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Original Article

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

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ABSTRACT

Objective: This randomized controlled trial investigated the efficacy of standardized *Vitex agnus-castus* extract in managing features of PCOS. The primary aim was to assess changes in oxidative stress markers; secondary outcomes included lipid profile, insulin resistance, and clinical signs such as hirsutism and menstrual frequency.

Methods: Sixty women with PCOS were randomly assigned to a *Vitex* group (5.8 mg daily, standardized to 0.42–0.82 mg Aucubin) or placebo for 12 weeks. Dietary habits and physical activity and physical activity were maintained throughout the study. Serum total antioxidant capacity, glutathione peroxidase, reduced glutathione, and other biochemical and clinical parameters were assessed pre- and post-intervention. Between-group differences were analyzed using independent t-tests and ANCOVA.

Results: Compared to placebo, Vitex significantly increased total antioxidant capacity (effect size = 13.01), glutathione peroxidase (3.35), reduced glutathione (3.88), total thiol (3.34), and HDL (5.74) (all $p<0.05$). It decreased total oxidant status (–6.49), oxidative stress index (–9.30), malondialdehyde (–5.29), fasting blood sugar (–5.10), HOMA-IR (–0.31), LDL (–2.85), ALT (–3.51), and mFG score (–5.38). Menstrual frequency improved (3.51), and left ovarian volume reduced (–0.80).

Conclusions: *Vitex agnus-castus* improved oxidative stress markers and insulin resistance and favorably modulated clinical manifestations of PCOS. These findings suggest a clinically meaningful benefit and support further investigation into Vitex as an adjunctive therapy.

Keywords: polycystic ovary syndrome, *Vitex agnus-castus*, oxidative stress, lipid profile, insulin resistance, randomized controlled trial

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder affecting 6-22% of women of reproductive age (Bargiota & Diamanti-Kandarakis, 2012). Defined by the Rotterdam criteria and endorsed by the 2018 International Evidence-based Guideline, PCOS diagnosis requires the presence of at least two of the following: hyperandrogenism (biochemical and/or clinical), chronic anovulation and menstrual dysfunction, and polycystic ovarian morphology on ultrasonography, after excluding other endocrine disorders (Bargiota & Diamanti-Kandarakis, 2012; Siddiqui *et al.*, 2022). This complex disorder poses a significant health burden, increasing the risk of metabolic and cardiovascular complications, including insulin resistance, obesity, dyslipidemia, gestational diabetes, type 2 diabetes, and cardiovascular disease (Vink *et al.*, 2006).

Although the exact etiology of PCOS remains incompletely understood, it is widely recognized that a complex interplay of genetic, hormonal, and environmental factors contributes to its pathogenesis (Vink *et al.*, 2006; Bednarska & Siejka, 2017; Siddiqui *et al.*, 2022). Among these, oxidative stress has emerged as a critical player in the pathophysiology of PCOS (Bednarska & Siejka, 2017). Insulin resistance and hyperglycemia, which are frequently observed in women with PCOS, are key drivers of elevated oxidative stress (Bannigida *et al.*, 2020). Conversely, oxidative stress exacerbates insulin resistance, creating a vicious cycle that fuels hyperandrogenism, hyperinsulinemia, dyslipidemia, and an altered luteinizing hormone/follicle-stimulating hormone ratio, ultimately contributing to PCOS development and progression (Zeng *et al.*, 2020). Recent PCOS interventions include lifestyle changes (diet, exercise) and more targeted drug therapies (metformin, Glucagon-like peptide-1 agonists, inositols). Individualized, multifaceted approaches integrating lifestyle and medication are increasingly emphasized for successful management of this chronic condition. The complex and multifactorial nature of PCOS poses significant challenges for its clinical management. Beyond herbal interventions, several recent studies have expanded our understanding of PCOS treatment by emphasizing the role of oxidative stress and inflammation in clinical outcomes, especially in the context of assisted reproductive technologies (ART). A systematic review by Moreira *et al*. compared the follicular fluid composition between women with PCOS and normo-ovulatory women, revealing significant differences in oxidative stress and inflammatory biomarkers that may adversely affect ART outcomes (Moreira *et al.*, 2023). Furthermore, a study by Vale-Fernandes *et al*. identified a positive association between elevated anti-Müllerian hormone (AMH) levels and increased oxidative stress in the follicular fluid of women with PCOS, suggesting that AMH may serve as a surrogate marker for oxidative imbalance and potential reproductive compromise. These findings underscore the importance of targeting oxidative stress in PCOS not only for metabolic and endocrine improvement but also for optimizing fertility-related outcomes (Vale-Fernandes *et al.*, 2024).

Given the well-established role of oxidative stress in PCOS pathogenesis and its associated complications, therapeutic strategies aimed at restoring oxidative balance hold considerable promise. In this context, herbal medicines

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have garnered increasing attention due to their long history of traditional use and perceived lower incidence of adverse effects compared to conventional pharmaceuticals (Li *et al.*, 2022; Masjedi *et al.*, 2024). Several studies have reported the benefits of herbal interventions in women's health, including improvements in oxidative stress status related to premenstrual syndrome and infertility (Li *et al.*, 2022; Puglia *et al.*, 2023).

One such herbal remedy is *Vitex agnus-castus* (*Vitex*), a medicinal plant widely used for managing various gynecological conditions, such as menopausal symptoms, menstrual disorders, and mastalgia (Shayan *et al.*, 2016; Wang *et al.*, 2018). Phytochemical analyses of *Vitex* extracts have identified a range of bioactive compounds with antioxidant properties, including vanillic acid, luteolin, quercetin, caffeic acid, resveratrol, and naringenin (Kavaz *et al.*, 2022). Preclinical and clinical studies suggest that *Vitex* may modulate menstrual irregularities and hyperandrogenism, both of which are hallmark features of PCOS (Alois & Estores, 2019). Despite these promising findings, robust clinical trials evaluating the efficacy of *Vitex* specifically for PCOS management remain limited.

To address this gap, we conducted a randomized, placebo-controlled trial to investigate the effects of a standardized *Vitex* extract on key parameters relevant to PCOS. Specifically, we evaluated its impact on oxidative stress markers (total antioxidant capacity, glutathione peroxidase, reduced glutathione, total thiol, total oxidant capacity, oxidative stress index, and malondialdehyde), lipid profile (cholesterol, low-density lipoprotein, and high-density lipoprotein), insulin resistance, ovarian volume, hirsutism score, and menstrual frequency in women with PCOS. This study aims to provide rigorous evidence regarding the therapeutic potential of *Vitex* in the management of this complex and multifactorial condition.

MATERIAL AND METHODS

Study registration and approval

This study was a double-blind, placebo-controlled, parallel-group clinical trial. The trial was conducted in accordance with the Declaration of Helsinki and was carried out from April 2023 to January 2024 in Ayatollah Taleghani Educational and Therapeutic Center, Arak, Iran. This study was reviewed and deemed exempt from ethics approval by the Ethics Committee of Arak University of Medical Sciences with the reference number: IR.ARAKMU.REC.1401.333, dated February 12th, 2023. The study was also registered in the Iranian Registry of Clinical Trials (registration code: IRCT20230222057493N1) on March 28, 2023. This study was conducted using CONSORT reporting guidelines. Both participants and outcome assessors were blinded to group assignments throughout the study. Written informed consent was obtained from all participants prior to their enrollment, and the consent form was approved by the Ethics Committees of Arak University of Medical Sciences, Iran.

Population (Inclusion and exclusion criteria)

Women aged 18-45 years diagnosed with PCOS according to the Rotterdam criteria were recruited. Inclusion criteria were: (1) PCOS diagnosis per Rotterdam criteria; (2) age 18-45 years; and (3) no intention to become pregnant during the study. Exclusion criteria were: (1) pregnancy or breastfeeding; (2) smoking; (3) hyperprolactinemia; (4) thyroid disorders; (5) congenital adrenal hyperplasia; (6) diabetes mellitus; (7) use of antioxidant or herbal supplements within the past three months; and (8) use of dopamine antagonists.

Sample size

Using GSH as a variable (Shokrpour & Asemi, 2019), and a two-sided significance level (α) of 0.05 and a power (1-β) of 80% (β = 0.20), according to the following equation, the minimum sample size was calculated 28 for each group:

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

$\alpha = 0.05$ $\beta = 0.20$ $\mu_1 = 483.8$ $\mu_2 = 519.4$ $\sigma \approx 47.7$ $n_1 = n_2 \geq 28$

Accounting for an estimated 10% dropout rate, we aimed to recruit 30 participants per group.

Randomization

Sequence generation

A randomization list was created prior to the commencement of the study. The permuted block randomization method with blocks of four was employed in this study. The *Vitex* group was labelled as A, and the placebo group as B. Six possible combinations—AABB, BBAA, BABA, ABBA, BAAB, and ABAB—were written on separate sheets of paper and placed into a container. Each time, one sheet was randomly drawn from the container, the combination on it was recorded, and the sheet was returned to the container. Given the sample size of 60, this process was repeated 15 times, with each combination being recorded sequentially. Subsequently, each letter (A or B) in the recorded sequence was assigned a number from one to 60.

Allocation concealment mechanism and blinding

Letters were individually placed into envelopes, each marked with a specific number. When a participant was recruited, an envelope was opened, and the number indicated on it was used to allocate the subject to either the *Vitex* group or the placebo group. Subjects and healthcare providers were blinded and unaware of the study grouping. A.M. generated the random allocation sequence, F.S. enrolled participants, and A.H. assigned participants to *Vitex* and placebo groups.

Intervention

Following randomization, participants were assigned to either the *Vitex* group (n=30) or the placebo group (n=30) for a 12-week intervention period. Participants were instructed to maintain their usual dietary habits without any modifications throughout the study period. They were explicitly advised against initiating any new dietary regimens, herbal medications, antioxidant supplements, during the 12-week intervention. Adherence to these guidelines was continuously monitored through telephone follow-ups. At the baseline visit and the end of the 12-week intervention, after a 12-hour overnight fast, 10 mL of venous blood was collected. Participants in the *Vitex* group received 5.8 mg daily of a standardized *Vitex* extract (standardized to 0.42-0.82 mg Aucubin) administered orally as *Agnugol* tablets (Goldaro Pharmaceutical Company, Isfahan, Iran) and this standardization was carried out according to the production and quality control processes of the mentioned company. The placebo group received matching placebo tablets containing cellulose acetate (Goldaro Pharmaceutical Company, Isfahan, Iran). All tablets were identical in appearance (shape, color, and smell) and were packaged in identical containers. During this study, patients were not deprived of the main medicine, which included metformin and an oral contraceptive (3 mg Drospirenone + 0.03 mg Ethinyl estradiol).

Laboratory Methods

Sample Collection and Preparation

Venous blood samples were collected from all participants after a 12-hour overnight fast. Samples were centrifuged at 3000 rpm for 10 minutes, and the resulting serum was stored at -80°C until analysis.

Biochemical Assays

Serum total thiol (TT) levels were measured using Ellman's reagent (DTNB) (Eyer *et al.*, 2003). Total antioxidant capacity (TAC) was quantified using the ferric reducing ability of plasma (FRAP) assay (Samimi *et al.*, 2024). Total oxidant status (TOS) was assessed by the ferric-xylenol orange method (Erel, 2005).

Catalase (CAT) activity was determined by incubating serum with hydrogen peroxide as a substrate, with the enzymatic reaction terminated by ammonium molybdate (Samimi *et al.*, 2024). Commercially available kits were used to evaluate activities of GPx and levels of GSH (Novin Navand Salamat Pishtaz Co. Urmia, Iran), and MDA (Malondialdehyde) (Kushan Zist Azma Parseh Co. Tehran, Iran).

Fasting blood sugar (FBS), triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels were measured using an automated analyzer (Hitachi 717, Japan) and commercially available enzymatic kits (Delta Darman Part, Iran). Serum insulin levels were determined by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Monobind, Iran). Insulin resistance was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR) index, as follows (Tahapary *et al.*, 2022):

Fasting insulin (μU/dL) × Fasting blood glucose (mmol/L) / 22.5.

A HOMA-IR value ≥2.5 was considered indicative of insulin resistance (Minh *et al.*, 2021).

Outcome measures

The primary outcome of this study was the change in oxidative stress markers, specifically TAC, GPx, GSH, TOS, oxidative stress index (OSI), and MDA levels. Secondary outcomes included changes in lipid profile (TG, TC, HDL-C, LDL-C), insulin resistance (HOMA-IR), ovarian volume assessed by ultrasonography, menstrual frequency, and hirsutism score measured using the Modified Ferriman-Gallwey (mFG) scoring system.

The mFG score (Willis *et al.*, 2020) was used to quantify the degree of hirsutism, and ultrasonography was used to measure the ovary volume. Menstrual frequency was calculated by dividing the observed number of menstrual periods during the 12-week intervention by the expected number of periods (n=3, assuming a regular 28-day cycle). The menstrual frequency was then compared between the *Vitex* and placebo groups (Kiel *et al.*, 2020).

Statistical analysis

Statistical analysis was performed using SPSS version 23.0 (IBM, New York, USA). The results of demographic factors were reported as Mean±SD, and the results of other variables were reported as Mean±standard error of the mean (SEM). The effect sizes (Cohen's d) were calculated to assess the magnitude of differences, beyond statistical significance. A *p*-value of less than 0.05 was considered statistically significant.

Normality of continuous variables was assessed using the Shapiro-Wilk test. Within-group changes from baseline to the end of the intervention were analyzed using paired t-tests. Independent samples t-tests were used to compare changes between the *Vitex* and placebo groups. Analysis of covariance (ANCOVA) was employed to adjust for baseline differences between groups when comparing outcomes at the end of the intervention.

RESULTS

One hundred and one women with PCOS were selected using convenience sampling. Thirty-nine subjects were excluded due to exclusion criteria, six subjects were excluded because they were not satisfied to participate in the study, and finally, 60 subjects were included in the study. These subjects were divided into two groups: *Vitex* and placebo, using the permuted block randomization method with blocks of four. None of the 60 subjects who were included in the study were subsequently excluded, and all remained in the study until its completion (Figure 1). The recruitment period began on April 4, 2023 and ended on January 20, 2024.

Table 1 presents the baseline and post-intervention anthropometric characteristics of the participants in both the *Vitex* and Placebo groups. At baseline, there were no statistically significant differences between the two groups in terms of age (p=0.87), height (p=0.88), weight (p=0.68), or BMI (p=0.76). This indicates that the groups were well-matched at the start of the study. After the intervention period, there were no statistically significant differences between the *Vitex* and Placebo groups in terms of weight (p=0.57) or BMI (p=0.82).

Tables 2 and 3 demonstrate that comparisons were made between and within groups at baseline and the end of the trial. Findings from the independent t-test at the trial's conclusion showed that the consumption of *Vitex* significantly increased the levels of TAC (p<0.001), GPx (p<0.05), GSH (p<0.05), TT (p<0.05), HDL (p<0.001) and Menstrual frequency (0.046).

Furthermore, values of TOS (p<0.001), OSI (p<0.001), MDA (p<0.001), FBS (p<0.001), HOMA-IR (p<0.05), ALT (0.05), mFG (0.001), and LDL (p<0.05) significantly decreased by the consumption of the *Vitex* compared to the placebo group. Values of TG, Chol, AST, right ovary volume, and left were decreased non-significantly in the *Vitex* group compared to the placebo group (p>0.05). In addition, CAT increased non-significantly in the *Vitex* group compared to the placebo group (p=0.83).

Based on ANCOVA, values of TAC (p<0.001), GPx (p<0.05), GSH (p<0.05), TT (p<0.05), HDL (p<0.001), and menstrual frequency (p<0.05) were significantly increased after the intervention compared to the placebo group. Furthermore, values of TOS (p<0.001), OSI (p<0.001), MDA (p<0.001), FBS (p<0.05), HOMA_IR (p<0.05), LDL (p<0.001), Chol (p<0.05), AST (p<0.001), ALT (p<0.001), ovary volume left (p<0.05). mFG score (p<0.001) were significantly decreased after supplementation compared to the placebo group. Consequently, the improvements observed in these variables may be attributed to the intervention, and after Covariance Analysis, AST, Chol, and left ovary volume showed a significant decrease. However, the ANCOVA for TG, CAT, and right ovary volume was non-significantly.

DISCUSSION

This study assessed the effects of a 12-week intervention with *Vitex* on oxidative stress markers, insulin resistance, lipid profile, menstrual frequency, ovarian volume, and hirsutism in women with PCOS. While earlier studies have highlighted the benefits of *Vitex* on menstrual irregularities such as oligomenorrhea (Shayan *et al.*, 2016), this is the first clinical trial to systematically investigate its multi-dimensional effects across metabolic and clinical parameters in this population.

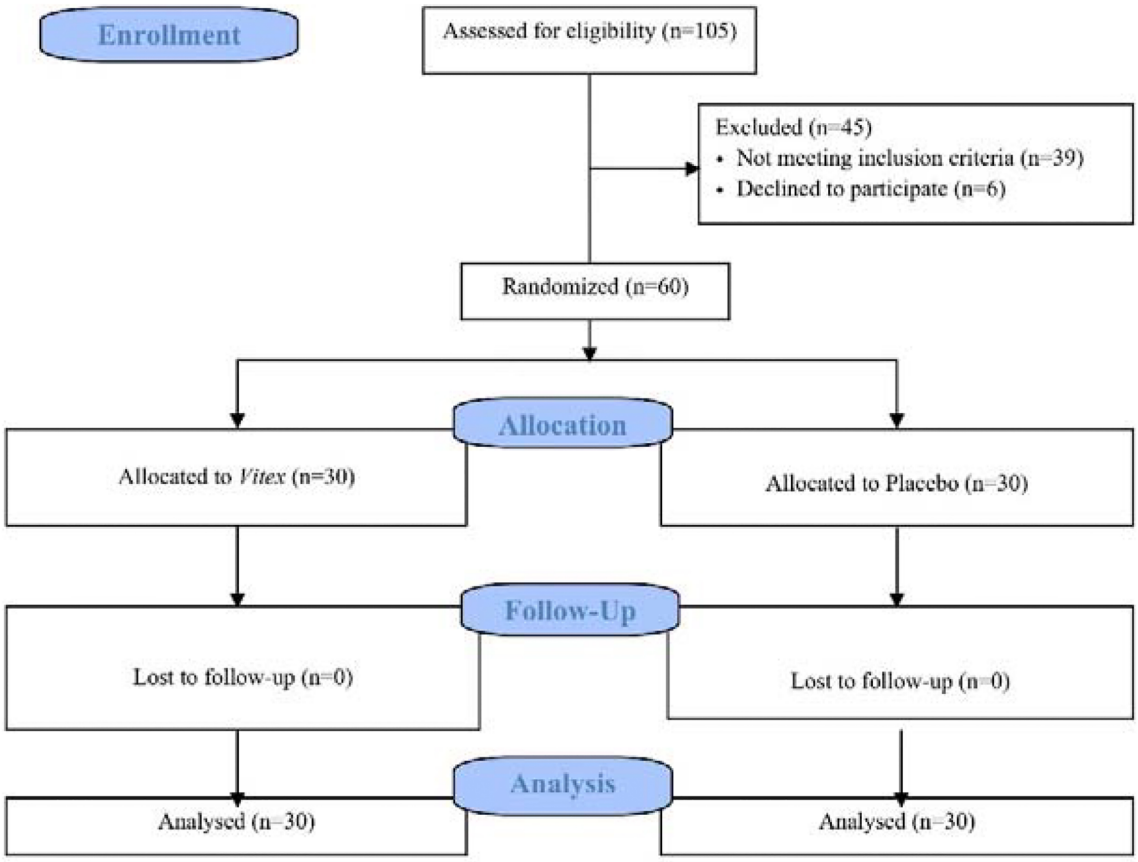

Figure 1. Consort diagram of the study population.

Our findings revealed that daily supplementation with 5.8 mg of *Vitex* extract significantly increased TAC, GPx, GSH, TT, and HDL-C, while decreasing TOS, OSI, MDA, FBS, HOMA-IR, total cholesterol, LDL-C, AST, and ALT levels compared to placebo. Additionally, it improved menstrual frequency, decreased left ovarian volume, and reduced hirsutism scores.

The observed improvements are likely mediated through *Vitex's* rich phytochemical profile, particularly phenolic acids (e.g., vanillic acid) and flavonoids (e.g., quercetin), which possess antioxidant properties (Kavaz *et al.*, 2022).These compounds may enhance cellular antioxidant defense by upregulating enzymes such as GPx and GSH, mitigating oxidative damage that is known to impair insulin signaling and ovarian function (Zamora & Villena, 2014; Mohammadi, 2019).

Vitex may also exert endocrine effects by modulating dopamine D2 receptors, reducing prolactin secretion, which in turn supports menstrual regularity and ovulation (Feyzollahi *et al.*, 2021). Furthermore, it may regulate the hypothalamic-pituitary-gonadal axis via modulation of KISS-1 gene expression, a pathway implicated in GnRH pulpability and reproductive hormone balance (Puglia *et al.*, 2023). Its hypoglycemic and insulin-sensitizing effects may be partly attributed to inhibition of carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase (Berrani *et al.*, 2021), reducing postprandial glucose excursions. These metabolic benefits may also lower circulating androgens, improving clinical manifestations such as hirsutism.

Our findings align with several animal studies reporting decreased MDA and improved antioxidant enzyme activity (SOD, CAT) following *Vitex* administration (Moreno *et al.*,

Table 1. Baseline and post-intervention anthropometric characteristics.

Variables	Vitex (n=30)	Placebo (n=30)	*p*-value†
Age (year)	26.37±5.53	26.60±5.71	0.87
Height (m)	1.60±0.06	1.63±0.06	0.88
Weight baseline (Kg)	66.86±11.80	68.20±13.26	0.68
Weight post-intervention (Kg)	65.70±10.41	67.30±11.60	0.57
BMI baseline (Kg/m^2)	26.09±4.87	25.70±5.26	0.76
BMI post-intervention (Kg/m^2)	25.64±4.43	25.37±4.68	0.82

Notes: Results are presented as Mean±SD.
† Independent t-test.
Adjusted *p*-values corrected using Bonferroni correction.

Table 2. Comparison of the effects of Vitex and placebo consumption on biochemical characteristics of women with PCOS.

Variables	*Vitex* (n=30)	Placebo (n=30)	*p*-value†	Effect size (Cohen's d)	*p*-value §
TAC (mM)					0.001
Before	0.60±0.02	0.65±0.02	0.09	-2.5	
After	1.10±0.04	0.64±0.03	0.001	13.01	
p-value‡	0.001	0.55			
TOS (µM)					0.001
Before	11.76±0.55	13.65±0.94	0.09	-2.45	
After	9.13±0.52	14.18±0.97	0.001	-6.48	
p-value‡	0.001	0.58			
OSI %					0.001
Before	2.00±0.10	2.12±0.14	0.51	-0.98	
After	0.84±0.04	2.44±0.24	0.001	-9.3	
p-value‡	0.001	0.15			
MDA (µM)					0.001
Before	4.64±0.81	4.12±0.57	0.60	0.74	
After	2.99±0.22	5.63±0.67	0.001	-5.29	
p-value‡	0.054	0.14			
CAT (KU)					0.939
Before	5.24±0.51	6.63±0.53	0.06	-2.67	
After	6.98±0.37	6.86±0.41	0.83	0.30	
p-value‡	0.01	0.74			
GPx (mU/ml)					0.02
Before	242.87±2.61	243.87±2.41	0.79	-0.39	
After	250.75±1.11	246.65±1.33	0.02	3.34	
p-value‡	0.008	0.33			
GSH (µM)					0.01
Before	4.42±0.56	5.54±0.71	0.22	-1.75	
After	23.94±2.63	15.20±1.79	0.008	3.88	
p-value‡	0.001	0.001			
TT (mM)					0.01
Before	0.139±0.008	0.146±0.006	0.50	-0.99	
After	0.178±0.014	0.142±0.006	0.023	3.34	
p-value‡	0.018	0.48			
FBS (mg/dl)					0.03
Before	100.23±2.15	105.13±1.57	0.071	-2.60	
After	84.90±1.15	90.97±1.23	0.001	-5.09	
p-value‡	0.001	0.001			
HOMA-IR					0.004
Before	6.40±1.29	6.72±0.72	0.83	-4.53	
After	0.983±0.06	1.33±0.09	0.004	-0.30	
p-value‡	0.001	0.001			
TG (mg/dl)					
Before	189.3±14.30	198.60±14.07	0.64	-0.88	0.57
After	177.83±15.29	190.60±13.53	0.53	-0.12	
p-value‡	0.001	0.09			
Chol (mg/dl)					0.016
Before	198.66±7.10	199.43±4.51	0.93	-0.12	
After	174.36±6.89	187.96±4.70	0.11	-2.30	
p-value‡	0.001	0.002			
HDL (mg/dl)					0.001
Before	50.00±1.64	47.16±1.07	0.15	2.05	
After	60.13±1.76	50.93±1.43	0.001	5.73	
p-value‡	0.001	0.002			

LDL (mg/dl) Before After *p*-value‡	 100.43±4.85 81.03±4.68 0.001	 96.20±4.04 93.37±3.96 0.001	 0.51 0.049	 0.94 -2.84	0.001
AST (U/l) Before After	 20.46±1.73 17.60±1.41	 21.43±1.66 20.16±1.48	 0.68 0.21	 -0.57 -1.77	0.012
p-value‡	0.001	0.052			
ALT (U/l) Before After	 22.73±1.77 18.70±1.39	 22.20±1.64 24.00±1.62	 0.83 0.016	 0.31 -3.51	0.001
p-value‡	0.001	0.001			

Note: Results are presented as Mean±SEM. A significance level of 0.05 was considered (*p*-value<0.05).
† Independent t-test
‡ Paired t-test
§ Based on ANCOVA
Abbreviations: TAC: total antioxidant capacity; TOS: total oxidant status; OSI: oxidative stress index; MDA: malondialdehyde; CAT: catalase; GPx: glutathione peroxidase; GSH: reduced glutathione; TT: total thiol; FBS: fasting blood sugar ; HOMA-IR: homeostatic model assessment-insulin resistance; TG: triglyceride; Chol: cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

Table 3. Comparison of the effects of Vitex and placebo consumption on clinical characteristics of women with PCOS.

Variables	***Vitex* (n=30)**	**Placebo (n=30)**	***p*-value†**	**Cohen's d**	***p*-value§**
OV. Right (CC) Before After	 14.58±0.75 12.06±0.53	 13.75±0.73 12.30±0.54	 0.43 0.76	 1.12 -0.44	0.1
p-value‡	0.001	0.001			
OV. Left (CC) Before After	 14.51±0.71 12.81±0.55	 13.58±0.70 13.28±0.63	 0.35 0.58	 1.31 -0.79	0.023
p-value‡	0.001	0.47			
mFG Score Before After	 10.70±0.70 7.83±0.58	 9.20±0.58 11.26±0.69	 0.11 0.001	 2.33 -5.38	0.001
p-value‡	0.001	0.001			
Menstrual frequency Before After	 0.200±0.06 0.955±0.02	0 .277±0.08 0.844±0.04	 0.49 0.046	 -1.08 3.51	0.027
p-value‡	0.001	0.001			

Results are presented as Mean±SEM. A significance level of 0.05 was considered (*p*-value<0.05).
† Independent t-test
‡ Paired t-test
§ Based on ANCOVA
OV: ovary volume; mF G: modified ferriman-gallwey.

2015; Ahangarpour *et al.*, 2016). However, unlike these studies, we did not observe a significant increase in catalase activity, possibly due to the shorter intervention period or differences in assay sensitivity.

In terms of lipid profile, our results are consistent with Berrani *et al*. (2021), who demonstrated improved HDL-C and reduced TG and LDL-C levels in diabetic rats treated with *Vitex*. However, dosages varied widely across studies. While we used a low dose (5.8 mg), other trials in PMS and mastalgia have employed doses between 20–40 mg (Roemheld-Hamm, 2005) indicating that therapeutic efficacy may follow a non-linear dose-response pattern. Highlighted such variability, with low doses improving ovarian histology in animals while higher doses worsened it.

Despite promising results, several limitations must be acknowledged. First, the short duration (12 weeks) limits our ability to assess long-term effects on fertility or metabolic outcomes. Second, the single-center design and convenience sampling may reduce external validity, and although randomization minimized intergroup bias, the sample may not reflect the heterogeneity of PCOS phenotypes. Third, we did not stratify participants based on PCOS phenotype, BMI, or insulin resistance severity—factors that may modulate response to antioxidant therapy. Fourth, adherence to supplementation was self-reported, which could introduce recall bias. Fifth, the study lacked mechanistic biomarkers such as sex hormone-binding globulin (SHBG), LH/FSH ratio, or prolactin levels, which would further clarify hormonal effects.

The statistically significant findings also bear clinical importance. Improvements in antioxidant status and insulin resistance may help reduce long-term risks such as type 2 diabetes and cardiovascular disease. Reductions in hirsutism and irregular menstruation directly improve quality of life and reproductive health.

Compared to conventional interventions such as metformin and oral contraceptives, *Vitex* offers a complementary and potentially safer alternative, particularly for women seeking herbal therapies with fewer side effects. For instance, while metformin improves insulin sensitivity, it is often associated with gastrointestinal side effects (Kiani *et al.*, 2022). In contrast, *Vitex*, through its antioxidant and endocrine-modulating properties, not only improves insulin resistance but also addresses menstrual irregularities and hirsutism without reported major adverse events.

Vitex thus presents as a promising complementary therapy for women with PCOS, especially those seeking natural alternatives. Future trials should include larger, diverse populations, multiple dosing arms, longer durations, and mechanistic endpoints to elucidate *Vitex's* full therapeutic potential—particularly in women planning pregnancy.

CONCLUSIONS

This study's outcomes demonstrate that *Vitex* supplementation effectively reduces oxidative stress and enhances antioxidant defense in women with PCOS. The significant improvements observed in insulin resistance, lipid profile, menstrual frequency, and clinical symptoms such as hirsutism suggest that *Vitex* may serve as a beneficial complementary therapy for managing PCOS-related complications. From a clinical standpoint, it is advisable for healthcare professionals to consider *Vitex* fruit extract—particularly in patients seeking natural alternatives—as part of an individualized treatment plan.

Future research should explore long-term effects, dose-response relationships, and its impact on hormonal biomarkers such as LH/FSH ratio and SHBG. Additionally, studies involving diverse PCOS phenotypes and stratified populations are needed to better understand the full therapeutic potential and safety profile of *Vitex* in reproductive and metabolic health.

Abbreviations:

PCOS	Polycystic ovary syndrome
IR	Insulin resistance
Vitex	*Vitex agnus-castus*
ANCOVA	Analysis of covariance
BMI	Body mass index
TT	Total thiol
TAC	Total antioxidant capacity
FRAP	Ferric reducing ability of plasma
TOS	Total oxidant status
FBS	Fasting blood sugar
TG	Triglyceride
Chol	Cholesterol
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
ELISA	Enzyme-linked immunosorbent assay
HOMA-IR	Homeostasis model assessment
mFG	Modified Ferriman-Gallwey

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Author contributions

All authors contributed significantly to the manuscript and approved the final version for submission. Specific contributions were as follows:

AH: Investigation, data curation, writing – original draft, visualization.

FS: Supervision, validation, writing - review & editing.

FJ-M: Project administration, validation, writing - review & editing, funding acquisition.

AK: Advisors, validation, writing - review & editing, conceptualization, resources.

AM: Formal analysis and methodology, writing - review & editing.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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