Basil Leaves *(Ocimum sanctum linn.)* Extract Decreases Total Cholesterol Levels in Hypercholesterolemia Sprague Dawley Rats Model

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Basil Leaves *(Ocimum sanctum linn.)* Extract Decreases Total Cholesterol Levels in Hypercholesterolemia Sprague Dawley Rats Model

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Abstract. Hypercholesterolemia is closely related to the severity of atherosclerosis which is one of the factors in the occurrence of cardiovascular and cardiovascular diseases. Cardiovascular disease is one third of all cases of death worldwide including in Indonesia. The death rate caused by cardiovascular disease is expected to continue to increase until 2030. One of the natural foods that has the potential to reduce cholesterol is the basil leaf *(Ocimum sanctum linn.)*. The content of polyphenol compounds such as flavonoids and tannins in basil leaves can reduce total cholesterol levels and inhibit fat oxidation which is the cause of atherosclerosis. This study aims to analyse the effect of the basil leaves extract on cholesterol levels in Hypercholesterolemia Sprague Dawley rats. Thirty six Sprague Dawley rats aged 8 weeks, weighing 150-200 g, were divided into 6 groups, namely P1) Normal rats, not treated (negative control), P2) Rats induced with *High Fat Diet* (HFD) without treatment, P3) Rats induced with HFD and intervened using statin medication of 0.18 mg/kgBW (positive control), P4) Rats induced with HFD with the basil leaves extract dose of 20 mg/kgBW/day, P5) Mice induced with HFD with the basil leaves extract dose of 40 mg/kgBW/day, P6) Rats induced with HFD with the basil leaves extract dose of 80 mg/kgBW/ day. The treatment lasted for 7 days. Collected data were analized using the one way ANOVA test and LSD post hoc test. The data showed that the basil leaf extract at a dose of 20 mg/kgBW/day, 40 mg/kgBW/day, and 80 mg/kgBW/day can reduce cholesterol levels significantly ($P<0.005$). The effect of reducing cholesterol levels depends on the dose of the basil leaves extract (dose dependent), with that the highest dose has the same effect as the positive control group (KP). In conclusion, Basil leaf extract with a dose of 20 mg/kgBW/day, 40 mg/kgBW/day and 80 mg/kgBW/day for 7 days can reduce cholesterol levels.

Keywords: Basil leaf extract, cholesterol level, hypercholesterolemia.

1. Introduction

Hypercholesterolemia is a clinical condition that is characterized by increase of total cholesterol level ≥ 200 mg/dl and has a strong relationship with the severity of atherosclerosis. Hypercholesterolemic atherosclerosis on the arterial wall is the main factors of the occurrence of heart and cardiovascular diseases [1]. Globalization has a negative impact on nutritional transitions in common society of various countries in the world that shift food consumptions from high carbohydrate and fibres to high fat, salt and cholesterol. A recent study reported that increased cholesterol levels in adult are associated with higher risk of CVD *(Cardiovaskuler Disease),* compared with those in normal or low cholesterol levels. Although changes in cholesterol levels cannot be determined, we assume that such decreased cholesterol levels will be achieved by lifestyle intervention or using statin medication. Lifestyle management for decreasing cholesterol levels such as diet, increased physical activity, and body weight control has been well established [2,3].

For a long period, basil leaves (*Ocimum sanctum L*) have been recognized as a food additive but some evidence shows that these leaves can also be used to overcome some human diseases, due to their phytochemical contents, like antioxidants, polyphenols and flavonoids. Essential oil of O. *sanctum* is dominantly found in the leaves. One study stated that chemical analysis of essential oil derived from *O. sanctum* leaves is rich in monoterpenes, sesquiterpenes and phenylpropane derivatives [4].

Nutritional compounds of basil leaves that show anti-inflammatory and antioxidant effects have a beneficial effect in reduction of cholesterol levels and prevention of oxidative stress-related injury. In general, polyphenolic compounds, especially flavonoids, are ubiquitous in dietary components from plant-derived foods. From medical viewpoint, flavonoids have potentially been applied to decrease cholesterol level and oxidative stress [5]. Basil leaves which contain flavonoids and tannins can reduce cholesterol levels by increasing cholesterol metabolism into bile acids and cholesterol excretion through feces. Some research conducted stated that optimal dosage of basil leaves can reduce LDL and cholesterol levels [6]. Another researcher reported a supplementation with *O. sanctum* led to a significant decrease in homocysteine, total cholesterol [7].

Based on the description above, the purpose of this study was to find out the effect of basil leaves extract on total cholesterol levels in hypercholesterolemic Sprague Dawley rats model.

2. Material and Methods

2.1. Extraction of basil leaves

Basil leaves were separated from stems and then washed with water flow. Cleaned basil leaves were dried in to an oven. After that, dried basil leaves were grinded using a blender and sieved. Simplicia of *O. sunctum* was extracted using a maceration method with 70% ethanol to attract the chemical components contained in basil. The dose of basil leaves extract given was divided into three, namely 20 mg/kg, 40 mg/kg, 80 mg/kg body weight/day. Giving once a day after rats were treated with HFD *(High Fat Diet).*

2.2. High Fat Diet (HFD)

The *High Fat Diet* (HFD) used in this research is a reference from Listianasari in 2014 with the ingredient of 100 g quail egg yolks mixed into 50ml of palm oil.

2.3. Eksperimental Design

This research used thirty six Sprague Dawley rats aged 8 weeks, weighing 150-200 grams, divided into 6 groups, namely P1) Normal rats, not treated (negative control), P2) Rats induced with HFD without treatment, P3) Rats induced with HFD and intervened using statin medication of 0.18 mg/kgBW (positive control), P4) Rats induced with HFD with the basil leaves extract dose of 20

mg/kgBW/day, P5) Rats induced with HFD with the basil leaves extract dose of 40 mg/kgBW/day, P6) Rats induced with HFD with the basil leaves extract dose of 80 mg/kgBW/ day. The treatment lasted for 7 days. Animals experiment were carried out at the Central Laboratory of Food and Nutrition Studies, Gadjah Mada University, Yogyakarta. This research is a laboratory experiment research using the Pre Test and Post Test Control Group Design. The examination of levels of total cholesterol was carried out in PSPG UGM laboratory. The total cholesterol level of rats was measured using a Microlab 300 spectrophotometer with the CHOD-PAP enzymatic photometric test method.

2.4. Statistical Analysis

The data were analysed using SPSS version 20. The normality test was done to see whether the data were normally distributed or not by looking at the results of Shapiro Wilk. The differences between the groups in the treatment were analysed statistically using one way analysis of variance (ANOVA) followed by the Tukey post-hoc test. The data that were not normally distributed were tested using the Kruskal Wallis test with the Mann Whitney advance test. The significance of differences between groups is if $p<0.05$.

3. Result and Discussion

Data were normally distributed and homogeneity. The difference in influence from the six treatment groups was analysed using the One Way Anova parametric statistical test for normal and homogeneous distributed data, followed by the Post Hock test with *Tukey High Significant Difference* (HSD). There is a significant the administration of basil leaf extract to reduce cholesterol levels ($p<0.005$). The results of HFD induction in rats experienced a significant increase in total cholesterol levels. The effects of basil leaves extract on decrease cholesterol levels, but there is no difference in the effect of giving the highest dose of basil leaves extract (P6) (107.28±2.02) in this research using statin medication treatment group (P3)(103.44±2.25). Statistical results are listed in Table 1.

Table 1. Basil Leaves Extract *(Ocimum sanctum Linn.)* Decreases Total Cholesterol Levels in Hypercholesterolemia Sprague Dawley Rats Model.

Treatment	Pre-test	Post-test
P1	82.19±1.37	86.33±2.46
P2	193.15±2.74	193.35±3.85
P3	186.53±1.04	103.44±2.25
P4	190.41±2.05	146.61±5.100
P5	188.35±2.98	120.82±2.46
P6	185.61±2.47	107.28±2.02

The effects of basil leaves extract on reducing cholesterol levels are shown in Table 1. The results of the statistical analysis showed a significant effect ($p<0.005$). This research which showed that basil leaves extract can reduce cholesterol levels in hyperlipidaemia rats [6]. Cholesterol levels before the treatment of basil leaves extract in the HFD-induced group had high cholesterol levels. It was shown in Table 1. HFD induced group can increase cholesterol levels significantly. HFD causes hypercholesterolemia which is characterized by the increased total cholesterol, LDL and VLDL. This increase in lipids occurs because the absorption of cholesterol in the intestine has increased [8]. Disorders achieved by HFD resemble metabolic syndrome in humans and this can also prolong the complications of cardiovascular events [9]. The results of this research support by researcher study stating that giving high-fat diets can increase total cholesterol, triglycerides and LDL levels [10].

Table 2. Basil Leaves Extract *(Ocimum sanctum Linn.)* Decreases Total Cholesterol Levels in Hypercholesterolemia Sprague Dawley Rats Model.

Treatment	Treatment	*p**
Normal	P2 (HFD Induction)	.001
	P3 (Statin drug)	.001
	P4 (20 ml/Kg BW)	.001
	P5 (40 ml/Kg BW)	.001
	P6 (80 ml/Kg BW)	.001
P2 (HFD Induction)	Normal	.001
	P3 (Statin drug)	.001
	P4 (20 ml/Kg BW)	.001
	P5 (40 ml/Kg BW)	.001
	P6 (80 ml/Kg BW)	.001
P3 (Statin drug)	Normal	.001
	P2 (HFD Induction)	.001
	P3 (20 ml/Kg BW)	.001
	P4 (20 ml/Kg BW)	.001
	P5 (40 ml/Kg BW)	.001
	P6 (80 ml/Kg BW)	.167
P4 (20 ml/kg BW)	Normal	.001
	P2 (HFD Induction)	.001
	P3 (Statin drug)	.001
	P5 (40 ml/Kg BW)	.001
	P6 (80 ml/Kg BW)	.001
P5 (40 ml/kg BW)	Normal	.001
	P2 (HFD Induction)	.001
	P3 (Statin drug)	.001
	P4 (20 ml/Kg BW)	.001
	P6 (80 ml/Kg BW)	.001
P6 (80 ml/kg BW)	Normal	.001
	P2 (HFD Induction)	.001
	P3 (Statin drug)	.167
	P4 (20 ml/Kg BW)	.001
	P5 (40 ml/Kg BW)	.001
	P6 (80 ml/Kg BW)	.001

*One way Anova Test

Basil leaves extract at a dose of 20, 40, 80 mg/kgBW for 7 days can decrease cholesterol levels. Significant decrease ($p<0.005$) can be seen in Table 2, where most significant decrease occurred in the group given statin drug at a dose of 0.18 mg/kgBW and the highest dose of basil leaves extract was 80 mg/kgBW. There is no difference in the effect of giving the highest dose of basil leave extract (P6) in this research using statin medication treatment group (P3) (Table 2.). Medication for statin is one of the most widely used drugs to reduce cholesterol levels in the blood by inhibiting the *enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase* (HMG CoA-reductase*)* mechanism [11].

Flavonoids can reduce plasma cholesterol levels by inhibiting the absorption of cholesterol in the intestine and can increase the reaction of bile acid formation from cholesterol to be excreted through

feces. Phenol and polyphenols play a role in reducing the secretion of lipoproteins found in the liver and intestines and reducing the cholesterol esterification process resulting in decreased levels of cholesterol ester [12]. Another results of the analysis research explained that the polyphenols contained in Olive Oil is very significant in reduce cholesterol levels, triglycerides, and very low density lipoprotein [13].

Flavonoids and beta carotene are known to decrease cholesterol levels, triglycerides, LDL and increase HDL levels because they can inhibit *3- Hydroxy-3-methylglutaryl Coenzyme A* (HMG-CoA) reductase which functions as a catalyst in the formation of cholesterol. The inhibition of *3- Hydroxy-3 -methylglutaryl Coenzyme A* (HMG-CoA) reductase results in cholesterol synthesis, triglycerides, LDL being slow which results in decreased cholesterol, triglyceride and VLDL formation processes. Flavonoids can also increase the activity of *Lechitin Cholesterol Acyl Taransferase* (LCAT). LCAT is an enzyme that can convert free cholesterol to a more hydrophobic cholesterol ester, so that cholesterol ester can bind to lipoprotein nucleus particles to form new HDL, which will increase serum HDL levels. Other compounds found in basil extract such as tannins can reduce cholesterol and LDL levels by increasing cholesterol metabolism into bile acids and increase excretion of bile acids through feces [9]. Leaves of *O. sanctum* are rich in essential oils. Eugenol has been shown to possess significant antioxidant property leading to inhibition of lipid peroxidation and hypocholesterolemia [14]. *O. Sanctum* oil treatment decrease lipid peroxidation and increase reduced glutathione conthent in blood. *O.Sanctum* oil, since contains both linoleic and linolenic acid, could be considered as a drying oil and is expected to behave similarly. Accordingly, *O. Sanctum* oil may absorb the oxygen and get it self preferentially oxidized or metabolized there by inhibiting the oxidation or metabolism of cholesterol [15].

4. Conclusion

Basil leaves extract at a dose of 20, 40, 80 mg/kgBW for 7 days can decrease cholesterol levels. Basil leaves extract at a dose of 80 mg/kgBW is the most effective dose to decrease cholesterol levels.

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