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Bisoprolol oral treatment of heart failure rats: Involvement of calmodulin

Xiaoyan Lv^a, Yuqin Gao^a, Yifan Zhang^a, Narendra Maddu^b, Zhanbin Feng^{a,*}^a Department of Cardiovascular Medicine, Ninth Hospital of Xi'an, No. 151 East Section of South Second Ring, Beilin District, Xi'an City, Shaanxi Province 710054, China^b Department of Biochemistry, Sri Krishnadevaraya University, Ananthapuramu 515003, Andhra Pradesh, India

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ABSTRACT

Objectives: The current study is to estimate the bisoprolol (BOP) oral treatment of rats against the myocardial infarction (MI) administrated by using isoproterenol (ISO).**Methods:** Treatment of rats are MI administration by subcutaneous for two successive days of ISO (85 mg/kg). The tissue of heart is removed without delay, it is washed used isotonic chilled saline in this study. Plasma is separated from the collected rat blood and further analysis of biochemical parameters were carried out.**Results:** Group of ISO-treated rats have increased levels of glucose in blood, also observed the activity of lipids in plasma along fibres of muscle with hyalinization, compared to BOP treated rats, and ISO + BOP treated rats. The parameter values of BOP administered rat were close to control rats. Reports clearly state that ISO + BOP treated rat tissue of heart damage is reduced when compared to ISO treated rats.**Conclusions:** Rats were treated with ISO + BOP to defend against hyperlipidaemia and tissue of cardiac damage and followed by MI. Hence, to investigate the biochemical and biophysical BOP mechanism against MI risk in rats.© 2020 The Authors. Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

The disproportion of myocardial demand of oxygen supply directs to necrosis of heart muscle known as MI. The higher levels of hypercholesterolemia and hyperlipoproteinemia of low density lipoprotein cholesterol (LDL-C), lower levels of high density lipoprotein cholesterol (HDL-C) are the leading risk factors for MI

Abbreviations: BOP, bisoprolol; MI, myocardial infarction; ISO, isoproterenol; LDL-C, cholesterol with lesser density; HDL-C, high density of lipoprotein cholesterol; CVD, cardiovascular diseases; LV, left ventricular; COPD, chronic obstructive pulmonary disease; CaM, calmodulin; NCLAS, national centre for laboratory animal sciences; AC, air condition; TG, triglycerides; CK, creatinine kinase; LDH, lactate dehydrogenase; AST, aspartate transaminase; ALT, alanine transaminase; NAD, nicotinamide adenine dinucleotide; DNPH, 2,4-dinitrophenylhydrazine; CaCaM, canonical Ca²⁺ loaded CaM.

* Corresponding author.

E-mail address: fzbxinnei@sina.com (Z. Feng).

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(Khader et al., 2003). The stress in the heart muscle is induced by ISO which is catecholamine that leads to necrosis of myocardium (Sushamakumari et al., 1989). The blockage in the arteries along with cardiovascular diseases (CVD) is caused by ISO elevates lipoproteins and lipids specifically LDL-C seen in blood circulation (Goldstein and Brown, 1984). MI is caused due to the release of oxygen free radicals by ISO (Singal et al., 1982). These oxygen free radicals and oxidative stress jointly leads to atherosclerotic lesions generation by formation of oxidized LDL from LDL, which is the base cause of MI (Libby, 2003). The failure of heart, neurohumoral axes gets active, along with the sympathetic nervous system. Myocardial remodelling, prognosis (Elser et al., 1997) and cardiac dysfunction levels have an effect on sympathetic activity. The sympathetic and inflammatory activities may partially antagonized by beta blockers, affecting hypertrophy and cardiomyocyte apoptosis which leads to attenuated development of ventricular remodelling and raise in ejection fraction (Fringierio and Roubina, 2005). Some experimental studies have established on how the beta-blockers shows an effect in falling mortality rate among patients with failure of heart in different functional classes (Domanski et al., 2003), and with recommend guidelines that left ventricular (LV) systolic dysfunction of the patient shall be treated with

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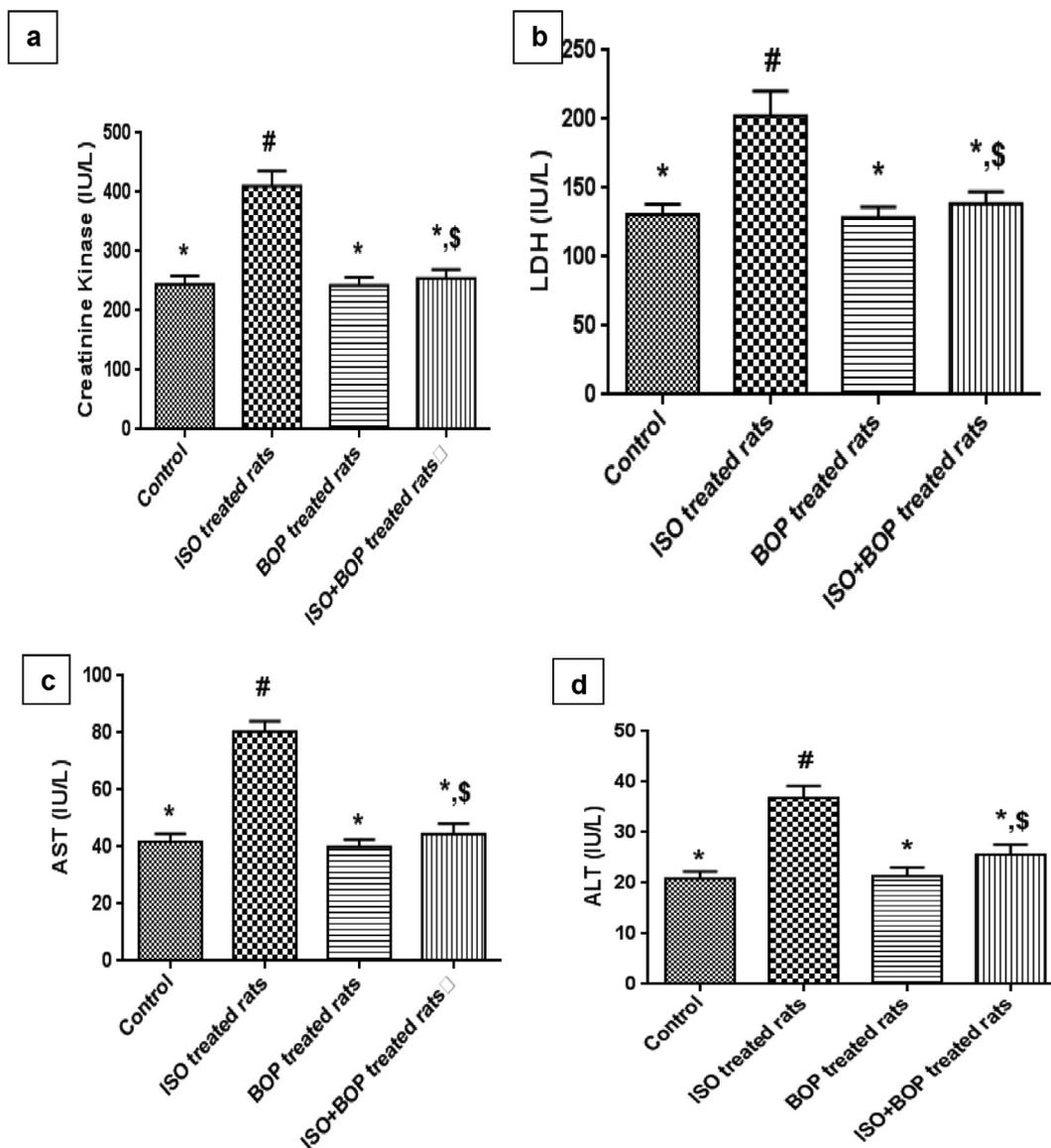


Fig. 1. Effect of isoproterenol and bisoprolol administration on cardiac marker enzymes. Values are expressed as Mean ± SEM, on each bar followed by the same symbol are not significantly different ($P \leq 0.05$) from each other according to Duncan's Multiple Range (DMR) test, $n = 8$, Control = Control rats.

beta-blockers (ACC/AHA, 2005). To control the high blood β_1 -adrenergic blocking agent is used which is BOP a cardiac selective (Albouaini et al., 2007). To minimise the multiple doses of anti-hypertensive drugs instead it can be used once in a day for a long half life. Generally BOP is well suitable, due to the β_1 -adrenergic selectivity receptor and acts as a substitute to non-selective and β -blocker drugs use to treat of hypertension induced zebeta. Report clearly mentioned this use alone or in combination/alone with another drugs to manage hypertension (Albouaini et al., 2007). The patients suffer with COPD (chronic obstructive pulmonary disease) is useful for selective receptors in patients. Clinical and hemodynamic status was improved by BOP therapy, and the heart failure of different etiologies, cardiac function in COPD patients, it includes Chagas' disease, though, improvement of exercise capacity and is not done. Other studies mentioned BOP was safe and tolerated well in different etiologies, in cardiac patients. Another study from the placebo-controlled and tolerated the medication rate is 76.5% (CIBIS, 1994) which is not different from the other

report. Medication in Chagas' disease tolerates the dose up to 10 mg, the symptoms are improved and functions of ventricular comparison to other etiologies patients. BOP and Chagas' disease is the first report to use and attention of possibility of myocardiopathy and interaction of BOP mechanism. Oxidative modifications in specific sites are identified Ca-ATPase expressed and calmodulin (CaM) in membrane sarcoplasmic reticulum, it arise oxidative stress response (Gao et al., 1998b; Viner et al., 1999). CaM is oxidized to their equivalent methionine sulfoxides, and biological aging methionine and loss of functions where as specific nitration of tyrosines in the Ca-ATPases and related sites. The oxidation of methionines and regulatory role in signaling of calcium coupling and calcium are two types of modifications post translation and CaM function and cellular redox conditions (Sun et al., 1999; Squier and Bigelow, 2000; Yermolaieva et al., 2000). The present study to investigate the ISO induced heart failure rats and BOP dose response in plasma lipid profile, cardio marker enzymes and involvement of CaM mechanism.

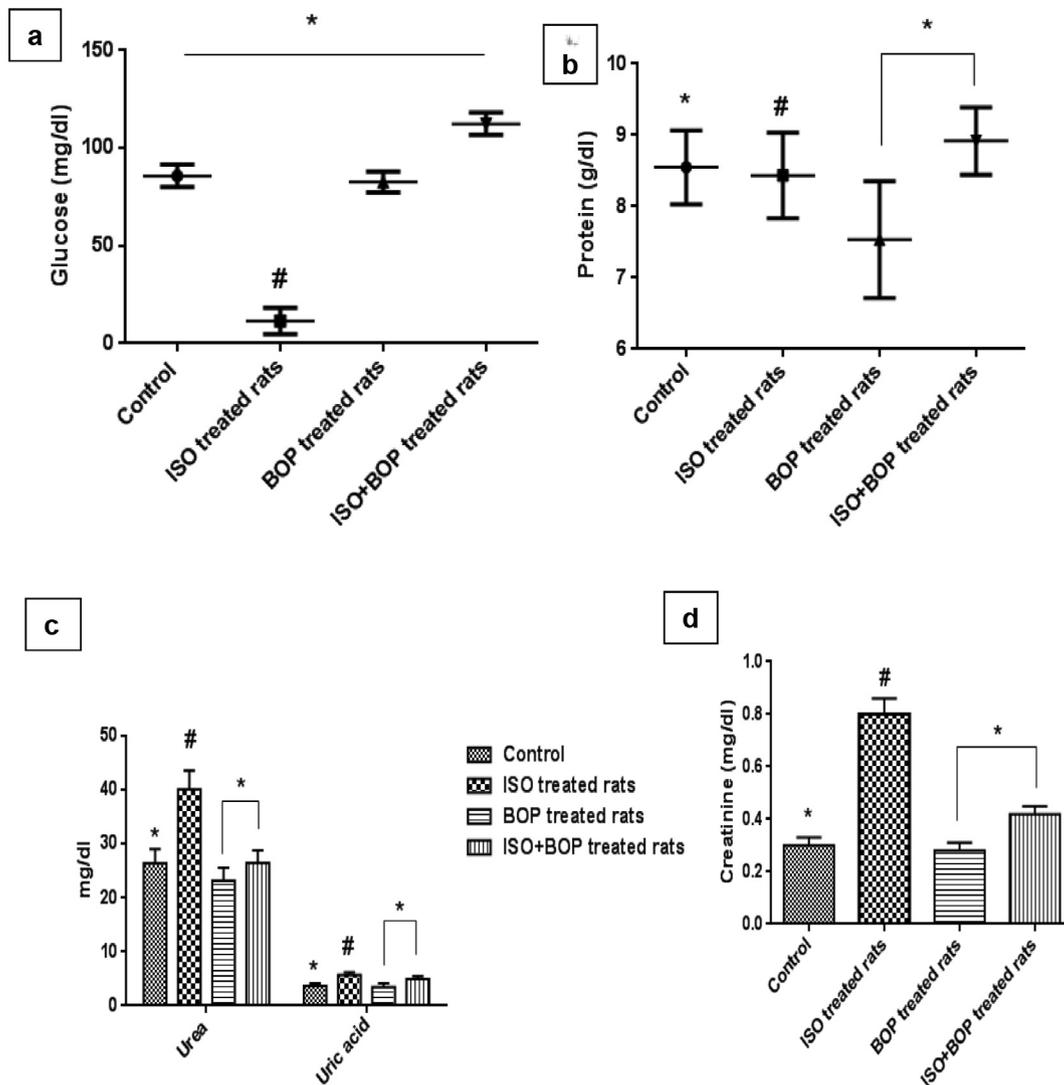


Fig. 2. Effect of isoproterenol and bisoprolol administration on plasma components Values are expressed as Mean \pm SEM, on each figure/bar followed by the same symbol are not significantly different ($P \leq 0.05$) from each other according to Duncan's Multiple Range (DMR) test, $n = 8$, Controls = Control rats.

2. Experimental methodology

2.1. Experimental design and procuring animals

60 days old thirty two albino Wistar strain of male rats, body weight ranging from 120–140, were taken from NCLAS, Hyderabad, Telangana, India and quarters in cage separately Air Condition (AC) room (25 ± 1 °C) during 7.00 a.m to 7.00p.m. Experimental animals divided into four groups in each one 8 rats. Group I consisted of normal/control rats equivalent amounts of distilled water instead of BOP (Antihypertensive Drug). Group II we design to develop a heart failure MI model in rats e. g. ISO induced heart failure model rats 85 mg/kg body weight/day (Rajadurai et al., 2006) do not consider this hypertrophy. Group III which received markedly available BOP is available under the following brand name: Zebeta was given 14 days or 30 min after MI, body weight per day 60 mg/kg). Group IV ISO administrated + BOP treated rats. All the rats were recorded daily weight and intake of food and water was followed on days of alternate. The finishes of the investigational period, in each group of rats were fasted overnight and then by using of cervical dislocation rats were sacrificed. Collected tissues and immediately for further analysis for processed. The

animal study, we are followed all procedures of including the whole surgical, feeding, and raising process were based on approved methods by the committee of animal ethical clearance for the treatment of animals and approved for the study protocol was obtained subsequent the guiding principle. Current study was also accepted by institutional ethical committee, Ninth Hospital of Xi'an, Ethic Number: 201917.

2.2. Blood collection and sample processing

Blood was collected from rats by cardiac puncture allows the collections of large amount of blood during the time at 7–10 a. m. in heparinised tubes, total proteins and albumins in plasma (Lowry et al., 1951), uric acid were analyzed using auto analyzer kit methods, urea, creatinine (Slot, 1965) were estimated using kit method in spectrophotometer. Plasma TG (triglycerides) using (Fossati and Principe, 1982) method Cholesterol estimation using by (Allian et al., 1974) estimated glucose was (Trinder, 1969). Phospholipids by (Connerty et al., 1961). Plasma Creatinine Kinase (CK), activity, was estimated by using (Sharma and Nand, 2006). Free fatty acids estimation by Moore and Stein (1948).

2.3. LDH AST and ALT activities

Estimation of (LDH) Lactate dehydrogenase by the method of (Goldberg et al., 1988). 1 ml of substrate buffer is taken in a tube and adds 0.1 ml of sample along with NAD solution after which it has to be incubated for 15 min. Added reagent of 1 ml DNPH arrest the reaction. Again it is incubated at 37 °C for 15 min and added serum 0.1 ml to empty test tube with DNPH reaction was arrested, colour developed after added 7 ml of sodium hydroxide solution at 420 nm measured. AST (Babson et al., 1962), ALT (Matsuzawa and Katunuma, 1966) activities are assayed in separate tubes; one ml of substrate buffer added to the sample of 0.1 ml incubates it for 30 min at 37 °C. The reaction is arrested after adding the reagent 1 ml DNPH. Let the tubes rest for 15 min and sodium hydroxide of 10 ml added and absorbed at 520 nm.

3. Results and discussion

Observed results that exposed in ISO treated rat leads to heart injury with significant increased change in the cardiac marker enzymes CK, LDH, AST and ALT. In ISO + BOP treated group IV results were nearly normal levels and BOP treated alone group also same when compared to controls rats in (Fig. 1). Blood glucose, serum urea, uric acid, creatinine in group II ISO treated rats has a significant increase and it showed a significant increase in the levels of these parameters. Plasma protein contents increase in experimental subjects of ISO, ISO + BOP rats but not BOP group III when compared to control group I (Fig. 2). Anandan et al. reported (2007) involvement of lipids plays important role in cardiovascular disease (CVD). Hypercholesterolaemia and hyperlipidaemia are the very important factors in cardiac risk and MI progress. ISO treated and MI includes elevated lipid levels are circulatory. Our reports, ISO treated rat's shows increase levels of TGs, Total cholesterol significantly in plasma levels with increase free fatty acids (Fig. 3). Cardiovascular disturbances and MI is the primary risk factors to increased level of TGs (Sushama Kumari et al., 1990). Increased levels significant in plasma phospholipids of ISO treated rats and ISO + BOP treated rats were clearly observed that the levels are decreased near to normal control range which might be appropriate to the decrease of hypolipidemic and hypocholesterolaemic activities in present reports are supported to previous results (Anandan et al., 2007). ISO create hypoxia or relative ischemia with the effect of coronary hyperactivity in myocardium and hypotension in heart (Bloom and Davis, 1991), the overload of cytosolic Ca²⁺ induces myocardial ischemia in ISO group (Singal et al., 1983). The level of glucose in blood increased while comparing with the controls. The difference increase may be appropriate to the improved lipolysis and glycogenolysis, with the start of phosphorylase kinase enzyme and β -adrenergic receptor. On testing enzymes in the heart tissue is at a low level and microscopic myocardial necrosis and gross is produced by ISO treated rats. Though there are many beneficial effects and drugs are well standardized model studies to cardiac functions in clinical and mimics of MI due to ischemia in rats induces ISO (Harada et al., 1993). In CVD lipids are major role played by not only development of atherosclerosis and hyperlipidaemia, and modification of cell composition and stability of structure of cell membranes (Rajadhurai and Prince, 2005). Increased lipid values in plasma that are administered rats with ISO. The ISO treated rat's shows increase in plasma triglycerides and cholesterol this might be due to increase in adenosine monophosphate in cardiac cycle followed by biosynthesis of lipids (Paritha and Devi, 1997). The key role of eukaryotic cells in deciphering the signalling of Ca²⁺ is due to the CaM that acts as primary indicator of intracellular calcium (Ca²⁺). Being the secondary messenger, the CaM interacts/reacts with large

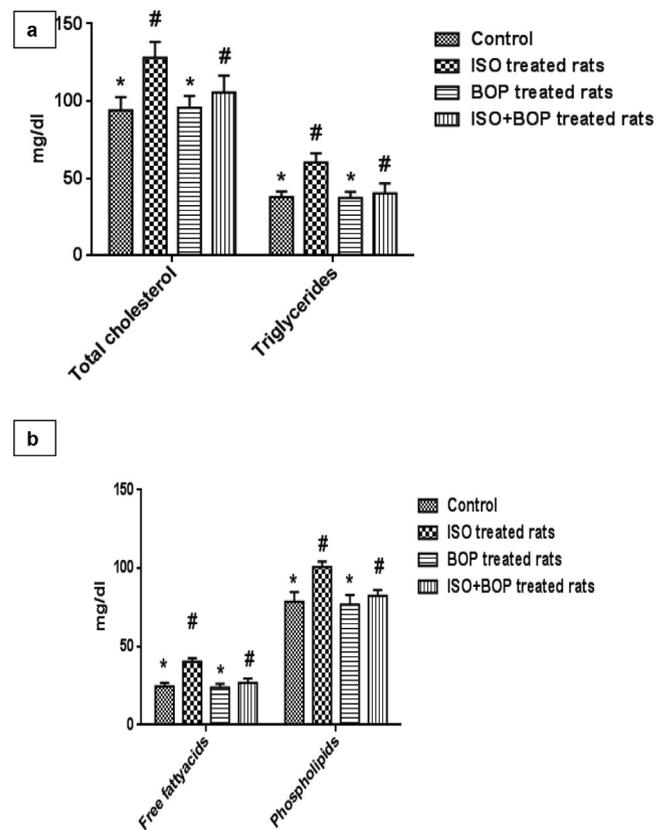


Fig. 3. Effect of isoproterenol and bisoprolol administration on plasma lipid profile. Values are expressed as Mean \pm SEM, on each bar followed by the same symbol are not significantly different ($P \leq 0.05$) from each other according to Duncan's Multiple Range (DMR) test, $n = 8$, Controls = Control rats.

number of proteins which is not unusual (Fig. 4). Every lobe is exposed with hydrophobic patches this firm changes are due to the binding of CaM and it helps to bind hydrophobic anchor residues in large that is present in canonical Ca²⁺ loaded CaM (CaCaM) which is a target binding sequences. The channels NaV1.5 are huge, consisting subunit (a) pore-forming (~220 kDa) and subunit (b) one of four auxiliary (b1–4, 30–35 kDa) in heterooligomeric assemblies which involve in function and channel trafficking. The cytosolic subunit (b) joins as the linker region in connecting DI and DII, adapt CaV1.2 voltage dependent activation and inactivation in

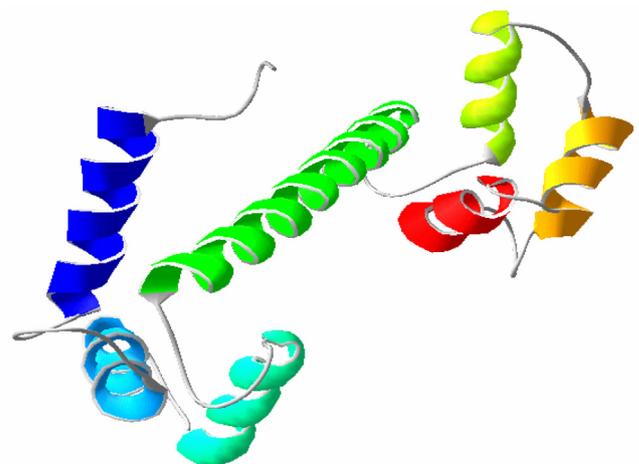


Fig. 4. 3D structure of Ca²⁺ bound calmodulin (PBD:10SA).

ISO + BOP administrated rats (Yamniuk and Vogel, 2004; Bodi et al., 2005; Nerbonne and Kass, 2005; Abriel, 2010; Catterall, 2011).

4. Conclusion

Rats were administered BOP parameters were close to control rats. Reports clearly stated that ISO treated rat tissue of heart damage was reduced when compared to treated ISO + BOP rats. The models were treated with ISO + BOP to defend against hyperlipidaemia and tissue of cardiac damage followed by MI. Hence, to investigate biochemical BOP mechanism against MI risk in rats. More in strength studies are needed to interact of BOP and ISO treated experimental animals.

5. Statistical investigation

The consequences are states that mean \pm SEM. (DMR) impact was set at ($P \leq 0.05$).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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