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Effect of *Phaleria Macrocarpa* (Scheff.) Boerl Leaf Ethanol Extract on Serum IL-6 and TNF-α Levels in Diabetic Rats

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ABSTRACT

Background: Hyperglycemia conditions in diabetes mellitus (DM) can turn on pro-inflammatory cytokines like IL-6 and TNF-α. These cytokines play a role in insulin resistance and the development of DM complications. People in Indonesia have used ***Phaleria macrocarpa*** to treat diabetes, but the leaf of this plant has not been studied to see if it can reduce inflammation. **Objective:** This study aims to analyze the effect of ethanolic extract of ***Phaleria macrocarpa*** leaves (EEPML) in serum IL-6 and TNF-α levels of diabetic rats. **Methods:** This study was an experiment with a post-test-only control group design. Thirty 8-week-old male Wistar rats were used in the study. They were split into six groups: K1 was the normal control group; K2 was the DM control group; K3, K4, and K5 were given EEPML at doses of 125, 250, and 500 mg/KgBW; and K6 was given metformin 45 mg/KgBW orally once a day for 14 days. A high-fat diet and a 30 mg/KgBWi.p injection of streptozotocin were used to make the diabetic rat model. ELISA method for measuring serum IL-6 and TNF-α levels. The Kruskal-Wallis and the Mann-Whitney test were used to examine the differences between the groups. **Results:** There were significant differences between treatment groups in the mean levels of serum IL-6 ($p=0.017$), but there were no significant differences in the mean levels of serum TNF-α ($p>0.05$). **Conclusion:** Administration of ***Phaleria macrocarpa*** leaf ethanol extract 125 mg/KgBW reduced serum IL-6 levels but could not significantly reduce serum TNF-α levels in diabetic rats.

Keywords: TNF-α, IL-6, serum, ethanol extract of Phaleria Macrocarpa leaf.

1. BACKGROUND

When someone has hyperglycemia, pro-inflammatory cytokines can be turned on. These cytokines play a role in insulin resistance and the development of diabetic complications. One way glucose can be harmful is through protein glycation, linked to cytokines like IL-6, IL-12, and TNF-α as solid factors in developing diabetic microangiopathy. Chronic inflammation in the disease is shown by a rise in IL-6 and TNF-α (1, 2).

People in Indonesia and Malaysia have used *Phaleria macrocarpa*, a Papua plant, as a traditional medicine to treat diabetes, allergies, liver disease, vascular disease, cancer, kidney failure, high blood pressure, and stroke (3). The *Phaleria macrocarpa* fruit has been tested for toxicity and mutagenicity, and the results show that it is safe to use as medicine (4). The fruit of *Phaleria macrocarpa* is the part that has been studied the most. On the other hand, the leaves have not been studied as much.

The leaves of *Phaleria macrocarpa* are said to have flavonoids, polyphenols, saponins, tannins, and steroids. Falerin is one of its anti-inflammatory substances that work (5, 6). It can also fight free radicals and stop tyrosinase from working (7, 8). The anti-inflammatory effect of low-dose extract *Phaleria macrocarpa* leaves contained in chitosan nanoparticles was able to suppress TNF-α expression in the colon tissue of mice (9). More research needs to be done to find out how the leaves of *Phaleria macrocarpa* affect pro-inflammatory cytokines in diabetes cases. This will make using these medicinal plants more theoretically and practically accurate.

2. OBJECTIVE

This study aims to analyze the effect of ethanolic extract of *Phaleria macrocarpa* leaves (EEPML) in IL-6 and TNF-α serum levels of diabetic rats.

3. MATERIAL AND METHODS

Animal and treatments

This study was an experiment with a post-test-only control group design. Thirty 8-week-old male Wistar rats weighing 200-230 grams were used in the study. They were split into six groups: K1 was the normal control group; K2 was the DM control group; K3, K4, and K5 were given EEPML at doses of 125, 250, and 500 mg/KgBW; and K6 was given metformin 45 mg/KgBW orally once a day for 14 days. A high-fat diet (HFD) and a 30 mg/KgBWi.p injection of streptozotocin (STZ) were used to make the diabetic rat model. They were kept in a controlled environment (room temperature of 22–24°C and relative humidity of 40–60%) for one week before the experiment. During that time, they had free access to food and distilled water. Each group's body weight and blood glucose level were examined every week. On the 14th day after the extract was given, blood was taken from each treatment group and checked for serum IL-6 and TNF-α levels using the ELISA method.

Procedure and ethical considerations

The study plan was approved by the Ethical Committee of Universitas Sumatera Utara, with the number 459/KEP/USU/2021. The researcher thought about how ethical it was to use animals in experiments, based on the Helsinki Declaration of 1983's standards for animal ethics.

Measures

Rat IL-6 ELISA BZ-08185310-EB kit and Rat TNF-α ELISA BZ 08184670EB kit were used to measure serum IL-6 and TNF-α levels. The Kit's instructions were followed to prepare for the TNF-α and IL-6 measurements. The OD values were measured with a microplate reader and a wavelength of 450 nm, and the results were turned into ng/L using a standard linear curve.

Statistical analysis

The SPSS software version 26.0 (IBM, NY, USA) was used to analyze the data statistically. Shapiro - Wilk was used to testing data normality. Data with a normal distribution were tested with one-way ANOVA test, while data with abnormal distribution were tested with Kruskal - Wallis test followed by a Mann-Whitney test. All values are presented as the mean ± SD; p <0.05 was considered statistically significant.

4. RESULTS

Sample characteristics

The diabetic rat model in this study successfully created used a combination of HFD and STZ induction. A total of 25 rats were weighed initially, with an average of 204.60 ± 3.24 grams. After being given HFD for 2 weeks, the rats' body weight increased to >20% of their initial body weight with an average of 247.10 ± 6.01 grams. STZ-induced rats had an initial mean fasting blood glucose level of 93.32 ± 8.89 mg/dL and experienced an increase in the average fasting blood glucose level of >200 mg/dL to 225.68 ± 20.80 mg/dL.

Groups	IL-6 (ng/L) (Mean ± SD)	TNF-α (ng/L) (Mean ± SD)
K1	2,16 ± 0,28	67,71 ± 6,31
K2	2,41 ± 0,30	74,32 ± 5,45
K3	2,06 ± 0,33	61,52 ± 5,61
K4	2,81 ± 0,53	68,16 + 16,07
K5	2,62 ± 0,20	66,19 ± 7,52
K6	2,66 ± 0,52	61,58 ±9,00
p-value	0,017 [a*]	0,266 [b]

Table 1. Differences in serum IL-6 and TNF-α levels between groups. [a]Kruskal Wallis test, *p <0,05 significantly different; [b]One-way ANOVA test

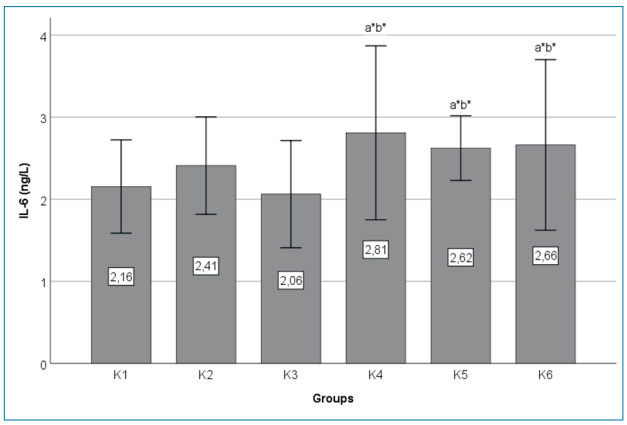

Figure 1. Comparison of mean serum IL-6 levels between groups. K1: normal control, K2: DM control, K3: EEPML 125 mg/KgBW, K4: EEPML 250 mg/KgBW, K5: EEPML 500 mg/KgBW, K6: Metformin 45 mg/KgBW. aMann Whitney post hoc with K1, bpost hoc Mann Whitney with K3, *p<0.05.

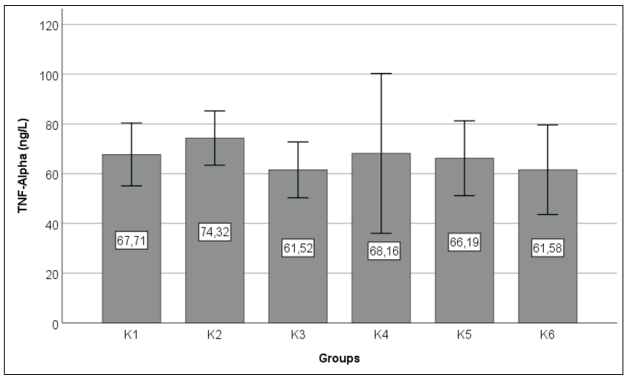

Figure 2. Comparison of mean serum TNF-α levels between groups. K1: normal control, K2: DM control, K3: EEPML 125 mg/KgBW, K4: EEPML 250 mg/KgBW, K5: EEPML 500 mg/KgBW, K6: Metformin 45 mg/KgBW.

The results measuring the average IL-6 level K1 group (2.41 ± 0.30 ng/L) were lower than the K2 group (2.16 ± 0.284 ng/L). This result proves that the DM condition increased levels of IL-6. The mean IL-6 level in the K3 group showed the lowest result (2.06 ± 0.33 ng/L). Statistical analysis using the Kruskal-Wallis test showed a significant difference in the mean IL-6 between treatment groups (p=0.017), as shown in Table 1. This shows

that the administration of EEPML affects the average serum IL-6 level in diabetic rats. A comparison graph of the average IL-6 levels can be seen in Figure 1. The Mann-Whitney test showed no significant difference between the average IL-6 level in K1 and K3 groups (p=0.251). This proves that EEPML 125 mg/KgBW's ability to reduce the average IL-6 level to close to the expected value is statistically significant. From the results of this study, it can be concluded that the administration of EEPML reduced the average serum IL-6 level in diabetic rats. The most effective dose in reducing IL-6 levels in diabetic rats is low-dose EEPML (125 mg/KgBW).

The highest average TNF-α level was in the K2 group (74.32 ± 5.45 ng/L). This shows that diabetic conditions increase TNF-α levels compared to the K1 group (67.71 ± 6.31 ng/L). The mean TNF-α levels in K3, K4, K5, and K6 groups were lower than in the K2 group, as shown in Table 1. This shows that administering EEPML and metformin in the diabetic rat group reduced their serum TNF-α levels. TNF-α levels in the K3 group showed the lowest levels (61.52 ± 5.61 ng/L) and were close to the K6 group (61.58 ± 9.00 ng/L). Nevertheless, statistical analysis using the one-way ANOVA test showed no significant difference in the mean TNF-α between treatment groups (p=0.266). A comparison graph of the average TNF-α levels can be seen in Figure 2. Based on the findings of this study, we concluded that the administration of EEPML reduced the average serum TNF-α level in diabetic rats, but the decrease was not statistically significant. The most effective dose in reducing the average serum TNF-α level in diabetic rats was low-dose EEPML (125 mg/KgBW).

5. DISCUSSION

Another study that used STZ induced diabetes rat model showed that diabetic rats had higher levels of IL-6 in their blood (10). Patients with type 2 diabetes mellitus (T2DM) also had an increase of about 3.3 times that of patients without T2DM (p< 0.05) (11). Hyperglycemia causes oxidative stress and markers of inflammation in cells and higher levels of IL-6, especially in patients with T2DM, because AGEs stimulate adipose tissue and leukocytes (2). In this study, giving diabetic rats EEPML reduced the average IL-6 level significantly (p=0.017). This result is similar to a study that showed intensive insulin therapy in critically ill people with high blood sugar lowered their IL-6 levels (p=0.023) (12). Metformin can also lower IL-6 in people with T2DM while raising the levels of IL-10 (13).

In diabetic rats, a low dose of EEPML (125 mg/KgBW) was the most effective way to lower the average serum IL-6. This is because the leaves of *Phaleria macrocarpa* have flavonoids as an antioxidant compound. Flavonoids protect the body from ROS's destructive effects, make radical products more stable, and do not react as much. Flavonoid compounds are primary antioxidants whose main job is to bind minimal amounts of free radical compounds. Giving many flavonoids changes their properties into pro-oxidants that speed up oxidative stress reactions (14).

The diabetic rats had higher TNF-α levels than the normal rats, but the difference was insignificant. Another study also shows that blood glucose levels and HOMA IR are unrelated to serum TNF-α levels in T2DM patients (15). However, a study found that the average TNF-α level in T2DM patients who were under control was much higher in those who were not under control. This shows how vital reasonable glycemic control is to keep complications from diabetes from happening (16). ROS production will increase when there is much glucose outside of cells. This will eventually lead to more TNF-α expression and worsening oxidative stress. TNF-α can trigger insulin resistance by reducing the autophosphorylation of insulin receptor substrates, restricting the activity of insulin receptor tyrosine kinase, lowering the GLUT, raising the circulation of fatty acids, changing how cells work, elevating glyceride levels, and lowering HDL levels (17). High TNF-α levels are linked to other cytokines, making it more likely that complications from T2DM will happen. Increased TNF-α, nitric oxide (NO) metabolites and fat indicate dysfunctional endothelium in T2DM (18).

In this study, giving EEPML to diabetic rats lowered the mean serum TNF-α levels, but the drop was not statistically significant (p=0.266). This result is similar to a study that showed intensive insulin therapy in critically ill people with high blood sugar could not lower their TNF-α levels (p=0.078) (12). The effects of herbal plants that contain flavonoids potentially decreased TNF-α expression in pancreatic tissue and repaired that damaged pancreatic tissue, and decreased the severity of insulitis (p<0.05) (19). Researchers have already looked at the effect of *Phaleria macrocarpa* extract on lowering TNF-α levels in Preeclampsia-Induced Human Umbilical Vein Endothelial Cell (HUVEC) cell cultures, which showed that *Phaleria macrocarpa* causes endothelial dysfunction (20).

Oxidative stress and inflammation in diabetics can be affected by how long they have had the disease. When diabetes has been going on for longer, there is a more significant rise in ROS. Lipid peroxide levels, which show the number of free radicals in plasma, also rise significantly in people with diabetes, whose disease has been going on for more than 5 years (21). The malondialdehyde level increases by 25% in people with diabetes after 4 to 6 weeks (22). The concentration of the test substance and how long it is also significant given affect how much TNF-α and IL-6 levels go down (23).

6. CONCLUSION

Administration of *Phaleria macrocarpa* leaf ethanol extract 125 mg/KgBW reduced serum IL-6 levels but could not significantly reduce serum TNF-α levels in diabetic rats.

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- **Author's contribution**: I.C.L contributed to the study's conception, design, and manuscript preparation. S.I. performed

data acquisition and experimental laboratory work. D.L. and T.W. contributed to data analysis and article drafting. All authors have approved the final version of the manuscript.

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REFERENCES

1. Singh G. Role of Adiponectin and Inflammatory Markers CRP, IL-6 and TNF-Alpha in Diabetes Mellitus. IOSR J Dent Med Sci. 2019; 18(10): 32–37.
2. Rehman K, Akash MSH, Liaqat A, Kamal S, Qadir MI, Rasul A. Role of interleukin-6 in development of insulin resistance and type 2 diabetes mellitus. Crit Rev Eukaryot Gene Expr. 2017; 27(3): 229–236.
3. Ali RB, Atangwho IJ, Kuar N, Ahmad M, Mahmud R, Asmawi MZ. In vitro and in vivo effects of standardized extract and fractions of Phaleria macrocarpa fruits pericarp on lead carbohydrate digesting enzymes. BMC Complement Altern Med. 2013; 13(1): 1-11.
4. Zaetun S. Uji Toksisitas Sari Buah Mahkota Dewa (Phaleria macrocarpa) pada Hewan Coba Mencit (Mus musculus). Media Bina Ilm. 2014; 8(6).
5. Lestari IC, Lindarto D, Ilyas S, Widyawati T, Eka S. Yield Analysis and Phytochemical Screening of Ethanol Extract of Phaleria macrocarpa Leaves. 2023; 8(1): 28-35.
6. Mamatha S, Reddy PP, Voruganti A, Reddy VA, Bakshi V, Boggula N. Phaleria macrocarpa (scheff.) Boerl: A Phytochemical and Pharmacological. Chem Res J. 2020; 5(3): 51–61.
7. Sumarawati T S, Chodidjah C C, Fatmawati D. Effect of Combination of Soybean and Phaleria macrocarpa Ethanol Extract on IL6, TNFα, VEGF and Fibroblasts in Mice Exposed to UVB. Pharmacogn J. 2023; 15(1) :6-13.
8. Nadri MH, Salim Y, Basar N, Yahya A, Zulkifli RM. Antioxidant Activities And Tyrosinase Inhibition Effects Of Phaleria macrocarpa. African J Tradit Complement Altern Med. 2014; 11(1): 107–111.
9. Kusmardi K, Rahmah RS, Estuningtyas ARI. Anti inflammatory effect of mahkota dewa (Phaleria macrocarpa) leaf extract loaded in chitosan nanoparticles in reducing tumor necrosis factor α expression on colon of dextran sodium sulfat-induced mice. Int J Pharm Res. 2019; 11: 624–631.
10. Mustika A, Fatimah N, Sari GM. The self-nanoemulsifying drug delivery system of Petiveria alliacea extract reduced the homeostatic model assessment-insulin resistance value, interleukin-6, and tumor necrosis factor-α level in diabetic rat models. Vet World. 2021; 14(12): 3229.
11. Yuli M. Hubungan Hiperglikemia Dengan Kadar Interleukin-6 (Il-6) Pada Penderita Diabetes Melitus Tipe 2. Universitas Andalas; 2014.
12. Wiryana M, Suastika K, Bagianto H, Bakta M. The Role of Intensive Insulin Therapy on Superoxide Dismutase (Sod), Tumor Necrosis Factor-α (Tnf-α), and Interleukin-6 (Il-6) on Hyperglycemia in Critically Ill Patients. Indones J Biomed Sci. 2010; 3(1): 224852.
13. Chen W, Liu X, Ye S. Effects of metformin on blood and urine pro-inflammatory mediators in patients with type 2 diabetes. J Inflamm. 2016; 13(1): 1–6.
14. Arifin B, Ibrahim S. Struktur, bioaktivitas dan antioksidan flavonoid. J Zarah. 2018; 6(1): 21–29.
15. Poltak P, Syafril S, Ganie RA. Hubungan kadar TNF-α dengan HOMA-IR pada pasien diabetes mellitus tipe-2 di Rumah Sakit Umum Pusat Haji Adam Malik, Medan, Indonesia. Intisari Sains Medis. 2019; 10(3): 580–583.
16. Yuniarti E. Perbedaan Kadar Tumor Necrosis Factor-Alfa Antara Diabetes Mellitus Tipe 2 Terkontrol Dengan Tidak Terkontrol. Bioscience. 2017; 1(1): 18–29.
17. Byrne CD, Wild SH. The metabolic syndrome. John Wiley & Sons; 2011.
18. Pereira FO, Frode TS, Medeiros YS. Mediators of Inflammation. Artic ID. 2006; 39062: 1–7.
19. Suryani N, Endang TH, Tinggi Ilmu Kesehatan Husada Borneo Banjarbaru S. Effect of Methanolic Swetenia mahagoni Seed Extracts in Increasing Insulin Level, Decreasing TNF-α Expression. J Kedokt Brawijaya. 2013; 27(3): 4784900.
20. Simanjuntak L, Siregar M, Mose JC, Lumbanraja SN. The Effects of Phaleria macrocarpa (Scheff.) Boerl Extract on Tumor Necrosis Factor-Alpha (TNF-α) Level in Preeclampsia-Induced Human Umbilical Vein Endothelial Cell (HUVEC) Culture. 18th World Congr Acad Hum Reprod. 2018;
21. Hsu WT, Tsai LY, Lin SK, Hsiao JK, Chen BH. Effects of diabetes duration and glycemic control on free radicals in children with type 1 diabetes mellitus. Ann Clin Lab Sci. 2006; 36(2): 174-178.
22. Bughdadi FA. Protective effects of vitamin E against motor nerve conduction deficit in diabetic rats. World Appl Sci J. 2013; 27: 28-32.
23. Aramita Z, Astuti TD, ST S, Irfani FN. Systematic Review: Pengaruh Pemberian Propolis Terhadap Kadar Interleukin 6 (Il-6) Dan Tumor Necrosis Factor A (Tnf-A) Pada Diabetes Melitus Tipe-2. Universitas 'Aisyiyah Yogyakarta; 2020.