The Egyptian Journal of Hospital Medicine (October 2018) Vol. 73 (10), Page 7650-7658

The Possible Protective Role of Vitamin C against Toxicity Induced by Lead Acetate in Liver and Spleen of Adult Albino Rats (Light and Electron Microscopic Study)

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ABSTRACT

Background: lead toxicity has been recognized as a major environmental health hazard worldwide affecting both humans and animals at all ages especially young children in humans. Lead does not have any beneficial biological effects to humans and its presence at high concentrations produce very undesirable toxic consequences to humans affecting all the body organs. Ascorbic acid is probably the most widely studied vitamin when it helps to prevent lead induced oxidative stress. Its property of quenching ROS along with metal chelation makes it a potential detoxifying agent for lead.

Aim of work: this study aimed to detect the possible protective effect of vitamin C against toxicity induced by lead acetate in liver and spleen of adult albino rats by light and electron microscope.

Material and Methods: 40 adult male albino rats were used in this work. They were categorized into four groups each group was consisted of ten rats as follows: group I (Control group): The rats received 1ml 0.9% sodium chloride orally every day for 28 days. Group II: rats received vitamin C in a dose of 27 mg/day orally every day for 28 days. Group III: rats received lead acetate in a dose of 10.8 mg/kg; orally every day for 28 days. C group IV: rats received vitamin C in a dose of 27 mg of one hour prior to administration of 10.8 mg/kg of lead acetate orally every day for 28 days. Finally, on the 29th day, the rats were anesthetized with ether and their abdomens were opened and their livers and spleens were excised and divided to small slices and prepared for light and electron microscopic examination.

Results: results of the present study revealed that administration lead acetate to rats produced harmful effects on the rat's liver and spleen; showed distortion of liver architecture with marked vacuolar degeneration of the swollen hepatocytes with cytoplasmic vaculations and condensed pyknotic nuclei. Central vein was dilated and congested, some of blood sinusoids were obliterated and others showed congestion and hemorrhage. Portal tract also showed congestion of the portal vein with mononuclear cellular infiltration in the portal tract area. Collagen deposition was detected around the central vein, between the cords of hepatocytes and in the blood sinusoids. Portal tracts expanded by thick collagen fibers also. And spleen showed distorted splenic architecture with massive hemorrhagic areas in the red pulps and highly reduced white pulps (diffusion of white pulp into the red pulp) and marked degeneration in the lymphocytes with necrotic foci with marked collagen deposition around the splenic arterioles and in the red pulps.

and these effects relatively improved by administration of vitamin C. Sections were examined by light microscopic examination.

Conclusion: lead acetate caused histological changes in liver and spleen of adult albino rats most probably through oxidative stress. Vitamin C therapy could ameliorate these changes in liver and spleen and this may be attributed to its antioxidant and free radicals scavenging properties. This may indicate the effectiveness of vitamin C in prevention of lead acetate toxicity on liver and spleen

Keywords: lead acetate; vitamin C; liver; spleen.

INTRODUCTION

Lead, a non-physiological heavy metal, is one of the first metals used by man. Its wide application was begun since over 8000 years ago [(1)]. The absorbed lead is conjugated in the liver and passed to the kidneys, where part of it is excreted in the urine and the rest of absorbed lead accumulates in various body organs, affecting many biological functions at the molecular, cellular and intercellular levels [(2)]. Ascorbic acid is well known for its antioxidant activity, acting as a reducing agent to reverse oxidation in liquids. When there are more free radicals (ROS) in the human body than antioxidants, the condition is called oxidative stress [(3)].

The present work aimed to evaluate the protective effect of vitamin C against toxicity induced by lead acetate on liver and spleen of adult albino rats.

MATERIAL AND METHODS

Forty adult male albino rats weighting 200-250 g were used. The rats were randomly categorized into four groups each group formed of ten rats:

1- The control group (I): rats were received 1 ml normal saline orally.

Received:7/9/2018
Accepted:26/9/2018

2- The vitamin C group (II): rats were received vitamin C in a dose of 27 mg/day orally.
3- The lead acetate group (III): rats were received lead acetate orally in a dose of 10.8 mg/kg of lead acetate; every day for 28 days. 4-The lead acetate and vitamin C group (IV): rats were received 27 mg of vitamin C one hour prior to administration of 10.8 mg/kg of lead acetate orally.

The experimental duration was 28 days, livers and spleens were dissected on the 29 day and examined by light and electron microscopes [(4)].

RESULTS

The results of the present study revealed that administration lead acetate to rats produced harmful effects on the rat's liver and spleen and these effects relatively improved by administration of vitamin C. Light microscopic examination of the liver sections from the control group showed classical hepatic architecture.

Hepatocytes are arranged in cords or plates radiating from the central vein separated by blood sinusoids containing Von Kupffur cells and portal tract in periphery of the lobule with minimal collagen deposition around the central vein indicate its basement membrane and minimally in the blood sinusoids and in the periportal area.

While, light microscopic examination of the spleen sections from the control group showed red pulp formed of cords of blood elements and blood sinusoids, the white pulps containing central or follicular arteriole, C.T trabeculae extend from the capsule into the substance of the spleen, thin collagen fibres which are supporting the wall of central arterioles and fine strands in the white and red pulps.

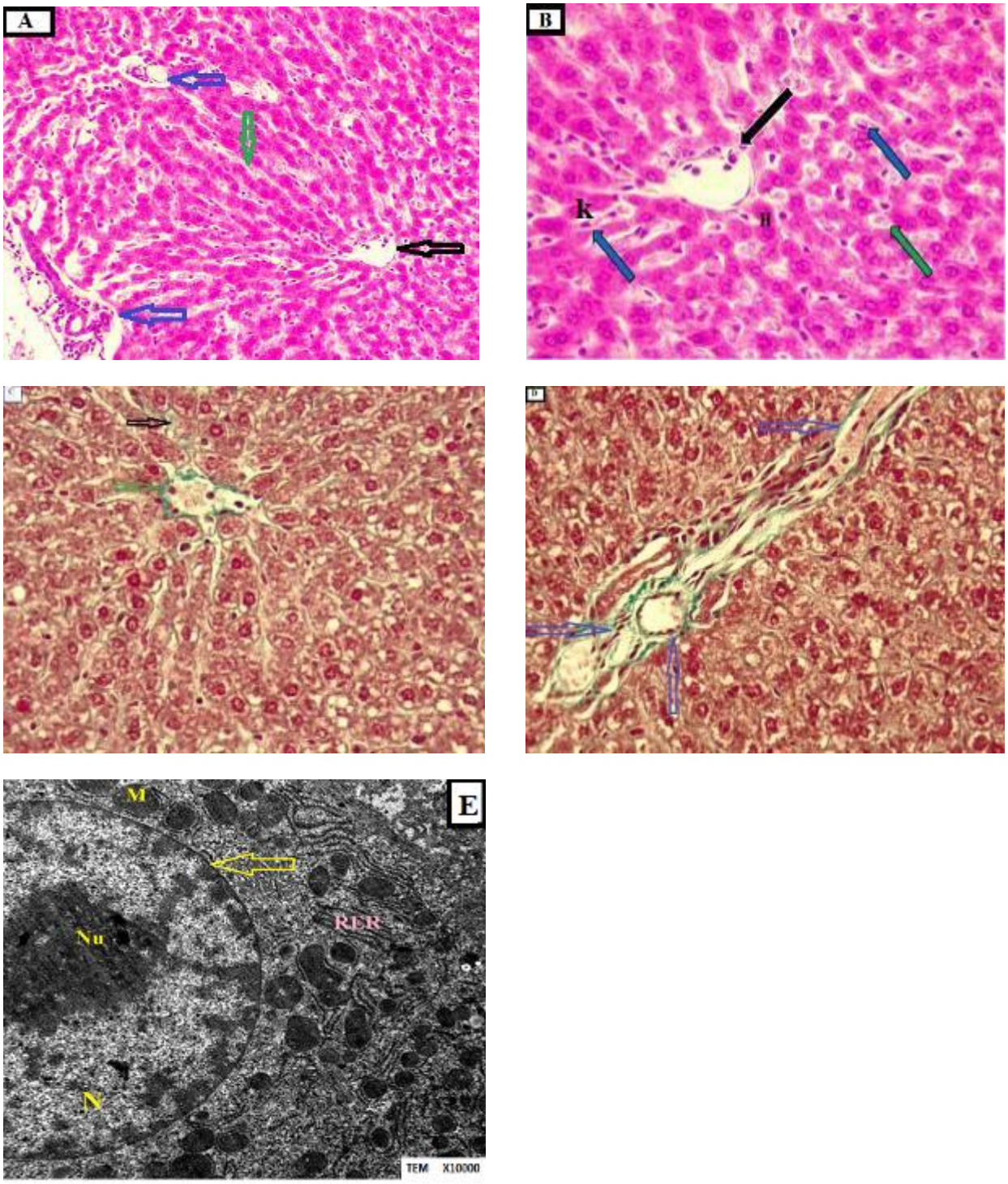
The same findings were appeared in the light microscopic examination of liver and spleen sections from group II which received vitamin C only. While, the light microscopic examination of the liver sections of group III which comprised rats treated with lead acetate only showed distortion of liver architecture with marked vacuolar degeneration of the swollen hepatocytes with cytoplasmic vacuolations and condensed pyknotic nuclei. Central vein was dilated and congested, some of blood sinusoids were obliterated and others showed congestion and hemorrhage. Portal tract also showed congestion of the portal vein with mononuclear cellular infiltration in the portal tract area. Collagen deposition was detected around the central vein, between the cords of hepatocytes and in the blood sinusoids. Portal tracts expanded by thick collagen fibers also.

Light microscopic examination of the spleen sections of group III which included rats treated with lead acetate only showed distorted splenic architecture with massive hemorrhagic areas in the red pulps and highly reduced white pulps (diffusion of white pulp into the red pulp) and marked degeneration in the lymphocytes with necrotic foci with marked collagen deposition around the splenic arterioles and in the red pulps.

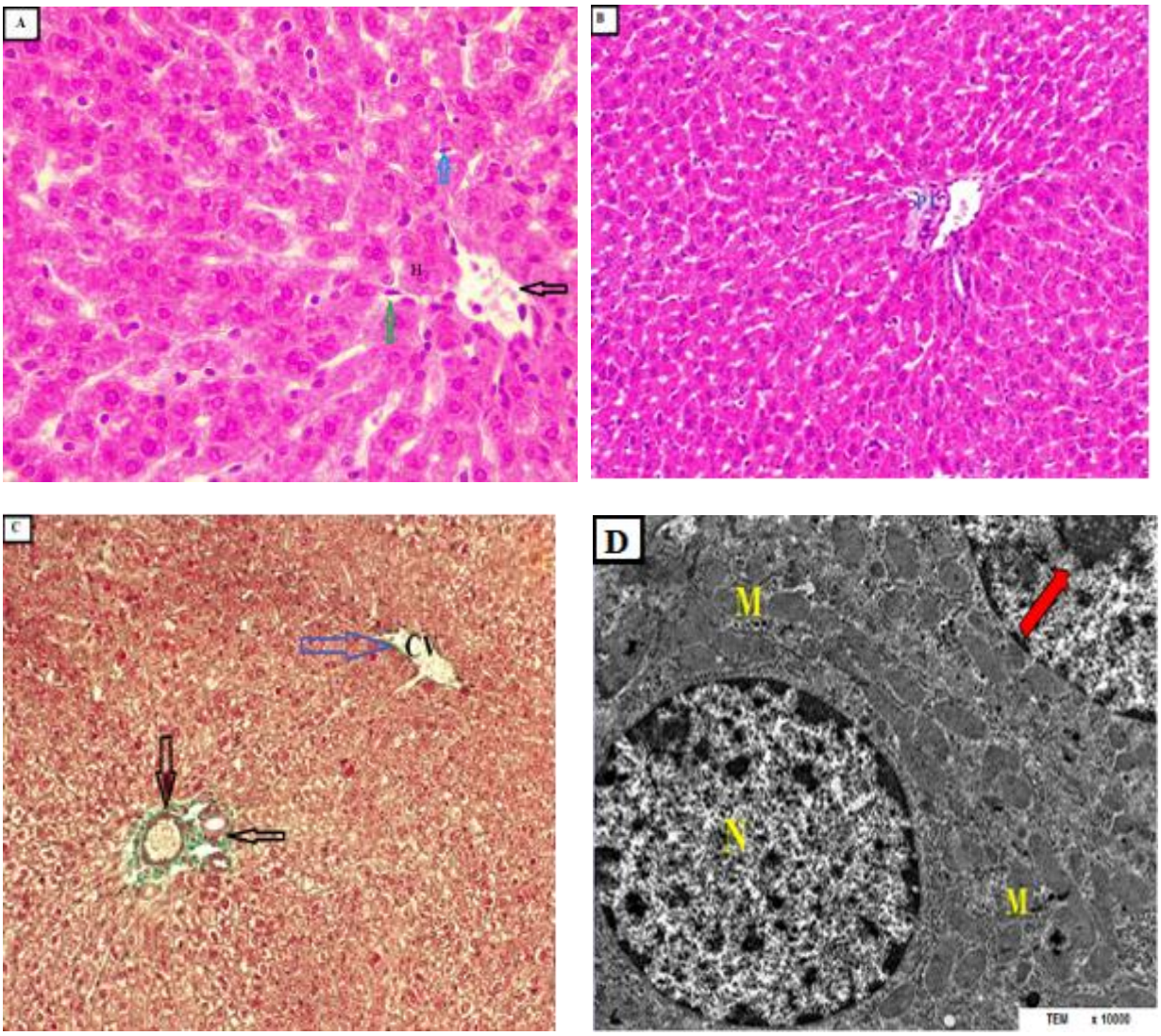
The changes occurred in the liver and spleen of the rats of group III which were administrated lead acetate only nearly not found in the liver and spleen of group IV of the rats treated with vitamin C before lead acetate. Examination of liver sections of the group IV by light microscope showed restoration of liver architecture; hepatocytes were arranged in cords and some of them appeared healthy, but the others showed vaculations. Few congested blood sinusoids were seen and showed minimal collagen distribution in portal tract and in the walls of central veins compared to lead acetate group.

The light microscopic examination of the spleen sections of group IV showed restoration of splenic architecture in the form of reappearance of white pulps with lymphatic nodules around the central arterioles and red pulps with blood sinuses appeared healthy with C.T. trabeculae and some necrotic foci areas were noticed and showed minimal collagen distribution in fibrous septa compared to lead acetate group.

LIVER

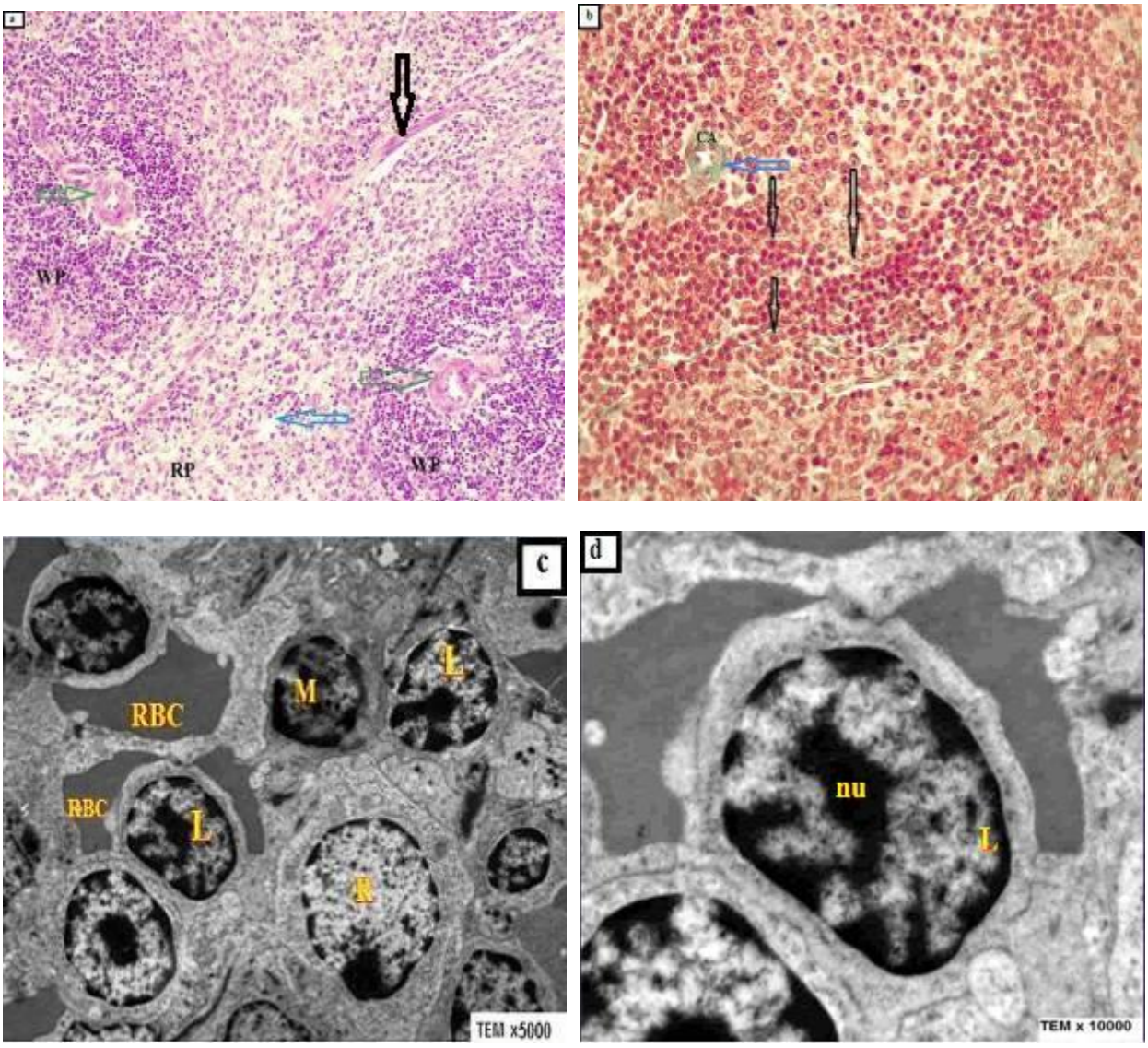


Group I: photomicrographic sections from the liver of adult albino rats, control group **A-** showing classical hepatic architecture. Hepatocytes are arranged in cords or plates radiating from the central vein (black arrow) separated by blood sinusoids (green arrow) and portal tract (blue arrows) in the periphery of the lobule **(Hx & E x 200).**
B- Higher magnification of **A**. Hepatocytes (H) are arranged in cords radiating from central vein (black arrow) separated by blood sinusoids (green arrow) containing Von Kupffur cells (k) (blue arrows) **(Hx & E x400).**
C- Showing deposition of collagen fibers (greenish color) in the basement membrane of central vein (CV) (green arrow) and very thin radiating fibers between the hepatic cords (black arrow) **(Masson trichrome stain x400).**
D- Showing thin layer of periportal greenish color indicates collagenous fibers (blue arrows) **(Masson Trichrome stain x400) E-** An electron photomicrograph of a liver section of group I showing: a hepatocyte with rounded nucleus (N), prominent nucleolus (Nu) with regular intact nuclear membrane (yellow arrow). The cytoplasm contains many normal mitochondria (M) and Rough Endoplasmic Reticulum (RER) **(TEM x10000).**

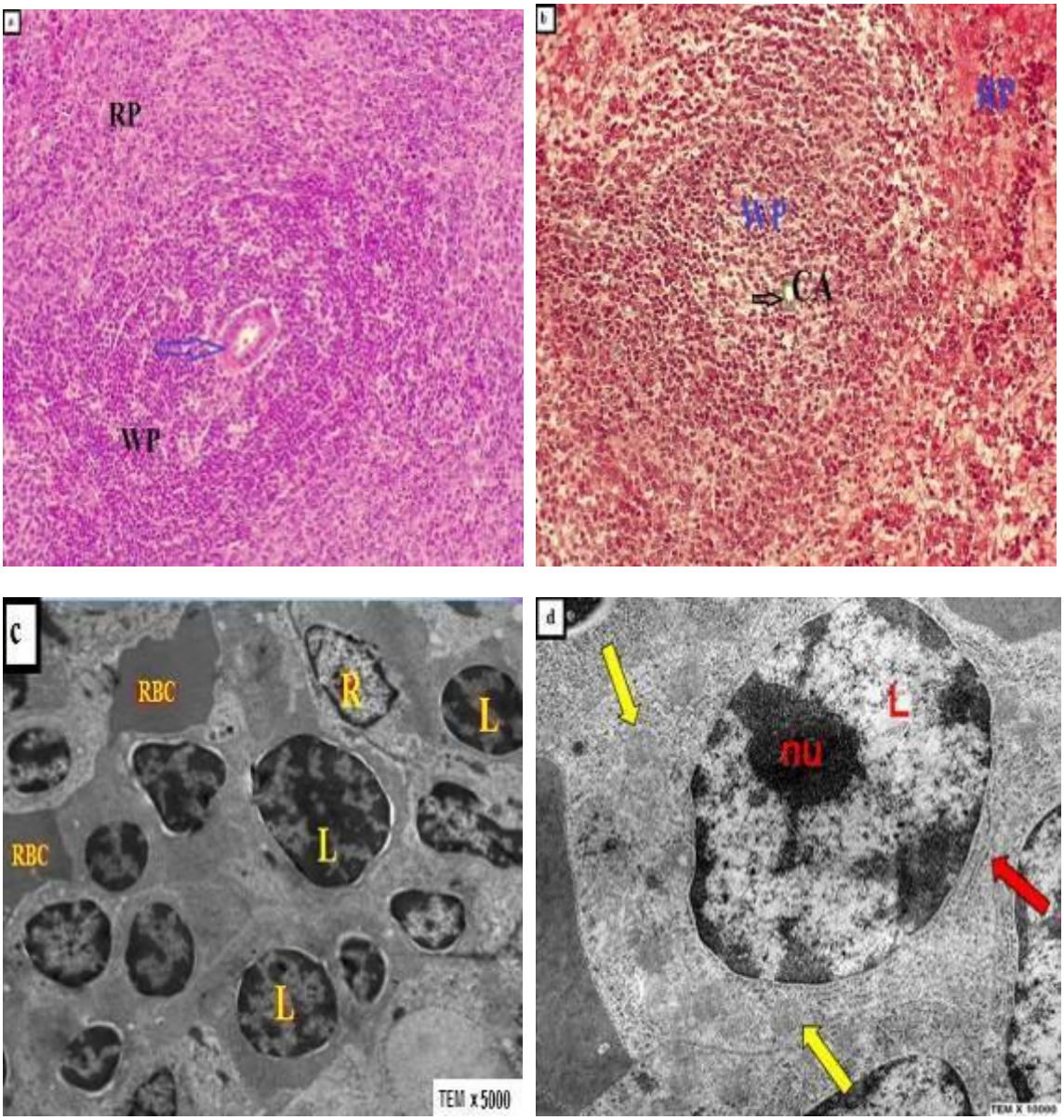


Group II: photomicrographic sections from the liver of adult albino rats, vitamin C group **A-** showing hepatocytes (H) are arranged in cords radiating from central vein (black arrow) separated by blood sinusoids (green arrow) containing Von Kupffur cells (blue arrow) **(Hx & E x400). B-** Showing: hepatocytes are arranged in cords separated by blood sinusoids and portal tract (PT) is observed clearly **(Hx & E x200). C-** showing: normal thin layer of periportal greenish color indicates collagenous fibers (black arrows) and also in wall of the central vein (CV) (blue arrow) **(Masson trichrome stain x200). D-** An electron photomicrograph of a liver section of group II showing: two adjacent hepatocytes with euchromatic nuclus (N) and prominent nucleolus (red arrow). Mitochondria (M) appear rounded or oval in shape **(TEM x10000).**

SPLEEN



Group I: photomicrographic sections from the spleen of adult albino rats, conrol group **a-** showing normal structure; background red pulp (RP) formed of cords of blood elements, blood sinusoids (blue arrow) and septa of C.T trabeculae (black arrow). Two white pulps (WP) containing central or follicular arteriole (green arrows) are present **(Hx & E x400). b-** Showing distribution of collagen fibers in wall of the central arterioles (CA) (blue arrow) and fine strands in the white and red pulps (black arrows) **(Masson trichrom stain x400). c-** Showing an electron micrograph spleen from the group I showing the cellular components of the white and red pulps. They contain lymphocytes (L) macrophage (M), reticular cells (R) and red blood corpuscles (RBC) **(TEM x5000). d-** Showing lymphocyte (L) with regular nuclear cell membrane and heterochromatic nucleus with prominent nucleolus (nu) **(TEM x10000).**



Group II: photomicrographic sections from the spleen of adult albino rats, viamin C group **a-** showing white pulp (WP) formed of collection of small lymphocytes containing central or follicular arteriole (blue arrow) and the surrounding structure is the red pulp (RP) **(Hx & E x200).b-** Showing normal distribution of collagen fibers in wall of the central arteriole (CA) **(Masson trichrom stain x200). c-** Showing an electron micrograph spleen from the group (II) showing the cellular components of the white and red pulps. The white pulp contains lymphocytes (L) and reticular cells (R) with red blood corpuscles (RBC) **(TEM x5000). d-** Showing the lymphocyte (L) with prominent nucleolus (nu), normal RER (red arrow) and normal mitochondria in the cytoplasm (yellow arrows) **(TEM x10000).**

DISCUSSION

Many heavy metals, including lead, are known to induce over production of reactive oxygen species and consequently enhance lipid peroxidation, decrease the saturated fatty acids and increase the unsaturated fatty acid contents of the membranes, which become a hindrance in membrane transport [(5)]. In addition, ROS are highly reactive to membrane lipids, protein and DNA and are believed to be the major contributing factors to stress injuries and lead to rapid cellular damage [(6)].

Based on the observations that free radical was generated during the pathogenesis processes induced by lead exposure, it was presumed that supplementation of antioxidants could be an alternative method for chelation therapy. Specifically, ascorbic acid, the known chelating agent with antioxidant features, was widely reported with the capability of protecting cells from oxidative stress [(7)]. The findings of our study are in agreement with results of [(8)] they stated that exposure of rats to lead acetate caused hepatotoxicity characterized by engorgement of blood vessels along with sinusoidal hemorrhage, infiltration, dilatation of central veins and vacuolar degeneration of hepatocytes. According to the results of [(9)] exposure to lead acetate causes

liver tissue toxicity by causing histological lesions, including necrosis, inflammation and hemorrhage. These alterations may be due to lipid peroxidation induced by the excessive production of free radicals under the influence of exposure to lead. In the present study, lead seemed to have accumulated in the liver tissue causing severe alterations characterized by congested and dilated portal veins and degeneration in hepatic cells. Most of the orally ingested lead is excreted, but a portion is absorbed and is transferred to the blood where it binds to hemoglobin in the erythrocytes. Lead is carried through the circulatory system by erythrocytes, virtually to all tissues in the body particularly hematopoietic and immune system [(10)]. The liver of treated albino rats showed dilated and congested central vein and sinusoids that indicator for liver alterations. Similar observations reported by [(11)]. The lymphocytic infiltration observed in this study following lead treatment show evidence of cell irritability, inflammation and hypersensitivity to the lead [(12)].

Our study showed collagen deposition around the central vein, between the cords of hepatocytes and in the blood sinusoids. Portal tracts expanded by thick collagen fibers by masson trichrome stain; these go in hand with results of **Hegazy and Fouad [(13)].**

Liver fibrosis represents the final common pathway of almost all types of chronic liver diseases characterized by excessive connective tissue deposition in extracellular matrix (ECM). ROS can activate fibrogenic gene expression and transforming growth factor (TGF-β1), which is known to play a major role in the activation of hepatic stellate cells (HSCs) in liver fibrosis [(14)].

These ultrastruractural alterations due to lead intoxication which involved mitochondria, RER, and lysosomes have been shown to have a strong affinity for mitochondria and are usually associated with changes in the parenchymal cells [(15)]. The destruction and decreased number of tubular arrays of the RER and mitochondrial- RER associates can be considered as an indication of impaired protein synthesis in the affected hepatocytes. The demonstrated shrunken dark hepatocytes may represent a form of degenerated hepatocytes. The appearance of these cells may indicate an evidence of single cell necrosis (apoptosis) or shrinkage necrosis as a consequence of lead toxicity. Shrinkage necrosis is considered as an expression of programmed cell death in response to pathological changes [(16)].

Some of the pleomorphism alterations seen in the present study were in agreement with **Jarrar** [(17)] who reported that apoptotic alteration might be followed by organelles swelling especially the mitochondria, endoplasmic reticulum and rupture of lysosomes which might lead to amorphous eosinophilic cytoplasm as an initial sign in the sequence of hepatocytes necrosis before shrinking and dissolution of nuclei**. Aldahmash and El-nager** [(4)] found that vitamin C before oral administration of lead acetate, maintained the normal histological structure of liver. However, some necrotic foci were present. There are many proofs that vitamin C had hepatoprotective effects against many hazardous chemicals as carbon tetrachloride, paracetomol and malathion induced hepatotoxicity, it is also as well effective against radiation induced [(18)]. In addition, **Hamadouche *et al.*** [(19)] reported that vitamin C could reduce hepatotoxicity induced by lead acetate and diminished most of histopathological changes induced by lead. All previous findings run in full agreement with the results of this study, when rats received (VC) before lead treatment, it might be due to the ability of vitamin C to chelate ROS by reducing oxidative stress and related complications.

The mechanism by which vitamin C decreases the hepatotoxicity induced by toxic agents is embodied in the fact that vitamin C might ameliorate the oxidative damage by decreasing lipid peroxidation and altering the antioxidant defense system or by denoting electrons to free radicals and quenching their reactivity [(20)]. In addition, it prevents hepatic glutathione depletion in chemical induced hepatotoxicity in rats, in which glutathione acted as intracellular free-radical scavengers and protected cells against radical mediated lipid peroxidation [(21)]. It was reported that lipid peroxides stimulated hepatic deposition of type I collagen in cultured human hepatic stellate cells [(22)]. This suggestion established a possible link between the lipid peroxidation (i.e. oxidative stress) and hepatic fibrosis. Studies demonstrating the beneficial effect of vitamin C supplementation in prevention of both enhancement of lipid peroxidation and synthesis of type I collagen might support this suggestion [(23)].

Aldahmash and El-nager [(4)] found that Spleen of mice treated with lead acetate showed marked changes represented by distorted spleen architecture, that it was ill-defined due to diffusion of white pulp into the red pulp in addition to appearance of necrotic foci; large macrophages were seen in the tissue with great number and hemorrhage. Also, **Sary *et al.*** [(24)] reported that the histopathological changes induced by lead in the spleen indicated exhaustion of lymphoid elements in the splenic pulp, necrosis of lymphocyte in the white pulp, vacuolar degeneration in tunica media of central artery, exhaustion of lymphoid elements with hemorrhage in the red pulp and necrosis and hemorrhage at the red pulp.

Aous *et al.* [(25)] reported the histopathological changes of spleen tissues showing depletion of lymphoid tissue which become more severe at the end of the experiment and that can be related to the effect of lead acetate which cause depression in immune system. Similar results were obtained by **Al- Naimi *et al.*** [(26)] who reported that the experimental findings of his research demonstrate the ability of lead acetate to cause vascular changes in the splenic arterioles and that agreed with the previous studies which concluded that lead causes endothelial injury

/dysfunction, hemorrhage, impede endothelial repair, reduce endothelial cells growth and there is a positive association between lead exposure and peripheral arterial diseases [27].

Mohamed and Nabela [28] showed some splenic white pulps of spleen suffered from lymphoid depletion and the others became hyperplastic with proliferation of megakaryocytes, beside hemosiderosis in red and white pulps, beside thickened splenic trabeculae in lead-treated rats.

Teijon *et al.* [29] also showed that spleen of lead-treated rats revealed an increase in the number of lymphocytes as well as edema, an expansion of lymphatic sinuses of red and white pulps, cell aggregation constituted by lymphocytes and specific cells of the red pulp which form a lattice.

Oxidative stress has been proposed to be a principle mechanism involved in lead toxicity. Lead exposure disturbs the pro-oxidant-antioxidant balance [30]. So, the histopathological changes observed in the current study as a result of lead treatment could be attributed to the formation of free radical damage through the generation of ROS and direct depletion of antioxidant reserves.

In the spleen, phagocytes (macrophages and polymorphonuclear cells) are responsible for slowing the propagation of an invading pathogen, while an antigen-specific adaptive immune response (antibody- or cell-mediated) is being established. Lead was reported to inhibit macrophage function possibly by overloading macrophages with cellular debris and inhibiting macrophage production of nitric oxide. In the context of adaptive humoral and cellular immune responses, lead increased both B-cell and T-cell in vitro proliferation [31].

Obidike *et al.* [32] found that samples of the spleen from lead acetate treated rats were also reported to show basophilic granular degeneration of erythrocytes, necrosis and perivascular cuffing by fibrous tissue. These pathological lesions were increased in magnitude with increase in the dose of the lead.

Ekanem *et al.* [1] found that there was evidence of haemolysis and congestion of blood vessels which overshadowed the red pulp. These reactions were more prominent in the rats that received the high dose of lead acetate.

The microscopic changes in the spleen of the lead treated rats suggested immune alterations and splenic damage. The histomorphological alterations in the spleen of rats exposed to lead acetate, suggested that exposure of the animals to lead (particularly high doses) stimulated the spleen to become reactive. Thus, the impact on human and animal health from the immune perspective, following environmental lead exposure, is of great health implications [35].

The same our results reported by EM examination also detected by **Mesure *et al.*** [33] they showed that red pulp of a rat in the lead acetate exposure group, venous sinusoids filled with erythrocytes, macrophages, and lymphoid cells located at neighboring sites, vacuolation in the cytoplasm of some cells were seen. Vacuolation in mitochondria also was seen.

Our results comply with earlier studies conducted on rats [34]. Vacuole formation of various sizes was also noted in our study. The number of vacuoles increased with lead exposure time. This finding also complies with studies of Deveci [35]. Nuclear changes clearly indicated cellular death. On the other hand, we could not clarify whether cellular death was due to lead exposure or if it was a result of normal cellular activity. It was probable that lead had played a role in cellular death [35].

Cytopathologic alterations and mitochondrial distortions observed during the study would inevitably impair cellular functions. Similar degenerative findings in mitochondria have been described by **Mesure *et al.*** [33]. Consequences of damaged oxidative phosphorylation on spleen functions and the immune system need further histochemical and immunological studies.

The retcular cell synthesizes type III collagen and uses it to produce retcular fibers. Our propositon is that lead acetate may interfere in the functioning of retcular cells and synthesize abnormal type III collagen to produce defective reticular fibers [35].

Aldahmash and El-nagar [4] reported that, spleen sections of mice received vitamin C before treatment with lead acetate showed relative improvement when compared to control sections manifested by well-defined sections, healthy lymphoid follicle was abundant, moreover other section showed scattered necrotic foci besides normal sized macrophages.

Sary *et al.* [24] reported that vitamin C plus vitamin E showed marked improvement in rat spleen, compared to lead acetate treated group.

The study carried out by **Deveci** [35] revealed the ameliorative effects of ascorbic acid on the spleen of experimental animals induced with mercury chloride toxicity.

Study has demonstrated that TGF-B is unregulated in the presence of fibrosis. Vitamin C has been shown in both animal and human studies to decrease TGF-B levels after treatment [24].

CONCLUSION

Lead acetate causes histological changes in liver and spleen of adult albino rats most probably through oxidative stress. Vitamin C therapy could ameliorate these changes in liver and spleen and this may be attributed to its antioxidant and free radicals scavenging properties. This may indicate the effectiveness of vitamin C in prevention of lead acetate toxicity on liver and spleen.

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