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Effect of Red Dragon Fruit Extract (*Hylocereus polyrhizus*) on Total Cholesterol Levels in Wistar Rats Model of Metabolic Syndrome

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ABSTRACT

Introduction: The consumption of high fat and sugar can increase the risk of metabolic syndrome. *Hylocereus polyrhizus* is known to be able to lower total cholesterol levels in the blood. This study aims to determine the effect of *Hylocereus polyrhizus* extract on total cholesterol levels in Wistar rats with the metabolic syndrome model.
Methods: This study used laboratory experimental research using a pretest and posttest with a control group design. The sample consisted of 30 Wistar rats according to the inclusion criteria which were divided into 5 groups: K1 (positive control), K2 (negative control), K3, K4, and K5 (treatment group). The treatment group was given *Hylocereus polyrhizus* extract with doses of 60, 120, and 180 mg/200grW. Total cholesterol levels were measured on the 8th, 36th, and 64th day. The data were analyzed using the parameterized One-way ANOVA test followed by Tukey's HSD posthoc test, paired T-test, and Pearson's correlation test.
Results: The average total cholesterol after being given by *Hylocereus polyrhizus* extract in K3 (137 ± 2.82 mg/dL), K4 (109 ± 2.31 mg/dL), and K5 (100 ± 2.38 mg/dL) was lower than the negative control group. Data analysis showed significant differences ($p<0.05$) in total cholesterol levels before and after treatment in all groups. The Pearson correlation test explained that the variation dosage of *Hylocereus polyrhizus* extract had a very strong correlation with a positive correlation with total cholesterol levels.
Conclusion: Induction of *Hylocereus polyrhizus* extract at doses of 60, 120, and 180 mg/200grW was able to significantly reduce total cholesterol levels.

Keywords: metabolic syndrome; total cholesterol; red dragon fruit

INTRODUCTION

High-fat and fructose diets are becoming popular in the globalization era. Consumption of these types of food is slowly increasing the risk of metabolic syndrome. Metabolic syndrome is a pathological condition caused by several risk factors such as central obesity, hypertension, microalbuminuria, hyperglycemia, and dyslipidemia[1]. The prevalence of metabolic syndrome in the world currently reaches 20-25%. The incidence is predicted to continue to increase until 2035 and will reach until 53%[2]. Metabolic syndrome occurs due to insulin resistance which can lead to dyslipidemia.

Currently, natural herbs are more widely chosen than conventional medicine due to their lack of side effects. Red dragon fruit (*Hylocereus polyrhizus*) contains antioxidant compounds including phenolic, anthocyanin, tocotrienol, ascorbic acid, and polyunsaturated fatty acid (PUFA)[3]. Flavonoids and tocotrienols can suppress the enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase,

while acyl-CoA cholesterol acyl transferase (ACAT) enzyme which plays a role in the formation and storage of cholesterol in the body [4-6]. Ascorbic acid and PUFA can inhibit lipid accumulation. The food fiber in this fruit facilitates the excretion of cholesterol through feces[7].

Previous study reported that red dragon fruit is beneficial to modulate obesity-related genes in metabolic syndrome rats model. Red dragon fruit can modulate balance homeostasis by modulating the anorectic, orexigenic and energy expenditure related genes[8]. In another study, red dragon fruit extract can lower total cholesterol in hyperlipidemia Wistar rats by its active compound such as anthocyanin[9]. However, the effect of fruit extracts on total cholesterol in metabolic syndrome rats has not been widely reported. This study aims to find out about the effect of red dragon fruit extract and determine the optimal dose of the extract itself to decrease total cholesterol in Wistar rats (*Rattus norvegicus)* induced metabolic syndrome.

METHOD

This study used laboratory experimental methods within pretest and posttest with a control group design. The research was conducted at the Laboratory of the Center for Food and Nutrition Studies of Gadjah Mada University Yogyakarta. The sample consisted of 30 Wistar rats which are healthy, male, 150-200 grams, and 2-3 months old. Meanwhile, rats that were sick, dead, and had used in previous studies will be excluded.

The sample selection used simple random sampling, while the sample size was calculated using the Federer formula. The number of samples of each group contained 5 rats plus 1 as a backup. The calculated sample size was divided into 5 groups K1 (positive control), K2 (negative control), and K5 (treatment). The K2-K5 group was fed a high-fat high-fructose diet which contained 2% cholesterol, 2 ml coconut oil, and 1% cholic acid in 200 ml of 0.5% CMC-Na on days 8-36. Then samples were induced by streptozotocin-nicotinamide (STZ-NA) on day 29 to achieve the condition of metabolic syndrome. *Hylocereus polyrhizus* was extracted using a maceration technique with 70% ethanol. *Hylocereus polyrhizus* extract with doses of 60, 120, and 180 mg/200gBB were sequentially given to K3-K5 on days 37-63. Total cholesterol level was measured 3 times on day 8 (before treatment), day 36 (after HFFD and STZ-NA induction), and day 64 (after giving *Hylocereus polyrhizus* extract).

Data were analyzed using SPSS 25.00 for Windows. The analysis began with the Saphiro-Wilk normality test and Levene's homogeneity test. Normally distributed and homogeneous data will be analyzed by One-way ANOVA parametric test with Tukey Honest Significant Differences (HSD) post-hoc test. Analysis of the linear correlation between total cholesterol levels and various doses of *Hylocereus polyrhizus* extract will be calculated by Pearson correlation test. The entire series of research conducted on Wistar rats both before and after the study has been approved and received a Certificate of Ethical Clearance No. 490/III/HREC/2023 by the Health Research Ethics Commission of Dr. Moewardi Surakarta Hospital.

RESULT

Metabolic Syndrome

Metabolic syndrome is achieved if at least 3 out of 5 criteria are met[9]. These criteria include hypertension, central obesity, fasting blood glucose levels >110 mg/dL, HDL <40 mg/dL, and increased triglyceride levels >150 mg/dL. Lee index was used to determine obesity in rats. In this study, rats reached the metabolic syndrome condition after being induced with HFFD and STZ-NA. Table 1 described the measurement of weight, lee index, fasting blood glucose and HDL as a parameter for metabolic syndrome

Table 1. Mean of Weight, Lee index, Fasting Blood Glucose, and HDL at Day-8 and Day-36

Group	Weight (gram) + standard deviation		Lee Index	Fasting blood glucose levels (mg/dL) + standard deviation		HDL levels (mg/dL) + standard deviation	
	Day-8	Day-36		Day-8	Day-36	Day-8	Day-36
K1	181 ± 2.80	212 ± 2.64	284 ± 3.43	67 ± 1.34	70 ± 1.87	86 ± 1.44	84 ± 1.47
K2	181 ± 2.58	234 ± 2.80	324 ± 6.59	66 ± 1.48	264 ± 4.34	86 ± 2.43	29 ± 1.27
K3	180 ± 3.33	233 ± 3.66	324 ± 2.26	66 ± 1.89	263 ± 5.75	87 ± 1.96	31 ± 1.17
K4	177 ± 4.17	231 ± 4.27	322 ± 3.61	68 ± 1.26	263 ± 3.35	86 ± 2.07	31 ± 2.06
K5	180 ± 2.43	233 ± 2.48	323 ± 2.99	68 ± 1.43	262 ± 4.12	87 ± 2.51	33 ±1.58

K1: positive control, K2: negative control, K3: treated group with 60 mg red dragon fruit extract, K4: treated group with 120 mg red dragon fruit extract, K5: treated group with 180 mg red dragon fruit extract, HDL: high density lipoprotein

Data on body weight and length will be used to calculate the Lee index as an indicator of obesity. *Rats* are classified as obese if the Lee index value is >300. The results showed that the positive control group (K1) with a standard diet of BR-2 pellets, while the negative control group (K2) and treatment (K3- K5) were classified as obese because the Lee index value was >300. The fasting blood sugar data of Wistar rats also showed a significant increase until >110 mg/dL both in the negative control (K2) and treatment groups (K3-K5). In addition, rats also experienced a significant decrease in HDL levels until <40 mg/dL in the negative control (K2) and treatment (K3-K5) groups. Thus, Wistar rats have achieved a model-like metabolic syndrome by covering 3 out of 5 criteria.

Total Cholesterol Level Analysis Data

Total cholesterol levels increased at day 36 after being given STZ-NA and HFFD. Meanwhile, cholesterol levels measured at day 64 decreased significantly after being given *Hylocereus polyrhizus* extract in the treatment group with consecutive doses of K3 60 mg/200, K4 120 mg/200gBB, K5 180 mg/200gBB. The data is described in Table 2 as follows.

Table 2. Mean of total cholesterol at day-8, day-36, and day-64

Group	Number of samples	Cholesterol total level (mg/dL)		
		Day-8	Day-32	Day-64
K1	6	88 ± 2.09	90 ± 2.29	90 ± 4.46
K2	6	87 ± 2.60	164 ± 4.33	166 ± 4.01
K3	6	87 ± 2.34	162 ± 3.28	137 ± 2.82
K4	6	88 ± 2.33	161 ± 3.72	109 ± 2.31
K5	6	88 ± 2.68	163 ± 2.73	100 ± 2.38

K1: positive control, K2: negative control, K3: treated group with 60 mg red dragon fruit extract, K4: treated group with 120 mg red dragon fruit extract, K5: treated group with 180 mg red dragon fruit extract

The data pretest was analyzed with the Saphiro-Wilk normality test and Levene's homogeneity test. As a result, all group data was normally distributed and homogeneous. Normally distributed and homogeneous were analyzed by *One-way* ANOVA parametric test. The results of the analysis were $p=0.000$ or $p<0.05$, indicating that there was a significant difference from each group. The analysis continued with the Tukey Honest Significant Differences (HSD) post hoc test. The results showed that there was a significant difference between K1 and other groups, while the results of K2-K5

showed no significant difference.

Data posttest was analyzed with the Saphiro-Wilk normality test and Levene's homogeneity test. As a result, all group data was normally distributed and homogeneous. Normally distributed and homogeneous data are analyzed by *a One-way* ANOVA parametric test. The results of the analysis were p=0.000 or p<0.05, indicating that there were significant differences from each group. The analysis continued with the Tukey *Honest Significant Differences* (HSD) *post hoc* test. The results showed that there were significant differences between pairs of all groups.

Both data were analyzed using a paired T-test. The results showed a value of p<0.05, which means that there is a significant gap between total cholesterol levels both before and after being given *Hylocereus polyrhizus* extract. The correlation between the variation of the extract dose and total cholesterol level was analyzed by the Pearson correlation test. The results showed a value of -0.944, which means there is a very strong correlation between the 2 variables that the higher the dose of *Hylocereus polyrhizus* extract consumed, the lower the total cholesterol level that will be achieved.

Table 3 Pretest and Posttest Data Analysis Result

Group	Value (p)								
	Normality Test		Homogenity test		One-way ANOVA Test		Post Hoc Tukey HSD Test		Paired T Test
	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest	
K1	0,981*	0,964*	0,229*	0,229*	0,000**	0,000**	K2(0,00)** K3(0,00)** K4(0,00)** K5(0,00)**	K2(0,00)** K3(0,00)** K4(0,00)** K5(0,00)**	0,503**
K2	0,504*	0,936*					K3(0,924) K4(0,818) K5(0,995)	K3(0,00)** K4(0,00)** K5(0,00)**	0,000**
K3	0,568*	0,980*					K4(0,999) K5(0,993)	K4(0,00)** K5(0,00)**	0,000**
K4	0,641*	0,979*					K5(0,958)	K5(0,002)**	0,000**
K5	0,679*	0,920*					-	-	0,000**

Note: N=6, *p>0,005=significant dan **p<0,005=significant

DISCUSSION

Increase in Total Cholesterol Level after HFFD and STZ-NA Induction

Based on the results of data analysis before and after being given by STZ-NA and HFFD, K1 has a significant difference compared to K2-K5. K1 data showed a less significant increase of 1% while K2-K5 experienced a high average increase of 86%. This is in line with the research of Yustisia *et al.* (2022) that HFFD can induce dyslipidemia, one of which is by increasing total cholesterol levels. In the previous study, HFFD and STZ-NA could increase the total cholesterol levels of rats in the control group reached 140 mg/dL, while the HFFD treatment group reached 181 mg/dL[11].

Consumption of a high-fat diet in experimental animals triggers oxidative stress of vascular endothelial cells. This will increase reactive oxygen species (ROS) which results in dysregulation of lipid metabolism. This study is in line with De Castro *et al.* (2013) who examined the effect of high fructose and fat diets on stimulating de novo lipogenesis which causes regulation of lipogenic enzymes to catalyze esterification, synthesis, and accumulation of cholesterol in hepatocytes, resulting in an increase in the lipid profile of experimental animals after being given HFFD[12]. A high-fat diet also affects the activation of inflammatory cytokines that trigger oxidative stress, resulting in dysregulation of lipids that are broken down into free cholesterol in the plasma[13]. STZ-NA triggers hyperlipidemia by damaging the normal function of pancreatic beta cells, resulting in insulin resistance[14]. Insulin resistance

will increase free fatty acids by triggering an increase in sensitive hormones in fat tissue, lipolysis of triglycerides, and the formation of free fatty acids in plasma. Free fatty acids will be converted into cholesterol. This is in line with the research of Fitri *et al* (2022) which states that STZ-NA induction will trigger insulin resistance and hyperlipidemia[15].

Effect of *Hylocereus polyrhizus* Extract on Total Cholesterol Levels

The results of the Tukey HSD post hoc test showed that K1 had a significant difference with K3-K5 after these groups were treated with *Hylocereus polyrhizus* extract. This explained that 3 different doses of *Hylocereus polyrhizus* extract at K3-K5 can reduce total cholesterol levels but cannot approach the levels of K1. The data also showed a significant difference between K2 and K3-K5, which explained the significant decrease in cholesterol in K3-K5 compared to K2. A comparison test between the pretest and posttest was calculated using a paired T-test. The results explained that *Hylocereus polyrhizus* extract doses of 60 mg/200gr, 120 mg/200gr, and 180 mg/200gr significantly reduced total cholesterol levels. Pearson correlation test showed that the correlation between the two variables was very strong.

The percentage of total cholesterol reduction after being given dragon fruit extract doses of 60 mg/200grW, 120 mg/200grW, and 180 mg/200grW was 16%, 32%, and 38%, respectively. This is in line with the results of research by Sigarlaki & Tjiptaningrum (2016) who gave dragon fruit extract doses of 30 and 60 mg/kg to hyperlipidemia model Wistar rats, able to reduce total cholesterol levels by 10% and 29% [9]. Betacyanin and flavonoids in *Hylocereus polyrhizus* work as anti-inflammatory by inhibiting inflammatory mediators[16]. These compounds can reduce TNF-α which plays a role in the oxidation process of FFA in the liver so that cholesterol synthesis is inhibited and insulin sensitivity increased[15]. Research conducted by Prakoso *et al.* (2017) showed similar results, that the decrease in total cholesterol levels in hyperlipidemia model Wistar rats was greater when using red dragon fruit than white dragon fruit with the same dose[7]. Its ascorbic acid is 33 mg/100g, which can bind bile salts so that cholesterol excretion also increases. Its food fiber is 3.2 g/100g *Hylocereus polyrhizus* plays a role in inhibiting HMG Co-A reductase as a catalytic enzyme for cholesterol synthesis[17]. The phenolic acts as an antioxidant that inhibits beta-oxidation, thus preventing the increase of FFA in plasma. Recent research by Maharani and Saktiningsih, (2022), also explained that red dragon fruit juice can reduce total cholesterol levels in women because of its tocotrienol content which suppresses the work of the HMG-Koa reductase enzyme as a catalyst for the formation of mevalonate in cholesterol synthesis[18]. The PUFA in *Hylocereus polyrhizus* can inhibit the synthesis of LDL and VLDL which is indirectly associated with a decrease in total cholesterol[19].

This research has been carried out optimally, but there are limitations. Researchers have not been able to accurately identify the percentage of active compound content in *Hylocereus polyrhizus* and determine the optimal dose of *Hylocereus polyrhizus* extract to reduce total cholesterol due to the limited variation in the dose determined, creating a central obesity model rat, so that one of the criteria for metabolic syndrome is replaced by obesity calculated by the Lee index, and can only show a decrease in total cholesterol in Wistar rats modeling metabolic syndrome, without explaining the exact reason for the decrease.

CONCLUSION

Based on the research that has been carried out, it can be concluded that the *Hylocereus polyrhizus*) extract with a dose variation of 60 mg/200grW, 120 mg/200grW, and 180 mg/200grW can significantly decrease total cholesterol levels. Dosage 180 mg/200grW is the most optimum dose to reduce total cholesterol levels. We suggest conducting research with a larger subject regarding the purification and isolation of active compounds in red dragon fruit to identify compounds that can reduce total cholesterol levels in metabolic syndrome.

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CONFLICT OF INTEREST

The author has no conflict of interest.

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