THE EFFECT OF RED DRAGON FRUIT (Hylocereus polyrhizus) ON ROS PLASMA OF OVERWEIGHT SPRAGUE DAWLEY RATS

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ABSTRACT
Red dragon fruit (Hylocereus polyrhizus) contains antioxidants, namely vitamin C, flavonoids, phenols, and betacyanins that can reduce or inhibit oxidative stress in the body. Excess body weight condition is associated with an increase in oxidative stress due to a decrease in antioxidants. This study was aimed to analyze the effects of red dragon fruit on the level of ROS (MDA) of overweight Sprague-Dawley rats. It used experimental analysis by using pre- and posttest design with control group. A total sample of 30 male white Sprague-Dawley rats aged 8-12 weeks old with 150-200g of body weight were divided into 6 groups, namely: normal, negative control (KN), positive control (KP), red dragon fruit juice 3.5 g/200gBW (P1), overweight + dragon fruit juice 7 g/200gBW (P2), overweight + dragon fruit juice 10.5g/200gBW (P3) and were observed for 14 days. ROS levels were measured in the beginning and at the end of the intervention by using MDA markers with TBARs method. The results of MDA level measurements were 3.83 ± 0.13 in the KP group, 7.18 ± 0.27 in the P1 group, 5.30 ± 0.30 in the P2 group, and 4.35 ± 0.42 in the P3 group. The results of one-way ANOVA test showed a significant difference in MDA levels in all intervention groups compared to those of the normal and KN groups (p = 0.05). In conclusion, red dragon fruit intake can reduce ROS levels in overweight Sprague-Dawley rats.

Keywords: Red dragon fruit, ROS, overweight Sprague-Dawley rats

INTRODUCTION
World Obesity Federation stated that obesity is an epidemic which becomes the most important health problem (de Mello et al., 2017). Globally, the prevalence of overweight has tripled, amounting to 1.9 billion of the world’s population. In Indonesia, around 13.6% of the population was recorded to be overweight in 2018 (Khadaee and Saidi, 2016; Rebello et al., 2020). The problems of overweight and obesity are the fifth leading causes of global risk of death (Sudargo et al., 2018). An increase in body mass index (BMI) of people aged 25-74 years old over the last five years indicates an increased risk of hypertension by 30% compared to those with normal BMI (Hall et al., 2019).

An increase in body weight can trigger insulin resistance in peripheral tissues through adipose tissue dysfunction or lipotoxicity, inflammation, mitochondrial dysfunction, hyperinsulinemia, and endoplasmic reticulum (ER) stress (Longo et al., 2019). In overweight, there is excessive accumulation of intra-abdominal fat, causing disruption of adipocytokines production including leptin, resistin, adiponectin, monocyte chemoattractant protein-1(MCP-1), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) (Mesquida et al., 2020). Obesity or overweight is associated with an increase in oxidative stress due to an imbalance between pro-oxidants and antioxidants (Yosika et al., 2020). High MDA level can be used as a marker of increased reactive oxidative stress (ROS) (Moazen et al., 2013). Being overweight is an early sign of obesity which was has an impact on the emergence of various degenerative diseases such as diabetes mellitus (Muhammad et al., 2019). So that treatment in overweight people is carried out as an early prevention.

The use of fruits to prevent the occurrence of free radicals has been widely studied. The content of bioactive compounds is a source of natural antioxidants. Antioxidants work by inhibiting or preventing ROS or as chain breakers of free radical reactions (Saklayen et al., 2018). In addition, antioxidants can also be obtained from chemical
synthesis. Synthetic antioxidants have several side effects, ranging from inflammation to liver damage and are carcinogenic if consumed in the long term (Parwata, 2016). Natural sources of antioxidants can be found in spices, whole grains, cereals, fruits, and vegetables.

Red dragon fruit (Hylocereus polyrhizus) has natural antioxidants such as betacyanins, flavonoids, polyphenols, lycopenes, vitamin C, vitamin B1, vitamin B3, vitamin B12, betacarotene, fiber and mineral phosphorus. The antioxidant activity of red dragon fruit is 67.45 ppm. It should be noted that a compound is said to have good antioxidant activity if the IC value is IC50<200 ppm (Kim et al., 2017).

**METHODS**

This research used a randomized control trial with pre- and post-test control group design. Samples in this study were male white Sprague-Dawley strain, aged 8-12 weeks old, with 150-200 g body weight, and in good health (normal activity, willing to eat, no anatomical abnormalities). The samples were never used in a scientific study and obtained from the Nutrition Laboratory of the Center for Food and Nutrition Studies of Inter-University Center (PAU), Gajah Mada University, Yogyakarta. The number of samples was calculated by using the Federer formula. Based on this formula, the number of samples was determined to be 5 rats in each group. To prevent drop-out, the number of samples was added by 20% so that the total number of samples obtained was 30 samples.

The samples were determined by simple random sampling and divided into 6 groups with 5 rats each, namely the normal weight group with standard feed, negative control (KN) group of overweight rats with intake of high fat high carbohydrate diet (HFHC), positive control group (KP) of overweight rats with intake of Orlistat 6.48 mg/200g of body weight, overweight rats group with red dragon fruit juice intake of 3.5 g/200g body weight (P1), overweight rats group with red dragon fruit juice intake of 7 g/200g body weight (P2), and overweight rat group with red dragon fruit juice intake of 10.5g/200g body weight (P3) (Picture 1). The standard Comfeed feed was AD II ad libitum. Each 100 g of standard AD-II feed contains 12% water, 7% ash, 15% crude protein, 3-7% crude fat, 0.9-11% calcium, 0.6-0.9% phosphorus, antibiotics, and up to 20 mg/day coccidiostats.

Modelling of overweight rats was carried out by giving them high-fat, high-carbohydrate (HFHC) diet. The composition of HFHC included 5% cheese, 10% egg yolk, 15% beef fat, 5% oil, 45% rice, and 20% standard feed (Ardiansyah et al., 2018). HFHC was given for 14 days ad libitum.

The dose of red dragon fruit was given by using therapeutic doses that have been tested on humans, namely 200 ml of fruit juice obtained from 100 grams of fruit (Nisa et al., 2019). The maximum volume of fluid administration for rats weighing 200 grams is 5 ml. The dose given referred to the dose in previous studies, namely 7.2 grams of dragon fruit which was reduced to 7 grams to see the effectiveness of giving dragon fruit at lower doses. The first dose was calculated by 0.5 x 7grams = 3.5 grams/200 grams. The second dose was 1 x 7 grams, and the third dose was 1.5 x 7 grams = 10.5 grams.

![Samples Grouping and Intervention Flow](Picture 1)
The overweight status in each rat was measured by using Lee index, which compares between the rat’s height and its weight (Lee et al, 2011). Rats were determined overweight if their Lee index value was > 300. Rat weight was measured with a scale, while their height was measured with a ruler.

Malondyaldehyde (MDA) levels were measured by using spectrophotometry with 532 nm wavelength. Blood samples used were plasma blood taken 2 times after overweight modeling on the 15th day post-administration of red dragon fruit juice. MDA levels were checked using the TBARS reagent kit method. Take 3 cc of venous blood and put it into a centrifuge tube that has been given EDTA. And than blood samples were centrifuged at 3000 rpm for 30 minutes, 200 L supernatant was put into an empty centrifuge tube. Add 2 ml of 15% TCA solution. Add 0.37% TBA solution in 0.25 N . HCL. Heat in a water bath at 80°C for 60 minutes. Centrifuge for 15 minutes at 3000 rpm. Take the supernatant and put it in the cuvette. Read the absorbance of the supernatant with a spectrophotometer at a wavelength of 532 nm with blanks in the form of TCA and TBA. MDA levels are obtained using the

$$\text{MDA (μmol/L) = } \frac{0.2422 + \text{absorbansi}}{0.0241}$$

The datas were analyzed with SPSS Version 16.0 and represented by the mean and standard deviation values. Before analyzed and first tested in terms of normality and homogeneity. To determine the effect of dragon fruit juice, it was analyzed with one-way ANOVA. The test results were considered statistically significant if the p value was less than 0.05.

This research has been approved by the Health Research Ethics Commission, Faculty of Medicine, Universitas Sebelas Maret Surakarta (KEPK UNS) No. 23/UN27.06.6.1/KEP/EC/2021.

RESULTS AND DISCUSSION

Table 1 shows the general difference in the average body weight of rats in all groups before and after HFHC induction. The results showed that there was no significant difference in weight at the beginning of the study (p=0.785). It indicated that the initial conditions of the rats were similar, before the treatment was given. The difference in the mean body weight of rats in all treatment groups induced by HFHC feed both on the 7th and 14th days showed a significant difference when compared to the normal rats group (p<0.001). The highest mean weight gain occurred in group P1 (213.6 ± 4.62) and the lowest was in group P3 (210.0 ± 5.70). The results of the average body weight of rats are shown in Table 1.

The results were in line with the of research by Wong (2018) which stated that administration of HFHC diet for 6 weeks could increase the body weight of obese rats. Another study on Sprague-Dawley rats showed that feeding a high-fat diet as much as 61% for 8 weeks could lead to obesity and increase visceral fat mass (Udomkasemsab and Prangthip, 2019). The presence of excess free fatty acids in adipose tissue increases the intracellular accumulation of acetyl-co-enzyme-A including diglycerides, triglycerides, monoglycerides, and ceramides which causes dephosphorylation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight (g) Δ (+)</th>
<th>Δ (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-test (Average ± SD)</td>
<td>Post-test (Average ± SD)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>183.2 ± 3.70</td>
<td>193.0 ± 2.92</td>
<td>376.2</td>
</tr>
<tr>
<td>KN</td>
<td>184.2 ± 3.77</td>
<td>210.6 ± 3.36</td>
<td>394.8</td>
</tr>
<tr>
<td>KP</td>
<td>186.4 ± 3.78</td>
<td>212.4 ± 4.45</td>
<td>398.8</td>
</tr>
<tr>
<td>P1</td>
<td>187.0 ± 3.54</td>
<td>213.6 ± 4.62</td>
<td>400.6</td>
</tr>
<tr>
<td>P2</td>
<td>184.8 ± 6.22</td>
<td>210.4 ± 7.23</td>
<td>395.2</td>
</tr>
<tr>
<td>P3</td>
<td>184.2 ± 6.10</td>
<td>210.0 ± 5.70</td>
<td>394.2</td>
</tr>
<tr>
<td>P</td>
<td>0.785b</td>
<td>&lt;0.001b</td>
<td></td>
</tr>
</tbody>
</table>

Source: Primary Data (2021). Data are presented in Average± SD.

a) Paired t Test. b) one-way Anova was significant if the p-value was less than 0.05
of protein kinase B/Akt. This process would trigger glucose transport to the tissues by glucose transporter-4 (GLUT-4). The occurrence of protein kinase B/Akt (PKB/Akt) dephosphorylation can inhibit the process of lipolysis and gluconeogenesis, causing an increase in body mass (Nissa and Madjid, 2016). Foods high in carbohydrates, animal protein, and fat can contribute to increased oxidative stress and the emergence of inflammation through the formation of white adipose tissue that secretes proinflammatory factors (Tan et al., 2018; Tan and Norhaizan, 2019). A marker of excess fat accumulation in ectopic tissue is an increase in intra-abdominal visceral fat, which is associated with abdominal obesity (Tchernof et al., 2013).

Furthermore, excessive intake of fat and carbohydrates can change oxygen metabolism which caused oxidation reactions. Excessive intake will cause enlargement of adipose tissue through adipocyte hypertrophy and hyperplasia. The imbalance between ROS and antioxidants causes an increase in oxidative stress, causing systemic inflammation. High ROS in the body will oxidize lipids and go through three phases including initiation, propagation, and termination. The initiation phase is marked by the removal of hydrogen atom from the lipid carbon which will then form lipid radicals. Propagation phase is marked by the rapid reaction of lipid radicals with oxygen to form lipid peroxyl radicals by taking hydrogen from other lipid molecules to produce new lipid radicals and form a chain reaction. Meanwhile, in the termination phase, the hydrogen atoms that have been lost will form lipid peroxidation (Thadeus et al., 2018).

Malondialdehyde MDA levels are the result of lipid peroxidation which is a marker of oxidative stress in the body (Susantiningsih, 2015). Lipid peroxidation is processed by withdrawing one electron from the PUFA double bond for the formation of radicals. Carbon lipid radicals will adapt themselves by rearranging the intra-molecular environment so that they can react quickly to form lipid peroxyl radicals. The presence of lipid peroxyl radicals will attract hydrogen atoms from other PUFA double bonds to form other lipid radicals. The reaction between lipid peroxyl radicals and hydrogen atoms will form lipid peroxidation (Thadeus et al., 2018).

Effect of Dragon Fruit Juice on Plasma ROS (Reactive Oxygen Species)

The results of one-way ANOVA showed that there were a difference in the ROS among the groups with red dragon fruit juice treatment (p<0.001). The results of this study were in line with a research by Ma’arif et al (2020) which stated that administration of 200 g of red dragon fruit for 14 days could reduce ROS levels by 0.645 nmol/ml in smokers. The intervention of red dragon fruit juice for 14 days at three doses decreased the ROS plasma in all intervention groups. The highest decrease was found in the group of rats given dragon fruit juice at a dose of 10.5 g/200 gBW (51.07%). This study were in line with Zahra et al (2019), which showed that the administration of red dragon fruit juice to hypercholesterolemic rats could significantly reduce MDA levels.

Table 2 shows that there was a decrease in MDA levels in all intervention groups. The most significant decrease in MDA was found in the KP group. The results showed that the use of Orlistat in overweight rats after 6 months could significantly reduce body weight, total cholesterol, LDL, and triglyceride levels, due to the reduction of the absorption of dietary fat. Orlistat inactivates gastrointestinal lipase. The lower the activity of enzymes produced by the intestine would result in, the less the reduction of the fat absorbed from the body. The weight loss was caused by Orlistat inhibiting the lipase enzyme which rendered the fat non-absorbable by the body and caused them to be excreted through feces. However, in addition to providing a good reduction effect, Orlistat has a high rate of side effects in the gastrointestinal tract (Ioannides-Demos et al., 2011).

Weight loss is associated with a reduced levels of ROS. Red dragon fruit contains higher vitamin C white dragon fruit. In addition, the fiber content of red dragon fruit (3.2g/100g) also greater than white dragon fruit (1.1g/100g) (Choo and Yong, 2011; Mahattanatawee et al., 2006). The presence of this nutrient can induce the hydrolyzation of bile salts and bind the bile salts. The increased of bile salts can cause the increased of cholesterol excretio. Water-soluble dietary fiber is more easily fermented by intestinal bacteria to produce short chain fatty acids (SCFA) (Hapsari
and Kusumastuti, 2014; Pareira, 2010). SCFA has an effect on lowering cholesterol by inhibiting the HMG-CoA reductase enzyme.

The antioxidant activity of red dragon fruit is $134.1 \pm 30.1$ g GA/g. The phenol content is $24.22 \pm 0.95$ mg GA/100g. Phenol compounds play a role in countering free radicals and radil peroxide which are effective in inhibiting lipid oxidation (Susanti and Panunggal, 2015).

In addition, antioxidant compounds in the form of flavonoids in red dragon fruit play a role in preventing cell damage due to oxidative stress by donating hydrogen ions and thereby reducing the toxic effects of free radicals. The content of antioxidants such as flavonoids, phenols, vitamin C, and betacyanin in dragon fruit plays a role in warding off free radicals and lowering cholesterol levels. The way flavonoids work in inhibiting radicals is by donating hydrogen ions to stabilize free radicals and indirectly stimulate the expression of antioxidant genes and can increase bile secretion which can reduce cholesterol levels in the body (Thadeus et al., 2018). This is in line with research by Karimah et al. (2014) showing that giving red dragon fruit juice can reduce cholesterol levels in hypercholesterolemic rats. In addition, research conducted by Titrlolobi et al. (2020) showed that giving red dragon fruit juice could significantly reduce blood sugar levels in people with diabetes mellitus.

## CONCLUSION

The administration of red dragon fruit juice can reduce the ROS levels compare. The highest decrease was found at a doses $10.5$ g/200 gBW. Red dragon fruit juice can be used as an alternative beverage that contains a source of antioxidants to tackle oxidative stress in people with overweight conditions. Further research on the antioxidant content of fruit juices and whole fruit is needed to see the difference in antioxidant activity by the different food processing. In addition, further research on the bioactive content in dragon fruit that plays a role in controlling and reducing oxidative stress.

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