Naringin Administration Mitigates Oxidative Stress, Anaemia and Hypertension in Lead Acetate-Induced Cardiorenal Dysfunction in Cockerel Chicks

Chinomso Gift Ebirim
PAU: Pan African University
Oluwaseun Esan
University of Ibadan Faculty of Veterinary Medicine
Moses Olusola Adetona
University of Ibadan College of Medicine
Ademola Adetokunbo Oyagbemi (ademola.oyagbemi778@gmail.com)
University of Ibadan Faculty of Veterinary Medicine https://orcid.org/0000-0002-8996-8610
Temidayo Olutayo Omobowale
University of Ibadan Faculty of Veterinary Medicine
Omolade Abodunrin Oladele
University of Ibadan Faculty of Veterinary Medicine
Adeolu Alex Adedapo
University of Ibadan Faculty of Veterinary Medicine
Olufemi Omoniyi Oguntibeju
Cape Peninsula University of Technology - Bellville Campus
Momoh Audu Yakubu
Texas Southern University

Research Article

Keywords: Lead toxicity, oxidative stress, anaemia, hypertension, antioxidant, naringin

Posted Date: October 20th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-925993/v1

Abstract

Lead is one of the major pollutants in the world which is deleterious to both animals and humans. It is found in every aspect of the environment such as the air, water and soil. This pollutant affect both wild and domestic birds. Naringin is a flavonoid that has been found to have medicinal properties mostly because of its antioxidant and metal chelating effects. This study was carried out to investigate the protective effect of naringin against lead-induced anaemia, cardio and nephrotoxicity, oxidative stress, and hypertension. Thirty -six cockerel chicks were used for this study, and randomly grouped into six chicks per group; Group A served as the control, Group B received Pb only (300 ppm), Group C (Pb and naringin; 80 mg/kg), Group D (Pb and naringin; 160 mg/kg), Group E (naringin 80 mg/kg) and Group F (naringin 160 mg/kg), respectively for eight weeks. Lead (Pb) was administered via drinking water while Naringin was administered via oral gavage. Lead acetate intoxication precipitated anaemia as indicated by significant reductions in the values of PCV, RBC, and Hb concentration in lead-treated chicks when compared with the controls. Also, lead administration induced hypertension together with increased oxidative stress, depletion of the antioxidant defense system, reduced nitric oxide production and increase in high blood pressure. Immunohistochemistry indicated high expressions of cardiac troponin, renal angiotensin converting enzymes, and renal neutrophil gelatinase associated lipocalin. Treatment with naringin corrected anemia, reduced oxidative stress, improved antioxidant system, reduced high blood pressure, and offered protection against lead acetate-induced cardio-renal dysfunction in cockerel chicks.

Introduction

Lead (Pb) is a blue–gray and highly toxic divalent metal that occurs naturally and is spread through various human activities in the environment, and its toxicity remains one of the most studied in environmental health and medicine (Hossain et al. 2014). Wild and domestic birds are quite sensitive to Pb exposure which can present as either acute or chronic responses, and about 120 species have been documented to be exposed to Pb poisoning (Haig et al. 2014). Main sources of Pb are the environment; getting into birds mainly due to anthropogenic activities, industrial emissions, wind carrying dust, water runoffs, and erosions in the soil (Hossain et al. 2014). Pb has been found to induce a range of dysfunctions both physiological, biochemical, and behavioral in animals and man by affecting the cardiovascular system and inducing renal damage, with its toxicity closely related to the route of exposure and duration, level of intake, absorption rate and efficiency of excretion (Fakunle et al. 2013, Matovic *et al.*, 2015). Pb is a non-redox metal and one of the mechanisms of its toxicity is by the induction of oxidative stress through generating reactive oxygen species (ROS) which induces oxidative stress and also by the depletion of the antioxidant defense system in various tissues (Matovic et al. 2015; Oyagbemi et al. 2015; Akinlolu et al. 2021; Nasiruddin et al. 2021; Gadde et al. 2021).

Pb toxicity manifests most times as anemia due to its ability to cause destruction of red blood cells and reduced production of red blood cells (Gargouri et al. 2020; Oyem et al. 2021). It does so by interfering with certain enzymes necessary for heme production such as delta-aminolaevulinic acid dehydratase

(Thuppil et al. 2013; Hossain et al. 2014; Dsouza et al. 2021; Azab 2021). The inhibition of the delta-aminolaevulinic acid dehydratase (δ-ALAD) is a reliable and sensitive indicator of Pb exposure, ALAD strongly decreases after Pb exposure and often remains depressed over an extended period in an otherwise apparently healthy bird (Golden et al. 2016; Krone 2018). Lead exposure has been documented to be associated with increased blood Pb levels which in turn caused hypertension and increased mean arterial pressure (Vaziri 2008; Wildemann et al. 2015; Broseghini-Filho et al. 2016; de Moura et al. 2021). Long-term lead exposure has been reported to alter cardiorespiratory and nervous Systems (Shvachiy et al. 2020). Several studies have identified oxidative stress, an impaired nitric oxide (NO) bioavailability, inflammation, and changes in cellular Ca_2 transport during lead exposure (Xu et al. 2015; Ileriturk et al. 2021). Chronic lead exposure causes oxidative stress, limits NO availability, impairs nitric oxide signaling, promotes inflammation, increases sympathetic activity, increases endothelial production, and alters cardiovascular functions (Liu et al. 2013; Ileriturk et al. 2021; Long et al. 2021; Ajarem et al. 2021; Kucukler et al. 2021).

Naringin is a naturally occurring flavonoid which is known to have a bioactive activity such as anti-inflammatory, free radical scavenging, antioxidant, anti-apoptotic, and immunomodulatory effect on animal and human health (Venkateswara et al. 2017; Baranowska et al. 2021; Hassan et al. 2021; Li et al. 2021; Deng et al. 2022). Naringin differs from other bioflavonoids because of its ability to improve the lipid profile (Chanet et al. 2012), bioactivity in the regulation of heart rhythm (Rani et al. 2013), cardioprotective (Amini et al. 2019) and its antiarterogenic action LDL cholesterol and arterial pulmonary hypertension (Wu et al. 2021). All of these contribute to the control of blood pressure and the prevention of the appearance of atherosclerosis. The present study sought to understand the neproprotective, cardioprotective and anti-hypertensive actions of metal chelating antioxidant naringin in lead acetate-induced toxicity in cockerel chicks and its possible molecular mechanism of action.

Materials And Method

Chemicals

Pb acetate trihydrate ((C□H□O□)2Pb.3H□O), Lead acetate, naringin, Trichloro acetic acid (TCA), sodium hydroxide, Odianisidine, and hydrogen peroxide (H_2O_2), xylenol orange (XO), potassium hydroxide, reduced glutathione (GSH), oxidized glutathione (GSSG), NaF, thiobarbituric acid (TBA), 1,2dichloro4nitrobenzene, were purchased from Sigma (St. Louis, MO, USA). Normal goat serum, Biotinylated, and antibody 2-step plus Poly-HRP Anti Mouse/Rabbit IgG Detection System with DAB solution were purchased from Elabscience Biotechnology®, China), anti- Angiotensin Converting Enzyme1 Polyclonal Antibody (E-AB-16159: 1:500 Dilution) and Anti-Lipocalin (TINGAL1) Polyclonal Antibody (E-AB-40223: 1:500 Dilution) for the Kidney, Cardiac Troponin (TNNC1) Polyclonal Antibody (E-AB-18400: 1:50 Dilution). All other chemicals used for this study were of analytical grade.

Experimental Animals And Design

Thirty-six-day old cockerel chicks were used for this study. They were randomly grouped into six of six chicks each; control, Pb (600 ppm), Pb and Naringin (80 mg/kg), Pb and Naringin (160 mg/kg), Naringin (80mg/kg) and Naringin (160mg/kg). The dosage of Pb acetate was chosen from the previous study of Amini et al. (2021) with slight adjustment. They were exposed and treated for eight weeks, respectively. The chicks were given poultry feed and supplied with water *ad libitum* and housed in poultry units of the Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ibadan. Lead was given via drinking water and Naringin via oral gavage. Ethical regulations were followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments (Garber et al. 2011).

Electrocardiogram (ECG)

The Electrocardiographic recordings were done using a 6/7 lead computer ECG machine (EDAN VE-1010, Shanghai, China) as earlier reported by Omobowale et al. (2017). Lead II parameters of the heart rate, P-duration, PR-, QRS-duration, QT duration and the Bazett's correction of the QT segment were recorded for each bird.

Blood Pressure Measurement

The blood pressure was measured using automated blood pressure monitor. Birds were manually restrained, and indirect blood pressure measurements were obtained by placing a cuff around the limb over the femoral artery. This was performed 24 hours before the termination of the experiment.

Hematology

Blood was collected from the right jugular vein using a sterile syringe and transferred into a lithium heparin anticoagulant bottle. The blood was analyzed for full blood count using the method described by Thomas and Merle (2016).

Blood Sample Collection And Serum Preparation

At termination of the study, the birds were humanely handled and about 5ml blood collected via the jugular vein. The clotted blood was centrifuged at 4, 000 rpm for 10 mins. The clear serum was harvested and stored until analysis. The kidney and heart were harvested and weighed for the determination of organ weight and relative organ weight respectively for biochemical assay.

Renal And Cardiac Post-mitochondrial Fractions Preparation

Tissue samples from kidney and heart were quickly excised, rinsed, blotted with filter paper, weighed, chopped into bits and homogenized with homogenizing buffer (0.1M phosphate buffer, pH 7.4) using a Teflon homogenizer. The homogenate obtained was centrifuged at 10,000 × g for 10 min with a cold centrifuge at − 4°C to obtain post-mitochondrial fractions (PMFs). The supernatants (PMFs) obtained were used for biochemical assays.

Biochemical Assays

Determination of antioxidant defense system

Superoxide dismutase (SOD) assay was carried out by the method of Misra and Fridovich with slight modification by Oyagbemi et al. (2015). The glutathione peroxidase (GPx) activity was also measured according to Beutler et al. (1963), glutathione S-transferase (GST) was estimated by the method of Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene as substrate, and the reduced glutathione (GSH) content was estimated by the method of Ellman (1959).

Determination Of Markers Of Oxidative Stress

Hydrogen peroxide (H_2O_2) generation was determined using the method described by Wolf's (1994). Malondialdehyde (MDA) as a product of lipid peroxidation was determined using the method described by Varshney and Kale (1990). Serum myeloperoxidase (MPO) was determined by the method described by Xia and Zweier (1997).

Determination Of Serum Total Protein And Nitric Oxide

Serum Nitric oxide was determined using the method described by Olaleye et al. (2007) while serum total protein was determined by Biuret's method as described by Gornal et al. (1949).

Immunohistochemistry (Ihc) Protocol

Immunohistochemistry was done as described by Oyagbemi et al. (2019). Antibodies against renal angiotensin converting enzyme (ACE), neutrophil gelatinase-associated lipocalin (NGAL), while cardiac troponin were probed in the heart with slight modification using 2-step plus Poly-HRP Anti Mouse/Rabbit IgG Detection System with DAB solution (Catalog number: E-IR-R217 from Elabscience Biotechnology®, China). The kidney and Heart samples were fixed with 10% paraformaldehyde, embedded in paraffin and sectioned at a thickness of 5 µm. The slides were subsequently dewaxed in xylene (100%) solution for 2 minutes and afterward, hydration was carried out in different concentrations of ethanol (100%, 90%, and 80%) for 2 minutes each. The hydrated slides were rinsed and put in a PBS buffer tank for 5 mins. The antigen retrieval was performed with citrate buffer solution containing 2.1 g of citric acid monohydrate

and 14.75 g of trisodium citrate dehydrate adjusted to pH 6.0 in microwave oven. Endogenous peroxide (H_2O_2 block) was carried out following manufacturer's instructions as directed on the kit (E-IR-217C). Drops of H_2O_2 were added to cover the sections and incubated in humidifying chamber at room temperature for 10 min. The slides were rinsed afterwards and put back in the PBS tank for 5 min. Goat serum (E-1R-R217A) was added onto the slides to prevent nonspecific binding and incubated in humidifying chamber at room temperature for 30 mins. After 30 mins of incubation, the tissues were probed with primary antibodies viz a viz Angiotensin Converting Enzyme1 Polyclonal Antibody (E-AB-16159: 1:500 Dilution) and Anti-Lipocalin (TINGAL1) Polyclonal Antibody (E-AB-40223: 1:500 Dilution) for the Kidney, Cardiac Troponin (TNNC1) Polyclonal Antibody (E-AB-18400: 1:50 Dilution) for the Heart and were incubated for 2 hours at room temperature. Following incubation, the slides were rinsed with PBS and secondary antibody labelled (E-1R-R217B) was added, and the slides were incubated in humidifying chamber at room temperature for 20 min. Thereafter, the slides were rinsed and immersed in PBS tank for 5 min. Finally, a few drops of the substrate diaminobenzidine (DAB) was added at room temperature for 10 s; 50 µL of DAB concentrate (E-1R-R217D) + 1 mL DAB solution (E-1R-R217E) in the dark. The reaction was terminated with deionized water and slides were immersed in hematoxylin for 3 s before rinsing with PBS. The slides were placed in 80%, 90%, and 100% of ethanol, and then xylene (100%) for 2 minutes each. Slides were removed, allowed to dry and a DPX mountant was applied. Sections were observed with light microscope (Leica LAS-EZ®) using Leica software application suite version 3.4 equipped with a digital camera.

Statistical analysis

All values were expressed as mean ± standard deviation (SD) and the test of significance between two groups was estimated with Student's t-test. The One-Way Analysis of Variance (ANOVA) with Turkey's post-hoc test of Graph pad prism 5.0 was also carried out with p-Values < 0.05 considered statistically significant.

Results

The effects of lead acetate intoxication on body weight

There was a significant decrease in final body weight in Pb exposed group when compared to their initial body weights. Also, the final body weights of the naringin treated chicks and those on naringin were significantly improved when compared to the initial body weights (Table 1).

Table 1
The effect of lead acetate and naringin on weekly body weights (g)

Weeks/ Groups	Control	Pb	Pb + Nar1	Pb + Nar2	Nar1	Nar2
Week 0	179 ± 22.2	181.6 ± 13.4	182.1 ± 11.9	178.5 ± 16.1	176.3 ± 7.8	178.1 ± 10.1
Week 1	271 ± 13.1[a]	398.8 ± 96.9[a]	257.6 ± 9.8[a]	267 ± 18.8[a]	256.1 ± 16.0[a,b]	273.8 ± 21.4[a]
Week 2	320.3 ± 45.8[a]	359 ± 73.5[a]	257.6 ± 48.2[a]	297.8 ± 10.4[a]	315.5 ± 6.5[a]	317.3 ± 29.3[a]
Week 3	370.5 ± 20.7[a]	326.3 ± 54.9[a]	253.6 ± 25.0[a]	337.6 ± 13.2[a,b]	345.1 ± 23.3[a]	328.1 ± 43.3[a]
Week 4	456.8 ± 54.3[a]	304.8 ± 52.3[a]	361 ± 108.2[a]	385.6 ± 50.6[a,b]	426.5 ± 41.5[a,b]	383.8 ± 67.4[a]
Week 5	479.8 ± 62.6[a]	279.3 ± 53.2[a]	385.3 ± 110.8[a]	407.1 ± 38.3[a,b]	443.6 ± 47.9[a,b]	409.6 ± 67.7[a]
Week 6	586.8 ± 48.8[a]	251.1 ± 14.2[a]	582.6 ± 183.7[a,b]	531.1 ± 46.9[a,b]	526.5 ± 61.2[a,b]	485.1 ± 50.8[a,b]
Week 7	654.5 ± 57.9[a]	239.3 ± 6.9[a]	595 ± 180.6[a]	537.1 ± 41.9[a,b]	529.3 ± 65.2[a,b]	521.8 ± 56.3[a,b]
Week 8	740.6 ± 69.7[a]	212.1 ± 13.4[a,b]	688.6 ± 215.4[a,b]	638.6 ± 59.1[a,b]	601 ± 78.3[a,b]	556 ± 61.6[a,b]

Superscript (a) indicates significant difference at $p < 0.05$ when compared with Group A. Superscript (b) indicates significant difference when compared with Group B. The results are shown in Mean ± SD (n = 6). Group A: Control, Group B: Pb (3.00ppm), Group C: Pb and Naringin (80 mg/kg), Group D: Pb and Naringin (160 mg/kg), Group E: Naringin (80 mg/kg), & Group F: Naringin (160 mg/kg).

Antioxidant Defense Status And Oxidative Stress

In both the renal and cardiac assays, there were significant ($p < 0.05$) reductions in the activities of GPx, GST, and SOD, with concomitant reductions in the GSH contents in the Pb intoxicated chicks when compared to the control. Treatment with naringin however produced significant improvement in the activities of the aforementioned renal and cardiac antioxidant defence system relative to the Pb administered chicks (Tables 2 and 3). Surprisingly, there was no significant difference in the GST activity of the cardiac tissues of chicks intoxicated with Pb and those treated with naringin, respectively (Tables 2 and 3). The observed improvement in the antioxidant enzymes and GSH content signified the antioxidant power of naringin as recorded in Tables 2 and 3, respectively.

Table 2
The effect of lead acetate and naringin on antioxidant defense system in the kidney

Groups/ Parameters	Control	Pb	Pb + Nar1	Pb + Nar2	Nar1	Nar2
GPx	55.62 ± 11.5	24.36 ± 3.16[a]	34.51 ± 3.38	35.37 ± 8.73	52.2 ± 2.69	53.02 ± 8.21
GSH	96.04 ± 0.88	91.58 ± 1.73[a]	93.26 ± 3.01	95.44 ± 1.53	96.34 ± 3.5	95.87 ± 4.05
GST	13.6 ± 4.53	7.28 ± 3.89[a]	10.07 ± 2.36	11.33 ± 5.14	11.16 ± 1.9	11.25 ± 2.69
SOD	63.37 ± 7.84	45.77 ± 4.64[a]	52.07 ± 7.5	57.2 ± 6.74	66.11 ± 3.7	68.77 ± 8.6

Superscript (a) indicates significant difference at $p < 0.05$ when compared with Group A. Superscript (b) indicates significant difference when compared with Group B. The results are shown in Mean ± SD (n = 6). Group A: Control, Group B: Pb (3.00ppm), Group C: Pb and Naringin (80 mg/kg), Group D: Pb and Naringin (160 mg/kg), Group E: Naringin (80 mg/kg), & Group F: Naringin (160 mg/kg). *SOD* superoxide dismutase (units/mg protein), *GPx;* glutathione peroxidase (units/mg protein), *GST;* glutathione S-transferase (mmole 1-chloro-2, 4-dinitrobenzene-GSH complex formed/min/mg protein), *GSH;* reduced glutathione (µmol/mg protein).

Table 3
The effect of lead acetate and naringin on antioxidant defense system in the heart

Groups/ Parameters	Control	Pb	Pb + Nar1	Pb + Nar2	Nar1	Nar2
GPx	65.01 ± 2.59	58.78 ± 1.41[a]	60.5 ± 3.81	61.04 ± 1.63	64.44 ± 0.85	62.19 ± 3.45
GSH	71.02 ± 7.2	65.7 ± 0.9[a]	68 ± 2.65	68.68 ± 0.6	73.14 ± 0.9	71.37 ± 3.8
GST	3.89 ± 0.24	3.80 ± 1.92	3.37 ± 0.55	3.56 ± 1.66	3.35 ± 0.44	3.87 ± 0.49
SOD	57.55 ± 6.59	52.44 ± 4.39[a]	54.51 ± 7.18	53.62 ± 8.31	55.77 ± 9.74	56.66 ± 6.96

Superscript (a) indicates significant difference at $p < 0.05$ when compared with Group A. Superscript (b) indicates significant difference when compared with Group B. The results are shown in Mean ± SD (n = 6). Group A: Control, Group B: Pb (3.00ppm), Group C: Pb and Naringin (80 mg/kg), Group D: Pb and Naringin (160 mg/kg), Group E: Naringin (80 mg/kg), & Group F: Naringin (160 mg/kg). *SOD;* superoxide dismutase (units/mg protein), *GPx;* glutathione peroxidase (units/mg protein), *GST;* glutathione S-transferase (mmole 1-chloro-2, 4-dinitrobenzene-GSH complex formed/min/mg protein), *GSH;* reduced glutathione (µmol/mg protein).

Cardiac and renal oxidative stress markers

The cardiac and renal oxidative stress markers (H_2O_2 and MDA) were observed to be significantly increased in the Pb exposed group when compared to the control. This is indicative of antioxidative and

free radical scavenging action of naringin. Meanwhile, a significant reduction in values was observed in the naringin co-treated with Pb acetate groups (Table 4).

Table 4
The effect of lead acetate and naringin on markers of oxidative stress in the kidney and heart

Groups/ Parameters	Control	Pb	Pb + Nar1	Pb + Nar2	Nar1	Nar2
Kidney						
H₂O₂	150.77 ± 3.04	187.58 ± 8.84[a]	136.23 ± 9.9[b]	127.82 ± 10.52[b]	149.64 ± 5.08	149.87 ± 7.93
MDA	3.05 ± 0.92	5.82 ± 0.15[a]	2.9 ± 0.74[b]	2.11 ± 0.78[b]	3.1 ± 0.99	3.12 ± 0.27
Heart						
H₂O₂	131.8 ± 15.91	142.34 ± 6.38[a]	139.14 ± 1.17[b]	135.35 ± 6.01[b]	128.93 ± 2.35	137.89 ± 7.95
MDA	8.44 ± 0.17	9.61 ± 1.33[a]	8.57 ± 0.91[b]	8.27 ± 1.14[b]	8.37 ± 1.1	8.33 ± 1.13

Superscript (a) indicates significant difference at $p < 0.05$ when compared with Group A. Superscript (b) indicates significant difference when compared with Group B. The results are shown in Mean ± SD (n = 6). Group A: Control, Group B: Pb (3.00ppm), Group C: Pb and Naringin (80 mg/kg), Group D: Pb and Naringin (160 mg/kg), Group E: Naringin (80 mg/kg), & Group F: Naringin (160 mg/kg). *H_2O_2;* hydrogen peroxide (µmol/mg protein). *MDA;* malondialdehyde (µmol of MDA formed/mg protein).

The effects of lead acetate and naringin on myeloperoxidase activity, nitric oxide content and serum total protein in chicks.

Our data also indicated a significant increase in the activity of serum MPO in Pb exposed chicks compared to the control. However, in the naringin treated groups (80 mg/kg and 160 mg/kg body weight), a significant decrease in MPO activity was observed; indicative of anti-oxidative, anti-inflammatory, and cardioprotective effects of naringin (Table 5). The serum NO bioavailability also decreased significantly in Pb intoxicated chicks (Table 5). Naringin co-administration however, significantly improved NO bioavailability in comparison to Pb only exposed chicks as indicated in Table 5. Furthermore, the values of serum total protein were also significantly reduced in Pb exposed chicks when compared to the control, and naringin treated chicks relative to Pb only treated group (Table 5).

Table 5
The effect of lead acetate and naringin on myeloperoxidase activity, nitric oxide content and serum total protein.

Groups/ Parameters	Control	Pb	Pb + Nar1	Pb + Nar2	Nar1	Nar2
MPO	20.13 ± 9.57	47.34 ± 3.87[a]	15.35 ± 0.18[b]	18.4 ± 2.7[b]	20.04 ± 1.2	20.3 ± 5.75
NO	1.18 ± 0.02	0.98 ± 0.18[a]	1 ± 0.19[b]	1.18 ± 0.09[b]	2 ± 0.26	1.33 ± 0.01
TP	3.73 ± 0.3	3.32 ± 0.04[a]	3.41 ± 0.3[b]	3.57 ± 0.21[b]	3.73 ± 0.18	3.78 ± 0.24

Superscript (a) indicates significant difference at $p < 0.05$ when compared with Group A. Superscript (b) indicates significant difference when compared with Group B. The results are shown in Mean ± SD (n = 6). Group A: Control, Group B: Pb (3.00ppm), Group C: Pb and Naringin (80 mg/kg), Group D: Pb and Naringin (160 mg/kg), Group E: Naringin (80 mg/kg), & Group F: Naringin (160 mg/kg). *NO;* Nitric oxide (µmol/mg protein), *TP;* Total protein (g/dl), *MPO;* Myeloperoxidase (nmol/mg protein).

Hematology

The hematological parameters showed marked reductions in the RBC, Hb concentration and PCV in the Pb exposed group when compared to the control. This is indicative of anemia in chicks. Furthermore, significant reductions in the values of MCH, MCV, and MCHC together with significant increase in WBC counts were also recorded in Pb intoxicated chicks when compared Pb acetate co-administered with naringin (Table 6). The observable low values of MCV and MCH precipitated by Pb intoxication are indicative of hypochromic microcytic anemia, which was interestingly corrected and restored in naringin treated chicks.

Table 6
The effect of lead acetate and naringin on hematological parameters

Parameters/ Groups	Control	Pb	Pb + Nar1	Pb + Nar2	Nar1	Nar2
PCV (%)	30.2 ± 4.2	19.8 ± 2.2[a]	27.2 ± 1.6[b]	27.8 ± 2.1[b]	29.4 ± 1.9	30.4 ± 1.8
RBC (10^{12}/L)	3.09 ± 0.24	2.22 ± 0.47[a]	2.66 ± 0.10[b]	2.94 ± 0.22[b]	3.07 ± 0.39	3.08 ± 0.20
WBC (10^9/L)	13.75 ± 0.84	17.92 ± 2.97[a]	15.87 ± 1.03[b]	16.56 ± 1.31[b]	13.95 ± 1.03	13.81 ± 2.14
HB (g/dl)	12.2 ± 0.38	6.88 ± 1.37[a]	7.32 ± 0.46[b]	8.74 ± 0.53[b]	10.82 ± 0.87	11.08 ± 0.99
mcv(fl)	97.34 ± 7.75	74.61 ± 5.63[a]	100.42 ± 1.45[b]	100.57 ± 0.24[b]	95.97 ± 4.43	97.97 ± 2.95
MCH (ρg)	34.21 ± 0.96	32.19 ± 1.17[a]	33.74 ± 1.59[b]	34.63 ± 0.43[b]	30.98 ± 1.32	33.03 ± 2.08
MCHC (g/dl)	33.22 ± 0.45	23.56 ± 0.54[a]	33.47 ± 1.47[b]	33.65 ± 0.96	32.64 ± 0.07	33.03 ± 1.01

Superscript (a) indicates significant difference at $p < 0.05$ when compared with Group A. Superscript (b) indicates significant difference when compared with Group B. The results are shown in Mean ± SD (n = 5). Group A: Control, Group B: Pb (3.00ppm), Group C: Pb and Naringin (80 mg/kg), Group D: Pb and Naringin (160 mg/kg), Group E: Naringin (80 mg/kg), & Group F: Naringin (160 mg/kg).

Table 7
The effect of lead acetate and naringin on white blood cells (WBC) differentials

Parameters/ Groups	Control	Pb	Pb + Nar1	Pb + Nar2	Nar1	Nar2
HET (%)	52.2 ± 21.97	30.4 ± 3.91[a]	32.8 ± 5.26[b]	34.8 ± 4.76[b]	50.6 ± 13.64	50.8 ± 11.07
EOS (%)	2.0 ± 0.70	4.6 ± 1.67[a]	3.4 ± 1.14[b]	3.8 ± 1.48[b]	1.8 ± 0.83	1.8 ± 0.83
BASO (%)	1.8 ± 0.44	5.8 ± 1.64[a]	5.0 ± 1.00[b]	5.6 ± 0.54[b]	1.4 ± 0.54	1.4 ± 0.54
MONO (%)	2.8 ± 0.83	5.4 ± 0.54[a]	3.2 ± 0.44[b]	3.6 ± 0.54[b]	2.6 ± 0.54	2.8 ± 1.09
LYM (10^9/L)	2.88 ± 0.53	3.64 ± 0.25[a]	2.89 ± 0.95[b]	2.56 ± 0.57[b]	2.20 ± 0.48	2.27 ± 0.32

Superscript (a) indicates significant difference at $p < 0.05$ when compared with Group A. Superscript (b) indicates significant difference when compared with Group B. The results are shown in Mean ± SD (n = 5). Group A: Control, Group B: Pb (3.00ppm), Group C: Pb and Naringin (80 mg/kg), Group D: Pb and Naringin (160 mg/kg), Group E: Naringin (80 mg/kg), & Group F: Naringin (160 mg/kg).

Electrocardiogram (ECG)

There was no significant difference in the values of heart rate, P-duration, PR intervals, QRS and QT durations, and QTc interval, respectively, in Pb intoxicated chicks, chicks co-administered with naringin, and chicks administrated only naringin (Fig. 1).

Blood Pressure

The results showed significant increases in the systolic, diastolic, and mean arterial blood pressures of cockerel chicks exposed to Pb acetate when compared to the Pb acetate treated groups and groups administered only naringin (Fig. 2). The anti-hypertensive effect of naringin at both 80 mg/k and 160 mg/kg was demonstrated with significant reduction in systolic, diastolic, and mean arterial blood pressures as indicated in Fig. 2.

Immunohistochemistry

The immunolocalization of renal neutrophil gelatinase-associated lipocalin (NGAL) and angiotensin converting enzyme (ACE) revealed higher expressions in the Pb exposed chicks when compared to the control and groups co-treated with naringin (Figs. 3 and 4). The reduction in the expressions of ACE and NGAL is indicative of anti-hypertensive and nephroprotective effects of naringin against Pb acetate toxicity. The immunohistochemistry results showed a higher expression of cardiac troponin in the heart of the Pb exposed chicks when compared to the control. The expression of cardiac troponin was lower in chicks co-administered with naringin, those administered with naringin alone (Fig. 5). The cardioprotective effect of naringin as a metal chelating antioxidant was also demonstrated as indicated by reduction in the cardiac troponin expression in naringin treated chicks.

Discussion

Pb poisoning has been identified as a significant public health risk, particularly in developing countries, and has been considered a global issue affecting the health of various bird species (Herring et al. 2018; González et al. 2019; Monclús et al. 2020; Descalzo et al. 2021). Pb exposure has a number of negative effects on the body's systems, most notably is increased oxidative stress which plays a significant role in disease manifestations (Amini et al. 2019).

Negative inotropism and electrocardiogram abnormalities, particularly conduction defects have been reported following lead exposure (Williams et al. 1983; Xie et al. 2019; Eum et al. 2011). In an earlier study on isolated rat hearts exposed to Pb, Reza et al. (2008) reported statistically significant ($p < 0.05$) increases in heart rate after 8 and 12 weeks of exposure to Pb. Although, Pb treated chickens in our study had higher heart rates, the differences were however not statistically ($p > 0.05$) significant. The difference

observed is probably due to differences in the length of treatment, species of animals involved and the methodology of the study.

Pb administration has been reported to cause decrease in body weight, anemia, marked increase in oxidative stress, inflammation, and apoptosis (Gargouri et al. 2020; Oyem et al. 2021; Ileriturk et al. 2021; Ajarem et al. 2021; Kucukler et al. 2021). Interestingly, it was observed that Pb intoxication precipitated anemia as indicated with significant decrease in PCV, RBC and Hb concentration; confirming previous studies that correlated lead-induced anemia with Pb toxicity (Dsouza et al. 2021; Azab 2021). Furthermore, we observed significant decreases in the values of MCV, MCH and MCHC in exposed chicks, indicative of microcytic hypochromic anaemia. The hematinic property of naringin was demonstrated by significant improvement in PCV, RBC and Hb concentration and restoration of MCV, MCH and MCHC values.

The pathogenesis and pathophysiology of Pb-induced toxicity has been associated with free radical generation, induction of oxidative stress and depletion of antioxidant defence system (Nasiruddin et al. 2021; Gadde et al. 2021). From our study, marked increase in hydrogen peroxide (H_2O_2) generation and lipid peroxidation product (MDA) content associated with increased oxidative stress and free radical generation were observed in Pb untreated birds. Our findings are in consonance with earlier studies that reported that Pb toxicity precipitated marked oxidative stress, inflammation and apoptosis (Gagan et al., 2012; Omobowale et al. 2014; Oyagbemi et al. 2014; Matovic et al. 2015; Markiewicz-Górka et al. 2015; Omobowale et al. 2016). Also, Pb intoxication led to significant decrease in reduced glutathione (GSH) content, and activities of glutathione S-transferase (GST), superoxide dismutase (SOD) and glutathione peroxidase (GPx), in the Pb administered untreated cockerel chicks. However, data from our study revealed naringin co-administration improved antioxidant defence system and attenuated aforementioned oxidative stress markers. These findings therefore agreed with the reports of other studies on the antioxidant properties of naringin (Baranowska et al. 2021; Hassan et al. 2021; Li et al. 2021; Deng et al. 2022).

According to this study, the observed decreased in serum nitric oxide (NO) indicated that Pb toxicity adversely effected NO production. NO is important for the maintenance of vascular tone, blood pressure regulation, thereby improving blood pressure in hypertensive individuals and animals (Ahmad et al. 2018). Therefore, the recorded decrease in serum NO bioavailability was inversely proportional to high blood pressure. The toxicity of Pb and its implications on the induction of hypertension had been earlier documented (Broseghini-Filho et al. 2016; de Moura et al. 2021). Similarly, reduction in serum NO availability by Pb acetate has also been positively correlated with hypertension (Long et al. 2021; Ajarem et al. 2021; Kucukler et al. 2021). Our data revealed that administration of naringin improved serum NO and normalized high blood pressure precipitated by Pb toxicity. This is indicative of the anti-hypertensive action of naringin against Pb-induced hypertension in cockerel chicks. We report for the very first time, the anti-hypertensive effect of naringin, a metal chelating flavonoid in cockerel chicks. MPO has been reported as a novel marker of inflammation, oxidative stress, renal damage, and a diagnostic marker of cardiomyopathy including cardiac arrest, heart attack (Khan et al. 2018; Veltman et al. 2021; Peng et al.

2021; Wei et al. 2021; Sandamali et al. 2021). In our study, the elevated MPO activity was observed in birds exposed to Pb alone in comparison to the groups administered naringin. The reduction in the activity of MPO in birds treated with naringin indicated the anti-inflammatory, nephroprotective and cardioprotective effects of naringin in Pb treated birds. Furthermore, marked reduction in serum total protein of Pb intoxicated birds has been associated with kidney and heart dysfunction as reported by Moussa and Bashandy (2008). In this study, naringin administration was found to significantly improve serum total protein concentration.

Naringin is a widely available flavonoid found in citrus fruits that has a variety of pharmacological benefits including antioxidant, anti-inflammatory, and anti-apoptotic properties. In this study Naringin was seen to exhibit its antioxidant, anti-inflammatory and anti-apoptosis properties against oxidative stress, inflammation and apoptosis in groups administered 80 mg/kg and 160 mg/kg naringin, significantly increased the activities of enzymatic levels and non-enzymatic antioxidants, mitigated oxidative stress, improved serum NO, and reduced MPO activity.

Immunohistochemistry showed higher expression of ACE in the kidney of Pb treated birds relative to other groups that received naringin in combination with Pb or naringin alone. ACE converts angiotensin I to angiotensin II which causes narrowing of blood vessel leading to hypertension (Puspita et al. 2021). The increased expression of ACE in the kidney signified toxicity associated with Pb-induced hypertension. It is important to note that naringin co-administration conferred antihypertensive effect as indicated by the reduction in the expression of renal ACE. Therefore, we propose that the antihypertensive mechanism of naringin is through the reduction in the expression of renal ACE. Neutrophil gelatinase-associated lipocalin (NGAL) is a member of the lipocalin superfamily of proteins that has been extensively studied in acute kidney injury. NGAL is one of the most robustly expressed proteins in the kidney following ischemic or nephrotoxic injury in animals and human (Jia et al. 2021; Kovacevic et al. 2021; Najafi et al. 2021; Al-Brakati et al. 2021). NGAL is a recent marker that have been used to aid diagnosis and it has been shown to be more sensitive and specific than classical markers such as creatinine and blood urea nitrogen (BUN). However, new biomarkers such as NGAL does not only serve as indicators of glomerular damage, but also tubular impairment (Najafi et al. 2021; Al-Brakati et al. 2021). In this study, the kidneys of Pb treated birds showed high expression of NGAL when compared with birds co-treated with naringin. The observed high expression of NGAL was indicative of acute kidney injury and impairment of glomerular filtration rate. Therefore, it can be extrapolated that Pb intoxication from the present study induced acute renal damage as indicated by higher expression of NGAL in Pb-intoxicated birds relative to birds from other groups that received naringin in combination with Pb. Again, it can be clearly deduced that naringin offered nephroprotection against Pb-induced nephrotoxicity as demonstrated by reduced expression of renal NGAL in Pb-intoxicated birds. The proposed mechanism of nephroprotection of naringin might be through its antioxidant, free radical scavenging and anti-inflammatory properties.

Troponins are a group of proteins found in skeletal and heart (cardiac) muscle fibers that regulate muscular contraction (Tang et al. 2021; Jamil et al. 2021). Measurement of cardiac-specific troponin in the blood or tissues helps in detecting myocardial injury and myocardial necrosis (Veltman et al. 2021;

Karaarslan et al. 2021; Yuan et al. 2021). From our study, the immunohistochemistry revealed increase in expression of cardiac troponin in Pb-intoxicated birds. We observed lower expression of cardiac troponin in Pb-intoxicated treated birds. This is therefore an indication of cardioprotective effect of naringin against Pb-induced cardiotoxicity. We speculate that the mechanism of Pb-induced cardiotoxicity might be through exaggerated increase in oxidative stress, free radical generation, and depletion of antioxidant defense system in cardiac tissues. Therefore, naringin administration in poultry feed or water might be applicable in preventing cardiovascular diseases associated with heavy metal intoxication such as Pb.

Conclusion

Combining all, it can be concluded that naringin as a metal chelating flavonoid has antioxidant, anti-inflammatory, cardio and nephroprotective effects against lead acetate toxicity. Furthermore, naringin co-admiration with lead mitigated lead-induced oxidative stress, lowered high blood pressure, significantly improved serum nitric oxide bioavailability and antioxidant defense system.

Declarations

Acknowledgements: The authors deeply thank the African union through the the Pan African University Institute of Earth and Life Sciences Institute (PAULESI) for funding this work and Cardio-renal laboratory, University of Ibadan for the facilities used.

Ethical approval: The study was conducted following guidelines approved by the Animal Care and Use Research Ethics Committee (ACUREC) of the University of Ibadan (Approval number: UI-ACUREC/ 021-0421/16).

Consent to Participate: Not applicable

Consent to Publish: Not applicable

Authors Contributions: The authors, Chinomso Gift Ebirim, Oluwaseun Esan, Ademola Adetokunbo Oyagabemi and Temidayo Olutayo Omobowale designed the experiment. Chinomso Gift Ebirim, Oluwaseun Esan and Ademola Adetokunbo Oyagabemi performed the immunohistochemistry and biochemical assays. The blood pressure and electrocardiogram was performed by Gift Ebirim, Oluwaseun Esan and Temidayo Olutayo Omobowale. Moses Olusola Adetona, Ademola Adetokunbo Oyagabemi, Temidayo Olutayo Omobowale Omolade Abodunrin Oladele, Adeolu Alex Adedapo, Oluwafemi Oguntibeju, Momoh Audu Yakubu supervised, proof-read and approved the submission.

Funding: The study was funded by the African Union

Competing Interests: The authors declare that they have no competing interest

Availability of data and materials: Data will be made available on request

References

1. Ahmad A, Dempsey SK, Daneva Z, Azam M, Li N, Li P, Ritter JK (2018) Role of Nitric Oxide in the Cardiovascular and Renal Systems. Int J Mol Sci 19:2605
2. Ajarem JS, Hegazy AK, Allam GA, Allam AA, Maodaa SN, Mahmoud AM (2021) Effect of Visnagin on Altered Steroidogenesis and Spermatogenesis, and Testicular Injury Induced by the Heavy Metal Lead. Comb Chem High Throughput Screen. 24(6):758-766
3. Akinlolu AA, Ameen MO, Oyewopo AO, Kadir RE, Ahialaka O, Tijani S, Ogungbesan O, Bebeyi R, Adebayo S, Amoo T, Abdulazeez M (2021) Anticancer effects of Morinda lucida and Annona muricata on immunomodulations of Melatonin, tumor necrosis factor-alpha and p53 concentrations in lead acetate-induced toxicity in rats. Int J Health Sci (Qassim). 15(4):20-28
4. Al-Brakati A, Alsharif KF, Alzahrani KJ, Kabrah S, Al-Amer O, Oyouni AA, Habotta OA, Lokman MS, Bauomy AA, Kassab RB, Abdel Moneim AE (2021) Using Green Biosynthesized Lycopene-Coated Selenium Nanoparticles to Rescue Renal Damage in Glycerol-Induced Acute Kidney Injury in Rats. Int J Nanomedicine 16:4335-4349
5. Amini N, Sarkaki A, Dianat M, Mard SA, Ahangarpour A, Badavi M (2019) Protective effects of naringin and trimetazidine on remote effect of acute renal injury on oxidative stress and myocardial injury through Nrf-2 regulation. Pharm Rep 71(6): 1059-1066
6. Azab FE (2021) Ameliorative Effect of Kiwifruit (Actinidia deliciosa) against Lead-Induced Oxidative Stress in Wistar Albino Rats. Pak J Biol Sci 24(5):599-611
7. Baranowska M, Koziara Z, Suliborska K, Chrzanowski W, Wormstone M, Namieśnik J, Bartoszek A (2021) Interactions between polyphenolic antioxidants quercetin and naringenin dictate the distinctive redox-related chemical and biological behaviour of their mixtures. Sci Rep 11(1):12282
8. Beutler E, Duron O, Kelly MB (1963) Improved method for determination of blood glutathione reduced. J Lab Clin Med 61: 882- 888.
9. Broseghini-Filho GB, Almenara CC, Vassallo DV, Padilha AS (2016) Blood Pressure Decreases Following Lead Treatment Cessation: Highest NO Bioavailability Involved. Biol Trace Elem Res 170(2):410-414
10. Chanet A, Milenkovic D, Deval C, Potier M, Constans J, Mazur A, BennetauPelissero C, Morand C, Bérard AM (2012) Naringin, the major grapefruit flavonoid, specifically affects atherosclerosis development in diet-induced hypercholesterolemia in mice. J Nutr Biochem 23:469-477
11. De'Moura Magalhães BAB, Rodrigues LF, De'Oliveira TF, Vassallo DV, Simões MR (2021) Lead and mercury 28-day exposure at small concentrations reduces smooth muscle relaxation by decreasing cGMP. Toxicol Appl Pharmacol 413 (15):115405
12. Deng M, Jia X, Dong L, Liu L, Huang F, Chi J, Ma Q, Zhao D, Zhang M, Zhang R (2022) Structural elucidation of flavonoids from Shatianyu (Citrus grandis L. Osbeck) pulp and screening of key antioxidant components. Food Chem 366:130605

13. Descalzo E, Camarero PR, Sánchez-Barbudo IS, Martinez-Haro M, Ortiz-Santaliestra ME, Moreno-Opo R, Mateo R (2021) Integrating active and passive monitoring to assess sublethal effects and mortality from lead poisoning in birds of prey. Sci Total Environ 750:142260
14. D'souza HS, Menezes G, Thuppil V (2021) Ameliorative effects of nutritional minerals on lead-induced hematological alterations in male Wistar albino rats. Drug Chem Toxicol 1:1-5
15. Eum KD, Nie LH, Schwartz J, Vokonas PS, Sparrow D, Hu H, Weisskopf MG (2011) Prospective cohort study of lead exposure and electrocardiographic conduction disturbances in the Department of Veterans Affairs Normative Aging Study. Environ Health Perspect 119(7):940-944
16. Fakunle PB, Ajibade AJ, Oyewo EB, Hannah AO (2013) A study of some effects of aqueous extract of neem (Azadiractha indica) leaves on the lead acetate induced neurotoxicity in the superior colliculus of adult wistar rats (*Rattus norvegicus*). Br J Pharm Res 3(2): 217-231
17. Fengwei L, Zhenjian Z, Jin Q, Chuanbin C, Wei Y, Wang N (2021) Naringin attenuates rat myocardial ischemia/reperfusion injury via PI3K/Akt pathway-mediated inhibition of apoptosis, oxidative stress and autophagy. Exp Ther Med 22(2):811
18. Gadde R, Betharia S (2021) N, N' bis-(2-mercaptoethyl) isophthalamide (NBMI) exerts neuroprotection against lead-induced toxicity in U-87 MG cells. Arch Toxicol 95(8):2643-2657
19. Gagan F, Deepesh G, Archana T (2012) Toxicity of lead: A review with recent updates. Interdiscip Toxicol 5(2):47-58
20. Gargouri M, Akrouti A, Magné C, El Feki A, Soussi A. (2020) Protective effects of spirulina against hemato-biochemical alterations, nephrotoxicity, and DNA damage upon lead exposition. Hum Exp Toxicol 39(6):855-869
21. Golden NH, Warner SE, Coffey MJ (2016) A review and assessment of spent lead ammunition and its exposure and effects to scavenging birds in the United States. In Reviews of Environmental Contamination and Toxicology, eds. W. de Voogt, 237: 123-191
22. González F, Camacho M, Tiburón NP, Peña MZ, Rueda LR, Luzardo OP (2019) Suitability of anodic stripping voltammetry for routine analysis of venous blood from raptors. Environ Toxicol Chem 38(4):737-747
23. Gornal AG, Bardawill JC, David MM (1949) Determination of serum proteins by means of Biuret reaction. J Biol Chem 177: 751-766
24. Habig WH, Pabst MJ, Jakpoby WB (1974) Glutathione transferase, a first enzymatic step in mercapturic acid formation. J Biol Chem 249: 7130-7139
25. Haig SM, D'Elia J, Eagles-Smith C, Fair JM, Gervais J, Herring G, Rivers JW, Schulz JH (2014) The persistent problem of lead poisoning in birds from ammunition and fishing tackle. The Condor: Ornithological Applications 116:408-428
26. Hassan RA, Hozayen WG, Abo Sree HT, Al-Muzafar HM, Amin KA, Ahmed OM (2021) Naringin and Hesperidin Counteract Diclofenac-Induced Hepatotoxicity in Male Wistar Rats via Their Antioxidant, Anti-Inflammatory, and Antiapoptotic Activities. Oxid Med Cell Longev 2021:9990091

27. Helander B, Krone O, Räikkönen J, Sundbom M, Ågren E, Bignert A (2021) Major lead exposure from hunting ammunition in eagles from Sweden. Sci Total Environ 795:148799
28. Herring G, Eagles-Smith CA, Varland DE (2018) Mercury and lead exposure in avian scavengers from the Pacific Northwest suggest risks to California condors: Implications for reintroduction and recovery. Environ Pollut 243(Pt A):610-619
29. Hossain MA, Mostofa M, Alam MN, Sultana MR, Rahman MM (2014) The ameliorating effects of garlic (allium sativum) against lead (pb) intoxication on body weight, dressing percentages, feed consumption and feed conversion ratio in lead induced broiler chickens. Bangl J Vet Med 12 (1): 1-7
30. Ileriturk M, Benzer F, Aksu EH, Yildirim S, Kandemir FM, Dogan T, Dortbudak MB, Genc A (2020) Chrysin protects against testicular toxicity caused by lead acetate in rats with its antioxidant, anti-inflammatory, and antiapoptotic properties. J Food Biochem 45(2):e13593
31. Jamil S, Jamil G, Mesameh H, Qureshi A, AlKaabi J, Sharma C, Aziz F, Al-Shamsi AR, Yasin J (2021) Risk factor comparison in young patients presenting with acute coronary syndrome with atherosclerotic coronary artery disease vs. angiographically normal coronaries. Int J Med Sci 18(15):3526-3532
32. Jia Z, Li W, Bian P, Yang L, Liu H, Pan D, Dou Z (2021) Ursolic acid treats renal tubular epithelial cell damage induced by calcium oxalate monohydrate via inhibiting oxidative stress and inflammation. Bioengineered 12(1):5450-5461
33. Karaarslan K, Abud B, Karacelik MCB (2021) Cardioplegia Application with A Hand-Squeezed Cardioplegia Bag. Is It Safe? Heart Surg Forum 24(4): E619-E623
34. Khan AA, Alsahli MA, Rahmani AH (2018) Myeloperoxidase as an Active Disease Biomarker: Recent Biochemical and Pathological Perspectives. Medical sciences (Basel, Switzerland) 6(2): 33
35. Kovacevic S, Ivanov M, Zivotic M, Brkic P, Miloradovic Z, Jeremic R, Mihailovic-Stanojevic N, Vajic UJ, Karanovic D, Jovovic D, Nesovic Ostojic J (2021) Immunohistochemical Analysis of 4-HNE, NGAL, and HO-1 Tissue Expression after Apocynin Treatment and HBO Preconditioning in Postischemic Acute Kidney Injury Induced in Spontaneously Hypertensive Rats. Antioxidants (Basel) 10(8):1163
36. Kucukler S, Benzer F, Yildirim S, Gur C, Kandemir FM, Bengu AS, Ayna A, Caglayan C, Dortbudak MB (2021) Protective Effects of Chrysin Against Oxidative Stress and Inflammation Induced by Lead Acetate in Rat Kidneys: A Biochemical and Histopathological Approach. Biol Trace Elem Res 199(4):1501-1514
37. Liu CM, Zheng GH, Ming QL, Sun JM, Cheng C. Protective effect of puerarin on lead-induced mouse cognitive impairment via altering activities of acetyl cholinesterase, monoamine oxidase and nitric oxide synthase. Environ Toxicol Pharmacol. 35(3):502-510
38. Long X, Sun F, Wang Z, Liu T, Gong J, Kan X, Zou Y, Zhao X (2021) Lactobacillus fermentum CQPC08 protects rats from lead-induced oxidative damage by regulating the Keap1/Nrf2/ARE pathway. Food Funct 12(13):6029-6044
39. Matović V, Buha A, Đukić-Ćosić D, Bulat Z (2015) Insight into the oxidative stress induced by lead and/or cadmium in blood, liver and kidneys Food Chem Toxicol 78:130-140

40. Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 217: 3170-3175
41. Monclús L, Shore RF, Krone O (2020) Lead contamination in raptors in Europe: A systematic review and meta-analysis. Sci Total Environ 15: 748:141437
42. Moussa SA, Bashandy SA (2008) Biophysical and Biochemical changes in the blood of rats exposed to lead toxicity. Romanian J Biophys 18(2): 123-133
43. Najafi L, Keshtkar Rajabi S, Pirsaheb S, Keyvani H, Khajavi A, Shati M, Hadavand F, Amouzegar A (2021) Assessment of Serum and Urine Neurophil Gelatinase- Associated Lipocalin (s-NGAL and u-NGAL) Level as a Predictive Factor of Disease Progression in Diabetic Nephropathy in Type 2 DM. Iran J Kidney Dis 15(4):270-278
44. Nasiruddin Rana M, Karim N, Changlek S, Atiar Rahman M, Tangpong J, Hajjar D, Alelwani W, Makki AA (2020) Thunbergia laurifolia leaf extract partially recovers lead-induced renotoxicity through modulating the cell signaling pathways. Saudi J Biol Sci 27(12):3700-3710
45. Olaleye SB, Adaramoye OA, Erigbali PP, Adeniyi OS (2007) Lead exposure increases oxidative stress in gastric mucosa of HCL/ethanol exposed rat. World J Gastreoenterol 13: 5121-5126
46. Omobowale TO, Oyagbemi AA, Akinleye SA, Ola-Davies OE, Adebowale BS, Olopade JO, Adedapo AA (2016) Effect of Exposure and Withdrawal on Lead-induced Toxicity and Oxidative Stress in Cardiac Tissues of Rats. Toxicol Intern 23(1): 12-17
47. Omobowale TO, Oyagbemi AA, Akinrinde AS, Sabab AB, Daramolaa OT, Ogunpolua BS, Olopade JO (2014) Failure of recovery from lead induced hepatoxicity and disruption of erythrocyte antioxidant defense system in Wistar rats. Environ Toxicol Pharm 37:1202–1211
48. Oyagbemi AA, Omobowale TO, Akinrinde AS, Saba AB, Ogunpolu BS, Daramola O (2015) Lack of reversal of oxidative damage in renal tissues of lead acetate-treated rats. Environ Toxicol 30:1235-1243
49. Oyagbemi AA, Omobowale TO, Akinrinde AS, Saba AB, Ogunpolu BS, Daramola O (2015). Lack of reversal of oxidative damage in renal tissues of lead acetate-treated rats. Environ Toxicol 30(11): 1235-1243
50. Oyagbemi AA, Omobowale TO, Awoyomi OV, Ajibade TO, Falayi OO, Ogunpolu BS, Okotie UJ, Asenuga ER, Adejumobi OA, Hassan FO, Ola-Davies OE, Saba AB, Adedapo AA, Yakubu MA (2019) Cobalt chloride toxicity elicited hypertension and cardiac complication via induction of oxidative stress and upregulation of COX-2/Bax signaling pathway. Hum Exp Toxicol 38(5): 519-532
51. Oyem JC, Chris-Ozoko LE, Enaohwo MT, Otabor FO, Okudayo VA, Udi OA. (2021) Antioxidative properties of Ocimum gratissimum alters Lead acetate induced oxidative damage in lymphoid tissues and hematological parameters of adult Wistar rats. Toxicol Rep. 10: 8:215-222
52. Pawlas K (2015) Effects of chronic exposure to lead, cadmium, and manganese mixtures on oxidative stress in rat liver and heart. Arh Hig Rada Toksikol 66: 52-62
53. Peng L, Li X, Li Y, Zhao W, Nie S, Yu H, Qi Y, Qin Y, Zhang H (2021) Increased concentrations of myeloperoxidase in serum and serum extracellular vesicles are associated with type 2 diabetes

mellitus. Clin Chim Acta 522:70-76

54. Puspita FM, Yunir E, Agustina PS, Sauriasari R (2021) Effect of Angiotensin Receptor Blocker and Angiotensin Converting Enzyme Inhibitor on Kidney Function and Blood Potassium Level in Indonesian Type 2 Diabetes Mellitus with Hypertension: A Three-Month Cohort Study. Diabetes Metab Syndr Obes 7(14):3841-3849
55. Rani N, Bharti S, Manchanda M, Nag T, Ray R, Chauhan S, Kumari S, Dharamvir A (2013) Regulation of Heat Shock Proteins 27 and 70, p-Akt/p-eNOS and MAPKs by Naringin Dampens Myocardial Injury and Dysfunction In Vivo after Ischemia/Reperfusion. PloS one. 8. e82577. 10.1371/journal.pone.0082577.
56. Sandamali JAN, Hewawasam RP, Jayatilaka KAPW, Mudduwa LKB. (2021) Cinnamomum zeylanicum Blume (Ceylon cinnamon) bark extract attenuates doxorubicin induced cardiotoxicity in Wistar rats. Saudi Pharm J 29(8):820-832
57. Shvachiy L, Geraldes V, Amaro-Leal Â, Rocha I. (2020) Persistent Effects on Cardiorespiratory and Nervous Systems Induced by Long-Term Lead Exposure: Results from a Longitudinal Study. Neurotox Res. 37(4):857-870
58. Tang X, Xu Y, Dai X, Xing Y, Yang D, Huang Q, Li H, Lv X, Wang Y, Lu D, Wang H (2021) The Long-term Effect of Dobutamine on Intrinsic Myocardial Function and Myocardial Injury in Septic Rats with Myocardial Dysfunction. Shock 56(4):582-592
59. Thomas A. Bailey and Merle M. Apo (2016) Pharmaceutical Products Commonly Used in Avian Medicine. Edited by: Jaime Samour. ISBN: 978-0-7234-3832-8. 637-678.
60. Thuppil V, Tannir S (2013) Treating Lead Toxicity: Possibilities beyond Synthetic Chelation. J Krishna Inst Med Sci Univ 2:4-31
61. Varshney R, Kale RK (1990) Effect of calmodulin antagonists on radiation induced lipid peroxidation in microsomes. Int J Radiat Biol 58:733-743
62. Vaziri ND (2008) Mechanisms of lead-induced hypertension and cardiovascular disease. Am J Physiol Heart Circ Physiol 295:H454-H465
63. Veltman D, Wu M, Pokreisz P, Claus P, Gillijns H, Caluwé E, Vanhaverbeke M, Gsell W, Himmelreich U, Sinnaeve PR, Janssens SP (2021) Clec4e-Receptor Signaling in Myocardial Repair After Ischemia-Reperfusion Injury. JACC Basic Transl Sci 6(8):631-646
64. Venkateswara RP, Kiran SDVS, Rohini P, Bhagyasree P (2017) Flavonoid: A review on Naringenin. J Pharmacog Phytochem 6(5): 2778- 2783
65. Wei H, Xue Q, Sun L, Lv J (2021) BRD4 inhibition protects against myocardial ischemia/reperfusion injury by suppressing inflammation and oxidative stress through PI3K/AKT signaling pathway. J Cardiovasc Pharmacol doi: 10.1097/FJC.0000000000001138
66. Wildemann TM, Mirhosseini N, Siciliano SD, Weber LP (2015) Cardiovascular responses to lead are biphasic, while methylmercury, but not inorganic mercury, monotonically increases blood pressure in rats. Toxicol 3(328):1-11

67. Wolff SF (1994) Ferrous ion oxidation in the presence of ferric ion indicator xylenol orange for measurement of hydrogen peroxides. Methods Enzymol 233:182–189
68. Wu Y, Cai C, Xiang Y, Zhao H, Lv L, Zeng C (2021) Naringin Ameliorates Monocrotaline-Induced Pulmonary Arterial Hypertension Through Endothelial-To-Mesenchymal Transition Inhibition. Front Pharmacol 12:696135
69. Xie J, Du G, Zhang Y, Zhou F, Wu J, Jiao H, Li Y, Chen Y, Ouyang L, Bo D, Feng C, Yang W, Fan G (2019) ECG conduction disturbances and ryanodine receptor expression levels in occupational lead exposure workers. Occup Environ Med 76(3):151-156
70. Xu L-H, Mu F-F, Zhao J-H, He Q, Cao C-L, Yang H, Liu Q, Liu XH, Sun XJ (2015) Lead Induces Apoptosis and Histone Hyperacetylation in Rat Cardiovascular Tissues. PLoS ONE 10(6): e0129091. doi:10.1371/ journal. pone.0129091
71. Yuan Z, Wang L, Chen J, Su W, Li A, Su G, Liu P, Zhou X (2021) Electrochemical strategies for the detection of cTnI. Analyst 146(18):5474-5495

Figures

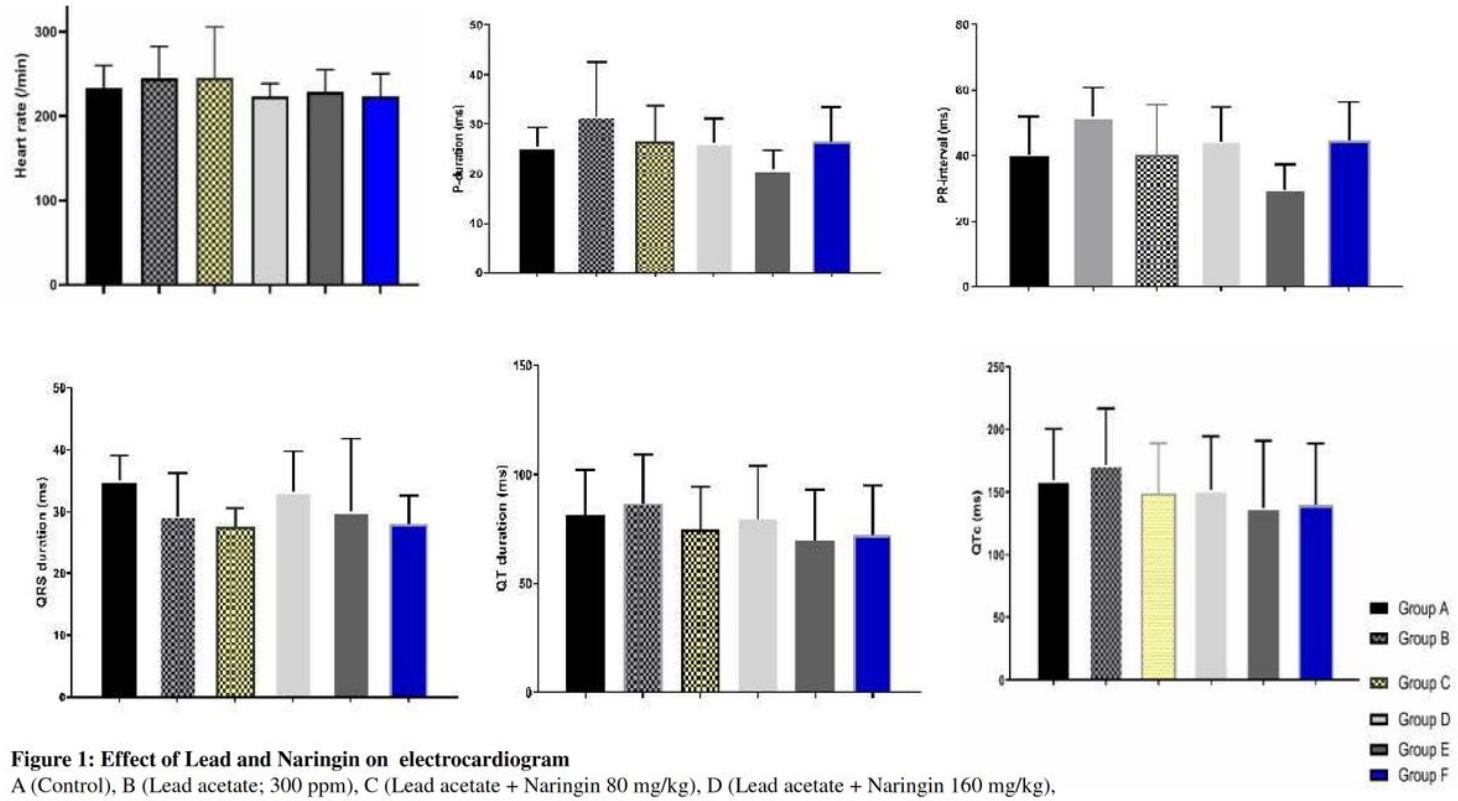

Figure 1: Effect of Lead and Naringin on electrocardiogram
A (Control), B (Lead acetate; 300 ppm), C (Lead acetate + Naringin 80 mg/kg), D (Lead acetate + Naringin 160 mg/kg), E (Naringin 80 mg/kg), F (Naringin 160 mg/kg).

Figure 1

See image above for figure legend.

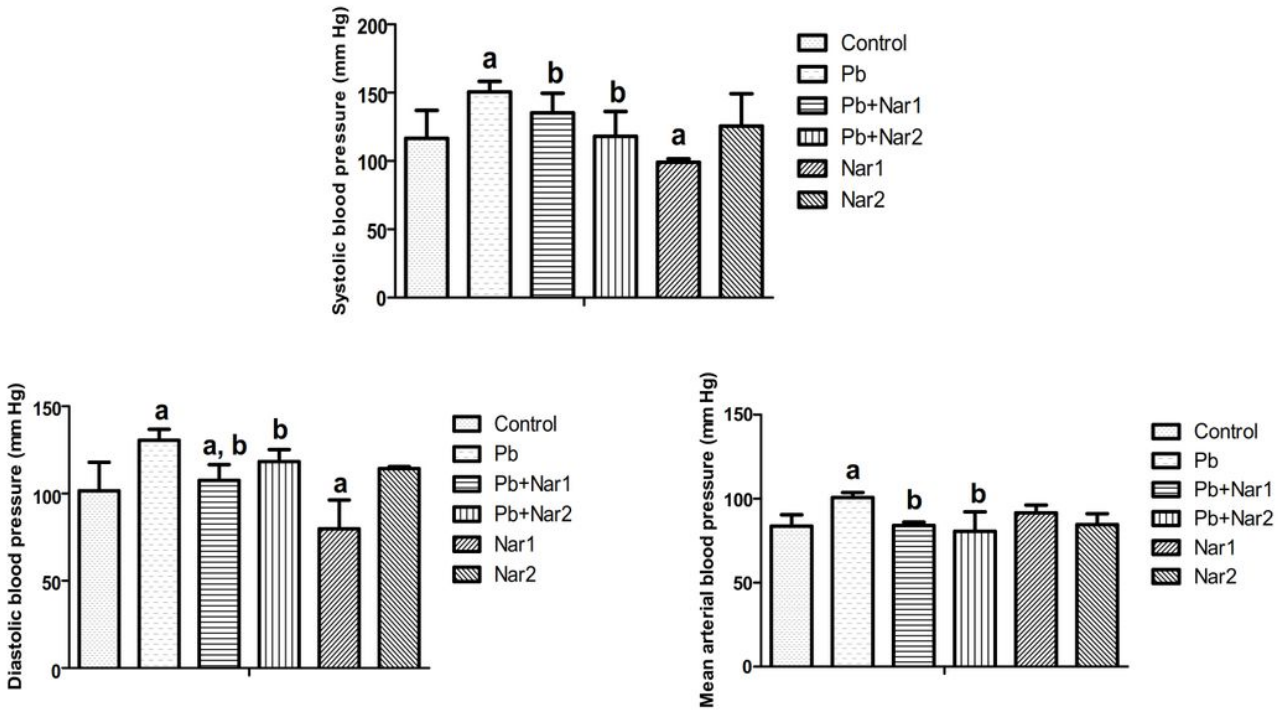

Figure 2: Effect of Lead and Naringin on blood pressure. A (Control), B (Lead acetate; 300 ppm), C (Lead acetate + Naringin 80 mg/kg), D (Lead acetate + Naringin 160 mg/kg), E (Naringin 80 mg/kg), F (Naringin 160 mg/kg).

Figure 2

See image above for figure legend.

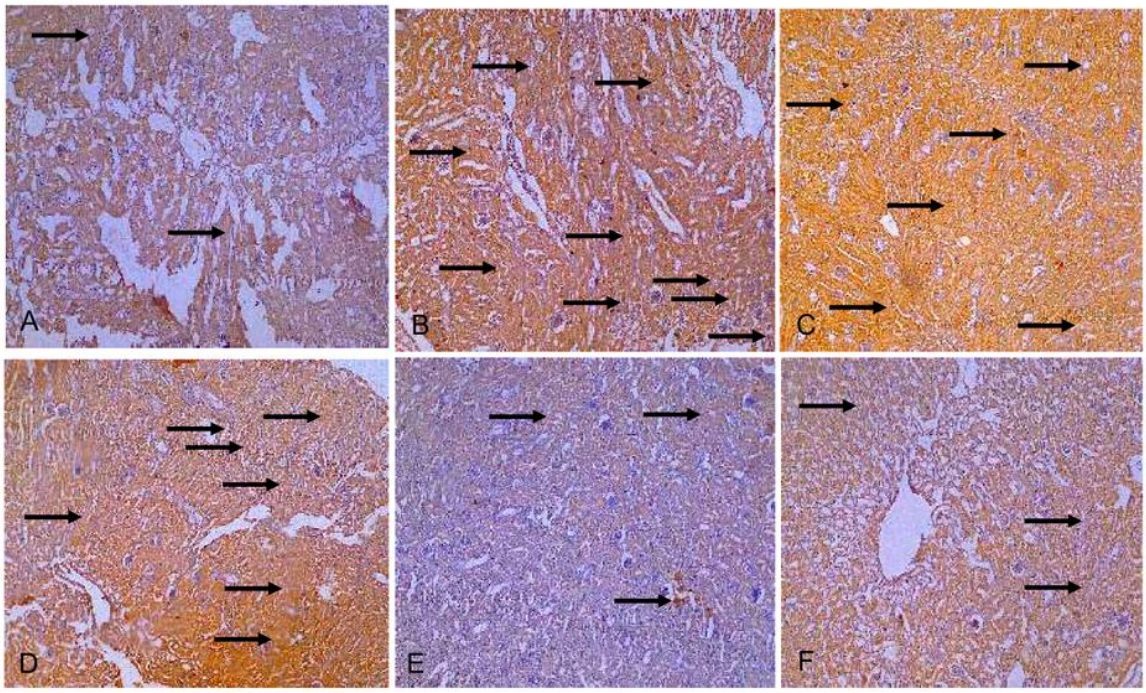

Figure 3: **The immunohistochemistry of renal Neutrophil gelatinase-associated lipocalin (NGAL).** A (Control), B (Lead acetate; 300 ppm), C (Lead acetate + *Naringin 8*0 mg/kg), D (Lead acetate + Naringin *16*0 mg/kg), E (Naringin 80 mg/kg), F (Naringin 160 mg/kg). Slides stained with high definition Heamtoxylin. (Magnification x 100)

Figure 3

See image above for figure legend.

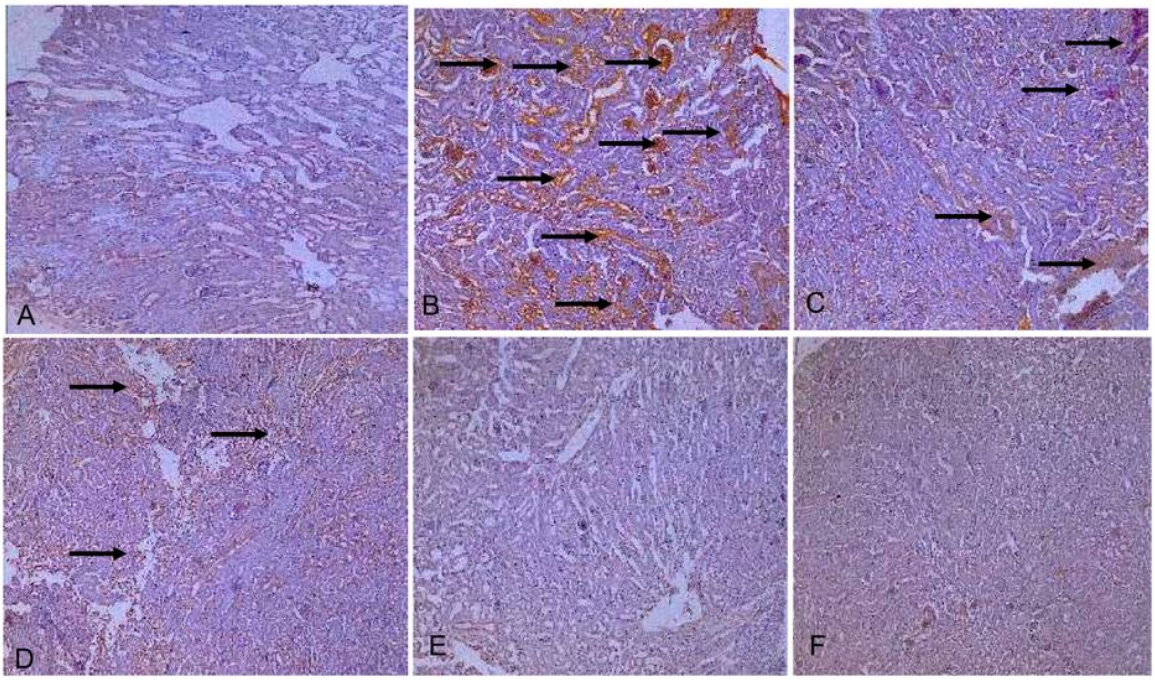

Figure 4: **The immunohistochemistry of renal angiotensin converting enzyme (ACE).** A (Control), B (Lead acetate; 300 ppm), C (Lead acetate + *Naringin 8*0 mg/kg), D (Lead acetate + Naringin *16*0 mg/kg), E (Naringin 80 mg/kg), F (Naringin 160 mg/kg). Slides stained with high definition Heamtoxylin. (Magnification x 100)

Figure 4

See image above for figure legend.

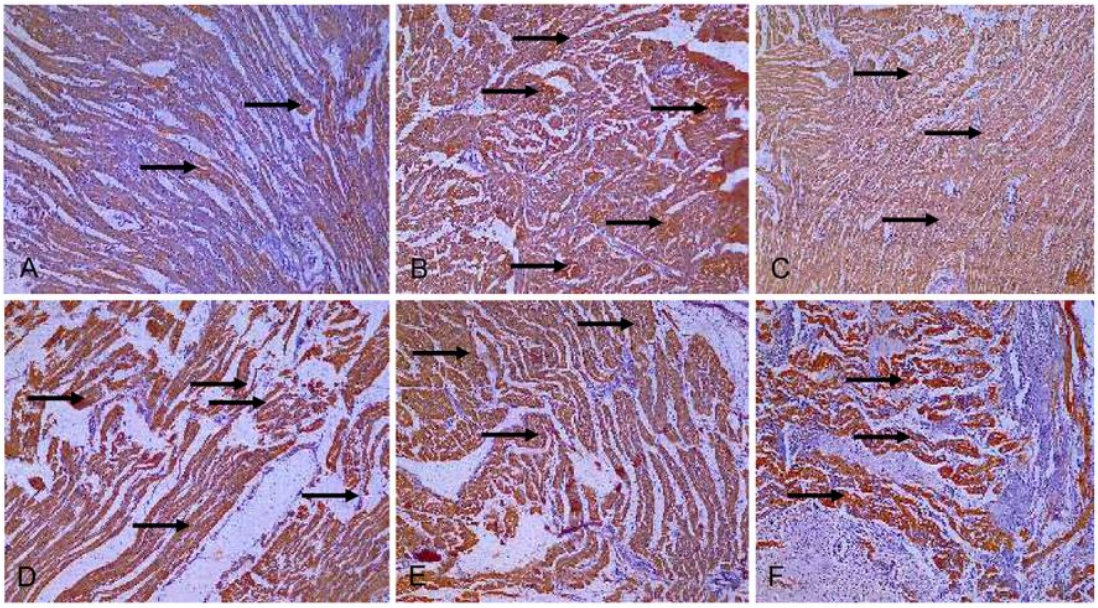

Figure 5: **The immunohistochemistry of cardiac troponin.** A (Control), B (Lead acetate; 300 ppm), C (Lead acetate + Naringin 80 mg/kg), D (Lead acetate + Naringin 160 mg/kg), E (Naringin 80 mg/kg), F (Naringin 160 mg/kg). Slides stained with high definition Heamtoxylin. (Magnification x 100)

Figure 5

See image above for figure legend.