Asian Journal of Health Research
Journal Homepage: https://a-jhr.com
Published by Ikatan Dokter Indonesia Wilayah Jawa Timur

Original Research

The Effect of Flavonoids from Mahkota Dewa Fruit Extract (*Phaleria macrocarpa*) on Inflammation Factors (IL-1β and IL-6) and Estrogen Receptor Alpha (ERα) in The Ovaries of Endometriosis Model Mice

Lovin Destikatari, Rahajeng, Irfani Baihaqi*

Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Brawijaya/dr. Saiful Anwar General Hospital, Malang, East Java, Indonesia

ARTICLE HISTORY
Received: 22 January 2025
Revised: 20 February 2025
Accepted: 17 March 2025

CORRESPONDING AUTHOR*
Irfani Baihaqi
irfanibaihaqidsog@gmail.com
Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Brawijaya/ dr. Saiful Anwar General Hospital, Malang, East Java, Indonesia

KEYWORDS
Endometriosis; IL-1β; IL-6; Estrogen Receptor α (ERα)

This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/)

ABSTRACT

Introduction: Endometriosis is a gynecological disorder characterized by the growth of endometrial tissue outside the uterus. The disease is marked by uncontrolled cell proliferation, increased cell aggressiveness, apoptosis defects, and an inflammatory microenvironment. *Phaleria macrocarpaa,* a native Indonesian plant, contains flavonoids that function as antioxidants and have anti-inflammatory and antimicrobial properties. Previous research has shown that flavonoids from *Phaleria macrocarpa* can inhibit endometriosis lesion growth by regulating cell apoptosis.

Material and Methods: This study is an experimental study using a randomized post-test-only control group design. Six groups were included: a negative control, a positive control, and four treatment groups receiving *Phaleria macrocarpa* flavonoid extract at concentrations of 3.75%, 7.5%, 11.25%, and 15%. The primary data obtained were then examined using SPSS 26.0.

Results: IL-1β, IL-6, and ERα levels in the treatment groups were lower than in the positive control group, with significant differences between each group. Higher extract doses were associated with a greater reduction in inflammatory mediators. Regression analysis demonstrated a strong correlation between *Phaleria macrocarpa* extract administration and decreased levels of IL-1β (86%), IL-6 (94%), and ERα (95%).

Conclusion: In endometriosis model mice, administering *Phaleria macrocarpa* fruit extract can lower the concentration of IL-1β, IL-6, and ERα. There is a correlation between the reduction of concentration of IL-1β, IL-6, and ERα in endometriosis model mice and various doses of *Phaleria macrocarpa* fruit extract.

Cite this as: Destikasari L, Rahajeng, Baihaqi I (2025) The Effect of Flavonoids from Mahkota Dewa Fruit Extract (*Phaleria macrocarpa*) on Inflammation Factors (IL-1β and IL-6) and Estrogen Receptor Alpha (ERα) in The Ovaries of Endometriosis Model Mice. *Asian J Heal Res.* 4 (1): 17–20. doi: 10.55561/ajhr.v4i1.220

INTRODUCTION

Endometriosis is a gynecological disorder in which tissue (endometrial glands and stroma) that normally lines the uterus (endometrium) develops outside of the uterus, often found in areas close to the uterus such as the ovaries and fallopian tubes. This condition can cause severe pain, menstrual disorders, and infertility in fertile women. It is estimated that the incidence rate of endometriosis is around 10-15% in women of reproductive age, and the prevalence increases up to 70% in women that have symptoms of prolonged pelvic pain, and females with infertility have a 35-50% prevalence of endometriosis. However, the exact incidence of this disorder is uncertain because a definitive diagnosis requires laparoscopy [1–4].

The involvement of immunosuppressive factors, exosome activation, and abnormal communication between the peritoneum play key roles in the development of endometriosis [5,6]. Uncontrolled cell proliferation, increased cell aggressiveness, defect of apoptosis, and inflammatory microenvironment are the

main characteristics of endometriosis, prior experiments show that pro-apoptotic and anti-apoptotic protein profile decreased [7,8].

Endometriosis lesions appear as prolonged inflammatory lesions that happen locally, and changes in cell-mediated immunity and inflammatory cytokine activation are involved. Previous studies have shown that increased serum levels of a large number of pro-inflammatory cytokines, such as IL-1, IL-6, and IL-8, which result in peritoneal macrophage migration, recruitment, activation, and increased monocyte proliferation observed in endometriosis patients [9].

The development of endometriosis is significantly influenced by the estrogen receptor (ER), specifically the two major receptor subtypes that are involved: the estrogen receptor α (ERα) and the estrogen receptor β (ERβ). Different genes, ESR1 and ESR2, respectively, encode these subtypes. Compared to normal endometrium, both ERα and ERβ exhibit aberrant expression and regulation in endometriosis [10]. Additionally, in endometriosis, both α and β receptors show different tissue expression patterns, localization patterns, also ligand specificities. The fact that ERα is thought to be the main mediator of estrogenic action in the stroma and endometrial glands helps to explain this. All endometrial cell components have a decrease in ERα levels during the secretory phase, whereas only endometrial epithelial cells have a decrease in ERβ levels [11].

Phaleria macrocarpa (Mahkota Dewa) is a native Indonesian plant that serves many purposes, including as a traditional medicine [12]. The standard extract of *Phaleria macrocarpa* also has the potential as an antiproliferative and anti-apoptotic [13]. Flavonoid compounds are thought to be one of the main antioxidant compounds in *Phaleria macrocarpa* [12]. Our research purpose is to analyze the effect of *Phaleria macrocarpa* flavonoid isolates on the development of endometriosis disease through changes in IL-1β, IL-6, and ERα titers.

MATERIAL AND METHODS

This study used a true experimental design conducted with a randomized post-test only with a control group design in vivo on *Mus musculus* females. This study aims to assess changes in levels of IL-1β, IL-6, and ERα against the administration of various doses of *Phaleria macrocarpa* fruit extract.

Inclusion criteria included female *Mus musculus* mice, aged 8-12 weeks, weight of 20-30 grams, and those with healthy, active, and no extremity disabilities. Exclusion criteria are mice with extremity disabilities, extremity infection, or sickness and death during the study.

In this study, there are 6 research groups, 1 group as a negative control consisting of mice that receive no treatment, 1 group as a positive control consisting of mice that underwent adenomyosis tissue implantation without treatment administration, and lastly 4 treatment groups consisting of mice that underwent adenomyosis tissue implantation that given different dose of *Phaleria macrocarpa* fruit extract (3,75 mg/day, 7,5 mg/day, 11,25 mg/day, and 15 mg/day).

The collected data underwent editing, coding, and tabulation and were then organized and processed using computer assistance using Statistical Product and Service Solution (SPSS) software, IBM SPSS Statistics 26 with a significance rate or probability of 0.05 (p=0.05) and 95% confidence interval.

RESULTS

The measurement data of IL-1β, IL-6, and ERα levels that have been obtained from six treatment groups will be calculated with descriptive and inferential statistics using SPSS 26.0.

IL-1β

Table 1 shows the analysis results of each group. Among those groups, the difference is statistically significant.

Table 1. IL-1β Analysis Result

Parameter	Analysis	
	Mean ± SD	p-value
Negative Control	20,78 ± 2,42	0.000
Phaleria macrocarpa 15 mg	29,89 ± 4,83	
Phaleria macrocarpa 11,25 mg	40,88 ± 2,85	
Phaleria macrocarpa 7,5 mg	54,19 ± 8,76	
Phaleria macrocarpa 3,75 mg	69,00 ± 11,44	
Positive Control	105,18 ± 11,04	

IL-6

Table 2 shows the analysis result of each group. Among those groups, the difference is statistically significant.

Table 2. IL-6 Analysis Result

Parameter	Analysis	
	Mean ± SD	p-value
Negative Control	18,65 ± 3,08	0.000
Phaleria macrocarpa 15 mg	31,09 ± 4,15	
Phaleria macrocarpa 11,25 mg	40,66 ± 3,75	
Phaleria macrocarpa 7,5 mg	54,03 ± 5,57	
Phaleria macrocarpa 3,75 mg	70,92 ± 5,16	
Positive Control	94,07 ± 4,44	

ERα

Table 3 shows the analysis result of each group. Among those groups, the difference is statistically significant.

Table 3. ERα Analysis Result

Parameter	Analysis	
	Mean ± SD	p-value
Negative Control	8,15 ± 1,34	0.000
Phaleria macrocarpa 15 mg	15,95 ± 3,04	
Phaleria macrocarpa 11,25 mg	22,89 ± 1,84	
Phaleria macrocarpa 7,5 mg	29,54 ± 2,43	
Phaleria macrocarpa 3,75 mg	37,35 ± 3,04	
Positive Control	44,79 ± 2,3	

Linear Regression Test

The data was analysed using a linear regression test to assess the result of administering *Phaleria macrocarpa* fruit extract on reducing IL-1β, IL-6, and ERα titres. The R-Square results for IL-1β, IL-6, and ERα, respectively, are 0.86, 0.94, and 0.95. The result above showed that the administration of *Phaleria macrocarpa* extract affects the reduction of IL-1β, IL-6, and ERα titres by 86%, 94%, and 95%, respectively.

Ethics Approval

This study was approved by the Ethical Committee of Medical Faculty at Universitas Brawijaya.

DISCUSSION

Endometriosis shows proliferation, implantation, and angiogenesis and is likely to survive in different environments. The pathology of endometriosis is complex and multifactorial, yet the cause and progression of endometriosis are not well-known [14,15]. Several studies have identified the involvement of IL-1 in the pathogenesis of endometriosis, namely through the process of inflammation and pain. IL-1β is a pro-inflammatory cytokine that can trigger inflammation and stimulate the release of other pro-inflammatory factors [16,17].

In the context of endometriosis, IL-6 has also been studied because it contributes to the progression of this disease. IL-6 has been associated with the regulation of cell invasion and spreading processes, which are paramount in the progression of ectopic endometrium [18]. Previous research using mouse models and cells obtained from endometriosis patients explains the role of ERα in endometriosis progression. ERα is needed for endometrial cell adhesion and proliferation when there are disturbances in estradiol that result in endometriosis-like lesion development [19,20].

Based on the statistical results of the effect of flavonoid extract of *Phaleria macrocarpa* fruit administration in IL-1β, IL-6, and ERα levels, a p-value of 0.000 was obtained; this result is smaller than a = 0.05 ($p < 0.05$). So, from this test, it can be concluded that there is a significant difference in the administration of flavonoid extract from *Phaleria macrocarpa* fruit on IL-1β expression. Post hoc tests were also carried out to assess each group, the results of the test showed significant differences in each group in the IL-1β and IL-6 groups, except in ERα, where the result of *Phaleria macrocarpa* 15 mg group is not statistically different from the negative control group. This is in line with research conducted by Sutrisno et al. [14] where genistein, one of the products of which is isoflavone, is able to modulate estrogen receptors and suppress angiogenesis and inflammation in a murine model of peritoneal endometriosis. This is in accordance with the review article conducted by Bartiromo et al. [19], where they conducted a study on the pharmaceutical effects of medicinal plants and phytochemicals on endometriosis. Medicinal herbs and their bioactive compounds show anti-angiogenic and antioxidant properties that have beneficial effects on the management of endometriosis.

From the research that has been completed, there are several important points that can be conveyed, this study has advantages and disadvantages. The advantage of this study is the use of the ELISA assessment method to assess the levels of IL-1β, IL-6, and ERα. The use of ELISA makes it easier for researchers to see changes in IL-1β, IL-6, and ERα levels quantitatively. From the ELISA results, it was found that there was a decrease in IL-1β, IL-6, and ERα levels in endometriosis model mice, even the value of the ERα parameter in the 4th treatment showed results equivalent to the negative control, this shows that the *Phaleria macrocarpa* fruit extract has good potential to reduce IL-1β, IL-6, and ERα levels in endometriosis model mice.

The weakness of this study is that this study still uses a mouse model, so the *Phaleria macrocarpa* fruit extract cannot yet be applied to human subjects. Further clinical trial research is needed so that it can be used immediately in humans.

ACKNOWLEDGMENT

This research paper is made possible by the support of all colleagues from the Obstetrics and Gynecology Department.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

REFERENCES

1. American College of Obstetricians and Gynecologists (ACOG). (2019) Endometriosis: ACOG Practice Bulletin.Page number 214.
2. Parasar P, Ozcan P, Terry KL. (2017) Endometriosis: Epidemiology, Diagnosis and Clinical Management. Curr Obstet Gynecol Rep. 6 (1): 34-41.
3. Zondervan, K. T., Becker, C. M., & Missmer, S. A. (2020) *Endometriosis. New England Journal of Medicine, 382(13), 1244–1256.*
4. Vempilly J.J. (2022) Endometriosis: Pathogenesis, clinical features, and diagnosis
5. Liang Y., Wu J., Wang W., Xie H., Yao S. (2019) Pro-endometriotic niche in endometriosis. RBMO. 38 (4): 549–559.
6. Maharani M, Lajuna L, Yuniwati C, Sabrida O, Sutrisno S. (2021) Phytochemical characteristics from *Phaleria macrocarpa* and its inhibitory activity on the peritoneal damage of endometriosis. J Ayurveda Integr Med. 12(2): 229-233. doi: 10.1016/j.jaim.2020.06.002. Epub 2020 Dec 4. PMID: 33288353; PMCID: PMC8185966.
7. Sbracia M., Valeri C., Antonini G., Biagiotti G., Pacchiarotti A., Pacchiarotti A. (2016) Fas and fas-ligand in eutopic and ectopic endometrium of women with endometriosis: the possible immune privilege of ectopic endometrium. Reprod Sci. 23 (1): 81–86.
8. Bilgic E., Guzel E., Kose S., Aydin M.C., Karaismailoglu E., Akar I. (2017) Endocannabinoids modulate apoptosis in endometriosis and adenomyosis. Acta Histochem. 119: 523–532.
9. Tsamantioti ES, Mahdy H. (2023) Endometriosis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Available from: https://www.ncbi.nlm.nih.gov/books/NBK567777/
10. Chantalat E, Valera M-C, Vaysse C, Noirrit E, Rusidze M, Weyl A, et al. (2020) Estrogen Receptors and Endometriosis. Int J Mol Sci. 21(8):2815.
11. Yilmaz BD, Bulun SE. (2019) Endometriosis and nuclear receptors. Hum Reprod Update. 25 (4): 473–85.
12. Hendra, R., Ahmad, S., Oskoueian, E., Sukari, A. and Shukor, M.Y. (2011) Antioxidant, anti-inflammatory, and cytotoxicity of *Phaleria macrocarpa* (Boerl.) Scheff fruit. *BMC complementary and Alternative Medicine*, *11*, pp.1-10.
13. Tjandrawinata R.R., Arifin P.F., Tandrasasmita O.M., Rahmi D., Aripin A. (2018) DLBS1425, a *Phaleria macrocarpa* (Scheff.) Boerl. Extract confers anti-proliferative and proapoptosis effects via the eicosanoid pathway. J Exp Therapeut Oncol. 8 (3): 187–201.
14. Sutrisno, S., Sulistyorini, C., Manungkalit, E. M., Winarsih, L., Noorhamdani, N., & Winarsih, S. (2017) The effect of genistein on TGF-β signal, dysregulation of apoptosis, cyclooxygenase-2 pathway, and NF-kB pathway in mice peritoneum of endometriosis model. Middle East Fertility Society Journal. https://doi.org/10.1016/j.mefs.2017.05.002
15. Jouhari S., Mohammadzadeh A., Soltanghoraee H., Mohammadi Z., Khazali S., Mirzadegan E. (2018) Effects of silymarin, cabergoline, and letrozole on a rat model of endometriosis. Taiwan J Obstet Gynecol. 57:83–85.
16. Akoum A, Al-Akoum M, Lemay A, Maheux R, Leboeuf M. (2008) Imbalance in the peritoneal levels of interleukin 1 and its decoy inhibitory receptor type II in endometriosis women with infertility and pelvic pain. Fertil Steril; 89:1618–1624.
17. Yu J, Francisco AMC, Patel BG, Cline JM, Zou E, Berga SL, Taylor RN. (2018) IL-1β stimulates brain-derived neurotrophic factor production in eutopic endometriosis stromal cell cultures: a model for cytokine regulation of neuroangiogenesis. Am J Pathol. 188:2281–2292.
18. Burns, K. A., Thomas, S. Y., Hamilton, K. J., Young, S. L., Cook, D. N., & Korach, K. S. (2018) Early Endometriosis in Females Is Directed by Immune-Mediated Estrogen Receptor α and IL-6 Cross-Talk. Endocrinology, 159(1), 103–118. https://doi.org/10.1210/en.2017-00562Beliard, A.; Noel, A.; Foidart, J.M. (2004) Reduction of apoptosis and proliferation in endometriosis. Fertil. Steril, 82, 80–85.
19. Bartiromo, Ludovica, Matteo Schimberni, Roberta Villanacci, Jessica Ottolina, Carolina Dolci, Noemi Salmeri, Paola Viganò, and Massimo Candiani. (2021) "Endometriosis and Phytoestrogens: Friends or Foes? A Systematic Review" *Nutrients* 13, no. 8: 2532. https://doi.org/10.3390/nu13082532
20. Béliard, A., Noël, A., & Foidart, J. M. (2004) Reduction of apoptosis and proliferation in endometriosis. *Fertility and sterility*, *82*(1), 80–85. https://doi.org/10.1016/j.fertnstert.2003.11.048