Mansoura Veterinary Medical Journal

Volume 24 | Issue 3 Article 2

2023

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How to Cite This Article

Mansour, Lubna A H; Elshopakey, Gehad E.; Hamidieh, Fatma M. Abdel; and Risha, Engy F M. (2023) "Naringenin attenuates hematobiochemical and histopathological alterations induced by lead intoxication in rats," *Mansoura Veterinary Medical Journal*: Vol. 24: Iss. 3, Article 2.
DOI: https://doi.org/10.35943/2682-2512.1011

ORIGINAL ARTICLE

Naringenin Attenuates Hematobiochemical and Histopathological Alterations Induced by Lead Intoxication in Rats

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Abstract

OBJECTIVE: The present study aimed to assess the protective effect of naringenin (NRG) against lead acetate (LA) toxicity in rats.
DESIGN: A randomized, controlled study.
ANIMALS: Forty adult male Wistar albino rats, weighing 140−160 g, were used in this study.
PROCEDURE: Forty adult male rats were divided into four groups (10 rats/group). First group (A): the control group was administered water and food pellets. Second group (B): LA was dissolved in distilled water orally at a dose of 500 mg/kg/day. Third group (C) was administered NRG at a dose of 50 mg/kg/day dissolved in 0.5 % carboxymethyl cellulose. Fourth group (D): LA plus NRG was administered for 4 weeks. All rats were killed and renal and splenic tissues were collected. The influence of NRG against LA toxicity on blood indices, nephrotoxicity, serum lipid profile, and histological changes was investigated.
RESULTS: LA induced a significant decrease in hemogram results, whereas the total leukocyte and lymphocyte counts were significantly elevated. Meanwhile, a marked elevation in renal function biomarkers and serum lipid profiles was observed. However, high-density lipoprotein cholesterol levels showed an opposite trend. In addition, significant histopathological alterations were observed in the renal and splenic tissues of LA-treated rats. Interestingly, we found that NRG markedly alleviated lead-induced hematological, biochemical, and histopathological alterations.
CONCLUSION AND CLINICAL RELEVANCE: NRG administration successfully ameliorated induced alterations in several health indicators in rats.

Keywords: Hematological, Hyperlipidemia, Lead acetate, Naringenin, Nephrotoxicity

1. Introduction

Lead is a colorless and tasteless metal that is extremely poisonous. It is a nonessential element that is present in varying degrees in both biological and environmental systems, and is an environmental pollutant with the longest half-life [1]. Lead exposure has several detrimental health effects, including growth retardation and respiratory, neurological, and visual impairment, as well as immunological, hematological, hepatic, renal, and reproductive problems [2]. Lead is absorbed through the gastrointestinal, respiratory, and possibly dermal tracts. Most of the lead content in blood (~99 %) is linked to red blood cells (RBC) [3]. Lead reduces the quantity of phospholipids in RBCs by binding to phosphatidylcholine in the cell membrane [4]. Absorbed lead is transported to soft tissues after binding to erythrocyte proteins and passing through divalent metal transporter 1 in the duodenum [5]. It is conjugated in the liver, excreted in trace amounts through the kidney in the urine, and then accumulates in different bodily tissues, where it harms macromolecules and ultimately results in cell death [6]. Nuclear inclusion bodies containing lead protein complexes, degenerative

Received 11 January 2023; revised 9 February 2023; accepted 12 February 2023.
Available online 15 December 2023

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https://doi.org/10.35943/2682-2512.1011

alterations in the tubular epithelium, and a considerable absence of tubular transport systems are indicative of acute or long-term nephrotoxic effects following prolonged lead exposure [7].

Flavonoids are the most common antioxidants, which have antioxidative, anti-inflammatory, antibacterial, antimutagenic, hepatoprotective, and nephroprotective properties that have recently gained attention [8]. Many studies have examined the biological effects of flavonoids found in plants and products generated from plants with a variety of chemical configurations [9]. Naringenin (NRG) is one of the most prevalent flavonoids in tomato, grape, and citrus fruits [10]. NRG acts as an effective scavenger because of its polyphenolic molecular structure, which is responsible for its radical-trapping properties and is effective against ROS-mediated damage [8]. It is well known that this natural product is safe and has a variety of pharmacological activities, including anti-inflammatory, anti-eNOS expression, antioxidant, anticancer, nephroprotective, and hepatoprotective effects [11].

Therefore, the purpose of this study was to explore the protective role of NRG against lead-induced toxicity in Wistar albino rats.

2. Materials and methods

2.1. Chemicals

$(CH_3CO_2)_2$ $Pb.3H_20$, 99.99 % lead acetate (LA; Product No, 316512), and 0.5 % carboxymethyl cellulose powder (Product No, 25698) were purchased from El-Nasr Pharmaceutical Chemical Co. (Cairo, Egypt); NRG 95 % (4′, 5, 7-trihydroxyflavanone) $C_{15}H_{12}O_5$ (Item No, N5893) was purchased from Sigma−Aldrich Company (St Louis, Missouri, USA). Additional chemicals and reagents were purchased from Diamond Diagnostics (Cairo, Egypt).

2.2. Animals and experimental design

In all, 40 healthy adult male Wistar albino rats were housed under conventional laboratory settings (25 ± 10 °C, 12/12 h cycle of light and dark), which were purchased from Benha Farm of Lab Animals (Ministry of Public Health). The rats weighed 140−160 g. As part of their acclimatization procedure, the rats received regular access to food and clean water for 2 weeks before the beginning of the experiment. All experimental procedures were approved by the Animal Research Ethics Committee of Mansoura University (M/18). After the acclimatization period, the rats were randomly distributed into four groups:

(1) The first group (negative control group) was administered water and food pellets.
(2) The second group was the positive control group, in which rats received LA orally at a dose of 500 mg/kg/day dissolved in distilled water for 4 weeks [12].
(3) The third group was the NRG control group, in which rats received NRG orally at a dose of 50 mg/kg body weight/day dissolved in carboxymethyl cellulose 0.5 % [13].
(4) The fourth group was treated with LA plus NRG in the same duration and dose as mentioned before.

2.3. Measuring hematological parameters

A hemocytometer was used for total leukocyte count and erythrocyte (RBCs) counts. Blood indices, including packed cell volume (PCV) and hemoglobin (Hb) concentration, were estimated. In addition, Giemsa staining was used to prepare and stain blood samples for differential leukocyte counts [14].

2.4. Biochemical tests

2.4.1. Assessment of renal function markers

Serum concentrations of uric acid, creatinine, and urea were measured using Spinreact (Barcelona, Spain). All values were estimated using a spectrophotometer (BM, Magdeburg, Germany, model 5010) according to manufacturer's instructions.

2.4.2. Assessment of lipid profile markers

Triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and total cholesterol (TC) levels were measured spectrophotometrically using commercial kits (Bio-diagnostic, Egypt), and very low-density lipoprotein cholesterol (VLDL-c) serum levels were calculated.

2.5. Histopathological examination

Hematoxylin and eosin was applied to paraffin-embedded slices with a thickness of 4−5 μm after the kidney and spleen tissues had been fixed in 10 % formaldehyde [15]. Using a biological Apex microscope (UK), the slides were examined, and an Apex Minigrab was used to take pictures (UK).

2.6. Statistical analysis

The data were analyzed using one-way analysis of variance as the mean ± SE of the mean, after which

values were entered into the SPSS software (version 26; SPSS Inc. Chicago, Illinois, USA), and Duncan multiple comparison tests were carried out. At P value less than 0.05, the findings were considered statistically significant and graphically displayed using Microsoft Excel 2013.

3. Results

3.1. Protective effect of the naringenin on hematological alterations in lead-intoxicated rats

RBCs (× 106/μl), PCV %, and Hb concentration (g/dl) were significantly lower in the LA-treated group than in all other groups ($P < 0.05$); however, the NRG and LA + NRG groups had restored their levels to the control group ($P < 0.05$). A similar trend was observed in the differential leukocyte counts, where the LA group showed a significant elevation in total leukocyte count and lymphocyte counts compared with all other groups, with no statistical changes in neutrophil and monocyte counts. However, NRG and LA + NRG groups restored their levels to those of the control group ($P > 0.05$) (Tables 1 and 2).

3.2. Protective effect of the naringenin on renal function markers in lead-intoxicated rats

The serum concentrations of uric acid, creatinine, and urea were significantly higher in LA-exposed rats than in the other groups ($P < 0.05$). However, all levels were restored to the control levels in rats treated with LA + NRG and NRG (Fig. 1).

3.3. Protective effect of the naringenin on lipid profile markers in lead-intoxicated rats

Levels of TG, LDL-c, VLDL-c, and TC were significantly decreased in the LA-exposed group compared with all other groups, with no statistical changes to the LA + NRG only for TC level.

The NRG and LA + NRG groups showed nonsignificant levels of these parameters compared with the control group.

However, LA-intoxicated rats showed a marked reduction in HDL-c levels compared with control rats. With the exception of the TC level, which remained slightly altered when compared with control or LA-intoxicated rats, treatment with NRG considerably restored the altered values of lipid profile parameters, reverting them to their normalcy (Fig. 2a–e, respectively).

3.4. Protective effect of naringenin on histopathological alterations in renal and splenic tissues of lead-intoxicated rats

3.4.1. Renal tissue

In Figs. 3 and 4 renal sections from the LA group show tubular dilation (black arrowheads), renal epithelial cell hydropic degeneration (black arrows), degenerated glomeruli (red arrows), and congested intratubular blood vessels (red arrowheads). Mononuclear cell infiltration (yellow arrow) is seen in the fibrous tissue. However, renal sections from the LA + NRG group showed tubular dilation (black

Table 1. Effects of naringenin on erythrogram in rats intoxicated with lead acetate at the fourth week posttreatment.

Groups	RBCs (× 10^6/μl)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
Control	8.01 ± 0.31^a	16.64 ± 0.44^a	42.04 ± 1.18^a	52.48 ± 0.89^a	20.77 ± 0.38^a	39.58 ± 0.30^a
LA	6.56 ± 0.45^b	12.50 ± 0.74^b	31.88 ± 1.52^b	48.59 ± 1.42^a	19.05 ± 0.36^a	39.20 ± 0.52^a
NRG	7.83 ± 0.23^a	16.81 ± 0.27^a	41.54 ± 0.43^a	53.05 ± 1.01^a	21.46 ± 0.36^a	40.46 ± 2.4^a
LA + NRG	7.48 ± 0.03^a	15.28 ± 0.76^a	38.92 ± 0.87^a	52.03 ± 1.00^a	20.42 ± 1.11^a	39.26 ± 0.23^a

Hb, hemoglobin concentration; LA, lead acetate; LA + NRG, lead acetate + naringenin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; NRG, naringenin; PCV, packed cell volume; RBC, red blood cell count.
Values are represented as mean±SEs for five rats per group.
Means with dissimilar superscript letters in the same column significantly varied at P value less than 0.05.

Table 2. Effects of naringenin on the leukogram in rats intoxicated with lead acetate in the fourth week posttreatment.

Groups	TLC (× 10^3/μl)	Lymphocyte (× 10^3/μl)	Neutrophil (× 10^3/μl)	Monocyte (× 10^3/μl)
Control	22.12 ± 2.06^b	14.48 ± 1.54^b	6.59 ± 0.44^a	1.05 ± 0.16^a
LA	28.66 ± 2.59^a	20.67 ± 1.98^a	7.00 ± 0.70^a	0.99 ± 0.24^a
NRG	21.78 ± 0.93^b	14.55 ± 1.05^b	6.24 ± 0.53^a	0.99 ± 0.08^a
LA + NRG	22.11 ± 1.78^b	14.70 ± 1.21^b	6.45 ± 0.51^a	0.96 ± 0.18^a

LA, lead acetate; LA + NRG, lead acetate + naringenin; NRG, naringenin; TLC, total leukocyte count.
Values are represented as mean±SEs for five rats per group.
Means with dissimilar superscript letters in the same column significantly varied at P value less than 0.05.

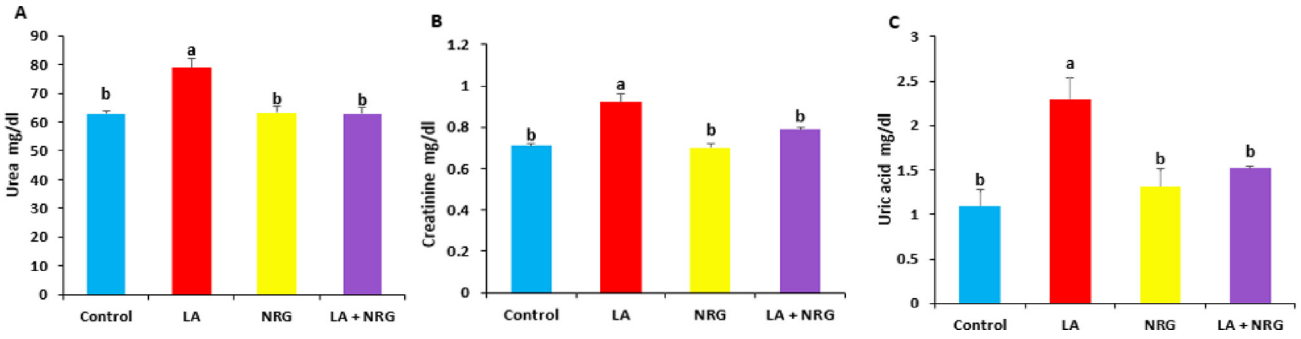

Fig. 1. Renal function parameters: (a) urea (mg/dl), (b) creatinine (mg/dl), and (c) uric acid (mg/dl) at the fourth week posttreatment with NRG in LA-intoxicated rats (mean ± SE) ($P < 0.05$).

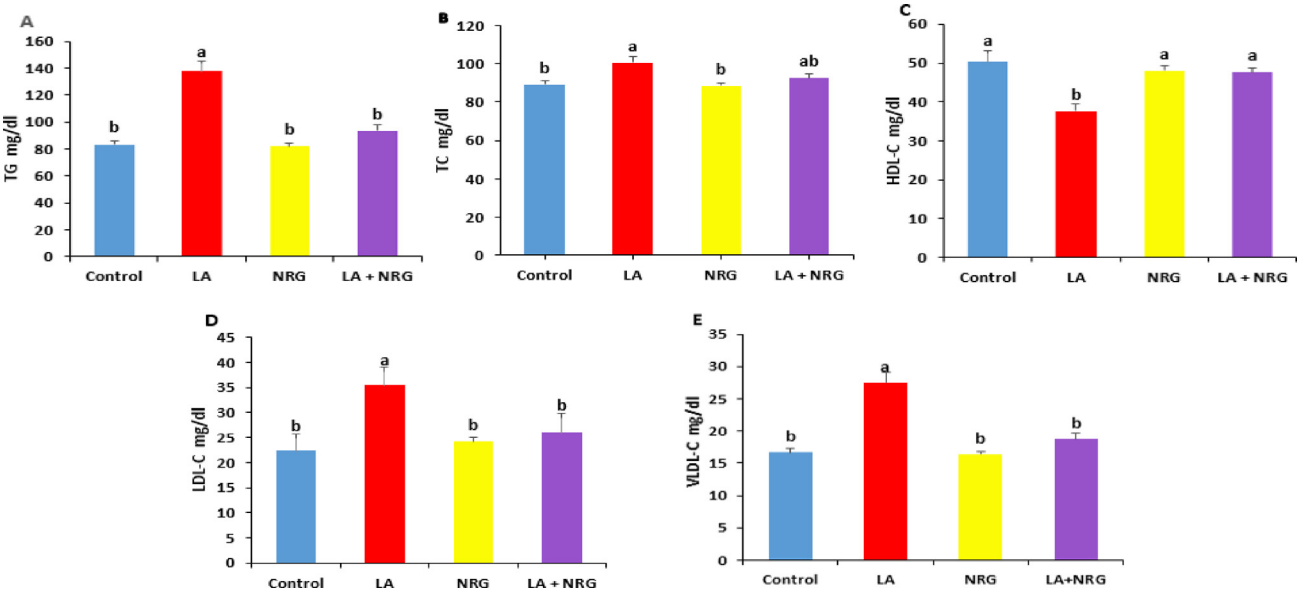

Fig. 2. Lipid profile parameters: (a) TG (mg/dl), (b) TC (mg/dl), (c) HDL-c (mg/dl), (d) LDL-c (mg/dl), and (e) VLDL-c (mg/dl) at the fourth week posttreatment with NRG in LA-intoxicated rats (mean ± SE) ($P < 0.05$). HDL-c, high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein-cholesterol; TC, total cholesterol; TG, triglycerides; VLDL-c, very low-density lipoprotein cholesterol.

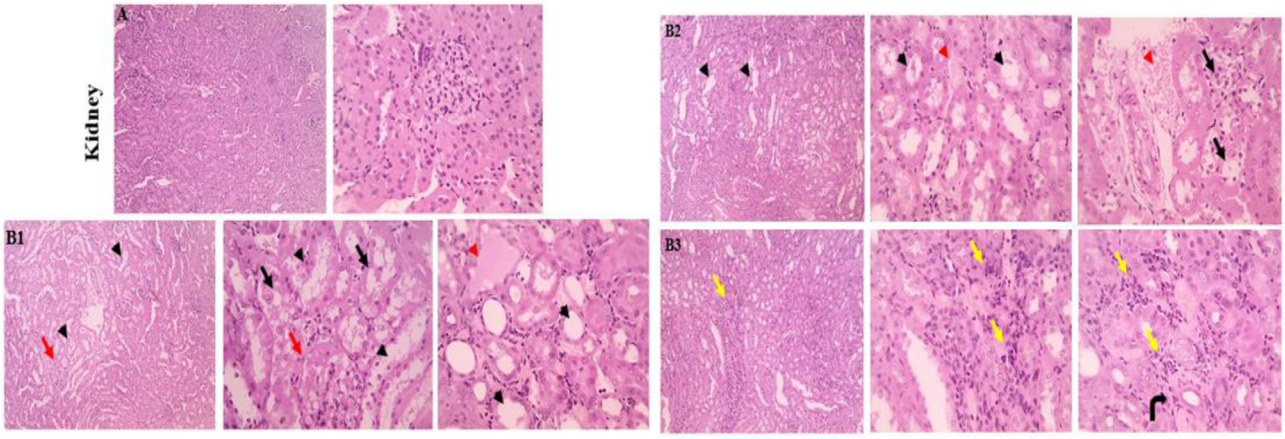

Fig. 3. Histopathological examination of renal sections showed normal glomeruli and tubules with minimal interstitial tissue in the control group (A). Microscopic pictures of hematoxylin and eosin-stained renal sections from group (B) showed tubular dilation (black arrowheads), hydropic degeneration of renal epithelial cells (black arrows), degenerated glomerulus (red arrows), and congested intratubular blood vessels (red arrowheads). Mononuclear cell infiltration (yellow arrow) is seen with fibrous tissue deposition (curved arrow) in the interstitial tissue. Low magnification: × 100 and high magnification: × 400.

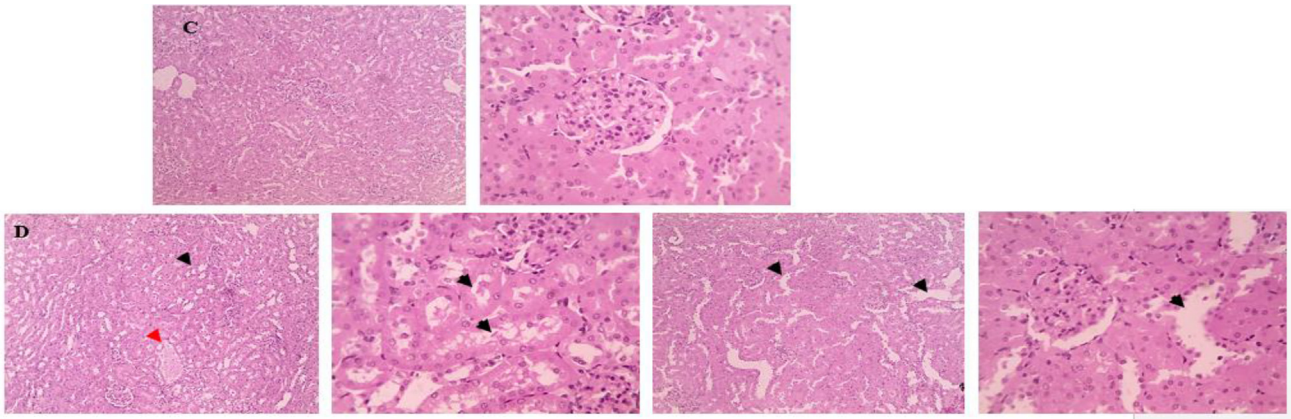

Fig. 4. Histopathological examination of renal sections showed normal glomeruli and tubules with minimal interstitial tissue in group (C). Microscopic pictures of hematoxylin and eosin-stained renal sections from group (D) showed tubular dilation (black arrowheads) with mildly congested intratubular blood vessels (red arrowheads). Low magnification: × 100 and high magnification: × 400.

arrowheads) with mildly congested intratubular blood vessels (red arrowheads).

3.4.2. Splenic tissue

Splenic sections showing normal-sized lymphoid follicles surrounded by normal red pulp in the control and NRG groups. Splenic sections from the LA group showed a marked decrease in the size of lymphoid follicles; however, the size of lymphoid follicles in the splenic sections was restored in the LA + NRG group (Fig. 5).

4. Discussion

This study aimed to distinguish the possible protective effect of NRG against lead toxicity in rats by assessing alterations in hematological and serum biochemical parameters. Histopathological alterations in the kidneys and spleen also ameliorated the effects of NRG.

Lead has a variety of pathological effects, such as neurotoxicity, kidney and liver damage, inflammation, and immune suppression, and is frequently linked to anemia, cardiac disorders, and hypertension [16,17]. The liver and kidney are the target organs for lead toxicity, and their toxic effects can be induced through different modes of action [18].

Natural substances with a variety of bioactivities are key sources of new therapeutics and offer a wide range of pharmacological potential. Flavonoids are antioxidants that are widespread in both plants and the human diet. Furthermore, they have been

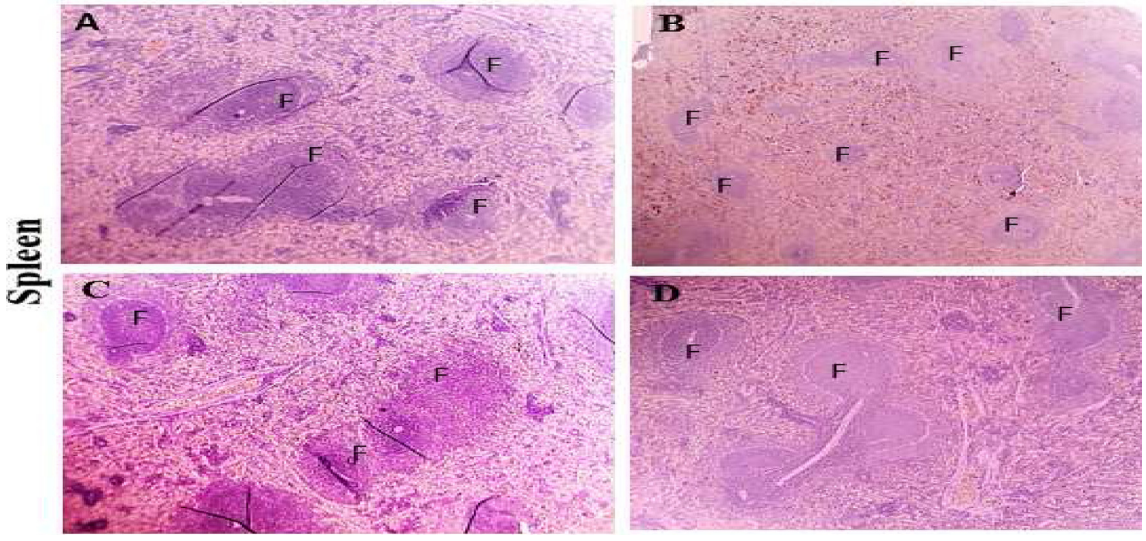

Fig. 5. Histopathological examination of splenic sections showed the normal size of lymphoid follicles (F) surrounded by the normal red pulp in the control group (A) and group (C). Splenic sections from group (B) showed a marked decrease in the size of lymphoid follicles (F). Splenic sections from group (D) showed restored size of lymphoid follicles (F).

reported to be efficient ROS scavengers because of their polyphenolic molecular structure, which is also responsible for their radical trapping characteristics [19]. According to van Acker et al. [20], they often have one or more aromatic hydroxyl groups in their structures, which give them antioxidant activity. They have also been demonstrated to exert positive effects on a variety of diseases by lowering the level of oxidative stress [21]. Therefore, they have recently gained a lot of interest because of their antioxidant activities, vasodilation, and nephroprotective effects [8]. NRG is one of the flavonoids most frequently found in citrus fruits, tomatoes, and grapes [22]. It is well recognized as a potent natural product with a variety of pharmacological properties, including antioxidant, anti-inflammatory, anti-eNOS, anticancer, nephroprotective, and hepatoprotective activities [23].

As demonstrated in our results, rats intoxicated with LA showed a significant drop in Hb concentration, RBC count, and PCV % compared with the control, indicating normocytic normochromic anemia. Mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration in all groups did not change significantly. One of the systems most vulnerable to lead toxicity is the hematological system. Our results agree with those of Othman et al. [24]. The observed anemia could be explained by the theory that RBC cell membranes are significantly damaged by toxic lead and become more fragile. In addition, lead toxicity inhibits ferrochelatase and delta-aminolevulinic acid dehydratase, two crucial enzymes involved in the heme biosynthesis pathway [25]. The inability of ferrochelatase to integrate iron into the protoporphyrin ring and aminolevulinic acid dehydratase to join two molecules of delta-aminolevulinic acid to produce porphobilinogen, which decreases heme has been studied. Similarly, Offor et al. [26] found the same findings when rats were orally administered LA at a dose of 60 mg/kg for 28 days.

Our current study showed leukocytosis and lymphocytosis, which might be attributed to the harmful effects of lead on the lymphoid organs involved in leukocytosis, leading to an increase in production from the germinal center of lymphoid organs [27]. In addition, inflammation caused by lead exposure has been linked to elevated leukocytes [28]. In addition, bone marrow destruction caused by lead toxicity revealed lymphocytosis and leukocytosis and carries a significant risk of developing lymphoproliferative neoplasms [29]. Our findings are in line with those of Abdelhamid et al. [30], who administered LA to rats through a stomach tube at a dose of 100 mg/kg body weight for 4 weeks.

With NRG treatment, our erythrogram and leukogram showed noticeable improvements in hematological parameters (white blood cells, RBCs, and Hb). NRG stimulates erythropoietin secretion, which stimulates bone marrow stem cells to produce RBCs [31]. This could be attributed to a decline in the level of lipid peroxide in the RBC membrane, which then leads to a decrease in the nonenzymatic glycosylation of membrane proteins. These results are in agreement with those of the Abdel-Magied and Shedid [32] study in which rats were orally administered 50 mg/kg body weight/day for 21 days.

In our investigation, LA treatment increased TGs, LDL-c, TC, and VLDL-c, while decreasing HDL-c levels, compared with the control group. The association between lead exposure and increased serum lipid levels may be explained by either decreased lipoprotein clearance due to altered cell surface receptors for these proteins or decreased hepatic lipoprotein lipase function [33]. Lead has also been shown to reduce cytochrome P450 function, which could disrupt the ability of the body to produce bile acids, the primary method of eliminating cholesterol. Our results are in line with those of Abdel-Moneim et al. [16], who injected LA at a dose of 25 mg/kg for rats for 7 days.

Upon NRG treatment, the levels of TC, LDL-c, total glycerides, and VLDL-c were markedly decreased in the (LA + NRG) group compared with the untreated group. These results are explained by the pharmacological effect of NRG as a potential hypolipidemic and hepatoprotective drug [34], which reduces the levels of plasma TC, TG, and FFA, preventing the deleterious side effects caused by the elevated levels of these lipids [35]. Notably, grapefruit and its juice contain significant amounts of NRG and its glycoside naringin, both of which have been shown to have hypolipidemic effects. Chtourou et al. [36] are in agreement with our results when rats were administered NRG orally at a dose of 50 mg/kg daily for 90 days.

As mentioned in our work, the LA-intoxicated group, in comparison to the untreated group, showed a marked increase in serum concentrations of uric acid, urea, and creatinine. These alterations reflect renal failure induced by LA, as the kidney is more susceptible to damage from lead due to larger perfusion and higher excretion of chemical concentrations in renal tubular cells [10]. Serum levels of creatinine and urea are used as biomarkers of renal function; therefore, it is recognized that higher blood urea levels are attributed to increased mammalian protein catabolism and/or the conversion of ammonia to urea as a result of increased arginase enzyme that forms urea in the body.

Adenosine and guanosine, two purine nucleotides, are primarily converted into uric acid. The body's response to increased endogenous oxygen species formation induced by lead can be represented by rising serum uric acid concentrations, as uric acid is a potent scavenger of peroxynitrite [37]. Our results are consistent with those of Al-Megrin et al. [38], who injected rats with LA at a dose of 20 mg/kg/I/P for 7 days.

NRG treatment in the (LA + NRG) group significantly improved urea, urine acid, and creatinine serum levels, protecting kidney function against lead toxicity because it protects against cisplatin-induced nephrotoxicity through its antioxidant capacity as it contains an aromatic hydroxyl group [23]. The present findings support earlier reports of rats supplemented with NRG orally at doses of 5 and 10 mg/kg for 10 weeks by Roy et al. [39].

Our findings showed that the coadministration of NRG and LA significantly enhanced hematobiochemical parameter distortion and diminished histopathological alterations in the kidney and spleen.

5. Conclusion

The current research suggests that concurrent administration of NRG protected rats from hematonephrotoxicity and hyperlipidemia caused by LA by improving kidney function, reestablishing normal levels of lipids in the plasma, and reducing kidney and spleen histopathological changes.

Author contributions

Lubna A.H. Mansour: methodology, formal analysis, data curation, writing original draft, review, and editing; Gehad E. Elshopakey, Fatma M. Abdel Hamid, and Engy F.M. Risha: conceptualization, validation, visualization, editing the final draft, supervision, and reviewing; Engy F.M. Risha: final review and preparing the manuscript for publication. All authors read and approved the final manuscript.

The authors declare that all data were generated in-house and that no paper mill was used.

Data availability statement

The authors confirm that the data supporting the findings of this study is available within the article.

Conflicts of interest

There are no conflicts of interest.

Acknowledgments

The authors acknowledge all members of the Clinical Pathology Department, Faculty of Veterinary Medicine, Mansoura University, Egypt for their help and support.

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