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Intake of *Moringa oleifera* Leaf Extract Decreases IL-1 and TNF-α Levels in Dyslipidemic Wistar Rat Model

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Abstract
Changes in consumption behavior to instant food cause various health problems, such as obesity, dislipidemia, and atherosclerosis. A study was conducted to investigate *Moringa oleifera* extract as an anti-inflammation product that decreases the levels of biochemical markers IL-1 and TNF-a. This experiment was done with randomized pre- and posttest control-group design, employing 40 Wistar rats separated into five groups: control group 0% *M. oleifera* leaf extract (P_0), treatment group 1 with 10% *M. oleifera* leaf extract (P_1), treatment group 2 with 15% *M. oleifera* leaf extract (P_2), treatment group 3 with 20% *M. oleifera* leaf extract (P_3), and treatment group 4 with 25% *M. oleifera* leaf extract (P_4). This research observed that intake of 20% *M. oleifera* leaf extract results in the highest significant decrease of 15.42% of IL-1 level (134.64 ± 1.98 to 113.87 ± 4.30 pg/mL) and decrease of 45.63% of TNF-α level (28.62 ± 1.25 to 15.56 ± 7.20 pg/mL). Therefore, it can be concluded that intake of *M. oleifera* leaf extract by Wistar rat has anti-inflammatory effects on chronic dyslipidemia through decrease of IL-1 and TNF-α levels and histopathology profile. Further research is required to determine whether the application of *M. oleifera* leaf extract (*daun kelor*) in humans will have similar anti-inflammation effects.

Keywords: Consumption habit of fast food; *Moringa oleifera* leaf extract (*daun kelor)*; Dislipidemia; Atherosclerosis.

1. INTRODUCTION

It has been understood that there is a significant correlation between inflammation levels and incidents of atheroschlerosis, a trigger of coronary heart disease. Coronary heart disease presents as a result of blood circulation disturbance and abnormality of cardiac electricity or other forms of arrhythmia. This leads to disorganized myocardial contraction, blood flow obstruction, and blood flow regurgitation. All these conditions result in the return of blood flow to the heart (shunts) in each contraction, blood flow abnormality, and heart failure [1]. Atherosclerosis is a slow, progressive disease, present in large to medium arterial muscle and elastic artery. The main sites of atherosclerosis are abdominal aorta, coronary artery, popliteal artery, thorax descending aorta, carotid internal artery, and willisi circulation. Risk factors such as hypertension, chronic hypercholesterolemia, immune system disturbance, toxin, and virus are also involved in arterial endothelial wall destruction. This damage induces permeability changes of endothelial cells and leads to increase of plasma constituents, such as lipoprotein that can easily enter the artery wall. A damage to these endothelial cells could also change the properties of thrombosistein lumen artery, which can lead to adhesion of thrombosite to the blood and induce inflammation. If this damaging process continues for a long time, it will be followed by continuous atherosclerosis and will lead to thickness of tunica intima and result in disturbance of blood flow in that site [2]. Managing consumption type is one way to overcome this condition. Plasma cholesterol can be increased by rising cholesterol turnover rate. Faster cholesterol replacement can be achieved through intake of *M. oleifera* extract acids. These acids in metabolism act as an antioxidant that could break down saturated fatty acid chains in hyphercholesterolemia patients [3].

Hypercholesterolemia, atheroschlerotic inducer, is a multifactorial diasase that also correlates to proinflammation cytokines, such as IFN-γ, IL-1β, IL-1, IL-,1 and TNF-α. Some researchers reported that atherogenic consumption could increase the formation of IL-1 and TNF-α; however, it did not significantly increase IL-1β [4-6].

Based on the background discussed above, this research was conducted to investigate *M. oleifera* leaf (*daun kelor*) extract (MOE) rich in octadecatrienoic acid is important as an anti-inflammatory agent, decreasing IL-1 and TNF-α levels in chronic dyslipidemia Wistar rats.

2. MATERIALS AND METHODS

This research applied a true experimental randomized pre- and posttest control-group design to determine the role of MOE as an anti-inflammatory agent. Research was conducted using 40 Wistar rats divided into five groups: P_0 for control with 0% MOE, P_1 for treatment with 10% MOE, P_2 for treatment with 15% MOE, P_3 for treatment with 20% MOE, and P_4 for treatment with 25% MOE. The rats were fed with a high-cholesterol diet for 18 weeks to achieve chronic dyslipidemia and atheroschlerosis and then were treated with various concentration of MOE for 6 weeks. IL-1 and TNF-α levels rats serum for chronic dyslipidemia

(pretest) and after treated (posttest) were then detected. All data obtained were analyzed statistically to determine the mean difference of treatment using one-way ANOVA at a 5% significant level.

3. RESULTS

3.1. Decrease of IL-1 Levels

Mean of pre- and posttest data of IL-1 serum levels are presented in Table 1.

Data in Table 1 were normally distributed with $p > 0.05$, and their variance was also homogeneous with $p > 0.05$. The mean difference of various MOE treatments can be performed on the basis of posttest data, if, however, all pretest data are comparable. It was obtained that all the pretest data were comparable with $p < 0.05$; therefore, mean difference of the treatment was obtained based on the posttest data and was analyzed using one-way ANOVA. There were significant differences in the treatment obtained with $p < 0.0$; then the data were analyzed using post hoc test to measure the difference. Post hoc test results are summarized in Table 2.

3.2. Decrease of TNF-α Levels

Pre- and Posttest data of the mean of TNF-α serum levels are presented in Table 3.

Data in Table 3 were normally distributed with $p > 0.05$, and their variance was also homogeneous with $p > 0.05$. All the pretest data were comparable ($p > 0.05$); therefore, the mean difference of various treatments of MOE can only be

Table 1: Serum level data for pre- and posttest mean of IL-1.

Treatment	IL-1 (pg/mL)	
	Pretest	Posttest
MOE 0% (control)	134.58 ± 2.21	133.15 ± 4.01
MOE 10%	134.24 ± 2.64	130.28 ± 3.59
MOE 15%	134.75 ± 2.51	127.20 ± 5.56
MOE 20%	134.64 ± 1.98	113.87 ± 4.30
MOE 25%	135.34 ± 4.57	120.87 ± 7.89

MOE = *M. oleifera* leaf extract.

Table 2: Resume of post hoc test of IL-1 levels.

Treatment		Mean difference of IL-1 (rg/mL)	*p**
MOE 0% (control)	- MOE 10%	2.87	0.232
	- MOE 15%	5.95	0.016
	- MOE 20%	19.28	0.001
	- MOE 25%	12.28	0.001
MOE 10%	- MOE 15%	−3.09	0.201
	- MOE 20%	16.41	0.001
	- MOE 25%	9.41	0.001
MOE 15%	- MOE 20%	13.33	0.001
	- MOE 25%	6.33	0.011
MOE 20%	- MOE 25%	−7.00	0.001

MOE = *M. oleifera* leaf extract.
*Significant $p < 0.05$.

Table 3: Date of the mean of TNF-α serum levels.

Treatment	TNF-α (pg/mL)	
	Pretest	Posttest
MOE 0% (control)	28.98 ± 6.00	28.11 ± 5.94
MOE 10%	29.12 ± 5.79	27.32 ± 5.01
MOE 15%	29.02 ± 5.34	24.42 ± 5.74
MOE 20%	28.62 ± 4.72	15.56 ± 7.20
MOE 25%	29.02 ± 5.06	26.02 ± 8.34

MOE = *M. oleifera* leaf extract.

performed based on the posttest data analyzed using one-way ANOVA. It was obtained that there were differences between all the treatments. Then, the data were analyzed using post hoc test (LSD) to determine the difference. The post hoc test results are presented in Table 4.

3.3. Histopathology Profile

Histopathology structure changes in the aortic tissue of Wistar rats with 100× zoom and HE (hematoxylin eosin) from normal artery (↓) (Figure 1a) without the presence of leucocyte cell infiltration and dyslipidemia (pretest consumtion of fat for 8 weeks) (Figure 1b). The figure reveals the change of artery structure due to protrusion to tunica intima, an indication of mechanical endotel injury. Figure 1c represents chronic dyslipidemia due to intake of fat for 18 weeks in Wistar rats initiated with chronic dyslipidemia, leading to a narrow hole in the aortic artery (↓); there are many artery protrudes to tunica intima, and thickening of tunica media and tunica adventitia occurred. Figure 1c indicates that there is thrombocyte aggregation in the artery (↓) due to intake of fat for 18 weeks. Initiated with chronic dyslipidemia and leading to a narrow hole in the aortic artery (↓), there are many artery protrudes to tunica intima, and thickening of tunica media and tunica adventitia occurred.

Figure 2 indicates that there is thrombolytic to aggregation in the artery (↓) due to intake of fat for 18 weeks. The aggregation was initially an acute change to chronic indicated by red color. Then intake of 0% MOE did not change structure. The presence of leukocyte cells, mainly monocyte cells infiltrated to the lower subendothelail tissue leads to endothelial cell destruction, that is, lumen artery experiencing inflammation compared to Figure 1a (pretest) and Figures 3-6 (picture of posttest artery histopathology structure of chronic dyslipidemia after intake of various concentration of MOE for 6 weeks (10, 15, 20, and 25%), Figure 3 indicates that the presence of thrombocyte aggregation in the artery (↓) thickens tunica media and wider tunica adventitia are indicated by red color. Intake of 15% MOE could not reduce thrombocyte aggregation. Figure 4 reveals the presence of a small thrombocyte aggregation in the artery (↓). Also, there is an aggregation in their tunica media indicated by red color. intake of 20% MOE. Figure 5 indicates no thrombocyte aggregation in the artery (↓); therefore, intake of 20% MOE results in the reduction of thrombocyte aggregation. Figure 6 indicates the appearance of thrombocyte aggregation (↓) anymore after intake of 25% SLO red color thicken of tunica media.

Table 4: Resume of post hoc test of TNF-α levels.

Treatment		Mean difference of TNF-α (pg/mL)	*p**
MOE 0% (control)	- MOE 10%	0.79	0.770
	- MOE 15%	3.69	0.177
	- MOE 20%	12.55	0.001
	- MOE25%	1.10	0.686
MOE 10%	- MOE 15%	2.90	0.287
	- MOE 20%	11.76	0.001
	- MOE 25%	0.30	0.911
MOE 15%	- MOE 20%	8.86	0.002
	- MOE 25%	−2.59	0.339
MOE 20%	- MOE 25%	−1.45	0.001

MOE = *M. Oleifera* leaf extract.
*significant $p < 0.05$.

Figure 1: (a) Histopathology profile pretest in normally Wistar rat (b) Histopathology profile pretest of consumption fat at 8 weeks in Wistar rat; (c) Histopathology profile pretest of consumption fat at 18 weeks in Wistar rat.

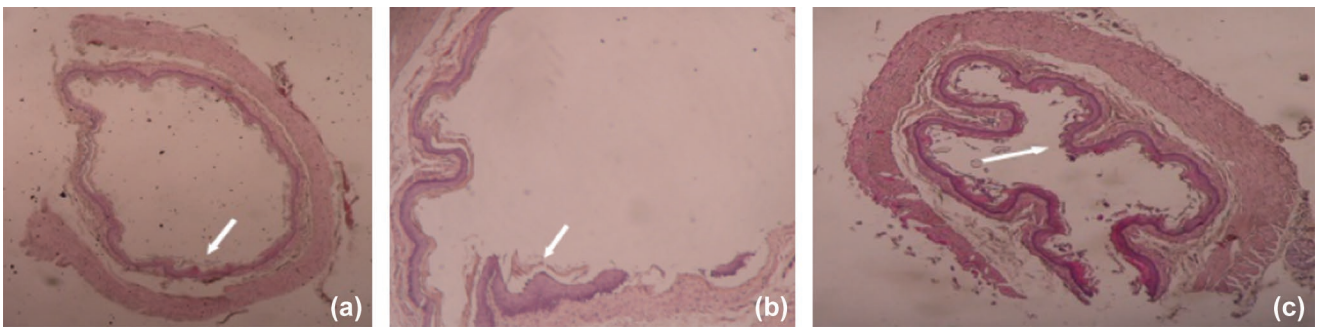


Figure 2: Histopathology profile posttest P_0.

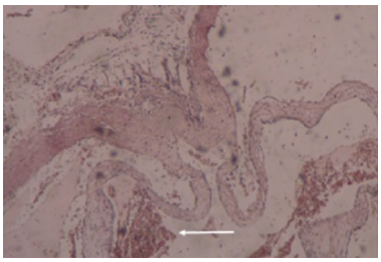


Figure 3: Histopathology profile posttest P_1.

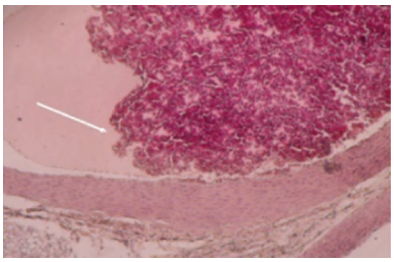


Figure 4: Histopathology profile posttest P_2.

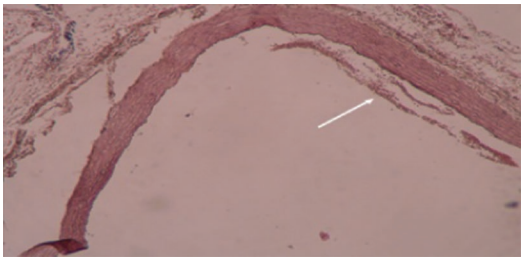


Figure 5: Histopathology profile posttest P_3.

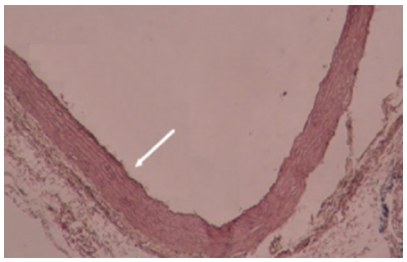
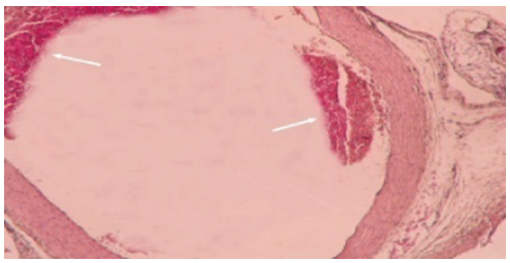


Figure 6: Histopathology profile posttest P_4.



4. DISCUSSION

The research results indicate that the highest decrease of 12.55 pg/mL of TNF-α was obtained for the intake of 20% MOE. The increase of MOE to 25% could not increase TNF-α levels. This condition indicates that concentration of 25% MOE has already saturated; therefore, it could not decrease TNF-α levels any further. This was supported by the findings of Chen and Goeddel [7]; they found that there was no transcription of NF-$\kappa\beta$, so there was no further production of TNF-α due to saturation.

Inflammation is a response of tissue damage during vascularization. This response is followed by an important process, such as endothelial process. Endothelium is an important part of blood vein that plays an important role in atheroschlerosis. Endothelium is the main target of mechanical and chemical damage due to dislipidemia risk factor. Chronic, continues, and prolong dislipidemia resulted in proinflammation response and prothrombic, which were initially acute but became chronic. This is followed by infiltration of leucocyte cells, mainly monocyte cells to lower subendothelial tissue to form macrophage cells. These cells destroy the rest of LDL-C and oxidized to form foam cells and change to ateroma [8].

The last two decades' research claims that *M. oleifera* is effective as an anti–inflammatory agent. This is because *M. oleifera* leaf is rich in octadecatrienoic acid. The fatty acids are a group of polyunsaturated fatty acids with double bond at the third carbon atom (from methyl group), which are known as omega-3 [4-6]. In this study, MOE which is rich in omega-3 was applied and proved to exceed an anti-inflammation effect. This anti-inflammation effect is due to activation of endothelium nuclear factor-kapa beta (ENF-$\kappa\beta$) on peripheral vein. ENF-$\kappa\beta$ is a transcription factor distributed in all endothelial cells that have a role in controlling vascularization.

Simopulus [9] presented that the role of omega-3 as an anti-inflammation is due to their action as an immunomodulator. In addition, their role as an anti-inflammation agent is a result of the effect of octadecatrienoic acids. These acids are a substrate for triggering the formation of cyclooxygenase and 5-lipooxyginase. These two oxygenases have vasodilator endothelium-dependent behavior to cause relaxation of ordinary coronary artery and paradoxal vasoconstruction in the atherosclerotic artery.

Data in Table 1 indicate that there is a decrease of IL-1 levels since the intake of 10% of MOE. Even though there is a decrease of IL-1 levels caused by the treatment of 10% MOE, which is about 2.87 pg/mL, the decrease is not statistically significant, with $p > 0.05$. Then, intake of 15% MOE resulted in a significant decrease of 5.95 pg/mL of IL-1 levels, with $p < 0.05$. In addition, intake of 20% MOE has also a similar significant decrease of 19.28 pg/mL of IL-1 levels, with $p < 0.05$. However, the increase of concentration to 25% MOE intake did not significantly decrease IL-1 levels, indicated by $p > 0.05$.

MOE is rich in octadecatrienoic acid. These acids are grouped to alfa linolenate that has an ability as an anti-inflammation. During endothelial cells experiencing activated inflammation, it will be followed by increase of selectin and VCAM-1 expression. VICAM-1 induces monocyte adhesion. This adhesion was also induced by proinflammation cytokines, such as 1L-1β and TNF-α. These cytokines were induced by CRP protein produced as a result of IL-1 response by protease-activated receptor signaling, uptake of oxLDL, through oxLDL receptor-1 (LOX-1), and by interaction of CD40/CD40 ligand in artery intima [10]. IL-1 has an important role in inflammation response, and this cytokine is secreted by activated macrophage, leading to phebric and known as pyrogen endogen. IL-1 was also initiated by phase acute response and marked by protein phase acute produced by hepatocyte [11,12].

5. CONCLUSIONS

1. Intake of 20% MOE decreases IL-1 serum levels in dyslipidemia Wistar rats around 15.42%, that is, from 134.64 $\pm$ 1.98 to 113.87 $\pm$ 4.30 pg/mL.
2. Intake of 20% MOE decreases TNF-α serum levels in dyslipidemia Wistar rats around 45.63%, that is, from 28.62 $\pm$ 4.72 to 15.56 $\pm$ 7.20 pg/mL.

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Author Contributions
Both authors contributed equally.

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Conflict of Interest
None

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