Niger. J. Physiol. Sci. 38 (December 2023): 239 – 246
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Full length Research Article

Immunohistochemical and Morphological Changes Associated with Hepatic Damage in Lead Acetate-Induced Toxicity and Mitigatory Properties of Naringin in Cockerel Chicks

Esan O.[a], Ajibade T.O.[b]*, Ebirim C.G.[c], Adetona M.O.[d], Oyagabemi A.A.[c], Omobowale T.O.[a], Oladele O.A.[a], Adedapo A.A.[e], Oguntibeju O.[f], Yakubu M.A.[g], Nwulia E.[h]

Departments of [a]Veterinary Medicine, [b]Veterinary Physiology and Biochemistry, [e]Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria
[c]Pan African University, Institute of Earth and Life Sciences Institute, Nigeria
[d]Department of Anatomy, Faculty of Basic Medical Sciences, University of Ibadan, Nigeria
[f]Phytomedicine and Phytochemistry Group, Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville 7535, South Africa
[g]Department of Environmental & Interdisciplinary Sciences, College of Science, Engineering & Technology, COPHS, Texas Southern University, Houston, TX, USA.
[h]Howard University, College of Medicine, Department of Psychiatry and Behavioral Sciences, Howard University Hospital, 2041 Georgia Avenue, Washington, DC 20060, USA.

Summary: Lead (Pb) toxicity constitutes a major health hazard to both humans and animals especially in the developing countries. It is a ubiquitous environmental contaminant found in the air essentially because of unregulated mining and other industrial activities. Lead can be found naturally in the soil thus, contaminating crops for human and animal food, as well as run-off water and air pollution. Intensively and extensively reared domestic chickens are exposed to contamination via inhalation and ingestion of contaminated food materials. Naringin, a product from citrus plant has been described to possess excellent metal chelating ability. Naringin is rich in flavonoid with attendant antioxidant, anti-autophagy, anti-inflammatory, hepatoprotective and cardio-nephroprotective properties. This study was conducted to investigate the hepatoprotective and modulation of oxidative stress in commercial cockerel chickens by Naringin. Thirty-six commercial cockerel chickens were randomly assigned into six groups A-F of six birds each viz: Group A served as control group while groups B, C, and D received Lead acetate at 300 ppm via drinking water continuously till the end of the experiment. In addition, groups C and D were treated with Naringin at 80 mg/kg and 160mg/kg, respectively, via oral gavage for 8 weeks. Groups E and F were administered naringin only at 80mg/kg and 160mg/kg respectively for eight weeks. Pb toxicity induced degenerative changes in the histological sections as well as, higher expression of hepatic caspase 3 as shown by immunohistochemistry. There was increased oxidative stress markers (H_2O_2, MDA) and depletion of the antioxidant defense system markers SOD, GPx, GSH, and GST. It concluded that Co- treatment with Naringin ameliorated oxidative stress, enhanced antioxidant defense system, reduced the expression of hepatic caspase 3 thus, offering protection against lead acetate-induced derangements in the liver of commercial cockerel chickens.

Keywords*: Lead acetate, Naringin, Oxidative stress, Immunohistochemistry*

**Authors for correspondence: toajibade@gmail.com, Tel: +234-9071842142*
Manuscript received- October 2022; Accepted: June 2023
DOI: https://doi.org/10.54548/njps.v38i2.13
©Physiological Society of Nigeria

INTRODUCTION

Lead (Pb) has been described as a toxic heavy metal causing significant health problems worldwide (WHO, 2021). It is found majorly as naturally occurring metal within the Earth's crust either in trace or large quantities as influenced by human activities such as mining and other industrial activities (Teerasarntipan, 2020). Also, Pb is one of the most frequently found metal in the environment considering its extensive industrial uses that include manufacture of different range of batteries, additives in paints, use in fuel to increase the octane ratings, ammunitions, and manufacture of radiation protection materials (Babalola and Areola, 2010).

Exposure to Pb in humans has been estimated to account for almost a million deaths as well as, loss of about 21.7 million years of healthy life worldwide with the highest burden in developing countries (IHME, 2019). IHME (2019) showed that lead exposure was ostensibly responsible for approximately 62.5% of the global burden

of developmental cognitive disability of unknown aetiology in children as well as 8.2% of global hypertensive heart disease in adults. Major sources of environmental Pb contamination to the avian species is through anthropogenic activities, polluted air carrying lead dust, water runoffs from mining sites, contaminated feed ingredients and erosions (De Francisco *et al*., 2003). Pb contamination is usually transferred to food animals through direct or indirect exposures and is mostly through the deposition of contaminants in soil and aquatic environment from emissions and human activities (Hossain *et al*., 2014). A low concentration of 1.0 mg/kg has been found to significantly cause problems in growth and reduction of blood δ-aminolaevulinic acid dehydratase enzyme (Hossain *et al*., 2014).

By affecting the central and peripheral nervous system, hematopoietic system, cardiovascular system, kidneys, liver, and reproductive system, Pb has been discovered to trigger a variety of physiological, biochemical, and behavioral dysfunctions in animals and humans. Toxicity studies is closely correlated with the route and duration of exposure, level of intake, absorption rate, and efficiency of excretion (Fakunle *et al*., 2013; Matovic *et al*., 2015).
Pb is a non-redox metal, and the principal mechanism of toxicity is via the induction of oxidative stress through the activities of reactive oxygen species (ROS) as well as, the depletion of the antioxidant defense system in various tissues (Matovic *et al*., 2015; Oyagbemi *et al*., 2015). Free radicals and ROS generated have been reported to precipitate lipid peroxidation and depletion of antioxidant status, by altering cellular membrane integrity and fatty acid composition (Oyagbemi *et al*., 2015; Omobowale *et al*., 2016). Pb toxicity has been shown to result in activation of Nuclear Factor Kappa β (NF-Kβ) that is a master regulator of pro-inflammatory cytokines (Omobowale *et al*., 2016; Vallverdú-Coll *et al*., 2019).

Pb can be absorbed into the bloodstream through either the respiratory or gastrointestinal tracts depending on the route of exposure and is eventually re-distributed into the bones and soft tissues, with significant amounts in the bones, liver, and kidneys (Oyagbemi *et al*., 2014). Pb affects the immune system of avian species causing imbalances between Th1 and Th2-type responses mounted by the separate classes of T-lymphocytes, leading to a depression of the Th1 responses associated with cell mediated immunity (Vallverdú-Coll *et al*., 2019).

A major cardinal step in disease prevention and management is neutralization of free radicals' activities/ROS by antioxidants deactivation or stabilization of these free radicals/ROS to prevent them from causing significant damage to biological systems (Munazza and Muhammad, 2018).

Naringin is a naturally occurring flavonoid glycoside that has a wide range of bioactive activities such as anti-inflammatory, free radical scavenging, antioxidant and immunomodulatory effect on animal and human health (Venkateswara *et al*., 2017). Flavonoids are important components of the human diet. Though not considered as nutrients, they are major class of plant secondary metabolites that serve a range of functions including antioxidant activities and other various health benefits (Venkateswara *et al*., 2017). Naringin is rapidly transformed into naringenin by the action of enzymes such as α-rhamnosidase and β-glucosidase and has been reported to have protective effect against radiation induced chromosomes damage in mouse bone marrow and influence the propagation reactions of free radicals and their formation by inhibiting the enzymes involved and by chelating transition metals (Cavia-Saiz *et al*., 2010).

Naringin has been shown to have neuroprotective properties and has been demonstrated to have an impact on iron-induced oxidative stress as well as chelate certain metals, such as nickel (Golechha *et al*., 2010; Ganesh and Tiyagura, 2011; Kulasekaran *et al*., 2011). In their studies, Ozkaya *et al*., (2016) and Wang *et al*., (2012) found that naringin and naringenin could lower oxidative damage in the liver and functioned as Pb chelating agents. Naringin used as a dietary supplement in poultry improved the quality, oxidative stability and nutritional value of broiler meat (Goliomytis *et al*.,2015; Hager-Theodoride *et al*., 2021) in their investigation.

MATERIALS AND METHODS

Chemicals/Reagents: Lead acetate trihydrate ($(C_2H_3O_2)2Pb.3H_2O$), Naringin, oxidized glutathione (GSSG), and thiobarbituric acid (TBA) were purchased from Sigma (St. Louis, MO, USA). Biotinylated secondary antibody and caspase 3 monoclonal antibody was purchased from Elabscience Biotechnology, China.

Animal housing and Experimental design: Thirty-six-day old cockerel chicks were reared together for six weeks before randomly distributed into six groups (A-F) of six chickens per group. Group A served as the control, Group B was administered (Pb; 300 ppm) only, Group C was administered (Pb and 80 mg/kg Naringin), Group D was administered (Pb and 160 mg/kg Naringin), Group E was administered (80 mg/kg Naringin) only and Group F was administered (160 mg/kg Naringin) only. The dosage of Pb administered was selected based on the previous study of Amini *et al*. (2021). The chickens were fed starter chick feed and later growers mash from Top® Feeds Limited, Ibadan as often as required, and water was made available *ad libitum*. Pb and NAR were co-administered via drinking water and oral gavage respectively for eight weeks. Ethical approval was sought and granted by the university of Ibadan animal care and use research committee with the approval number UI-ACUREC/ 021-0421/16.

Sample Collection and Preparation: Twenty-four hours after the last administration, chickens were euthanized by quick cervical dislocation. The whole liver was rapidly dissected out on ice, rinsed with distilled water, blotted with filter paper, and weighed for the determination of organ weight as well as relative organ weight respectively for biochemical assay. Subsequently, about 2 g each of the liver sample was collected inside universal bottle and frozen until biochemical assays while, samples for histology were fixed in 10% formalin.

Preparation of hepatic post-mitochondrial fractions: The harvested liver samples were chopped into bits and homogenized in homogenizing buffer (0.1M phosphate buffer, pH 7.4) using a Teflon® homogenizer. The

homogenate was centrifuged at 10,000 × g rpm for 10 minutes with a cold centrifuge at −4°C to obtain post-mitochondrial fractions (PMFs). The supernatants obtained (PMFs) obtained were used for biochemical assays.

Biochemical Assays

Determination of antioxidant defense system: Superoxide dismutase (SOD) was determined according to Misra and Fridovich's method with slight modification by Oyagbemi *et al*., (2015). Additionally, the glutathione peroxidase (GPx) activity was determined in accordance with Beutler *et al*., (1963), glutathione S-transferase (GST) activity was calculated using Habig *et al*. (1974) method using 1-chloro-2,4-dinitrobenzene as substrate, and reduced glutathione (GSH) content was calculated using Ellman method (1959).

Determination of markers of oxidative stress: The method outlined by Wolf's (1994) was used to measure the formation of hydrogen peroxide (H_2O_2) while Malondialdehyde (MDA), a product of lipid peroxidation was determined using the technique outlined by Varshney and Kale (1990).

Determination of serum Total protein: Serum total protein was determined by Biuret's method as described by Gornal *et al.,* (1949).

Histopathology: Hepatic tissues were fixed in 10% formalin embedded in paraffin wax, and sections of 5-6 mm in thickness were made and thereafter stained with Hematoxylin and Eosin (H&E) as previously described by Drury and Walington, (1976).

Immunohistochemistry: Caspase 3 Monoclonal Antibody in the liver was assayed using an immunohistochemistry procedure as described by Oyagbemi *et al*. (2019) with a small modification using a 2-step plus Poly-HRP Anti Mouse/Rabbit IgG Detection System with DAB solution (Catalog number: E-IR-R217 from Elabscience Biotechnology®, China). The liver samples were embedded in paraffin, fixed with 10% paraformaldehyde, and sectioned at a thickness of 5 m. The slides were therefore dewaxed for 2 minutes in a 100% solution of xylene before being hydrated for 2 minutes in each of three distinct concentrations of ethanol (100%, 90%, and 80%). Thereafter, the slides were rinsed and immersed for 5 minutes in a PBS buffer tank. A citrate buffer solution containing 2.1 g of citric acid monohydrate and 14.75 g of trisodium citrate dehydrate, adjusted to pH 6.0, was used to perform the antigen retrieval in a microwave oven. Endogenous peroxide (H_2O_2 block) was done in accordance with the kit's instructions (E-IR-217C) from the manufacturer. The sections were covered with drops of H_2O_2 before being incubated in a humidifying chamber at room temperature for 10 minutes. The slides were rinsed before placing once more in the PBS tank for five minutes at room temperature. To prevent nonspecific binding, goat serum (E-1R-R217A) was applied to the slides, which were then incubated for 30 min at room temperature in a humidifying chamber. Following incubation, the tissues were probed with primary antibodies, specifically Caspase 3 Monoclonal Antibody, and incubated for 2 hr at room temperature in a humidifying chamber. Thereafter, 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. The slides were then rinsed and submerged for five minutes in a PBS tank. Finally, a few drops of the substrate diaminobenzidine (DAB) were added after it had been prepared by mixing 50µL of DAB concentrate (E-1R-R217D) with 1 mL of DAB solution (E-1R-R217E) in the dark for 10 seconds. The reaction was stopped with deionized water, and slides were submerged in hematoxylin for 3 seconds prior to rinsing with PBS. The slides were immersed for two minutes each in 70%, 80%, 90%, 100%, and 100% ethanol, followed by 100% xylene. Slides were taken out, given enough time to dry, and then a DPX mountant and cover slip was used. Sections were examined using a digital camera and a Leica software application package version 3.4 light microscope (Leica LAS-EZ®).

Statistical analysis

The Student's t-test was used to evaluate the test of significance between the groups. All values were expressed as mean ± standard deviation. Additionally, the One-Way Analysis of Variance (ANOVA) using GraphPad Prism 5.0's Turkey's post-hoc test was performed, with p-Values less than 0.05 regarded as statistically significant.

RESULTS

Antioxidant defense status and oxidative stress markers: There was significant increase in the valves of makers of oxidative stress, H_2O_2 and MDA, when compared with the control (Group A) and this was ameliorated in both groups treated with different doses of naringin as indicated in figures 1 and 2 respectively.

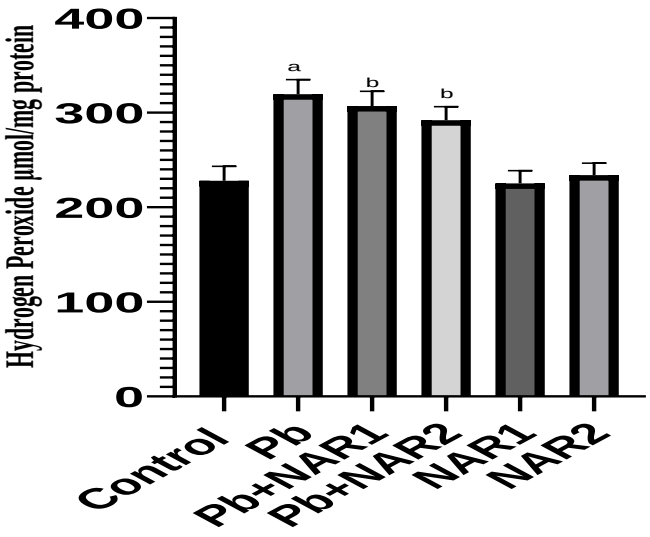

Figure 1:
Effect of Lead toxicity and Naringin on hepatic hydrogen peroxide (H_2O_2) generation. A (Control), Pb (Lead acetate; 300 ppm), Pb+Nar1 (Lead acetate + Naringin 80 mg/kg), Pb+Nar2 (Lead acetate + Naringin 160 mg/kg), Nar1 (Naringin 80 mg/kg), Nar2 (Naringin 160 mg/kg). Notes: Superscript (a) indicates significant difference at $p<0.05$ when compared with Control. Superscript (b) indicates significant difference when compared with Pb.

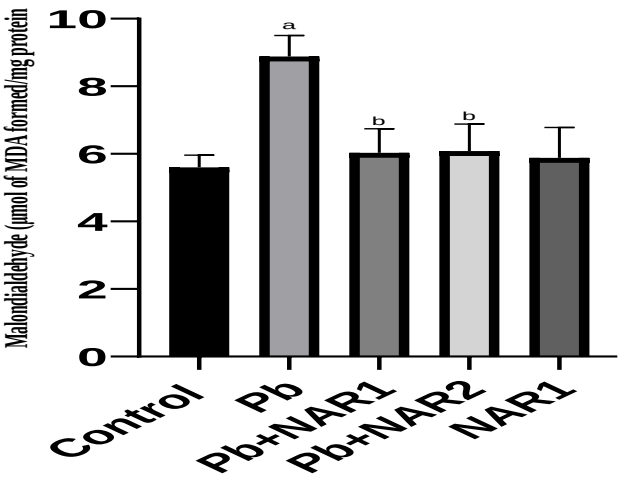

Figure 2:
Effect of Lead toxicity and Naringin on hepatic Malondialdehyde (MDA) content. A (Control), Pb (Lead acetate; 300 ppm), Pb+Nar1 (Lead acetate + Naringin 80 mg/kg), Pb+Nar2 (Lead acetate + Naringin 160 mg/kg), Nar1 (Naringin 80 mg/kg), Nar2 (Naringin 160 mg/kg). Notes: Superscript (a) indicates significant difference at $p<0.05$ when compared with Control. Superscript (b) indicates significant difference when compared with Pb.

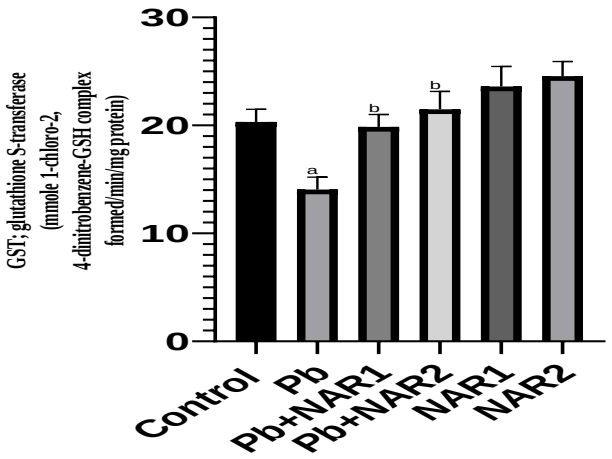

Figure 4:
Effect of Lead toxicity and Naringin on hepatic Glutathione S-transferase (GST) activity. A (Control), Pb (Lead acetate; 300 ppm), Pb+Nar1 (Lead acetate + Naringin 80 mg/kg), Pb+Nar2 (Lead acetate + Naringin 160 mg/kg), Nar1 (Naringin 80 mg/kg), Nar2 (Naringin 160 mg/kg). Notes: Superscript (a) indicates significant difference at $p<0.05$ when compared with Control. Superscript (b) indicates significant difference when compared with Pb.

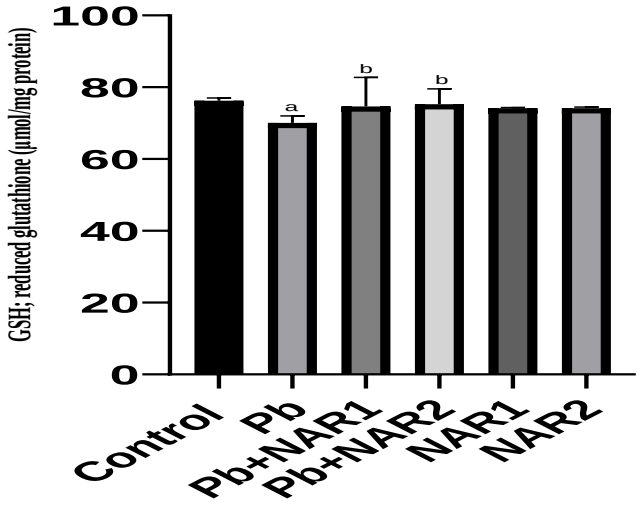

Figure 3:
Effect of Lead toxicity and Naringin on hepatic reduced glutathione(GSH) content. A (Control), Pb (Lead acetate; 300 ppm), Pb+Nar1 (Lead acetate + Naringin 80 mg/kg), Pb+Nar2 (Lead acetate + Naringin 160 mg/kg), Nar1 (Naringin 80 mg/kg), Nar2 (Naringin 160 mg/kg). Notes: Superscript (a) indicates significant difference at $p<0.05$ when compared with Control. Superscript (b) indicates significant difference when compared with Pb.

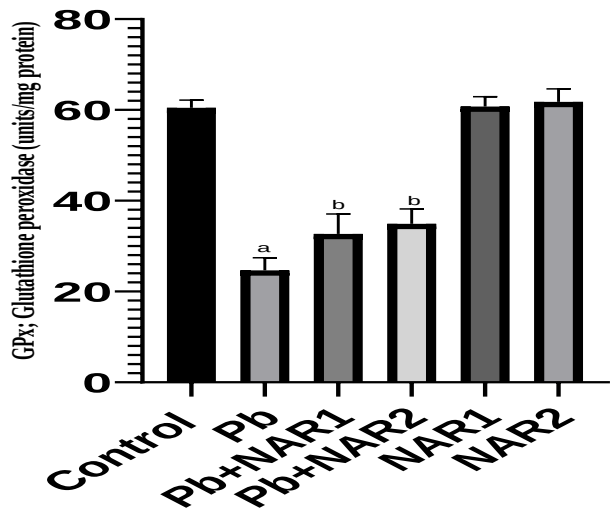

Figure 5:
Effect of Lead toxicity and Naringin on hepatic Glutathione peroxidase (GPx) activity. A (Control), Pb (Lead acetate; 300 ppm), Pb+Nar1 (Lead acetate + Naringin 80 mg/kg), Pb+Nar2 (Lead acetate + Naringin 160 mg/kg), Nar1 (Naringin 80 mg/kg), Nar2 (Naringin 160 mg/kg). Notes: Superscript (a) indicates significant difference at $p<0.05$ when compared with Control. Superscript (b) indicates significant difference when compared with Pb.

There were remarkable significant ($p<0.05$) reductions in the activities of GSH (Figure 3), GST (Figure 4), GPx (Figure 5), and SOD (Figure 6) in the Pb intoxicated chicks when compared to the control across all the experimental groups. Co-administration of Pb and two different doses of naringin however produced significant improvement in the activities of the hepatic antioxidant defence system relative to the Pb alone with the group with the higher dose of naringin (160mg/kg) showing optimum valves in the experimental birds.

Histology: At X40 magnification, group A which served as the control had no observable lesion while severe locally extensive hepatocellular necrosis and inflammation were observed in the group treated with Pb only (Group B) as shown in Figure 7. There was moderate centrilobular hepatocellular degeneration in group C while Group D showed centrilobular plate atrophy and bridging fibrosis (Figure 7). Groups E and F that were administered naringin at 80mg/kg and 160mg/kg respectively had no observable lesions.

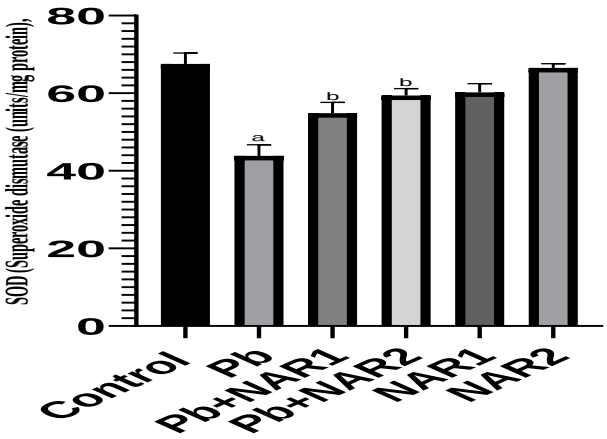

Figure 6:
Effect of Lead toxicity and Naringin on hepatic superoxide dismutase (SOD) activity. A (Control), Pb (Lead acetate; 300 ppm), Pb+Nar1 (Lead acetate + Naringin 80 mg/kg), Pb+Nar2 (Lead acetate + Naringin 160 mg/kg), Nar1 (Naringin 80 mg/kg), Nar2 (Naringin 160 mg/kg). Notes: Superscript (a) indicates significant difference at $p<0.05$ when compared with Control. Superscript (b) indicates significant difference when compared with Pb.

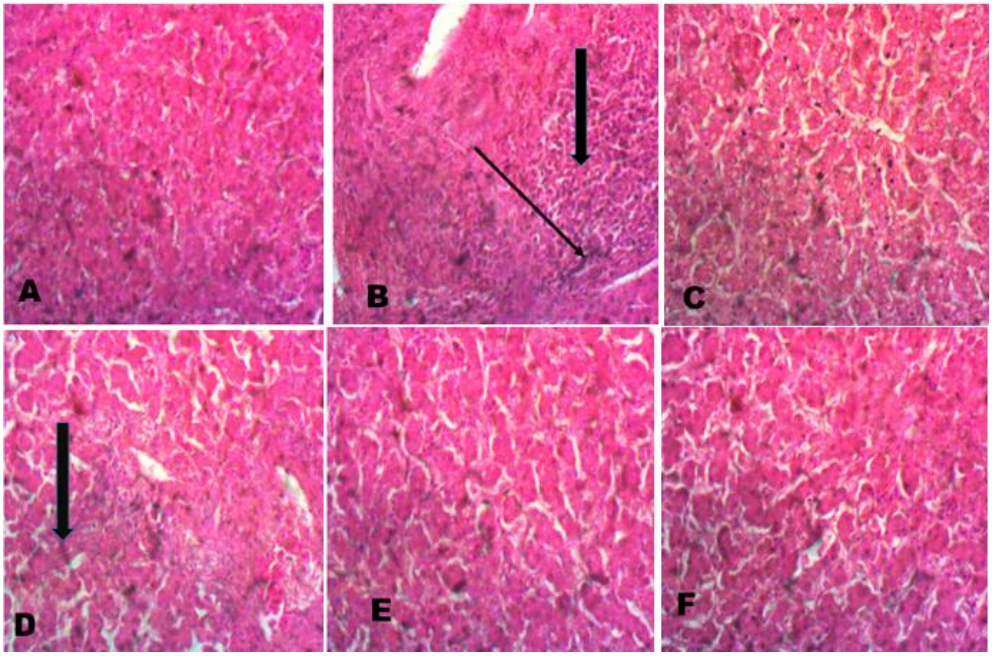

Plate 1.
The Histology of the Liver.
A (Control), B (Lead acetate, 300 ppm), C (Lead acetate, 300 ppm + Naringin 80 mg/kg), D (Lead acetate, 300 ppm + Naringin 160 mg/kg), E (Naringin 80 mg/kg), F (Naringin 160 mg/kg).

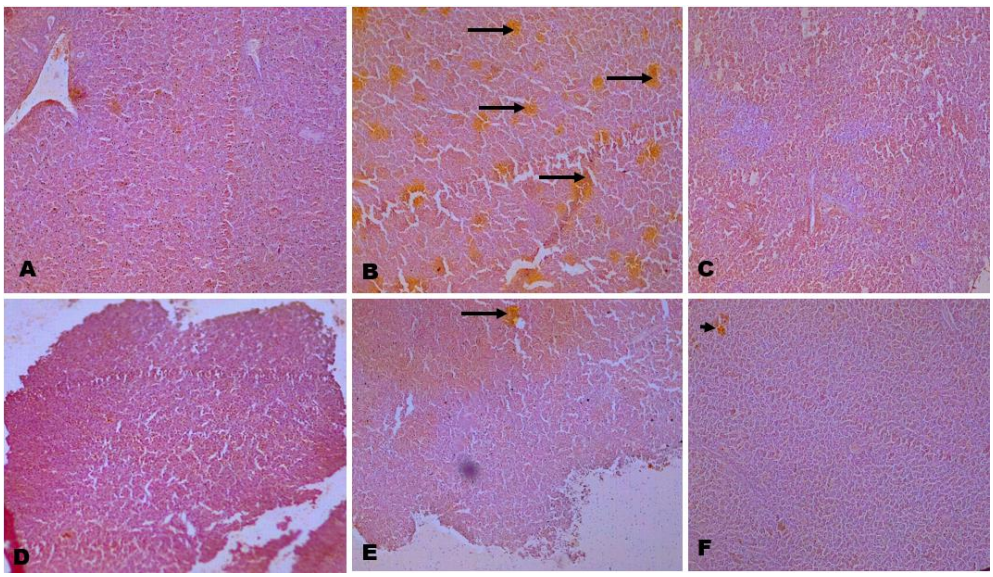

Plate 2.
The immunohistochemistry of liver caspase 3. A (Control), B (Lead acetate; 600 ppm), C (Lead acetate + *Naringin 8*0 mg/kg), D (Lead acetate + Naringin 80 mg/kg), E (Naringin 80 mg/kg), F (Naringin 100 mg/kg). Slides stained with high definition Heamtoxylin. (Magnification x 100)

Immunohistochemistry: The immune localization of Caspase 3 in the hepatocytes revealed higher expressions in the Pb exposed group when compared to the control and groups co-treated with naringin (Figure 8). The reduction in the expressions of Caspase 3 is indicative of amelioration of apoptosis and hepatoprotective effects of naringin against Pb acetate toxicity as shown by the reduction in the expression of caspase 3 in the groups treated with naringin and groups administered naringin only thus.

DISCUSSION

According to several studies (Herring *et al*., 2018, González *et al*., 2019, Moncls *et al*., 2020, Descalzo *et al*., 2021), Pb poisoning continues to be one of the most serious public health issues, especially in underdeveloped nations. Pb exposure has a variety of detrimental impacts on the body's systems, but the most notable is increased oxidative stress, which is crucial for the development of disease (Amini *et al*., 2019). In soft tissues, the chicken liver was found to have the second highest Pb content after the kidney. The liver, which has been regarded as the greatest Pb store of soft tissues in animals, oversees detoxifying a variety of compounds (Wang *et al.,* 2021).

The Pathogenesis and pathophysiology of Pb-induced toxicity have been linked to the production of free radicals that generate oxidative stress and deplete the antioxidant defense system (Nasiruddin *et al*., 2020; Gadde *et al.,* 2021). According to our research, the birds in the Pb- induced toxicity group showed a noticeably higher level of hydrogen peroxide (H_2O_2) production, lipid peroxidation product (MDA) content, and oxidative stress in the liver of the experimental birds. Our results are consistent with earlier findings that showed Pb poisoning caused a significant rise in oxidative stress, inflammation, and apoptosis (Gagan *et al.,* 2012; Omobowale *et al.,* 2014; Oyagbemi *et al*., 2015; Matovic *et al*., 2015; Omobowale *et al*., 2016).

Furthermore, we found that Pb intoxication resulted in considerable reductions in reduced glutathione (GSH) levels as well as in the activities of glutathione S-transferase (GST), superoxide dismutase (SOD), and glutathione peroxidase (GPx). But, naringin co-administration enhanced the antioxidant defense system and reduced the oxidative stress markers described above. Therefore, these results are consistent with the conclusion of other studies on the antioxidant capabilities of naringin (Baranowska *et al*., 2021; Deng *et al*., 2022).

Compared to the control and treatment groups, there was a significant decrease in serum total protein in Pb-intoxicated birds. Naringin treatment considerably increased the serum total protein concentration as shown by the findings of this study. As observed by Moussa and Bashandy (2008), a decrease in serum total protein has been linked to kidney and cardiac failure. Additionally, in this study, birds exposed to Pb alone showed higher MPO activity when compared to the control and groups given naringin. MPO, however, has been described as a novel sign of oxidative stress, kidney damage, inflammation, and a diagnostic marker of cardiomyopathy including cardiac arrest and heart attack (Khan *et al.,* 2018; Veltman *et al*., 2021; Peng *et al*., 2021; Wei *et al*., 2021; Sandamali *et al*., 2021). The decrease in MPO activity in naringin-treated birds suggested that naringin had anti-inflammatory, hepatoprotective, and cardioprotective effects in Pb-exposed birds.

With numerous pharmacological advantages, such as antioxidant, anti-inflammatory, and anti-apoptotic qualities, naringin is a commonly accessible flavonoid that is found in citrus fruits. In this study, naringin was administered to groups at doses of 80 mg/kg and 160 mg/kg, which significantly increased the levels of enzymatic and non-enzymatic antioxidants, reduced oxidative stress, improved serum NO, and decreased MPO activity. Naringin was also found to have antioxidant, anti-inflammatory, and anti-apoptotic properties.

Exposure to lead has been shown to cause fatty changes in the liver parenchyma, degraded hepatocytes, and nuclear pyknosis, which results in an increase in the number of apoptotic cells in the liver (Ashrafizadeh *et al*., 2018). This was consistent with our observations of severe inflammation and hepatocellular necrosis in the untreated group. Additionally, lead toxicity, as observed by Chi *et al.,* (2017) may result in cellular oedema that causes mitochondria to rupture, vacuolize, and wrinkle. Naringin was co-administered in the treatment groups, and this resulted in varied degrees of improvement for all these pathologies. According to Ashrafizadeh *et al.,* (2018), lead toxicity may be responsible for the development of intracellular inclusions, localized atrophy, and intracytoplasmic calcification in the proximal tubules of rats. According to reports, lead destroys the morphologic structures of hepatocytes by causing cellular disruptions, necrosis, and the infiltration of inflammatory cells in the liver, as was the case in the untreated group (Chi *et al*., 2017). The dilatation of the liver portal vein and the scaffolding of the hepatic lobules and pyknotic regions identified in the degenerating hepatocytes have also been reported in lead intoxication in chickens (Haleagrahara *et al.,* 2010).

In contrast to other groups that received naringin in conjunction with Pb or naringin alone, immunohistochemistry revealed increased expression of Caspase 3 in the liver of Pb-treated birds. The conserved family of proteins known as caspase 3, which is widely expressed, is known for its active proteolytic functions in the execution of apoptosis in cells in response to extrinsic or intrinsic inducers of this method of cell death (Eskandari and Eaves 2022). In multicellular creatures, however, caspase 3 also appears to serve important functions in controlling the development and upkeep of both healthy and pathological cells and tissues, according to mounting data (Sudhakar *et al*., 2008).

Additionally, Walsh *et al*., (2008) reported that caspase 3 is the predominant executioner caspase, although caspase 7 may play multiple roles depending on the substrate. This discovery is consistent with our study's finding that caspase 3 is expressed more abundantly in Pb-induced toxicity. Caspase3, the main enzyme responsible for apoptotic death, makes cell death more effective (Eskandari and Eaves 2022).

As a result, our study has shown that co-administration of a naturally occurring substance with a high flavonoid content, such as naringin, can reverse oxidative stress, reduce the production of free radicals, and significantly improve the antioxidant defense system.

Overall, it can be said that naringin, a metal chelating flavonoid, protects the liver from lead acetate toxicity by acting as an antioxidant and an anti-inflammatory agent, and as a metal chelator. Additionally, co-admiration of naringin with lead reduced the oxidative stress caused by lead and markedly enhanced the antioxidant defense system and serum nitric oxide bioavailability. Therefore, the naringin administration either in chicken feeds or water act as a heavy metal chelator, which could be advantageous to the poultry industries.

Acknowledgements
The African Union, through the Pan African University Institute of Earth and Life Sciences Institute (PAULESI), provided money for this work, and the Cardio-renal Laboratory at the University of Ibadan provided the facilities. The authors are extremely grateful for both.

REFERENCES

Ahmad, A., Dempsey, S.K., Daneva, Z., Azam, M., Li, N., Li, P. and Ritter. J.K. (2018). Role of Nitric Oxide in the Cardiovascular and Renal Systems. Int. J. Mol. Sci. 19:2605

Ajarem, J.S., Hegazy, A.K, Allam, G.A., Allam, A.A, Maodaa, S.N. and Mahmoud A.M. (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

Amini, N., Sarkaki, A., Mahin, D., Seyyed, A. M., Akram A. and Mohammad B. (2019). Protective effects of naringin and trimetazidine on remote effect of acute renal injury on oxidative stress and myocardial injury through Nrf-2 regulation. Pharmacol. Rep. 71(6):1059-1066.

Ashrafizadeh, M., Rafiei, H. and Ahmadi, Z. (2018). Histological Changes in the Liver and Biochemical Parameters of Chickens Treated with Lead Acetate II. Iran J. Toxicol. 12: 6.

Babalola, O.O. and Areola J.O. (2010). Interactive roles of terpenoid extract from the leaves of neem plant (Azadirachta indica, A. Juss) on lead induced toxicity in pregnant rabbits. J. Med. Plants Res. 4 (12):1102-1107.

Baranowska, M., Koziara, Z., Suliborska, K., Chrzanowski, W., Wormstone, M., Namieśnik, J., and Bartoszek, A. (2021). Interactions between polyphenolic antioxidants quercetin and naringenin dictate the distinctive redox-related chemical and biological behavior of their mixtures. Sci Rep. 11(1): 12282.

Beutler, E., Duron, O. and Kelly, MB (1963) Improved method for determination of blood glutathione reduced. J Lab Clin Med. 61: 882- 888.

Brentnall, M., Rodriguez-Menocal, L., De Guevara, R. L., Cepero, and Boise, L. H. (2013). Caspase-9, caspase-3 and caspase-7 have distinct roles during intrinsic apoptosis. BMC Cell Biol. **14**, 32.

Broseghini-Filho, G.B., Almenara, C.C., Vassallo, D.V. and Padilha A.S. (2016). Blood Pressure Decreases Following Lead Treatment Cessation: Highest NO Bioavailability Involved. Biol. Trace Elem. Res. 170(2): 410-414.

Cavia-Saiz, M., Busto, D.M., Maria, C.P., Natividad, O., Perez-Mateos, M. and Pilar, M. (2010). Antioxidant properties, radical scavenging activity and biomolecule protection capacity of flavonoid naringenin and its glycoside naringin: a comparative study. J. Sci. Food Agric. 90: 1238–1244.

Chi, Q., Liu, T., Sun, Z., Tan, S., Li, S. and Li, S. (2017). Involvement of mitochondrial pathway in environmental metal pollutant lead-induced apoptosis of chicken liver: perspectives from oxidative stress and energy metabolism. Environ. Sci. Pollut. Res.. 2017 24 (36):28121-31.

De Francisco, N., Ruiz, T. J. D. and Agüera, E. I. (2003). Lead and lead toxicity in domestic and free-living birds. Avian Pathology, 32 (1): 3-13.

Deng, M., Jia, X., Dong, L., Liu, L., Huang, F., Chi, J., Ma, Q., Zhao, D., Zhang, M. and Zhang, R. (2021). Structural elucidation of flavonoids from Shatianyu (Citrus grandis L. Osbeck) pulp and screening of key antioxidant components. Food Chem. 366:130605.

Descalzo, E., Camarero, P.R, Sánchez-Barbudo, I.S., Martinez-Haro, M., Ortiz-Santaliestra, M.E., Moreno-Opo, R. and 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.

Drury, R.A., (1976). Wallington E.A. Carlton's Histopathological Techniques. 4th ed. Oxford University Press, London, pp 139-142

Ellman, G. L. (1959). Tissue sulfhydryl groups. Arch Biochem Biophys. 82(1):70

Eskandari, E. and Eaves, C. J. (2022). Paradoxical roles of caspase-3 in regulating cell survival, proliferation, and tumorigenesis. J. Cell Biol. 221 (6): e202201159.

Fakunle, P.B., Ajibade, A.J., Oyewo, E.B. and Adeyemi, O.H. (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.

Gadde, R. and 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.

Gagan, F., Deepesh, G. and Archana, T. (2012). Toxicity of lead: A review with recent updates. Interdiscip Toxicol. 5(2):47–58.

Ganesh, C.J. and Reddy, T.K. (2011). Alleviation of iron induced oxidative stress by the grapefruit flavanone naringin in vitro. Chemico-Biological Interactions. 190: 121–128.

Golechha, M., Chaudhry, U., Bhatia, J., Daman, S. and Singh D. (2011). Naringin Protects against Kainic Acid-Induced Status Epilepticus in Rats: Evidence for an Antioxidant, Anti-inflammatory and Neuroprotective Intervention. Biol. Pharm. Bull. 34(3) 360—365.

Goliomytis, M., Kartsonas, N., Charismiadou, M.A., Symeon, P.E. and Deligeorgis, S.G. (2015). The Influence of Naringin or Hesperidin Dietary Supplementation on Broiler Meat Quality and Oxidative Stability. PloS one. 10: e0141652.

González, F., Camacho, M., Tiburón, N.P., Peña, M.Z., Rueda, L.R. and Luzardo, O.P. (2019). Suitability of anodic stripping voltammetry for routine analysis of venous blood from raptors. Environ. Toxicol. Chem. 38(4):737-747.

Gornal, A.G., Bardawill J.C. and David M.M. (1949). Determination of serum proteins by means of Biuret reaction. J. Biol. Chem. 177: 751–766.

Habig, W.H., Pabst, M.J. and Jakpoby, W.B. (1974) Glutathione transferase, a first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139.

Hager-Theodorides, A.L., Massouras, T., Simitzis, P.E., Moschou, K., Zoidis, E., Sfakianaki, E., Politi, K., Charismiadou, M., Goliomytis, M. and Deligeorgis, S. (2021). Hesperidin and Naringin Improve Broiler Meat Fatty Acid Profile and Modulate the Expression of Genes Involved in Fatty Acid β-oxidation and Antioxidant Defense in a Dose Dependent Manner. Foods. 10, 739.

Haleagrahara, N., Jackie, T., Chakravarthi, S., Rao, M. and Kulur, A. (2010). Protective effect of Etlingera elatior (torch ginger) extract on lead acetate-induced hepatotoxicity in rats. The Journal of toxicological sciences. 35(5): 663- 71.

Herring, G., Eagles-Smith, C.A. and Varland, D.E. (2018). Mercury and lead exposure in avian scavengers from the Pacific Northwest suggest risks to California condors: Implications for reintroduction and recovery. Environ Pollut. 243:610-619.

Hossain, M.A., Mostofa, M., Alam, M.N., Sultana, M.R., and Rahman, M.M. (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.

Khan, A.A., Alsahli, A.M., and Rahmani, A.H. 2018. Myeloperoxidase as an Active Disease Biomarker: Recent Biochemical and Pathological Perspectives. Med. Sci. 6:33

Kucukler, S., Benzer, F., Yildirim, S., Gur, C., Kandemir, F.M., Bengu, A.S., Ayna, A., Caglayan, C. and Dortbudak, M.B. (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. 199:1501-1514.

Kulasekaran, G., Dharmalingam, P. and Ganapasam, S. (2011). Neuroprotective effect of naringin, a dietary flavonoid against 3-Nitropropionic acid induced neuronal apoptosis. Neurochem. Inter. 59, 1066–1073.

Long, X., Sun, F., Wang, Z., Liu, T., Gong, J., Kan, X., Zou, Y. and 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

Matović, V., Aleksandra, B., Đukić-Ćosić, D., Zorica, B. (2015). Insight into the oxidative stress induced by lead and/or cadmium in blood, liver and kidneys. Food and Chemical Toxicology. 78:130-40.

Monclús, L., Shore, R.F. and Krone, O. (2020). Lead contamination in raptors in Europe: A systematic review and meta-analysis. Sci. Total Environ. 748:141437.

Moussa, S.A. and Bashandy, S.A. (2008). Biophysical and Biochemical changes in the blood of rats exposed to lead toxicity. Romanian J. Biophys. 18: 123–133.

Munazza, S. and Muhammad, S.A. (2018). Neem (*Azadirachta indica*) And Its Potential for Safeguarding Health, Prevention and Treatment of Diseases. Matrix Sci. Med. 2(1):04-08.

Nasiruddin, R. M., Karim, N., Changlek, S., Atiar, R. M., Tangpong, J., Hajjar, D., Alelwani, W., Makki, A.A. (2020). Thunbergia laurifolia leaf extract partially recovers lead-induced renotoxicity through modulating the cell signaling pathways. Saudi J. Biol. Sci. 27(12):3700-3710.

Omobowale, T.O, Oyagbemi, A.A, Akinleye, S.A, Ola-Davies, O.E, Saba, A. B., Olopade, J.O. and Adedapo, A.A. (2016). Effect of Exposure and Withdrawal on Lead-induced Toxicity and Oxidative Stress in Cardiac Tissues of Rats. Toxicol. Intern. 23(1): 12-17

Omobowale, T.O., Oyagbemi, A.A., Akinrinde, A.S., Saba, A.B., Daramola O.T., Ogunpolu B.S., Olopade J.O. (2014). Failure of recovery from lead induced hepatoxicity and disruption of erythrocyte antioxidant defence system in Wistar rats. Environ. Toxicol. Pharmacol. 37:1202–1211.

Oyagbemi, A.A, Omobowale, T.O., Awoyomi, O.V., Ajibade, T.O., Falayi, O.O., Ogunpolu B.S., Okotie, U.J., Asenuga, E.R., Adejumobi, O.A., Hassan, F.O., Ola-Davies, O.E., Saba, A.B., Adedapo, A.A. and Yakubu, M.A. (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

Oyagbemi A.A., Omobowale T.O., Akinleye S.A., Adebowale B.S., Blessing S.O., Oluwabusola D. (2014). Lack of Reversal of Oxidative Damage in Renal Tissues of Lead Acetate-Treated Rats. Environ. Toxicol. Environ Toxicol. 30(11):1235-43.

Ozkaya, A., Zafer, S., Uzeyir, D. and Mustafa, O. (2016). Effects of Naringenin on Oxidative Stress and Histopathological Changes in the Liver of Lead Acetate Administered Rats. J Biochem. Mol. Toxicol. 30: 5.

Sandamali, J.A.N., Hewawasam, R.P., Jayatilaka K.A.P.W. and Mudduwa L.K.B. (2021). *Cinnamomum zeylanicum* Blume (*Ceylon cinnamon*) bark extract attenuates doxorubicin induced cardiotoxicity in Wistar rats. Saudi Pharm. J. 29(8):820-832

Sudhakar, C., Jain N. and Swarup G. (2008). Sp1-like sequences mediate human caspase-3 promoter activation by p73 and cisplatin. FEBS J. 275: 2200 –2213

Teerasarntipan, T., Chaiteerakij, R., Prueksapanich, P. and Werawatganon D. (2020). Changes in inflammatory cytokines, antioxidants and liver stiffness after chelation therapy in individuals with chronic lead poisoning. BMC Gastroenterol. **20,** 263 (2020).

Vallverdú-Coll, N., Mateo, R., Mougeot F. and Ortiz-Santaliestra. M.E. (2019). Immunotoxic effects of lead on birds. Sci. Total Environ. 689:505-515.

Varshney, R. and Kale, R.K. (1990). Effect of calmodulin antagonists on radiation induced lipid peroxidation in microsomes. Inter. J. Rad. Biol. 58: 733–743.

Veltman, D., Wu, M., Pokreisz, P., Claus, P., Gillijns, H., Caluwé, E., Vanhaverbeke, M., Gsell, W., Himmelreich, U., Sinnaeve, P.R. and Janssens S.P. (2021). Clec4e-Receptor Signaling in Myocardial Repair After Ischemia-Reperfusion Injury. JACC Basic Transl. Sci. 6(8):631-646.

Venkateswara, R. P., Kiran, S.D.V.S., Rohini, P. and Bhagyasree P. (2017). Flavonoid: A review on Naringenin. J. Pharmacog Phytochem. 6(5): 2778- 2783.

Walsh, J.G., Cullen, S.P., Sheridan, C., Luthi, A.U., Gerner, C. and Martin, S.J. (2008). Executioner caspase-3 and caspase-7 are functionally distinct proteases. Proc. Natl Acad. Sci. USA.105 (35): 12815-12819.

Wang, J., Yang, Z., Lin, L., Zhao, Z., Liu, Z., Liu, X. (2012). Protective effect of naringenin against lead-induced oxidative stress in rats. Biol. Trace Elem. Res. 146: 354–359.

Wang, S., Li, Q., Gao, Y., Zhou Z. and Li, Z. (2021). Influences of lead exposure on its accumulation in organs, meat, eggs and bone during laying period of hens, Poul. Sci. 100: 101249.

Wei, H., Xue, Q., Sun, L. and 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.

Wolff S.F. (1994). Ferrous ion oxidation in the presence of ferric ion indicator xylenol orange for measurement of hydrogen peroxides. Methods Enzymol. 233:182–189.

Xia, Y. and Zweiler, J.L. (1997). Measurement of myeloperoxidase in leucocyte containing tissue. Anal biochem. 245:93-96.