Pharmacogn J. 2025; 17(3): 277-281
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

The Effect of Tender Coconut Water on the Expression of Vascular Endothelial Growth Factor (VEGF) and Macrophage Type-1 (M1) in Hyperlipidemic Rats

Winda Noer Oktavia[1], Siti Thomas Zulaikhah[2]*, Agung Putra[3], Mochamad Navi Suharto[1], Raja Faisal Manganju Sitorus[4]

Winda Noer Oktavia[1], Siti Thomas Zulaikhah[2]*, Agung Putra[3], Mochamad Navi Suharto[1], Raja Faisal Manganju Sitorus[4]

[1]Postgraduate Magister Biomedical Science Study Program, Faculty of Medicine, Universitas Islam Sultan Agung (Unissula), Semarang, INDONESIA.

[2]Department of Public Health, Faculty of Medicine, Universitas Islam Sultan Agung (Unissula), Semarang, INDONESIA.

[3]Magister Biomedical Science Study Program, Faculty of Medicine, Universitas Islam Sultan Agung (Unissula); Semarang, INDONESIA.

[4]Komisi XIII Dewan Perwakilan Rakyat Republik, INDONESIA.

Correspondence

Siti Thomas Zulaikhah

Department of Public Health, Faculty of Medicine, Universitas Islam Sultan Agung (UNISSULA), Kaligawe Streat Km 4, 50112, Semarang, Central Java, INDONESIA.

E-mail: sitithomas@unissula.ac.id

History

- Submission Date: 20-04-2025;
- Review completed: 21-05-2025;
- Accepted Date: 27-05-2025.

DOI : 10.5530/pj.2025.17.34

Article Available online

http://www.phcogj.com/v17/i3

ABSTRACT

Background: Individuals with hyperlipidemia have an increased risk of developing cardiovascular disease compared to those with normal total cholesterol levels. High-fat intake can increase cholesterol esters, especially low-density lipoprotein (LDL), triglycerides, and Reactive Oxygen Species (ROS) levels. This causes adipocyte death, infiltration of macrophage type-1 (M1) expression and release of pro-inflammatory cytokines from M1 such as Interleukin 6 (IL-6). The effect leads to apoptosis of vascular and neuronal cells as well as regulates the expression of Vascular Endothelial Growth Factor (VEGF). Tender coconut water contains antioxidants with the ability to prevent ROS, as well as reduce PKC activation, AGEs formation, and VEGF expression. **Objective:** To determine the effect of tender coconut water on VEGF and M1 expression in hyperlipidemic male Wistar rats. **Method:** This research used an experimental design and a post-test Only Control Group Design. The number of samples was 24 rats divided into Groups K1 (healthy rats), K2 (hyperlipidemic rats), K3 (hyperlipidemic rats+ Tender coconut water 4 mL / 200grBW / day), and K4 (hyperlipidemic rats+ coconut water 8 mL / 200grBW / day), with treatment duration of 21 days. VEGF expression data were analyzed by a one-way ANOVA test. **Results:** The highest and lowest average number of M1 was in K2 (28.72) and K1 (3.61). The average in K3 was lower when compared to K2 (14.66 >< 28.72). Furthermore, M1 expression in K4 was lower when compared to K3 (6.72 >< 14.66). The highest and lowest average VEGF expression was in K2 (4.58) and K1 (1.00). The average K3 was lower when compared to K2 (2.85 >< 4.58), and VEGF expression in K4 was lower than K3 (1.76 >< 2.85). **Conclusion:** Tender coconut water affected VEGF expression and M1 quantity in hyperlipidemic rats
Keywords: Tender coconut water, hyperlipidemia, VEGF expression, M1 quantity.

INTRODUCTION

Hyperlipidemia is a condition of abnormal body fat levels causing an increase in low-density lipoprotein (LDL), total cholesterol, and triglycerides, as well as a decrease in high-density lipoprotein (HDL). This condition is the risk factor for various cardiovascular and cerebrovascular diseases, including stroke and myocardial infarction.[1] Furthermore, hyperlipidemia causes increased fat accumulation in the liver, leading to high levels of Reactive Oxygen Species (ROS), which trigger oxidative stress and tissue damage. High-fat intake causes adipocyte accumulation to induces obesity. High fat increases cholesterol esters, especially LDL, triglycerides, and ROS levels.[2] An imbalance between intake and energy used in physical activity increases fat tissue deposits, leading to obesity and visceral fat. Accumulation of visceral fat leads to adipose hypertrophy and causes hypoxia in the endoplasmic reticulum of cells, adipocyte death, and infiltration of macrophage type 1 (M1) expression. This continuation increases the release of pro-inflammatory cytokines from M1, such as Interleukin 6 (IL-6), leading to local and systemic inflammation.[3]

Accumulation of visceral fat leads to continuous destruction of adipose tissue. This condition causes M1 to be abundant in the body, while the production of macrophage type 2 (M2) is formed in small quantities.[4] The role of M2 in the inflammatory process is significant in reducing and stopping inflammation by inhibiting the NF-κB pathway. Therefore, the promotion of M1 to M2 is crucial in reducing the inflammatory process. Inflammatory obesity is triggered by the presence of hypertrophied adipose tissue and an increase in a number of factors, such as FFA (Free Fatty Acid) and LPS (Lipopolysaccharide). In addition, LPS binds and activates the TLR 4 receptor. The activated M1 expression releases pro-inflammatory cytokines. Activated macrophages secrete maximal pro-inflammatory mediators, including TNF-α, PGE2, IL-1, IL-6, ROS, and Nitric Oxide (NO).[5] Vascular Endothelial Growth Factor (VEGF) is essential proteins in the body, regulating cell defense, migration, and differentiation during vasculogenesis and angiogenesis. VEGF is balanced with factors inhibiting angiogenesis under normal conditions. Increased expression of the protein is found in several disease conditions, such as cancer, ischemic cardiovascular disease, and diabetes mellitus (DM). Molecular sensors from cells stimulate the production of angiogenic growth factors, especially VEGF when there is a decrease in oxygen.[6]

Tender coconut water contains L-arginine, polyphenols, vitamin C, selenium, and minerals (Cu, Mg, Mn, K, Na, Zn). L-arginine can significantly reduce free radicals. Oxidative stress is reduced with decreased free radicals in the body. Furthermore,

Cite this article: Oktavia WN, Zulaikhah ST, Putra A, Suharto MN, Sitorus RFM. The Effect of Tender Coconut Water on the Expression of Vascular Endothelial Growth Factor (VEGF) and Macrophage Type-1 (M1) in Hyperlipidemic Rats. Pharmacogn J. 2025;17(3): 277-281.

L-arginine inhibits inflammation as indicated by a decrease in pro-inflammatory cytokine levels such as CRP, TNF-α, IL-6, and IL-8.[7] Tender coconut water has also been shown to reduce the levels of TNF-α, IL-1, and IL-6 as inflammatory parameters in DM rats.[8] Clinical trials show that Tender coconut water contains a source of natural antioxidants for protecting the body against inflammation caused by ROS. Therefore, this research aimed to determine the effect of Tender coconut water on VEGF and M1 expression in male hyperlipidemic Wistar rats.

METHOD

This research was designed as an experimental design with a Post-Test Control Group Design.

Ethical clearance

This research received ethical clearance from the Medical / Health Bioethics Research Commission of the Faculty of Medicine, Unissula Semarang, number 468/XI/2022/Komisi Bioetik.

Administration of Tender Coconut Water.

Tender coconut water from green coconuts was obtained from the Yogyakarta area and the surroundings around 5-7 months old. In this context, 4mL/200g BB/day and 8mL/200g BB/day doses were administered to the treatment group for 14 days. The dose was adjusted based on previous research and was given orally using a sonde for 2 weeks.[8]

Conditioning Hyperlipidemic Rats

The rats are given a high-fat diet, namely quail egg yolk to achieve hyperlipidemia. The quail egg yolk was shaken first and presented orally by being sucked for 14 days at a dose of 1 g/head/day.[9]

Treatment Administration

The subjects were male Wistar rats that met the inclusion criteria of 2 months old. The rats weighed 150–200 grams, showing healthy physical appearance and active movement, maintaining normal feeding and drinking behavior, and reporting no signs of injury or physical impairment. A total of 24 rats were randomly divided into 4 groups, each comprising 6 rats.

Blood was taken to examine VEGF and macrophage type 1 (M1) expression after the treatment completion.

Blood Collection Method

The equipment used was sterile microhematocrit tubes, blood collection bottles, and sterile cotton. Blood was taken by inserting the microhematocrit tube into the ophthalmic vein at the corner of the eyeball periorbitally, and rotating slowly until blood is ejected. The ejected blood was collected in 2cc of ependroph. The microhematocrit tube was removed when the required blood was sufficient. The remaining blood in the corner of the eyeball was cleaned with sterile cotton.[9]

M1 and VEGF Examination Method

1. VEGF-A expression was analyzed using Reverse Transcription Polymerase Chain Reaction (RT-PCR) through cDNA samples, Taq master mix (dNTPs), Taq DNA Polymerase, reaction buffer, and MgCl2, VEGF-A gene-specific primers.
2. Observation of M1 number used Immunohistochemistry (IHC) staining of adipose tissue, namely deparaffination with Xylene 1 and 2, rehydration using absolute ethanol 1, absolute 2, ethanol 70%, 80%, 90%, Citrate Buffer 1x pH 6.5, blocking with 3% H2O2, counterstaining using Hematoxilin Eosin. The number of M1 was indicated by the black color accumulating in the cytoplasm and diffusing out of the cell. The number of M1 was calculated by the quantity of cytoplasm in cells positively stained in 5 fields of view at 400x magnification on an Olympus Cx23 microscope photo.

Statistical Analysis

VEGF and M1 expression results were tested for normality using *Shapiro-Wilk* and homogeneity through *Leuvene's Test*. Statistical analysis was conducted using *one-way Anova* since all data were confirmed to be normally distributed and homogeneous. This was followed by a *Post Hoc Least Significant Difference* (LSD) Test to identify differences between groups. The decision to reject or accept the hypothesis was based on alpha 5%.[10]

RESULTS & DISCUSSION

Table 1 shows the effect of Tender coconut water on VEGF expression and M1 expression in male hyperlipidemic Wistar rats.

Table 1 and Figure 1 show that the average VEGF expression was highest and lowest in K2 (4.58) and K1 (1.00). The average in K3 was lower when compared to K2 (2.85 >< 4.58), and VEGF expression in K4 was lower than in K3 (1.76 >< 2.85). The one-way ANOVA test analysis obtained a p-value of 0.007 since there was a difference in the average VEGF expression in all groups.

The average number of M1 was highest and lowest in K2 (28.72) and K1 (3.61). In K3, the average was lower when compared to K2 (14.66 >< 28.72). Furthermore, M1 expression in K4 was lower when compared to K3 (6.72 >< 14.66). The results using one-way ANOVA test obtained a p-value of <0.0001 (<0.05) since there was a difference in the average number of M1 in all groups. The LSD Post Hoc Test was used to analyze the differences in average VEGF expression and M1 quantity between groups.

The significant differences in VEGF and M1 expression between K2 and K3, K2 and K4 as well as K3 and K4 are as follows.

1. There is a difference in the average expression of VEGF in the group of rats given a high-fat diet (K2) with the group given a high-fat diet and Tender coconut water at a dose of 4 mL/200g BW for 2 weeks (K3). A difference is also reported in the average expression of VEGF in the group of rats only given a high-fat diet (K2) and the group given a high-fat diet and Tender coconut water at a dose of 8 mL/200g BW for 2 weeks (K4). The average expression of VEGF in K4 was lower when compared to K3. This is because the group of rats given a high-fat diet and Tender coconut water at a dose of 8 mL/200g BW for 2 weeks had a better effect on VEGF expression compared to those receiving a dose of 4 mL/200g BW for 2 weeks.
2. There is a difference in the average number of M1 in the group of rats only given a high-fat diet (K2) with the group receiving a high-fat diet and Tender coconut water at a dose of 4 mL/200g BW for 2 weeks (K3). Furthermore, there is a difference in the average number of M1 in the group of rats only given a high-fat diet (K2) and those receiving a high-fat diet and Tender coconut water at a dose of 8 mL/200g BW for 2 weeks (K4). The average number of M1 in K4 is lower when compared to K3 since the group of rats given a high-fat diet and Tender coconut water at a dose of 8 mL/200g BW for 2 weeks had a better effect on the number of MI.

Figure 2 shows the IHC staining results of M1 quantity. Observations using a binocular microscope show that the black arrow represents positive M1.

Figure 2 shows that the number of M1 in group K2 appears to be greater than K1. In group K3 (administration of Tender coconut water at a dose

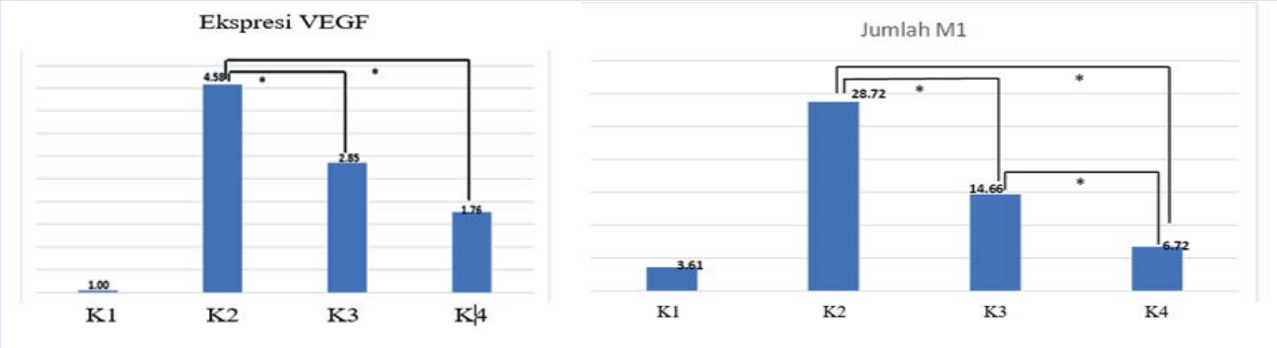

Figure 1. Results of the differences in average VEGF expression and M1 quantity between groups using LSD Post Hoc Test.

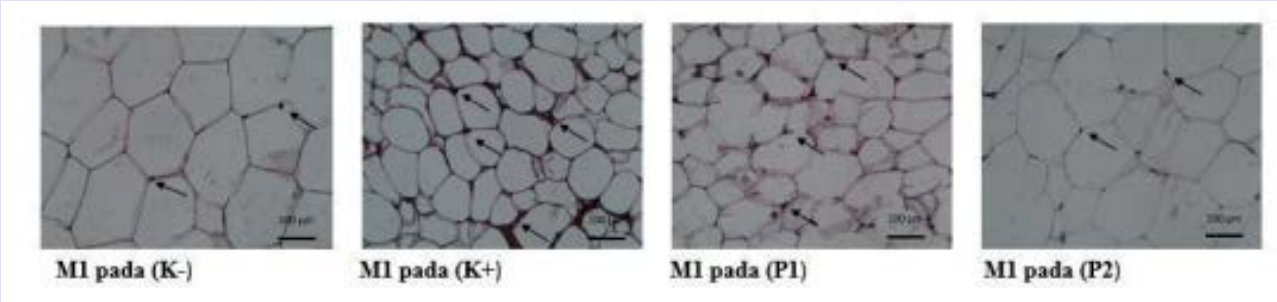

Figure 2. Immunohistochemistry (IHC) staining results of M1 quantity.

Table 1. Group Division.

Group 1 (K1):	The healthy rat group consisted of male white rats of the Wistar strain receiving a standard diet and ad libitum drink.
Group 2 (K2):	The negative control group consisted of male white rats of the Wistar strain receiving a standard feed diet, ad libitum drink, and a high-fat diet.
Group 3 (K3):	Treatment group 1 consisted of male white rats of the Wistar strain receiving a standard feed diet, ad libitum drink, and a high-fat diet. The rats were given Tender coconut water at a dose of 4 mL/200 g BW for 2 weeks.
Group 4 (K4):	Treatment group 2 consisted of male white rats of the Wistar strain receiving a standard feed diet, ad libitum drink, and a high-fat diet. The rats were given Tender coconut water at a dose of 8 mL/200 g BW for 2 weeks.

Table 2. Results of Average Analysis, Normality Test, Homogeneity Test on VEGF Expression and M1 Quantity.

Variable	K1 N=6	K2 N=6	K3 N=6	K4 N=6	Sig.(p)
Ekspresi VEGF					
Average	1.00	4.58	2.85	1.76	
Std. deviation	0.85	1.38	1,55	0.95	
Shapiro Wilk	0.00	0.516*	0.251*	0.314*	
Lavene Test					0.267*
One Way Anova					0.007**
M1 Quantity					
Average	3.61	28.72	14.66	6.72	
Std. deviation	2.13	2.85	1.13	1.46	
Shapiro Wilk	0.479*	0.311*	0.576*	0.219*	
Levene Test					0.019*
One Way Anova					<0.0001**

Note: * Normal and homogeneous p>0.05 **Significant p<0.05

of 4mL/200grBW), the number is greater than K4 (administration of Tender coconut water at a dose of 8mL/200grBW). The number of M1 in K4 is reduced, as reported by the decreasing black color.

The healthy control group (K1) was with standard feed and distilled water. The negative control was only given a high-fat diet (K2), while treatment group 1 (K3) was given Tender coconut water with a dose of 4 mL/200 gr BB/day and given a high-fat diet. Furthermore, treatment group 2 (K4) was given coconut water with a dose of 8 ml/200 gr BB/dray and a high-fat diet. On the 36th day, the levels of Triglycerides, HDL, LDL, Lee index, and BW of rats were examined and randomized. Adipose tissue was taken on the 49th day to analyze VEGF levels and the number of M1 macrophages. Groups K3 and K4 showed decreased VEGF gene expression after being given coconut water with doses of 4

mL/200 g BB/day and 8 mL/200 g BB/day in hyperlipidemia conditions. The results of M1 examination in group K4 given Tender coconut water at a dose of 8mL/200grBW showed a significant increase compared to K3. According to Tham (2020), processed coconut ingredients can reduce insulin resistance in obesity by increasing the number of macrophages.[11] Budhy (2021) also stated that coconut caused a higher number of macrophages compared to the control group of active smokers.[12]

In hyperlipidemia, endothelial dysfunction and decreased NO bioavailability occur.[13] Tender coconut water contains L-arginine, vitamin C, methionine, and minerals.[14] Arginine is the primary substrate of the enzyme Nitric Oxide Synthase (NOS) that produces NO. Additionally, NO is essential in maintaining normal vascular function, inhibiting leukocyte adhesion and platelet aggregation, as well as inducing vasodilation. Arginine supplementation also increases NO levels and restores endothelial function. In this context, NO has anti-inflammatory effects, which affect macrophage polarity and VEGF expression acting as an essential factor in angiogenesis. VEGF production can be increased by NO because the compound stimulates the expression through activation of PI3K/Akt and HIF-1α pathways. Hyperlipidemia triggers tissue hypoxia and oxidative stress. As compensation, VEGF expression increases, and the response can strengthen NO from arginine.[15] M1 has a pro-inflammatory function that is dominant in hyperlipidemia. This MI produces TNF-α, IL-6, and ROS. NO can inhibit M1 activation and support the transition to anti-inflammatory M2. Arginine plays a role in macrophage modulation by increasing NO, inhibiting NF-κB, and reducing the expression of M1 markers such as iNOS and IL-1β. The protein can improve endothelial dysfunction and increase NO bioavailability, suppressing inflammation and reducing M1 expression.[16]

Tender coconut water contains vitamin C, which is a powerful antioxidant for reducing ROS and protecting endothelial cells from damage. Vitamin C regulates the stability and activity of HIF-1α, reducing VEGF expression. This vitamin is needed as a cofactor for prolyl hydroxylase, which is an enzyme facilitating the degradation of HIF-1α to reduce excessive VEGF expression. In hyperlipidemia, macrophages tend to be M1 phenotype, producing pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, as well as a high quantity of ROS and NO. Vitamin C inhibits M1 activation by (1) reducing ROS to prevent activation of the NF-κB pathway that induces pro-inflammatory gene transcription, (2) decreasing iNOS expression to reduce excess NO production in M1 macrophages, and (3) increasing anti-inflammatory expression, to enhance transition to M2.[17] The mineral content in Tender coconut water is Potassium (K), Magnesium (Mg), Zinc (Zn), Copper (Cu), Iron (Fe), and Manganese (Mn). Furthermore, potassium maintains endothelial function and blood pressure. The deficiency leads to endothelial dysfunction, potentially increasing VEGF expression in response to oxidative stress. Low K levels increase the activation of NLRP3 inflammasome, which plays a role in M1 macrophage polarity. Mg has anti-inflammatory and antioxidant effects, which can decrease VEGF expression by reducing oxidative stress. In addition, Zn regulates gene expression through transcription factors such as MTF-1. Cu is required for the activity of angiogenic enzymes such as lysyl oxidase to increase VEGF expression and angiogenesis. Fe plays a role in regulating HIF-1α, which can increase VEGF expression under hypoxic conditions. Mn acts as a cofactor for antioxidant enzymes such as superoxide dismutase (SOD) to reduce oxidative stress and VEGF expression. These minerals can also inhibit NF-κB activation, the production of pro-inflammatory cytokines such as TNF-α and IL-1β, as well as promote M1 polarity towards M2.[18,19]

CONCLUSION

In conclusion, the administration of Tender coconut water decreased VEGF expression and M1 quantity in hyperlipidemic male Wistar rats. The dose of Tender coconut water was inversely related to VEGF expression and M1 quantity.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Zhao, L., Chen, H., Liu, C., *et al.* "Hyperlipidemia and cardiovascular diseases: From pathogenesis to clinical practice." *Journal of Cardiology Research. 2021; 34*(2), 78-92
2. Kolderup, A. & Svihus, B. Fructose Metabolism and Relation to Atherosclerosis, Type 2 Diabetes, and Obesity. *J. Nutr. Metab.* 2015
3. Item F, Konrad D. Visceral fat and metabolic inflammation: The portal theory revisited. *Obes Rev.* 2012;13(SUPPL.2):30–39.
4. Lu Z, Li Y, Song J. Characterization and treatment of inflammation and insulin resistance in obese adipose tissue. *Diabetes, Metab Syndr Obes Targets Ther.* 2020; 1(13): 3449–3460
5. Wardhana, I. M. W. & Wangko, S. Interaksi antara Makrofag dan Jaringan Adiposa pada Obesitas. *J. Biomedik.2012;* 3, 111–118
6. Syafuadi, R. Pengaruh Terapi Preventif dan Kuratif Kombinasi Curcumin dan Vitamin E dibandingkan Doxorubicin Terhadap Vascular Endothelial Growth Factor (VEGF) dan Gambaran Histopatologi Tikus Jantan Galur Wistar (Rattus norvegicus) Model Kanker Mammae. 2017. *Thesis. Repos.*
7. Zulaikhah ST, Aini HFN, Rini AS, Abiyyu BH, Dewi EAT, Pratama AA. Tender Coconut Water (Cocos nucifera L.) Can Increase Antioxidant Enzymes and Decrease MDA Levels: Experimental Study on Cigarette Smoke-Exposed Rats. *Pharmacogn J.* 2022;14(5):469–476.
8. Zulaikhah ST, Wahyuwibowo J, Suharto MN, Enggartiasto BH, Ortanto MIR, Pratama AA. Effect of tender coconut water (TCW) on TNF-α, IL-1 and IL-6 in streptozotocin (STZ) and nicotinamid (NA) induced diabetic rats. *Pharmacogn J.* 2021;13(2):500–505.
9. Zulaikhah S, Pertiwi D, Bagus SA, Nuri S, M BJE, Alfiza NS. Effect of Tender Coconut Water on Blood Lipid Levels in Hight Fat Diet Fed Male Rats. *J Krishna Inst Med Sci Univ.* 2017;6(2):63–68.
10. Ratnawati R, Zulaikhah ST, Hussaana A, Pratama AA. Effect of Tender Coconut Water Powder Enriched with Vitamin E in Preventing Oxidative Stress of Diabetes Male Wistar Rats. *Pharmacogn J* . 2024;16(5):1062–8.
11. Tham, Y. Y., Choo, Q. C., Muhammad, T. S. T. & Chew, C. H. Lauric acid alleviates insulin resistance by improving mitochondrial biogenesis in THP-1 macrophages. *Mol. Biol. Rep. 2020;* 47 (12): 9595–9607 doi: 10.1007/s11033-020-06019-9. Epub 2020 Dec 1.
12. Budhy, T. I., Arundina, I., Surboyo, M. D. C. & Halimah A N. The Effects of Rice Husk Liquid Smoke in Porphyromonas gingivalis-Induced Periodontitis. *Eur. J. Dent. 2021;* 15 (4): 653–659 doi: 10.1055/s-0041-1727554. Epub 2021 May 26.
13. Saleh NA, Yaseen H, Kinaneh S, Khamaisi M, Abassi Z. Combination of hyperglycaemia and hyperlipidaemia induces endothelial dysfunction : Role of the endothelin and nitric oxide systems. 2021;(July 2020):1884–1895.
14. Zulaikhah ST. Health Benefits Of Tender Coconut Water (TCW). *Int J Pharm Sci Res.* 2019;10(2):474–480.
15. Shen Y, Wang H, Xie H, Zhang J, Ma Q, Wang S, *et al.* L-arginine promotes angio-osteogenesis to enhance oxidative stress-inhibited bone formation by ameliorating mitophagy. *J Orthop Transl* [Internet]. 2024;46(May):53–64. Available from: https://doi.org/10.1016/j.jot.2024.03.003
16. Wu G, Fuller W Bazer, Teresa A Davis, Sung Woo Kim, Peng Li, J Marc Rhoads, *et al.* Arginine metabolism and nutrition in growth, health and disease. *Amino Acids.* 2009 May;37(1):153-168. doi:10.1007/s00726-008-0210-y.

17. Ang A, Pullar JM, Currie MJ, Vissers MCM. Vitamin C and immune cell function in inflammation and cancer. *Biochem Soc Trans*. 2018;46 (5):1147–1159.

18. Alghamdi M, Gutierrez J, Komarnytsky S. Essential Minerals and Metabolic Adaptation of Immune Cells. *Nutrients*. 2023;15(1):1–19.

19. Díez-tercero L, Delgado LM, Perez RA. Modulation of Macrophage Response by Copper and Magnesium Ions in Combination with Low Concentrations of Dexamethasone. *Biomedicine*s. 2022;10(4): 1-16

Cite this article: Oktavia WN, Zulaikhah ST, Putra A, Suharto MN, Sitorus RFM. The Effect of Tender Coconut Water on the Expression of Vascular Endothelial Growth Factor (VEGF) and Macrophage Type-1 (M1) in Hyperlipidemic Rats. Pharmacogn J. 2025;17(3): 277-281.