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The Effect of Basil Leaves Extract (*Ocimum basilicum*) on the Triglyceride Level in Atherosclerosis Rats

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ABSTRACT

Atherosclerosis is the main cause of cardiovascular disease (CVD) characterized by fat accumulation and transformation, inflammation, smooth muscle cell proliferation, and necrotic cell remnants in the tunica intima of blood vessels. Basil leaves are known to contain active biochemical compounds that have the potential to reduce lipids. This is an experimental study that investigated the effect of basil leaves on rat triglyceride levels in atherosclerotic models. This research consisted of five groups of rats that were induced atherosclerosis by partial carotid ligation and a high-fat diet for 7 days. The treatment group was given basil leaves extract ethanol with 100 mg/kgBW, 200 mg/kgBW dose, and simvastatin 1.5 mg/rat/day. Examination of triglyceride levels by enzymatic calorimetry GPO-PAP method using Micro Lab 300. The data were analyzed using One-way ANOVA and Post Hoc Bonferroni. The results showed that there was an average decrease in triglyceride levels in rats given basil leaves extract with 100 mg/kg BW dose and indicated that the *Ocimum basilicum* leaves extract affected triglyceride levels in rats induced by atherosclerosis.

Keywords: Atherosclerosis; Basil Leaves Extract; High Fat diet; *Ocimum basilicum*; Triglyceride

INTRODUCTION

Cardiovascular disease (CVD) is a non-communicable disease and a leading cause of mortality globally each year (Lopez et al., 2022). According to the World Health Organization (WHO), approximately 17.9 million people succumbed to cardiovascular diseases in 2019, accounting for 32% of the total global deaths (WHO, 2021). In 2018, based on the findings of the Riskesdas' study, the diagnosed prevalence of cardiovascular diseases by physicians in Indonesia reached 1.5%. Notably, the province of West Sumatra is among the top 10 provinces surpassing the national prevalence, registering an incidence rate of 1.6% (Kementrian Kesehatan RI, 2018).

The main cause of CVD is atherosclerosis (Lopez et al., 2022). The development of atherosclerosis is based on inflammation causing lesions in blood vessels characterized by fat accumulation and transformation, inflammation, smooth muscle cell proliferation, and necrotic cell remnants in the tunica intima of blood vessels (Björkegren & Lusis, 2022). Dyslipidemia is a risk factor playing an major role in the pathogenesis of atherosclerosis (Shahawy & Libby, 2016). The condition of dyslipidemia is associated with a major cause of oxidative stress. Dyslipidemia is a disorder of lipid metabolism indicated by abnormal lipid profile levels in the form of increased levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides (TG) and decreased levels of high-density lipoprotein (HDL) (PERKENI, 2019). Triglyceride levels in the blood that exceed normal values are called hypertriglyceridemia. Untreated hypertriglyceridemia contributes to a significantly increased risk of CVD, even after LDL cholesterol reduction targets have been achieved (Bazarbashi & Miller, 2022).

The principle of atherosclerosis management is to control the risk factors causing atherosclerosis. Several conventional pharmacological therapies have been used to control blood lipid levels and blood pressure. Statins have been shown to be effective in reducing total cholesterol, LDL, and triglyceride levels, and increasing HDL levels. It works through the

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mechanism of inhibiting the HMG-CoA reductase enzyme which plays a role in cholesterol biosynthesis. Nevertheless nutrition and lifestyle modification are also important aspects as non-pharmacologic therapies for atherosclerosis (Perhimpunan Dokter Spesialis Kardiovaskular Indonesia, 2013).

Ocimum basilicum, commonly known as basil, is a plant frequently encountered and extensively utilized by the Indonesian community as a culinary and salad seasoning. *Ocimum basilicum* harbors a diverse array of biochemically active compounds exhibiting pharmacological activities such as antimicrobial, anti-inflammatory, and antioxidant properties (Shahrajabian et al., 2020). Additionally, basil is reported to contain bioactive compounds with the potential to address hyperlipidemia, closely associated with atherosclerosis occurrence (Dhama et al., 2021). Previous research has identified polyphenols, flavonoids, quercetin, rosmarinic acid, caffeic acid, vanillic acid, rutin, and apigenin as constituents present in basil leaves extract (Dhama et al., 2021; Shahrajabian et al., 2020). Studies have reported that the administration of basil extract to hypercholesterolemic rat models resulted in a significant 39% reduction in triglyceride levels (Harnafi et al., 2009). However, limited research has been conducted on the impact of basil leaves extract on triglyceride levels in atherosclerosis-induced rat models. The purpose of this study was to determine the effect of basil leaves extract on triglyceride levels in Wistar rats induced by atherosclerosis.

MATERIALS AND METHODS

Tools and Materials

The tools in this study include arterial ligation instruments, an oral sonde, vacuum distillation, rotary evaporator, microhematocrit, micropipette, centrifugator, and Microlab 300 spectrophotometer. The materials used were male Wistar rats, distilled water, anesthesia, standard rat diet, high-fat rat diet, basil leaves extract *(Ocimum basilicum)*, simvastatin, and triglyceride assay kit.

Methods

This study was an experimental research design using the post-test-only control group design method consisting of five groups of experimental animals. These groups consist of 1 negative control group, 1 positive control group, and 3 treatment groups. Ethical approval for this research has been obtained from the Ethics Committee of the Faculty of Medicine, Universitas Andalas, with No.853/UN.16.2/KEP-FK/2022.

The experimental subjects consisted of male Wistar rats (*Rattus norvegicus*) aged 6-8 weeks, with body weights ranging from 180 to 250 grams. Twenty-nine male Wistar rats were divided into five groups. Each group received distinct treatments as follows: 1. Negative control (K-): Rats provided with standard diet and water. (n=6); 2. Positive control (K+): Rats modeled for atherosclerosis. (n=7); 3. Treatment 1 (P1): Rats modeled for atherosclerosis were administered with *Ocimum basilicum* leaves extract at a dose of 100 mg/kgBW for 14 days after atherosclerosis induction. (n=5); 4. Treatment 2 (P2): Rats modeled for atherosclerosis were administered with *Ocimum basilicum* leaves extract at a dose of 200 mg/kgBW for 14 days after atherosclerosis induction. (n=5); 5. Treatment 3 (P3): Rats modeled for atherosclerosis were administered with simvastatin at a dose of 1.5 mg/rat/day for 14 days after atherosclerosis induction. (n=6).

Extract Preparation

The sample used was basil (*Ocimum basilicum*) leaves simplisia. Basil leaves powder was macerated with 96% ethanol solvent for 2 days at room temperature. The macerate was collected then evaporated by vacuum distillation and concentrated with a rotary evaporator at temperatures below 55°C.

Induction of Atherosclerosis

In the positive control group and treatment group, experimental animals were induced to develop atherosclerosis through partial ligation of the carotid artery. Rats were anesthetized with ketamine (80 mg/KgBW) before the surgery, and an incision was made along the midline of the rat's neck. Carotid arteries were ligated using a 6–0 silk suture. Additionally, the atherosclerotic response was triggered by administering a high-fat diet, achieved by mixing 300 grams of pork fat, 200 grams of duck egg yolk, 100 ml of distilled water, and 1 ml of 0.5% Carboxymethyl Cellulose (CMC). Rats were fed a high-fat diet and were given orally once a day for 7 days (Nam et al., 2010).

Determination of Triglyceride

Blood samples were collected from the retroorbital sinus of the experimental rats using a hematocrit capillary and centrifuged at a speed of 2500 rpm for approximately 20 minutes until serum and plasma were separated. The assessment of rat triglyceride levels was conducted using the GPO-PAP method employing the Microlab 300.

Table I. Effect of Basil Leaves Extract (Ocimum basilicum) on Triglyceride Level in Atherosclerosis

Group	Triglyceride level (mg/dL) (Mean ± SD)
K- (Normal)	67.22 ± 10.06[bc]
K+ (Atherosclerotic)	100.49 ± 14.03[a]
P1 (Atherosclerotic + 100 mg extract)	79.00 ± 7.47[b]
P2 (Atherosclerotic + 200 mg extract)	85.00 ± 6.72
P3 (Atherosclerotic + 1.5 mg Simvastatin)	90.28 ± 13.22

Values with [a] superscript were significantly different (p<0,05) from the K- group; Values with [b] superscript were significantly different (p<0,05) from the K+ group; Values with [c] superscript were significantly different (p<0,05) from the P3 group.

Data Analysis

Triglyceride levels were analyzed, and the results were presented as the mean ± standard deviation. Data were statistically evaluated using the Statistical Package for the Social Science (SPSS) program. Differences in triglyceride levels among groups were examined using the parametric one-way ANOVA statistical test, followed by the Bonferroni post-hoc test. Statistical significance was considered if the p-value was obtained <0.05.

RESULTS

The results of measuring the average triglyceride levels of Wistar rat blood samples after treatment in each group are presented in the following table I.

Table I shows that the mean serum triglyceride levels of rats in the negative control group (K-) and positive control group (K+) were 67.22 ± 10.06 mg/dL and 100.49 ± 14.03 mg/dL, respectively. This result shows that the partial carotid artery ligation treatment followed by a high-fat diet in the positive control group (K+) caused an increase in the average triglyceride level when compared to the negative control group (K-).

The average serum triglyceride levels of rats in the treatment groups (P1, P2, and P3) with the administration of basil extract at a dose of 100 mg/kgBW and 200 mg/kgBW and simvastatin administration for 14 days respectively resulted 79.00 ± 7.47 mg/dL, 85.00 ± 6.72 mg/dL, and 90.28 ± 13.22 mg/dL. The administration of basil leaves extract (*Ocimum basilicum*) with two different doses (group P1 and group P2) and simvastatin was able to reduce triglyceride levels compared to the K+ group. Statistical analysis using Bonferroni post hoc showed that 100 mg/kgBW treatment of basil extract significantly reduced triglyceride levels of experimental animals (p<0.05). A dose of 100 mg/kgBW reduced serum triglycerides by 21,39% compared to the control group. The results of this study are shared with a research by Ezeani et al., who found that the administration of basil extract at a dose of 100 mg/kgBW significantly reduced triglyceride levels by 39.54% compared to the control group, respectively 76.0 mg/dL and 125.7 mg/dL (Ezeani et al., 2017).

DISCUSSION

Ocimum basilicum extract significantly inhibited the increase in blood triglyceride concentration. Based on these results, it suggests that the basil can restore, at least partially, lipid catabolic metabolism due to the presence of phenolic compounds, flavonoids, and tannins in basil leaves (*Ocimum basilicum*) (Harnafi et al., 2009). Flavonoid compounds contained in basil leaves extract can increase the activity of plasma lipoprotein lipase (LPL) enzymes and liver lipase (Harnafi et al., 2009; Unnikrishnan et al., 2013). The triglyceride transporter, VLDL, will be hydrolyzed into fatty acids and glycerol when LPL enzyme activity increases. The released fatty acids will then be absorbed by muscles and other tissues, then oxidized to produce energy, and stored as reserves by adipose tissue (Mutia et al., 2018).

Flavonoid compounds also play a role in inhibiting pancreatic lipase enzymes (Rusmini et al., 2021). Reduced pancreatic lipase enzyme activity can reduce triglyceride deposits that enter the small intestine. The tannin content in basil leaves extract is known to play a role in reducing lipid levels through the same mechanism, namely by inhibiting pancreatic lipase enzymes. This causes the body to absorb less lipids. In addition, tannins are known to interact with mucosal proteins and intestinal epithelial cells to inhibit fat absorption in the intestine (Oliveira et al., 2015; Prahastuti et al., 2011).

Based on the results of the study, a decrease in triglyceride levels in the P2 group was also found. It is the group using basil leaves extract at a dose of 200 mg / kgBW compared to the K + group, but the decrease in occuring triglyceride levels was not statistically significant. The results of this study are in line with research conducted by Ezeani et al. which showed a dose of 200 mg/kgBW basil extract did not have a beneficial effect on triglyceride levels (Ezeani et al., 2017). This result may be caused by the presence of side compounds in the ethanol extract of the plant affecting the desired response. When these by-product compounds are in small concentrations, they are still in small amounts and do not affect the intended results. However, in larger concentrations, the effects of these compounds will be significant and can interfere with the response (Hidayat, 2011).

Table I shows that serum triglyceride levels in the P3 group decreased compared to the K+ group. This result indicates that the administration of simvastatin at a dose of 1.5 mg/kgBW can reduce triglyceride levels in group P3 but not statistically significant. The insignificant decrease in triglyceride levels might happen because within 14 days, simvastatin has not yet reached its maximum therapeutic effect. Generally, simvastatin reaches its maximum therapeutic effect in the fourth to sixth week (Bryant & Knights, 2011).

CONCLUSION

The administration of basil leaves extract demonstrated a reduction in triglyceride levels in the atherosclerosis-induced rat model. Specifically, the administration of basil leaves extract at a dose of 100 mg/kgBW significantly decreased triglyceride levels. This result indicates that the basil extract has a potential to be an anti-hyperlipidemia therapy.

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