



The Effect of Flavonoid from Extract of Phaleria Macrocarpa on Levels IL-2, IL-17a and IL-33 in Endometriosis Mice

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Article History	Abstract
Received: 16 June 2023 Revised: 12 Sept 2023 Accepted: 21 Oct 2023	<p>Endometriosis is a inflammatory disease especially in the state of reproductive age. <i>Phaleria macrocarpa</i> is a plant that has a population in Papua, Irian Jaya. Endometriosis therapies available have still many weakness, including being expensive, only relieving pain, and continuous treatment to prevent endometriosis from recurring. The fruit and seeds of the <i>Phaleria macrocarpa</i> have a wide range of important biological activities in the form of extracts such as antimicrobial, anti-inflammatory, and antioxidant activities. This aim of this was to analyze the effect of flavonoid from extract of <i>phaleria macrocarpa</i> on IL-2, IL-17A, and IL-33 levels in endometriosis mice. This research is using total of 40 female mice (<i>Mus musculus</i>) model endometriosis were grouped into 8 groups, including of healthy mice (without any treatment), the endometriosis group, and the endometriosis group treated flavonoids of <i>Phaleria macrocarpa</i> with dose of 3.75 mg/day; 7.5 mg/day; 11.2 mg/day; 15 mg/day. The level of IL-2, IL-17A, IL-33 were measured using the ELISA method. There was an increase in IL-2, IL-33, and IL17A levels in mice model of endometriosis. Flavonoid of <i>Phaleria macrocarpa</i> can reduce levels of proinflammatory cytokines such as IL-2, IL-33, and IL17A.</p>
CC License CC-BY-NC-SA 4.0	<p>Keywords: <i>Phaleria macrocarpa</i>, flavonoid, endometriosis, IL-2, IL-17A, IL-33</p>

1. Introduction

Endometriosis is an inflammatory disease that can be found in the endometrial glands and stroma outside the uterine cavity, especially in the pelvic peritoneum and ovarium (1,2). The process of development of endometriosis tissue is very dependent on the hormone estrogen. Deviation of synthesis and estrogen metabolism is pathogenesis (3,4). According to WHO, globally, endometriosis affects about 10% (190 million) women of reproductive age (5). Women of endometriosis have symptoms from pelvic pain, menstrual pain (dysminore), dyspareunia, and pain during defecate (6, 7).

The reason cause of endometriosis is still unknown, although there are several theories that have been put proposed (8). In endometriosis, there is a local inflammatory process in the pelvis with changes in the function of immune cells in the peritoneal environment, decreased cell apoptosis and T cell abnormal. Inflammatory response will cause pain symptoms in the presence of cytokines. When an inflammatory process occurs, in the peritoneum there will be a loss of homeostasis due to an increase in pro-inflammatory cytokines (IL-2, IL-6, IL-8, IL-10, IL-17A, IL-33, IFN- γ , TNF- α) and anti-inflammatory (IL-4 and IL-10). Interleukin (IL-33) has a significant influence on the innate and adaptive immune system in inflammatory disorders (9,10).

Pathophysiology of endometriosis consists of inflammatory response, cell survival, proliferation, migration, adhesion, invasion, and neoangiogenesis. The most important factors in the pathophysiology of endometriosis are hormonal dysregulation of estrogen and progesterone resistance CpG hypomethylase 14 can change the balance of estrogen receptors from ER α to ER β . Estrogen produced locally in endometriosis tissue, especially estradiol has an important role in endometrial cell proliferation and a stimulator of pro-inflammatory cytokines (11).

Treatment of endometriosis includes surgery and medical therapy. However, the therapies available have many weaknesses, including being expensive, only relieving pain, and continuous treatment to prevent endometriosis from recurring. Herbal therapies are still being developed today, one of which comes from *Phaleria macrocarpa* (12, 13)

Phaleria macrocarpa is a plant that has a population in Papua, Irian Jaya. The fruit and seeds of the *Phaleria macrocarpa* have a wide range of important biological activities in the form of extracts such as antimicrobial, anti-inflammatory, and antioxidant activities. (14,15). There is study to suggest that inflammatory cytokines have potential as therapeutic targets for treating inflammatory diseases. The effects of flavonoids on inflammatory mediators, especially by modulating cytokines, are relevant for developing alternative treatments for inflammation-related diseases (16).

Therefore, this study aims to analyze the effect of dose flavonoids of *Phaleria macrocarpa* fruit extract on IL-2, IL-17A, IL-33 levels in mice endometriosis.

2. Materials And Methods

Animal

This study used healthy female mice (*Mus musculus*), age 12 weeks old, weight 20-30 grams. A total of 40 mice were divided into 8 groups, including healthy mice (n = 5), endometriosis mice (n = 5), endometriosis mice received *Phaleria macrocarpa* at a dose of 3.75mg/day (n = 5), endometriosis mice received *Phaleria macrocarpa* at a dose of 7.5 mg / day (n = 5), endometriosis mice received *Phaleria macrocarpa* at a dose of 11.25 mg / day (n = 5), mice model of endometriosis received *Phaleria macrocarpa* at a dose of 15 mg/day (n = 5). Flavonoids of *Phaleria macrocarpa* fruit extract were administered orally for 14 days. Doses are given based on previous studies (17).

Endometriosis Model

Mice models of endometriosis was according previous study. Mice models of endometriosis was evaluated visually and using immunohistochemical methods by examining the expression of estrogen receptor A and estrogen receptor B (18).

Preparation of ekstrak

Two thousand five hundred grams of *Phaleria macrocarpa* flour soaked in 30 L of 96% ethanol, then stirred (\pm 30 minutes) until well mixed. The mixture then allowed to stand for five nights until settled. Next, filtering with the Buncher funnel gets the filtrate.

Preparation of Flavonoid

The separation of flavonoid compounds to obtain n-hexane and n-butanol partitions. The ethanol extract was dissolved in n-hexane (1L). After obtaining a precipitate, then the n-hexane solution is removed, and the ethanol precipitate is evaporated at a temperature of 45 C. Separation is continued with n-butanol. The ethanol solution was mixed with n-butanol (centrifuged at 3000 RPM for 10 min). The supernatant is then taken and evaporated at 60° C to obtain a concentrated flavonoid solution.

Ethic

Health Research Ethics Commission Brawijaya University No 67/EC/KEPK/03/2023

Analysis of IL-2, IL-17A, IL-33

Measurement of IL-2, IL-17A, IL-33 levels using the Enzym-linked immunosorbent assay (ELISA) method. ELISA Kit IL-2 (Cat.No.KTE7006) and IL-17A (Cat.No.KTE7012) and IL-33 (Cat.No.E1182Mo) with content components, namely 48 well strip microplate, antibody, sample diluent, assay buffer, streptavidin HRP, HRP substrate, stop solution, wash buffer, plate cover.

Statistical Analysis

Data were presented as the mean \pm SD, and the ANOVA test analyzed differences between treatment groups. The analysis was performed with the SPSS 26.0 statistical package for Windows programs. The probability values (p < 0.05) expressed significantly different.

3. Results and Discussion

Lesions in endometriosis tissue can be cured as in the group of healthy mice at doses of 3.75mg / day and 7.5mg/ day. Peritoneal fluid mean levels between groups there was an increase in levels in all endometriosis groups compared to the healthy group ($p>0.05$). Flavonoids of *Phaleria macrocarpa* fruit extract have a significant effect on IL-17A and IL-33 levels with an optimum dose of 3.75mg/ day, while in IL-2 the optimum dose that has a significant effect is 7.5mg / day.

Phaleria macrocarpa is a medicinal plant that is widely used in traditional Indonesian medicine to treat several diseases (19). This plant contains tannins, terpenoids, alkaloids, and flavonoids. Flavonoids are secondary metabolites found in plants, contribute to plant growth and development and have prominent applications in food and medicine (20).

Structurally, flavonoids are constructed from a 15-carbon skeleton and consist of two benzene rings connected by a 3-carbon link chain so that they are represented as C6-C3-C6 compounds. Some types of flavonoids are flavonols, flavones, flavones, and isoflavones. This compound produced as a secondary metabolite by plants as a defense mechanism. In addition, it has been widely proven that flavonoids have various health benefits due to their antibacterial, antioxidant and anti-inflammatory (21, 22). Flavonoids are strong inhibitors of prostaglandin production, a group of powerful proinflammatory signaling molecules (23).

Interleukin (IL) is cytokine that was first thought to be expressed by leukocytes alone but was later found to be produced by many other body cells. It important role in the activation and differentiation of immune cells, as well as proliferation, maturation, migration, and adhesion. Interleukin also have pro-inflammatory and anti-inflammatory properties. Therefore, the primary function of interleukins is to modulate growth, differentiation, and activation during inflammatory and immune responses (24).

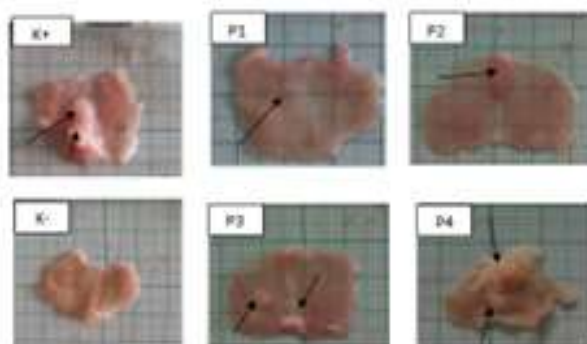


Figure 1. Peritoneal tissue of mice in each group. (K-) mice without treatment, (K+) yaitu mice EMT without treatment, (P1) mice EMT with treatment flavonoid of *Phaleria macrocarpa* dose 3.75 mg/day, (P2) mice EMT with treatment flavonoid of *Phaleria macrocarpa* 7.5 mg/day, (P3) mice EMT with treatment flavonoid of *Phaleria macrocarpa* 11.25 mg/day, (P4) mice EMT with treatment flavonoid of *Phaleria macrocarpa* 15 mg/mencid/day |

Interleukins and cytokines as communication for innate and adaptive immune cells as well as non-immune cells and tissues (25). IL-2, IL-17A, and IL-33 are proinflammatory cytokines secreted in a wide variety of cells. IL-2 is a pro-inflammatory cytokine secreted by T cells. When an inflammatory process occurs in the peritoneum, there will be a loss of homeostasis due to an increase in pro-inflammatory cytokines (26). IL-17A is a pro-inflammatory cytokine secreted by TH17 cells and can be found elevated in the peritoneal fluid of women with endometriosis (27). Interleukin IL-33 is a new member of the IL-1 superfamily, primarily upregulated by stromal cells after proinflammatory stimulation. IL-33 acts on a variety of innate and immune cells including dendritic cells and T and B lymphocytes, NK cells, neutrofil and makrofag (28,29). Significant increase in IL-33 concentrations in serum and peritoneal fluid in patients with endometriosis (30).

Flavonoids contribute to the repair of endometriosis (31). This study using flavonoid doses of crown of god fruit extract as much as 3.75mg / day, 7.5mg / day, 11.25mg / day, 15mg / day. In this study, the formation of lesions in the peritoneal tissue is a marker that endometriosis occurs. The presence of lesions in the peritoneal tissue is visually evidenced as in figure 1. The following is a picture of the results of peritoneal tissue dissection of negative control mice, positive control, and treatment groups that have been given flavonoids from *Phaleria macrocarpa* fruit extract at various doses for 14 days.

Levels of IL-17A and IL-33 experience a rapid decrease if given flavonoids *Phaleria macrocarpa* fruit extract at a dose of 3.75mg / day. In Maharani's study, a dose of 7.5mg/day can suppress the growth of granulomas in endometriosis so that it can cause a decrease in IL-2 levels in endometriosis mice.

4. Conclusion

According to the findings of this study, giving flavonoid isolates from extract *Phaleria macrocarpa* in endometriosis mice model can reduce IL-2, IL-17A, IL-33 levels So, *Phaleria Macrocarpa* Flavonoids can be used as an option to prevent endometriosis from developing.

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