International Journal of Pharmacognosy and Pharmaceutical Sciences

**ISSN Print:** 2706-7009
**ISSN Online:** 2706-7017
IJPPS 2020; 2(2): 09-13
www.pharmacognosyjournal.net
Received: 11-07-2020
Accepted: 13-08-2020

**Brindha Elangovan**
Assistant Professor, Department of Biochemistry, Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu, India

**Abhirami S Ajayakumar**
Student, M.Sc Biochemistry, Department of Biochemistry, Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu, India

**Ranjani Priya Anandraj**
Ph.D., Research Scholar, Department of Biochemistry, Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu, India

**Corresponding Author:**
**Brindha Elangovan**
Assistant Professor, Department of Biochemistry, Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu, India

# Cardioprotective role of *Ageratum conyzoides* L. on cardiac mitochondrial enzymes during isoproterenol-induced myocardial infarction in rats

**Brindha Elangovan, Abhirami S Ajayakumar and Ranjani Priya Anandraj**

**DOI:** https://dx.doi.org/10.33545/27067009.2020.v2.i2a.64

**Abstract**
The present study is to evaluate the preventive role of *Ageratum conyzoides* L. on mitochondrial enzymes such as isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH), and α-ketoglutarate dehydrogenase (α-KGDH) and respiratory chain enzymes such as NADH dehydrogenase and cytochrome c oxidase in isoproterenol (ISO)- induced myocardial infarction (MI) in male albino Wistar rats. Rats were orally pre-treated with *Ageratum conyzoides* L. (100 and 200 mg/kg) to ISO-induced rats daily for a period of 56 days. After the treatment, rats were subcutaneously injected with ISO (85 mg/kg) at an interval of 24 h for 2 days. ISO induction also showed a significant ($p$<0.05) decrease in the activities of enzymes such as ICDH, SDH, MDH, α-KGDH, NADH dehydrogenase, and cytochrome c oxidase. Pre-treatment with *Ageratum conyzoides L.* significantly ($p$< 0.05) altered all the biochemical parameters and regulated the normal mitochondrial function. Transmission electron microscopic (TEM) study also correlated with these biochemical findings. Thus, the present study findings demonstrate that *Ageratum conyzoides* L. possesses excellent cardioprotective activity by preventing alterations in ISO-induced MI in rats.

**Keywords:** α-ketoglutarate dehydrogenase, *Ageratum conyzoides* L.

## Introduction
Cardiovascular disease (CVD) is long established as the leading cause of loss or death all over the world, especially in developed countries [1]. MI followed by myocardial ischemia is the most lethal manifestation of CVD that causes cessation of blood flow to the myocardium leads to myocardial necrosis. MI is characterized by varying degrees of chest pain, discomfort, sweating, weakness, nausea, vomiting, arrhythmias and sometimes causing loss of consciousness. Chest pain is the most common characteristic caused by MI [2]. The consequence of MI is lipid peroxidation [3], hyperlipedemia [4], and loss of membrane integrity. Isoproterenol-induced MI is the most widely used experimental model to study cardiac functions. Isoproterenol (1, [3, 4 dihydroxyphenyl]-2-isopropyl amino ethanol hydrochloride) is a synthetic catecholamine and β-adrenergic agonist, which has been found to cause severe stress in the myocardium resulting in infarct like necrosis of heart muscles [5, 6] Auto-oxidation of ISO, produce quinones react with oxygen to form the highly reactive products, free radicals such as superoxide anions ($0_2$-) and $H_2O_2$ that is highly toxic oxygen derivatives, which are detrimental to extracellular and intracellular enzymes and proteins. Furthermore, these free radicals generated could initiate the peroxidation of membrane-bound PUFAs, altering the membrane fluidity leading to both functional and ultra-structural myocardial injury [7] ISO-induction induces MI and a distinct increase in marker enzymes, lipid peroxides, and a decrease in anti-oxidants. Environmental factors, especially dietary factors play a vital role in the development of various diseases including CVD. Recent studies suggested the cardioprotective effect of natural phytochemicals can be used for the prevention and blocking of the progression of the disease by targeting oxidative stress [8, 9]. It is already well established that phenolic compounds represent one of the important groups of natural plant products, having physiological properties such as antiallergenic, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, cardioprotective, and vasodilatory effects [9].

These compounds possess one or more aromatic rings bearing hydroxyl substituents that act as antioxidants as a result of the reactivity of the phenolic moiety. Phytic acid (Myo-inositol hexaphosphate), is a plant component existing in most grains, legumes, wheat, rice bran, and virtually every kind of mammalian cell contributes 1 to 7 of its dry weight [11, 12]. It is usually a mixture of calcium/magnesium/ potassium salts of inositol hexaphosphoric acid and is the primary source of phosphorus soy and corn is shown to adversely impact mineral bioavailability and protein solubility when present in animal feeds [12]

## Materials and Methods

### Experimental Animals

This experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Animal Ethical Committee of Bharathidasan University (Approval no. BDU/IAEC/2011/31/29.03.2011). All the experiments were carried out with healthy male albino Wistar rats weighing 140–160 g, obtained from the Central Animal House, Rajah Muthiah Institute of Health Sciences, Annamalai University, Annamalai Nagar, Tamil Nadu, India. They were housed in polypropylene cages (47 × 34 × 20 cm) lined with husk, renewed every 24 h under a 12h light and12 h dark cycle at around 22◦ C, and had free access to tap water and food. The rats were fed on a standard pellet diet. (Pranav Agro Industries Ltd., Maharashtra, Pune, India). The pellet diet consists of 21% protein, 5% lipids, 4% crude fiber, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamins and 55% nitrogen-free extract. The diet provides metabolizable energy of 3,600 kcal. Throughout the study, animals were maintained under normal laboratory conditions.

### Drugs and Chemicals

Isoproterenol hydrochloride, phytic acid, cytochrome c, N-phenyl p-phenylene diamine, oxaloacetate, sodium succinate, potassium ferricyanide, and α- ketoglutarate were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used in the study were of analytical grade.

### Induction of Experimental Myocardial Infarction

Myocardial infarction was induced by dissolving Isoproterenol (85 mg/kg) in normal saline and injected subcutaneously to rats at an interval of 24 h for two consecutive days of the experimental schedule [13].

### Experimental Design

The rats were randomly grouped into ten animals each. Two rats from each group were used for TEM studies. Group 1: Normal control rats; Group2; normal rats treated with *Ageratum conyzoides L.* (25 and 50 mg/kg); Group3; ISO (85 mg/kg) control rats; Group 4; rats pre-treated with *Ageratum conyzoides L.* (100 and 200 mg/kg) and then subcutaneously injected with ISO. *Ageratum conyzoides* L. was dissolved in distilled water and administered to rats orally using an intragastric tube daily for a period of 56 days [13].

At the end of the experimental period, after 12 h of the second ISO injection, all the rats were anesthetized with sodium pentobarbital and sacrificed by cervical decapitation. The heart tissue was excised immediately from the animals and washed off the blood with ice-chilled physiological saline.

### Isolation of Heart Mitochondrial Fractions

Heart mitochondria were isolated by the method of Takasawa *et al*. (1993) The heart tissue was put into ice-cold 50 mM Tris–HCl (pH 7.4), containing 0.25 M sucrose, and homogenized. The homogenates were centrifuged at 700×g for 20 min, and then the supernatant obtained was centrifuged at 9000×g for 15 min. The pellets were then washed with 10 mM Tris–HCl (pH 7.8), containing 0.25 M sucrose, and finally resuspended in the same buffer and used for the estimation of various biochemical parameters.

### Biochemical Estimations

The concentration of mitochondrial thiobarbituric acid reactive substances (TBARS) was estimated (Fraga *et al*., 1998). Activities of ICDH, SDH, MDH, α-KGDH, NADH dehydrogenase, and cytochrome c oxidase were assayed. Protein content in the mitochondrial fraction was estimated by the method of Lowry *et al*. (1951).

### Statistical Analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using Statistical Package for the Social Sciences (SPSS) software package version 9.05. Results were expressed as mean ± S.D. for eight rats in each group. p values < 0.05 were considered significant.

## Results

Isoproterenol-induced myocardial infarction was confirmed by elevated levels of TBARS in rats. Table 1 shows levels of mitochondrial TBARS in the heart of normal and experimental rats. Rats induced with ISO showed a significant ($p< 0.05$) increase in the levels of mitochondrial TBARS when compared with normal control rats. Oral pre-treatment with *Ageratum conyzoides* L. (100 and 200 mg/kg) to ISO-induced rats daily for a period of 56 days significantly ($p< 0.05$) decreased the levels of mitochondrial TBARS in the heart when compared with ISO-alone induced rats.

**Table 1:** Effect of *Ageratum conyzoides* L. on the levels of heart mitochondrial TBARS in normal and isoproterenol (ISO) – induced myocardial infarcted rats.

| Groups | **Mitochondrial TBARS (nmoles/mg ptn)** |
|---|---|
| Normal control | 3.14 ± 0.27 a |
| Normal + *A. conyzoides* L. leaf extract (100 mg/kg) | 3.02 ± 0.23 a |
| *A. conyzoides* L. leaf extract (200 mg/kg) | 3.11 ± 0.20 a |
| ISO (85 mg/kg) control | 8.32 ± 0.47 b |
| *A. conyzoides* L. leaf extract (100 mg/kg) + ISO | 5.31 ± 0.40c |
| *A. conyzoides* L. leaf extract (200 mg/kg) + ISO | 4.62 ± 0.31d |

Each value is mean ±S.D. for 6 rats in each group. Columns not sharing a common letter (a, b, c and d) differ significantly from each other ($p<0.05$, DMRT).

**Table 2:** Effect of *Ageratum conyzoides* L. on the activities of the heart mitochondrial isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH), and α-ketoglutarate dehydrogenase (α-KGDH) in normal and isoproterenol (ISO)-induced myocardial infracted rats.

| Groups | ICDH | SDH | MDH | α-KGDH |
|---|---|---|---|---|
| Normal control | 648.4±37.2a | 222.3±17.1a | 315. ±17.2 a | 104.2±5.3a |
| Normal + *Ageratum conyzoides L.* (100 mg/kg) | 652.2±43.5a | 224.2±15.2a | 316.3±21.1a | 104.5±6.7a |
| Normal + *Ageratum conyzoides L.* 200 mg/kg) | 653.1±32.3a | 224.5±13.2a | 317.4±19.8a | 104.2±7.4a |
| ISO (85 mg/kg) control | 412.6±23.5b | 121.1±10.3b | 190.4±10.2b | 69.2±4.1b |
| *Ageratum conyzoides L.* (100 mg/kg) + ISO | 543.7±41.3c | 181.3±14.2c | 263.2±17.2c | 84.2±4.3c |
| *Ageratum conyzoides L.* (200 mg/kg) + ISO | 612.1±35.2d | 208.2±16.8d | 296.4±16.2d | 92.3±4.2d |

Activity is expressed as nmoles of NADH oxidized/h/mg protein for ICDH; nmoles of succinate oxidized/min/mg protein for SDH; nmoles of NADH oxidized/min/mg protein for MDH; nmoles of ferrocyanide formed/h/mg protein for α-KGDH. Each value is mean ± S.D. for 6 rats in each group. Values not sharing a common superscript (a, b, c, d) differ significantly from each other $p< 0.05$ (DMRT).

Activities of the heart mitochondrial TCA cycle enzymes such as ICDH, SDH, MDH, and α-KGDH were decreased significantly in ISO-induced rats when compared with normal control rats. Oral pretreatment with *Ageratum conyzoides* L. (100 and 200 mg/kg) to ISO-induced rats significantly increased the activities of these enzymes when compared with ISO- alone-induced rats (Table 2).

**Table 3:** Effect of *Ageratum conyzoides* L. on the activities of the heart mitochondrial NADH-dehydrogenase and cytochrome-c-oxidase in normal and isoproterenol (ISO)-induced myocardial infarcted rats.

| Groups | NADH-dehydrogenase | Cytochrome c-oxidase |
|---|---|---|
| Normal control | 161.5±10.0a | 0.451±0.01a |
| Normal + *Ageratum conyzoides* L. (100 mg/kg) | 163.1±12.3a | 0.453±0.01a |
| Normal + *Ageratum conyzoides* L. 200 mg/kg) | 165.5±13.9a | 0.454±0.02a |
| ISO (85 mg/kg) control | 84.7±4.8b | 0.287±0.02b |
| *Ageratum conyzoides* L. (100 mg/kg) + ISO | 120.7±7.8c | 0.358±0.01c |
| *Ageratum conyzoides* L. (200 mg/kg) + ISO | 146.4±7.8d | 0.411±0.01d |

Activity is expressed as nmoles of NADH oxidized/min/mg protein for NADH-dehydrogenase; nmoles/min/mg protein for cytochrome-c-oxidase. Each value is mean ± S.D. for 6 rats in each group. Values not sharing a common superscript (a, b, c, d) differ significantly from each other $P < 0.05$ (DMRT).

The activities of the heart mitochondrial respiratory chain enzymes such as NADH-dehydrogenase and cytochrome-c-oxidase in normal and ISO-induced rats are shown in Table 3. The activities of these enzymes were significantly decreased in ISO-induced rats when compared with normal control rats. Oral pre-treatment with *Ageratum conyzoides* L. (100 and 200 mg/kg) to ISO-induced rats significantly increased the activities of these enzymes when compared with ISO-alone induced rats.

## Discussion

Thus, ISO produces relative ischemia or hypoxia due to myocardial hyperactivity and coronary hypotension18 and induces myocardial ischemia due to cytosolic Ca2+ overload [14]. Grimm *et al*. (1998) have reported that a toxic dosage of ISO caused characteristic myocardial damage that subsequently resulted in heart failure. ISO administration causes ischemic necrosis in rats, which closely resembles histological damage seen in human MI. Free radicals could initiate the peroxidation of membrane-bound polyunsaturated fatty acids (PUFA), leading to both functional and structural myocardial injury [15]. The effects of isoproterenol (ISO) on the heart are mediated through β1 and β2 adrenoceptors. Both β1 and β2 adrenoceptors mediate the positive inotropic and chronotropic effects of adrenoceptor agonists [16].

Increased levels of mitochondrial free radical production experiential under pathological conditions, such as ischemia, are associated with impairment of mitochondrial structure and function. We observed increased levels of heart mitochondrial TBARS in ISO-induced rats indicating increased lipid peroxidation, which could be credited to the deficiency of the antioxidant system [17]. Pre-treatment with *Ageratum conyzoides* L. to ISO-induced rats daily for a period of 56 days decreased the levels of heart mitochondrial TBARS. Flavonoid activities mainly depend on their antioxidant and chelating properties, which are responsible for the inhibitory effect of flavonoids on lipid peroxidation. In this context, Jagetia and Reddy have reported that *Ageratum conyzoides L.* treatment reduced the levels of TBARS in radiation-induced lipid peroxidation.We have observed a decrease in the activities of tricarboxylic acid cycle enzymes, such as ICDH, SDH, MDH, and α-KGDH, in the heart mitochondria in ISO-induced rats. These enzymes are located on the outer membrane of mitochondria and could be pretentious by excessive production of free radicals by ISO. Our results are in agreement with previous reports [18-19]. Pre-treatment with *Ageratum conyzoides* L. to ISO-induced rats significantly increased the activities of tricarboxylic acid cycle enzymes. This could be due to its ability to prevent the free radical formation and free radical scavenging properties of phytic acid.

Biological membranes and subcellular organelles rich in PUFA are the major sites for free radical-mediated damage. Activation of lipid peroxidation in mitochondria corresponds with changes in lipid composition, which includes a decrease in the levels of total and readily oxidizable lipid, cardiolipin. Cytochrome c oxidase and NADH dehydrogenase are present in the inner mitochondrial membrane and are involved in the synthesis of the high-energy compound ATP. These enzymes have an absolute requirement of cardiolipin. The activities of these enzymes were decreased in the heart mitochondria of ISO-

induced rats. This could be due to enhanced phospholipids degradation resulting in the non-availability of cardiolipin for their functional activity. Previous studies also reported diminished activities of cytochrome c oxidase and NADH dehydrogenase in rats induced with ISO [20, 21]. Pre-treatment with *Ageratum conyzoides L.* increased the activities of NADH dehydrogenase and cytochrome c oxidase in ISO-induced rats. Our results indicate that pre-treatment with *Ageratum conyzoides L.* substantially prevented the excessive impairment of these enzyme activities. Furthermore, our study suggests that *Ageratum conyzoides* L. may restore the energy status of the mitochondria, thereby maintaining membrane integrity. This could be due to the inhibition of PLs degradation in the biological membranes and maintaining the levels of cardiolipin in the membrane PLs.

The transmission electron micrograph of the mitochondria of the heart in ISO-induced rats showed swelling morphology of mitochondria and loss of cristae with vacuolation. The swollen morphology is typical for mitochondria that have been subjected to ischemic and hypoxic conditions [27], which could be due to the accumulation of lipid peroxide products as a result of GSH depletion [28]. Rats pre-treated with *Ageratum conyzoides L.* (100 mg/kg) in ISO-induced rats showed mild swelling with dissociation of cristae and rats pre-treated with *Ageratum conyzoides* L. (200 mg/kg) in ISO-induced rats showed mild dissociation of cristae without swelling and vacuolation. *Ageratum conyzoides* L. (100 and 200 mg/kg) treated normal rats heart mitochondria showed no pathological changes, which indicates that *Ageratum conyzoides* L. does not possess any adverse effects under normal conditions. The anti-lipid peroxidative and antioxidant property of *Ageratum conyzoides* L. could have reduced the mitochondrial lipid peroxidation and maintained the normal functioning of mitochondria in the myocardium.

## Conclusion

The results obtained from our study specified that *Ageratum conyzoides* L. Offers protection to the cardiac mitochondria by decreasing the levels of lipid peroxides and maintaining the activities of mitochondrial enzymes in ISO-induced rats. This could be due to its antioxidant as well as membrane-stabilizing effects. TEM study also supports these biochemical findings in ISO-induced rats. Restoration of cellular normalcy accredits the cytoprotective role of phytic acid, as *Ageratum conyzoides* L. possessed a protective effect on mitochondria, which is a crucial element involved in both triggering and mediating the cardioprotective responses in myocardial cells. Thus, this study may have a significant impact on the clinical treatment of myocardial diseases. On the basis of the previous and present findings, we speculate that *Ageratum conyzoides* L. Could be an effective chemo preventive agent against ISO-induced myocardial infarction and associated oxidative stress.

## References


1. Davis TM, Fortun P, Mulder J, Davis WA, Bruce DG. Silent myocardial infarction and its prognosis in a community-based cohort of Type 2 diabetic patients: The Fremantle Diabetes Study. Diabetologia. 2004;47:395-399,.
2. Malik MA, Alam Khan S, Safdar S, Taseer IU. Chest Pain as a presenting complaint in patients with acute myocardial infarction (AMI). Pak J Med Sci. 2013 Apr;29(2):565-8. doi: 10.12669/pjms.292.2921. PMID: 24353577; PMCID: PMC3809224.
3. Lu J, Chen B, Chen T, Guo S, Xue X, Chen Q, Zhao M. Comprehensive metabolomics identified lipid peroxidation as a prominent feature in human plasma of patients with coronary heart diseases. Redox Biol. 2017 Aug;12:899-907. doi: 10.1016/j.redox.2017.04.032. Epub 2017 Apr 26. PMID: 28472752; PMCID: PMC5415551.
4. Wexler BC, Greenberg BP. Protective effect of clofibrate on isoproterenol-induced myocardial infarction in arteriosclerotic and non-arteriosclerotic rats. Atherosclerosis. 1978;29:373-375,.
5. Thompson JA, Hess ML. The oxygen free radical system: a fundamental mechanism in the production of myocardial necrosis. Prog Cardiovasc Dis. 1986;28:449-462.
6. Ahmed KK, Rana, AC, Dixit VK. Effect of Caltropis Procera latex on isoproterenol-induced myocardial infarction in albino rats. Phytomedicine. 2004;11:327-330.
7. Karthikeyan K, Sarala Bai BR, Niranjali Devaraj S. Grape seed proanthocyanidins ameliorates isoproterenol-induced myocardial injury in rats by stabilizing mitochondrial and lysosomal enzymes: An *in vivo* study. Life Sci. 2007a;81:1615-1621.
8. Pop RM, Popolo A, Trifa AP, Stanciu LA. Phytochemicals in Cardiovascular and Respiratory Diseases: Evidence in Oxidative Stress and Inflammation. Oxid Med Cell Longev. 2018 Aug 9;2018: 1603872. doi: 10.1155/2018/1603872. PMID: 30159110; PMCID: PMC6109561.
9. Brindha E, Mutational analysis of FOG2 gene in patients with congenital Heart disease . Gene Technol, 9(2). No:1000150 9:2. Doi: 10.24015/2329-6682.20.9.150
10. Coulibaly A, Kouakou B, Chen J. *Ageratum conyzoides L.* in cereal grains: structure, healthy or harmful ways to reduce *Ageratum conyzoides L.* in cereal grains and their effects on nutritional quality. Am. J Plant. Nutr. Fert. Technol. 2011;1:1-22. Linear 1994.
11. Brindha E**,** Rajasekapandiyan M, Preventive effect of phytic acid on lysosomal hydrolases in normal and isoproterenol-induced myocardial infarction in Wistarrats.Toxicol Mech Methods. 2015 Jan 5, 1-20.
12. Brindha E, Rajasekapandiyan M. Preventive effect of phytic acid on isoproterenol- induced cardiotoxicity in Wistar rats: Int J Biomed Sci. 2015;11(1);35-41.
13. Bloom S, Davis DL. Calcium as mediator of isoproterenol-induced myocardial necrosis. Am J Pathol. 1972;69:459-470.
14. Grimm D, Elsner D, Schunkert H, Pfeifer M, Griese D, Bruckschlegel G. Development of heart failure following isoproterenol administration in the rat: role of renninangiotension system. Cardiovasc Res. 1998;37:91-100.
15. Thompson JA, Hess ML. The oxygen free radical system: a fundamental mechanism in the production of myocardial necrosis. Prog Cardiovasc Dis. 1986;28:449-462.
16. Brodde OE. β1 and β2 adrenoceptors in the human heart: properties, function and alteration in chronic heart failure. Pharmacol Rev. 1991;43:203-242.

17. Raghavendran HRB, Sakthivel A, Devaki T. Antioxidant effect of Sargassum polycystum (Phaeophyceae) against acetaminophen induced changes in hepatic mitochondrial enzymes during toxic hepatitis. Chemosphere 2005;61:276–281.
18. Jagetia GC, Reddy TK. Modulation of radiation induced alteration in the antioxidant status of mice by phytic acid. Life Sci. 2005;77:780-794.
19. Yogeeta SK, Gnanaprakasam SS, Kumar R, Subashini T, Devaki T. Synergistic interaction of ferulic acid with ascorbic acid: Its cardioprotective role during isoproterenol induced myocardial infarction in rats. Mol Cell Biochem 2006;283:139-146.
20. Prabu S, Jainu M, Sabitha KE, Devi CS. Effect of mangiferin on mitochondrial energy production in experimentally induced myocardial infarction in rats. Vasc Pharmacol 2006;44:519–525.
21. Chagoya de Sanchez V, Munoz RH, Barrera FL, Yanez L, Vidrio S, Suarez J. Sequential changes of energy metabolism and mitochondrial function in myocardial infarction induced by isoproterenol in rats: a long –term and integrative study. Can J Physiol Pharmacol. 1997;75:1300-1311.
22. Hegstad AC, Ytrehus K, Myklebust R, Jorgensen L. Ultrastructural changes in the myocardial myocytic mitochondria: a crucial step in the development of oxygen radical-induced damage in isolated rat hearts? Basic Res Cardiol. 1994;89:128-138.