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Antioxidant Effect of Dayak Onion Extract (*Eleutherine Americana* Merr.) on Serum MDA Levels in Mice (*Mus Musculus*) Exposed by Lead Acetate

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Abstract: Phenols and flavonoids are the antioxidants contained in Dayak bulb extract that can eliminate the exposure of free radicals. Lead is one of the heavy metals toxic used by industries. It can increase ROS in the body due to pollution that occurs in the environment. The lead that enters the body is excreted throughout the body tissues. The lead accumulated in the blood can inhibit δ -ALAD enzyme function in the synthesis of heme to form a complex bond between the δ -ALAD enzyme and oxyhemoglobin that results in reactive bonds such as hydrogen peroxide, superoxide radicals and also produces ROS in the body. The formation of ROS can damage lipid peroxide from cell membranes by spreading a chain reaction. MDA is an indicator of free radicals emerging in the body. This study aims to find out whether the extract of Dayak onion can prevent the increase of serum MDA levels in mice exposed by lead acetate. Dayak fresh bulbs extracted with 96% ethanol solvent were administrated orally at 65 mg/kgBW, 130 mg/kgBW, and 260 mg/kgBW for 21 days, along with the administration of lead acetate for 14 days on the 8th – 21st day. The serum MDA levels were examined using the TBA test method. The expected results showed that the serum level tended to experience a decrease in the treatment group rather than in the control group ($p < 0.05$). It can be concluded that the extract of Dayak onion can prevent the increased serum MDA levels in mice exposed by lead acetate.

1 INTRODUCTION

The biodiversity belonging to our country, Indonesia, has more than 30,000 species of plant, 940 species of which are known as medicinal plants (Supriatna, 2008). Nowadays, traditional medicinal plants are developing in terms of use and demand so that the amount of research into traditional medicines is also increasing. Antioxidant compounds have a very important role in humans' health. Various scientific evidence suggests that antioxidant compounds can reduce certain diseases because the main character of the compound has the ability to capture free radicals (Prakash, 2001). Free radicals are molecules that have one extra or one less electron resulting in instability. Free radicals try to steal or give their electrons to other molecules which can change their chemical structure (Michael, 2013). Oxidative stress is a condition where the unbalanced volume of oxidants and pro-oxidants in the body,

which causes free radical molecular activity or ROS (Reactive Oxygen Species), attack lipids, glucose, proteins, and DNA, causing cellular and genetic damage (Harahap, 2014).

Lead is a toxic heavy metal used in the industrial world. Lead exists in the environment of daily life, for instance in canned food, cosmetic materials, motor vehicle fuel, wall paint, plant fertilizer, and so on. The lead that enters the body through the digestive tract accumulates in the blood and inhibits the enzyme δ -ALAD (Amino Levulinic Acid Dehydratase), which acts on heme synthase (Palaar, 2008). From a series of heme synthetases, this inhibition results in an increase in ALA substrate levels that lead to the formation of reactive bonds and ROS compounds (Komousani, 2011). The ROS mechanism results in cellular malfunction, and cell membrane lipid peroxidation causes calcium homeostasis on mitochondria and endoplasmic reticulum. MDA as biological biomarker of ROS

reaction on lipid peroxydation produces aldehyde compound (Hendromartono, 2000).

There are many methods to prevent losses arising from exposure to free radicals, especially in this case (lead), one of which uses the antioxidant content contained in the Dayak onion (*Eleutherine americana Merr.*). The previous research by Yuswa (2017) proved the total content of phenol and high flavonoid levels in this plant. The presence of antioxidants in the body is expected to balance the free radical reactions generated from various cells (Al-Muqsih, 2013). Phenol is a natural chemical compound that acts as an antioxidant through the mechanism of a reducer, a free radical capturer, a metal chelator, a reducer of reactive bond formation and electron donors. Flavonoids as antioxidants can protect cells from oxidative stress and reduce the risk of degenerative diseases associated with oxidative damage, and almost all flavonoids have strong antioxidant effects because they contain chemical elements responsible for antioxidant activity (Patel, 2008; Sandhar et al, 2011 cited from Soewono & Khaerunisa, 2016).

Thus, based on the explanation above, the researchers carried out a study on the effects of antioxidants contained in Dayak onion extract (*Eleutherine americana Merr.*) on serum MDA levels of mice (*Mus musculus*) exposed to lead acetate.

2 METHODS

This is a laboratory experimental research using Post-test Only Control Group Design. It consists of a negative control group treated by oral placebo every day for 21 days. The positive control group was treated by placebo for 21 days and lead acetate of 50 mg/kgBW orally, daily from the 8th to the 21st day. The P1 group was treated by Dayak onion extract with 65 mg/kgBW dosage for 21 days and lead acetate of 50 mg/kgBW orally, daily from the 8th to the 21st day. The P2 group was treated by Dayak onion extract with 130 mg/kgBW dosage for 21 days and lead acetate of 50 mg/kgBW orally, daily from the 8th to the 21st day, and the P3 group was treated by Dayak onion extract with 260 mg/kgBW dosage for 21 days and lead acetate of 50 mg/kgBW orally, daily from the 8th to the 21st day.

On the 22nd day, blood was intracardially taken from all mice, previously anesthetized by using ketamine. Then the examination of serum MDA levels was carried out by using the TBA test method (thiobarbituric acid).

3 RESULTS

Giving Dayak onion extract (*Eleutherine americana Merr.*) orally is expected to prevent the increase of the serum MDA levels of mice exposed to lead acetate. Thus, in this case the serum MDA levels are expected to decrease in the treated group compared to the control group.

Based on the prior research by Kusumaningtyas (2016), giving an antioxidant in the form of vitamin E can decrease the serum MDA levels in mice exposed to lead acetate. In this research, the antioxidant contained in the Dayak onion extract in various dosages can capture free radicals caused by exposure to lead acetate.

4 DISCUSSION

This study used Dayak onion extract (*Eleutherine americana Merr.*), which was administered in multilevel dosage to each treatment group (P1, P2, and P3). The content of phenols and flavonoids contained in the tubers of this plant work as an antidote to free radicals due to oxidative stress. The antioxidant activity of Dayak onion extract resulted from the total phenol content and high flavonoid content in 96% ethanol solvent. The higher total value of phenol causes the higher antioxidant activity that resulted. The antioxidant activity is in flavonoid and phenol compounds with -OH groups attached to aromatic ring carbon (Pereira et al, 2009).

The effect of hematotoxicity of lead in inhibiting the conversion of δ -ALAD enzymes into porphobilinogen and inhibiting the conversion from iron (Fe) into portofirin IX to form hemoglobin. The inhibition of enzyme δ -ALAD results in the accumulation of δ -ALA and protoporphyrin, causing an obstacle to the occurrence of hemoglobin synthesis. In addition, lead can inhibit the enzyme of ferochelataze so that iron (Fe) ions cannot bind to the protoporphyrin chain. Therefore, there is competition between lead and iron (Fe) (Siagian, 2008).

Previous research by Ponche-Canchihuaman et al. (2010) proved that in the administration of 25 mg/0.5ml lead acetate by injection intraperitoneally, there was a significant increase in MDA levels. Therefore, it can be said that lead acetate causes an increase in the occurrence of Reactive Oxygen Species (ROS). That is, administering Dayak extract to mice exposed to lead acetate can prevent elevated

serum MDA levels due to the accumulation of lead acetate in the blood. It is expected that, in this study, the antioxidant content found in the Dayak extract can be used as a preventive effort against the exposure to free radicals that are unconsciously absorbed in the everyday environment.

The physiological system of the body has the ability to reduce the level of destruction of cells by peroxidation. However, if the body is weak or when exposure to ROS is too much, the mechanisms of additional body protection are needed. One of these additional forms of protection is through the consumption of many antioxidants contained in natural materials. All antioxidants can prevent lipid peroxidation. Antioxidants are compounds that are able to neutralize or stabilize free radicals by supplementing electron deficiencies in these free radicals. Antioxidants are able to prevent the occurrence of chain reactions from the formation of free radicals that can cause oxidative stress

There are three mechanisms by which antioxidants can work against free radicals: primary antioxidants, secondary antioxidants, and tertiary antioxidants. The content of antioxidants in Dayak onion extract works on the secondary antioxidant mechanism, which is via vitamin C, vitamin E, vitamin B, beta-carotene and phytochemical compounds (flavonoid, carotenoid) that work by chelating the metal acting as prooxide, for instance the lead, capturing free radicals and preventing a chain reaction. Secondary antioxidants can be called a *chelating agent*.

5 CONCLUSION

It is expected that the distribution of Dayak onion extract, whether 65 mg/kgBB, 130 mg/kgBB or up to the 260 mg/kgBB dosage, can prevent the increase in the levels of MDA serum of mice exposed to acetate lead. The levels of MDA serum in the treated groups (P1, P2 and P3) decreased compared to the positive control group.

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