THE EFFECT OF UNRIPE BERLIN BANANA FLOUR ON SUPEROXIDE DISMUTASE (SOD) IN DYSLIPIDEMIC RATS

(Pengaruh Tepung Pisang Berlin Mentah terhadap Kadar Superoxide Dismutase (SOD) Pada Tikus Dislipidemia)

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ABSTRACT

Dyslipidemia is the occurrence of conditions that have abnormal lipid profile levels in the blood. Consumption of food sources of flavonoids and resistant starch is expected to play a role in dyslipidemia. Unripe Berlin banana flour (UBF) is source of flavonoids and resistant starches that benefit in dyslipidemia. This study aimed to determine the effect of giving UBF on SOD levels in dyslipidemic rats. This research used true experimental method with Pretest-Posttest and Control Group Design. This study used 30 rats divided into 5 groups K-, K +, P1 (UBF 0.144g /rat/day), P2 (UBF 0.288g /rat/ day), and P3 (UBF 0.576g/rat/day). The data were analyzed with one-way Anova and paired T-test. The results of the study showed that the values of SOD levels pre and post-intervention were not significantly different between groups. The mean of SOD levels pre-intervention showed that the K+, P1 and P3 groups were lower than K- group, then post-intervention at P1, P2 and P3 had higher SOD levels than K+ group. Meanwhile, the SOD levels of each group pre and post-UBF intervention in the P1 group were significantly different ($p < 0.05$), while in the P2 and P3 groups were not significantly different. This study concluded that giving raw Berlin banana flour at a dose of 0.144 g / day could increase the SOD levels in dyslipidemic rats.

Keywords: *Dyslipidemia, superoxide dismutase (SOD), Unripe Berlin Banana Flour*

INTRODUCTION

Dyslipidemia is one of the causes of cardiovascular disease (Stahel et al., 2018). The prevalence of dyslipidemia in Indonesia's population over 15 years is 7.6% with high cholesterol levels, 24.3% with low High Density Lipoprotein (HDL) levels, 9% with high Low Density Lipoprotein (LDL) levels, and 3.4 % with very high LDL levels (Kemenkes, 2018). The condition of dyslipidemia is also related to oxidative stress, which is a fundamental process that contributes to cardiovascular pathogenesis (Rivera-Mancía et al., 2018).

A high-fat diet can cause dyslipidemia (Susetyowati et al., 2019). High fat intake can directly increase cholesterol, LDL, and triglyceride levels, while reducing HDL levels. This can indirectly worsen the lipid profile through insulin resistance (Maulana & Ridwan, 2021). A high-fat diet can lead to a decreased effect on the activity of antioxidant enzymes such as superoxide dismutase-1 (SOD1), as observed in the liver, heart, and plasma of rat (Bai et al., 2015). SOD, as an endogenous antioxidant, can combat free radicals as the first line of defense (Yin. et al., 2018).

Nutrients from food have the potential to naturally change the biological function of cells by increasing endogenous antioxidant mechanisms or altering the signaling status of oxidation reactions in cells. This is often associated with the composition of various antioxidant compounds found in different food ingredients. Antioxidants are beneficial in pathological conditions as they play a crucial role in combating oxidative stress (Csonka et al., 2016).

Unripe banana flour is a source of flavonoids and resistant starch that play a positive role in dyslipidemia (Agustin et al., 2019). The flavonoid content in unripe Berlin banana flour is 241 mg/100 g, and the resistant starch content is 40.01% (Febriyatna et al., 2019). In addition to

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their capacity as antioxidants, flavonoids also play a role in improving lipid profiles and have anti-inflammatory, antiplatelet, and antithrombotic effects (Csonka et al., 2016). Foods containing resistant starch can reduce fat accumulation, improve lipid profiles, and increase antioxidant enzyme activity (Zhang et al., 2015) (Chen et al., 2023). The aim of this study was to determine the effect of unripe Berlin banana flour on SOD levels in dyslipidemic rats.

METHODS

This research was conducted from August to November 2019 at the Biomedical Laboratory of the Faculty of Dentistry and the Biosciences Laboratory of the Dental and Oral Hospital, Jember University. The research was a true experimental study with a pre- and post-test design with a control group. The research used 30 male Wistar rats aged 2-3 months with a body weight of 150-200 grams. The minimum sample size was determined based on the Federer (1963) formula with an additional estimated drop out rate of 10% of the minimum sample size. The rats were divided into the negative control group (K-), positive control group (K+), P1 group with a dose of unripe Berlin banana flour (UBF) 0.144 g/day, P2 group with a dose of UBF 0.288 g/day, and P3 group with a dose of UBF 0.576 g/day. Dose of 0.144 g/day, based on research results from Agustin et al. (2019), showed that this dose can improve the lipid profile in dyslipidemic rats.

Rats were adapted for 7 days (Obernier & Baldwin, 2006). However, it was found that the rats' body weight did not meet the inclusion criteria, so an additional 13 days were required to reach the appropriate body weight. The adaptation period involved treating the rat with Rat Bio feed at a dosage of 30 g/rat/day. Additionally, the rat in the K- group continued to receive standard food, while the K+, P1, P2, and P3 groups were fed a high-fat diet (HFD) of 30 g/day for 8 weeks. The HFD was a mixture of standard Rat Bio brand feed, beef tallow, margarine, and coconut milk. Subsequently, blood samples were collected from the rat to analyze their lipid profile (total cholesterol, LDL, and HDL) using the CHOD-PAP method, as well as superoxide dismutase (SOD) as pretest data or before the intervention was administered.

After the rats were induced by HFD, rats in groups P1, P2, and P3 were intervened with the addition of UBF in the form of feed according to the dose for each group for 14 days. The intervention feed was prepared by mixing standard feed with UBF according to the dose, i.e., P1 0.144 g P2 0.288 g, and P3 0.576 g. Each rat was given 30 g/rat/day. Blood was collected from the rats to analyze lipid profiles as posttest data (cholesterol, LDL, and HDL), as well as for SOD analysis.

Data were analyzed with SPSS. Tests for differences between groups were performed using the One-Way ANOVA test. Meanwhile, the pre- and post-tests for each group utilized a paired t-test.

RESULT AND DISCUSSION

Lipid profile after HFD for 8 weeks (Table 1). Total cholesterol, LDL, and HDL showed no significant difference between groups.

High fat diet showed no significant effect on increasing cholesterol and LDL levels, and decreasing HDL. However, the total cholesterol and LDL levels in the K- group were lower than those in the K+ and P groups.

The high fat diet used fat sources from beef tallow, margarine, coconut milk which are high in saturated fat. The saturated fatty acid content of beef tallow is greater than that of pork and chicken tallow (Hermanto et al., 2008).

Saturated fatty acids can increase total cholesterol, LDL and increase HDL (Papotti et al, 2021). Margarine is an emulsion of vegetable oil and water containing at least 80% fat (Ulfa et al., 2017). The coconut milk used in this study is coconut milk powder with a fat content of 37% (Ariningsih et al., 2021). Coconut milk is also a source of saturated fat, but in the form of medium-

Table 1. Lipid Profile Analysis.

Groups	Total Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
K-	85.3 ± 16.78	68.7 ± 18.23	35.1 ± 3.60
K+	108.5 ± 17.56	100.6 ± 33.44	46.5 ± 6.30
P1	93.8 ± 10.66	104.1 ± 13.88	40.7 ± 5.50
P2	89.7 ± 23.95	80.7 ± 29.47	38.7 ± 5.40
P3	96.1 ± 28.00	80.4 ± 19.59	38.7 ± 5.10

Note : (*) significant difference ($p<0.05$)

chain saturated fatty acids (MCFAs) (Raghavendra & Raghavarao, 2010). The increase in HDL levels in the study after feeding high-fat diets may be due to the effect of the medium-chain fatty acids (MCFA) contained in high-fat diets, which can increase HDL levels. MCFAs increase mRNA expression of ATP-binding cassette transporter A1 (ABCA1) in the liver, thereby increasing HDL particle biogenesis (Panth et al., 2018).

SOD Analysis

The results of the superoxide dismutase (SOD) analysis can be seen in Table 2, showing that the SOD levels between groups at pre- and post-intervention did not show a significant difference. The p-values for pre-intervention were p = 0.376 and for post-intervention were p = 0.476. The mean pre-intervention SOD levels in the K+, P1, and P3 groups were lower compared to the K- group. The mean SOD levels in the group of dyslipidemic rats that received UBF intervention (P1, P2, P3) were higher compared to the K+ group.

The SOD results for each pre-intervention and post-intervention group showed a significant difference in the P1 group, but not in the K-, K+, P2, and P3 groups. However, based on the statistical analysis of changes in SOD or delta levels in Table 2, it was found that there was no significant difference between groups.

Pre-intervention data were obtained after the rats were given a high-fat diet in the K+ and P groups, while the K- group was given standard food. SOD levels in the group given high-fat diet were lower than those in the K- group (Table 2). High fat diets contain saturated fats which affect mitochondrial metabolism and increase the accumulation of reactive oxygen species (ROS) (Leamy, A. K., Egnatchik, R. A., & Young, 2013).

SOD is the only enzyme that plays a role in preventing potential toxicity by controlling the level of presence of ROS molecules and reactive nitrogen species (RNS), as well as regulating signaling in cells (Yin. et al., 2018). Decreased SOD levels make cells susceptible to diseases associated with oxidative stress (Lobo et al., 2010). The body's most important form of compensation when there are high levels of free radicals in the body is by producing antioxidants. The body's first line of antioxidants to be produced are SOD, catalase, and glutathione peroxidase (GSH). However, hyperlipidemia can cause an increase in ROS levels, which makes it difficult for the body to compensate, resulting in a decrease in antioxidant activity, one of which is SOD (Ighodaro & Akinloye, 2018).

SOD levels increased significantly after intervention in the treatment group receiving a dose of UBF 0.144 g (Table 2). In contrast, the other groups did not show significant changes in SOD levels. The administration of UBF can improve the lipid profile in dyslipidemic rats, possibly due to the effects of resistant starch and flavonoid content in unripe Berlin banana flour (Agustin et al., 2019). The administration of flavonoid-rich fractions along with a high-fat diet led to a significant increase in SOD enzyme activity, indicating that flavonoid compounds protect tissues from lipid peroxidation through their antioxidant abilities, thereby reducing lipid peroxidation (Kaviarasan et al., 2008). This increase in SOD levels may also be attributed to the role of SOD as a first-line enzyme in cellular defense against oxidative injury, as they can be rapidly induced to decompose O_2 and H_2O_2 to prevent the formation of more reactive hydroxyl radicals (Xie et al., 2022).

In contrast to the group given UBF at 0.288g/rat/day (P2) and 0.576g/rat/day (P3), which did not have a significant effect on increasing SOD levels before and after the intervention. This could be caused by the higher dose of UBF, resulting in a higher resistant starch content in the feed. The meta-analysis results show that resistant starch (RS) has no significant effect on SOD levels (Wei et al., 2022).

Table 2. Superoxide Dismutase (SOD) Analysis

Groups	SOD Levels (mean±SD)			p-*value**
	Pre	*Post*	Delta (Δ)	
K-	0.77±0,18	0.92±0.18	0.10 ± 2.42	0.125
K+	0.59±0,10	0.76±0.19	0.27 ± 1.53	0.463
P1	0.68±0,05	1.05±0.24	2.80 ± 1.62	0.028*
P2	0.84±0,21	0.89±0.22	-0.28 ± 4.47	0.796
P3	0.65±0,34	0.77±0.15	3.60 ± 0.68	0.507
*p-value***	0.376	0.476	0.920	

Note: (*) Significant difference on pre and post intervention at paired t-test (p<0.05) (**) Significant difference at ANOVA test (p<0.05)

Flavonoids can inhibit the α-amylase enzyme when they interact with patients. Flavonoids can interact not only with α-amylase but also with other components in the food consumed, such as proteins, polysaccharides and lipids that form complexes with these compounds. Considering these interactions, it is thought to be difficult to maintain the high concentrations of free flavonoids required to inhibit α-amylase activity in the intestine (Takahama & Hirota 2018). In addition, the higher the carbohydrate component, especially fructose, which is contained in raw Berlin bananas, it can reduce SOD levels (Phillips et al., 2021) (Nagalievska et al. 2022).

CONCLUSION AND SUGGESTION

Unripe berlin banana flour dose 0,144 g/day on dyslipidemia rats can increase SOD levels Further analysis is required to determine the content of nutrients and bioactive compounds at a dose of 0,144 g/day of unripe banana flour.

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