The effect of ascorbic acid on histopathological, biochemical, pharmacological, and immunological toxicity of chronic lead acetate exposure on the spleen in a rat model

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Objective
To evaluate the effect of vitamin C on histopathological, biochemical, and immunotoxicity of chronic lead exposure in the spleen of a rat model.
Methods
The rats were divided into five groups of 10 rats each: group I received normal saline orally as a control group; groups II and III received lead acetate for 4 and 8 weeks, respectively; and groups IV and V received lead acetate and vitamin C for 4 and 8 weeks, respectively. The spleen was excised and processed for light, electron microscopic, histopathological, and biochemical analyses. Quantitative assessments of matrix metalloproteinase-2 (MMP-2), MMP-9, interleukin-2 (IL-2), IL-6, and tumor necrosis factor-alpha gene expressions were performed by real-time PCR.
Results
The examination of control and vitamin C with lead acetate supplemented groups revealed normal splenic architecture. In contrast, the spleen of lead-intoxicated groups exhibited degenerative changes in the spleen, with a significantly decreased expression of IL-2, glutathione peroxidase, superoxide dismutase, and hemoglobin ($P<0.05$), with significantly increased proinflammatory cytokine (IL-6 and tumor necrosis factor-alpha) expressions, concomitantly with increased oxidative products (malondialdehyde) and protease enzymes (MMP-2 and MMP-9) in the spleen tissues. The coadministration of vitamin C with lead for 4 weeks markedly resolved these changes.
Conclusion
This study may specify the efficiency of vitamin C in lead toxicity prevention in the spleen, represented by the reduced splenic harmful changes produced by lead administration.

Keywords:
histopathological, immunotoxicity, lead acetate, spleen, vitamin C

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Introduction

Heavy metal pollution represents a significant environmental problem owing to its toxic effect and accumulation throughout the food chain, leading to severe biological and health problems [1]. Lead acetate has been distinguished as an accidental poisoning source [2]. The WHO has published a list of 10 chemicals that concern human health, such as lead. Additionally, the United States Agency for Toxic Substances and Disease Registry ranked lead as second on the priority list of dangerous substances [3]. Lead poisoning affects children on a massive scale, according to a report launched by United National International Children's Emergency Fund. The report states that approximately one in three children have blood lead levels at or above 5 μg/dl in up to 800 million globally, thereby requiring action [4]. The manufacturing of pigments, batteries, and ceramic and industries associated with mining contribute to increased exposure to metallic lead and lead salts. Additionally, ingestion and inhalation denote the primary ways of contact [5]. After lead absorption, it is carried through the circulatory system by erythrocytes and is distributed and collected in soft tissues, bones, blood, liver, and kidney [6,7]. Lead facilities poisonous effects on numerous tissues such as the hematopoietic, immune, digestive, hepatic, central nervous, and renal systems [8]. Lead can induce anemia by hemoglobin synthesis inhibition.

Lead produces oxidative damage and causes hyperproduction of free radicals and decreased availability of antioxidant reserves. The imbalance of oxidants/antioxidants in the body was the chief toxic mechanism of lead-induced organ poisoning. It also changes calcium homeostasis and interrupts structural protein synthesis. Lead has been associated with several cancer forms in humans [9]. Acute and chronic lead exposure may increase the incidence of infectious diseases (could lower host resistance to bacterial and viral infections), allergy, and autoimmunity (might produce immunogenic self-peptides) [10]. Lead might inhibit the macrophage function and induce dysregulation of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1α (IL-1α), and IL-6, and the synthesis of T helper 1 cytokines (interferon-γ and IL-2). Lead exposure enhances B-cell production, activates responses mediated by T helper 2 cells, and encourages antibody production, including autoantibodies [11,12] Ascorbic acid (vitamin C) is structurally related to glucose and is absorbed from the gut. Its property of metal chelation creates a possible detoxifying agent for lead [10]. Ascorbic acid has good antioxidant activity and also facilitates lead excretion from the body [5]. Environmental pollution and occupational contact to lead still denote a thoughtful problem for the health of humans, and overall pathophysiologic hostile nature of lead continues to be a great challenge. Therefore, this study aimed to assess the influence of vitamin C on histopathological, biochemical, and immunological toxicity of chronic lead exposure in the spleen of a rat model.

Materials and methods

The present study aims and procedures were permitted by the institutional Ethics Committee (Fayoum University Ethics committee), with NO: R233.

Animals and experimental design

This study used 50 adult male albino rats weighing 180–220 g. Mice were obtained from the animal house, Faculty of Medicine, Cairo University. The rats were preserved under standard environmental and laboratory conditions with standard rat food in distinct cages. Rats were randomly divided into five groups, of 10 rats each. The first group served as the control group and received saline orally. The second group included rats that received oral administration of lead acetate (50 mg/kg) once daily and were killed after 4 weeks. The third group included rats that received oral administration of lead acetate (50 mg/kg) once daily and were killed after 8 weeks. The fourth group included rats that received oral lead acetate (50 mg/kg) and vitamin C (500 mg/kg) once daily and were killed after 4 weeks. The fifth group included rats that received oral lead acetate (50 mg/kg) and vitamin C (500 mg/kg) once daily and were killed after 8 weeks [13,14].

Chemicals

Lead acetate was purchased from Sigma-Aldrich Chemical Co. (St Louis, Missouri, USA) and was administered to animals in their drinking water at 50 mg/kg for 4 and 8 weeks [13].

Vitamin C was supplied by Sigma-Aldrich Chemical Co..

Vitamin C salt was orally received by animals in their drinking water at 500 mg/kg once daily [14,15] for 4 and 8 weeks.

Methods

The spleen was excised and processed for the following studies:

(1) Tissue preparations for quantitative assessments of matrix metalloproteinase-2 (MMP-2), MMP-9, IL-2, IL-6, and TNF-α gene expression by real-time PCR.

Total RNA was extracted from spleen tissue homogenate using the Qiagen extraction kit (Qiagen, Valencia, California, USA) following the manufacturer's instructions. RNA concentrations and purity were assessed by a Nanodrop ND-2000 spectrophotometer (Thermo Scientific Inc., Waltham, Massachusetts, USA) and kept at −80°C. Reverse transcription of RNA was carried out using QuantiTect reverse transcription kit (Qiagen) as described in the manufacturer's protocol. The expression of target genes was done by RT-thermal cycler (MJ Research Inc., Watertown, Massachusetts, USA) with a FastStart DNA Master SYBR Green I kit (Roche Diagnostics, Indianapolis, Indiana, USA) in a total volume of 20 µl comprising 2 µl of cDNA, 10 µl of SYBER Green PCR Master Mix, and 1 µl of each primer following the manufacturer's protocol. B-actin was used as an internal control for data normalization. The steps of cycling were as follows: 95°C for 4 min; 35 cycles of 95°C for 30 S, 55°C for 30 S, and 72°C for 30 S; and a final elongation step was performed at 72°C for 10 min. Melting curves were implemented to emphasize specificity of PCR products. The expression of studied genes was calculated by the $2^{-\Delta Ct}$ method.

(1) Histopathological study
   (a) Light microscopic study using hematoxylin, eosin stain, and Masson's trichrome stain.
   (b) Electron microscopic examination.
   (c) Histochemistry of Perl's ferric iron.
(2) Measurement of blood hemoglobin: it was assessed as previously studied by Okonkwo *et al*. [16], using Sysmex KX-21N Haematology Analyzer. One milliliter of blood samples was drained into test tubes that contain EDTA, and the blood hemoglobin was measured.
(3) Biochemical analysis: antioxidant enzymes, that is, glutathione peroxidase (GPX) and superoxide dismutase (SOD), and lipid peroxidation marker, that is, malondialdehyde (MDA), were measured in spleen macrophages [17]:

The spleen sections were homogenized in a potassium phosphate buffer (50 mM, pH 7.4). The homogenate was divided into two portions. The first portion was centrifuged at 700*g* for 20 min to determine the levels of MDA. The second portion was centrifuged at 8500*g* for 30 min to determine the activity of SOD and GPX in the supernatant. Spleen MDA concentration was measured with a BIOXYTECH MDA-586TM Assay kit. SOD and GPX activities were analyzed following the kit's instructions, with the BIOXYTECH SOD-525TM and BIOXYTECH GSP-340TM Assay kits (OXIS International Inc., Portland, Oregon, USA).

Statistical analysis

The data were arranged and statistically analyzed using the Statistical Package for the Social Sciences statistical software computer package, version 22 (SPSS Inc., Chicago, Illinois, USA). The mean and SD were calculated. One-way analysis of variance was used to test the difference in mean values of measured variables among groups, and multiple comparisons between pairs of groups were performed using a post-hoc test. Results were considered significant at P values less than or equal to 0.05.

Results

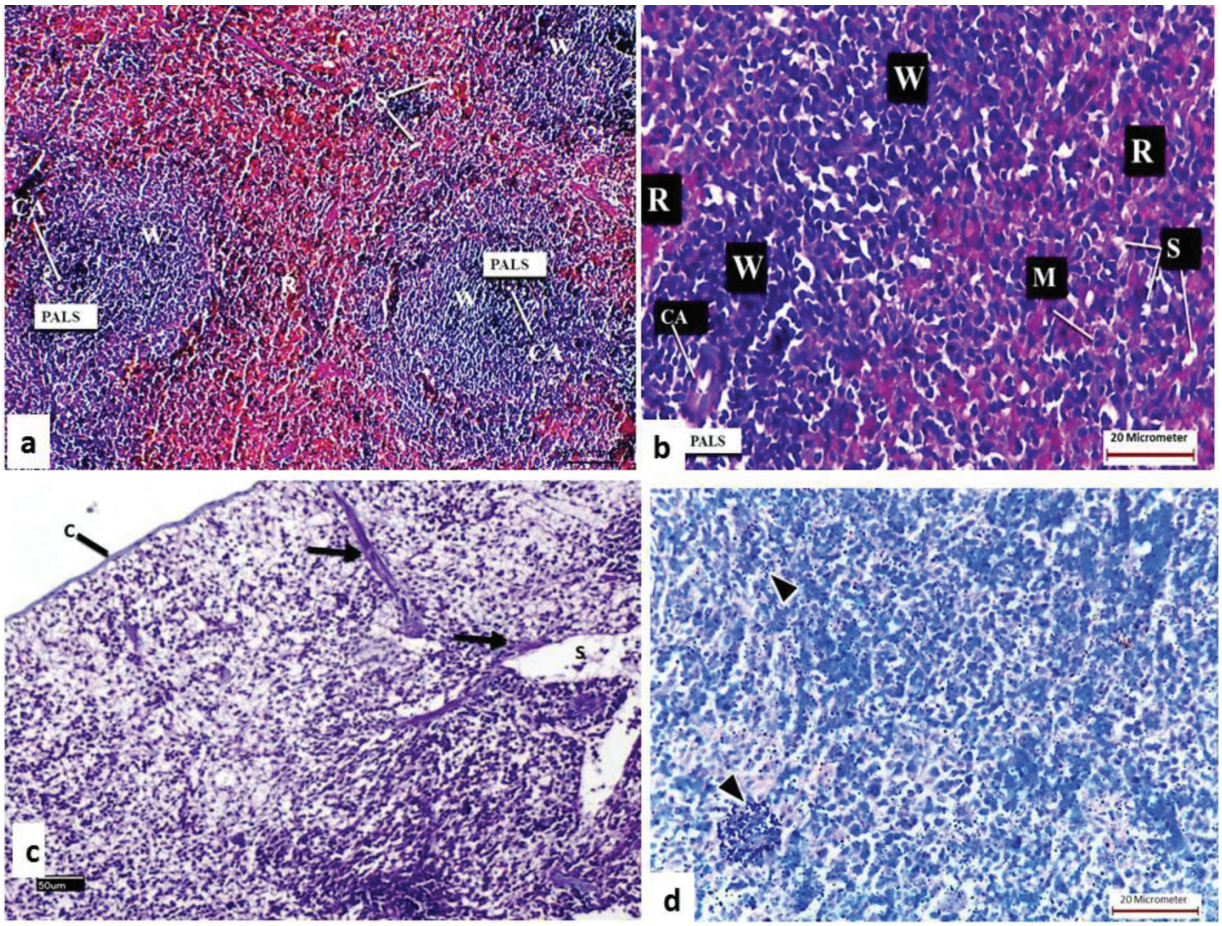
The effect of vitamin C on the histopathological alternation on lead-induced rats

Histopathological examination of the lead acetate administration groups for 4 and 8 weeks revealed variable and marked signs of degeneration in the spleen tissue compared with the control group. Contrarily, cotreatment with vitamin C for 4 and 8 weeks reduced these degenerative changes as follows: in the spleen specimens of the control animals (group I) stained with hematoxylin and eosin stain, normal splenic architecture in the form of white pulps containing lymphocytes, central arteriole, and periarterioral lymphatic sheath was shown. The red pulps contained cellular cords and blood sinusoids, and the macrophage could be observed. Masson's trichrome-stained sections displayed normal collagen fiber deposition in connective tissue trabeculae. Small-sized isolated blue granules and fine deposits in reticuloendothelial cells were displayed in Prussian blue-stained sections (Fig. 1a–d). Electron microscopic examination of the same group revealed lymphocytes containing a heterochromatic nucleus with peripheral condensation of chromatin material forming a rim, a prominent nucleolus and intact nuclear envelope, intact mitochondria with intact cristae, and parallel cisterns of rough endoplasmic reticulum (Fig. 6a).

The examination revealed notable degenerative changes represented by wide red pulps, congested sinusoids, degenerated white pulps, hemorrhage in the splenic parenchyma, degenerated lymphocytes with cytoplasmic vacuolation and pyknotic nuclei, spindle-shaped fibroblasts and large phagocytic cells, and a moderate increase in collagen fiber deposition in connective tissue trabeculae and around blood sinusoids with moderate thickness of fibrous capsule by Masson's trichrome stain in the lead-intoxicated group for 4 weeks (group II). Multiple fine deposits were seen in reticuloendothelial cells and around blood sinusoids in Prussian blue stain (Fig. 2a–d). Electron microscopic examination revealed lymphocytes with a heterochromatic nucleus with less margination of chromatin material and prominent nucleoli and, cytoplasmic vacuolation, and the nuclear envelope was indented by inclusion bodies and a ballooned mitochondria with damaged cristae. A red blood cell (RBC) could be observed (Fig. 6b). Additionally, macrophages with a heterochromatic nucleus, a prominent nucleolus, intact mitochondria with intact cristae, inclusion bodies, and electron dense lysosomes were detected. An RBC can be observed (Fig. 7a).

Additionally, the histopathological examination of spleen specimens revealed markedly disturbed splenic architecture, thickened splenic capsule, degenerated lymphocytes with cytoplasmic vacuolation and pyknotic nuclei, hyperplasia of lymphocytes in white pulps and large phagocytic cells, marked increase in collagen fiber deposition in connective tissue trabeculae and around blood sinusoids, and pronounced reaction in the form of frequent fine deposits in reticuloendothelial cells, around blood sinusoids and along connective tissue trabeculae in the lead-intoxicated group for 8 weeks (group III)

Figure 1



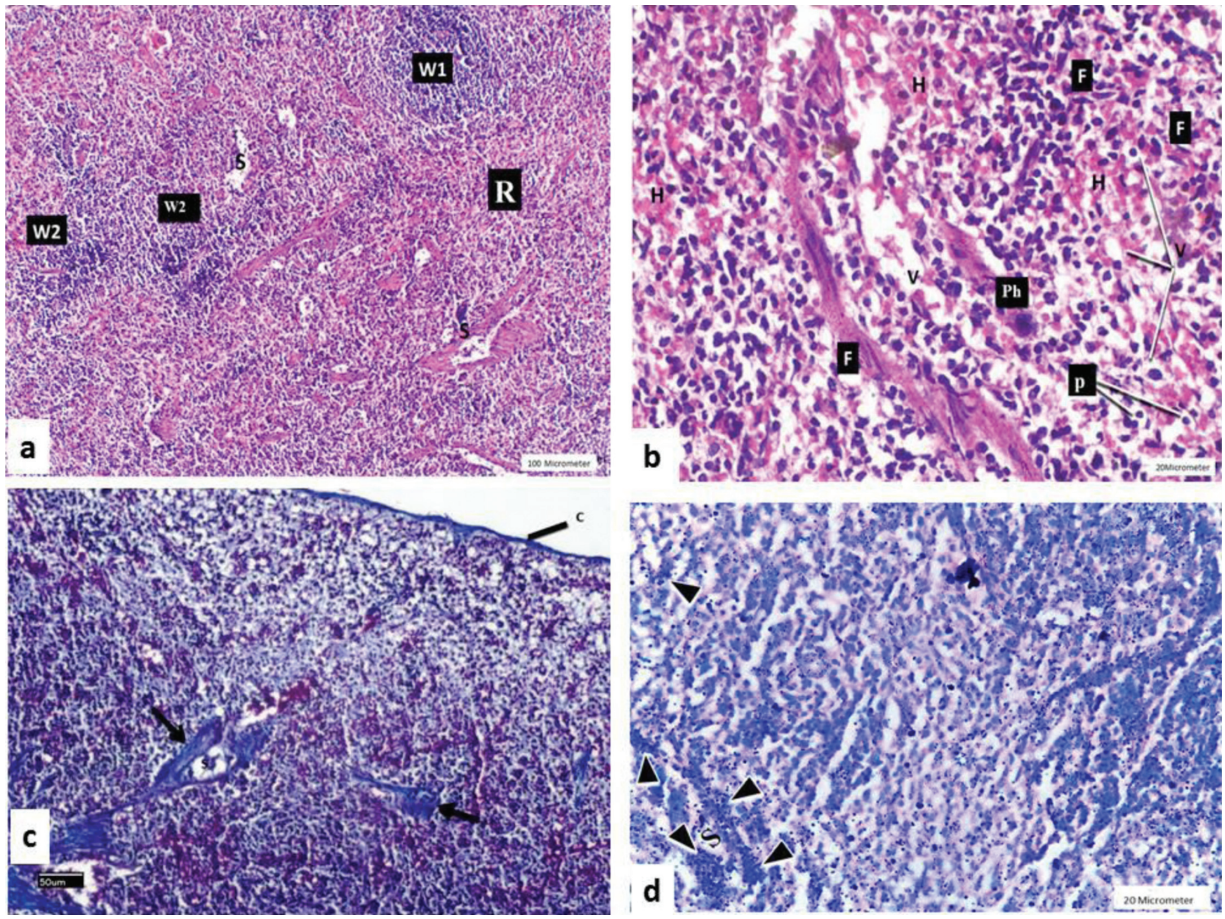
A photomicrograph of a section of a rat spleen from group I (normal control) (a H&E, ×100): showing normal architecture of the spleen; white pulps (W) consisting of lymphocytes, central arteriole (CA), and periarterioral lymphatic sheath (PALS), and the red pulps (R) consisting of cellular cords and blood sinusoids (S). (b H&E, ×400): higher magnification showing a macrophage (M) in the red pulp. (c Masson, ×200): displaying normal collagen fiber deposition (arrow) in connective tissue trabeculae and around blood sinusoids (S) with thin fibrous capsule (C). (d Prussian, ×400): displaying normal Prussian blue reaction (arrowhead) in the form of small sized isolated blue granules and fine deposits in reticuloendothelial cells.

(Fig. 3a–d). The electron microscopic examination revealed degenerated white pulp lymphocytes with extremely karyolitic nuclei, absent cytoplasmic organelle, absent margination of chromatin material with nuclear protrusion, a prominent nucleolus, indented nuclear envelope, and ballooned mitochondria with damaged cristae. Additionally, macrophages with a heterochromatic nucleus, intact mitochondria with intact cristae, inclusion bodies, and pseudopodia were found (Figs 6c, d, 7b).

Contrastingly, cotreatment with vitamin C reduced the degenerative changes as follows. The examination showed normal splenic architecture; white pulps with central arteriole and peri-arteriolar lymphatic sheath and red pulps, minimal collagen fiber deposition in a thin fibrous capsule, connective tissue trabeculae and around blood sinusoids, normal reaction in the form of small-sized isolated blue granules and fine deposits in reticuloendothelial cells in rats treated with lead and vitamin C for 4 weeks (group IV) (Fig. 4a–d). Electron microscopic examination revealed: a normal lymphocyte with a heterochromatic nucleus with peripheral condensation of chromatin material forming a rim, a prominent nucleolus, and intact nuclear envelope. The nucleus was surrounded by a thin rim of cytoplasm containing intact mitochondria with intact cristae (Fig. 6e).

Rats treated with lead and vitamin C for 8 weeks (group V) showed wide red pulps, congested blood sinusoid, normal white pulps, degenerated lymphocytes with cytoplasmic vacuolation, some spindle-shape fibroblasts, areas of hemorrhage and moderately thickened fibrous capsule, a moderate increase in collagen fiber deposition in connective tissue trabeculae and around blood sinusoids with

Figure 2



A photomicrograph of a section of a rat spleen from group II (lead administration 4 weeks) (a H&E, ×100): showing apparently normal white pulps (W1), wide red pulps (R) in between degenerated white pulps (W2) and congested sinusoid (S). (b H&E, ×400): showing hemorrhage in splenic parenchyma (H), degenerated lymphocytes with cytoplasmic vacuolation (v), pyknotic nuclei (p), spindle-shaped fibroblasts (F) and large phagocytic cells (Ph). (c Masson, ×200): displaying moderate increase in collagen fiber deposition (arrow) in connective tissue trabeculae, around blood sinusoids (S) and moderate thickness of fibrous capsule (C). (d Prussian, ×400): displaying moderate Prussian blue reaction (arrowhead) in the form of fine deposits in reticuloendothelial cells and around blood sinusoids (S).

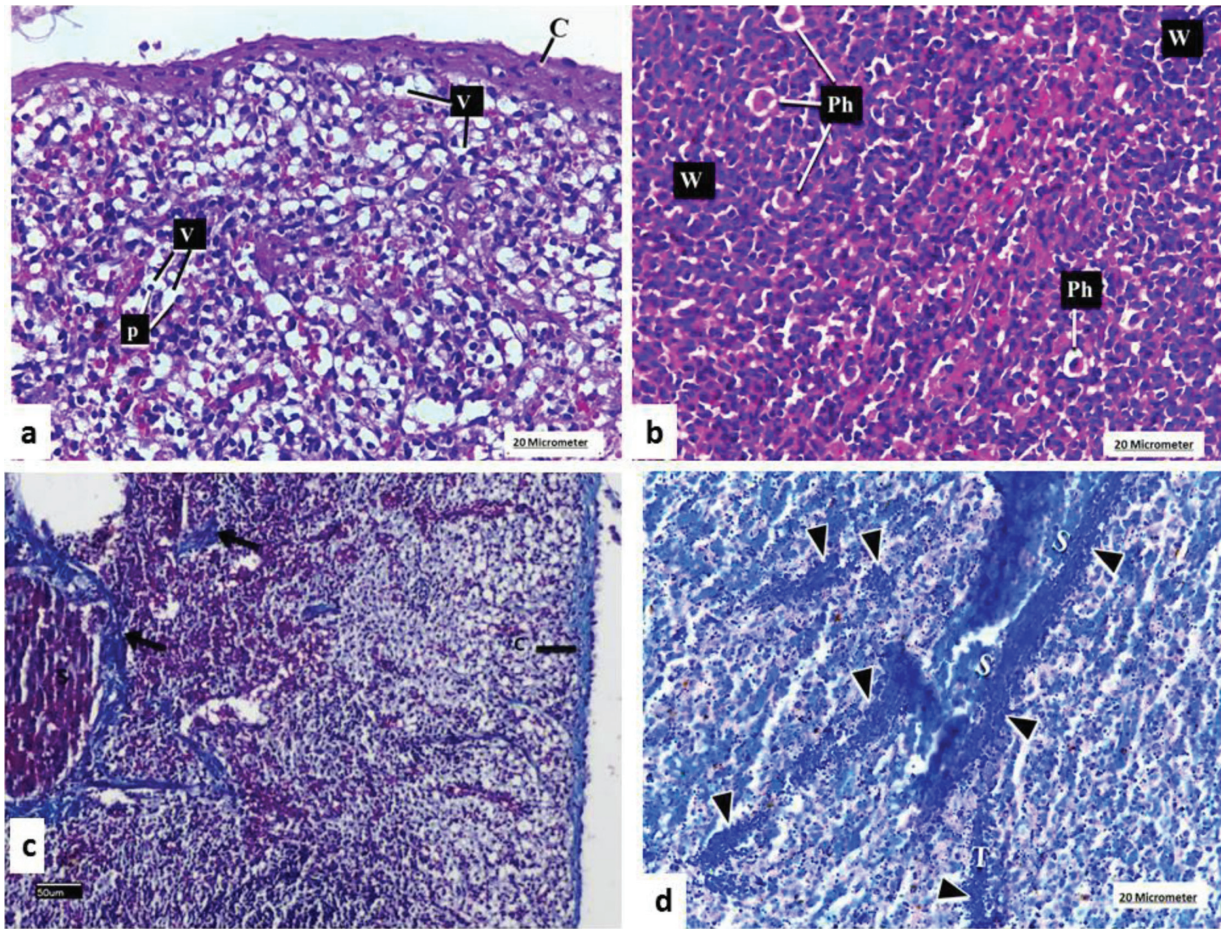
moderate thickness of fibrous capsule, moderate reaction in the form of small-sized isolated blue granules, and frequent fine deposits in reticuloendothelial cells and along connective tissue trabeculae (Fig. 5a–d). Electron microscopic revealed lymphocyte with a heterochromatic nucleus with peripheral condensation of chromatin material forming a rim, a prominent nucleolus, and intact nuclear envelope. The nucleus is surrounded by a thin rim of cytoplasm containing intact mitochondria with intact cristae and parallel cisterns of rough endoplasmic reticulum (Fig. 6f). However, the examination of other spleen specimens of the same group revealed lymphocytes with a heterochromatic nucleus, deeply indented nuclear envelope, and some ballooned mitochondria with damaged cristae. Macrophages with a heterochromatic nucleus, a prominent nucleolus, deeply indented nuclear envelope, intact mitochondria with intact cristae, inclusion bodies, and cisterns of rough endoplasmic reticulum were observed (Figs 6g, Fig. 77c).

Moreover, our results found a statistically significantly increased in collagen area (%) in lead-intoxicated groups (groups II and III) when compared with the control group (P=0.008, and P<0.0001, respectively). However, there was a statistically significant decrease in collagen area (%) between the cotreatment group by lead and vitamin C (group V) and the lead-intoxicated group (group III) for 8 weeks (P<0.0001) (Fig. 8).

The effect of vitamin C on the levels of interleukin-2, interleukin-6, and tumor necrosis factor-alpha in the lead-induced groups

Lead significantly promoted proinflammatory cytokines (IL-6 and TNF-α) in the spleen tissues concomitantly with decreases in IL-2. The coadministration of vitamin C with lead for 4 weeks

Figure 3



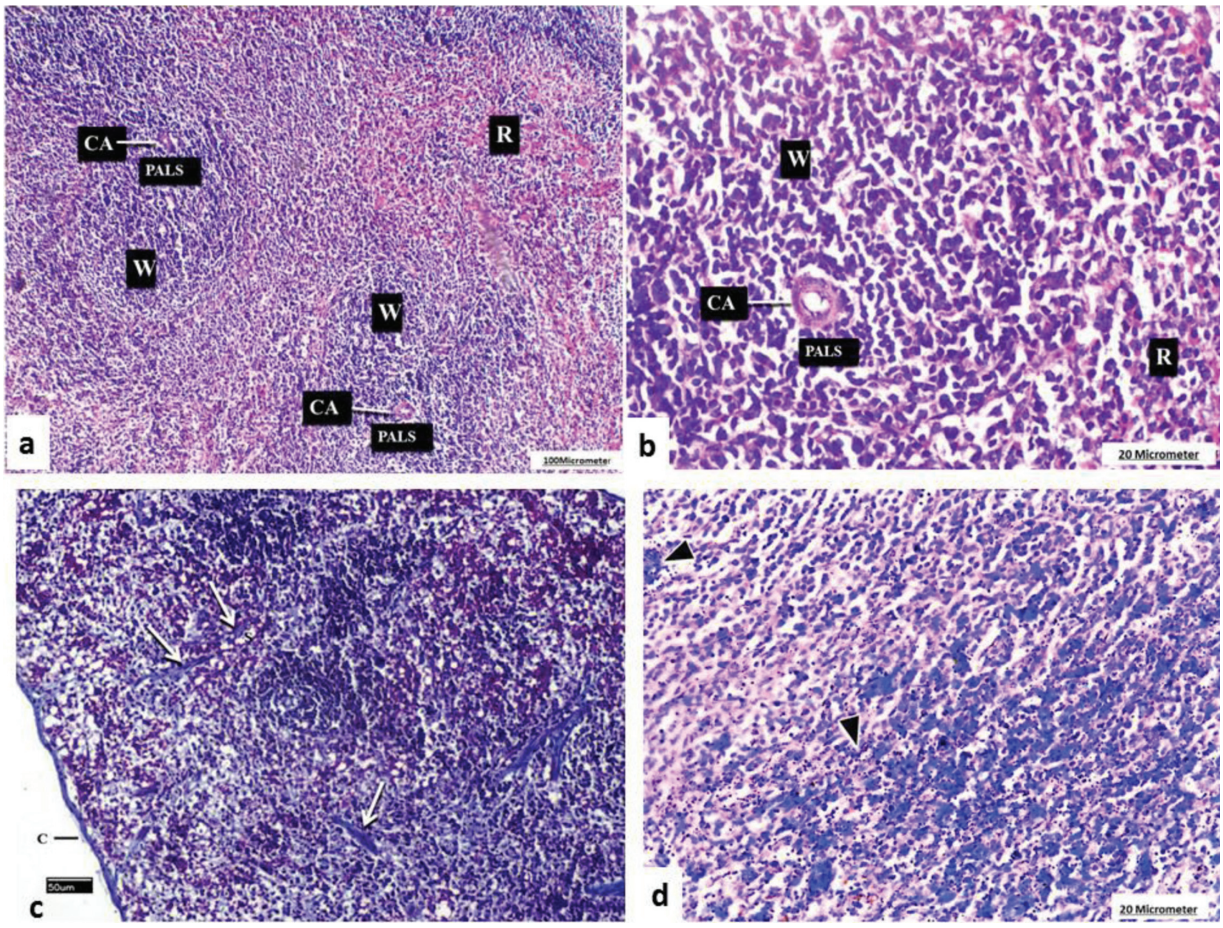
A photomicrograph of a section of a rat spleen from group III (lead administration 8 weeks) (a H&E, ×400): showing disturbed splenic architecture, markedly thickened splenic capsule (C), extremely degenerated lymphocytes with cytoplasmic vacuolation (v) and pyknotic nuclei (p). (b H&E, ×400): showing: hyperplasia of lymphocytes in white pulps (W) and large phagocytic cells (Ph). (c Masson, ×200): displaying marked increase in collagen fiber deposition (arrow) in connective tissue trabeculae and around blood sinusoids (S). (d Prussian, ×400): displaying pronounced Prussian blue reaction (arrowhead) in the form of frequent fine deposits in reticuloendothelial cells, around blood sinusoids (S) and along connective tissue trabecuale (T).

markedly resolve these changes. IL-2 mRNA concentrations were statistically significantly decreased ($P<0.05$) in groups II, III, and V compare with the controls. Additionally, a statistically significant difference was found between groups IV and V ($P=0.001$). Moreover, mRNA concentrations of IL-6 and TNF-α were statistically significantly greater ($P<0.05$) in groups II, III, and V as compared with the control group (Fig. 9b–d).

The effect of vitamin C on oxidative biomarkers of lead-induced rats

Our results showed that lead significantly decreased the antioxidative markers (GPX and SOD) and concomitantly increased lipid peroxidation marker (MDA) and proteases enzymes (MMP-2 and MMP-9) in the spleen tissues. The coadministration of vitamin C with lead markedly diminished splenic injuries compared with controls. Compared with control, SOD and GPX were statistically significantly lower in groups II, III, IV, and V ($P<0.05$), whereas statistically significantly higher in groups IV and V compared with group III ($P<0.0001$). MDA in groups II, III, IV, and V were statistically significantly increased compared with the control group ($P<0.0001$, $P<0.0001$, $P=0.033$, and $P<0.0001$, respectively). Additionally, it was statistically significantly decreased ($P<0.05$) in groups IV and V compared with group III (Fig. 10). The present study also found that MMP-2 and MMP-9 were statistically significantly higher ($P<0.05$) in groups II, III, IV, and V, whereas statistically significantly lower ($P<0.05$) in group IV compared with group III (Fig. 11). Additionally, hemoglobin was statistically significantly lower in groups II ($P<0.0001$), III ($P<0.0001$), IV ($P=0.001$), and V

Figure 4



A photomicrograph of a section of a rat spleen from group IV (lead administration and vitamin C 4 weeks) (a H&E, ×100) showing apparently normal splenic architecture; white pulps (W) with central arteriole (CA), and peri-arteriolar lymphatic sheath (PALS) and red pulps (R). (b H&E, ×400): higher magnification showing apparently normal splenic architecture. (c, Masson×200): displaying minimal collagen fiber deposition (arrow) in connective tissue trabeculae, around blood sinusoids (S) and thin fibrous capsule (C). (d Prussian, ×400): displaying apparently normal Prussian blue reaction (arrowhead) in the form of small sized isolated blue granules and fine deposits in reticuloendothelial cells.

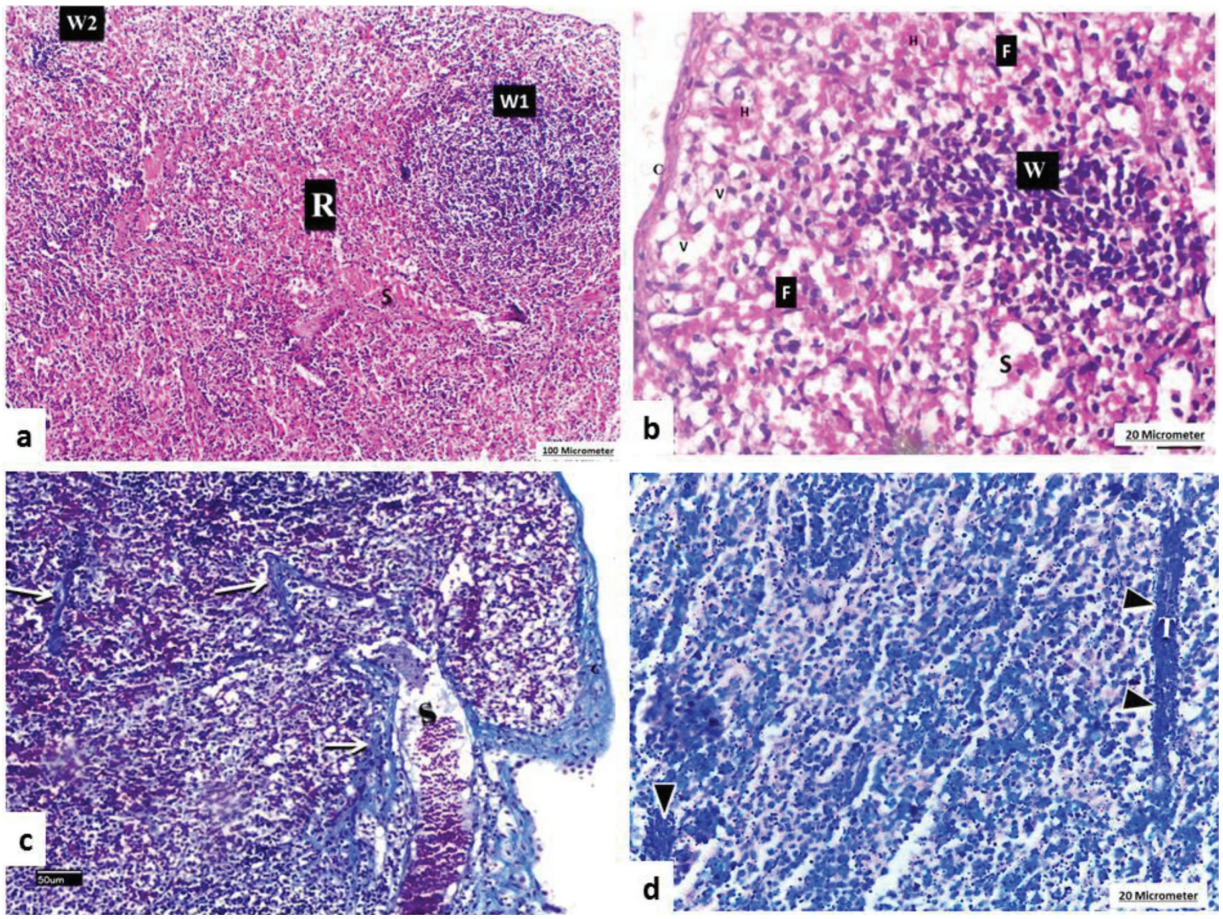
($P<0.0001$) compared with the control group. However, no statistically significant difference was found between groups III and V ($P=0.054$) (Fig. 9a).

Discussion

The current work recorded the manifestations of the pathological effect of lead acetate on the spleen rats. Histopathological examination revealed disturbed splenic parenchymal architecture, marked lymphocyte disintegration, and increased collagen tissue deposition around the blood sinusoids, adding to the appearance of big phagocytic cells. Similar findings were reported by Uchewa *et al.* [18], who observed that lead acetate exposure resulted in splenic architecture disruption, revealing deterioration of white pulps with necrotic lymphocytes. They also reported that lead acetate can hinder macrophage function by overcapacity of these cells with cellular remains. Additionally, the appearance of large macrophages in the spleen of rats that are exposed to lead acetate may be due to the inflammation caused by toxicity and the creation of the dead cell debris. Al-Mzaien *et al.* [19] reported the histopathological variations of the spleen in form of lymphoid tissue reduction that can be connected to the effects of lead acetate. Comparable results were found by Al-Naimi *et al.* [20], who reported the ability of lead acetate to cause vascular changes in the splenic arterioles in the form of endothelial injury and hemorrhage. They added a positive relationship between lead exposure and peripheral arterial diseases.

Our results showed increased collagen tissue deposition in rats treated with lead acetate. This was agreed by Deveci [21], who demonstrated that the reticular cell synthesizes type III collagen and uses it to produce reticular fibers. Lead acetate may affect the functions of reticular cells and make abnormal type III collagen that produces defective reticular fibers, thereby forming

Figure 5

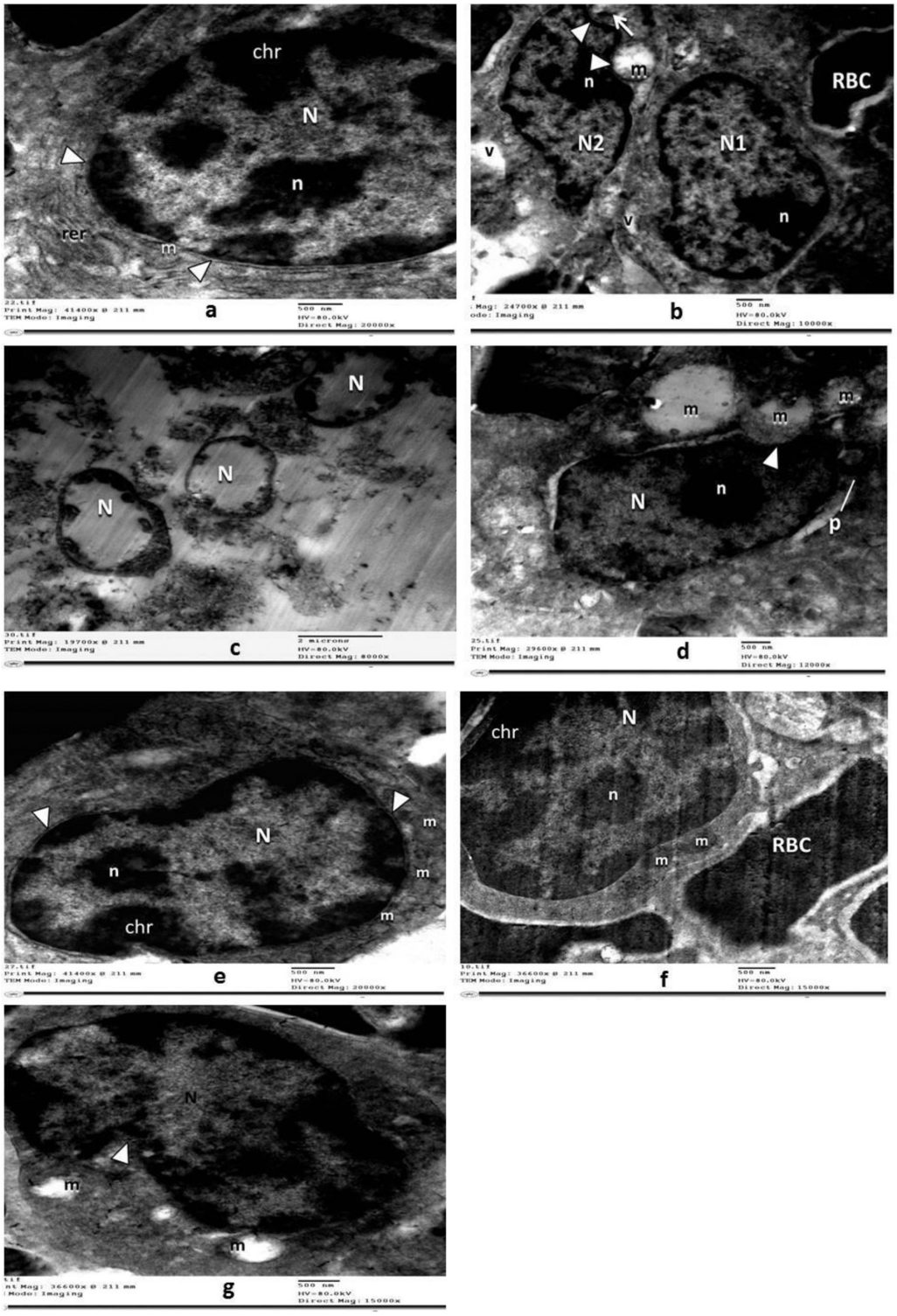


A photomicrograph of a section of a rat spleen from group V (lead administration and vitamin C 8 weeks) (a H&E, ×100) showing apparently normal white pulps (W1), wide red pulps (R) in-between degenerated white pulps (W2), and congested blood sinusoid (S). (b H&E, ×400): showing apparently normal white pulps (W), degenerated lymphocytes with cytoplasmic vacuolation (v), some spindle shape fibroblasts (F), areas of hemorrhage (H), and moderately thickened fibrous capsule (C). (c Masson, ×200): displaying moderate increase in collagen fiber deposition (arrow) in connective tissue trabeculae, around blood sinusoids (S) and moderate thickness of fibrous capsule (C). (d Prussian, ×400): displaying moderate Prussian blue reaction (arrowhead) in the form of small-sized isolated blue granules, frequent fine deposits in reticuloendothelial cells and along connective tissue trabeculae (T).

fibrous tissue. Hyperplasia of white pulps and hemorrhage in the spleen parenchyma in groups treated with lead acetate were observed. Hashem and El-Sharkawy [22] found that some splenic white pulps showed lymphoid reduction and others developed hyperplastic with the megakaryocyte propagation with hemosiderosis in white and red pulps, in addition to thickened splenic trabeculae in lead-treated rats. The current study revealed that electron microscopic examination of rat spleen specimens treated with lead acetate showed degenerated white pulp lymphocytes and an increased number of macrophages. Mesure *et al.* [23] agreed with our results and reported that the number of vacuoles amplified with lead exposure time and nuclear changes designated cellular death. Moreover, mitochondrial alterations would certainly impair cellular immune functions. Our results found that cotreatment with vitamin C for 4 and 8 weeks reduced the splenic degenerative changes. In agreement with these findings, Autifi *et al.* [24] reported that the spleen sections of rats that received vitamin C displayed healthy lymphoid follicles and decreased fibrosis. Additionally, the study conducted by Deveci [21] revealed the protective properties of ascorbic acid on the spleen of experimental animals encouraged with mercury chloride toxicity.

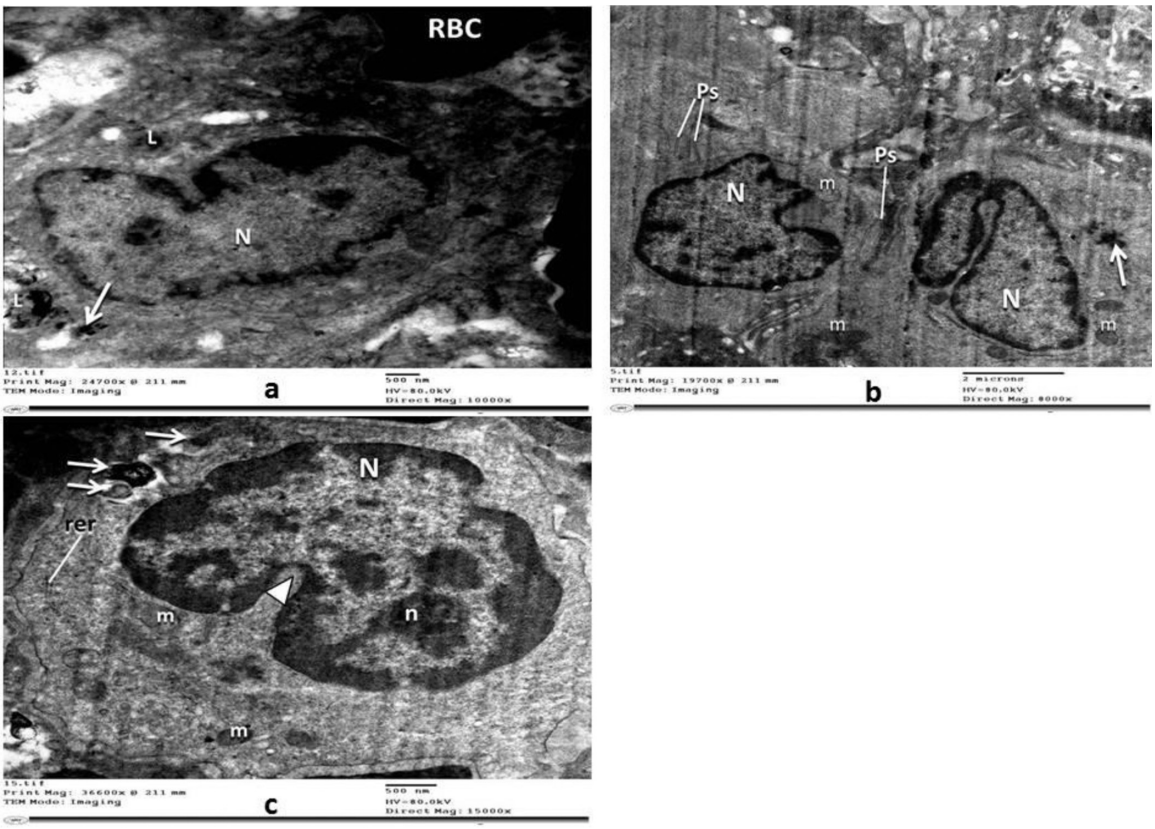
Metal toxicants that disturb the immune system may contribute to amplified infectious diseases and cancer occurrence, by host resistance reduction against infectious agents and tumor cells [8]. In our study, lead acetate administration resulted in reduced IL-2 expression and augmented IL-6 and TNF expression. This was in agreement with Chibowska *et al.* [25] and colleagues who stated that lead can cause generalized inflammation in the body as it exerts a harmful effect by increasing early inflammatory mediators such as

Figure 6



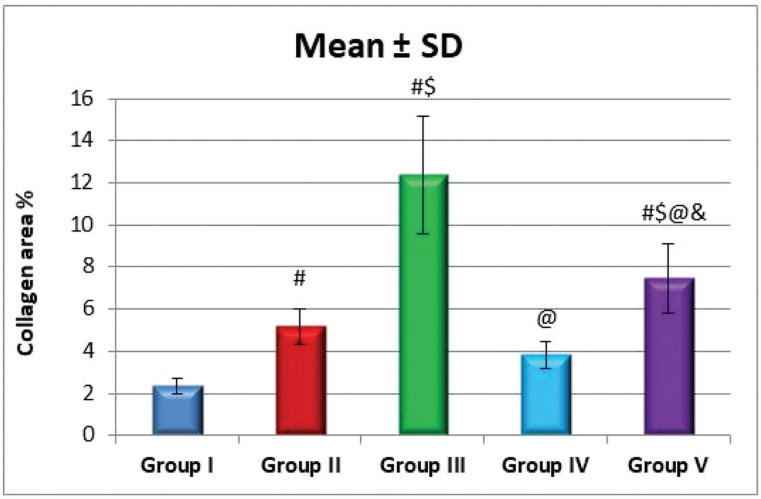
An electron micrograph of a section of a rat spleen from group I (a EM ×20 000) displaying lymphocytes with a heterochromatic nucleus (N) with peripheral condensation of chromatin material (chr) forming a rim, a prominent nucleolus (n), and intact nuclear envelope (arrowhead). The nucleus is surrounded by a thin rim of cytoplasm featuring intact mitochondria (m) with intact cristae and parallel cisterns of rough endoplasmic reticulum (rer). Group II (b EM, ×10 000) displaying lymphocytes with a heterochraomatic nuclei (N1, N2) with less margination of chromatin material and prominent nucleoli (n), and cytoplasmic vacuolation (V). The nuclear envelope in N2 is indented (arrowhead) by inclusion bodies (arrow) and a ballooned mitochondria (m) with damaged cristae. A red blood cell (RBC) can be observed. Group III (c, EM, ×8000) displaying degenerated lymphocytes with extremely karyolitic nuclei (N) and absent cytoplasmic organells. Group III (d EM ×12 000) displaying a lymphocyte with decreased margination of chromatin material in nucleus (N) with nuclear protrusion (p), a prominent nucleolus (n), and indented nuclear envelope (arrowhead) with ballooned mitochondria (m) with damaged cristae. Group IV (e EM, ×20 000) displaying lymphocyte with a heterochromatic nucleus (N) with peripheral condensation of chromatin material (chr) forming a rim, a prominent nucleolus (n), and intact nuclear envelope (arrowhead). The nucleus is surrounded by a thin rim of cytoplasm containing intact mitochondria (m) with intact cristae. Group V (f EM ×15 000) displaying lymphocyte with a heterochromatic nucleus (N) with peripheral condensation of chromatin material (chr) forming a rim and a prominent nucleolus (n). The nucleus is surrounded by a thin rim of cytoplasm featuring intact mitochondria (m) with intact cristae. A red blood cell can be observed (RBC). Group V (g, EM ×15 000) displaying a lymphocyte with a heterochromatic nucleus (N), deeply indented nuclear envelope (arrowhead), and some ballooned mitochondria (m) with damaged cristae.

Figure 7



An electron micrograph of a section of a rat spleen from group II (a EM, ×10 000) displaying a macrophage with a heterochromatic nucleus (N), inclusion bodies (arrow), and electron dense lysosomes (L). A red blood cell (RBC) can be observed. Group III (b EM, ×8000) displaying macrophages with a heterochromatic nucleus (N), intact mitochondria (m) with intact cristae, inclusion bodies (arrow), and pseudopodia (Ps). Group V (c EM ×15 000) displaying macrophages with a heterochromatic nucleus (N), a prominent nucleolus (n), deeply indented nuclear envelope (arrowhead), intact mitochondria (m) with intact cristae, inclusion bodies (arrow), and cisterns of rough endoplasmic reticulum (rer). #Significant from control, $^{\$}$significant from group II, $^{@}$significant from group III, and $^{\&}$significant from group IV.

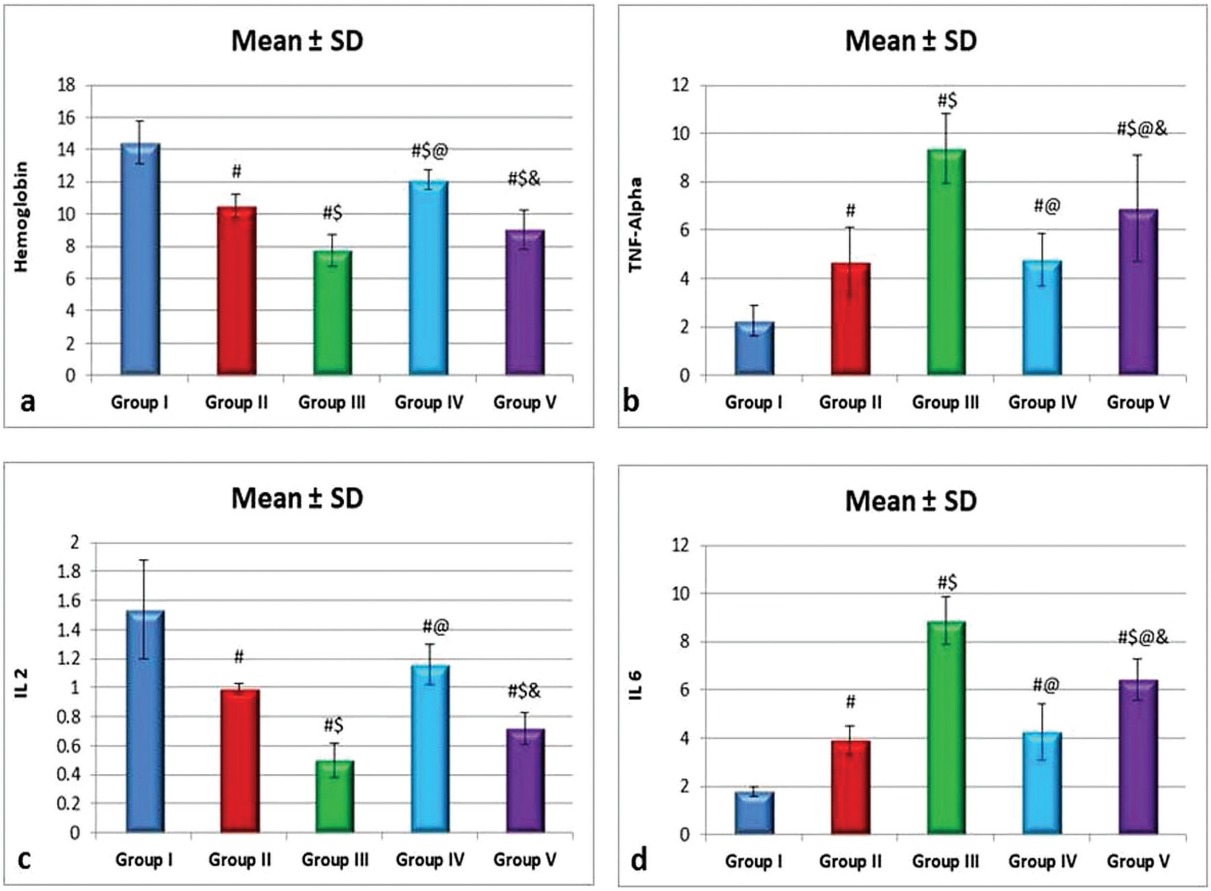
Figure 8



significant from control,$ significant from group II,@ significant from group III& significant from group IV

A histogram illustrating mean values of collagen area percent in rat spleen specimens obtained from different groups of the examined animals. $^{\#}$significant from control, $^{\$}$significant from group II, and $^{@}$significant from group III, and $^{\&}$significant from group IV.

Figure 9



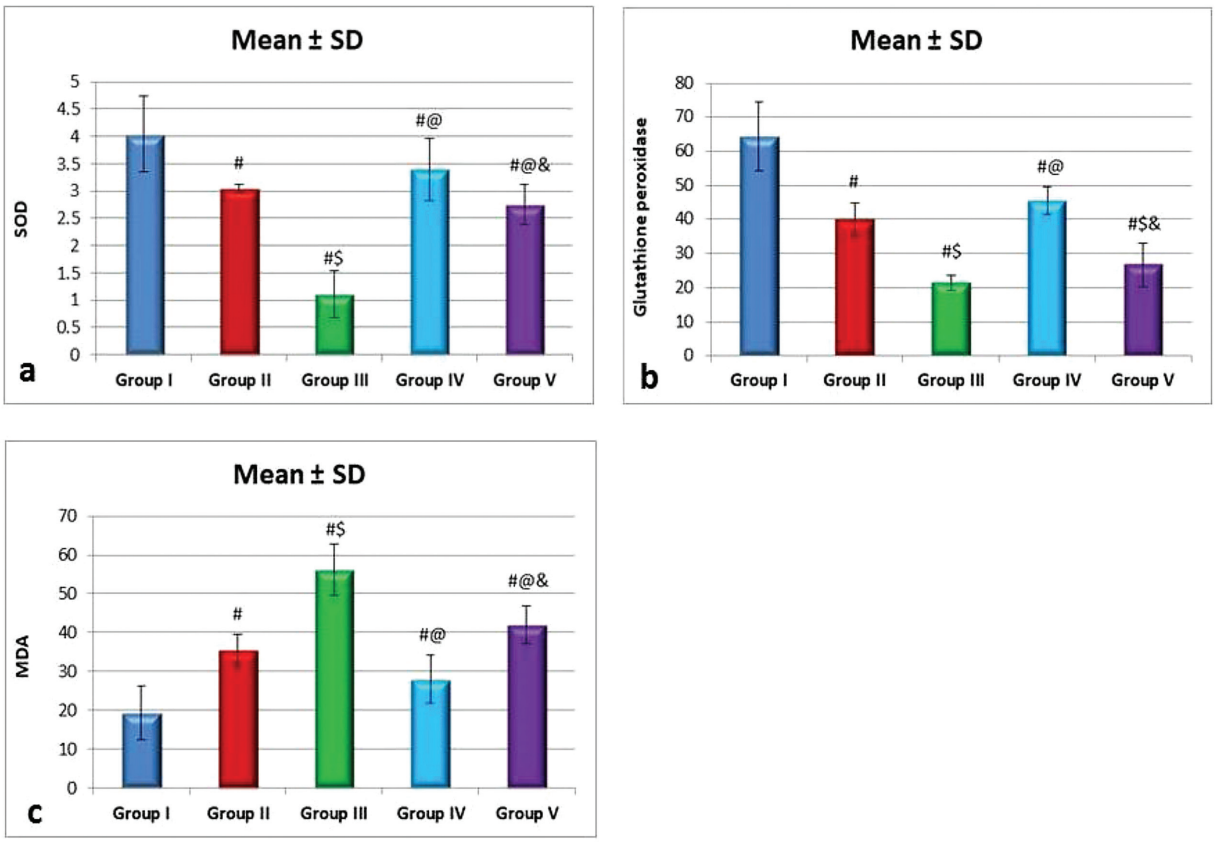
significant from control,$ significant from group II,@ significant from group III& significant from group IV

A histogram (a) illustrating mean values of hemoglobin (HB) in rat blood samples obtained from different groups of the examined animals, (b) illustrating mean values of tumor necrosis factor-alpha (TNF-α), (c) illustrating mean values of interleukin (IL)-2, and (d) illustrating mean values of IL-6. #Significant from control, $significant from group II, @significant from group III, and &significant from group IV.

TNF-α and IL-6 and secondary inflammatory mediators such as reactive oxygen species. They added that the immune system is a sensitive target of lead as it adversely affects the function, immune cell regulation, cytokine production, enzyme activity, and receptor expression contributing to the inflammation processes. The coadministration of vitamin C with lead for 4 weeks, in our study, markedly resolve these immunological changes. Autifi *et al.* [24], Gorkom *et al.* [26], and Mousavi *et al.* [27] reported that a basal concentration of vitamin C is needed for a well-functional host defense mechanism, and the vitamin C application is supposed to recover immune function. Experimentally encouraged vitamin C deficiency decreases humoral and cellular immune functions. Additionally, high doses of vitamin C not only motivated murine immune cells, mainly dendritic cells, but also stimulated proliferation, differentiation, and function of macrophages and T and B cells in addition to its anti-inflammatory properties; thus, it is used in ulcerative colitis and Crohn's disease treatment. Vitamin C administration during sepsis altered the regulatory T-cell activity by improving cell proliferation and impeding different transcription factor expression, and cytokines. Furthermore, vitamin C is fundamentally elaborate in the biosynthesis and repair of collagen, and the absence of ascorbic acid weakens mucosal epithelia, basement membranes, and connective tissue integrity. Additionally, vitamin C is required for proper wound healing and bone development.

Lead can substitute the zinc ions that act as vital cofactors for these antioxidant enzymes and deactivate them. Lipid peroxidation occurs due to the action of reactive oxygen species (ROS) on lipid membranes. The produced free radical arrests electrons from the lipids that exist inside the cell membranes and harms the cell [28].

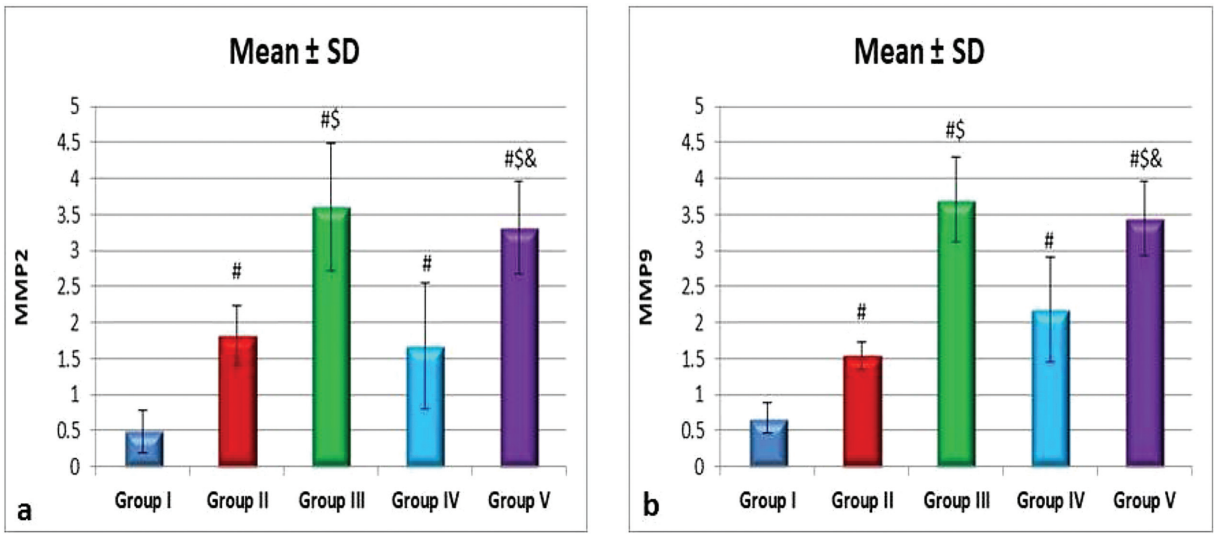
Figure 10



significant from control,$ significant from group II,@ significant from group III& significant from group IV

A histogram (a) illustrating mean values of superoxide dismutase (SOD) in rat spleen specimens obtained from different groups of the examined animals, (b) illustrating mean values of glutathione peroxidase, and (c) illustrating mean values of malondialdehyde (MDA). #Significant from control, $significant from group II, @significant from group III, and &significant from group IV.

Figure 11



significant from control,$ significant from group II,@ significant from group III& significant from group IV

A histogram (a) illustrating mean values of MMP-2 in rat spleen specimens obtained from different groups of the examined animals and (b) illustrating mean values of MMP-9. MMP, matrix metalloproteinase.

Our study revealed that the lead acetate administration caused decrease activity of GPX and SOD and increased levels of MDA. Offor *et al.* [29] explained this by two mechanisms: first, the ROS generation, and second, the exhaustion of antioxidant reserves. Our results revealed that vitamin C treatment improved the biochemical modifications encouraged by lead acetate administration. This was in agreement with Jewo *et al.* [30], Nimse and Pal [31], Ghanwat *et al.* [32], and Kandeil *et al.* [33], who reported that uptake of vitamins can carry defense against lead toxicity, and vitamin C can normalize changes of oxidative stress biomarkers initiated by lead; thus, its supplementation might be the greatest chelation therapy for lead intoxication. They added that the vitamin C antioxidant effect is due to hydrogen atoms, which couple with unpaired electrons of the free radicals, changing them to nonfree radicals. Khordad *et al.* [34] reported that vitamin C cleared ROS and free radicals to protect against lead toxicity. They added that vitamin C acts as a chelating agent for lead, thereby enhancing its execration from the body and reducing its accumulation in tissues and blood, and its effect is nearly equal to EDTA.

Additionally, the present study found that MMP-2 and MMP-9 expressions were increased. This was in agreement with Liu *et al.* [35] who found that MMPs are vital parts of extracellular matrix proteasome that destroy tight junction and basement membrane proteins. They observed increased expression of MMP-2 and MMP-9 24–48 h following lead administration. Finally, lead acetate administration resulted in a marked decrease in blood hemoglobin levels. Similar results were observed by Alwaleedi [36], who reported that hematological changes may be credited by the lead toxicity on cell metabolism, and some enzymatic activities inhibition such as aminolevulinic acid dehydratase, which shows a chief role in heme biosynthesis. The effects of lead acetate on the enzyme activity in heme biosynthesis may be due to iron metabolism failure. The lead acetate inhibitory effect on the conversion of coproporphyrinogen III to protoporphyrin IX results in a shortened erythrocyte life span and a reduced hemoglobin production. The decreased hematological standards may be credited to the binding of lead to RBCs, which increases membrane fragility and RBC destruction. Our results revealed that vitamin C supplementation increased the blood hemoglobin level. This was explained by Ghanwat *et al.* [37] and Ray [37], who found vitamin C had the ability to increase iron absorption and reduce lipoperoxidative damage of the erythrocyte membrane.

Ebuehi *et al.* [38] studied the effect of the administration of vitamin C and vitamin E for 7 weeks on lead-induced hepatotoxicity and oxidative stress in the rats' brains and found that the administration of vitamin C and vitamin E significantly improves the hepatic damage and significantly reduced the oxidative stress. These findings come in contrary to our results, which revealed rats treated with lead and vitamin C for 8 weeks showed degenerative changes in the spleen; this may be because with the prolonged vitamin C administration some toxicity developed, and so, we recommend further studies to explain this result.

Conclusion

Ascorbic acid could normalize alteration of oxidative stress biomarkers induced by lead. Moreover, its ability to reduce free radicals and chelate heavy metals makes it a unique antioxidant. This may postulate vitamin C efficiency in lead acetate toxicity prevention in the spleen, and vitamin C therapy could improve the changes caused by lead acetate toxicity in the spleen of adult albino rats.

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Conflicts of interest

There are no conflicts of interest.

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