Evaluation of Cardioprotective Activity of *Cleome gynandra* and *Barleria gibsonii* Against Isoproterenol Induced Cardiotoxicity in Rats

C. Sunitha, C. Rajaram*, S. Nelson Kumar, R. Manohar, S. Kalpana

Department of Pharmacology, P. Rami Reddy Memorial College of Pharmacy, Kadapa - 516 003, Andhra Pradesh, India.

Professor & Head, Department of pharmacy practice, P. Rami Reddy Memorial College of Pharmacy, Kadapa - 516 003, Andhra Pradesh, India.

Corresponding Author: Dr.C.Rajaram
rajaram212121@gmail.com

Article History: Received: 02-07-23 **Revision:20-07-23** **Accepted:25-07-23**

ABSTRACT

This study evaluates the cardioprotective effects of a combination of *Cleome gynandra* (CG) and *Barleria gibsonii* extract (BG) on rats with isoproterenol (ISO)-induced myocardial necrosis. Significant myocardial necrosis, depletion of the endogenous antioxidants superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), and increases Cardiac markers and serum marker enzymes aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK) were observed in ISO-treated rats compared with normal rats. Coadministration of CG & BG at two doses (50 and 75 mg/kg) for 30 days to rats treated with ISO (85 mg/kg, sc) on the 29th and 30th days significantly decreased ISO-induced serum marker enzyme elevations and significantly decreased ISO-elevated myocardial lipid peroxidation marker malondialdehyde (MDA). In the hearts of the treatment groups, a substantial recovery of ISO-depleted activities and levels of CK-MB, AST, LDH, GSH, SOD, and CAT, was observed. The combination of CG 75 mg/kg and BG 100 mg/kg was more protective than the combination of CG 50 mg/kg and BG 100 mg/kg. The oral administration of CG-BG to rodents exposed to ISO demonstrates significant cardiac protection, and restores antioxidant activities. However, the combination did not enhance the cardioprotective activity of either herb when used separately.

Key Words: Cardioprotective, cardiotoxicity, Antioxidant and LDH

DOI: 10.48047/ecb/2023.12.si9.268

INTRODUCTION

The prevalence of cardiovascular disease (CVD) as the leading cause of death keeps rising. Cardiovascular disease has replaced diabetes as the major cause of global disability

attributable to an epidemiological shift that occurred in the twentieth century. According to the World Health Organization, it will still be the leading cause of death worldwide in 2030 [1]. Changes in lifestyle, environment, and genetic predisposition lead to CVD and its atherothrombotic effects. Cardiovascular disease is an umbrella term for a variety of conditions affecting the heart and blood vessels. Coronary heart disease (CHD), peripheral artery disease (PAD), angina, congestive heart failure (CHF), and myocardial infarction (MI) are all part of this category [1]. Human mortality is primarily due to hypertension [2]. Cardiovascular disease (CVD) is more common in the South Asian region than in other parts of the world, according to studies.

Due to drastically transformed lifestyles, a number of emerging nations, including Bangladesh, Iran, Afghanistan, and India, are quickly catching up to this epidemic [2]. Cardiovascular disorders are made worse by fast food, additives, preservatives, genetically modified foods, and a sedentary lifestyle [3]. Although synthetic drugs are highly effective in treating cardiovascular diseases, their utility is constrained due to their side effects [4]. The common ischemia condition known as MI is brought on by severe cardiac tissue damage. The mismatch between blood supply and demand for oxygen in cardiomyocytes with or without atherosclerotic plaque is the cause of its development [5]. Endocardial lipid peroxidation is caused by excessive reactive oxygen species (ROS) production. Such damage triggers apoptosis, an increase in oxidative stress, and the loss of cardioprotective antioxidants [5]. Catecholamine also affects the heart in an inotropic and chronotropic manner. MI is brought on by excessive catecholamine's effect on coronary vasoconstriction, which raises myocardial oxygen demand and lowers myocardial blood supply [6].

Large dosages of the synthetic catecholamine and s-adrenergic agonist isoproterenol (ISO) have been shown to cause myocardial infarction. When ISO autooxidizes, it produces extremely cytotoxic free radicals that have been shown to accelerate the peroxidation of membrane phospholipids and seriously harm the cardiac membrane [7].

Due to improvements in our understanding of the mechanisms through which herbs improve health and quality of life, herbal medicine is becoming more and more popular with both the general public and medical experts.

Extracts of Cleome gynadra leaves have been found to possess cardioprotective, antiasthmatic, antidiabetic, hepatoprotective, and potent central nervous system activities. Native peoples in Asia, South America, India, and East Africa all use their leaves to treat cardiac problems and its complications.

Gibson's barleria, or *barleria Gibsonii* (Acanthaceae), is a plant used for its antihyperglycemic, antihyperlipidaemic, anti-inflammatory, cardioprotective, and antioxidant properties in the treatment of cardiovascular and metabolic diseases. The presence of bioactive chemicals including phenols, flavonoids, and tannins in various plant tissues is responsible for these effects.

By mixing CG extract with BG phytosome (BGP), significant cardioprotective action is anticipated. This goal motivated the design of the current investigation, which used an in vivo rat model to examine the synergistic cardioprotective efficacy of BGP and CG combination therapy in ISO-induced cardiac necrosis (ischemia-reperfusion damage). To

comprehend the underlying mechanism(s) of its cardioprotective action, the authors looked at the chronic effects of oral BGP-CG combo treatment on cardiac antioxidants, lysosomal enzymes, and histoarchitectural alterations in the rat myocardium.

MATERIALS & METHODS

Experimental Animals

We used Wistar albino rats (150–200 g, either sex) that were used in research. They were kept in sanitary polypropylene cages with a standard food (Pavan agro, Bangalore, India) and water available at all times, under conditions of humidity (50 5%), temperature (25 2°C), and light (12-hour cycle). All of the animals were treated with compassion. The Institutional Animal Ethics Committee (1423/PO/Re/S/11/CPCSEA) examined and approved the experimental protocols, and they complied with the Indian National Science Academy Guidelines for the Use and Care of Experimental Animals in Research.

Preparation of BGP, CG, and ISO Solutions

In distilled water, *barleria Gibsonii* phytosomes were dissolved. *Cleome gynadra* leaf extract dry powder was diluted with distilled water and utilized right away. Immediately after being dissolved in distilled water, isoproterenol was injected subcutaneously. 85 mg/kg was chosen as the toxicant dose in the current investigation because previous experiments for dose determing show ISO 85 mg/kg given subcutaneously twice at an interval of 24 hours generates moderate necrosis in rat heart and a significant modification in biochemical parameters.

Experimental Procedure

Wistar albino rats were divided into five groups of six animals each after acclimatized in for 6-7 days in the animal quarters and were then given the following treatments.

Group I – Termed as normal control, received distilled water (1mL/kg, po) daily for 30 days

Group II- Termed as ISO control, received two injections of ISO (85 mg/kg, sc) at an interval of 24 hours.

Group III- Termed as Standard, received BGP (100 mg/kg, po) daily for 30 days and in addition received ISO (85 mg/kg, sc) on the 29th and 30th days at an interval of 24 hours.

Group IV- Termed as Standard treatment-1, received CG (50 mg/kg, po) and BGP (100 mg/kg, po) and in addition received ISO (85 mg/kg, sc) on the 29th and 30th days at an interval of 24 hours.

Group V- Termed as Standard treatment-2, received CG (75 mg/kg, po) and BGP (100 mg/kg, po) and in addition received ISO (85 mg/kg, sc) on the 29th and 30th days at an interval of 24 hours.

Twenty-four hours following the last subcutaneous ISO injection, rats were weighed and euthanized. Good Laboratory Practices were followed during the blood collection process. Light ether anesthetic was used to perform the heart puncture and collect the blood, which was then left to clot for 30 minutes at room temperature. The creatine kinase-myocardial band (CK-MB), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH), and creatine

phosphokinase (CPK) marker enzymes were estimated from the serum by centrifugation at 2500 rpm for 15 minutes at 30°C.

Right away, the hearts were removed, cooled, and infused with ice-cold saline. The hearts were cleaned with ice-cold saline, dried with a towel, weighed, and split in half. Half was used to create a homogenate that was 10% (w/v) in phosphate buffer (50 mM, pH 7.4). The amount of lipid peroxidation (LPO) in the homogenate was assessed using an aliquot of the mixture. Superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) assays were performed on the supernatants after centrifuging the homogenates at 7000 g for 10 minutes at 4°C. Half of it was employed for histology research after being fixed in buffered formalin at 10% (w/v) concentration.

Histoarchitectural Studies

Heart tissue samples kept in buffered formalin at a 10-percent (w/v) concentration were embedded in paraffin, sectioned at a thickness of 5 m, and stained with hematoxylin and eosin. Under a light microscope, these sections were checked for histoarchitectural modifications.

Statistical Analysis

Results from six animals in each group were used to calculate the mean and standard error of the cardioprotective and antioxidant activities. One-way ANOVA was used to statistically assess the results, and p 0.05 was set as the significance threshold for the Tukey-Kramer post-test for individual comparisons. We used GraphPad Software for statistical analysis.

RESULTS

Table No. 1 shows the effects of oral BGP and CG treatments during a 30-day period on the blood marker enzymes AST, LDH, ALP, ALT, and CK-MB. When compared to the normal rat group, rats treated with ISO displayed a much higher ($p < 0.001$) rise in the activity of blood marker enzymes. When rats were pretreated with BGP 100 mg/kg, CG 50 mg/kg, and CG 75 mg/kg for 30 days, then ISO was subcutaneously injected on the 29th and 30th days, the ISO-induced elevated activities of AS, AL, ALP, LDH, and CK-MB were significantly ($p < 0.001$) reduced.

The effects of BGP and CG oral treatments for 30 days on antioxidant catalase, GSH, and LPO are summarized in Table The ISO-treated group showed a significant ($p<0.001$) increase in LPO compared with normal untreated rats. A significant diminution ($p<0.001$) of ISO-induced LPO elevation was observed in all pre-treated experimental groups.

Myocardial GSH levels decreased significantly ($p<0.001$) in ISO-treated rats. These levels were restored significantly by pretreatment with BGP 100 mg/kg, CG 50 mg/kg, BGP 100 mg/kg + CG 75 mg /kg, Significantly. CAT, and GSHGR activities were restored significantly ($p<0.001$) in all experimental groups compared with the ISO-only group.

The myocardial cell membrane was clearly intact upon histopathological analysis of the myocardium of normal rats (Figure 3). The pericardium and endocardium were both within typical ranges. There was no evidence of inflammatory cell infiltration. The group of rats given ISO treatment exhibited mild to severe myocytic necrosis and mild lymphocyte and macrophage infiltration. Papillary muscles and the endocardium both showed more obvious

GROUPS	On 31st Day				
	CK-MB (IU/L)	**LDH (IU/L)**	**AST (IU/L)**	**ALT (IU/L)**	**ALP (IU/L)**
I	87.33±1.145	526.3±1.174	81.00±0.577	38.67±0.988	43.17±0.980
II	183.2±0.980###	932.3±0.881###	184.5±1.335###	69.00±0.730###	70.4±1.033###
III	90.17±1.046***	524.3±0.714***	80.90±0.856***	39.33±0.0.210***	43.50±0.885***
IV	124.3±0.749***	705.5±1.384***	119.3±1.022***	51.50±0.619***	60.00±0.683***
V	81.67±0.843***	623.3±0.988***	103.2±0.980***	42.00±0.774***	49.83±0.749***

alterations. The BGP100 group's cardiac sections showed mild-to-moderate localized myonecrosis and moderate diffuse infiltration of lymphocytes.

Table no 1: Effect of BGP and CG on Treatment on Myocardial Marker Enzymes

The above values are expressed in mean ±SEM and n=6. ###p<0.001 when compared with normal group, *** indicates p<0.001 when compared with ischemic control group.

CK-MB: Creatine phosphokinase – MB, LDH: Lactate dehydrogenase, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP:Alkaline phosphate

Table no 2: Effect of BGP and CG on tissue antioxidant levels

GROUPS	**CAT (H_2O_2consumed/gram tissue)**	**GSH (µg of GSH/mg)**	**LPO (µM /mg)**
I	51.86±0.66	21.15±0.52	1.85±0.08
II	22.36±0.62###	5.23±0.07###	9.67±0.26###
III	52.48±0.34***	23.68±0.271***	1.83±0.065***

IV	61.69±0.59***	17.15±0.39***	5.68±0.26***
V	74.74±0.29***	23.80±0.52**	3.46±0.14***

The above values are expressed in Mean ± SEM and n=6. ### indicates P<0.001 when compared to normal group. **indicates p<0.01, *** indicates P<0.001 when compared to control group
CAT: Catalase, **GSH:** Reduced glutathione, **LPO :** Lipidperoxidation.

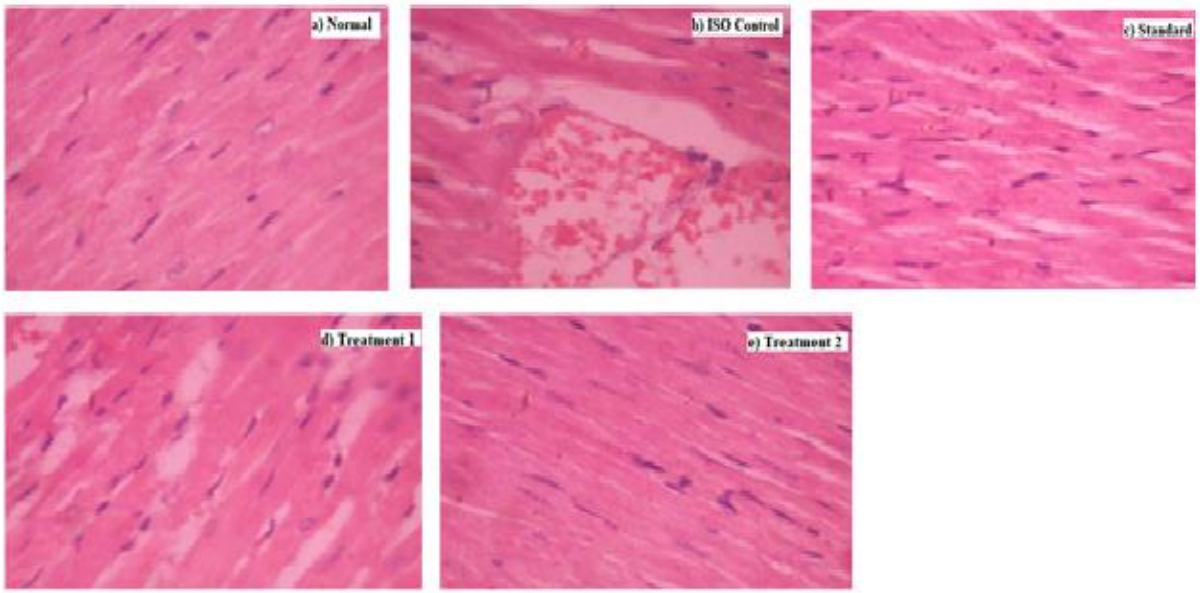

Figure 1: Effect of BGP & CG on histoarchitectural studies

DISCUSSION

By increasing the myocardial oxygen demand and causing positive inotropic and chronotropic effects, the synthetic beta-adrenergic agonist isoproterenol causes ischemic necrosis of the myocardium in rats. Numerous pathophysiological processes, such as altered permeability, enhanced norepinephrine turnover, and production of cytotoxic free radicals on catecholamine autooxidation, have been postulated to explain the ISO-induced cardiac injury. The main causes of cardiotoxicity brought on by ISO are free radical-mediated lipid peroxidation and subsequent changes in membrane permeability [8]. Oxidative stress raises cAMP levels via depleting ATP and reducing sarcolemmal Ca^{+2} transport, which causes intracellular calcium overload and contractile failure in the rat heart's ventricles [9,10].

Lesions caused by ISO in the rat heart resemble those caused by myofibrillar degeneration in ischemic heart disease (IHD) in humans [11]. Therefore, research into ISO-induced myocardial necrosis and its underlying mechanisms may offer new information and

improved understanding of the pathophysiology of IHD.

Due to the leakage of marker enzymes from a damaged heart into the circulation, ISO-control rats were shown to have elevated serum AST, ALP, ALT, LDH, and CK-MB activity as compared to healthy animals. The combination of BGP (100 mg/kg) and CG (50 mg/kg and 75 mg/kg) treatments considerably reduced the raised serum levels of the marker enzymes AST, ALP, ALT, LDH, and CK-MB and dramatically increased their myocardial activity in rats exposed to ISO. These results point to the cardioprotective effect of BGP-CG by demonstrating its capacity to preserve cardiac integrity, primarily by preventing myocardial damage brought on by lipid peroxidation.

CONCLUSION

It is possible to draw the conclusion that giving rats with ISO challenges BGP-CG oral medication for 30 days significantly protects their hearts, reduces lipid peroxidation, and restores antioxidant activities.

Acknowledgement

The authors are thankful to Management, PRRM College of Pharmacy, Kadapa, A.P. for encouragement and availing of the laboratory facilities during the course of investigation.

Conflict of Interest

Authors disclose no conflicts of interest for publication of the manuscript.

REFERENCES

[1] Khan, I.A.; Hussain, M.; Syed, S.K.; Saadullah, M.; Alqahtani, A.M.; Alqahtani, T.; Aldahish, A.A.; Asiri, S.; Zeng, L.-H. Pharmacological justification for the medicinal use of Plumeria rubra Linn. in cardiovascular disorders. Molecules 2022, 27, 251.

[2] Khan, I.A. Pharmacotherapeutic modifications in cardiopulmonary patients during COVID-19 outbreak. J. Coll. Phy. Surg. Pak. 2020, 30, 15–17.

[3] Ashraf, N.; Rashid, A.; Naz, U.; Ashraf, M.M.; Khaliq, S.; Khan, I.A.; Nazir, A.; Sarwar, A.; Asif, A. Detection of antibiotics residues in protein containing diets (meat and eggs) of human through different methods. J. Univ. Med. Coll. 2018, 9, 1–11.

[4] Quiñones, M.; Miguel, M.; Aleixandre, G. Beneficial effects of polyphenols on cardiovascular disease. Pharmacol. Res. 2013, 68, 125–131.

[5] Khan, I.A.; Hussain, M.; Munawar, S.H.; Iqbal, M.O.; Arshad, S.; Manzoor, A.; Shah, M.A.; Abbas, K.; Shakeel, W.; Syed, S.K. Jasminum sambac: A Potential candidate for drug development to cure cardiovascular ailments. Molecules 2021, 26, 5664.

[6] Manzoor, A.; Khan, I.A.; Sadiq, M.; Iqbal, M.O.; Munawar, S.H. Evaluation of cardioprotective potential of hydroalcoholic leaf extract of Citrullus colocynthis against doxorubicin induced oxidative stress in rats. Pak. J. Zool. 2022, 25, 1–8.

[7] Rona G, Chappel CI, Balazs T, Gaudry R. An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. AMA Arch Pathol 1959;67:443- 455.

[8] Noronha-Dutra AA, Steen EM, Woolf N. The correlation between catecholamine and lipid peroxidation induced damage in heart cells. Basic Res Cardiol 1985;80:133-136.

[9] Bhagat B, Sullivan JM, Fischer VW, et al. cAMP activity and isoproterenol-induced myocardial injury in rats. Recent Adv Stud Cardiac Struct Metab 1976;12:465-470.

[10] Tappia PS, Hata T, Hozaima L, et al. Role of oxidativestress in catecholamine-induced

changes in cardiac sarcolemmal Ca2+ transport. Arch Biochem Biophys 2001;387:85-92.
[11] Milei J, Nunez RG, Rapaport M. Pathogenesis of isoproterenol-induced myocardial lesions: its relation to human coagulative myocytolysis. Cardiology 1978;63:139- 151.