The Preventive Effect of Rome Beauty Variety Apple Skin Extract on Blood Urea Nitrogen (BUN) levels of Rat (*Rattus norvegicus*) Induced by Lead (Pb)

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Abstract. Lead (Pb) is a heavy metal that can cause poisoning in living things. Pb contamination that enters the body through the digestive system will bind to erythrocytes and trigger an increase in ROS the body resulting in a decrease in organ function such as the kidneys followed by an increase in levels of BUN. Apple is an example of a fruit that rich in antioxidant. Apple skin contains flavonoids which act as antioxidants and as chelating agent for metal ion. The parameters observed in this study were BUN levels. This study consist of 5 groups with each group consisting of 4 white rats, namely negative control, positive control with 10 mg Pb / head / day of Pb administration for 14 days, and preventive therapy group with Rome Beauty apple skin extract 28 mg/200g, 56 mg/200g, and 112 mg/200g for 21 days and giving lead at a dose of 10 mg/rat/day for 14 days. BUN levels were analyzed using one way ANOVA method which was followed by Turkey. The results showed that the preventive group of apple skin extract of Rome Beauty variety with a dose of 112 mg/200g was able to prevent an increase in BUN levels.

1. Introduction

Lead or plumbum (Pb) is a heavy metal which primarily come from motor vehicle exhaust gas that can pollute the environment. If Pb is inhaled or swallowed by living being, the Pb will be distributed following the blood vessel, and reabsorbed into kidney and brain, also deposited in bone and teeth. Living being can be contaminated with Pb in the way of airborne, waterborne, and foodborne [1].

Plumbum (Pb) also commonly become intoxication factor in ruminant. Pb intoxication happens commonly because animal feed that has been contaminated by Pb [2]. Cellularly, Pb is known to cause the abundant production of Reactive Oxygen Species (ROS) and can cause the increase of lipid peroxidation in membrane cell. Increase of ROS production is a result of stress oxidative within cell. The Pb dosages are adjusted based on previous research, namely the provision of Pb as much 10 mg / mouse / day for 14 days that can cause increase of degeneration and necrosis in organ cell [3].

Damage to kidney function parameters can be known one of them by examining BUN levels. Blood Urea Nitrogen (BUN) is the final product of protein metabolism that is synthesized in the liver and secreted by almost 75% through the kidneys without undergoing molecular changes. Normal BUN levels in the blood vary based on age and are affected by kidney function [4].

One fruit that is rich in antioxidants is apple, both flesh and peel. Apple peel contains a greater phenolic compound than apple flesh. The total phenolic and flavonoid content is mostly found in apple peel followed by fruit flesh [5].
Flavonoid is a secondary metabolic compound which is contained in green plants, except algae. Flavonoid is also potential compound as antioxidant. Flavonoid also function well as protector from free radical because flavonoid tend as chelating agent against metal ion, so Pb will bind to flavonoid first rather than erythrocyte or even enzyme which has sulphydryl group [6].

Apple variety Rome Beauty contains antioxidant, which proven from experimetal that has been done by Octaviany [7] which apple variety Rome Beauty can decrease SGPT enzyme of rat (*Rattus norvegicus*) which is induced by CCl$_4$ until normal value.

2. Materials and Methods

The material needed in this experiment is serum of the Rat. This experimental design use complete randomized trial design because the sample and environmental factor in this experiment is assumed same or homogenous. This trial is divided into 5 groups and 4 times of repeat. It is needed as much as 20 rat and each group contains 4 rat. (A) Negative Control: without intervention; (B) Positive Control: Induction plumbum 10 mg/mouse/day; (C) Intervention 1: Extract of apple peel 28 mg/200 g BW + Plumbum 10 mg/mouse/day; (D) Intervention 2: Extract of apple peel 56 mg/200 g BW + Plumbum 10 mg/mouse/day; (E) Intervention 3: Extract of apple peel 112 mg/200g BW + Plumbum 10 mg/mouse/day. Pb was given in form of Pb acetate which is dilluted in 1 cc aquades and given with feeding tube for 14 days, started from day 14 until day 28. Apple peel extract was given in 21 days with feeding tube which dilluted with 1 cc aquades. Measurement of BUN serum data is analyzed with Analysis One Way Analytical of Variance (ANOVA), which continued by Tukey Test $\alpha = 5\%$ to analyze the difference between treatment group.

3. Result and Discussion

The results can be seen in the following table:

| Group | Average BUN ± SD (mg/dL) | Increase | Decrease |
|-------|--------------------------|----------|----------|
| A - Control (-) | 18.02 ± 1.25$^a$ |          |          |
| B – Control (+) | 33.42 ± 1.68$^b$ | 85.4 % |
| C – Dosage 28 mg/200 g BW | 29.42 ± 6.50$^b$ | 11.9 % |
| D – Dosage 56 mg/200 g BW | 18.45± 3.67$^a$ | 44.8 % |
| E – Dosage 112 mg/200 g BW | 13.12 ± 2.23$^a$ | 60.7 % |

Notes: Notation (a,b) which different shows that there are significant difference (P<0.05) between treatment group.

Based on analysis result using One Way Anova test shows that apple peel Rome Beauty (*Malus sylvestris Mill*) give significant difference (p<0.05) against BUN serum in rat (*Rattus norvegicus*). The average BUN serum of positive control group (B) experienced an increase compared to the negative control group (A). BUN serum in positive control group has significant difference (p<0.05) with negative control group (A), which mean the Pb treatment significantly (p<0.05) increase the BUN serum in rat (*Rattus norvegicus*). The difference in dose of Rome Beauty (*Malus sylvestris Mill*) apple peel extract have a significant effect (p <0.05) in reducing BUN levels. The higher the preventive dose given, the lower the BUN level in rat (*Rattus norvegicus*). The decrease in BUN levels proves that the content in Rome Beauty (*Malus sylvestris Mill*) apple peel extract can reduce BUN levels in rats (*Rattus norvegicus*) induced by Pb.

The increase of BUN serum in positive control (B) could be happened because of Pb inside the body. Plumbum (Pb) will binds to erythrocytes, causing interference in the synthesis of hemoglobin. Hemoglobin synthesis begins with the reaction of sucinyl co-A with glycin which will form the Amino Levulinic Acid (ALA) which catalyzed by ALA synthetase. Then, ALA will be dehydrated into...
porphobilinogen with enzyme Delta Amino Levulinic Acid Dehidratase (D ALAD). After several reaction process, porphobilinogen compound will change comformationally into protoporphyrin –IX, which then changed into Heme. Heme will react with Globin and metal ion Fe$^{2+}$ and with the help of ferrochelatase enzyme will make hemoglobin. Pb compound inside the body will attach with sulphydryl (SH) group from D ALAD enzyme inside cytosol and inhibits ferrochelatase inside mytochondria. The bound between Pb and D ALAD group will stop the continuum of hemoglobin synthesis process and will accumulate ALA itself [8]. Increase of ALA will cause the formation of hydrogen peroxide, radical superoxide and also interaction between two of them will result a hydroxyl radical, a free radical which is very reactive [6]. Steps of oxidation couple from ALA and oxyhemoglobin will make ROS. ALA become electron donor to molecular oxygen together with electron transfer from oxyhemoglobin to oxygen. H$_2$O$_2$ and O$_2$ which is made then interact to form HO radical which is very reactive. The amount of abundant free radical will form the condition of stress oxidative [9].

Stress oxidative condition will trigger the process of lipid peroxidation. Lipid peroxidation is a reaction process of ROS with PUFA at cell membrane which can induce cell degeneration, that is a condition about the loss of normal cell structure and cause damage to tissue. Kidney damage will cause decrease in glomerulus filtration. Everything which reduce the average speed of glomerolus filtration will impact the increase of urea reabsorbtion in tubulus so that the amount of BUN serum will be increased [10].

Based on experiment result shows that treatment of Rome Beauty (Malus sylvestris Mill) apple peel extract in group C, D, and E can prevent the increase of BUN serum which induced by Pb because of flavonoid contain. The flavonoid content inside Rome Beauty (Malus sylvestris Mill) apple peel extract can act as antioxidant because it can donor one or more electron to free radical, so that free radical reaction can be hampered. Flavonoid also act as barrier against free radical because flavonoid act as chelating agent against metal ion, so Pb will bind to flavonoid first rather than erythrocyte or even enzyme which has sulphydryl group [6].

4. Conclusion
The treatment of Rome Beauty (Malus sylvestris Mill) apple peel extract in dosage 28 mg / 200 g BW, 56 mg/200 g BW, and 112 mg/200 g BW can prevent the increase of BUN serum in rat (Rattus norvegicus) which induced by Pb significantly, with the best dosage which is 112 mg / 200 g BW.

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