Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

Dr Odunayo Ibraheem Azeez
Department of Veterinary
Physiology and Biochemistry
Faculty of Veterinary Medicine
University of Ibadan, Ibadan,
Nigeria

Dr Ifeoluwa Opeyemi Adegboyega
Department of Veterinary
Physiology and Biochemistry
Faculty of Veterinary Medicine
University of Ibadan, Ibadan,
Nigeria

Ameliorative effects of aqueous and ethanol extracts of *Curcuma longa* rhizome on lead-induced toxicity in adult male Wistar rats

Azeez OI and Adegboyega IO

DOI: https://doi.org/10.22271/phyto.2023.v12.i5c.14734

Abstract
Lead as very common in the environment as heavy released through burning of various fuels, mining, and manufacturing of lead batteries. Therefore, constant exposure to lead predisposes people to lead induced toxicity with acute and prolonged exposure. This study investigated the ameliorative roles of *Curcuma longa* rhizome extracts (aqueous and ethanolic) on the haematological, erythrocyte osmotic fragility, plasma biochemistry and indicators of associated oxidative stress in the rats.

Thirty-five adult male Wistar rats that weigh 150-190 g used in this experiment were randomly divided into seven groups, A to G which consist five rats in each group. The positive control (group A) was given distilled water, Group B rats were administered a dose of 150 mg/kg lead acetate only. Groups C-D rats were given 150 mg/kg lead acetate plus 100 and 200 mg/kg aqueous extract of *Curcuma longa* respectively, while group E and F rats were exposed to a dose of 150 mg/kg lead acetate with 100 and 200 mg/kg of *Curcuma longa* ethanol extract, respectively. Finally group G rats were administered a dose of 150 mg/kg lead acetate and 100 mg/kg Gallic acid, a known antioxidant, for a period of twenty-eight (28) days. All extracts were dissolved in 5% Tween 80, which was also given to the control groups A, B and G. At days 14 and 28, blood was collected for haematological parameters, erythrocyte osmotic fragility and plasma biochemist. At day 28, kidneys, liver and testes were collected for histology and determination of markers/indicators of oxidative stress.

Exposure to lead acetate resulted in mild macrocytic hypochromic anaemia as indicated by decreased values of Hb concentration, PCV and RBC with increased MCV and decreased MCHCH as compared to the untreated control. The osmotic fragility in lead only treated group B was higher than were those of the control and those treated with aqueous and ethanol extract of *Curcuma longa* as well as Gallic acid. Concomitant exposure to lead and *Curcuma longa* showed a degree of response to the anaemia. The extracts also modulated the elevated erythrocyte osmotic fragility seen in lead only treated group B. Furthermore, signs of mild liver and kidney damages in lead toxicity were observed, following elevated AST and ALT levels. The LDL was also elevated while HDL was reduced. The damages were however ameliorated by *Curcuma longa* aqueous and ethanol extract, especially at 200 mg/kg dosage and Gallic acid.

Oxidative stress indicators such as H_2O_2 and MDA values were higher, while antioxidant enzymes, GSH, GPx and SOD were depleted in lead only treated rats in the liver, kidneys, and testes. Degenerative changes, congestion and were also observed in the liver, kidneys and testes in Group B, but the extracts were able to ameliorate the oxidative stress damages and histopathological changes in those treated concurrently with the toxicant and *Curcuma longa* aqueous and ethanol extracts, both at 100 mg/kg and 200 mg/kg dosages.

This study further established the toxic effects of exposure to lead by causing anaemia, increased erythrocyte membrane fragility and multiple organ damage by increasing oxidative stress and exhaustion of endogenous antioxidants. The effect of lead was however corrected by *Curcuma longa* extracts in a manner that is similar to that of gallic acid. These effects of *Curcuma longa* extract may be linked with flavonoids and phenolic compounds with antioxidant effects in *Curcuma longa*.

Keywords: Heavy metals, lead, oxidative stress, turmeric

Introduction

Continual presence of heavy metals including Lead in the ecosystem is still going on unabated among artisanal miners (Rakete *et al*., 2022 and Rabiu *et al*., 2020) [33, 32], lead battery workers (Ravibabu *et al*., 2020) [34] among others, especially in developing countries. According to Dignam *et al*. (2019) [12], a review of the efforts to reduce exposure over long term period from 1970-2017 in the United States reportedly yielded a 93.6% reduction in lead exposure, yet an estimated 500,000 children still have a blood lead level that is above the reference value of 5 µg/dL acceptable internationally.

Corresponding Author:
Dr. Odunayo Azeez
Department of Veterinary
Physiology and Biochemistry
Faculty of Veterinary Medicine
University of Ibadan, Ibadan,
Nigeria

On the contrary, however, continual exposure is still a common challenge in developing countries through illegal artisanal mining, iron smelting, recycling of batteries etc. For example, a recent survey of 207 miners by Rakete *et al.* (2022) [33] that is not limited to developing countries with artisanal miners alone. Regular monitoring of lead exposure and prevention or mitigation of the toxicity is therefore very important, because of its deleterious effects on the blood, bone marrow and the brain especially in growing children. Lead has been widely reported to cause musculoskeletal dysfunction and bone demineralization, anaemia, oxidative stress, impaired blood coagulation, male reproductive dysfunction, and impaired calcium balance (Ravibabu *et al.*, 2020) [34]. In children with rapid brain development, lead toxicity has been shown to affect brain development, leading to general cognitive impairments (Ortega *et al.*, 2021 and Goodchild *et al.*, 2021) [29, 16].

The pathogenesis of lead toxicity has been associated with increased generation of reactive oxygen species (ROS) and reduction of endogenous antioxidants levels in the body. Lead denatures glutathione by joining to sulfhydryl group in GSH, which prevents GSH renewal, thereby increasing the oxidative stress (Flora *et al.*, 2012) [15]. Lead also reportedly disrupts the activities of SOD and catalase leading ultimately destruction of membrane lipid bilayer, integral and peripheral proteins as well as DNA through lipid peroxidation. Lead exposures has also been linked to no regenerative anaemia by blocking 5-aminolevulinic acid dehydratase resulting in oxidation of haemoglobin and erythrocyte lysis.

Curcuma longa, also known as Turmeric, is a tropical rhizome of the ginger family. It contains several metabolites such as curcuminoid, oil, flavonoids, phenolics, some important amino acids, protein and alkaloids, which have been suggested to be responsible for its pharmacological actions (Kulyal *et al.*, 2021 and Salem *et al.*, 2022) [21, 37]. For example, Curcumin from the extracts has been shown to have antifungal, anti-bacterial, anti-parasitic, antimutagen and antimicrobial activities (Sahoo *et al.*, 2021) [35]. It also protects against renal damages, allergies, arthritis and Alzheimer's disease (Mishra and Goel 2020) [27].

Following its popularity as a spice and the claims of the medicinal values of tumeric, this study was designed to determine the effectiveness of its aqueous and ethanol extracts to ameliorate lead acetate induced toxicity in adult male Wistar rats.

Materials and methods
Extraction
Fresh rhizomes obtained from a local market in Ibadan, South West, Nigeria were rinsed, air dried and then grinded into fine powder. The aqueous extract was obtained by soaking the powdery material in water at atmospheric temperature and then allowed to stay overnight. The extract was thereafter filtered and centrifuged at 5000 rpm for 15 min before storage at 4 °C. The ethanol extract was made by soaking the weighed turmeric powder in ethanol for two days with regular stirring. The mixture was thereafter filtered using muslin cloth and Whitman's filter paper to produce the final filtrate. The solvent was removed from the filtrate by concentrating with rotary evaporator at a temperature of 45 °C and stored at 4 °C, until required for use.

Experimental Animals
The Adult male Wistar rats used as the experimental animal were purchased from a standard farm in the neighbouring community of University of Ibadan. The average weight was between 150 - 190 g and housed in conducive plastic cages at tropical 12 hours light and dark cycles in the experimental animal house, Department of Veterinary Physiology and Biochemistry, University of Ibadan, Ibadan, Nigeria. The animals were allowed to rest and get used to the environment for about eight (8) days before the experiment commenced. They were fed with the normal rat chows and provided with potable water *ad libitum*.

Experimental Design
Thirty-five male Wistar rats used for the experiment were randomly divided to seven groups A-G, made up of five rats in each group. The control (Group A), was given distilled water, Group B contained rats that were administered 150 mg/kg Lead acetate only while Groups C and D were given 150 mg/kg Lead acetate plus 100 and 200 mg of *Curcuma longa* aqueous extract, respectively. Similarly, Groups E and F were given a dose of 150 mg/kg Lead acetate and 100 and 200 mg/kg of *Curcuma longa* ethanol extracts, in that order. Finally, group G rats were administered a dose of 150 mg/kg Lead acetate and 100 mg/kg gallic acid, a known antioxidant; all for a period of twenty-eight (28) days.

Blood Sample Collection and Haematology
Blood sample (5 ml) was collected from each rat into lithium heparin tubes via the retro-orbital venous plexus. From each blood collected were determined, Packed Cell Volume (PCV), the Haemoglobin Concentration (Hb), Red Blood Cell count (RBC), total White Blood Cells count and erythrocyte osmotic fragility as previously described (Azeez and Braimah 2020) [7]. Erythrocyte indices-MCV, MCH and MCHC were thereafter determined according to standard procedure. The plasma was thereafter obtained after centrifugation of the blood in a macro centrifuge (MSE England) at 3000 revolutions per min for 10 minutes for determination of plasma biochemical parameters, using standard methods. The plasma was also collected for plasma biochemistry after the blood samples were centrifuged at 4000 rev/min for 10 minutes, then stored immediately at 20 °C.

Plasma Biochemistry
From the plasma, urea and creatinine were measure using spectrophotomet try as described by Colum be and Farreau, and Taussky respectively (Kuribayashi *et al.*, 2017 and Onuegbu *et al.*, 2014) [22, 28], total protein and albumin measure according to the procedures of Bradford *et al.* (1976) [10] and Doumas *et al.* (1971) [13], respectively. Globulin levels in the plasma was calculated by subtracting albumin from the total protein. Plasma ALP, ALT and AST activity were determined by the methods of Bessey (Bilal *et al.*, 2018) [8] and Reitman and Frankel (Malhi *et al.*, 2018) [25]. Total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL) and triglyceride (TG) were measure spectrophotometric ally using Randox® kits using the manufacturer's instructions.

Tissue preparation
The rats were culled at the end of the experiment at 28 days under ether anaesthesia. The liver, kidneys and testes were removed rinsed in normal saline and weighted before storage at 20 °C. The organs were cut into small pieces and homogenized in twenty volumes of homogenizing buffer (consisting of 0.1M phosphate buffer at pH 7.4) using a Teflon homogenizer (PHD 710, New Jersey, US). To obtain

the post mitochondrial fraction, the homogenate was the centrifuged at 10,000 rpm for 10 minutes using a refrigerated ultra centrifuge (80-2, Lemfield medical, UK) at -4 °C for determination of markers of oxidative stress.

Markers of Oxidative Stress

Lipid peroxidation was determined as malondialdehyde (MDA) levels according to the method of Farombi *et al*. (2000) [14]. The H_2O_2 generation was evaluated according to the method of Wolff (1994) [41], reduced glutathione (GSH) concentrations by the method previously described by Jollow *et al*. (1974) [17] while Glutathione-S-transferase (GST) activity was measured using the method of Farombi *et al*. (2008) [42]. Protein level was determined by Biuret method (Oyagbemi *et al*., 2017) [30]. Glutathione peroxidase activity was evaluated using the method of Sahu *et al*. (2016) [36].

Histopathology

Liver, heart, kidneys and testes samples were fixed in 10% buffered formalin, dehydrated in graded alcohol and then embedded in paraffin at 60 °C. Sectioning of embedded tissues was done using a microtome at 5µm thickness and each section floated in water bath at 45 °C and then floated on the glass slide which were thereafter stained with haematoxylin and counterstained with eosin. All slides were viewed on Olympus light microscope at x10 and x40 magnifications.

Statistical Analysis

Except where stated, values are presented as mean and standard deviation. One Way ANOVA with Tukey post hoc test was used to compare the means comparison across groups Prism Graphpad version 9.0. A P value ≤ 0.05 was considered significant at 95% CI.

Results
Subacute Exposure to Lead
Erythrocytes parameters

The effects of sub-acute exposure of lead toxicity to Wistar rats for 14 days on the haematological parameters are shown in Table 1. We observed a generalized significant reduction in erythrocyte indices in the lead acetate only treated group (group B) followed by significant increases in the extract and gallic acid treated groups. For example, the PCV was significantly reduced in group B, than in the untreated control, while marginal increases were seen in the other groups, as compared to group B, but the PCV value in group E, (Pb+100 mg of ethanol extract of *Curcuma longa* (CL) was higher than that of the toxicant only. The haemoglobin concentration (Hb) in the lead only (group B) was significantly lower ($p<0.05$) than that of the untreated control (group A). It was also lower than the Hb values in groups C, D, E and G which received 150 mg of Lead acetate and 100 and 200 mg/kg of aqueous extract, 100 mg/kg ethanol extract of *Curcuma longa* (CL) with 100 mg/kg gallic acid respectively. Although the Hb values in groups E and F were higher than that of the toxicant only, they were significantly lower ($p<0.05$) than that of the untreated control. Meanwhile, the total red blood cell counts in group B (Pb only) was significantly lower than the RBC values obtained in groups F (Pb + 200 mg/kg ethanol CL extract) and G (Pb +100 mg/kg gallic acid). However, the RBC count in group D was significantly lower than those in groups A, E and G. Group C (Pb + 100 mg/kg of aqueous CL extract) and F (200 mg/kg ethanol CL extract) had significantly lower RBC values ($p<0.05$) than those obtained

in groups E and G. The mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) values also showed significant variation, as shown in Table 1. For example, the MCV and MCH values in groups F and G and were significantly lower ($p<0.05$) than the values in groups A, B, C, D and E. However, the MCV in group E was significantly higher the values in groups A and B. Meanwhile, only the mean corpuscular haemoglobin concentration (MCHC) in the untreated control, (group A) was higher than that of group B (Pb only).

Leucocytes Parameters

The white blood cell parameters of those rats exposed to sub-acute lead toxicity with concurrent amelioration with aqueous and ethanol extract of CL, as well as gallic acid are shown in Table 2. Total white blood cell counts were significantly lower ($p<0.05$) in the entire Pb treated groups (B-G) when compared to the untreated control (group A). However, the total WBC values improved in groups D, F and G when compared with Pb only treated group B, although the variations were statistically significant in group D ($p<0.05$). We also determined the effects of lead toxicity on the white blood cell differential counts. The neutrophil, lymphocytes, eosinophil and monocyte and basophil counts in group B was lower ($p<0.01$) than the values in the untreated control. Following treatment with the extracts, there were significant increases in the differential counts in the groups treated with lead + the extracts, as well as lead + gallic acid group there were significant improvement in the neutrophil, eosinophil, monocytes and basophils, especially in groups D, F or G as compared with group B (Pb only), other groups including C and E, statistically significant elevations especially in monocytes and eosinophil counts while others such as lymphocytes and eosinophils in groups C and E, were not statistically significant as compared to the toxicant only group.

Erythrocyte osmotic fragility

The effects of sub-acute lead acetate exposure and concurrent administration of aqueous and ethanol extracts of *Curcuma longa* and antioxidant gallic acid on adult male Wistar rats are shown in Fig 1. From the figure, the erythrocyte osmotic fragility of group G rats was significantly higher than that of group A or B at 0.5% NaCl concentration. Meanwhile, the result shows a generally lower erythrocyte osmotic fragility in groups A and B when compared to those animals exposed to lead and extracts, at 0.5%, 0.7% and 0.9% NaCl, though the difference were marginal with no statistical significance.

Sub Acute Study
Erythrocytes Parameters

The effect of sub chronic exposure of the adult male Wistar rats to lead acetate for 28 days on the erythrocytic indices are shown in Table 3. In a manner that is similar to the observations in the sub-acute study, there was a general and significant ($p<0.05$) reduction in the mean PCV, Hb and RBC values in the group 2 (Pb only) as compared to the untreated control (group A). The PCV values in groups C, D, E and G were also significantly higher ($p<0.05$) than the value in the toxicant group. Similarly, the Hb concentration was elevated significantly ($p<0.05$) in group C and D when compared with toxicant only group; whereas, groups E, F and G had Hb concentrations that were similar to the value in group B but lower than that of the untreated control. The RBC count only increased significantly ($p<0.05$) in E and G, which were

higher than the RBC values in the groups B, C, D and F. Thus, Group B (Pb only) also had similar RBC count with those from group C, D and E. The MCV values in groups C, D and F were significantly higher ($p<0.05$) than those of groups A, E and G, whereas the highest MCH was seen in group C, it was higher significantly ($p<0.05$) than those of groups A, E and G. The lowest MCH was observed in group E, which was significantly lower than the values in groups C, D and F. The MCHC also followed a similar pattern, with group E having the lowest MCHC value, which was significantly lower ($p<0.05$) than that of groups A and C. All other MCHC values though lower than that of group C, the differences were not statistically significant.

Leucocytes Parameters
Table 4 shows the impacts of subacute lead exposure on the white blood cell parameters of adult male Wistar rats and the modulatory/corrective effects of aqueous and ethanol extracts of *Curcuma longa* and gallic acid. We observed a slight elevation of the total white blood in groups B and C, although the differences were not significant, while group G had significantly lower ($p<0.05$) total WBC than all the other groups. The neutrophil count in group B reduced significantly when compared to the control while restoration was observed in the extract and Gallic treated groups, although only groups C and E were significantly higher ($p<0.05$) than the Pb only group. The lymphocyte on the other hand was elevated significantly in group B than in group A, which was reduced considerably in all the treated groups. In fact, the absolute lymphocyte counts in the extracts and Gallic treated groups were either lower or comparable to the untreated control. Similarly, the eosinophil counts were considerably lower in all the lead exposed group, whether treated or untreated, than the value in the untreated control group A. The monocyte count however was higher significantly ($p<0.05$) in group C than in all the others.

Erythrocyte osmotic fragility
As shown in Fig 4.2, the erythrocyte osmotic fragility of those rats in group B was higher significantly ($p<0.05$) at 0.5% concentration than the values in all the other groups. The erythrocyte osmotic fragility was however similar at all the other concentrations.

Plasma Biochemistry
The results of the plasma biochemistry of the rats following subchronic exposure to lead and the treatment with *Curcuma longa* extracts and gallic acid are shown in Tables 5 – 8. As shown in the plasma electrolytes (Table 5), there is a generalized reduction in the plasma sodium, potassium and chloride values in the lead only treated group when compared with the control (group A) and some of the extract-treated groups. For example, the plasma Na^+ in Pb only treated group was lower significantly ($p<0.05$) in group B than the values in either group C, D, E or F. Rats in group G also had lower Na+ than the values in groups E and F. Plasma K^+ and Cl^- in group B were slightly lower than the untreated control but lower ($p<0.05$) than the values in groups E or F. Meanwhile, the bicarbonate ion was significantly elevated in group B than in the control, but the values dropped generally in all the treated groups, though non-significantly.
As shown in Table 6, the plasma urea and creatinine levels were lower marginally in group B than in the control, but was significantly elevated ($p<0.05$) in those treated groups, except in the group treated with gallic acid which was similar to that

of group B. For example, urea levels in group B was lower than the values in groups D, E and F, in fact the urea concentrations were higher in groups D, E and F than in the untreated control and in gallic acid treated rats (group G). Creatinine on the other hand was higher in groups than the values obtained in groups A, B and G. The total protein in the lead only group was similar to that of the control and group G, but significantly lower than the values groups C, D, E and F. Group G also had significantly lower total protein than the values in groups C, D, E and F. Albumin levels were however similar across the entire groups.
As shown in Table 7, plasma AST was significantly higher in groups D and E, than the values obtained in the gallic acid treated group. Similarly, the Alt and ALP were found to be higher in group E than that of groups F and G, while ALP was additionally higher than the value in group C.
Table 8 shows the lipid profile of adult male Wistar rats following sub chronic exposure to lead and concomitant treatment with *Curcuma longa* extracts and gallic acid. There was a generalized elevation of triglyceride, high density and low-density and total cholesterol lipoprotein in group F which received lead acetate and 200 mg/kg ethanol extract of *Curcuma longa* as compared to all the other groups. Total cholesterol in group C was however lower significantly ($p<0.05$) than the value in group A. Similarly, LDL value in group A was higher than the values in groups C, D and E.

Markers of oxidative stress
The impacts of lead on free radical production and endogenous antioxidant enzymes in the liver, kidneys and testes were also determined in this study, the results of which can be seen in Tables 9-11. As shown in Table 9, the values of Kidney H_2O_2 generation and MDA in group B rats were higher significantly ($p<0.05$) than the corresponding values in the untreated control. Meanwhile the extracts and gallic acid treated groups C, D, E, F and G were significantly lesser than the values in group B. Furthermore, GSH, SOD as well as glutathione peroxidase (GPx) were significantly reduced in group B, than were the untreated control, while values of these enzymes showed significant improvement in the extract and gallic acid treated groups.
Similarly in the liver (Table 10), the H_2O_2 generation and MDA value in lead treated group B rats were significantly elevated ($p<0.05$) than the values obtained in the untreated control (group A), however the values of extracts and gallic acid treated groups C, D, E, and G had significantly lower values ($p<0.05$) than the values seen in group B. Meanwhile, the GSH and glutathione peroxidase, GPx were significantly lesser in group B, compared with the untreated control (group A) while values of same enzymes showed significant improvement in the extract and gallic acid treated groups.
Finally in the testes, as shown in Table 11, the H_2O_2, MDA and Protein values obtained in lead acetate only treated group B rats were significantly more ($p<0.05$) than the values obtained in group A (untreated control), which received distilled water only. The values in extracts and Gallic treated groups C, D, E, F, and G were lower significantly ($p<0.05$) than that obtained in lead only treated group B. Also, the GPx and SOD values reduced in group B as compared to the values in the untreated control while it improved significantly ($p<0.05$), in the extracts and gallic acid treated groups especially group D rats that received 200 mg/kg of aqueous *Curcuma longa* extract and 150 mg/kg of lead acetate.

Histopathology
Figures 1-3 show the histology (H & E) sections of the testes, liver as well as the kidney of the rats following subacute exposure to lead acetate and treatment with *Curcuma longa* extracts. Exposure to lead resulted in degeneration of the hepatocytes, glomerular tufts and seminiferous tubules in the liver, kidneys and testes, respectively. Congestion of central vein and amyloid casts were also observed in the liver and kidneys, respectively, lesions which were not observed in the extract treated groups.

Discussion
Haematology
Lead exposure has been previously reported to cause anaemia kidney damages, brain damage especially in young growing animals and humans (Ab Latif Wani and Usmani 2015) [1]. This study showed that exposure to lead as lead acetate in the Wistar rats resulted in reduction in the values of PCV, RBC, Hb concentration and a corresponding increase in the osmotic fragility values both in the sub-acute and subchronic phases of the study. This indicates that there was a mild anaemia (Ahur *et al*., 2018) [3], which may be regenerative as evidenced by the macrocytic (increased MCV) and a relatively hypochromic (reduced MCHC) pattern shown in the sub-acute and sub chronic studies at 14 and 28 days of lead acetate treatment respectively. The lowering of PCV, Hb, and RBC counts might be due to inhibition of haeme or porphyrin synthesis. Lead is known to inhibit aminolaevulinic acid dehydratase, and alter haeme synthesis (Patil 2006) [31]. However, significant increases in the PCV and RBC values observed in groups treated with ethanol and aqueous *Curcuma longa* extracts shows its modulatory effects in Lead toxicity following sub-acute and subchronic exposures. The modulatory effect observed in both phases of this study supports the erythropoiesis-boosting (hepatoprotective) effect of *Curcuma longa* rhizome extracts as reported by Abdelhamid *et al*. (2020) [2] who observed that Curcumin treatment improves the haematological, biochemical, and histopathological alterations induced by Lead acetate.

Plasma Biochemistry
Changes in plasma electrolyte concentrations, urea, and creatinine levels serve as clinical indicators of the health status of the kidneys while liver enzymes AST and ALT serve as indicators of liver damage (Kuatsienu *et al*., 2017) [20]. These elevations were observed in lead only treated group in the present study, which is an indication that sub-chronic exposure to lead could result in liver and kidney damage. Mild hyponatremia, hypokalaemia, hypochloremia and a reduction in values of bicarbonate in plasma of lead treated groups when compared to the control may also serve as indicators of renal insufficiency. The good cholesterol, HDL (high density lipoprotein) values, which helps to clear other forms of cholesterol from the bloodstream (Ma and Shieh 2006) [23] reduced in lead only treated group B significantly, while the bad LDL (low density lipoprotein) values increased. These imbalances were however corrected significantly in the rats treated with ethanol and aqueous extracts of *Curcuma longa* as well as gallic acid. This must be due to the antioxidant properties of *Curcuma longa* on the liver and kidney as described earlier by Tanvir *et al*., (2017) [39] who

explained in their study, that the flavonoid and phenolic compounds in turmeric are useful sources of natural antioxidant and confers significant protection against damages by free radicals. It could also be further inferred from this study that the extracts possess a good anti-obesity or antilipidaemic property in concurrence with Song *et al*., (2016) [38] who observed that *Curcuma longa* induced lipolysis and leptin regulation in adipose tissues and rats.

Markers of oxidative stress and histopathology
Oxidative stress plays significant and important roles in the pathogenesis of several conditions and toxicity such as seen in aging, environmental stress, pollution and even some cardiovascular and metabolic diseases such as hypertension and diabetes. In our study, lead treatment was shown to increase oxidative stress by increasing hydrogen peroxide levels and lipid peroxidation, but was ameliorated by the extract and gallic acid. Oxidative stress can be reduced by antioxidant treatment through consumption of natural antioxidants (Chaturvedi *et al*., 2017) [11]. These antioxidants help to delay or prevent the oxidation of an oxidisable lipids and proteins and DNA. *Curcuma longa* has been previously reported to possess a high contents phenol and flavonoids, with very good free radical scavenging ability (Meizura *et al*., 2011; Ak and Gülçin 2011; Mekonnen and Desta 2021) [24, 43, 26].

Furthermore, exposure to lead acetate resulted in considerable and consistent oxidative stress and histopathology lesions in the kidneys, liver and testes as a result of increased hydrogen peroxide generation, lipid peroxidation and depletion of antioxidant enzymes GPx, GSH and SOD. Lead is known to cause multiple organ damage and failure especially in kidney, liver and testes, via generation of ROS and other free radicals (Assi *et al*., 2016) [6]. The oxidative stress was however corrected by the concurrent administration of *Curcuma longa* ethanol and aqueous extracts in a manner similar to that of gallic acid. Ortega *et al*. (2021) [29] also reported that oxidative stress is probably the most important mechanism associated with lead-induced toxicity, resulting in increased generation of potent ROS including hydroxyl radical (OH⁻), and reduction in levels antioxidant enzymes in tissues. These unstable and highly reactive ROS attack various biomolecules in cells including membrane lipids, proteins, RNA and DNA, resulting in pathological tissue damage or apoptosis (Juan *et al*., 2021) [18].

Several flavonoids reportedly possess protective effects on parenchymatous organs including liver and kidneys, which is attributable to their antioxidant properties (Akbari *et al*., 2022) [5]. Tannins have also been shown to reduce lipid peroxidation in these organs and protect their cells from the destructive effects of ROS as a result of its potent antioxidant and anti-inflammatory properties (Kola *et al*., 2022) [19]. Phenolic compounds were also reported as the important active ingredients of natural products that are responsible for the antioxidant effects (Bodoira and Maestri 2020) [9], including *Curcuma longa* (Yang *et al*., 2020) [40]. These assumptions were confirmed by the observed lower values of oxidative stress markers observed in groups treated concurrently with 100 mg/kg and 200 mg/kg of ethanol and aqueous extracts of *Curcuma longa* in this study.

Table 1: Erythrocyte parameters of adult male Wistar rats exposed to sub-acute treatment with Lead acetate only or concurrently with aqueous and ethanolic extracts of *Curcuma longa* rhizomes. Values are mean and standard deviation while n = 5.

Parameter/ Groups	Group A	Group B	Group C	Group D	Group E	Group F	Group G
PCV (%)	48.40±1.49^{ab}	45.33±2.35^{a}	47.75±2.59	45.80±1.60	48.00±1.89^{b}	46.40±1.20	47.40±1.20
HB (g/dl)	16.96±0.84^{abd}	12.32±0.58^{acdef}	15.52±0.84^{c}	15.10±0.77^{ad}	16.38±0.98^{beg}	13.86±1.74^{bg}	15.46±1.56^{f}
RBC (x10^6/µl)	6.52±0.22^{c}	6.03±0.86^{ab}	6.21±0.22	5.54±0.14^{cde}	5.48±0.24^{af}	11.70±2.29^{fdg}	8.4±0.98^{beg}
MCV (fl)	74.21±2.00^{abc}	75.24±5.77^{def}	76.96±5.05^{gh}	83.05±1.52^{ij}	86.78±5.30^{adkl}	41.05±9.65^{begikm}	57.01±5.71^{cfhjlm}
MCH (ρg)	26.00±1.30^{af}	20.76±1.07^{bgl}	24.99±0.97^{ch}	27.31±2.25^{dil}	29.99±1.9^{ej}	12.53±4.56^{abcdek}	18.79±3.93^{fghijk}
MCHC (g/dl)	35.06±2.09^{a}	28.22±1.35^{a}	32.56±1.90	32.90±2.99	33.63±2.83^{a}	29.82±3.47	32.66±3.98

Values with the same superscript alphabets along the same row are significantly different at $P < 0.05$

Legend to all Tables

Group A = Distilled water

Group B = 150 mg/kg Lead acetate only

Group C = 150 mg/kg Lead acetate + 100 mg/kg aqueous extract of *Curcuma Longa*

Group D = 150 mg/kg Lead acetate + 200 mg/kg aqueous extract of *Curcuma Longa*

Group E = 150 mg/kg lead acetate + 100 mg/kg ethanol extract of *Curcuma Longa*

Group F = 150 mg/kg Lead acetate + 200 mg/kg ethanol extract of *Curcuma Longa*

Group G = 150 mg/kg Lead acetate + 100 mg/Kg Gallic acid

Table 2: Leukocyte parameters of adult male Wistar rats treated with lead acetate and aqueous and ethanolic extracts of *Curcuma longa* rhizomes sub-acutely. Values are mean and standard deviation with n=5

Parameters	Group A	Group B	Group C	Group D	Group E	Group F	Group G
WBC (x 10^3/µl)	30.08±3.64^{abcde}	19.51±1.31^{ag}	23.13±0.81^{b}	30.70±7.60^{cg}	22.86±5.20^{d}	29.42±7.04^{e}	29.12±10.44^{f}
Neutrophil (x 10^3/µl)	4.41±0.66^{a}	1.82±0.97^{abcd}	3.38±1.32	5.80±1.10^{b}	3.29±0.71	6.17±1.48^{c}	4.57±1.77^{d}
Lymphocyte (x 10^3/µl)	20.35±2.34^{a}	16.34±2.16^{abcd}	15.39±5.30	20.11±5.17^{b}	15.38±3.61	17.81±4.18^{c}	18.47±6.70^{d}
Monocyte (x 10^3/µl)	2.90±1.11^{a}	1.02±0.38^{abcdef}	3.18±1.12^{b}	2.02±0.85^{c}	3.28±0.72^{d}	2.41±0.87^{e}	3.82±1.56^{f}
Eosinophil (x 10^3/µl)	1.59±0.29^{a}	0.24±0.25^{abcd}	0.57±0.12	1.13±0.57^{b}	0.34±0.22	1.33±0.41^{c}	0.83±0.49^{d}
Basophil (x 10^3/µl)	0.83±0.31^{a}	0.10±0.16^{abcdef}	0.61±0.29^{b}	1.63±0.43^{c}	0.57±0.28^{d}	1.74±0.44^{e}	1.42±0.65^{f}

Table 3: Erythrocyte parameters of adult male Wistar rats following sub-chronic treatment with Lead acetate and modulation with aqueous and ethanolic extracts of *Curcuma longa*. Values are means and standard deviation while number of animals, n = 5

Parameters	Group A	Group B	Group C	Group D	Group E	Group F	Group G
PCV (%)	48.25±0.83^{a}	45.00±0.82^{abcde}	47.40±1.65^{b}	49.40±4.22^{c}	48.00±0.82^{d}	44.25±2.78	47.75±1.92^{e}
HB (g/dl)	16.72±1.62^{aefg}	14.07±0.50^{abcd}	16.88±1.55^{bhij}	15.23±0.33^{ck}	12.58±1.11^{dehk}	14.48±0.96^{fi}	14.12±0.69^{gj}
RBC (x10^6/µl)	7.48±0.72^{abcd}	5.83±0.70^{ahi}	5.25±0.82^{bfj}	5.14±0.71^{cek}	7.23±0.62^{efgh}	5.29±0.61^{dgl}	7.49±0.78^{ijkl}
MCV (fl)	66.06±7.29^{ab}	77.27±11.69	92.98±20.02^{acd}	97.57±14.38^{bef}	65.24±6.18^{ce}	87.53±11.93^{g}	61.78±4.08^{dfg}
MCH (ρg)	34.80±4.69^{a}	31.45±1.41	35.98±4.49^{abc}	30.15±3.91^{de}	26.92±3.67^{bdf}	32.32±1.52^{fg}	29.18±2.32^{ceg}
MCHC (g/dl)	22.70±4.66^{a}	25.73±0.97	33.91±6.42^{b}	28.86±5.41	17.62±3.22^{ab}	28.60±53.68	19.09±2.80

Values with the same superscript alphabets along the same row are significantly different at $p < 0.05$

Table 4: Leucocyte parameters of adult male Wistar rats following sub-chronic treatment with Lead acetate and modulation with aqueous and ethanolic extracts of *Curcuma longa*. Values are means and standard deviation while number of animals, n = 5

Parameters	Group A	Group B	Group C	Group D	Group E	Group F	Group G
Total WBC (x 10^3/µl)	16.4±3.70^{a}	18.87±5.43^{b}	19.5±5.18^{cg}	15.60±2.00^{d}	13.57±2.48^{eg}	11.11±1.47^{f}	10.00±6.3^{abcdef}
Neutrophil (x 10^3/µl)	2.27±0.55^{a}	1.25±0.27^{abc}	2.59±0.81^{b}	1.86±0.36	2.45±0.36^{c}	1.97±0.11	1.75±1.11
Lymphocyte (x 10^3/µl)	11.43±3.00^{a}	16.50±3.90^{bcde}	12.80±3.60^{fg}	9.27±1.71^{b}	6.54±0.81^{cf}	5.39±1.93^{adg}	6.97±3.93^{e}
Eosinophil (x 10^3/µl)	0.74±0.22^{abcdef}	0.19±0.04^{a}	0.40±0.18^{b}	0.15±0.02^{c}	0.47±0.24^{d}	0.27±0.13^{e}	0.26±0.13^{f}
Monocyte (x 10^3/µl)	1.56±0.24^{a}	0.84±0.21^{b}	3.32±0.80^{abcdef}	1.92±0.39^{c}	1.03±0.19^{d}	1.34±0.74^{e}	1.34±0.74^{f}
Basophil (x 10^3/µl)	0.49±0.15	0.22±0.02	0.40±0.10	0.37±0.12	0.65±0.17	0.48±0.25	0.65±0.62

Values with the same superscript alphabets along the same row are significantly different at $p < 0.05$

Table 5: Effects of lead acetate toxicity on plasma electrolytes in male Wistar rats as modulated by ethanolic and aqueous extracts of *Curcuma longa*.

Parameters	A	B	C	D	E	F	G
Na$^+$ (mmol/L)	139.60±1.53	136.33±1.53^{abcd}	140.00±1.00^{a}	141.33±1.15^{b}	143.67±2.1^{ce}	142.33±1.53^{df}	137.33±2.52^{ef}
K$^+$ (mmol/L)	4.00±0.10	3.60±0.20^{ab}	3.93±0.23	4.00±0.27	4.23±0.15^{a}	4.23±0.12^{b}	3.83±0.21
Cl$^-$ (mmol/L)	106.70±2.89	101.67±2.89^{ab}	106.67±2.89	108.33±2.89	110.00±0.00^{a}	110.00±0.00^{b}	103.33±2.89
HCO$_3^-$ (mmol/L)	22.00±1.00^{a}	25.00±1.00^{a}	22.33±1.53	21.33±1.53	21.33±1.53	21.33±0.58	23.33±2.2.08

Values with the same superscript alphabets along the same row are significantly different at $p < 0.05$

Table 6: Effects of lead toxicity on plasma proteins and metabolites in male Wistar rats modulated by aqueous and ethanolic extracts of *Curcuma longa*.

Parameters	A	B	C	D	E	F	G
Urea	28.67±2.52^{abc}	24.33±3.51^{def}	37.00±5.57^{g}	43.67±6.11^{adi}	51.50±6.36^{begj}	48.00±4.24^{cfk}	24.67±2.52^{ijk}
Creatinine	0.63±0.06^{a}	0.57±0.06^{bc}	0.73±0.06	0.87±0.06	1.17±0.38^{abd}	1.03±0.15^{ce}	0.57±0.06^{de}
Total Pro (g/dl)	6.70±0.15	6.70±0.20^{abcd}	7.10±0.05^{dae}	7.10±0.15^{bf}	7.20±0.15^{ceg}	7.20±0.05^{dcfh}	6.70±0.20^{efg}
Albumin (g/dl)	3.80±0.20	3.70±0.20	4.00±0.05	4.10±0.05	4.00±0.20	4.10±0.10	3.70±0.20

Values with the same superscript alphabets along the same row are significantly different at $p<0.05$

Table 7: Effects of lead toxicity on liver enzymes activities and bilirubin in male Wistar rats as modulated by curcuma longa.

Parameters	A	B	C	D	E	F	G
TB (mg/dl)	0.5±0.2	0.4±0.15	0.5±0.26	0.6±0.10	0.8±0.3	0.6±0.15	0.40±0.10
CB (mg/dl)	0.2±0.05	0.2±0.15	0.2±0.05	0.3±0.10	0.4±0.15	0.3±0.11	0.20±0.10
AST (iu/l)	14.00±1.00	14.33±2.08	14.00±2.00	16.00±1.00 [a]	17.00±2.00 [b]	14.67±1.53	11.00±1.00[ab]
ALT (iu/l)	11.00±1.00	11.67±1.53	11.33±2.52	12.50±0.70	13.50±0.70 [ab]	9.00±1.41 [a]	8.67±1.53 [b]
ALP (iu/l)	51.33±4.16	51.00±2.80	47.00±1.4 [a]	59.00±3.61 [b]	64.00±2.40 [abc]	55.50±10.60	41.00±7.81 [c]

Values with the same superscript alphabets along the same row are significantly different at $p< 0.05$

Table 8: The effect of lead toxicity on lipid profiles in male Wistar rats as modulated by *Curcuma longa* extracts

Parameters	A	B	C	D	E	F	G
TC (mg/dl)	82.50±10.60 [ag]	66.33±6.67 [b]	55.67±5.69 [cg]	63.50±4.90 [d]	67.00±8.48 [e]	113.33±7.64[abcdef]	64.00±8.19 [f]
TG (mg/dl)	33.00±4.24 [a]	28.33±3.06 [b]	24.00±4.20 [c]	27.00±1.41 [d]	38.33±5.90 [e]	53.00±3.61[abcdef]	29.50±2.10 [f]
HDL (mg/dl)	23.00±5.29 [a]	17.50±0.70 [a]	20.00±1.00 [a]	24.33±2.08	29.33±8.33	37.00±6.08[abc]	29.67±3.51
LDL (mg/dl)	46.33±4.51[abc]	39.00±5.65 [d]	27.00±1.41 [ae]	31.33±2.08[bf]	30.50±2.12 [cg]	60.00±9.89[defgh]	36.50±6.36 [h]

Values with the same superscript alphabets along the same row are significantly different at $p< 0.05$

Table 9: The effect of lead toxicity on markers of oxidative stress in the kidney of male Wistar rats as modulated by ethanol and aqueous extract of *Curcuma longa*. Values are presented in means and standard deviation.

Parameters	A	B	C	D	E	F	G
Protein	4.68±0.89	4.94±1.49	5.26±0.51	4.85±0.41	4.89±1.57	4.07±1.41	4.43±0.74
H_2O_2	117.42±12.06[a]	135.87±20.35[abcdef]	72.45±9.77[bgh]	100.00±13.42[ch]	99.15±13.37[d]	96.16±14.48[e]	97.62±5.83[f]
GSH	93.15±18.16	81.84±3.94[a]	94.57±12.43	80.96±5.4[b]	90.23±11.80	106.70±18.36[ab]	89.99±8.00
MDA (x10^{-6})	1.67±0.52[a]	12.63±7.94[abcde]	1.88±0.79	1.11±0.22[b]	1.58±0.59[c]	1.91±0.41[d]	2.16±1.01[e]
SOD	103.92±65.13[abcdef]	47.06±6.19[a]	51.26±15.99[b]	43.55±4.46[c]	36.22±4.42[d]	39.41±1.85[e]	36.89±3.55[f]
GPX	40.39±8.56[d]	28.14±2.15[abc]	35.67±3.44	38.39±3.82[e]	46.52±4.21[b]	56.88±11.37[bde]	49.32±8.23[c]

Values with the same superscript alphabets along the same row are significantly different at $p<0.05$

Table 10: The effect of lead toxicity on markers of oxidative stress in the liver of male Wistar rats as modulated by ethanol and aqueous extract of *Curcuma longa*. Values are presented in means and standard deviation.

Parameters	A	B	C	D	E	F	G
Protein	5.81±0.95	5.84±1.68	4.92±1.05	5.57±1.35	5.74±1.30	5.98±0.62	4.89±0.562
H_2O_2	205.76±25.00[ab]	290.19±73.45[acdefg]	158.85±26.39[c]	158.25±18.82[d]	175.93±11.08[e]	158.43±34.94[f]	106.14±7.90[bg]
GSH	105.84±13.79	83.82±15.14[a]	101.98±23.55	56.13±43.24[b]	93.57±14.65	91.94±10.08	148.61±51.48[ab]
GPX	43.43±7.98[a]	37.15±8.93[b]	57.72±5.69[abcdef]	54.51±5.42[c]	50.20±5.01[d]	43.22±2.17[e]	52.02±3.92[f]
MDA (x10^{-6})	2.25±0.29	4.14±1.89	3.27±0.82	3.48±0.79	3.52±2.74	3.74±1.52	3.03±1.22
SOD	48.38±9.69	40.05±6.91	45.08±9.55	51.94±10.4	51.93±10.46	41.59±5.96	47.78±12.54

Values with the same superscript alphabets along the same row are significantly different at $p<0.05$

Table 11: The effect of lead toxicity on markers of oxidative stress in the testes of male Wistar rats as modulated by ethanol and aqueous extract of *Curcuma longa*. Values are presented in means and standard deviation.

Parameters	A	B	C	D	E	F	G
Protein	2.99±0.55	3.91±0.69[a]	2.96±0.52	3.21±0.53	3.77±0.60[b]	2.44±0.25[ab]	3.02±0.79
H_2O_2	74.51±4.39[a]	97.31±5.49 [abcdef]	69.08±4.42[b]	75.19±9.99[c]	77.65±7.47[d]	72.45±9.76[e]	69.53±5.56[f]
GSH	53.30±8.04	36.23±6.01	62.01±45.32	46.79±20.56	42.22±12.78	53.05±36.55	29.22±4.08
GPX	46.23±10.54[ab]	30.69±4.64[cd]	53.26±9.47[c]	46.34±7.94[ef]	44.03±4.05[gh]	69.83±8.84[aeg]	64.63±10.87[bdfh]
MDA (x10^{-6})	4.95 ± 0.94	11.60 ±9.98[abcde]	2.82 ±0.76[a]	1.51±0.62[b]	0.96±0.30[c]	2.53±0.66[d]	1.67±0.91[e]
SOD	35.20±2.90[a]	31.75±7.49	33.48±3.00	34.00±4.00[b]	33.24±6.27	30.00±2.31	24.66±3.34[ab]

Values with the same superscript alphabets along the same row are significantly different at $p<0.05$
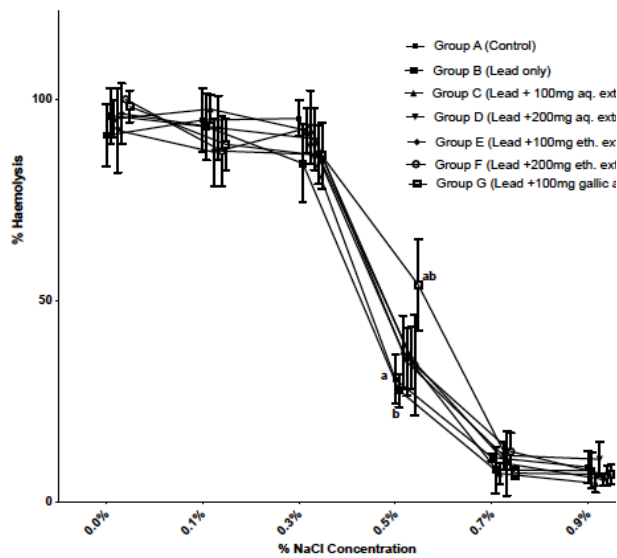
Fig. 1. Erythrocyte osmotic fragility of adult male Wistar rats following sub-acute treatment with lead acetate only and concurrently with aqueous and ethanol extracts of *Curcuma longa* and gallic acid. Values are means while vertical bars represent standard deviation while n = 5 animals in each group.

Values with the same superscript alphabets at the same concentration are significantly different at P < 0.05.
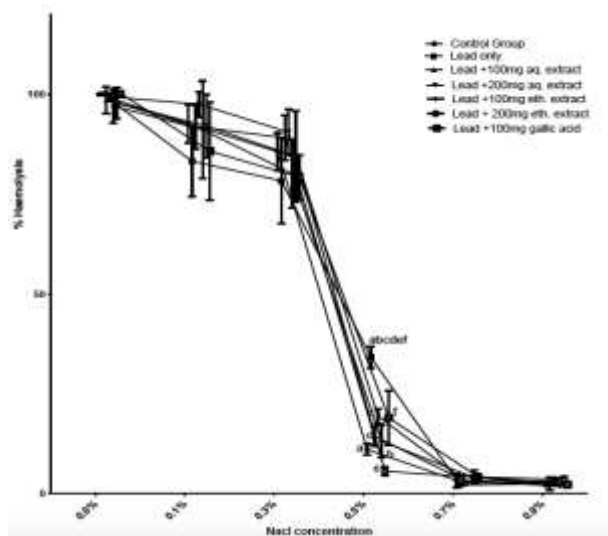

Fig. 2. Erythrocyte osmotic fragility of adult male Wistar rats following sub-chronic treatment with lead acetate only and concurrently with aqueous and ethanol extracts of *Curcuma longa* and gallic acid. Values are means while vertical bars represent standard deviation while n = 5 animals in each group.

Values with the same superscript alphabets at the same concentration are significantly different at P < 0.05.
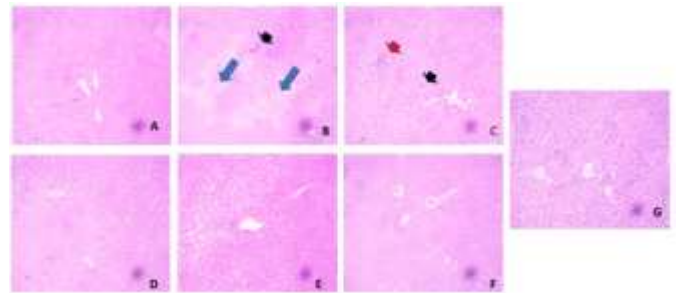

Fig. 3. Histology of the liver in adult male wistar rats following exposure to lead acetate treated with aqueous and ethanol *Curcuma longa* rhizome extracts. Group A showed no visible lesion while group B showed diffuse areas of degeneration of hepatocytes with loss of the hepatic chords (blue arrows) and congestion of the central vein (Black arrow). Group C showed slight congestion of the central vein (black arrow) infiltration of the portal triad by mononuclear leucocytes (red arrow). No significant lesions were seen in the other groups. Magnification X 400.
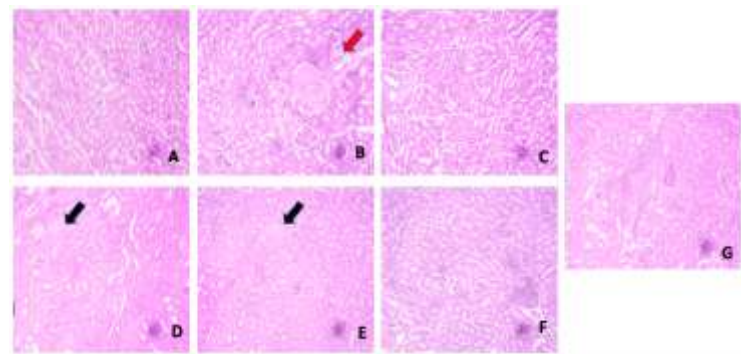

Fig. 4. Histology of the kidney (H & E) in adult male wistar rats following exposure to lead acetate treated with aqueous and ethanol *Curcuma longa* rhizome extracts. Group A (control) showed no visible lesion. Group B showed degeneration and shrinkage of the glomerular tuft (red arrow). Groups D and E also showed areas of amyloid protein cast infiltration (black arrow). No significant lesions were however seen in groups C, F and G. Magnification X 400.
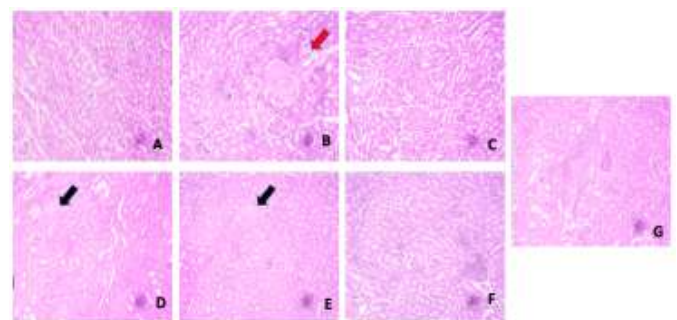

Fig 5. Histology of the testes (H & E) in adult male wistar rats following exposure to lead acetate treated with aqueous and ethanol *Curcuma longa* rhizome extracts. Group A (control) showed no visible lesion. Group B showed significant disruption of the architecture of the seminiferous tubules (Black arrows), whereas, no significant lesions were however seen in the other groups and control (group A). Magnification X400.

Conclusion

This study further reinforce the damaging effects of lead acetate on haematological parameters and its multi-organ damaging effects through oxidative stress. Moderate concentrations used in this study however shows that, *Curcuma longa* ethanol and aqueous extracts have considerable ameliorative effects on the lead acetate induced toxicity and could be employed as supplement to curb the damages associated with lead toxicity through its antioxidant effects.

References

1. Ab Latif Wani AA, Usmani JA. Lead toxicity: a review. Interdiscip Toxicol. 2015;8(2):55-64.
2. Abdelhamid FM, Mahgoub HA, Ateya AI. Ameliorative effect of curcumin against lead acetate–induced hemato-biochemical alterations, hepatotoxicity, and testicular oxidative damage in rats. Environmental Science and Pollution Research. 2020;27:10950-10965.
3. Ahur VM, Anika SM, Onyeyili PA. Age-sex dimorphisms in the estimation of median lethal dose (LD50) of lead diacetate in rabbits using up-and-down procedure (Arithmetic method). Sokoto Journal of Veterinary Sciences. 2018;16(4):64-72.
4. Ak T, Gülçin İ. Antioxidant and radical scavenging properties of curcumin. Chemico-biological interactions. 2008;174(1):27-37.
5. Akbari B, Baghaei-Yazdi N, Bahmaie M, Mahdavi Abhari F. The role of plant-derived natural antioxidants in reduction of oxidative stress. BioFactors. 2022;48(3);611-633.
6. Assi MA, Hezmee MNM, Abd Wahid Haron MYM, Sabri MAR. The detrimental effects of lead on human and animal health. Veterinary world. 2016;9(6);660-671.
7. Azeez OI, Braimah SF. Protective Effect of Vitamin E on Potassium Dichromate-induced Haematoxicity and Oxidative Stress in African Catfish (*Clarias gariepinus*). Asian Journal of Environment and Ecology. 2020;13(2):18-31.
8. Bilal MS, Freed MZ, Ashraf MB, Khan. Activity of acetylcholinesterase and acid and alkaline phosphatases in different insecticide treated *Helicoverpa armigera* (Hubner). Environmental Science and Pollution research. 2018;25(23):22903-22910.
9. Bodoira R, Maestri D. Phenolic compounds from nuts: extraction, chemical profiles, and bioactivity. Journal of Agricultural and Food Chemistry. 2020;68(4);927-942.
10. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-254.
11. Chaturvedi A, Tiwari AK, Mani RJ. Traditional practices of using various medicinal plants during *postnatal* care in Chitrakoot district. Indian Journal of Traditional Knowledge. 2017;16(4):605-613.
12. Dignam T, Kaufmann RB, LeStourgeon L, Brown MJ. Control of lead sources in the United States, 1970-2017: public health progress and current challenges to eliminating lead exposure. Journal of public health management and practice. 2019;25(1);S13-S22.
13. Doumas BT, Waston WA, Biggs AG. Biuret method for quantitative estimation of total protein in serum or plasma. Clin Chim Acta. 2018;31:87-91.
14. Farombi EO, Tahnteng JG, Agboola AO, Nwankwo JO, Emerole GO. Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kolaviron-a garcinia kola seed extract. Food Chem Toxicol. 2000;38:353-541.
15. Flora G, Gupta D, Tiwari A. Toxicity of lead: a review with recent updates. Interdisciplinary toxicology. 2012;5(2):47-58.
16. Goodchild CG, Beck ML, VanDiest I, Czesak FN, Lane SJ, Sewall KB, *et al*. Male zebra finches exposed to lead (Pb) during development have reduced volume of song nuclei, altered sexual traits, and received less attention from females as adults. Ecotoxicology and Environmental Safety. 2021;210:111850.
17. Jollow DJ, Michell JR, Zampaglione N, Gillete JR. Bromobenzene induced liver necrosis: protective role of GSH and evidence for 3,4- bromobenzene oxide as the hepatotoxic metabolite. Pharm. 1974;11:15-169.
18. Juan CA, Pérez de la Lastra JM, Plou FJ, Pérez-Lebeña E. The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. International Journal of Molecular Sciences. 2021;22(9);4642. https://doi.org/10.3390/ijms22094642
19. Kola P, Metowogo K, Manjula SN, Katawa G, Elkhenany H, Mruthunjaya KM, et la. Ethnopharmacological evaluation of antioxidant, anti-angiogenic, and anti-inflammatory activity of some traditional medicinal plants used for treatment of cancer in Togo/Africa. Journal of Ethnopharmacology. 2022;283:114673.
20. Kuatsienu EL, Ansah C, Adinortey MB. Toxicological evaluation and protective effect of the ethanolic leaf extract of Launaea taraxacifolia on gentamicin induced rat kidney injury. Asian Pac J Trop Biomed. 2017;7:640-646.
21. Kulyal P, Acharya S, Ankari AB, Kokkiripati PK, Tetali SD, Raghavendra AS, *et al*. Variable Secondary Metabolite Profiles Across Cultivars of *Curcuma longa* L. and C. aromatica Salisb. Frontiers in Pharmacology. 2021;12:659546.
22. Kuribayashi T, Sato K, Joh T, Kaneoke M. Breeding of a non-urea-producing sake yeast carrying a FAS2 mutation, Myosciences. 2017;58(4):302-306.
23. Ma H. Shieh KJ. Cholesterol and human health. The Journal of American Science. 2006;2(1):46-50.
24. Maizura M, Aminah A, Wan Aida WM. Total phenolic content and antioxidant activity of kesum (*Polygonum minus*), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) extract. International Food Research Journal. 2011;18(2):526-531.
25. Mallhi TH, Qadir MI, Khan YH. Determination of phytoconstituents of n-hexane extract of leaves of Morus nigra and elevation of their effects on biochemical and histopathological parameters in paracetamol intoxicated mice liver. Brazilian Journal of Pharmaceutical Sciences. 2018;54(3):1-5.
26. Mekonnen A, Desta W. Comparative study of the antioxidant and antibacterial activities of Rumex abyssinicus with commercially available Zingiber officinale and *Curcuma longa* in Bahir Dar city, Ethiopia. Chemical and Biological Technologies in Agriculture. 2021;8:1-11.
27. Mishra S, Goel B. Pharmaceutical and Nutritional Properties of Turmeric (*Curcuma longa*): A Mini Review. Advances in Zoology and Botany. 2020;8(3):83-86.

28. Onuegbu JA, Ubuo KA, Nnamdi KM, Joseph EA, Gladys LA, Christian EO, *et al*. Evaluation of nephrotoxic effect of lead exposure mong automobile repairers in Nnewi Metropolis. Int J of Res Med Sci. 2014;2(3):1107-1111.

29. Ortega DR, Esquivel DFG, Ayala TB, Pineda B, Manzo SG, Quino JM, *et al*. Cognitive impairment induced by lead exposure during lifespan: Mechanisms of lead neurotoxicity. Toxics. 2021;9(2):23-53. https://doi.org/10.3390/toxics9020023.

30. Oyagbemi AA, Omobowale TO, Asenuga ER, Adejumobi AO, Ajibade TO, Ige TM, *et al*. Sodium fluoride induces hypertension and cardiac complications through generation of reactive oxygen species and activation of nuclear factor kappa beta. Environmental toxicology. 2017;32(4):1089-1101.

31. Patil AJ, Bhagwat VR, Patil JA, Dongre NN, Ambekar JG, Jailkhani R, *et al*. Effect of lead (Pb) exposure on the activity of superoxide dismutase and catalase in battery manufacturing workers (BMW) of Western Maharashtra (India) with reference to heme biosynthesis. International journal of environmental research and public health. 2006;3(4):329-337.

32. Rabiu S, Abubakar MG, Sahabi DM, Makusidi MA. Oxidative Stress Markers in Individuals Environmentally Exposed To Lead. Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT). 2020;14(2):05-14. e-ISSN: 2319-2402, p- ISSN: 2319-2399

33. Rakete S, Moonga G, Wahl AM, Mambrey V, Shoko D, Moyo D, *et al*. Biomonitoring of arsenic, cadmium and lead in two artisanal and small-scale gold mining areas in Zimbabwe. Environmental Science and Pollution Research. 2022;29:4762-4768. Available at: https://doi.org/10.1007/s11356-021-15940-w.

34. Ravibabu K, Barman T, Bagepally BS. Assessment of bone turnover biomarkers in lead-battery workers with long-term exposure to lead. The International Journal of Occupational and Environmental Medicine. 2020;11(3):140.

35. Sahoo JP, Behera L, Praveena J, Sawant S, Mishra A, Sharma SS, *et al*. The golden spice turmeric (*Curcuma longa*) and its feasible benefits in prospering human health-A review. American Journal of Plant Sciences. 2021;12(3):455-475.

36. Sahu BD, Kumar JM, Kuncha M, Borkar RM, Srinivas R, Sistla R, *et al*. Baicalein alleviates doxorubicin-induced cardiotoxicity via suppression of myocardial oxidative stress and apoptosis in mice. Life sciences. 2016;144:8-18.

37. Salem MA, El-Shiekh RA, Fernie AR, Alseekh S, Zayed A. Metabolomics-based profiling for quality assessment and revealing the impact of drying of Turmeric (*Curcuma longa* L.). Scientific reports. 2022;12(1):10288.

38. Song WY, Choi JH. Korean *Curcuma longa* L. induces lipolysis and regulates leptin in adipocyte cells and rats. Nutrition research and practice. 2016;10(5):487-493.

39. Tanvir EM, Hossen M, Hossain M, Afroz R, Gan SH, Khalil M, *et al*. Antioxidant properties of popular turmeric (*Curcuma longa*) varieties from Bangladesh. Journal of Food Quality. Article ID 8471785. 2017, 8.

40. Yang QQ, Cheng LZ, Zhang T, Yaron S, Jiang HX, Sui ZQ, *et al*. Phenolic profiles, antioxidant, and antiproliferative activities of turmeric (*Curcuma longa*). Industrial crops and products. 2020;152:112561.

41. Wolff L. Inventing Eastern Europe: The map of civilization on the mind of the Enlightenment. Stanford University Press; c1994.

42. Farombi EO, Shrotriya S, Na HK, Kim SH, Surh YJ. Curcumin attenuates dimethylnitrosamine-induced liver injury in rats through Nrf2-mediated induction of heme oxygenase-1. Food and Chemical toxicology. 2008;46(4):1279-87.

43. Göçer H, Gülçin İ. Caffeic acid phenethyl ester (CAPE): correlation of structure and antioxidant properties. International journal of food sciences and nutrition. 2011;62(8):821-5.