The Effectiveness of Faloak Extract on Superoxide Anion Radical (O_2^-) Activity in Kidney Organ of Tilapia Exposed to Mercury

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Abstract: ***Research has been conducted to determine the radical content of superoxide anion (O_2^-) on kidney organs exposed to mercury before and after the administration of faloak (Stercullia quadrifida R.Br) extract as an antioxidant. The mercury concentration used in this study was 0.018 ppm. The activity of radical compounds was measured using Electron Spin Resonance (ESR) equipment, with the output of the Lissajous curve displayed on the oscilloscope. The extent of the curve illustrates radical activity, the wider the curve the more reactive radicals are. Faloak extract was varied in seven different concentrations, namely 0.25 mg/ml; 0.50 mg/ml; 1.00 mg/ml; 2.00 mg/ml; 4.00 mg/ml; 8.00 mg/ml and 16.0 mg/ml. After measurement, it was found that faloak extract was able to reduce O_2^- radical activity, starting from 1.00 mg/ml to 16.0 mg/ml. O_2^- radical activity was no longer identified after the administration of faloak extract at a concentration of 4.00 mg/ml; 8.00 mg/ml and 16.0 mg/ml.***

Keywords: Antioxidant, Faloak extract, Mercury, Superoxide Anion radicals (O_2^-)

1. Introduction

Mercury is one of the heavy metal types which is often found in river water as industrial and mining waste. The content of mercury in river water will cause environmental changes and will have a negative impact on exposed organisms. The accumulation of mercury in the body of an organism can occur in two forms, namely organically and inorganically. Organic accumulation of mercury can cause visual and hearing impairments, while the inorganic one may cause proteinuria, Acute Respiratory Infection (ARI) and kidney failure [1][2]. Damage to the body tissues of exposed organisms begins with the appearance of radical compounds. Radical compounds in the body will bind with other compounds which will then lead to oxidative stress, causing damage to cells, tissues, and organs [3]. One type of radicals that can form due to exposure to mercury is O_2^- radicals.

O_2^- radicals are formed naturally due to the body's metabolic processes, but in small amount and can be directly eliminated by the body. The amount and activity of O_2^- radicals can increase when exposed to mercury. Radical compounds in small amount can be directly inactivated in the body's metabolic processes, but in large amount antioxidant intake from outside the body is needed [3][4]. Antioxidants are compounds that are able to donate electrons to radicals, thus reducing the activity of radical compounds. The use of natural antioxidants is recommended because it does not pose a risk of side effects that have negative impacts on health. Compounds that have the potential as natural antioxidants include flavonoids and phenols [4].

Faloak (*Stercullia quadrifida R.Br.*) is a kind of local plants that grows in East Nusa Tenggara (NTT) province, especially on the island of Timor, Indonesia. The spread of faloak plants are found on parts of the northern coast of Australia [5]. The bark (klika) of faloak is often used by people in the city of Kupang, Indonesia as an herbal medicine believed to cure hepatitis. Previous researches have identified bioactive compounds in faloak klika extract. The results obtained show that in the faloak klika extract, there are phenolic compounds, flavonoids, alkaloids and terpenoids. These compounds are antioxidant compounds, and have the potential to become hepatoprotectors and anticancers [5][6][7], but it has not been investigated regarding the effect of faloak klika extract on O_2^- radical activity arising due to exposure to mercury in the environment. Since radical compounds are the initial cause of damages from cells, tissues to the organs of organisms, researchers, therefore, are interested in researching the potential of faloak bark extract in reducing activities of superoxide anion radicals. This study aims to identify the radical content of O_2^-, in the kidney organs of tilapia exposed to mercury

2. Methods

This type of research is quantitative research with experimental research design [8]. Tilapia was chosen as a bio-indicator in this study because tilapia is a freshwater organism that is most sensitive to environmental changes due to environmental pollution [9]. The fish selected as samples are local tilapia aged around 1 month with body length of 8-10 cm and mass of 10-15 grams. The tilapia samples used were 60 fish, divided into two groups, 10 for control group and the remaining 50 for experimental group. Fish were kept in an aquarium and mercury was added as a pollutant with a concentration of 0.018ppm. Tilapias were then maintained for 14 days. The reason of 14 day maintenance refers to a previous research which found that 14 days was the period needed for maximum accumulation of heavy metals in the fish's body [4].

After maintenance in a polluted environment for 14 days, the fish were then transferred to a normal environment. A total of 5 fish were taken randomly to examine the superoxide anion radical activity in the kidney organs, while the other samples were divided into 7 different groups and maintained for 14 days with faloak extract. Determination of treatment group samples was carried out randomly. The technique of administering faloak extract to fish was by adding it to fish pellets. The extract was mixed with distilled water and divided into 7 different concentrations before being added to fish pellets. Extract concentration varied from 0.25mg/ml, 0.50mg/ml, 1.00mg/ml, 2.00mg/ml, 4.00mg/ml, 8.00mg/ml to 16.0mg/ml

Identification of O_2^- radical content was carried out with Leybold-Heracus Electron Spin Resonance (ESR) equipment. Each kidney of the fish was taken and then put into a 2 cm glass tube which was then inserted in ESR equipment. The magnetic field generated around the sample by ESR equipment would cause a Zeeman effect on a sample containing a radical compound, then forming a curve which would be shown on the oscilloscope.

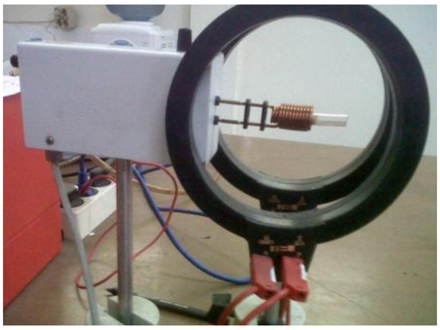

Figure 1: Glass tube contain samples of ESR equipment

The magnitude of the magnetic field and the wave frequency value were used to calculate the value of the sample g factor. The calculation was done using the equation

$$g = \frac{h.f}{\mu_B.B}$$

The frequency range (f) used in this study ranges from 22.7MHz - 75MHz. μ_B Bohr's magnet on constant is 9.273x10-24, while h is a planck constant of 6.63x10-34 Ws-2. B is the magnitude of the magnetic field in the tesla unit. The value of the identified g factor can indicate the type of radical in the sample, while the area of the curve seen on the oscilloscope shows a radical content. The wider the curve, the more reactive the radical compounds are and also the higher the radical contents are in the test samples [9]. Curve images on the oscilloscope are shown in Figure 2 below

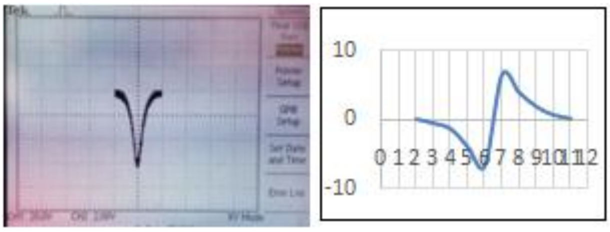

Figure 2. (a) Display of the Lissajous curve on the oscilloscope indicating the presence Radical compounds in the sample (b) radical curve derivatives

The Lissajous curve formed shows energy splitting, the wider the energy splitting formed the more reactive the radical found in the sample is [9]. In this study the radical activity of superoxide anions can be determined by measuring the area of the Lissajous curve formed on the oscilloscope.

3. Result and Discussion

Test results of tilapia kidney organ samples showed that a Lissajous curve was formed at a frequency of 36.3 MHz, which was then identified as O_2^- radical. The area of the radical curve is at 1.38mm2. Mercury found in the environment can enter the body of fish through the mouth, respiratory tract and digestive tract [10]. Mercury as a pollutant that enters the fish's body, causes O_2^-radical production to increase from normal condition, and is difficult to be inactivated by the body [11], therefore it is necessary to consume antioxidants from the outside.

The provision of faloak klika extract as an antioxidant showed an influence on the radical content as shown in Figure 3 below.

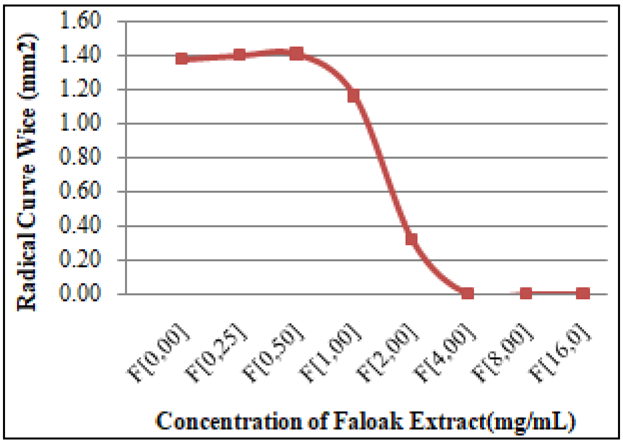

Figure 3: The radical activites of superoxide anion radicals in the kidney of tilapia given various doses of faloak extract.

Figure 3, shows that the faloak klika extract performed its expected effect as an antioxidant began to be seen at the concentration of 1.00mg / ml, where the area of the radical curve previously at the point 1.38 mm^2, decreased to 1.16mm^2. The decrease in radical activity continued to occur along with the addition of faloak klika extract concentrations. At the concentration of 4.00mg/ml to 16.00mg/ml, O_2^- radical activity was no longer detected because the Lissajous curve was no longer formed. When the radicals are no longer reactive, radicals no longer have enough energy to bind to other molecules in the body, so that the adverse effects of body cell damage can be avoided.

4. Conclusion

Exposure to mercury at a concentration of 0.018 ppm causes the formation of O_2^- radicals in the kidney organ with an area of 1.38 mm^2 curves. The provision of faloak klika extract was proven to be able to inactivate O_2^- radical activity, where a decrease in activity was observed in the 1.00 mg/ml extract. The area of the radical curve at the concentration of 1.00 mg/ml is at the point of 1.16 mm^2, down from the 1.38 mm^2. The decrease continues to occur along with the addition of extract concentration. At the concentration of 4.00 mg/ml to 16.0 mg/ml the curve is no longer observed in other words, the area of the radical curve is at the point of 0 mm^2.

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