like peroxidase and remove the stable peroxides from the system (Jagetia et al., 2004). Similar results were reported earlier by Thomes et al., (2010) and Cheng et al. (2012), their studies showed that overproduction of reactive oxygen radicals, such as superoxide and hydrogen peroxide, inhibit the activities of antioxidant enzymes. The results of the current study revealed that ISO-administration enhanced lipid peroxidation and decreased antioxidant status which is in line with the above reports. In the current investigation, pretreatment with BBM significantly increased the antioxidant status compared to ISO alone induced rats, which could be attributed to the antioxidant activity of BBM. Increased antioxidant status in diabetic rats was reported earlier in ethanolic root extract of Berberis aristata (Singh and Kakkar, 2009).

Hyperlipidemia results in the development of coronary heart disease (CHD) (Abhilash et al., 2010). ISO-administered rats showed significantly increased levels of total cholesterol and triglyceride in both serum and tissue. An increase in total cholesterol and triglycerides in serum and cardiac tissue were reported earlier by Baldissara et al. (2017), which was attributed to cyclic adenosine monophosphate mediated lipid synthesis in ISO administered rats. Several reports have shown that pretreatment with natural compounds such as neferine (Laithia et al., 2013), curcumin (Nirmala and Puvanakrishnan, 1996) prevent ISO induced hyperlipidemia. In the current study, the levels of cholesterol and triglycerides in serum and tissue were decreased significantly in BBM pretreated ISO administered rats. Thus, results of the present study corroborate with above reports.

Increased cholesterol content affects membrane fluidity adversely, which in turn leads to the inhibition of activities of the membrane-bound enzymes and membrane phospholipid degradation (Yeagle, 1985). The current study demonstrated that ISO-administration increases the level of serum phospholipids followed by a decreased level of heart phospholipids compared to control; this might be due to membrane phospholipid breakdown. In rats pretreated with BBM, the level of serum phospholipids was reduced significantly with a significant increase in heart tissue phospholipids.

Lipoprotein is an independent risk factor for the development of atherosclerotic disease (Rajadurai and Stanely Mainzen Prince, 2006). An increase in serum LDL-C and VLDL-C fraction along with a concurrent decrease in HDL-C was observed in ISO-induced rats compared to control rats. Hypolipidemic effect of fish oil administration in experimentally induced MI in rats was reported earlier by Vijaya Padma et al. (2006). Razzaq et al. (2011) has reported the hypolipidemic effect of Berberis asiatica plant extract reduced the elevated levels of total cholesterol in streptozotocin-induced diabetic rats. In the present study, BBM pretreatment prevented the ISO induced alterations in the lipid profile which suggest that our results are in line with the above reports.

TTC staining is a well-accepted method to measure infarct size (Krishnamurthy et al., 2012). The LDH of the viable cells converts the TTC stain to formazan precipitate which gives intense red color to the tissue (Panda et al., 2013). In ISO-induced rats, due to the membrane damage and consequent leakage of LDH in infarcted tissue, it fails to stain with TTC. Therefore the unstained region represents infarcted tissue. Pretreatment with BBM in ISO administered rats significantly decreased the unstained region which indicates reduced infarct size. Our results are consistent with the observation of Roy and Prince (2012).

Nitric oxide plays a vital role in host defense and cellular homeostasis. Recent experimental studies have shown that NO at low concentrations is beneficial in regulating cardiac function through the endothelial nitric oxide synthase (eNOS) pathway (Remya et al., 2013; Ribeiro et al., 2009). Whereas, excessive amounts of NO, mediated by the inducible nitric oxide synthase (iNOS) may suppress the myocardial contractility and further increase the ROS generation thus may result in cardiomyocyte apoptosis (Haywood et al., 1996; Wollert and Drexler, 2002). In the present study, ISO-administration caused endothelial dysfunction as evidenced by high iNOS and low eNOS expression levels. The observations that ISO upregulated iNOS and downregulated eNOS were established previously in myocardial infarcted rats (Elhemely et al., 2014). In the present study, results from RT-PCR for iNOS demonstrated a strong iNOS expression in ISO-treated rats compared to the control rats. We have also observed reduced eNOS mRNA expression and increased level of nitrite in ISO-induced group. Interestingly, pretreatment with BBM down-regulated iNOS and upregulated eNOS and decreased the serum nitrite levels which might result in enhancement of the endothelial...
function and the coronary blood flow in ISO administered rats. These results are in line with the study of Dianat et al. (2016).

In conclusion, the results of the present study suggest that BBM offers a significant protective effect against ISO-induced myocardial infarction, by maintaining the myocardial antioxidant status and inhibition of iNOS expression and oxidative stress.

Conflict of interests

The authors have no conflict of interests to declare.

Novelty of the research

The present study provides the evidence for protective effect of berberine for the first time against isopropenol induced cardiotoxicity.

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