Effect of Giving Michelia Champaca Linn Extract on Histopathology of Uterus and Ovary in Menopausal Rats

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Abstract. *Purpose*: This study was conducted to determine the effect of giving Michelia Champaca Linn Extract on the histopathology of the uterus and ovaries in menopausal rats.

Methodology: This was an experimental design with a posttest only control group design approach. This study used 28 menopausal Rattus Novergicus Strain Wistar rats, which were divided into 4 groups selected randomly. The control group was given a placebo, three treatment groups were given the extract of Michellia Champaca L dose one, dose two and dose three.

Results: Our analysis showed on histopathological observation of mucosal inflammatory cells in the uterus, it was shown that the p-value of 0.000. So does in observation of uterine bleeding and uterine necrosis. These results showed that there were a significant effect of giving Michelia Champaca Linn Extract. On the other hand, in observation of uterine congestion, it was shown that p-value of 0.125 proves that there was no significant effect of giving Michelia Champaca L extract on uterine congestion. Meanwhile, on histopathological observation of hemorrhage in the ovaries, it was shown p-value of 0.236 and observation of vacuolization of the ovarium, it shown p-value 0.306. So there were no significant effect of giving Michelia Champaca Linn Extract. Different result found at observation of ovarian congestion and oocytes in the ovaries, each of them showed p-value of 0.022 and 0.021, proves that there were a significant effect of giving Michelia Champaca Linn Extract.

Applications/Originality/Value: Michelia Champaca Linn extract has the potential as a natural estrogen replacement which begins to disappear during menopause.

Keywords: Michelia Champaca Linn · Histopathology · Uterus · Ovaries · Menopause

1 Introduction

The World Health Organization (WHO) states that every year, 25 million women worldwide experienced with menopause. In 2030 it is predicted that the number of women in the world who will experience menopause will reach 1.2 billion people [1]. Based on

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data from the Indonesian Demographic and Health Survey in 2017, the percentage of women aged 30–49 years who had experienced menopause reached 16.1% with a total of 28,767 women [2].

Women will experience menopause between the ages of 45 to 55 years. Menopause is a natural process of biological aging. Menopaue process will stop the production of estrogen from the ovaries, resulting in disruption lack of estrogen [3]. Several conditions can arise from increased cholesterol levels and triglycerides, tissue reduction bones leading to osteoporosis psychological disorders, fatigue to depression [1, 4, 5].

The menopausal phase is a natural phase caused by changes in hormone levels in a woman's body. Menopause is a series of stages in a woman's life that is marked by the end of their reproductive period. Towards the end of the age of 30 years, the performance of the ovaries will decrease. Then, at the age of 50 years, the ovaries will stop producing the hormone estrogen [1, 6] In addition, estrogen deficiency can cause several symptoms [7] before menopause to post menopause. One of the signs seen in women who experience menopause is a disturbance in the menstrual cycle, this causes various complaints accompanied by changes, both physical and psychological [5, 8]. To minimize the complaints that occur, some women use hormone therapy, one of them is hormone replacement therapy. Estrogen without progesterone will cause thickening of the lining of the uterine wall (endometrium). In some cases, the thickened cells become hyperplasia which eventually triggers the occurrence of uterine cancer [9].

Giving Extract Michelia Champaka L is one of the alternative efforts to replace hormone replacement therapy. It is because of Cempaka flowers contain various benefits, including anti-oxidant, anti-microbial, anti-inflammatory, analgesic, anti-fertility, anti-cancer and so on. Apart from being an anti-oxidant, Cempaka Flower extract is expected to be useful as a phytoestrogen [6, 10, 11].

The structure and function of phytoestrogens are similar to those of endogenous estrogens, therefore phytoestrogens themselves have an attachment to the estrogen receptor. One of the functions of phytoestrogens it self is to reduce complaints during menopause [8].

2 Methods

This research used an experimental design with a posttest only control group design approach. This study used 28 Rattus Novergicus Strain Wistar rats, which were divided into four groups selected randomly. The control group was given a placebo, 3 treatment groups were given the extrach of michellia champaca L dose one, dose two and dose three.

The extraction of Michelia Champaca Linn was carried out by maceration method. It was given orally to the control group at dose one (100 mg/KgBW), dose two (200 mg/KgBW) and dose three (300 mg/KgBW) for 15 days in menopausal rats. The rats used were postmenopausal rats, i.e. rats aged between 450–540 days, the weight between 250–300 g.

This research was conducted at the Pathology Laboratory, Faculty of Veterinary Medicine, Gadjah Mada University. Histopathology of the ovaries and uterus was seen from the results of the Hematoxylin Eosin (HE) staining examination. Ethical

approval number 015/IV/AUEC was granted by the Ethics Committee Board of 'Aisyiyah University of Surakarta.

3 Results

This study was conducted to determine the effect of giving The extract of Michelia Champaka L on ovarian and uterine histopathology of menopausal rats. Histopathological observations of the uterus studied included: Mucosal Inflammatory Cells, Hemorrhage, Necrosis and Congestion. While, the histopathology of the ovaries studied were Hemorrhage, Congestion, Vacuolization, and Oocytes. The testing process was carried out using the Chi-Square test. The testing process was carried out using SPSS software.

3.1 The Effect of Giving Michelia Champaca Linn Extract on Histopathology of Uterus in Menopausal Rats

The following are the results of testing the effect of giving the extract of Michelia Champaka L on uterine histopathology in menopausal rats using the Chi-Square test (Table 1):

On histopathological observation of mucosal inflammatory cells in the uterus, it was shown that from 7 samples of the control group, all samples contained mucosal inflammatory cells. Meanwhile, in all groups giving Michelia Champaka L extract at doses one, two and three, no mucosal inflammatory cells were found. The P-value of 0.000 ($p < 0.05$) proves that there is a significant effect of giving extract Michelia Champaka L extract on mucosal inflammatory cells in the uterus (Fig. 4) (Table 2).

On histopathological observation of uterine bleeding, it was shown that 7 samples in the control group, all samples had uterine bleeding. Meanwhile, in the group giving Michelia Champaka L extract dose one, there is 1 (3.6%) positive samples. Then at dose two and dose three there was no bleeding. P-value of 0.000 ($p < 0.05$) proves that there was a significant effect of giving Michelia Champaka L extract on uterine bleeding (Table 3).

On histopathological observation of uterine necrosis, it was shown that from 7 samples of the control group, all samples had uterine necrosis. Meanwhile, in the group giving Michelia Champaka L extract dose one, there was 1 (3.6%) positive sample. Then at dose two and dose two there was no necrosis. P-value of 0.000 ($p < 0.05$) proves

Table 1. Effect of giving Michelia Champaca L Extract on Mucosal Inflammatory Cells in Menopausal Rat Uterus

Mucosal Inflammatory Cells			
Group	Negative	Positive	P-Value
Control	0	7 (25%)	0.000
Dose 1	7 (25%)	0	
Dose 2	7 (25%)	0	
Dose 3	7 (25%)	0	

Table 2. Effect of giving Michelia Champaca L Extract to Hemorrhagic in Menopausal Rat Uterus

Uterus Hemorraghic			
Group	Negative	Positive	P-Value
Control	9	7 (25%)	
Dose 1	6 (21.4%)	1 (3.6%)	
Dose 2	7 (25%)	0	0.000
Dose 3	7 (25%)	0	

Table 3. Effect of giving Michelia Champaca L Extract to Necrosis on Menopausal Rat Uterus

Uterus Necrosis			
Group	Negative	Positive	P-Value
Control	0	7 (25%)	
Dose 1	6 (21.4%)	1 (3.6%)	
Dose 2	7 (25%)	0	0.000
Dose 3	7 (25%)	0	

that there was a significant effect of giving Michelia Champaka L extract on uterine necrosis (Table 4).

On histopathological observation of uterine congestion, it was shown that from 7 samples of the control group, all samples had uterine congestion. Meanwhile, in the group giving Michelia Champaca L extract dose 1, there were 5 (17.9%) positive samples. Then at Dose 2 and Dose 3, there were 4 (14.3%) and 3 (10.7%) positive samples, respectively. P-value of 0.125 ($p > 0.05$) proves that there was no significant effect of giving Michelia Champaca L extract on uterine congestion. In general, the effect of Cempaka flower extract on the uterus showed poor results due to the occurrence of fibrosis in all treatment groups.

Table 4. Effect of giving Michelia Champaca L extract to congestion on Menopausal Rat Uterus

Uterus Congestion			
Group	Negative	Positive	P-Value
Control	0	7 (25%)	0.125
Dose 1	2 (7.1%)	5 (17.9%)	
Dose 2	3 (10.7%)	4 (14.3%)	
Dose 3	4 (14.3%)	3 (10.7%)	

3.2 The Effect of Giving Michelia Champaca L Extract on Ovarian Histopathology in Menopausal Rats

The following are the results of testing the effect of giving Michelia Champaca L extract on ovarian histopathology in menopausal rats using the Chi-Square test (Table 5).

On histopathological observation of hemorrhage in the ovaries, it was shown that of the 7 samples of the control group, there were 3 (10.7%) positive samples. In the group giving Michelia Champaca L extract dose 1, 2 (7.1%) samples were positive. At Dose 2, 1 (3.6%) was positive and at Dose 3, all samples were negative for hemorrhage. P-value of 0.236 ($p > 0.05$) proves that there was no significant effect of giving Michelia Champaca L extract on hemorrhage in the ovaries (Table 6).

On histopathological observation of ovarian congestion, it was shown that from 7 samples of the control group, there were 5 (17.9%) positive samples. In the group giving Michelia Champaca L Extract dose 1, 1 (3.6%) positive sample was obtained. At Dose 2 there were 3 (10.7%) positive and at Dose 3 all samples were negative for congestion. The P-value of 0.022 ($p < 0.05$) proved that there was a significant effect of giving Michelia Champaca L Extract on congestion in the ovaries (Table 7).

On histopathological observation of vacuolization of the ovarium, it was shown that of the 7 samples of the control group, there were 5 (17.9%) positive samples. In the group giving Michelia Champaca L Extract dose 1, 5 (17.9%) positive samples were obtained. At Dose 2, 4 (14.3%) were positive and at Dose 3, all samples were positive for vacuolization. P-value of 0.306 ($p > 0.05$) proves that there was no significant effect of giving Michelia Champaca L Extract on vacuolization of ovarium (Table 8).

Table 5. The Effect of Giving Michelia Champaca L Extract on Hemorrhage in the Ovaries of Menopausal Rats

Ovarium Hemorrhage			
Group	Negative	Positive	P-Value
Control	4 (14.3%)	3 (10.7%)	0.236
Dose 1	5 (17.9%)	2 (7.1%)	
Dose 2	6 (21.4%)	1 (3.6%)	
Dose 3	7 (25%)	0	

Table 6. Effect of Giving Michelia Champaca L Extract on Congestion in Menopausal Rat Ovary

Ovarian Congestion			
Group	Negative	Positive	P-Value
Control	2 (7.1%)	5 (17.9%)	0.022
Dose 1	6 (21.4%)	1 (3.6%)	
Dose 2	4 (14.3%)	3 (10.7%)	
Dose 3	7 (25%)	0	

Table 7. Effect of Giving Michelia Champaca L Extract on Vacuolization in Menopause Rat Ovary

Ovarium Vacuolization			
Group	Negative	Positive	P-Value
Control	2 (7.1%)	5 (17.9%)	
Dose 1	2 (7.1%)	5 (17.9%)	0.306
Dose 2	3 (10.7%)	4 (14.3%)	
Dose 3	0	7 (25%)	

Table 8. Effect of Giving Michelia Champaca L Extract to Oocytes in Menopause Rat Ovaries

Ovarium Oocytes			
Group	Negative	Positive	P-Value
Control	3 (10.7%)	4 (14.3%)	
Dose 1	4 (14.3%)	3 (10.7%)	
Dose 2	0	7 (25%))	0.021
Dose 3	0	7 (25%)	

On histopathological observation of oocytes in the ovaries, it was shown that from 7 samples of the control group, there were 4 (14.3%) positive samples. In the group giving Michelia Champaca Linn Extract dose 1, 3 (10.7%) positive samples were obtained. In Dose 2 and Dose 3, all samples were positive for oocytes. P-value of 0.021 ($p < 0.05$) proves that there was a significant effect of giving Michelia Champaca L Extract on oocytes in the ovaries.

4 Discussion

Michelia Champaca Linn is known as a plant that has many benefits, one of which is as an antioxidant. Detection of antioxidants in Michelia Champaca L can be done through checking the levels of MDA (Malondialdehyde), which can be used as the first step in the assessment of free radicals [10, 12–14].

Another study stated that one of the substances that are often used as antioxidants are flavonoids. The mechanism of action of flavonoids as antioxidants is to capture free radicals and bind metal ions. Flavonoids also work indirectly as antioxidants through other mechanisms such as inhibiting pro-oxidant enzymes and inducing antioxidant enzymes [6].

This was related with another study which stated that compounds contained in Michelia Champaca L, Flavonoids, have been shown to have antioxidant activity with a significant decrease in MDA levels, its around 81.44% [13] The same results were also found that there were high antioxidant properties in flavonoid compounds as free radical scavengers [2].

Apart from being an antioxidant, Michelia Campaka Linn also has a role as a phytoestrogen. Phytoestrogens are bound to estrogen receptors, because phytoestrogens have characteristics and benefits similar to endogenous estrogens [14]. Another benefit obtained from phytoestrogens is to reduce the level of discomfort felt in postmenopausal women [8].

Another study stated that Michelia Campaca Linn can be used as a contraceptive [15]. Michelia Champaca Linn also can be used as an anti-fertility, traditionally used for fertility regulation of women in Chhattisgarh, the state of India [16].

Michelia Champaca Linn is also known to be used as an anticancer. This is confirmed by the findings of several studies, both in vitro and in vivo [17]. Besides, Michelia Champaca Linn also has anticancer activity in almost all parts of the plant, although the active content is not the same [18].

So far, no study has been found regarding the effect of Michelia Champaca Linn extract on the histopathology of the uterus and ovaries. Uterine histopathology in this study was assessed from mucosal inflammatory cells, hemorrhage, necrosis and congestion.

Based on the results of this study showed a significant effect (p-Value 0.000) of giving Michelia Champaca Linn extract on inflammatory cells of the uterine mucosa. The statistical results also found a significant effect (p-Value 0.000) of giving Michelia Champaca Linn extract on uterine bleeding and necrosis. However, no significant effect was found of giving Michelia Champaca Linn extract on uterine congestion.

According to another study, a mucosal tissue has special features that are useful as protection against infection and other environmental hazards. The occurrence of inflammation of the uterine mucosal cells most likely can be caused by infection and other factors [19]. Based on the results of this study, the control group showed the presence of mucosal inflammatory cells (Fig. 2). Meanwhile, in the group giving Michelia Champaca Linn extract, there were no mucosal inflammatory cells. Likewise for the first, second and third doses.

The same thing was found in the results of the assessment of hemorrhage and necrosis that occurred in the uterus of menopausal rats in the treatment group. In the treatment group, in the first dose, there was 1 rat that showed bleeding and necrosis (Fig. 1). Meanwhile, in the second and third doses, there were no samples that showed hemorrhage and necrosis in the uterus. It can be concluded that the second and third doses have a better effect on the uterus than the first dose. The results of this study was in accordance with previous study which states that Michelia Champaca Linn Extract was useful as an antioxidant that has the ability to repair cell damage due to free radical damage [20].

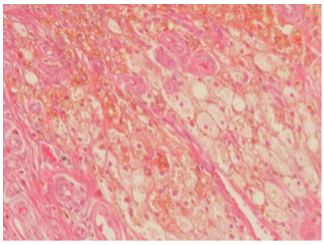

Fig. 1. Degen, Necrosis and Hemosiderin on Uterus in the first dose group

Necrosis can occur due to several things such as lack of blood supply, toxins, no nerve innervation, temperature, radioactive rays, and mechanical trauma[20]. The antioxidant content of Michelia Champaca Linn Extract can play a role in protecting cell membrane damage caused by free radicals. In addition, the antioxidant content plays a role in preventing oxidative stress, apoptosis or necrosis.

In ovarian histopathology, the study was assessed from hemorrhage, congestion, vacuolization and oocytes found in the ovaries. The results showed that there was a significant effect of giving Michelia Champaca Linn Extract on ovarian congestion and oocytes (Fig. 3 & 4). On the other hand, there was no significant effect on ovarian hemorrhage and vacuolization. In line with another study, Michelia Champaca Linn was useful as an antioxidant and phytoestrogen [13, 14, 20].

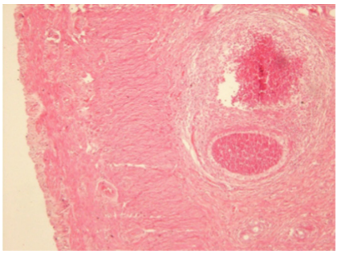

Fig. 2. Inflammation of the uterus in the control group

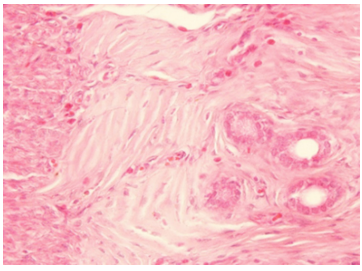

Fig. 3. Ovarian oocytes in the second dose group

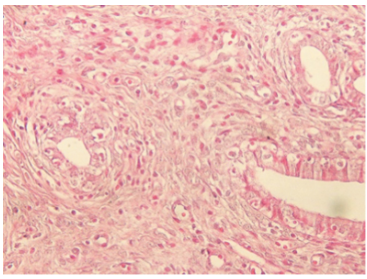

Fig. 4. Ovarian oocytes in the third dose group

5 Conclusion

Michelia Champaca Linn Extract can be used as a substitute for natural estrogen which begins to disappear during menopause. It can be seen from the potential generated, the first potential as an antioxidant, which can be shown by the absence of inflammation, hemorrhage and necrosis of the uterus. While the second opotential as a phytoestrogen, it can be shown by an increase in the number of oocytes in the ovaries of the treatment group at doses one, two and three.

6 Conflicts of Interest

The authors have no conflicts of interest to declare.

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Author's Contribution. 1. Endang Sri Wahyuni: Conceptualization of research and article writing.
2. Maryatun: Investigation and setting research methodology.
3. Siska Ningtyas Prabasari: article writing and grammar.
4. Riyani Wulandari: Formal analysis of research data.
5. Lely Firrahmawati: Curation of research data.
6. Endah Sri Wahyuni: Acquisition of research funding.
7. Asti Nur Hayati: research project administration as well as implementing resource.

Data Availability. All relevant data and supporting information files are within the manuscript. Additionally, the data sets used to support the findings of this study are available from the corresponding author upon request.

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