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Prenatal, lactation and postnatal effects of lead acetate on histological, histochemical and hepatic indices of liver of male offspring wistar rats

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Abstract---The aim of this study was to investigate the possible effects of maternal administration to lead acetate on the morphological structure of the liver; AST, ALT, ALP and GGT levels and glycogen content of hepatocytes of male offspring wistar rats. Female rats were exposed to 0.2% lead acetate in drinking water and the study was performed on their male offspring (7 groups). To evaluate the levels of liver enzymes, blood samples were taken from rats after anesthesia. After dissection, to study its morphological structure and glycogen content of hepatocytes, liver tissue was isolated and transferred to 10% formalin. To measure the levels of hepatic enzymes; AST, ALT, ALP and GGT, serum samples were evaluated. For histological examinations, liver tissue samples were embedded in paraffin after tissue processing and 3 μ m thickness sections were prepared from them and then H&E and PAS staining was performed. The results showed that exposure to 0.2% lead acetate induced significant changes in liver enzyme levels in different experimental groups. Histological studies also showed that lead acetate induced changes such as central vein and sinusoidal congestion, hydropic degeneration of hepatocytes, multifocal lymphocytic infiltration, note the no distinct portal triads. PAS staining also showed that lead acetate reduced the glycogen content of hepatocytes in different experimental groups. We concluded that lead acetate in the amount of 0.2% induces various changes in the levels of enzymes, tissue structure and glycogen content of hepatocytes in the prenatal to lactation periods of male rats.

Keywords---*lead acetate, liver structure, hepatic enzymes, glycogen content, prenatal, lactation.*

Introduction

Environmental pollution is recognized as a fundamental issue for humans and other animals. Lead and cadmium are the two most abundant toxic elements in the environment. Lead and cadmium sources are diverse in nature, including natural and anthropogenic processes like combustion of coal and mineral oil, iron smelting, mining and alloy processing plants, and paint industries. The continued rise of environmental pollutants due to increased urbanization, industrialization and through scientific and technical advances has led researchers to study toxic substances and their effects on the biological systems (1). Lead is present in plastics, paints, ceramics, glass, water pipes, insecticides and gasoline. Lead toxicity is still an important health issue since the discovery of this element

thousands of years ago (2). No report has been yet made on the amount of lead to be allowed, and it has been found that small quantities are also harmful to humans and other organisms.

Lead has been found to have wide-ranging effects on various organs of the body such as the nervous system, hematopoietic system, skeletal system, kidney, circulatory system, and endocrine system (3). Due to the presence of lead in different parts of nature, this element can have effects on humans through various foods, drinking water and dust. Exposure to lead or lead toxicity has caused a wide range of physiological, biochemical, and behavioral defects in humans and other organisms (4). The liver has been recognized as a target organ for the toxic effects of lead (5-7). Heavy metals such as lead are the oldest known toxins for humans, and the organ that receives the most impact is the liver (8). Autopsy studies have shown that among the soft tissues of lead-exposed humans, the largest storage organ (33%) in the liver followed by the kidney (9). Lead damage in liver tissue involves defects in its structure and function (10). According to Taupeau *et al.* (11) lead is an estrogenic compound that may affect fetal development by crossing the blood-placental barrier. Maternal milk has been Proposed as a significant potential source of infant lead exposure (12). Therefore, the purpose of this study was to explore the lead acetate-induced changes in the histologic architecture of the liver, the glycogen content of Hepatocytes and levels of *AST*, *ALT*, *ALP* and *GGT* which are the most important indicators of liver damage.

Materials and methods

Experimental animals

In this study, male and female adult wistar rats, 150-170 g of weight with 8-11 weeks aged, were purchased from the Pasteur Institute of Iran and kept in the embryology laboratory under the following conditions: 12 h light-12 h darkness, 20-26 °C, and adequate food and water.

At all stages of this study, ethical standards were observed for the maintenance and use of laboratory animals (ethical code: 7612032). After adaptation of animals to the environment, for mating animals, two females and one male are considered in each cage, and after 12 h. vaginal plaque was investigated. Then fertile rats were divided into different groups randomly. 0.2% lead acetate (Sigma-Aldrich) was used in drinking water with 0.5 ml/liter glacial acetic acid to prevent the deposition of lead acetate (13-15).

Experiment design

To investigate the effects of lead acetate on histological characteristics and levels of physiological factors in perinatal and lactation periods, rats were divided into seven groups: first and second; control and sham groups, third; pre-pregnancy group, fourth; pregnancy group, fifth; lactation group, sixth; pregnancy lactation group, and 7; pre-pregnancy pregnancy lactation group (6 rats for each group). Maternal and neonatal rats in the control and sham groups from pre-pregnancy to puberty had access to drinking water and acetic acid_ drinking water

composition, respectively. In the pre-pregnancy group, lead acetate was used in drinking water for 30 days prior to mating. Pregnant rats were treated with lead acetate in drinking water during pregnancy (21 days). Lactation group rats during the 21 days of lactation were treated with 0.2% lead acetate in drinking water. In the pregnancy lactation group, combined lead acetate acetic acid was used during the period (42 days). The pre-pregnancy-pregnancy-lactation group was exposed to a combination of lead acetate and acetic acid during this period. All rats, except those of the control group, received a combination of drinking water acetic acid from the end of the lead acetate treatment until the end of the period (63 days after birth).

In all groups, after the lactation period, pups were separated from mothers and kept in separate cages. At the end of the experiment, the animals were euthanized and all rats were treated with 300 mg/kg, 10% ketamine and 30 mg/kg Xylazine.

Histopathology

After anaesthetizing the rats, their liver tissue was removed and immediately transferred to 10% formalin. The tissues were embedded into the paraffin and 3 μ m thickness sections were prepared from them. The sections were stained with H&E (hematoxylin and eosin) and PAS (periodic acid Schiff's) and then studied by light microscopy as previously described (16,17).

For H&E staining, the sections were deparaffinized in xylene, hydrated in descending ethanol, stained with hematoxylin, embedded in acid alcohol and sodium carbonate solutions respectively, stained with eosin, dehydrated in ascending ethanol and finally cleared with xylene and mounted. For PAS staining, after deparaffination and hydration, sections were embedded in acid periodic 1%, then followed by: rinse with water, Schiff's reagent, rinse with water, staining with hematoxylin, rinse with water, dehydration, clearing and finally mounting.

Biochemical study

The activity of ALT, AST, ALP and GGT of all serum samples from rats have been determined. The levels of alanine aminotransferase (*ALT*), aspartate aminotransferase (*AST*), alkaline phosphatase (*ALP*) and Gamma-glutamyl transferase (*GGT*) were measured using standard techniques and commercial kits (Pars Azmoon Co., Iran) with an auto-analyzer (Gcsan Chem 2000, Spain).

2.5 Statistical analysis

All data are expressed as mean $\pm$ St.D. Data analysis was done by using SPSS (version 22). Differences among groups of the present study were tested by one-way analysis of variance (ANOVA) with Tukey as a post hoc test. Probability values less than 0.05 were considered significant.

Results

Histopathological Findings

H&E staining

Histopathological studies showed that the control and sham groups had regular hepatic structures. Hepatocytes were polygonal in shape with a large round nucleus and acidophilic cytoplasm. These cells were arranged in radial cords around the central vein. Portal triads, sinusoidal structures, kupffer cells and central veins were clearly seen in these groups (Fig 1a-b). In the pre-pregnancy group, central vein and sinusoidal congestion were observed (Fig 1c). The pregnancy group samples had distinct central and portal vein congestion. The hepatic sinusoidal dilatation was moderate. Hydropic degeneration of hepatocytes was also observed in this group (Fig 1d).

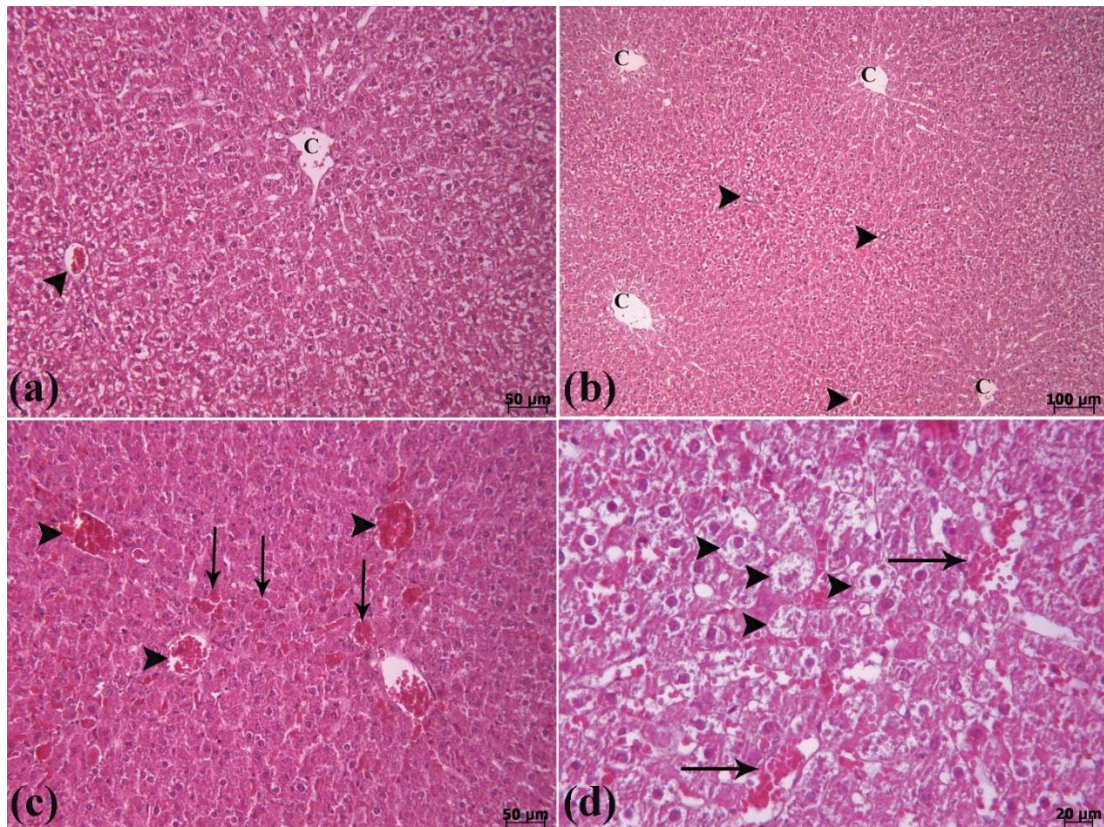


Fig. 1 (a-d): Histopathological findings of liver in the control (a), sham (b), pre-pregnancy (c) and pregnancy group (d). (a-b) Normal structures of liver, C (central vein) and arrowheads (Portal triad). (c) Central vein (arrowheads) and sinusoidal (arrows) congestion. (d) Sinusoidal congestion (arrows) and hydropic degeneration of hepatocytes (arrowheads), H&E.

In the lactation group, changes such as central vein congestion, hepatic sinusoidal dilatation and hydropic degeneration of hepatocytes were also observed. Multifocal lymphocytic infiltration was also seen. There was no distinctive triad structure in this group (Fig 2a-b). The Pregnancy-lactation group showed loss of lobular organization. Central vein congestion, hydropic degeneration of hepatocytes and multifocal lymphocytic infiltration (Fig 2c). In the

pre-pregnancy-pregnancy-lactation group, irregular hepatic lobules (no distinct portal triads) were observed (Fig 2d).

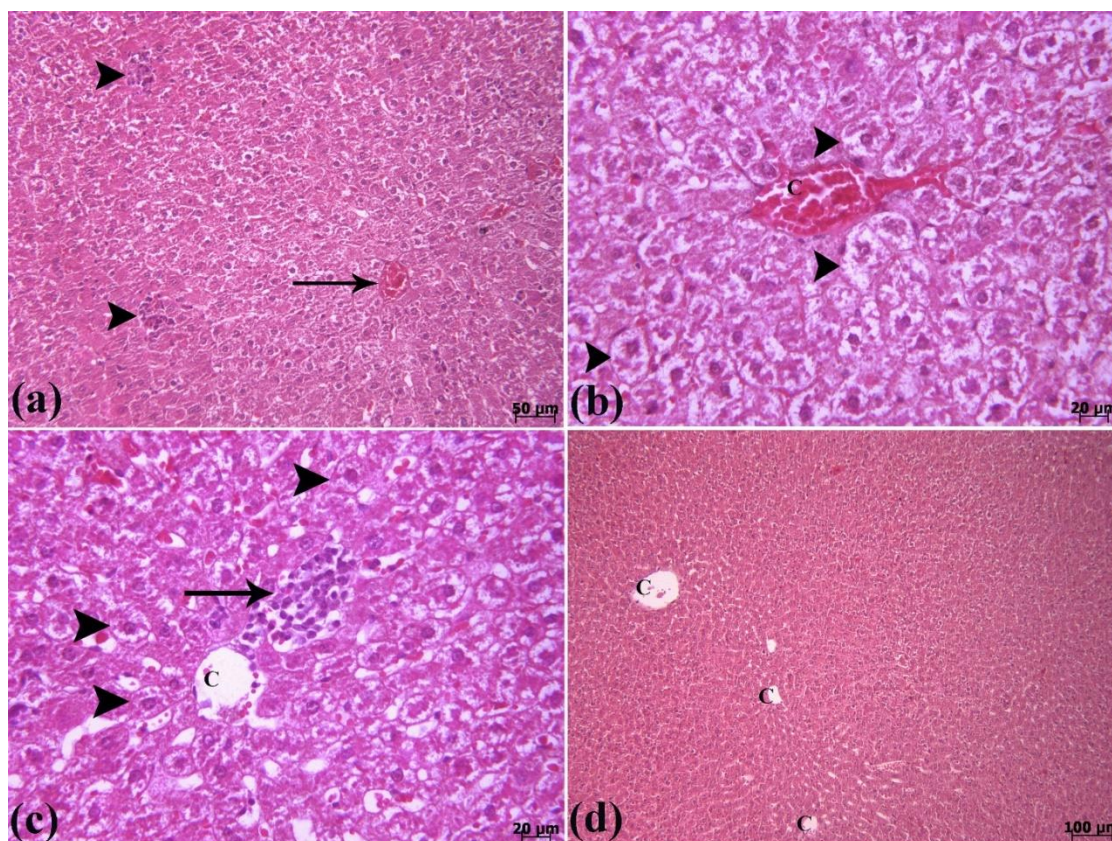


Fig. 2 (a-d): Histopathological findings of liver in the lactation (a-b), pregnancy-lactation (c) and pre-pregnancy-pregnancy-lactation (d) group. (a) Central vein congestion (arrow) and multifocal lymphocytic infiltration (arrowheads). (b) Hydropic degeneration of hepatocytes (arrowheads). (c) Multifocal lymphocytic infiltration (arrow) and hydropic degeneration of hepatocytes (arrowheads). (d) Note the no distinct portal triads, C (central vein), H&E.

PAS staining

Liver specimens from the sham group were showed glycogen storage in a deeply red-purple color by using periodic acid-Schiff (PAS) (Fig 3a). PAS staining was confirmed low glycogen depletion in the pre-pregnancy and pregnancy group, mild glycogen depletion in lactation and pregnancy-lactation group and moderate glycogen depletion in the pre-pregnancy-pregnancy-lactation group (Fig 3b-f).

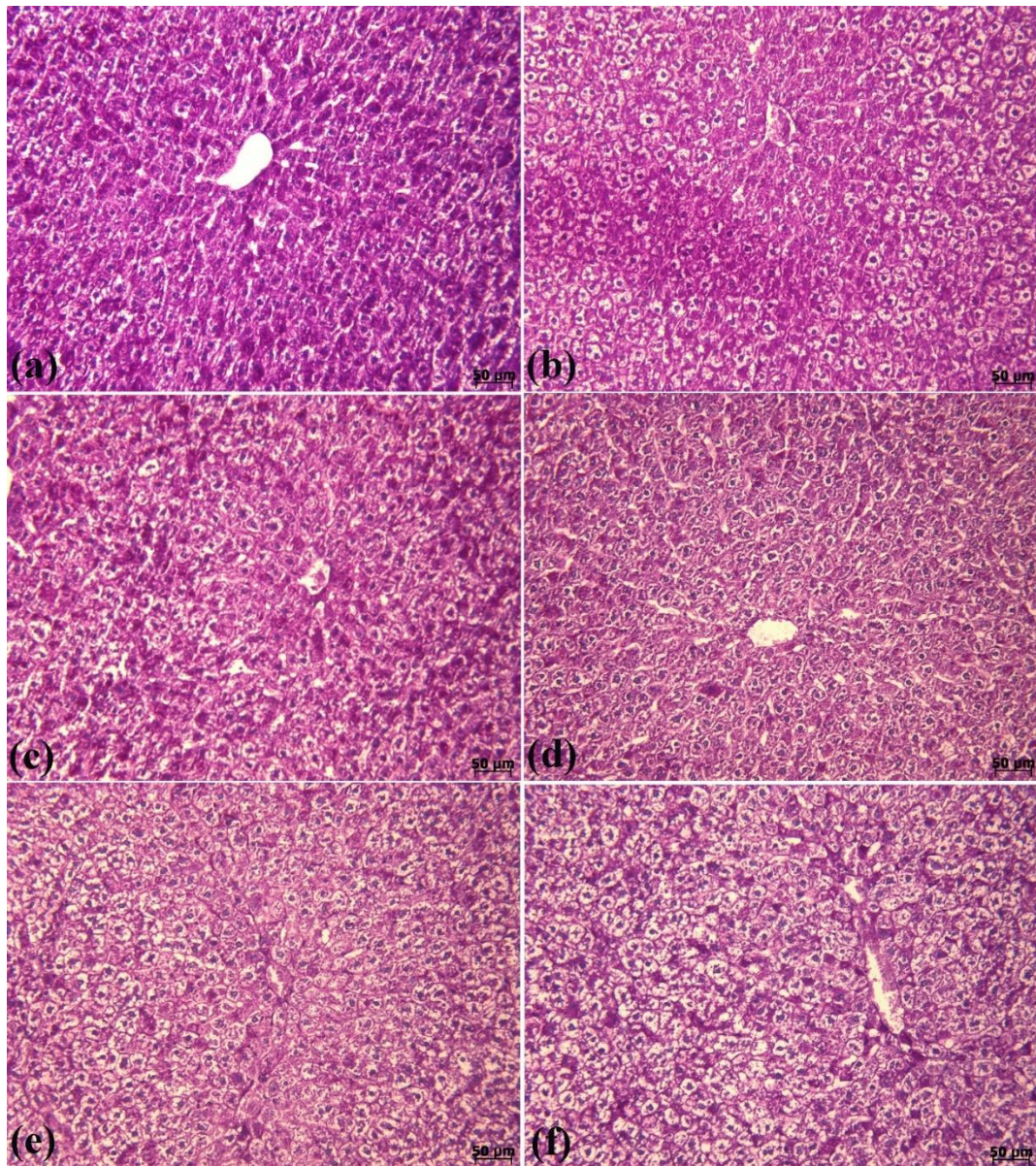


Fig. 3 (a-f). PAS staining of glycogen storage in the liver of sham (a), pre-pregnancy (b), pregnancy (c), lactation (d), pregnancy-lactation (e) and pre-pregnancy-pregnancy-lactation (f) group.

3.3 Biochemical results

In terms of AST, there was a significant difference between the control groups and pre-pregnancy, pregnancy and pregnancy-lactation groups. Between the other groups, there was a significant difference between pregnancy and pregnancy-lactation with pre-pregnancy- pregnancy- lactation and lactation groups. There

was also a significant difference between the lactation group and the pre-pregnancy and pregnancy groups (Table 1).

There was a significant increase in *ALT* levels between the pregnancy -lactation, pregnancy and pre-pregnancy treatments in comparison with other groups. But this difference between pre-pregnancy-pregnancy-lactation and lactation groups was not significant (Table 1).

ALP measurements showed a significant difference between the control groups and other treatments. In other words, the increase in *ALP* was significant in the other groups compared to the control and sham groups. But there was no significant difference between *ALP* levels in pre-pregnancy-pregnancy-lactation, lactation and pregnancy groups (Table 1).

There was no significant difference in *GGT* values between the control groups and other treatments except pre-pregnancy treatment. In other words, there was a significant increase in *GGT* values for the pre-pregnancy group compared to other treatments (Table 1).

Table 1, The effect of lead acetate on serum Aspartate transaminase (*AST*), Alanine transaminase (*ALT*), alkaline phosphatase (*ALP*) and Gamma-glutamyl transferase (*GGT*) activities in control, sham and different lead acetate treatments.

Mean $\pm$ St.D

	1	2	3	4	5	6	7
<i>AST</i>	30.75 $\pm$ 2.5 ^a	31 $\pm$ 1 ^a	148.75 $\pm$ 45 ^b	175 $\pm$ 30 ^b	161.5 $\pm$ 6.24 ^b	205 $\pm$ 57 ^b	182.25 $\pm$ 27 ^b
<i>ALT</i>	15.25 $\pm$ 7 ^a	13.75 $\pm$ 5 ^a	200.25 $\pm$ 10 ^c	142 $\pm$ 13 ^b	153 $\pm$ 3 ^b	116.5 $\pm$ 8 ^b	223.5 $\pm$ 14.4 ^c
<i>ALP</i>	104 $\pm$ 1.41 ^a	100 $\pm$ 1.35 ^a	714 $\pm$ 35 ^d	560 $\pm$ 16 ^c	572.75 $\pm$ 10.5 ^c	446 $\pm$ 24 ^b	578.5 $\pm$ 27.5 ^c
<i>GGT</i>	5.5 $\pm$ 0.57 ^{ab}	5.6 $\pm$ 0.48 ^{ab}	7.5 $\pm$ 1.73 ^b	4.6 $\pm$ 0.73 ^a	5.5 $\pm$ 0.57 ^{ab}	4.25 $\pm$ 0.95 ^a	4.25 $\pm$ 0.95 ^a

Groups: 1, control; 2, sham; 3, Pre-pregnancy; 4, pregnancy; 5, lactation; 6, Pregnancy- lactation; 7, Pre pregnancy- pregnancy- lactation. Different letters indicate significant differences.

4. Discussion

4.1 Histopathology

Exposure to lead, due to occupational activities and from the environment, is a global concern (9). Absorbed lead in the body can be stored in soft tissues such as the liver (18). Guyton and Hall (19) believe the liver is the first organ to be exposed to absorbed food, that this exposure is done through the portal vein.

In the present study, the liver of the control and sham groups had a normal structure with hepatocyte cords, central vein, sinusoids, Kupffer cells, and distinct portal area. Muhammed *et al.* (3) and Ibrahim *et al.* (20) in their studies reported similar results for the liver tissue structure of the control group. Our results also showed, in lead-exposed treatments, several changes such as kupffer cell necrosis, mild to severe dilation of sinusoids, and the mild influx of inflammatory cells was observed. Muhammed *et al.* (3) stated that, in the lead-exposed group, some defects such as necrosis and apoptosis of Kupffer cells, the influx of inflammatory cells and dilation of sinusoids were observed. Also, Ibrahim *et al.* (20) reported that exposure to lead acetate induce several negative change in liver histologic structure such as: marked midzonal and periportal vacuolar degeneration, focal areas of coagulative necrosis represented by nuclear pyknosis, infiltration of mononuclear inflammatory cells, distention in the portal area, congestion of portal vein, perivascular edema and mononuclear cell infiltration, and hepatocyte vacuolization. In the other similar study, Abdelhamid *et al.* (21) reported that exposure to lead acetate induced many pathologic changes such as hydropic degeneration, hepatocytes necrosis, bile duct hyperplasia, and portal vein and central vein congestion in liver structure.

On the other hand, the results of the present study were in line with studies of muhammed *et al.* (3) and Ibrahim *et al.* (20). Liver histological study of lead-treated animals by Kubo *et al.* (22) showed damage to the liver structure, necrosis, and inflammatory cells infiltration. Similar results have been reported by the observation of immune cells in the liver parenchyma in this study, as Jarrar & Taib (23) stated, may be due to the interaction of lead with the interstitial liver proteins and enzymes that interfere with the antioxidant defence mechanism and may produce ROS. That ROS production can in turn trigger an inflammatory response.

In the present study, the samples of the pregnancy group had more distinct central veins than the pre-pregnant group, but central vein congestion was observed in some of the veins in this group. There was also congestion in the portal vein. The dilatation of sinusoids in this group was less than the pre-pregnancy treatment. Hepatocyte oedema was also observed in the pregnancy group. In the lactation group, changes such as central vein congestion and sinusoidal dilatation were also observed, but these dilations were milder than those of the pregnancy treatment. Lymphocyte aggregation was also seen in this group. There was no distinct triad structure in this group and the pregnancy treatment (triad without arteriole was also seen).

Ramesh *et al.* (24) reported the histopathological results of liver tissue in lead-treated rats on the 14th and 28th days after a treatment: Group 2 on the 14th day showed mild congestion of central vein, low inflammatory cell invasion, sinusoidal dilatation, and vacuolar degeneration, which were similar to those reported by Suradkar *et al.* and Hegazy & Fouad (25,26). On the 28th day, marked degenerative changes, hepatocyte necrosis, along inflammatory cell invasion were observed around the portal area by Ramesh *et al.* (24).

In our study, hydropic degeneration and kupffer cell apoptosis were observed in lactation treatment. Also, Muhammed *et al.* (3) reported a similar result. In the

present study, mild leukocyte invasion was observed in the parenchyma of hepatic tissue in all lead-exposed treatments. But no inflammatory cell invasion was observed around the portal triad. Furthermore, was not observed obvious increase in kupffer cells in sinusoids. Ramesh *et al.* (24) reported that, on the 14th day, low leukocyte invasion was observed around the portal triad and Kupffer cells were increased in liver parenchymal sinusoids. On the 28th day, inflammatory cell invasion increased in the hepatic parenchyma and around the portal triad. Accordingly, the results of our study are not in line with the results reported by Ramesh *et al.* (24). In the pregnancy-lactation treatment of the present study, loss of lobular organization as well as loss of sinusoidal structure was observed. Also in this group, hepatocyte oedema and moderate hepatocyte necrosis, central vein congestion, central vein enlargement, and kupffer cell necrosis was seen. Mehana *et al.* (27) reported different liver complications such as hydropic degeneration, steatosis, Blood vessels congestion, oedema, necrosis, Kupffer cell activation, and fibrosis in the rat. Ozkaya *et al.* (28) reported similar results to their study, although they did not report Kupffer cell activity. In the study of Hasanein *et al.* (8) it has been reported that exposure to lead also dilates the portal vein. Also, Sharma *et al.* (29) reported that mice exposed to lead nitrate for 40 days also extend portal and central veins in addition to other tissue damages. The results of the present study are in line with these results. In other words, exposure of lead acetate to Wistar male rats in this study also dilated central asteroids and ports. Changes in Kupffer cells such as hyperplasia also reported by Neyrinck *et al.* (30) may be related to long-term exposure to lead acetate that induces oxidative stress mechanism. The results of the present study showed the accumulation of mononuclear inflammatory cells in the liver tissue after treatment with lead, which was lined with findings of Mabrouk *et al.* (31). They also reported the occurrence of hydropic degeneration and hypertrophy of hepatocytes. In the present study, the results from lactation and pregnancy-lactation groups were similar to the results stated by Mabrouk *et al.* (31). Hydropic degenerations may be accompanied by the leakage of lysosomal enzymes that result in the destruction of cytoplasmic elements and macromolecules (32). Finally, the strong effects of lead exposure were observed in pre-pregnancy- pregnancy- lactation treatment. In other words, no hepatic lobe was observed and no distinct triad structure was observed. The sinus spaces were not easily recognizable due to severe necrosis of the hepatocytes and a severe dilatation of the vein was seen in the triad structure. Changes such as hydropic degeneration, cell vacuolation, and cellular necrosis due to lead exposure were observed in this study which according to Jarrar & Taib (23) may be due to increased cellular activity and loss of nucleus during the detoxification mechanism. Ibrahim *et al.* (20) stated that induced changes in liver structure of lead administrated rats to the disruption of the body's antioxidant balance.

4.2 Glycogen content

The results of this study showed a decrease in glycogen content in liver parenchyma cells of samples from different lead treatments. Similar results have been reported by Jarrar & Taib and Hegazy & Fouad (23,26). Decreases in glycogen content of hepatocytes may be due to the effect of lead acetate on glucose uptake or on the enzymes involved in the process of glycogenesis or glycolysis or both of them. According to Jarrar & Taib (23) heterogeneity in the

glycogen content of hepatocyte cells may indicate a difference in the glucose release of these cells.

4.3 Biochemical studies

Adverse effects of lead on tissues have been shown to be primarily due to stress oxidative processes, interference with lipid peroxidation, cell membrane integrity, and extensive tissue damage (33). Factors that indicate liver damage have different types. According to El-Tantawy (34) *ALT* and *AST* activities are indicators of liver tissue damage. One of the goals of this study was to evaluate the activity of *AST*, *ALT*, *ALP* and *GGT* which are the most important indicators of liver damage. Liver tests, such as measurement of liver enzymes (*ALT*, *AST*, *ALP*, and *GGT*) along with measurement of total bilirubin levels, help to identify liver and gallbladder injury status (35). Aminotransferases are known as markers of hepatocyte organelles and bile duct. Therefore, the activity of these enzymes in serum indicates the physiological status of the liver and their activity in the blood is highly dependent on the cellular damage (36). *GGT* is a sensitive marker of hepatic inflammation (35). The effect of lead on *AST*, *ALT*, *ALP* and *GGT* activities has been investigated in several studies. Our results showed significant differences for *ALT*, *AST* and *ALP* activities between controls and lead treated groups. *GGT* values in the present study were significantly increased in pre-pregnancy treatment compared to other treatments, which were different from the Al-Neamy *et al.* (37) findings. Also, Al-Neamy *et al.* (37) reported that *AST* increased in the lead-treated group but this increase was not significant. They reported opposite but nonsignificant results for *ALT*. In the case of *GGT*, they also reported that it was higher in the lead group than in the control group, but this difference was not significant. Finally, they reported significantly higher *ALP* levels in the lead-treated group than the control group. Hasanein *et al.* (8), Ibrahim *et al.* (20) and Abdelhamid *et al.* (21) reported that the lead administration was found to cause significant increases in serum *ALT*, *AST*, and *ALP*. Our results for *ALT*, *AST* and *ALP* were in line with their findings. This significance effects may be due to the accumulation of lead in liver as one of the major sites for the excretion and presence of lead-binding proteins in this organ (20). The results of Ramesh *et al.* (24) in a study on male Wistar rats showed a significant difference in the plasma *ALT* and *AST* concentrations in the treated groups (33 mg/kg lead) compared to the control group (deionized water). Damage to hepatocyte membranes will cause the release of these enzymes into the bloodstream (38). *ALP* is an enzyme that binds to the cell membrane and affects its permeability. This enzyme also disrupts metabolite transport (33). Increasing levels of *AST* and *ALT* in the plasma of treated rats is mainly due to the leakage of these enzymes from the liver cytoplasm into the bloodstream. The *AST* level rises significantly in plasma as a result of enzyme leakage from the injured hepatic cells into the bloodstream. *ALT* also increases in plasma when cellular degeneration or destruction occurs in the organ (39). Similar results for serum liver enzyme activity have been found by Abdel-Kader *et al.* (40) and Abdelhamid *et al.* (21) that they stated that these changes are due to the loss of plasma membrane integrity of hepatocytes as an effect of lead exposure. Sivarprasad *et al.* (41) observed an increase in serum transaminases levels after exposure of male Wistar rats to 0.02% lead acetate in water for 5 weeks. Gill *et al.* (42) showed that lead acetate II increased the concentration of aminotransferases. *AST* and *ALT* are involved in the metabolism

of carbohydrates and amino acids and mediate the interaction between the substrates of the citric acid cycle (33).

4.4 Conclusion

Based on the results of the present study, it can be concluded that due to the ability of lead as one of the heavy metals to cross important membranes and physiological barriers of the body such as blood testes barrier and embryonic membranes, this compound can have negative effects on liver tissue structure such as hepatic physiologic indices and glycogen content of rat hepatocytes in the prenatal and lactation periods. As reported in previous studies, exposure to low amounts of lead acetate can also cause serious damage to target organs such as the liver.

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Conflict of interest:

The authors declare no conflict of interest.

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