

Cardioprotective Effect of Ethanolic Extract of Leaves of *Amaranthus cruentus* in Isoprenaline-Induced Myocardial Infarction in Rats

Priya Bisen, Aman Chaturvedi, Aditya Ganeshpurkar*, Nazneen Dubey

ABSTRACT

Objectives: The present study is focused to evaluate cardioprotective activity of ethanolic extract of *Amaranthus cruentus*. **Methods:** *Amaranthus cruentus* extract (Dose 200mg/kg and 400mg/kg) in isoprenaline induced myocardial infarction in rats. The ethanolic extract of *Amaranthus cruentus* was prepared and subjected to acute toxicity in albino rats. The extract was given orally at two different doses 200mg/kg and 400mg/. Isoprenaline was administered subcutaneously (85mg/kg s.c.). **Results:** The histopathological examination revealed mild infarction and inflammation in isoprenaline treated rats. The ethanolic extract of *Amaranthus cruentus* showed significant cardio protective effect by decreasing the serum level of LDL and VLDL cholesterol levels. The biochemical parameters (AST, ALT and ALP) and HDL cholesterol levels were restored. **Conclusion:** The outcome of the present study suggested cardioprotective activity of *Amaranthus cruentus* extract.

Key words: *Amaranthus cruentus*, Isoprenaline, Cardiotoxicity, Myocardial infarction, Cholesterol, Lipid, Cardiac markers.

Priya Bisen, Aman Chaturvedi, Aditya Ganeshpurkar*, Nazneen Dubey

Shri Ram Institute of Technology-
Pharmacy, Jabalpur, Madhya Pradesh,
INDIA.

Correspondence

Dr. Aditya Ganeshpurkar

Assistant Professor, Shri Ram Institute of
Technology- Pharmacy, Jabalpur-482002,
Pradesh, INDIA.

Phone no: +91 9993821431

E-mail: adityaganeshpurkar@gmail.com

History

- Submission Date: 25-11-2019;
- Review completed: 24-03-2020;
- Accepted Date: 20-05-2020.

DOI : 10.5530/fra.2020.1.5

Article Available online

<http://www.antiox.org>

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INTRODUCTION

Cardiovascular disease (CVD) remains the principal cause of death in developed and developing countries, Claiming 17.1 million lives a year. According to WHO report the most important cause of mortality in India by 2020.¹ Among the several cardiovascular diseases, the myocardial infarction becoming a worldwide problem. Myocardial infarction is commonly known as heart disease or heart attack.² It is an acute condition of necrosis of the myocardial cells that result due to the imbalance between coronary blood supply and myocardial damage. The symptoms myocardial infarction includes chest pain, sweating, palpitation and anxiety. Some of the important risk factor myocardial infarction are hypercholesterolemia, poor diet, smoking etc.³ Isoproterenol is a synthetic catecholamine and β -adrenergic agonist. It is cause severe oxidative stress in the myocardium resulting in infarct like necrosis of heart muscles. Experimental induction of myocardial infarction by isoprenaline in animal is a well-established model to study the protective role different cardio protective agents.⁴ Medicinal plant constituents an important source of active natural product which differs in term of structure and biological properties and play a important role in department of various human disease. *Amaranthus cruentus* belong to family Amaranthaceae. *Amaranthus cruentus* possess antioxidative effect. It is rich source of phytochemicals like alkaloids, glycosides, flavonoid,

saponin.⁴ *Amaranthus cruentus* leaf extract is found to be beneficial in experimentally induced anaemia.⁵ The present study was aimed to evaluate cardio protective effect of *Amaranthus cruentus*.

MATERIALS AND METHODS

Plant material

The fresh leaves of *Amaranthus cruentus* was collected from Balaghat (21.8129° N, 80.1838° E), M.P., India, during the month of January 2018 and was authenticated by Collage of Agriculture, Murjhad, Balaghat, M.P. (Reference Letter No./COA/2016-17-1989B).

Extraction

The leaves of *Amaranthus cruentus* after drying were coarsely powdered. The dried powdered leaves were first extracted with petroleum ether to remove fat and were further extracted with ethanol (95%). All the studies were performed on ethanolic extract.

Drug and Chemicals

Isoprenaline HCl (Samarth Life Science, Solan, India), ethanol (Changshu Hongsheng Fine Chemical Co.Ltd., China) diethyl ether, formaldehyde, sodium citrate, petroleum ether (Central Drug House, New

Cite this article: Bisen P, Chaturvedi A, Ganeshpurkar A, Dubey N. Cardioprotective Effect of *Amaranthus cruentus* Extract on Isoprenaline Induced Myocardial Infarction in Rats. Free Radicals and Antioxidants. 2020;10(1):23-8.



Delhi) were used in the study. All the other chemicals used in this study were of analytical grade.

Experimental Animal

Albino Wistar Rats (100-180gm) of either sex were in the present study. The animal were housed in clean polypropylene cages in an air conditioned room and were kept under standard condition of humidity (50±5%) temperature (25±2)°C and light (12 hr. light /12 hr. dark cycle). The bedding material of cages was changed every day. The rats were offered standard diet water *ad libitum*. They were initially acclimatized to the laboratory environment for seven days. The experimental protocol was approved by the Institutional Animal Ethics Committee.

Induction of Myocardial Infarction

Isoprenaline (85mg/kg) was dissolved physiological saline solution and was injected subcutaneously to rats daily two consecutive days to induce firstly tachycardia and finally induce experimental myocardial infarction.⁶

Experimental Design

Animal were randomly divided into five groups of six animals.

- Group I : Normal Control (Saline 2 ml/kg)
- Group II : Isoprenaline HCl (85mg/kg, s.c.)
- Group III : Isoprenaline HCl (85mg/kg, s.c.) + Atorvastatin (10mg/kg.s.c.)
- Group IV : Isoprenaline HCl (85mg/kg, s.c.) + *Amaranthus cruentus* extract (200mg/kg/day)
- Group V : Isoprenaline HCl (85mg/kg, s.c.) + *Amaranthus cruentus* extract (400mg/kg/day)

The duration of treatment was 30 days. At the end of the treatment, blood was collected through retro-orbital sinus from all the groups and was allowed to clot at room temperature.⁷ The serum was separated by centrifugation (2500 rpm for 10 min). Later, animals were sacrificed and heart-tissues were excised immediately, rinsed in ice chilled saline and were processed for biochemical estimation and histopathological studies.

Biochemical estimation

The serum was analysed for the presence of different enzymes related to myocardial infarction like AST, ALT and ALP by commercial kits (Span Diagnostics). The levels of VLDL, LDL and HDL cholesterol were also estimated by commercial kits (Accurex Kits). The levels of total protein were also determined (HiMedia BCA Kit).

Statistical analysis

The results were expressed as mean ± SEM. Statistical analysis was carried out by using One-way ANOVA followed by Dennett's test and $p < 0.05$, $p < 0.01$, $p < 0.001$ was considered significant.

RESULTS

Effect *Amaranthus cruentus* extract on cholesterol level

The rats treated with isoprenaline revealed an increased cholesterol and triglycerides level in the serum. The ethanolic extract of *Amaranthus cruentus* caused a significant decrease the level of cholesterol and triglycerides in the serum of isoprenaline induced myocardial infarcted rats (Figure 1, Figure 2).

Isoproterenol induced myocardial infarcted rats demonstrated a significant increase in the level of serum LDL and VLDL cholesterol with a decline in the level of serum HDL cholesterol. Pre-treatment with *Amaranthus cruentus* extract significantly decreased the levels of LDL and VLDL

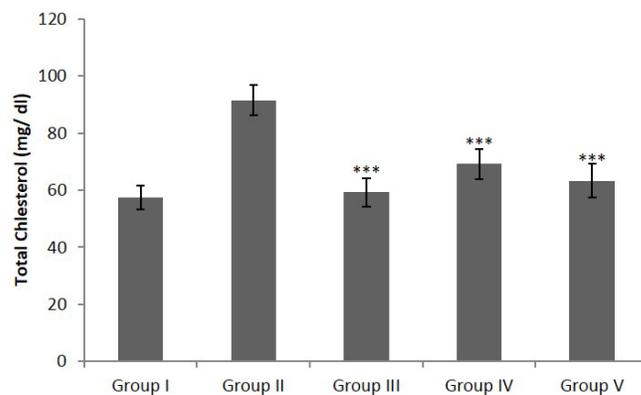


Figure 1: Effect of *Amaranthus cruentus* extract on total cholesterol against isoproterenol induced myocardial infarction in Wistar Albino rats Each value is mean ± S.E.M. ($n = 6$). Data was analyzed by One-way ANOVA followed by Dunnett's test. Significance at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ when compared to Group II.

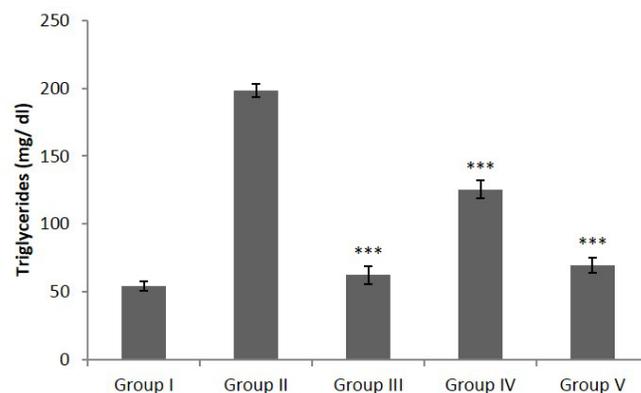


Figure 2: Effect of *Amaranthus cruentus* extract on triglycerides level against isoproterenol induced myocardial infarction in Wistar Albino rats Each value is mean ± S.E.M. ($n = 6$). Data was analyzed by One-way ANOVA followed by Dunnett's test. Significance at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ when compared to Group II.

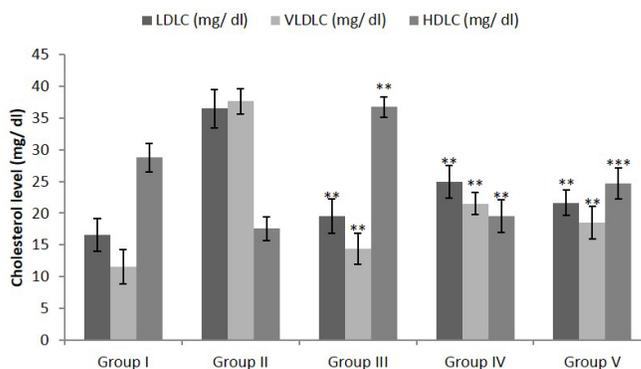


Figure 3: Effect of *Amaranthus cruentus* extract on cholesterol levels against isoprenaline induced myocardial infarction in Wistar Albino rats. Each value is mean ± S.E.M. ($n = 6$). Data was analyzed by One-way ANOVA followed by Dunnett's test. Significance at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ when compared to Group II.

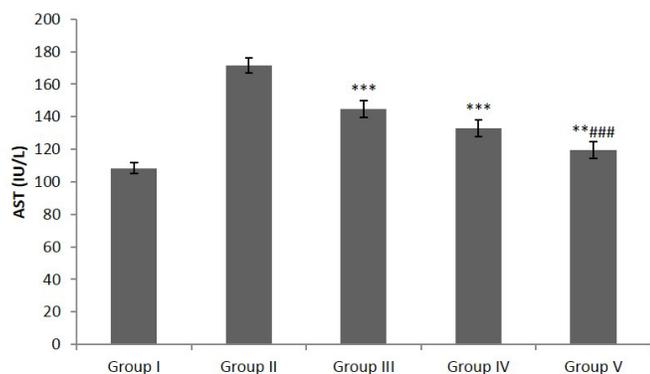


Figure 4: Effect of *Amaranthus cruentus* extract on AST levels against isoproterenol induced myocardial infarction in wistar albino rats each value in mean \pm SEM ($n=6$). Data was analysed by one way ANOVA followed by Dunnett's Test. Significance of at $*p<0.05$; $**p<0.01$ $***p<0.001$ when compared to Group II.

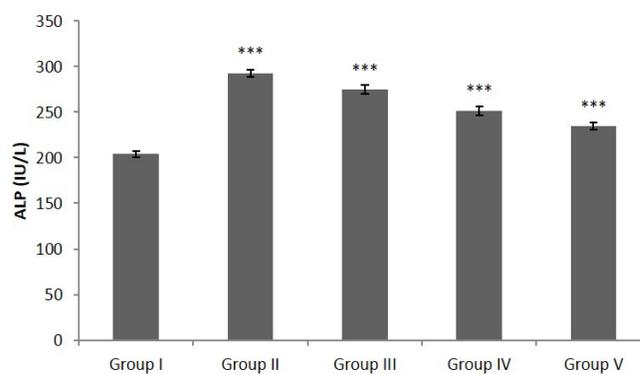


Figure 6: Effect of *Amaranthus cruentus* extract on ALP levels against isoproterenol induced myocardial infarction in wistar albino rats. Each value is mean \pm SEM ($n=6$). Data was analysed by one way ANOVA followed by Dunnett's Test. Significance of at $*p<0.05$; $**p<0.01$ $***p<0.001$ when compared to Group II.

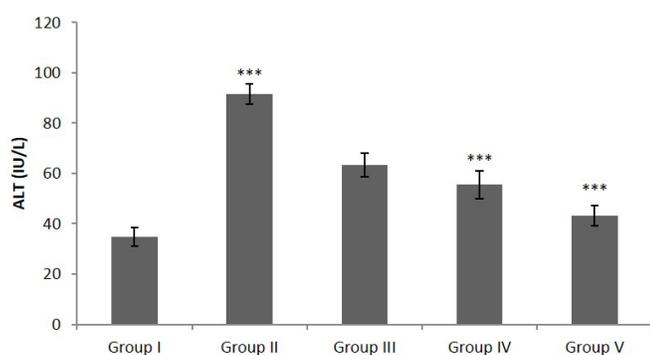


Figure 5: Effect of *Amaranthus cruentus* extract on ALT levels against isoproterenol induced myocardial infarction in wistar albino rats each value in mean \pm SEM ($n=6$). Data was analysed by one way ANOVA followed by Dunnett's Test. Significance of at $*p<0.05$; $**p<0.01$ $***p<0.001$ when compared to group -II.

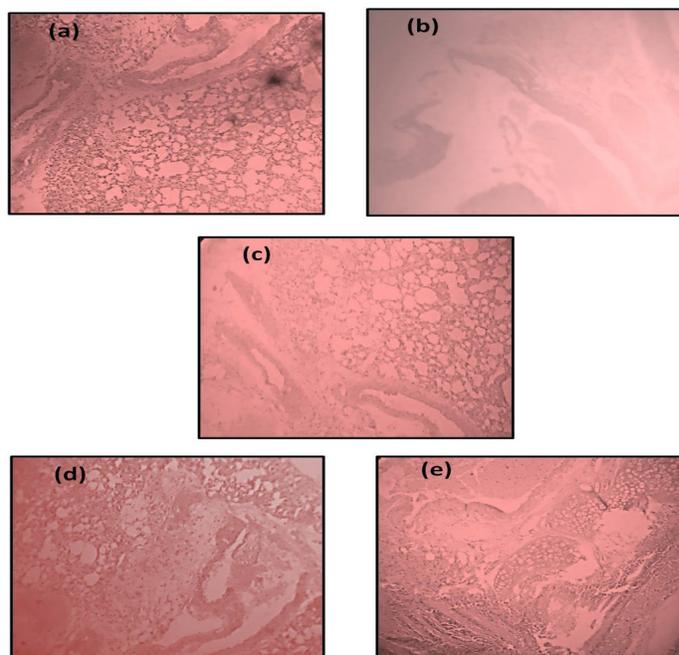


Figure 7: Evidence for the protective effect of *Amaranthus cruentus* extract in rats treated with isoprenaline; (a) normal control, (b) Isoprenaline, (c) Atrovastatin (d) Hesperidin 25 mg/kg p.o. (e) *Amaranthus cruentus* 200 mg/kg p.o., (f) *Amaranthus cruentus* 400 mg/kg p.o.,

cholesterol and significantly increased the levels of serum HDL cholesterol in isoprenaline infarcted rats (Figure 3).

Effect *Amaranthus cruentus* extract on cardiac enzymes

Isoprenaline treatment in the experimental animals resulted in notable increase cardiac marker enzyme ALT, AST and ALP when compared with control groups. Oral treatment with *Amaranthus cruentus* extract restored all the alteration in serum to normal levels (Figure 4-6).

Effect *Amaranthus cruentus* extract on total protein level

Isoprenaline treated rats revealed a consequent decrease in total protein level as compared to Group I (control rats). Orally administered ethanolic extract of *Amaranthus cruentus* to isoprenaline injected rats demonstrated a significant increase in total protein levels (Table 1).

Histopathological Studies

The histological images of group I animals revealed a normal cardiac architecture with normal cardiac muscle fibers without any infarction and cardiac impulses. The animals treated with isoprenaline showed the presence of lipid droplets, inflammatory cell, oedema, myocardial infarction and damage to cardiocyte damage. Isoprenaline and

Table 1: Effect of ethanolic extract of *Amaranthus cruentus* on total protein level.

Groups	Total Protein
Group I	9.17 \pm 0.54
Group II	5.74 \pm 0.73
Group III	6.45 \pm 0.51 $***$
Group IV	7.52 \pm 0.68 $***$
Group V	8.21 \pm 0.48 $***$

Each value is mean \pm SEM (N=6). Data was analysed by one way ANOVA followed by Dunnett's Test. Significance of at $*p<0.05$; $**p<0.01$ $***p<0.001$ when compared to Group II

Atorvastatin treated group revealed edema with slight inflammatory cellular infiltration. *Amaranthus cruentus* extract (200mg) revealed the presence of edema and moderate inflammatory cell with decrease area of coagulative inflammation in myocardial fibres. *Amaranthus cruentus* extract (400mg) treated groups showed mild oedema but no infraction and inflammation (Figure 7).

DISCUSSION

Myocardial infarction is the commonly known as ischemic heart disease. Myocardial infarction cause the necrosis of the myocardium which is due to imbalance between coronary blood supply and myocardium demand.^{8,9} Myocardial infarction remains a leading cause for death worldwide and prompt treatment for a heart attack is essential to save the life. Recent studies suggest that increased free radical formation and subsequent oxidative stress are associated with the occurrence of a relative deficit in the endogenous antioxidants, may be one of the mechanisms for the development of heart failure after myocardial infarction.¹⁰ Herbal medicine is getting an increased acceptance from the public due to the fact that the herbs positivity modulate the health^{11,12} and quality of life.¹³ The present study was designed to investigation the cardioprotective effect of ethanolic extract of *Amaranthus cruentus* against isoprenaline induced functional and structural damage of cardiocytes mediated through oxidative stress and lipid peroxidation. Isoprenaline is a β -adrenergic agonist that causes a severe oxidative stress in myocardium resulting infarct-like necrosis of cardiocytes which proceeds towards the development of myocardial infarction in large dose. Isoprenaline-induced myocardial necrosis is a authenticated model of myocardial infarction in rats.¹⁴ Catecholamines are important regulators of myocardial contractility and metabolism. They are responsible for cellular damage, observed in clinical condition like angina, myocardial hypoxia, sub-endocardial infarct.¹⁵ There are considerable epidemiological evidence suggesting that consumption of fruit and vegetables, particularly, green leafy vegetables is associated with a lower risk of coronary heart disease. The beneficial effect of vegetables and fruits could be due to the presence of compound with antioxidant properties.¹⁶⁻¹⁸ The formation of reactive oxygen species plays a key role in cardiac pathophysiology. Therefore, by targeting oxidative stress the treatment of myocardial infarction can be substantially improved.¹⁹ Ayurveda play a very important role in maintaining good vigor. Medicinal plants play an important role with respect to cardio-protection. Several medicinal plant and there parts like bark, leaves, stem, flowers and fruits have been evaluated for prophylactic and therapeutic effect and thus provide relief in cardiovascular diseases.⁶ These include *Hibiscus sabdariffa*,²⁰ *Mangifera indica*,²¹ *Emblica officinalis*,²¹ *Moringa oleifera*,²² and many more. In the present study, administration of *Amaranthus cruentus* extract in isoprenaline treated animals revealed a significant protection to cardiocytes. There was decrease in levels of VLDL and LDL cholesterol along with partial restoration of HDL cholesterol levels. There was restoration of ALT, AST and ALP. Further total protein content of cardiocytes increased significantly due to administration of *Amaranthus cruentus* extract.

CONCLUSION

The outcomes of the present study revealed a cardio protective effect of *Amaranthus cruentus* extract. There was an increase in the overall integrity of targets studied. However, more studies are necessary to explore the mechanism behind this effect.

ACKNOWLEDGEMENT

Authors are thankful to Dr. Sharad Bisen, Assist. Professor, Collage of Agriculture, Murjhad, Balaghat, M.P. for identifying plant samples.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest

ABBREVIATIONS

MI: Myocardial infraction; **CVD:** Cardiovascular disease; **AST:** *Aspartate transaminase*; **ALT:** Alanine amino transferase; **ALP:** Alkaline phosphate; **CH:** Cholesterol; **VLDL:** Very low density lipid; **LDL:** Low density lipid; **HDL:** High density lipid.

SUMMARY

The present study establishes cardioprotective effect of *Amaranthus cruentus* on isoprenaline induced cardiac damage in rats. The outcomes of the present study would be useful in identifying cardioprotective constituents

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Cite this article: Bisen P, Chaturvedi A, Ganeshpurkar A, Dubey N. Cardioprotective Effect of Ethanolic Extract of Leaves of *Amaranthus cruentus* in Isoprenaline-Induced Myocardial Infarction in Rats . *Free Radicals and Antioxidants.* 2020;10(1):24-8.