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Histopathological of Mice (*Mus musculus*) Liver Induced by Lead (Pb) Orally

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Abstract

Lead (Pb) is a prominent heavy metal emitted by motor vehicle exhausts, factory and mining fumes. Its presence in the atmoshpere can endure for up to seven days, posing a considerable risk of contaminating surrounding food and beverages. Lead enters the body through inhalation and the skin. Lead can also enter the human body via the oral route and accumulate in the body. It causes health problems such as oxidative stress and damage human organs such as the kidneys and liver. This research aims to examine the effect of oral lead exposure on the liver histopathology of Swiss Webster strain mice (*Mus musculus*). Employing a non-probability sampling technique, 25 male mice were divided into 5 groups: negative control, K2, K3, K4 and K5. These mice were administered a daily oral dose of 0.5 mL and subsequently euthanized in CO_2 chamber the following week for liver dissection. The findings reveal signs of hydropic degeneration characterized by cellular swelling, irregular shapes, and disrupted organelles in groups K2, K3, K4, and K5. In addition, the mean degree of liver damage was observed as 0 for the negative control, 1 for group K2, 1 for group K3, 2 for group K4, and 3 for group K5. In conclsuin, this study confirms that lead exposure can result in dentrimenal liver histopathology changes in mice.

Keywords

Histopathology, Liver, *Mus musculus*, Lead (Pb), Oral administration.

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INTRODUCTION

The number of motorized vehicles in Indonesia is increasing rapidly, which harms the concentration of air emissions produced (1,2). Motor vehicles are the main cause of all toxic air emissions. Around 70% of air pollution in Indonesia is currently caused by vehicle emissions that emit smoke containing

hazardous substances which harm body health (3) Other pollution factors caused by lead, namely mining, coal burning, and environmental pollution from the air such as smoke, dust, industrial installations, and even poor food hygiene, are factors that support lead poisoning in living things (4).

Lead (Pb) exposure has been reported to be associated with liver-related diseases. Lead is toxic to living things, even in small amounts (1). Lead can enter the body through various intermediaries, like the respiratory system and be absorbed directly through the skin. In the body, it acts on enzymes related to heme synthesis, DNA transcription and neurotransmitter release, which regulate cellular growth and memory (5). The more lead enters the body over a long period, it will accumulate to harm human health and cause progressive poisoning (6). The liver metabolizes heavy metals and excretes them into the intestines through bile. About 5% of the bile substance is removed through the faeces and the rest can be reabsorbed through the enterohepatic circulation. As a result, liver cells are exposed to chemicals, which leads to liver dysfunction, cell damage, and organ failure. Considering that aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) from damaged hepatocytes are released into the blood, these are useful markers for liver injury. Some effects of exposure to lead can be permanent. If caught early, healthcare providers, and communities can act to prevent further exposure and reduce damage. The most important thing that parents and caregivers, healthcare providers, and public health professionals should be aware of is the higher the level of lead poisoning, the more severe the liver damage will be. The liver is the largest gland in the body and is located at the top of the abdominal cavity under the diaphragm (7).

The liver acts as a metabolic and detoxification process to neutralize toxic compounds that enter the body (8). Most of the toxic substances that enter the body after being absorbed by the epithelial cells of the small intestine will be transported to the liver through the portal vein (9). Chronic exposure to subtoxic concentrations of lead produced changes in the hepatocytes, portal triads and the sinusoids. The hepatocyte alterations were mainly anisokaryosis, nuclear vesiculation, binucleation, cytoplasmic inclusions, cytoplasmic swelling, hydropic degeneration, necrosis and reduction in glycogen content. In addition, portal triads mild chronic inflammation, Kupffer cells hyperplasia and occasional fatty change were seen together with hemosiderosis (10). The purpose of this study was to determine the effect of lead on the appearance of the liver of mice so that the mechanism of damage caused by Lead in the liver could be clearly described.

MATERIALS AND METHODS

The study was conducted at the Therapeutic and Pharmacology Laboratory of Padjajaran University and the Sitohistological Specialist Laboratory of the Bandung Health Polytechnic in July 2022. This study uses experimental research methods. The study focused on Swiss Webster strain male mice (*Mus musculus*) aged between 8-12 weeks, with an average weighed o approximately 30 grams. Mice were selected as the experimental subjects due to their physiological similarities to humans, compact size and body weight, and easy to handle (11). There were 25 mice used in this study, which were divided into five groups, namely the negative control (KN), dose 2 (K2), dose 3 (K3), dose 4 (K4) and dose 5 group (K5). The mice were adapted for one week to their new environment and did not experience stress. Sample calculation obtained by Federer formula. The sampling technique used was non-probability sampling with consecutive sampling technique (12). The mice used were healthy males with normal activities, aged 8-12 weeks and weighing 20-35 grams (13).

The primary data analysed in this study was the score of the degree of liver damage from all treatment groups. In all groups, adaptation was carried out for 7 days by being given mice food ratio. The following week, the KN group only received normal feed without any treatment, while the K2, K3, K4 and K5 groups received treatment with induction of lead via oral with a dose of 0.5 ml, once a day for 35 days. Each group will undergo surgery to harvest liver organs weekly. The surgery for the control group was carried out together with the K5 group in the last week (on the 28th day). The liver that has been taken was put into a 10% neutral buffered formalin (NBF) solution and will be made for histological preparations.

The preparation and colouring of these preparations were carried out at the Bandung Health Polytechnic Integrated Laboratory. The preparation is done by using fixation, dehydration, clearing, embedding, and impregnation, blocking, trimming, cutting and mounting. Meanwhile, the preparation was stained by Hematoxylin Eosin (HE) staining (14).

The obtained results are presented in the form of an overview. The data was statistically analysed using descriptive, in the form of information from researchers and several literature sources (15).

Data was obtained from the results of liver histology scores. The score was determined by observing the damage to the histological appearance of the mice's liver. The anatomical pathologist would give a score of 0 for no liver cell damage occurs; 1: Liver cell damage reaches 0-1.5%; 2: Liver cell damage reaches 6-25%; 3: Liver cell damage reaches 26-50%; and 4: Liver cell damage reaches 50% (13).

Furthermore, the data from the statistical percentage test results were analyzed using the Kruskal-Wallis test and then continued with the homogeneity test. If the results of the Kruskal-Wallis test show a significant difference, then proceed with the Mann-Whitney test (α = 0.05) at a 95% confidence level. All experiments were performed with ethical approval from the Health Research Ethics Committee Politeknik Kesehatan Kementrian Kesehatan Bandung (No.78/KEPKEC/ III//2023).

Dosage Calculation

The determination of the lead dose in this study was derived from the conversion of the maximum dose relevant to human subject to that suitable for mice. This conversion was accomplished using an animal dose calculation table based on Laurence and Bacharach's method (1964). The World Health Organization (WHO) states that the tolerance limit for lead (Pb) in the human body is 50 mg/adult body weight in a week. The conversion factor of 70 kg human to 20 g mice = 0.0026. The dosage calculation for the lead dose = 50 mg/BB with route of administration = oral – maximum volume of 1 mL is as below:

Conversion factor = 0.0026 (mice mice)

Total = 25 heads

Body weight = 20 – 30 g

$$\frac{VAO}{Exposure\ Volume} = \frac{\frac{1}{2}}{maximum\ volume}$$

$$\frac{\frac{1}{2}}{1} = 0.5\ ml$$

Dose conversion:

$$Dose\ conversion = \frac{human\ dose}{conversion\ factor}$$

$$= 50\,\frac{mg}{BW} \times 0.0026$$

$$= 0.13\ mg$$

Experimental Animal Treatment

In this study, 25 male mice were prepared for in vivo experiment. The mice were divided into five treatment groups, consisting of 5 Swiss Webster strain mice for each group. The distribution of treatment groups is explained in Table 1.

Table 1. Distribution of Treatment Groups

Group	Day	Treatment
KN	1 – 7	Adaptation.
	8 – 35	No specific treatment was administrated to the mice. They were provided with only aquadest and regular feed. Subsequently, the livers of mice were extracted to observe the histological images.
K2	1 – 7	Adaptation.
	8 – 14	The adaptation process involved the daily administration of 0.5 mL of lead acetate solution over the course of one week. Following this regimen, the livers of the mice were extracted for the purpose of examining the histological images.

K3	1 – 7	Adaptation
	8 – 21	The experimental process included the daily provison of 0.5 mL of lead acetate solution over a two-weeks. Subsequently, the livers of the mice were collected to visualisze the histological images.
K4	1 – 7	Adaptation
	8 – 28	The mice were administered of 0.5 mL of lead acetate solution daily for three consecutive weeks. Following this protocol, the livers of the mice were extracted for the purpose of conducting the histological images.
K5	1 – 7	Adaptation
	8 – 35	The experimental procedure involved the daily provision of 0.5 mL of lead acetate solution for a duration of four weeks. Following this extended exposure, the livers of mice were collected to the histological images.

Histopathological Examination

The collected samples were fixed in 10% NBF solution for a period 24 hours. Subsequently, they underwent a dehydration process using a series of graded alcohol solutions, starting from 70% and gradually progressing to 80% and 96%. Post dehydration, the sample were cleared using xylol and then subjected to the impregnation stage, being soaked in liquid paraffin for a duration of 12 hours. Tissue were cleaned with xylene and embedded in paraffin. Using a rotary microtome, sections were cut at a nominal thickness of 5–6 μm and stained with hematoxylin and eosin to observe microscopic histopathological alterations and their severity (16). Histopathological observations of the liver were performed after staining using Hematoxylin and Eosin staining (HE staining) by taking 400x and 200x magnification photos under a trinocular microscope Olympus U TV0.5XC-3, T7 Tokyo, Japan. The analysis of 400x magnification photos was followed by observations from pathological anatomical specialist, dr. Komala, Sp.PA., an anatomical specialist from Cimacan Hospital, Indonesia.

Data Analysis

The obtained results were organized and presented in the form of an overview. The data was statistically analysed using descriptive, in the form of information from researchers and several literature sources. The data was statistically analyzed using a descriptive approach, primarily focusing on the liver histology scores. The statistical analysis encompassed the utilization of the Kruskal-Wallis test, a non-parametric method for comparing multiple independent samples. In instances where Kruskal-Wallis indicated a notable difference, the analysis further involved the application of the Mann-Whitney test (α = 0.05) at a 95% degree of confidence (17).

RESULTS

Histology of Lead-Induced Mice Liver

Following the treatment procedure, liver

tissue samples were collected and subsequently preserved. The tissue processing consists of 8 stages, fixation, dehydration, clearing, embedding, blocking, cutting, colouring and mounting. Following these preparatory steps, the tissue samples underwant staining using the HE method. Figure 1 shows the histology of lead-induced mice liver, with HE staining conducted at 40x magnification. The histological images represent the following groups: KN (negative control), K2 (dose group II), K3 (dose group III), K4 (dose group IV), K5 (dose group V).

The Measure of Degree of Damage

The degree of damage according to the Mitchel method (15) is the degree of 0 is no cell damage; 1 is liver cell damage has reached 0.1-5%; 2 is liver cell damage has reached 6-25%; 3 is liver cell damage has reached 26-50%; and 4 is liver cell damage reaches 50%.

Table 2. Processing of Liver Histology Preparatory Scores

Mice	Group Treatmen				
	KN	K2	K3	K4	K5
1	0	1	1	2	3
2	0	1	1	2	3
3	0	1	1	2	3
4	0	1	1	2	3
Amount	0	4	4	8	12
Average	0	1	1	2	3

Degree of Damage Mitchel Method (Gufron, 2001) (18)

The score of each liver histology preparation in the treatment group, namely the negative control, no damage occurred, the mean was 0; groups K2 and K3 experienced damage with an average score of 1; group K4 experienced damage with an average score of 2; then finally group K5 experienced damage with an average score of 3 (Table 2).

The results of histopathology observations of the mice's liver under a microscope showed damage to cells that experience hydropic degeneration, however, the rest are normal. The average percentage of liver cell damage is calculated and presented in Figure 2.

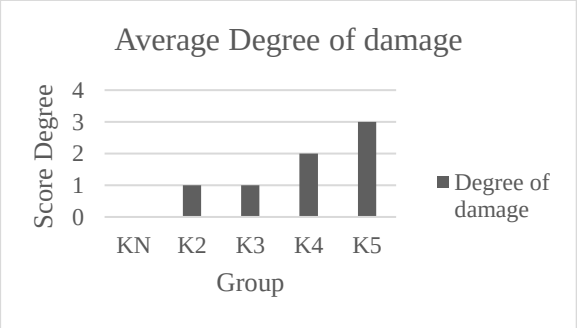
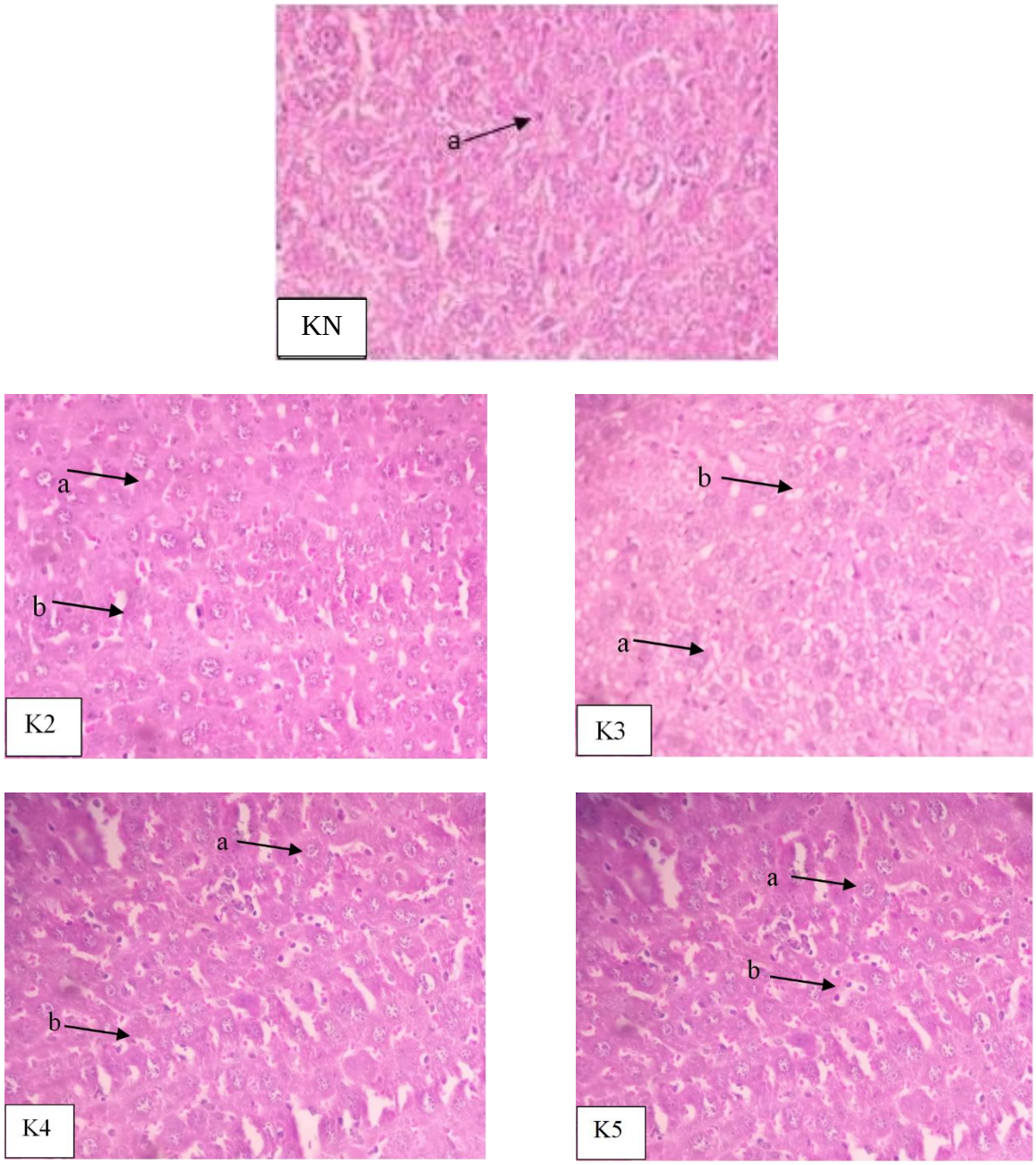


Figure 2. Average score of liver cell damage degrees

DISCUSSION

Observations of the negative control group revealed no evidence of damage, the cells appeared normal, displaying a tidy cell structure with healthy hepatocytes and regular cell nuclei, without any signs of degeneration. These findings were expected as the negative control group was not subjected to lead induction but received only standard food and water. This description analysis underscores the detrimental impact of lead on health, particulary its potential to cause damagen to liver tissues.



Description: a. normal hepatocyte cells, b. hydropic degeneration

Figure 1. Histology of lead-induced mice liver, and Hematoxilin Eosin (HE) staining was performed with 40x magnification. KN (negative control), K2 (dose group II), K3 (dose group III), K4 (dose group IV), K5 (dose group V).

In the K2 and K3 treatment groups, there was a picture of cells that experienced hydropic degeneration and were not so visible that many cell nuclei experienced pyknotic conditions. That condition occurred because the duration of lead exposure had not spread to all cells. The liver cell damage development begins with a degeneration process characterized by swelling of the cells. Liver cells are the particular tissue that becomes the target of increased concentrations of free radicals because the

liver is where the metabolism of xenobiotic compounds occurs. It causes normal cells to be damaged (19).

In the K4 treatment group, there was a wider area of degenerative (hydropic) damage than in groups K2 and K3 due to the longer exposure time that caused more accumulation of lead levels in the blood. The result was in line with research conducted by Arifuddin et al (18) that found liver cell damage occurs due to exposure to lead at higher doses. Large amounts of lead (3 g/L) will result in the accumulation of lead in the blood and liver tissue, which causes loss of the liver cell structures and arranged in strands. Lead exposure also results in histological changes in liver tissue, including necrotic cells with vacuolization and swelling of cells, pycnotic nuclei and dilated central veins, and sinusoids (20).

The statistical anlaysis of percentage test data involved the application of the Kruskal-Wallis test. Followed by a homogeneity test. The SPSS Homogeneity Test output yielded a significance value (sig) of 0.00, suggesting that the variants among the five treatment groups under comparison were significantly different and not homogenous. Another sig value of 0.000 was obtained, wleading to the rejection of the null hypothesis, indicating an influential effect of each treatment group on the liver histology of the mice. Further analysis was then performed to identify which treatment groups exhibited significant difference.

The Mann-Whitney test result indicated a notable discrepancy in liver damage between the treatment groups, with a p-value of <0.05, except for the comparison between groups K2 and K3, where no significant difference was observed.

The influence lead intoxication as prominently manisfested in the significant alterations observed in the nuclei of hepatocyte. These alterations may have resulted from an escalation in cellualar acitivity and disruptions in the mechanisms of lead detoxification within the nucleus. This study found that the level of liver tissue damage increased with lead exposure time, which was induced in the mice. This condition showed that lead has a real harmful impact on tissues, especially the liver. damage the largest occurred in the K5 group, where the exposure was longer for 35 days and the highest degree of hydropic degeneration was obtained with an average score of 3.

The hepatocytes in the periportal zones were particularly susceptible to necrosis, as evidenced by the blue staining of the cytoplasm in these cells. When the influx of toxic compounds surpasses the liver's detoxification capacity and continues to accumulate over an extended period, it can lead to progressive tissue degeneration (21).

Chronic lead exposure also increased the activities of alkaline phosphatase and α-glycerophosphate-dehydrogenase, which might be an adaptation to the metabolic, structural and functional changes in the organelles of hepatic cells due to lead intoxication. The findings revealed that chronic exposure to lead produced significant histological and histochemical changes in the liver of the Wistar albino rats (10). There is also necrosis, which can damage the tissue.

Lead in the blood can damage various organs, including the liver (22). This is due to the ability of lead to form free radicals in the body and reduce the ability of antioxidants, which naturally cause oxidative stress (23). It has been reported that Pb binds to the –SH group of glutathione (GSH), thereby reducing the antioxidant activity and increasing the ROS production.

Sandhir and Gill and Omobowale et al. (24) observed that the depletion of GSH and the activity of the antioxidant defense system are significantly reduced in rats exposed to Pb (25). The in vivo studies have shown that the accumulation of oxidative stress is due to mercury exposure and the destruction of the balance between Reactive Oxygen Species (ROS) and the antioxidant defence system (25).

The liver plays an essential role in the metabolism of drugs or exogenous toxicants. In addition, the majority of drugs are biologically transformed in the liver. The pathological state of the liver can affect drug metabolism, thus changing the efficacy and the ADRs, whilst the metabolic products of drugs can cause liver damage (26). Furthermore, it is known from several studies that lead can directly cause interference with the normal biochemical system (hepatobiliary) and can also cause necrosis of liver cells.

CONCLUSIONS

Based on the findings of the study, it can be inferred that the K5 group, subjected to the longest duration lead administration over four weeks, exhibited the most substansial liver damage. The administration of lead orally to the mice cwas correlated with an escalation in the degree of liver damage, as evidenced by alterations in cell nucleus morphology, notably including instances of hydropic degeneration.

AUTHOR CONTRIBUTIONS

Kodariah Liah: conceptualization, methodology, formal analysis, investigation, writing-original draft. Pakpahan Suyarta Efrida: co-writer. Nugraha Aditya: data curation, writing-original draft preparation, visualization, and writing-reviewing. Rena Nurzal Zabila: data curation, writing- original draft preparation, visualization, and writing-reviewing.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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