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Effects of Vitamin C and Jamblang Leaf Extract (*Syzygium cumini*) on Catalase Enzyme Activity in Lead Acetate-Induced Rats

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ABSTRACT

Lead is a toxic heavy metal that causes oxidative stress and suppresses endogenous antioxidants, such as the catalase enzyme. Vitamin C and jamblang leaves are exogenous antioxidants to inhibit oxidative stress. This research aimed to determine the effect of jamblang leaf extract and vitamin C on the catalase enzyme activity of mice that had been induced by acetate lead. This research was true experimental research with a randomized post-test-only control group design using mice divided into 7 groups, namely, group K- (group was given a standard diet), acetate lead 40 mg/kgBW induced K+, P1 was induced by acetate lead 40 mg/kgBW and jamblang leaves extract 75 mg/kgBW, P2 was induced by acetate lead 40 mg/kgBW and jamblang leaves extract 150 mg/kgBW, P3 was induced by acetate lead 40 mg/kgBW and vitamin C 75 mg/kgBW, P4 was induced by acetate lead 40 mg/kgBW and jamblang leaves extract 75 mg/kgBW as well as vitamin C 35 mg/kgBW, P5 was induced by acetate lead 40 mg/kgBW and jamblang leaves extract 150 mg/kgBW as well as vitamin C 75 mg/kgBW for 30 days. On the 31st day, the mice were killed to check their catalase enzyme activity. In this research, there was a significant ($P<0.05$) increase in catalase enzyme activity between the K- with K + group and between the K+ group with P4. The conclusion was that jamblang leaves extract was able to increase catalase enzyme activity after being induced by lead acetate.

Keywords: Lead acetate, jamblang leaf extract, vitamin C, catalase enzyme activity, rats.

INTRODUCTION

Lead (lead = plumbum), symbolized by Pb, is a metal chemical element in group IVA (14) with atomic number 82. Lead belongs to the heavy metal group which has a higher density than other materials. Lead is soft, malleable, relatively inert, difficult to react, and has a low melting point.[1]

Lead is widely distributed throughout the world due to its physical and chemical properties. Lead has many benefits for humans so the risk of exposure to lead will

also be high. Humans are exposed to lead mainly through food. Ingested lead is absorbed by the body causing adverse effects on cognitive, postnatal growth, central nervous system, peripheral nervous system, cardiovascular system, kidney and fertility problems.[2]

Lead acts as an oxidant or free radical when it is in the body. Free radicals will produce Reactive Oxygen Species (ROS) which are harmful to the body. Free radicals are small molecules that can spread and are very reactive because they have unpaired electrons.[3]

Under normal circumstances, levels of free radicals and antioxidants are in a state of balance. An imbalance between oxidant and antioxidant levels in the body will result in oxidative stress.[4] The body has its own defence system to ward off free radicals, namely in the form of endogenous antioxidants that already exist in the body naturally, such as glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase (CAT). Catalase enzyme is one of the endogenous antioxidants that can be a marker of oxidative stress. When Reactive Oxygen Species (ROS) are formed in excess and endogenous antioxidants are unable to counteract these free radicals, additional antioxidants are needed from outside the body (exogenous antioxidants) to minimize health risks due to free radicals.[5]

Sources of exogenous antioxidants can be obtained from jamblang leaves and vitamin C. The most abundant antioxidant compounds in jamun are phenolic compounds and flavonoids. One part of jamblang that contains these compounds is jamblang leaves.[6] Jamblang is one of Indonesia's local plants. Jamblang is often used as herbal medicine in several countries apart from being a food ingredient. It is proven that jamun has antioxidant activity which is classified as strong to very strong.[7] Based on research conducted by Rauza and Elmatris in 2021 on rats induced by lead acetate at a dose of 40 mg/kgBB and given *Syzigium cumini* at a dose of 150 mg/kgBW for 30 days, an increase in catalase enzyme activity was found compared to rats induced only by lead acetate at a dose 40 mg/kgBB.[8] Exogenous antioxidants besides jamblang leaves, namely vitamin C. Vitamin C as an exogenous antioxidant works by acting as an electron donor and becoming a free radical reducing agent so that vitamin C undergoes oxidation and other compounds do not become oxidized. This reaction forms dehydroascorbic acid (DHA), which is more stable and less reactive than free radicals.[9] Based on the above background, it is known that vitamin C and jamblang leaf extract have the potential to be good antioxidants for the body so researchers are interested in examining the combined effect of vitamin C and jamblang leaf extract on catalase enzyme activity in experimental animals that have been induced by lead.

MATERIALS & METHODS

MATERIALS

Standard feed, husks, water, lead acetate, distilled water, rotary evaporator, jamblang leaf extract, 96% ethanol, vitamin C, potassium dichromate, glacial acetic acid, pure sulfuric acid, H_2O_2, rat blood samples, phosphate buffer, alcohol, antiseptic hand washing soap, were used during the experiment.

METHODS

Preparation of *Syzygium cumini*'s leaves extracts

Jamblang leaf extract was made at the Pharmacology Laboratory, Faculty of Pharmacy, Andalas University. The sample of this research is jamblang leaves (*Syzygium cumini*) which are still fresh. Dirt and other foreign materials are separated from the jamblang leaves. Jamblang leaves are washed with water until clean and free from other impurities. Then, the jamblang leaves are cut into small pieces. After that, the jamblang leaves are dried and blended until smooth to form a powder. Making jamblang leaf ethanol extract, using the maceration method using 96% ethanol for 5 days.[10] Next, the macerate was filtered using a flannel cloth, then remaceration of the

dregs was carried out for 2 days. This maserate is collected and then evaporated by vacuum distillation, then concentrated using a rotary evaporator at 40°C so that a thick jamblang leaf extract is produced.[11]

Animal Test Preparation

The experimental animals used were male Wistar strain white rats aged 2-3 months with body weight ranging from 150-250 grams. Prior to the study, the experimental animals were acclimatized. Then, 42 experimental animals were taken that met the inclusion and exclusion criteria and were divided into 7 treatment groups, namely the negative control group (K-), which were only given a standard feed diet without being given lead acetate, jamblang leaf extract, and vitamin C for 30 day; positive control group (K+), group, which was induced by lead acetate 40 mg/kgBB for 30 days; treatment group 1 (P1), which was induced by lead acetate at a dose of 40 mg/kgBW and given jamblang leaf extract at a dose of 1, which was 75 mg/kgBW for 30 days; treatment group 2 (P2), which was induced by lead acetate at a dose of 40 mg/kgBW and given jamblang leaf extract at a dose of 2, which was 150 mg/kgBW for 30 days.[8] Then, treatment group 3 (P3), which was induced by lead acetate at a dose of 40 mg/kgBW, and was given vitamin C at a dose of 75 mg/kgBW for 30 days.[12] Next, treatment group 4 (P4), which was induced by lead acetate at a dose of 40 mg/kgBW and given jamblang leaf extract at a dose of 1, which is 75 mg/kgBW and vitamin C at a dose of 35 mg/kgBW for 30 days; treatment group 5 (P5) which had been induced by lead acetate at a dose of 40 mg/kgBW and was given jamblang leaf extract at a dose of 2, which was 150 mg/kgBW and vitamin C at a dose of 75 mg/kgBW for 30 days. This research has passed the ethical test of the Faculty of Medicine, Andalas University with number870/UN.16.2/KEP-FK/2022.

Administration of Lead Acetate, Jamblang Leaf Extract, and Vitamin C

Administration of lead acetate to rats was carried out orally using a sonde. Mice were held in such a position. Vitamin C and jamblang leaf extract are inserted through the edge of the palate while being pushed gently backwards into the esophagus. The time for giving treatment to these experimental animals was tried to remain consistent between 9.00-11.00 WIB. In groups P1, P2, P3, P4, P5, the rats were induced with lead acetate 4 hours before being given vitamin C and jamblang leaf extract.[13]

Measurement of Catalase Enzyme Activity

The process of taking blood samples from the rats was carried out after the rats were anaesthetized using cotton soaked in diethyl ether solution. After the rats lost consciousness, the rats were removed from the tube and then fixed and blood samples were collected using a microhematocrit through the retro orbital plexus. After that, the rat blood was collected in a centrifugation tube and centrifuged at 3000 rpm for 10 minutes. Then, the serum is separated from the blood, then put into a micro tube.

Add 4 ml of H_2O_2 solution to the tube (hydrogen peroxide, 0.2 M), followed by 5 ml of phosphate buffer. After that, add 1 ml of serum slowly then homogenize. Then add 2 millilitres of glacial acetate. This procedure was repeated at 60-second intervals in each tube. The tube is heated in boiling water for 10 minutes so that the blue precipitate disappears and produces a green solution. Absorbance was measured at a wavelength of 570 nm. Determine how much H2O2 is left in the serum when the reaction is stopped by acetic acid using the standard curve. The amount of protein consumed is determined by the activity of the enzymes.

STATISTICAL ANALYSIS

Data is presented in the form of SEM averages. This study used the One-Way ANOVA test and continued with several

comparison tests. The data is significant if the p-value <0.05.

RESULT

Based on the research results shown in Figure 1, it shows that there was a significant decrease in catalase enzyme activity in rats ($p<0.05$) after being induced with lead acetate for 30 days at a dose of 40 mg/kg BW. Lead is a heavy metal that is commonly found in everyday life. Lead acts as an oxidant or free radical when it is in the body. Exposure to lead increases the amount of oxidants in the body and reduces the activity of endogenous antioxidant enzymes, one of which is the activity of the catalase enzyme. [14]

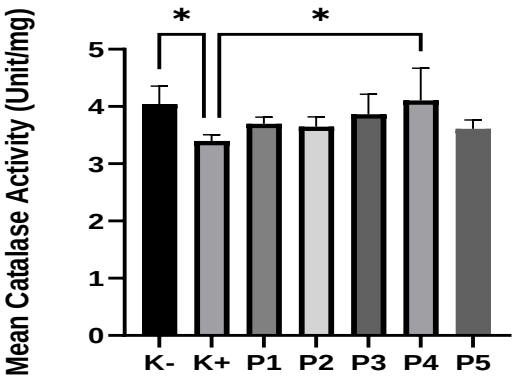

Figure 1. Mean catalase activity ± SEM (Unit/mg) in all groups

In addition, based on the results of the research shown in Figure 1, it was also found that there was an increase in catalase enzyme activity in rats in treatment group 1 (P1) induced by lead acetate at a dose of 40 mg/kgBW and given jamblang leaf extract at a dose of 75 mg/kgBW and treatment group 2 (P2) induced by lead acetate at a dose of 40 mg/kgBW and given jamblang leaf extract at a dose of 150 mg/kgBW. However, statistically the increase in catalase enzyme activity in the 1st and 2nd treatment groups did not show a significant difference ($p>0.05$).

DISCUSSION

The increase in the average activity of the catalase enzyme in treatment groups 1 and 2 proved that jamblang leaf extract could ward off free radicals produced by lead acetate. Jamblang leaf extract contains flavonoids, phenolics, tannins and terpenoids. Phenolic compounds and flavonoids have high antioxidant activity.[15] Flavonoids as antioxidants work by delaying, preventing or eliminating target molecules from oxidative damage. Flavonoids also enhance and stimulate enzymatic antioxidants that already exist in the body such as superoxide dismutase (SOD), catalase and glutathione reductase.[16] The phenolic contained in jamblang leaf extract is known as a direct antioxidant, but also exhibits indirect antioxidant activity by inducing protective enzymes produced by the body, such as the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione (GSH).[17] Phenolic compounds donate hydrogen atoms to free radicals because phenolics are easily oxidized during oxidation reactions to form stable phenoxy radicals. This causes phenolics to have the potential to be antioxidants.[18]

Based on Figure 1, it was also seen that there was an increase in catalase enzyme activity in rats in treatment group 3 (P3) induced by lead acetate at a dose of 40 mg/kgBW and given vitamin C at a dose of 75 mg/kgBW. However, statistically the increase in catalase enzyme activity in the treatment group 3 did not show a significant difference ($p>0.05$). This shows that giving vitamin C at a dose of 75 mg/kg BW can

ward off free radicals produced by lead acetate. Vitamin C as an antioxidant works by neutralizing oxidative stress through electron transfer or donation processes. Vitamin C prevents other compounds from being oxidized by donating electrons and scavenging superoxide anions, hydroxyl radicals, and lipid hydroperoxides.[19]

This study also used two different combination doses of jamblang leaf extract and vitamin C. The treatment group 4 (P4) was induced by lead acetate at a dose of 40 mg/kg and given 75 mg/kg of jamblang leaf extract and 35 mg/kg of vitamin C, while the control group rats in treatment 5 (P5) were induced with lead acetate at a dose of 40 mg/kgBW and given jamblang leaf extract 150 mg/kgBW and vitamin C 75mg/kgBW. Based on the research results shown in Figure 1, it was found that there was an increase in the activity of the catalase enzyme in rats in the group of mice in treatment 4 (P4) and treatment 5 (P5). Statistically, the increase in catalase enzyme activity between the positive control group and the treatment group 4 (P4) showed a significant difference ($p < 0.05$), while the increase in catalase enzyme activity between the positive control group and the treatment group 5 (P5) did not show a significant difference ($p<0.05$). On the other hand, the comparison of statistical data between the negative control group (K-) and the treatment group 4 (P4) and treatment 5 (P5) did not show a significant difference in the statistical test ($p> 0.05$).

The result above shows that the combination of jamblang leaf extract and vitamin C synergizes in increasing antioxidant properties. The ability of jamblang leaf extract was strengthened by vitamin C in the group of rats in treatment 4 (P4) and in treatment 5 (P5) by increasing the antioxidants in the rats as seen in the increased activity of the catalase enzyme so that the immune function in protecting cells from the dangers of free radicals also increased. The selection of two different doses in the combination aims to compare which combination dose is more effective in counteracting free radicals. The choice of combination dose at P4 is derived from the effective dose of jamblang leaf extract and vitamin C because antioxidants have the potential to become pro-oxidants at high doses, causing toxicity.[20]

CONCLUSION

Lead acetate can cause oxidative stress in the body resulting in a decrease in the activity of the catalase enzyme in the body. Giving jamblang leaf extract and vitamin C has potential as an antioxidant that can overcome oxidative stress due to lead. The combination of jamblang leaf extract at a dose of 75 mg/kg BW and vitamin C at a dose of 35 mg/kg BW is the most effective for overcoming oxidative stress due to lead.

Declaration by Authors

Ethical Approval: Approved

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Conflict of Interest: The authors declare no conflict of interest.

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