Effect of Lead Toxicity on Liver of Male Albino Mice Ultrastructural Study

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دراسة دقيقة حول تأثير سمية نترات الرصاص على كبد الفئران البيض

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الخلاصة:

تهدف هذه الدراسة بالكشف عن التغيرات التي يمكن ان تحدثها سمية الرصاص في خلايا الفئران البيض. تم تعريض مجموعتين من الفئران البيض كل مجموعه تحوي ٦ حيوانات إلى تركيز ٢٥% من نترات الرصاص في مياه الشرب لمدة ٤اشهر بينما مجموعة التحكم احتوت على ماء مقطر فقط. لقد أوضحت خلايا الكبد للحيوانات المعاملة بالرصاص وبالمقارنة مع مجموعة التحكم تغيرات شديدة في التراكيب الدقيقة التي تشمل المايتوكندريا والشبكة الاندوبلازمية واللايسوسوم.

أظهرت النتائج ان التعرض لتراكيز منخفضة من الرصاص عن طريق مياه الشرب قادرة على احدث تغيرات شديدة في التراكيب الدقيقة لعينات خلايا هذه الأنسجة.

Abstract

Lead is a multi targeted toxicant causing effects in different organs of the body. The present ultrastructural study was undertaken on the hepatic tissue of male albino mice.Two groups of male albino mice, 6 animals each were used. The treated group was exposed to 0.25% lead nitrate in drinking water for 4 months,and control group, in comparison with respective control mice. Chronic exposure to subtoxic doses of lead produced ultrastructural alterations in hepatocytes involving mitochondria, endoplasmic reticulum, lysosomes and nuclei. The present study shows chronic exposure to low subtoxic doses of lead, it can induce adverse subcellular alterations in the hepatic tissues.

Introduction

Lead is being an ubiquitous environmental contaminant due to its significant role in modern industry.However, both occupational and environmental exposures remain a serious problem in many developing and industrializing countries [1]. It has many undesired effects, including neurological behavioura[2,3], immunological [4, 5, 6, 7], renal [8, 9, 10], hepatic [9], and haematological dysfunctions [11, 12]. Lead toxicity is closely related to its accumulation in certain tissues such as hepatic tissue and however, interfere with hepatic functions and limited ultrastructural changes(8, 9**)**. Investigations have been done on the tissues of experimental animals exposed to lead (8,10). Studies the alterations at the ultra structural level in the hepatic tissues due to chronic lead exposure are rather limited and need more information about this effect. With this objective, the present ultra structural study was undertaken on hepatic tissue of male albino mice exposed to lead nitrate in the drinking water.

Key words: hepatocyte-lead nitrate-toxicity

Materials and Methods

In this study were used 12 male albino mice of the same age Animals were randomly divided into two groups of 6 mice. Each animals were caged at room temperature, lead nitrate was administered in drinking water at the rate of 0.25% for the treated group while the control group was given distilled water. The animals from each group were killed by dislocation of the neck.

Electron microscopy:

Small pieces of liver from each experimental mice of both groups were transferrd immediately to buffered gluteraldehyde fixative for 3hr , washed in 2 changes of phosphate buffer (PH7.2) at 4c^{o}. Tissue specimens were post fixed in 1%. osmium tetroxide in phosphate buffer for 2hr at 4c^{o}, then washed in phosphate buffer . Tissue specimens were then dehydrated in ascending grades of ethanol alcohol , cleared 2 changes of propylene Oxide ,infiltrated gradually in resin and embedded Epon 812 at 60 c^{o} for 2 days . Ultra thin sections (0.5micron) were cut using class knife and stained with uranyl acetate and lead citrate . Tissue sections were then examined and photographed by transmission electron microscope Philips (CM10).

Results

Examination under electron microscope of the hepatic tissues of control mice,show the normal ultrastructural pattern scattered mitochondria and rough endoplasmic reticulum (Fig. 1) . The ultrastructural changes induced by lead chronic exposure in the hepatocytes of the treated mice were as follows:

The mitochondrial ultra structural abnormalities were evident at 4 months of lead nitrate treatment. There was a general reduction in the number of mitochondria in hepatocytes in comparison with those of the control mice , and the mitochondria became swollen and

it matrix was of variable density. In the swollen mitochondria, cristae were marginated and short if compared to the well-preserved parallel ones of the control mice. In many of the affected mitochondria, there was a loss of cristae structures and low density or destructed rough endplasmic reticulum (RER),due to fragmentation structures representing the damaged mitochondria were noticed(fig2&3).

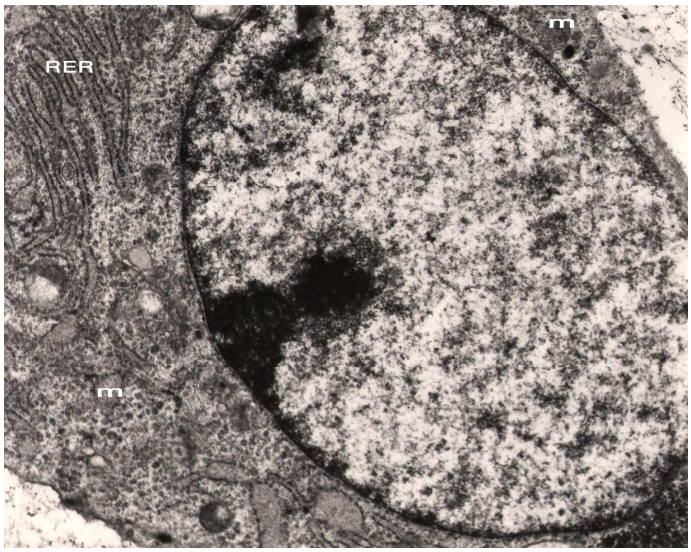

Fig1.Electron micrograph of h epatocytes of control mice Demonstrating the normal ultra structural pattern. Microchondri (m) have distinct cristae.Note the RER(r).X14000

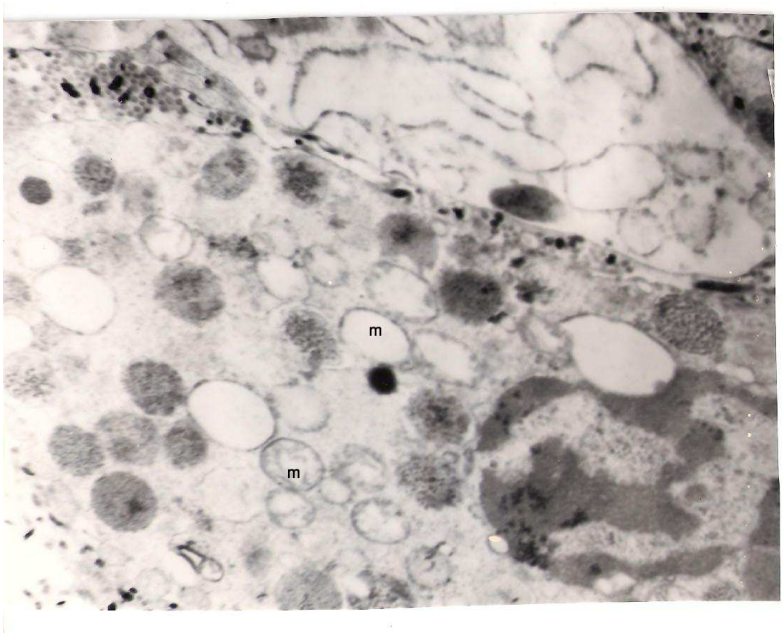

Fig2. Electron micrograph of hepatocytes from treated mice recived 0.25% lead nitrate for 4 months.The mitochondria (m) are swollen, often vacuolated with distruptedcristae X25.000.

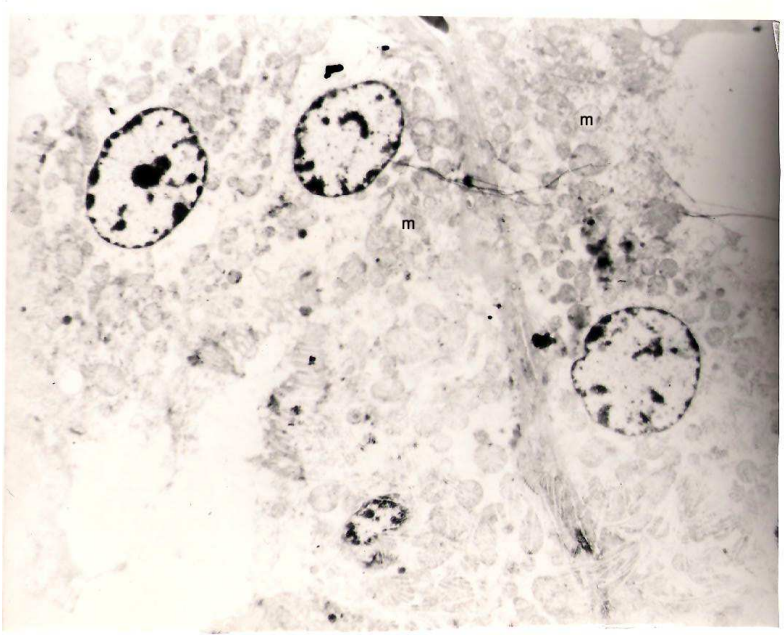

Fig3. Electron micrograph of hepatocytes from treated mice recevid 0.25% lesd nitrate for 4 months .The hepatocyte shows distruction of cristae of mitochondria(m) and almost complete destruction of RER.X8000.

and in (fig.4) show increase in the number of lysosome within the hepatocytes of lead treated mice at 4 monthes,some of the lysosome containing hepatocytes had autophagic vacuoles and showed indistinct outlines due to partial cell destruction.And affected

hepatocytes frequently contained large number of droplets. These droplets varied in size and more observed at the peripherey of cells (Fig.5). Occasionally, some hepatocytes appeared as shrunken condensed cells which had irregular outlines (fig. 6).

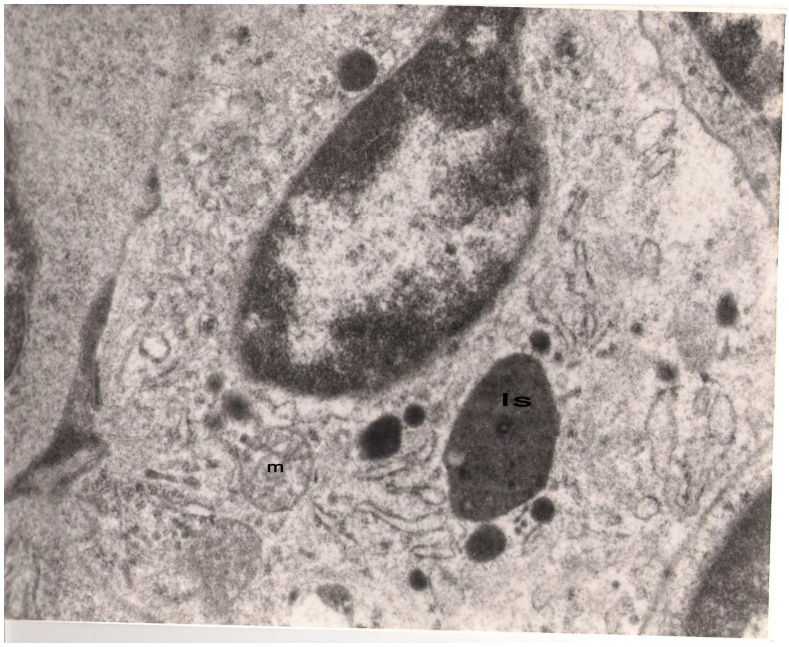

Fig4. Electron micrograph of hepatocytes from treated mice received0.25%lead nitrate for 4 months .Numerous of lysosomal structures(ls)of variable size are seen.X25000.

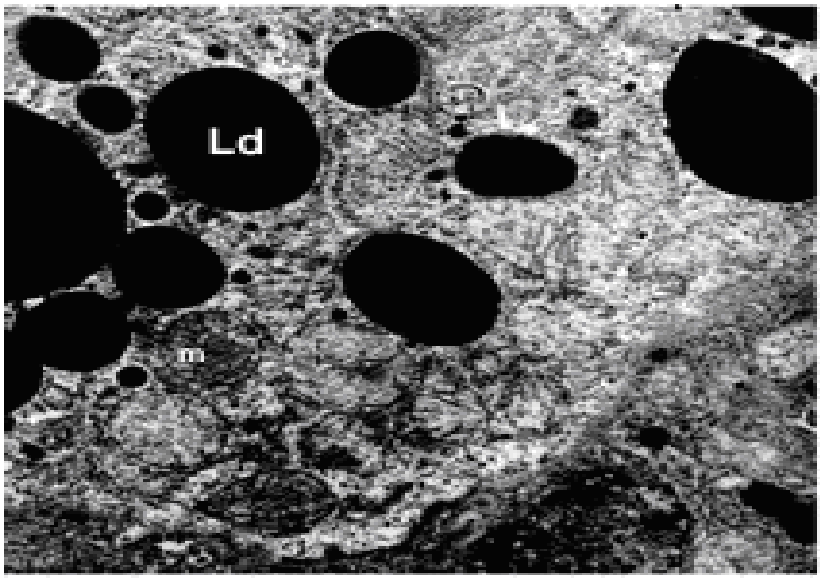

Fig5. Electron micrograph of hepatocytes from treated mice received0.25%lead nitrate for 4 months. Note the cytoplasmic lipid (Ld) X27000.

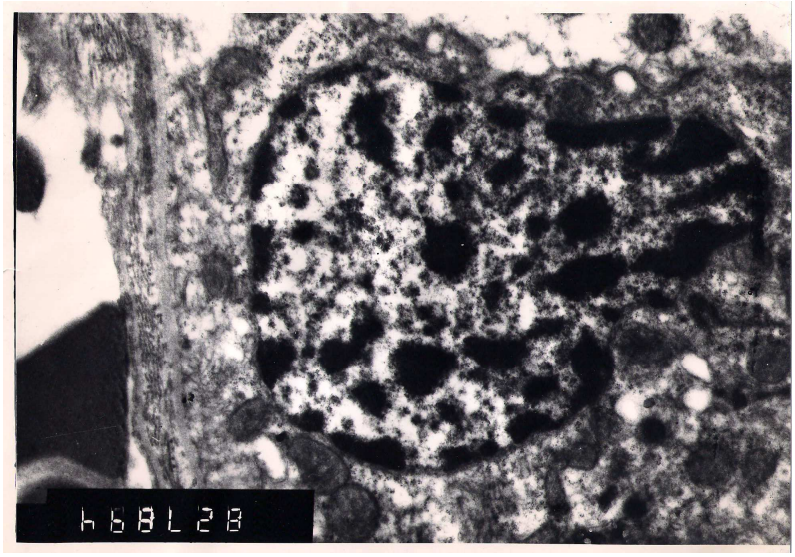

Fig6. Electron micrograph of hepatocytes fromtreated mice received 0.25%lead nitrate for 4 months demonestratating clumped of adjacent to heterochromatin irregular nuclear membrane (arrow) (X25000).

Discussion

These ultrastruractural alterations due to lead intoxication wich involved mitochondria, RER, and lysosomes. has been shown to have strong affinity for mitochondria and is usually associated with changes in the parenchymal cells (12,13) The results of the present investigation indicate clearly that the mitochondria are highly susceptible to lead toxicity and mitochondrial swelling was a frequent ultrastructural finding.This swelling is thought to be related to change in osmolarity that leads to an influx of salts and water through the inner mitochondrial membrane which becomes distended. Consequently, the outer membrane eventually ruptures due to osmotic swelling as a result of water and ion accumulation (11) .this alteration may be related to expansion of mitochondrial matrix as evidenced by the decreased metrical electron density. The high degree of swelling of mitochondria might indicate impaired electron transport as a consequence to interaction of lead with the mitochondrial membranes Regular spacing and orientation of the mitochondrial cristae were lost with no changes in the lysosomal membrane system in the cardiac papillary muscle of rats given lead at the rate of 1000ug/1 in drinking water for 59 weeks (14).Similar mitochondrial swelling had been observed in other tissues of lead exposed animals such as the heart, cerebellum and kidney (15). The disorientation and fragmentation of the mitochondrial cristae might indicate a special lead affinity for mitochondrial membranes which play a key role in the functional integrity of this organelle (8,16). Cells with noticeable mitochondrial changes, as observed here, are unable to perform efficient functions specially the oxidative phosphorylation and ATP production (17, 18) The demonstrated increase in the number of lysosomal-related structures in affected hepatocytes might be an indication of an increase in the autophagic activity, as a removal mechanism of defective organelles in the affected cells.. The increase in the lysosomes number might also reflect an increase of the synthesis of hydrolytic and detoxifying enzymes (19).In this respect, Some previous studies showed that lead poisoning increases the number of lysosomes in the renal tubular cells (10). The destruction and decreased number of tubular arrays of the RER and mitochondrial- RER associates can be considered as an

indication of impaired protein synthesis in the affected hepatocytes. The demonstrated shrunken dark hepatocytes may represent a form of degenerated hepatocytes. The appearance of these cells may indicate an evidence of single cell necrosis(apoptosis) or shrinkage necrosis as a consequence of lead toxicity. Shrinkage necrosis is considered as an expression of programmed cell death in response to pathological changes(20*)* The accumulation of fat droplets in the cytoplasm of the affected hepatocytes may suggest lead interference with lipid removal from these cells through impairment of ATPdependent fatty acids(21).

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