Indonesian Journal of Biology and Education
Vol. 2, No. 1, 2019, pp: 40-44
pISSN:2654-5950, eISSN: 2654-9190
Email: ijobe@untidar.ac.id
Website: jurnal.untidar.ac.id/index.php/ijobe

Protective Effect of Red Dragon Fruit *(Hylocereus polyrhizus)* Juice on Myocardium Structure of Albino Rat Treated By Used Cooking Oil

Tamara Ismi Claudya[1], Novi Febrianti[2*],
Department of Biology Education, Faculty of Teacher Training & Education, Universitas Ahmad dahlan
Email: [1]tamaraismiclaudya@gmail.com,
[2]novifebrianrti@pbio.uad.ac.id,

Article History

Received : 12 – 03 – 2019
Revised : 20 – 03 – 2019
Accepted : 15 – 04 – 2019

***Corresponding Author**
Novi Febrianti
Department of Biology Education
Universitas Ahmad Dahlan
Jl. Lingkar Selatan, Tamanan, Bantul, Yogyakarta, 55191
novifebrianti@pbio.uad.ac.id

Keywords:
Red dragon fruit, myocardium, protective effect, used cooking oil

Abstract

Used cooking oil contain free radicals that are carcinogenic. These radicals able to damage myocardium structure. Red dragon fruit has many types of antioxidants. The objective of this study was to analyze the effect of red dragon fruit juice on the histopathological image of albino rat (Rattus norvegicus) myocardium treated with used cooking oil.This study used thirty male albino rat divided into 6 groups namely control healthy group (K), used cooking oil group (K-), vitamin C group (K+), 2.5 g red dragon fruit juice /kgbody weight (P1), 5g red dragon fruit juice/kg body weight (P2), and 10g red dragon fruit juice 10g/ kg body weight (P3). Each groups (except K) were given 2.5ml used cooking oil after one hour treated with various dosage of red dragon juice for 30 days. On the 31st day, a surgical action was performed and the heart stained by Haematoxylin-eosin (HE). The quantitative data of parenchymal degeneration, hydropic degeneration, and myocardium necrosis were analyzed by One-Way ANOVA and LSD. The results showed that red dragon fruit juice can reduce parenchymal degeneration, hydropic degeneration, and necrosis due to the used cooking oil treatment. The most effective dose of red dragon fruit juice that reduce myocardium damage was 10 g/ kg body weight.

1. INTRODUCTION

One of the problems in society is the repeated use of cooking oil or generally called used cooking oil. The used cooking oil is cooking oil that used more than three times in a frying process. The used cooking oil is not good for human health because the repeated heating process will produce a lot of free radicals. In addition, the levels of unsaturated fats contained in the oil will surely decrease, thus the remaining fats will be saturated causing damages to human body organs, such as the heart.

The tissue damages on heart due to used cooking are indicated by the presence of vacuoles that accumulated on the epithelial cell wall. Vacuoles deposited fats known as the *foam cells* (Rukmini, 2007). The presence of these fat deposits can cause various degenerative diseases.

Actually, the degenerative diseases can be prevented by consuming antioxidants. Fruits are the important source of natural antioxidants. The red dragon fruit *(Hylocereuspolyrhizus)* contains various antioxidant compounds such as phenols, flavonoids, and ascorbic acid. Febrianti et al. (2018) stated that the portion of the peel and flesh of red dragon fruit contains various amounts of vitamin C (ascorbic acid). The dragon fruit has many benefits such as; can reduce cholesterol levels, balance blood sugar levels and can prevent heart disease (Heryani, 2016).

Based on the previous information regarding the negative effects of the use of a used cooking oil and various antioxidant content in the red dragon fruit, a study to find out the effect of the red dragon fruit juice on the histopathology of white myocardium in a experimented mouse that is experimented using a used cooking oil.

2. RESEARCH METHODS

a. Collection and Preparation of Red Dragon Fruit Sample

The dragon fruit samples were collected from the Teguh dragon fruit farm in Wonoroto, Gadingsari, Sanden District, Bantul, Yogyakarta. The selected red dragon fruits were approximately one month old starting from the blooming of the

dragon fruit flowers. Red dragon fruits were firstly washed under running tap water and air-dried. Then, the fruits were peeled in order to separate the flesh from the peel. The flesh was minced and pressed using a juice extractor to produce filtrates and pomaces. The filtrate was took and used in experiment groups.

b. Production Used Cooking Oil

The used cooking oil came from a five times cassava frying processes. According to Zaki *et al*. (2015), the frying process begins by adding cooking oil to a frying pan of ± 1 liter, then heating it to 200ºC then frying 50 grams of cassava until cooked. The used cooking oil that was given to a group of P1, P2, and P3 with 2.5 ml each.

c. Treatment of Red Dragon Fruit Juice

Thirty male albino rats weighing 200-250 g with free access to water and food and *ad libitum* were randomly divided into five groups including healthy group (K group), used cooking oil group (K- group), vitamin C 0,04545 g/day group (K+ group), dragon fruit juice with a dose of 2.5 g / kg body weight with a volume of 2 ml each (P1 group), dragon fruit juice with a dose of 5 g/ kg of the body weight with a volume of 2 ml each (P2 group, dragon fruit juice with a dose of 10 g / kg of the body weight with a volume of 2 ml each (P3 group). Each groups (except K group) were given 2.5 ml used cooking oil after one hour treatment with various dosage of red dragon juice for 30 days. On the 31st day, a surgical action was performed and heart stained by *Haematoxylin-eosin* (HE).

d. Statistical analysis

All experiments were performed in triplicate. The data were presented as a mean ± standard deviation. Statistical significance was set at p=0.05. The differences of damages between six groups were analyzed using one-way Anova and LSD test.

3. RESULTS AND DISCUSSION

a. Myocardium Histopathological Image

Myocardium histopathological image observed by using a microscope with a 400X magnification (Figure 1). Based on the image results it can be explained that there are some damages in the myocardium tissue. It was being suspected that the damage was due to the used cooking oil toxic that caused the peroxide levels and the free radicals increased. The free radicals are naturally formed in the body. According to Novianti and Febrianti (2015), basically, the free radicals that were naturally formed in the body through metabolic processes and the negative effects of those free radicals can be prevented by antioxidant compounds. Thus, the amount of free radicals can naturally increase due to the consumption of chemicals such as a free radicals compound in the used cooking oil.

b. Scoring of Myocardium Damages

Based on the myocardium histopathological images of the albino rat, it was found percentage of parenchymal degeneration. The parenchymal degeneration then be scored according to Amalina *et al*. (2014) method to analyze the number of parenchymal degeneration. The damage of the parenchymal degeneration was shown in Figure 1. The observation of the myocardium damages was also being done to analyze hydropic degeneration and necrosis damages. The scoring process was also being done according to Amalina *et al*., (2014). the percentage of hydropic degeneration and necrosis is shown in Figure 2 and Figure 3.

The normal myocardium is indicated by a core of a nucleus cell and surrounded by a pale cytoplasm. However, a parenchymal degeneration was still being found in the control group which was 8.61% (Figure 2), the percentage of hydropic degeneration damage was 9.53% (Figure 3), and the percentage of necrotic damage was 7.69% (Figure 4). All of the three results showed that the damage category was still normal (<10%). This was because in the control group, the albino rats were only given food and drink without any treatments, so the cell structure in the body of the white rat was normal.

The highest percentage of parenchymal degeneration, hydropic degeneration and necrosis were found in the negative control group at 30.3%, 31.89%, and 37.32% respectively.. The percentage of the parenchymal degeneration was indicated by the presence of a nucleus core that was being pushed to the edge from the cytoplasm cells, then the cell cavity was empty because of the cells were swollen (Figure 1). The hydropic degeneration that was indicated by vacuoles cells that was containing water in the cytoplasm, so that the myocardium was brighter (Figure 1) that showed the damage category was medium (10-33%), while the percentage of necrosis which was indicated by a shrinking nucleus which was broken into fragments and undergone a lysis showed a high damage category (around 34-66%). This condition was found in the negative control group that the mouse was only given a used cooking oil and food.

The used cooking oil is an oil that has been used several times, the repeated heating affected the peroxide value to be increased. According to Aisyah et al (2014) the used cooking oil to the mouse body contained a lot of toxic compounds, one of them was peroxide which will trigger a creation of free radicals, thus will be resulting in damage to the myocardium.

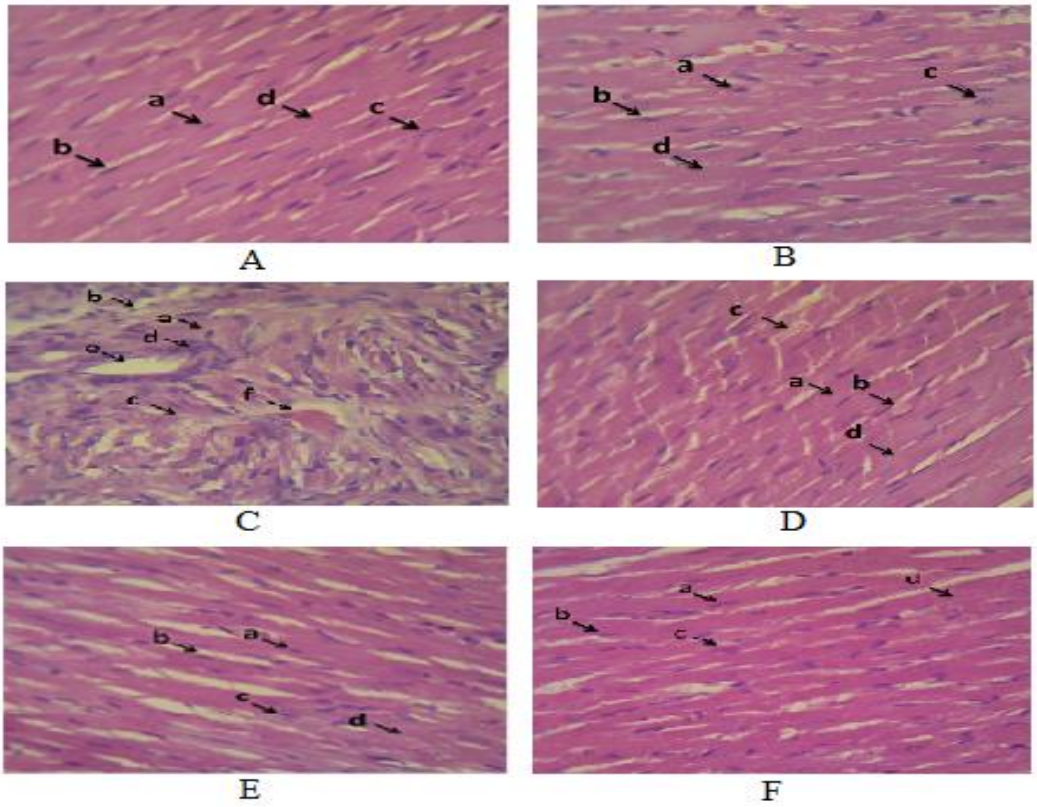

Figure 1. Histopathological images of albino rat myocardium with HE staining (Magnification 400x). A. Control (K), B. P1 Group, C. Used cooking oil goup (K-), D. P2 group, E. Red draguit only (K+), F. P3 group.
a. Normal, b. Parenchymal degeneration, c. Hydropic Degeneration,
d. Necrosis, e. Fat Infiltration, f. Hemorrhage.

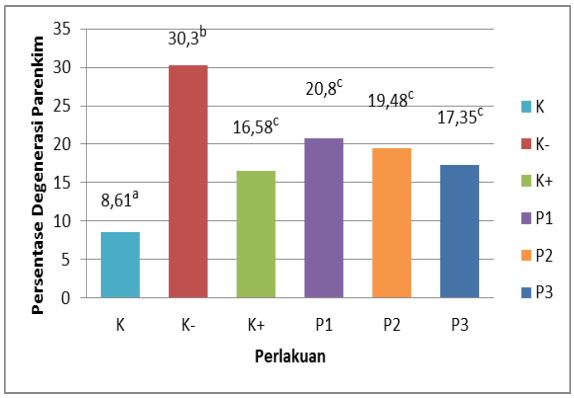

Figure 2. Percentage of Parenchyma degeneration

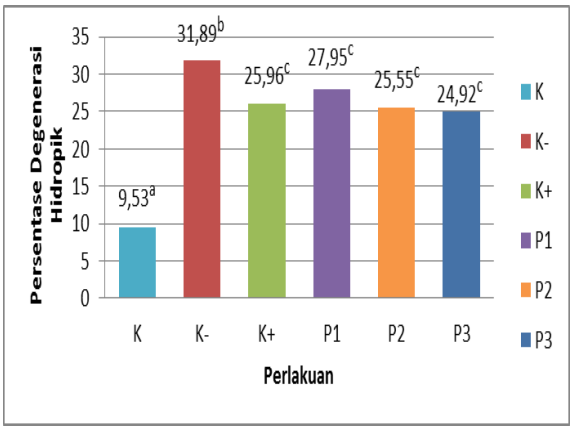

Figure 3. Percentage of hydropic degeneration

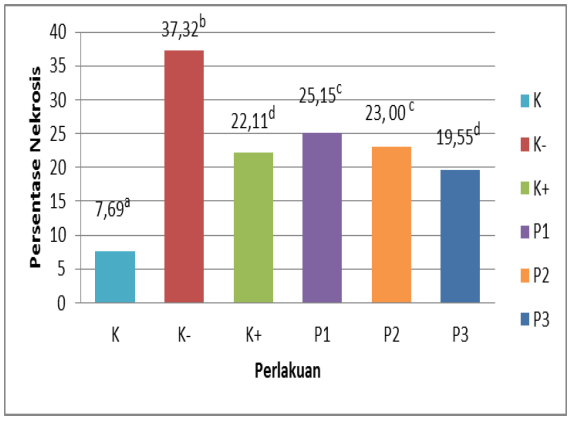

Figure 4. Percentage of necrosis

Based on the myocardium histopathology result of the negative control group, it was found that the undertaken damage was in the form of fats and hemorrhagic infiltrations (Figure 1). The fat cell infiltration was indicated by the penetrating of a fat cells into the cell membranes and then accumulated between the parenchymal cells in the heart muscle. According to Aisyah et al (2014), the fat infiltration occurs due to the changes in the fats metabolism in a mouse due to the excessive fats intake that is resulting in an accumulation of triacylglycerol in the myocardium. Other than that, it was also found that a hemorrhage (bleeding) damage was occurred. The hemorrhage was indicated by the blood cells that are visibly clumping in the myocardium. According to Assiam et al. (2014: 236-246), hemorrhage is the presence of blood outside the blood vessels which microscopically will show blood cells that are in the tissue.

In the positive control group, the albino rats were given used cooking oil and vitamin C. It was found that the cells were normal as 147 cells, but there were damages in the form of parenchymal degeneration, hydropic degeneration and necrosis. The percentage results of the parenchymal degeneration in positive control group were 16.58% (Figure 2), the percentage of hydropic degeneration damage was 25.96% (Figure 3), and the percentage of necrotic damage was 22.11% (Figure 4). The three indicators indicated that the percentage of damage was in the medium category (10-33%). The vitamin C that was given to the positive control group had a significant effect in decreasing the myocardium damage compared to the negative control group. Presumably because of the ability of the vitamin C as a part of the body's defense system against a reactive oxygen compounds in cells. In accordance to Werdhasari (2014) which stated vitamin C as a secondary antioxidant has the ability to counteract free radicals by working as an electron donor, so that it can convert free radicals into more stable compounds.

Percentages of treatment group 1, 2, and 3 showed a parenchymal degeneration, hydropic degeneration, and necrosis decreased due to use of used cooking oil compared to the negative control group and positive control group. The observation of the histopathological image from the white mouse. Treatment Group 1, Treatment Group 2, and Treatment Group 3 parenchymal degeneration of the myocardium parenchyma was respectively 20.80%, 19.48%, and 17.35%. The white mouse myocardium hydropic degeneration showed an average percentage in a row in such result 27.95%, 25.55%, and 24.92%, and necrosis showed an average percentage of 25.15%, 23.00%, and 19.55%. These results were considered a medium damage category (In average 10-33%). The smallest damage of the parenchymal degeneration, hydropic degeneration, and necrosis in the treatment Group 3, thus the white mouse were given used cooking oil and after one hour they were given a red dragon fruit juice with a dose of 10 g / kg of the mouse weight. In one hand, after being analyzed using Post Hoc LSD between RD1, RD2, and RD3 groups, there were no significant difference found. In the other hand, when compared with the negative control group, there was a significant difference of the damage percentages. It showed that the antioxidant compounds in red dragon fruit juice with different volume of doses can provide a protective effect on free radicals from toxic compounds contained in used cooking oil

CONCLUSIONS

Consumption of red dragon fruit juice can minimize a myocardium damage in the form of parenchymal degeneration, hydropic degeneration, and necrosis due to the usage of used cooking oil. The most effective dose of red dragon fruit juice to reduce the rate of damage to white mouse myocardium is 10 g/kg body weight of albino rat

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