



The Effect of Giving Red Fruit Oil (*Pandanus Conoideus Lam.*) on Degeneration and Necrosis Levels of Mice Hepatocyte (*Mus musculus*) Exposed to Plumbum

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Abstract

Plumbum exposure is known can induce excessive production of Reactive Oxygen Species (R.O.S.) and cause oxidative stress on the cell. Oxidative stress can cause disorders in the body's organs, one of which is the liver organ. The study aims to determine the effect of giving red fruit oil as a protection against the exposure of plumbum at the rate of degeneration and cell necrosis of the liver. Red fruit oil contains antioxidant compounds beta carotene and tocopherol that function as antidotes to free radicals to prevent oxidative stress. This study was conducted for 14 days with four treatments. Namely P0 (negative control), P1 (positive control given exposure to plumbum at a dose of 0, 01mg), P2 (0.3 ml oil of red Fruit and exposure to plumbum at a dose of 0, 01mg), and P3 (0.8 ml of red fruit oil and exposure to plumbum at a dose of 0, 01mg). Determination of the level of degeneration and necrosis of liver cells is done by looking at the hematoxylin-eosin (HE) preparation of mice's liver under the microscope with magnification 400x. This study showed that the red fruit oil at a dose of 0.3ml and 0.8ml increased the effect on degeneration and necrosis of a mice's liver cell exposure to plumbum.

Keywords: degeneration, mice, necrosis, plumbum, red fruit oil

A. Introduction

The rapid development of industry and transportation equipment from year to year, both in terms of number and type, has an impact in the form of pollution on the environment and

affects the survival of humans and other living creatures (Supraptini, 2002). One of the resulting and hazardous contaminants for living beings is Plumbum (Pb), also known as timbal (Sudarmaji et al., 2006). The entry of Pb into the body can be through the respiratory system, food and drink, and penetration through skin tissue. Plumbums that enter the body will be distributed by the blood throughout the organs and tissues of the body and will accumulate in soft tissues (bone marrow, nervous system, kidneys, liver) and hard tissues (bones, nails, hair, teeth) (Palar, 2004).

Plumbum induces excessive Reactive Oxygen Species (R.O.S.) production and results in oxidative stress at the cell level. Reactive Oxygen Species is a molecule with unpaired electrons that is highly reactive and can damage cell membranes (Hamadouche et al., 2012; Yuslianti, 2018). One of the body's organs damaged due to exposure to Pb is the liver. Intraperitoneal Pb acetate in mice causes an impaired balance of oxidants and antioxidants. It causes increased oxidative stress, inducing lipid peroxidase that can damage cell membranes resulting in changes in cell structure and function (Gajawat et al., 2006). Research conducted by Suprijono et al. (2012) showed that induction of Pb 10 mg/day in white rats for 14 days could lead to degeneration and necrosis of liver cells. Another study by Syahrizal (2008) found that exposure to Pb at a dose of 20 mg /day on mice for seven days intraperitoneally led to increased degeneration and necrosis of mice's liver cells.

Body protection mechanisms are essential in reducing liver damage due to Pb exposure. In the event of increased R.O.S., the body needs additional protection mechanisms through antioxidant consumption to avoid oxidative stress. Many plants contain antioxidants, one of which is Red Fruit (*Pandanus* considers lam.) Red Fruit is an endemic fruit of Papua containing high tocopherol and β -carotene. B-carotene in red Fruit reaches 378.29 ppm, and tocopherol content reaches 10,319 ppm (Budi, 2000). Both compounds can prevent and treat liver diseases caused by R.O.S., Carotene, and tocopherol in fruits and vegetables. Moreover, other plants contain hydroxy or polyhydroxy groups that play an essential role in hepatoprotection action (Bass, 1999). The impact of Pb exposure on liver damage and red fruit content that has the potential to prevent liver damage became the basis of this research.

B. Methodology

1. Animals and Treatment

The study used mice animals trying as research objects. The mice used is female sex with a weight of about 20 g, amounting to 20 tails. The study has conducted at the Laboratory of Anatomical Pathology of The Veterinary Hospital of the Faculty of Veterinary Medicine, Pendidikan Mandalika University, and the preparation of hematoxylin-eosin (HE) of mice's liver was conducted at the Pathology Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga Surabaya.

This type of research is experimental with a randomized posttest-only control group design. The design used in this study was a Complete Randomized Design with four treatments; in each group, there were five replays. Red fruit oil and Pb are administered orally with gastric sonde for 14 days. Group 1 administered equates to as much as 0.5 ml/day per tail. Group 2 administered Pb per tail as much as 0.01 mg/day, group 3 administered red fruit oil 0.3ml and Pb 0.01mg/day, and group 4 administered red fruit oil 0.8ml and Pb 0.01mg/day, continuing with Ningtyas et al. (2019)

2. Preparation and Observation of the Hematoxylin Eosin (He) Preparation of Mice's Liver

The collected mice's liver organs are fixated using 10% formalin, then dehydrated with storeyed alcohol, then done clearing using xylol. After going through the clearing stage, the next stage is the infiltration and embedding stage using liquid paraffin. Paraffin blocks are then sectioned (cutting) and embedded glass objects. Preparations are then colored with hematoxylin-eosin (HE) staining.

Degeneration and necrosis in liver cells were observed under a microscope with 400x magnification in five viewing fields starting from the left, right, upper, lower, and middle corners of the hematoxylin-eosin (HE) preparation of the mice's liver. The scoring rate of degeneration and necrosis uses the Mordue et al. (2001) scoring method on a scale of 0 to 4. Score 0 if no degeneration and necrosis are found, score 1 when there is degeneration and necrosis 1-20%, score 2 when there is degeneration and necrosis 21-50%, score 3 when there is degeneration and necrosis 51-75% and score 4 when there is degeneration and necrosis more than 75%.

3. Data Analysis

The data was then analyzed with the ANOVA test ($\alpha= 0.05$) after a normality test with the Shapiro-Wilk test, then conducted with the Duncan test. Test analysis is performed using the SPSS for Windows applications.

C. Result and Discussion

The results from five different viewing microscopes with Methode Mordue et al. (2001) we analyzed with ANOVA, data analysis of the rate of degeneration and necrosis of mice's liver cells given red fruit oil and exposure to Pb using the ANOVA test ($\alpha= 0.05$) showed a significant difference between P0 with P1, P2, and P3 ($p<0.05$). However, Duncan's further tests found that the noticeable difference was only shown between P0 with P1, P2, and P3, while P1 with P2 and P3 had no noticeable difference.

Table 1. The mean rate from five field view of degeneration and necrosis of the liver cell of mice given ret fruit oil and exposure to plumbum

Variable	Treatment	$\bar{X}\pm SD$
Degeneration	P0	0.000 ^a \pm 0.000
	P1	2.160 ^b \pm 0.699
	P2	2.520 ^b \pm 0.502
	P3	2.360 ^b \pm 0.589
Necrosis	P0	0.000 ^a \pm 0.000
	P1	1.240 ^b \pm 0.740
	P2	1.480 ^b \pm 0.502
	P3	1.640 ^b \pm 0.589

Description: Different superscripts in the same column showed a noticeable difference ($p<0.05$). P0 = control group, P1 = given exposure PB 0.01mg/day, P2 = first given red fruit oil 0.3 ml/day after one hour given Pb 0.01mg/day, P3 = given red fruit oil 0.8 ml/day after one hour given Pb 0.01mg/day.

Histopathological images of mice's liver cells given red fruit oil and exposure to Pb show that mice's liver cells experience degeneration and necrosis, a sign of damage to the mice's liver.

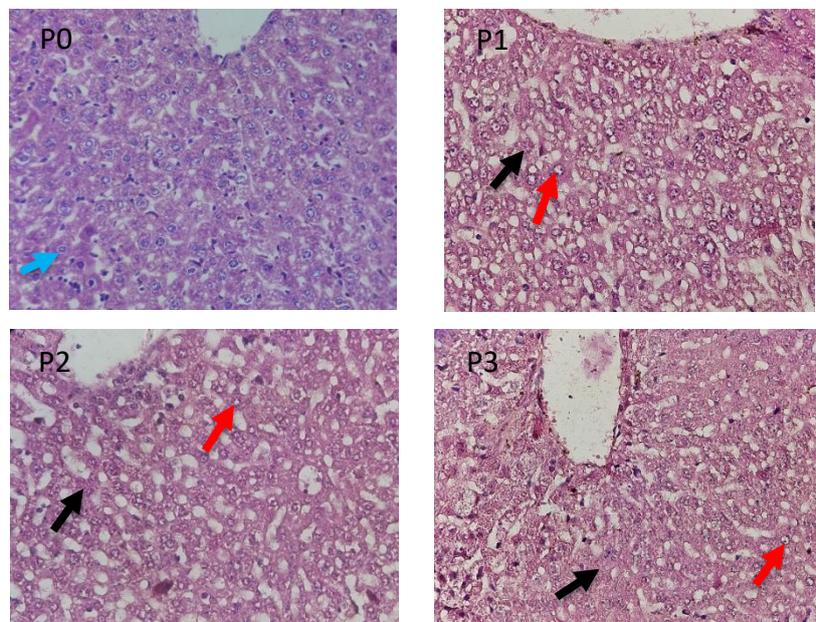


Figure 1. Histopathological images of liver cells increase by 400x, \rightarrow = normal liver cells, \rightarrow = degenerating liver cells, \rightarrow = liver cells that have necrosis

Degeneration and necrosis are histological signs of liver damage. Degeneration is an early manifestation of reversible liver cell damage, while necrosis is irreversible damage to liver cells. Necrosis will occur if the damage is severe enough and lasts so long that liver cells cannot compensate and run metabolism (Maulina, 2018). The results of this study show that degeneration and necrosis occur in the P1, P2, and P3 groups.

The P1 group that was only given exposure to Pb experienced degeneration and necrosis due to P.B. accumulation, leading to increased R.O.S. levels resulting in oxidative stress. Oxidative stress will cause ionic homeostasis disorders and membrane integrity that can lead to degeneration ballooning, where liver cells become swollen, rounded, and pale in color (Jaishankar et al., 2018). Oxidative stress that occurs continuously causes liver cells to not return to normal so that irreversible changes occur in the form of necrosis. Oxidative stress can cause damage to molecules in cells. Lipid molecules that experience oxidative stress will experience auto-oxidation or lipid peroxidation. Oxidized proteins malfunction, and D.N.A. oxidizes into mutagens, carcinogens, or causing cell death (Ercal et al., 2001).

The P2 and P3 groups were given Pb, and red fruit oil with doses of 0.3 ml and 0.8 ml experienced higher levels of degeneration and necrosis than P1, given only Pb. The P2 group, given a dose of red fruit oil lower than P3, experienced the highest rate of degeneration, while the P3, given the highest dose of red fruit oil, experienced the highest rate of necrosis. We assume that red fluid oil with higher doses can cause harmful effects on life. Such as giving high defects than P1 that just gave Pb. Such higher levels of degeneration and necrosis are possible because the dose of red fruit oil given is too high to cause damage to the liver, which is a storage place of tocopherol (vitamin E) and β -carotene (vitamin A) that exceeds the need (Ramanathan et al., 2009; Gee, 2011).

Tocopherol and Beta carotene are red fruit oil ingredients that serve as antioxidants (Budi, 2000). Tocopherol as vitamin E is absorbed in the intestine and regulated by Scavenger Receptor class B type 1 (SR-B1) after forming micelles assisted by bile acids and pancreatic enzymes. Vitamin E is most widely absorbed in erythrocytes. It enters the body's circulation through the lymphatic system and is absorbed alongside lipids through chylomicrons (Ball, 2006). Chylomicrons will undergo lysis due to the enzyme lipoprotein lipase, which enters the blood circulation and forms a chylomicron remnant. After lipolysis, vitamin E is transferred to High-Density Lipoprotein (HDL), which is accelerated by phospholipid transfer proteins to be carried into the circulatory system. Vitamin E in unused chylomicrons is carried by parenchyma cells to the liver to be stored as a backup of vitamin E (Gee, 2011).

Beta carotene is absorbed in the intestine in whole form or broken down in the intestinal tract and dissolved with the help of bile acids as a fat emulsifier. β -carotene that has been broken down is then deciphered in the intestinal mucosa. It was by carotene dioxygenase, which produces two retinaldehyde molecules. The reductase enzyme aldehyde diesterification will reduce retinaldehyde to retinol. Retinol is then degraded into small chylomicrons containing retinol. The chylomicrons are then carried to blood vessels, and the liver is combined with palmitic acid and stored in the form of retinyl palmitate. When required by the body's cells, retinyl-palmitate is bound by retinol-binding protein (R.B.B.) and then transferred to transthyretin to be carried to tissue cells. Excessive vitamin A is not required of the body's cells stored in the liver, kidneys, and adipose tissue.

The excretion of vitamin A from the slow-walking body can lead to hypervitaminosis, characterized by anorexia, jaundice, vomiting, and blurred vision (Ramanathan et al., 2009). Excessive accumulation of vitamin A stored in stellate liver cells will lead to activation and hypertrophy, excess collagen production, fibrosis, and liver injury (NCBI, 2013). The exact mechanism of vitamin A toxicity is unknown, but several theories have been put forward. One of them is that vitamin A activates kupffer cells through the production of gamma interferons by activating T-lymphocytes. Activation of kupffer cells indicates the potential for liver toxicity even at low levels of hepatotoxins (Ramanathan et al., 2009).

D. Conclusion

Based on the results of this study, the administration of red fruit oil with doses of 0.3 ml and 0.8 ml increases the effect on degeneration and necrosis of mice's liver cells compared with positive control given exposure to plumbum at a dose of 0,01 mg.

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