The Effect of Red Dragon Fruit Extract (Hylocereus Polyrhizus) on Membrane Lipid Peroxidation and Liver Tissue Damage Triggered by Hyperlipidemia in White Rats (Rattus Norvegicus)

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Abstract—This study aims to prove that the administration of red dragon fruit extract (Hylocereus polyrhizus) has an effect on membrane lipid peroxidation and liver tissue damage triggered by hyperlipidemia in white rats (Rattus norvegicus). This study used true-experimental design with using 30 male Wistar rats which were divided into 5 groups. Group K1 - K4 was given treatment for 8 weeks and group K5 for 16 weeks. The group consists of K1 negative control (standard diet); K2 positive control (standard diet and quail eggs 10 ml / kgBW); K3 (standard diet, quail eggs 10 ml / kgBW and simvastatin 0.72 mg / day); K4 (standard diet, quail eggs 10 ml / kgBW and red dragon fruit extract dose 60 mg / day) for 8 weeks and K5 (standard diet, quail eggs 10 ml / kgBW and red dragon fruit extract dose 60 mg / day) for 16 weeks. Data was analyzed by One Way ANOVA test, followed by the Wilcoxon Man-Whitney Post Hoc test (P-value <0.05) in 8 weeks treatment. K3 and K4 groups showed improvement in MDA concentration and improvement of liver tissue (P-value <0.05) compared to K2. Red dragon fruit extract 60 mg/day has a potential effect in repairing lipid peroxidation damage on liver cell membrane in hyperlipidemia conditions with high fat diet.

Keyword: Red Dragon Fruit Extract (*Hylocereus Polyrhizus*), Hyperlipidemia, Membrane Lipid Peroxidation, Rattus Norvegicus.

1. INTRODUCTION

Hyperlipidemia has become a major cause of degenerative disease such as coronary heart disease, stroke, and liver damage in developed and developing countries. The prevalence of high cholesterol levels occurs more in the European region (54%), followed by American (48%), and Southeast Asia (39%). High-income countries were reported to have a higher incidence of hypercholesterolemia than low-income countries (WHO 2016).

Hyperlipidemia that occurs in the body is a free radical that will cause the formation of lipid peroxidation products such as malondialdehyde (MDA). MDA levels are widely used as biomarkers for assessing oxidative stress in the biomedical field. Lipid peroxidation is an indicator of tissue damage caused by free radical activity (Zorawar Singh, 2014).

Management of hyperlipidemia includes diet and drug administration. Traditional medicine in general is considered safer than the use of modern medicine. This is because traditional medicine has relatively few side effects compared to modern medicine. Research on plant extracts, especially from fruits, continues to be carried out. One of the fruits that is reported

to have antioxidant effects and is easy to grow in Indonesia and has been known by the public is red dragon fruit (*Hylocereus polyrhizus*) (Prakoso, 2017).
Red dragon fruit has a dietary fiber content of 3.2 gr / 100 gr of fruit, vitamin B3 content (niacin) (Liniawati 2011, pp. 50; Pareira 2010, p. 8). In addition, dragon fruit seeds contain unsaturated fat MUFA (monounsaturated fatty acids), and PUFA (polyunsaturated fatty acids) (Mahattanatawee et al, 2006).
This study aims to analyze the effect of red dragon fruit extract (*Hylocereus polyrhizus*) on membrane lipid peroxidation and liver tissue damage caused by hyperlipidemia in white rats (*Rattus norvegicus*)

2. MATERIALS AND METHOD

The study used white mice (*Rattus norvegicus*), Wistar strain, male, aged ± 8 weeks with a weight of ± 300-400 grams. There were 30 rats which were divided into 5 groups by simple random sampling. Red dragon fruit extract was obtained from the market, then extracted at Ballitro.

Hypercholesterol induction was carried out by giving raw quail eggs to K1 (Negative Control) rats that received standard feed, Na-CMC and given aquadest; K2 (Positive Control) that gets standard feed, quail eggs and given aquadest; K3 which received standard feed was added with quail eggs and simvastatin was given a dose of 0.72 mg by dilution in 2 ml of aquadest; K4 which received standard feed added with quail eggs and was given red dragon fruit extract dose of 60 gr / 200 grBB/day for 8 weeks and K5 which received standard feed supplemented with quail eggs and given red dragon fruit extract with a dose of 60 gr/200 grBB/day for 16 weeks.

After the treatment, blood was taken to check the cholesterol levels and MDA and liver tissue for damage analysis of steatosis and necrosis. Data were analyzed by One-way ANOVA test. The normality test used the Shapiro-Wilk test, then it was continued by Post Hoc Tukey's HSD (Honestly Significant Difference) test, because the assumptions were not fulfilled, Kruskal-Wallis non-parametric alternative tests were performed (Sing, 2013). While the analysis test between the red dragon fruit extract group for 8 weeks and 16 weeks used the Mann-Whitney U non parametric test.

3. RESULTS AND DISCUSSION

a. Cholesterol Levels

Table 1. Average total cholesterol levels in rats

Group	Treatment	Number of samples (n)	Average Cholesterol Levels ± SD
K 1	Negative Control	6	64,83 ± 10,19
K 2	Positive Control	6	101,17 ± 6,34
K 3	Simvastatine	6	73,17 ± 9,81
K 4	Red dragon fruit 8 weeks	6	54,17 ± 2,32
K 5	Red dragon fruit 16 weeks	6	71,17 ± 22,59

There were significant differences in avarage cholesterol levels between treatment groups. It can be concluded that giving quail eggs at a dose of 10 mg / day for 8 weeks is more influential in increasing rat cholesterol levels. This is due to the high saturated fatty acids contained in quail egg yolks trough beta oxidation metabolic processes produce acetyl CoA which is a precursor from cholesterol (Kusuma, 2016) and after negative control (K1) compared to positive control (K2) there was a significance of .000 (P-value <0.05).

The two groups that were intervened (K3 & K4) compared with positive controls (K2) showed a significance value of .000 (P-value <0.05). It showed that both treatments could reduce cholesterol levels in mice induced by high-fat feed. This is because simvastatin works by inhibiting the work of the HMG-CoA reductase enzyme (Arsana et al, 2015) so that HMG-CoA cannot be change to mevalonate and finnaly cholesterol synthesis cannot perform (Murray et al, 2009) and cholesterol levels decrease with differences an average of 28.00 in the simvastatin (K3) treatment group.

Red dragon fruit has various ingredients that can reduce cholesterol levels such as alkaloids, saponins, antioxidants (phenols, flavonoids, ascorba acid, betasianin), triterpenoids, niacin, unsaturated fiber and fatty acids (Prakoso, 2017), so that dragon fruit can reduce levels cholesterol with an average difference of 47.00.

Saponins (Shi et al, 2014) and triterpenoids (Prakoso, 2017) work to inhibit HMG-CoA reductase. Phenol, betasianins (Prakoso, 2017) and ascorbic acid (Elon and Polancos, 2015) neutralize free radicals and peroxide radicals so that oxidative stress decreases. Flavonoids directly donate hydrogen ions to stabilize free radicals and indirectly stimulate antioxidant gene expression. In addition, flavonoids increase the secretion of the bile that can reduce cholesterol levels in the body (Prakoso, 2017).

If the effect of red dragon fruit extract is compared to the use of statin (simvastatin) in reducing total cholesterol levels, the significance is .002 (P-value <0.05), the administration of red dragon fruit is more significant in reducing cholesterol levels than use simvastatin, this is contrary to the results of a study conducted by Sharan, 2017 which states that the potential statin dose of 10 mg / day for 28 days is stronger than red dragon fruit extract at a dose of 120 mg / day in reducing cholesterol levels. This happened because in this study a longer dragon fruit extract was given for 8 weeks and this study induced high-fat feed and intervened red dragon fruit extract simultaneously, so that it could only test the effectiveness of red dragon fruit as a preventive strategy in avoiding increases blood cholesterol level. Red dragon fruit has a content that works like simvastatin, namely saponin (Shi et al., 2014) and triperpenoid (Prakoso, 2017) which can degrade and inhibit the work of the HMG-CoA reductase enzyme so that it can lower rat blood cholesterol level.

Cholesterol levels in the treatment group given red dragon fruit extract for 8 weeks (K4) and 16 weeks (K5) using the Mann-Whitney U test showed a significance value of .15 (P-value <0.05). This showed a decrease in the effectiveness of red dragon fruit extract in controlling cholesterol levels after administration for 16 weeks. In this study, there were no deaths in the group of mice given chronic dragon fruit extract. Previous research examining the toxic effects of red dragon fruit extract on acute (14 days) and subchronic administration (28 days) stated that administration of red dragon fruit extract with dose intervals of 250 mg / day, 500 mg / day, and 1000 mg / day relative safely administered orally. As long as it does not exceed the lethal doses, which exceeds 1000 mg / day (Hor et al, 2012).

So it can be concluded that the administration of red dragon fruit is still effective in controlling cholesterol levels and is not toxic.

b. Malondialdehyde (MDA)

Table 2. Descriptive Malondialdehyde Serum Levels (MDA)

Group	Mean ± SD	Min	Max	Saphiro-Wilk	Kruskal - Wallis
K1	9.6875 ± 11.27601	.00	30.00	.170	0,031*
K2	195.1682 ± 95.22596	8.90	259.46	.012*	
K3	39.1450 ± 78.67722	2.77	199.59	.000*	
K4	44.3417 ± 87.60978	5.39	223.04	.000*	
K5	8.3183 ± 3.77292	3.52	13.11	.751	

Malondialdehyde (MDA) is the result of lipid peroxidation and is used as a biomarker or to estimate the peroxidation process (Marjani, 2010). Determination of MDA levels in blood plasma or a tissue is one method that is useful for predicting oxidative stress levels due to the degree of damage due to membrane lipid peroxidation or lipoprotein (Susantiningsih, 2015).

Table 3 Comparison of P values MDA levels between groups

GROUP	*P - value*
K1 – K2	0.010*
K2 – K3	0.016*
K2 – K4	0.025*
K2 – K5	0.016*

The comparison of the negative control group (K1) with positive control (K2) showed a P-value of 0.010 (<0.05) which meant that there was significance of comparison data between the two groups, thus concluding that hyperlipidemia increased damage to membrane lipid peroxidation. The peroxidation process begins with the withdrawal of one electron containing one electron from the PUFA double bond to form a lipid radical. Carbon lipid radicals tend to stabilize themselves by doing intra-molecular rearrangement so they can quickly react with oxygen to form peroxyl lipid radicals, where these radicals will attract hydrogen atoms from other PUFA double bonds so that a chain reaction forms the other lipid radicals. The reaction between peroxyl radicals and hydrogen atoms will produce lipid peroxides (Susantiningsih, 2015).

The comparison of the positive control group (K2) with the treatment group simvastatin (K3), the group of red dragon fruit 8 weeks (K4) and the group of red dragon fruit 16 weeks (K5) all showed P-value <0.05, this stated that there was significance difference between the positive control group and the simvastatin group through simvastatin worked by inhibiting the action of the HMG-CoA reductase enzyme (Arsana et al, 2015) so that HMG-CoA could not be reduced

to mevalonate thereby repairing damage to lipid peroxidation.The red dragon fruit group also showed improvement in damage to lipid peroxidation when compared with the positive control group.

Oxygen and H202 free radicals are formed by body cells such as polymorphonuclear cells, monocytes and macrophages. Hydrogen peroxide is a strong oxidant because it is able to react with various compounds, therefore catalase converts it to water and oxygen, besides that the flotation peroxidase enzyme also converts it to water. If H202 reacts with Cu2 + and Fe2 +, hydroxyl free radicals will form, where unsaturated fatty acids contained in cell membranes such as phospholipid, glycolipids and cholesterol are very sensitive to them because the methylene bridges owned by PUFA are the target. Furthermore, when this reaction occurs, the chain of fatty acids will be cut off and form aldehyde compounds such as malondialdehyde, ethane, and pentane which are very damaging to other body cells such as DNA and protein (Susantiningsih, 2015).

c. Damage to liver tissue

1) Steatosis (fatty liver)

The fatty liver or steatosis found in this study is characterized by changes in the shape of lipids in hepatocytes in the form of microvascular steatosis because the nucleus of hepatocytes is surrounded by small vacuoles that are clearly shaped, according to Sanyal's statement (2002). Hepatic steatosis can be reversible or develop into steatohepatitis, depending on whether the cause is persistent or not. Steatohepatitis consists of macrovesicular steatosis, core glycogenation, portal and lobular inflammation, fibrosis, bubbling hepatocytes, apoptotic cells, and Mallory hyaline. Inflammatory severity is not always related to the degree of steatosis (Charlton, 2009).

The liver plays an important role in lipid and carbohydrate metabolism. Fat accumulation manifests as fatty / steatosis if there is an imbalance between the delivery or synthesis of fatty acids with the capacity of the liver to oxidize or remove it (Anstee and Goldin; 2006). Increasing free fatty acids in the liver will lead to increased oxidation and esterification (Schreuder et al., 2008; Charlton, 2009).

Table 4. Distribution of Treatment Groups Based on Steatosis Abnormalities.

GROUP	No steatosis (degree 0)	Steatosis < 33% (degree 1)	Steatosis 33-66% (degree 2)	Steatosis > 66% (degree 3)	Total	Kruskal-Wallis
K1	5 (83,3)	1 (16,7)	0 (0,0)	0 (0,0)	6 (100%)	
K2	0 (0,0)	0 (0,0)	4 (66,7)	2 (33,3)	6 (100%)	
K3	2 (33,3)	4 (66,7)	0 (0,0)	0 (0,0)	6 (100%)	0,001
K4	1 (16,7)	3 (50,0)	2 (33,3)	0 (0,0)	6 (100%)	
K5	1 (16,7)	4 (66,7)	1 (16,7)	0 (0,0)	6 (100,%)	

Increased incidence of steatosis based on the length of the treatment group due to the administration of used oil increases fat absorption and affects lipid metabolism (Thadeus, 2005). This is also stated by Anstee (2006) and Henryk (2010) that increasing the delivery and synthesis of fatty acids in hepatocytes, can be triggered by the consumption of high-fat diets

or increased release of fatty acids from adipose tissue. Steatosis can also be formed due to an increase in de novo synthesis of fatty acids and triglycerides in the liver, which is based on an increase in the expression of activated SREBP-1c and ChREBP and lipogenic genes (Browning, 2004; Anania, 2007). Fat accumulation can also occur due to a decrease in VLDL synthesis and a decrease in the release of triglycerides from the liver (Anania, 2007; Tacer, 2011).

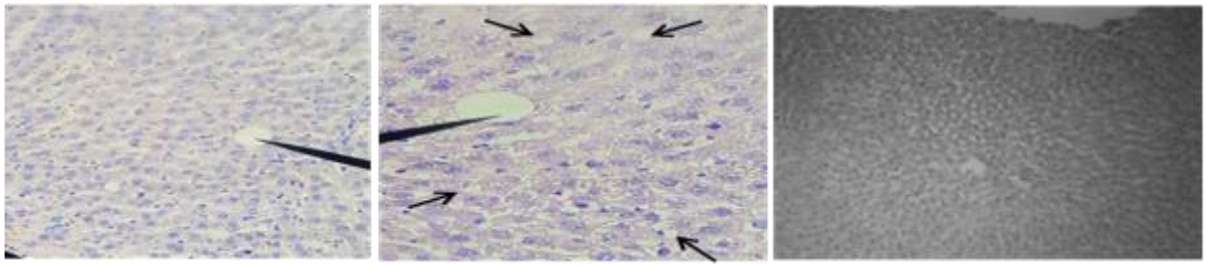

Figure 1. Degree of Steatosis (A = degree 1, B = degree 2, C = degree 3)

Hepatocytes are very unique cells. The potential for hepatocytes to proliferate appears during moments of cell mass loss, called the fasa prima or fasa replicative competency which is generally triggered by Kupffer cells through secretion of IL-6 and TNF-α cytokines. In this phase, hepatocytes enter the cell cycle from phase G0 to G1 (Fujiyoshi, 2010). Tumor necrotizing factor-alpha can have a proliferative or apoptotic effect, depending on ROS and glutathione, at least 4 transcription factors are activated before the hepatocytes enter the proliferative phase, namely NFκB, STAT-3, AP-1 and C / EBP-beta (Fausto et al., 2010).

When steatosis has formed, the liver is more easily sensitized and an inflammatory response will occur which can be precipitated by various kinds of stimulus. Oxidative damage to inflammation is a key player in "second hits" (Anstee, 2006; Hübscher, 2006).

Cell lipid peroxidation produces toxic by-products of aldehydes, including MDA and HNE which more persistent than ROS, and causes further damage to intracellular organelles and decreases hepatocyte glutathione (Browning, 2004; Anstee, 2006). Furthermore, aldehyde will also increase the production of NF-β-dependent proinflammatory cytokines (TNF-, IL-6, IL-1), increase TGF-1 expression, encourage the entry of inflammatory cells into the liver, and activate hepatic stellate cells (Ito cell) which is fibrogenic. These effects can directly trigger hepatocyte death and necrosis, inflammation, and liver fibrosis, which are the characteristic of NASH (Browning, 2004; Anstee, 2006). When hepatocyte damage occurs, the enzymes contained in it will be released into the systemic circulation, so that ALT levels often increase persistently in NAFLD patients.

Fatty liver cause an increase in oxidation and esterification of fat which is focused on mitochondrial liver cells, resulting in damage to mitochondria (second hits) (Hübscher, 2006). Then followed by various reactions in liver cells such as progressive inflammation, liver cell swelling, liver cell death, and fibrosis processes (McAvoy et al., 2006; Setiawan, 2014).

2) Necrosis

Table 5. Distribution of Treatment Groups Based on Necrosis Disorders

GROUP	No inflammation (degree 0)	Portal inflammation without necrosis (1st degree)	Lobular necrosis is focal (degree 2)	Bridging necrosis (degree 3)	Total	Kruskal-Wallis
K1	3 (50,0)	2 (33,3)	1 (16,7)	0 (0,0)	6 (100%)	0,037
K2	0 (0,0)	1 (16,7)	3 (50,0)	2 (33,3)	6 (100%)	
K3	1 (16,7)	4 (66,7)	1 (16,7)	0 (0,0)	6 (100%)	
K4	2 (33,3)	3 (50,0)	1 (16,7)	0 (0,0)	6 (100%)	
K5	0 (0,0)	4 (66,7)	2 (33,3)	0 (0,0)	6 (100,%)	

In addition to increasing the number of hepatocytes with fat degeneration, the condition of hyperlipidemia causes hepatocyte damage. This can be seen from changes in the cell nucleus to picnotics (the nucleus shrinks, irregular and dark colored borders, and varying hepatocyte size) initially triggered by mitochondrial damage (second hit) (Hübscher, 2006). It is then followed by various reactions in liver cells such as progressive inflammation, liver cell swelling, liver cell death, and fibrosis processes (McAvoy et al., 2006; Setiawan, 2014). This means that hyperlipidemia affects the occurrence of liver cell damage, because it causes oxidative damage resulting in an increase in ROS which ultimately causes hepatocyte death or necrosis (Nakamoto, 2009).

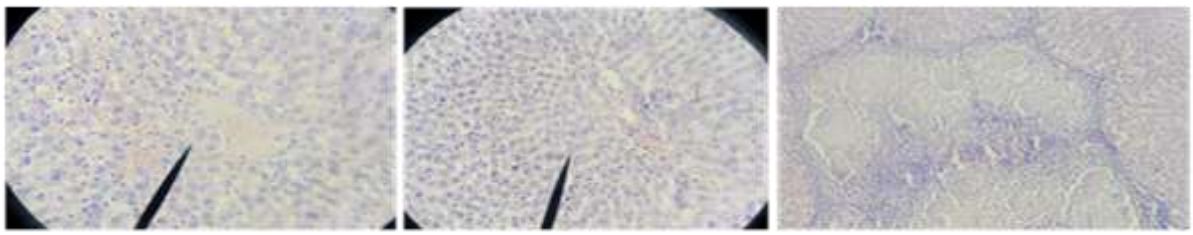

Figure 2. Degree of Necrosis (A = degree 1, B = degree 2, C = degree 3)

Long exposure to injury will lead to the development of more severe necrosis in the form of bridging necrosis, which is a cell death initially starting from the continuing edge to the middle, which bridges the port area with the central vein and forms bridging, and eventually will result whole necrosis (Neil, 2012).

CONCLUSION

Red dragon fruit (Hylocereus polyrhizus) has the potential as an antioxidant by repairing oxidative stress trough improving MDA levels and damage to liver tissue (steatosis and necrosis).

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