EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 7
702-705
Year: 2018

CARDIOPROTECTIVE EFFECT OF *CARDIOSPERMUM HALICACABUM* ON ISOPROTERENOL INDUCED CARDIAC HYPERTROPHIC RATS

Doss V. A.*, Vineetha Krishnamoorthy[1] and Dharaniyambigai Kuberapandian[2]

Department of Biochemistry, PSG College of Arts & Science, Coimbatore, Tamil Nadu, India.

***Corresponding Author: Dr. Doss V. A.**
Department of Biochemistry, PSG College of Arts & Science, Coimbatore, Tamil Nadu, India.

Article Received on 18/05/2018 **Article Revised on 08/06/2018** **Article Accepted on 28/06/2018**

ABSTRACT
Cardiac hypertrophy is regarded as a compensation mechanism to overcome the increased workload when the stress or injury is transient and turns detrimental on chronic conditions. The cardioprotective potential of the aqueous extract of *Cardiospermum halicacabum* was evaluated against cardiac hypertrophy induced by isoproterenol in male Albino Wistar rats. Isoproterenol (10 mg/kg b.w., subcutaneous., 7 days) induced cardiac hypertrophy in experimental rats which were simultaneously treated with the standard drug, Losartan (50 mg/kg b.w., oral.,7 days) and plant extract of *C.halicacabum* (400 mg/kg body weight for 7 days). Biochemical estimations of glucose, protein, cholesterol, cardiac marker enzymes namely serum glutamate oxaloacetate transaminase [SGOT], serum glutamate pyruvate transaminase [SGPT], lactate dehydrogenase [LDH] and enzymic antioxidants (SOD, catalase, glutathione peroxidase) in serum and heart tissues thus collected followed by histopathological analysis at the end of 7 days. Isoproterenol administered rats exhibited detrimental values of biochemical parameters and antioxidants which were significantly ($P<0.05$) restored toward normal level by the treatment of *Cardiospermum halicacabum* which also had similar effect at the histological level. Hence treatment with *Cardiospermum halicacabum* extract showed curative effect in isoproterenol administered rats.

KEYWORDS: Cardiac hypertrophy, Isoproterenol, Losartan, *Cardiospermum halicacabum*.

INTRODUCTION
Cardiac hypertrophy induce heart failure is one of the major origin of morbidity and mortality in the world. Cardiac hypertrophy is identified by a chronic physiological increase in heart muscle mass developing from systolic or diastolic wall stress. This happens normally during development, pregnancy and in sustained exercise.[1] At cellular level, cardiac hypertrophy is distinguished by an increase in cell size, protein synthesis and reactivation of the foetal gene program finally induce heart failure.[2] The treatment is usually based on the figured echocardiographic or magnetic resonance imaging estimate of the left ventricular mass.[3] The medication methods for cardiac hypertrophy contains exercise, monitoring blood pressure and usage of anti hypertensive drugs.[4] Spectral myectomy is acknowledged as a surgical treatment for cardiac hypertrophy that implies removing of a part of the septum which was hindering the blood stream from the left ventricle to the aorta.[5]

In recent years, the identification and characterisation of the molecular pathways leading to cardiac hypertrophy has highlighted a number of potent therapeutic targets. There is a huge and increasingly global burden of cardiovascular disease. Approximately 14 million individuals died of cardiovascular disease in 1990, and this is projected to rise to about 25 million by 2020.

Losartan, a well-known drug used for the treatment of cardiac hypertrophy. Commonly reported side effects of losartan include asthenia, chest pain, diarrhea, fatigue and hypoglycaemia, hypotension. Recently there has been a change in universal trend from synthetic to herbal medicine, which we can state “Return to Nature”. India has recently been referred to as the medicinal garden of the world.[6]

Cardiosperum halicacabum Linn (Family: Sapindaceae) is a climbing plant widely distributed in tropical and subtropical Africa and Asia. Often found as a weed along roads and rivers. Indian system of medicine prescribes *Cardiosperum halicacabum* leaves for medications of rheumatism, stiffness of limbs and snake bites.[7] Root is administered for bleeding piles, and nervous disease[8] and as a diaphoretic, diuretic, emetic, emmenagogue, laxative, refrigerant, stomachic, sudorific.[9]

This plant showed various therapeutic uses such as antibacterial, antifungal, anti-parasitic, anti-diarrhoeal, anxiolytic, rubefacient, antipyretic, anti-inflammatory,

anticonvulsant, anti-carcinogenic, anti-rheumatic, anti-oxidant, neuro protective and hepato- protective.[10]

MATERIALS AND METHODS

Chemicals

Isoproterenol (ISO) was purchased from Sigma-aldrich. All the chemicals and drug were of analytical grade.

Plant Collection and Extraction

Cardiosperum halicacabum was collected in the month of December 2017 from in and around the Coimbatore, Tamil Nadu. The plant was identified and certified (BSI/SRC/5/23/2017/Tech/1956) by the Taxonomist, Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore, Tamil Nadu, India. Collected whole plants were shade dried and ground to a coarse powder. The coarse powder was extracted using water (1:3)[11] cold maceration method for 48 hours which was then filtered and the extracts were condensed to dryness using rotator evaporator and crystals were obtained. Crystals were weighed, dissolved in distilled water and administrated orally to the experimental animals for the treatment of cardiac hypertrophy.

Animals

Male wistar rats, weighing around 100-200g procured from scientific suppliers were used for the study. The ethical clearance for handling experimental animals was obtained from Institutional Animal Ethics Committee (IAEC) (388/2018/IAEC). The animals were maintained under standard laboratory conditions with controlled humidity and temperature and acclimatized for 3 days before the start of the experiment. The animal house was well ventilated and protected in large spacious hygienic cages during the experiment.

Experimental Groups

The animals (male Albino Wistar rats) were divided into 4 groups with each group containing six rats.

Table 1: Experimental groups of induction and treatment of cardiac hypertrophy.

Groups	Experimental animals
Group I	Normal control rats
Group II	Isoproterenol (10 mg/kg b.w., s.c.,7 days)[12]
Group III	Isoproterenol + Losartan (50 mg/kg b.w., oral.,7 days)[13]
Group IV	Isoproterenol + aqueous extract of *C. halicacabum* (100 mg/kg b.w., oral., 7 days)[14]

At the end of the experiment, the animals were sacrificed under mild chloroform anaesthesia. Blood was collected by cardiac puncture and the serum was separated by centrifugation at 3000 rpm for 20 minutes and placed under -20^{o} C for further studies. The excised heart tissue was washed several times in ice-cold saline and was subsequently homogenized in Tris-buffer (pH 7.4). The resultant homogenate was centrifuged at 3000 rpm for 15 mins. The tissue debris was separated, and the homogenate was stored at -20^{o}C until further use.

Biochemical Parameters

Estimation of SGOT and SGPT activities by modified IFCC method (Microlyn) and determination of LDH activity by optimised kinetic assay method (AUTOSPAN) followed by estimation of SOD[15], catalase[16] and glutathione peroxidase.[17]

Histopathological Analysis

The cleaned excised rat hearts were initially preserved in 10% formalin immediately until tissue processing as transverse, 5-µm-thick paraffin, mid-ventricular sections. These sections were stained with haematoxylin and eosin (H&E) and the cellular architecture of the heart tissues were investigated by scanning the H&E-stained slides (4X).[18]

Statistical Analysis

Data obtained was expressed as mean ± SD. Statistical analysis was performed by using the method of distribution statistics (Standard descriptive analysis) and analysis of means (Student ‘t’ test) using R- Statistical Computing and Graphical Tools (formerly AT & T, Lucent technology). A probability of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Phytochemical Evaluation

The phytochemical qualitative analysis of aqueous extract of *C.halicacabum* indicates the presence of major phytoconstituents including alkaloids, flavonoids, phenols, saponins, tannins, amino acids, cardiac glycosides, sterols and important nutrient such as proteins, carbohydrates.

Effects of *C.halicacabum* on Cardiac Marker Enzymes

Levels of various cardiac marker enzymes namely SGOT, SGPT, LDH in the serum and heart tissue samples were found to be drastically elevated in isoproterenol induced animals when compared to that of control animals indicating cardiac necrosis produced by isoproterenol and these increased levels were decreased significantly by *C.halicacabum* treatment.

Table 1: Activity of SGOT, SGPT and LDH.

Groups	SGOT		SGPT		LDH	
	Serum	Heart	Serum	Heart	Serum	Heart
I	0.685±0.05	0.587±0.048	0.086±0.012	0.185±0.011	1.2082±0.017	1.006±0.02
II	0.795±0.005^{a*}	0.41±0.01^{a}	0.254±0.05^{a*}	0.055±0.001^{a*}	1.3532±0.005^{a*}	0.776±0.009^{a*}
III	0.654±0.01^{b}	0.535±0.025^{b*}	0.107±0.004^{b}	0.071±0.026^{b*}	1.00±0.012^{b}	1.09±0.003^{b*}
IV	0.608±0.01^{c*}	0.486±0.02^{c*}	0.087±0.02^{c*}	0.067±0.016^{c*}	0.857±0.008^{c*}	1.075±0.005^{c*}

Table values are expressed by Mean±SD of 6 samples per group. Group comparison: a – Normal (I) Vs ISO (II) ; b – ISO (II) Vs Drug (III); c – ISO (II) Vs Plant extract (IV). Statistical significance indicated by *

Increased activities in serum accompanied by their concomitant reduction in heart homogenate confirm the onset of cardiac necrosis.[19] The above findings clearly pointed out that C.*halicacabum* could be highly cardioprotective against the cardiac hypertrophy. This potential of the plant may be due to the rich phenols, flavonoids and saponins present in it. Rutin is a flavonoid compound reported to have cardioprotective activity.[20]

Effects of *C. halicacabum* on Antioxidant Enzymes

There was a significant decrease in the activity of SOD, CAT, GPx in the isoproterenol administered animals when compared to that of normal. However, plant extract administered rats showed a significant increase in the activity of SOD, CAT, GPx when compared to untreated cardiac hypertrophic rats.

Table 2: Activity of SOD, CAT and GPx.

Groups	SOD		CAT		GPx	
	Serum	Heart	Serum	Heart	Serum	Heart
I	4.05±0.42	5.26±0.89	3.23±0.56	2.48±0.08	29.67±0.10	24.12±0.18
II	1.86±0.58^{a*}	2.47±0.87^{a*}	2.12±0.01^{a*}	1.74±0.80^{a*}	13.62±0.87^{a*}	14.44±0.04^{a*}
III	5.25±0.75^{b}	4.23±0.54^{b*}	3.29±0.03^{b}	2.29±1.97^{b*}	28.60±0.15^{b}	19.06±0.07^{b*}
IV	4.62±0.56^{c*}	4.12±0.65^{c*}	3.17±0.01^{c*}	2.34±0.25*	18.47±0.76^{c*}	24.88±0.984^{c*}

Table values are expressed by Mean±SD of 6 samples per group. Group comparison: a – Normal (I) Vs ISO (II); b – ISO (II) Vs Drug (III); c – ISO (II) Vs Plant extract (IV). Statistical significance indicated by *

Histopathological Observations

Histopathological analysis of the scanned heart tissues of experimental groups revealed detrimental impact of isoproterenol during the induction of cardiac hypertrophy which all were restored similar to normal on treatment using losartan and plant extract as shown in figure 1.

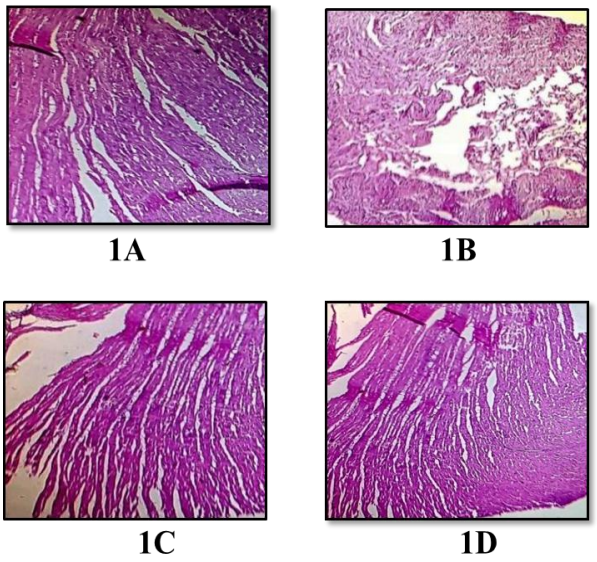

Figure 1: Histopathological observation of experimental groups. 1A: Group I (Normal): Control rats exhibited clear, intact homogenous myofibril arrangements in heart tissue with no sign of inflammation. **1B: Group II (ISO):** Administration of ISO caused degenerative myofibrillar network, cellular necrosis, with presence of inflammatory cell infiltration. **1C: Group III (ISO+Losartan):** Losartan treatment rejuvenated the myofibrils and less cellular infiltrations were seen. **1D: Group IV (ISO+Plant extract):** Treatment with plant extract reduced the cellular necrosis causing tissue rejuvenation and myofibirillar arrangement similar to that of normal.

CONCLUSION

The present work implies that the aqueous extract of *Cardiosperumum halicacabum* whole plant showed a significant decrease in the elevated cardiac marker and its inverse with respect to antioxidant enzymes which may be due to the phytochemical, Rutin present in the plant. To conclude, the *Cardiosperumum halicacabum* and its phytocompounds can be considered as a potent therapeutic lead against isoproterenol induced cardiac hypertrophy, due to the enhanced antioxidant potential of the plant. Further research is needed to isolate and characterize the specific bioactive compounds such as rutin that are responsible for the cardioprotective (anti-hypertrophic) action and to evaluate its mechanism of action.

ACKNOWLEDGEMENT

Thanks to the Botanical Survey of India, for the plant authentication. Thanks to PSG Institutional Animal Ethics Committee (PSGIMS&R), Coimbatore, India and

the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for providing animal ethical clearance. Thanks to Ponmani & Co Pvt. Ltd., Coimbatore for providing Isoproterenol (Sigma).

Conflict of Interest

There is no conflict of interest among the authors.

REFERENCES

1. Lorell BH, Carabello BA. Left ventricular hypertrophy: Pathogenesis, detection, and prognosis. Circulation, 2000; 102: 470-79.
2. Yung CK, Halperin VL, Tomasell GF, Winslow RL. Gene expression profiles in end-stage human idiopathic dilated cardiomyopathy:altered expression of apoptotic and cytoskeletal genes. Genomics, 2004; 281-297.
3. Buss SJ, Riffel JH, Malekar P, Hagenmueller M, Asel C, Zhang M *et al.*, Chronic Akt blockade aggravates pathological hypertrophy and inhibits physiological hypertrophy. Am J Physiol Heart Circ Physiol, 2012; 302: H420-H430.
4. Van Zwieten PA. The influence of antihypertensive drug treatment on the prevention and regression of left ventricular hypertrophy. Cardiovasc Res, 2000; 45: 82-91.
5. Ommen SR, Maron BJ, Olivotto I, Maron MS, Cecchi F. Long term effects of surgical septal myectomy on survival in patients with obstructive hypertrophic cardiomyopathy. J AM Coll Cardiol, 2005; 46: 470-76.
6. Jain SK. Contribution to Indian ethanobotany, scientific publishers, jodhpur, 1991; 302: 78-92.
7. Kankanamalage TN, Oharmadasa RM, Wijesekara RG. A Survey on medicinal materials used in traditional systems of medicine in sri lanka. Journal of ethnopharmacology, 2014; 155: 679-681.
8. Kapoor BSS and Kumar S. Ethnomedicinal plants of barmer District, rajasthan used in herbal and folk remedies. Indian journal of pharmaceutical and biological research, 2013; 1: 61-66.
9. Menichini F, Losi L, Bonesi M, Tundis R. Chemical profiling and invitro biological effects of cardiospermum halicacaum. L aerial parts and seeds for application in neurodegenerative disorders. Journal of enzyme inhibition and medicinal chemistry, 2014; 29(5): 677-685.
10. Savitha G, Vishnupriya V, Krishnamohan S. *Cardiospermum halicacabum*. L- A Review. Asian journal of pharmaceutical and clinical research, 2017; 10(10): 23-26.
11. Murali B, Upadhyaya UM and Goyal RK. Effect of chronic treatment with *Enicostemma littorale* in non-insulin dependent diabetic (NIDDM) rats. *J* Ethnopharmacol, 2002; 81: 199.
12. Zhang S, Tang F, Yang Y, Lu M, Luan A, Zhang J, Yang J and Wang H. Astragaloside IV protects against isoproterenol-induced cardiac hypertrophy by regulating NF-κB/PGC-1α signalling mediated energy biosynthesis. Plosone, 2015; 1-18.
13. Shimada JY, Passeri JJ, Baggish LA, Callaghan OC, Lowry PA, Yannekis G, Abbara S, Ghoshhajra BB, Rothman DR, Ho YC, Januzzi LJ, Seidman EC and Fifer AM. Effects of losartan on left ventricular hypertrophy and fibrosis in patients with nonobstrucutve hypertrophic cardiomyopathy. JACC Heart Fail, 2013; 1(6): 480-487.
14. Asha VV. Antipyretic activity of *Cardiospermum halicacabum*. Indian Journal of Experimental Biology, 1999; 37: 411-414.
15. Kakkar P, Das B and Vishwanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys, 1984; 21: 130 – 132.
16. Sinha AK. Colorimetric assay of catalase. Anal Biochem, 1972; 47: 389-394.
17. Rotruck JT, Pope AL, Ganther HE, Swasson AS, Haseman DG and Howkstra WG. Selenium: Biochemical role as a component of glutathione peroxidise. Science, 1973; 779: 588 – 590.
18. Al-Rasheed NM, Al-Oteibi MM, Al-Manee RZ, Al-Shareef NM, Hasan IH, Mohamad RA and Mahmoud AM. Simvastatin prevents isoproterenol-induced cardiac hypertrophy through modulation of the JAK/STAT pathway. Drug Design, Development and Therapy, 2015; 9: 3217-3229.
19. Saxena R, Wijnhoud A D *et al.*, Effect of diaspirin crosslinked haemoglobin on endothelium-1 and blood pressure in acute ischemic stroke in man. J. Hypertens., 1998; 16: 1459-1465.
20. Ali S, Mudagal M P, Goli D.Cardioprotective effect of tetrahydrocurcumin and rutin on lipid peroxides and antioxidants in experimentally induced MI in rats. Pharmazie, 2009; 64(2): 132-136.