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A Study of the Modulating Action of Quercetin on Biochemical and Histological Alterations Induced by Lead Exposure in the Liver and Kidney of Rats

Azza Sedky[1] and Hany Elsawy[2, 3]

[1]Zoology Department-Faculty of Science, Alexandria University, Alexandria, Egypt
[2]College of Science, King Faisal University, Al-Ahsaa, P.B. Box 380, Hufof 31982, Saudi Arabia
and
[3]Chemistry Department-Faculty of Science, Tanta University, Tanta, Egypt

Abstract

Lead is a highly toxic metal and a very potent poison. Lead poisoning is a serious condition but can be treated. Quercetin is a flavonoid with many beneficial uses. The aim of the present study was to investigate the possible modulating action of quercetin as a model of an antioxidant against the toxic effects of lead acetate on liver and kidneys of rats. Rats were randomly divided into four groups: (i) saline group (control); (ii) lead group received i.p. lead acetate (20 mg/kg b.w.); (iii) quercetin group received i.p. quercetin (50 mg/kg b.w.); (iv) lead and quercetin group received i.p. lead acetate (20 mg/kg b.w.) followed by i.p. quercetin (50 mg/kg b.w.) for 4 weeks. The lead concentrations were determined in the liver and kidney tissues. Liver marker enzymes, bilirubin, albumin, total protein, creatinine, uric acid and urea, were assessed in the serum and light microscopic studies were performed. The results showed that lead acetate administration was associated with an increase in serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities, total bilirubin, creatinine, uric acid, urea levels. Lead accumulation in kidneys and liver tissues was also found, but were associated with decrease in albumin and total protein in comparison with the respective mean values of the control. Lead acetate caused numerous histological alterations in the liver, including chronic inflammation, bilary hyperplasia, edema, congestion, Kupffer cells hyperplasia and hemosiderosis, and in the kidney, including tubular dilation, atrophy of glomerular tuft, widening of urinary space and mild fibroblast. In contrary, administration of lead acetate along with quercetin partially restored the studied parameters to normal values and improved structure of liver and kidney with significant decreases in the severity of histopathological changes when compared with the lead acetate group. In conclusion, treatment with quercetin may provide a modulating action against the toxic effects induced by lead acetate in the liver and kidney of male rats.

Key Words: biochemical alteration, histological alteration, lead toxicity, quercetin

Introduction

Lead exposure causes serious health hazards in animals and humans (15). Accumulation of lead produces damaging effects in the hematopoetical, renal and gastrointestinal systems (9). Lead has been associated with various forms of cancer, nephrotoxicity, central nervous system effects and cardiovascular diseases in human (30). The most common sources of lead are storage batteries, the production of solder for electrical devices, formulation of metal alloys, manufacture of pipes, cable sheeting, radiation shielding

Corresponding author: Dr. Hany Elsawy, College of Science, King Faisal University, Al-Ahsaa, P.B. Box 380, Hufof 31982, Saudi, Arabia, Tel: +966548927297, Fax: +96635899556, E-mail: hmostafa@kfu.edu.sa
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and ammunition, pigments stabilizers or binders in paints, ceramics, glass, plastic and mortar industries (17). Other sources of lead poisoning are found in automobile radiators, storage of drinking water in tanks, wrapping the food in newspapers (ink), canned food and the use of lead utensils (17).

Liver is the main target organ for lead toxicity (3). Lead acetate induces liver toxicity through an increase in bilirubin and serum liver enzymes and decrease in serum albumin and total protein (32). The kidney is also affected by lead (2). Lead induces injury of the renal tissue evidenced by increase in lead concentration in the kidney, and increase in uric acid, urea and creatinine (1).

Quercetin is a typical flavones-type flavonoid distributed in vegetables and fruits, such as onion, broccoli and apples (34). It has been found that quercetin prevents injury caused by oxidant stress and cell death by several mechanisms, such as protecting against lipid peroxidation and chelating ions (24) and scavenging oxygen radicals (19). The aim of this study was to evaluate the possible protective action of quercetin as a model of antioxidant on biochemical and histological alterations induced by lead exposure in the liver and kidney of rats.

Materials and Methods

Animals

All experimental procedures were carried out according to NIH guidelines of animal care. Twenty adult male rats weighing about 180-200 g (3 months old) were obtained from the animal house of Faculty of Medicine, Alexandria University, Egypt. All animals were housed in plastic cages, five animals per cage, and kept under the same laboratory conditions of temperature (25°C) and lighting (12 h light/12 h dark) for one week prior to start of experiments for acclimatization. The rats had free access to standard commercial rat chow and water. Institutional Animal Care and Use Committee (IACUC) at the Alexandria University approved the experimental protocol of this study.

Chemicals

Lead acetate and quercetin were purchased from Sigma chemicals (St. Louis, MO, USA). Reagent kits for assay of ALP, ALT, AST, total protein, urea and creatinine were obtained from BIOMED diagnostic, Hannover, Germany. Reagent kit for assay of bilirubin was obtained from Diamond Diagnostics, Holliston, MA, USA. Reagent kits for assay of uric acid and albumin were obtained from SPECTRUM, Cairo, Egypt.

Experimental Design

Animals were randomly divided into four groups, with 5 animals in each group. Group I: the control group injected (i.p.) daily with normal saline (0.9 % NaCl). Group II: lead acetate group injected (i.p.) daily with lead acetate (20 mg/kg b.w.) (14). Group III: quercetin group injected (i.p.) daily with quercetin (50 mg/kg b.w.) (12). Group IV: the lead acetate and quercetin group injected (i.p.) daily by lead acetate (20 mg/kg b.w.) followed by quercetin (50 mg/kg b.w.). The duration of the experiments was 4 weeks. At the end of the experimental period, all the animals were sacrificed; blood samples and selected tissues were collected for analysis.

Determination of Bioaccumulation of Lead

Half a gram of the selective tissue was homogenized and digested with nitric acid at 120°C for 2 h. The level of lead was estimated in the digested solution according to the described method (21).

Biochemical Studies

Determination of the levels of liver marker enzymes, bilirubin, albumin, total protein, creatinine, uric acid and urea in serum were done using relevant commercial kits.

Histopathological Examination

Specimens of liver and kidney were fixed in 10% neutral buffered formalin for 24 h, rinsed with water, dehydrated in alcohols, cleared in xylene and embedded in paraffin. Tissue blocks were cut into 5-micron sections and routinely stained with haematoxylin and eosin (H&E) stain (7) before examination under a light microscope (Olympus Microscope BX-51, connected with a Cool-Snap Prodigital camera and Image-Pro Plus image with analysis Software version 6.0). Histological sections were examined under X10 and X40 objective lenses.

Statistical Analysis

Results were presented as mean ± standard error of the mean (SE) obtained from five animals. SPSS program was used for statistical analysis of data with one–way ANOVA to compare between groups. In all the cases, a difference was considered significant when $P < 0.05$.

Results

Bioaccumulation of Lead

The lead concentrations were higher ($P < 0.05$) in

Table 1. Lead levels in the chosen organs in all experimental groups

	Kidney (μg/g)	Liver (μg/g)	Blood (μg/ml)
Group I	$0.16^{c} \pm 0.02$	$0.14^{c} \pm 0.02$	$0.12^{c} \pm 0.02$
Group II	$4.74^{a} \pm 0.21$	$3.84^{a} \pm 0.15$	$2.78^{a} \pm 0.17$
Group III	$0.18^{c} \pm 0.04$	$0.15^{c} \pm 0.02$	$0.12^{c} \pm 0.02$
Group IV	$2.60^{b} \pm 0.13$	$1.96^{b} \pm 0.10$	$1.63^{b} \pm 0.17$
F (*P*)	313.062*(<0.001*)	370.293*(<0.001*)	111.925*(<0.001*)

Groups I to IV are as defined in the text. Normally distributed data are expressed in mean ± SE and are compared using F test (ANOVA); *Post Hoc* Test (LSD) is 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 $P < 0.05$.

Table 2. Hepatic biochemical parameters in sera of all experimental groups

	ALP (U/L)	ALT (U/L)	AST (U/L)	Total protein (g/dl)	Albumin (g/dl)	Bilirubin (mg/dl)
Group I	$51.20^{c} \pm 2.52$	$39.26^{c} \pm 1.62$	$62.80^{c} \pm 2.96$	$7.55^{a} \pm 0.05$	$3.26^{a} \pm 0.8$	$0.48^{a} \pm 0.05$
Group II	$147.0^{a} \pm 9.91$	$89.25^{a} \pm 3.83$	$164.0^{a} \pm 4.44$	$5.96^{c} \pm 0.14$	$1.36^{b} \pm 0.11$	$0.87^{b} \pm 0.10$
Group III	$49.40^{c} \pm 3.87$	$43.75^{c} \pm 2.48$	$65.96^{c} \pm 0.82$	$7.52^{a} \pm 0.09$	$3.22^{a} \pm 0.14$	$0.55^{a} \pm 0.07$
Group IV	$83.80^{b} \pm 2.85$	$66.0^{b} \pm 3.65$	$126.60^{b} \pm 2.73$	$6.84^{b} \pm 0.13$	$3.14^{a} \pm 0.10$	$0.63^{c} \pm 14.08$
F (*P*)	65.149*(<0.001*)	57.565*(<0.001*)	264.446*(<0.001*)	38.835*(<0.001*)	70.957*(<0.001*)	0.994(0.421)

See footnote to Table 1.

Table 3. Renal biochemical parameters in sera of all experimental groups

	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Group I	$31.50^{c} \pm 0.50$	$0.39^{c} \pm 0.01$	$31.50^{c} \pm 0.50$
Group II	$55.92^{a} \pm 1.23$	$0.80^{a} \pm 0.02$	$55.52^{a} \pm 0.92$
Group III	$32.62^{c} \pm 0.80$	$0.39^{c} \pm 0.02$	$32.62^{c} \pm 0.80$
Group IV	$41.92^{b} \pm 0.71$	$0.63^{b} \pm 0.01$	$41.92^{b} \pm 0.71$
F (*P*)	176.210*(<0.001*)	189.234*(<0.001*)	220.514*(<0.001*)

See footnote to Table 1.

tissues of the lead group than the control group. In the lead and quercetin group, the lead concentrations were reduced ($P < 0.05$) in the selected tissues in comparison to the lead group (Table 1). The lead levels of all groups increased in the following order: kidney > liver > blood.

Biochemical Indicators of Liver Function

The activities of serum ALT (alanine transaminase), ALP (alkaline phosphatase) and AST (aspartate transaminase), and the bilirubin level were higher ($P < 0.05$) in the lead group relative to those obtained from the control group (Table 2), but the activities of ALP, ALT and AST and bilirubin level in the lead acetate and quercetin group were lower ($P < 0.05$) compared to the lead group. Also, exposure to lead acetate lowered ($P < 0.05$) the concentrations of total protein and albumin compared with the concentrations values obtained for the control group (Table 2). There was an increase ($P < 0.05$) in total protein and albumin concentrations in the lead acetate and quercetin group compared with the lead group. No changes in the studied parameters were observed among the quercetin group when compared with the control group.

Biochemical Indicators of Kidney Function

Exposure to lead acetate increased ($P < 0.05$) the levels of uric acid, urea and creatinine relative to the control. Also, there was a decrease ($P < 0.05$) in uric acid, urea and creatinine levels in the lead acetate and quercetin group compared with the lead acetate

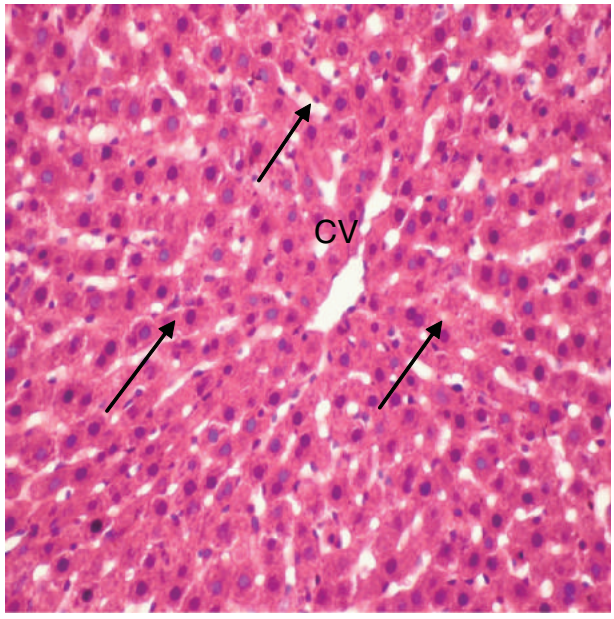

Fig. 1. Light micrograph of a control liver section of a male rat. Central vein (CV), sinusoids in between hepatocytes strands (arrows) (H&E, X10).

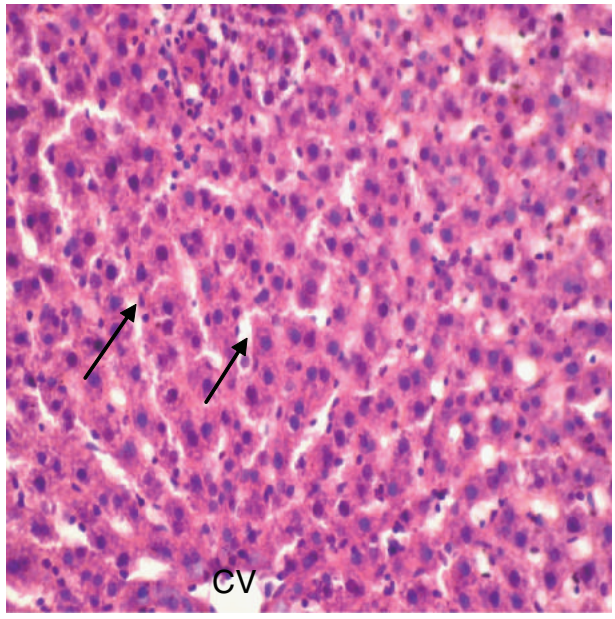

Fig. 2. A liver section of a male rat injected with quercetin for four weeks. Normal liver architecture with anastomosing blood sinusoids (arrows) and central vein (CV) is shown (H&E, X10).

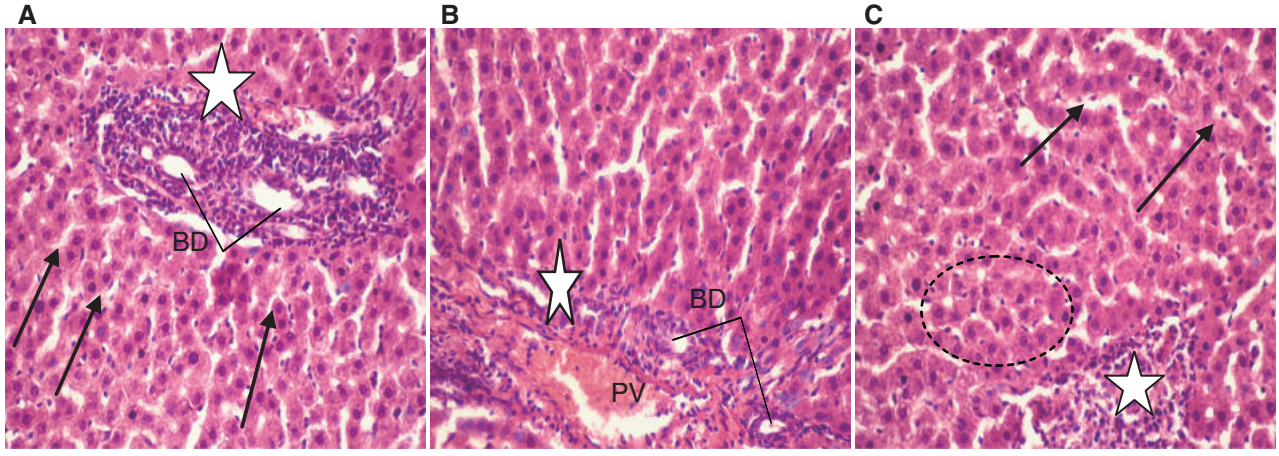

Fig. 3. A liver section of a male rat injected with lead acetate for four weeks. A: increased number of binucleated hepatocyte (arrows), cellular infiltration (star), congested portal vein (PV),bile ductule proliferation (BD) (H&E Stain, X10); B. cellular infiltration (Star) in the portal area, highly congested portal vein (PV), bile ductule proliferation (BD) (H&E Stain, X10); C. deformed liver (circle) with disappearance of normal liver architecture, shrinked blood sinusoids, increased number of Kupffer cells (arrows), focal area of mononuclear inflammatory cells (star) (H&E, X10).

group. No changes in creatinine, urea and uric acid levels were observed among the quercetin group when compared with the control (Table 3).

Liver Histopathology

Light micrographs revealed that the structural components of the liver in both the control and the quercetin groups both showed normal hepatic cytoarchitecture. They formed of polyhedral hepatocytes which were radically arranged in anatomizing and branching plates separated by vascular blood sinusoids (Figs. 1 and 2). Chronic exposure to lead acetate for 4 weeks (group II) induced alteration in the hepatic architecture including hepatocytes, the sinusoids and portal triads. The alterations in the hepatocytes involved the appearance of some hepatocytes with acidophilic cytoplasm hypereosinophilic cytoplasm, binucleation and necrosis (Fig. 3A). In addition, portal triads with chronic inflammation, biliary hyperplasia,

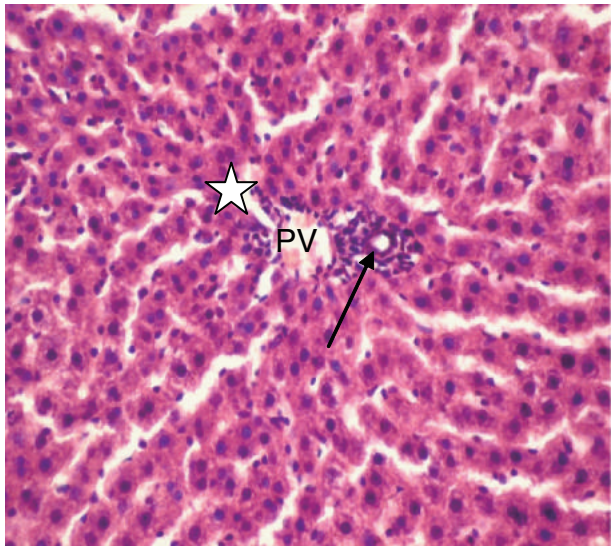

Fig. 4. A liver section of a male rat injected with lead acetate and quercetin for four weeks. Strands of hepatocytes, portal area with less congested portal vein (PV), minor cellular infiltration (*), bile ductule (arrow) (H&E, X10).

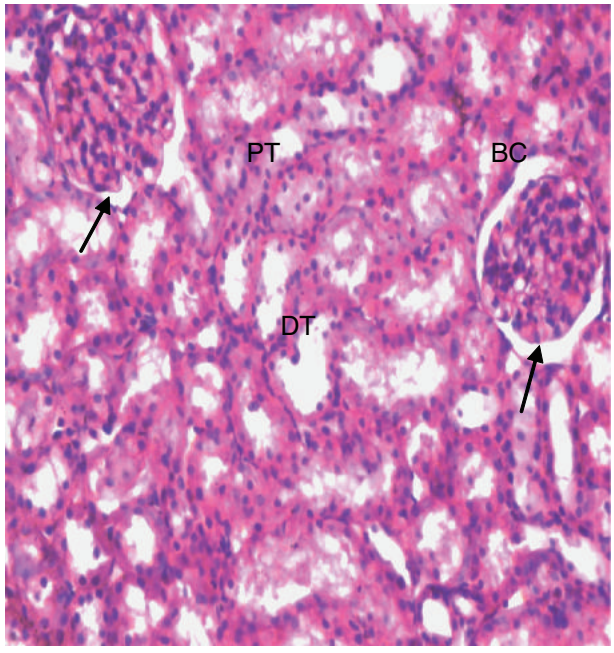

Fig. 5. A control kidney section of a male rat. Bowman's capsule (BC) with normal urinary space (arrows), renal tubules consist of proximal tubules (PT) with narrow lumen, while distal tubules (DT) possess large lumen (H&E, X40).

edema, congestion were noticed (Fig. 3, A and B). It is noticeable that the mononuclear inflammatory cells in the portal triad and different parts of the liver were characterized by the disappearance of normal liver architecture with shrunk blood sinusoids (Fig. 3, A, B and C). In sinusoids, Kupffer cells hyperplasia were seen together with hemosiderosis (Fig. 3C). Histopathological examination of liver sections of the lead acetate and quercetin group demonstrated less pathological changes in its histological structure in comparison with the lead acetate group, in which the liver appeared with strands of hepatocytes, portal area having less congested portal vein, minor cellular infiltration and organized bile ductule (Fig. 4).

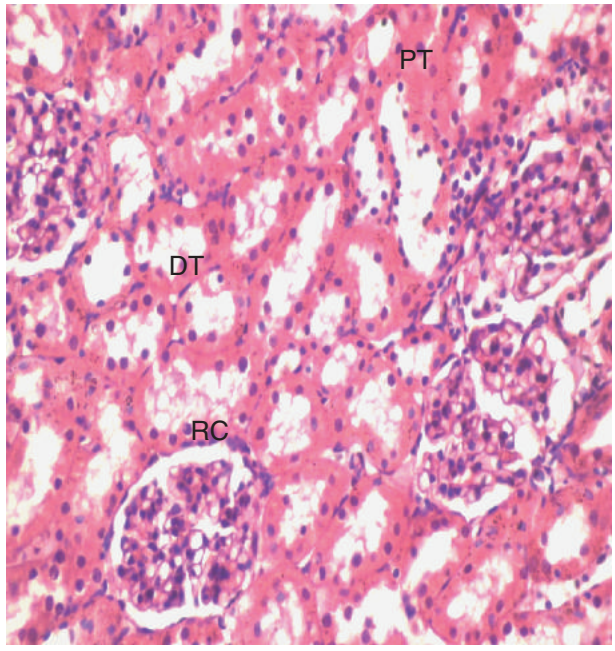

Fig. 6. A kidney section of a male rat injected with quercetin for four weeks. Well-organized renal corpuscles (RC) with normal architecture of glomeruli and urinary space. Note, normal structures of proximal tubules (PT), and distal tubule (DT) (H&E, X40).

Kidney Histopathology

The histopathological studies of the kidneys of the control and the quercetin groups revealed normal glomerulus surrounded by the Bowman's capsule, and distal and proximal convoluted tubules (Figs. 5 and 6); moreover, sections of the quercetin rats showed more vital appearance than the control rats. On the other hand, kidneys sections of the animals in the lead acetate group revealed extensive degenerative changes in tubular epithelial cells, including hyper chromatic nuclei, hyperplasia and desquamated of renal tubular epithelium leading to tubular dilation. In addition, swollen renal corpuscles with atrophy of glomerular tuft in focal areas were hyalinized with widening of urinary space (Fig. 7A). In some areas, renal tubules showed severe vacuolation (Fig. 7B) and mild fibroblast proliferation in between tubules with lysis of tubules by the end of the fourth week in all animals compared

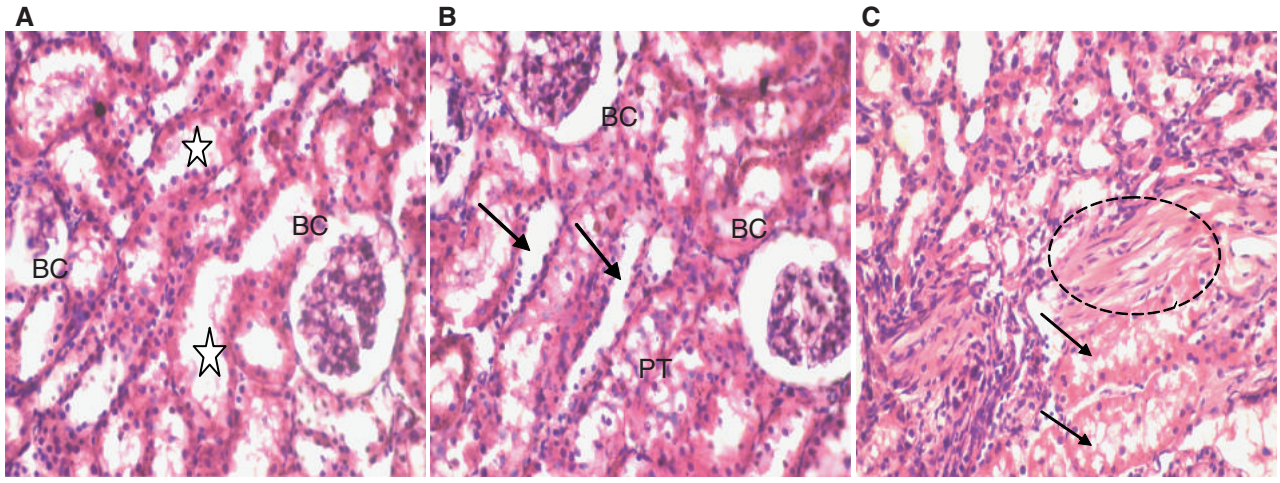

Fig. 7. A kidney section of a male rat injected with lead acetate for four weeks. A: Bowman's capsule (BC) with atrophied glomeruli and wide urinary space, desquamated renal tubular epithelium led to tubular dilation (stars) (H&E, X40); B: Bowman's capsule (BC) with wide urinary space, and vacuolated proximal tubules (PT) or with dilated lumen (arrows) (H&E, X40); C: Fibrosis in interstitial tissue (dashed circle) and highly vacuolated proximal tubules (arrows) (H&E, X40).

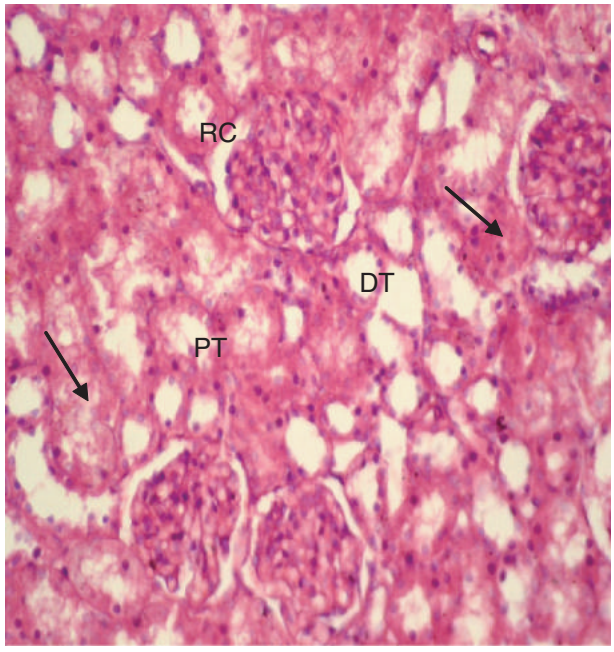

Fig. 8. A kidney section of a male rat injected with lead acetate and quercetin for four weeks. Improved renal corpuscles (RC), many improved proximal tubules (PT) and distal tubules (DT) with few tubules lumen filled with eosinophilic casts (arrows) (H&E, X40).

to the normal histological structure (Fig. 7C). In the present study, improved structure with significant decreases in the severity of histopathological changes was observed in animals treated with lead acetate and quercetin when compared with the lead acetate group. It was clear that in most areas of the lead acetate and quercetin-group, nearly normal tubules and renal corpuscles were detected but in some areas, few tubules were still affected, which had protein acious casts (Fig. 8).

Discussion

In the present study, the data showed a significant increase in concentrations of lead in the liver and kidney of rats treated with lead acetate, as were previously reported (27). The results of this study revealed that lead acetate induced liver toxicity through increase in serum liver enzymes and bilirubin but decrease in serum albumin and total protein, in agreement with results obtained by many investigators who studied the effects of lead on liver functions (4). Elevated levels of serum enzymes ALP, AST and ALT indicated organ dysfunction (36) and may be due to either loss of enzyme molecules from tissues, or defective *de novo* synthesis (35). Increase in ALP activity is a threat to the survival of cells that are dependent on phosphate esters for vital cellular processes (31).

Lipid peroxides have been shown to impair tissue membranes (22). It was also shown that lead acetate increased malondialdehyde level in hepatic tissues (2, 6). This suggests that lead acetate may act as a plasma membrane labilizer. In the current study, the group of rats treated with lead acetate followed by quercetin showed a decrease in ALP, AST and ALT enzyme activities, probably due to the ability of quercetin to inhibit lipid peroxidation (25). The reduction of lead concentrations in the studied tissues of rats treated with quercetin may be due to the chelating property of quercetin. Quercetin chelates lead by forming a coordination band with the lead ions through its orthophenolic groups located on the quercetin B ring (8). The decrease in total protein and albumin observed here also agreed with previous studies (2, 23), and could

occur by lead interference with protein synthesis or by the binding of lead to metal binding proteins and removal of such proteins through detoxification processes (37). Inclusion of quercetin in the diet improved total protein level and quantitative measurement of protein oxidation in ethanol-treated rats along with quercetin revealed that supplementations of quercetin decreased protein oxidation, or increase the protein synthesis, and thus maintaining the normal endogenous protein content (26).

In agreement with previous results (2, 4), the present study showed that exposure to lead increased the levels of serum urea, uric acid and creatinine which may indicate the presence of glomerular injury and renal dysfunction where the level of creatinine displays increase in renal disorders (12). The presence of lead may have caused brush-border impairment of the epithelial cells making them impermeable to creatinine and urea. These caused elevated levels of creatinine and urea in the blood impairing kidney functions (28).

In the present study, supplementation of quercetin in lead acetate-treated rats led to partial restoration to the normal levels of serum creatinine, urea and uric acid. The protective effects of quercetin on the liver and kidney involve enhancing antioxidant enzyme activity and decreasing pro-oxidant effects (2, 7) where lead acetate decreases antioxidant enzymes (glutathione S-transferase, superoxide dismutase and glutathione peroxide) levels and increases lipid peroxides in rats ingested lead acetate (16).

The present study showed that lead acetate treatment caused many histological alterations in the liver involving congestion of portal vein, Kupffer cells hyperplasia, focal area of inflammatory cells, shrinkage of blood sinusoids and binucleation of hepatocytes as previously reported (10, 29). The appearance of inflammatory cells in the liver on lead exposure may be due to the interaction of lead with enzymes and proteins of the hepatic interstitial tissue, which interferes with the antioxidant mechanism leading to the generation of reactive oxygen species. These may imitate an inflammatory response (20). Binucleation observed in the present study might represent a consequence of cell injury and is usually seen in regenerating cells (14). The data of the present study show that lead activates the phagocytic activity of the sinusoidal cells through increasing the number of Kupffer cells as a result of increased autophagy throughout the hepatic tissue to help in removing the accumulated lead and its metabolites. The produced Kupffer cells hyperplasia represents a defense mechanism of detoxification (18). Histopathological investigations of the liver display changes which reflect damages in hepatic tissues possibly due to cycling of heavy of heavy metal. It has been presented that heavy metals form mercaptides with the SH groups of cysteine and less stable complexes with other amino acid chain and these changes reflect damages in hepatic tissues (13).

The liver of rats treated with lead acetate followed by quercetin demonstrated marked improvement in its histological structure in comparison to the lead acetate group with less pathological changes. Similar ameliorative effects of quercetin on lead-induced changes in the liver of rats were demonstrated (23). Our histological investigation of renal tissue revealed that lead acetate treatment resulted in glomerular alterations. These alterations involved the atrophy of glomeruli and degeneration of tubular epithelial cells, in agreement with previous investigations (4, 33). The cytoplasmic degeneration observed might be due to the leakage of lysosomal hydrolytic enzymes (11).

The administration of quercetin in lead-treated rats partially restored the studied parameters to normal values and improved the structure with decreases in the severity of histopathological changes. Therefore, our results suggest that quercetin, at 50 mg/kg bodyweight, could produce modulating action on the liver and kidney of rats intoxicated with lead, suggesting the clinical possibility that quercetin may be used in therapy for human individuals subjected to lead environmental pollution.

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References

1. Abd El Raheim, A., Maged, M., Nahed, M. and Rokaya, M.A. Blood, serum glucose and renal parameters in lead-loaded Albino rats and treatment with some chelating agents and natural oils. *Turk. J. Bio.* 31: 25-34, 2007.
2. Abd El-Kader, M.A., El-Sammad, N.M. and Taha, H. The protective role of Rosemary (*Rosmatinus officinalis*) in lead acetate induced toxicity in rats. *J. Appl. Sci. Res.* 8: 3071-3082, 2012.
3. Abdel-Wahhab, M.A., Abdel-Galil, M.M., Hassan, A.M., Hassan, N.H., Nada, S.A., Saeed, A. and Elsayed, M.M. Zizphus spina-christi extract protects against alfatoxin B1-initiated hepatic carcinogenicity. *Afr. J. Tradit. Complement. Altern. Med.* 4: 248-256, 2007.
4. Abdou, H.M. and Hassan, M.A. Protective role of omega-3-polyunsaturated fatty acid against lead acetate-induced toxicity in liver and kidney of female rats. *Biomed. Res. Int.* 2014: 435857, 2014.
5. Analia, P.M., Possa, M.N., Augusto, M.C. and Francisca, L.S. Quercetin prevents oxidative stress in cirrhotic rats. *Dig. Dis. Sci.* 52: 2616-2621, 2007.
6. Attia, A.M.M., Ibrahim, F.A.A., Nabil, G.M. and Aziz, S.W. Antioxidant effects of ginger (*Zingiber officinale Roscoe*) against lead acetate-induced hepatotoxicity in rats. *Afr. J. Pharm. Pharmacol.* 7: 1213-1219, 2013.
7. Bancroft, D. and Gamble, M. The theory and practice of histo-

logical technique, 5th edition. Churchil Living Stone. pp. 75, 2002.
8. Bravo, A. and Anacona, J.R. Metal complexes of the flavonoid quercetin: antibacterial properties. *Trans. Met. Chem*. 26: 20-23, 2001.
9. Correia, P.R.M., Oliveira, E. and Oliveira, P.V. Simultaneous determination of Cd and Pb in food staffs by electrothermal atomic absorption spectrometry. *Anal. Chem. Acta* 405: 205-211, 2000.
10. Dehkordi, K.K., Dehkordi, S.K. and Dehkordi, R.A. Histopathological study of the rat liver exposed with lead acetate as a microscopic survey. *Animal Vet. Sci*. 3: 141-143, 2015.
11. Del Monte, U. Swelling of hepatocytes injured by oxidative stress suggests pathological changes related to macromolecular crowding. *Med. Hypotheses*. 64: 818-825, 2005.
12. Eraslan, G., Kanbur, M. and Silici, S. Evaluation of propolis effects on some biochemical parameters in rats treated with sodium fluoride. *Pestic. Biochem. Phys*. 88: 273-283, 2007.
13. Gajawat, S., Sancheti, G. and Goyal, P. Vitamin C against concomitant exposure to heavy metal and radiation: a study on variations in hepatic cellular counts. *Asian J. Exp. Sci*. 19: 53-58, 2005.
14. Gerlyng, P., Abyholm, A., Grotmol, T., Erikstein, B., Huitfeld, T.H.S., Stokke, T. and Seglen, P.O. Binucleation and polyploidization patterns in developmental and regenerative rat liver growth. *Cell Prolif*. 26: 557-565, 2008.
15. Gray, O.K. and Blair, M.J. Effect of diet on the response in rats to lead acetate given orally or in drinking water. *Biol. Trac. Elem. Res*. 17: 167-173, 2007.
16. Haleagrahara, N., Jackie, T., Chakravarthi, S., Rao, M. and Kulur, A. Protective effect of *Etlingera elatior* (torch ginger) extract on lead acetate-induced hepatotoxicity in rats. *J. Toxicol. Sci.* 35: 663-671, 2010.
17. Henretig, F.M. Lead In: *Gold Frank's Toxicological Emergencies,* Lewis, G., N. Lewis, Howland, M.A. and Nelson, L. (Eds.). 7th Edn., McGraw Hill Co., New York, pp. 1200-1237, 2002.
18. Ilić, S., Stojiljković, N., Veljković, M., Veljković, S. and Stojanović, G. Protective effect of quercetin on Cisplatin-induced nephrotoxicity in rats. *Med. Biol.* 16: 71-75, 2014.
19. Inal, M.E., Akgun, A. and Kahraman, A. Radioprotective effects of exogenous glutathione against whole-body gamma-ray irradiation: age- and gender-related changes in malondialdehyde levels, superoxide dismutase and catalase activities in rat liver. *Methods Find. Exp. Clin. Pharmacol*. 24: 209-212, 2002.
20. Johar, D., Roth, J.C., Bay, G.H., Walker, J.N., Kroczak, T.J. and Los, M. Inflammatory response, reactive oxygen species, programmed (necrotic-like an apoptotic) cell death and cancer. *Rocz. Akad. Med. Biolynst*. 49: 31-39, 2004.
21. Khan, M., Ahmed, M.J. and Bhanger, M.I. A simple spectrophotometric method for the determination of trace level lead in biological samples in the presence of aqueous micellar solutions. *Spectroscopy* 20: 285-297, 2006.
22. Knowles, S.O. and Donaldson, W.E. Dietary lead alters fatty acid composition and membrane peroxidation in chick liver microsomes. *Poult. Sci*. 75: 1498-1500, 1996.
23. Koriem, K.M.M. Lead toxicity and the protective role of Cupressus sempervirens seeds growing in Egypt. *Rev. Latinoamer Quim*. 37: 230-242, 2009.
24. Laughton, M.J., Evans, P.J., Moroney, M.A., Hoult, J.R. and Halliwell, B. Inhibition of mammalian 5-lipoxygenase and cyclooxygenase by flavonoids and phenolic dietary additives: relationship to antioxidant activity and to iron-reducing ability. *Biochem. Pharmacol*. 42: 1673-1681, 1991.
25. Lee, E.S., Lee, H.E., Shin, J.Y., Yoon, S. and Moon, J.O. The flavonoid quercetin inhibits demethylnitrosanine-induced liver damage in rats. *J. Pharm. Pharmacol*. 55: 1169-1174, 2003.
26. Liu, J.L., Du, J., Fan, L.L., Liu, X.Y., Gu, L. and Ge, Y.B. Effects of quercetin on hyper-proliferation of gastric mucosal cells in rats treated with chronic oral ethanol through the reactive oxygen species-nitric oxide pathway. *World J. Gastroenterol*. 14: 3242-3248, 2008.
27. Moustafa, A.A, Mohamed, T.M., Ali, E.M. and Ahmed, A.A. Effect of soybean on bone and gonad hormones in lead intoxicated rats. *J. Biol. Res*. 9: 25-34, 2008.
28. Oloyede, O.B., Adeymi, O., Sunmonu, T.O. and Bakare, A.A. The effect of polluted water on selected rat enzymes. *NISEBJ* 3: 91-97, 2003.
29. Omotoso, B.R., Abiodun, A.A., Ijomone, O.M. and Adewole, S.O. Lead-induced damage on hepatocytes and hepatic reticular fibresin rats; protective role of aqueous extract of Moringa oleifera leaves (Lam). *J. Biosci. Med*. 3: 27-35, 2015.
30. Pitot, C.H. and Dragon, P.Y. Chemical carcinogenesis. In: *Casarett and Doulls Toxicology*, 5th ed. NewYork: Mc Graw Hill, pp. 201-206, 1996.
31. Sangai, N.P. and Verma, R.J. Quercetin ameliorates Bisphenol-A induced toxicity in mice. *Acta Pol. Pharm*. 69: 557-563, 2012.
32. Shaban, M.G., Mostafa, M.S., Hassouna, M.M., El-Nabi, S.E. and El-Refaie, A. Amelioration of lead toxicity on rat liver with vitamin C and silymatin supplements. *Toxicology* 206: 1-15, 2005.
33. Sharma, S. and Singh, B. Lead acetate induce histopathological alterations in renal tissue of Balb-C mice. (Mus musculus). *Int. J. Appl. Biol. Pharmaceut. Technol*. 5: 23, 2014.
34. Takahama, U. Scavenging of active oxygen by flavonoids. *Protein Nucleic Acid, Enzyme* 33: 2994-2999, 1988.
35. Umezawa, H. and Hooper, I.R. Aminoglycoside antibiotics. Springer-verlag, Berlin, Heidelberg, New York, 1982.
36. Wells, R.M., Mclntyre, R.H., Morgan, A.K. and Davie, P.S. Physiological stress responses in big gamefish after capture: observations on plasma chemistry and blood factors. *Comp. Biochem. Physiol. A. Comp. Physiol*. 84: 565-571, 1986.
37. Yousef, M.I. Aluminum-induced changes in hemato-biochemical parameters, lipid peroxidation and enzymes activities of male rabbits. Protective role of ascorbic acid. *Toxicology* 199: 47-57, 2004.