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Histological of the Epididymis after Lead (Plumbum) Exposure in Albino Mice *Mus musculus*

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Abstract
Lead or Plumbum (Pb) is a type of heavy metal-based chemicals that our body discontinue require for its proper functioning. When these chemicals enter the body in excessive amounts, they can harm the body's systems and increase the risk of cancer. Here we use albino mice *Mus musculus*. These small mammals often used in laboratorium research.This study to learn the effect of lead administration on the epididymal epithelium of albino *Mus musculus* mice. There are 18 mice divided into 3 treatment groups with 6 mice in each group (n=6). Every mouse in each group was given an intraperitoneal injection of $Pb(NO^3)^2$ at a dose of 0 mgPb/kgBB, 5 mgPb/kgBBand 10 mgPb/kgBB. After 35 days, the treated mice were euthanized using the neck dislocation technique, and the epididymal epithelium was examined histologically. The findings of the study indicate that there was a reduction in the thickness of the epididymal epithelium, or thinning of the epididymal epithelium, in the group that received Pb when compared to the control group. The average tubule diameter in the control group was 7.125µm, in 5 mbPb/kgBB it was 5.2083 µm, whereas in the 10 mgPb/kgBB treatment group, it was 4.3333 µm. This means that there was a significant difference in the thickness of the epididymal epithelium between the control group and the group given 5 mgPb/kgBB, 10 mgPb/kgBB. The results suggest that the administration of Pb is not safe for the epididymal epithelium at a dosage of 5mgPb/kgBB and 10 mgPb/kgBB.

Keywords: epididymis, heavy metal, mice, *Mus musculus*, plumbum.

Introduction

Numerous environmental pollutants considering contribute to human illness, affecting human health and climate change, following in high number sorrow and mortality (Shetty *et al.* 2023). The air, water, and land are all contaminated at an alarming level. Some examples of pollutants found in the environment are carbon monoxide (CO), nitrogen oxide (NO), sulfur dioxide (SO2), and particulate matter. Additionally, there are particles such as dust and lead that are commonly found in the environment (Darmono 2009).

Lead can be released into the atmosphere through gas and particulate forms (Zhang *et al.* 2005). It is a heavy metal chemical that is not required by the body and can have negative effects on physiological functions if it enters living organisms in excess amounts. Charles (2016) states that heavy metals, including lead, can cause disruptions to the body's system and lead to subjective fatigue. They are also carcinogenic. The toxic and accumulative properties of lead can cause harm to various organs in the body, including the male animal's reproductive system. Studies have shown that lead poisoning can lead to a decline in the number of spermatozoa in mice.

Studies adverse effects of Pb on humans and male reproductive organs have extensively researched in various rodent species (Apostoli *et al.* 1998). A study on experimental mice showed that administering Plumbum in doses of 40 mgPb/kgBB, 50 mgPb/kgBB, and 60 mgPb/kgBB for 35 days resulted in significant changes in epididymal tubular epithelial thickness, serum testosterone levels, and prostate gland weight in the group given 60 mgPb/kgBB compared to the group given 40

mgPb/kgBB and 50 mgPb/kgBB (Adriyoso 2007). Another study by Zen (2003) involved intraperitoneal injections of Pb to mice at doses of 40 mgPb/kgBB, 50 mgPb/kgBB, and 60 mgPb/kgBB. The results showed that Pb can affect the lumen diameter of the seminiferous tubules, decrease the number of primary spermatocytes, decrease the number of mature spermatids and the number of spermatozoa in the tubules. It can be concluded that the higher the dose of lead given, the greater the changes in the histology of the seminiferous tubules, resulting in more significant obstacles to the spermatogenesis process.

Methods

The study was conducted in experimental research where male albino mice *Mus musculus* were used as experimental animals. The research design used for grouping and providing treatment to the test animals was a Completely Randomized Design (CRD). The control group and treatment group in this study were mice that were given the compound $Pb(NO^3)^2$ intraperitoneally.

The research was conducted at the Medical Biology Laboratory of the Faculty of Medicine (Fakultas Kedokteran or FK) Sriwijaya University, Palembang, and the Anatomical Pathology Laboratory of FK Unsri Palembang/Public Hospital Mohammad Hoesin (Rumah Sakit Mohammad Hoesin or RSMH) Palembang.

The test animals used in this research were sexually mature male albino mice *Mus musculus*. A total of 12 animals were used, which were 12-14 weeks old with a bodyweight of 35-42 grams.The samples were divided into two treatment groups and one control group, and given various doses based on the time of data collection.

Results and Discussion

The study was conducted on twelve adult male Swiss Webster mice, which were divided into two groups - a control group and a treatment group (Table 1). Data was collected through histological examination, which included measuring the thickness of the epididymal tubular epithelium (Fig. 1).

Table 1. The dose given to the treatment groups varied according to the time of data collection.

No	Dose (mg/kgBB)	35 days
1	Control was awarded Aquabidest	6 tails
2	Treatment 1, dose 5 mg/kgBB	6 tails
3	Treatment 2, dose 10 mg/kgBB	6 tails
Total		12 tails

As per the data from table 1, it is evident that the epididymal epithelium thickness reduced or got thinner in the group given Pb in comparison to the control group. The average tubule diameter in the control group was 7.1250 µm, while in the 5 mgPb/BB treatment group, it was 5.2083 µm and 10 mgPb/kgBB treatment group, it was 4.3333 µm (Table 2).

Table 2. Average Epididymal Epithelial Thickness of Albino Mice *Mus musculus* before and after 35 days of Pb administration.

Dose Pb/kg BB	Before/Control (µm)	After (µm)	Std. Deviation	*p*Value
5 mg	7.1250	5.2083	1.10019	0.000
10 mg	7.1250	4.3333	0.58452	0.000

The male Albino mice used in this study were between 10-12 weeks of age and weighed 40-45 grams.The thickness of the epididymal epithelium decreased after the 10 mgPb/kgBB treatment, as evidenced by the 4.333 µm measurement.

There is a noticeable difference in the average epididymal epithelial thickness between the control group and the group of mice that were given Pb (Table 3). This difference becomes more apparent as the dose of Pb increases. The difference in epithelial thickness between the control group and the group given a 5 mg dose of Pb was 1.91667 µm, while the difference between the control group and the group given a 10 mg dose of Pb was 2.79167 µm.

Table 3. After 35 days of Pb administration, the epididymal epithelial thickness of albino mice *Mus musculus* was measured using an Average Difference Test in a Post Hoc Analysis.

Pb Dose	Mean Difference	*P*value
0 mg ----- 5 mg	1.91667	0.000
0 mg ----- 10 mg	2.79167	0.000
5 mg ----- 10 mg	0.87500	0.068
10 mg------ 15 mg	1.16667	0.018

There is a significant correlation between the administration of Plumbum (Pb) at increasing levels and the thinning of the epididymal epithelium, which is an early symptom of degeneration of epithelial cells. This is evidenced by the results of the p value from the Post Hoc Test obtained between the control group and each group given Pb doses of 5 mg and 10 mg. This p value is smaller than the significance limit of 0.05, indicating that the statistical decision is Ho. epididymis in accordance with increasing Pb levels administered. In simpler terms, it means that the administration of Pb at increasing levels from 0 mgPb/kgBB, 5 mgPb/kgBB, up to a dose of 10 mgPb/kgBB has caused damage to the epididymal epithelium due to the process of cell degeneration.

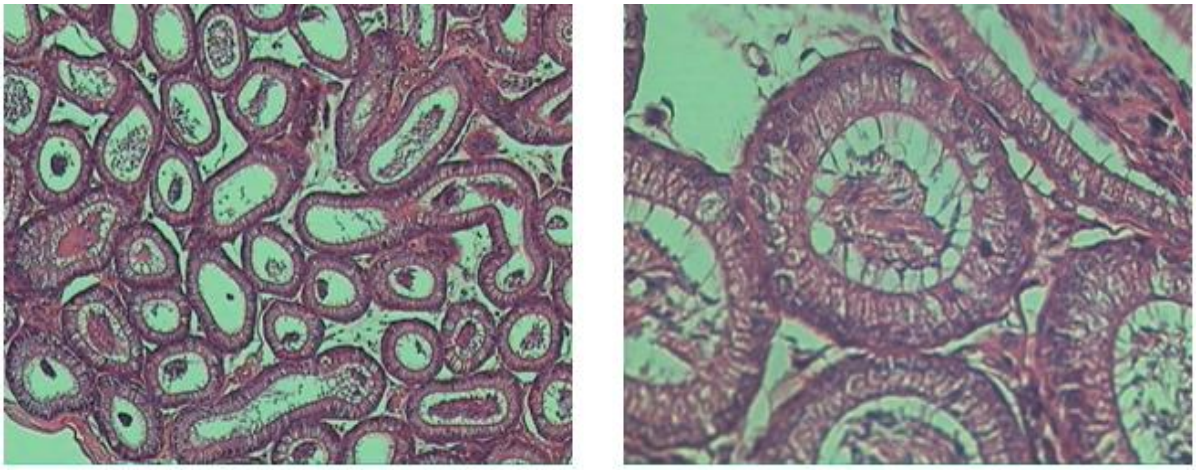


Figure 1A and 1B. Two images show of the epididymal epithelium in the control cauda area taken at different magnifications before treatment. The first image (A) was taken at 10 x 10 objective magnification and shows the thickness of the epithelium. The second image (B) was taken at 10 x 40 objective magnification and shows intact epididymal epithelial cells. The epididymal duct is lined by pseudostratified columnar cells with high columnar cells and low basal cells. The cells show two rows of elongated cell nuclei.

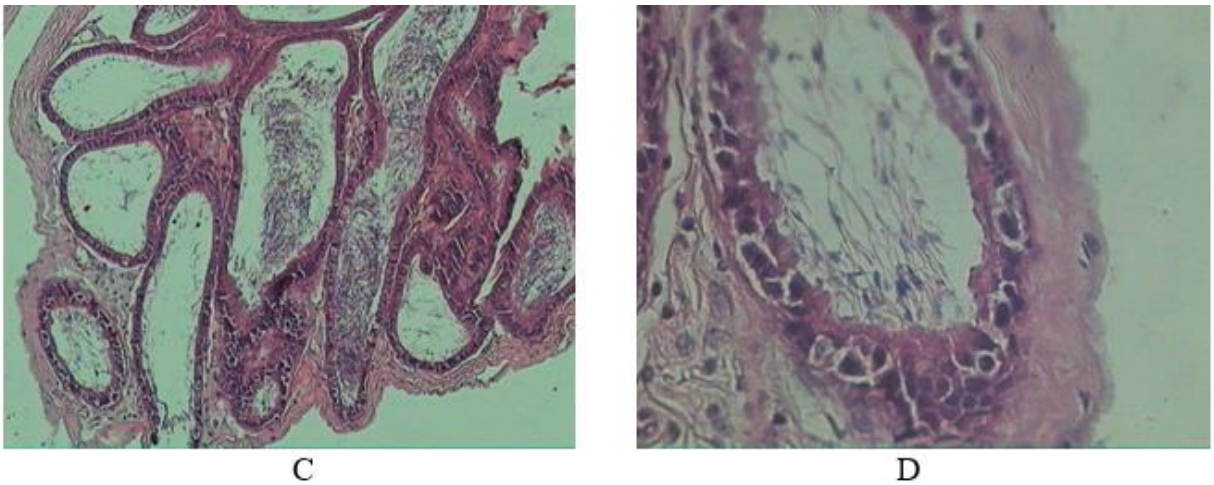
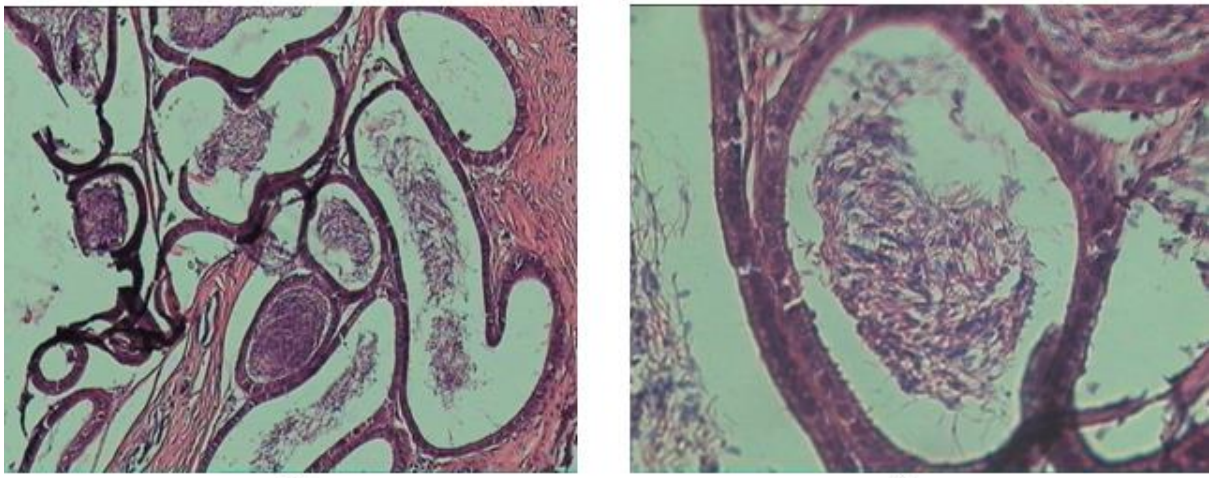


Figure 1C and 1D. The figures show the thickness of the epididymal epithelium in the cauda area after being treated with Pb at a dose of 5mgPb/kgBW. The magnification for the objective used was 10 x 10 and 10 x 40 respectively. The image portrays intact epididymal epithelial cells which are lined by pseudostratified columnar cells. These cells have high columnar cells and low basal cells, showing 2 rows of elongated cell

nuclei.



E F

Figure 1E and 1F. The thickness of the epididymal epithelium in the cauda area after being treated with 10 mgPb/kgBW of Pb at 10 x 10 objective magnification (E). The thickness of the same epithelium area after being treated with 10 mgPb/kgBW of Pb at 10 x 40 objective magnification (F). The epithelial structure appears to have difficulty in proliferating, and individual cells are rather blurry. Moreover, the structure seems to be partially damaged, and the connective tissue begins to break down.

Microscopic images of the epididymal tubules were observed to compare the effects of lead (Pb) exposure on mice. The first group of mice was given a dose of 0 mgPb/kgBB by intraperitoneal injection with sterile aqubidest. The microscopic images of their epididymal epithelial cells showed that they remained intact. On the other hand, the second group of mice was given a dose of 10 mgPb/kgBW. The microscopic images of their tubule lumen cells showed damage, and there was degeneration of the epithelial cells. The distance between the tubules was looser, and some of the tubules experienced thinning of the epithelial cells.

Lead is a heavy metal that is highly toxic to cells (Lee *et al.* 2017). The histological analysis revealed that after 35 days of lead administration, the epithelial tissue suffered damage. Cell damage occurs due to the mechanism of lead toxicity, as well as the mechanism of toxicity of other heavy metals, specifically, the activity of glutathione reductase (Rang *et al*. 1999).

Glutathione depletion occurs due to inhibition of glutathione reductase activity. Glutathione acts as an antioxidant that suppresses oxidative stress. When lead enters cells, it can cause an increase in Reactive Oxygen Species (ROS), which can suppress epithelial tissue. If there is a lack of glutathione, ROS levels can increase, leading to lipid peroxidation. This can damage epithelial cells (Astuti 2004).

The study found that the thickness of the epididymal tubular epithelium significantly decreased ($p<0.05$) when doses of 5 mgPb/kgBB and 10 mgPb/kgBB were administered, in comparison with the control group. This indicates that the administration of Pb is not safe for the epididymal epithelium, even at doses of 5 mgPb/kgBB or higher, since there is a significant decrease in the thickness of the epididymal epithelium.

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