



Research Article

EVALUATION OF CARDIOPROTECTIVE ACTIVITY OF FULVIC ACID AGAINST ISOPROTERENOL INDUCED OXIDATIVE DAMAGE IN RAT MYOCARDIUM

T. S. Shikalgar *, N. S. Naikwade

Department of Pharmacology, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India

*Corresponding Author Email: tabbu_shikalgar@rediffmail.com

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ABSTRACT

The aim of study was to evaluate cardio protective activity of fulvic acid in Isoprenaline induced cardiac toxicity. In current investigation, female/male wistar rats were divided into five groups, normal, control, Fulvic acid 100mg/kg, Fulvic acid 200mg/kg, and Fulvic acid 300 mg/kg. Isoprenaline 85 mg/kg was administered on 29th and 30th day during study period to all except normal group. Fulvic acid was administered to respective groups once daily for total 30 days, on the last day of study, the animals were anesthetized to record ECG and BP (by cannulating carotid artery), blood was collected from carotid artery and SGOT, LDH And CK-MB were estimated, Animals were sacrificed to isolate heart and preparation of tissue homogenate. The antioxidant status is analyzed by measuring MDA content, SOD CAT, GSH activity. The tissue sample is also preserved for histological studies. Isoprenaline causes cardiac damage which was manifested by alteration in serum cardiac markers, antioxidant markers, ECG and hemodynamic and histological changes. These alterations were restored due to treatment with fulvic acid 300mg/kg. With data obtained in study it have been concluded that fulvic acid treatment for 4 weeks protect the heart of rat from cardiotoxicity as a result of isoprenaline administration.

Key words: Cardio protective, Isoprenaline, fulvic acid, Antioxidant, cardiotoxicity

INTRODUCTION

Of the 56.4 million deaths worldwide in 2015, more than half (54%) were due to the top 10 causes. Ischemic heart disease and stroke are the world's biggest killers, accounting for a combined 15 million deaths in 2015. These diseases have remained the leading causes of death globally in the last 15 years¹. Cardiovascular disease are leading cause of death in 21st century in both developed and developing countries. This burden will increase to 25 million people by 2030 mainly from heart diseases such as myocardial infarction (MI) and stroke².

Isoproterenol is a synthetic β -adrenoreceptor agonist that its subcutaneous injection has been reported to induce myocardial infarction in rats by many researchers³. Isoprenaline produces irreversible cellular damage and ultimately infarct-like necrosis to cardiomyocytes^{4,5}.

Amongst various mechanisms proposed to explain isoproterenol induced cardiac damage, generation of highly cytotoxic free radicals through auto-oxidation of catecholamines has been implicated as one of the important causative factor⁶. The acute phase of myocardial necrosis and dysfunction induced by isoproterenol mimics changes in blood pressure, heart rate, electrocardiogram (ECG), and left ventricular dysfunction similar to that occurs in patients with myocardial infarction. The rat model of isoproterenol induced myocardial infarction offers a reliable non-invasive technique for studying the effects of various potential cardioprotective agents⁷.

Isoproterenol [1-(3, 4-dihydroxyphenyl)-2-isopropylamino ethanol hydrochloride] (ISO), a synthetic catecholamine and β -adrenergic agonist, causes severe oxidative stress in the myocardium, resulting in infarct-like necrosis of the heart muscle.

It is also known to generate free radicals and to stimulate lipid peroxidation, which may be a causative factor for irreversible damage to the myocardial membrane⁸.

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Now a day, long term prevention of CVD are associated with healthy diet rich in fresh fruits, vegetables or plants rich in antioxidants. Hence tremendous increase in research regarding finding a natural product which is probably superior in terms of safety and efficacy in contrast to different synthetic analogues⁹.

Fulvic Acid is stated as a natural antioxidant by many nutritional specialists. The various basis have been proposed which include that Fulvic Acids greatly enhance the bioavailability of important trace minerals. It is also claimed to modify the damage or toxic compounds such as heavy metals and free radicals, Enhance the permeability for digestive, circulatory, and cell membranes. Fulvic acid minerals are thought, by leading natural health experts, to be one of the most important "missing links" in the modern food chain. Medical and agricultural research continues to conclusively point to one fact: fulvic acid minerals either directly or indirectly may hold the keys and solutions to many of the world's health problems. Fulvic mineral complexes are the world's finest electrolyte, which improves energy function, increases assimilation, stimulates metabolism, restores electrochemical balance, reduces high blood pressure, enhances nutrients, and helps rebuild the immune system. Fulvic acid is yet

not well known or understood by medical community regarding its health benefits to heart. It has extremely complex nature. Various claims of encouraging health benefits by the public have been simply remarkable. Many of these health assertions have also shown that they could be disease preventative in nature and may dramatically increase longevity. Yet until now, fulvic acid has been entirely overlooked by the majority of alternative health concerns as well¹⁰.

Fulvic acid is one of the ingredients present in humus and has been well documented for having antithyroid and Immunomodulating activity¹¹. Shilajit has been reported for various biological activities which is due to high fulvic acid constituents¹². Humic substances also have been reported for having anti-inflammatory as well as immunomodulatory activity and it is safe upto 1.8 g/day¹³. In China, humic and fulvic acids had been used in hospitals and among the general population for the treating of a wide range of diseases with success and where it is referred to as 'gold medicine' or 'Wu Jin San'¹⁴.

Till now there is no or little available data on the cardioprotective properties of fulvic acid further its recommendations by nutritionalist and some pharmacological properties of interest motivate to investigate its approaching cardioprotective ability in isoprenaline induced cardiac stress.

MATERIALS AND METHODS

Drugs and Chemicals

Fulvic acid was purchased from Mana life (Wanek Medical Center, Greensboro NC). Isoprenaline was procured from Research-lab Fine Chem Industries Mumbai. Urethane (Himedia India), DTNB, heparin, n-butanol, Pyridine, Sodium dodecyl sulphate, Conc. HCL, Hydrogen peroxide, Ammonium acetate, Potassium hydrogen phosphate, formalin, Potassium dihydrogen phosphate, Trichloro Acetic Acid (all from Research-lab Fine Chem Industries Mumbai), Pyrogallol (Sigma Aldrich, Pvt Ltd, Bangalore) Thiobarbituric acid (Loba chemicals (Mumbai)) All chemicals were of analytical grade, the diagnostic kits from Corals clinical systems were used for serum SGOT, LDH and CK-MB analysis.

Experimental Animals

Wistar albino rats of either sex weighing 200 ± 30 gm were randomly selected from animal house of Appassheb Birnale College of pharmacy Sangli. They were housed in air conditioned room in polypropylene cages lined with rice husk. The animals were fed on conventional diets and had free access to water. They were maintained under standard conditions of humidity (45%-55%), temperature (25 ± 2 °C) and light/dark cycle (12 h: 12 h). The experiments were carried after approval and clearance from Institutional animal ethics committee constituted in accordance of CPCSEA, India (Protocol no- ABCP/IAEC/ 03/2012-13)

Preparation of drug solution

Fulvic acid is dissolved in chlorine free water; Isoprenaline is prepared in normal saline.

Experimental Design and protocol

After a week-long acclimation A total no. of 30 Animals were randomly divided into five groups contain six animals in each.

Group I-served as normal, received water 2 ml/Kg of body weight p.o. daily for 30 days

Group II-Control animal received with water 2 ml/Kg of body weight p.o. daily + Isoprenaline 85 mg/kg s.c, on 29th,30thday.
Group III- received fulvic acid 100mg/kg up to 30 days daily + Isoprenaline 85mg/kg s.c, on 29th,30th day.
Group IV - received fulvic acid 200mg/kg up to 21 days+ Isoprenaline 85mg/kg s.c, on 29th,30th day.
Group V - received fulvic acid 300 mg/Kg up to 21 days + Isoprenaline 85mg/kg s.c, on 29th, 30thday.

During the experimental period, the rats body weights were recorded regularly and the doses were modulated accordingly. All animals were observed for whole study period for appearance, behavior, occurrence of necrosis at the site of administration, and mortality. Before and after completion of experimental period body weights were recorded. Heart/body weight ratio was also calculated using formula heart weight/body weight X1000

Electrocardiogram

24 hr after last dose of Isoprenaline, animals were anesthetized by injecting urethane 1.25 mg/kg i.p. and then taken for ECG recording using lead II. This recording are done using digital, 4 channel data acquisition system Biopac MP35. Santa Barbara California USA. The ECG records include ST elevation, QT interval, and QRS complex duration.

Hemodynamic parameters

24 h after last dose of Isoprenaline, animals were anesthetized by injecting urethane 1.25 mg/kg i.p., The animals are dissected further and left carotid cannulation is performed using PE 50 cannula. The cannula is filled with heparinised saline (100 IU/ml) and connection is made to pressure transducer to measure the systolic BP, diastolic BP and heart rate parameters. This recording are done using digital, 4 channel data acquisition system Biopac MP35. Santa Barbara California USA.

Serum Parameters

Blood was collected from carotid cannulation in plane tubes. Serum was separated after centrifuging the samples at 3000 rpm for 20 min. The serum samples were analyzed for determination of levels of SGOT, CK-MB; LDH using standard kit according to manufactures instructions using semi auto analyser (Mispal Plus).

Antioxidant parameters

Animals were euthanized and hearts tissue were quickly dissected out and washed in ice cold phosphate buffer, dried on filter paper and quickly weighed. A 10% w/v tissue homogenate is prepared in ice cold 0.05 M phosphate buffer using tissue homogenizer. The chilled tissue homogenate was used for estimation of level of MDA, Glutathione, SOD activity, CAT activity, and total protein.

Measurement of lipid peroxidation.

The thiobarbituric acid reactive substance (malondialdehyde) was measured as a marker of lipid peroxidation by method of Okhawa. In a mixture of 0.4 ml of 10% tissue homogenate 1.5 ml 20% acetate buffer (pH 3.5) and 1.5 ml of 0.8 % TBA solution were added. The mixture was heated at 95° C for 60 min. the solution is then cooled to room temperature. After cooling, 5 ml of n-butanol-pyridine (15:1) was added. The mixture is vortexed thoroughly and allowed to stand until the organic and aqueous layers get separated. Further absorbance of organic layer was measured at 532 nm on UV/Visible spectrophotometer (Jasco V-550)¹⁵. The MDA level was calculated using molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

Measurement of Superoxide Dismutase

SOD activity was measured by determining the ability of sample to inhibit auto oxidation of pyrogallol by Improved Pyrogallol Autooxidation Method. One unit of SOD activity one unit of SOD activity is defined as amount of enzyme required to inhibit the rate of pyrogallol autooxidation by 50%. At pH 7.4, 50 μ L of sample solution was mixed with 2950 μ L of Tris-HCl buffer (0.05 M, pH 7.4, 37 °C) containing 1 mM Na₂EDTA and 50 μ L of pyrogallol (60 mM in 1 mM HCl, 37 °C) and then rapidly shaken by hand at 37 °C.

The absorbance was measured against the Tris-HCl buffer every 30 s for 5 min at 325nm using UV visible double beam spectrophotometer (Jasco V- 550) ¹⁶. The oxygen radical scavenging ability was calculated as

$$\{\Delta A (\text{control}) - \Delta A (\text{test})\} \Delta A (\text{control}) / T \times 100$$

Here, ΔA (control) is the increase in Absorbance at 325 nm of the mixture without the sample and ΔA (sample) is that for the mixture with the sample; T = 5 min ,

Measurement of Catalase activity

Catalase enzyme degrades hydrogen peroxide (H₂O₂) into oxygen and water. Ultraviolet absorption of H₂O₂ can be measured at 240 nm. In the presence of catalase, absorption decreases due to degradation of H₂O₂. 0.1 ml of tissue homogenate is mixed with 1.0 ml freshly prepared hydrogen peroxide and 1.9 ml phosphate buffer into cuvette. Blanks were similarly prepared without tissue homogenate. Absorption of test was measured at 240 nm against blank. On using UV visible double beam spectrophotometer (Jasco V- 550) for at least 3 min at 240 nm^{17, 18}. Activity of CAT was expressed in unit/mg of protein and calculated using molar extinction coefficient 43.6 M⁻¹ cm⁻¹

Measurement of glutathione activity

GSH reacts with Ellman's reagent (5, 5-dithio bis Nitrobenzoic acid or DTNB) to produce a chromophore Thio Nitrobenzoic acid (TNB) that give maximal absorbance at 412 nm. Absorbance value can give the estimation of enzyme value. 1 ml of 10% tissue homogenate is mixed with 1 ml 20% trichloroacetic acid (TCA) containing 1mM EDTA and mixture is centrifuged for 10 min at speed 1000 rpm. In 1 ml supernatant 0.5 ml DTNB solution and 3 ml Phosphate Buffer were added. In 0.2 ml of supernatant is added to new set of test tubes containing 1.8 ml of Ellmans reagent (0.1mM DTNB(5,5'-dithio bis-2-nitrobenzoic acid) prepared in 0.3 M Phosphate buffer containing sodium citrate). It was mixed well. Absorbance is measured the at 412 nm using UV visible double beam spectrophotometer (Jasco V- 550) against blank^{19,18}. The amount of glutathione is calculated using the molar extinction co-efficient 13600M⁻¹ cm⁻¹

Total protein

Total protein is estimated using method of lawry. 0.5 ml of tissue homogenate is mixed with 0.5 ml of 10% TCA and centrifuged for 10 min. Precipitate is dissolved in 0.1N NaOH. 0.1 ml of aliquot from above solution was mixed with 5 ml alkaline copper reagent and allowed to stand at room temperature for 10 min. 0.5 ml of Folin's phenol reagent was added and kept for 20 min to develop blue color. Absorbance was read at 640 nm. The protein concentration was determined by using standard curve which prepared by using standard sample of bovine serum albumin^{18, 20}.

Histology of heart

The heart tissues were excised and placed overnight in 10% buffered formalin. Dehydration and further impregnation is carried out in paraffin wax. Specimen were cut into thin sections and stained with hematoxyline and eosin. The sections were mounted by diestrene phthalate xylene. The slides were prepared by professional technically expert person. The heart sections were further assessed for infiltration of inflammatory cells, necrosis and other inflammatory changes. The slide micrograph was taken using Saglo soft microimaging software from Saglo research equipments Pvt ltd. India.

Statistical Analysis

The data was expressed as mean $\pm$ SEM. Statistical analysis was performed by one way analysis of variance followed by Sidak's multiple comparison test Using graph pad prism 6 software for windows (GraphPad software, San Diego CA, USA). P value < 0.05 was considered statistically significant.

RESULTS

Chronic administration of Fulvic acid in done daily in different doses, all animals from all experimental groups were survived with complete 4 weeks treatment. Change in Body weights and general appearance of all groups of animals were observed through experimental course. At the end of study, levels of cardiac biomarker enzymes and endogenous antioxidant were analyzed by methods described above. The results are additionally supported by finding related to histology and hemodynamic changes.

Heart weight to body weight ratio.

No significant differences in the body weights observed at the baseline time point or at the end of experimental period between the groups (Table 1), while the heart to body weight ratio of control differs significantly (P<0.01) as compared to normal. The rats pretreated with fulvic acid 300 mg/kg and 200 mg/kg produced significant decrease (P<0.05) in heart weight to body weight ratio. Whereas group treated with FA 100mg/kg produce nonsignificant decrease in heart weight to body weight ratio. All animals were survived till completion of experimental period (Table 1)

Electrocardiographic changes and Hemodynamic changes

Figure 1 (Graphs) and 2 (ECG) shows the effect of fulvic acid on electrocardiographic changes in different groups. The ISO control group showed significant increase in ST voltage, QT interval and shortens QRS duration as compared to normal. Treatment with Fulvic acid 300 mg/kg produced significant modification in ST voltage, QT interval and QRS segment. The Figure 2 (E) Also showed this alteration in deflections due to treatment with fulvic acid 300 mg/kg, which suggesting its cardioprotective efficacy. Even though figure no. 2 (C) (Fulvic acid 200 mg/kg) and 2 (D) (Fulvic acid 100 mg/kg) indicating mild changes in ST elevation due to treatment but the values are statistically less significant (p<0.05).

The ISO control group animals showed significant reduction in systolic BP (p< 0.01) and diastolic BP(p< 0.001) compared to normal group (Table 2). The treatment with fulvic acid 300 mg/kg, significantly (p<0.01) raise level of systolic and diastolic BP when compared to control. While other group treated with 200 mg/kg and 100 mg/kg also caused increase in value but showed non significant systolic and diastolic BP elevation.

Heart rate in control group is increased significantly ($p < 0.01$) (Table no.2) in contrast to normal group. The group treated with fulvic acid 100 mg/kg produced non significant decrease in heart rate, while group 200 mg/kg and 300mg/kg produced significant decrease ($p < 0.05$ and $p < 0.01$ respectively) in heart rate.

Evaluation of serum parameters

Figure 3 shows the effect of fulvic acid on cardiac markers, ISO Control group showed significant alteration ($p < 0.0001$) in level of serum SGOT, LDH, and CK-MB when compared to normal group. The oral treatment with fulvic Acid 200 mg/kg and 300 mg/kg significantly reduces elevated level of these marker enzymes. While fulvic acid 100 mg/Kg also significantly ($p < 0.05$) reduces level of cardiac markers but with comparatively less intense than fulvic Acid 200 mg/kg and 300 mg/kg treated groups. The group received 300 mg/kg on daily basis reduced the levels to considerable extent i.e. ($p < 0.0001$) statistically for CK-MB, and LDH and $P < 0.01$ for SGOT.

Evaluation of antioxidant parameters

Malondialdehyde (MDA)

There was increase in the heart tissue lipid peroxidation due to isoprenaline administration in control group which results in Significant ($p < 0.0001$) increase in Malondialdehyde levels (MDA). Groups treated with fulvic acid 300 mg/kg, 200 mg/kg, 100 mg/kg significantly reduced ($p < 0.0001$) this elevated level of MDA.

Glutathione Reductase

There was a significant decrease ($P < 0.0001$) in the glutathione levels in isoprenaline treated control group when compared to normal group. However treatment with fulvic acid 300 mg/kg, 200 mg/kg, significantly ($p < 0.001$, $p < 0.05$ respectively) enhance these levels (Table 3)

Superoxide Dismutase

There was significant reduction ($p < 0.0001$) in level of superoxide dismutase in ISO treatment. The non significant increase in SOD levels were found in groups treated with 100mg/kg. Whereas the group treated with 200 mg/kg and fulvic acid 300 mg/kg has shown significant increase ($p < 0.001$, $p < 0.0001$ respectively) in the level of SOD as compared to control. (Table 3)

Catalase

The control group showed significant decrease ($p < 0.001$), in level of catalase due to ISO treatment. However treatment with fulvic acid 300mg/kg significantly restore ($p < 0.01$) the level of enzyme while groups treated with 200mg/kg and 100 mg/kg showed non significant increase in level of catalase (Table 3).

Histological Findings

Figure 4 illustrates the histological findings in normal, control, and treated groups.

The histology of heart of normal animal showed absence of infiltration of inflammatory cells, and myocytolysis. The control animals due to Isoprenaline treatment showed cellular infiltration of inflammatory change as well as myocytolysis due to necrosis in most of regions and at certain level we have also noticed hyaline change, hyperemia in control slide.

The group treated with 100 mg/kg and 200 mg/kg showed less occurrence inflammatory changes and necrosis when observed visually. The Fulvic acid 300 mg/kg has reduced the myocytolysis and inflammatory cell infiltration, at most side normal cellular structure is preserve even though exposed to Isoprenaline.

Table 1: Effect of treatment of fulvic acid on Isoprenaline- induced changes in body weight, heart weight, Heart/Body weight ratio and %mortality

Groups	Body weight	Heart weight	Heart/Body weight ratio	Mortality %
Normal	208±1.95	0.712±0.0255	3.31±0.14	0
Control	215±0.428	0.808 ± 0.00792	3.78±0.0512**	0
Fulvic acid (100mg/kg)	225±3.97	0.753 ±0.0148	3.35±0.0844ns	0
Fulvic Acid (200mg/kg)	221±5.63	0.738±0.0197	3.34±0.0903*	0
Fulvic Acid (300mg/kg)	219±4.13	0.737 ± 0.0143	3.37±0.059*	0

Data are expressed as mean± SEM (n=6) and analyzed by one way ANNOVA followed by Sidak's multiple comparison test, * $P < 0.05$, ** $P < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared with positive control group, # $P < 0.05$, ## $P < 0.01$, ### $p < 0.001$, #### $p < 0.0001$ compared with Normal group

Table 2: Effect of fulvic acid on BP and Heart rate parameters

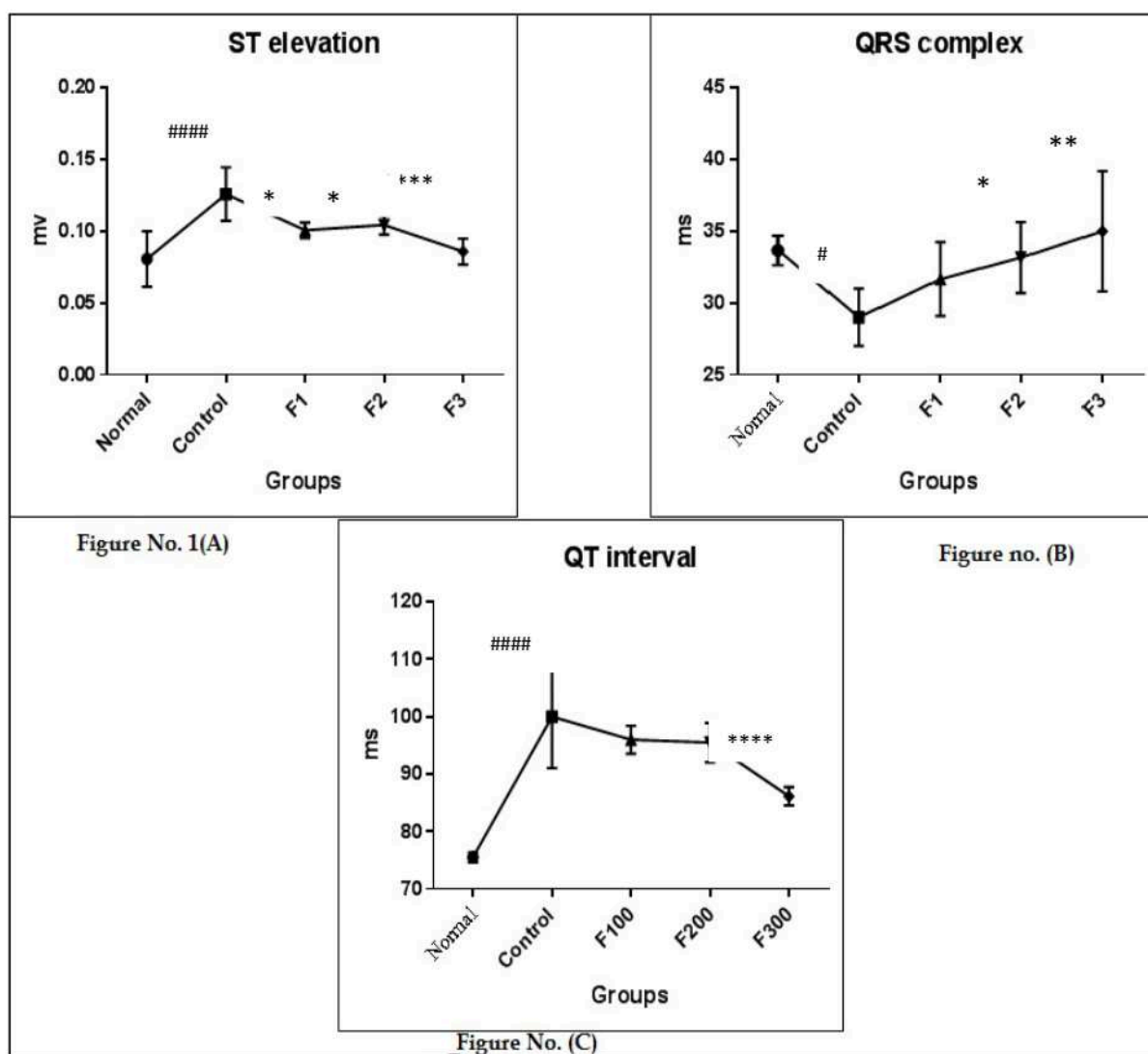
Groups	Systolic BP	Diastolic BP	Heart Rate
Normal	132±0.253	95.4±1.98	356±0.401
Control	124±2.46##	86.3 ±1.99###	366±3.57##
Fulvic acid 100mg/kg	127±2.21 ns	90.3±0.633 ns	358±3.88ns
Fulvic Acid 200mg/Kg	127±1.81 ns	91.9±0.663*	357±0.667*
Fulvic Acid 300mg/Kg	122±0.985**	93.1±1.05**	356±2.86*

Data are expressed as mean± SEM (n=6) and analyzed by one way ANNOVA followed by Sidak's multiple comparison test, * $P < 0.05$, ** $P < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared with positive control group# $P < 0.05$, ## $P < 0.01$, ### $p < 0.001$, #### $p < 0.0001$ compared with Normal group

Table 3: Effect of fulvic acid on tissue Malondialdehyde, Glutathione reductase, Catalase, Superoxide dismutase

Groups	Malondialdehyde(μ Mol MDA/g of tissue)	Glutathione (nMol/g of tissue)	Superoxide dismutase(units /mg of protein)	Catalase (units /mg of protein)
Normal	30.3 $\pm$ 1.22	36.5 $\pm$ 1.02	245 $\pm$ 14.2	34.9 $\pm$ 4.08
Control	77.9 $\pm$ 1.7****	17.7 $\pm$ 1.59****	130 $\pm$ 10.6****	20.1 $\pm$ 1.67***
Fulvic acid 100mg/kg	60.7 $\pm$ 2.6****	23.7 $\pm$ 1.2ns	137 $\pm$ 9.64ns	19.1 $\pm$ 2.19ns
Fulvic Acid 200mg/Kg	44.5 $\pm$ 0.836****	25.4 $\pm$ 1.26*	218 $\pm$ 4.59***	27.7 $\pm$ 0.615ns
Fulvic Acid 300mg/Kg	37.5 $\pm$ 1.86****	30.4 $\pm$ 0.584***	224 $\pm$ 19.7****	31.3 $\pm$ 0.721**

Data are expressed as mean $\pm$ SEM (n=6) and analyzed by one way ANNOVA followed by Sidak's multiple comparison test, *P < 0.05, **P < 0.01, ***p < 0.001, ****p < 0.0001 compared with positive control group # P < 0.05, ##P < 0.01, ###p < 0.001, ####p < 0.0001 compared with Normal group

**Figure 1: Graphical representation in ECG changes**

(A): ST elevation (mv), (B): QRS duration(ms), (C): QT interval(ms) occur in Normal, control, FA 100mg/kg, FA 200mg/kg, FA 300mg/kg groups

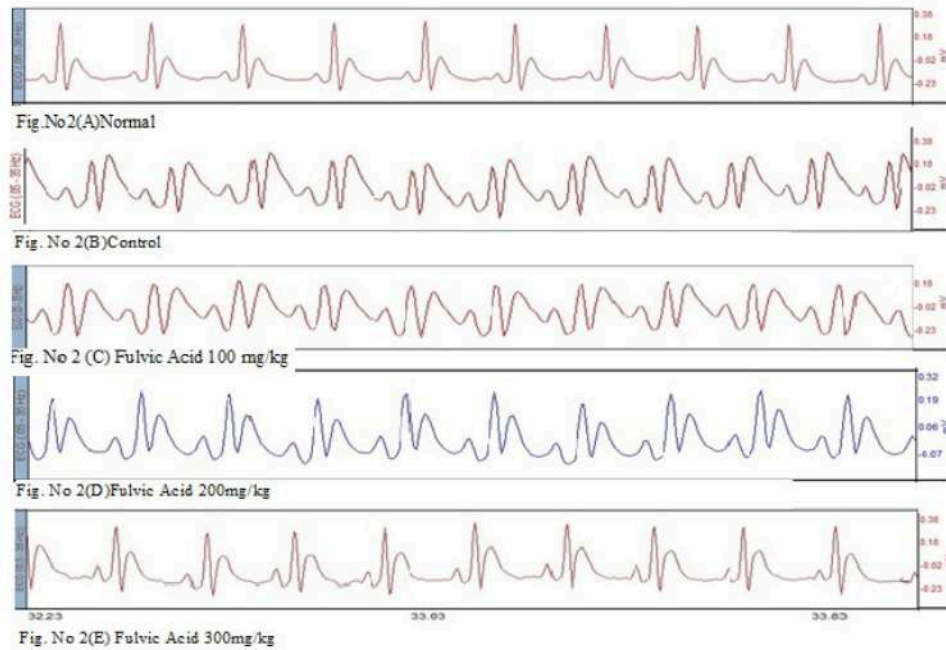


Figure 2: ECG changes in (A) Normal, (B) control (C) FA 100 mg/kg (D) FA 200 mg/kg (E) FA 300 mg/kg

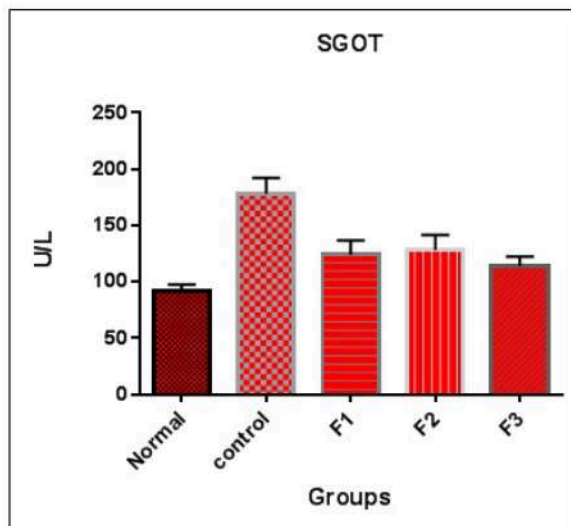


Figure No. 3(A)

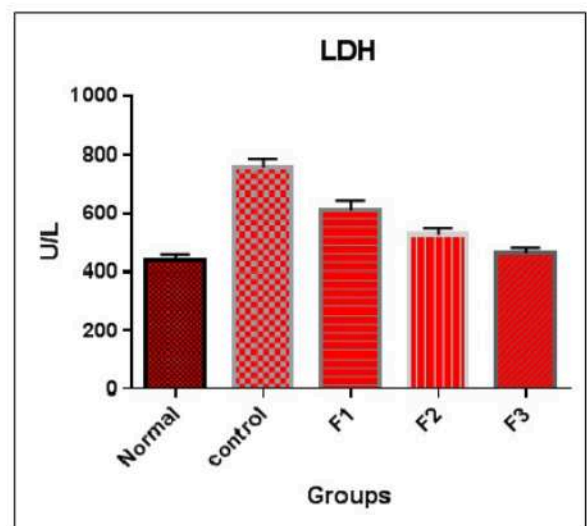


Figure no.3(B)

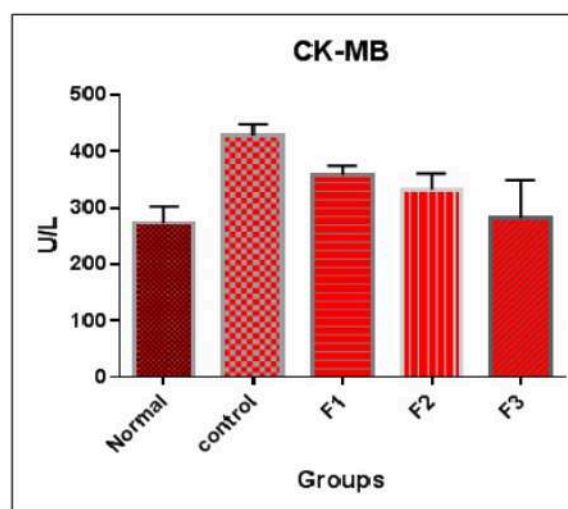


Figure no 3 (C)

Figure 3: Markers of myocardial damage (SGOT, CK-MB and LDH) in Normal, control, FA 100mg/kg, FA 200mg/kg, FA 300mg/kg

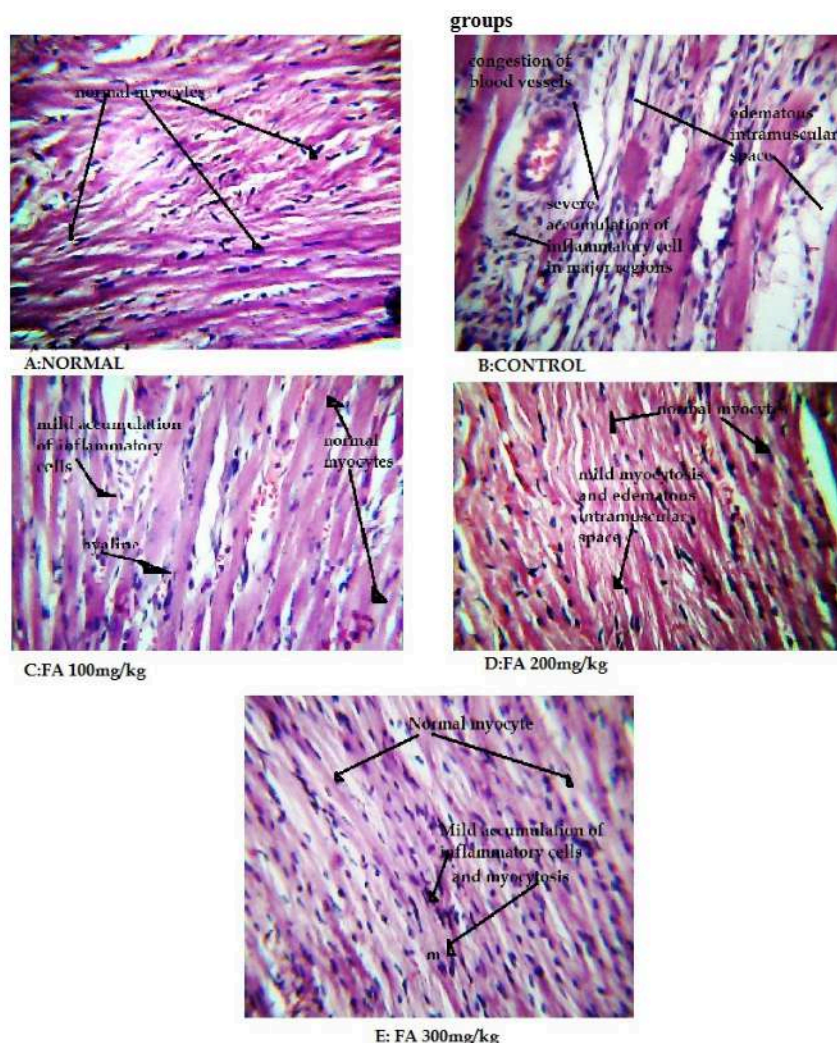


Figure 4: A light microscopic photographs of cardiac muscle tissue stained with H&E, original magnification of 100X. (A) (n) shows normal cardiac muscle structure, (B) shows ISO control cardiac muscle structure, (m) myocytolysis, hyaline change, necrosis, (C) shows cardiac muscle structure in group treated with Fulvic acid 100mg/kg, (D) shows cardiac muscle structure in group treated with 200 mg/kg, (E) shows cardiac muscle structure in group treated with 300 mg/kg.

DISCUSSION

The investigation was objected to evaluate the cardio protective effect fulvic acid regarding its efficacy to revert various biochemical, hemodynamic, electrocardiographic and histological alteration caused by Isoprenaline induced cardiac oxidative stress. Supramaximal doses of isoprenaline induced subendocardial myocardial ischemia, hypoxia, necrosis, and finally fibroblastic hyperplasia with decreased myocardial compliance and inhibition of diastolic and systolic function²².

Isoprenaline induces morphological and functional alterations in the heart leading to myocardial necrosis. It also produces excessive production of free radicals resulting from oxidative metabolism of catecholamines. Although cardiotoxicity occurs primarily via adrenoceptor activation, there is increasing evidence that it may also occur through oxidative mechanisms. It has been reported that excess catecholamines affect calcium transport mechanism primarily via oxidation reactions involving free radical mediated damage²³.

A subcutaneous injection of a high dose of isoproterenol have been reported to produce electrocardiographic, hemodynamic, and structural changes in the heart very similar to that occurs in

patients with myocardial infarction. Fulvic acid is a humic substance which has been described by many reports for having beneficial antioxidant activity. fulvic acid is natural products derived from the humidification process of plant materials. The fulvic used in current investigation is a peat derived which was reported and proposed to have variety of medical applications. Fulvic acid is called as miracle molecule in different literatures, it was considered as one of missing link of life²⁴.

A article by Prof. Dr. Renate Klocking and et.al summaries the different medical application of fulvic acid where they mentioned about its antiviral, bone generating capabilities²⁵. Furthermore its in-vitro antioxidant activity has been also reported²⁶. Isoprenaline was administered at dose 85 mg/kg s.c. for two consecutive days, which have been used and reported by many researchers for induction of lesion like myocardial infarction.

Isoproterenol, at such a dose is responsible for cellular damage muscle, alters membrane permeability, which bring about the loss of function and integrity of myocardial membrane as observed in clinical conditions such as angina, transient myocardial hypoxia, acute coronary insufficiency and sub-endocardial infarct²⁷. In present investigation animals heat weight to body weight ratio is determined. The animals which received only Isoprenaline have

shown increase in this ratio. The observed increase in the heart to body weight ratio due to isoprenaline in rats might be due to the increased water content, edematous intramuscular space and extensive necrosis of cardiac muscle fibers followed by the invasion of damaged tissues by the inflammatory cells²³. Further increase in protein content is also one of the possibility results in increased heart weight. These findings are in accordance with Md. Khalil et al²⁷.

ECG interpretation is one of the reliable methods to observe Isoprenaline induced cardiac changes which resembles much with Myocardial infarction. Many investigations done previously emphasis ECG studies to determine protective actions of different compounds.

After completion of experimental period, we have recorded ECG as described in part material and method. The control animals have shown elevation in ST segment. ST elevation is one of the significant indications used to diagnose acute myocardial ischemia in human too. The ECG finding also showed Reduction in QRS complex and increase in QT interval. These ECG findings indicate development of myocardial infarction. These ECG findings of ISO-MI are in accordance with earlier studies^{28,2}. Such abnormalities in the ISO-induced MI model might be due to ISO-induced generation of free radicals which further enhance oxidative stress. This Increase in oxidative stress causes loss of cell membrane function leading to elevation of ST segment, conduction disturbances and tachycardia²⁹. Further, it has been demonstrated that an increase in heart rate is responsible for increased oxygen consumption leading to accelerated myocardial necrosis.²⁸ Our finding also indicate increase in HR in ISO-control animals the increase in probably causes cardiac dysfunction and contractile failure in heart. The QRS complex shows total duration of ventricular depolarization. Alteration in its duration reveals unusual functioning in hearts activity. QT interval prolongation in ISO control group is related with vagal dysfunction also indicating arrhythmias, cardiac dysfunctioning and sudden cardiac collapse².

Cardiomyocyte damage was manifested by elevated levels SGOT, LDH, CK-MB, which reflects cardiac damage due to isoprenaline. The significant hike in serum levels of CK-MB, LDH & AST was occurred due to ISO-induced cardio toxic effect and destruction of cell membranes of myocardial cells. The role of these enzymes as diagnostic markers of myocardial tissue damage is well documented in different articles.

The quantity changes of these enzymes in the serum reveal the alteration in plasma membrane integrity or permeability and extend of damage due to isoprenaline. Pre-treatment with Fulvic acid 300 mg/kg resulted in significant reduction in cardiac markers in serum which indicate the protective action on FA on cardiac cells. FA probably reduces leakage of enzymes and maintains membrane integrity.

The other groups, Fulvic acid 200 mg/kg and Fulvic acid 100mg/kg also produce significant reduction but less than Fulvic acid 300 mg/kg. These effects of fulvic Acid on cardiac enzymes might be explained by their antioxidant effects which is probably responsible for its protective action on myocardium.

The hearts tissue homogenate is also analyzed for determining levels of different oxidative stress markers. LPO is a well-established mechanism of cellular injury and has been used as an indicator of oxidative stress that is responsible for the pathogenesis of MI.³⁰ The extend of LPO has been evaluated by estimating MDA content, In our investigation, ISO treatment resulted in a significant increase in the levels of MDA content a

products of lipid peroxidation in the heart tissue homogenate. The control animals who received Isoprenaline have shown raise in MDA content as a result of increase in lipid peroxidation as well as concomitant plunge in activity of antioxidant enzymes such as SOD, GSH and catalase. Antioxidants constitute the defence system that limit the toxicity associated with free radicals, and thus are expected to be consumed by enhanced radical reactions³¹. The oxidative stress may be exerted through quinine metabolites of ISO that react with oxygen to produce superoxide anions and other reactive oxygen species (ROS) that interfere with antioxidant enzymes³².

The presence of the endogenous antioxidant enzymatic defense is highly important for the neutralization of oxygen-free-radical-mediated tissue injury. SOD, catalase (CAT), are the primary free radical scavenging enzymes, which involved in the cellular defense against oxidative injury, as prevent conversion of oxygen (O₂) and hydrogen peroxide (H₂O₂) before their interaction to form the more reactive hydroxyl radical³³.

Fulvic acid pretreatment significantly reduces the levels of lipid peroxides in ISO-treated rats. The retreatment also resulted into increase in activities of antioxidant enzymes such as GSH, SOD and CAT. Which possibly indicate that reduction in generation of free radicals and their less utilization by antioxidant enzymes. Among the group treated the animals treated at dose FA 300mg/kg were significantly restore level of MDA, GSH, SOD, and CAT. Whereas the FA 100 mg also showed non significant modification in levels of these antioxidant markers, while dose group 200mg/kg bring back the levels moderately and produce less intense activity as compare to group treated with 300 mg/kg. Histopathological illustration is also important parameter to notify the destruction of myocardium. Fig no. 3 a represent part of normal animals slide, which represent normal architecture of cells which are intact, non edematous. The ISO-control animals have shown various destructive manifestations in the form of presence of inflammatory cell infiltration, edematous intramuscular space and myocytolysis. These changes are in line with the finding reported by Zhou R et al²², and Md. Ibrahim Khalil et al²⁷. The animals pretreated with fulvic acid 300 mg/kg retain normal morphology of cardiomyocytes with limited occurrence of inflammatory cell infiltration and myocytolysis. While other groups treated with fulvic acid 100 mg/kg and 200 mg/kg have shown moderate to mild cellular alteration and edematous space. These finding are again correlated with biochemical, hemodynamic and electrocardiographic modification by different doses of Fulvic acid which further assure its ability for being a potential cardioprotective agent.

CONCLUSION

The overall data obtained after hemodynamic, electrocardiographic, biochemical and histological investigation conclude that Fulvic acid can protect the heart cells integrity and maintain activity of various enzyme of heart like CKMB, LDH, SGOT, keep pace of antioxidant enzymes GSH, SOD, and CAT, reduce lipid peroxidation, restore Electrocardiographic alteration and prevent pathological alteration due to Isoprenaline.

These findings might be rational to understand the cardio protective effect of fulvic acid in isoprenaline induced myocardial injury, in which oxidative stress was a contributing factor in pathogenesis.

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