Journal of Veterinary and Animal Sciences pISSN 2550-1283; eISSN:2622-0571
DOI: https://doi.org/10.24843/JVAS.2023.v06.i02.p02 Agustus 2023 Vol. 6 No.2: 77-86

Distribution and Elimination of Lead in Rat (*Rattus norvegicus*) Tissues

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Abstract. This study aims to determine the distribution and elimination of lead levels in various tissues of rats (*Rattus norvegicus*). The study used 32 rats which were divided into 2 groups, namely the control group and the group given 2.00 ppm Pb-acetate. Treatment by administering Pbacetat is carried out orally every day for 30 days (phase 1). On day 31 (phase 1), 8 rats from each group were taken their blood plasma for measurement of lead levels. Measurement of lead levels was carried out using the atomic absorption spectrophotometry (AAS) method. Then the rats were necropsied and the liver, kidneys, spleen, lungs, intestines, heart muscle and brain tissue were taken for histopathological preparation. Histopathological preparations were made according to the hematoxylin eosin (HE) staining. The remaining 8 rats from each group were kept continuously for the next 30 days (phase 2), without giving Pb-acetate solution. This phase aims to determine the level of lead elimination from rat tissues. On day 61, all remaining rats were taken their blood for measurement of lead content. Then the rats were necropsied to take liver, kidney, spleen, lung, intestine, myocardium and brain tissues, the same as in phase 1.The histopathologically examination were categorized based on thehaemorrhage, inflammatory and necrotic lesions. The average of measurement result of lead content in the blood of rats in phase 1 was 0.27±0.06 ppm. Whereas the average of lead content in phase 2 was 0.12±0.03 ppm. This result showed significantly difference by variance of analize. Based on the tissue lesions, the liver, kidneys, spleen and lungs were the main tissues undergoing histopathological changes. Up to phase 2, the liver tissue still has lesions. It can be concluded that lead contamination in 30 days can significantly decrease in the next 30 days. Lead distribution can cause lesions in liver, kidney, spleen and lung tissues. But in phase 2, liver lesions were still found, indicating that liver tissue had the lowest elimination power compared to other tissues.

Keywords: Blood Plasma; Distribution; Elimination; Lead; Tissue

I. INTRODUCTION

Lead contamination has been increasing and widespread in the environment, plants and animals. The cattle reared in landfills are very susceptible to exposure by lead, as happened to cattle reared at the Denpasar City landfill [1], at the Jatibarang landfill , Piyungan landfill

[2], in Nigeria [3]. As is known, urban waste generally consists of organic and inorganic materials. The cattle have ability to select feed, but degradation of inorganic materials including lead in the waste can be ingested [4].

Intensively reared dairy cattle have also been reported to be exposed by lead [5]. It is suspected that the plants used as animal feed were exposed to lead. The fodder plants may get Pb exposure from the soil, water or air pollution in the vicinity. The chain of lead exposure among the plants, animals and humans who consume it, is a common chain that also occurs in other contaminants [6].

Characteristics of lead which are accumulative due to not being metabolized by the body, causing lead to be very risky to human health [7]. Lead poisoning can cause brain degeneration [8], anemia, decreased immunity to infection [9], gastroenteritis and encephalopathy [10]. Brain disorders in the form of cognitive decline, especially in children [11]. The impact of lead poisoning on animals and humans is very serious and deserves attention.

The distribution pattern of lead in the bodies of animals and humans naturally has no clear mechanism and pathogenesis [12]. However, based on the characteristics of lead, which can substitute for iron (Fe) in hemoglobin, it is assumed that the lead distribution in the body follows the pattern of blood circulation. Some tissues of cattle can also be exposed by lead such as liver, kidney and spleen tissues with the highest content.

Several mechanisms to eliminate lead contamination from animal bodies include dietary regulation and the addition of phytochemical additives [4]. But these efforts are more of a preventive nature. How long the presence of lead in the animal's body, a series of further studies are needed after exposure to lead. There is a possibility that lead content will decrease or even become non-existent, after stop the lead expose.

II. MATERIAL AND METHODS

The study used 32 Wistar rats (Rattus norvegicus), male, 2 month, 250-300 gr body weight. The samples taken were whole blood, liver, kidney, spleen, lungs and intestines. Blood samples were taken for measurement of lead content. The tissue samples were taken to make histopathological preparations.

The research design used a completely randomized design of the 32 rats randomly divided into 2 groups, namely the control and the treatment group given 2.00 ppm Pb-acetate. The treatment Pb-acetate 2.00 ppm was administered orally, every day for 30 days (phase 1). On the 31st day after treatment, then 8 rats each

from two treatment group were taken their blood from the orbital vein and stored in a tubes. Those 16 rats were necropsied to take liver, kidney, spleen, lungs, intestine, myocard and brain tissues. The blood samples was taken for measurement of lead content. Those tissues taken were made histopathological preparations according to the hematoxylin eosin (HE) staining procedure. Meanwhile, the remaining 8 tails from each treatment group were kept continuously until day 61 (phase 2), but without the administration of Pb acetate solution. On day 61, all experimental animals were euthanized and their blood plasma was taken. Furthermore, the experimental animals were necropsied for tissue collection and processed in the same way as in phase 1.

Preparation of a 2.00 ppm Pb-acetate solution was carried out by dissolving 2 mg Pb acetate powder in 1 liter of distilled water. Then shaken until dissolved and homogeneous, then given to experimental animals.

Measurement of lead content in the blood was carried out using by the Atomic Absorption Spectrophotometry (AAS) method [13] Amount 1 ml of blood plasma from each experimental animal was divided into two parts, 0.5 ml for the positive control and 0.5 ml for the sample. Then add 0.25 ml of 1mg/l standard solution to the sample to make spiked or positive control. Spiked was evaporated on a hot plate at a temperature of 100ºC until dry. The sample and spiked were put into an ashing furnace and half-covered. The temperature of the ashing furnace was gradually increased by 100ºC every 30 minutes until it reached 450ºC and maintained for 18 hours. Samples and spikes were removed from the ashing furnace and left at room temperature. After cooling, 1 ml of 65% HNO3 was added, shaking slowly until all the ash was dissolved in the acid. Then evaporated on a hot plate at a temperature of 100ºC until dry. After drying, the sample is spiked and put back into the ashing furnace. The temperature was gradually increased to 100ºC every 30 minutes until it reached 450ºC and maintained for 3 hours. After the ash was completely white, the sample and spiked were cooled to room temperature. Next, add 5 ml of 6 M HCl to each sample and the spikes are shaken slowly until all the ash is dissolved in the acid. Evaporated on a hot plate at 100ºC until dry. 10 ml of 0.1 M HNO3 was added and cooled at room temperature for 1 hour, the solution was transferred to a 50 ml polyproylene volumetric flask and a matrix modifier was added, adjusting it to the mark using 0.1 M HNO3. Standard working solutions of Pb were prepared respectively. -each at least five concentration points. The

working standard solution, sample, and spiked were read on a graphite fumace atomic absorption spectrophotometer at a wavelength of 288.3 nm for Pb metal. Pb concentration in g/g is calculated by the following formula (SNI 2354.5:2011) :

$$Concentrate = \frac{(D - E)\ x\ Fp\ x\ V}{W}$$

Description:
D: Concentration of sample in µg/l from AAS reading
E: Concentration of blank sample in µg/l from AAS reading
Fp: Dilution factor
V: Final volume of prepared sample solution (in ml), converted to liters
W: Weight of sample (in grams)

The histopathological preparation of the tissue according to the method of Kiernan [14]. The tissues were fixed by immersing the organs in 10% Neutral Formalin Buffer solution for ± 48 hours at room temperature. The fixed tissue is then trimmed to a size of 1 x 1 x 1 cm so that it can be inserted into a tissue cassette for processing in a tissue processor. The next step is dehydration by immersing the tissue in alcohol successively with alcohol concentrations of 70%, 80%, 90%, absolute alcohol I, absolute alcohol II, each for ± 2 hours. The next step is clearing to clean the remaining alcohol from the tissue. After cleaning, the tissue is ready to be inserted into the paraffin block. The next stage is embedding and blocking with paraffin blocks. Organs were placed on paraffin, then made in paraffin blocks, cooled and stored in the refrigerator for 24 hours. The paraffin blocks were then cut with a microtome with a thickness of 4-5 m. The cut tissue was then floated in a water bath at a temperature of 600C to avoid folding of the tissue slices after cutting. The preparation was transferred to the object glass. Then dried at room temperature 26-27oC. The next process is staining of tissue preparations using the Haris Hematoxylin-Eosin (HE) method. The staining procedure includes the deparaffination step, which is soaking the slide on the slide in xylol grades I-III for five minutes each. After that, dehydration with the aim of providing water to the tissue is by immersing the preparations in absolute alcohol solution and then transferred to 95% alcohol solution with a duration of five minutes each. Then rinsed with running water for 1 minute. The preparations were then immersed in Harris hematoxylin solution for 15 minutes. Dip in distilled water for 1 minute by lifting and lowering, then dip into 1% acid-alcohol mixture quickly 5-7 dips. Then rinse in distilled water for 1 minute and rinse again with distilled water for 15 minutes. Dip 3-5 times in lithium carbonate solution for 15-30 seconds until the pieces are bright blue and then wash with running water for 15 minutes. The preparations were then immersed in eosin

for 2-3 minutes. The next step is dehydration by incorporating the preparation in graded alcohol from 80%, 90% and 95% to absolute alcohol I-III. After that, clearing is done by inserting the preparation in xylol I-II and drying. Next, the mounting process is carried out, namely closing the preparation with a cover glass using a permount as an adhesive. The histopathological preparations were then observed under a microscope.

Histopathological examination of liver, kidney, spleen, lung and intestine tissue was based on the presence of bleeding, inflammatory and necrotic lesions. Bleeding is a common lesion in the acute poisoning process. Meanwhile, inflammation and necrosis are further lesions of cell or tissue damage due to poisoning.

Data from the measurement of heavy metal levels of Pb in the blood between phase 1 and phase 2 were analyzed by the one ay anova test. While the results of tissue histopathology were analyzed descriptively.

III. RESULT AND DISCUSSION

Based on the measurement of lead content in the blood of experimental animals, the results are as presented in table .1. From the table, there is an average of 0.27±0.58 ppm of lead content in the blood circulation in phase 1 and 0.13±0.31 ppm in phase 2. When viewed from the content of lead given as much as 2. 00 ppm, means that only 13.5% enters the blood circulation. Meanwhile, during the 30 days of phase 2, 14.3% of lead contamination was eliminated from the experimental animal's body.

Table 1. The results of measuring Pb levels in the blood plasma of experimental animal

Treatment	Replikation	Level of Pb (ppm)	
		Phase 1 (30 days)	Phase 2 (60 days)
P0 (Control)	1	0.00	0.00
	2	0.00	0.00
	3	0.00	0.00
	4	0.00	0.00
	5	0.00	0.00
	6	0.00	0.00
	7	0.00	0.00
	8	0.00	0.00
P1 (Pb asetat 2.00 ppm)	1	0.34	0.14
	2	0.26	0.12
	3	0.33	0.10
	4	0,18	0.12
	5	0.24	0.20

	6	0,30	0.11
	7	0,32	0.11
	8	0,22	0.13

The results of the one way ANOVA analysis showed that there was a significant difference between the lead exposure period (phase 1) compared to the lead elimination period (phase 2). It means that lead contamination is eliminated from the experimental animal's blood during the second 30 days (phase 2) after lead administration in phase 1 (Table 2).

Table .2. *One Way Anova Test*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,084	1	,084	38,890	,000
Within Groups	,030	14	,002		
Total	,114	15			

The results of histopathological examination of liver, kidney, spleen, lungs, intestines are, myocard and brain tissues as presented in table 3.

Table 3. Histopathological changes data due to lead administration

Tissue	Treatment	Severity of histopathological lesions						Lession level	
		Hemorrhages		Imflammation		Necrosis			
		Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II
Liver	P0	-	-	-	-	-	-	Normal	Normal
	P1	++	++	+++	++	+	-	Severe	mild
Kidney	P0	-	-	-	-	-	-	Normal	Normal
	P1	++	-	+++	+	-	-	Severe	mild
Spleen	P0	-	-	-	-	-	-	Normal	Normal
	P1	++	+	+++	+	+	-	Severe	mild
Lungs	P0	-	-	-	-	-	-	Normal	Normal
	P1	++	-	++	-	+	-		
Intestine	P0	-	-	-	-	-	-	Normal	Normal
	P1	+	-	-	-	-	-		
Myocard	P0	-	-	-	-	-	-	Normal	Normal
	P1	-	-	-	-	-	-		
Brain	P0	-	-	-	-	-	-	Normal	Normal
	P1	-	-	-	-	-	-		

P0=without Pb; P1= Treatment with Pb 2,0 ppm

N = normal; + = mild; ++=Medium; +++= severe

The impact of the administration of the lead based on the severity of histopathological lesions seen in liver, kidney and spleen tissues were the most dominant in phase I administration. These results are in accordance with the report of research that the accumulation of heavy metals including the lead is common in the liver and kidneys [15]. These results are also in accordance with the report of research that in cattle reared in landfills, the liver and kidneys contain the lead heavy metal [1]. The liver as the central organ of metabolism will always try to metabolize various organic and inorganic materials that enter the body. While the kidney is an organ that plays a role in excretion, it is very susceptible to toxic materials including heavy metals.

The spleen as an organ for forming blood cells (erythropoiesis) has a different pattern than the liver and kidneys. Lead heavy metal in the spleen can cause damage to the structure of the spleen tissue [9,16]. Lead heavy metal particles in the circulation will cause peroxidation or toxicity in every tissue that is passed. As a result of this toxicity, it causes the formation of free radicals and damages the cell structure resulting in bleeding, inflammation and necrosis in varying degrees of severity [10,17]. Chronic toxicity of heavy metal Pb in lymphoid tissue, especially the spleen, can cause immunotoxicity [18, 20].

In the lungs there was bleeding and moderate inflammation in phase I indicating that there were heavy metal Pb particles passing through the pulmonary circulation [6]. The circulatory system is very intensive, causing the lungs to be very sensitive to contamination with inorganic materials.

In the intestine there are changes in the form of bleeding and mild inflammation, indicating that at a concentration of 2.0 ppm heavy metal Pb can cause lesions in the intestine. Leadheavy metal in the intestine is selected, where small particles will be absorbed and other particles excreted [10]. The presence of high levels of heavy metal Pb in the intestines can cause behavioral changes in animals [19, 21].

IV. CONCLUSION

From the results of the study it can be concluded as follows the level of Pb in the blood of experimental animals was highest compared to the tissue and significantly decreased at 30 days after Pb administration was discontinued. Liver, kidney, spleen and lung tissue, respectively, were the worst tissues with pathological damage lesions due to Pb contamination and a marked

decrease in lesions occurred 30 days after discontinuation of Pb administration.

ACKNOWLEDGMENT

Thank you to the Rector of Udayana University cq Institute for Research and Community Service of Udayana University for the 2021 PNBP funds that have funded this research.

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