

Ameliorative Potentials of Quercetin against Lead-Induced Hematological and Testicular Alterations in Albino Rats

Mohammed A. Al-Omair¹, Azza Sedky², Awatef Ali², and Hany Elsayy^{1,3,*}

¹Chemistry Department, College of Science, King Faisal University, Al-Ahsaa, King Saudi Arabia

²Zoology Department, Faculty of Science, Alexandria University, Alexandria, Egypt
and

³Chemistry Department, Faculty of Science, Tanta University, Tanta, Egypt

Abstract

Lead is one of the oldest environmental and occupational toxins. Health hazards from increased lead exposure as a result of industrial and environmental pollution are recognized. The aim of the present study was to investigate the protective effects of quercetin as a model of an antioxidant drug against the toxic effects of lead acetate on the blood and the testis of rats. The lead concentrations were determined in blood and the testis. Testosterone (T), luteinizing hormone (LH) and follicle stimulating hormone (FSH) were assessed in serum. Hemoglobin (Hb) content, packed cell volume (PCV), white blood cell (WBC) and red blood cell (RBC) counts were evaluated in the whole blood. Our results showed that administration of lead acetate was associated with an increased lead levels in blood as well as in the testis. Lead acetate administration also caused a decrease in testicular function, Hb content, PCV and RBC count in comparison to the respective mean values of the control. In addition, lead acetate increased WBC count and induced alterations in sperm count, sperm motility and sperm abnormality and histopathology. In the contrary, administration of lead acetate along with quercetin partially restored the studied parameters to normal values. In conclusion, the treatment with quercetin may provide a partial protection against the toxic effects induced by lead acetate in blood and the testis of rats.

Key Words: blood, lead acetate toxicity, quercetin, testicular alteration

Introduction

Lead is a heavy metal environmental pollutant which has wide industrial applications in the modern society. Lead is being used in production of paints, batteries, water pipes, gun bullets, X-ray and atomic radiation protection, eye cosmetics, base metal utensils, and also as an additive in gasoline (21). One of the main targets of lead toxicity is the hematological system. Lead has been shown to alter red blood cell (RBC) membrane flexibility and increase membrane fragility, leading to increased risk of hemolysis (41). Ex-

posure to different heavy metals like lead, cadmium and mercury causes irreversible toxic insult to the male reproductive system and produces cellular impairments at the structural and functional levels, resulting in a variety of harmful effects on cells, tissues, or organs (28). The exposure to lead results in the inhibition of testicular function as manifested by decreased levels of serum Testosterone (T), follicle stimulating hormone (FSH) and luteinizing hormone (LH) (17).

One possible mechanism explaining lead-induced toxicity is the disturbance of the balance between the pro-oxidant and anti-oxidant by generating reactive

Corresponding author: Dr. Hany Elsayy, College of Science, King Faisal University, Al_Ahsaa, P.B. Box 380, Hufuf, 31982, Saudi Arabia; Telephone: +966548927297, Fax: +96635899556, E-mail: alomair@kfu.edu.sa, hmostafa@kfu.edu.sa
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oxygen species (ROS), thereby stimulating the process of lipid peroxidation. Lipid peroxides have been shown to impair tissue membranes, which is a risk factor in a variety of diseases (33). In recent years, studies have begun to focus on the use of antioxidants as protectants against the harmful effects of lead (44). Flavonoids are polyphenolic compounds widely distributed in plant foods. Quercetin (3,5,7,3',4'-pentahydroxyflavon) seems to be the most powerful flavonoids for protecting the body against ROS, which are produced during the normal oxygen metabolism, or are induced by exogenous damage (14). Quercetin is abundant in food including green leafy vegetables, fruit and beverages (9, 25). By acting as an antioxidant, quercetin exhibits several beneficial effects, such as anti-inflammatory, anti-allergic, antiviral as well as anticancer activities (40).

The aim of this study was to evaluate possible protective effects of quercetin as a model of powerful therapeutic drug on hematological and testicular alterations induced by lead exposure.

Materials and Methods

Chemicals

Lead acetate and quercetin were purchased from Sigma Chemicals (St. Louis, MO, USA). Reagent kit for determination of hemoglobin (Hb) was purchased from Biodiagnostics, Cairo, Egypt. Kit for determination of T was purchased from K-assay, WA, USA. LH and FSH kits were obtained from Biovender, Tokyo, Japan.

Animals

Twenty adult male rats (*Rattus norvegicus*) weighing about 180-200 gm were purchased from the animal house of faculty of medicine, Alexandria University, Egypt. All animals were housed in plastic cages (5 per cage) and kept under the same laboratory conditions of temperature (25°C), humidity (60%) and lighting (12 h light/12 h dark) for one week prior to start of the experiments for acclimatization. The rats were allowed free access to food and water. They were fed with standard commercial rat chow.

Experimental Design

Animals were randomly divided into four groups of five animals each. Group I served as the control group: rats were given daily normal saline (0.9% NaCl) intraperitoneally (i.p.) as vehicle. Group II served as lead acetate-treated group: rats were injected (i.p.) daily by lead acetate dissolved in normal saline at a concentration of 20 mg/kg bodyweight (b.w.) (16). Group III served as the quercetin-treated group: rats

were injected (i.p.) daily by quercetin dissolved in normal saline at concentration of 50 mg/kg b.w. (15). Group IV served as the lead acetate and quercetin co-treated group: rats were injected (i.p.) daily by lead acetate (20 mg/kg b.w.) followed by quercetin (50 mg/kg b.w.). At the end of the 4-week experimental period, the animals were sacrificed and blood samples and testes were collected for analysis. The collected blood from each animal was divided into two parts. Ten percent ethylenediaminetetraacetic acid (EDTA) was added to one part for evaluation of lead levels and hematological parameters while the other part was centrifuged at 5,000 rpm for 10 min; the separated sera were subjected to biochemical analysis.

Evaluation of Lead Level

The lead concentration was determined in blood and the testis using Atomic Absorption Spectroscopy.

Hematological Analysis

In the whole blood, Hb content was determined using a commercial kit according to supplier's protocol. Packed cell volume (PCV), white blood cells (WBC) and RBC counts were determined according to reported method (20).

Biochemical Parameters

Biochemical markers of testicular function in the serum were determined. T was determined as previously reported using a rat T enzyme-linked immunosorbent assay (ELISA) kit (Cat. No. KT-29533). The levels of LH was determined according to the method in Shibayagi's rat LH ELISA kit while the levels of FSH were estimated following the method described in rat FSH ELISA kit.

Determination of Sperm Characteristics

The testis was minced in pre-warmed saline (37°C) and the resulted suspension was used in the following analyses:

Determination of Sperm Motility: One drop of sperm suspension was placed on a glass slide to analyze 200 motile sperm in 4 different fields. The motility of the sperms was evaluated microscopically within 2-4 min of their isolation from the testis and data were expressed as percentage motility (37). Non-motile sperm numbers were first determined, followed by counting total sperm. Sperm motility was expressed as a percent of motile sperm from the total sperm counted.

Determination of Sperm Count: The sperms were counted using a hemocytometer following the method of Freud and Carol (22).

Table 1. Lead acetate concentrations in blood and the testis of rats after lead treatment

Group No.	N	Blood ($\mu\text{g/ml}$)	Testis ($\mu\text{g/gm}$)
Group I	5	$0.12^c \pm 0.02$	$0.14^c \pm 0.02$
Group II	5	$2.78^a \pm 0.17$	$2.04^a \pm 0.11$
Group III	5	$0.12^c \pm 0.02$	$0.14^c \pm 0.02$
Group IV	5	$1.63^b \pm 0.17$	$0.72^b \pm 0.02$
	F (P)	111.925* (<0.001*)	218.166* (<0.001*)

Normally distributed data were expressed in mean \pm SE and were compared using the F test (ANOVA); *Post Hoc* Test (Fisher least significant difference, LSD) was used for comparison between groups. The groups have the same superscript letter have no significant difference between them while groups have significant difference have different superscript letter. *Statistically significant at $P \leq 0.05$.

Determination of Sperm Abnormality: Percentage of morphologically abnormal sperm was determined by the method described by Evans and Maxwell (18). Briefly, the percentage of live and dead spermatozoa was estimated by preparing a stained film using a drop from the sperm suspension with two drops from an Eosin-nigrosin stain. The smears were made by placing a drop from the sperm suspension and one or two drops of the previously warmed (37°C) eosin-nigrosin stain at one end of clean slide. The smears were allowed to dry in the air and then examined using a high power (100x) oil immersion objective. At least 200 sperm cells from different fields on the slide were examined to determine the live and dead spermatozoa. Normally live spermatozoa had completely unstained heads. Partially or completely red stained heads were classified as dead. Having calculated the dead/live ratio, the same stained slide was used to determine the percentage of abnormal spermatozoa. At least 200 sperm cells from different fields were examined and the number of abnormal ones was calculated as a percentage.

Histopathological Examination

Specimens of the testis were collected from all experimental groups and fixed in 10% neutral buffered formalin for 24 h. After proper fixation, the tissues were rinsed with water and dehydrated in ascending grades of alcohols, cleared in xylene and embedded in paraffin. Tissue blocks were cut into thin sections ($5 \mu\text{m}$) routinely stained with haematoxylin and eosin (H&E) stain (8) and examined by light microscopy.

Statistical Analysis

Results are presented as mean \pm standard error of the mean (SE) of data obtained from five animals in each groups. SPSS program was used for the statistical analysis of data with one-way analysis of variance

(ANOVA) to compare data between the groups. In all the cases, a difference was considered significant when $P \leq 0.05$.

Results

Bioaccumulation of Lead in Blood and the Testis

The lead acetate group showed a significant ($P \leq 0.05$) increase in the blood lead level in comparison to the control group (Table 1). The blood lead level in the lead and quercetin group was also significantly ($P \leq 0.05$) higher when compared to that of the control, but it was significantly ($P \leq 0.05$) lower than that of the lead acetate group. In the testis, the lead acetate group showed a significant ($P \leq 0.05$) increase in the lead level whereas the lead level in the lead and quercetin group was also significantly ($P \leq 0.05$) higher when compared to the control group, but the testicular lead level was significantly ($P \leq 0.05$) lower than in the lead acetate group.

Hematology

Exposure to lead acetate (Group II) significantly ($P \leq 0.05$) lowered the values of the Hb concentration, PCV and RBC count relative to values obtained for the control group (Table 2). Exposure to lead acetate followed by concomitant treatment with quercetin significantly ($P \leq 0.05$) increased the values of Hb content, PCV and the RBC count when compared with the lead acetate group. Treatment with quercetin only did not affect significantly ($P \leq 0.05$) the hematological parameters when compared with the control group.

Biochemistry

The mean serum T, FSH and LH levels were significantly lower ($P \leq 0.05$) in rats treated with lead

Table 2. Hematological parameters of the experimental rats

Group No.	N	Hb (gm/dl)	PCV %	RBC (10^6 cells/cm ²)	WBC (cells/cm ²)
Group I	5	13.04 ^a ± 0.20	40.36 ^a ± 1.50	4.60 ^a ± 0.10	8400.0 ^{bc} ± 284.60
Group II	5	9.54 ^c ± 0.23	31.00 ^c ± 0.71	3.10 ^c ± 0.14	11540.0 ^a ± 213.5
Group III	5	12.72 ^a ± 0.14	40.40 ^a ± 0.75	4.50 ^a ± 0.13	7960.0 ^c ± 312.4
Group IV	5	11.82 ^b ± 0.22	36.20 ^b ± 0.86	3.93 ^b ± 0.05	9300.0 ^b ± 659.6
F (P)		61.649* (<0.001*)	19.587* (<0.001*)	32.269* (<0.001*)	15.419* (<0.001*)

See footnote to Table 1.

Table 3. Testis hormones and sperm parameters in the experimental rats

Group No.	N	Testis					
		Hormones			Sperm parameters		
		T (pg/ml)	LH (ng/ml)	FSH (ng/ml)	Sperm counts (million cells/ml)	Sperm motility (%)	Sperm abnormality (%)
Group I	5	4.92 ^a ± 0.10	0.39 ^a ± 0.04	1.32 ^a ± 0.07	82.60 ^a ± 0.81	87.80 ^a ± 1.39	5.80 ^c ± 0.86
Group II	5	1.61 ^c ± 0.12	0.18 ^b ± 0.01	0.86 ^b ± 0.04	27.00 ^c ± 1.26	25.80 ^c ± 1.07	30.80 ^a ± 2.01
Group III	5	4.76 ^a ± 0.19	0.36 ^a ± 0.02	1.30 ^a ± 0.06	82.80 ^a ± 2.15	87.20 ^a ± 1.02	4.60 ^c ± 0.51
Group IV	5	2.96 ^b ± 0.08	0.34 ^a ± 0.05	1.24 ^a ± 0.02	64.20 ^b ± 1.74	51.00 ^b ± 1.41	17.80 ^b ± 0.86
F (P)		150.092* (<0.001*)	8.075* (0.002*)	19.226* (<0.001*)	277.445* (<0.001*)	594.447* (<0.001*)	103.813* (<0.001*)

See footnote to Table 1.

acetate relative to that of the control group (Table 3). On other hand, the levels of these hormones in the rats treated with lead acetate and concomitantly treated with quercetin were significantly higher ($P \leq 0.05$) compared to those obtained from rats treated with lead acetate only. Treatment with quercetin did not affect significantly ($P \leq 0.05$) these parameters when compared with the control group.

Sperm Quality

There was a significant ($P \leq 0.05$) decrease in sperm count and sperm motility while there was a significant ($P \leq 0.05$) increase in the percentage of sperm abnormality in the rats treated with lead acetate. In rats co-treated with lead acetate and quercetin, these altered parameters were ameliorated compared to rats exposed to lead acetate. Treatment with quercetin only did not affect significantly ($P \leq 0.05$) these parameters when compared with the control group (Table 3).

Light Microscope Observations of the Testis

Histological cross sections of testes of the rats of the control group revealed seminiferous tubules sep-

arated by the interstitial tissue (Fig. 1A). Each seminiferous tubule was lined with spermatogenic epithelium consisting of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. The lumina of seminiferous tubules were occupied by the late spermatids and spermatozoa (Fig. 1B).

Pathological examination of the testicular tissues showed that in the Group II lead-poisoned animals showed deformities in the testis architecture where the seminiferous tubules appeared with a wavy outline and surrounded by irregular basement membrane. The interstitial tissue was degenerated. The irregular basement membranes of seminiferous tubules were lined with one or two layers of small acidophilic cells with dark nuclei. The lumen was free from spermatozoa (Fig. 1C). Most of the seminiferous tubules of the testes in this group showed a clear absence of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa indicating loss of the spermatogenesis process. Shrunken spermatogonia with pyknotic nuclei and sparse spermatocytes with large and dark-stained nuclei (Fig. 1D).

The quercetin group showed healthier testicular tissues than those of the control group with a slight increase in both the intestinal and epithelial tissues

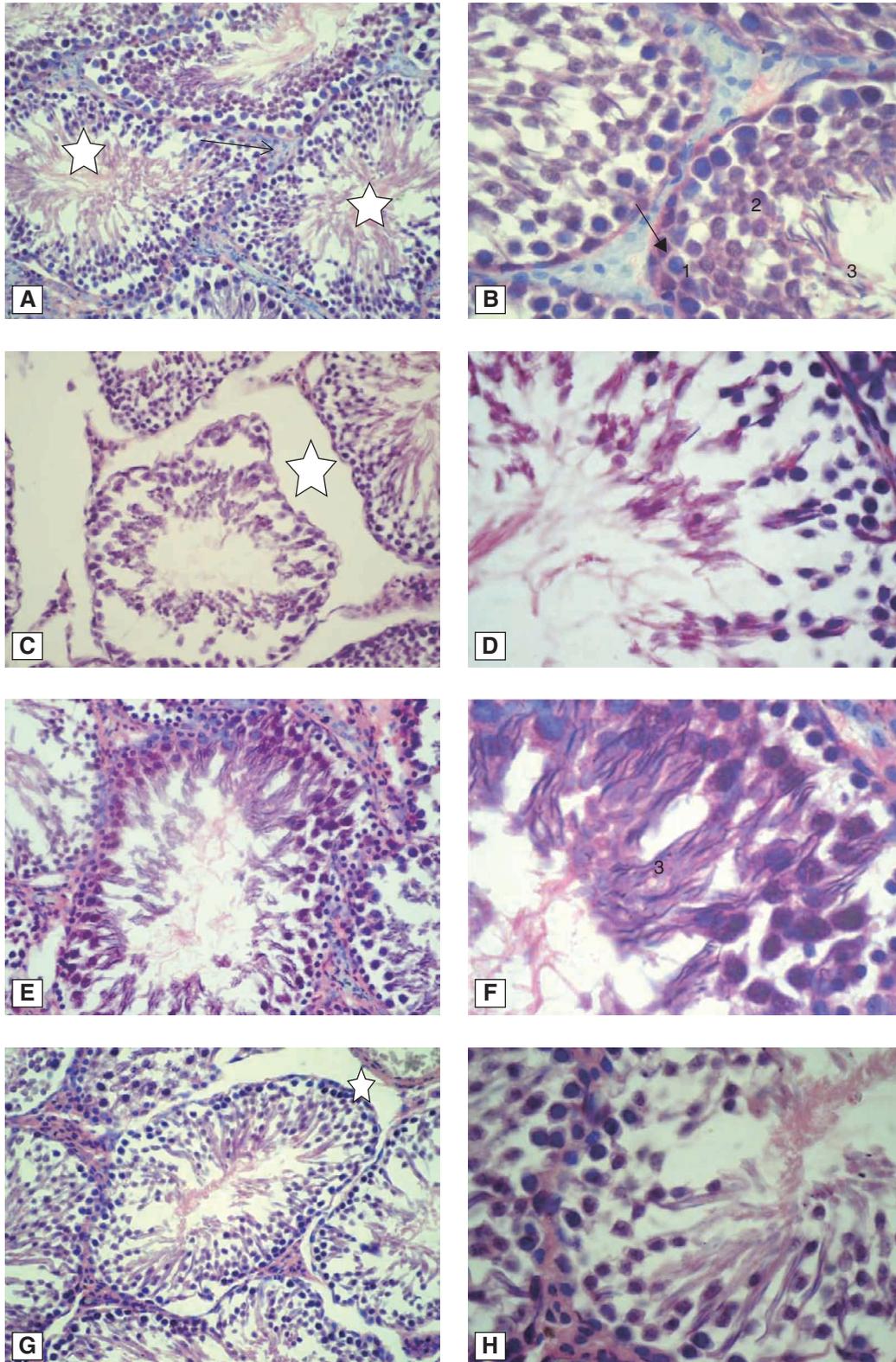


Fig. 1. Light microscopy of testicular tissues of rats treated with lead in the absence or presence of quercetin (H&E stain). (A, B) In the control, normal testicular tissue with seminiferous tubules (indicated by the star) was separated by interstitial tissue (arrow) (10X) (A), and the germ cells included spermatogonia (arrow), spermatocyte (1), spermatids (2) and spermatozoa (3) (40X) (B); (C, D) In the lead-treated group, severe testicular damage with marked winding of interstitial space (indicated by a star) (10X) (C) and reduction of spermatogenesis are shown (40X); (E, F) In the quercetin group, healthy testicular tissue (10X) (E) and normal number of spermatozoa (3) are displayed (40X); (G, F) In the lead and quercetin co-treated group, mild improvement in the seminiferous tubules structure with narrow interstitial space (indicated by the star) (G) (10X) and restoration of spermatogenesis (40X) (H) are evident.

(Fig. 1E). There was regular and compact arrangement of the seminiferous tubules with large spermatozoa number (Fig. 1F). Comparison between the quercetin and lead co-treated group and lead only group indicated that administration of quercetin reversed the damage caused by lead poisoning in which case the testicular tissues showed vast improvement in the seminiferous tubules, which were closer to each other, and the restoration of spermatogenesis, which led to more spermatozoa in the tubule lumen (Fig. 1G). Focal proliferation of the spermatogonia, more spermatocytes and spermatids were observed (Fig. 1H).

Discussion

Ameliorative Potentials of Quercetin against Lead-Induced Hematological Alterations

Lead is one of the persistent ubiquitous heavy metals (27) with broad spectrum of toxic effects in animal systems (45). The hematological system is the major target of low levels of lead exposure (46). In the present study, lead acetate was shown to cause significant increases in WBC count and significant decreases in RBC count, Hb and PCV levels in treated rats compared to the control group. These results were in accordance with previous reports (1, 4). Lead exposure induces severe oxidative damage in RBCs by inhibiting Hb synthesis and changing erythrocyte morphology and survival as a result of direct interaction of lead with RBC membranes, inducing lipid peroxidation (42). The decrease in haemoglobin content due to lead exposure may be due to the ability of lead to inhibit the enzyme δ -aminolevulinic acid dehydratase (ALAD), which is highly sensitive to lead poisoning (19). In the present study, reduction in RBC counts may be due to increased rate of RBC breakdown or reduction rate of RBC formation (39). The low hematological parameters of Hb, PCV and RBCs showed that lead acetate administration caused anemia (6). Lead has been shown to alter RBC membrane flexibility and to increase RBC fragility, leading to increased risk of hemolysis (41).

In the present study, quercetin administration along with lead acetate partially restored the haemoglobin content, PCV and RBC count to normal values. The result of the present study agreed with that of Koriem (34), who reported a protective role of quercetin in rats intoxicated with lead and in rabbits intoxicated with doxorubicin (40). The lack of significant decreases in Hb, PCV and RBC count in rats co-treated with lead acetate and quercetin was an indication of the alteration of lead acetate-evoked anemia by the antioxidant quercetin.

It is well known that lead induces oxidative stress demonstrated by decrease in superoxide dismutase and catalase and stimulation of lipid peroxidation (30).

Oxidation of erythrocyte membranes by free radicals induces the oxidation of lipids and proteins eventually resulting in hemolysis (43). The mature human erythrocyte, when submitted to oxidative stress, demonstrated oxidation of the Hb molecule. Quercetin protected against oxidative damage of the erythrocyte membrane (32). Hb oxidation leads to the formation of hemichrome, which binds to the membrane and causes RBC removal by the reticuloendothelial system. Quercetin has previously been shown to reduce the levels of the membrane-bound hemichrome and to suppress lipid peroxidation (12).

Ameliorative Potentials of Quercetin against Lead-Induced Testicular Alterations

In the present study, treatment of rats with lead acetate led to a considerable increase of accumulation of the metal in the blood and testis compared with the normal group. However, administration of lead acetate along with quercetin decreased the level of lead in these tissues, in agreement with previous reports (17, 36). Our results showed that lead acetate-treated rats showed a significant increase in the testicular lead level in comparison to the control, suggesting that lead accumulated in the testis, as was found by Moustafa *et al.* (38). Our data showed that quercetin reduced the amount of lead in the testis.

There was also a decrease in the levels of T, LH and FSH levels in lead-intoxicated rats (17, 35, 36), probably attributed to a decrease in the number of LH binding sites in the Leydig cells (38).

Possible Protective Effects of Quercetin as an Antioxidant

Alterations in sperm parameters were observed following lead administration (7, 24). One possible explanation is that lead acetate is toxic to the testicular structure. Moreover, the decrease in sperm motility can be due to indirect effects of lead, like increase of ROS generation in sperm cells. By causing lipid oxidation, ROS alters the integrity and the fluidity of cellular membrane structures, particularly of cell membrane which is essential for sperm motility, structural integrity, and ultimately for sperm viability (47). It was previously reported that lead acetate has the ability to induce oxidative stress in testes of adult rats (3).

Data of the present study showed that quercetin partially brought back the T, LH and FSH levels to the control level. This may be due to the possible protective effect of quercetin, as an antioxidant. The protective effect of quercetin against sodium fluoride-induced decrease in plasma T level was reported (13), as was a report against 2,3,7,8-tetrachlorodibenzo-p-dioxin (29).

Quercetin prevents antioxidant injury and cell

death *via* several mechanisms, such as scavenging oxygen radicals (11), protecting against lipid peroxidation and chelating metal ions (5). Previous studies have strongly emphasized that quercetin is considered as a surpass free-radical scavenging antioxidant (23) owing to a high number of hydroxyl groups and an ability to donate electrons or hydrogens, and scavenges hydroxyl groups, hydrogen peroxide, and superoxide anions (26). Quercetin is able to chelate lead by forming a coordination bond with the lead ions through its ortho-phenolic groups located on the quercetin B ring (10).

Histological examination of testis treated with lead showed that degenerated seminiferous tubules surrounded by irregular basement membrane and separated by degenerated interstitial tissue, as was found by (2, 31). Our data showed that quercetin improved the histological alterations made by lead, in agreement with previous report (2).

In conclusion, this study showed that quercetin at 50 mg/kg bodyweight could produce protective effects in rats intoxicated with lead, and this response was reflected on the blood and testis. Data of this work suggest potential preventive or therapeutic applications of quercetin for individuals subjected to lead environmental pollution.

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