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Original Research Article

Histological and Histochemical Study of the Toxic Effects of Lead Acetate on Liver and Kidney Tissues in Laboratory Mice: Application of PAS and Silver Staining Techniques

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Abstract: Lead (Pb) is a hazardous substance due to its ability to bioaccumulate in living organisms; therefore, lead is highly detrimental to human health and the environment. The objective of this study was to examine the histopathological effects of lead acetate treatment on liver and kidney tissues in mice .METHODS: Mice (n = 20, 10 per group) were randomly assigned to either a control group (standard laboratory conditions) or lead acetate-treated group (50 mg/kg) for 30 days. All mice were housed under controlled temperature and light-cycle conditions and provided standard chow and tap water. After the experimental period, the liver and kidney tissues were removed and immediately immersed in 10% neutral buffered formalin (NBF). The tissues were embedded in paraffin blocks and sectioned at 5 µm. The sections were stained using Hematoxylin-Eosin, Periodic Acid-Schiff, and silver stains. Results obtained from all examinations indicated alterations in tissue structure and metabolism. Both liver and kidney tissues demonstrated destruction of normal architecture and degenerative cytoplasmic changes. Kidney tissues showed necrotic renal tubular cells, degenerative changes in the epithelial lining of the renal tubules, and deposition of cellular debris within the glomeruli. PAS staining indicated a significant reduction in glycogen content within hepatic cells. Tissue injury was also evident due to the disorganized appearance of tissues and fragmentation of extracellular matrix components, which were assessed using silver stains. To summarize, lead acetate induced histopathological and histochemical changes in liver and kidney tissues associated with disruption of normal organ structure and function.

Keywords: Lead Acetate, Histopathology, Liver, Kidney, Oxidative Stress, Mice, PAS Staining, Silver Staining, Tissue Damage.

INTRODUCTION

Environmental contamination from a wide range of human activity (industrial processes, burning leaded fuel, use of lead based paints and coatings for homes etc.) has made lead one of the most damaging heavy metals to human health globally. Polluted soil and water significantly increase the total amount of lead in the environment. Continuous exposure to heavy metals, including lead, causes a gradual accumulation of these substances in the human body; this means that low level continuous exposure can result in the development of toxicity across multiple organs within the body, particularly the liver and kidneys because of their role in metabolizing and excreting heavy metals (Tchounwou *et al*., 2021; Kumar *et al*., 2022). Additionally, long-term exposure to lead or any other heavy metal is associated with the high incidence of neurological disorders (e.g., dementia, multiple sclerosis, etc.), hematological disorders (e.g., aplastic anemia, leukemia, etc.), and immune disorders (e.g., autoimmune disorders, etc.). The most common methods for the human population to be exposed to lead are through the lungs or digestive tract; however, it is possible to become exposed to lead through the skin, in which case some of the lead will also enter the bloodstream. Once in the bloodstream, lead will circulate within the body, often being deposited into the bones and/or soft tissue, which places people at risk for chronic toxicity from lead.

Oxidative stress results from free radicals, disrupting membrane integrity with an imbalance in antioxidant protection, and impeding critical enzyme function. It(blocks) Processes controlled by calcium-dependent signaling, inhibiting proper function (Abdel-Daim, Genchi, 20). Since the liver acts primarily to detoxify foreign substances, major effects can be seen as structural damage to the hepatocyte. Tubular injury to the kidney is due to lead accumulation, which will result in inflammation and, possibly, tubulointerstitial scarring (Nasef, Renu, 23). Thus, both of these organs represent key locations for evaluating toxicity from systemic exposures.

According to Suvarna *et al*., (2019), fixation, embedding and sectioning are all used with the goal of preserving their native architecture (also known as tissue preservation) which is where the histological analysis of a sample begins. Histological staining methods such as H&E, PAS and argentaffin (silver staining) provide information regarding the overall morphology, glycogen level and reticular fibre content of a sample of tissue (Fischer *et al*., 2021). The goal of the present studies is to correlate the histological changes caused by lead acetate exposure with available data concerning the toxic effect of lead acetate on tissues. There is still very little literature describing the early biomarker of tissue injury due to lead acetates. Twenty laboratory mice were used in this experiment, and they were allocated randomly into two both control (raw clinical group) and as lead acetate (50 mg/kg orally for 30 days) fractionally to standard laboratory conditions).

Experimental Procedures:

Experimental Design and Lead Administration

Methods: Twenty laboratory mice were randomly divided into two equal groups (n=10) of mice per treatment. The experimental protocol was performed in parallel with control group receiving the same volume of distilled water (diluent) per day under identical conditions as in treated groups. Controlled water (normal control) and lead acetate (purity > 99%) in distilled water at a dose of 50 mg/kg body weight for 30 days.

30-day single gavage, once a day oral dose. Weekly animal weighings ensured the correct dose was delivered. The volume of the dose administered was adjusted, based on the change in body weight (dosing took place every 3 days) using weights. In order to limit circadian and biological variation, the treatments were uniformly administered in the morning (10:00 AM). The animals were kept in a controlled laboratory environment maintained at 25±2°C, normal humidity, a 12 hour light/dark cycle and chow diet (standard pellet) with free access to water.

Sample Collection:

Once the test was completed, euthanization was performed on each animal using an appropriate anesthetic protocol to minimize suffering and pain. Immediately after killing, the liver and kidney were excised from animals to minimize postmortem changes. Before performing tissue fixation, the collected tissues were thoroughly washed with saline solution to eliminate all retained blood and debris.

Fixation:

Undoubtedly, fixation of tissue samples for 24 hours in 10% neutral buffered formalin (NBF) is the prior and most relevant factor responsable for preserving the overall structure and shape of the tissue preventing the damage of tissue aggraveted by autolysis, stabilizing cellular components allowing subsequent processing by histological methodology.

Tissue Processing:

Standard histological processing steps were then performed on the fixed samples. The tissues were dehydrated in an ascending series of alcohol (70%, 80%, 90% and absolute) take the water out from the samples. The samples were then cleared in xylene for 2 hours to remove the alcohol and clear the tissues. TRIZM-154 content of the samples were processed at three (3) leading steps, and then embedded in paraffin to furnish sufficient support for cutting.

Sectioning of paraffin-embedded tissue blocks was performed using a rotary microtome, samples were cut at 5 to 10 microns thickness. The sections were mounted onto clean glass slides and allowed to dry before staining.

Staining:

Various histological stains were applied for the evaluation of tissue alterations. Hematoxylin and eosin (H&E) staining was used to assess the overall structure of the tissue and cell shape. We utilized Periodic Acid-Schiff (PAS) staining to determine the presence of glycogen and carbohydrates in the cells. Silver Staining: The reticular fibers were present and connective tissue organization was changed.

The integrative methods provided a more thorough evaluations regarding the structural and histochemical damages of liver and kidneys due to lead acetate exposure.

Statistical Analysis

Quantitative results were expressed as mean ± standard deviation (SD). Normality of data was examined with the Shapiro-Wilk test and for homogeneity of variances, Levene's test. The effects of treatment and organ type were compared using a two-way analysis of variance (ANOVA), followed by Tukey post-hoc test to analyze differences between multiple comparisons. The Kruskal-Wallis test was carried out for semi-quantitative histological results, and Dunn's post-hoc test was performed on group data. Statistical analyses were completed using the Statistical Package for Social Sciences (SPSS) version 25, using the benchmark of $p < 0.05$ to determine statistical significance.

RESULTS

Histopathologic evaluation of liver and kidney sections in the control group remained intact. In the controls group, hepatocytes were arranged in a radial pattern with well-defined sinusoidal spaces and normal defined cell form in the liver. In contrast, the treated group showed multiple significant hepatic alterations that could be classified into: 1) swelling of vacuolated liver cytoplasm; 2) loss of normal radial arrangement and organization within periportal areas; 3) destruction around the cord. Additionally, those sections analyzed in the treated group showed signs of vascular congestion and mild inflammatory cell infiltration (i.e. tissue damage).

The kidney tissues of control animals showed normal renal structure with intact glomeruli, well defined Bowman's capsules and regularly arranged renal tubules. On the other hand, lead-treated animals showed markedly severe histopathological changes in renal tissues such as tubular necrosis; degeneration of epithelial lining cells accompanied by widening and occasional narrowing of tubular lumens; partial destruction of glomerular structures. Consequently, the rather significant impairments of both structure and function of kidneys due to lead exposure are not surprising.

Table 1: Semi-quantitative scoring of histopathological changes (H&E Staining)

Histopathological Parameter	Control Group (n=10)	Lead-Treated Group (n=10)	P-value
Liver: Hepatocellular swelling	0.10 ± 0.05	2.70 ± 0.45*	< 0.05
Liver: Cytoplasmic vacuolation	0.05 ± 0.02	2.25 ± 0.38*	< 0.05
Kidney: Tubular necrosis	0.00 ± 0.00	2.85 ± 0.52*	< 0.05
Kidney: Glomerular damage	0.12 ± 0.06	1.90 ± 0.42*	< 0.05

Data are presented as Mean ± SD. Statistical analysis was performed using Two-way ANOVA followed by Tukey's post-hoc test. (*) indicates a significant difference vs. control ($p < 0.05$). Normality was assessed using the Shapiro–Wilk test.

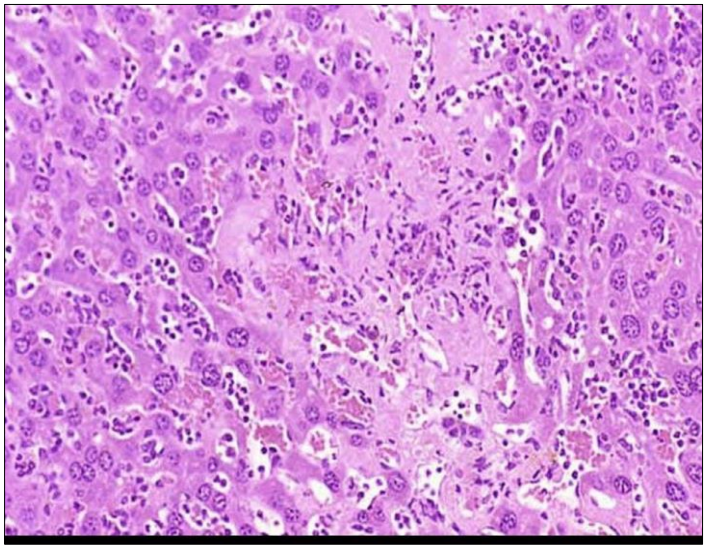

Figure 1: Histological section of liver tissue stained with Hematoxylin and Eosin (H&E) showing structural alterations in control and lead-treated groups

There were notable differences between the two groups concerning the presence and distribution of glycogen as determined through Periodic Acid-Schiff (PAS) staining. The control group had strong, uniform magenta staining throughout the hepatocytes indicating normal glycogen storage and metabolic function. On the other hand, lead-treated animals exhibited significantly less PAS positivity indicating that there was a breakdown of glycogen stores, which also reflects an impairment in carbohydrate metabolism of the hepatic cells.

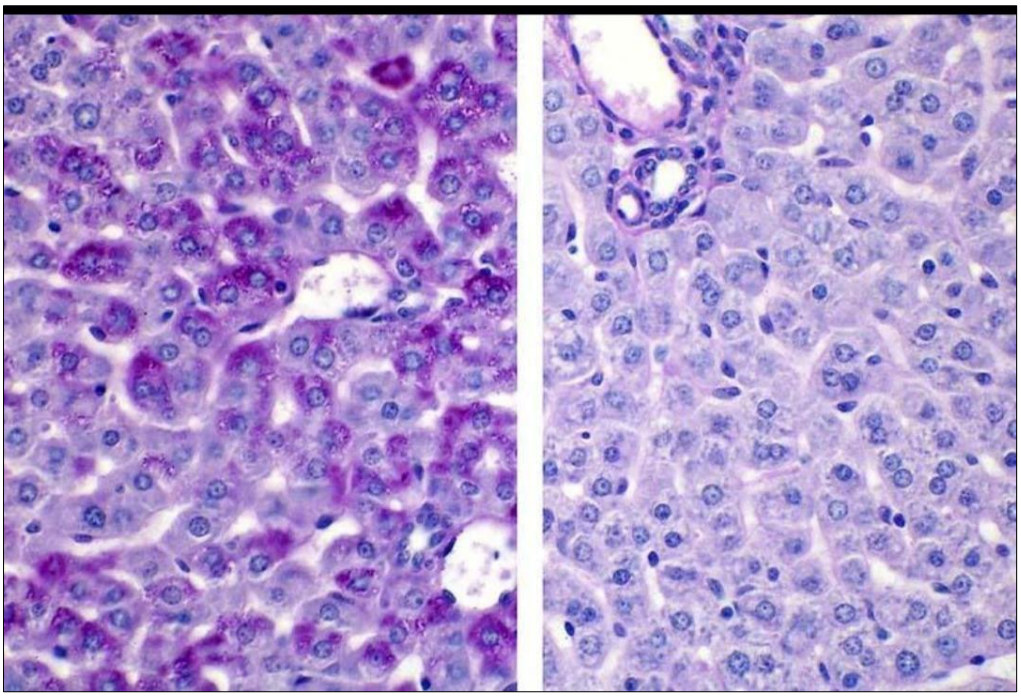

Figure 2: Histological section of kidney tissue stained with Hematoxylin and Eosin (H&E) illustrating normal and pathological changes following lead exposure

Silver staining revealed notable changes in the reticular fiber network. In the control group, reticular fibers appeared as a dense, continuous, and well-organized black meshwork supporting the structural framework of liver and kidney tissues. In contrast, all these fibers exhibited disintegration, discontinuation and uneven scattering in the lead-treated group, especially renal fibrocytes. Indicates degradation of the extracellular matrix and loss of normal tissue support architecture.

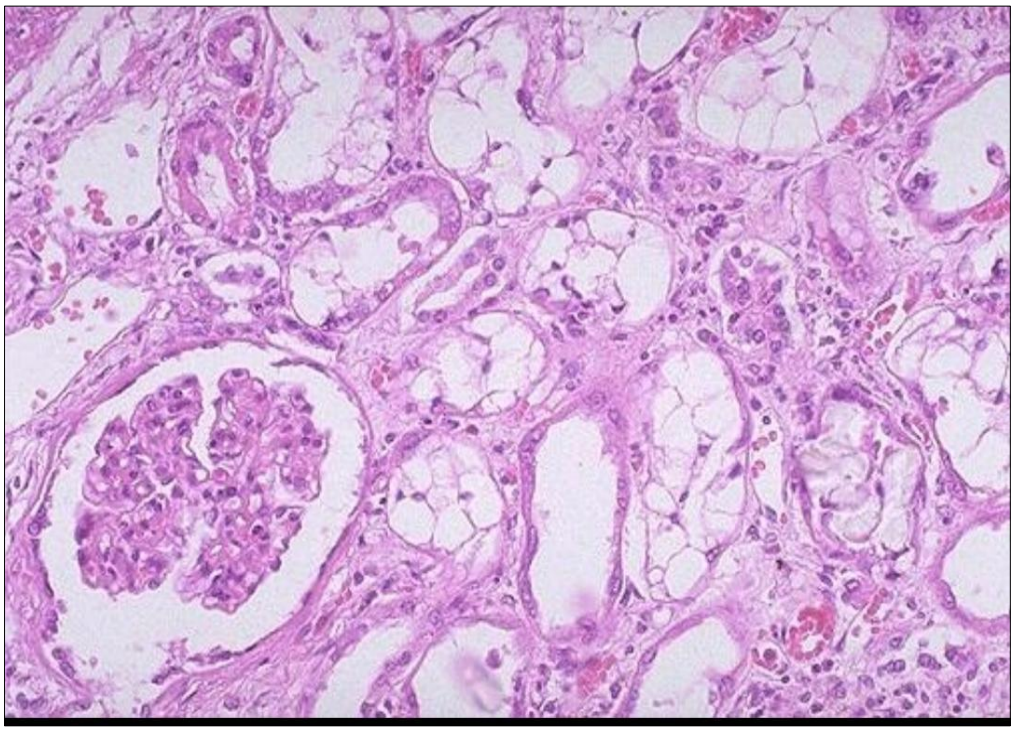

Figure 3: PAS & Silver stains showed glycogen distribution and reticular fibers integrity of control and lead-treated Groups

Table 2: Histochemical analysis (PAS and Silver Staining)

Parameter	Control Group (n=10)	Lead-Treated Group (n=10)	P-value
PAS Staining Intensity (Glycogen)	2.95 ± 0.05	1.10 ± 0.28*	< 0.05
Reticular Fiber Integrity (Silver)	2.88 ± 0.12	1.15 ± 0.35*	< 0.05

Scoring system: 0 (Normal), 1 (Mild), 2 (Moderate), 3 (Severe). (*) $p < 0.05$.

Overall, the histological and histochemical findings demonstrate that lead acetate exposure induces severe structural, cellular, and biochemical alterations in both liver and kidney tissues.

DISCUSSION

Findings from the current research study were that lead acetate resulted in severe alterations in both the underlying histological and biochemical profile of the liver and kidney. These results align with previous work which has shown that metals (such as lead, Pb) possess several pathways of toxicity, in addition to oxidative stress, through disruption of fundamental enzymes that are necessary for the maintenance of cellular homeostasis (Flora *et al*., 2012; Tchounwou *et al*., 2021; Renu *et al*., 2021). The silver-stained samples contained high levels of fragmentation and abnormality of reticular fibers, such as collagen, which is one of the primary proteins of the extracellular matrix (ECM), providing strength to the liver, kidneys, and other organs. Therefore, lead toxicity may not only indicate an adverse effect at the cellular level but also could be a precursor to true necrosis or serve as a clinical indicator of catastrophic injury due to changes in organ structure.

The liver's glycogen stores are nearly depleted, and there is a very significant decrease in metabolic function when glycosylation levels measured by PAS are altered. Inhibiting glucose metabolism through lead poisoning is thought to be the mechanism leading to the decrease in hepatic glycogen due to decreased glycogen production and increased hepatic energy expenditure as ATP. Several published studies have shown that lead disrupts metabolic homeostasis and decreases the availability of energy in the liver (Tchounwou *et al*., 2021).

Furthermore, altered collagen formation as shown by silver staining indicates that lead has a direct toxic effect on the extracellular matrix. Lead appears to accelerate the loss of normal tissue architecture by increasing the breakdown of structural proteins (including reticulin fibers) along with organ atrophy. Our findings are analogous with a number of recent studies indicating that heavy metals can induce pathological remodeling of connective tissue molecules (Abdullah *et al*., 2021; Genchi *et al*., 2020). Additionally, in this methodological aspect, it is important to have consistent histological processing (fixation with buffered formalin and embedding in paraffin) in order to eliminate or at least minimize artifacts and maintain the integrity of the tissue; processing changes described by Suvarna *et al*., The reported transformations create multiple structural alterations that may interfere with histological analyses (Biliu *et al.,* 2019).

Statistical correlations can exist between differences arising from different treatment controls and differences due to pathology and/or modification of tissue biochemical qualities, thereby increasing the naïve state of tissues (for example, lead poisoning can change the way pH mediators are absorbed/infiltrated into the body). The alteration of any type of tissue can potentially alter the dye's ability to interact with tissue, thereby impacting the staining properties that are necessary to view stained tissue through the microscope. In fact, an extensive review of the current literature shows that toxicity as a result of environmental contaminants can alter the pH of the tissue and as a result impact the staining reactivity and histochemical reactions that are normally expected within this environment (e.g., Flora *et al*., 2012; Fischer *et al*., 2021).

What has been concluded from all of these experiments is that there can be changes due to lead acetate, and those changes can vary depending on the duration of exposure to lead. Therefore, it can range from molecular abnormalities to microscopic findings of tissue damage. Consequently, all of these alterations make lead acetate an ideal experimental model to study the impact of heavy metals on living biological tissues.

CONCLUSIONS

- Liver and kidneys sustained severe histological damage with alterations in normal functions following exposure to lead acetate.
- Pathological changes in liver tissue induced by lead, such as hepatocellular swelling, cytoplasmic vacuolar degeneration, vascular congestion and inflammatory cell infiltration.
- Lead acetate produced clear renal impairment by way of tubular necrosis, degeneration of the epithelial cells and glomerular damage.
- The glycogen content in liver cells decreased significantly according to PAS staining, reflecting disorder of carbohydrate metabolism and energy balance.
- Reticular fibers were fragmented and lost structural support within the tissues as seen by silver Staining.

- Lead toxicity is largely due to bioaccumulation in the liver and kidneys, as well as its damage caused by oxidative stress to cellular membranes and essential enzymes.

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