



## EVALUATION OF MARKERS AND ANTIOXIDANTS IN *BOERHAVIA DIFFUSA* FOR CARDIOPROTECTIVE ACTIVITY

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Article Received on 04/04/2018

Article Revised on 25/04/2018

Article Accepted on 15/05/2018

### ABSTRACT

Cardiovascular disease (CVD) is the principle cause of death in both developed and developing countries. CVD includes coronary heart disease, congestive heart failure, stroke and Myocardial infarction (MI). MI is the interruption of blood supply to the heart, causing heart cells to die, due to occlusion of coronary artery. Several medicinal plants are available that exhibit significant curative properties for the treatment of cardiovascular disorders. Various research studies on medicinal plants have been going on to prevent the mortality caused by cardiovascular disease. The objective of the present study was to evaluate the cardioprotective role of ethanolic extract of *Boerhavia diffusa* in increasing concentrations. Myocardial infarction (MI) was experimentally induced by isoproterenol in rats. Serum markers, lipid profile, lipoproteins and antioxidants were analyzed in experimental rats. Administration of *Boerhavia diffusa* ethanolic extracts restored the levels of marker enzymes like Aspartate Transaminase (AST), Alanine Transaminase (ALT), Acid Phosphatase (ACP) and Alkaline Phosphatase (ALP) in increasing concentration. Lipid profile and the level of lipoproteins exhibited significant changes in serum of plant co treated experimental rats. Also, a significant ( $P < 0.05$ ) increase in antioxidants like SOD, Catalase, Glutathione peroxidase and Glutathione reductase were observed in the heart tissue of plant treated groups. The present study can be concluded that the ethanolic extracts of *Boerhavia diffusa* exhibited significant cardioprotective potential in increasing concentration which might be due to its antioxidant property and membrane stabilizing action.

**KEYWORDS:** *Boerhavia diffusa*, antioxidants, cardioprotective, Isoproterenol.

### INTRODUCTION

Cardiovascular diseases (CVD) are the most widespread global diseases responsible for death and disability. According to WHO, that CVD is the major causative factor which claims 17 million deaths annually.<sup>[1]</sup> CVD is defined as a group of disorders of the heart which includes coronary heart disease, congestive heart failure, stroke and congenital heart defects, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, deep vein thrombosis and pulmonary embolism.

Myocardial Infarction (MI) is a type of cardiovascular disease, known as heart attack commonly. It is the death of cardiac tissue mainly due to lack of oxygen and blood supply. Oxidative stress, hyperlipidemia, hyper tension, loss of membrane stability are the major deleterious effects.<sup>[2]</sup>

Oxidative stress is the vital risk factor of MI that leads to the development of free radicals synthesis. The free radicals generation damages the membrane integrity and

affects the functions of macromolecules. MI induced by isoproterenol (beta adrenergic agonist) causes myocardial injury in experimental animals and initiates the mechanism of lipid peroxidation and cell membrane damage.<sup>[3]</sup>

Medicinal plants serves as a reservoir of various active molecules with therapeutic activity. People make use of herbal based drugs due to high effectiveness, low cost and less side effects.<sup>[4]</sup> Traditional medicinal plants provide a platform for the development of new drugs. Several research works has been employed on plant extracts for the management of cardiovascular diseases.<sup>[5]</sup> Secondary metabolites present in medicinal plants possess cardioprotective activity due to its antioxidant property, which can be evaluated using suitable pharmacological screening approach.

*Boerhavia diffusa* is a species of flowering plant belongs to Nyctaginaceae family. It is commonly known as "punarnava", spreading hogweed in English and

Mukkurttaikkodi in tamil. *Boerhavia diffusa* is a perennial herb, widely dispersed, distributed throughout India.<sup>[6]</sup> *Boerhavia diffusa* contain chemical constituents like of  $\beta$ -Sitosterol,  $\alpha$ -2-sitosterol, palmitic acid, ester of  $\beta$ -sitosterol, tetracosanoic, hexacosanoic, stearic, arachidic acid, urosilic acid,  $\beta$ -Ecdysone, triacontanol etc.<sup>[7]</sup>

It is taken as an herbal drug for pain relief and other uses. The leaves of *Boerhavia diffusa* are regularly used as a green vegetable in many parts of India. In Ayurvedic herbal medicine, the whole plant of *Boerhavia diffusa* is widely used for the treatment of various disorders. The root is primarily used to treat gonorrhoea, internal inflammation of all kinds, dyspepsia, oedema, jaundice, menstrual disorders, anaemia, liver, gallbladder and kidney disorders, enlargement of spleen, abdominal pain, abdominal tumours, and cancers.<sup>[8]</sup>

The present study was carried out to evaluate the cardioprotective activity of ethanolic extract of *Boerhavia diffusa* in isoproterenol induced Myocardial infarction.

## MATERIALS AND METHODS

### Collection of Plant Material

The roots of *Boerhavia diffusa* was collected from the local villages around Tiruchirappalli and was authenticated by plant taxonomist, Department of Botany, St. Joseph's College, Tiruchirappalli. The collected parts were washed thoroughly with water, rinsed with distilled water to remove soil and foreign material if any. The plant was shade dried and powdered. About 1 g of the powdered material was then subjected to extractions using Soxhlet apparatus using ethanol for 6 hours. The extract was finally filtered and used for analysis.

### Animals

Healthy albino male wistar rats weighing between 150-200g were used for the study after securing the ethical clearance from Institutional Animal Ethical Committee. All the animals were individually housed in polyethylene cages, maintained under standard conditions, fed with standard rat pellet diet and water *ad-libitum*. The animals were divided in to five groups (n=6).

### Experimental Design.

Group I: Control ((normal rats received 0.9 % saline)  
 Group II: received Isoproterenol subcutaneously twice (85mg/kg SC) at an interval of 24 hours.  
 Group III: received Isoproterenol (85mg/kg SC) and *Boerhavia diffusa* Ethanolic Extract (100 mg/kg body weight orally) for 15 days.  
 Group IV: received Isoproterenol (85mg/kg SC) and *Boerhavia diffusa* Ethanolic Extract (200 mg/Kg body weight orally) for 15 days.

Group V: received Isoproterenol (85mg/kg SC) and *Boerhavia diffusa* Ethanolic Extract (300 mg/Kg body weight orally) for 15 days.

The animals were sacrificed by cervical decapitation and blood was collected and separated. The heart was dissected and washed in ice cold saline, homogenized and used for various experiments.

## Biochemical Assessment in Serum

### Estimation of serum markers:

Blood samples were taken and serum was separated for analysis of different cardiac biomarkers like aspartate transaminase (AST), alanine transaminase (ALT), acid phosphatase (ACP) and alkaline phosphatase (ALP) by standard procedure.

### Estimation of Lipids

Among lipids, total cholesterol, triglycerides, Phospholipids, free fatty acids were determined in serum of experimental animals.

### Estimation of Lipoproteins

Lipoproteins like LDL, VLDL and HDL were also estimated in serum of experimental animals.

## Biochemical Assessment in Tissue

### Estimation of Antioxidant Enzymes

The animal's heart portion was taken and homogenized in 10% ice cold phosphate buffer (pH = 7) and the mixture was centrifuged and supernatant was collected for the estimation of antioxidants like SOD, Catalase, Glutathione peroxidase and Glutathione reductase.

### Statistical Analysis

The statistical analysis was performed by ANOVA under one way classification followed by Bonferroni multiple comparison test, changes were considered significant at the P-value of < 0.05 level of significance. The values were expressed as mean  $\pm$  SD.

## RESULTS AND DISCUSSION

### Serum markers

Table I depicted the level of serum marker enzymes such as AST, ALT, ACP and ALP in experimental groups. Isoproterenol (Group II) treated rats produced significant increase in serum markers as compared to normal group. As a result of destruction of myocardial cells, the serum markers were released into blood, served as a indicator of cardiac damage and altered plasma permeability.<sup>[9]</sup> Co treatment with *Boerhavia diffusa* in different concentrations restored the level of marker enzymes significantly. The phytoconstituents present in the plant extract are responsible for safeguarding the membrane stability of cardiac tissue membrane, thus blocking the leakage of markers similar to the observations made by Nimbale and Koti.<sup>[10]</sup>

### Serum Lipid profile

Data in table II represented the levels of total cholesterol, triglyceride, free fatty acids, and phospholipids in serum

of the control and experimental rats. The increased levels of lipid profile could be due to enhanced lipid biosynthesis by cardiac cyclic AMP.<sup>[11]</sup> Hyperlipidemia plays an important role in cardiovascular diseases and the development of atherosclerosis.<sup>[12]</sup>

A significant elevation in the serum lipid profile was observed isoproterenol treated rats compared to control rats. The observed changes in lipid profile were similar to the results recorded by Eman and Ghada.<sup>[13]</sup> Cardiac ischemia induced by isoproterenol showed alterations in membrane permeability and loss of function of myocardial membrane. Medicinal plants exhibit potent hypolipidemic effect which may be due to the presence of active phytoconstituents.<sup>[14]</sup> Co treatment with *Boerhavia diffusa* in increasing concentration significantly altered the levels of lipid profile compared to group II rats.

#### Serum Lipoprotein level:

The level of serum lipoproteins LDL, VLDL and HDL were represented in table III in experimental groups. There was a significant increase in LDL and VLDL in isoproterenol induced rats followed by decrease in HDL level when compared to control rats. High concentration of LDL in serum is an important risk factor for heart diseases.<sup>[15]</sup> LDL molecules are oxidized by reactive oxygen free radicals by a series of reactions and damage the artery wall.

Treatment with ethanol extracts of *Boerhavia diffusa* in increasing concentration significantly (P <0.05)

decreased the LDL and VLDL level and the HDL level was return back to normal when compared to isoproterenol treated group. The presence of phytochemicals in *Boerhavia diffusa* extract inhibits cAMP thus maintaining the fluidity function of the tissue membranes. An increase in LDL and along with a decrease in HDL was observed in myocardial infarcted rats.<sup>[16]</sup>

#### Level of Cardiac antioxidants

Table IV represented the levels of antioxidants SOD, catalase, Glutathione peroxidase and glutathione reductase in the heart tissue of control and isoproterenol administered rats. The level of antioxidants was found to be decreased significantly (P<0.05) in isoproterenol treated rats when compared to control groups. Experimental rats co treated with *Boerhavia diffusa* in different concentrations brought back the level of antioxidants to near normal. The decreased level of antioxidants was mainly due to lipid peroxidation and production of free radicals. Restoration of antioxidants by the plant extract proved the presence of rich phytochemicals present in the extract. Antioxidants play vital role in providing protection against oxidative and degenerative diseases.<sup>[17]</sup> Previous studies reported by Ganesan<sup>[18]</sup> described that natural antioxidants provide membrane stability and significant free radical scavenging ability. Treatment with plant extracts increased the level of endogenous antioxidants in cardiovascular diseases as reported by Mohanty.<sup>[19]</sup>

**Table I: Effect of serum marker enzymes in different experimental groups. Values are expressed as mean±SD for 6 rats in each group.**

Groups	AST(IU/L)	ALT(IU/L)	ACP(IU/L)	ALP(IU/L)
I	24.89 ± 0.67	75.75 ± 0.67	34.70±1.71	186.56 ± 1.67
II	47.60 ± 0.42*	189.65 ± 1.06*	54.47± 0.21*	289.23 ± 2.59*
III	39.35 ± 0.35**	84.10 ± 0.90**	48.14 ± 0.27**	234.01 ± 2.17**
IV	31.56 ± 0.09**	88.05 ± 1.24**	45.00±0.26**	219.45 ± 1.99**
V	28.66 ± 0.04**	80.45 ± 0.24**	42.65±0.91**	204.23 ± 1.36**

Statistically significant variations at P<0.05\* group II vs group I, \*\* group III, IV, V vs group II

**Table II: Effect of serum lipid profile in different experimental groups. Values are expressed as mean±SD for 6 rats in each group.**

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Free fatty acids (nmoles/mg/protein)	Phospholipids (mg/dl)
I	60.78 ± 0.39	68.37 ± 0.29	45.06 ± 0.31	8.99 ± 0.05
II	165.94 ± 0.34*	179.13 ± 0.53*	80.79 ± 0.38*	15.69 ± 0.37*
III	137.31 ± 0.50**	138.80 ± 0.63**	71.08 ± 0.41**	13.69 ± 0.12**
IV	102.99 ± 0.78**	102.52 ± 0.71**	61.23 ± 0.29**	11.69 ± 0.20**
V	69.83 ± 0.91**	75.72 ± 0.48**	48.39 ± 0.41**	9.56 ± 0.09**

Statistically significant variations at P<0.05\* group II vs group I, \*\* group III, IV, V vs group II.

**Table III: Effect of Serum Lipoproteins in different experimental groups. Values are expressed as mean±SD for 6 rats in each group.**

Groups	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I	26.25 ± 0.24	70.85 ± 0.17	13.67 ± 0.06
II	9.93 ± 0.08*	120.19 ± 0.17*	35.83 ± 0.11*
III	12.71 ± 0.32**	96.84 ± 0.30**	27.76 ± 0.13**
IV	17.33 ± 0.87**	85.16 ± 0.96**	20.50 ± 0.14**
V	25.23 ± 0.48**	72.46 ± 1.05**	15.14 ± 0.10**

Statistically significant variations at P<0.05\* group II vs group I, \*\* group III, IV, V vs group II.

**Table IV: Effect of Tissue Antioxidants in different experimental groups. Values are expressed as mean±SD for 6 rats in each group.**

Groups	SOD (U/mg protein)	GPx(U/mg protein)	GR(U/mg protein)	Catalase ( U/mg protein)
I	5.04 ± 0.08	3.22 ± 0.09	1.47 ± 0.04	2.92 ± 0.05
II	0.57 ± 0.11*	0.29 ± 0.03*	0.30 ± 0.02*	0.74 ± 0.03*
III	1.71 ± 0.04**	0.88 ± 0.03**	0.44 ± 0.03**	1.04 ± 0.02**
IV	2.35 ± 0.08**	2.23 ± 0.05**	0.97 ± 0.04**	1.62 ± 0.04**
V	3.51 ± 0.03**	2.85 ± 0.09**	1.09 ± 0.02**	2.46 ± 0.05**

Statistically significant variations at P<0.05\* group II vs group I, \*\* group III, IV, V vs group II

## CONCLUSION

The present study clearly depicts that co treatment of *Boerhavia diffusa* ethanolic extract in increasing concentration exhibited preventive effect against isoproterenol induced myocardial infarction. Treatment with *Boerhavia diffusa* changed the isoproterenol induced alterations in the activities of serum markers, lipid profile, lipoproteins and tissue antioxidants. Counteraction of free radicals and blockage of lipid peroxidation by the antioxidant activity of *Boerhavia diffusa* visibly shows powerful cardioprotective activity. Thus the ethanolic extract of *Boerhavia diffusa* can be used as a proficient drug choice for the treatment of myocardial infarction due to the presence of rich antioxidant property.

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