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Research Article

EFFECT OF GIVING RED DRAGON (*SELENICEREUS COSTARICENCIS*) FRUIT EXTRAC ON SUPEROXIDE DISMUTASE (SOD) AND MALONDIALDEHYDE LEVELS INDUCED BY MAXIMUM PHYSICAL ACTIVITY IN RATS

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Abstract

Antioxidants as inhibitors work to inhibit oxidation by reacting with reactive free radicals to form non-reactive free radicals that are relatively stable so that they can protect cells from the harmful effects of reactive oxygen free radicals. Medicinal plants are a precious gift of nature that plays an essential role in the health care system of developing countries and cure various diseases in the world. Red dragon fruit is rich in antioxidants needed by the human body. This study aims to determine the red dragon fruit extract (RDFE) has antioxidant activity. Extraction was carried out using 96% ethanol solvent by the maceration method. Measurement of MDA and SOD levels was carried out once at the end of the study using the spectrophotometer method and histopathological examination. The increased SOD activity in the extract group at a dose of 300 mg/kg BW was significantly higher ($p<0.05$) than that given maximum physical activity. The 200mg/kg BW rats group increased SOD activity significantly ($p<0.05$) compared to the negative group. The group of rats with a dose of 300 mg/kg BW had lower MDA activity than 200 mg/kg BW and 100 mg/kg BW in the negative group. The results obtained that red dragon fruit extract has an effect in increasing the activity of SOD levels and reducing MDA and the histological picture of the kidney given maximum physical activity, and the extract undergoes cell necrosis.

Keywords: SOD, MDA Antioxidant, *Selenicereus costaricensis.*

INTRODUCTION

The imbalance of the number of free radicals with the number of endogenous antioxidants produced by the body, such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), is called oxidative stress. This situation can cause cell damage that can cause various diseases such as cancer, heart disease, cataracts, premature ageing, and other degenerative diseases. Free radicals can be in the body because of the by-products of the oxidation and burning of cells that take place during breathing, cell metabolism, excessive or maximal exercise or physical activity, inflammation, and exposure to pollution from outside the body such as vehicle smoke, cigarette smoke, food, heavy metals, industry and solar radiation. So that free radicals are not rampant to damage and interfere with health, the body will spontaneously produce antioxidants. Antioxidants as inhibitors that work to inhibit oxidation by reacting with reactive free radicals to form non- reactive free radicals that are relatively stable so that they can protect cells from the harmful effects of reactive oxygen free radicals. Finding and developing ways to predict and prevent disease and disorders caused by oxidative stress and free radicals has been of paramount importance in disease prevention and control over the past few decades. The risk assessment method has been widely applied. In clinical practice, risk models identify patients at risk for free radical-induced events, and healthy lifestyle therapies reduce disease and mortality rates.

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The use of natural food ingredients as primary prevention in diseases caused by free radicals needs to be prioritized. Vitamin E is a well-known antioxidant, effective in scavenging free radicals generated by radiation exposure, as a powerful breaker in the leading lipid chains found in the body4, inhibits the production of reactive oxygen species (ROS) molecules when fats are oxidized and during the propagation of free radical reactions. Oxidative stress plays an essential role in producing injury to organs such as the liver and others. Therefore, by targeting oxidative stress components, vitamin E appears as an antidote5, The role of oxidants in muscle fatigue has been investigated in various animal models in vitro and in situ during exercise. Medicinal plants are a precious gift of nature that plays an important role in the health care systems of developing countries and are a source of potent medicines for curing various diseases in the world. It plays an important role in maintaining our health. Medicinal plants have believed to be much safer. Nowadays, the use of herbal products has become the main choice for people worldwide because of its curative treatment without side effects. The World Health Organization (WHO) also recommends and recommends traditional medicines to treat various diseases. Still, the safety aspect of using traditional medicines must be prioritized in selecting a traditional medication. Red dragon fruit is rich in antioxidants needed by the human body. Anthocyanin levels range from 8.8mg/100g of dragon fruit. Red dragon fruit also contains betacyanin which has been proven in vitro. Red dragon fruit is rich in antioxidants needed by the human body. Anthocyanin levels range from 8.8mg/100g of dragon fruit. Red dragon fruit also contains betacyanin which has been proven in vitro.

METHODS

This type of research is experimental research, namely to determine the effect or relationship of the independent variable with the dependent variable. For example, the independent variable was red dragon fruit extract. In contrast, the dependent variable was a decrease in MDA levels and an increase in SOD levels in experimental rats induced by doxorubicin free radicals and maximum physical exercise. The research phase includes the collection of test materials, manufacture of simplicia and extracts, characterization of simplicia and extracts, phytochemical screening of simplicia and extracts, and in vivo antioxidant activity test.

Animals

Animals used in this study were male Wistar rats (*Rattus norvegicus*) weighing 150-200 g. Before starting this study, the test animals were acclimatized for one week at room temperature (22-25°C), under a 12-hour light/dark cycle, given pellets and drinking tap water ad libitum. 30 male Wistar rats were purchased from the Medan Bintang street animal market. Rats are healthy (active and can eat). This researchhas been approved by the health research ethics commission from FKKGIK, Universitas Prima Indonesia with no ethical clearance : 004/KEPK/UNPRI/III/2022.

Extract preparation

Red Dragon Fruit is collected from traditional markets in Medan, as much as 1.5 kg to 2 kg. The collected Red Dragon Fruit has been cleaned with clean water and then wiped dry with a clean towel to remove dirt and dust. After that, the leaves were dried in an oven at 55 °C ± 1 °C for 72 hours and the dried leaves were made into a coarse powder. The dried red dragon fruit was chopped and dried in a drying cabinet for 3 days. The ethanol extract of red dragon fruit was made by maceration with 96% ethanol as solvent. A total of 500 grams of red dragon fruit simplicia powder was put into a glass container, added 96% ethanol as much as 3.75 L, closed, left for 5 days protected from light while frequently stirring, shredded, squeezed, washed the dregs with enough liquid to obtain 4 L. Transfer to a closed vessel, leave in a cool place, protected from light for 2 days and poured or filtered. The results obtained were concentrated with a Rotary Evaporator until most of the solvent evaporated and continued the evaporation process on a water bath until a thick extract of red dragon fruit was obtained.

In vivo testing of antioxidant activity and oxidative stress of red dragon fruit extract

This test was carried out using male Wistar rats as subjects. The in vivo test in the experiment used the Federer formula to determine the number of mice treated (n-1) (t-1) ≥ 15, (n)=number of repetitions, (t)=number of groups, with the result n ≥ 5. Healthy mice with body weight were about 170 g ± 10%, and as many as 30 rats were divided into 5 groups consisting of 6 rats. Induction of organ damage using maximum physical activity was given by giving swimming treatment for ± 10 minutes, then extract suspension was given every day at a dose of 100 mg/kg BW, 200 mg/kg BW, and 300 mg/kg BW. On day 21, before being executed, the rats fasted for approximately 18 hours (not fed, but still given water). The rats were anaesthetized with chloroform and then tethered to surgical boards on all four limbs. The chest cavity was dissected, and 2 mL of blood in the heart was taken using a 3 mL syringe. The blood was then transferred into a blood tube and centrifuged for 10 minutes at a speed of 3000-4000 rpm to produce 2 layers, namely serum/supernatant and the precipitate. The serum layer was taken, then accommodated in a microtube and stored in a refrigerator at -4°C. Blood serum was used to check MDA and SOD.

Measurement of MDA and SOD levels

Measurement of MDA and SOD levels was carried out once at the end of the study using the spectrophotometer method. Increasing the concentration of MDA proves the presence of a lipid peroxide reaction in the body of experimental animals. MDA measurement has long been used as an indicator of oxidative damage to unsaturated fats as well as an indicator of the presence of free radicals.

Histopathological Examination and Assessment of Changes in Kidney Tissue (Tubules)

Renal tubule microscopy is a microscopic examination of the renal tubules using a light microscope with 40x and 400x magnification. Observations were made by divided into five parts to the renal tubules in the form of hydropic degeneration and necrosis. Hydrofic degeneration is characterized by swelling o cells due to the accumulation of fluid in the cytoplasm. Necrosis is characterized by nuclear degeneration in the form of *Karyopyknosis* (small and solid nucleus), *Karyolysis* (pale and dissolved nucleus) and *Karyorexis* (nuclear rupture into several lumps). The percentage of renal tubular damage from the 5 parts is then added up and divided by five.

Statistical Analysis

The research data were analyzed using the SPSS (Statistical Product and Service Solution) version 22 program. The data were analyzed using the Shapiro Wilk method to see the normality of the data. If the data is normally distributed (P> 0.05), it is continued using the One Way ANOVA method to determine the average difference between groups. If there is a difference (P< 0.05), the Post Hoc Tukey HSD test is followed to see the real difference between treatments. But if the data is not normally distributed, then the Kruskal-Wallis test is used.

RESULTS

Examination of SOD Levels in Rat Blood Serum

In the group that was given the extract, the SOD activity increased in line with the increase in the dose given to the rats. In the dose group of 300 mg/kg BW with 200 mg/kg BW, doses of 300 mg/kg BW with 100 mg/kg BW and 200 mg/kg BW with 100 mg/kg BW, the statistical values had significantly different values (p<0,05). In the extract group at doses of 100, 200 and 300 mg/kg BW, SOD activity increased compared to that given maximum physical activity. The increased SOD activity in the extract group at a dose of 300 mg/kg BW was significantly higher (p<0.05) than that given maximum physical activity. The 200 mg/kg BW rats group increased SOD activity significantly (p<0.05) compared to the negative group. In the rat group, the extract dose of 100 mg/kg

BW increased SOD activity significantly ($p<0.05$) compared to the negative group. The group of rats with a dose of 300 mg/kg BW had a higher SOD activity than 200 mg/kg BW and 100 mg/kg BW in the maximal physical activity group, which can be seen in Fig. 1.

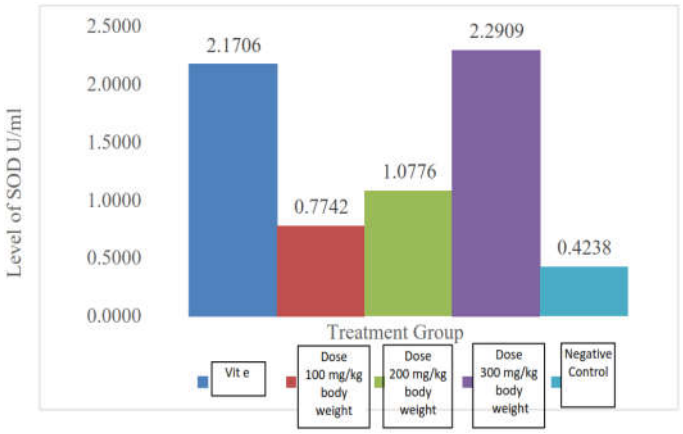

Fig 1. Graph of the average number of SOD activities with standard deviation.

Examination of MDA Levels in Rat Blood Serum

In the extract group, at doses of 100, 200 and 300 mg/kg, BW MDA activity decreased compared to the negative group. MDA activity decreased in the extract group at a dose of 300 mg/kg BW, significantly lower ($p<0.05$) than in the negative group. The 200 mg/kg BW rat group decreased MDA activity significantly ($p<0.05$) compared to the negative group. In the rat group, the extract dose of 100 mg/kg BW decreased SOD activity significantly ($p<0.05$) compared to the negative group. The group of rats with a dose of 300 mg/kg BW had lower MDA activity than 200 mg/kg BW and 100 mg/kg BW in the negative group. The graph of the average number of MDA activities with a standard deviation can be seen in Fig. 2.

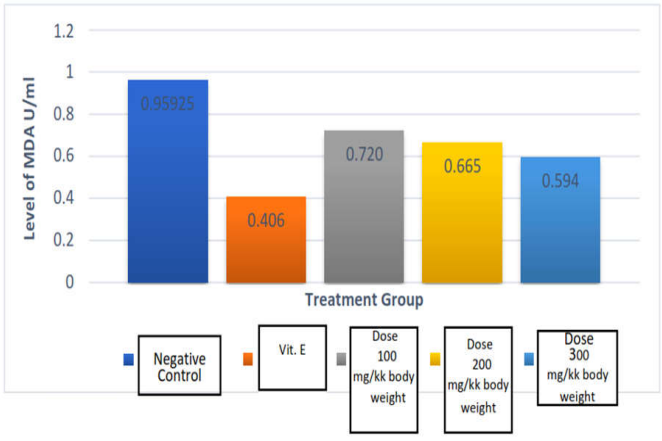

Fig 2. Graph of the average number of MDA activities with standard deviation.

Histological Examination of Rat Kidney Tissue

Histological examination of rat kidney tissue was performed with Hematoxylin Eosin (HE) staining. Hematoxylin is alkaline and will stain acidic (basophilic) tissue elements, namely the cell nucleus. While eosin is acidic, it functions to colour the alkaline cytoplasm (acidophilic). The results of the kidney histology Vexamination can be seen in Fig. 3.

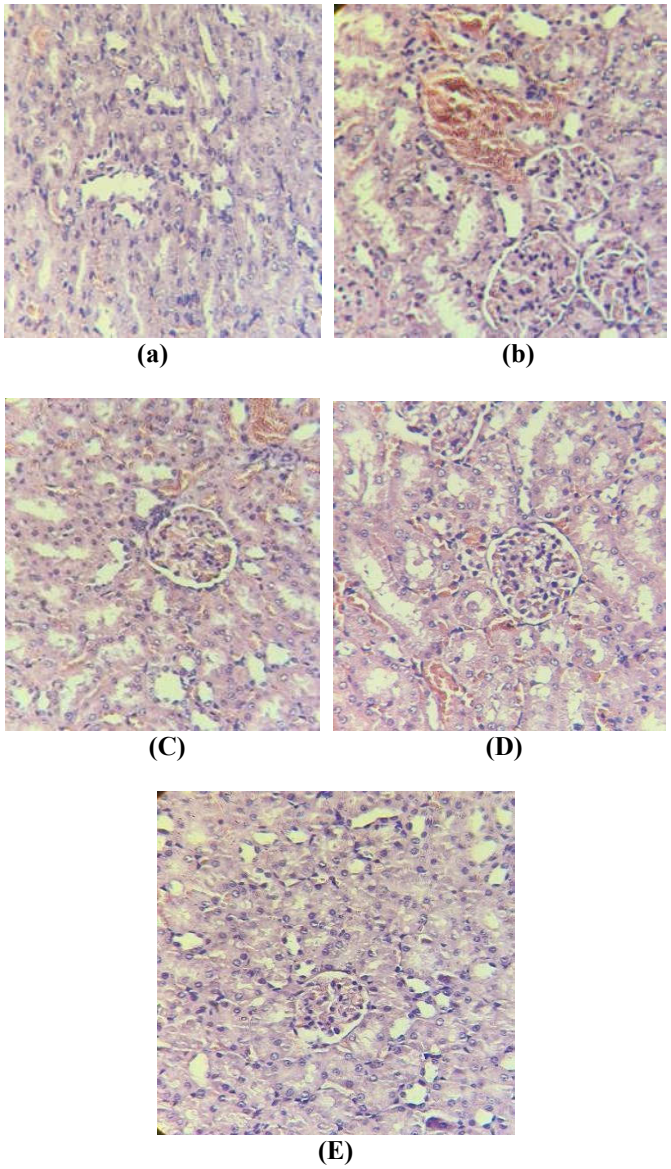

Fig 3. Histology of rat kidney with Hematoxylin and Eosin staining in various treatments (40x magnification). (a) group negative (b) positive group given vitamin E 1% BW (c) 100 mg/kgBW extract group (d) 200 mg/kgBW extract group, (e) 300 mg/kgBW extract group.

DISCUSSION

The group given the extract at a dose of 100 mg/kg BW had higher SOD levels than the negative group, given only maximal physical activity without treatment. Still, the levels were lower than the SOD levels in the positive control group given vitamin E. Based on these results, it was seen that there was a decrease in SOD levels in the group that was given maximum physical activity compared to the positive control group, which had been given vitamin E. This decrease could be seen especially in the RDFE group at doses of 300, 200 and 100 mg/kg BW. the negative control group indicated an increase in free radicals. Increased ROS can cause oxidative stress conditions, namely an imbalance between free radicals and antioxidants, where the number of free radicals is more than antioxidants. Quantitatively, SOD activity also increased with increasing doses. This increasing effect is thought to be because the flavonoid compounds that have antioxidant properties in RDFE not only work to neutralize free radicals formed due to stress but can also affect the activity of the SOD enzyme. The group given the extract at a dose of 300

mg/kg BW had lower MDA levels than the negative group, which was given only maximum physical activity without any treatment. Still, the levels were higher than the MDA levels in the positive control group given vitamin E. Based on these results, it can be seen that there was an increase in MDA levels in the group that was given maximum physical activity compared to the positive control group, which has given vitamin E. This decrease could be seen especially in the extract group at doses of 100, 200 and 300 mg/kg BW. the negative control group indicated an increase in free radicals. Increased ROS can cause oxidative stress conditions, namely an imbalance between free radicals and antioxidants, where the number of free radicals is more than antioxidants. Based on the results of the histological examination above, it can be seen that in the negative group, the composition of the kidney tissue in all rats was still in good condition good. However, in some parts, there is cell degeneration. In all groups of animals that were given maximum physical activity and RDFE, it was seen that there were cells undergoing cell necrosis. If observed microscopically, evident signs of cell death are in the nucleus. Usually, the cells that have died core shrink, appear denser, have irregular boundaries and are dark in colour (hyperchromatic), this process is called pyknosis, and the core is called pyknosis. In some circumstances, the dead cell nucleus loses the ability to be stained so that it becomes pale and disappears just like that or is not accurate, and this process is. Called karyolysis. Some cells also undergo apoptosis, characterized by condensed cells and cell nuclei and shrinks in size. At this stage, apoptosis cells exhibit dark-stained nuclei (pyknotic nuclei) that are easily recognized by light microscopy.

Conclusion

The red dragon fruit extract increases the activity of SOD levels and reduces MDA. The histological picture of the kidney given maximum physical activity and the extract undergoes cell necrosis.

Conflict of interest

The authors declare no conflict of interest in conducting this study.

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