## Research Article

## PROTECTIVE EFFECT OF ELLAGIC ACID AGAINST LEAD INDUCED MYOCARDIAL TOXICITY

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**ABSTRACT**

Chronic exposure of lead is responsible for life threatening cardiac manifestations. Ellagic acid (EA), by virtue of its antioxidant potential, is responsible for cardio-protective activity. The present study was under taken to evaluate protective effect of EA against lead induced myocardial toxicity. Rats were treated with high (50 mg/kg, p.o.) and low (25 mg/kg, p.o.) dose of EA for 12 weeks. Apart from normal controls, all other groups were exposed to lead acetate (100 mg/litre) in drinking water for 12 weeks. Effects of different treatments were evaluated by changes in electrocardiographic parameters, serum biomarkers and tissue antioxidant levels and histological studies. Compared with the only lead exposed group both high and low dose of EA exhibited a significant decrease in serum biomarkers and increase in tissue antioxidant levels. EA treatment was also responsible for significant improvement in ECG parameter and histological score. The present findings clearly suggest that the EA reported dose dependent beneficial effect against lead induced myocardial toxicity.

**Key words:** Lead acetate, Myocardial toxicity, Ellagic acid

## INTRODUCTION

Environmental contaminations of heavy metals have become an increasing ecological and global public health concern. Lead is one of the earliest heavy metals discovered by human. Unique properties of lead, like low melting point, softness, malleability, ductility, and resistance to corrosion have resulted in its widespread usage in different industries such as paint, ceramics, automobiles, plastics, etc. [1] Chronic exposure of lead is responsible for life threatening detrimental effects on major organs such as brain, heart, liver, kidney, bones, reproductive organs. Lead exposure is responsible for different cardiac manifestation such as atherosclerosis, prolonged AV node and His bundle conduction times, reduced coronary blood flow, lowered heart rate and altered cardiac energy metabolism which are accused of increased mortality rate.[2] Probable reasons for Lead exposure associated myocardial dysfunction may be due to generation of huge amount of free radicals, consequently reduction in nitric oxide production and inhibition, down regulation of soluble guanylate cyclise, reduction in cGMP production.[3]

With the traditional background herbal medicines are getting popularity throughout the world due to their potency and apparent safety profile. The Beneficial effects of polyphenol in heavy metal toxicity have already been noticed. Polyphenols were also reported for detoxification and removal of heavy metals. [4] The probable reason by which it may show the protection is scavenging of reactive oxygen species, generated by lead and other heavy metals. Moreover, it is reported that they are also responsible for detoxification by removal of accumulated heavy metals from major organs. Apart from that, Polyphenols attenuated ROS-mediated inflammatory cytokines secretion through ERK/JNK/p38 pathways which is responsible for protection against lead induced inflammatory reactions. [5, 6]

Ellagic acid, is a polyphenolic phyto-chemical and is important component of fruits, nuts and vegetables, and it is well known for several medicinal properties. [7] It is abundantly found in pomegranates, black raspberries, raspberries, blackberries, strawberries, red guava, white guava, beefsteak fungus, Cranberries, walnuts and almonds. [8, 9] Ellagic acid already has been proved for having potential antioxidant, hepato-protective, Neuro protective, Antiulcer, Anti inflammatory, Cardioprotective, Anticancer, Antiviral and Anti-cataract activities. [10-19] So the present study has been designed to evaluate protective effect of Ellagic acid against lead induced myocardial toxicity.

## MATERIALS AND METHODS

### Chemicals

All chemicals used were of analytical grade and purchased from standard companies. Lead acetate was purchased from Loba chemicals (Mumbai, India). Biochemical kits were procured from Crest Biosystems (Goa, India).

**Phyto-chemicals**

Ellagic acid sample was obtained from Yucca Enterprises (Mumbai, India).

**Experimental Animals**

Healthy adult Male Wistar albino rats weighing 170-200 g, were housed in polypropylene cages, maintained under standardized condition (12 h L:D cycles, 25° ± 5°C) with paddy husk bedding at the Central Animal House, Soniya Education Trust's College of pharmacy, Dharwad, were provided with standard pellet food and had free access to purified drinking water. The guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)[Reg No. 112/PO/ReS/1999/CPCSEA. Dtd 19-05-1999, under the rule 5(a) of the "Breeding of and Experiments on Animals (control and supervision) Rules 1998"]. Ministry of Social Justice and Empowerment, Government of India were followed and prior permission was sought from the Institutional Animal Ethics Committee for conducting the study.

**Dose selection of Ellagic acid**

Based on the earlier literature rat dose of Ellagic acid was selected as 50 mg/kg and 25 mg/kg through oral route and termed as high and low dose respectively. [20]

**Experimental design**

After one week of acclimatization, the animals were randomly divided into 6 groups of 8 animals in each. Group I Served as normal control and received normal saline 5 ml/kg body weight through oral route. Group II Served as toxic control and animals were treated with lead acetate (100 mg/litre) in drinking water for 12 weeks. [21] Group III & IV animals received Ellagic acid 50 & 25 mg/kg p.o. respectively for 12 weeks along with that lead acetate was administered as in group II. [20]

Twenty four hours after the last treatments, the animals were anesthetised with ketamine (75 mg/kg, ip) and xylazine. (8 mg/kg, ip) and blood was withdrawn by retro-orbital puncture. Serum was separated by centrifugation for the estimation of enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), Alkanine phosphate (ALP) and bio-markers creatine kinase-MB (CK-MB), creatine kinase-NAC (CKNAC), lactate dehydrogenase (LDH) by semi-autoanalyser (robonik, Mumbai). Electro cardiogram recordings were made for each animal using digital physiograph (model no- DI-2, INCO, Ambala city, India). Thereafter the animals were sacrificed; four hearts were used for the preparation of homogenate to estimate antioxidant super oxide dismutase (SOD), Catalase, thiobarbituric acid reactive species (TBARS). Remaining four hearts were embedded in formal saline (0.7%) for histological examination. [22, 23]

*Preparation of Heart Tissue Homogenate*

The hearts removed after the experiment was made free of the adjacent vessels and fatty tissue mass with the help of scissors. Hearts were then cut open, rinsed with saline (0.9% NaCl) and dried using filter paper. The weight of the heart was then recorded. Thereafter the heart was homogenized in ice cold 0.25M sucrose solution using a mortar and pestle. The homogenate obtained was centrifuged at 5000 rpm for 15 min. The supernatant was decanted and used for the estimation of SOD, Catalase and TBARS. [22, 23]

*Histological analysis*

Heart sections were prepared from the remaining half of the heart samples in each group, stained with Hematoxylin and Eosin (H&E) and changes in histology were observed. The myocardial damage was determined by scoring method based on the severity: no change - 0 score, mild - 1 score (focal myocytes damage, small multifocal degeneration with slight degree of inflammation), moderate - 2 score (extensive myofibrillar degeneration) and marked - 3 score (necrosis with diffuse inflammation). [22, 23]

**Statistical analysis**

Results are expressed as mean +/- SEM. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey-Karmer multiple comparison tests. $P<0.05$ was considered significant.

**RESULTS**

**Effect on serum enzymes**

Effect on AST, ALT, ALP
Toxic control (only lead acetate treated) group demonstrated extremely significant ($P <0.001$) increase in serum AST, ALT, ALP values compared to normal control. Treatment groups EA 50 and EA 25 showed extremely significant ($P <0.001$) decrease in AST, ALT, ALP values compared to toxic control. **(Table 1)**

**Effect on serum biomarkers**

Effect on serum CK-MB, CK-NAC, LDH
Toxic control group result showed extremely significant ($P <0.001$) increase in serum CK-MB, CK-NAC, LDH values compared to normal control.
Treatment groups such as EA 50 demonstrated extremely significant ($P <0.001$) whereas EA 25 showed moderately significant ($P <0.01$) decrease in CK-MB, CK-NAC, LDH values compared to toxic control group. **(Table 1)**

**Effect on antioxidants in Heart tissue homogenate (HTH)**

*Effect on SOD and Catalase*
Toxic control group reported extremely significant ($P <0.001$) decrease in SOD and Catalase activity compared to normal control.
Experimental groups EA 50 demonstrated extremely significant ($P <0.001$) whereas for EA 25 treated groups it was found to be moderately significant ($P <0.01$) increase in SOD and Catalase values compared to toxic control group. **(Table 2)**

*Effect on TBARS*
Toxic control group demonstrated extremely significant ($P <0.001$) increase in TBARS activity compared to normal control. Treatment groups such as EA 50 showed extremely significant ($P <0.001$) whereas for EA 25 treated groups it was found to be moderately significant ($P <0.01$) decrease in TBARS activity compared to toxic control group. **(Table 2)**

**Effect on histological score**

Toxic control group demonstrated extremely significant ($P <0.001$) increase in histological score compared to normal control.
Treatment groups such as EA 50 showed extremely significant ($P <0.001$) whereas for AE EA 25 treated groups it was found to be moderately significant ($P <0.01$) decrease in histological score compared to toxic control group. **(Table 2)**

**Table 1: Effect on serum enzymes & biomarkers against lead acetate induced myocardial toxicity**

| Treatment | Blood serum level (U/L) | | | | | |
|---|---|---|---|---|---|---|
| | AST | ALT | ALP | CK-MB | CK-NAC | LDH |
| Normal control | 68.55±0.36 | 35.96±0.72 | 95.82±0.72 | 79±0.82 | 85.56±0.17 | 156.54±0.71 |
| Toxic control | 124.56±0.86*** | 99.27±0.57*** | 181.62±0.41*** | 137±0.79*** | 151.39±0.59*** | 240.73±0.89*** |
| EA 50 | 81.82±0.81### | 58.64±0.29### | 125.34±0.89### | 91±0.53### | 97.59±0.62### | 183.29±0.52### |
| EA 25 | 96.59±0.32### | 72.12±0.62### | 143.45±0.71### | 112±0.38## | 115.92±0.79## | 207.11±0.45## |

All values are mean ± SEM, n=8, ***P <0.001, when compared to normal control; ###P <0.001, ##P <0.01 compared to Toxic control group.

**Table 2:Effect on antioxidants in HTH and histological score against lead acetate induced myocardial toxicity**

| Treatment | Heart Tissue Homogenate (Units/mg of protein) | | | Histological score |
|---|---|---|---|---|
| | SOD | CATALASE | TBARS | |
| Normal control | 30.34±0.34 | 21.45±0.56 | 27.38±0.39 | 0.53±0.03 |
| Toxic control | 11.82±0.78*** | 8.82±0.39*** | 59.35±0.91*** | 2.19±0.06*** |
| EA 50 | 24.56±0.45### | 19.98±0.20### | 35.73±0.25### | 1.12±0.02### |
| EA 25 | 21.39±0.56## | 15.65±0.49## | 41.29±0.46## | 1.76±0.04## |

All values are mean ± SEM, n=8, ***P <0.001, when compared to normal control; ###P <0.001, ##P <0.01 compared to Toxic control group.

**Table 3: Effect on electro cardiograph patterns against lead acetate induced myocardial toxicity**

| Treatments | Heart rate (Beats/Min) | QT interval (ms) | RR interval (ms) | PR interval (ms) | QRS interval (ms) |
|---|---|---|---|---|---|
| Normal control | 351.91±1.23 | 95.83±1.72 | 210.47±1.89 | 85.91±1.23 | 95.83±1.72 |
| Toxic control | 427.29±1.74*** | 201.91±1.29*** | 315.11±2.67*** | 187.29±1.74*** | 201.91±1.29*** |
| EA 50 | 373.30±1.38### | 116.73±1.37### | 226.23±1.39### | 103.30±1.38### | 116.73±1.37### |
| EA 25 | 381.75±1.57## | 169.39±1.82## | 257.91±1.91## | 127.75±1.57## | 139.39±1.82## |

All values are mean ± SEM, n=8, ***P <0.001 when compared to normal control; ###P <0.001, ##P <0.01 compared to Toxic control group.
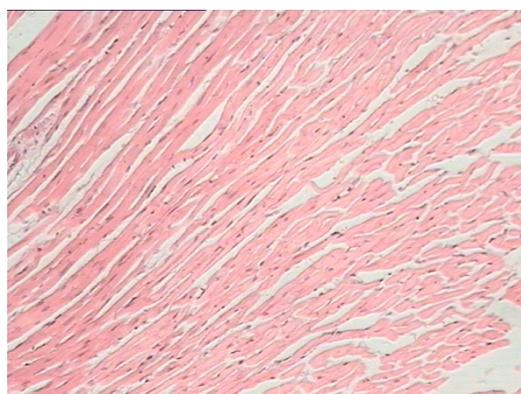


**Figure 1a: (H&E) (x400) stained microscopic section of normal control.**
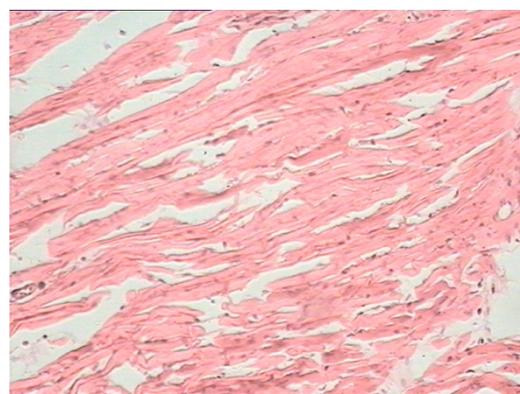**1. Normal texture of cell**



**Figure 1b: (H&E) (x400) stained microscopic section of toxic control.**
**1. Myocardial Oedema 2. Separation of Fibres 3. Loss of Striation 4. More interstitial space**
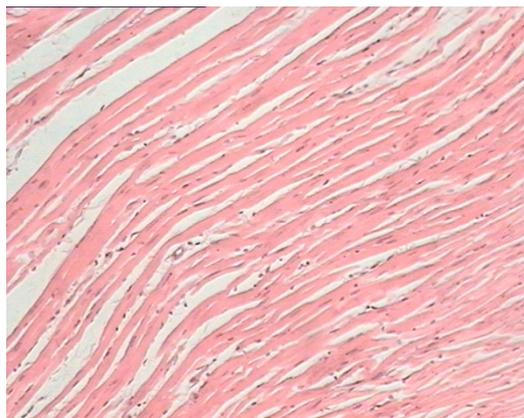
**Figure 1c: (H&E) (x400) stained microscopic section of EA 50. 1. Less interstitial space 2. Reduced separation of Fibres 3. Reduced loss of Striation**
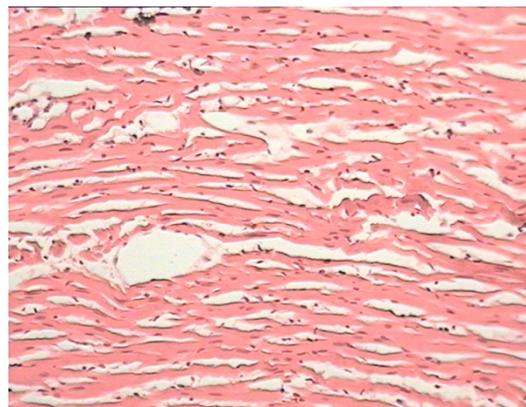


**Figure 1d: (H&E) (x400) stained microscopic section of EA 25. 1.Myocardial Oedema 2. Reduced separation of Fibres 3. Reduced loss of Striation 4. More interstitial space**

**Figure 1: Haematoxylin and eosin (H&E) stained section of heart in lead acetate induced myocardial damage. Photographed at magnification 400X**
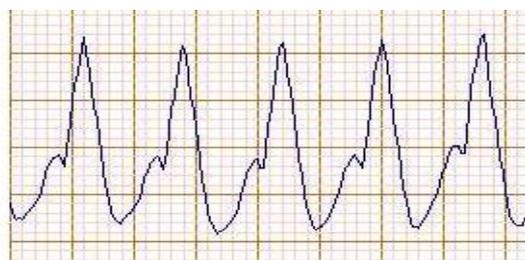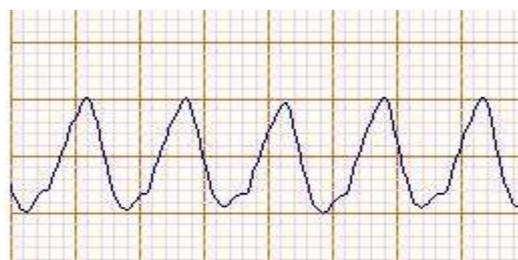


**Figure 2a: ECG of normal control rat at 25mm/sec**



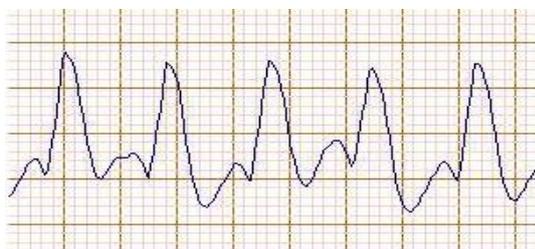**Figure 2b: ECG of toxic control rat at 25mm/sec**


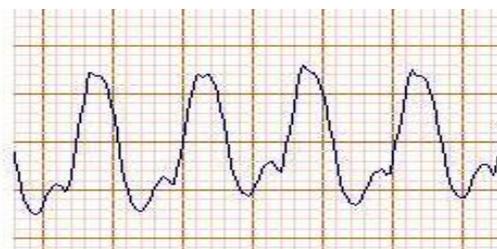
**Figure 2c: ECG of EA 50 treated rat at 25mm/sec**



**Figure 2d: ECG of EA 25 treated rat at 25mm/sec**

**Figure 2: Effect of drugs on electrocardiographic parameters in lead acetate induced myocardial damage**

**Effect on Electocardiographic parameters**

Toxic control group demonstrated extremely significant increase (P <0.001) in QT, RR, PR, QRS intervals and in heart rate compared to normal control.

Treatment groups such as EA 50 showed extremely significant (P <0.001) whereas EA 25 treated groups it was found to be moderate significant (P <0.01) decrease in heart rate, QT, RR, PR, QRS intervals compared to toxic control group. **(Table 3)**

**DISCUSSION**

The aim of the present study was to investigate protective effect of ellagic acid (EA) against lead acetate induced cardiac toxicity. Observed results suggested dose dependent beneficial effects for EA against lead acetate induced myocardial toxicity.

Lead exposure is responsible for generation of huge amount of free radicals which is responsible for development of oxidative stress. Enormous amount of oxidative stress associated with lead exposure is responsible for severe damage in cardiac myocytes.

Excessive damage in myocardial cell is responsible for leaking of cardiac specific biomarkers to serum. [3, 24] In our present study also only lead exposed animal reported extremely significant increase in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), Alkanine phosphate (ALP) and bio-markers creatine kinase-MB (CK-MB), creatine kinase-NAC (CKNAC), lactate dehydrogenase (LDH).

Reactive oxygen species (ROS) are effectively neutralized by the antioxidant defense system. But during pathophysiological conditions such as lead exposure result in enormous increased production of ROS and/or impaired antioxidant capacity, which culminate in oxidative stress.[25]

Results of this study witnessed extremely significant decrease in superoxide dismutase (SOD), catalase and increase in thio barbituric acid reactive substances (TBARS) in myocardial tissue. This may be due to excessive damage produced by lead exposure.

Four phenolic groups and two lactones present in EA can act as hydrogen-forming sides and electron acceptors delay, inhibit or prevent the oxidation of compounds, trapping free radicals and reduce oxidative stress. EA reported for inhibiting the generation of superoxide anions, hydroxyl radicals and prevent lipid peroxidation associated with damage of cell membrane.[10,26] The antioxidant effect associated with EA may be due to direct action on free radical scavenging and indirect action through the induction of antioxidant enzymes. In addition, EA is able to inhibit PGE2 produced and reduce the COX-2, thus, decreased TNF-α level. [27]

In our present study also EA showed significant decrease in serum AST, ALT, ALP, LDH, CKMB & CKNAC levels which reflect protection against lead induced myocardial toxicity. Moreover EA treated groups showed significant increase in SOD, catalase and decrease in TBARS levels.

We found only lead exposed group was responsible for abnormal changes in electrocardiogram readings which include increase in heart rate, QT, RR, PR & QRS intervals.

Increase in heart rate may be due to altered cellular metabolism of $Ca^{+2}$. Prolongation of PR interval associated with lead exposure might be due to AV block. Change in parasympathetic tone and conduction system deformation can be the reason for AV block. Hypokalemia is suspected for QT interval elongation. [28]

EA dose dependently restored the ECG parameters towards the normal. Histological study also in coordination with the findings of other parameters. For the normal heart, myocardial fibers were found to be of uniform size, shape, and configurations with no inflammatory cell infiltrates.

Only lead exposed group caused enormous changes in the myocardial cell associated with degeneration of myocardial tissue, vacuolization of the cardiomyocytes, infiltration of inflammatory cells, and myofibril loss. Treatment with EA dose dependently inhibited lead induced cardiac damage by decreasing fragmentation of myofibrils and inflammation.

**CONCLUSION**

It can be concluded from the present study that EA exhibited dose dependent protection against lead induced cardiotoxicity. Findings of the present study can be important for those who are chronically exposed to high level lead. Ellagic acid in the dietary source or in the form of formulation can keep their heart healthy and safe. Further studies can be carried out to establish the fact clinically.

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