Therapeutic Effect of *Phaleria macrocarpa* Flavonoid Extract on TGF-β Modulation in Endometriosis Mice Model

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Abstract

Endometriosis is a chronic estrogen-dependent inflammatory disorder characterized by ectopic endometrial-like tissue growth and dysregulated immune responses. Transforming Growth Factor Beta (TGF-β), although classically known as a profibrotic cytokine, also exhibits context-dependent anti-inflammatory activity, creating uncertainty regarding its net impact on endometriotic progression. This gap in the literature highlights the need to investigate agents that may modulate TGF-β in a controlled experimental setting. Phaleria macrocarpa, a flavonoid-rich medicinal plant, has demonstrated immunomodulatory potential, yet its relationship with TGF-β regulation in endometriosis remains poorly understood. This study aimed to evaluate the effect of Phaleria macrocarpa flavonoid extract on TGF-β levels in a Balb/C mouse model of endometriosis. A true experimental post-test only controlled design was conducted using 30 mice divided into six groups: negative control, positive control, and four treatment groups receiving 3.75, 7.5, 15, or 30 mg/mouse/day for 14 days. TGF-β levels were quantified using ELISA and analyzed with ANOVA followed by Tukey's post-hoc test. The results demonstrated a non-linear dose–response pattern, with the 7.5 mg/mouse/day dose producing the highest increase in TGF-β, while higher doses showed attenuated or reduced responses. These findings indicate a biphasic modulatory effect of Phaleria macrocarpa flavonoids on TGF-β, suggesting a dose-dependent immunoregulatory influence that warrants further investigation.

Keywords: Phaleria macrocarpa, TGF-β, Endometriosis, Mice.

INTRODUCTION

Endometriosis is a chronic gynecological disorder affecting approximately 10% of women of reproductive age worldwide, with clinical manifestations primarily involving chronic pelvic pain, dysmenorrhea, and infertility (World Health Organization, 2023). The prevalence is notably higher among women with dysmenorrhea (40–60%), infertility (21–47%), and chronic pelvic pain (71–87%) (Falcone & Flyckt-Rebecca, 2018). Endometriosis is characterized by the ectopic growth of endometrial-like tissue outside the uterine cavity, which undergoes cyclic hormonal stimulation and triggers chronic inflammation, fibrosis, and adhesions (Zhou et al., 2019; Garcia-Garcia et al., 2023). Despite advancements in its clinical management, recurrence occurs in more than 75% of cases within two years after surgery, and existing pharmacological therapy often causes significant side effects and is contraceptive in

nature. Consequently, the need for safer and more effective therapeutic alternatives remains substantial (Young et al., 2017).

The pathogenesis of endometriosis is complex and multifactorial, involving hormonal imbalance, impaired immune clearance, and dysregulation of inflammatory mediators (Oală et al., 2024). Transforming Growth Factor Beta (TGF-β) is one of the most important cytokines implicated in endometriosis. TGF-β exhibits dual and context-dependent roles: it suppresses inflammation by inhibiting NF-κB activation and pro-inflammatory cytokines, yet paradoxically promotes fibrosis, extracellular matrix deposition, angiogenesis, and lesion progression, making it a key driver of peritoneal remodeling in endometriosis (Baba et al., 2022; Sikora et al., 2018; Young et al., 2017). This duality creates a major biological paradox—while TGF-β may help regulate inflammation, its overexpression may worsen lesion persistence. Understanding factors that modulate TGF-β is therefore critical to clarifying the immunopathology of endometriosis.

Phaleria macrocarpa is an Indonesian medicinal plant rich in bioactive flavonoids, including naringenin and 8-prenylnaringenin, which have been widely studied for anti-inflammatory and immunomodulatory properties (Putri et al., 2023; Maharani et al., 2021). Previous studies demonstrate its ability to inhibit NF-κB activation and reduce pro-inflammatory cytokines; however, its specific influence on anti-inflammatory cytokines, particularly TGF-β, has been rarely investigated. Given the complex and conflicting role of TGF-β in endometriosis, examining whether *Phaleria macrocarpa* modulates this cytokine represents a significant knowledge gap in the current literature (Maharani et al., 2021).

Therefore, this study aimed to evaluate the effect of *Phaleria macrocarpa* flavonoid extract on TGF-β levels in a Balb/C mouse model of endometriosis. By addressing the modulation of a key cytokine involved in both inflammatory suppression and fibrotic progression, this research provides insights into the potential therapeutic or adverse implications of *Phaleria macrocarpa* in endometriosis.

METHODS

This study was a true experimental in vivo laboratory study using a post-test only controlled group design. The research was conducted on female Balb/C mice (*Mus musculus*), aged 8–10 weeks and weighing 25–30 g, housed under standardized conditions (12-hour light–dark cycle, temperature 22–24°C, and ad libitum access to food and water). All procedures involving animals were approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Medicine, Brawijaya University (Approval No.: 131/EC/KEPK-S2/05/2025), and were conducted in accordance with ARRIVE guidelines.

Endometriosis was induced following the standardized autologous endometrial implantation method. Donor uterine horns were harvested aseptically, and endometrial tissue was separated, minced into 1×1 mm fragments, and implanted into the peritoneal cavity through a small midline incision under ketamine–xylazine anesthesia. The induction was performed only once, and lesion establishment was allowed for 14 days prior to treatment.

Thirty mice were randomly assigned into six groups (n=5 per group): K− (healthy control), K+ (endometriosis-induced control), P1 (3.75 mg/mouse/day), P2 (7.5 mg/mouse/day), P3 (15 mg/mouse/day), and P4 (30 mg/mouse/day). Flavonoid extract of *Phaleria macrocarpa* was administered orally using an oral gavage tube for 14 consecutive days. The dosage selection was based on prior pharmacological studies and adjusted for mouse body weight; however, a formal power calculation was limited by the availability of preliminary cytokine data, which is acknowledged as a limitation.

At the end of treatment, mice were anesthetized, and peritoneal fluid samples were collected through lavage. TGF-β levels were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Brand: Elabscience®, Catalog No.: E-EL-M0048; total TGF-β). All measurements were performed in duplicate according to the manufacturer's protocol.

Data analysis included normality testing (Shapiro–Wilk) and homogeneity testing (Levene's test). Since assumptions for parametric analysis were met, one-way ANOVA followed by Tukey's post hoc test was performed using SPSS version 26. The significance level was set at $\alpha = 0.05$ with a 95% confidence interval.

RESULT

Table 1. TGF-β levels in endometriosis model mice

Group	N	Min	Max	Mean ±SD (ng/L)
K-	5	0,21	0,28	0,25 ± 0,03
K+	5	0,21	0,28	0,24 ± 0,03
P1	5	0,14	0,24	0,21 ± 0,04
P2	5	0,28	0,33	0,31 ± 0,02
P3	5	0,23	0,30	0,26 ± 0,03
P4	5	0,21	0,29	0,23 ± 0,03

Based on Table 1, a total of 30 mice were included in the analysis, with five animals in each of the six groups (K−, K+, P1, P2, P3, P4). The descriptive statistics of TGF-β levels are presented in Table 1. The highest mean concentration of TGF-β was observed in Group P2 (0.31 ± 0.02 ng/L), followed by P3 (0.26 ± 0.03 ng/L), while the lowest level was found in P1 (0.21 ± 0.04 ng/L). Both control groups showed lower TGF-β concentrations compared to most treatment groups, with K− at 0.25 ± 0.03 ng/L and K+ at 0.24 ± 0.03 ng/L.

Tabel 2. Results of the Normality and Homogeneity Test of TGF-β Levels

Variable	Sig. *Shapiro-Wilk*	Data Distribution	Sig. *Levene test*	Data Homogeneity
K-	0,391	Normal	0,942	Homogenous
K+	0,980			
P1	0,103			
P2	0,257			
P3	0,332			
P4	0,093			

Description: The Sig. value for both the Shapiro-Wilk and Levene Tests shows a value >0.05, which indicates that the data is normally distributed and homogeneous.

The data analysis showed that normality testing using the Shapiro–Wilk test showed that all groups had p-values > 0.05, indicating normally distributed data. The Levene test also demonstrated homogeneity of variance (p = 0.942). Thus, the data met the assumptions required for parametric analysis.

Tabel 3. Results of one-way ANOVA test of TGF-β levels in endometriosis model mice

Sample Group	N	Mean ± SD (ng/L)	Sig.
K-	5	0,25 ± 0,03*	0,003
K+	5	0,24 ± 0,03*	
P1	5	0,21 ± 0,04*	
P2	5	0,31 ± 0,02*	
P3	5	0,26 ± 0,03*	
P4	5	0,23 ± 0,03*	

Description : There was a significant difference in TGF-β levels between groups (p = 0.003). The lowest mean level was in group P1 (3.75 mg/mouse/day), while the highest was in group P2 (7.5 mg/mouse/day, 0.31 ± 0.02). TGF-β levels in treatment groups varied compared to the positive control (K+)

Figure/Graphic/Illustration

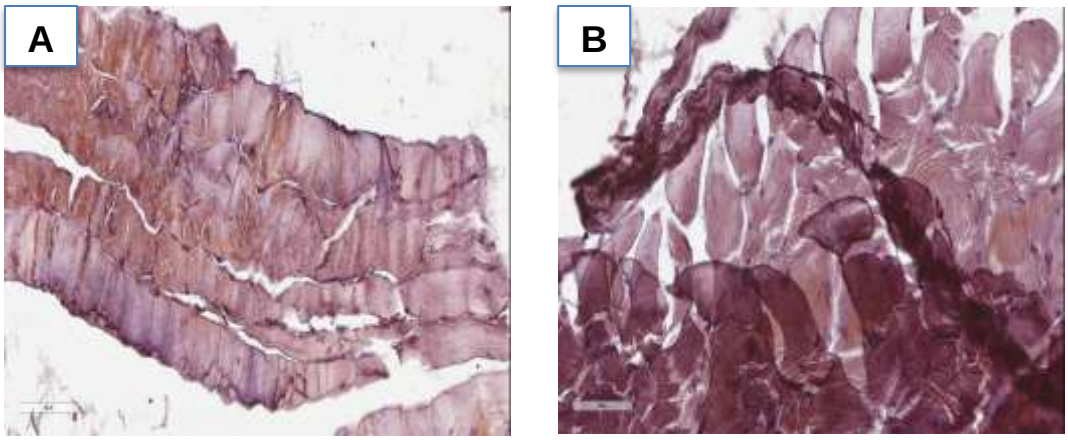

Figure 1. Illustration of ERβ expression in endometriosis model mice

Caption: A. Peritoneal tissue in healthy mice (K-)

B. Strong ERβ expression in the cytoplasm of endometriosis lesion stromal cells in the peritoneal tissue, characterized by a homogeneous brown color.

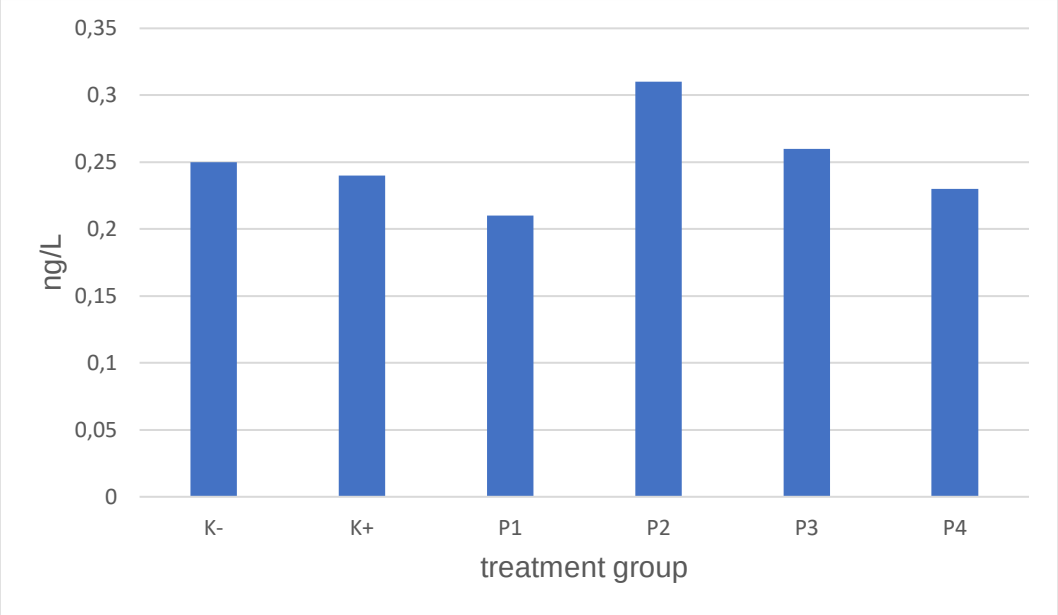

Figure 2. Histogram of the effect of Phaleria macrocarpa flavonoid extract on increasing Transforming Growth Factor Beta (TGF-β) levels in endometriosis model mice.

Based on the figure, the mean TGF-β levels differed significantly between groups. Group P2 (7.5 mg/mouse/day) showed the highest TGF-β level (0.31 ± 0.02), surpassing both control groups (K− and K+). In contrast, P1 (3.75 mg/mouse/day) had the lowest level (0.21 ± 0.04). Higher doses in P3 and P4 tended to reduce TGF-β levels. Interestingly, the TGF-β level in the untreated endometriosis group (K+) was slightly lower than that in healthy mice (K−), indicating suppressed TGF-β expression during disease progression.

DISCUSSION

The administration of *Phaleria macrocarpa* flavonoid extract demonstrated a modulatory effect on TGF-β levels in mice with induced endometriosis, a finding that must be interpreted within the complex molecular role of TGF-β in the disease. TGF-β is a multifunctional cytokine involved in immunoregulation, extracellular matrix remodeling, fibrosis, epithelial–mesenchymal transition, and immune evasion, making its elevation potentially beneficial or detrimental depending on the biological context. Flavonoids such as naringenin and 8-prenylnaringenin have been shown to influence TGF-β signaling by interacting with SMAD pathways, reducing oxidative stress, attenuating NF-κB activity, and promoting anti-inflammatory cytokine expression, which may partially explain the observed modulation. The biphasic dose–response pattern, with 7.5 mg/mouse/day producing the highest TGF-β level, suggests receptor saturation or feedback regulation typical of phytochemical immunomodulation.

However, the absence of molecular assays—including SMAD phosphorylation, macrophage polarization, regulatory T-cell assessment, or fibrosis markers—prevents

determining whether TGF-β elevation reflected anti-inflammatory compensation or pro-fibrotic stimulation. Additional limitations include the measurement of only one cytokine, a small sample size (n=5/group), short intervention duration, lack of lesion histopathology, and no evaluation of long-term progression. Thus, while the extract influences TGF-β expression, its biological and therapeutic significance remains uncertain and requires further mechanistic and longitudinal investigation.

To support the interpretation that increased TGF-β may exert anti-inflammatory effects in this model, several recent studies have demonstrated its immunosuppressive role in inflammatory conditions. TGF-β is known to inhibit the activation of nuclear factor kappa B (NF-κB) and suppress the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, thereby contributing to the resolution of inflammation (Zhou et al., 2019). In addition, TGF-β plays a crucial role in promoting regulatory T cell (Treg) differentiation, which further suppresses excessive immune responses and maintains immune tolerance (Li et al., 2018).

In experimental animal models, elevated TGF-β levels have been associated with reduced inflammatory cell infiltration and decreased expression of pro-inflammatory mediators, indicating a protective anti-inflammatory mechanism (Yoshimura & Muto, 2017). Furthermore, TGF-β signaling has been reported to shift macrophage polarization toward the M2 phenotype, which is linked to anti-inflammatory and tissue repair processes (Chen et al., 2020). These findings suggest that the increase in TGF-β observed in this study, particularly in the P2 group, may reflect an anti-inflammatory compensatory response rather than purely a pro-fibrotic effect. However, without additional molecular markers, this interpretation remains speculative and requires further validation.

Based on the findings of this study, the administration of high-dose flavonoid extract of *Phaleria macrocarpa* (30 mg/mouse/day) demonstrated reduced effectiveness compared to the optimal dose due to a biphasic or non-linear dose–response relationship, in which increasing the dose does not necessarily result in enhanced therapeutic effects. The dose of 7.5 mg/mouse/day was identified as the most optimal in increasing the levels of anti-inflammatory cytokines TGF-β, whereas higher doses are presumed to induce receptor saturation or desensitization of immune signaling pathways, leading to diminished cellular responses. Furthermore, the pleiotropic nature of TGF-β, which exhibit both anti-inflammatory and profibrotic properties, may contribute to a shift in biological effects at higher doses, thereby reducing their overall efficacy in modulating inflammation in the endometriosis mouse model.

CONCLUSION

The administration of *Phaleria macrocarpa* flavonoid extract demonstrated a modulatory effect on TGF-β levels in a mouse model of endometriosis, with the 7.5 mg/mouse/day dose producing the most notable response; however, given the dual role of TGF-β in both anti-inflammatory regulation and pro-fibrotic lesion progression, the findings must be interpreted cautiously. The increase in TGF-β cannot be assumed to be therapeutic without confirming whether it reflects beneficial immune modulation or potentially adverse fibrotic activation. This study is further limited by its measurement of only a single cytokine, absence of molecular and histopathological analyses (such as SMAD signaling, macrophage polarization, fibrosis markers, and lesion morphology), small sample size, and short duration, all of which restrict the generalizability and biological interpretation of the results. Therefore, although preliminary findings suggest that *Phaleria macrocarpa* flavonoids influence TGF-β expression, more comprehensive mechanistic and longitudinal studies are required before any therapeutic relevance in endometriosis can be established.

REFERENCES

Baba, A. B., Rah, B., Bhat, G. R., Mushtaq, I., Parveen, S., Hassan, R., Hameed Zargar, M., & Afroze, D. (2022). Transforming Growth Factor-Beta (TGF-β) Signaling in Cancer-A Betrayal Within. *Frontiers in Pharmacology*, *13*(February), 1–16. https://doi.org/10.3389/fphar.2022.791272.

Chen, W., Ten Dijke, P., & others. (2020). Immunoregulation by members of the TGF-β superfamily. Nature Reviews Immunology, 20(6), 1–17. https://doi.org/10.1038/s41577-019-0263-6

Falcone, T., & Flyckt-Rebecca, R. (2018). Clinical management of endometriosis. *Obstetrics and Gynecology*, *131*(3), 557–571. https://doi.org/10.1097/AOG.0000000000002469

Garcia-Garcia, J. M., Vannuzzi, V., Donati, C., Bernacchioni, C., Bruni, P., & Petraglia, F. (2023). Endometriosis: Cellular and Molecular Mechanisms Leading to Fibrosis. *Reproductive Sciences*, *30*(5), 1453–1461. https://doi.org/10.1007/s43032-022-01083-x.

Li, M. O., Wan, Y. Y., & Flavell, R. A. (2018). T cell-produced transforming growth factor-β1 controls T cell tolerance and regulates Th1- and Th17-cell differentiation. Immunity, 26(5), 579–591. https://doi.org/10.1016/j.immuni.2007.03.014

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. *Journal of Ayurveda and Integrative Medicine*, *12*(2), 229–233. https://doi.org/10.1016/j.jaim.2020.06.002.

Oală, I. E., Mitranovici, M. I., Chiorean, D. M., Irimia, T., Crișan, A. I., Melinte, I. M., Cotruș, T., Tudorache, V., Moraru, L., Moraru, R., Caravia, L., Morariu, M., & Pușcașiu, L. (2024). Endometriosis and the Role of Pro-Inflammatory and Anti-Inflammatory Cytokines in Pathophysiology: A Narrative Review of the Literature. *Diagnostics*, *14*(3). https://doi.org/10.3390/diagnostics14030312.

Putri, D., Wirasutisna, K. R., Mariani, R., & Wibowo, D. P. (2023). Literature Review Ethnopharmacognosy, Chemical Content and Pharmacological Activity of Mahkota Dewa Plant (Phaleria macrocarpa (Scheff) Boerl): Literature Review. *Jurnal Kolaboratif Sains*, *6*(12), 1660–1668. https://doi.org/10.56338/jks.v6i12.4458

Sikora, J., Smycz-Kubańska, M., Mielczarek-Palacz, A., Bednarek, I., & Kondera-Anasz, Z. (2018). The Involvement of Multifunctional TGF-β and Related Cytokines in Pathogenesis of Endometriosis. *Immunology Letters*, *201*, 31–37. https://doi.org/10.1016/j.imlet.2018.10.011

World Health Organization. (2023). *Penyakit Endometriosis*. https://www.who.int/news-room/fact-sheets/detail/endometriosis

Yoshimura, A., & Muto, G. (2017). TGF-β function in immune suppression. Cytokine & Growth Factor Reviews, 31, 1–8. https://doi.org/10.1016/j.cytogfr.2016.10.002

Young, V. J., Ahmad, S. F., Duncan, W. C., & Horne, A. W. (2017). The role of TGF-β in the pathophysiology of peritoneal endometriosis. *Human Reproduction Update*, *23*(5), 548–559. https://doi.org/10.1093/HUMUPD/DMX016

Zhou, W. J., Yang, H. L., Shao, J., Mei, J., Chang, K. K., Zhu, R., & Li, M. Q. (2019). Anti-inflammatory cytokines in endometriosis. *Cellular and Molecular Life Sciences*, *76*(11), 2111–2132. https://doi.org/10.1007/s00018-019-03056-x