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Original Article

Effect of Zinc on Lead Acetate Induced Liver and Stomach Injury in Adult Mice: Electron microscopic and Biochemical Study

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ABSTRACT

Background: Lead is one of the common heavy metals to which people are exposed daily. It is found in drinking water, and occurs naturally in soil but at low levels in Earth's crust, as lead sulfide. Lead is considered dangerous and can cause environmental pollution due to its toxic effects on livings. Gastrointestinal ingestion is the most common source of lead intake in the body. Chronic exposure to lead even in low doses can induce liver damage as it is considered as one of the target organs affected by lead toxicity. Zinc is an essential trace element, exerting a protective effect as anti-inflammatory, antiapoptotic and antioxidant but these hepatoprotective properties have not been fully elucidated.

Aim of the work: The present study was designed to detect the possible protective effect of Zinc against the toxic effects of lead acetate on liver and stomach of mice.

Material and Methods: Sixty healthy adult mice were randomly divided into 3 groups of 20 mice each. Group I (Control group) were given distilled water by orogastric tube. Group II (Experimental group) were given lead acetate in a dose of 4mg/kg body weight by orogastric tube for 2 weeks. Group III (Experimental group) were given lead acetate and Zinc in a dose of 25 mg/kg/body weight by orogastric tube for 2 weeks. After two weeks biochemical and electron microscopic examinations of the liver and stomach were done.

Results: Significant increase of liver enzymes SGPT and SGOT was observed in experimental groups (group II and III) but more increased in group II. Electron microscopic examination group II revealed marked changes involving both the cytoplasm and the nucleus. The cytoplasm showed many vacuoles, multiple lipid droplets, areas of necrosis, dilated rough endoplasmic reticulum, dilated smooth endoplasmic reticulum and pleomorphic mitochondria with dense matrix. The nuclei of most of the hepatocytes showed dilated perinuclear cisternae, variability in shape and size of nuclei was noticed with vesiculation of their chromatin content. Many abnormal Kupffer cells were seen lining the blood sinusoids, distorted bile canaliculi and areas of necrosis were seen. Electron microscopic examination of group III revealed moderate changes of most liver cells. The cytoplasm exhibited pleomorphic mitochondria with dense matrix, dilated rough endoplasmic reticulum and dilated smooth endoplasmic reticulum, many lysosomes and glycogen. The nuclei are binucleated and irregular. Stomach of mice group II revealed pocket in nucleus, dialysis of organelles and abnormal Golgi, the lumen with no microvilli and dark spots of lead in it, two cells with membrane in between irregular nucleus and lysis of rough endoplasmic reticulum and disturbed smooth endoplasmic reticulum. The stomach of mice group III revealed Nucleus with membrane slightly interrupted, the lumen with few microvilli, multiple secretory granules and disturbed rough Endoplasmic Reticulum.

Conclusion: Lead acetate induces chronic inflammation, oxidative stress and toxic injury in the liver and can change the structure of stomach mucosa. Zinc supplementation partially attenuated lead -induced liver and stomach injury as measured by biochemical, and electronmicroscopic studies and differences in structure has been observed between the Zinc and lead, lead and the control groups. Present study results demonstrate the protective effect of Zinc in decreasing but not a complete protective effect in lead induced significant toxic pathological changes in the liver and stomach of the albino mice.

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Key Words: Electronmicroscopic, hepatocytes, lead acetate, zinc.

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INTRODUCTION

Exposure of Lead is one of the heavy metals is common in daily life. It is found in drinking water ,and found naturally in soil in low levels in Earth's crust, as lead sulfide. Moreover it is used in agriculture , however, lead is widely spread in the environment as a result of human activity, such as mining, smelting, etc. Lead is present in the environment, once released as an element and can be transported by atmospheric emissions to most remote regions of the world ***(Hernberg 2000 & IARC 2006)***.

Lead is used mainly in modern industries, and its occupational exposure can also occur during the production of lead-acid batteries, plumbing materials, alloys, glass and crystal. It is also present in the application of paints, cable sheathing and processing of materials painted with lead-containing paints.. Processing and recycling of electronics, and the use of leaded gas are other forms of occupational exposure ***(Patrick 2006 & Xu et al 2009)***.

Lead is considered dangerous and can cause environmental pollution due to its toxic effects on livings as plants, animals, and humans. Chronic exposure to lead even in low doses can induce kidney and liver damage, due to metal accumulation leads to neurological and hepatobiliary disorders. Researchers have found that exposure in more than permissible limits can cause haematological, immunological disorders, and even liver damage. They have proved the production of free radicals ***(Verity 1990 & Landrigan 2002 & Siposet et al 2003)***.

Lead enters the body through many routes: inhalation, eating and drinking. The limit to dietary lead ranges from 2.8 to 4.13 μg/kg of body weight per week. However, in smokers or people living in polluted areas their rate of exposure is increased. ***(Satarug & Moore 2004 & Kaczmarek-Wdowiak et al 2004)***.

Lead enters the body through two common routes: inhalation and ingestion. Inhalation is the most significant as pulmonary absorption is efficient. Lead is absorbed in the plasma. It can cross membranes such as the blood brain barrier and placenta, and accumulates in soft tissues and bones. Lead that is stored in bones has a long half-life of up to 28 years and can be mobilized intermittently at times of stress, lactation or hormonal imbalances. Gastrointestinal ingestion is the most common source of lead intake in the body as lead does not have a feedback mechanism to inhibit its uptake ***(Needleman 2004 & Pearce 2007)***.

The liver is one of the major organs involved in the biotransformation and detoxification of toxic substances it is considered as one of the target organs affected by lead toxicity as it is the site of storage after exposure. Also, absorbed lead is stored in soft tissues mainly in the liver via the portal vein, so that it is the first organ for which the histological analysis can be used to examine the morphological changes that reflect possible lead effects on cells ***(Metwally et al 2015)***.

The doses used in this study were higher than the current estimates of daily exposure of the general population to lead through diet, but were lower than that heavely exposed to lead as smokers or polluted areas.

Zinc is an essential trace element, exerting its effect through its protective effect as anti-inflammatory, antiapoptotic and antioxidant. Zinc supplementation is important for growth and development. Zinc is necessary for proper liver function while, the liver is important for the regulation of zinc homeostasis. Lead was proved to disturb enzymes dependent on zinc, and it is also necessary for metabolic processes by causing imbalanced metabolism ***(Sakata et al 2007)***.

Zinc could protect the liver from some diseases, but these hepatoprotective properties have not been fully elucidated. ***(Zhou et al 2005 & Sidhu et al 2005 & Mahran et al 2011)***.

For this reason possible protective effect of Zinc against lead should be studied. The present study was designed to detect the possible protective effect of Zinc against the toxic effects of lead acetate on liver and stomach in adult mice.

MATERIAL AND METHODS

I. Tested Article

Lead acetate and Zinc were used as a tested metalloid and mineral respectively. Lead was obtained from El-Gomhorreya Company (Arsenic), Alexandria, Egypt. Zinc was obtained as Zinc capsules (Zinctron) from Future Pharmaceutical Company.

II. Tested Species

Sixty healthy adult mice aged 3 months or weighted 25-35 gm, were acclimated for a week in the animal house of Human Anatomy& Embryology department, Faculty of Medicine,

University of Alexandria. The animal procedures were performed in accordance with Guidelines for Ethical Conduct in the Care and Use of Animals, maintained at room temperature of 25 ± 2°C with 12-hour dark-light cycle. They were fed with standard rodent diet. There was no water and light restriction throughout the experimental period. They were randomly divided into 3 groups of 20 mice each:-

Group I (Control group): included 20 mice, that were given distilled water by orogastric tube for two weeks.

Group II (Experimental group): included 20 mice, that were given lead acetate in a dose of 4mg/kg body weight by orogastric tube for two weeks.

Group III (Experimental group): included 20 mice, that were given lead acetate and Zinc in a dose of 25 mg/kg/body weight by the orogastric tube for two weeks.

The dose of lead was prepared by dissolving 0.6 mg of lead acetate trihydrate into 1000 ml of distilled water according to ***(Jin et al 2008)***.

Zinc capsules were opened and dissolved in distilled water.

After two weeks of exposure the following was done:

I- Biochemical examination of liver function

Blood samples were collected from the tail vein. Blood was collected from the 3 groups in sterilized dry centrifuge tubes. The mouse-tail was warmed by immersion in water (40°C) for 2 minutes. Blood was collected from the tip of the tail by squeezing the tail gently after it was cut using a new scalpel blade.. Blood samples were allowed to clot for 30-40 min at room temperature. Centrifugation was done at 2500 rpm for 15 min, serum was separated, and biochemical parameters of serum aspartate aminotransferase (AST), and serum alanine aminotransferase (ALT) were estimated by using ELISA (Enzyme Linked Immunosorbant Assay) technique ,at the Clinical Pathology department, Faculty of Medicine, University of Alexandria ***(Siest et. al., 1981 & Arneson et. al., 2007)***.

II- Gross anatomical study

After scarification of mice the stomach and liver and were extracted, examined and weighted. Relative liver mass (%) was calculated as a ratio of liver weight and body weight.

III- Ultrastructural study

After the sacrification of mice the stomach, right and left liver lobes from all animals were excised, and cut into specimens.

The specimen was immediately removed after sacrification and immediately fixed in 3% phosphate buffered glutaraldehyde (Ph 7.4) for 2 hours at 4ºC, and further processed for EM examination, and photography of the ultrastructure by Joel -100 CX transmission electron microscope in Faculty of Science, Alexandria University ***(Glauret 1986 et al & Bozzola et. al., 1992 & Trevor et. al., 1996)***.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. The values of AST and ALT of the 3 groups were presented as mean and standard deviation. The absolute and relative liver weights (ratio) were presented as mean and standard deviation. Data were analyzed using one way analysis of variance (ANOVA) If the ANOVA was significant, a Tukey-B was performed. Values of $p>0.05$ were considered nonsignificantly different, while those of $p<0.05$ and $p<0.01$ were significantly and highly significantly different respectively. (PC – STAT 1995)

RESULTS

During the experiment all mice were carefully monitored for behavior, appearance, palpable tumors, and infections. No mortality was detected in all groups during the study period. There was no rejection of the diet in both control and experimental groups.

I- Biochemical results

Serum AST and ALT

Mice exposed to lead acetate showed a significant increase in Serum AST and ALT concentrations as compared with the control group (Table I).

II. Gross anatomical structure of the liver: (Figure 1) and (Table 2)

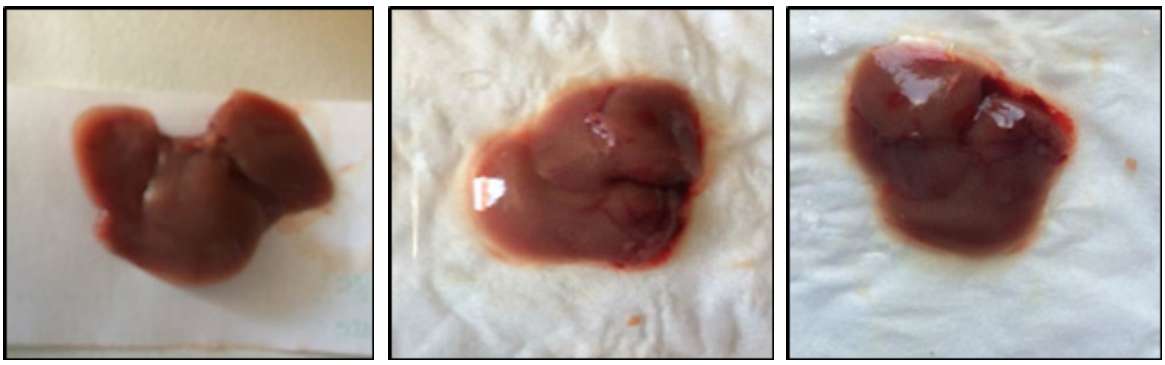
The body weight of the control group was 30± 5 gram ranging from 25 to 35 gram and 30± 5 after 2 weeks, while group II the body weight was 27 ± 6 gram ranging from 21 to 33. In group III the body weight was 29 ± 5 ranging from 24- 34. The weight of liver was significantly increased in group II and III as compared with control group (Table 2).

The exposure to Lead resulted in increased absolute liver mass in mice from the Lead (II) and Lead and Zinc groups (III) compared to the control group. Moreover, there was a difference in relative liver mass between the tested groups and control groups (Table 2).

III- Ultrastructural Results

The liver of control mice (group I) revealed normal hepatocytes with rounded regular euchromatic nuclei and prominent nucleoli. The cytoplasm of these cells showed numerous rounded to oval mitochondria, multiple parallel arrays of rough endoplasmic reticulum, smooth endoplasmic reticulum, glycogen particles and few lysosomes. Bile canaliculi were seen as narrow spaces limited by short microvilli of two adjacent hepatocytes. Kupffer cells with irregular nuclei and lysosomes were seen lining blood sinusoids (Figures 2a, b). Sections of liver of mice of group II revealed marked changes involving both the cytoplasm and the nucleus. The cytoplasm showed many vacuoles, multiple lipid droplets, areas of necrosis, dilated rough endoplasmic reticulum, dilated smooth endoplasmic reticulum and pleomorphic mitochondria with dense matrix. The nuclei of most of the hepatocytes showed dilated perinuclear cisternae and other showed vesiculation. Variability in shape and size of nuclei was noticed with vesiculation of their chromatin content. Many abnormal Kupffer cells were seen lining the blood sinusoids, and, distorted bile canaliculi with areas of necrosis (Figures 3a, b, c, d). Electron microscopic examination of group III revealed moderate changes of most liver cells. The cytoplasm exhibited pleomorphic mitochondria with dense matrix, dilated rough endoplasmic reticulum and dilated smooth endoplasmic reticulum, many lysosomes and glycogen. The nuclei are binucleated and irregular (Figures 4 a,b,c,d).

The stomach of mice group I revealed normal lumen of the stomach, regular nucleus, normal Golgi apparatus, multiple rough endoplasmic reticulum and secretory granules (Figure 5). Stomach of mice group II revealed pocket in nucleus, dialysis of organelles and abnormal Golgi, the lumen showed absence of microvilli ,and dark spots of lead in it, two cells with membrane in between irregular nucleus and lysis of rough endoplasmic reticulum (Figures 6a,b,c) The stomach of mice group III revealed Nucleus with slightly interrupted membrane, few microvilli in the lumen, multiple secretory granules and disturbed rough Endoplasmic Reticulum (Figures 7 a, b, c).

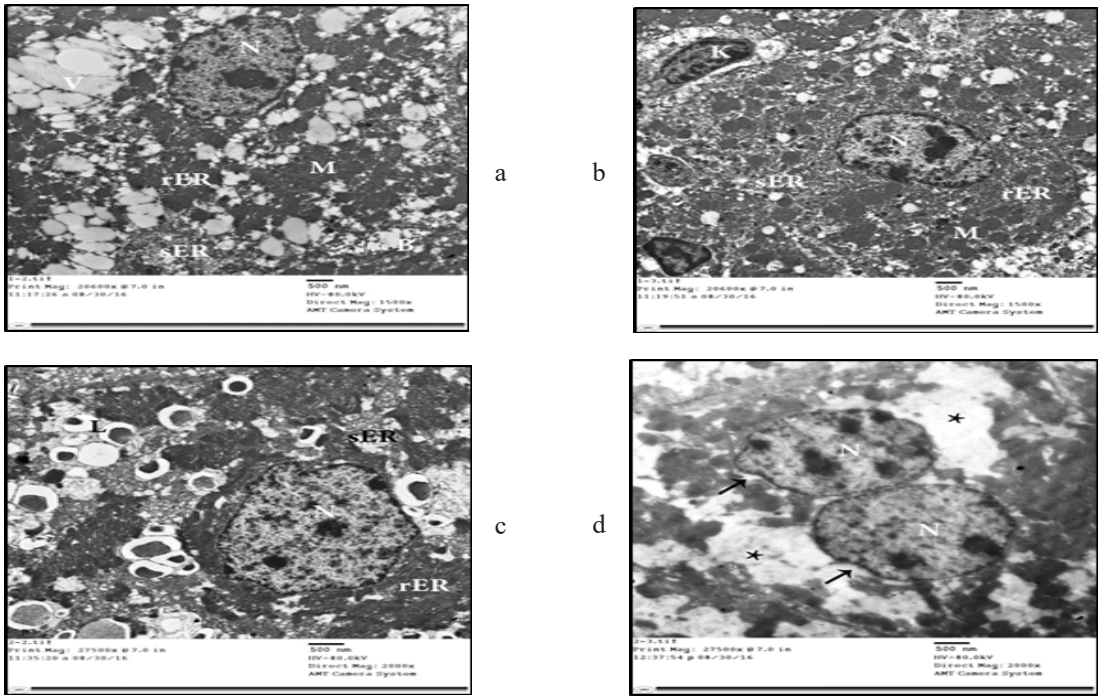


a b c

Fig. 1 (a, b, c): Photograph of morphology of the mice liver.
(a) Showing normal liver in mice in control group I.
(b) Showing disturbed liver with fat deposition group II.
(c) Showing liver with partial fat deposition in group III.

a b

Fig. 2: Electron micrograph of control rat liver (group I) showing:
(a): A hepatocyte with euchromatic nucleus (N) of regular outline. The cytoplasm contains numerous mitochondria (M), multiple arrays of rough endoplasmic reticulum (rER). Bile canaliculus (B) ,blood sinusoid (S).
(b): A large nucleus (N) and prominent nucleolus are noticed in its cytoplasm. The cytoplasm contains numerous mitochondria (M), multiple arrays of rough endoplasmic reticulum (rER). Glycogen in the form of rosettes are seen (G) (Mic. Mag. × 1500).



a b

c d

Fig. 3 (a, b, c, d): Electron micrograph of mice liver (group II) showing:
(a): Hepatocytes with irregular nucleus (N), swollen mitochondria with dense matrix (M), dilated rough endoplasmic reticulum (rER) and smooth endoplasmic reticulum (sER) the cytoplasm shows multiple vacuoles (V) of different sizes and distorted bile canaliculi (B). (Mic. Mag. × 1500).
(b): Hepatocyte with irregular nucleus (N), multiple pleomorphic mitochondria with dense matrix (M), dilated rough endoplasmic reticulum (rER) and multiple Kupffer cells (K). (Mic. Mag. × 1500).
(c): Hepatocyte with irregular dense and small nucleus (N), multiple lipid droplets (L) occupying most of the cytoplasm, multiple dilated smooth endoplasmic reticulum (sER) and rough endoplasmic reticulum (rER). (Mic. Mag. × 2000).
(d): Binucleated(N) hepatocyte with the nucleus shows vesiculation with dilated perinuclear cisternea (↑) and the cytoplasm showing areas of necrosis (*). (Mic. Mag. × 2000).

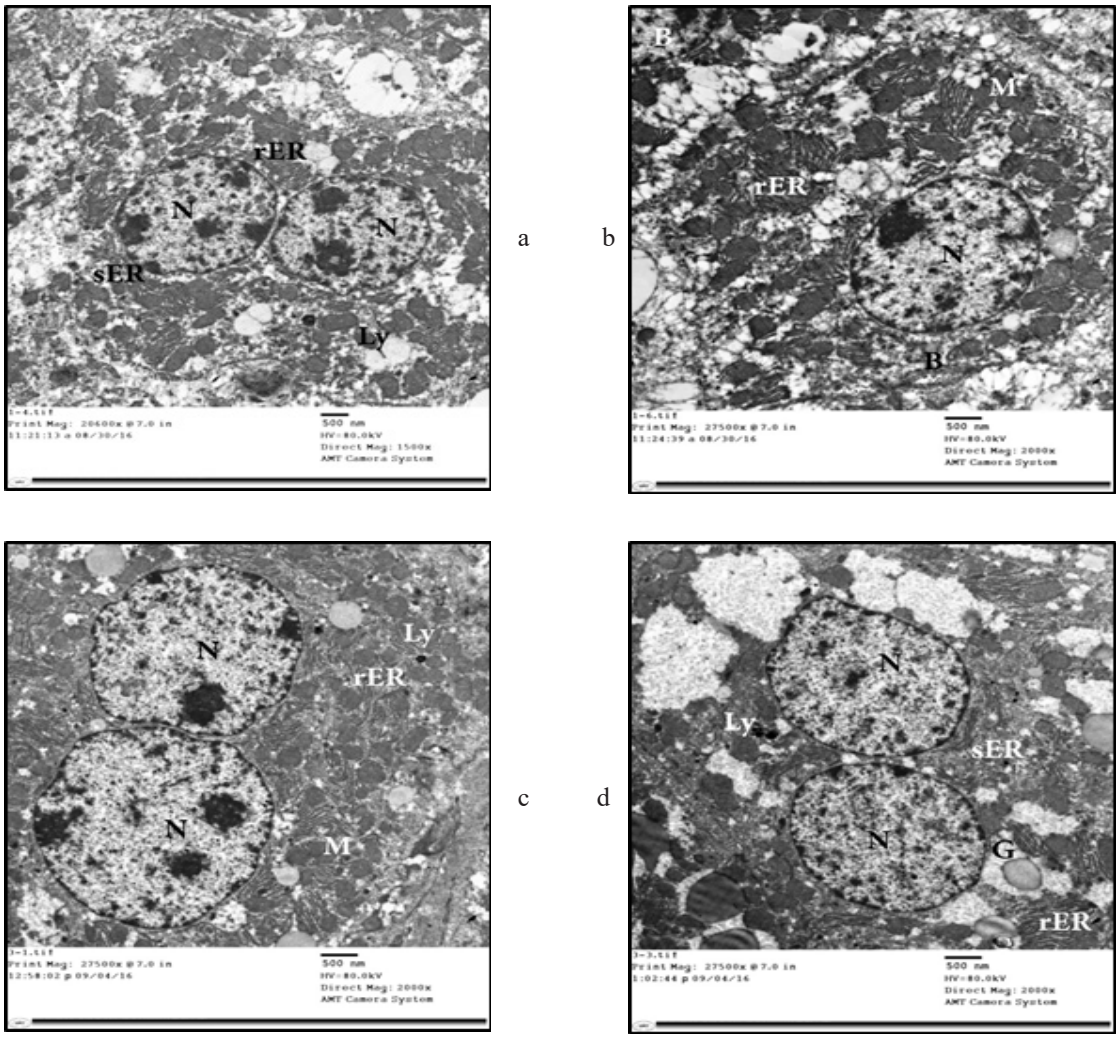


Fig.4 (a, b, c, d): Electron micrograph of mice liver (group III) showing:
(a) Binucleated (N) hepatocyte with the cytoplasm containing multiple rough endoplasmic reticulum (rER), dilated profiles of smooth endoplasmic reticulum (sER),) and lysosomes (Ly). (Mic. Mag. × 1500)
(b) Regular nucleus (N), the cytoplasm contains multiple rough endoplasmic reticulum (rER), vacuoles (V), narrow bile canaliculi (B) and multiple irregular mitochondria (M). (Mic. Mag. × 2000).
(c) Binucleated(N) hepatocyte with irregular nucleus multiple rough endoplasmic reticulum (rER), Mitochondria (M) and lysosomes (Ly). (Mic. Mag. × 2000)
(d) Binucleated (N) hepatocytes enclosing multiple rough endoplasmic reticulum (rER), dilated profiles of smooth endoplasmic reticulum (sER), lysosomes (Ly) and glycogen (G). (Mic. Mag. × 2000).

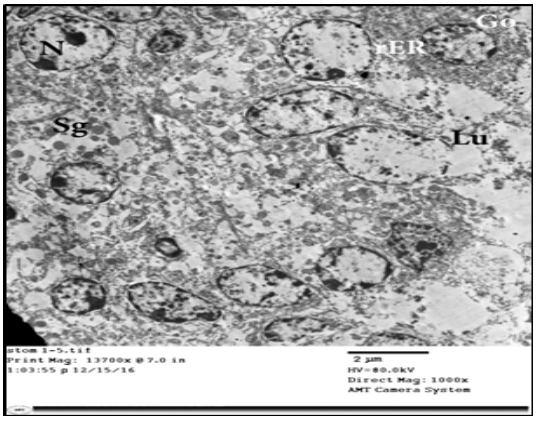
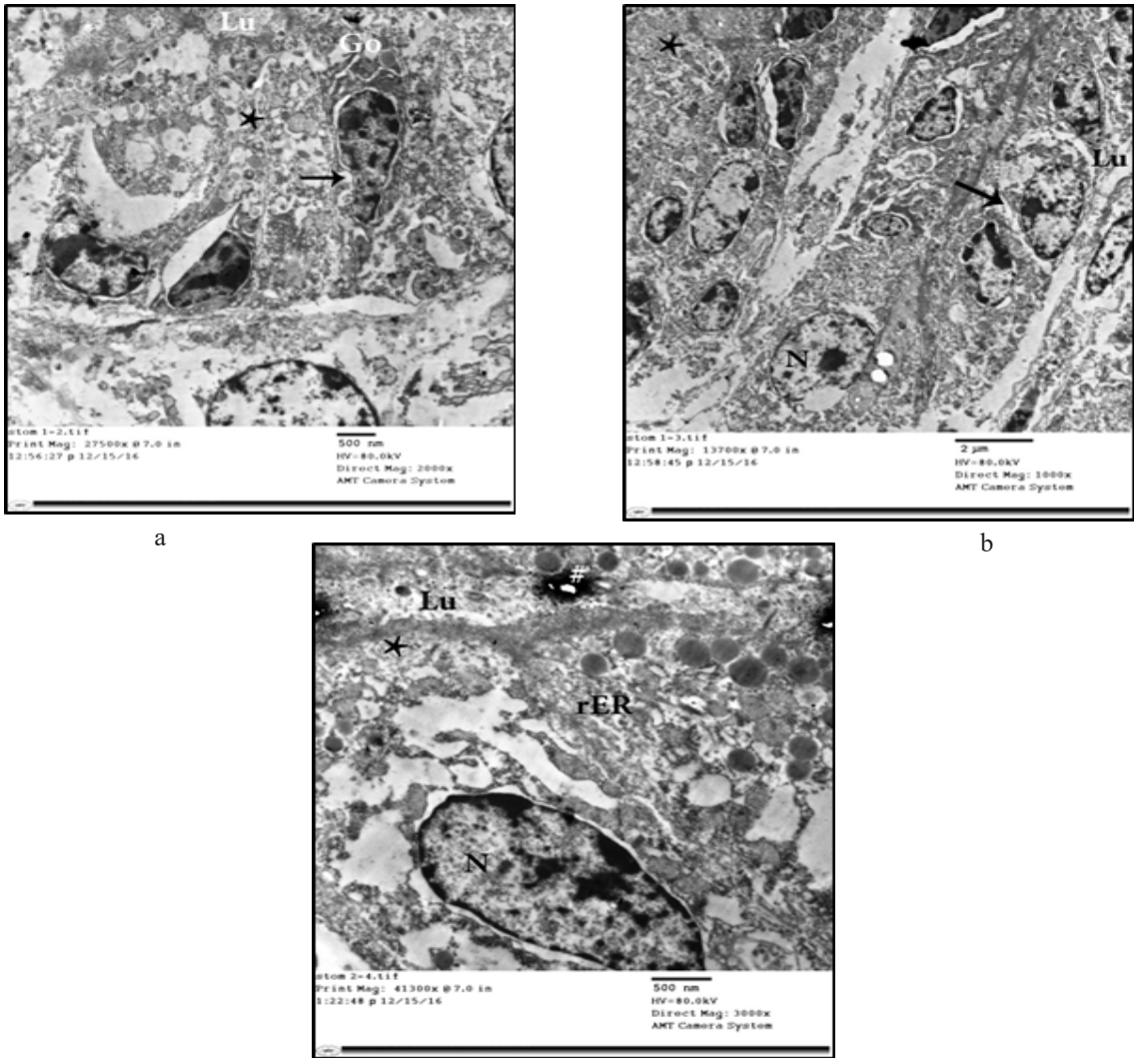
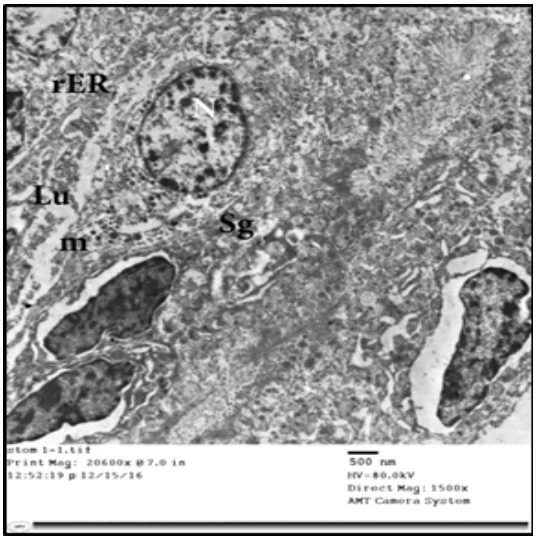


Fig. 5: Electron micrograph of the mouse stomach group I (control group) showing normal lumen of the stomach (Lu), regular nucleus (N), normal Golgi apparatus (Go), multiple rough endoplasmic reticulum (rER) and secretory granules (Sg). (Mic. Mag. × 1000).

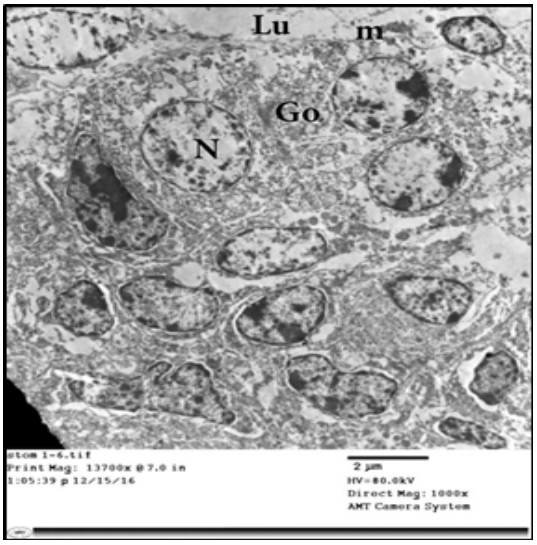


a b c

Fig. 6 (a, b, c): Electron micrograph of the mouse stomach group II showing:
(a) Pocket in nucleus (↑), lysis of organelles (*) and abnormal Golgi (Go) & lumen (Lu). (Mic. Mag. × 2000)
(b) Two cells with membrane in between (arrow), irregular nucleus (N) and lysis of organelles (*), lumen (Lu) with no microvilli .(Mic. Mag. × 1000)
(c) The lumen (Lu) with no microvilli and dark spots of lead in it (#), irregular nucleus (N) and lysis of organelles (*), disturbed rough endoplasmic reticulum (rER). (Mic. Mag. × 3000)



a



b

Fig. 7 (a, b): Electron micrograph of the stomach group III showing:
(a) The Nucleus with slightly interrupted membrane (N), the lumen(Lu), with few microvilli(m) multiple secretory granules (Sg), disturbed rough Endoplasmic Reticulum (rER). (Mic. Mag. × 1500)
(b) The lumen (Lu), with few microvilli (m),Golgi (Go) and slightly irregular nucleus (N). (Mic. Mag. × 1000)

Table1: Values of AST and ALT in control and experimental mice

	Control	group II a Experimental	group III b Experimental
ALT [IU/L]	38.2 ± 5.4	57.7 ± 6.5 *	52.2 ± 9.2 *
AST [IU/L]	52.4 ± 6.2	154.6 ± 43.3 **	109.0 ± 14.0 *

Data are expressed (15 mice in each group) as Mean ± SD
a. group taking lead acetate
b. group taking zinc and lead acetate
* Statistically significant at $p \leq 0.05$
** Statistically significant at $p \leq 0.01$

Table 2: Body weight, Absolute and Relative Liver Weight

	Control group I	Group II a (Experimental)	Group IIIb (Experimental)
Body weight	30± 5	27 ± 6	29 ± 5
Absolute weight [g]	1.72 ± 0.02	2.13 ± 0.40*	1.98 ± 0.09*
Relative liver weight	0.06±0.002	0.08±0.001	0.07±0.001

Data are expressed (20 mice in each group) as Mean ± SD
a. group taking lead acetate
b. group taking zinc and lead acetate
* Significantly different from control at $P \leq 0.05$.
# absolute organ weight (g)/ terminal body weight (g)

DISCUSSION

Chronic exposure to lead even in low doses can induce kidney and liver damage due to metal accumulation can cause neurological and hepatobiliary disorders ***(Verity 1990 & Landrigan 2002 & Siposet et. al., 2003)***.

In the present study, mice in group II and III treated with lead acetate ,and zinc with lead acetate respectively showed a significant increase in serum aspartate aminotransferase (AST), and alanine aminotransferase (ALT) concentrations ,as compared with the control group. These aminotransferases are liver enzymes and form a major constituent of the liver cells. ,in lesser concentration in muscles cell. Damage or injury to the liver cells lead to leak of these enzymes into the blood stream, rising their blood levels. Hence raised blood levels of AST and ALT signifies liver disease or injury. In the present study their level was increased in both experimental groups as compared to the control one, but they were increased more significantly in the group exposed only to lead acetate. AST is normally present in a number of tissues such as heart, liver, muscle, brain and kidney. It is released into the blood stream whenever any of these tissues gets damaged. For instance, blood AST level is increased in conditions of muscle injury and heart attacks. Hence, it is not highly specific in liver tissue damage as it can be elevated in other conditions. By contrast, ALT is normally present in large concentrations in the liver. So it acts as a specific indicator for liver injury when its level in the blood rises. ***(Upasani & Balaraman 2001 & Blazovic 2001)***.

Heavy metals inducing oxidative stress change physiological processes through emission of free radicals, and lead to cell damage by accumulation of toxic metals ***(Valko et al 2005)***. Increase AST with lead toxicity in the present study was in agreement with the studies done by ***(Khan et al 2008, Moussa & Bashandy 2008, Herman et al 2009, Allonche et. al., 2011, Azoz & Raafat 2012)***.

Serum AST and ALT were less increased or decreased in group III treated with Zinc and lead although all the mice received similar amounts of lead in the diet. This was in agreement with the studies done by ***(Borah et. al., 2011 & Mahran et. al., 2011)***.

In the present study, the weight of the liver was significantly increased in group II as compared with control group. ***Ibrahim et. al., 2012***, explained this due to reticular cell hyperplasia as a defense mechanism to the necrosis and apoptosis which could be attributed to the accumulation of the lipids in the organ due to lead exposure. These results are in accordance with ***(Upasani & Balaraman 2001)***, they found that lead treatments produced a significant accumulation of lipids in kidney cells of rats. The increased weight of liver and other organs was attributed to tumorigenicity of lead salts in general, and revealed that lead acetate is carcinogenic in rats especially to the kidney as a target organ ***(Valko et. al., 2006)***. The weight of the liver in group III was slightly decreased than the control group. Zinc and copper are essential components of antioxidant enzymes of the body that play an important role in the prevention of free radical – induced damage to tissues ***(Evans & Halliwel 2001)***. Moreover zinc protects the peroxidation of membranes lipids, possibly by displacing bound transition metal ions ***(Bettiger et. al., 1980)***.

In the present study; EM examination of sections of liver of group II revealed marked degenerative changes and necrosis of liver cells involving both the cytoplasm and the nucleus. The cytoplasm showed many vacuoles, lipid droplets, areas of necrosis, dilated rough endoplasmic reticulum, dilated smooth endoplasmic reticulum and pleomorphic mitochondria with dense matrix. The nuclei of most of the hepatocytes were irregular. Variability in shape and size of nuclei, irregular nuclear membrane, perinuclear cysterna, binucleation accompanied by dense nucleolus indicating coiling of their chromatin content. Many Kupffer cells were seen lining the blood sinusoids.

These findings in agreement with those of ***(Jerrar and Taib 2012)*** who observed that chronic exposure to subtoxic concentrations of lead, produced changes in the hepatocytes, portal triads and the sinusoids. They detect many cytoplasmic inclusions, lipid droplets, cytoplasmic swelling, hydropic degeneration, necrosis and reduction in glycogen content. In addition, portal triads showed mild chronic inflammation, Kupffer cells hyperplasia and occasional fatty change were seen, hyperplasia and occasional fatty change were seen together with hemosiderosis. This is also consistant with the findings of ***(Mahran et. al., 2011)*** who observed a blurred trabecular structure, vacuolar degeneration and increased density of nuclear chromatin with very compact nuclear structure in hepatocytes with mononuclear cell infiltrations and single cells necrosis.

In the present study many vacuoles were seen in the group II. Cell vacuolation is an indicator of hepatic toxicity and it is a cellular defense mechanism against injurious substances. These substances were segregated in vacuoles, and thus prevented from interfering with cellular metabolism. It has also been suggested that cytoplasmic vacuolation is mainly a consequence of disturbances in lipid inclusions and fat metabolism ***(Mollendorf 1984)***. This vacuolation might also result from disturbance in the oxidative phosphorylation in the mitochondria with suppression of ATP production, and failure of the ATP dependent sodium pump at the cell membrane, resulting in accumulation of sodium intracellularly, and consequent entry of water into the different cellular compartments leads to cellular swelling. For several years, a special attention has been paid to oxidative stress; situation of an excessive production of reactive oxygen species. The hepatocytes necrosis due to chronic lead exposure might indicate oxidative stress on these cells by glutathione depletion. ***(Haouas et. al., 2014)***.

Moreover, hepatotoxins rapidly induces proinflammatory cytokines, such as TNF-α and IL-1β by Kupffer cells, and recruit stromal cells of the liver to participate in this inflammatory response through paracrine production of cytokines to attract circulating immune cells, further amplifying an inflammatory response ***(Aykin-Burns 2003 & Haouas et. al., 2014)***. Moreover, Kuppfer cells produce Chemokines in cases of liver damage play an important role in inflammatory responses as mediators of leukocyte activation and maturation ***(Haouas et. al., 2014)***.

In the present study the nuclei of hepatocytes showed many changes. Considerable alterations induced by lead intoxication were seen in the nuclei of the hepatocytes. They were mainly in the form of irregular nucleus, binucleation, perinuclear cysterna and prominent nucleolus. This is consistent with the findings of ***Mahran et. al., 2011*** which observed anisokaryosis, nuclear vesiculation, binucleation, This may be due to increased cellular activity and nuclear interruption as a mechanism in lead detoxification. Some of the pleomorphism alterations seen in the present study were in agreement with ***(Abd El-aal 1989, Nehru & Kaushal 1993, Jarrar et. al., 2006, Jarrar & Taib, 2012). (Zusman et. al., 1991)*** in their studies indicate that nuclear polymorphism is seen in hepatic dysplasia and carcinomatous lesion. Cell necrosis and vacuolization induced by lead toxicity as shown by the present work were described previously by other studies ***(Abd El-aal et. al., 1989, Nehru & Kaushal 1993)***.

The cytoplasmic swelling with hydropic degeneration as seen in the results of the present study might be accompanied by the leakage of lysosomal hydrolytic enzymes that explain cytoplasmic degeneration and macromolecular crowding ***(Del Monte 2005)***.

The data of the present study showed that lead activates the phagocytic activity of the sinusoidal cells by increasing the number of Kupffer cells. Similar findings were reported by other investigators ***(Nehru & Kaushal 1993)***. This might be a result of increased autophagy throughout the hepatic tissue to help in removing the accumulated lead and its metabolites, where lysosomes are involved in the intracellular breakdown into small metabolic products. The produced Kupffer cells hyperplasia might be correlated with the amount of injury to the hepatic tissue induced by lead intoxication and represent a defense mechanism of detoxification and might be contributed to hepatic oxidative stress ***(Neyrinck 2004)***.

In the present study the lead group showed marked effect on the hepatocytes. Experimental data have demonstrated that activation of Kupffer cells is a major event in initiation of liver injury. The activation of Kupffer cells is an important source for induced inflammatory mediators such as TNF-α, IL-1β, IL-6, and IL-8, which in turn contribute to generation of free radicals in the liver ***(Ahamed 2007)*** Moreover, recent evidences indicated a cross-talk between liver macrophage/ Kupffer cell and Hepatic stellate cells, located in the space of Disse's and comprise approximately one-third of the non-parenchymal cell population, leading to their activation ***(Bartosz 2008 & Al-Fara 2010 & Almansour et. al., 2009)***. Liver fibrosis represents the final common pathway of almost all types of chronic liver diseases characterized by excessive connective tissue deposition in extracellular matrix. Reactive Oxygen Species can activate fibrogenic gene expression and transforming growth factor (TGF-β1) signaling pathway, which is known to play major role in the activation of in liver fibrosis ***(Bartosz 2008)***. In a healthy liver, hepatic stellate cells contain the largest reservoir of vitamin A in the body ***(Pande 2002)***. When the liver is injured due to viral infection or any hepatic toxicity, they receive signals secreted by

damaged hepatocytes and immune cells, causing them to trans-differentiate from a resting vitamin A-rich cell into active, proliferating, fibrogenic, and contractile cell leading to hepatic fibrosis. Hepatic stellate cells when activated cause the accumulation of extracellular matrix materials, including type I collagen ***(Del Monte 2005)***.

In the present study the electron microscopic examination of Group III, revealed more or less preserved hepatic cells. Moderate changes of most liver cells were seen. The cytoplasm exhibited pleomorphic mitochondria with dense matrix, dilated rough endoplasmic reticulum and dilated smooth endoplasmic reticulum, many lysosomes and glycogen. The nuclei were slightly irregular and showed binucleation. This is consistent with the findings of ***(Hu et. al., 2011)***, who concluded that Zinc supplementation (50 mg/kg/d) for 5 d of mice treated with Lead acetate (1.5 g/kg, ip) could reduce their mortality rate, restore liver pathomorphological changes, maintain zinc content, inhibit the lipid peroxidation, hasten the protein synthesis, and improve liver function.

Zinc is essential components of antioxidant enzymes of the body, that play an important role in the prevention of free radical – induced damage to tissues ***(Evans & Halliwel 2001)***, in addition zinc protects the peroxidation of membrane lipids, possibly by displacing bound transition metal ions ***(Bettiger et. al., 1980 & Patora & Swarup 2004)*** recorded that administration of lead significantly decreased zinc and copper concentration in cardiac tissue of calves, where it leads to reduction in the absorption of micronutrients from gastrointestinal tract, besides interaction of lead and trace mineral at tissue level . Lead nephropathy, possibly because of high lead burden, was found to be coupled with lower protective factors, notably of Zinc ***(Satarug et. al., 2004). (Mahran et. al., 2011)*** found that Zinc partially alleviated the damage observed in both the liver and kidney, and differences in histological structure has been observed between the Zn-Cadmium and the cadmium groups. Zinc supplement could abate the death of D-galactosamine intoxicated hepatocytes, decrease malonaldehyde content, and maintain reduced glutathione so zinc has protective effects on D-galactosamine -induced liver damage. Its effects may be owing to inhibition of lipid peroxidation and hastening of protein synthesis. ***(Sidhu et. al., 2005)*** found the protective effects of zinc on the enzymes involved in oxidative stress induced in liver of protein-deficient rats. The effects of zinc treatment in conditions of protein deficiency were studied on rat liver antioxidant enzymes, which included catalase, glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), and glutathione-S-transferase (GST). Protein deficiency in normal rats resulted in a significant increase in hepatic activities of catalase, glutathione peroxidase, glutathione reductase, and glutathione-S-transferase and the levels of lipid peroxidation. A significant inhibition in the levels of reduced glutathione and the enzyme activity of superoxide dismutase has been observed after protein deficiency in normal rats. Zn treatment to protein-deficient animals lowered already raised activity catalase, glutathione peroxidase, and glutathione-S-transferase and levels of lipid peroxidation to significant levels when compared to protein-deficient animals. Also, Zn treatment to the protein-deficient animals resulted in a significant elevation in the levels of superoxide dismutase (SOD), activity as compared to their respective controls, thereby indicating its effectiveness in regulating their levels in adverse conditions. It has also been observed that concentrations of zinc, copper, iron, and selenium were found to be decreased significantly in protein-deficient animals. The levels of these elements came back to within normal limits when zinc was administrated to protein-deficient rats. One concluded that zinc has the potential to regulate the activities of oxidative stress enzymes as well as essential hepatic elements. Lead administration cause excessive production of reactive oxygen species (ROS) affects parameters indicating oxidative stress (increased glutathione levels) and causes Zinc depletion as Zinc deficiency has been implicated in the pathogenesis of liver diseases ***(Zhou 2005 & Alcaraz et. al., 2011 & Haouas et. al., 2014)***. That is why Zinc was used to replace the depletion and as an antioxidant that reduces glutathione level and other oxidative enzymes ***(Sidhu et. al., 2005)***.

Few literatures study effect of lead on the stomach.In the present study the Stomach of mice group II treated with lead revealed pocket in nucleus, lysis of organelles and abnormal Golgi, the lumen with no microvilli and dark spots of lead in it, two cells with membrane inbetween irregular nucleus (blebbing, pyknotic) and lysis of rough endoplasmic reticulum. The stomach of mice group III treated with lead and zinc revealed Nucleus with membrane slightly interrupted, Nucleus slightly irregular, the lumen with few microvilli, multiple secretory granules,

and lysis of organelles due to the swelling of intracellular organelles especially mitochondria and endoplasmic reticulum. Apoptotic alteration might be followed by organelle swelling especially the mitochondria, endoplasmic reticulum and rupture of lysosomes ***(Rosser and Gores, 1995)***

(Tomaszewska et. al., 2015) found intensive negative changes in jejunal epithelium and liver, probably caused by the high lead and cadmium intake (in relation to body mass), which resulted in the increased liver mass, and liver content of heavy metals. It was found found that Injury of intestinal mucosa can be caused by infringement of the epithelial barrier by inducing apoptosis and desquamation of premature enterocytes. Moreover, the junctions between the cells of the intestinal epithelium were disturbed ***(Tomkzoc et. al., 1988)***.

CONCLUSION

Over viewing the present results, one can concluded that exposure to lead acetate induces chronic inflammation, oxidative stress and toxic injury in the liver, and could change the structure of stomach mucosa. Based on the results obtained, Zinc supplementation attenuated lead -induced liver injury as measured by biochemical, and electronmicroscopic studies. Zinc partially alleviated the damage observed in both the liver and stomach, and differences in histological structure has been observed between the Zinc and lead, lead only and the control groups. The present results demonstrate the protective effect of Zinc in decreasing but not a complete protective effect in lead induced significant toxic pathological changes in the liver and stomach of the albino mice.

CONFLICT OF INTERESTS

There are no Conflicts of Interest.

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تأثير الزنك على سمية خلات الرصاص في الكبد والمعدة في فئران التجارب: دراسة ميكروسكوبية وبيوكيميائية

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ملخص البحث

خلفية البحث: الرصاص هو أحد المعادن الثقيلة الشائع تعرض الناس لها يوميا. فهو يوجد في مياه الشرب ويتوافربصورة طبيعية في التربة و فى المستويات المنخفضة في قشرة الأرض في صورة كبريتيد الرصاص ويعتبر الرصاص خطير ويمكن أن يسبب التلوث البيئي بسبب آثاره السامة على الأحياء. الابتلاع هو المصدر الأكثر شيوعا لتناول الرصاص في الجسم. عن طريق الجهاز الهضمي التعرض المزمن للجرعات المنخفضة يمكن أن يحدث تلفاً في الكبد كما لأنه يعتبر واحدا من الأعضاء المستهدفة المتضررة من التسمم بالرصاص. الزنك هو عنصر أساسي أثر، وتأثير وقائي كمضاد للالتهابات، ومضاد للأكسدة ولكن هذا التاثير الخصائص على الكبد لم يتم توضيحها من قبل كاملة.

الهدف من العمل: دراسة التأثير الوقائي المحتمل للزنك ضد التأثيرات السمية لخلات الرصاص على الكبد والمعدة في الفئران البيضاء.

المواد والطرق: تم تقسيم ستين من الفئران البيضاء الأصحاء البالغين عشوائيا إلى 3 مجموعات تتكون كل مجموعة من 20 من الفئران. أعطيت المجموعة الأولى (مجموعة التحكم) الماء المقطر عن طريق أنبوب معدة. أعطيت المجموعة الثانية (مجموعة ضابطة) خلات الرصاص في جرعة من 4 ملجم/ كجم من وزن الجسم عن طريق أنبوب معدة لمدة اسبوعين. أعطيت المجموعة الثالثة (مجموعة ضابطة) خلات الرصاص والزنك في جرعة من 25 ملجم / كجم / يوميا بواسطة أنبوب معدة لمدة اسبوعين. تم قياس انزيمات الكبد و اجرى الفحص المجهري بالميكروسكوب الإلكتروني للكبد والمعدة.

النتائج: لوحظ زيادة كبيرة في انزيمات الكبد في المجموعات التجريبية (المجموعة الثانية والثالثة) ولكن كانت الزيادة أكثر في المجموعة الثانية. أظهر الفحص المجهري بالميكروسكوب الإلكتروني تغييرات ملحوظة تضم كلا من السيتوبلازم والنواة. أظهر السيتوبلازم العديد من الفجوات، وقطرات الدهون، وتوسع في الشبكة الاندوبلازمية الخشنة والملساء. وظهرت الميتوكوندريا في أشكال متعددة مع وجود الأشكال مصفوفة كثيفة. وأظهرت النواة في خلايا الكبد أشكال مختلفة كصهاريج متسعة حول النواة، مع اختلاف في حجم النواة. وشوهد العديد من خلايا كوبفر غير طبيعية في بطانة الجيوب الدموية، ونفيق الصفراء.وأظهر الفحص المجهري بالميكروسكوب الإلكتروني في المجموعة الثالثة من الفئران تغييرات معتدلة في معظم خلايا الكبد. بالنسبة للميتوكوندريا و اتساع فى الشبكة الإندوبلازمية الخشنة والملساء، كما ظهرت العديد من جسيمات الجليكوجين. ونوى غير منتظمة أو نواتين. أظهرت خلايا المعدة في مجموعة الفئران الثانية جيوب في النواة، وجولجي غير طبيعي، والبقع الداكنة من الرصاص في ذلك، واثنين الخلايا مع غشاء المنتصف ونواة غير منتظمة وتحلل في الشبكة الإندوبلازمية الخشنة والملساء. وأظهرت خلايا المعدة في مجموعة الفئران الثالثة تغير في شكل النواة مع وجود تجاويف، والقليل من الزغيبات الصغيرة.

الاستنتاجات: هذا البحث أظهر أن خلات الرصاص تسبب الالتهاب المزمن، والاكسدة والسمية للكبد ويمكن أن تغير الغشاء المخاطي في المعدة. كما وجد أن مكملات الزنك تخفف جزئياً من تأثير سمية الرصاص على الكبد والمعدة عن طريق الفحوص الكيميائية والفحص المجهري بالميكروسكوب الإلكتروني حيث وجدت فروقات بين مجموعة خلات الرصاص فقط ومجموعة الزنك وخلات الرصاص ومجموعة التحكم, أثبتت النتائج التأثير الوقائي الجزئي في تخفيف سمية الرصاص و هذا التاثير الوقائى ليس واقيا نهائيا ضد السمية والتغيرات الباثولوجية في الكبد والمعدة لفئران التجارب.