Research in Pharmaceutical Sciences, April 2026; 21(2): 206-214
Received: 24-08-2025
Peer Reviewed: 21-09-2025
Revised: 21-10-2025
Accepted: 25-10-2025
Published: 24-04-2026

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

The effects of *Vitex agnus-castus* supplementation on inflammatory markers in women with PCOS: a randomized, double-blind, placebo-controlled trial

Aniseh Hatami[1], Hanieh Taheri[1], Ali Khosrowbeygi[2,3], Fateme Seidi[4], Mohadeseh Azimi[1], and Farideh Jalali-Mashayekhi[3,2,*]

[1]Student Research Committee, Arak University of Medical Sciences, Arak, Iran. [2]Department of Biochemistry and Genetics, School of Medicine, Arak University of Medical Sciences, Arak, Iran. [3]Molecular and Medicine Research Center, Arak University of Medical Sciences, Arak, Iran. [4]Department of Obstetrics and Gynecology, School of Medicine, Arak University of Medical Sciences, Arak, Iran.

Abstract

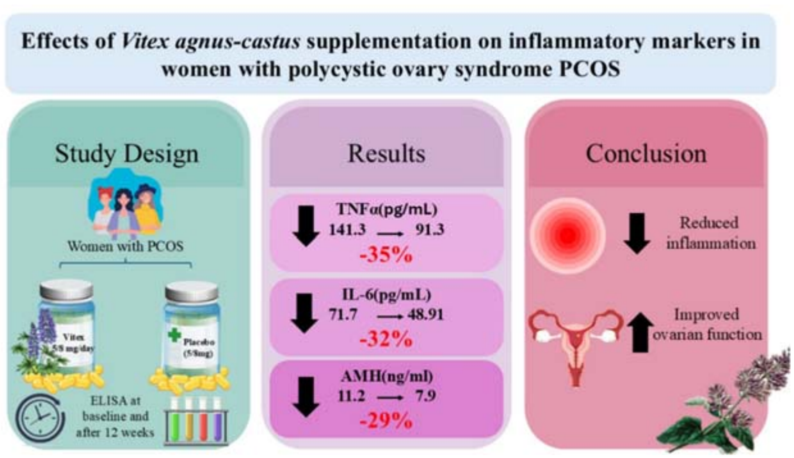
Background and purpose: To evaluate the effects of *Vitex agnus-castus* supplementation on serum levels of inflammatory cytokines (primary outcomes: tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6); secondary outcome: anti-Mullerian hormone (AMH)) in women with polycystic ovary syndrome (PCOS).

Experimental approach: This secondary analysis included 40 serum samples (20 *Vitex* and 20 placebo) randomly selected from a previously registered randomized, double-blind, placebo-controlled trial in women with PCOS. Participants received either *Vitex* (5.8 mg/day, standardized to 0.42-0.82 mg aucubin) or a placebo for 12 weeks. Serum TNF-α, IL-6, and AMH were measured at baseline and week 12 using ELISA. Within-group changes were assessed with paired t-tests, and between-group differences were analyzed using independent t-tests and ANCOVA using SPSS v23.0 software.

Findings/Results: *Vitex* supplementation significantly reduced serum TNF-α (141.27 ± 24.27 to 91.28 ± 19.57 pg/mL), IL-6 (71.72 ± 13.63 to 48.91 ± 24.19 pg/mL), and AMH (11.21 ± 3.89 to 7.88 ± 4.87 ng/mL) levels, whereas the placebo group showed no significant changes. The between-group differences were significant for all markers. These findings suggest potential benefits in reducing inflammation and improving ovarian function, pending confirmation in future clinical outcome studies.

Conclusion and implications: *Vitex* supplementation may offer a complementary approach to managing inflammation and hormonal disturbances in women with PCOS.

Keywords: Anti-Mullerian hormone; Interleukin-6; Polycystic ovary syndrome; TNF-α; *Vitex*.



*Corresponding author: F. Jalali-Mashayekhi
Tel: +98-9188614706, Fax: +98-8634173526
Email: mashayekhi@arakmu.ac.ir,faridehjalalimashayekhi@gmail.com

Access this article online
Website: http://rps.mui.ac.ir
DOI: 10.4103/RPS.RPS_152_25

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting 6-20% of women of reproductive age worldwide (1). PCOS is characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology, leading to reproductive, metabolic, and psychological challenges (2). Diagnosis based on the Rotterdam criteria requires at least two of the following: oligo- or anovulation, hyperandrogenism (biochemical and/or clinical), and polycystic ovaries on ultrasound (3). PCOS significantly impairs the quality of life and increases the risk of infertility, metabolic syndrome, cardiovascular disease, and endometrial cancer (4). Its etiology involves genetic, environmental, and lifestyle factors (5).

Chronic low-grade inflammation is a key driver, with elevated pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) contributing to insulin resistance and ovarian dysfunction by impairing granulosa cell function, follicular maturation, and ovulation, thus exacerbating hyperandrogenism (6-8). Anti-Mullerian hormone (AMH), produced by granulosa cells of preantral and small antral follicles, is a marker of ovarian reserve and is often elevated in PCOS due to increased follicle numbers and altered granulosa cell function (9,10).

AMH is clinically relevant for assessing fertility treatment responses and reproductive lifespan, suggesting that its modulation may be beneficial (10).

Current PCOS treatments, such as lifestyle changes, oral contraceptives, and metformin, often fail to address chronic inflammation (11,12). Concerns about their long-term side effects have increased interest in herbal medicines, such as *Vitex,* for PCOS management (8). Ample evidence demonstrates the protective role of antioxidants against oxidative damage induced by reactive oxygen species (13-15). Antioxidants, including *Vitex*, have shown promise in alleviating PCOS symptoms by reducing inflammation and oxidative stress (16). *Vitex*, a widely used herbal remedy, contains bioactive compounds (*e.g.*, flavonoids and iridoids) that inhibit pro-inflammatory cytokines (TNF-α and IL-6) *via* nuclear factor kappa B (NF-κB) suppression and modulate dopaminergic activity, affecting prolactin and ovarian function (17,18). While *Vitex* has been studied for its hormonal effects in PCOS (*e.g.*, prolactin and luteinizing hormone (LH) / follicle-stimulating hormone (FSH) ratios) (19), its impact on TNF-α, IL-6, and AMH remains unexplored.

While prior randomized trials have evaluated *Vitex agnus-castus* for outcomes such as prolactin levels or menstrual symptoms (19), to our knowledge, no randomized, placebo-controlled trial has specifically assessed its effects on inflammatory cytokines or AMH in women with PCOS.

Given the role of inflammation in PCOS and the anti-inflammatory properties of *Vitex* constituents (*e.g.*, casticin) *via* NF-κB suppression (15), this randomized, double-blind, placebo-controlled trial aimed to determine whether *Vitex* reduces circulating TNF-α, IL-6, and AMH levels in women with PCOS.

MATERIALS AND METHODS

Study design and setting

This study was a secondary analysis of serum samples collected as part of a randomized, double-blind, placebo-controlled clinical trial (registered on March 28, 2023) conducted at the Arak University of Medical Sciences (20). The original trial investigated the effects of *Vitex* supplementation in women with PCOS. This present sub-study specifically assessed inflammatory and hormonal markers, including TNF-α, IL-6, and AMH.

Participants

The parent trial (20) initially screened 105 women aged 18-45 years who were diagnosed with PCOS according to the Rotterdam criteria. For this sub-study, serum samples were first screened for adequate remaining volume (≥ 1 mL) to allow measurement of TNF-α, IL-6, and AMH. Only 2-3 samples were excluded due to insufficient volume. From the remaining eligible samples, a total of 40 participants

(20 per group) were randomly selected using a computer-generated random number sequence. This approach ensured sufficient sample volume for all assays while introducing randomness into the selection process.

Following blood collection, serum was immediately separated *via* centrifugation and aliquoted into five individual microtubes to prevent degradation caused by repeated freeze-thaw cycles. All aliquots were immediately stored continuously at -80 ℃. The serum samples selected for the sub-study assays (TNF-α, IL-6, and AMH) were retrieved from the freezer approximately 2.5 months after initial storage. Critically, only one aliquot per participant was used for the final batch analysis, ensuring the assays were performed on samples that had undergone zero freeze-thaw cycles.

The inclusion criteria were as follows: women aged 18-45 years, diagnosed with PCOS according to the Rotterdam criteria, and not planning pregnancy during the study period.

Exclusion criteria included pregnancy, lactation, smoking, hyperprolactinemia, thyroid dysfunction, diabetes mellitus, recent use (within 3 months) of antioxidant, anti-inflammatory, immunosuppressive, or other herbal supplements, or dopamine antagonists, or starting any of these medications during the intervention period.

Exit criteria were criteria for withdrawal from the intervention included withdrawal of informed consent, occurrence of a severe adverse event, or failure to comply with the protocol (defined as taking less than 80% of the prescribed daily dose of *Vitex* or placebo).

Randomization and blinding

In the parent trial, participants were randomized using permuted block randomization (block size = 4). Allocation concealment was ensured using sequentially numbered, opaque, sealed envelopes. Both the participants and healthcare providers were blinded to the group assignments.

Intervention

The *Vitex* group received 5.8 mg/day of standardized *Vitex agnus-castus* extract (Agnugol tablets; *Goldaru* Pharmaceutical Company, Isfahan, Iran) for 12 weeks, while the placebo group received identical cellulose acetate tablets for the same duration. The placebo tablets were also prepared by Goldaru Pharmaceutical Company (Isfahan, Iran) to be visually, olfactorily, and physically identical to the active *Vitex* tablets to maintain the double-blind nature of the trial. Both groups continued their prescribed metformin and combined oral contraceptive regimens (3 mg drospirenone + 0.03 mg ethinyl estradiol). Adherence was monitored biweekly through pill counts and phone calls.

Sample size

Based on TNF-α (7), with a two-sided significance level (α) of 0.05 and power (1 - β) of 80% (β = 0.20), the minimum sample size per group was calculated as 17 using the following equation:

$$n \geq 2\,(Z_{\alpha} + Z_{\beta})^2\,\sigma^2 / (\mu_1 - \mu_2)^2$$

$\alpha = 0.05$, $\beta = 0.20$, $\mu_1 = 5.27$, $\mu_2 = 4.48$, $\sigma \approx 0.81$, $n_1 = n_2 \geq 17$

Considering an estimated dropout rate of 10%, we aimed to recruit 20 participants per group.

Sample collection and laboratory assays

After a 12-h overnight fast, venous blood samples were collected and centrifuged at 3000 rpm for 10 min. Serum aliquots were stored at -80 °C until analysis. Anthropometric measurements (weight and height) were recorded at baseline and after the intervention.

Serum AMH concentrations were measured in duplicate using a commercial ELISA kit (Pishtaz Teb, Kerman, Iran; Cat. No. PT-AMH-96) with a sensitivity of 0.08 ng/mL and inter- / intra-assay CVs of < 6 - 8% and < 6 - 9%, respectively. TNF-α was measured using a commercial ELISA kit (Karmania Pars Gene, Kerman, Iran; Cat. No. KPG-HIL6P) with a sensitivity of 2 pg/mL and inter-/intra-assay CVs of < 8% and < 3%. IL-6 was measured using a commercial ELISA kit (Karmania Pars Gene; Cat. No. KPG-HIL6), with a sensitivity of 2 pg/mL and inter- / intra-assay CVs of < 8-10% and < 3-4%, respectively. All assays included positive and negative controls.

Statistical analysis

Analyses were performed using SPSS v23.0 (SPSS Inc., Chicago, IL). The Shapiro-Wilk test was used to assess normality. Continuous variables are presented as mean ± SD. Within-group changes were analyzed using paired t-tests, and between-group comparisons were performed using independent t-tests. ANCOVA was applied to adjust for baseline values. Given that multiple comparisons were conducted across three primary outcomes (TNF-α, IL-6, and AMH), we applied the Holm-Bonferroni sequential correction to control the Family-Wise error rate (FWER) and minimize the risk of type I error. This approach was chosen because it is less conservative than the standard Bonferroni method, thereby retaining greater statistical power while still reducing the likelihood of false-positive results. Both raw and Holm-Bonferroni adjusted *P*-values are presented in the Results section. *P*-values ≤ 0.05 were considered significantly different.

RESULTS

For this secondary analysis, 40 serum samples (20 *Vitex*, 20 placebo) were randomly selected from a previously registered randomized, double-blind, placebo-controlled trial involving 105 women with PCOS. In the original trial, 45 women were excluded (39 due to predefined exclusion criteria and 6 due to personal reasons), leaving 60 eligible participants who were randomized to receive *Vitex* or a placebo. For the present sub-study, 40 participants (20 per group) were randomly selected from the serum sample repository. All selected samples were analyzed with no exclusions or losses noted. Figure 1 provides a CONSORT flow diagram illustrating the parent trial enrollment, randomization, and sub-study sample selection process.

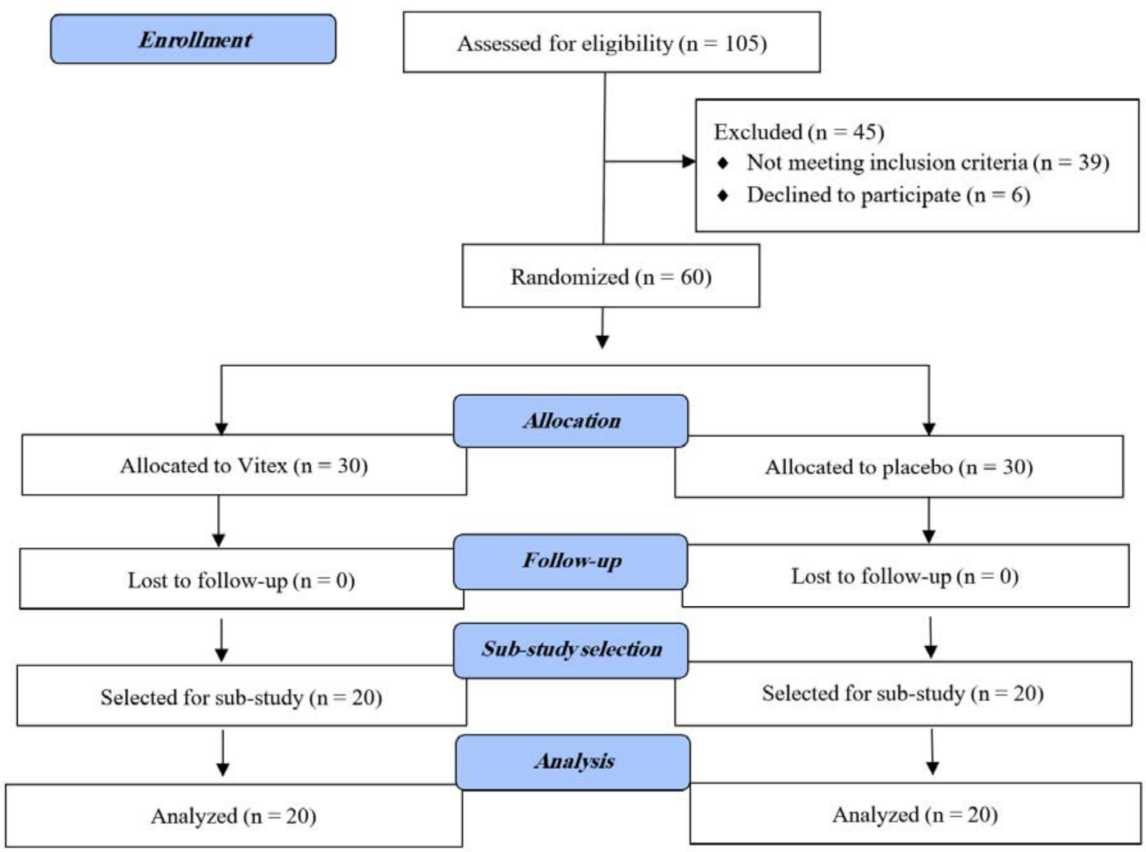


Fig. 1. Consort diagram of the study population, CONSORT diagram showing screening (n = 105), randomization in parent randomized controlled trial (n = 60), and sub-study sample selection (n = 40).

Table 1 presents the participants' demographic characteristics. No statistically significant differences were observed between the *Vitex* (n = 20) and placebo (n = 20) groups in terms of age, height, weight (both before and after intervention), and BMI (both before and after intervention) (all $P > 0.05$). The recruitment period began on April 4, 2023, and ended on January 20, 2024.

No significant adverse effects were observed or reported in either the *Vitex* or placebo group during the 12-week intervention period. Figure 2A-C illustrates the serum levels of AMH, TNF-α, and IL-6 at baseline and post-intervention, along with their respective changes in the two groups. In the placebo group, the mean AMH level increased from 10.97 ± 4.25 ng/mL to 11.87 ± 2.99 ng/mL ($P = 0.290$). Conversely, in the *Vitex* group, the mean AMH level significantly decreased from 11.21 ± 3.89 ng/mL to 7.88 ± 4.87 ng/mL. The between-group difference in the change in mean AMH levels was also significant.

Table 1. Baseline and post-intervention anthropometric characteristics. Data are presented as mean ± SD.

Variable	*Vitex* (n = 20)	Placebo (n = 20)	*P*-values[a]
Age (year)	26.30 ± 6.01	28.35 ± 5.12	0.25
Height (m)	1.60 ± 0.05	1.62 ± 0.06	0.27
Weight before (kg)	65.90 ± 11.91	67.45 ± 14.01	0.71
Weight after (kg)	64.85 ± 9.69	66.60 ± 12.05	0.62
BMI before (kg/m^2)	25.71 ± 4.50	25.60 ± 4.82	0.94
BMI after (kg/m^2)	25.33 ± 3.89	25.31 ± 4.18	0.99

BMI: Body Mass Index; [a], Based on an independent t-test

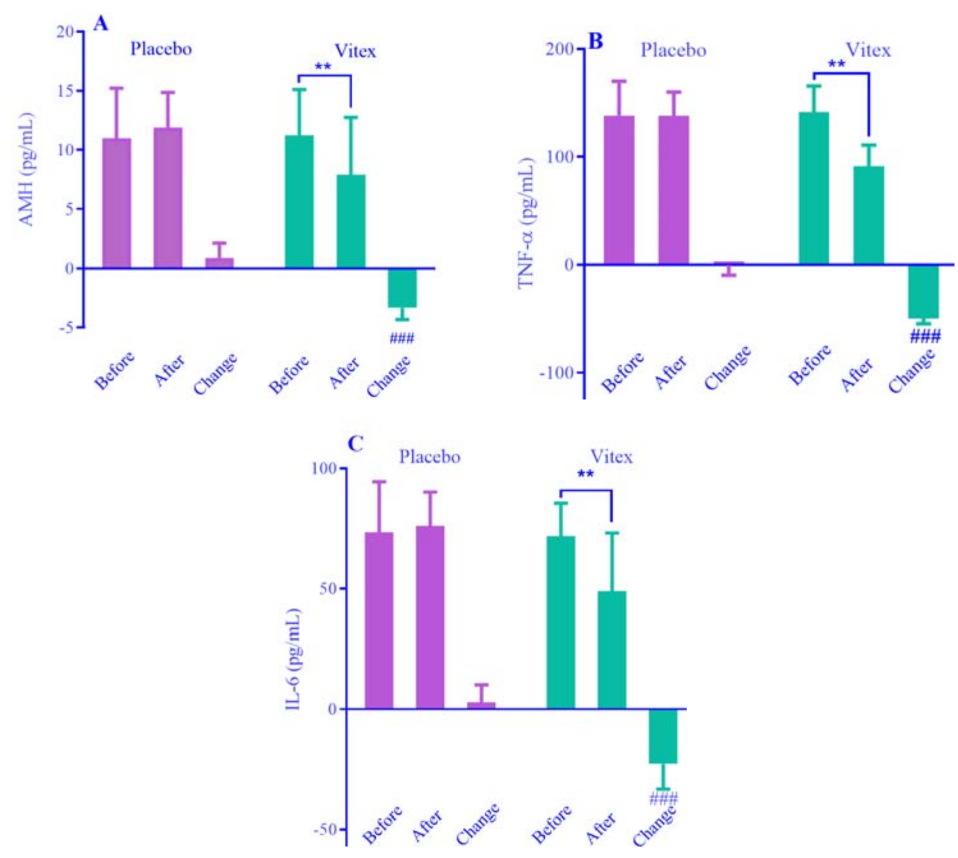


Fig. 2. Comparison of serum concentrations for (A) AMH, (B) TNF-α in panel, and (C) IL-6 in women with PCOS before and after a 5.8 mg *Vitex* dry extract or placebo intervention. For each group, the initial serum level (Before), the final level (After), and the net difference (Change, calculated as After - Before) are shown. The data are presented as mean ± SD. $^{**}P < 0.01$ indicates significant differences between designated groups; $^{\#\#\#}P < 0.01$ shows differences between the groups "Change". TNF-α, Tumor necrosis factor-alpha; IL-6, interleukin-6; AMH, anti-Müllerian hormone.

At baseline level, there was no significant difference between the mean of TNF-α of the placebo and *Vitex* groups (P = 0.760). Following the intervention, a statistically significant decrease was observed in the *Vitex* group, while the placebo group remained relatively stable (P = 0.970). The between-group difference in the change from baseline was significant.

Baseline IL-6 levels were similar between the *Vitex* and placebo groups (P = 0.760). Post-intervention, a statistically significant decrease was observed in the *Vitex* group. The placebo group showed a slight, non-significant increase (P = 0.400). The between-group difference in change from baseline was statistically significant.

DISCUSSION

This study found that 12 weeks of *Vitex agnus-castus* supplementation was associated with significant reductions in serum TNF-α, IL-6, and AMH levels in women with PCOS. These findings suggest potential benefits for both inflammatory and hormonal profiles, supporting Vitex as a complementary approach for PCOS management. Importantly, after applying the Holm-Bonferroni sequential correction for multiple comparisons, these reductions remained statistically significant, reinforcing the robustness of our finding.

Several phytochemicals in Vitex, including casticin, flavonoids, and iridoid glycosides, may contribute to these effects. Casticin suppresses NF-κB-mediated signaling, reducing TNF-α and IL-6 (21,22). Vitex's dopaminergic agonist activity may modulate prolactin and gonadotropin release, influencing folliculogenesis and AMH expression (18). The observed reduction in pro-inflammatory cytokines is particularly notable, as chronic low-grade inflammation drives insulin resistance in PCOS. TNF-α impairs insulin receptor substrate-1 signaling, whereas IL-6 promotes hepatic glucose output and peripheral insulin resistance (22-25). Additionally, antioxidant compounds in Vitex, such as quercetin and vanillic acid, may attenuate oxidative stress, further supporting its anti-inflammatory actions (17,26).

Our findings are consistent with animal studies showing reduced TNF-α, IL-6, and C-reactive protein levels in letrozole-induced PCOS models (27,28), although extrapolation to humans requires caution. Beyond PCOS, vitexin, a major flavonoid constituent, has shown neuroprotective effects in Parkinson's and Alzheimer's disease models by attenuating microglial activation and suppressing pro-inflammatory cytokines (29,30). Casticin has been reported to downregulate NF-κB signaling and reduce immune cell infiltration in inflammatory joint disorders (31). Moreover, Vitex extracts improved systemic inflammation and metabolic parameters in experimental models of diabetes and metabolic syndrome (32). Collectively, these studies support Vitex as a plant-based therapeutic agent with broad anti-inflammatory potential.

The significant decline in AMH observed in this trial is clinically relevant, given that elevated AMH reflects excessive small antral follicles and disrupted folliculogenesis in PCOS (10,33). Pro-inflammatory cytokines, such as TNF-α and IL-6, can interfere with follicular maturation and oocyte quality (34,35). By reducing these cytokines, Vitex supplementation may indirectly support granulosa cell function and normalize AMH expression (36). Although these findings are promising, their direct implications for ovarian function, ovulation, and fertility outcomes remain uncertain and warrant further investigation.

This study had several limitations. Despite meeting the recalculated sample size requirement (≥ 17 per group), the relatively small number (n = 20 per group) limited the statistical power to detect smaller but clinically relevant effects. The 12-week duration may also be insufficient for assessing long-term outcomes. All participants continued metformin and oral contraceptive use, which are known to influence inflammatory and hormonal markers. Although randomization likely balanced these factors, residual confounding cannot be excluded. Additionally, a few participants were excluded due to insufficient serum volume, introducing potential selection bias. Future studies with larger sample sizes, power calculations specific to IL-6 and AMH, and wash-out or factorial designs are warranted to confirm these results and isolate the independent effects of Vitex.

CONCLUSION

To our knowledge, this is the first randomized, double-blind, placebo-controlled trial to specifically investigate the effects of Vitex on inflammatory cytokines (TNF-α and IL-6) and AMH in women with PCOS. By focusing on biomarkers closely linked to PCOS pathophysiology, our study extends the evidence base beyond prior trials that mainly evaluated prolactin or menstrual symptoms. The observed biochemical improvements confirmed the measurable biological activity of Vitex; however, the clinical implications remain uncertain, and these results should be considered hypothesis-generating. Larger, longer-term trials incorporating reproductive and metabolic endpoints are needed to determine whether these biochemical changes translate into meaningful clinical benefits. In conclusion, this study demonstrated that Vitex supplementation has a significant modulating effect on key reproductive and inflammatory markers. Vitex significantly reduced the serum levels of TNF-α and IL-6, two pro-inflammatory cytokines implicated in various pathological conditions. Furthermore, Vitex supplementation significantly decreased serum AMH levels. These results indicate that Vitex may have a positive impact on reproductive health and inflammatory responses.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the Research Council of Arak University of Medical Sciences through Grant No. 4524. The authors express their sincere gratitude to all the participants for their valuable contribution to this study.

Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

A. Hatami and H. Taheri conducted the investigation, were responsible for data curation, and wrote the original draft of the manuscript. F. Seidi provided supervision and performed validation. F. Jalali-Mashayekhi was responsible for project administration, performed validation, created the visualization, and conducted the formal analysis and methodology. A. Khosrowbeygi served as an advisor and performed validation. M. Azimi conducted the investigation and was responsible for data curation. F. Seidi, F. Jalali-Mashayekhi, A. Khosrowbeygi, and M. Azimi participated in writing, reviewing, and editing the article. All authors read and approved the final version of the manuscript.

All authors have read and approved the finalized article. Each author has fulfilled the authorship criteria and affirmed that this article represents honest and original work.

Trial registration and ethical considerations

The parent trial was approved by the Ethics Committee of Arak University of Medical Sciences (IR.ARAKMU.REC.1403.061), and the trial was registered at the Iranian Registry of Clinical Trials (IRCT20230222057493N1). Written informed consent was obtained from all participants, including permission for the storage and future use of biological samples. This sub-study adhered to the principles of the Declaration of Helsinki.

Data availability

The data associated with the current study are available from the corresponding author upon a reasonable request.

AI declaration

To improve readability and language, ChatGPT was used. After using this tool, the author(s) reviewed and edited the content and take full responsibility for the content of the publication.

REFERENCES

1. Rodriguez-Paris D, Remlinger-Molenda A, Kurzawa R, Głowińska A, Spaczyński R, Rybakowski F, *et al*. Psychiatric disorders in women with polycystic ovary syndrome. Psychiatr Pol. 2019;53(4):955-966. DOI: 10.12740/PP/OnlineFirst/93105.
2. Vink JM, Sadrzadeh S, Lambalk CB, Boomsma DI. Heritability of polycystic ovary syndrome in a Dutch

twin-family study. J Clin Endocrinol Metab. 2006;91(6):2100-2104.
DOI: 10.1210/jc.2005-1494.
3. Siddiqui S, Mateen S, Ahmad R, Moin S. A brief insight into the etiology, genetics, and immunology of polycystic ovarian syndrome (PCOS). J Assist Reprod Genet. 2022;39(11):2439-2473.
DOI: 10.1007/s10815-022-02625-7.
4. Stańczak NA, Grywalska E, Dudzińska E. The latest reports and treatment methods on polycystic ovary syndrome. Ann Med. 2024;56(1):2357737,1-21.
DOI: 10.1080/07853890.2024.2357737.
5. Bednarska S, Siejka A. The pathogenesis and treatment of polycystic ovary syndrome: What's new? Adv Clin Exp Med. 2017;26(2):359-367.
DOI: 10.17219/acem/59380.
6. Rudnicka E, Suchta K, Grymowicz M, Calik-Ksepka A, Smolarczyk K, Duszewska AM, *et al*. Chronic low grade inflammation in pathogenesis of PCOS. Int J Mol Sci. 2021;22(7):3789,1-12.
DOI: 10.3390/ijms22073789.
7. Tarkun I, Çetinarslan B, Türemen E, Cantürk Z, Biyikli M. Association between circulating tumor necrosis factor-alpha, interleukin-6, and insulin resistance in normal-weight women with polycystic ovary syndrome. Metab Syndr Relat Disord. 2006;4(2):122-128.
DOI: 10.1089/met.2006.4.122.
8. Li Y, Zheng Q, Sun D, Cui X, Chen S, Bulbul A, *et al*. Dehydroepiandrosterone stimulates inflammation and impairs ovarian functions of polycystic ovary syndrome. J Cell Physiol. 2019;234(5):7435-7447.
DOI: 10.1002/jcp.27501.
9. Eilertsen TB, Vanky E, Carlsen SM. Anti-Mullerian hormone in the diagnosis of polycystic ovary syndrome:can morphologic description be replaced. Hum Reprod. 2012;27(8):2494-2502.
DOI: 10.1093/humrep/des213.
10. Rudnicka E, Kunicki M, Calik-Ksepka A, Suchta K, Duszewska A, Smolarczyk K, *et al*. Anti-Müllerian hormone in pathogenesis, diagnostic and treatment of PCOS. Int J Mol Sci. 2021;22(22):12264,1-12.
DOI: 10.3390/ijms222212507.
11. Morgante G, Massaro MG, Di Sabatino A, Cappelli V, De Leo V. Therapeutic approach for metabolic disorders and infertility in women with PCOS. Gynecol Endocrinol. 2018;34(1):4-9.
DOI: 10.1080/09513590.2017.1370644.
12. Ortiz-Flores AE, Luque-Ramírez M, Escobar-Morreale HF. Pharmacotherapeutic management of comorbid polycystic ovary syndrome and diabetes. Expert Opin Pharmacother. 2018;19(17):1915-1926.
DOI: 10.1080/14656566.2018.1528231.
13. Samimi F, Baazm M, Eftekhar E, Rajabi S, Goodarzi M, Jalali Mashayekhi F. Possible antioxidant mechanism of coenzyme Q10 in diabetes: impact on Sirt1/Nrf2 signaling pathways. Res Pharm Sci. 2019;14(6):524-533.
DOI: 10.4103/1735-5362.272561.
14. Mafi A, Mokhtari Z, Hosseini E, Alimohammadi M, Aarabi MH, Askari G. Effect of saffron (*Crocus sativus*) supplementation on oxidative stress, inflammatory indices, and renal and liver function parameters in patients with type 2 diabetes mellitus:a GRADE-assessed systematic review and meta-analysis of randomized clinical trials. Nutr Rev. 2025;83(6):971-987.
DOI: 10.1093/nutrit/nuae121.
15. Roustaei Rad N, Movahedian A, Feizi A, Aminorroaya A, Aarabi M. Antioxidant effects of astaxanthin and metformin combined therapy in type 2 diabetes mellitus patients: a randomized double-blind controlled clinical trial. Res Pharm Sci. 2022;17(2):219-230.
DOI: 10.4103/1735-5362.335179.
16. Li W, Liu C, Yang Q, Zhou Y, Liu M, Shan H. Oxidative stress and antioxidant imbalance in ovulation disorder in patients with polycystic ovary syndrome. Front Nutr. 2022;9:1018674,1-10.
DOI: 10.3389/fnut.2022.1018674.
17. Kavaz A, Işık M, Dikici E, Yüksel M. Anticholinergic, antioxidant, and antibacterial properties of *Vitex agnus-castus L*. seed extract: assessment of its phenolic content by LC/MS/MS. Chem Biodivers. 2022;19(10):e202200143,1-10.
DOI: 10.1002/cbdv.202200143.
18. Feyzollahi Z, Kouchesfehani HM, Jalali H, Eslimi-Esfahani D, Hosseini AS. Effect of Vitex agnus-castus ethanolic extract on hypothalamic KISS-1 gene expression in a rat model of polycystic ovary syndrome. Avicenna J Phytomedicine. 2021;11(3):292-301.
PMID: 34046325.
19. Shahnazi M, Khalili AF, Hamdi K, Ghahremaninasab P. The effects of combined low-dose oral contraceptives and *Vitex agnus* on the improvement of clinical and paraclinical parameters of polycystic ovarian syndrome: a triple-blind, randomized, controlled clinical trial. Iran Red Crescent Med J. 2016;18(12):e37987,1-11.
DOI:10.5812/ircmj.37510.
20. Hatami A, Seidi F, Khosrowbeygi A, Moslemi A. The effect of *Vitex Agnus - Castus* plant on some markers of oxidative stress, lipid profile and insulin resistance in women with polycystic ovary syndrome: a randomized, double-blind controlled clinical trial study. JBRA Assist Reprod. 2025 (Advanced View).
DOI: 10.5935/1518-0557.20250165.
21. Gao L, Gu Y, Yin X. High serum tumor necrosis factor-alpha levels in women with polycystic ovary syndrome: a meta-analysis. PLoS One. 2016;11(10):e0164021,1-18.
DOI: 10.1371/journal.pone.0164021.
22. Oróstica L, Poblete C, Romero C, Vega M. Pro-inflammatory markers negatively regulate IRS1 in endometrial cells and endometrium from women with obesity and PCOS. Reprod Sci. 2020;27(1):290-300.
DOI: 10.1007/s43032-019-00026-3.
23. Oróstica L, García P, Vera C, García V, Romero C, Vega M. Effect of TNF-α on molecules related to the insulin action in endometrial cells exposed to hyperandrogenic and hyperinsulinic conditions, characteristics of polycystic ovary syndrome. Reprod Sci. 2018;25(7):1000-1009.
DOI: 10.1177/1933719117732157.

24. Thathapudi S, Kodati V, Erukkambattu J, Katragadda A, Addepally U, Hasan Q. Tumor necrosis factor-alpha and polycystic ovarian syndrome: a clinical, biochemical, and molecular genetic study. Genet Test Mol Biomarkers. 2014;18(9):605-609. DOI: 10.1089/gtmb.2014.0151.
25. Yu H, Gao X, Ge Q, Tai W, Hao X, Shao Q, *et al*. Tumor necrosis factor-α reduces adiponectin production by decreasing transcriptional activity of peroxisome proliferator-activated receptor-γ in calf adipocytes. J Dairy Sci. 2023;106(7):5182-5195. DOI: 10.3168/jds.2022-22919.
26. Al-Ajwady HAN, Al-Zobidy AMH. Effect of *Vitex agnus-castus* ethanolic extract and clomiphene citrate on oxidative stress and immunological parameters in female rats with polycystic ovary syndrome induced by letrozole. Reprod Heal East Eur. 2024;14(3):389-395. DOI: 10.34883/PI.2024.14.3.009.
27. Kariyawasam KWJC, Sirisena PDNN, Nanayakkara HLC, Ratnasooriya WD, Handunnetti SM. *Vitex negundo L*. leaf extract inhibits IL-6 and TNF-α secretion and phagocytosis in human leukocytes. J Herb Med. 2020;21:100341,1-7. DOI: 10.1016/j.hermed.2020.100341.
28. Enechukwu CI, Onuegbu AJ, Olisekodiaka MJ, Eleje GU, Ikechebelu JI, Ugboaja JO, *et al*. Oxidative stress markers and lipid profiles of patients with polycystic ovary syndrome in a Nigerian tertiary hospital. Obstet Gynecol Sci. 2019;62(5):335-343. DOI: 10.5468/ogs.2019.62.5.335.
29. Hu M, Li F, Wang W. Vitexin protects dopaminergic neurons in MPTP-induced Parkinson's disease through PI3K/AKT signaling pathway. Drug Des Devel Ther. 2018;12:565-573. DOI: 10.2147/DDDT.S156920.
30. Yahaya MAF, Zolkiffly SZI, Moklas MAM, Hamid HA, Stanslas J, Zainol M, *et al*. Possible epigenetic role of vitexin in regulating neuroinflammation in Alzheimer's disease. J Immunol Res. 2020;2020:9469210,1-7. DOI: 10.1155/2020/9469210.
31. Li X, Wang Y. Cinnamaldehyde attenuates the progression of rheumatoid arthritis through down-regulation of PI3K/AKT signaling pathway. Inflammation. 2020;43(5):1729-1741. DOI: 10.1007/s10753-020-01246-5
32. Ahangarpour A, Oroojan AA, Khorsandi L, Najimi SA. Pancreatic protective and hypoglycemic effects of *Vitex agnus-castus L*. fruit hydroalcoholic extract in D-galactose-induced aging mouse model. Res Pharm Sci. 2017;12(2):137-143. DOI: 10.4103/1735-5362.202452.
33. Abduljabbar HA, Mahmood MM, Al-Jbooriay MJ, Al-obaidi MT. Concurrent autoimmune and endocrine dysregulation in polycystic ovary syndrome. Middle East Fertil Soc J. 2025;30(1):23,1-10. DOI: 10.1186/s43043-025-00236-7.
34. Su P, Chen C, Sun Y. Physiopathology of polycystic ovary syndrome in endocrinology, metabolism and inflammation. J Ovarian Res. 2025;18(1):34,1-10. DOI: 10.1186/s13048-025-01621-6.
35. Adams J, Liu Z, Ren YA, Wun WS, Zhou W, Kenigsberg S, *et al*. Enhanced inflammatory transcriptome in the granulosa cells of women with polycystic ovarian syndrome. J Clin Endocrinol Metab. 2016;101(9):3459-3468. DOI: 10.1210/jc.2015-4275.
36. Vale-Fernandes E, Carrageta DF, Moreira MV, Guerra-Carvalho B, Rodrigues B, Sousa D, *et al*. Follicular fluid profiling unveils anti-Müllerian hormone alongside glycolytic and mitochondrial dysfunction as markers of polycystic ovary syndrome. Mol Cell Endocrinol. 2025;602:112536, 1-9. DOI: 10.1016/j.mce.2025.112536.