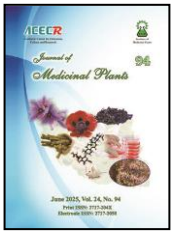
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Research Article

The effect of herbal combination therapy compared to pharmaceutical treatment on hormonal changes, ovarian cysts, hirsutism, and hair loss in patients with PCOS

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is the most common cause of anovulation and one of the main causes of infertility. Management varies with emphasis on the role of herbal medicines in the treatment of polycystic ovary syndrome (PCOS) compared to medical therapy. **Objective:** The aim of this study is to compare the herbal regimen *Vitex agnus-castus* var. + *Foeniculum vulgare* Mill. +*Portula caoleracea* L) with a medical regimen (spironolactone + cyproterone acetate with ethinyl estradiol) in polycystic ovarian syndrome patients. **Method:** The clinical trial, which compared two groups of women with polycystic ovary syndrome (PCOS), included 64 participants aged 18 to 42 years, all referred to a gynecological clinic. In a clinical study, the division of patients into case and control groups using random assignment so that both groups are as similar as possible. The control group receives a medical regimen that is compared to the herbal regimen of the case group. **Results:** The study did not show any statistically significant difference in Follicle-Stimulating Hormone (FSH), Thyroid-Stimulating Hormone (TSH), or Dehydroepiandrosterone Sulfate (DHEA-S) levels between the control and case groups after the intervention (P > 0.05)." the LH level has declined significantly after the intervention in the case group" is a common finding in this research (P = 0.001). The severity or existence of hirsutism (excessive hair growth in androgen-dependent areas of the female body) did not vary significantly between the two groups being compared (P> 0.05). The case group showed a significant reduction in the rate of hair loss (P = 0.001).Ovarian ultrasound Showed Formation of dominant follicles in case and control groups. **Conclusion:** The Herbal regimen was as effective as the phamacological regimen. *Vitex agnus-castus* and Fennel, known for their phytoestrogenic properties, induce negative feedback of luteinizing hormone with long-term use, leading to decrease in testosterone production. The synergistic effect of *Vitax agnus-castus*, Fennel and Purslane combination reduce testosterone levels and accelerates the treatment of PCOS.

Abbreviations: PCOS, Polycystic ovary syndrome; PMS, Premenstrual syndrome; TSH, Thyroid-stimulating hormone; FSH, Follicle Stimulating Hormone; LH, Luteinizing Hormone; DHEA-S, Dehydroepiandrosterone sulfate

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1. Introduction

Polycystic ovary syndrome (PCOS) is a common condition that causes anovulation and infertility [1].

The combination of androgens excess and anovulation is considered to be the main feature of polycystic ovary syndrome [2].

Polycystic ovaries have a third criterion, in addition to oligo- or anovulation and symptoms of hyperandrogenism. The Rotterdam criteria specifically define polycystic ovaries as having 12 or more follicles measuring 2 to 9 mm in size in at least one ovary or an ovarian volume greater than 10 ml.

The diagnosis of Polycystic Ovary Syndrome (PCOS) is definitive when two of the following three criteria are present: oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries at ultrasound [1].

In addition to anovulation, limited fertility, scanty or absent menstrual bleeding, and hyperandrogenism, polycystic ovary syndrome (PCOS) may be associated with dermatological issues such as hirsutism, acne, and androgenetic alopecia, as well as acanthosis nigricans, which is more commonly associated with hyperinsulinism [3].

Vitex agnus-castus, *Cinnamomum* genus, *pomegranate*, *Tribulus terrestris* Muhl., *Mentha species*, *Nigella sativa* L. [4], *Inonotus obliquus* (Ach.ex Pers.) [5] were effective for the management of PCOS in clinical and pre-clinical experiences.

Hirsutism is actually defined as excessive hair growth in a male-pattern in women, which usually affects 5 to 10 percent of women of reproductive age [6].

The described condition, characterized by increased androgen activity in hair follicles, can be caused by increased androgen levels (either naturally occurring or exogenously) or by increased sensitivity of hair follicles to normal levels of androgens.

Hirsutism, which is excessive hair growth in a male-like pattern in women, is a common and prominent symptom of androgen excess and is a key feature of polycystic ovary syndrome [7].

Anti-androgen medications or herbal remedies are used in the management of polycystic ovary syndrome (PCOS) to reduce androgen levels and other symptoms.

Chaste tree (*Vitex agnus-castus*), from the Verbenaceae family, is considered a herbal product due to the medicinal use of its fruit and dried leaves [8].

The European Medicines Agency and German health authorities have recognized the beneficial effects of *Vitex agnus-castus* in regulating the menstrual cycle and treating premenstrual syndrome (PMS) and mastalgia [8, 9, 10].

It is considered a proper alternative to pharmaceutical products for treating various disorders, including acne, digestive issues, infertility, and for supporting lactation [8-11].

Fennel, scientifically known as *Foeniculum vulgare*, is a versatile herb from the Umbelliferae (Apiaceae) family, used for its edible seeds, leaves, and fruits. Fennel has a long history of use in traditional medicine for various diseases related to the digestive, endocrine, reproductive, and respiratory systems [12].

Portulaca oleracea, commonly known as purslane, is actually a widely used medicinal plant with both edible and traditional medicinal applications [13].

This plant is used as an herbal medicine in many countries and has pharmacological activities including antinociceptive, antibacterial, muscle relaxant, wound healing, anti-inflammatory, free radical scavenger, and anticonvulsant [14].

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Spironolactone is a synthetic steroid that is structurally similar to aldosterone and was actually used as an early medical treatment for hirsutism. Since the serendipitous discovery 20 years ago that spironolactone given to a woman for PCOS and associated hypertension also improved hirsutism, it has been used as a primary medical treatment for hirsutism [15].

Cyproterone compound (Ethinylestradiol/cyproterone acetate) (EE/CPA) acts as an oral contraceptive by suppressing ovulation It is a reliable option for women suffering from symptoms of androgenization; it has strong antiandrogen properties and can be used to treat disorders associated with androgenization, including acne and hirsutism. Studies have shown that cyproterone compound is highly effective in treating moderate to severe acne and hirsutism. [16].

2. Materials and Methods

2.1. Study design

This randomized controlled trial has been approved by the Ethics Committee of Jahrom University of Medical Sciences. The ethical and IRCT codes are IR.JUMS.REC.1395.169 and IRCT2016062121653N7 respectively. Informed consent is obtained from participants, which means that participants in this research study are fully informed about the purpose, procedures, risks, and benefits of the study before agreeing to or participating.

2.2. Patient selection

Based on Rotterdam criteria, 64 PCOS women were included in this study among the patients referring to gynecology and Obstetrics and Infertility clinic affiliated to Jahrom university of medical sciences(each group included 32 patients).

The study's inclusion criteria specified female participants between the ages of 18 and 45 years, without any endocrine disorders (thyroid, parathyroid, diabetes mellitus, Cushing syndrome, congenital adrenal hyperplasia and hyperprolactinemia), virilizing tumors, renal or liver diseases, and without taking medication.

The study period is three months.

Exclusion criteria included severe gastrointestinal symptoms, pregnancy, lactation and inability to continue medication like allergic reaction.

Transvaginal ultrasound parameters and various hormone tests were evaluated (TSH, FSH, LH, DHEA-S) before and after treatment.

BMI was matched in both groups and abnormal cases were excluded from the study.

2.3. Randomization

All eligible patients were divided into two groups: case (herbal) and control (medication) using simple random sampling.

2.4. Treatment protocol

Each group received its special drug regimen. Case Group (1): six capsules of herbal regimen (Purslane + Fennel + Vitex combination). The patients received a total of six capsules every 24 hours, with each capsule containing 549 mg of fresh powder. The powder composition was 25% Purslane (137 mg), 25% Fennel (137 mg), and 50% Vitex (275 mg) and Control Group (2) received one tablet of Spironolactone(100mg)+ one tablet of Cyproterone acetate with Ethinyl estradiol (Cyproterone Acetate 2 mg + Ethinyl estradiol 35 μg) per day for three months.

In this clinical trial, the Ferriman-Gallwey (FG) score was used to measure hirsutism in areas sensitive to hair growth [17].

This study assessed hirsutism in the human body by focusing on four specific areas: the chin, chest, abdomen, and the area under the lip.

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The highest possible score is 16. The scoring system is as follows: 0 to 3 indicates non-hirsutism, 4 to 7 represents mild hirsutism, 8 to 11 corresponds to moderate hirsutism, and a score of 12 to 16 is classified as severe hirsutism.

The Numeric Rating Scale (NRS) is a pain assessment tool used to determine the severity of menstrual pain, with scores ranging from 0 to 10. A score of 0 indicates no pain, while 10 indicates the most severe pain imaginable [18].

The Global Acne Grading System (GAGS) categorizes acne severity based on a numerical score, with ranges for each severity level: 1-18 (mild), 19-30 (moderate), 31-38 (severe), and above 39 (severe/very severe). This assessment is based on clinical observations and examinations [19].

In this study, the hair extension test was utilized to assess hair loss. To achieve optimal results, patients are advised to wash their hair 24 hours prior to the examination.

A hair pull test, performed during a medical examination, involves examining approximately 40 to 60 strands of hair, usually by holding them between the second and third fingers and the thumb.

The strands are then gently stretched with continuous pressure, pulling the fingers along the hair shaft. If 6 or more hair strands (over 10%) are pulled out, the test is considered positive, indicating hair loss [20]. In current study, menstrual dysfunction was measured using the Smith-Dijulio criteria during clinical interview.

2.5. Collection, identification and preparation of herbal medicine

The plants used in this study included *Vitex agnus-castus*, Purslane and Fennel. *Vitex* was collected from Maraveh Tappeh to Ghazanghaie, Fennel from Shahrood to Sabzevar and Purslane from Andimeshk to Ahvaz.

These plants were identified through the Medicinal herbarium Code by the Institute of Forests and Rangelands (TARI), 87604 for Vitex, 45544 for fennel and 58452 for Purslane, respectively, and after ensuring the proper selection of plants, seeds were dried at room temperature and away from sunlight. After complete drying by electric milling, their seeds are prepared separately in powder form.

2.6. The pharmaceutical regimen

The control group was administered a daily regimen of 100 mg spironolactone combined with Cyproterone acetate 2 mg + Ethinyl estradiol 35 mcg for a duration of three months. Both drugs were processed in Aboureihan pharmaceutical company.

2.7. Statistical analysis

Data were analyzed using SPSS software version 21 using independent t-test, chi-square. T-test is used to compare the means of two groups.To analyze gonadotropin hormone levels before and after treatment, a paired t-test is appropriate to compare within each group, and an independent t-test is suitable to compare between the two groups, both before and after treatment.

The chi-square test is a statistical method used to compare hormonal indices reported as numbers and percentages. A significance level was considered $P < 0.05$.

3. Results

There was no statistically significant difference between the case and control groups in terms of age, weight, body mass index (BMI), and endometrial thickness ($P > 0.05$)

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This means that any changes observed in these parameters between the two groups were likely due to random chance, rather than a true difference between the study groups.

No significant difference was observed in serum levels of FSH, TSH, and DHEA-S (dehydroepiandrosterone sulfate) before and after the intervention in either group, but a significant decrease was observed in in serum levels of LH in the case group after the intervention (P = 0.006).

In the control group, there was no significant difference in serum LH levels before and after the intervention (P = 0.557) (Figure 1).

Also, there was no significant difference in hirsutism score between the case and control groups.

The acne reduction rate in the case and control groups was 1 (3.1%) and 8 (25%), respectively, with a significant difference between the two groups (P = 0.026).

The improvement in menstrual cycle was similar in both groups (P = 0.316), the reduction in menstrual pain and hair loss was greater in the case group compared to the control group (P = 0.001) (Table 1).

Ovarian ultrasound status in case and control groups before and after treatment is shown in Figure 2 and 3. In our study, the total number of small ovarian follicles detected on ultrasound decreased in both case and control groups, and dominant follicles were formed after treatment, indicating the beneficial effects of both treatments in improving the condition of ovarian follicles.

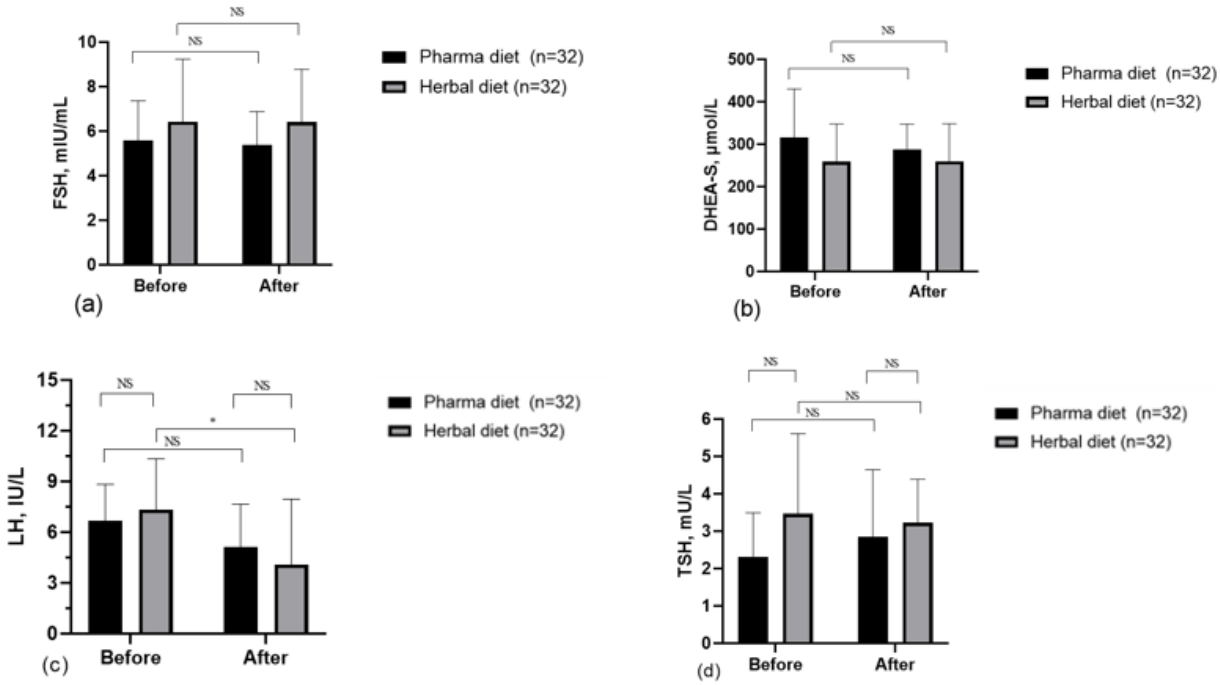


Fig. 1. Comparison of the FSH (a) , LH (b), DHEA-S (c) and TSH (c) levels between two groups (before and after the intervention). *: significant difference before and after intervention in one group (P < 0.05), NS: not significant.

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Table 1. Comparison of hirsutism, acne, menstrual cycle improvement, menstrual pain and hair loss in case and control groups

Variable		Group		P-value
		Pharma diet (n = 32)	Herbal diet (n= 32)	
Hirsutism (Ferriman-Gallway score)	Non reductio	17 (43%)	18 (56%)	0.317
	Reduction	15 (56.3%)	14 (43.8%)	
Acne (GAGS score)	Non reductio	24 (75%)	31 (96.9%)	0.026
	Reduction	8 (25%)	1 (3.1%)	
Menstrual cycle improvement	Non improvement	19 (54.4%)	15 (46.9%)	0.316
	Improvement	13 (40.6%)	17 (53.1%)	
Menstrual pain situation	Non reduction	32 (100%)	18 (56.3%)	0.001
	Reduction	0 (0%)	14 (43.8%)	
Hair loss situation	Non reduction	26 (81.3%)	11 (34.4%)	0.001
	Reduction	6 (18.8%)	21 (65.6%)	



Fig. 2. Polycystic ovary before treatment in both groups

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Fig. 3. Polycystic ovary after treatment (Formation of dominant follicles.)

4. Discussion

In this study, we compared the effect of herbal regimen consisting of *Vitex agnus-castus* + Fennel + Purslane with pharmaceutical regimen consisting of spironolactone + Cyproterone acetate with ethinyl estradiol on PCOS women with hirsutism in Jahrom (Fars province, Iran).

The statement shows that while the levels of specific hormones (TSH, DHEA-S, and FSH) remained stable after treatment in both groups, the level of luteinizing hormone (LH) in the case group decreased significantly after treatment. This indicates a specific effect of the treatment on LH regulation in the case group.

In other word, herbal regimen used in the case group impacts LH levels and possibly reduces the rate of androgen synthesis in the ovaries.

Spironolactone (SPA) reduces ovarian androgen production and is a very effective agent in reducing hirsutism score [21].

The combination of Ethinyl estradiol and cyproterone acetate (cyproterone compound) is actually an effective and widely used contraceptive medication for women with polycystic ovary syndrome who experience hyperandrogenism [16].

The goal of the described combination therapy is to improve the symptoms of polycystic ovary syndrome (PCOS) by targeting multiple hormonal pathways. This approach involves inhibiting LH secretion from the pituitary gland, reducing androgen production in the ovaries, reducing free androgen levels, and increasing sex hormone binding globulin (SHBG), ultimately limiting the effects of peripheral androgens on the body [22].

Ruan et al. (2017) used 2 mg of cyproterone acetate plus 35 µg of Ethinyl estradiol for the treatment of moderate to severe acne associated with androgen sensitivity (with or without seborrhea) and/or hirsutism, in women of reproductive age. Cyproterone acetate/Ethinyl estradiol (CPA/EE) is an effective treatment for hyperandrogenic skin symptoms associated with polycystic ovary syndrome (PCOS). Specifically, studies have shown that this medication is effective in treating acne, hirsutism (excessive hair growth), and seborrhea (oily skin) in women with PCOS [16].

Akha et al. (2014) conducted a study on the effectiveness of 3% fennel (*Foeniculum vulgare*) gel for reducing hair thickness in women with mild to moderate idiopathic

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hirsutism. The study concluded that the fennel gel treatment was indeed effective in decreasing hair thickness [23].

Foeniculum vulgare essence changed follicles number in PCOS women [24]. *Foeniculum vulgare* increased ovarian follicles number in the rats that treated with Bisphenol [25].

Vitex agnus-castus and *Foeniculum vulgare* have therapeutic effect on acne vulgaris in teenagers [26, 27].Our finding is in line with these studies.

Hamza et al. (2019) investigated the potential of chasteberry and a medicinal product containing chasteberry in rats with polycystic ovary syndrome (PCOS).

They showed that both the chasteberry extract and the supplement could potentially improve PCOS symptoms by regulating hormonal levels, lipid profiles, and oxidative stress [28].

In our research, herbal regimen reduces LH level in PCOS women.

In a previous study, *Vitex agnus-castus* treatment decreased follicular cyst, LH hormone and increased dominant folliculs in PCOS rats [29].

Vitex agnus-castus improved all the symtoms of menstrual cycle disorders including menstrual pain [9]. Furthermore, *Foeniculum vulgare* is used to decreases menstrual pain via reduction of prostaglandin level in blood circulation [30]. In our study, herbal regimen alleviates menstrual pain significantly (Table 1).

There was no significant difference in hirsutism score between the case and control groups ($P > 0.05$).

In a study, it was determined that taking spironolactone at a daily dose of 100 or rarely 200 mg for 6–9 months effectively decreasing hirsutism, seborrhea, effluvium capillorum [31] and our treatment period was three months.

Probably one of the reasons that did not have a significant effect on hirsutism is the shortness of our research period which is 3 months.

Menstrual cycle improvement was similar in both groups ($P = 0.316$) which shows similar results in both groups.

Portulaca oleracea [32], *Foeniculum vulgare* [33] and *Vitex agnus-castus* [34] have antioxidant activity.

Oxidative stress interacts with polycystic ovary syndrome features such as obesity, insulin resistance, hyperandrogenism and chronic inflammation.

Oxidative stress is indeed considered a significant factor in the development and progression of Polycystic Ovary Syndrome (PCOS).

Many studies believed that PCOS patients have high level of oxidative stress. Natural antioxidant scavenges free radicals and improves symptoms of PCOS [35, 36].

All mentioned studies are compatible with our results and support the effectiveness of herbal regimen in management of PCOS manifestations.

5. Conclusion

The results showed that the Vitex, Purslane and fennel combination was as effective as the pharmacological treatment in PCOS women. These plants may help to improve different features of PCOS. These plants can be used to improve PCOS after further clinical studies and ensuring the absence of side effects.

Author contribution

A.R.J.; Participated in study design and all experimental work and overall supervision. Gh. B.Kh; Prepared plants extract, T.N.; Contributed in experimental work, F.Sh.S; Participated in experimental work, S.N.; participated in study design and all experimental work and overall supervision. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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