The Effect of *Ocimum tenuiflorum* Ethanolic Extract on Cholesterol Level of Diabetic Mice

Pande Ayu Naya Kasih Permatananda[11], *Catherine* Cababa[2], *I Gde Suranaya* Pandit[3], *Sri* Masyeni[1]

[1]Faculty of Medicine and Health Science, Universitas Warmadewa, Denpasar 80239, Indonesia
[2]Lyceum of the Philipines, University (LPU), Cavite 4107, Philippines
[3]Faculty of Agriculture, Universitas Warmadewa, Denpasar 80239, Indonesia

Abstract. Dyslipidemia are conditions that frequently occur with Diabetes Melitus. Several previous studies have reported suboptimal management of dyslipidemia. The effect of Ocimum tenuiflorum or holy basil as anti-dyslipidemia has not been widely reported. This study was conducted to evaluate the effect of Ocimum tenuiflorum extract on the colesterol leve lof diabetic mice. In this study, we prepared 18 diabetes mice and divided into 3 groups based on the treatment, as follow: treatment 1 (56 mg/kgBW of Ocimum tenuiflorum extract), treatment 2 (112 mg/kgBW of Ocimum tenuiflorum extract), and control. Cholesterol level was measured after 2 weeks of treatment. The lowest colesterol level after treatment was in Group 2 (112 mg/kgBW), followed by Group 1 (56 mg/kgBW), and the last, or the highest colesterol level was had by control group. The difference in mean of colesterol level after treatment between groups was found to be significant based on the One Way Anova and Post hoc Tamhane tests with a P value <0.05. These findings can support the potential of Ocimum tenuiflorum as a new pharmacotherapeutic agent for the management of diabetes-induced dyslipidemia. Additional research is needed to to evaluate this potency in humans.

1 Introduction

Persistent elevation of blood glucosa levels or chronic hyperglycemia due to Diabetes Melitus (DM) leads to various damages, dysfunctions, and irreversible failures in several organs, including the eyes, kidneys, nerves, heart, and blood vessels. Dyslipidemia such as hypertryglyceridemia and hypercholesterolemia are conditions that frequently occurs with DM. The prevelance of dyslipidemia in DM varies greatly. However it is often to be higher in patients with type 2 DM, which can reach 91.4% [1].

Dyslipidemia is recognized to elevate the risk of cardiovascular disease (CVD) in diabetic patients by 3 – 4 times compared to non-diabetic patients. Diabetes and Dyslipidemia could induce inflammation and oxidative stress, which can lead to the occurence of aterosclerosis. The aterosclerosis can decrease blood flow and and increase the risk of

[1] Corresponding author: nayakasih@warmadewa.ac.id

thrombosis, which affects the occurence of cardiovascular diseases. Regarding the cardiovascular risk, unfortunately, in type 2 DM, these complications are independent of glycemic control. In other words, good glycemic control in type 2 DM does not inherently diminish the incidence of cardiovascular evento [2].

Previous studies have reported suboptimal management of dyslipidemia with lipid-lowering medications. Individual variations in response to treatment as well as side effects such as muscle pain and gastrointestinal symptoms, are mentioned to affect the effectiveness of dyslipidemia therapy [3]. *Ocimum tenuiflorum*, or holy basil, has active metabolites that offer as antidiabetic, antioxidant, and anti-inflammatory properties[4]. However, its effect as a lipid-lowering agent in diabetic mice has not been widely reported. This study was conducted to evaluate the effect of *Ocimum tenuiflorum* extract on the colesterol level of diabetic mice. In this study, we used 56 mg/kgBW and 112 mg/kgBW of *Ocimum tenuiflorum* extract, and aquades as control.

2 Method

This research is belong to post-test only experimental study which aimed to analyze the effect of *O. tenuiflorum* extract on cholesterol level of diabetic mice.

2.1 Preparation of *Ocimum tenuiflorum* extract

The sample used was Tulsi (*O. tenuiflorum*) with ratio between leaves and stems 3:1. After drying with oven at temperature around 50ºC, the samples were mashed with a blender and macerated with ethanol solvent 70% with ratio 1:5 (w/v) for 3 days. The filtrate then concentrated with evaporator until produced crude extract.

2.2 Experimental Animal

Male mice, 2-3 months old, body weight 30-40 grams, and healthy were used as experimental animal in this research. Prior experiment, all mice undergone acclimatization period for 7 days. All mice were provided with standard diet and cage. 18 mice that survived the acclimatization period, then injected intraperitoneally with STZ dose 40 mg/kgBW for 5 days. Mice with fasting blood glucose levels less than 150 mg/dl after STZ induction were drop out. At the end, we got 18 mice that divided into 3 groups, as follow: group 1 treated with *O. tenuiflorum* extract dose 56 mg/kgBW, group 2 treated with the extract at dose 112 mg/kgBW, and group 3 treated with aquades as a control.

2.3 Measurement of Cholesterol Level

After 2 weeks of treatment, cholesterol levels in mice from each group were tested. The measurement was conducted using Autocheck cholesterol strips, with blood samples taken from the tail vein.

2.4 Data Analysis

Cholesterol levels are a numerical variable, so they are presented in the form of mean and standard deviation. The statistical test used is one-way ANOVA, followed by the Tamhane post hoc test if the data variation is normal.

2.6 Ethical Clearance

Permission was obtained from the Ethics Committee of the Faculty of Medicine and Health Sciences at the University of Warmadewa with approval letter: 67/Unwar/FKIK/EC-KEPK/VII/2024.

3 Result and Discussion

This study used 18 diabetic mice induced by STZ 40 mg/kgBW for 5 days. Then the mice were randomized into 3 groups, namely Group 1 and Group 2 that received treatment with *O. tenuiflorum* extract at 56 mg/kgBW and 112 mg/kgBW, respectively, while the control group received only aquades. Treatment were given for 2 weeks. After 2 weeks, cholesterol tests were conducted, with the mean of cholesterol level for each group presented in Figure 1.

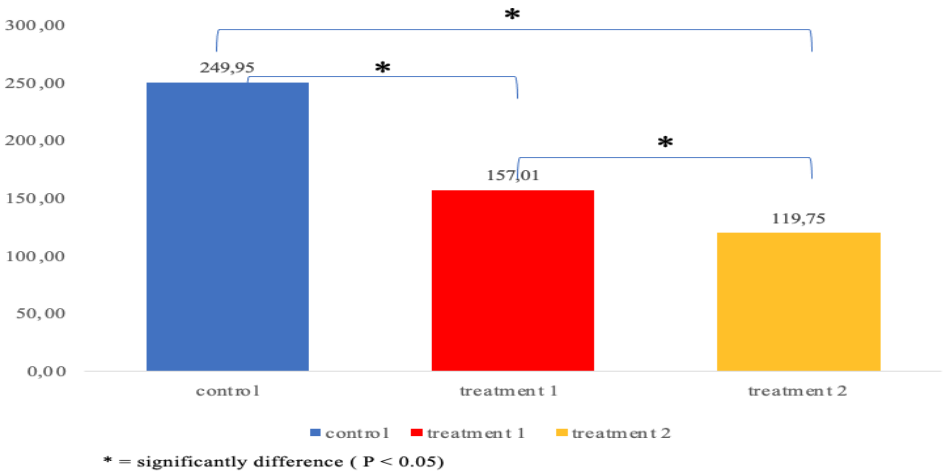

Fig 1. Post-hoc Analyses of Cholesterol Level (mg/dl) After Treatment

In this present study, we used STZ as diabetogenic agent. Streptozotocin is not only capable of inducing diabetes but also capable of increasing various dyslipidemia parameters. The decrease in bioavailability or insulin resistance that happened in Diabetes, triggers lipolysis through the activation of lipases and results in an increase in free fatty acids. This phenomenon is an early stage of diabetes-induced dyslipidemia [5,6].

As shown in figure 1, the lowest cholesterol level after treatment was in Group 2 (112 mg/kgBW), with amount 119.75 ± 10.90 mg/dl, followed by Group 1 (56 mg/kgBW) with cholesterol level 157.01 ± 11.64 mg/dl, and the last, or the highest cholesterol level was had by control group with number 249.95 ± 21.78 mg/dl. The difference in mean of cholesterol level after treatment between groups was found to be significant based on the One Way Anova and Post hoc Tamhane tests with a P value <0.05.

This study succesfully demonstrated the potential of *O. tenuiflorum* extract as lipid-lowering agent in diabetic mice, similar with some previous studies [5,7]. The extract of *O. tenuiflorum* is known to contain a number of secondary metabolites such as flavonoids and polyphenols [8]. In this study, ethanol was chosen as the solvent to extract O. tenuiflorum. This conclusion is linked to the research results of Hariantha et al. (2024), which state that the antioxidant activity of ethanol extract of *O. tenuiflorum* is higher compared to chloroform

extract. The strong antioxidant activity of *O. tenuiflorum* extract using ethanol solvent is probably because it contains a lot of polyphenols, flavonoid, and polar compounds [9].

Besides having effects as antidiabetic, antioxidant, and anti-inflammatory agents, Polyphenols and Flavonoid compounds are also known to help improve lipid profiles by reducing lipoprotein oxidation and enhancing lipid metabolism. The flavonoid content is also known to inhibit the enzyme HMG-CoA reductase, which plays a role in cholesterol synthesis [4,10].

From the GCMS study, the extract of *O. tenuiflorum* is known to contain several volatile compounds, with one of the largest percentages being eugenol. In addition to being found in the extract of *O. tenuiflorum*, eugenol is also abundantly present in cloves oil. FAO recommends eugenol as a safe compound for consumption as a food additive at doses up to 2.5 mg/kg body weight in humans [11]. By lowering the gene expression of sterol regulatory element binding protein 1 (SREBP1), a membrane-bound transcription factor that controls de novo lipogenesis in the liver, eugenol stops lipids from building up in the hepatocytes of mice with fatty liver. This process depends on calcium and involves the phosphorylation of AMP-activated protein kinase by Ca2+-calmodulin-dependent protein kinase kinase [12].

4 Conclusion

This study successfully demonstrated that the administration of *Ocimum tenuiflorum* extract significantly reduced total cholesterol levels in diabetic mice. These findings can support the potential of *Ocimum tenuiflorum* as a new pharmacotherapeutic agent for the management of diabetes-induced dyslipidemia. Additional research is needed to evaluate this potency in humans.

This experiment was supported by Ministry of Higher Education, Research, and Technology of Indonesia (Contract number 110/E5/PG.02.00.PL/2024)

References

1. Al Quran TM, Bataineh ZA, Al-Mistarehi AH, Alaabdin AMZ, Allan H, Al Qura'An A, et al. Prevalence and Pattern of Dyslipidemia and Its Associated Factors Among Patients with Type 2 Diabetes Mellitus in Jordan: A Cross-Sectional Study. Int J Gen Med. **15**, 7669 (2023). https://doi.org/10.2147/ijgm.s377463
2. Vekic J, Stromsnes K, Mazzalai S, Zeljkovic A, Rizzo M, Gambini J. Oxidative Stress, Atherogenic Dyslipidemia, and Cardiovascular Risk. Biomedicines. **11**, 2897 (2023). https://doi.org/10.3390/biomedicines11112897
3. Htira Y, Dridi M, Hadj Ali Z, Trabelsi N, Ben Mami F. Efficacy, Safety and Protective Effects of Lipid Lowering Drugs in Patients with Diabetes. European Journal of Medical and Health Sciences. **4**, 89 (2022). https://doi.org/10.24018/ejmed.2022.4.2.1220
4. Permatananda PANK, I Wayan Putu Sutirta Yasa, I Wayan Sumardika, Made Ratna Saraswati, I Gde Suranaya Pandit. From Tradition to Translation: A Systematic Review on the Pharmacological Actions of Eugenol Extracted from Ocimum Plants in Oxidative Stress, Inflammation, and Diabetes Mellitus. Bioscientia Medicina : Journal of Biomedicine and Translational Research. **9**, 7648 (2025). https://doi.org/10.37275/bsm.v9i6.1302

5. Husain I, Chander R, Saxena JK, Mahdi AA, Mahdi F. Antidyslipidemic Effect of Ocimum sanctum Leaf Extract in Streptozotocin Induced Diabetic Rats. Indian Journal of Clinical Biochemistry. **3**, 72 (2015). https://doi.org/10.1007/s12291-013-0404-2
6. Permatananda PANK, Sumardika IW, Yasa IWPS, Saraswati MR, Pandit IGS, Masyeni S. Antioxidant Effects of Ocimum tenuiflorum on Catalase and Glutathione Peroxidase In Vivo. Journal of Angiotherapy. **8**, 1 (2024). https://doi.org/10.25163/angiotherapy.81110040
7. Parasuraman S, Balamurugan S, Christapher PV, Petchi RR, Yeng WY, Sujithra J, et al. Evaluation of antidiabetic and antihyperlipidemic effects of hydroalcoholic extract of leaves of Ocimum tenuiflorum (Lamiaceae) and prediction of biological activity of its phytoconstituents. Pharmacognosy Res. **7**, 156 (2015). https://doi.org/10.4103/0974-8490.151457
8. Sintya E, Aryastuti SA, Permatananda PANK, Indrayani AW. Relative GC-MS Examination of Biological Activity Constituents of Ocimum tenuiflorum Extracts. Res J Pharm Technol. **16**, 4377 (2023). https://doi.org/10.52711/0974-360X.2023.00716
9. Hariantha MCS, Aryastuti AASA, Permatananda PANK, Sintya E, Udiyani DPC. Antioxidant Activity of Ocimum tenuiflorum Extract with Ethanol and Chloroform Solvent. Bali Medical Journal. **13**, 100 (2024). https://dx.doi.org/10.15562/bmj.v13i2.5322
10. Zeka K, Ruparelia K, Arroo R, Budriesi R, Micucci M. Flavonoids and Their Metabolites: Prevention in Cardiovascular Diseases and Diabetes. Diseases. **5**, 19 (2017). https://doi.org/10.3390/diseases5030019
11. Kaur G, Athar M, Sarwar Alam M. Eugenol precludes cutaneous chemical carcinogenesis in mouse by preventing oxidative stress and inflammation and by inducing apoptosis. Mol Carcinog. **49**, 290 (2010). https://doi.org/10.1002/mc.20601
12. Harb AA, Bustanji YK, Almasri IM, Abdalla SS. Eugenol Reduces LDL Cholesterol and Hepatic Steatosis in Hypercholesterolemic Rats by Modulating TRPV1 Receptor. Sci Rep. **9**, 14003 (2019). https://doi.org/10.1038/s41598-019-50352-4