European Journal of Biomedical Research
Vol 3 | Issue 5 | November 2024
ISSN 2796-0056

RESEARCH ARTICLE

The Effect of Green Gedi Leaves Ethanol Extract (Abelmoschus Manihot) Towards TNF-Alpha Levels, Amount Foam Cells, Aorta Wall Thickness and Lee Index in Male Wistar Rats Induced by High-Fat Diet

Lisye Marrilyn Lukas[1,*], Ni Made Linawati[2], I Wayan Sumardika[3], Ida Ayu Alit Widhiartini[4], I Wayan Weta[5], and Luh Putu Iin Indrayani Maker[6]

ABSTRACT

CHD is a chronic disease of narrowing of the heart's blood vessels due to atherosclerosis. The cause of atherosclerosis is by consuming a high-fat diet (HFD) for a long period of time will triggers inflammatory processes and oxidative stress. Green gedi leaves (*Abelmoschus manihot*) have the potential to inhibit this process because consist of rich antioxidants in the form of flavonoids, tannins, etc. This study aims to prove the effect of ethanol extract of green gedi leaves toward TNF-α levels, foam cells, aortic wall thickness and Lee index. This is an experimental study *posttest control group design*. 18 male Wistar white rats (*Rattus novergicus*) aged 2–3 months were induced by an initial adrenalin injection of 0.006 mg/ 200 gr of rat weight and HFD consisted of diet 594, pork fat and duck egg yolk. Samples were divided into 3 groups: the normal group (KN) with a standard diet, the negative control group (P0) WITH adrenaline injection and HFD, and the treatment group (P1) with adrenaline injection, HFD and ethanol extract of green gedi leaves 200 mg/kg body weight. After 21 days the body weight and naso-anal length of the rats were measured, blood sample was taken for TNF-α level results. And abdominal aorta tissue was fixed and stained using Hematoxylin Eosin to count the amount of foam cells and aortic thickness. From *One Way Anova* parametric test showed that the P1 group had a lower mean of TNF-α level 77.82 ± 0.96 pg/mL compared to the P0 group 264.26 ± 0.46 pg/mL and KN group 189.14 ± 0.96 with ($p < 0.001$). From the statistical results of the *Mann-Whitney* test, there are no significant differences for the amount of foam cells P1 groups mean 3.17 ± 2.13 /PVF ($p = 0.557$), groups KN 3.33 ± 3.26 /PVF and P0 group 3.5 ± 3.5 /LPF. P aortic wall thickness P1 group mean was 79.45 ± 7.61 μm lower than the P0 group 99.83 ± 14.04 ($p = 0.04$) and KN group 79.76 ± 6.71 μm. Index Lee KN group mean 0.27 ± 0.01 gr/mm and P1 group 0.29 ± 0.01 gr/mm and P1 group 0.28 ± 0.01 gr/mm. Extract ethanol green gedi leaves with dossage 200 mg/rat body wight can decreased TNF-α level, aorta thickness and index Lee towards rats induced by high fat diet.

Keywords: *Abelmoschus manihot*, foam cell, gedi leaves, TNF-α.

Submitted: May 20, 2024

Published: November 27, 2024

10.24018/ejbiomed.2024.3.5.96

[1] *Master Program in Biomedical Science, Concentration in Anti-Aging Medicine, Faculty of Medicine, Udayana University, Indonesia.*
[2] *Department of Histology, Faculty of Medicine, Udayana University, Indonesia.*
[3] *Head of the Bachelor Medical Study Program, Faculty of Medicine, Udayana University, Indonesia.*
[4] *Department of Pharmacology, Faculty of Medicine, Udayana University, Indonesia.*
[5] *Department of Nutritional Sciences, Faculty of Medicine, Udayana University, Prof. I.G.N.G Ngoerah Hospital, Indonesia.*
[6] *Department of Anatomical Pathology, Faculty of Medicine, Udayana University, Indonesia.*

Corresponding Author:
e-mail: ct.gregorius@gmail.com

1. Introduction

Obesity or overweight is a disease characterized by an increase in body fat mass above normal. Obesity is a serious problem because it has the potential to trigger chronic metabolic-degenerative diseases as a companion. Comorbidities from obesity such as metabolic syndrome (central obesity, insulin resistance, diabetes, dyslipidemia, hypertension) which will lead to cardiovascular disease are increasing in prevalence globally [1]. Every year there are 74,000 deaths due to CHD which is the main cause of death due to non-communicable diseases in the world, this shows that 200 people die every day due to CHD [2]. Considering the pathogenesis of the formation of atherosclerosis lesions mentioned above, it is necessary to explore natural ingredients that have the potential to prevent or control inflammation and oxidative stress. The government is encouraging the use of natural ingredients because Indonesia is rich in natural ingredients with side effects minimal. One of the natural ingredients that is often consumed by people in Sulawesi as vegetable is green gedi leaves (*Abelmoschus manihot*).

The content components obtained and identified from the flowers, seeds, stems and leaves of the gedi plant *(Abelmoschus manihot)* are flavonoids, amino acids, nucleosides, polysaccharides, organic acids, steroids and essential oils [3]. Flavonoids contain polyphenolic structures, the majority of which are found in various kinds of fruits, vegetables and certain drinks. Because they contain various biochemical and antioxidant effects, flavonoids are known to play an important role and provide protective effects on the cardiovascular system [4]. The antioxidants contained in flavonoids work by neutralizing free radicals by providing one hydrogen atom to compounds that are oxidants so that the oxidant compounds become stable [5].

2. Materials and Methods

This study compares the normal group (KN) normally diet with positive control (P0) rats induced by initial injection of adrenalin 0.006 mg/kg body weight and HFD, treatment group (P1) rats induced by initial injection of adrenalin 0.006 mg/kg body weight, HFD and Extract Ethanol of green gedi leaves (Abelmoschus manihot) 200 mg/kg body weight. In inhibiting the increase of TNF-α levels, amount of foam cells, aortic thickness and Lee index. 18 male Wistar rats were divided into 3 groups and treated according to each group's guidelines. In the first day, the body weight and naso-anal length of the rats were measured and recorded. After the last exposure 21 days, the body weight and naso-anal length of the rats were measured, blood sample was taken to count TNF-α levels. And abdominal aorta tissue was fixed and stained using Hematoxylin Eosin to count amount of foam cells and aortic wall thickness.

2.1. Initial Adrenalin Injection

Initial adrenalin given just one time on the first day with dosage 0.006 mg/200 gr rat weight by intramuscular injection in the posterior rat thighs.

2.2. High Fat Diet

Rats were induced with a high-fat diet that combination consisted of 80% of animal feed 594, and 15% of lard. The other 5% of duck egg yolk is given by an orogastric tube (OGT).

2.3. Blood and Histopathologic Examination

On the first day, the body weight and naso-anal length of all groups of rats were measured and recorded. Also, an initial injection of adrenalin 0.006 mg/200 gr rat body weight was given to rats in the P0 and P1 groups just one time in the first day. 3 groups of samples KN, P0 and P1 each treated according to each group's guidelines. Food and water consumption were recorded daily. At the end of the experiment after 21 days body weight and naso-anal length of all groups of rats again were measured and recorded, the rats were anesthetized by injecting Ketamine, blood samples for TNF-α examination were taken with a pipette at the retro-orbital plexus and then rats killed by cervical dislocation. Then the abdominal aorta was fixed with 10% phosphate-buffered saline Formalin for 24 hours. Then stained with Hematoxylin-Eosin staining. Hematoxylin-Eosin staining by observing the number of foam cells with 400x magnification in 5 fields of view, observations and calculations were made by shifting the preparation from left to right then photographed and averaged. And aorta wall thickness with 100x magnification in 4 zones clockwise at 9, 12, 3 and 6 o'clock, observing and calculating the aorta wall thickness in the 4 zones clockwise then photographing and averaging. record each result and then test with SPSS version 25.

3. Results and Discussion

Table I shows that there is a significant difference in the TNF-α levels in each group between the normal group (KN) with a standard diet, the negative control group (P0) with adrenaline injection 0.006 mg/200 gr rat weight and HFD, and the treatment group (P1) with adrenaline injection, HFD and ethanol extract of green gedi leaves 200 mg/kg body weight ($p < 0.001$). The mean of TNF-α on group KN was 189.149 pg/mL P0 was 264.2622 pg/mL and P1 was 77.8273 mg/dL shown in Fig. 1. Aortic wall thickness shown in Fig. 2. where there are thicker in group P1 than group KN and P1. The P1 group appear to have the thinnest aorta wall thickness than the others group.

Based on the results of the Kruskal Wallis test for foam cells (Table II), aorta wall thickness and Lee index, a value of $p = 0.793$ ($p > 0.05$) was obtained for the number of foam cells and it was concluded that there was no difference in the number of foam cells observed in the KN, P0 and P1 groups. Meanwhile, the values obtained from the aorta wall thickness and Lee index obtained significant

TABLE I: Mean Difference in the Numbers of TNF-α Levels between Groups Sample

	Groups	*N*	Mean	SD	*p-value*
TNF-α	KN	6	189.1495	1.22978	0.001
	P0	6	264.2622	0.46158	
	P1	6	77.8273	0.96759	

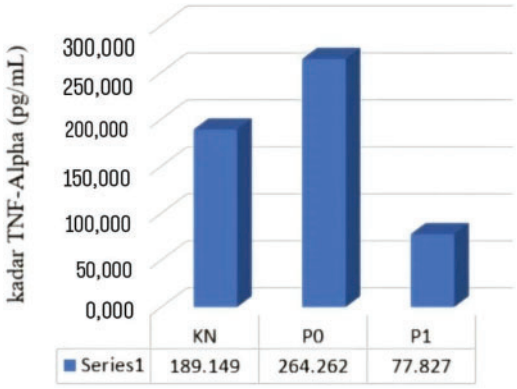

Fig. 1. Mean number of TNF-α levels.

TABLE II: Kruskal Wallis Test of Foam Cells, Aorta Thickness and Lee Index

	Foam cells	Aorta thickness	Lee index
Chi-square	0.464	10.819	12.312
Df	2	2	2
Asymp sig	0.793	0.004	0.002

values, namely p = 0.004 and p = 0.002 (p < 0.05). So, it can be concluded that in the aorta wall thickness and Lee index, there were differences observed in the KN, P0 and P1 groups.

Figs. 2A–2C show histological features of the aortic abdominal of rats after treatment (Haematoxylin eosin staining, magnification 100x). The measure shown in Table III. Aortic wall shows on indicates the aorta thickness is more visible in group P0 (B) than KN (A) and P1 (C). Group P1 show the thinnest thickness of aortic wall thickness. Figs. 2D–2F show histological features of the number of foam cells in rats after treatment (Haemotoxylin eosin staining, with 400x magnification). The arrow indicates the form of a foam cell. The measure is show in Table III. where the highest mean of foam cells is group P0 (E). The second is group P1 (D) and the last is group KN (F).

From the Table III, we can conclude ethanol extract green gedi leaves (Abelmoschus Manihot) give results by inhibiting TNF-α levels, aorta wall thickness and Lee index.

3.1. Effect of Ethanol Extract of Green Gedi Leaves Towards TNF-α, Foam Cells, Aortic Thickness and Index Lee

In conditions of obesity, inflammatory processes occur, including excessive lipogenesis, inhibition of lipolysis, and increased adipocyte apoptosis. Adipocyte cells in obesity can produce cytokines through inflammatory mediators which will ultimately increase the release of reactive oxygen species (ROS) and will cause a condition called oxidative

TABLE III: Mean Difference in the Amount of Foam Cells, Aorta Thickness and Lee Index Between Groups Sample

	Group	N	Mean rank
Foam cells	KN	6	8.75
	P0	6	10.67
	P1	6	9.08
	Total	18	
Aortic thickness	KN	6	6.17
	P0	6	15.33
	P1	6	7.00
	Total	18	
Lee index	KN	6	4.33
	P0	6	14.50
	P1	6	9.67
	Total	18	

stress. Oxidative stress is a condition when the number of free radicals in the body exceeds the body's ability to neutralize them [6].

TNF-α is a cytokine produced mainly by activated macrophages and dendritic cells as well as by T lymphocytes and mast cells during an inflammatory reaction [7]. Excessive fat consumption can cause hyperlipidemia by increasing blood fat levels obtained from food intake/HFD. Hyperlipidemia can disrupt endothelial function by increasing the formation of oxygen free radicals. Changes in the chemical process of fat are triggered by free radicals produced by macrophages or endothelial cells in the arterial walls which will produce oxidized LDL which will then be captured by macrophages until the macrophages turn into foam cells which will trigger the migration of smooth muscle cells from the tunica media into the tunica intima and trigger cell proliferation. smooth muscle in the intima resulting in thickening of the walls of blood vessels [8].

The results of this study were that administration of ethanol extract from green gedi leaves was proven to inhibit the increase of TNF-α, aorta wall thickness and Lee index which was statistically significant for the normal control group, the negative control and the treatment group had a value of (p < 0.05). In general, antioxidants facilitate the treatment of atherosclerosis through various mechanisms, including inhibition of LDL oxidation, reduction of reactive oxygen species produced, inhibition of cytokine secretion, prevention of atherosclerotic plaque formation and platelet aggregation, prevention of mononuclear cell infiltration, improvement of endothelial dysfunction and vasodilation, increasing NO bioavailability, modulates the expression of adhesion molecules such as VCAM-1 and ICAM-1 on endothelial cells, and inhibits foam cell formation [8].

4. Conclusion

Based on the results of the study, the ethanol extract of green gedi leaves (Abelmoschus Manihot) 200 mg/kg body weight inhibited the increase of TNF-α, aorta thickness and Lee index. This is because gedi leaves can act as an antioxidant and enhance the role of cardioprotective in protecting the heart and preventing

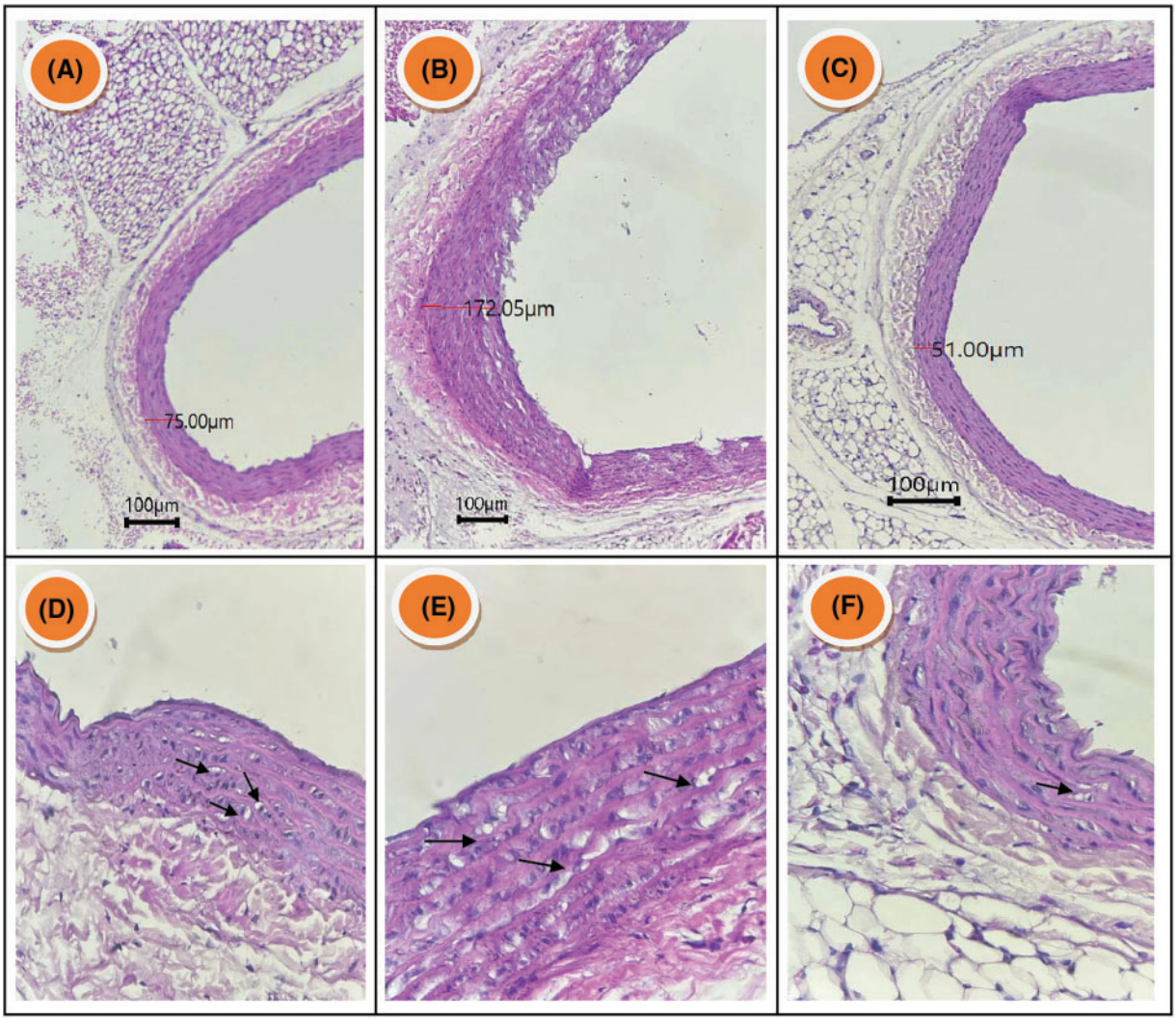

Fig. 2. Aorta thickness and amount of foam cells: (A) KN, aortic thickness H&E 100x, (B) P0, aortic thickness H&E 100x, (C) P1, aortic thickness H&E 100x, (D) KN, H&E foam cells 400x, (E) P0, H&E foam cells 400x, and (F) P1, HE foam cells 400x.

damage from exposure to a high-fat diet. For foam cells, the result is not significant and can be predicted the reason is research time is too short and foam cells have not yet formed.

Acknowledgment

Authors would like to thank the Rector of Udayana University, Prof. Ir. Ngakan Putu Gede Suardana, M.T., Ph.D.; the Dean of the Faculty of Medicine, Udayana University, Prof. Dr. Komang Januartha Putra Pinatih, M. Kes; and the Coordinator of the Master of Biomedical Sciences Study Programme, Faculty of Medicine, UdayanaUniversity, Dr. dr. I Made Muliarta, and M. Kes, the examining lecturers who have provided valuable input, suggestions, rebuttals, guidance, and corrections, allowing this scientific work to be completed properly.

Funding

The costs of this research were covered by the personal expenses of the researchers.

Conflict of Interest

Authors declare that they do not have any conflict of interest.

References

[1] Weta I. *Orasi Ilmiah: Obesitas Pembuka Pintu Berbagai Penyakit*. Universitas Udayana; 2020, pp. 3–33.

[2] Gerhard-Herman MD, Gornik HL, Barrett C, Barshes NR, Corriere MA, Drachman DE, *et al.* 2016 AHA/ACC guideline on the management of patients with lower extremity peripheral artery disease: a report of the American college of cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation*. 2017;135:726–79.

[3] Luan F, Wu Q, Yang Y, Lv H, Liu D, Gan Z, *et al.* Traditional uses, chemical constituents, biological properties, clinical settings, and toxicities of Abelmoschus manihot L.: a comprehensive review. *Front Pharmacol*. 2020;11(August):1–28.

[4] Tordjman S, Najjar I, Bellissant E, Anderson GM, Barburoth M, Cohen D, *et al.* Advances in the research of melatonin in autism spectrum disorders: literature review and new perspectives. *Int J Mol Sci*. 2013;14:20508–42.

[5] Hilma H, Putri NAD, Lely N. Penetuan total fenol dan total flavonoid ekstrak daun kelengkeng (Dimoncarpus longan Lour). *J Ilm Farm Bahari*. 2021;12(1):80.

[6] Susantiningsih T. Obesitas dan stress Oksidatif. *J Kesehat Univ Lampung*. 2015;5(9):219–25.

[7] Kumar, Abbas A, Aster J, Turner J. *Robbins & Cotran Pathologic Basis of Disease Tenth Edition*. 10th ed. New York: Elsevier Inc; 2021, pp. 112.
[8] Malekmohammad K, Sewell RDE, Rafieian-Kopaei M. Antioxidants and atherosclerosis: mechanistic aspects. *Biomolecules*. 2019;9(8):1–19.