Egyptian Journal of Zoology (EJZ), Vol. 80: 35-49 (December, 2023) p-ISSN: 1110-6344
Publisher: The Zoological Society of A. R. Egypt e-ISSN: 2682-3160
DOI: 10.21608/ejz.2023.166927.1087

RESEARCH ARTICLE

THE AMELIORATIVE EFFECTS OF LACTOFERRIN AGAINST LEAD ACETATE TOXICITY IN FEMALE ALBINO RAT

Mohamed G. Shalan; Nada A. Al-Bakry; Hanan M. Rashwan*
Zoology Department, Faculty of Science, Arish University, North Sinai, Egypt

Article History:
Received: 4 October 2022
Revised: 22 January 2023
Accepted: 28 January 2023

Published Online:
1 February 2023

Keywords:
Female rat
Histopathology
Lactoferrin
Lead acetate
Oxidative stress

***Correspondence:**
Hanan Rashwan
Zoology Department
Faculty of Science
Arish University
North Sinai, Egypt
E-mail:
h_rashwan2008@yahoo.com

ABSTRACT

This study investigated the protective role of lactoferrin (LF) against lead acetate poisoning in female albino rats (*Rattus norvegicus*). Four equal groups of animals (6 rats/group) were treated orally/daily by gastric tube for eight weeks. The first group was the control group, the second group received LF (10 mg/kg of body weight), the third group received lead acetate (100 mg/kg of body weight), and the fourth group received both LF and lead acetate with the same doses. After eight weeks, blood and tissue samples were taken for hematological, biochemical and histological analyses. The results showed that lead exposure induced significant elevations (P<0.05) in the activity of plasma alanine aminotransferase and aspartate aminotransferase, plasma creatinine concentration, plasma lead level, hepatic cytochrome P450, and the brain malondialdehyde. Brain superoxide dismutase activity was reduced significantly (P<0.05), in response to the lead toxicity. Lead acetate caused many pathological changes in the liver, brain, and kidneys tissues. Severe DNA fragmentation in the brain tissue was observed after lead treatment. On the other hand, LF was found to modulate significantly (P<0.05) the deteriorations in most parameters under investigation. In conclusion, these data suggest that LF has a protective function in the molecular and histological processes causing neurodegeneration, as well as renal and hepatocyte injury induced by lead.

INTRODUCTION

Lead is the most toxic heavy metal in the environment[1]. Lead exposure is widespread and has an impact on public health[2]. It is used to produce many products, including lead crystal tools, printing pigments, hair coloring pigments, and cosmetics[2]. Exposure to lead occurs through lead-related professions and in industrial processes such as lead smelting, combustion, pottery making, paint, battery recycling dyes, book printing, house dust, and polluted soil[2]. Occupational exposure to lead occurs in smelters, mines, and battery factories, as well as welding of lead-coated metal. The magnitude of the response to lead toxicity depends on the dose, age of the person exposed, duration of exposure, nutritional status and occupational exposure[2]. Lead spreads to all organs and systems in the body, but the prime target of lead toxicity is the central nervous system[2]. It can affect the neurological and biological functions of the animal body. It causes several problems in various organs, such as mental retardation, behavioral problems, violence, and growth delay, damage to the central nervous system and learning abilities,

damage to sensory nerves, and impairment in cognitive function, hearing and vision[2]. Lead can cause adverse health effects in three ways inhalation, ingestion and skin contact[3]. It also causes toxic effects on genes, especially in the brain, bone marrow, lungs, and liver[4].

Lactoferrin (LF) is a functional glycoprotein, namely transferrins, containing about 690 amino acid remnants[5]. LF is associated with many biological and physiological activities, such as antitumor, antibacterial, antiviral, anti-inflammatory, anti-stress, antioxidant, and immune regulation, and iron-regulating properties[6]. Little attention has been paid to the role of LF in ameliorating lead-induced toxicity in female rats. So, the objective of this study was to evaluate the protective role of LF against lead-induced toxicity in the liver, kidney, and brain tissues.

MATERIAL AND METHODS

Chemicals

Pure lead acetate was purchased from Adwik, El Naser Pharmaceutical Chemicals Company (Cairo, Egypt). The LF was obtained from Green Field for Hygint Pharmaceutical Company (Alexandria, Egypt).

Experimental animals and design

The experiment was conducted following the Faculty of Science Animal Ethics Committee guidelines, Suez Canal University, Egypt (approval number: REC110/2022). Twenty-four adult female Wistar albino rats (*Rattus norvigicus*) weighing (100-120 g) were obtained from the animal house of the Faculty of Science, Suez Canal University, Egypt. The rats were kept in plastic cages with a 12 hours light-dark cycle. All rats had free access to food and water during the study period. The rats were allowed to acclimatize for one week before the investigation under standard laboratory conditions in the animal house of the Faculty of Science, Arish University, North Sinai, Egypt.

After acclimatization, rats were divided randomly into four groups (A, B, C, and D), with six animals in each group and treated orally by oral gavage for 8 weeks as follows: group A (normal control group) orally received vehicle (distilled water), group B (LF-treated group) received 10 mg LF/kg body weight dissolved in distilled water[7], group C (lead acetate-treated group) received a sub-lethal dose of 100 mg lead acetate/kg body weight dissolved in distilled water[8], which equals 1/6 of LD_{50}[9], and group D (LF + lead acetate-treated group) received 10 mg LF/kg body weight and 100 mg lead acetate/kg body weight. All treatments were given orally/ daily for eight weeks by gastric tube.

The weight of the rats was evaluated weekly. At the end of the experiment (week 8), all rats were sacrificed under ketamine anesthesia (50 mg/kg body weight, intramuscular injection). Two blood samples were taken for every animal, one collected in EDTA tube and used immediately for hematological analysis and the second collected in centrifuge tube then centrifuged for 15 minutes at 1000 ×*g*. Serum was separated and stored at −30ºC in a deep freezer till further biochemical measurements. The tissues were excised for biochemical and histopathological examinations.

Hematological indices analysis

The hemoglobin content (Hb), red blood corpuscles (RBCs) count, hematocrit value (HCT), mean cell volume (MCV), and mean cell hemoglobin (MCH) were done using a complete blood cell count DIAGON Ltd-D-Cell 60 fully automatic hematological analyzer.

Preparation of tissues for microscopical and gel examinations

After dissecting rats, the liver, kidney and brain were taken, blotted on filter paper and weighed. Portion of 200 mg of the brain was taken immediately for gel examination. Representative specimens of the liver, kidney and brain were placed in 10%

formalin solution and processed to paraffin for histological section and staining. The remaining portions were stored at −30ºC.

Histopathological methods

Five-micron thick histological sections were prepared and stained with hematoxylin and eosin. Microscopic analysis of the specimens was done blindly[10].

DNA extraction and visualization

Tissue homogenates of the brain (200 mg) were subjected to DNA extraction using the Zymoresearch Quick-g DNA™ MiniPrep kit, (Catalog Number: D3024; Irvine, CA, USA). Tissue homogenates were centrifuged at 12,000 ×*g* for 10 minutes at 4ºC. Residual supernatants were used for DNA isolation. The DNA product was isolated and visualized by agarose gel electrophoresis under a UV transilluminator using a 100 bp DNA ladder (Jena Bioscience, Germany).

Measurement of Cytochrome P450

Cytochrome P450 was measured using an ELISA kit specific for rat (Catalog Number: MBS760819; MyBiosource, San Diego, CA, USA) based on sandwich ELISA technology.

Biochemical analysis

Oxidative stress parameters such as superoxide dismutase (SOD) activity and malondialdehyde (MDA) concentration were determined as described previously[11,12] using BioDiagnostic kits (Giza, Egypt). Plasma lead concentration was measured using T80 UV/visible double beam spectrophotometer[13]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured as described previously[14]. Creatinine was quantified using Diamond Diagnostics kits (Holliston, MA, USA) as described previously[15].

Statistical analysis

The results were expressed as mean ± standard error of means, and were statistically analyzed using One-way ANOVA followed by LSD and Tukey multiple range test. For all data, a probability of less than 0.05 was used as the criterion for statistical significance.

RESULTS

Effects of lactoferrin and lead acetate on body weight, organs weight, and hematological indices

The results showed insignificant changes in the body weights among different groups at different treatment periods. In addition, liver, brain and kidney weights, as well as organ's somatic index after eight weeks of rats' treatment showed insignificant changes among groups (Table 1). Moreover, insignificant differences were observed in Hb content, RBCs count, and blood indices among all groups (Table 2).

Ameliorative effects of lactoferrin against lead acetate toxicity

Chronic lead intake led to significant elevations ($P<0.05$) in the plasma ALT, AST activities, plasma creatinine concentration (Table 3), plasma lead concentration (Figure 1) and brain MDA concentration after eight weeks compared with the control group. However, the brain SOD activity was diminished significantly ($P<0.05$) compared with controls (Table 3). The LF was found to alleviate significantly the above mentioned toxicity of lead.

Hepatic cytochrome P450 was found to increase significantly ($P<0.05$) in response to chronic lead acetate toxicity compared with the control group after eight weeks of treatment. However, LF supplementation was found to ameliorate significantly this effect (Figure 2).

Figure "3" showed the optical density of apoptotic bands at 260 nm in brain cells of different groups. The optical density of apoptotic bands decreased significantly in rats treated with lead acetate compared with the control rats, while LF retained the level to approach the normal value. Lead acetate induced DNA fragmentation in rat brains (Figure 4). However, providing LF amended lead acetate toxicity and was influential in restoring DNA.

Table 1: Effects of lead acetate (100 mg/kg of body weight) and lactoferrin (LF; 10 mg/kg of body weight) on body weights at different treatment periods, as well as organs weight and organs somatic index after eight weeks of treatment.

Weights (g)	Control	LF	Pb	LF+Pb
Body weight (g) at 2 W	102.4±7.2	108.6±7.6	100.2±9.4	103.2±5.6
Body weight (g) at 4 W	107.0±5.4	110.8±8.6	105.8±7.9	108.4±5.7
Body weight (g) at 6 W	113.8±5.9	113.2±9.8	110.6±8.4	113.1±5.5
Body weight (g) at 8 W	119.9±4.8	115.1±8.3	117.0±7.3	115.3±7.6
Liver weight (g) at 8 W	6.0±0.2	5.2±0.4	5.1±0.2	5.2±0.3
Brain weight (g) at 8 W	1.3±0.1	1.2±0.1	1.4±0.1	1.4±0.1
Kidney weight (g) at 8 W	1.6±0.1	1.3±0.1	1.3±0.1	1.4±0.1
Hepatosomatic index (g)	4.7±0.3	4.2±0.3	3.8±0.1	3.8±0.1
Brainsomatic index (g)	1.0±0.1	1.0±0.1	1.0±0.04	1.0±0.02
Kidneysomatic index (g)	1.2±0.1	1.0±0.1	1.0±0.04	1.0±0.05

Values are expressed as means ± their standard errors (n = 6). No significant differences ($P \geq 0.05$) were observed in body weights, as well as organs weight and organs somatic index among all groups.

Table 2: Effects of lead acetate (100 mg/kg of body weight) and lactoferrin (LF; 10 mg/kg of body weight) on some hematological indices after eight weeks of treatment.

	Control	LF	Pb	LF+Pb
Hb (g/dL)	11.9±0.4	12.3±0.9	12.5±1.1	13.3±0.8
RBCs (cells $\times 10^6$/mL)	5.6±0.3	6.0±0.4	6.5±0.3	6.9±0.3
HCT (%)	33.8±1.7	34.8±2.5	36.8±0.9	39.4±1.2
MCV (fL)	59.5±0.8	57.3±0.9	56.3±0.9	56.2±0.8
MCH (pg)	20.4±0.3	20.3±0.2	19.4±0.4	19.1±0.3

Data were presented as means ± standard error (n = 6). Hb: hemoglobin content, RBCs: red blood corpuscles, HCT: hematocrit, MCV: mean cell volume, MCH: mean cell hemoglobin. No significant differences ($P \geq 0.05$) were observed in Hb content, RBCs count, and blood indices among all groups.

Table 3: Effects of lead acetate (100 mg/kg of body weight) and lactoferrin (LF; 10 mg/kg of body weight) on plasma activity of aminotransferases, plasma creatinine concentration, and brain oxidative stress parameters after eight weeks of treatment.

	Control	LF	Pb	LF +Pb
Plasma ALT (U/L)	103.4 ± 6.9^{c}	91.4 ± 6.7^{c}	177.2 ± 6.3^{a}	155.8 ± 6.5^{b}
Plasma AST (U/L)	26.0 ± 1.5^{c}	24.2 ± 1.6^{c}	57.4 ± 3.0^{a}	42.0 ± 2.3^{b}
Plasma creatinine (mg/dL)	0.75 ± 0.06^{c}	0.67 ± 0.05^{c}	1.45 ± 0.04^{a}	1.05 ± 0.10^{b}
Brain MDA(nmol/g)	6.5 ± 0.4^{c}	5.81 ± 0.3^{c}	$13.89\pm0.\ 6^{a}$	11.9 ± 0.6^{b}
Brain SOD (U/g)	157.4 ± 8.9^{a}	168.5 ± 10.2^{a}	102.2 ± 4.8^{b}	123.1 ± 5.7^{b}

Data were presented as means ± standard error (n = 6), the different letters in the same raw represented significant different $P<0.05$). ALT: alanine aminotransferase, AST: aspartate aminotransferase, MDA: malondialdehyde, SOD: superoxide dismutase.

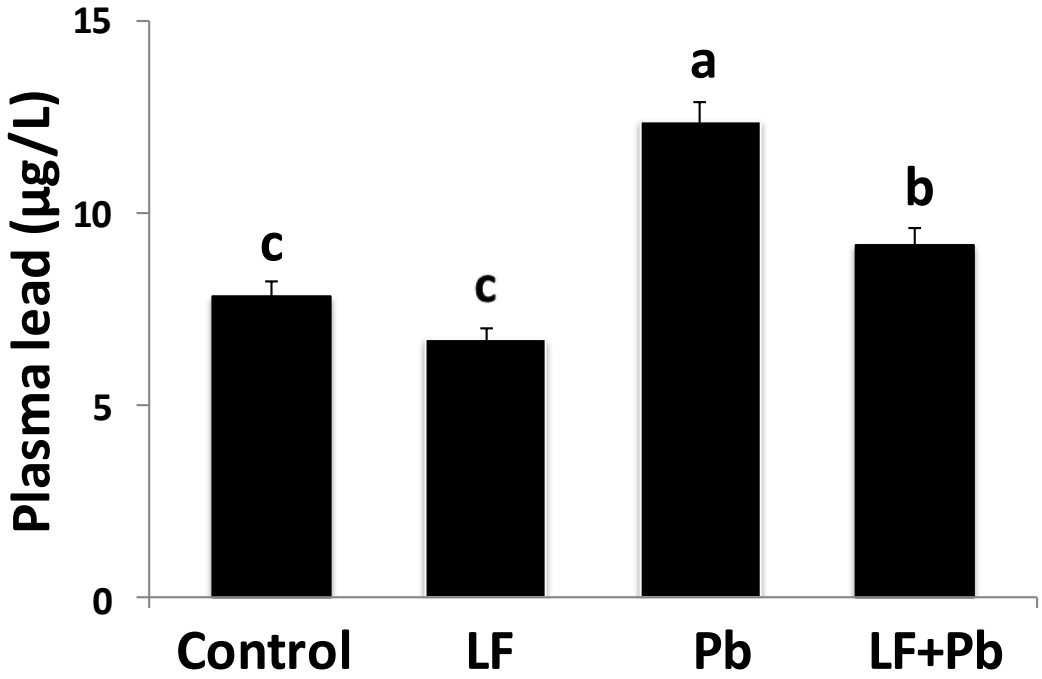


Figure 1: Effects of lead acetate (100 mg/kg of body weight) and lactoferrin (LF; 10 mg/kg of body weight) on plasma lead concentration (µg/L) after eight weeks of treatment. Data were presented as means ± standard error (n = 6). The different letters represented significant different (P<0.05).

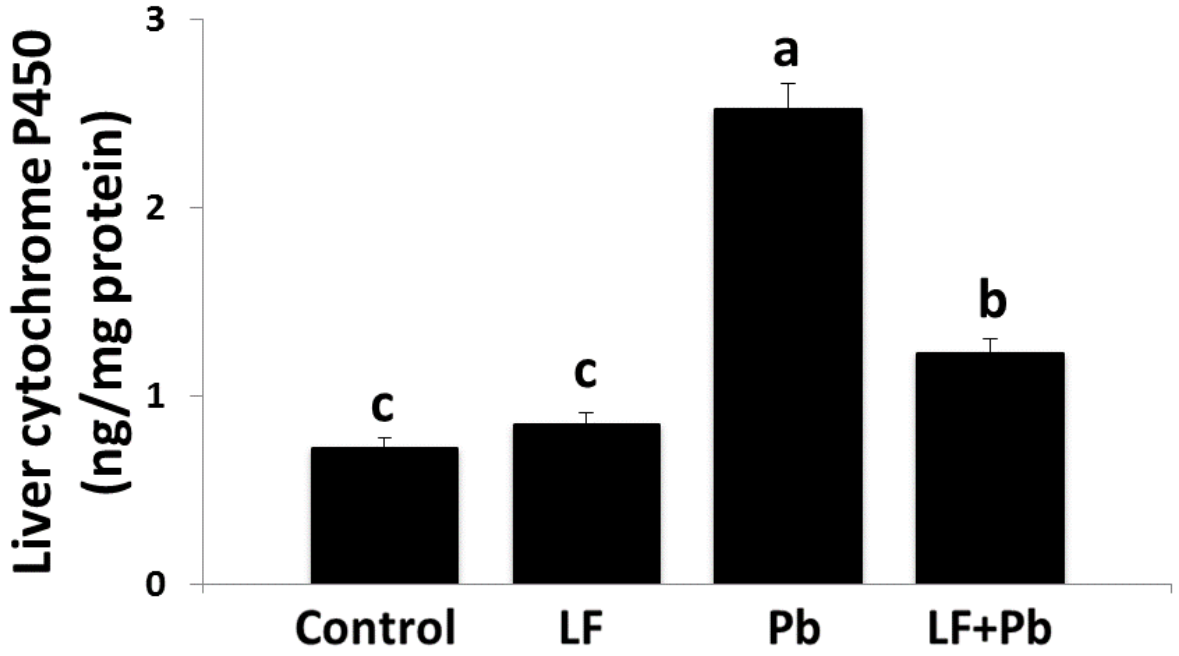


Figure 2: Effects of lead acetate (100 mg/kg of body weight) and lactoferrin (LF; 10 mg/kg of body weight) on hepatic cytochrome P450 (ng/mg protein) after eight weeks of treatment. Data were presented as means ± standard error (n = 6), the different letters represented significant different (P<0.05).

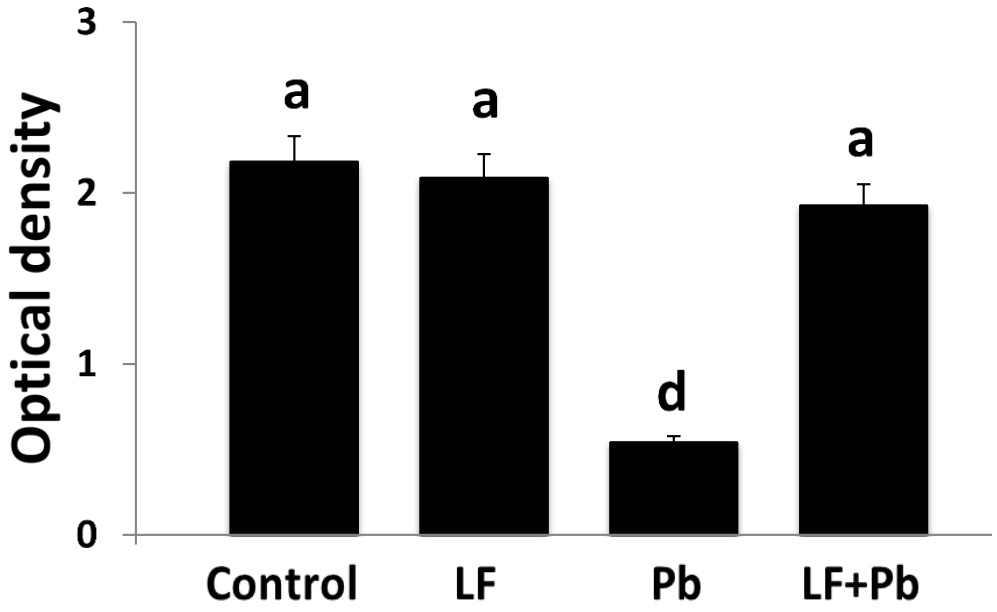


Figure 3: Effects of lead acetate (100 mg/kg of body weight) and lactoferrin (LF; 10 mg/kg of body weight) on optical density of apoptotic bands at 260 nm in brain tissues of rats after eight weeks of treatment. Data were presented as means ± standard error (n = 6). The different letters represented significant different (P<0.05).

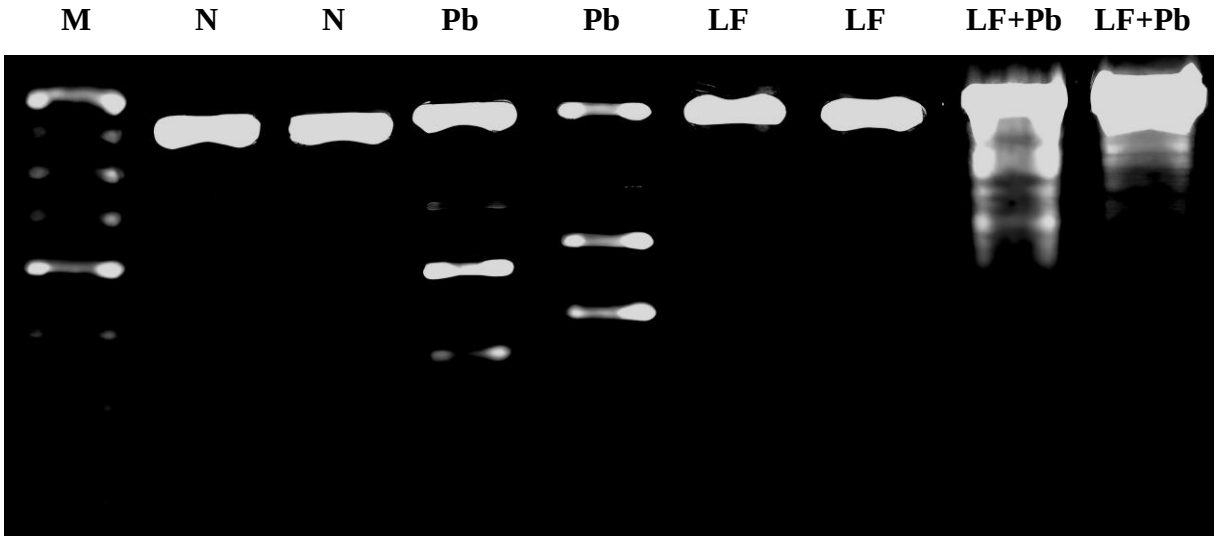


Figure 4: Apoptotic DNA fragmentation in the brain of rats exposed to lead acetate and the protective role of lactoferrin. M: one kilo ladder, N: normal controls, Pb: lead acetate-treated group, LF: lactoferrin-treated group, LF+Pb: lactoferrin + lead acetate-treated group. Lead-induced apoptotic DNA fragmentation in brain tissues of rats more than lactoferrin supplemented lead acetate group.

Histopathological finding

Figure "5" showed diminished cytoplasmic volume with dilated sinusoids and distorted hepatocytes arrangement in lead acetate-treated rats. Figure "6" showed atrophy and surrounded vacuolation of the cerebral cortex of lead acetate-treated rats. Atrophy of the glomeruli, signs of tubular cell lining degeneration, and thinning of the tubular wall were observed after lead acetate exposure (Figure 7). On the other hand, LF was found to induce significant improvement in the affected tissues.

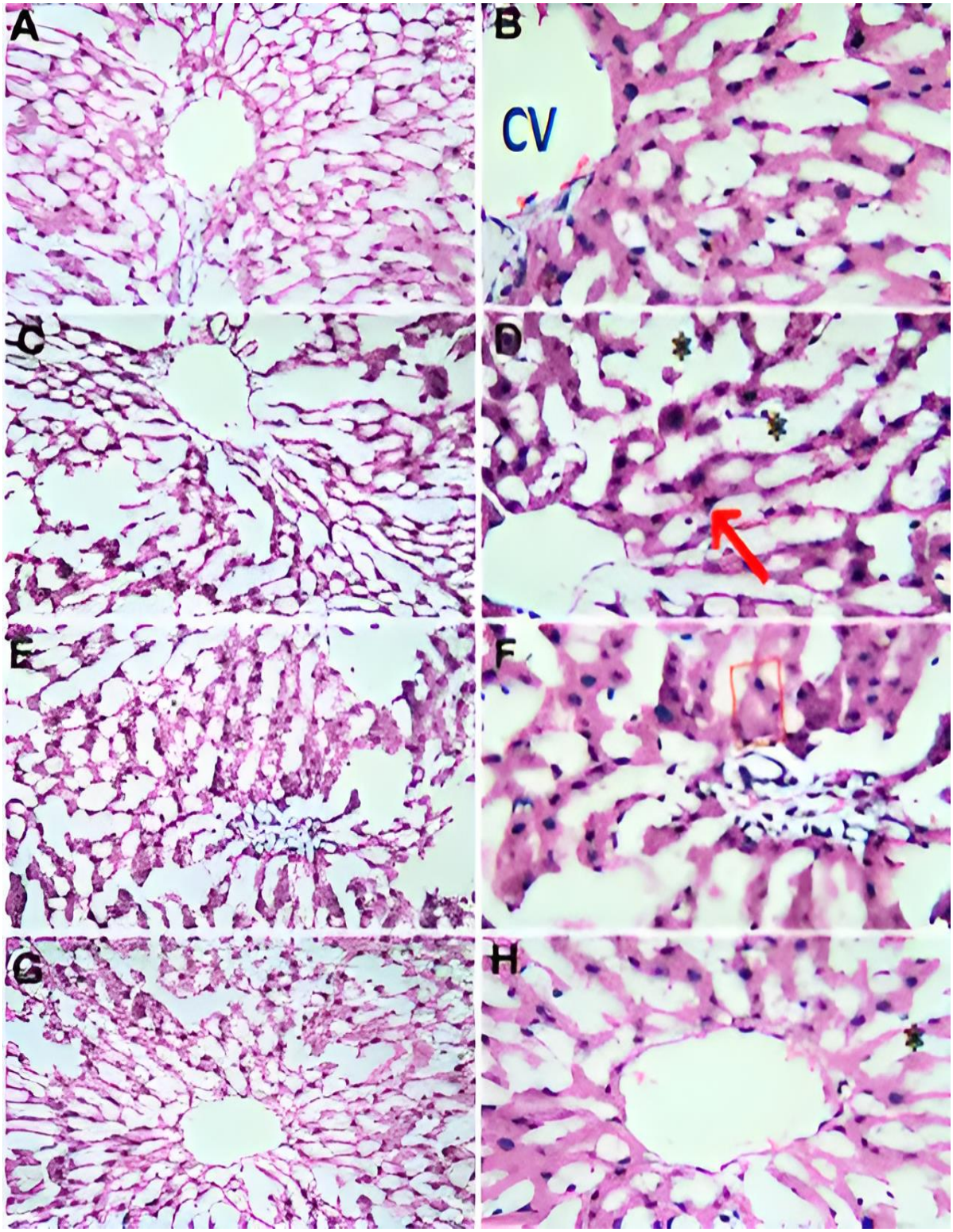


Figure 5: Histological sections of rat liver (hematoxylin and eosin staining) showing the liver of the control (A and B) with a normal arrangement of the hepatocytes around the central veins (CV). The lead acetate group (C and D) showed a marked affection of the hepatocytes with diminished cytoplasmic volume (arrow), dilated sinusoids (*), and distorted arrangement. The lactoferrin group (E and F) showed almost normal liver parenchyma. The lead acetate + lactoferrin group (G and H) still shows less marked affection of the hepatocytes with the preserved arrangement of the hepatocytes and narrow sinusoidal spaces compared to the lead acetate group (magnification: A, C, E, and G = 200× and B, D, F, and H = 400×).

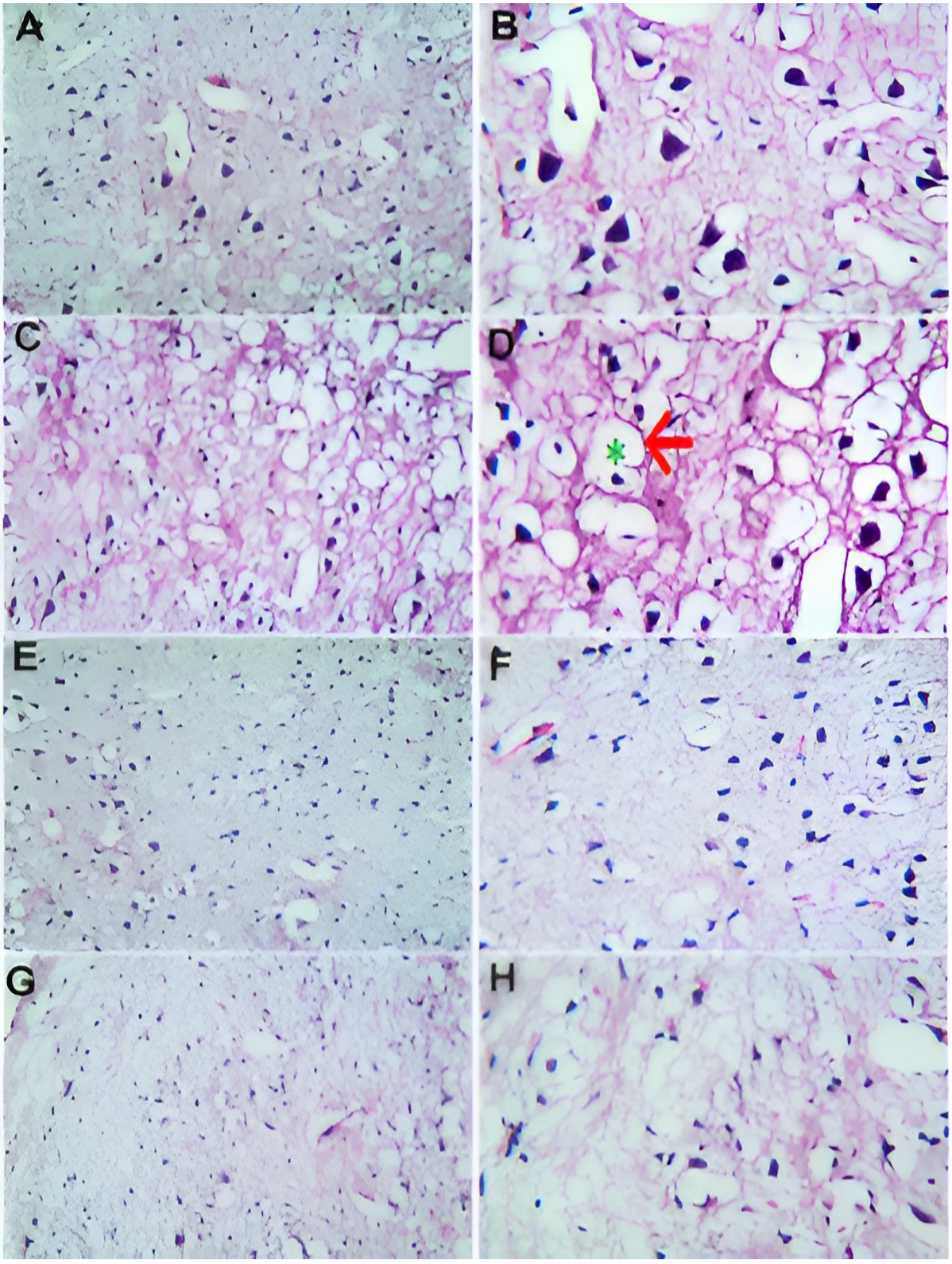
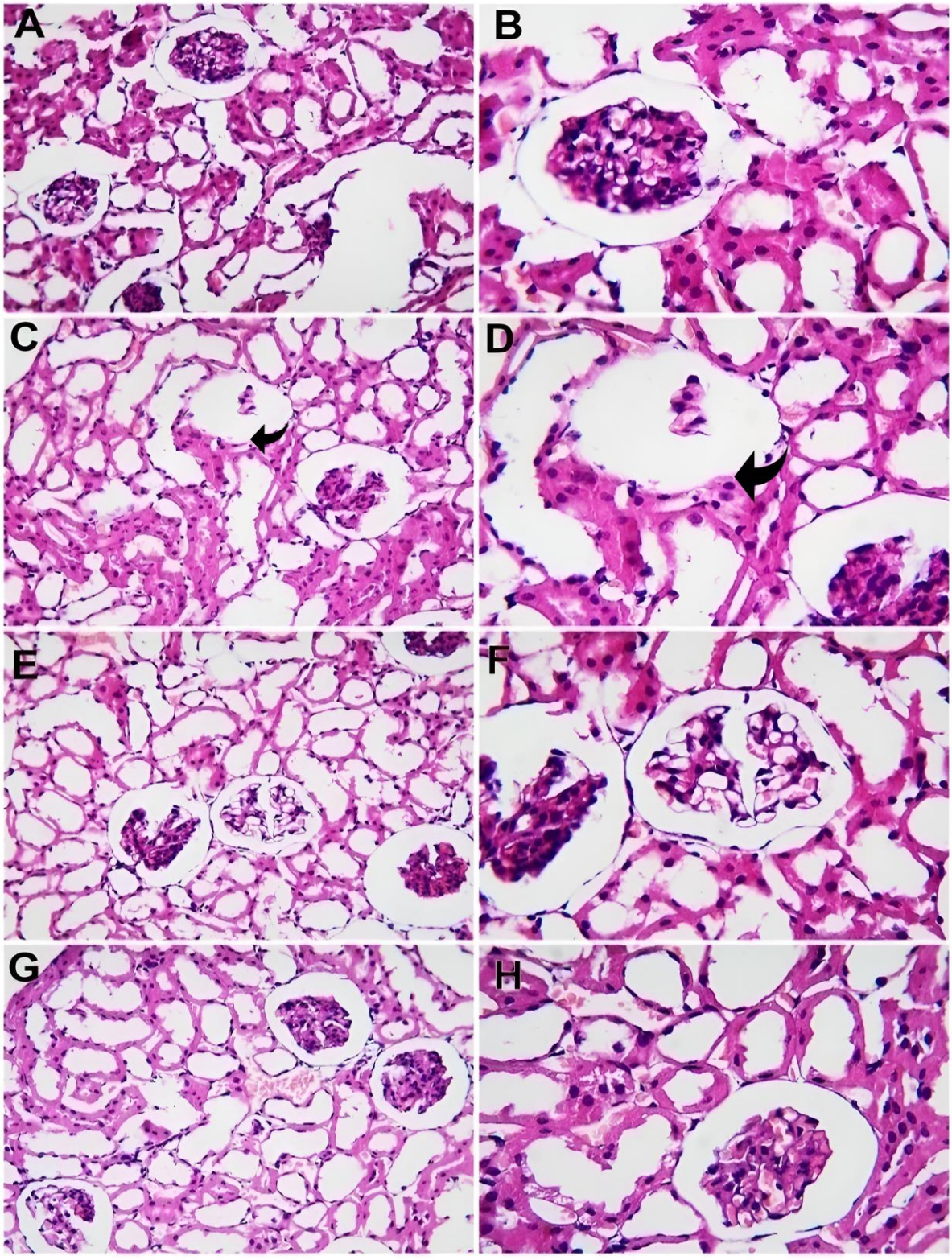


Figure 6: Histological sections of rat brain (hematoxylin and eosin staining) showing the cerebral cortex of the control (A and B) rat with the normal structure. The lead acetate group (C and D) showing a marked affection of the brain cells with signs of cell atrophy (arrow) and surrounded vacuolation (*****). The lactoferrin group (E and F) showing almost normal cerebral cortex nerve cells. The lead acetate + lactoferrin group (G and H) showed minimal affection of the brain cells with almost normal histological appearance (magnification: A, C, E, and G = 200× and B, D, F, and H = 400×).



]

Figure 7: Histological sections of rat kidney showing the cortex of the control rat (A and B) with the normal structure. The lead acetate group (C and D) showing marked affection of the renal tubules in the form of atrophy of the glomeruli and signs of tubular cell lining degeneration and thinning of the tubular wall (black arrow). The lactoferrin group (E and F) showing almost normal glomeruli and tubules. The lead acetate + Lactoferrin group (G and H) showing minimal affection for the renal tubules and more or less normal glomeruli (magnification: A, C, E, and G = 200× and B, D, F, and H = 400×).

DISCUSSION

Environmental pollution is the presence of a pollutant in an environment such as air, water, soil, and consequently in food which may be toxic and will cause harm to living things in the polluted environment. Lead in the environment is considered one of the most dangerous toxins and pollutants that cause neurological disorders[16]. In addition, lead acetate caused liver toxicity in mice[17]. In the present study, there was an in-significant change in body weight, which differs from the previous study[18] reported that exposure of male rats to lead at 1/20 LD_{50} every two days for 14 weeks caused weight loss. This difference may be due to the dose of lead acetate used in the present work, where the effect of lead acetate is affected by doses[19]. Another study[20] was in line with our results, and found that lead acetate exposure [1.0 mmol as the sole drinking fluid for 4 weeks] does not affect the weight gain of female Wistar rats. Therefore, undernutrition can be ruled out as a causative agent for the observed changes.

Although lead has no significant change in organs weight (Table 1), this was accompanied by alterations in the histo-logical structure of the organs (Figures 5 and 6), leading to serious health problems such as liver and brain damage. Lead acetate enhanced lipid peroxidation and the production of ROS[17]. Lead acetate can interfere with cell physiology and increase the brain's enzymic antioxidant and lipid peroxidation products[21]. Although lead penetrates all tissues, some components of the brain, for example, the hippocampus, showed more significant damage in exposure to lead, resulting in malfunctions[4,21]. It is interesting to mention here that LF supplementation can improve memory, learning, and information processing[22].

Lead exposure induced elevations in plasma activities of ALT and AST (Table 3). It was reported that the significant increases in plasma AST and ALT, combined with increased creatinine levels, might be associated with hepatic failure[23]. Elevated creatinine in liver cirrhosis indicated an alteration in renal perfusion due to splanchnic vasodilation associated with portal hypertension, which leads to hepatorenal syndrome[24]. It was shown that in chronic liver disease, a reduction in serum creatinine pool occurred due to decreased hepatic production of creatine, accumulation of extracellular fluid, edema, ascites, malnutrition and loss of muscle mass[25]. Researchers suggest that up to 40% of those with end-stage liver disease (liver failure) will develop hepatorenal syndrome[26]. It is of interest to mention here that lead induced elevations in hepatic enzymes, creatinine, and hepatorenal dysfunction on both structural and functional levels. This may indicate the failure of antioxidants to overcome ROS under lead toxicity. This effect was accompanied by hepatic inflammation and failure, resulting in increased lead plasma burden[17,23].

The lead-induced cellular damage and decreased the availability of antioxidants[27]. Lead also oxidizes hemoglobin, which directly causes hemolysis of RBCs, leading to the generation of hydroxyl radicals. This eventually causes the cell to be severely exposed to oxidative stress and may lead to cell death[27]. In the present investigation, Hb, RBCs, HCT, MCV, and MCH indices were insignificantly changed in response to chronic lead toxicity. Lead acetate at present dose and duration of exposure failed to affect blood indices. In line with the present investigation, Peng *et al*.[28] found that lead exposure did not affect hematological parameters. In contrast other research indicated significant changes in different blood indices in response to lead toxicity including anemia, leukocytosis, thrombocytosis, and others[29,30]. These findings suggested that the effect of lead on hematologic parameters is possibly not linear.

The significant increase in brain MDA content and the decrease in brain SOD activity in response to lead toxicity (Table 3) indicates antioxidant deactivation effect

of lead in brain tissue; this agrees with other previous reports[21,27]. Lead promotes oxidative stress through two different pathways acting simultaneously; the first comes from the production of ROS, such as hydroperoxides, monooxygenase, and hydrogen peroxide; the second comes from the depletion of antioxidant reserves[21,27]. A few other famous antioxidant enzymes are rendered inactive by lead as SOD. A decrease in the concentration of SOD reduces the removal of superoxide molecules[31].

Lead induced apoptotic DNA fragmentation in brain tissue versus the control group (Figures 3 and 4). This effect was in line with that reported previously[32]. ROS causes inflammation in vascular endothelial cells, damages nucleic acids and inhibits their repair, and initiates lipid peroxidation in cellular membranes by inhibiting the production of sulfhydryl antioxidants, and thus inhibits enzyme reactions involved in heme production[33], which is improved by LF. It has been exhibited that LF acts as a transcription factor[34]. The LF is believed to be critical in supporting neural development and cognitive function[22].

Results showed that lead-induced drastic histopathological effects on the liver, brain, and kidney of rats. Lead-induced hepatocyte proliferation and fibrosis after chronic lead toxicity showed diminished cytoplasmic volume with dilated sinusoids and distorted arrangement (Figure 5). LF suppressed hepatocellular necrosis and revealed a direct cytoprotective function via its antioxidant activity[35]. Cytochrome P450 is a drug-metabolizing enzyme in hepatic tissue[36]. Elevated Cytochrome P450 in the liver under lead acetate exposure indicated an increased lead acetate metabolic rate, which may be associated with the drastic effects of lead in hepatic tissue. The LF reduced the elevation in liver cytochrome P450 in lead acetate-treated rats and thus it may reduce the metabolism of lead acetate in the liver.

Results showed that lead-induced atrophy surrounded vacuolation in the cerebellar cortex and damaged brain cells (Figure 6). Lead-induced apoptosis altered neurotrophic factor mRNA in the developing rat cortex[32]. Lead also inhibits the activity of synaptotagmin I, a protein found at synapses that appears to be required for transmitter release, in addition to influencing neurotransmitter storage and release. Lead also alters neurotransmitter receptors, which may lead to atrophy of the cerebral cortex[37].

Lead acetate caused glomerular atrophy, as well as signs of tubular cell lining degeneration and tubular wall thinning. Our results agreed with those of previous studies regarding lead-induced renal toxicity[38,39]. Nausheen *et al*.[38] showed that the alterations in the proximal tubular cells induced by lead acetate indicated its nephrotoxic effects on renal cortical tissue.

The LF is an antioxidant with the capacity to reduce oxidative stress. It can bind to the LF receptor (LFR), resulting in iron transport across the plasma membrane[40]. LF is only synthesized in the brain by activated microglia. The LFRs are found in blood vessels, different tissues and cell types, as well as nigral dopaminergic neurons[40]. The LF has been shown to possess hepatoprotective activity against acetaminophen toxicity[41]. The LF controls ROS production's physiological balance and elimination rate through iron sequestration. LF can prevent the harmful effects of oxidative stress by sequestering Fe^{3+}. Many studies have shown that it contributes to general homeostasis by interfering with the production of these dangerous radicals[42-44]. LF inhibits the reactivity of free ferric ions with superoxide molecules, limiting the formation of ferrous salt and ground state oxygen[45]. This effect prevents the Fenton reaction in which the ferrous ion is oxidized by hydrogen peroxide to a ferric ion, forming a hydroxyl radical and a hydroxide ion[45].

In conclusion, lead causes damage to the liver, brain and kidney tissues. It has drastic effects at biochemical, molecular and histopathological levels. LF is protective against lead acetate-induced toxicity in liver

and brain tissues. It is recommended to use LF as a safe protective agent against lead acetate-induced toxicity, especially for those expected to be occupationally exposed.

FUNDING SOURCE DISCLOSURE
This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST
The authors have no potential financial conflict of interest.

AUTHORS' CONTRIBUTIONS
MGS and HMR designed the work. Bioassays were performed by MGS, NAA, and HMR. All authors reviewed and approved the manuscript.

REFERENCES

[1] Ungureanu, L. E. and Mustatea, G. (2022). Toxicity of Heavy Metals, Chapter 2 (DOI: 10.5772/intechopen. 102441). In: Environmental Impact and Remediation of Heavy Metals (Saleh, H. M. and Hassan, A. I. eds), IntechOpen, London, UK.

[2] Wani, A. L.; Ara, A. and Usmani, J. A. (2015). Lead toxicity: a review. Interdiscip Toxicol, 8(2): 55-64.

[3] Pan, T.-L.; Wang, P.-W.; Al-Suwayeh, S. A. *et al.* (2010). Skin toxicology of lead species evaluated by their permeability and proteomic profiles: a comparison of organic and inorganic lead. Toxicol Lett, 197: 19-28.

[4] Sanders, T.; Liu, Y.; Buchner, V. *et al.* (2009). Neurotoxic effects and biomarkers of lead exposure: a review. Rev Environ Health, 24: 15-45.

[5] Zhang, Y.; Lu, C. and Zhang, J. (2021). Lactoferrin and its detection methods: a review Nutrients, 13(8): 2492 (DOI: 10.3390/nu13082492).

[6] Elzoghby, A. O.; Abdelmoneem, M. A.; Hassanin, I. A. *et al.* (2020). Lactoferrin, a multi-functional glycoprotein: active therapeutic, drug nanocarrier & targeting ligand. Biomaterials, 263: 120355 (DOI: 10.1016/j.biomaterials.2020.120355).

[7] Lozovska, Y. V.; Andrusishina, I. M.; Lukianova, N. Y., *et al.* (2019). The influence of lactoferrin on elemental homeostasis and activity of metal-containing enzymes in rats with Walker-256 carcinosarcoma. Exp Oncol, 41: 20-25.

[8] Gazwi, H. S. S.; Yassien, E. E. and Hassan, H. M. (2020). Mitigation of lead neurotoxicity by the ethanolic extract of Laurus leaf in rats. Ecotoxicol Environ Saf, 192: 110297 (DOI: 10.1016/j.ecoenv.2020.110297).

[9] Augustine, I. O.; Gertrude, O. N.; Martin, E. *et al.* (2021). Cerebellar and hypocampal changes induced by lead in Wistar rats: the role of *Ocimum gratissimium* leaves extract. J Bio Med Sci, 10(3): 56.

[10] Mohamed, A. I. E. (2022). Lecture Notes On: Routine Histological Techniques. University of El-Imam El-Mahdi, Khartoum, Sudan.

[11] Nishikimi, M.; Appaji, N. and Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun, 46(2): 849-854.

[12] Ohkawa, H.; Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95(2): 351-358.

[13] Zaky, M.; Amin, A. S. Elgendy, Kh. *et al.* (2017). Direct spectrophotometric determination of lead (II) in industrial samples using 1,3-benzenediamine, N, N'-bis(2-furanylmethylene) in presence of surfactant. BFSZU, 2017: 134-148.

[14] Schumann, G. and Klauke, R. (2003). New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: preliminary upper reference limits obtained in hospitalized subjects. Clin Chem Acta, 327: 69-79.

[15] Heinegård, D. and Tiderström, G. (1973). Determination of serum creatinine by a direct colorimetric method. Clin Chim Acta, 43(5): 305-310.

[16] Saleh, S. M. and Meligy, F. Y. (2018). Study on toxic effects of lead acetate on cerebellar cortical tissue of adult albino rats and the role of vitamin E as a protective agent. Ain Shams J Forensic Med Clin Toxicol, 31: 110-118.

[17] Aldahmash, B. A. and El-Nagar, D. M. (2016). Antioxidant effects of captopril against lead acetate-induced hepatic and splenic tissue toxicity in Swiss albino mice. *S*audi J Biol Sci, 23(6): 667-673.

[18] Ibrahim, N. M.; Eweis, E. A.; and El-Beltagi, H. S. *et al.* (2012). Effect of lead acetate toxicity on experimental male albino rat. Asian Pac J Trop Biomed, 2: 41–46.

[19] Ibrahim, N. M.; Eweis, E. A.; El-Beltagi, H. S. *et al*. (2011). The effect of lead acetate toxicity on experimental male albino rat. Biol Trace Elem Res, 144: 1120-1132.

[20] Mello, C. F.; Kraemer, C. K.; Filippin, A. *et al*. (1998). Effect of lead acetate on neurobehavioral development of rats. Braz J Med Biol.Res, 31(7): 943-950.

[21] Bokara, K. K.; Blaylock, I.; Denise, S. B. *et al.* (2009). Influence of lead acetate on glutathione and its related enzymes in different regions of rat brain. J Appl Toxicol, 29(5): 452-458.

[22] Wang, B. (2016). Molecular determinants of milk lactoferrin as a bioactive compound in early neurodevelopment and cognition. J Pediatr, 173: S29-36.

[23] Cárdenas, A. and Ginès, P. (2009). A patient with cirrhosis and increasing creatinine level: what is it and what to do? Clin Gastroenterol Hepatol, 7(12): 1287-1291.

[24] Carrier, P.; Debette-Gratien, M. and Loustaud-Ratti, V. (2018). Serum creatinine in cirrhotic patients: a cornerstone. AME Med J, 3: 109 (DOI: 10.21037/amj.2018.10.07).

[25] Slack, A.; Yeoman, A. and Wendon, J. (2010). Renal dysfunction in chronic liver disease. Crit Care, 14(2): 214 (DOI: 10.1186/cc8855).

[26] Wadei, H. M.; Mai, M. L.; Ahsan, N. *et al.* (2006). Hepatorenal syndrome: pathophysiology and management. Clin J Am Soc Nephrol, 1(5): 1066-1079.

[27] Flora, G.; Gupta, D. and Tiwari, A. (2012). Toxicity of lead: a review with recent updates. Interdiscip Toxicol, 5(2): 47-58.

[28] Peng, S.; Zhang, C.; Wang, C. *et al*. (2002). The investigation on the changes of hematological parameters in the occupationally lead exosed workers. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi, 20(5): 334-335.

[29] Mugahi, M. N.; Heidari, Z.; Sagheb, H. M. *et al.* (2003). Effects of chronic lead acetate intoxication on blood indices of female adult rat. DARU, 11(4): 147-151.

[30] El-shater, A. A.; Ali, R. A. and Fawy, M. A. (2022). Study on toxic effects of lead acetate on hematological parameters of adult albino rats and the role of mesenchymal stem cells and flax seeds oil as therapeutic agents. Egypt Acad J Biolog Sci, B. Zoology, 14(2): 123-136.

[31] Sudjarwo, S.-A.; Eraiko, K.; Sudjarwo, G. W. *et al.* (2017). Protective effects of piperine on lead acetate induced-nephrotoxicity in rats. Iran J Basic Med Sci, 20(11): 1227-1231.

[32] Chao, S. L.; Moss, J. M. and Harry, G. J. (2007). Lead-induced alterations of apoptosis and neurotrophic factor mRNA in the developing rat cortex, hippocampus, and cerebellum. J Biochem Mol Toxico, 21(5): 265-272.

[33] Patrick, L. (2006). Lead toxicity part II: the role of free radical damage and the use of antioxidants in the

pathology and treatment of lead toxicity. Altern Med Rev, 11(2): 114-127.

[34] Abrink, M.; Larsson, E.; Gobl, A. *et al.* (2000). Expression of lactoferrin in the kidney: implications for innate immunity and iron metabolism. Kidney Int, 57(5): 2004-2010.

[35] Hessin, A.; Hegazy, R.; Hassan, A. *et al.* (2015). Lactoferrin enhanced apoptosis and protected against thioacetamide-induced liver fibrosis in rats. Open Access Maced J Med Sci, 3(2): 195-201.

[36] Pan, Y.; Tiong, K. H.; Abd-Rashid, B. A. *et al.* (2014). Effect of eurycomanone on cytochrome P450 isoforms CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2E1 and CYP3A4 *in vitro*. J Nat Med, 68(2): 402-406.

[37] Lidsky, T. I. and Schneider, J. S. (2003). Lead neurotoxicity in children: basic mechanisms and clinical correlates. Brain, 126(Pt 1): 5-19.

[38] Nausheen, Q. N.; Ali, S. A.; Syed, S. M. *et al.* (2022). Amelioration of hepato-renal impairment by natural chelators in lead-induced poisoning in rats. IJPER, 56(4): 1123-1133.

[39] Aldhalemi, M. A.; Alsallami, Z. T.; Al-Fatlawi, A. J. S.; *et al.* (2022). Effects of parsley administration preventing lead-induced toxicity to liver and kidney tissues. REDVET, 23(3): 344-352.

[40] Wang, J.; Bi, M.; Liu, H. *et al.* (2015). The protective effect of lactoferrin on ventral mesencephalon neurons against MPP + is not connected with its iron binding ability. Sci Rep, 5: 10729 (DOI: 10.1038/srep10729).

[41] Yin, H.; Cheng, L.; Holt, M. *et al.* (2010). Lactoferrin protects against acetaminophen-induced liver injury in mice. Hepatology, 51(3): 1007-1016.

[42] Actor, J. K.; Hwang, S.-A. and Kruzel, M. L. (2009). Latoferrin as a natural immune modulator. Curr Pharm.Des, 15(17): 1956-1973.

[43] Ahmed, K. A.; Saikat, A. S. M.; Moni, A. *et al.* (2021). Lactoferrin: potential functions, pharmacological insights, and therapeutic promises. J Adv Biotechnol Exp Ther, 4(2): 223-237.

[44] Zimecki, M.; Actor, J. K. and Kruzel, M. L. (2021). The potential for lactoferrin to reduce SARS-CoV-2 induced cytokine storm. Int Immunopharmacol, 95: 107571 (DOI: 0.1016/j.intimp.2021.107571).

[45] Superti, F. (2020). Lactoferrin from bovine milk: a protective companion for life. Nutrients, 12(9): 2562 (DOI: 10.3390/nu12092562).

How to cite this article:
Shalan, M. G.; Al-Bakry, N. A. and Rashwan, H. M. (2023). The ameliorative effects of lactoferrin against lead acetate toxicity in female albino rat. Egyptian Journal of Zoology, 80: 35-49 (DOI: 10.21608/ejz.2023.166927.1087).

التأثيرات المُحسنة للاكتوفيرين ضد التسمم بخلات الرصاص في إناث الجرذان المهقاء

محمد جابر محمد شعلان، ندى عبدالله محمد البكري، حنان محمد رشوان

قسم علم الحيوان كلية العلوم، جامعة العريش، شمال سيناء ، جمهورية مصر العربية

أجريت هذه الدراسة لمعرفة الدور الوقائي للاكتوفيرين ضد التسمم بخلات الرصاص في إناث الجرذان المهقاء (*Rattus norvegicus*). عوملت أربع مجموعات متساوية من الحيوانات (6 فئران/مجموعة) عن طريق الانبوب المعدي يومياً لمدة ثمانية أسابيع. المجموعة الأولى عبارة عن مجموعة ضابطة، والمجموعة الثانية تم معاملتها باللاكتوفرين (10 مجم/كجم من وزن الجسم)، والمجموعة الثالثة عوملت بخلات الرصاص (100 مجم/كجم من وزن الجسم)، والمجموعة الرابعة تم معاملتها باللاكتوفيرين وخلات الرصاص بالجرعات نفسها. بعد مرور ثمانية أسابيع، تم أخذ عينات الدم والأنسجة للتحاليل الدموية والبيوكيميائية والنسيجية. وأظهرت النتائج أن التعرض للرصاص أدى إلي ارتفاعات ذات دلالة إحصائية ($P<0.05$) في نشاط الإنزيمات الناقلة لمجموعة الأمين، وتركيز الكرياتينين في بلازما الدم، ومستوى الرصاص في بلازما الدم، والسيتوكروم الكبدي "P450"، وتركيز المالونديألدهيد في الدماغ. كما انخفض نشاط إنزيم سوبر أكسيد ديسميوتاز في الدماغ بمقدار ذو دلالة إحصائية ($P<0.05$) نتيجة للتسمم بالرصاص. كما تسببت خلات الرصاص في العديد من التغيرات المرضية في أنسجة الكبد والدماغ والكُلى. كما لوحظ حدوث تجزئة شديدة للحمض النووي في أنسجة الدماغ بعد المعاملة بالرصاص. من ناحية أخرى، وجد أن اللاكتوفيرين يعدل بمقدار ذو دلالة إحصائية ($P<0.05$) التدهور في معظم المعاملات قيد الدراسة. والخلاصة، تشير هذه النتائج إلى أن اللاكتوفيرين له وظيفة وقائية في العمليات الجزيئية والنسيجية التي تؤدي إلى التنكس العصبي، وكذلك إصابة الكُلى والكبد، التي يسببها الرصاص.