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RESEARCH ARTICLE

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The effects of propolis supplementation on high-sensitivity C-reactive protein, testosterone hormone, and metabolic profile in women with polycystic ovary syndrome: A randomized, triple-blinded, placebo-controlled clinical trial

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Abstract

One of the most prevalent ovulation disorders is polycystic ovarian syndrome (PCOS). According to the anti-inflammatory and beneficial effects of propolis, this triple-blind controlled trial was designed to evaluate the effect of propolis on metabolic factors, high-sensitivity C-reactive protein, and testosterone in women with PCOS. Recruited patients from the gynecologist clinic were randomized based on a stratified permuted four-block randomization procedure to supplement with propolis tablets, two tablets/day (500 mg propolis/day) ($n = 30$) or identical placebo tablets ($n = 30$) for 12 weeks in 2021 until 2022. Data were collected using a demographic questionnaire, blood samples, and a checklist to record the measured parameters. A total of 57 patients completed the trial. ANCOVA test showed that hip circumference (HC)) $p = 0.03$), fasting insulin ($p = 0.007$), homeostatic model assessment for insulin resistance ($p = 0.004$), testosterone ($p = 0.004$), and low-density lipoprotein (LDL)/high-density lipoprotein (HDL) ($p = 0.02$) were significantly decreased in the propolis versus the placebo group after adjustment for confounders. Although fasting blood glucose ($p = 0.04$) decreased significantly in the propolis group compared to the placebo, after adjusting for confounders, significance was lost ($p = 0.09$). Supplementation with propolis elicited positive effects on fasting insulin and insulin resistance, in addition to reducing the testosterone level, LDL/HDL, and HC, in PCOS women.

KEYWORDS
clinical trial, inflammation, metabolic parameters, polycystic ovarian syndrome, propolis, testosterone

Abbreviations: BMI, body mass index; DASS-21, depression, anxiety and stress Scales; DBP, diastolic blood pressure; FBG, fasting blood glucose; GSH, glutathione; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; LDL, low-density lipoprotein; MDA, malondialdehyde; MET, metabolic equivalent; PCOS, polycystic ovarian syndrome; SBP, systolic blood pressure; SOD, superoxide dismutase; TAC, total antioxidant capacity; TC, total cholesterol; TG, triglycerides; WC, waist circumference; WHOQOL-BREF, WHO quality of life questionnaire 26 questions; WHR, waist to hip ratio.

1 | INTRODUCTION

Polycystic ovarian syndrome (PCOS) is the most common ovulatory syndrome, impacting around 7% of reproductive-age women ("American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Gynecology", 2018). PCOS is related to several clinical

complications, including hirsutism, acne, baldness, endometriosis, amenorrhea, oligomenorrhea, and premature menopause. This condition is also associated with the augmented hazard of long-term metabolic issues, such as insulin resistance, diabetes mellitus, lipid profile disorders, and cardiovascular diseases (Qin et al., 2013; Sohrabvand et al., 2007). In PCOS women, testosterone and estrogen levels are high (Janssen et al., 2004). Of all PCOS cases, approximately 40%–50% are overweight or obese, which aggravates many related symptoms (Liou et al., 2009). The production of inflammatory cytokines from visceral fat has a significant role in insulin resistance and decreased ovulation (Tehrani et al., 2017); indeed, PCOS patients tend to develop higher serum levels of total cholesterol (TC), low-density lipoprotein (LDL), and triglycerides (TG) (Anderson et al., 1999), and lower high-density lipoprotein (HDL) (Murri et al., 2013). Due to the high costs of treatment for PCOS and complications such as infertility, menstruation disorders, and diabetes, knowledge about the treatment of this syndrome would improve health outcomes and reduce the economic burden on healthcare systems (Harrison et al., 2010).

Multiple factors contribute to the etiology of PCOS, such as genes and environmental factors (Raja-Khan et al., 2011). Adherence to a healthy diet along with significant weight loss may be effective in curing PCOS (Douglas et al., 2006); however, it is difficult to adhere to a weight loss diet for extended periods and maintain the reduced weight for most patients. Furthermore, because of the side effects of conventional drugs and surgery, alternative plant-based medicine has become very popular (Hernandez-Rodas et al., 2015).

Propolis is a natural product made by honeybees from the petals and buds of plants. It contains a wide range of plant antioxidants, and to date, more than 300 phytochemicals of propolis have been identified that directly and indirectly scavenge free radicals; the most important of them are polyphenolic compounds, terpenes, and flavonoids (Huang et al., 2014). Propolis has been shown to prevent oxidative damage and destruction of the structure of lipids, proteins, and DNA by increasing the body's antioxidant capacity (Siheri et al., 2017). In addition to the antioxidant properties of this substance, anti-inflammatory, antimicrobial, anti-carcinogenic, and immunomodulatory effects have also been reported (Miryan et al., 2020; Sforcin, 2016). Some studies have also shown that propolis can reduce oxidative stress and increase HDL levels in healthy subjects (Mujica et al., 2017). In Samadi et al., it was presented that consuming 900 mg per day of raw propolis for 12 weeks reduced fasting blood glucose (FBG), hemoglobin A1c (HbA1c), TC, and LDL-c in patients with type 2 diabetes mellitus (T2DM) (Samadi et al., 2017). A recent study by Soleimani et al., on Iranian propolis extract, showed that a daily intake of 500 mg of propolis extract resulted in a decrease in liver steatosis and fibrosis and serum high-sensitivity C-reactive protein (hs-CRP) levels (Soleimani et al., 2021).

According to the high prevalence of PCOS and its negative impacts on female fertility as well as accompanying metabolic diseases, studies to find the effective treatment for PCOS will likely improve the reproductive status of patients and reduce treatment costs. Considering the anti-inflammatory and antioxidant properties of propolis and its beneficial effects on metabolic parameters as well as endometriosis, it seems that propolis supplementation might have valuable effects on the indicators of inflammatory and glycemic indices in PCOS patients. As far as we know, the effects of propolis on patients with PCOS have not yet been evaluated.

Therefore, the current triple-blind clinical experiment was conducted to evaluate the effect of propolis supplementation on hs-CRP, testosterone hormone, and metabolic profile in women with PCOS.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

This study was a parallel, randomized, triple-blind, placebo-controlled clinical trial. The study was approved by the Ethical Committee of Isfahan University of Medical Sciences (code: IR.MUI.RESEARCH.REC.1399.587(, Isfahan, Iran, and registered in the Iranian Registry of Clinical Trials (ID: IRCT20121216011763N51; 7 March 2021). Participants were asked to fill out a written informed consent from before being enrolled in the trial.

Females with PCOS were voluntarily recruited from the gynecologist clinic of Shahid Beheshti Hospital, a referral center for infertility cases in Isfahan, Iran from November 2021 until February 2022. If the patients were between 18 and 45 years old and had PCOS according to the international Rotterdam criteria (Lauritsen et al., 2019) (based on this criterion, having two of these three features is necessary to diagnose PCOS: (1) Oligomenorrhea and anovulation (2) Hyperandrogenism (3) Polycystic ovary) and confirmed by the cooperator gynecological doctor, did not follow a specific diet or exercise program in the last 3 months, did not take any medicine other than metformin, did not perform laparoscopic ovarian surgery, and did not assist reproductive technology during the last year, they were enrolled. If participants were breastfeeding, used hormone therapy, took birth control pills (OCPs), consumed tobacco or alcohol, had malignancy or other long-lasting diseases, such as type 2 diabetes mellitus (T2DM), cardiovascular disease, liver, or renal dysfunction, pancreatic, asthma, neoplasms or gastrointestinal disease (We assessed these criteria according to their medical records in the gynecologist clinic of Shahid Beheshti hospital, Isfahan, Iran), or had Bee products intolerance, they were excluded. Participants who were reluctant to stay in this study or who had poor compliance with taking propolis or placebo (less than 80%) (Soleimani et al., 2021) were excluded from the follow-up.

2.2 | Sample size

To calculate the sample size, a former study, which assessed the effect of Iranian propolis supplement on type 2 diabetes mellitus patients for 3 months (Zakerkish et al., 2019), was used by considering the variable of homeostatic model assessment for insulin resistance (HOMA-IR) as the main outcome variable, according to the following formula.

$$N = \left(\frac{1+\varphi}{\varphi}\right)\left[\frac{\left(Z_{1-\frac{\alpha}{2}}+Z_{1-\beta}\right)^2}{\Delta^2}+\frac{Z^2_{1-\alpha/2}}{\varphi}\right]$$

We required 23 patients in each group based on a significance level of 5% ($\alpha = 0.05$), statistical power of 80% ($1-\beta$), standardized difference (Δ) of 1, and with the ratio of intervention to control group of 1 (φ). The false positive rate is set to α and the power for is set to $1-\beta$, where β is the false negative rate. The standardized difference is the absolute difference ($|\ \mu_1-\mu_2\ |$) divided by the standard deviation. To compensate for the potential loss of participants through follow-up, the ultimate sample size increased by 25%; therefore, a total of 30 patients were considered necessary in each group.

2.3 | Randomization and blinding

Patients were randomly allocated to propolis supplementation or placebo group, based on a stratified permuted four-block randomization procedure. Each block had to include subjects with similar age categories to match the groups. An authoritative website was used to generate the distribution sequence randomly (https://www.sealedenvelope.com/simple-randomiser/v1/lists). Except for the study pharmacist, the assignments of the experimental group were hidden from patients, medical doctors, and researchers (registration, evaluation, and analysis) until the end of the trial. Both Propolis and placebo tablets were analogous in dye, scent, taste, form, size, and mass.

2.4 | Intervention

According to the following points in the present study, patients were randomly allocated to supplement with propolis tablets, two tablets/day; each tablet contained 250 mg propolis (total, 500 mg propolis/day) ($n = 30$), or identical placebo tablets ($n = 30$) for 12 weeks.

The study by Zakerkish et al. (Zakerkish et al., 2019) reported that consuming 1000 mg (280 mg of the polyphenolic compound) of Iranian propolis per day for 3 months decreased insulin resistance in patients without any considerable side effects. In other clinical trials, the dose of propolis varied from 226.8 to 1500 mg for 2–3 months without any unfavorable adverse effects (Fukuda et al., 2015; Hesami et al., 2019).

The intervention group received propolis tablets, two 350 mg tablets/day, each tablet contained 250 mg Iranian green propolis extract and 100 mg of a safe and ineffective combination of microcrystalline cellulose as a supplemental formulation (totally 500 mg propolis/day) ($n = 30$), and the control group received a similar amount of identical placebo tablets that contained inert microcrystalline cellulose (Avicel) ($n = 30$), two times a day, for 12 weeks. The origin of the propolis was the honeybee colonies of Rasht, the northern area of Iran. The Reyhan Naghsh Jahan Pharmaceutical Company, Isfahan, Iran, prepared the propolis and placebo tablets. Propolis tablets were standardized for the amount of total polyphenols and flavonoids based on Bankova's recommendation (Bankova, 2005). Each propolis tablet contained 90 mg of polyphenols and 67 mg of flavonoids, and no side effects were reported in a recent clinical trial. Soleimani et al. have reported the amount of total polyphenols and flavonoids by spectrophotometric method using a standard curve in mg/ml (Soleimani et al., 2021).

Patients were requested to follow their normal diet, physical activity, and medication until the end of the trial. Also, changes in physical activity and dietary intake were assessed to control for potential confounding. Patients were followed up through weekly telephone calls and intermediate clinic visits to assess compliance with treatment and side effects. To examine how the participants followed the study process, they were asked to deliver unfilled containers of pills at the termination point.

2.5 | Primary and secondary outcomes

The initial outcomes of the study were FBG, HOMA-IR, insulin, HDL, LDL, TG, TC, systolic blood pressure (SBP), diastolic blood pressure (DBP), hs-CRP, testosterone, weight, body mass index (BMI), waist circumference (WC), hip circumference (HC), and waist to hip ratio (WHR), while the secondary outcomes were energy and activity changes.

2.6 | Data collection and management

2.6.1 | Biochemical measurements

Blood samples (10 mL) were drawn into EDTA tubes at the beginning and end of the study, after 10–12 h of fasting in the early morning, to determine the serum levels of testosterone, triglycerides, cholesterol, HDL, LDL, and FBG by using the enzymatic approach. Serum hs-CRP and insulin levels were measured using ELISA marketable kits (Pars Azmoun kit, Tehran, Iran), and we calculated the HOMA-IR using the following formula:

$$\mathrm{HOMA-IR} = (\text{fasting plasma glucose}(\mathrm{mmol/l}) \times \text{fasting insulin}(\mathrm{IU/ml}))/22.5$$

2.6.2 | Anthropometrics measurements

Anthropometric indices, including weight, BMI, HP, and WC, were evaluated by a skilled nutritionist who was blinded to the prescribing supplement, during pre-and post-intervention. Weight was measured to the nearest 100 g with participants in minimal clothing and unshod using a digital Seca scale (Saca 831, Hamburg, Germany). Height was measured using a stadiometer (Seca, Hamburg, Germany) to the nearest 0.5 cm, with participants standing upright, with shoulders in a typical position, and without shoes. We calculated BMI as weight (kg)/height squared (meters). Furthermore, WC and HC were measured using an inelastic tape according to standard anthropometric guidelines, to the nearest 0.5 cm. Then, the WHR was calculated as WC/HC.

2.6.3 | Blood pressure measurement

Blood pressure (SBP and DBP) was measured with a mercury sphygmomanometer from the right arm after at least 10 min of rest in the sitting position.

2.6.4 | Measurement of dietary intakes

As a "gold standard" for dietary assessment, a 3-day food record (2 weekdays and 1 weekend day) (Mahan & Raymond, 2016), was used to assess the changes in dietary intakes at the beginning, the middle (6-week mid-intervention), and after 12 weeks of intervention. The food records were analyzed by a nutritionist who was blind to the prescribed treatment. Calorie intakes were calculated utilizing Nutritionist IV software (First Databank Inc., Hearst Corp., San Bruno, CA, USA).

2.6.5 | Measurement of physical activity

Participants' physical activity in terms of Metabolic Equivalent (MET) per hour per day (MET/h/d) was obtained by completing a 3-day physical activity registration questionnaire. Participants were instructed to report the amount of their activities, including walking, exercising, sleeping, watching TV, doing housework, learning, bathing, etc. We obtained the total MET value using the following formula: the rate of recurrence * time * intensity of each physical activity in 24 h. Then, from the recording average, the final number was reported as the MET/h/d.

2.6.6 | Demographic questionnaire

At the onset of the study, demographic parameters including age, height, weight, marital status, metformin intake, and physical activity were collected using a questionnaire.

2.6.7 | Other questionnaires

The overall quality of life of applicants was obtained using the World Health Organization Quality of Life Questionnaire, which contained 26 questions (WHOQOL-BREF) (World Health, 1996). This questionnaire has four subscales and a general score. These subscales include physical and mental health, environmental health, social relationships, and overall score. Patients with a higher quality of life acquired a higher score. Previous analysis of this questionnaire indicated acceptable reliability and validity in different groups of Iranian people (Nejat et al., 2006).

The depression, anxiety, and stress Scales (DASS-21) questionnaire was used to measure the depression, anxiety, and stress of the participants. DASS-21 has 21 items, 7 items for depression, 7 items for anxiety, and 7 items for stress. The reliability and validity of the scale were previously confirmed (Henry & Crawford, 2005), and its validity and dependability in Iran have been assessed by Samani et al. (Samani & Joukar, 2007).

2.7 | Statistical analyses

Data were analyzed using SPSS software (Version 16, SPSS Inc., Chicago, IL, USA). The normality of the variable distribution was evaluated using the Kolmogorov–Smirnov test. Normal quantitative variables were represented as mean ± standard deviation, abnormal quantitative variables as median ± quartile amplitude, and qualitative variables as frequency percentages, respectively. The distribution of qualitative variables between and within the groups was checked using the Chi-Square test. For intergroup and intragroup comparisons of quantitative variables with normal distribution, two Independent Sample T-Test, and Paired T-Test were used, respectively, and if the distribution of variables were abnormal, rank tests of Mann–Whitney *U* and Wilcoxon were done, respectively. The effects of confounders, such as weight, energy intake, fat, selenium, and baseline values, were accounted for using analysis of covariance (ANCOVA). The significance level was considered as p-value <0.05. This study was not done based on intention-to-treat analysis.

3 | RESULTS

Out of 60 females who were randomized into the trial groups, 57 patients completed the trial (intervention group, $n = 28$, control group, $n = 29$, Figure 1). There was no co-intervention in this study, and no unfavorable adverse effect was stated throughout the study in any group. A comparison of baseline characteristics of patients in the propolis and control groups with the Independent T-Test showed that the mean age in the intervention and placebo groups was 31.07 ± 5.01 and 33.59 ± 9.74 years, respectively ($p = 0.22$). Qualitative variables such as marital status and metformin use did not show significant differences between the two groups. A total of 78.6% ($n = 22$) of patients in the propolis group and 72.6% ($n = 21$) of patients in the control group were married. Respectively, the percent of patients using metformin was 46.4 ($n = 13$) and 51.7 ($n = 15$) in the intervention and placebo groups, respectively. At the beginning of the trial, the initial characteristics of both groups were almost similar, without any significant differences between the two groups ($p > 0.05$).

3.1 | Anthropometric indices and nutrient intake

Assessments of baseline values between the two groups revealed that the weight of the intervention and placebo groups was 76.71 ± 15.86 and 67.16 ± 10.94 kg, respectively, which showed a significant body weight difference between the groups ($p = 0.01$). No significant differences were found in baseline values of age, BMI, WC, HC, WHR,

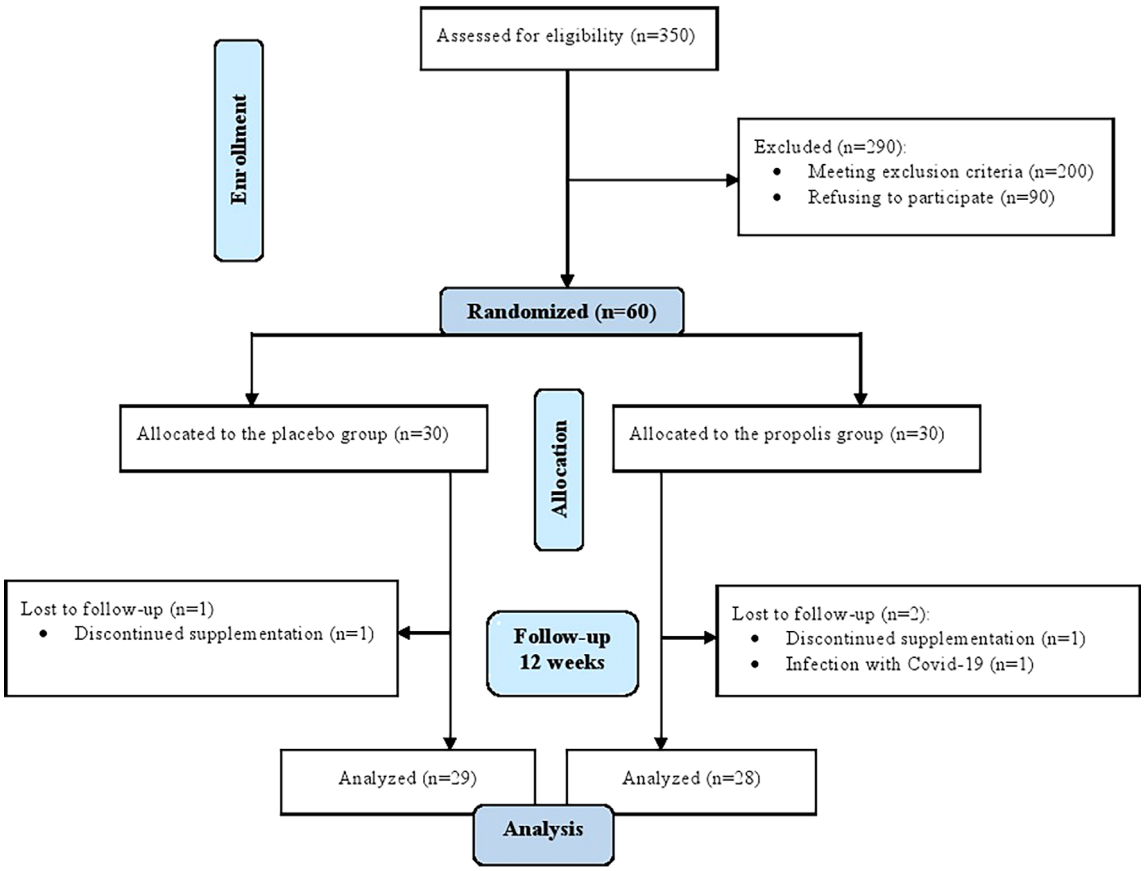

FIGURE 1 Study flow chart of enrolment, allocation, intervention, and assessment.

blood pressure, energy intake, or physical activity. After 12 weeks of supplementation, the body weight, BMI, WC, WHR, and blood pressure of the patients in the two groups did not significantly change. Although, hip circumference decreased significantly in the intervention group in comparison to the control group ($p = 0.03$) (Table 1). The patients' micronutrient intakes and physical activity remained unchanged in the propolis group compared with the control group after 12 weeks of intervention, except for selenium, which significantly increased in the propolis group (13.9 ± 28.17 vs. −3.58 ± 28.55 mg/day, $p = 0.01$). Also, energy (66.91 ± 304.93 vs. 5.13 ± 293.72 kcal/day, $p = 0.04$), protein (5.51 ± 17.94 vs. −2.63 ± 19.33 gr/day, $p = 0.006$), and fat (0.14 ± 12.26 vs. −2.17 ± 10.48 gr/day, $p = 0.007$) intake were significantly increased in the intervention group as compared with the control group, although, this increase was not actionable from a nutritional perspective.

3.2 | Glycemic status, testosterone, and hs-CRP

After 12 weeks, a significant decrease in insulin ($p = 0.05$), HOMA-IR ($p = 0.02$), and testosterone ($p = 0.001$) was seen in the propolis group versus the control group. Although we observed a decrease in FBG ($p = 0.04$) in the intervention group compared to the placebo, after adjusting for confounders, the significance was lost ($p = 0.09$). At post-intervention, however, compared to the control group, changes in the hs-CRP level were not significant in the intervention group. Nevertheless, in the propolis group, there was a significant reduction versus baseline (−1.8 (−3.67, −0.65) mg/l, $p = 0.001$), while in the control group, there was no significant difference versus baseline level (Paired Student T Test; Table 2).

3.3 | Lipid profile status

As presented in Table 2, in contrast to the placebo, a significant decline in LDL/HDL ($p = 0.01$) was detected in the intervention group after 12 weeks of supplementation. However, we observed no significant changes in HDL-c, LDL-c, TC, and TG in the intervention group as compared with the control group, before and after adjustment for confounders. Further, none of these mentioned lipid profiles were significantly altered in the intervention group compared with the start point.

3.4 | Quality of life and depression, anxiety, and stress of the participants

As shown in Table 3, after 12 weeks of supplementation with propolis, the physical and mental health scores of patients in the propolis group significantly increased compared with the placebo group

TABLE 1 Comparison of anthropometric properties, blood pressure, energy and, physical activity within and between the propolis supplement and placebo groups in patients with polycystic ovarian syndrome.

	Propolis group ($n = 28$)				Placebo group ($n = 29$)					Effect size	
Variables	Before	After	Changes	p^a	Before	After	Changes	p^a	p^c	Changes (mean ± SE)	p^d
Weight (kg)	76.71 ± 15.86	76.5 ± 15.47	−0.21 ± 2.15	0.6	67.15 ± 10.93	67.45 ± 11.56	0.29 ± 1.4	0.27	0.38	−0.51 ± 0.55	0.37
BMI (kg/m^2)	28.35 ± 5.97	28.28 ± 5.9	−0.07 ± 0.77	0.63	26.16 ± 4.42	26.27 ± 4.58	0.11 ± 0.53	0.29	0.33	−0.17 ± 0.21	0.42
WC (cm)	97.11 ± 13.75	96.89 ± 13.91	−0.21 ± 3.34	0.74	92.03 ± 11.48	91.76 ± 10.89	−0.27 ± 3.14	0.64	0.73	0.10 ± 0.98	0.99
HC (cm)	108.98 ± 10.27	106.89 ± 9.96	−2.09 ± 3.54	0.004	104.9 ± 7.7	104.83 ± 7.64	−0.07 ± 2.15	0.86	0.03	−1.93 ± 0.89	0.03
WHR	0.89 ± 0.07	0.9 ± 0.09	0.01 ± 0.04	0.03	0.87 ± 0.08	0.87 ± 0.07	0.0 ± 0.02	0.76	0.05	0.015 ± 0.01	0.12
SBP (mmHg)	11 (10, 12)	11 (10, 12)	0.0	0.16[b]	11 (10, 12)	11 (10, 12)	0.0	0.65[b]	0.2	−0.23 ± 0.20	0.27
DBP (mmHg)	7 (7, 8)	7 (7, 8)	0.0 (−1.0, 0.0)	0.02[b]	8 (7, 8)	8 (7, 8)	0.0 (−1.0, 0.0)	0.44[b]	0.08	−0.32 ± 0.17	0.09
Energy (kcal/day)	1543.39 ± 325.69	1610.3 ± 257.91	66.91 ± 304.93	0.26	1389.44 ± 309.52	1394.57 ± 322.77	5.13 ± 293.72	0.93	0.04	–	–
Physical activity (MET/h/day)	36.9 ± 3.11	38 ± 3.83	1.1 ± 3.77	0.13	36.67 ± 2.76	37.65 ± 3.66	0.98 ± 2.43	0.04	0.83	–	–

Note: Variables are reported as mean ± SD or median (interquartile range).

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HC, hip circumference; SBP, systolic blood pressure; WC, waist circumferenceWHR, waist to hip ratio.

[a]*p* values were obtained from the paired-sample *t*-test.

[b]Wilcoxon rank-sum test.

[c]*p* values a were obtained from the ANCOVA test with baseline values as covariates.

[d]Data were obtained from the ANCOVA test with baseline values and baseline weight, energy, fat, and selenium intake as covariates.

TABLE 2 Comparison of laboratory tests within and between the propolis supplement and placebo groups in patients with polycystic ovarian syndrome.

	Propolis group ($n = 28$)				Placebo group ($n = 29$)					Effect size	
Variables	Before	After	Changes	p[a]	Before	After	Changes	p[a]	p[b]	Changes (mean ± SE)	p[c]
FBG (mg/dL)	112 (106, 116)	103.5 (100, 114.75)	−3.5 ± 7.29	0.01	110 (103, 121.5)	110 (106, 118.5)	2 (−2.25, 5.25)	0.39	0.04	−3.81 ± 2.23	0.09
Fasting insulin (uIU/m)	8.94 (7.13, 13.55)	8.89 (5.43, 13.93)	−0.64 (−2.8, 0.77)	0.35	10.65 (6.66, 11.81)	12.46 (8.8, 14.19)	0.0 (0.0, 4.5)	0.001	0.05	−3.90 ± 1.38	0.007
HOMA-IR	2.4 (1.95, 3.67)	2.25 (1.36, 4)	−0.31 (−0.78, 0.46)	0.23	2.72 (1.64, 3.45)	3.2 (2.25, 3.98)	0.27 (0.0, 1.48)	0.001	0.02	−1.19 ± 0.39	0.004
Testosterone (ng/mL)	0.97 (0.78, 1.27)	0.88 (0.73, 1.51)	−0.1 (−0.23, 0.37)	1	0.58 (0.4, 0.88)	0.45 (0.39, 0.68)	−0.07 (−0.2, 0.0)	0.001	0.001	0.31 ± 0.10	0.004
hs-CRP (mg/L)	7.2 (5.12, 9.85)	5.6 (2.17, 8.77)	−1.8 (−3.67, −0.65)	0.001	4.3 (2.55, 7.6)	3.8 (2.1, 6.9)	0.0 (−1.55, 0.15)	0.2	0.38	−1.05 ± 0.94	0.27
LDL-c (mg/dL)	120.93 ± 25.48	118.75 ± 17.57	−2.18 ± 22.63	0.61	122.59 ± 14.95	123.27 ± 17.2	0.69 ± 7.61	0.63	0.32	−2.85 ± 4.20	0.5
HDL-c (mg/dL)	48.43 ± 8.8	48.64 ± 9.66	0.21 ± 8.38	0.89	48.41 ± 7.71	46.72 ± 8.11	−1.69 ± 5.46	0.11	0.29	2.01 ± 2.06	0.33
TC (mg/dL)	193.96 ± 42.68	192.46 ± 31.15	−1.5 ± 32.93	0.81	195.65 ± 20.86	202.31 ± 24.91	6.65 ± 14.4	0.02	0.11	−5.74 ± 6.45	0.38
TG (mg/dL)	147 (90.5, 215)	140.5 (98.5, 204.25)	−1 (−74.25, 14)	0.36[d]	155 (87, 218.5)	146 (105.5, 235)	0.0 (−9, 39.5)	0.38[d]	0.26	−51.13 ± 30.30	0.1
LDL/HDL	2.53 ± 0.34	2.47 ± 0.26	−0.06 ± 0.42	0.48	2.55 ± 0.23	2.66 ± 0.25	0.11 ± 0.22	0.01	0.01	−0.18 ± 0.07	0.02
TC/HDL-c	4.02 ± 0.62	4.03 ± 0.61	0.01 ± 0.75	0.94	4.1 ± 0.49	4.39 ± 0.6	0.3 ± 0.43	0.001	0.03	0.30 ± 0.17	0.08

Note: Variables are reported as median (interquartile range) or mean ± SD.

Abbreviations: FBG, fasting blood glucose; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of assessment-insulin resistance; hs-CRP, high sensitivity C- reactive protein; LDL-c, low-density lipoprotein cholesterol; TC, total cholesterol, TG, triglyceride.

[a]p values were obtained from the paired-sample t-test.

[b]p values a were obtained from the ANCOVA test with baseline values as covariates.

[c]Data were obtained from the ANCOVA test with baseline values and baseline weight, energy, fat, and selenium intake as covariates.

[d]Wilcoxon rank-sum test.

TABLE 3 Comparison of quality of life and psychological status within and between the propolis supplement and placebo groups in women with polycystic ovarian syndrome.

Variables	Propolis group ($n = 28$)				Placebo group ($n = 29$)				p^b	Effect size	
	Before	After	Changes	p^a	Before	After	Changes	p^a		Changes (mean ± SE)	p^c
Physical health	61.22 ± 16.83	64.79 ± 15.51	3.57 ± 4.12	0.001	70.81 ± 13.9	70.94 ± 13.08	0.12 ± 2.78	0.81	0.01	3.51 ± 1.24	0.046
Mental health	56.99 ± 13.84	60.71 ± 13.67	3.72 ± 3.46	0.001	62.07 ± 13.1	62.5 ± 11.89	0.43 ± 3.22	0.48	0.001	2.55 ± 1.06	0.005
Social health	64.88 ± 17.62	64.58 ± 17.51	−0.3 ± 1.57	0.33	66.38 ± 19.09	66.09 ± 18.76	−0.29 ± 1.55	0.33	0.93	−0.47 ± 0.45	0.38
Environmental health	56.47 ± 15	58.37 ± 13.53	1.9 ± 2.73	0.001	64.55 ± 11.88	65.19 ± 10.69	0.65 ± 2.11	0.11	0.51	−0.17 ± 0.63	0.99
Overall health	62.05 ± 16.48	68.3 ± 11.01	6.25 ± 9.32	0.001	69.4 ± 14.01	70.69 ± 13.06	1.29 ± 6.96	0.33	0.16	4.82 ± 2.89	0.09
Depression	12.5 ± 11.03	11.57 ± 10.35	−0.93 ± 1.49	0.003	10.96 ± 8.08	11.17 ± 8.34	0.21 ± 1.88	0.56	0.02	−0.77 ± 0.58	0.14
Anxiety	12.93 ± 9.28	11.89 ± 8.62	−1.03 ± 1.57	0.002	8.62 ± 6.55	9.1 ± 6.01	0.48 ± 1.74	0.15	0.01	−1.81 ± 0.58	0.01
Stress	19.57 ± 9.03	17.71 ± 8.3	−1.86 ± 1.32	0.001	18.48 ± 8.36	18.69 ± 7.56	0.21 ± 2.35	0.64	0.001	−2.06 ± 0.68	0.001

Note: Variables are reported as mean ± SD.

[a]*p* values were obtained from the paired-sample *t*-test.

[b]*p* values a were obtained from the ANCOVA test with baseline values as covariates.

[c]Data were obtained from the ANCOVA test with baseline values and baseline weight, energy, fat, and selenium intake as covariates.

($p = 0.01$) and ($p = 0.001$), respectively. Although, social, environmental, and overall health scores of patients did not significantly change in the propolis group. Subsequently, the crude score of anxiety and stress of women decreased significantly in the propolis group compared with the placebo group (-1.03 ± 1.57 vs. 0.48 ± 1.74, $p = 0.01$, and, -1.86 ± 1.32 vs. 0.21 ± 2.35, $p = 0.001$), respectively. Before adjustment, the crude score of depression showed a significant decrease; however, no significant changes were found in depression after adjustment for confounders (Table 3). After rating the scores, the levels of anxiety stayed moderate from baseline until the end of 12 weeks in the intervention group, but the levels of stress reduced in the intervention group from moderate to mild. In addition, depression levels stayed mild in the intervention group. In the placebo group, the levels of anxiety, stress, and depression stayed mild during the study.

4 | DISCUSSION

Based on the outcomes of this trial, 12 weeks of supplementation with 500 mg propolis in females with PCOS significantly reduced the levels of testosterone, insulin, HOMA-IR, LDL/HDL, HC, stress, and anxiety and increased physical and mental health scores of patients in relation to the control group. The levels of hs-CRP reduced significantly in the propolis group, but this reduction was not significant compared to the control group. Although FBG and depression decreased significantly in the propolis group compared to the placebo group, significance was lost after adjusting for confounders. Changes in body weight, BMI, WC, WHR, DBP, SBP, HDL-c, LDL-c, TC, and TG of patients were not significant between the two groups. As far as we know, this study represents the first trial to have evaluated the properties of propolis on metabolic parameters, testosterone, and hs-CRP in PCOS women.

Propolis has several health benefits due to many identified ingredients, such as flavonoids, lignans, aromatic aldehydes, esters, amino acids, fatty acids, minerals, vitamins, and phenolic acids (Braakhuis, 2019). Empirical evidence has highlighted the anti-inflammatory, antioxidant (Sforcin, 2016), and protective influence of propolis and its components on improving metabolic parameters (Samadi et al., 2017). Considering PCOS' inflammatory and autoimmune features (Patel, 2018), findings of the useful effects of propolis in the management of the levels of lipid profile, glycemic, and inflammatory factors, especially CRP, in patients with PCOS, could not only improve the symptoms of PCOS but also diminish the healthcare burden of comorbidities. So, this study introduces a novel therapy for PCOS women because there is no currently available, effective drug to treat PCOS. In addition, due to its safety and low price, it can be widely used.

Findings of a previous meta-analysis on 14 trials indicated a significant reduction in FBG and insulin. Additionally, a marginally significant decrease in insulin resistance was evident following propolis supplementation. The effect of propolis on weight and BMI, TG, TC, LDL-c, and HDL-c, however, was not significant (Hallajzadeh et al., 2021); these findings are in line with the outcomes of our study. Furthermore, Samadi et al. reported that consumption of 900 mg of propolis for 12 weeks reduced FBG in people with T2DM with no concomitant changes in insulin, insulin resistance, HDL-c, or TG (Samadi et al., 2017). In another study, after 60 days of supplementation with 1000 mg of propolis, Alassaf et al. observed a significant decrease in FBG (Alassaf et al., 2021). Our findings for FBG are consistent with the aforementioned results. As a possible mechanism, the expression and activity of glucose-6 phosphatase may be reduced by propolis (Kang et al., 2010). In addition, intestinal glucose uptake may be reduced due to decreased digestion of carbohydrates since propolis extract inhibits intestinal a-glucosidase and sucrase (Zhang et al., 2015).

Zakerkish et al. conducted a study that revealed that serum levels of insulin and HOMA-IR were significantly reduced in response to the consumption of 1000 mg/day of propolis for 90 days in T2DM patients (Zakerkish et al., 2019). In Afsharpour et al. (Afsharpour et al., 2017), 8 weeks of consumption of 1500 mg/day of propolis in T2DM reduced FBG, insulin, and HOMA-IR (Afsharpour et al., 2017). The results of our trial pertaining to insulin and HOMA-IR are similar to those of these previous studies. According to empirical data, propolis, with its antioxidant properties, reduces fasting insulin levels and, thus, reduces insulin resistance (Shahinozzaman et al., 2018). Possible mechanisms involved in the beneficial effects of propolis and its ingredients on blood glucose might be related to reducing insulin resistance, managing oxidative stress, reducing the construction of inflammatory parameters, cumulative level of adiponectin, facilitation of the glucose transfer into tissues, and inhibiting the activity of enzymes such as alpha-amylase and alpha-glucosidase (Cao et al., 2019). Propolis has also been shown to increase glucose transport through glucose transporter 4 by decreasing insulin resistance (Afsharpour et al., 2017). It has been reported that acute increases in insulin levels due to insulin resistance in women with PCOS subsequently induced rises in androgen levels (Diamanti-Kandarakis et al., 1998); therefore, insulin reduction, as a result of the increase in insulin sensitivity by propolis supplementation, may elicit a reduction in testosterone levels.

Moreover, in contrast with our findings, Soleimani et al. observed that 500 mg of propolis supplementation for 4 months in NAFLD patients significantly reduced hs-CRP (Soleimani et al., 2021). This reduction in hs-CRP was similar to Zakerkish et al. (Zakerkish et al., 2019). Some available evidence has suggested that propolis administration can reduce inflammation by down-regulating nuclear transcription factor-kb (NF-kB) and the expression of terminal c-Jun-N kinase and cyclooxygenase 2 (Natarajan et al., 1996). Additionally, the propolis-mediated reduction of inflammation might be related to its antioxidant components, such as flavonoids and polyphenols (Siheri et al., 2017). One of the reasons for the discrepant findings in the present versus previous studies on the propolis effects on hs-CRP levels might be attributable to the shorter period of propolis supplementation and smaller sample size.

Reportedly, propolis supplementation (30 drops/day for 12 weeks) increased HDL-c but did not decrease the body weight, BMI, TC, or LDL-c significantly (Mujica et al., 2017). A meta-analysis of five RCTs reported a significant upsurge in HDL-C and a reduction in TG after propolis supplementation, which is in contrast with our

results in the current study. However, for BMI, weight, TC, and LDL-C, no significant alterations were noted, which is similar to our study (Salehi-Sahlabadi et al., 2020). Gheflati et al. conducted a meta-analysis on 6 RCTs, which indicated that propolis had a non-significant effect on TG, TC, LDL-c, and HDL-c (Gheflati et al., 2021), which was analogous to our results. Propolis might have favorable effects on lipid profiles with a possible role in increasing expression of ATP-binding cassette transporters, which are related to the foundation of HDL and flow in peripheral tissue in liver proteins (Yu et al., 2011). Also, the stimulation of pre-β HDL-C may cause the positive effect of propolis on HDL-c (Gheflati et al., 2021). Although we did not identify any significant increase in HDL-c levels, LDL/HDL was significantly reduced.

We observed that propolis could improve physical and mental health. This is consistent with the data of the study conducted by Sibona et al., which showed that the 90-day consumption of Boswellia resin extract and polyphenols derived from propolis improves the quality of life of diabetic men. (Sibona et al., 2019). Evidence has shown that elevated levels of reactive oxygen species in skeletal muscle decrease muscle strength, increase fatigue, and impair cellular function. This situation can reduce the body's ability and ultimately negatively affect the quality of life. Polyphenol and CAPE components identified in propolis have been indicated to reduce muscle damage and ameliorate physical performance by inhibiting the NF-kB signaling pathway and increasing the activity of antioxidant enzymes. (Shen et al., 2013). Moreover, Usman et al. showed that honey and propolis have the potential to reduce stress-related hormones in women experiencing mild stress, including glucocorticoids and cortisol (Usman et al., 2020). These findings are similar to those in our study.

This study had some limitations, which should be acknowledged. First, due to financial limitations, we did not assess the other indicators of inflammation and oxidative stress markers. Second, failure to check different doses of propolis and time limitation precluded some further insight into our findings. The absence of biomarkers of adaptation for propolis consumption is another limitation of the current study; however, compliance with treatments was accurately assessed by counting the returned tablets. Therefore, due to inconsistencies between this study and various other studies, further studies are needed to confirm the above results. Despite the noted limitations, some of the strengths of this clinical trial included using the stratified permuted block randomization to balance BMI between two groups, as well as using a triple-blind and placebo-controlled strategy, and controlling the outcomes for possible confounding variables. Therefore, we restricted the impact of bias, and the study results are reliable. In addition, although treatment adherence is often problematic, we enhanced the supplement consumption adherence rates via SMS. Further research with diverse doses and with higher sample sizes is now needed to evaluate the effectiveness of propolis in PCOS women.

5 | CONCLUSION

In conclusion, the findings of this study demonstrated the promising effects of propolis in the reduction of glycemic indices and insulin resistance in PCOS. Moreover, supplementation with 500 mg/day of propolis for 12 weeks resulted in the reduction of the testosterone level, LDL/HDL, and HC in women with PCOS. Also, supplementation improved physical and mental health, along with reducing stress.

AUTHOR CONTRIBUTIONS

Elahe Abbasi: Conceptualization; data curation; formal analysis; investigation; software; writing – original draft; writing – review and editing. **Mohammad Bagherniya:** Conceptualization; data curation; formal analysis; methodology; validation; visualization; writing – original draft; writing – review and editing. **Davood Soleimani:** Conceptualization; data curation; formal analysis; methodology; resources; writing – original draft; writing – review and editing. **Hatav Ghasemi-Tehrani:** Conceptualization; investigation; resources; writing – original draft. **Mohammadreza Abbaspour:** Investigation; resources; validation; writing – original draft. **Cain C. T. Clark:** Data curation; formal analysis; investigation; writing – original draft. **Gholamreza Askari:** Conceptualization; funding acquisition; methodology; project administration; software; supervision; validation; visualization; writing – original draft.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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