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Evaluation of Cardioprotective Activity of flowers of *Butea monosperma* against Isoproterenol induced Cardiac Hypertrophy in Wistar Albino Rats

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Abstract

The objective of the present study was to investigate the effect of pretreatment with *Butea monosperma* on isoproterenol (ISO)-induced cardiotoxicity and cardiac hypertrophy in rats. **Methods:** Albino male Wistar rats (250–300 g) were evenly divided into following groups: Control group (2% acacia solution (2ml/kg p.o.) orally for 18 days and water IP from days 9–18). ISO group (olive oil 2 mL/kg orally for 18 days and ISO 1 mg/kg IP from days 9–18); *BM (300mg/kg)+ ISO:* Animals were administered with BM (300mg/kg p.o.) for 18 days and injected with Isoproterenol from 9th day (1ml/kg IP). *BM (600mg/kg) + ISO:* Animals were administered with BM (600mg/kg p.o.) for 18 days and injected with Isoproterenol from 9th day (1ml/kg IP). Co Q10 (100mg/kg) + ISO: Animals were administered with Co Q10 (100mg/kg p.o.) for 18 days and injected with Isoproterenol from 9th day (1ml/kg IP). And the twenty-four hours after the last dose of water or ISO, the rats were anesthetized, and an ECG was recorded. Blood was withdrawn by retro-orbital puncture for estimation of serum creatine kinase-MB (CK-MB) isoenzyme levels, lactate dehydrogenase (LDH) levels, and aspartate aminotransferase activities. The animals were euthanized using an overdose of ether. The hearts of 6 animals from each group were used for estimation of superoxide dismutase (SOD) activity, reduced glutathione (GSH) concentration, lipid peroxidation (LPO), malondialdehyde (MDA), and total protein concentration. **Results:** A total of 30 rats (6 in each group) were used in this study; all rats survived to study end. Compared with the control group, the ISO-treated rats had a significant change in the endogenous antioxidants (ie, significantly higher myocardial MDA concentration [$P < 0.001$]; significantly lower myocardial GSH concentration [$P < 0.001$] and SOD activity [$P < 0.01$]); and significantly higher serum activities of marker enzymes (eg, CK-MB [$P < 0.001$] and LDH [$P < 0.001$]). Compared with the ISO group, the BM 300 mg/kg + ISO group had a significant change in the endogenous antioxidants (ie, significantly lower MDA concentration [$P < 0.001$]; significantly higher myocardial GSH concentration [$P < 0.001$] and SOD activity [$P < 0.001$]); and significantly lower serum activities of marker enzymes (eg, CK-MB [$P < 0.05$] and LDH [$P < 0.001$]. **Conclusion:** Pretreatment with BM (1300 mg/kg) for 18 days was associated with moderate protection against ISO-induced cardiotoxicity and cardiac hypertrophy, and with lower myocardial injury by preserving endogenous antioxidants and reducing LPO in rat heart.

Keywords
LPO, SOD, MDA

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INTRODUCTION

Cardiac hypertrophy can be defined as a physiologic and pathologic response which is due to increase the functional demand or specific hormonal stimulation. Cardiac hypertrophy is itself a predictor of cardiovascular morbidity and mortality, independent of hypertension and coronary diseases (2, 7). Cardiac hypertrophy is an adaptive response of the heart muscle to a wide variety of intrinsic and extrinsic stimuli.

Isoproterenol is a potent, non-selective beta-adrenergic agonist. It possesses inotropic and chronotropic actions enhance normal myocardial function at the expense of elevating myocardial oxygen consumption, and consequently, coronary blood flow. Isoproterenol decreases protein degradation rates and content of ATP which accounts for increase in heart weight and also produces myocyte necrosis, even when applied in low doses with chronic infusion. This necrosis is followed by increased fibrosis and hypertrophy. Isoproterenol-induced myocardial hypertrophy is accompanied by enhanced NHE-1 activity and expression (3). Hypertrophy may be due to increased myocardial loading, which is a direct result of adrenergic hormone stimulation of the heart, or as a compensation for myocyte loss (1).

Butea monosperma (Lam.) is commonly known as Flame of forest, palas, palash, mutthuga, belongs to the family Fabaceae. It is seen throughout India, Burma and Ceylon except in very acrid parts. This plant is a host tree for lac insect (4, 6). It is well known traditional plant and reported to have hepatoprotective acitivity (14), antioxidant activity (9), anti-convulsive activity, nootropic activity (5) and anti oestrogenic activity (12). The plant was known to possess antioxidant activity, but cardio protective was not reported. Hence the present study reports the cardio protective activity of Butea monosperma flowers against Isoproterenol induced cardiac hypertrophy in rats.

MATERIALS AND METHODS

PLANT MATERIAL

Flowers of *Butea monosperma* were collected locally in Hyderabad, AP, India. The material was authentified by Dr. K Madhava Chetty, Sri Venkateshwara University, Tirupati, AP, India. The flowers were washed under running water, air dried and finely powdered using herbal grinder. The material was stored at room temperature in zipped plastic bags and used as test drug.

CHEMICALS

Isoproterenol HCL was purchased from Samarth Pvt Ltd (Mumbai, India). Coenzyme Q 10 was purchased from Universal Medicare Pvt Ltd (Mumbai, India). All other reagents and chemicals used in entire study were of analytical grade.

EXPERIMENTAL ANIMALS

Male rats of wistar strain weighing between 200-250gms were used for study. All the rats were purchased from National Institute of Nutrition (NIN). The animals were fed ad libitum with standard pellet diet and had free access to water.

EXPERIMENTAL PROTOCOL

All the rats were grouped into 5 groups consisting of six animals in each group.

Group-1: Control group: Animals were administered with 2% acacia solution (2ml/kg p.o.) for 18 days and distilled water from 9th day (1ml/ kg IP).

Group-2: Isoproterenol group: Animals were injected with Isoproterenol from 9th day (1ml/kg IP).

Group-3: BM (300mg/kg)+ ISO: Animals were administered with BM (300mg/kg p.o.) for 18 days and injected with Isoproterenol from 9th day (1ml/kg IP).

Group-4: BM (600mg/kg)+ ISO: Animals were administered with BM (600mg/kg p.o.) for 18 days and injected with Isoproterenol from 9th day (1ml/kg IP).

Group-5: Co Q10 (100mg/kg)+ ISO: Animals were administered with Co Q10 (100mg/kg p.o.) for 18 days and injected with Isoproterenol from 9th day (1ml/kg IP).

On 18th day of treatment period all the animals were anestheized with anesthetic ether and blood samples were collected from retro orbital plexus using micro capillary technique. The serum was separated by centrifugation method and used for estimation of cardiac biomarkers (SGOT, CKMB). Animals from all groups were sacrificed by cervical dislocation and hearts were dissected out. They were immediately washed with ice cold saline and weighed immediately on Single Pan Electronic Balance (Precisa 205 ASCS). The hearts were then minced and homogenized in chilled tris hydrochloride buffer (10mM, pH 7.4) to a concentration of 10% w/v. The homogenate was centrifuged at 7000 rpm at 25 minutes using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the determination of MDA, GSH and AST.

BIOCHEMICAL ESTIMATIONS

Reduced glutathione (GSH) content was estimated using Trichloroacetic acid (TCA) and 5,5'-dithiobis (2-nitrobenzoic acid) reagent (DTNB) previously described by Moron *et al.* (1979). Homogenate of myocardial tissue in Thiobarbituric acid (TBA) was

used for estimation of malondialdehyde content (MDA content) according to Slater and Sawyer (1971).

HISTOPATHOLOGICAL STUDIES

At the end of the study the lower portions of the heart were excised and fixed in 10% neutral formalin solution. These were embedded in paraffin and sections of 5 µm thickness were cut and stained with hematoxylin and eosin (H and E). Then these sections were examined under light microscope and photomicrographs were taken.

STATISTICAL ANANLYSIS

The statistical analysis for biochemical parameters was performed using statistical software Graph pad Prism 3.0. The results were expressed as mean±SEM. These results were analyzed using One way ANNOVA followed by Dunnett't' test. The values were statistically significant at $p<0.01$.

RESULTS:

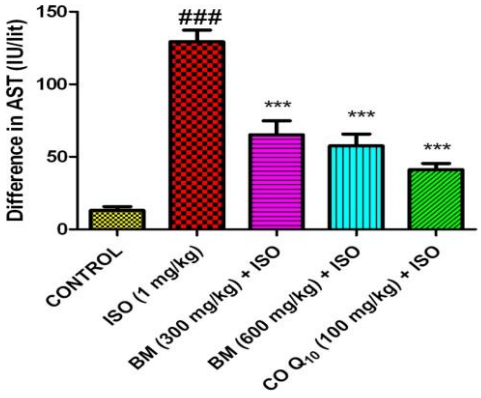
It has been found that, there is a change in biochemical parameters like SGOT, serum creatine kinase, lipid peroxidation, reduced glutathione and heart weight. Treatment with BM(300mg/kg) have good and quite comparable with that of standard drug on reduction of SGOT. When concerned with SGPT both the treatments [BM (300mg/kg) and BM (600mg/kg)] have reduction nearer to standard drug. In case of lipid peroxidation, treatment with BM (600mg/kg) have good effects. The heart to body weight ratio in the treatment with BM (600mg/kg) is having good results and quite comparable with the standard drug.

From this investigation it is concluded that the treatment with BM (600mg/kg) of *Butea monosperma Linn* possess cardioprotective activity and was not with that of positive control.

Table -1. Effect of BM on serum Glutamate Oxaloacetate Transaminase (SGOT) levels in isolated Heart of male wistar rats in Isoproterenol induced cardiac hypertrophy.

Group	IU/L
Control group (n=6)	8.166667±11.72035
ISO group (n=6)	141.8571±7.266311###
BM (300mg/kg) +ISO (n=6)	64.33333±22.8444***
BM (600mg/kg) +ISO (n=6)	58.33333±20.48089***
CoQ_{10}(100mg/kg) +ISO (n=6)	46.16667±13.39278***

Figure -1. Effect of BM on serum Glutamate Oxaloacetate Transaminase (SGOT) levels in isolated Heart of male wistar rats in Isoproterenol induced cardiotoxicity & cardiac hypertrophy.

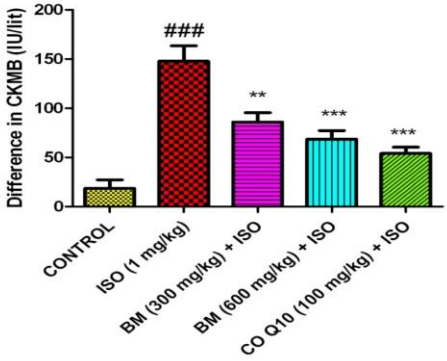


Values are expressed as mean ± SEM. Data analyzed by one-way ANOVA followed by Bartlett's test for equal variances. ### $p<0.001$ as compared with control group. ***$p<0.0001$ as compared with ISO group.

Table-2. Effect of BM on serum Creatinine kinase (CKMB) levels in isolated Heart of male wistar rats in Isoproterenol induced cardiotoxicity & cardiac hypertrophy.

Group	IU/L
Control group (n=6)	18.66667±20.85825
ISO group (n=6)	188±82.87822###
BM (300mg/kg) + ISO (n=6)	80.33333±43.99394**
BM (600mg/kg) +ISO (n=6)	68.5±21.47324***
CoQ_{10} (100mg/kg) + ISO (n=6)	54±15.70987***

Figure-2. Effect of BM on serum Creatinine kinase (CKMB) levels in isolated Heart of male wistar rats in Isoproterenol induced cardiotoxicity & cardiac hypertrophy.

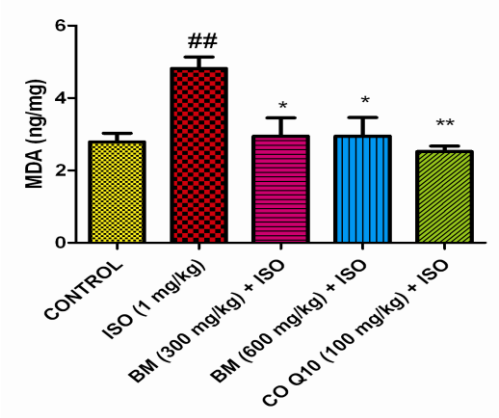


Values are expressed as mean ± SEM. Data analyzed by one-way ANOVA followed by Tukey's Multiple Comparison Test. ### p<0.001 as compared with control group. ***p<0.0001 as compared with ISO group. **p<0.001 is compared with ISO group.

Table-3. Effect of on lipid peroxidation (MDA) concentration in isolated Heart of male wistar rats in Isoproterenol induced cardiotoxicity & cardiac hypertrophy.

Group	MDA (nMol/mg of protein)
Control group (n=6)	2.786±0.476834
ISO group (n=6)	4.815±0.636372 ##
BM (300mg/kg)+ ISO (n=6)	2.946±1.011353 *
BM (600mg/kg)+ISO (n=6)	2.946±1.035135 *
CoQ_{10} (100mg/kg) + ISO (n=6)	2.521±0.305012 **

Figure -3. Effect of BM on lipid peroxidation (MDA) concentration in isolated Heart of male wistar rats in Isoproterenol induced cardiotoxicity & cardiac hypertrophy

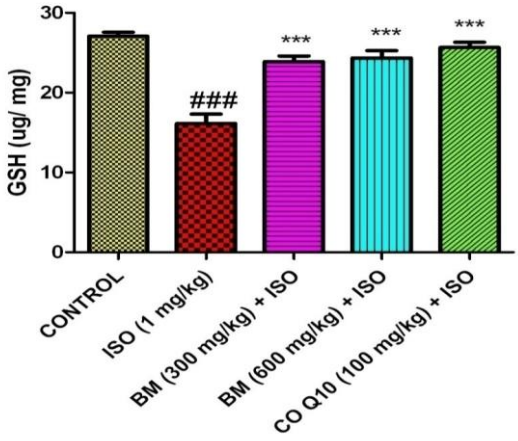


Values are expressed as mean ± SEM. Data analyzed by one-way ANOVA followed by Tukey's Multiple Comparison Test. ## p<0.001 as compared with control group. **p<0.0001 as compared with ISO group. *p<0.001 is compared with ISO group.

Table-4. Effect of BM on reduced glutathione (GSH) concentration in Hearts of male wistar rats in Isoproterenol induced cardiotoxicity & cardiac hypertrophy.

Group	GSH (µg/mg of protein)
Control group (n=6)	27.057±1.032146
ISO group (n=6)	16.136±2.3254 ###
BM (300mg/kg) + ISO (n=6)	23.88±1.399331 ***
BM (600mg/kg) +ISO (n=6)	24.3375±1.28586 ***
CoQ_{10} (100mg/kg) + ISO (n=6)	25.66895±1.28586 ***

Figure -4. Effect of BM on reduced glutathione (GSH) concentration in isolated Heart of male wistar rats in Isoproterenol induced cardiotoxicity & cardiac hypertrophy

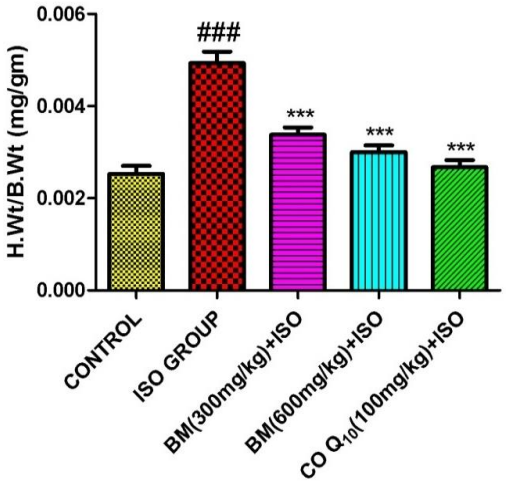


Values are expressed as mean ± SEM. Data analyzed by one-way ANOVA followed by Tukey's Multiple Comparison Test. ### p<0.001 as compared with control group. ***p<0.0001 as compared with ISO group.

Table-5. Effect of BM (300 and 600mg/kg) on Heart weight/Body weight ratio in male wister Rats in Isoproterenol induced cardiotoxicity & cardiac hypertrophy.

Group	Heart weight/ Body weight Ratio (mg/g)
Control group (n=6)	0.002528±0.000425
ISO group (n=6)	0.004932±0.000679###
BM(300mg/kg)+ ISO (n=6)	0.003375±0.000419***
BM (600mg/kg)+ISO (n=6)	0.002797±0.000241***
CoQ_{10} (100mg/kg) + ISO (n=6)	0.002728±0.000405***

Figure -5. Effect of BM on Heart weight to Body weight ratio in isolated Heart of male wistar rats in Isoproterenol induced cardiotoxicity & cardiac hypertrophy



Values are expressed as mean ± SEM. Data analyzed by one-way ANOVA followed by Bartlett's test for equal variances. ### p<0.001 as compared with control group. ***p<0.0001 as compared with ISO group.

DISCUSSION

Cardiac hypertrophy is an adaptive response of the heart muscle to a wide variety of intrinsic and extrinsic stimuli. Cardiac hypertrophy due to an increase in ventricular wall thickness is a common feature for various cardiovascular conditions like

hypertension, myocardial infarction and type-2 diabetes. ISO is a beta-adrenergic agonist has been reported to cause oxidative stress in myocardium resulting in infarct like necrosis of the heart muscle. In ISO induced myocardial infarction there is enhanced free radical formation due to auto oxidation of ISO and leads to peroxidation of membrane bound polyunsaturated fatty acids leading to permeability changes in the myocardial membrane, intracellular calcium overload and irreversible damage. Among various transducers of intracellular signaling pathways implicated in hypertrophic response, the role of calcineurin (Ca2+ - calmodulin dependent protein phosphatase) in cardiac hypertrophy is rapidly gaining importance in view of the well-recognized significance of reversible protein phosphorylation in hypertrophy (10). The vulnerability of Fe3+ in the bimetallic active centre of calcineurin to reactive oxygen species (15) offers an opportunity to evolve alternate strategies for controlling cardiac hypertrophy by using antioxidants. We thus examined the effect of BM to alleviate Isoproterenol induced cardiac hypertrophy. Isoproterenol (1 mg/kg for 10 days) treated rats significant increased MDA content, SGOT levels and CKMB levels in cardiac tissues and the highly significant reduction in cardiac tissue levels of protective biological antioxidant enzymes like GSH indicated oxidative stress. These results correlate with previous studies which have demonstrated the involvement of oxidative stress and lipid peroxidation in Isoproterenol induced cardiotoxicity and cardiac hypertrophy. The result indicated that the treatment with BM (300mg/kg) and BM (600mg/kg) significantly increases the GSH levels and decrease the MDA, SGOT and CKMB level, thus indicating a cardioprotective effect of BM. Almost the results of BM (300mg/kg) and BM (600mg/kg) are comparable. Our study demonstrated that BM reduced the Isoproterenol induced cardiac hypertrophy in male wistar rats. BM significantly reduced lipid peroxidation increased the level of GSH in Isoproterenol cardiac hypertrophy.

CONCLUSION

The results showed that administration of BM (300mg/kg) extract improved the biochemical marker levels indicating decrease in oxidative stress as evident by increased level of GSH, and with decreased production of lipid peroxidation (MDA). Also, BM (300mg/kg) reduced the SGOT and CKMBMB levels which are increased due to ISO administration. Antioxidant and cardioprotective activities were observed in the ISO model by CoQ_{10} (100mg/kg) and BM but CoQ10(100mg/kg) (standard) showed better effect than BM.

It is concluded that BM (300mg/kg) may moderately protect the myocardial tissue against Isoproterenol induced cardiac hypertrophy and Pressure Overload Induced cardiac hypertrophy in male wistar rats.

REFERENCES:

1. Benjamin IJ, Jalil JE, Tan LB, Cho K, Weber KT and Clark WA. (1989). isoproterenol-induced myocardial fibrosis in relation to myocyte necrosis. Circulation Research. 65, 657-670.
2. Drazner MH, Rame JE, Marino EK, Gottdiener JS, Kitzman DW, Gardin JM, Manolio TA, Dries DL and Siscovick DS (2004). Increased left ventricular mass is a risk factor for the development of a depressed left ventricular ejection fraction within five years: The Cardiovascular Health Study. J Am Coll Cardiol. 43, 2207-2215.
3. Irene LE, Ennis, Eduardo ME, Gloria MC, Gisela C, Cesar GD, Randolph WS, María C, Horacio EC 2003. Regression of Isoproterenol-Induced Cardiac Hypertrophy by Na+/H+ Exchanger Inhibition. Hypertension.41, 1-6.
4. Kapoor LD. Handbook of Ayurvedic Medicinal Plants, Herbal Reference Library Edition (Replica Press Pvt. Ltd., India, 2005) pp.86.
5. Kasture VS, Kasture SB and Chopde CT (2002). Anticonvulsive activity of Butea monosperma flowers in laboratory animals. Pharmacol. Biochem. Behav. 72, 965-72.
6. Kirtikar KR,Basu BD, Indian medicinal plants, (Lalit mohan Basu, Allahabad, India, 1935) Vol. I, 2nd edition, pp. 785-88.
7. Levy D, Larson MG, Vasan RS, Cannel WB and Ho KK. (1996). The progression from hypertension to congestive heart failure. JAMA. 275, 1557-1562.
8. Maria S. Morona, Joseph W. Depierrea and Bengt Mannervika. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica et Biophysica Acta (BBA). 582, 67-78.
9. Mishra M, Shukla YN and Kumar S (2000). Euphane triterpenoids and lipid constituents form Butea monosperma. Phytochemistry. 54(8), 835-38.
10. Molkentin J, Lu J, Antos CL, Markham B, Richardson J, Robbins J, Grant SR and Olson EN. (1998). A calcenurin dependent trascriptional pathway for cardiac hypertrophy. Cell. 93, 215-228.
11. Rona G. (1985). Cathecholamine cardiotoxicity. J Mol Cell Cardiol. 17, 291-306.
12. Shah KG, Bakxi AJ, Sukla VJ, Dave KK and Ravishankar B (1990). Phytochemical studies and antiestrogenic activity of Butea frondosa (Butea monosperma) flowers. Ind. J. Pharm. Sci. 52, 272-75.
13. Slater TF and Sawyer BC. (1971). The Sitmulatory Effects of Carbontetrachloride and other Halogenoalkanes on Peroxidative Reactions in rat Liver Fractions in vitro. Biochem J. 123, 805-814.

14. Wagner H,Geyer B, Fiebig M, Kiso Y and Hikino H (1986). Isobutrin and Butrin, the antihepatotoxic principles of Butea monosperma flowers. Planta Med. 52(2), 77- 79.

15. Wang X, Valeria C.Culotta, Claude B. Klee. (1996). Superoxide dismutase protects calcenurin from inactivation. Nature. 383, 434-437.