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Effects of Iodine Solution on Histopathologic Features of Lead Acetate-Induced Rat Hepar

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Abstract

Background: Lead is a heavy metal that can be found in the environment. Lead can be naturally occurring or produced from human activities. Lead entering the body can cause oxidative stress which can then cause cell damage. Iodine in the body can act as an antioxidant which then prevents oxidative stress.
Methods: This study is an experimental study on 18 *Rattus novergicus* rats conducted for 19 days. Rats were divided into three groups: negative, positive control group, and treatment group. On the 20th day the rats were killed then the hepatic organs were taken and preparations were made. The preparations were assessed using Knodell's Score. Data were analyzed using Kruskal-Wallis and then followed by Mann-Whitney Test.
Results: The negative control group showed that the dominant is in the form of normal hepatocytes whilst the positive control group hepatic cells experienced cell damage in the form of inflammation, degeneration, and necrosis. In the treatment group, damage to hepatic cells was lower than the positive control group.
Conclusion: There is an effect of iodine solution administration in preventing damage to rat hepatic cells induced by lead acetate.

Keywords: iodine solution, lead acetate, hepar histology

Introduction

Nowadays, there is rapid development in urban development and industrial centers, including the transportation sector. Developments in the transportation sector have resulted in a significant increase in the number of motorized vehicles. Based on the records of the Central Statistics Agency (BPS), in 2019 the number of motorized vehicles in Indonesia reached 133,617,012 units. The operation of motorized vehicles requires fuel oil. The combustion activity produces exhaust emissions which are a pollutant to the air so that the rapid increase in the number of motorized vehicles causes an increase in air pollution. One of the pollutants produced by the combustion activity of motor vehicle fuel oil is lead or Plumbum.[1]

Lead is an element that is classified as a heavy metal.[2] Heavy metals are natural metal elements that can be found in the environment that can be single elements or paired with other elements. Heavy metals can easily evaporate and are carried by fine particles widely and on a large scale. Heavy metals are unable to be degraded or destroyed, and are easily absorbed. There are various heavy metals that can be found in nature, including lead, mercury, and cadmium.[3,4]

Lead, or metal with atomic number 82, is naturally found in soil and is odorless and tasteless. Although lead is naturally occurring in nature, most of the lead concentrations are the result of human activities, with more than 50% of lead emissions coming from fuels. In the environment, lead can be found in various media such as water,

soil, dust, and air. In addition, in everyday life, lead can be found in ceramics, food can linings, pipes, some traditional medicines, and cosmetics. Environmental experts are unanimous in their opinion that lead is considered to be the largest contaminant of all metal dust in the air.[3]

Based on research conducted by Mustafiroh et al, on the relationship between air lead levels and blood lead levels of workers in the painting section of the car body industry in Semarang, it was found that the average value of lead levels in the blood of workers was 34.4 ⌈g /dl. This value is categorized as abnormal or exceeds the Treeshold Value.[5] In addition, in a study conducted by Fibrianti and Azizah (2015) it was proven that 30% of respondents who were Aki Home Industry workers had blood lead levels above the ATSDR (Agency for Toxic Substance and Disease Regitsry) standard of ≥ 10 µg/dL.[6]

Lead can enter the human body through several ways, namely through the respiratory tract, digestive tract, and skin. Inhaled lead will enter the body through the respiratory tract, enter the pulmonary blood vessels, and bind to the blood. Lead that enters orally will also be absorbed in the gastrointestinal tract and then bind to the blood. On the skin, lead can be absorbed because it can dissolve in fats and oils. Lead that has entered the body and binds to the blood will accumulate highly in soft tissues such as the brain, heart, lungs, liver, kidneys, and testes.[5,7]

The effects of prolonged exposure to lead are chronic so that the cumulative dose increases progressively which will then disrupt various organs. One of the soft organs that undergoes considerable changes due to lead exposure is the liver. The damage to the liver that occurs as a result of lead toxicity is the induction of free radical formation and a decrease in the body's antioxidant system resulting in oxidative stress.[8]

Hepar is actively involved in various metabolic functions and is one of the organs targeted by various toxins including lead. Damage to the liver is associated with distortion of metabolic functions including detoxification, digestive function, and protein synthesis. Hepatotoxic effects caused by the induction of lead manifest in the form of inflammation in hepatocyte cells and damage to hepatic reticular fibers.[9]

Lead toxicity in body cells can damage the balance of reactive oxygen species (ROS) production, where ROS levels increase while antioxidant levels decrease. Increased levels of ROS can cause damage to cell structures, nucleic acids, proteins, membranes, and lipids.[3]

Assessment of the degree of hepatic cell damage can use the Knodell Score method. The Knodell Score method is a system of assessing hepatic cell damage and is widely regarded as a benchmark for objective, semi-quantitative, and reproducible descriptions of various morphological lesions of hepatic cells.[10]

Iodine in the physiology of the human body plays a role in thyroid function. Iodine in the body also has a role in various other organs and tissues. Iodine in extrathyroidal and non-endocrine functions, acts as an antioxidant that can effectively fight free radicals. This ability depends on the iodine levels in the target organs.[11]

Studies conducted by Aceves et al, show that iodine acts as an antioxidant by competing free radicals for membrane lipids, proteins, and Deoxyribo Nucleic Acid (DNA), as well as increasing the activity of antioxidant enzymes and inactivating proinflammatory pathways. Iodine as an antioxidant and as an antiproliferative and differentiation agent, is able to maintain the integrity of several organs in the body.[12]

Based on research by Vidal et al, pregnant women who have optimal iodine levels (150-200 µg/L) in their bodies have optimal antioxidant status and low oxidative stress.[13] Iodine is needed by the human body approximately 150-300 µg per day. This need can be met from the consumption of iodine-rich foods such as marine fish and dairy products. In addition, iodine solution, namely lugol's solution, is a source of iodine that is widely used in meeting iodine needs. [14]

Based on the background that the author has mentioned above, it is necessary to conduct research on differences in the histopathological picture of rat liver in the administration of iodine solution induced by lead acetate.

Methods

This study is an experimental study that uses the object of research in the form of white rat (Rattus norvegicus). This experiment was conducted in the laboratory of the Faculty of Pharmacy, Andalas University.

The rats were divided into three groups: negative control group (only given standard diet), positive control group (given standard diet and lead acetate), and treatment group (given standard diet, lead acetate and iodine solution). The sample size to be used in this study was determined based on the minimum sample size criteria by the World Health Organization (WHO), namely 5 rats for each group. The treatment of the experimental animals was carried out for 19 days.

On the 20th day the rats were killed then the hepatic organs were then made into preparations using hematoxylin and eosin (HE) staining. Examination of hepatic preparations using HE staining will be viewed using a light microscope at 40 times objective lens magnification by identifying the most significantly damaged areas. Images of hepatic preparations were taken using a light microscope with an Olympus BX5 camera connected to the software, DP2-BSW. Hepatic damage will be assessed based on the Knodell score system in 5 different fields of view. The Knodell score method includes several assessments, including inflammation of hepatocyte cells, degeneration, fibrosis, and cell necrosis.

Data from the observation of hepatic microscopic preparations were tested for normality with the Saphiro-Wilk test and data were tested for homogeneity with the Levene statistical test. Furthermore, data analysis was carried out using parametric statistical tests (One Way ANOVA followed by Post Hoc test) or nonparametric (Kruskal-Wallis followed by Mann- Whitney test).

This research has passed the ethical test with ethical eligibility letter number 626/UN.16.2/KEP-FK/2022. The institution that issued no. permission to review the ethics of this research is the Faculty of Medicine, Andalas University.

Results

The results of observation of rat hepatic preparations are presented in Figure 1.

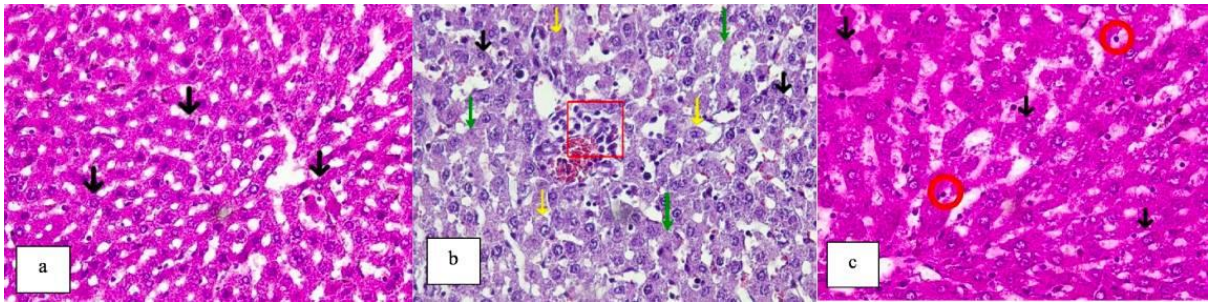

Figure 1. Histopathological picture of rat hepar in negative control group (a), positive control group (b), and treatment group (c). Normal hepatic cells (black arrow), inflammatory netrophil (square), inflammatory lymphocyte (red circle), degeneration (yellow arrow), necrosis (green arrow). Magnification 40 x 10 and hematoxylin eosin staining.

The histopathological picture of the hepar of rats in the negative control group showed normal hepatocyte cells. Whereas the histopathology picture of the hepatic rat of the positive control group showed a picture of damaged cells in the form of inflammation, degeneration, and cell necrosis. The treatment group showed a varied picture, but fewer hepatic cells were damaged compared to the positive control group.

The histopathological picture of the hepatic rat negative control group (K-) has a Knodell score ranging from 1 to the highest score of 2, the positive control group (K+) has a damage degree score ranging from 5 to 511 while the treatment group (KP) has a damage degree score ranging from 1 to the highest score of 3.

Each group has a varying number of Knodell scores. To determine the difference in scores from each group, the average value of all Knodell scores was calculated.

The recorded and averaged Knodell scores of each group of experimental rats are shown in Table 1.

Table 1. Average Knodell score in each group

Groups	Hepar Cell Damage Score					
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Average
K-	1	1.4	1.4	1.2	1.2	1.24
K+	9.6	8.6	10	10.2	6.2	8.92
P	2.2	2.4	2.2	2.8	1.6	2.24

Table 1 shows the difference in the degree of hepatic damage between groups. The highest hepatic damage score was found in the positive control group (K+), while the lowest hepatic damage was found in the negative control group (K-).

This study used the Shapiro-Wilk Test normality test and showed p>0.05, therefore the data was normally distributed. In the homogeneity test, the results obtained p <0.05 for each group mean that the data is not homogeneous so that the requirements for the One Way ANOVA test are not met. Therefore, data analysis was continued with a non-parametric test, namely the Kruskal-Wallis test.

Based on the Kruskal-Wallis test, it was found that there was a significant difference where the p value = 0.002 (p < 0.05). The analysis shows that there is an effect of administering iodine solution on the histopathological picture of the liver of rats induced lead acetate. Data were further analyzed using the Mann-Whitney test for differences in significance in each group. The results of the Mann-Whitney test can be seen in table 2.

Table 2. Mann-Whitney test of Knodell scores of the study groups.

Groups		Sig
K (-)	K (+)	0,009*
	Treatment	0,008*
K (+)	K (-)	0,009*
	Treatment	0,009*
Treatment	K (-)	0,008*
	K (+)	0,008*

Note: *Meaningful or significant difference (p < 0.05)

The Mann-Whitney test shows the comparison of scores from each group of experimental animals. Based on table 2, there is a significant difference between group K- with group K+, and there is a significant difference between group K+ with group with each p value <0.05. Thus, it can be said that there is an effect of administering iodine solution on the histopathological picture of the liver of rats induced by lead acetate.

Discussions

Histopathologic Features of Hepar of Lead Acetate-Induced Rats

Table 1 shows that the positive control group (K+) treated with lead acetate at a dose of 100 mg/L for 19 days showed the highest Knodell score compared to the other two groups. The histopathological picture of the positive control group showed that the picture was dominated by cells that experienced inflammation and necrosis. This picture has similarities with research conducted by Hegazy where the group exposed to lead acetate showed damage to the microscopic structure of the liver. The study showed fibrosis and necrosis in hepatic cells of experimental animals.[15] This supports the results of research conducted by Omotoso where the administration of lead acetate caused hepatotoxic effects that manifested in the form of cell inflammation, hepatic damage, and loss of hepatic reticular fibers. This may be due to oxidative stress arising from lead exposure.[9]

Lead is one of the most widely studied toxins. Based on studies that have been conducted on lead, it is found that lead affects various body functions, including the nervous, kidney, cardiovascular, reproductive system, immune system, and hematological system.[16]

Lead as a heavy metal that is potentially toxic to humans can cause damage to excretory and detoxification tissues, namely the kidneys and liver.[3] Lead can induce various biochemical and structural changes in hepatic cells which is an indication of lead toxicity to the liver.[9]

Exposure to lead in the environment is a health concern because lead is a toxic metal that is widely distributed in the environment. Lead can be absorbed by the human body through the digestive tract, respiratory tract, and skin. Lead is usually absorbed by the body from contaminated drinking water or when inhaling polluted air containing lead.19 Lead intoxication of rats caused structural and histopathological changes in the liver where cytoplasmic vacuolation was found due to degeneration of cellular organelles.[17,18]

Lead has been shown to cause free radical damage through two different pathways. The first pathway is known to produce ROS which cause lipid peroxidation, DNA damage, and depletion of sulfide groups such as sulthydryl (- SH) in enzymes in the body. The second pathway is through direct reduction of antioxidant reserves in the body.[9]

Lead can interfere with enzymes that work to maintain cell membrane integrity, which can cause cell damage such as cell degeneration. ROS imbalance can cause disturbances in cell proteins which can cause endoplasmic reticulum stress so that cells experience death.[19,20]

Histopathologic Features of Hepar of Rats Induced by Lead Acetate and Iodine Solution

This study showed a decrease in Knodell score in the group of rats that received lead acetate exposure of 100 mg/L and were given iodine solution at a dose of 0.225mg compared to the group of rats that were only given lead acetate. Statistical tests showed significant differences. This shows that there is an effect of iodine solution in preventing histopathological damage to the liver of rats induced by lead acetate. This is supported by the results of research conducted by Aceves where the administration of iodine solution was able to prevent hepatic damage due to lead acetate exposure.[21]

Iodine's role as an antioxidant was shown to be able to reduce the accumulation of oxidative stress arising from toxin exposure and reduce the occurrence of lipid peroxidation in cells. ROS in cells can be neutralized by iodine, which is an antioxidant that works in the form of iodide (-). Iodine in the form of I- acts as an electron donor. The more use of I- in oxidizing free radicals, the less free radicals that can potentially damage cells.[12]

Effects of Iodine Solution Administration on Histopathology of Lead Acetate-Induced Rat Hepar

Table 1 shows that there are differences in the degree of rat hepatic damage between groups. There was a decrease in the damage score in the treatment group compared to the positive control group.

The results of Mann-Whitney analysis as presented in table 2, showed that there was a significant difference ($p<0.05$) between the positive control group, which was given lead acetate, and the treatment group, which was given lead acetate and iodine solution.

The administration of iodine solution showed a lighter picture of cell damage compared to samples that were only given lead acetate. This can occur because the iodine solution acts as an antioxidant so that it can reduce hepatic cell damage caused by lead acetate exposure.[21]

Conclusions

Based on the results of this study, it can be concluded that the administration of iodine solution has the effect of preventing damage to rat hepatic cells induced by lead acetate.

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Declarations of competing interest

The authors declare none.

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