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DOI: [10.31965/infokes.Vol22.Iss2.1554](https://doi.org/10.31965/infokes.Vol22.Iss2.1554)Journal homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/infokes>**RESEARCH****Open Access****The Effect of Flavonoids of *Phaleria macrocarpa* Fruit Extract on Aortic Diameter Mice Menopause Model****Ani Khoirinda^{1a*}, R.A. Rahmawati Nurul Fadilah^{1b}, Sutrisno^{2c}, Yahya Irwanto^{2d}, R.A. Rose Khasana Dewi^{3e}**¹ Master Program of Midwifery, Department of Midwifery, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia² Department of Obstetric and Gynecology, Brawijaya University, Malang, East Java, Indonesia³ Department of Anatomical Pathology, Brawijaya University, Malang, East Java, Indonesia^a Email address: anikhairinda@gmail.com^b Email address: rahmawatinurulfadilah@gmail.com^c Email address: snospog@gmail.com^d Email address: yahyairwanto50@gmail.com^e Email address: rosenade.dr@gmail.com

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Abstract

Menopause is a phase experienced by women with an age range of 45-55 years. Menopause is a condition where menstruation stops for a minimum of 12 consecutive months due to the decline in ovarian function, leading to a reduction in estrogen levels. A decrease in estrogen can lead to impaired fat metabolism resulting in atherosclerosis. This study aimed to illustrate the influence of flavonoid extract derived from *Phaleria Macrocarpa* on the enlargement of the aorta's diameter of mice with a menopause condition. The method of this study is a genuine experimental laboratory setting with a research design of a Randomized Post Test Only Control Group setting. Using 32 female mice divided into 6 groups: K- (without ovariectomy and flavonoid extract *Phaleria Macrocarpa*), K+ (ovariectomy without treatment), P1 (ovariectomy + dose 3.75 mg/mice/day), P2 (ovariectomy + dose 7.5 mg/mice/day), P3 (ovariectomy + dose 11.25 mg/mice/day), and P4 (ovariectomy + dose 15 mg/mice/day). Administration of flavonoid extract *Phaleria Macrocarpa* was carried out for 14 days. Data analysis using statistical analysis. The results showed that in a post-hoc test, namely the administration of *Phaleria macrocarpa* flavonoid extract at a dose of 11.25 mg/mice/day and 15 mg/mice/day showed that it could increase the dilation of the aortic diameter of mice model menopause. The study concludes that the flavonoid fruit extract from *Phaleria Macrocarpa* has the ability to increase the width of the aorta in mice with a menopause condition. In future studies, it is recommended to investigate various variables in order to identify the factors that contribute to the narrowing of the aorta. Additionally, it is suggested to perform further research specifically focusing on women going through menopause.

Keywords: Menopause, *Phaleria macrocarpa*, Diameter.***Corresponding Author:**

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1. INTRODUCTION

Menopause is a physiological state that occurs in women. Menopause is characterized by the cessation of menstruation for a continuous period of 12 months and is attributed to the irreversible decline in ovarian follicular activity (WHO, 2022; Zhu et al., 2020). According to WHO, the average age at which women experience menopause ranges from 45 to 55 years. While the age in early menopause women is <40 years which can be caused by abnormalities such as chromosomes, or immune or other unknown causes (WHO, 2022). The number of menopause women in Indonesia increased from 15.8 million people in 2017 to 30.3 million people in 2020 (BPS, Bappenas dan UNFPA Indonesia, 2008). This suggests that the number of menopause women in Indonesia is increasing. Menopause is a condition that marks the start of the disease's onset (Macpherson & Quinton, 2022).

Ovarian follicular dysfunction results in a reduction in estrogen or hypoestrogenemia. Hypoestrogens are associated with fat metabolism disorders that have a risk of lipid peroxidation so that it can cause atherosclerosis as a risk of Cardio Vascular disease (CVD) (Newson, 2018). The incidence rate of CVD caused by atherosclerosis or so-called Therosclerotic Cardiovascular Disease (ASCVD) in women with an average age of 69 years in Canada from April 1, 2002, to March 31, 2018, was 432,300 from 1,042,621 (Hopper et al., 2021).

Menopause women may experience hypercholesterolemia due to a reduction in estrogen, resulting in elevated levels of low density lipoprotein (LDL) (Pratiwi & Damayanty, 2020). The progressive elevation of LDL levels and its interaction with reactive oxygen species (ROS) leads to the oxidation of LDL, resulting in the formation of Ox-LDL. Continuous increase in ROS results in oxidation stress or tissue damage such as endothelial dysfunction and decreased nitrite (NO) oxidation (Javadifar et al., 2021; Sargowo, 2015). Oxidized low-density lipoprotein (Ox-LDL) can stimulate the expression of lectin-1 (LOX-1) receptors for oxidized LDL (Li & Mehta, 2009). The binding of oxidized low-density lipoprotein (Ox-LDL) to lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) causes an upregulation in the production of adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), and cytokines, including monocyte-chemoattractant-protein-1 (MCP-1). This, in turn, leads to the attachment of monocytes to endothelial cells (Kattoor et al., 2019). The increase in monocyte recruitment to the tunica intima and differentiation into macrophages is due to an increase in LDL in the subendothelium (Javadifar et al., 2021). Scavengers found in macrophages can recognize Ox-LDL and engulf it into foam cells. So that the pile of foam cells will become atheromatous plaques. An increase in atheromatous plaques causes thickening of the tunica intima media and narrowing of the diameter of the aorta which leads to atherosclerosis (Khatana et al., 2020; Sargowo, 2015).

Hormone Replacement Therapy (HRT) is a treatment given to someone who has hormonal disorders, namely a decrease in estrogen levels, one of which is menopause women (Khoudary et al., 2020). Hormone Replacement Therapy (HRT) has the capacity to decelerate the process of adipose accumulation and atherosclerosis in women experiencing menopause. HRT in postmenopause women remains controversial in the prevention of Therosclerotic Cardiovascular Disease (ASCVD) due to long-term side effects, including increased incidence of stroke, lipid metabolism, pulmonary embolism, breast and uterine cancer, vaginal bleeding, and impaired liver function (Goldštajn et al., 2023; Nayak et al., 2022; Yousefzadeh et al., 2020).

Thus, it is necessary to explore alternate preventive measures, particularly natural substances that have minimal adverse effects and function similarly to estrogen. One such group of molecules is phytoestrogens. Chemically, phytoestrogens are phenolic or polyphenolic phytochemicals. It is the largest and most widely distributed phytochemical category in the

plant world (Bacciottini et al., 2007). One of the phytoestrogens is the flavonoid extract *Phaleria macrocarpa* which is widely found in nature, especially in Indonesia (Ahmad et al., 2023). Flavonoids in *Phaleria macrocarpa* fruit have potential as anti-microbial, anti-bacterial, antifungal, anti-allergic, antioxidant, and vasodilator (Fitriana et al., 2023). In the flesh of *Phaleria macrocarpa*, there are six kinds of flavonoid compounds. 70% ethanol extract of the *Phaleria macrocarpa* fruit has the largest relative flavonoid content of 45.734 µg/mg (Maharani & Sutrisno, 2021). By attaching to oestrogen receptors, phytoestrogens in plants can replicate or alter the effects of endogenous oestrogens. The endogenous estrogen is 17β-estradiol, mainly by binding to the estrogen receptor (ER). Phytoestrogens have an impact on oestrogen receptors, but they can also have antioxidant properties (Forslund & Anderson, 2017; Hasanah et al., 2020; Kuhnle et al., 2009).

Novelty in this study is looking at the effect of giving *Phaleria macrocarpa* fruit extract where flavonoids isolated specifically can be one of the active substances for phytoestrogens. Other studies on the effects of flavonoids of *Phaleria macrocarpa* fruit extract gave good results such as endometriosis and diabetes but until now there have been no studies related to menopause. The objective of this study was to examine the impact of flavonoids derived from the extract of *Phaleria macrocarpa* on the aortic diameter of menopause mice.

2. RESEARCH METHOD

This study employed a real experimental laboratory design with a research design of Randomized Post Test Only Control Group Design. The sample used was mice (*Mus musculus*) with a menopause model. Healthy mice were obtained from the Farma Veterinary Center (Pusvetma) of East Java Province. The number of mice used can be determined by counting the sample size using the formula $(t-1)(n-1) \geq 15$, where t represents the number of groups and n represents the number of mice used. This study utilizes a total of 32 mice, with 30 of them being allocated to 6 different treatment groups. The remaining 2 mice will be used for FSH checking, with one mice assigned to the control group and the other to the treatment group. FSH checking aims to check whether mice are already in menopause or not, if there is an increase in FSH in mice in the treatment group compared to the control group, then mice can be tied already in menopause (Rodríguez-Landa, 2022). The place to conduct research is the Embryology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga as a place for maintaining mice (*Mus musculus*), making menopause model mice, and giving flavonoid treatment of *P. macrocarpa* extract and the Anatomical Pathology Laboratory of the Faculty of Medicine, Universitas Brawijaya for aortic diameter examination.

Six groups in this study consisted of K- (control group, mice were not ovariectomy and were not given flavonoid extract *P. macrocarpa*), K+ (positive group, mice ovariectomy and not given flavonoid extract *P. macrocarpa*), P1 (ovariectomy and given flavonoid extract *P. macrocarpa* dose 3.75 mg/mice/day), P2 (ovariectomy and given flavonoid extract *P. macrocarpa* dose 7.5 mg/mice/day), P3 (ovariectomy and given flavonoid extract *P. macrocarpa* dose 11.25 mg/mice/day), and P4 (ovariectomy and given flavonoid extract *P. macrocarpa* dose 15 mg/mice/day) (Maharani & Sutrisno, 2021). Mice were ovariectomy in the 8th with a recovery period of 28 days. Ovariectomy is done by removing both ovaries of mice or called bilateral ovariectomy. After the recovery period, 2 mice were surgically removed for FSH examination. The mice are positioned on the underpad and then observed to assess the complete drying of the sutures. Once dry, the underpad is substituted with husks, the mice are treated with antibiotics, and they are provided with daily food and water. After FSH rose, mice were given treatment, namely *P. macrocarpa* flavonoid extract with a dose according to the group for 14 days. After treatment for 14 days, the termination and removal of the aortic and femoral organs was carried out.

P. macrocarpa fruit is obtained from Batu City, East Java and processed in the Batu materia medica laboratory. The part of the fruit used in this study is the peel and the ripe flesh is characterized by a maroon color on the skin of the fruit, from which part of the seed is removed. After the *P. macrocarpa* fruit is mashed and soaked in 96% ethanol for \pm 30 minutes and allowed to stand for 5 days until settled. Next, filter or filter the marinade with a *buncher* funnel to get maserat. Maserat from the *P. macrocarpa* which still contains ethanol solvent, then the solvent is evaporated with an evaporator for 8 hours with a temperature of 60 ° C to obtain a thick extract. It is then fractionated by a liquid-liquid process using n-hexane and n-butanol partitions (Maharani et al., 2021).

After 14 days of administration of *P. macrocarpa* extract, the next stage is termination for organ harvesting and preparation making. Before surgery, mice are injected with ketamine. Then dissected and cut the *descending thoracic aorta*, the organs are placed in different bottles containing 10% formalin for 7-24 hours and make sure all the organs are submerged. After that, the organ is cut in the aorta. Checking to see the diameter of the aorta is done by *Hematoxylin-Eosin* (HE) staining. Each organ will be cut with a thickness of 3-4 μ m. Aortic measurement is done by drawing a line from the intima to the intima by measuring the largest and smallest diameters. The colored organs were scanned using Aperio CS2 Leica and calculated using the ImageScopex64 application.

Analysis statistics the data from the comparison of the treatment group and the control group's aorta diameter in menopause mice model. Normality test using the Shapiro-wilk Test, homogeneity test using *Leven Test* method, One-way ANOVA Test, and *Post hoc* test using tukey HSD method.

Research actions are in accordance with the applicable research code of ethics and have been approved by the Faculty of Medicine, Brawijaya University, Malang, Indonesia, by issuing an ethics number: 26/EC/KEPK-S2/01/2024.

3. RESULTS AND DISCUSSION

a. Diameter of mice in a menopause model

The results of staining the thickness of the diameter of the aorta of mice menopause model with Hematoxylin-Eosin staining and observations were made using the ImageScopex64 application with a magnification of 400X given flavonoid treatment of *P. macrocarpa* fruit extract with 4 different doses, the following results were obtained:

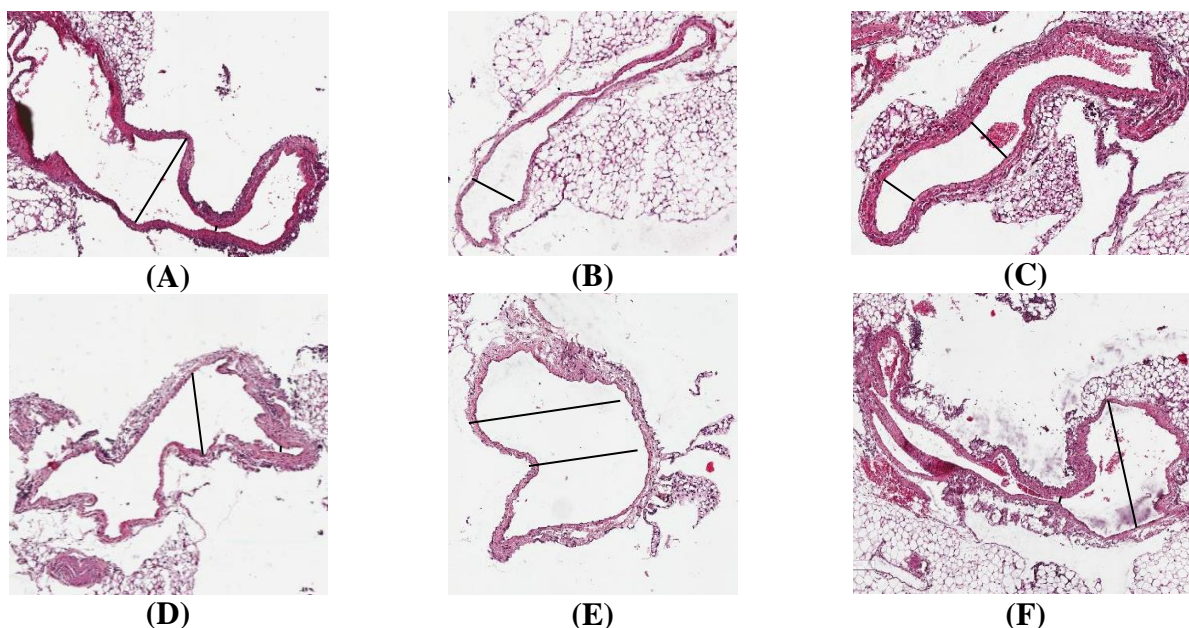


Figure 1. Histopathology of the diameter of the mice aorta. Image with 40x magnification with HE coloring. (A) K- (without ovariectomy and without administration of flavonoid extract *P. macrocarpa*), (B) K+ (ovariectomy and without administration of flavonoid extract *P. macrocarpa*), (C) P1 (ovariectomy and administration of flavonoid extract *P. macrocarpa* dose of 3.75 mg/mice/day), (D) P2 (ovariectomy and administration of flavonoid extract *P. macrocarpa* dose 7.5 mg/mice/day), (E) P3 (ovariectomy and administration of flavonoid extract *P. macrocarpa* dose 11.25 mg/mice/day), (F) P4 (ovariectomy and administration of flavonoid extract *P. macrocarpa* dose 15 mg/mice/day).

Table 1. Normality and Homogeneity Test on Aortic Diameter Mice Menopause Model

Group	N	<i>p-value</i> <i>Shapiro- Wilk test</i>	Data distribution	<i>p-value</i> <i>Levene test</i>	Data Homogeneity
KN	4	0.403	Normal	0.226	Homogen
KP	4	0.422			
P1	4	0.158			
P2	4	0.764			
P3	4	0.198			
P4	4	0.444			
Total	24	0.365			

The Shapiro-Wilk test was conducted to assess the normality of the data from the six groups. The obtained result, with a significance level of 0.365 ($p > 0.05$), indicates that the data can be considered normally distributed. After the data was distributed normally, the homogeneity test continued with the *Levene test* method and obtained the result sig = 0.226 ($p > 0.05$) which showed that the data variant was homogeneous. Both tests are a requirement to perform the *One-way ANOVA test*.

The average results of aortic diameter from untreated (K-) and treated mice samples in the positive control group and treatment group with 4 different doses of *P. macrocarpa* flavonoid extract are shown in the table as follows:

Table 2. One-Way ANOVA Test of Aortic Diameter in Mice Menopause Model

Treatment Group	Average \pm SD (Thickness (μ m))	<i>p-value</i> One-way ANOVA
K-	298.500 \pm 132.8100	0.000
K+	47.625 \pm 13.1617	
P1	119.875 \pm 33.5941	
P2	182.375 \pm 70.1134	
P3	218.000 \pm 33.5286	
P4	282.875 \pm 54.2776	

In table 1 of the results of this study with the Hematoxylin and eosin (HE) test, the average aortic diameter in the negative control group (K-) was 298,500 \pm 132.8100 or in mice without higher treatment when compared to the average aortic diameter in the positive control group (K+) of 47.625 \pm 13.1617 or in mice that were varietomized without being given *P. macrocarpa* extract. This study is in line with other studies that explain that there was a decrease in the positive control group (K+) of 101.93 \pm 12.27 compared to the negative control group (K-) of 152.42 \pm 5.66 (Yuliawati & Astuti, 2021). In addition, another study explained that there was a difference in aortic diameter in the ovariectomy group with the negative control group (K-) with values of 1.31 \pm 0.05 and 1.28 \pm 0.07 respectively (Halim et al., 2021).

This is because mice that are divariectomy experience differences in estrogen and FSH levels in menopause mice, namely estrogen in a decreased state and FSH in an increased state. Both of these things are very influential on vascularity, namely narrowing the diameter of the

aorta. The decrease in estrogen has a significant impact on the lipid profile, namely on the levels of HDL and LDL. When there is a reduction in HDL and an elevation in LDL (Moiety et al., 2015). Increased LDL in the blood can cause a buildup of LDL in the intima cells by penetrating the endothelium. LDL can affect endothelial permeability. LDL contained in the intima and binding to local ROS (reactive oxygen species) makes Ox-LDL. The overproduction of Ox-LDL stimulates the activation of LOX-1 on VSMC (Wu et al., 2017). Ox-LDL interacts with LOX-1, leading to the activation of CAM such as VCAM-1 and MCP-1. VCAM-1 and MCP-1 cause monocytes to be attached to endothelial cells and monocytes differentiate into macrophages (Kattoor et al., 2019).

Macrophages that enter the intima will eat Ox-LDL with the help of surface receptors called scavengers to recognize Ox-LDL. Ox-LDL eaten by macrophages will turn into foam cells. The buildup of foam cells will become atheroma plaques or called fatty streaks. Atheroma plaque is one of the causes of the thickening of the tunica intima, causing the narrowing of the diameter of the aorta (Khatana et al., 2020; Sargowo, 2015).

The test yielded a p-value of 0.000 ($p < 0.05$), showing that there are statistically significant variations in aortic diameter among the six treatment groups. The administration of flavonoid extract *P. macrocarpa* affects 4 treatment groups, namely P1, P2, P3, and P4. However, these results could not determine which group differed significantly among the 6 observation groups. Consequently, the analysis proceeded with the implementation of the Post Hoc Test, specifically utilizing the Tukey HSD test.

Table 3. Post hoc HSD Test of Aorta Diameter in Mice Menopause Model

p-value	K-	K+	P1	P2	P3	P4
KN	-	0.001*	0.017*	0.205	0.567	0.999
K+	-	-	0.670	0.104	0.024*	0.001*
P1	-	-	-	0.784	0.362	0.033*
P2	-	-	-	-	0.974	0.338
P3	-	-	-	-	-	0.757
P4	-	-	-	-	-	-

*p-value < 0.05 is significant

The HSD post hoc test indicated a statistically significant distinction (p -value = 0.024) between the K+ treatment group and the P3 treatment group. When comparing K+ with P4, there is a notable distinction with a p -value of 0.001. It may be inferred that administering quantities of *P. macrocarpa* fruit extract containing 11.25 mg/mice/day in treatment group 3 (P3) and a dose of 15 mg/mice/day in group (P4) of flavonoids has a notable impact. Furthermore, along with prior research, it has been demonstrated that administering *Vigna unguiculata*, a substance rich in antioxidants, to mice who have undergone ovariectomy can substantially augment the size of their aortas (Yulinda et al., 2014). Flavonoids consumed from *P. macrocarpa* fruit can help endogenous antioxidants by preventing cell damage caused by excess free radicals in the aorta. Radical clearance by flavonoids that inhibit LDL oxidation for the formation of atherosclerosis (Simanjuntak, 2012).

4. CONCLUSION

Flavonoids *P. macrocarpa* fruit extract, which includes phytoestrogens, is a botanical remedy that may serve as a substitute for reducing the likelihood of atherosclerosis in women experiencing menopause. The administration of a dose of 11.25 mg/mice/day in the treatment group 3 (P3) and a dose of 15 mg/mice/day in group (P4) can lead to an increase in the diameter of the aorta. Future research should focus on examining the amounts of flavonoid antioxidants in *P. macrocarpa* extract, as these antioxidants play a crucial role in influencing the diameter

of the aorta. Additionally, it is suggested to perform further research specifically focusing on women going through menopause.

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