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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

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Abstract

Background: Polycystic ovarian syndrome (PCOS) is the most common hormonal disorder in reproductive-age women. This syndrome is associated with many complications, so treatment of the disorder is usually warranted.

Objectives: To evaluate the effects of a combined low-dose oral contraceptive (LD) and *Vitex agnus* on the improvement of clinical and paraclinical parameters of PCOS.

Methods: This triple-blind, controlled trial was performed in 2015 in 70 women aged 18 - 45 years with PCOS in the clinics of the Alzahra and Taleghani hospitals and at other health centers in Tabriz, Iran, using a purposive sampling method. The women were randomized to either the LD or *Vitex agnus* groups. Regulation of menstrual cycle length, free testostrone, DHEA-S (Dehydroepiandrosterone sulfate), prolactin levels were assessed both before and after three cycles.

Results: The socio-demographic characteristics of the two groups were homogeneous before the intervention began. No significant statistical differences were identified between the LD and *Vitex agnus* groups three months after the intervention in terms of normalization of the menstrual cycle duration, means of free testostrone levels, DHEA-S, prolactin serum levels, and side effects. Three months after the intervention, the menstrual cycle duration in about 68.6% of the LD group members and 60% of the *Vitex agnus* group members became normal. The average levels of serum free testosterone and prolactin in both the LD and *Vitex agnus* groups had no differences three months after the intervention compared to prior to the intervention. The mean of the serum DHEA-S level in both the LD (Mean Difference (MD) = -0.52; [95% CI: -0.85 to -0.18]) and the *Vitex agnus* groups (MD = -0.60; [95% CI: -0.79 to -0.40]) decreased significantly three months after the intervention when compared with before the intervention.

Conclusions: This study showed that LD and *Vitex agnus* were both effective in normalizing the menstrual cycle and reducing DHEA-S, but they had no impact on the serum free testosterone or prolactin levels. The effects of LD and *Vitex agnus* on the normalization of the menstrual cycle and the means of serum prolactin, free testosterone, and DHEA-S levels in the women with PCOS were similar. Therefore, *Vitex agnus* can be used in place of LD.

Keywords: Vitex, Polycystic Ovarian Syndrome, Combined Oral Contraceptives, Amenorrhea, Oligomenorrhea

1. Background

Polycystic ovarian syndrome (PCOS) affects women of reproductive age and is a series of symptoms associated with hormonal imbalance (1) that impairs ovulation and promotes the formation of small cysts in the ovaries (2). This syndrome is the most common hormonal disorder in women (3) and the most likely cause of chronic anovulation, which is present in 4% - 6% of women of reproductive age (2).

A wide range of symptoms is manifested in women with PCOS. The three major symptoms are low ovulation or chronic anovulation, polycystic ovaries, and hyperandrogenism; the clinical manifestations include hirsutism, acne, and androgenetic alopecia (2, 4, 5). Other PCOS symptoms are irregular menstrual cycles (1, 2), overweight and obesity, pelvic pain, acanthosis nigricans, and fatigue (3). Laboratory and sonographic signs of PCOS include increased luteinizing hormone (LH) levels, a mild reduction in or unchanged follicule stimulating hormone (FSH) levels, an increased LH-FSH ratio, elevated levels of dehydroepiandrosterone sulfate (DHEA-S), excess androgens, such as testosterone and androstenedione, mildly increased prolactin levels, reduced levels of progesterone due to luteal phase defects, decresed levels of sex hormone

binding globulin (SHBG), and increased levels of estradiol and estrone (6). Upon ultrasonographic evaluation, the ovary has 10 or more cysts 2 - 8 mL in diameter, which is termed a polycystic ovary (7).

Different therapeutic methods have been recommended for PCOS treatment, including lifestyle changes, exercise, surgery, and medications. The medical treatment suggested for PCOS varies based on an individual's symptoms and may include metformin, clomiphene citrate, periodic progesterone, anti-androgens (spironolactone) (8), and gonadotropin (5). Combined oral contraceptive (COC) pills are the most common therapeutic strategy for menstrual disorders and skin diseases caused by hyperandrogenism and PCOS (7). COCs reduce ovarian androgen production and increase the steroid-binding capacity and SHBG levels. They have anti-androgenic effects and compete with androgens to bind with androgen receptors (9).

However, COC use has limitations in some health conditions and may also increase the risk of specific complications, such as menstrual bleeding pattern changes, headache, dizziness, nausea, breast tenderness, weight changes, mood changes, acne, and slight hypertension (10). Therefore, alternative treatements have been investigated.

The use of herbal supplements is widely varied due to the side effects caused by the consumption of chemical materials (11). *Vitex agnus* is one plant recommended to treat PCOS (12-14), and it has been used for many hormone-dependent disorders in women (11). The fruit of *Vitex agnus* contains flavonoids, glycosides, iridoids, and essential oils. The deficiency in progesterone and the dominance of estrogen in PCOS is the cause behind cyst formation in the ovaries. *Vitex agnus* leads to a mild inhibition in the release of FSH and the stimulation of LH release and ovulation, and it also increases progesterone levels (11, 13, 15). This compound selectively binds to estrogen receptor β and reduces circulating estrogen (15); it balances the estrogen-to-progesterone ratio (16, 17). *Vitex agnus* inhibits prolactin production through a direct connection with dopamine receptors (15).

Most PCOS patients present with hyperprolactinemia; this prolactin production inhibition may be useful in these patients (18, 19). *Vitex agnus* has been recognized only fairly recently for its anti-androgenic properties, which are suitable for PCOS (19). Studies have shown that *Vitex agnus* has significant beneficial effects on irregular menstrual cycles caused by hyperprolactinemia, corpus luteum defects, oligomenorrhea and secondary amenorrhea (15), breast pain (mastalgia) (20), premenstrual syndrome and dysmenorrheal symptoms (21), and the vasomotor symptoms of menopause (22-24); the substance can also reduce menstrual bleeding (25) and treat periodic mastalgia (15). This plant is considered to be a safe drug with very few side effects (26).

The world health organizaton's (WHO) statistics show that PCOS is one of the four major risk factors for cardiovascular diseases and myocardial infarction (27). The metabolic disorders that accompany PCOS include dyslipidemia (lower high-density lipoprotein (HDL) cholesterol and increased total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides), insulin resistance, and compensatory hyperinsulinemia. Therefore, women with PCOS are at risk for cardiovascular disease and type 2 diabetes (3). However, no study thus far has investigated the effect of *Vitex agnus* on PCOS treatment and its comparison with low-dose (LD) contraceptive pills.

2. Objectives

The aim of this study was to compare the effects of LD contraceptives and *Vitex agnus* in improving the clinical and paraclinical parameters of PCOS.

3. Methods

This was a randomized, triple-blind clinical trial with a placebo-controlled parallel design that was carried out in Tabriz, Iran. Sampling began after approval of the ethics committee of Tabriz University of Medical Sciences under the code 92151, registration in the IRCT under the code IRCT201306116709N13, and after compliance with the ethical guidelines of the Declaration of Helsinki. The inclusion criteria were as follows: women from 18 - 45 years of age with oligomenorrhea or amenorrhea (intervals of menstruation exceeding 35 days or no menstruation during the past three months) with polycystic ovaries diagnosed via ultrasound, the ability to read and write, a willingness to participate in the study, and a body mass index (BMI) between 18 - 35 kg/m^2. This study had the following exclusion criteria: other androgenic disorders, such as adrenal hyperplasia or an androgen-producing tumor; contraindications for LD contraceptives, including female smokers older than 35; active liver disease; a systolic blood pressure of 140 or higher or a diastolic blood pressure of 90 or higher; a diagnosis of diabetes for more than 20 years or existing damage to the arteries, vision, kidneys, or nervous system caused by diabetes; gallbladder disease; a history of stroke; blood clots in the shins and lungs; heart attack and other heart problems; breast cancer; migraine headaches with aura; an upcoming surgery that hindered walking for a week; consuming anticonvulsants (barbiturates, carbamazepine, lamotrigine, oxcarbazepine, phenytoin, prim-

idone, topiramate, rifampicin, rifabutin, etc.); lupus; antiphospholipid antibodies of thyroid gland diseases; Cushing's Syndrome; consumption of hormonal contraceptives or the use of other hormonal treatments; pregnancy; lactation; a previous surgery on one or both ovaries; and the use of a dopaminergic antagonist (antidopaminergic), such as tyrocidine, promazine, metoclopramide, hydroxyzine, etc.

The primary outcome measure was to compare the mean serum levels of DHEA-S (ng/mL) after three cycles of intervention. The secendary outcome measures included assessing the regulation of the menstural cycle length (21 - 35 days), the mean serum levels of free testostrone (ng/ml) and prolactin (ng/mL), and any side effects after three intervention cycles. We measured the mean serum levels of DHEA-S, free testosterone, and prolactin in ng/mL. Regulation of the menstural cycle length was determined by the calendar that the participants completed for each sample.

The minimum sample size was set at 35 individuals for each group when a 10% sample loss was predicted and assuming a confidence interval (CI) of 95% and a statistical power of 90% by comparing the means obtained using the Pukak Formula and also considering the 7% change between the two groups and the mean (standard deviation) serum level of DHEA-S in an earlier study (28) in both the intervention group (M1 = 5.2) and the control group (M2 = 4.8).

3.1. Sampling

The clinics of Alzahra and Taleghani hospitals (specialized referral governmental hospitals) and health centers in Tabriz were selected as the sampling centers. We included women from 18 - 45 years of age with PCOS and oligomenorrhea or amenorrhea. These women received an explanation about the procedures of the study, any probable risks, its potential benefits, and the voluntary nature of participation in the research. Written informed consent was provided by all participants. Initially, 115 women were assessed for eligibility; 35 of these were excluded because 9 (25.1%) women did not meet the eligibility criteria and 26 (74.2%) declined to participate in the study. Therefore, 70 women were randomly allocated into the LD or *Vitex agnus* groups.

Basic information about the women, such as their demographic, anthropometric and obstetric history, were collected after the participants had provided their informed consent. In addition, the women received a checklist to use to record their daily consumption of medications and any side effects. The adequacy of the questionnaire was determined by 10 specialists in obstetrics and gynecology, midwifery, and reproductive health using both face validity and content validity.

3.2. Randomization and Intervention

The women were randomly assigned to the LD or *Vitex agnus* group by a staff member not involved in the research. The allocation sequence was determined by a computerized random number table generator and block randomization, stratified by gravidity (first, second, or more) with block sizes of 4 and 6 and an allocation ratio of 1: 1.

Each participant who met the inclusion criteria (oligomenorrhea or amenorrea and having polycystic ovaries on sonography) was given a closed, translucent packet with the same shape and size bearing a number between 1 and 70. The capsules had been prepared by a pharmacist from the committee of pharmaceutical research at Tabriz Medical Sciences to have the same color, size, and appearance by grinding and pouring the substances into capsule coatings manufactured by the Kimia Shimi Company. In the LD group, 21 out of 28 capsules contained an LD pill, and the remaining 7 capsules were a placebo. Capsules for the *Vitex agnus* group contained *Vitex agnus* with a constant dosage for 28 days, and there were no placebo pills.

The capsules were placed in similar opaque packets that were numbered sequentially. Participants were given one packet containing three 28-piece LD or *Vitex agnus* capsules. One pill was to be consumed on the first day of menstruation during three cycles. In individuals with amenorrhea, bleeding was initiated with the injection of two 50-mg doses of medroxyprogesterone.

The LD oral contraceptives (containing 30 mcg ethyinyl estradiol (EE)/150 mcg levonorgestrel (LGN)) were produced by the Iran Hormone Pharmaceutical Company in Tehran, Iran), and *Vitex agnus* (containing a total fruit extract of *Vitex agnus* that had been standardized by Aucubin with as much as 2, 1 - 3, and 3 mg) was manufactured by the Poursina pharmaceutical company. An individual who was not involved in the research carried out the random allocation and placed the LD and *Vitex agnus* capsules inside the packages for allocation concealment and to preserve the blindness of the study. Package number 1 was given to the first individual who entered the study, and the allocation of sequentially numbered packages to sequential participants was continued for all the samples.

The participants were taught how to take their medication; it was also emphasized that they should not forget their other regularly prescribed medications and should also record their use on the consumed capsules sheet and the daily recording checklist. These checklists were collected from participants at the end of the intervention to ensure that the medication was taken. Additionally, to track medication consumption, telephone calls were made to participants during the three month-study. The participants, health care providers, data collectors, and outcome

assessors were masked as to which women received the LD or *Vitex agnus*.

3.3. Data Collection

A self-report questionnaire was used to collect data about socio-demographic characteristics and fertility information. BMI was measured before the intervention and also three months after the intervention. The heights and weights of the individuals were assessed to an accuracy of 0.1 kg and 0.1 cm, respectively, using a scale-stadiometer (Seca, Germany). The proper functioning of the scale was frequently evaluated with a 500-gram control weight. During measurement, the individuals wore light clothing and no shoes. Their BMI was calculated by dividing their weight in kilograms by their height in meters squared. A venous blood sample (5 cc) was obtained from the brachial area before the intervention and also three months after the intervention. After centrifuging the samples at 3000 rpm, the serum was transferred to 1.5-mL microtubes and stored at -70°C until the time of analysis. The serum levels of free testosterone, DHEA-S, and prolactin were measured using the enzyme-linked immunosorbent assay (ELISA) technique. Regulation of the menstural cycle length (to identify amenorrhea and oligomenorrhea) was determined with the calendar completed for each sample. All observations and laboratory experiments were carried out by one person.

3.4. Statistical Analysis

The Kolmogorov-Smirnov test was used to examine the normal distribution of continuous variables. Chi-square and Fisher exact tests were used to compare the qualitative variables between the two groups. T-tests and paired t-tests were used for the quantitative variables between the two groups and within a group, respectively. An analysis of covariance (ANCOVA) was employed to compare the clinical and paraclinical characteristics between the two groups three months after the intervention by controlling the effect of the score before the invention and also adjusting for the BMI.

All analyses were based on an intention-to-treat approach. $P < 0.05$ was considered statistically significant. All statistical analyses were conducted using the statistical package for the social sciences (SPSS), version 13 (SPSS Inc., Chicago, IL, USA).

4. Results

4.1. Study Population

The participants were recruited from March 2014 until March 2015 and were followed-up through September 2015. Forty females were randomly allocated to the LD group, and 40 were randomly assigned to the Vitex agnus group. Four individuals (1 member of the LD group and 3 from the Vitex agnus group) were excluded due to a lack of interest in continuing to participate in the study. Two participants dropped out because of nausea and digestive problems (LD group), 3 were lost due to pregnancy (1 in the LD group and 2 in the Vitex agnus group), and 1 discontinued participation due to spotting (LD group); all these participants were excluded from the study. Finally, 35 individuals (87.5%) in both study groups remained in the study until the end of the intervention (Figure 1).

4.2. Socio-Demographic Characteristics

The two groups were homogeneous in regards to their demographic, anthropometric, and midwifery characteristics. The mean (standard deviation) ages of the LD and *Vitex agnus* groups were 27.60 (7.49) and 23.37 (7.21) years, respectively. Most individuals in the LD (42.9%) and Vitex agnus groups (45.7%) had a secondary school education level. About 77.1% of the LD group members and 60.0% of the *Vitex agnus* group members were housekeepers; 77.1% of the members of both groups reported that they had an average income. Tables 1 and 2 show the remaining socio-demographic and fertility characteristics, respectively.

4.3. Menstrual Cycles

Before treatment, 62.9% of the LD and *Vitex agnus* group members reported oligomenorrhea, while 37.1% experienced amenorrhea. Three months after the intervention, the menstrual cycle duration of 68.6% of the LD group members and 60% of the *Vitex agnus* participants normalized. No statistically significant difference was observed between the two groups in this aspect ($P = 0.45$) (Table 3).

4.4. Mean Hormone Levels

Before treatment, the means of the free serum testosterone level in the LD group and the *Vitex agnus* group were 1.76 (1.62) and 2.17 (1.66), respectively. After treatment, these averages had been reduced in the LD group and the *Vitex agnus* group to 1.54 (1.14) and 1.98 (1.51), respectively. No significant difference was noted between the two groups in terms of the free serum testosterone level either at the time participants entered the study or after three months of treatment (Table 4).

Before treatment, the mean serum DHEA-S levels in LD and *Vitex agnus* groups were 1.96 (1.52) and 1.77 (0.98), respectively. The means after treatment were 1.44 (1.22) and 1.17 (0.93) in the LD and *Vitex agnus* groups, respectively. No significant difference was identified between the two groups in terms of the mean serum DHEA-S levels upon

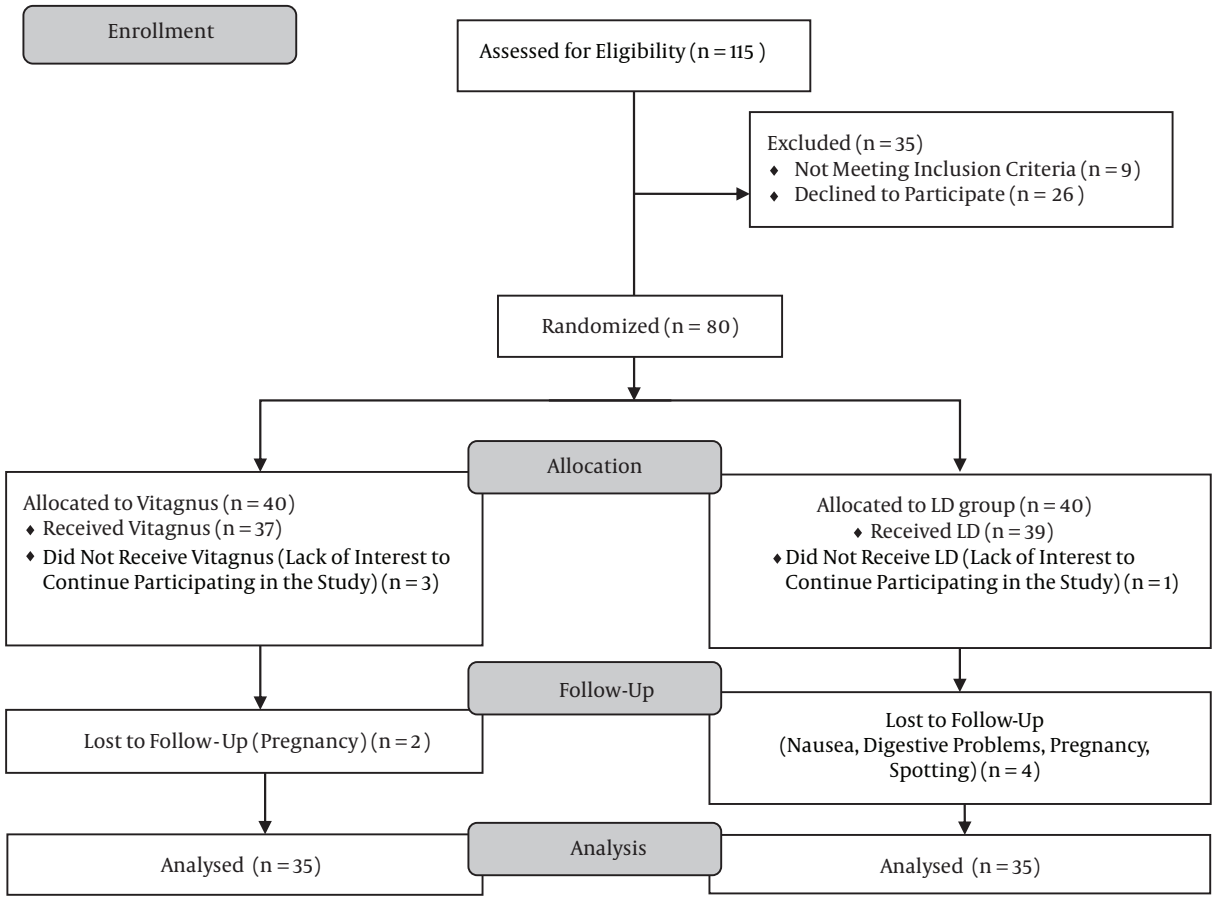

Figure 1. Consort Flowchart

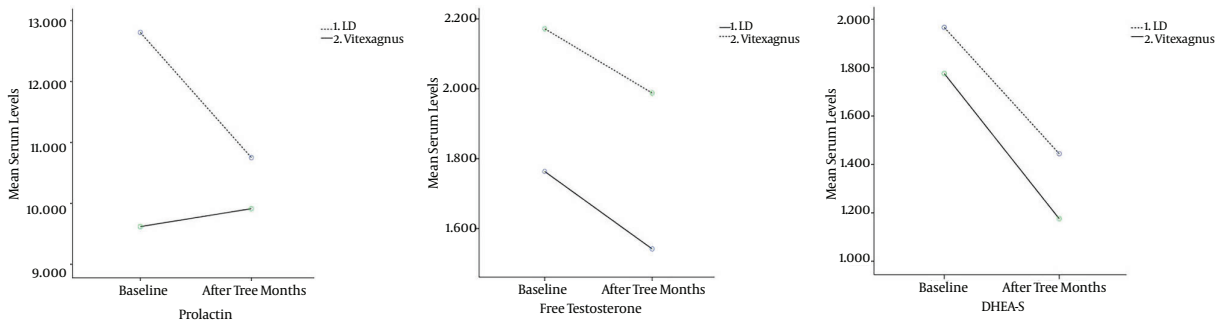

Figure 2. A Comparison of the Means of Free Testostrone (ng/mL), DHEA-S (ng/mL), and Prolactin (ng/mL) Serum Levels of the Samples in the LD-Receiving and *Vitex aignus*-Receiving Groups Three Months After the Intervention

entering the study or three months after treatment. The mean serum DHEA-S level in both the LD group (MD = -0.52; [95% CI: -0.85 to -0.18]) and the *Vitex agnus* group (MD = -0.60; [95% CI: -0.79 to -0.40]) had been reduced significantly three months after the intervention compared with before the intervention began (Table 4).

Before treatment, the means of the prolactin serum level were 12.80 (6.64) and 9.61 (2.07) in the LD and *Vitex agnus* groups, respectively. After treatment, these averages had changed to 10.57 (7.00) and 9.91 (2.73) in the LD

Table 1. Comparison of the Socio-Demographic Characteristics of the Samples in the LD-Receiving and *Vitex agnus*-Receiving Groups[a]

	LD n = 35	*Vitexagnus* n = 35	Statistical Indicators
Age, y			
18 - 25	15 (42.9)	13 (37.1)	
26 - 30	7 (20.0)	10 (28.6)	T-test = 0.387-
31 - 35	8 (22. 9)	6 (17.1)	df = 68
36 - 40	4 (11.4)	3 (8.6)	P = 0.70
41 - 45	1 (2.9)	3 (8.6)	
Mean (SD)	27.37 (7.21)	27.60 (7.49)	
Marital status			χ^2 = 0.254
Single	11 (31.4)	13 (37.1)	df = 1
Married	24 (68.6)	22 (62.9)	P = 0.91
Education level			
Elementary	4 (11.4)	3 (8.6)	
Guidance	10 (28.6)	7 (20.0)	χ^2 for trend = 1.120[b]
High school	15 (42.9)	16 (45.7)	df = 1
University	6 (17.1)	9 (25.7)	P = 0.29
Job			χ^2 = 2.386
Housewife	27 (77.1)	21 (60.0)	df = 1
Works outside the home	8 (22.9)	14 (40.0)	P = 0.12
Level of income			
Insufficient	7 (20.0)	7 (20.0)	χ^2 for trend = 0[b]
Relatively sufficient			df = 2
Sufficient	1 (2.9)	1 (2.9)	P = 1.00
BMI			
18.5 - 24.9	16 (45.7)	16 (45.7)	
25.2 - 9.9	12 (34.3)	12 (34.3)	χ^2 for trend = 0.001[b]
30 - 34.9	6 (17.1)	5 (14.3)	df = 1
38 - 39.9	1 (2.9)	2 (5.7)	P = 0.98
Mean (SD)	25.81 (4.43)	25.78 (5.06)	
Acne			χ^2 = 059
Yes	21 (60.0)	20 (57.1)	df = 1
No	14 (40.0)	15 (42.9)	P =0.81
Hair loss			χ^2 = 0.26
Yes	25 (71.4)	23 (65.7)	df = 1
No	10 (28.6)	12 (34.3)	P = 0.61

[a] All data with the exception of those specified as mean (standard deviation) are reported as the number (percent).
[b] Exact chi-square.

and *Vitex agnus* groups, respectively. No significant difference was noted between the two groups in terms of the mean serum prolactin level when the study began or three months after treatment (Table 4).

4.5. Side Effects

Three months after the intervention, no significant difference was present between the two groups as far as side effects were concerned (Table 5).

Table 2. A Comparison of the Fertility Characteristics of the Samples in the LD-Receiving and *Vitex agnus*-Receiving Groups[a]

	LD n = 35	Vitagnus n = 35	Statistical Indicators
Menarche (age)			
Mean (SD)	13.26 (1.52)	13.57 (1.39)	T-test = 0.90; df = 68; P = 0.37
Marriage age			
Mean (SD)	19.71 (4.27)	20.61 (4.90)	T-test = -0.67; df = 45; P = 0.50
Pregnancy age			
Mean (SD)	21.93 (2.84)	21.47 (2.90)	T-test =-0.45; df = 28; P = 0.66
Previous pregnancy			
No	20 (57.1)	19 (54.3)	Exact χ^2 = 1.619
1	0	1 (2.9)	df = 4
2	8 (22.9)	6 (17.1)	P = 0.93
3	4 (11.4)	5 (14.3)	
More than 3	3 (8.6)	4 (11.4)	
Children			
No	22 (62.9)	20 (57.1)	χ^2 = 0.317
1	8 (22.9)	10 (28.6)	df = 2
2	5 (14.3)	5 (14.3)	P = 0.85

[a] All data with the exception of those specified as mean (standard deviation) are reported as the number (percent).

Table 3. A Comparison of the Normalization of the Menstrual Cycle Duration of the Sample in the LD-Receiving and *Vitex agnus*-Receiving Groups Three Months After the Intervention

	LD n = 35	Vitagnus n = 35	Statistical Indicators
Before the Intervantion			
Oligomenorrhea	22 (62.9)	22 (62.9)	df = 1; P =1.00; χ^2 = 0.560
Amenorrhea	13 (37.1)	13 (37.1)	
After the intervention			
Normal menstural cycle[a]	24 (68.6)	21 (60.0)	df = 1
Anormal menstural cycle[b]	11 (31.4)	14 (0.0)	P = 0.45

[a] 21 - 35 day cycle.
[b] oligomenorrhea and amenorrhea.

5. Discussion

Our study findings revealed that *Vitex agnus* and LD were effective at normalizing the menstrual cycle duration and reducing the mean DHEA-S serum level in women with PCOS. Based on our literature review, this is the first study to investigate the effect of *Vitex agnus* on PCOS and to compare its effects with those of LD pills. COCs reduce ovarian androgen production, raise the steroid-binding capacity, increase SHBG levels, have anti-androgenic effects, and compete with androgens to bind with androgen receptors (9).

The studies of Banaszewska et al. (29) and Falsetti et al. (30), which were conducted to determine the effects of OCPs and ethinylestradiol together with cyproterone acetate in women with PCOS, respectively, revealed that a therapeutic intervention reduced the DHEA-S serum level; the results of both studies were consistent with our study's findings.

The studies of Bergmann et al. (31), Westphal et al. (32), Bohnert (33) and Propping (34) investigated women with abnormal menstrual patterns and ovulation disorders and confirmed that *Vitex agnus* led to the normalization of ovulation and increased progesterone levels, which resulted in

Table 4. A Comparison of the Means of Free Testostrone (ng/mL), DHEA-S (ng/mL), and Prolactin (ng/mL) Serum Levels of the Samples in the LD-Receiving and *Vitex aignus*-Receiving Groups Three Months After the Intervention

	LD Mean (SD)	Vitagnus Mean (SD)	Adjusted MD[b] (95%CI)	P0[c]	P1[b]
Free Testosterone					
Before intervantion	1.76 (1.62)	2.17 (1.66)			
After intervention	1.54 (1.14)	1.98 (1.51)	-0.25 (-0.78, 0.28)	0.98	0.35
MD (95%CI)[a]	-0.22 (-0.62, 0.17)	-0.18 (-0.73, 0.36)			
P[a]	0.26	0.50			
DHEA-S					
Before intervantion	1.96 (1.52)	1.77 (0.98)			
After intervention	(1.22) 1.44	1.17 (0.93)	0.14 (-0.18, 0.47)	0.25	0.38
MD (95%CI)[a]	-0.52(-0.85, -0.18)	-0.60 (-0.79, -0.40)			
P[a]	< 0.001	< 0.001			
Prolactin					
Before intervantion	12.80 (6.64)	9.61 (2.07)			
After intervention	10.57 (7.00)	9.91 (2.73)	-1.11 (-3.32, 1.11)	< 0.001	0.32
MD (95%CI)[a]	-2.05 (-4.16, 0.05)	0.29 (-0.67, 1.26)			
P[a]	0.56	0.54			

[b] ANCOVA.
[c] Independent T-test.
[a] Paired T-test.

menstrual cycle normalization.

Jolodar aimed to examine the effect of a hydroalcoholic extract of the fruit of the Vitex plant to change sex hormones by inducing PCOS in a rat model and showed that the use of the Vitex plant had no significant effect on the DHEA hormone rate (35), which is not consistent with our study's results. This discrepancy might be due our study's human PCOS patients compared with the use of PCOS-induced female rat samples in Jolodar's study. Moreover, our intervention took 12 weeks, but that in Jolodar's study lasted for only 30 days; there was also a difference of 365 mg/kg in the therapeutic dosage of *Vitex agnus* between the studies. In addition, this study showed that *vitex agnus* and LD had no significant difference in improving the mean of serum free testosterone and prolactin levels.

Banaszewska et al's. (29) and Hoeger et al's. (36) investigations of women with PCOS showed that OCPs reduced the serum free testosterone level; these results were not consistent with our study's findings. This difference was likely due to variations in the relevant therapeutic dosages used (the LD pills in our study contained 150 mcg of levonorgestrel and 30 mcg of ethinyl estradiol, but the Yasmin pills prescribed in previous studies contained 150 mcg desogestrel and 30 and 20 mcg of ethinyl estradiol, respectively). The measurement methods were also different, as we used an ELISA technique to assess the serum free testosterone level, while Banaszewska's study used a direct Coat-A-Count radioimmunoassay (RIA). Most of the participants in our study were between 18 and 25 years of age, while the majority in Hoeger's study ranged in age from 12 - 18 years old. The intervention lasted for 12 weeks in our study, but it ran for 24 weeks in Hoeger's study. The BMIs of the samples in our study were within the normal range, but the participants in the Hoeger study were obese. We used the ELISA technique for measuring the serum free testosterone level, but Hoeger's study used RIA.

The studies of Jolodar et al. (35), Nasri et al. (37), Webster et al. (38), and Malaivijitnond et al. (39) showed that the *Vitex agnus* plant reduced the serum testosterone content in mice, which was inconsistent with our findings and might be due to the differences in human and animal samples.

Banaszewska studied 48 women with PCOS and concluded that OCPs never lead to a significant reduction in the serum prolactin level (29), which was consistent with the results of this study, which found that LD consumption never lead to the significant reduction in the serum prolactin level.

The study of Milewicz aimed to examine the effect of *Vitex agnus* extract on treating 53 women with a men-

Table 5. A Comparison of the Side Effects of the Samples in the LD-Receiving and *Vitex agnus*-Receiving Groups Three Months After the Intervention

	LD n = 35	*Vitex agnus* n = 35	Statistical Indicators
Itching			Fisher's exact test = 2.05
Yes	2 (5.7)	0	df = 1
No	33 (94.3)	35 (100)	P = 0.49
Nausea			Fisher's exact test=0.34
Yes	2 (5.7)	1 (2.9)	df = 1
No	33 (94.3)	34 (97.1)	P = 1.00
Vomiting			Fisher's exact test = 1.01
Yes	1 (2.9)	0	df=1
No	34 (97.1)	35 (100)	P = 1.00
Headache			Fisher's exact test = 0.00
Yes	1 (2.9)	1 (2.9)	df = 1
No	34 (97.1)	34 (97.1)	P = 1.00
Digestive disorder			Fisher's exact test = 1.01
Yes	0	1 (2.9)	df = 1
No	35 (100)	34 (97.1)	P = 1.00
Skin rash			Fisher's exact test = 1.01
Yes	0	1 (2.9)	df = 1
No	35 (100)	34 (97.1)	P = 1.00
Dizziness			Fisher's exact test=1.01
Yes	1 (2.9)	0	df = 1
No	34 (97.1)	35 (100)	P = 1.00
Breast tenderness			Fisher's exact test = 5.38
Yes	5 (14.3)	0	df = 1
No	30 (85.7)	35 (100)	P = 0 .05
Weight change			Fisher's exact test = 0.0
Yes	1 (2.9)	1 (2.9)	df = 1
No	34 (97.1)	34 (97.1)	P = 1.00
Mood changes			Fisher's exact test = 0.00
Yes	3 (8.6)	3 (8.6)	df = 1
No	32 (91.4)	32 (91.4)	P = 1.00
Spotting			Fisher's exact test = 0.12
Yes	4 (11.4)	5 (14.3)	df = 1
No	31 (88.6)	30 (85.7)	P = 1.00

strual cycle disorder due to hyperprolactinemia and a deficiency in luteal phase hormones; the present study showed a considerable reduction in the prolactin level between the intervention and placebo groups because *Vitex agnus* tended to bind with dopamine receptors, normalize the luteal phase duration and progesterone levels, and significantly reduce PMS symptoms. However, the serum testosterone levels displayed no considerable reduction (40). Milewicz's findings were consistent with those of the present study regarding the serum testosterone level but were different regarding prolactin. This inconsistency was due to the difference in the measurement time and the measurement method of the serum prolactin level; the present study use an ELISA technique, while Milewicz em-

ployed a specific chemiluminescence assay method). In addition, the lack of an effect on testosterone in this study compared with other studies was likely the result of the low sample size in this study and variations in the times when blood samples were obtained from patients.

The study of Bohnert investigated the effect of *Vitex agnus* extract on 37 women with menstrual disorders, luteal phase defects, and hyperprolactinemia and concluded that the *Vitex agnus* plant extract significantly reduced their serum prolactin levels by decreasing thyrotropin-releasing hormone and binding to dopaminergic receptors (33), which was not consistent with the present study. This inconsistency was likely due to the low sample size in Bohnert's study compared with our study and also the difference in the measuring methods used for the serum prolactin level.

The investigations of Jarry et al. (41) and Sliutz et al. (42) aimed to examine the effects of *Vitex agnus* extract on inhibiting prolactin secretion in vitro in mouse pituitary cells and reported that the extract inhibits prolactin secretion by binding to dopamine receptors. However, the results of their studies were inconsistent with our findings; this inconsistency might be due to the respective differences in human and animal samples.

Our study results proved that the *Vitex agnus* plant has mild side effects, which was consistent with the results of other studies in this area (15, 17, 19, 21, 24, 32, 34, 40).

Menstrual disorders, including amenorrhea and oligomenorrhea, cause problems and pathological conditions that affect women's general health, such as infertility or reduced fertility, malignancies, cardiovascular disease, bone fractures, diabetes, hirsutism, and acne (43-45). Increased DHEA-S serum levels cause a deepening of the voice, acne, hirsutism, male pattern hair loss, a decreased breast size, and lower muscle mass (46). *Vitex agnus* can be as helpful as LD pills at normalizing the menstrual cycle's duration and reducing the DHEA-S serum level.

This randomized, controlled trial was conducted amongst women referred to the Alzahra and Talegani hospitals and other health care centers who may have had a poorer socio-economic status than women who visited private centers. Thus, our sample was not representative of all Iranian women, which reduces the generalizability of these results. It also lasted for a period of three months, and the FSH, LH, progesterone, and estrogen levels were not assessed due to financial constraints. Since the effect of herbal medications increases over time, it is recommended that futher studies conducted for a longer period of time that measures all of these hormones.

The *Vitex agnus* plant is as effective as LD contraceptive pills at improving the menstrual cycle duration and mean serum DHEA-S level in women with PCOS. Therefore, it can be recommended for women with PCOS who are willing to use herbal substances.

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Footnotes

Authors' Contribution: Study concept and design: Mahnaz shahnazi, Parvaneh Ghahremaninasab and Kobra Hamdi; acquisition of data: Parvaneh Ghahremaninasab and Kobra Hamdi; analysis and interpretation of data: Mahnaz Shahnazi, Azizeh Farshbafkhalili and Parvaneh Ghahremaninasab; drafting of the manuscript: Mahnaz Shahnazi, Azizeh Farshbafkhalili and Parvaneh Ghahremaninasab; critical revision of the manuscript for important intellectual content: Mahnaz Shahnazi and Azizeh Farshbafkhalili; statistical analysis: Mahnaz Shahnazi, Azizeh Farshbaf Khalili and Parvaneh Ghahremaninasab; administrative, technical, and material support: Mahnaz Shahnazi, Azizeh Farshbafkhalili and Parvaneh Ghahremaninasab; study supervision: Mahnaz Shahnazi, Azizeh Farshbafkhalili and Parvaneh Ghahremaninasab.

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