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Lead and Aluminum Toxicity Induce Loss and Death of Hepatorenal Cells in Mice

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Abstract

Lead and aluminum are natural components of the Earth's crust and are environmental contaminants. This study was undertaken to evaluate histological alterations induced by a chronic intoxication by aluminum chloride and lead acetate. After 12 weeks in vivo study, an increase in the activity of markers of liver function (AST and ALT) was found in blood of treated groups by these metals. Hypocalcaemia has been reported in mice poisoned by lead and hypocalcemia in aluminum-intoxicated group, implying that both metals impair calcium homeostasis. Histological study at the liver level showed hepatocyte lesions with presence of dilated sinusoids, ballooning, inflammatory infiltrates, nuclear pyknosis, Kupffer cells, vascular congestions and increased number of binucleated hepatocytes. The kidneys have dilated renal tubules, inflammatory infiltrates and congestions. The biochemical parameters confirmed the histological changes observed in the liver and kidneys. Lead and aluminum severely affect the liver and kidneys.

Keywords: Aluminum; Lead; Hepatoxicity; Nephrotoxicity; Cell loss.

1. Introduction

ATSDR[1] [1] has received reports of Lead contamination from various sources, including the deterioration of lead-based paint, the use of car batteries, smoke, gasoline, and plumbing systems where Lead can leach from pipes, faucets, and solder. Additionally, workplaces, particularly those involved in production processes, contribute to lead exposure. The agency has also highlighted the presence of Lead in certain commercial products such as imported jewelry, candy, children's toys, cosmetics, and traditional healing remedies in folk medicine.

White *et al.* [2] reported that Lead serves as a potent neurotoxin, exerting adverse effects on the nervous system. This metal, considered a xenobiotic, lacks any recognized vital function in cellular growth, proliferation, or signaling. Exposure to lead triggers heightened oxidative stress, leading to substantial damage to biomolecules, ionic mechanisms, and apoptosis. These effects extend across nearly all major organ systems of the body, potentially resulting in irreversible damage and contributing to various disease manifestations [3].

Concerning Aluminum, this latter constitutes 8% of the Earth's surface, it predominantly exists in natural sources such as silicates, cryolite, and bauxite rock. Despite its abundance in these natural sources, Aluminum can also act as a contaminant in drinking water and food items [4].

Yokel [5] pointed out that human exposure to Aluminum happens through multiple sources; including ingestion of food, consumption of water, inhalation of airborne dust, and the use of various cosmetic and pharmaceutical products such as: antacids, antiperspirants, vaccines, and allergy immunotherapy.

Even though abundant in environment, Aluminum lacks essentiality for living organisms and does not participate in any enzymatic reactions. In contrast, it interferes with the homeostasis of crucial metals like Magnesium, Calcium, and Iron. Aluminum mimics

[1] Agency for Toxic Substances and Disease Registry

these metals in their biological functions, resulting in a range of biochemical alterations [6].

The previous works of Gadouche *et al*. [7, 8] and Zerrouki *et al*. [9, 10] revealed that Aluminum and Lead are implicated in neurological disorders, demonstrated by alterations in the neurological behavior of mice and deterioration of architecture of their cerebral cortex and hippocampus, but also their involvement via intoxication in Alzheimer's disease .

Given the ubiquitous presence of Aluminum chloride and Lead acetate in the environment, the aim of this study is to assess the hepatic and nephritic histological aspects resulting from chronic exposure to both substances.

2. Materiel and methods

2.1. Animals

21 Swiss albino mice weighing 18.74±1.83 g and aged 4 weeks, provided by Pasteur Institute of Algiers in Algeria were used for *in vivo* trial.

Mice were housed in cages labeled with the batch number, treatment, and dates of experimentation. The cages were lined with wood shavings cleaned daily. The mice were exposed to standard animal facility conditions: a temperature of 22°C, a natural photoperiod of 12 hours of light and 12 hours of darkness. They were fed a standard diet and had unrestricted access to water.

Two groups of mice were exposed daily to drinking water containing 1000 ppm of lead acetate [11] and 500 mg/kg of aluminum chloride [12] for 90 days compared to a control group, which received only drinking water.

2.2. Biomarkers of liver function and Calcium

The mice were euthanized, and their blood was gathered in heparin tubes to analyze some biochemical markers; ALT, AST (utilizing the SPINREACT Kit), and Calcium (using the Biomaghreb kit).

Histological study

Histological sections were conducted at the Anatomo-pathology laboratory of Oran Military Hospital. After euthanasia, kidney and liver were promptly immersed in 10% formalin. The samples were then dehydrated in alcohol baths (70°, 80°, 90° and pure alcohol), which was later substituted by xylene, a paraffin-miscible solvent. Once dehydrated, the tissue was embedded in paraffin melted at 65°C. Once the fabric has hardened and stiffened, the mould was removed. Histological sections were then prepared in ribbon form using a microtome and stained with Hematoxylin and Eosin.

2.3. Statistical study

The data collected through this experimentation were subject to descriptive statistical analysis using Excel software. In order to compare the experimental groups, the data were analyzed using analysis of variance (ANOVA) followed by t-test. Significance was determined at $P<0.05$.

3. Results and discussion

3.1. Assay of hepatic transaminases and Calcium

Analysis of liver markers showed a significant increase in AST (444.50±28.99 U/L) ($P<0.05$) and ALT (109±35.35) ($P>0.05$) in Lead-treated mice, compared to the control groups (AST: 338±12.73; ALT: 73.50±13.43 U/L) ($P<0.05$) (Table 1).

Furthermore, the results showed that Lead induced high significant levels of Calcium compared to the controls (119.50±1.41 mg/L) ($P<0.05$).

Table 1
Levels of ALT, AST and Calcium in mice.

	Group (C)	Group (Pb)	Group (Al)
AST U/L	338±12.73	444.50±28.99 *	432.50±84.14
ALT U/L	73.50±13.43	109±35.35	220±125.86
Calcium (mg/L)	95.85±2.90	119.5±1.41*	79.85±0.92 *

C: control; Al: aluminum exposed mice (500 mg/kg); Pb: lead exposed mice (100 ppm); (Al vs. control); (Pb vs. control);
*P < 0.05

The determination of transaminases; AST and ALT indicated that hepatic function was impaired due to chronic Aluminum intoxication in the Al treated group (AST; 432.50±84,14 U/L; ALT; 220±125.86 U/L) compared to control mice (AST: 338±12.73 U/L;

ALT: 73.50±13.43 U/L). Calcium determination revealed significant hypocalcaemia (79.85±0.92) compared to control mice (95.85±2.9) $P<0.05$.

Transaminases are commonly used as markers for assessing liver function. Alterations in their levels in the bloodstream often signal damage to the liver's parenchymal cells. These changes can present diagnostic challenges for clinicians, particularly when evaluating conditions affecting the liver or other organs [13].

Our findings are in agreement with those of Ibrahim *et al*. [14] and El-Tantawy [15], who demonstrated that Lead ingestion significantly, enhances the activity of AST and ALT. These observations are consistent with the earlier study of Wardani *et al*. [16], suggesting that Lead exhibits hepatotoxic effects, explaining that oxidative stress plays a pivotal role in the liver damage caused by lead acetate.

Aspartate aminotransferase and alanine aminotransferase are enzymes primarily found in the liver, and alterations in their blood levels are indicators of liver damage. Elevated levels of these enzymes in the bloodstream are may be due to cellular injury or changes in cell membrane permeability [17].

In our study, Administration of Aluminum significantly increased the leakage of AST transaminases ($P<0.05$) and ALT ($P>0.05$) into the bloodstream. Similarly, Administration of aluminum resulted in a significant increase in the leakage of ASAT transaminases ($P<0.05$), while ALAT leakage showed a non-significant increase ($P>0.05$) into the bloodstream. Bhadauria [18] similarly observed elevated levels of these enzymes in rats following oral administration of $AlCl_3$. In addition, Shati & Alamri, [19], mentioned that Al has a hepatotoxic effect, evidenced by an increasing in biochemical markers of liver such as: cholesterol levels, triglycerides, GGT, ALT, AST, ALP, lipid peroxidation, and presence of hyperglycemia. Moreover, changes of AST and ALT in case of hepatotoxicity or hepatocellular injury suggest hepatic injury following membrane disruption.

Regarding Calcium, various hormones, such as parathyroid hormone, calcitonin and calcitriol, manage the balance of this element in the body. [20], malignant tumours, hypoadrenocorticism, renal insufficiency and primary hyperparathyroidism show calcium overload.

According to Song [22], hypocalcaemia can result from various factors, including chronic renal insufficiency, calcium sequestration in bones, chelation therapies for heavy metal removal, and calcium saponification in acute pancreatitis. Our study suggests that both Aluminum and Lead exposure contribute to calcium imbalance, potentially associated with renal insufficiency, as evidenced by several lesions observed in renal tissue via H&E staining in our study.

3.2. Histological structure of liver and kidneys

Microscopic examination of the liver of control mice showed a normal histological structure (Figure 1A). For mice treated with 1000 ppm of lead acetate during 90 days; we noted a loss of liver architecture including: dilated sinusoids, dilated centrilobular veins, irregular ballooned hepatocytes, inflammatory infiltrates, nuclear pyknosis, Kupffer cells, vascular congestions and increased number of binucleated hepatocytes (Figure 1B). The results of H&E staining indicated that chronic intoxication by Aluminum induced severe alterations of the histological architecture of the liver including marked enlarged areas of ballooning degeneration of hepatocytes, infiltration inflammatory cell, dilated sinusoids, dilated centrilobular veins and vascular congestion (Figure 1C). Our results corroborate the hepatic section observations in rats that received 0.13% of lead acetate in drinking water for 4 weeks, reported by Hegazy *et al*. [23].

The study found a slight infiltration of inflammatory cells surrounding the congested central vein, accompanied by hepatocyte vacuolation. Conversely, individuals administered the same dosage over 8 weeks showed marked disruption to liver architecture, evidenced by necrotic areas and the presence of pyknotic nuclei. Our results align with those of Pal *et al*. [24], who reported that lead induced liver cell death and dysfunction.

Our results are in line with those obtained by El-Sayed *et al*. [25] who found extensive injuries in $AlCl_3$ mice's liver tissues stained with H&E techniques. These damages included disruption of normal parenchymal tissue architecture, infiltration of inflammatory cells, congestion of sinusoids and blood vessels, cellular

degeneration accompanied by nuclear pyknosis, and the presence of necrotic region.

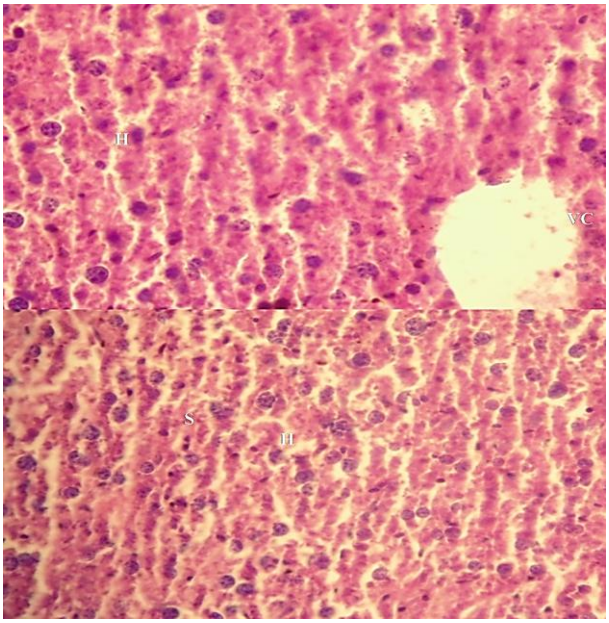

Figure 1 A: Photography of a section in the liver of control mice (H&E staining, Gr×40). Hepatocyte (H); central vein (CV); sinusoids (S)

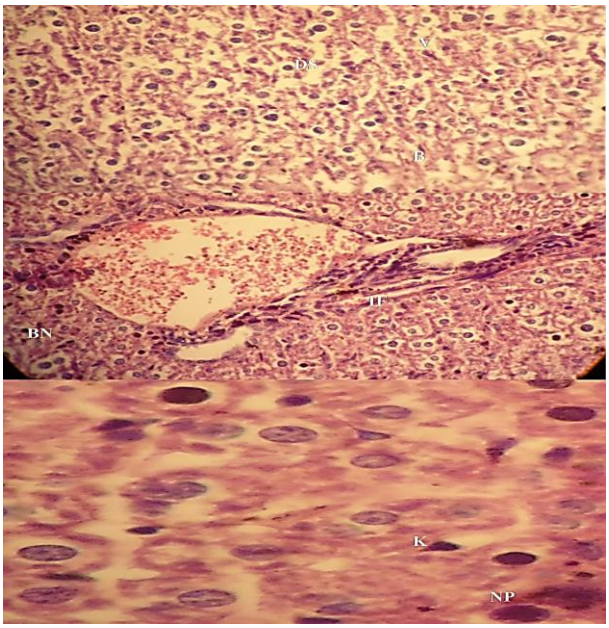

Figure 1 B: Photography of a section in the liver ofmice treated by lead acetate (C) (H&E staining, Gr×40). Normal cell (NC); Central vein (CV); sinusoids (S); Infiltrate inflammatory (IF); Dilated sinusoid (DS); Congested blood (CB); Vacuolated cells (V); Ballooning cell (B); b) Nuclear pyknosis(NP); kupffer cells (K); Binucleated hepatocytes (BN)

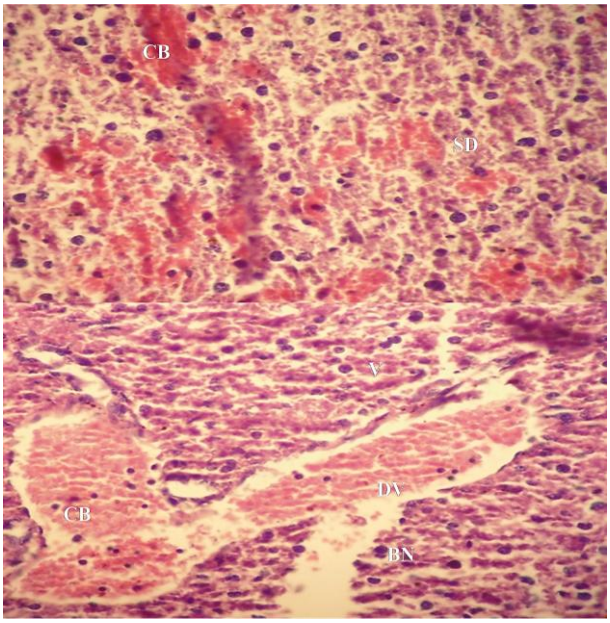

Figure 1C: Photography of a section in the liver of micetreated by aluminum (H&E staining, Gr×40). Infiltrate inflammatory (IF); Dilated sinusoid (DS); Congested blood (CB); Vacuolated cell (V); Ballooning cell (B); Dilated vein (DV); Binucleated hepatocytes (BN).

Concerning the kidney structure, control mice showed normal renal tubules and glomerulus with H&E staining (Figure 2A). Exposure to Lead showed a histological architecture, rich in aggregates of inflammatory cells, dilatation of the renal tubules and hypertrophy of the glomeruli (Figure 2B). While the kidneys of Aluminum-intoxicated mice revealed dilatation of the renal tubules, an infiltrate of inflammatory cells and blood congestion (Figure 2C). Tubular disorganization was observed in mice exposed to both metals.

Our observations are consistent with those of Offor *et al*. [26] who noted degeneration and necrosis of renal parenchymal cells, accompanied by significant infiltration of inflammatory cells in the kidneys of rats under a daily dose of 60 mg/kg of lead acetate over period of 28 days. Additionally, Missoun *et al*. [27] concluded that prolonged administration of lead acetate to rats for more than 8 weeks resulted in the development of nephropathy and related disorders.

Kutlubay *et al*. [28] demonstrated also that Aluminum induces the generation of reactive oxygen species, resulting in oxidative damage to cellular lipids, proteins, and DNA, ultimately leading to nephrotoxicity

characterized by the degeneration of renal tubular cells. Al-Dera [29] found that Aluminum induces renal oxidative stress and inflammation, indicating a potent pro-oxidant effect of $AlCl_3$ despite its non-redox nature.

This oxidative stress contributes to notable impairment in kidney function and architectural integrity following xposure to $AlCl_3$. Bhasin *et al*. [30] reported that Aluminum induces changes in liver histoarchitecture characterized by the disruption of hepatic cords and heightened vacuolization. These observations are widely demonstrated in our study.

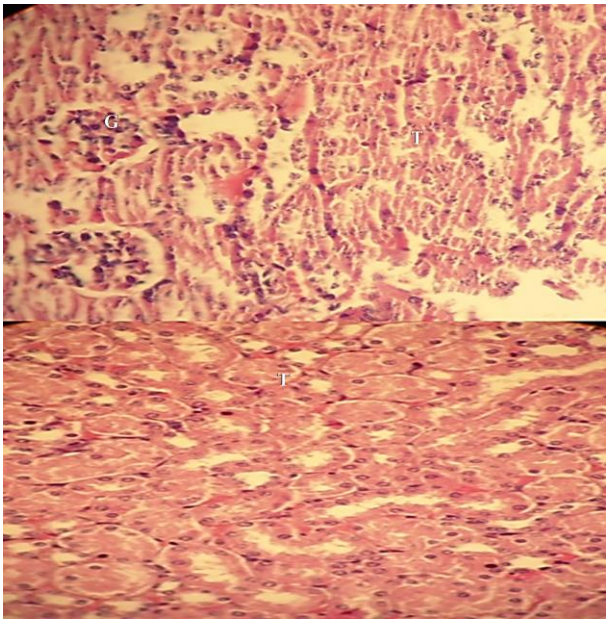

Figure 2 A: Microscopy of Kidneys of control mice (H&E staining, Gr×40). Tubules (T); Glomeruli (G).

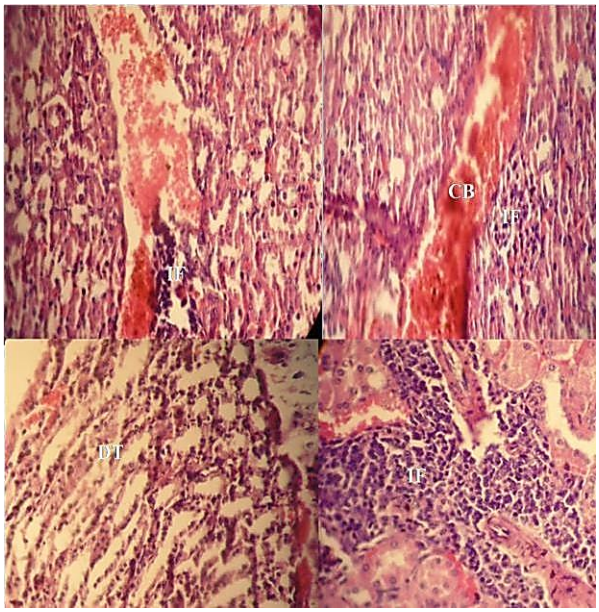

Figure 2 B: Microscopy of Kidneys of mice treated by lead acetate (H&E staining, Gr×40). Infiltrate inflammatory (IF); Dilated renal tubes (DT); Congested blood (CB).

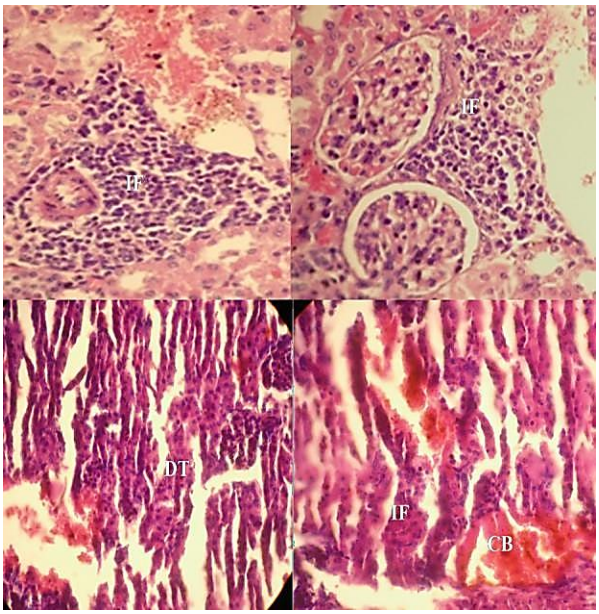

Figure 2C: Microscopy of Kidneys of mice treated by aluminum (H&E staining, Gr×400). Infiltrate inflammatory (IF); Dilated renal tubes (DT); Congested blood (CB).

4. Conclusion

This investigation showed that Lead and Aluminum cause severe hepatoxicity and nephrotoxicity. It is essential to raise awareness about the harmful effects of these xenobiotics on public health, explore preventive measures, and take steps to clean up our environment to address their widespread distribution. Adopting a proactive approach is very important to safeguard public health and the quality of our environment. Additional research is also necessary to document the dangers of these two metals for humans, by extending the concepts of the study to other compartments of the animal body.

Conflict of Interest

The authors declare no conflict of interest

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