

The Relationship between Blood MDA Levels and Aortic Foam Cells in Menopausal Wistar Rats Treated with Cempaka Flower Extract

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ABSTRACT

Background Menopause is the natural end of the menstrual cycle which results in decreased levels of estrogen (hypoestrogen). The number of free radicals can increase due to decreased estrogen as a natural antioxidant in the body. The effects of increasing free radicals can be reduced by consuming antioxidants. Cempaka flower (*Michelia champaca* L) contains flavonoids that function as antioxidants. *Purpose;* Proved that there was a relationship between blood MDA levels and aortic foam cells in menopausal wistar rats treated with Cempaka flower extract. *Method;* using experimental design with post test only control group design approach. The sample used 24 female rats (*Rattus norvegicus* strain Wistar) with a menopausal age (450-540 days). *Result;* The normality assumption test with Saphiro Wilk obtained a p-value of 0.537 more than $\alpha = 0.05$ ($p > 0.05$). The homogeneity test of variance with the Levene test obtained a p-value of 0.271 more than 0.05 ($p > 0.05$). Testing the effect of giving Cempaka flower extract on MDA levels using ANOVA obtained a p-value of 0.000 smaller than $\alpha = 0.05$ ($p < 0.05$). Testing the effect of giving Cempaka flower extract on the number of foam cells with the Kruskal-Wallis test obtained a p-value of 0.004, smaller than $\alpha = 0.05$ ($p < 0.05$). Testing the correlation between MDA levels and the number of foam cells used the Spearman correlation test with a correlation coefficient of 0.380 and a p-value of 0.067. P-value more than 0.05 ($p > 0.05$). *Conclusion;* There is a significant effect of giving Cempaka flower extract on blood MDA levels and aortic foam cells of menopausal wistar rats. There was no significant relationship between blood MDA levels and the number of aortic foam cells.

INTRODUCTION

The human physiology cycle is the occurrence of the aging process or aging. In a woman, one of the signs of aging is menopause or the cessation of menstruation, which women usually get every month (Fazdria and Ishak, 2020); Parsanezhad *et al.*, 2013). Based on data from the Central Statistics Agency (BPS), an increase in the number of menopausal women is predicted to reach 60 million in 2025 (Pusparatri, 2020).

Menopause is caused by a significant reduction in the number of follicles in both ovaries. This causes the production of the hormone estrogen to decrease significantly (Sullivan, Shannon D., Philip M. Sarrel, 2016). While estrogen is a natural antioxidant to prevent cell damage due to free radicals (Muchtadi, D., 2013). Various short-term and long-term problems will arise as a result of the decrease in the hormone estrogen, one of which is the ease of degenerative diseases due to an increase in the number of free radicals in the body (Fajria Maulida and Sri Wahyuni, 2018); (Fritz, M. A., 2011).

As a result of the increase in free radicals, it triggers various degenerative diseases such as heart disease, osteoporosis, Alzheimer's, hypertension, and others to appear in postmenopausal women. In menopausal women there is an increased risk of heart disease and atherosclerosis, due to

experiencing (Wend, Wend and Krum, 2012); (Murray, R. K., Granner, D. K. & Rodwell, 2012)

Supplement as an anti-inflammatory is needed to counteract the negative effects of hypoestrogens in menopausal women, one of which is by utilizing the natural resources around them as a living pharmacy. The tendency of the community to reuse natural resources "back to nature" in the field of medicine is quite large. One of the natural resources used is plants. Apart from being easy to obtain raw materials, this medicinal plant is also easy to mix and quite effective (Jideani *et al.*, 2021); (Khan and Priyamvada, 2018).

Yellow chrysolite flower (*Michelia champaca*) is a tropical flower that is often found in the archipelago. Based on research results, cempaka kuning flowers are rich in flavonoids and function as anti-inflammatory, antimicrobial, anticancer, antidepressant, and other benefits. So far, no research has been done on the role of cempaka kuning flower as an antioxidant in reducing free radicals (seen by MDA levels) and preventing damage to endothelial cells of blood vessels in menopausal mice (seen by assessing the presence of foam cells in the aorta) (Gowda, Amoolya, 2014); (Yeh *et al.*, 2011); (Wahyuni and Purwaningsih, 2016); (Wahyuni and Purwaningsih, 2016).

Jeumpa flower contains tannins, flavan-3-ols, saponins, flavonoids, carbohydrates, anthraquinones, polyphenols, glycosides. Jeumpa flower extract is reported to be active as an antihyperlipid in vivo at a dose of 500 mg/kgBW. Jeumpa flower leaves are reported to contain alkaloids, flavonoids, glycosides, tannins and sterols. Jeumpa flower leaf extract was reported to be active as an antihyperglycemic agent in vivo at a dose of 200 mg/kgBW. The bark of the Jeumpa flower contains triterpenoids, steroids, and fatty acids and has excellent antimalarial activity in vitro against *P. falciparum* strain 3D7. Research on Jeumpa flower as an antioxidant varies widely, between 100-500 mg/Kg.BB. Other studies have shown that Bunga Jeumpa acts as an antidiabetic with doses of 100 mg/KgBB, 200 mg/KgBB and 300 mg/KgBB (Partiwisari, 2014); (Gowda, Amoolya, 2014).

METHODS AND MATERIALS

The research design used is an experimental design with a post test only control group design approach. This study used an experimental design by adding a control group, namely after the treatment, observations were made in the treatment group, while the control group was only observed. The research variables that were

observed or measured included: Independent variables: Provision of Cempaka flower extract (*Michelia Champaca*L) , Dependent variable: The number of foam cells in the aortic endothelium. This study used 24 rats of *Rattus norvegicus* strain Wistar, which were divided into 4 randomly selected groups, 3 treatment groups with 3 doses of cempaka flower extract and 1 control group.

Extraction of Cempaka flowers was carried out by maceration method. Cempaka flower extract was given orally to treatment group 1 (100 mg / KgBB), treatment 2 (200 mg / KgBB) and treatment 3 (300 mg / KgBB) for 15 days in menopausal model rats.

The mice used were postmenopausal mice, namely rats aged between 450-540 days, weighing between 250-300gr.

After the treatment period is over, the experimental animals are terminated at the Physiology Laboratory of FK Universitas Brawijaya. Taking blood serum from the heart of the rats after being euthanized then centrifuged for 15 minutes at a speed of 1000 rpm. MDA examination using metode asam tiobarbiturat (TBA), and measuring MDA levels using spectrophotometric techniques.

The stages of this research are as follows:

1. Phytochemical test: Phytochemical tests were carried out to determine the active ingredients or compounds contained in

- cempaka flowers, especially antioxidants which were carried out in the laboratory. Unsyiah Chemistry FKIP
2. Cempaka flower extract making: Conducted in the Unsyiah Chemistry FKIP laboratory with the maceration method
 3. Order animal try: Experimental animals are ordered in the lab. There are 35 FK Physiques of Universitas Brawijaya
 4. Experimental animal adaptation: Performed for 14 days in the lab. Faculty of Medicine Universitas Brawijaya
 5. Measurement of serum estrogen levels: Measurement of estrogen levels through serum taken from rat tails in the Lab. Faculty of Medicine Universitas Brawijaya
 6. Menopausal age rats: Mice with serum estrogen levels $<100\mu\text{g}$ were declared as Lab menopause model mice. Faculty of Medicine Universitas Brawijaya
 7. Randomisasi: Rats were randomized and grouped into 4 groups: control (without Cempaka flower extract), treatment 1 (Cempaka flower extract at a dose of 100mg/Kg.BW/day , treatment 2 (Cempaka flower extract at a dose of 200mg/Kg.BW/day), treatment 3 (Cempaka flower extract dose of 300mg/Kg.BW/day in the Faculty of Medicine, Universitas Brawijaya.
 8. Giving Cempaka flower extract: Cempaka flower extract was given to the treatment group orally according to the dose. Cempaka flower extract was weighed and dissolved with sesame oil for 15 days in the Lab. Faculty of Medicine Universitas Brawijaya.
 9. Trial animal termination: After the treatment period is over, the experimental animals will be terminated in the Lab. Faculty of Medicine Universitas Brawijaya
 10. Aortic organ harvesting: After the rats were terminated and the aortic organs were removed for further tissue cutting, and HE (Hemotoxin-Eosin) staining was performed in the Lab. PA FK Universitas Brawijaya
 11. Pemeriksaan jumlah sel busa pada endotel: Counting the number of foam cells in the aortic endothelium using a light microscope at the PA FK Lab, Universitas Brawijaya
- The data collection technique in this research is by observation (observation). Observations were made by counting the aortic endothelial cells that had been stained with HE in the control group and the treatment group using Olivia software and ImageJ software on slides that had been scanned with a laser microscope.

In this study, the data analysis technique was carried out in 3 stages of calculation. There are 4 successive stages, namely (1) normality test of sample data using the Shapiro-Wilk test, (2) to compare the averages, the One Way Anova test (F test), (3) Pearson correlation test, all calculations are carried out with the help of software. (software) SPSS for Windows 19.0.

RESULTS AND DISCUSSION

a. Testing the Assumptions Underlying the ANOVA

Testing of MDA levels using 1 control group (K) and 3 levels of doses of Cempaka Flower Extract (100 mg / kg.BB / day, 200 mg / kg.BB / day, and 300 mg / kg.BB / day) was carried out using ANOVA. Before testing using ANOVA, first testing the assumptions underlying ANOVA. There are two assumptions that underlie ANOVA, namely the assumption of normality and homogeneity of variance. Testing the normality assumption is carried out using the Saphiro-Wilk test, with the following results:

Table 1. Normality Assumption Test

Variabel	Koefisien	p-value	Explanation
Kadar MDA	0.965	0.537	Normal

Based on Table 1, the MDA level variable obtained a p-value of more than $\alpha = 0.05$ ($p > 0.05$). This shows that the assumption of normality has been fulfilled. The assumption of homogeneity of variance was tested using the Levene test. The following are the results of testing the assumption of homogeneity of variety:

Tabel 2. Asumi Homogeneity Test of Variety

Variabel	Koefisien	p-value	Explanation
Kadar MDA	1.403	0.271	Homogen

Based on the test results of the assumption of homogeneity of variance in Table 2, it is shown that the MDA level variable obtained a p-value of more than 0.05 ($p > 0.05$). Therefore, the assumption of homogeneity of variance on these variables has been fulfilled and the process of testing the hypothesis on the MDA level variable was carried out using ANOVA and the 5% LSD test. While the variable number of foam cells in aortic preparations of menopausal rats is a variable with an ordinal measuring scale. Therefore, the statistical testing process for the number of foam cells variable was carried out with a nonparametric statistical approach using the Kruskal-Wallis test and the Dunn test.

b. Testing the Effect of Cempaka Flower Extract on MDA Levels with ANOVA

Following are the results of testing the effect of giving Cempaka Flower Extract with several dosage levels on MDA levels using ANOVA and 5% LSD test.

Table 3. Testing the Effect of Cempaka Flower Extract on MDA Levels with ANOVA and 5% LSD Test

Treatment	Mean ± SD	p-value
Kontrol	1.39 ± 0.67	a
D1	4.31 ± 0.33	b
D2	4.54 ± 0.49	b
D3	1.14 ± 0.67	a

Note: On average ± SD, if it contains different letters, it means that there is a significant difference ($p < 0.05$) and if it contains the same letter, it means that there is no significant difference ($p > 0.05$).

Based on the results of the analysis using ANOVA, it was found that the p-value was 0.000, smaller than $\alpha = 0.05$ ($p < 0.05$). So from this test it can be concluded that there is a significant effect of giving Cempaka flower extract on MDA levels..

Based on the results of the 5% LSD test in Table 3 above, in the comparison between the control group (K) and the

treatment, it was shown that a significant increase in MDA levels was shown in the treatment group giving Cempaka Flower extract at a dose of 100 mg / kg.BB / day (D1) and 200 mg / kg.BB / day (D2). This is indicated by the mean ± sd values of the treatment groups D1 and D2 which are higher and contain different letters from the control group (K). Whereas in the group giving Cempaka Flower extract at a dose of 300 mg / kg.BB/ day (D3), the average MDA level was not statistically significant different from the control group (K) although descriptively there was a decrease in the average MDA level. This is indicated by the mean ± sd value of the D3 treatment group containing the same letters as the control group (K).

When compared between treatment groups, it was shown that the average MDA levels in the treatment group were not significantly different. This is indicated by the mean ± sd values in the treatment groups D1 and D2 containing the letters that are same. The average MDA levels in the control group and the complete treatment is shown in the following histogram:

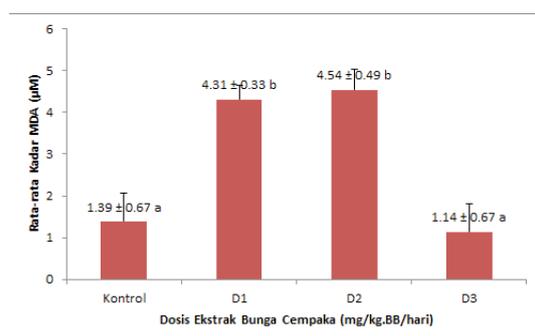


Figure 1. Histogram of Average MDA Levels

Figure 1 shows the average histogram of MDA levels for all control and treatment groups. Starting from group K, it was seen that the average MDA level increased significantly in the treatment group giving Cempaka Flower extract at a dose of 100 mg / kg.BB / day (D1) and 200 mg / kg.BB / day (D2). Whereas at 300 mg / kg.BB / day (D3), when compared to the control group, there was a decrease in MDA levels but it was not statistically significant.

Scientifically, when MDA levels fall, it is usually accompanied by an increase in antioxidant levels (Situmorang, Utara and Utara, 2020); (Veri *et al.*, 2021). Sources of antioxidants derived from plants are safer for the body than synthetic antioxidants, so the use of cempaka flower extract is used as an alternative (Veri *et al.*, 2021). However, in this study, the decrease in MDA levels was not statistically significant, which

means that the treatment with Cempaka flower extract at the given dose was not able to significantly reduce MDA levels.

In the control group described that the menopausal model mice contained foam cells in all samples. Foam cells can be formed, one of which is triggered by high levels of free radicals in the body that cause oxidative stress. In menopausal rats, oxidative stress was triggered due to decreased estrogen levels. After receiving therapy with cempaka flower extract in treatment groups 1 and 2 there was a decrease but not significant. In the third treatment group, there was a decrease in the number of foam cells in the aortic endothelium of menopausal rats. This confirms that cempaka flowers are able to reduce the number of foam cells in menopausal models of rats. The content of Cempaka flowers contains tannins, flavonoids, saponins, polyphenols, glycosides. Cempaka flower leaves are reported to contain alkaloids, flavonoids, glycosides, tannins and sterols. In general, flavonoids have 3 general functions in the body that are very beneficial. Flavonoids are antioxidants that are very effective at binding free radicals (Wahyuni and Purwaningsih, 2016).

Mice in menopausal conditions will experience hypoestrogens caused by the cessation of the ovaries to produce estrogen. While estrogen has a function as a natural antioxidant in the body to reduce or stabilize free radicals in the body. This imbalance in the number of free radicals and antioxidants triggers oxidative stress (Wahyuni, 2017). 17-estradiol is one of the estrogen groups that can increase antioxidant protection mechanisms and prevent cell death and lipid peroxidation, stimulate eNOS and subsequent NO generation through estrogen receptors and increase the work of endogenous antioxidant enzymes that prevent tissue damage by stabilizing ROS levels. In addition, estrogen also plays a role in the migration and proliferation of endothelial cells in the angiogenesis process and inhibits the expression of Tumor Necrosis Factor- α (TNF- α) which induces apoptosis. Oxidative stress can be overcome with endogenous and exogenous antioxidants to reduce free radicals in the body. Exogenous antioxidants found in herbal ingredients or medicinal plants are relatively safe and have been widely used for a long time, one of which is the cempaka flower which grows a lot in Indonesia (Fajria Maulida and Sri Wahyuni, 2018).

Estrogen has an action, one of which is to protect lipoproteins from oxidation.

Research states that low levels of estrogen in the long term can cause oxidative stress, and high doses of progesterone are a source of oxidants for the body. Triggers for oxidative stress in the body are dyslipidemic conditions such as increased levels of LDL cholesterol. Under conditions of oxidative stress, it facilitates the oxidation of LDL by ROS, because free fatty acids (PUFA) are more prone to be damaged by ROS and produce highly reactive radicals. The risk of atherosclerosis is more at risk in women after they experience menopause. Hypoestrogen conditions also trigger an increase in blood LDL levels which trigger an increase in the number of foam cells in blood vessels. LDL plays a role in the accumulation of cholesterol in macrophages, smooth muscle cells, and extracellular matrix in blood vessels so that it is atherogenic (Doshi, Sejal B., 2013); (Chainy and Sahoo, 2020); (Ashok Agarwal, 2012).

c. Testing the Effect of Cempaka Flower Extract on the Number of Foam Cells with the Kruskal-Wallis Test

Following are the results of testing the effect of giving Cempaka Flower Extract with several levels of doses on the number of foam cells using the Kruskal-Wallis test and the Dunn test.

Table 4. Testing the Effect of Cempaka Flower Extract on the Number of Foam Cells with the Kruskal-Wallis Test and the 5% Dunn Test

Group	Number of foam cells			p-value	
	Negatif	Positif 1	Positif 2		
Kontrol	0 (0%)	6 (100%)	0 (0%)	b	0.004
D1	1 (16.7%)	4 (66.7%)	1 (16.7%)	b	
D2	2 (33.3%)	4 (66.7%)	0 (0%)	b	
D3	6 (100%)	0 (0%)	0 (0%)	a	

Explanation:

On the mean \pm SD, if it contains different letters, it means that there is a significant difference ($p < 0.05$) and if it contains the same letter, it means that there is no significant difference ($p > 0.05$).

Based on the results of the analysis using the Kruskal-Wallis test, the p-value was 0.004, smaller than $\alpha = 0.05$ ($p < 0.05$). So from this test it can be concluded that there is a significant effect of giving Cempaka flower extract on the number of foam cells.

Based on the results of the 5% Dunn test in Table 4, in the comparison between the control group (K) and the treatment, it was shown that a significant reduction in the number of foam cells was found in the treatment group giving Cempaka Flower extract at a dose of 300

mg / kg.BB / day. This is indicated by the letter notation value of the D3 treatment group containing different letters when compared to the control group (K). As shown in Table 4, in the control group all samples had foam cells 1 (positive 1), while in the D3 group, all of them were negative. The complete percentage of foam cells in the control and treatment groups is shown in the following histogram:

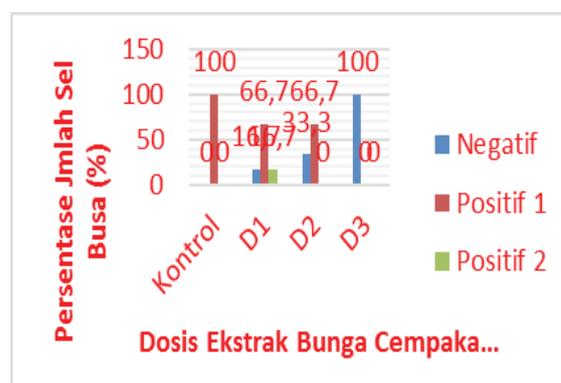


Figure 2. Histogram Average Number of Foam Cells

Figure 2 shows the histogram of the percentage of foam cells of all control and treatment groups. Starting with group K, it was seen that all samples (100%) had 1 foam cell (positive 1). In groups D1 and D2, there was a decrease in the number of foam cells by 66.7%. A significant reduction in the number of foam cells was found in the group giving Cempaka Flower Extract at a dose of 300 mg/kg.BB / day, where at that dose 100% of the samples were negative.

d. Testing the Correlation of MDA Levels with the Number of Foam Cells

To determine the relationship between MDA levels and the number of foam cells, the test can be done using the Spearman correlation test. Following are the results of the Spearman correlation test, the relationship between MDA levels and the number of foam cells:

Tabel 5. Results of Correlation Test Between MDA Levels and the Number of Foam Cells

Relation	Koefisien Korelasi	p-value
MDA levels with the number of foam cells	0.380	0.067

Based on the results of the Pearson correlation test in Table 5, the correlation

coefficient is 0.380 and the p-value is 0.067. The p-value of more than 0.05 ($p > 0.05$) proved that there was no significant relationship between MDA levels and the number of foam cells.

CONCLUSIONS AND SUGGESTIONS

Based on the results of the research that has been carried out. In this study, it can be concluded that there is no significant relationship between MDA levels and the number of foam cells in menopausal wistar rats treated with cempaka flower extract.

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