ORIGINAL ARTICLE

Cardioprotective effects of *Butea monosperma* (Lam.) following myocardial infarction in rats with isoproterenol-induced heart failure

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Abstract

Objective: The objective of this study was to evaluate the protective role of *Butea monosperma* (Lam.) aqueous extract (BMAE) on the various risk factors of ischemic heart disease. **Methods:** The male albino rats of Wistar strain were randomly divided and treated with BMAE (200, 400, and 600 mg/kg per oral) or normal saline or vitamin E for 30 days with concomitant subcutaneous injection of isoproterenol (ISO 85 mg/kg) on 29th and 30th day, at 24 h intervals. The effects of BMAE on cardiac marker enzymes, namely, creatine kinase (CK) and lactate dehydrogenase (LDH), antioxidant enzymes, namely, superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and glutathione peroxidase (GPx), and lipid profile, namely, cholesterol (CH), triglyceride (TG), low-density lipoproteins (LDL), and high-density lipoproteins (HDL), along with histopathological changes were accessed in ISO-induced myocardial infarction (MI) in male Wistar rats. **Results:** ISO-treated rats exhibited a significant ($p<0.05$) enhancement in the levels of CH, TG, LDL, CK, and LDH; also, there was a significant decrease in SOD and CAT, GSH, and GPx activity when compared to normal control rats. However, pretreatment with BMAE 600 mg/kg per oral for 30 days significantly ($p<0.05$) reversed the effects of ISO when compared to ISO-treated rats which were further endorsed by the histopathological studies. **Conclusion:** The current findings suggest that BMAE mitigates the cardiotoxic effects of ISO and may be of worth in the treatment of MI.

Keywords: Antioxidant enzymes, *Butea monosperma*, cardiac marker enzymes, lipid profile

INTRODUCTION

Myocardial infarction (MI) is described as an imbalance of coronary blood supply and demand, which can result in myocardial ischemic injury and damage the cardiomyocytes.[1] Studies have demonstrated that during ischemic damage, oxidative stress produced by the generation of reactive oxygen species (ROS) which is the leading cause of the development of MI. Ischemia surpasses a serious level in an extended period in MI and proceeds to permanent myocardial cell injury or death.[2] MI is the most clinically encountered ischemic heart disease and remains the foremost reason of death and disability worldwide which is further manifested by hemodynamic, biochemical, and histopathological alternations, along with altered arterial pressure indices, heart rate, ventricular impairment, and preload as well as diminished endogenous antioxidants, escape of cardiac injury marker enzymes, and lipid peroxidation.[3,4] These changes are consequential to the augmented increase in ROS such as superoxide anion and hydroxyl radicals in ischemic tissues resulting in oxidative damage to membrane lipids, proteins, carbohydrates, and DNA.[5] As a result, antioxidants may be valuable in averting the initiation and additional consequences of ischemic heart diseases.[6,7] Numerous synthetic antioxidants have revealed limitations in showing pro-oxidant, toxic, and/or mutagenic properties, thus, shifting the attention of researchers toward the naturally derived antioxidants.

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Since ancient times, the Indian spices and medicinal plants are believed to exhibit antioxidant activity and demonstrated to play an important role in the management of various diseases in humans, including cardiovascular diseases.[8] *Butea monosperma* (Lam.) (BM) is a well-known traditionally used herbal medicine which has the capability to treat many ailments. The plant is popularly known as "Flame of the forest," belonging to the family *Fabaceae*. The BM consists of chemical constituents such as triterpene, butein, butin, isobutrin, coreopsin, isocoreopsin (butin 7-glucoside), sulphurein, chalcones, aurones, monospermoside (butein 3-e-D-glucoside), isomonospermoside, flavonoids (palasitrin, prunetin), and steroids.[9] In numerous experimental studies, BM showed to exhibit aphrodisiac, astringent, tonic, and diuretics.[10-12] Several studies have shown that BM is a potent blocker of lipid peroxides formation and scavenger of superoxide anions and hydroxyl radicals.[13] The free radicals generated oxidative stress plays a critical role in the pathogenesis of MI and BM is reported to exert potent antioxidant and free radical scavenging activity.[14] Hence, the present investigation was designed to assess the potential of BM as a cardioprotective agent in isoproterenol (ISO)-induced MI in male rats. ISO is a synthetic catecholamine and β-adrenergic agonist that causes severe biochemical, functional, and structural changes in the heart[15] and recapitulates to the human MI. The present study also elucidates the mechanism of its therapeutic efficacy, by substantiating the biochemical changes with histopathological studies.

MATERIALS AND METHODS

Plant Material and Preparation of BM aqueous extract (BMAE)

The flowers of BM were collected during the month of April from the fields of Moha village, Taluka Yavatmal, Dist. Yavatmal, Maharashtra, India. It was identified and authenticated by Krishi Vigyan Kendra- Yavatmal division, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (Voucher No.KVK/Ytl/254/2021). The flowers were dried under shade thoroughly and powdered. About 500 g of the dry flower powder was extracted thrice with water. The combined extracts were concentrated in a rotary evaporator at reduced pressure to obtain about 63.20 g (12.64%, w/w) and stored at 4°C until further use.[16]

Animals

Male albino rats of Wistar strain, 150–180 g of body weight, were selected under hygienic conditions and kept at standard environmental conditions (temperature: 24 ± 1°C, light/dark cycle: 12/12 h) in the central animal facility of P. Wadhwani College of Pharmacy, Yavatmal. The protocol for the study was approved by the Institutional Animal Ethical Committee (IAEC) (IAEC Approval No.: 650/PO/Re/S/2002/CPCSEA/2021/07). The rats were fed with a standard pellet diet and water *ad libitum*.

Chemicals and Reagents

Formalin (SDFCL), thiobarbituric acid (Loba chemie), dithiobisnitrobenzoate (Sigma Co.), trichloroacetic acid (TCA)–(SRL) disodium hydrogen phosphate (Qualigen), liquid paraffin (Nice), ISO (Micro labs) chemical Kits – cholesterol (CH), triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) (Ambika diagnostics).

Experimental Designs

Rats randomly selected were divided into six groups, comprising six rats each. The BMAE was suspended in distilled water and animals were pretreated with the extract for 30 days. MI was induced in the experimental rats by concomitant subcutaneous injection of ISO (85 mg/kg,) twice at 24 h intervals.

- Group I-served as a normal control group received distilled water (5 mL/kg body weight, p. o) as a vehicle for 30 days
- Group II-ISO-treated group, received ISO (85 mg/kg,) twice at 24 h intervals
- Group III-BMAE 200 mg/kg + ISO-treated group, rats were pretreated with BMAE for 30 days (200 mg/kg body weight, p. o.) and ISO (85 mg/kg,) twice at 24 h intervals
- Group IV-BMAE 400 mg/kg + ISO-treated group, rats were pretreated with BMAE for 30 days (400 mg/kg body weight, p. o.) and ISO (85 mg/kg,) twice at 24 h intervals
- Group V-BMAE 600 mg/kg + ISO-treated group, rats were pretreated with BMAE for 30 days (600 mg/kg body weight, p. o.) and ISO (85 mg/kg,) twice at 24 h intervals
- Group VI-Vitamin E + ISO-treated group, rats were pretreated with vitamin E for 30 days (100 mg/kg body weight, p. o.) and ISO (85 mg/kg,) twice at 24 h intervals.[15,16,17]

Biochemical Studies

Blood was obtained from all the animals by the puncturing retro-orbital plexus. Collected blood was centrifuged (2000 rpm for 10 min) to get clear serum and was used to estimate various biochemical markers such as endogenous antioxidants, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and lipid profile – TG, CH, LDL, very LDL (VLDL), HDL and cardiac marker enzymes – creatine kinase (CK), and lactate dehydrogenase (LDH).

Histopathology

The heart was excised from the animals and washed with normal saline. The materials were stored in 10% buffered neutral formalin and then subjected to histopathological examination.

Statistical Analysis

Statistical analysis was performed using analysis of variance ANOVA and a least significant difference (LSD) *post hoc* test was used to compare individual means. The results were expressed as the mean ± SD of six rats in each group, and a statistical probability of $P < 0.05$, $P < 0.001$ was considered to be significant.

RESULTS

Effect of BMAE on Cardiac Marker Enzymes

Administration of ISO produced a significant decrease in myocytes grievance indicator enzymes, CK, and LDH enzymes in the heart as compared to the normal control group [Table 1]. Although, pretreatment with BMAE (200, 400, and 600 mg/kg) has significantly prevented the depletion of myocardial enzymes as compared to ISO-treated group.

Effect of BMAE on the Antioxidant Enzymes Activities

In the ISO control group, the administration of ISO injections significantly decreased the activities of antioxidant enzymes; SOD, CAT, reduced glutathione (GSH), and GPx in the heart as compared to the normal control group [Table 2]. However, pretreatment with BMAE (200, 400, and 600 mg/kg) significantly prevented the reduction in the activities of antioxidant enzymes; SOD, CAT, GSH, and GPx in a dose-dependent manner when compared to ISO-treated group.

Effect of BMAE on Lipid Profile

ISO administration produced a significant increase in the level of CH, TG, LDL, VLDL, and a significant decrease in the level of HDL when compared to the normal control group. However, pretreatment with BMAE (200, 400, and 600 mg/kg) significantly decreased the level of CH, TG, LDL, VLDL, and significantly increased the level of HDL as compared to ISO-treated group [Table 3].

Effect of BMAE on Histopathology of the Heart

The histopathological examination was scored and graded on the basis of severity of changes, as presented in Figure 1.

Table 1: Effect of BMAE on cardiac marker enzymes in ISO-induced myocardial infarction in rats

Treatments	CK (IU/mg Protein)	LDH (IU/mg protein)
Normal	185.26±14.56	263.65±19.34
ISO	79.59±9.83[a]	123.34±13.45[a]
BMAE 200 mg/kg+ISO	135.86±10.61[b]	236.43±14.62[b]
BMAE 400 mg/kg+ISO	151.82±12.24[b]	198.12±18.26[b]
BMAE 600 mg/kg+ISO	168.23±13.42[b]	238.33±19.46[b]
Vitamin E+ISO	176.11±16.64[b]	248.13±18.56[b]

The data are expressed as mean±SD and analyzed using one-way analysis of variance (ANOVA) followed by *post hoc* test. [a]=$P<0.05$, when compared to normal control (n=6); [b]=$P<0.05$, when compared to ISO-treated rats (n=6), ISO: Isoproterenol, BMAE: *Butea monosperma* (Lam.) aqueous extract, CK: Creatine kinase, LDH: Lactate dehydrogenase

Table 2: Effect of BMAE on antioxidant enzymes in the ISO-induced myocardial infarction in rats

Treatments	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	GSH (µmol/g tissue)
Normal	12.56±2.63	25.46±3.08	2.63±0.43	3.41±0.37
ISO	5.46±1.42[a]	14.16±1.52[a]	0.46±0.12[a]	1.35±0.13[a]
BMAE 200 mg/kg+ISO	7.83±2.24[b]	18.12±2.31[b]	1.21±0.21[b]	1.46±0.20[b]
BMAE 400 mg/kg+ISO	8.67±2.40[b]	20.64±2.16[b]	1.83±0.29[b]	2.24±0.65[b]
BMAE 600 mg/kg+ISO	9.13±2.52[b]	22.16±2.53[b]	1.9±0.41[b]	2.89±0.59[b]
Vitamin E+ISO	10.16±1.97[b]	24.16±3.15[b]	2.06±0.34[b]	3.12±0.56[b]

The data are expressed as mean±SD and analyzed using one-way analysis of variance (ANOVA) followed by *post hoc* test. [a]=$P<0.05$, when compared to normal control (n=6); [b]=$P<0.05$, when compared to ISO-treated rats (n=6), ISO: Isoproterenol, BMAE: *Butea monosperma* (Lam.) aqueous extract, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, GSH: Glutathione

Table 3: Effect of BMAE on lipid profile in the ISO-induced myocardial infarction in rats

Treatments	CH (mg/dl)	TG (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)
Normal	83.29±9.15	193.09±14.75	13.14±1.3	28.61±0.95	39.82±2.18
ISO	282.41±15.12[a]	263.91±17.5 [a]	68.88±7.31 [a]	59.44±2.06 [a]	12.08±2.07 [a]
BMAE 200 mg/kg+ISO	261.32±13.97[b]	245.32±16.44[b]	55.16±3.37[b]	53.06±2.28[b]	12.15±0.93[b]
BMAE 400 mg/kg+ISO	264.09±15.19[b]	182.3±12.79[b]	52.31±5.77[b]	52.46±2.55[b]	13.61±1.08[b]
BMAE 600 mg/kg+ISO	204.21±15.32[b]	145.3±12.28[b]	50.89±4.73[b]	49.06±1.39[b]	13.98±0.95[b]
Vitamin E+ISO	172.42±14.54[b]	141.78±14.54[b]	27.37±2.56[b]	36.62±1.09[b]	18.42±2.65[b]

The data are expressed as mean±SD and analyzed using one-way analysis of variance (ANOVA) followed by *post hoc* test. [a]=$P<0.05$, when compared to normal control ($n=6$); [b]=$P<0.05$, when compared to ISO-treated rats ($n=6$), LDL: Low-density lipoproteins, VLDL: Very LDL, HDL: High-density lipoproteins, CH: Cholesterol, TG: Triglyceride

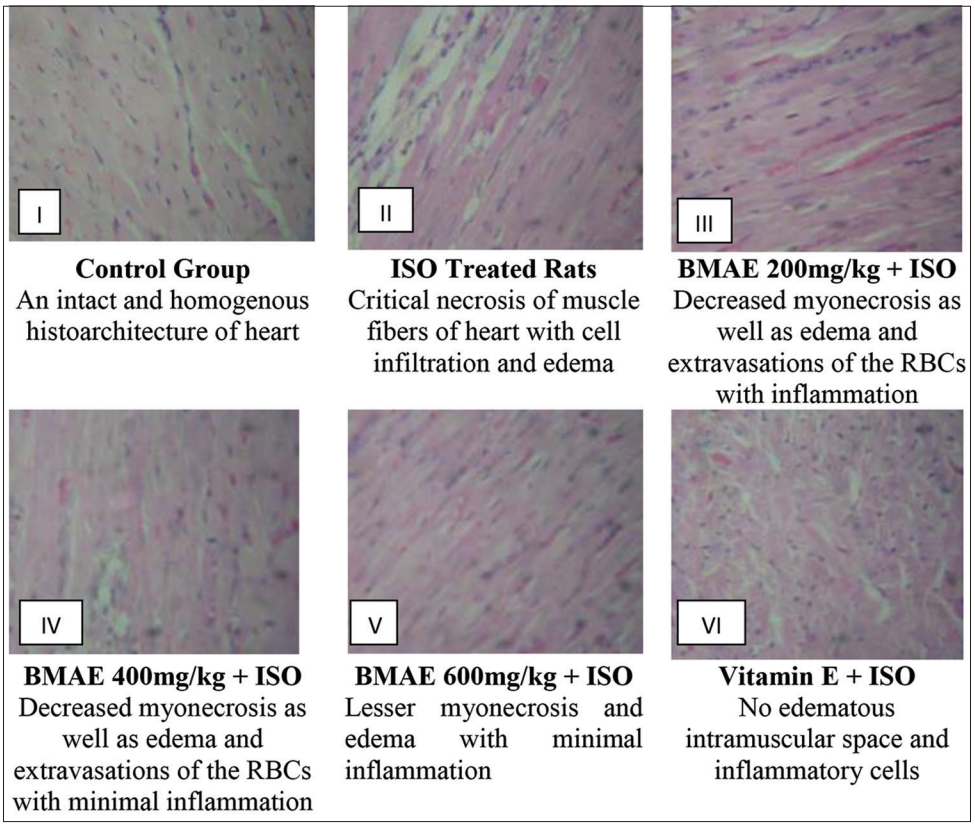

Figure 1: Effect of BMAE on histopathology of heart in the ISO-induced myocardial infarction in rats

The heart of normal control group showed an intact and homogenous histoarchitecture without edema, necrosis, and inflammation. In ISO-treated rats, heart tissue revealed confluent critical necrosis of muscle fibers with cell infiltration, edema, increased connective tissue among myocardial fibers, and myophagocytosis, along with extravasations of RBCs, whereas the rats treated with BMAE 200, 400, and 600 mg/kg followed by ISO administration showed protection from myocardial injury evidenced by decreased myonecrosis as well as edema and extravasations of the RBCs with minimal inflammation. The cardioprotection was observed with BMAE against ISO-induced myocardial necrosis in a dose-dependent manner.

DISCUSSION

The present investigation reveals that BMAE exerts cardioprotection against ISO-induced MI by restraining endogenous antioxidant defense system, along with histological and ultra-structural preservation of cardiomyocytes reflected by reduced leakage of myocytes injury marker enzymes. MI induced by ISO in experimental animals is a widely adopted animal model for the experimental evaluation of cardioprotective agents as it is clinically significant in reviewing the aspects of human MI.[15] There is an imbalance between oxygen supply and demand by the cardiomyocytes through increasing the chronotropic and inotropic activity

to evident myocardial function and increase the calcium overload in the myocardium after ISO administration.[7] Furthermore, the metabolites and auto-oxidation of ISO are also involved in the pathogenesis of myocardial ischemia by generating free radicals.[6] After the administration of ISO, a vigorous fall in the activities of endogenous antioxidant systems of the heart leads to the gradual loss of pro-oxidant/antioxidant balance that accumulates in cardiomyocytes and manifests as oxidative damage. The endogenous antioxidant enzyme systems consisting SOD, CAT, GSH, and GPx are the first line of cellular defense against oxidative stress and counter the formation of several ROS including superoxide anions and hydroxyl radicals.[18,19] The enormous decline in the activities of SOD, CAT, GSH, and GPx following ISO administration signifies the irresistibility of free radicals, which cause oxidative damage to the myocardial cells. In addition, it was also observed a significant diminution in the activities of GPx enzyme and GSH in ISO-treated animals, whereas the treatment with BMAE has improved the actions of SOD and CAT and prohibited the consumption of GSH, along with restoration in the GPx activity, which points to the potential antioxidant and free radical scavenging activity of BMAE. In the previous studies, BMAE has been described as one of the efficacious antioxidant and free radical scavengers by sustaining enzymatic antioxidants, the first line of defense.[13] The present study showed the antioxidant activity of BMAE and endorses its cardioprotective effect mediated by its antioxidant effect in the myocardium. An ISO administration causes debilitation to the antioxidant protective mechanisms and rendered the myocardium more vulnerable to lipid peroxidation evidenced by an elevated level of CH, TG, LDL, and VLDL. BMAE has shown involvement in the scavenging of ROS and confers defense against lipid peroxidation in accordance with the previous observations demonstrated antioxidative mechanism against the free radical-induced oxidative damages of body organs.[20]

ISO administration also enhances the release of the cardiac marker enzymes; CK and LDH from heart which serves as a sign of myocardial injury. CK and LDH are present in the myocardium and released into the blood after the myocytes injury and breakup of the subcellular and cellular compartments.[17] Although, BMAE treated rats showed a significant reinstatement of CK and LDH enzymes stipulated that BMAE preserved myocytes membrane and rendered cardiomyocytes less leaky accredited to stabilization of myocytes membranes consequent to inhibition of lipid peroxidation and membrane disruption. The histopathology of myocardial tissue in normal control animals demonstrated an intact and united cell membrane with no sign of edema, inflammation, and infiltration of inflammatory cells. Whereas histological examination of ISO-treated rats, myocardium unveiled coagulative myonecrosis, edema, and infiltration of inflammatory cells. On the other hand, rats treated with BMAE exhibited condensed myonecrosis, edema, and reduced permeation of inflammatory cells. Assimilating together the histological salvage with biochemical parameters, BMAE appears non-toxic to the cardiomyocytes most likely by reclamation of the endogenous antioxidant defense against ISO. The phytoconstituents present in BMAE are sterols, phenolic acids, flavonoids, and flavonols which are considered to be potential cardioprotectant against overt oxidative damage.[21]

CONCLUSION

The present biochemical and histopathological findings authenticate that BMAE 600 mg/kg conserves the integrity of the myocardial cell membrane by maintaining the activities of CK and LDH. Thus, the current study suggests that BMAE 600 mg/kg has the ability and potential to protect against oxidative stress-mediated cardiac dysfunction in experimental MI induced by ISO in rats. The outcome of the present study results may have future therapeutic value, particularly for patients who are vulnerable to develop ischemic heart disease.

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AUTHORS' CONTRIBUTIONS

The Corresponding author, A. V. Shrirao performed the experiment and wrote the manuscript. N. I. Kochar supported in the laboratory work. A. V. Chandewar supervised the work and reviewed the manuscript.

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