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Ageratum conyzoides L. Protects rat myocardium during isoproterenol-induced myocardial infarction in rats: A biochemical study

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Abstract
This study aimed to evaluate the preventive effect of *Ageratum conyzoides L.* on cardiac Troponin T (cTnT), lactate dehydrogenase (LDH)-isoenzyme, cardiac marker enzymes, blood glucose, total proteins, albumin/globulin (A/G) ratio, serum uric acid, serum iron, and plasma iron binding capacity in isoproterenol (ISO)-induced myocardial infarction (MI) in male Wistar rats. Rats treated with ISO(85 mg/kg**,** administered subcutaneously twice at an interval of 24 h for 2 days) showed a significant increase in the degrees of cardiac troponin T (cTnT), the intensity of the bands of LDH1 and LDH2, and the activities of cardiac diagnostic marker enzymes such as creatine kinase-MB (CK-MB), creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) in serum with a subsequent decrease in the activities of CK, LDH, AST and ALT in the heart. ISO-induced rats showed a significant increase in blood glucose, serum uric acid, and serum iron and a decrease in the levels of total proteins, A/G ratio, and iron binding capacity. Pre-treatment with *A. conyzoides L.* (100 and 200 mg/kg) daily for a period of 30 days significantly altered the levels of cTnT, the intensity of the bands of LDH1, and LDH2-isoenzyme and the activities of cardiac marker enzymes, and other biochemical parameters. Thus, *A. conyzoides L.* possesses a cardioprotective effect in ISO-induced oxidative stress in rats.

Keywords: *Ageratum conyzoides*, isoproterenol, myocardial infarction, antioxidant, free scavenging activity, auto oxidation

Introduction
Healthy human life is always cardinal for human beings starting from his birth to throughout life. The number of diseases plays a key role in disturbing healthy human life. In the 21st century, several complementary and alternative medications for the management of cardiovascular diseases (CVD). Myocardial Infarction (MI) (also known as a heart attack) is defined as insufficient oxygenated blood flow to the myocardium (via the coronary artery) that ultimately leads to demand for oxygen/nutrients and results in myocardial injury [1]. The induction of catecholamine at a toxic concentration to the animal models trigger the onset of acute MI [2]. Isoproterenol (ISO) also known as isoprenaline, is a synthetic catecholamine and β-adrenergic agonist, which is a significant controller of myocardial metabolism and an ideal model to study the effects of various drugs on cardiac function. Various mechanisms have been put forward to explain the ISO-induced MI. The complex entities of ISO-induction are hypoxia, ischemia, coronary insufficiency, alterations in metabolism, intracellular ca^{2+} overload, and oxidative stress [3] Spontaneous Autooxidation of catecholamine has adverse cytotoxic effects. The oxidized products have the ability to interact with the sulfhydryl groups of proteins resulting in the formation of free radicals. Administration of ISO accompanied by hyperglycaemia, hyperlipidaemia, and an increase in cardiac marker enzymes such as creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST), and alanine transaminase (ALT) in serum.
Phenolic compounds form a substantial part of plant foods and a vital role in human health. Most of these phenolic compounds are antioxidants, which may protect against CVD [4]. Phenolic acids have received much attention because of their role in the prevention of many human diseases, particularly atherosclerosis and cancer due to their antioxidant properties.[5] *A. conyzoides L.* (Myo-inositol hexaphosphate, IP_6) is widely found in cereals, nuts,

legumes, oil seeds, pollen, and spores. However, recently, *A. conyzoides L.* has been reported to be an antioxidant [6] anticarcinogenic [7], and hypoglycaemic or hypolipidemic effects. A previous scientific report has shown that *A. conyzoides L.* inhibits oxidation of low-density lipoprotein *in vitro* and might therefore protect against CVD. *Ageratum conyzoides* (AC) is unique for its antioxidant activity and protective role to tissues.[8] However this property is yet to be demonstrated in animals administered toxic alcohol concentrations.A detailed study is necessary to know, whether the antioxidant activity of *A. conyzoides L.* plays any role in the lipid peroxide–mediated cardiac damage in MI. In this context, an attempt has been made to determine the protective effect of *A. conyzoides L.* in ISO-induced cardiac damage with reference to cardiac enzymes and other biochemical parameters.

Materials and Methods

Experimental animals and diet

All the experiments were performed with healthy male Albino Wistar rats (Rattus norvegicus) weighing 170-200 g obtained from the IAEC, Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu, India. They were housed in polypropylene cages (47 cm × 34 cm × 20 cm) (4 rats per cage) lined with husk, renewed every 24 hours under a 12:12-hour light-dark cycle at around 22ºC and had free access to tap water and food. The rats were fed on a standard pelleted diet (Pranav Agro Industries, Maharashtra, India). The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India, and approved by the Institutional Animal Ethics Committee, Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu, India (Approval No. KAHE/IAEC/2021/11-09/009).

Drug and chemicals

A. conyzoides L., isoproterenol hydrochloride, butylated hydroxyl toluene, nitro blue tetrazolium, phenazine methosulfate, glutathione, nicotinamide adenine dinucleotide, and 1-chloro-2, 4-dinitrobenzene were purchased from Sigma Chemical, St Louis, MO, USA. All the other chemicals used in this study were of analytical grade.

Induction of experimental myocardial infarction

Isoproterenol (85 mg/kg) was dissolved in normal saline and subcutaneously injected into rats at intervals of 24 h for two consecutive days [4]. ISO-induced MI was confirmed by elevated activities of serum creatine kinase, CK–MB, and LDH in rats.

Study design

In this experiment, a total of 36 rats, were grouped into six each. The six groups were as follows: Group I: Normal control rats; Group II, and III: Normal rats treated with *A. conyzoides* L. (100 and 200 mg/kg respectively); Group IV: ISO (85 mg/kg) control rats, Group V and VI: Rats pre-treated with *A. conyzoides* L. (100 and 200 mg/kg respectively) and then subcutaneously injected with ISO. *A. conyzoides* L. was dissolved in water and administered to rats orally using an intragastric tube daily for a period of 56 days. At the end of the experimental period, after 12 h of the second ISO injection, all the rats were sacrificed by cervical decapitation. Blood was collected in two different tubes, i.e., one with anticoagulant for the separation of plasma and another without anticoagulant for the serum. Plasma and serum were separated by centrifugation and used for various biochemical estimations.

Processing of heart tissue

The heart tissue was excised immediately from the animals, washed off the blood with ice-chilled physiological saline, and stored for further biochemical estimation. A known weight (200 mg) of the heart tissue was homogenized in 5 ml of 0.1 M Tris-HCl (PH-7.4) buffer solution. The homogenate was centrifuged at 3000 rpm for 5 min.[10] The supernatant was used for the estimation of various biochemical parameters.

Biochemical assays

Estimation of cTnT and assay of CK-MB

The levels of cTnT in serum were estimated using a standard kit by electrochemiluminescence immunoassay (Catalogue no. 12017423, Roche Diagnostics, Switzerland). CK-MB activity was assayed in serum using a commercial kit (Product no. 11207001) obtained from Agappe Diagnostics, Kerala, India.

Separation and quantification of LDH-isoenzymes

LDH-isoenzymes were separated by agarose gel electrophoresis (Mckenzie and Henderson, 1983). Agarose gel (1%) was prepared and applied immediately to the glass slide. After the gel sets properly,10 ml of serum samples were applied into wells. After the run, the gels were removed and stained by the following method. The staining solution contained 1.0 ml of 1 M lithium lactate, 1.0 ml of 1 M sodium chloride, 1.0 ml of 5 mM magnesium chloride, 2.5 ml of 0.1% nitroblue tetrazolium, 0.25 ml of 0.1% phenazine methosulphate, 2.5 ml of 0.5 M phosphate buffer (pH 7.5) and 10 mg of NAD in a total volume of 10 ml. The gels were incubated with the staining solution at 37 ◦C in the dark for a suitable period. The gels were washed with acetic acid (7.5%) and preserved in 5% acetic acid. Finally, the gels were quantified using a Sebia, scanning densitometer, France. The activity of CK was assayed in serum and the heart tissue by the method of Okinawa *et al*. [14]. About 0.75 ml of double distilled water, 0.05 ml of sample, 0.1 ml of ATP, 0.1 ml of magnesium-cystine reagent, and 0.1 ml of creatine (240 mM) containing tubes were incubated at 37 ◦C for 20 min. The tubes were centrifuged and the supernatant was used for the estimation of phosphorus by Fiske and Subbarow [15] method. About 1.0 ml of the supernatant was made up to 4.0 ml with distilled water and added 1.0 ml of 2.5% ammonium molybdate. This was incubated at room temperature for 10 min and 0.4 ml of ANSA was added. The color developed was read spectrophotometric ally at 640 nm after 20 min.

Serum iron content was estimated by the method of Ramsay [12]. Equal volumes of serum, 0.1 M sodium sulphite, and dipyridyl reagent were mixed in glass stoppered centrifuge tubes. The tubes were heated in a boiling water bath for 5 min. The contents were cooled and 12.0 ml of chloroform was added to each tube. The tubes were stoppered and mixed vigorously for 30 s and centrifuged for 5 min at 1000 rpm. The color intensity was measured at 520 nm.

The plasma iron binding capacity was determined by this method of Ramsay [12]. A 4.0 ml of ferric chloride was

added to 2.0 ml of plasma. After standing for 5 min, 400 mg of magnesium carbonate was added and mixed well. The mixture was incubated for one hour at room temperature with frequent shaking. The contents were centrifuged and 4.0 ml of supernatant was taken for the estimation of iron.

Statistical Analysis: Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using the SPSS software package, version 9.05. P values <0.05 were considered significant, and all the results were expressed as mean ± S.D for 6 rats in each group.

Results

Table 1: Effect of *A. conyzoides L.* on the levels of cardiac troponin T (cTnT) and the activity of creatine kinase-MB (CK-MB) in serum in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

Groups	CK-MB (IU/L)	CTnT (ng/ml)
Normal control	182.1±10.08[a]	94.4±5.0[a]
Normal + *A. conyzoides L.* (100 mg/kg)	183.4±13.50[a]	93.2±4.7[a]
Normal + *A. conyzoides L.* 200 mg/kg)	179.0±10.01[a]	95.4±5.7[a]
ISO (85 mg/kg) control	392.3±20.92[b]	206.7±10.0[b]
A. conyzoides L. (100 mg/kg) + ISO	266.0±17.20[c]	140.3±9.6[c]
A. conyzoides L. (200 mg/kg) + ISO	222.1±18.30[d]	105.0±9.1[d]

Each value is mean±S.D. for six rats in each group. values not sharing a common letter (a–d) differ significantly from each other (p< 0.05, DMRT).

Table 1 shows the levels of serum cTnT and the activity of serum CK-MB in normal and experimental rats. Rats treated with ISO showed a significant (P < 0.05) increase in the levels of cTnT and the activity of CK-MB in serum when compared to normal control rats. Pre-treatment with *A. conyzoides L.* (25 and 50 mg/kg, respectively) daily for a period of 56 days significantly (P < 0.05) decreased the levels of cTnT and the activity of CK-MB in serum in ISO-induced rats.

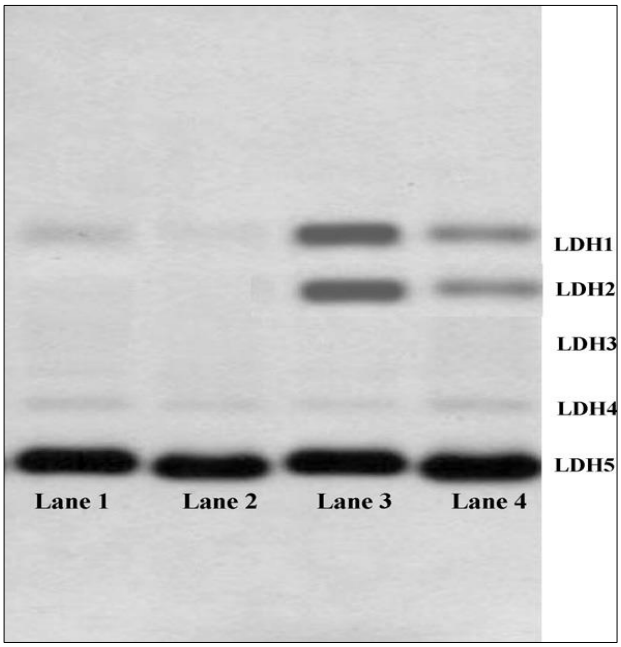

Fig 1: Effect of *A. conyzoides L.* on lactate dehydrogenase (LDH)-isoenzymes in serum of normal and ISO-induced rats. Lane 1: normal control; Lane 2: normal + *A. conyzoides L.* (200 mg/kg); Lane 3: ISO-control (85 mg/kg) rat; Lane 4: *A. conyzoides L.* (200 mg/kg) + ISO. Lane 1-normal; Lane 2-normal + *A. conyzoides L.* (100mg/kg); Lane 3-ISO+control Lane 4-*A. conyzoides L.* (100mg/kg) +ISO

Agarose gel electrophoretic separation of serum LDH-isoenzyme patterns. ISO-induction caused an increase in the intensity of bands of LDH-isoenzyme. Predominantly LDH1 and LDH2 when compared to normal control rats. The highest effective dose of *A. conyzoides L.*Agarose gel electrophoretic separation of serum LDH-isoenzyme patterns is shown in Fig 1. ISO-induction caused an increase in the intensity of bands of LDH-isoenzyme. Predominantly LDH1 and LDH2 when compared to normal control rats. The highest effective dose of *A. conyzoides L.* (100 mg/kg) was only taken for the study of the LDH-isoenzyme pattern. *A. conyzoides* L. pre-treatment (50 mg/kg) showed a decrease in the intensity of LDH1 and LDH2 bands.

Table 2: Effect of *A. conyzoides L.* on the activities of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST), and alanine transaminase (ALT) in serum in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

Groups	CK (IU/L)	LDH (IU/L)	AST (IU/L)	ALT (IU/L)
Normal control	222.38±12.88[a]	112.80 ±7.48[a]	31.60±1.98[a]	21.29±1.28[a]
Normal + *A. conyzoides L.* (100 mg/kg)	218.92±16.06[a]	111.28 ±8.30[a]	30.88±2.33[a]	19.18±1.38[a]
Normal + *A. conyzoides L.* 200 mg/kg)	218.38±12.45[a]	110.02 ±7.38[a]	30.95±2.01[a]	19.01±1.15[a]
ISO (85 mg/kg) control	428.27±23.01[b]	180.52±12.67[b]	60.96±3.24[b]	34.08±2.06[b]
A. conyzoides L. (100 mg/kg) + ISO	307.13±21.56[c]	135.62 ±9.81[c]	41.70±2.90[c]	24.36±1.92[c]
A. conyzoides L. (200 mg/kg) + ISO	254.78±17.54[d]	124.71 ±7.32[d]	35.40±2.66[d]	21.97±1.48[d]

Each value is mean±S.D. for six rats in each group.
Values not sharing a common superscript (a–d) differ significantly from each other ($P < 0.05$, DMRT).

Table 3: Effect of *A. conyzoides L.* on the activities of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST), and alanine transaminase (ALT) in the heart in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats

Groups	CK	LDH	AST	ALT
Normal control	19.01±1.07[a]	95.98±5.88[a]	46.01±2.75[a]	20.11±1.13[a]
Normal + *A. conyzoides L.* (100 mg/kg)	17.82±1.28[a]	96.67±1.12[a]	45.48±2.20[a]	19.83±1.28[a]
Normal + *A. conyzoides L.* 200 mg/kg)	18.06±1.02[a]	96.31±4.01[a]	45.48±1.87[a]	19.97±1.19[a]
ISO (85 mg/kg) control	10.11±.15[b]	59.98±4.11[b]	22.58±1.30[b]	10.81±0.12[b]
A. conyzoides L. (100 mg/kg) + ISO	13.79±1.02[c]	80.915±.79[c]	32.01±2.31[c]	15.91±1.08[c]
Normal + *A. conyzoides* (200 mg/kg) + ISO *L.* 200 mg/kg)	15.31±.02[d]	88.43±4.15[d]	40.36±2.42[d]	16.45±1.03[d]

Each value is mean±S.D. for six rats in each group. Values not sharing a common superscript (a–d) differ significantly from each other ($p< 0.05$, DMRT). CK activity: nmol of phosphorus liberated/min/mg protein. LDH, AST, and ALT activity: nmol of pyruvate liberated/min/mg protein.

Tables 2 and 3 represent the effect of *A. conyzoides L.* on the activities of serum and the heart CK, LDH, AST, and ALT in normal and ISO-induced rats. Rats induced with ISO, showed a significant ($p< 0.05$) increase in the activities of these enzymes in serum and a decrease in the heart. Pre-treatment with *A. conyzoides L.* (25 and 50 mg/kg, respectively) significantly ($p<0.05$) minimized the alterations in the activities of these enzymes in ISO-induced rats.

Table 4: Effect of *A. conyzoides L.* on the levels of blood glucose, plasma total proteins, and albumin/globulin (A/G) ratio in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats

Groups	Blood glucose (mg/dl)	Plasma total proteins (g/dl)	A/G ratio
Normal + *A. conyzoides L.* (100 mg/kg)	74.69 ±5.20[a]	6.71 ±0.32[a]	1.37±0.07[a]
Normal + *A. conyzoides L.* 200 mg/kg)	73.98 ± 3.83[a]	6.74 ±0.27[a]	1.38±0.07[a]
ISO (85 mg/kg) control	72.96 ±5.65[a]	6.60 ±.35[a]	1.38±0.06[a]
A. conyzoides L. (85 mg/kg) + ISO	146.63±8.13[b]	4.76 ±0.30[b]	0.67±0.04[b]
A. conyzoides L. (100 mg/kg) + ISO	107.31±6.36[c]	5.80 ±0.41[c]	1.11±0.08[c]
Normal + *A. conyzoides L.* (200 mg/kg)	86.40 ±4.45[d]	6.26 ±0.27 [d]	1.23±0.07[d]

Value is mean±S.D. for six rats in each group. Values not sharing a common superscript (a–d) differ significantly from each other ($p< 0.05$, DMRT).

Table 4 depicts the levels of blood glucose, plasma total protein, and A/G ratio in normal and ISO-induced rats. In ISO-induced rats, the levels of blood glucose were increased significantly ($P < 0.05$) and the levels of plasma total protein and A/G ratio were decreased. Oral pre-treatment with *A. conyzoides* L. (25 and 50 mg/kg, respectively) in ISO-induced rats significantly decreased the levels of blood glucose and increased the levels of plasma total protein and A/G ratio in ISO-induced rats.

Table 5: Effect of *A. conyzoides* L. on the levels of serum uric acid, serum iron, and plasma iron binding capacity in normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats

Groups	Serum uric acid (mg/dl)	Serum iron (µg/dl)	Plasma iron binding capacity (µg/dl)
Normal control	2.32±0.12[a]	45.21±2.87[a]	40.53±2.52[a]
Normal + *A. conyzoides L.* (100 mg/kg)	2.23±0.15[a]	44.00±3.19[a]	40.08±2.18[a]
Normal + *A. conyzoides L.* (200 mg/kg)	2.18±0.12[a]	45.20±2.05[a]	41.20±2.21[a]
ISO (85 mg/kg) control	4.57±0.18[b]	83.81±5.12[b]	26.45±1.64[b]
A. conyzoides L. (100 mg/kg) + ISO	2.87±0.15[c]	63.56±4.65[c]	32.54±2.37[c]
A. conyzoides L. (200 mg/kg) + ISO	2.57±0.14[d]	36.0 ±3.75[d]	36.27±2.66[d]

Each Value is mean±S.D. for six rats in each group.
Values not sharing a common superscript (a–d) differ significantly with each other ($p<0.05$, DMRT).

Table 5 shows the levels of serum uric acid, iron, and plasma iron binding capacity in normal and ISO-induced rats. The levels of uric acid and serum iron were increased significantly with a subsequent decrease in the levels of plasma iron binding capacity in ISO-induced rats when compared with normal control rats. *A. conyzoides L.* (100 and 200 mg/kg, respectively) pre-treatment with ISO-induced rats significantly minimized these alterations.

For all the parameters studied pre-treatment with *A. conyzoides L.* 100 mg/kg showed a better effect than *A. conyzoides L.* 200 mg/kg to ISO-induced rats. *A. conyzoides L.* treatment to normal rats did not show any significant effect.

Discussion

The oxidation of hydroxyl groups in catecholamines leading to the conversion into quinones and the subsequent formation of adrenochromes most probably account for the hazardous effects of catecholamines. During this reaction, highly toxic oxygen-derived free radicals are generated which are detrimental to extra and intracellular enzymes and proteins. Adrenochrome and other oxidation metabolites of catecholamines can cause cell necrosis and contractile failure in the rat's heart which have reported that autooxidation of catecholamines result in the generation of highly cytotoxic free radicals. Furthermore, free radicals could initiate the A supramaximal dose of ISO (85mg/kg) can elicit excessive stress in the myocardium resulting in myocardial dysfunction and elevation in lipid peroxidation products. Increased levels of troponin predict the risk of both cardiac death and subsequent infarction. Our results are reliable to a previous report by Acikel *et al*. [17] CK-MB isoenzyme activity is useful not only as an indicator of early diagnosis of MI but any type of myocardial injury. Pre-treatment with *A. conyzoides* L. for a period of 56 days showed declined levels of cTnT and CK-MB in serum of ISO-induced rats due to a potential reduction of the degree of damage in the myocardium. The increased activity of serum CK-MB observed in ISO-induced rats might be due to cardiac damage induced by ISO [18].

Lactate dehydrogenase is a cytosolic enzyme, present in all the tissues involved in glycolysis and exists in five different isoforms designated as LDH1–LDH5, in which LDH1 and LDH2 are predominantly present in the myocardium. Hence, detection of elevated concentrations of this enzyme released into the bloodstream from the damaged tissue has become a definitive diagnostic and prognostic criterion for various diseases and disorders and a study of its isoenzymes has found importance in the location of tissue damage [19].

In the present study, we have observed an increase in the intensity of LDH1 and LDH2-bands in ISO-induced rats, which is supported by the previous findings of Levinson and Hobbs [20]. Voet and Donald [21] have reported that the release of the cardiac-specific isoenzymes LDH1 and LDH2 into the circulation might be due to the necrosis induced by ISO. *A. conyzoides* L. pre-treatment reduced the ISO-induced activity of CK-MB and the intensity of LDH1 and LDH2-bands and densitometric scan peaks. This could be due to the protective effect of *A. conyzoides L.* in the myocardium thereby preventing the leakage of CK-MB and LDH.

Serum CK, AST, ALT, and LDH are well-known markers of MI. During ISO treatment myocardial cells are damaged due to deficient oxygen supply or glucose, the cardiac membrane becomes permeable or may rupture, which results in leakage of enzymes. These enzymes enter the bloodstream thus increasing their concentration in the serum [22]. Activities of these enzymes in serum decreased in *A. conyzoides* L. pre-treated ISO-induced group probably due to the protective effect of *A. conyzoides* L. on the myocardium, which had reduced the extent of myocardial damage induced by ISO and thereby restricting the leakage of these enzymes from the myocardium.

Glycemia is a major risk factor for heart diseases [23]. Stress hyperglycaemia induced by ISO has deleterious effects on the myocardium. The serum glucose levels are comparatively higher in ISO-induced rats than in the A. conyzoides treated rats. An increase in the levels of blood glucose in ISO-induced rats is due to the enhanced glycogen breakdown and less utilization by the peripheral tissues in MI in rats [24]. Pre-treatment with *A. conyzoides L.* significantly decreased the levels of blood glucose in ISO-induced rats. An *in vivo* study has demonstrated that *A. conyzoides L.* elicits significant amelioration of hyperglycaemia in streptozotocin-induced diabetic rats [25].

A decrease in the levels of plasma total proteins and the A/G ratio observed by us in ISO-induced rats could be due to excessive concentration of free radical production by ISO administration. Pre-treatment with *A. conyzoides L.* significantly increased the levels of plasma total proteins and the A/G ratio [26]. This could be due to the ability of *A. conyzoides L.* to scavenge free radicals and inhibit lipid peroxidation.

An increase in serum uric acid levels indicates the presence of MI. During hypoxia in the myocardium, ATP depletion occurs, which leads to the accumulation of hypoxanthine. Xanthine oxidase catalyzes the conversion of hypoxanthine to xanthine, uric acid, and superoxide. The dehydrogenase to oxidase conversion occurs in ischemic or hypoxic tissue [27]. This could be the reason for the elevated levels of serum uric acid in ISO-induced rats. Pre-treatment with *A. conyzoides* L. significantly reduced the levels of serum uric acid in ISO-induced rats.

Prominent levels of serum iron and decreased plasma iron binding capacity were observed in ISO-induced rats. Heme iron is directly related and total iron-binding capacity is inversely related to the risk of MI [28]. During ischemia, free iron is unconfined from heme-dependent proteins like haemoglobin and myoglobin and decreases the iron binding capacity and thus increasing prostaglandin metabolism and *in vivo* lipid peroxidation. Oral pre-treatment with *A. conyzoides L.* to ISO-induced rats decreased the levels of serum iron and increased the plasma iron binding capacity. This effect might be due to the free radical scavenging and antioxidant property of *A. conyzoides L*. Antioxidant activity of *A. conyzoides L.* is due to its chelating ability of iron [27]. Moreover, the increased plasma iron binding could have prevented haemolysis. and iron-catalysed lipid peroxidation.

Conclusion

In conclusion, *A. conyzoides L*, secluded the myocardium against ISO-induced cardiac damage by maintaining the cardiac marker and other biochemical parameters by scavenging free radicals, and their antioxidant effect. The results of our study show that *A. conyzoides L.* is an effective and safe antioxidant in animal models. The results of the present investigation may generate a renewed interest in the use of *A. conyzoides L.* for MI.

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