

Therapeutic effect of vitex-angus castus extract on polycystic ovary syndrome induced by letrozole in experimental female rats

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Abstract—Polycystic ovary syndrome (PCOS) is a multifaceted endocrine disorder characterized by a wide range of clinical symptoms. While its precise etiology remains uncertain, evidence points to an interplay of genetic susceptibility, environmental factors, and lifestyle influences in its development. This study aimed to investigate the therapeutic potential of Vitex agnus-castus (VAC) ethanol extract in alleviating Letrozole-induced PCOS, with particular attention to biochemical and physiological recovery. **Methodology:** The research was conducted at the College of Veterinary Medicine/University of Kerbala, from October 2024 to February 2025. The animals were divided into three groups. Each group consists of 8 female rats negative control group (Group I), positive control group (Group II) and VAC ethanol treatment group (Group III). To assess its effects, study analyzed key reproductive hormones like, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone; alongside inflammatory markers Interleukin-6 (IL-6) and Interleukin-8 (IL-18) across experimental groups. **Results:** The study found that, there were significant differences ($p = 0.0001$) in follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels between the negative control (GI) and the positive control (GII) groups, as well as between (GII) and the treatment group (GIII), which administered VAC extract. As Similar, according to the levels of both IL-6 and IL-18. The comparisons between the negative (GI) and positive (GII) control groups, as well as between (GII) and the treatment group (GIII), revealed statistically significant variations ($p = 0.0001$). **Conclusion:** These findings suggested that, VAC ethanol extract exhibited promising therapeutic effects in managing PCOS, likely throughout modulation of reproductive hormones and inflammatory responses. The results align with existing literature that supporting the plant's role in hormonal balance and anti-inflammation activity. This study added to the growing evidence for the potential integration of herbal medicine, such as VAC into PCOS strategies of treatment.

Keywords: PCOS, LH, FSH, Vitex-Angus Castus, Interleukin

INTRODUCTION

Polycystic ovary syndrome (PCOS) represented as the one of the most prevalent endocrine disorders, which affecting women of reproductive ages. This complex condition was clinically characterized by most three hallmark features: ovulation dysfunction, clinical or biochemical hyperandrogenism, and morphology of polycystic ovarian as visualized by ultrasound (1). While the pathophysiology involves the elevated luteinizing hormone (LH) to follicle-stimulating hormone (FSH) ratio, and increased pulsatility of gonadotropin-releasing hormone (GnRH), the precise etiological mechanisms still incompletely understood. Importantly, PCOS is related with significant long-term health consequences, including increased risks of cardiovascular disease, diabetic-type 2, metabolic disorders, and psychiatric comorbidities such as anxiety and depression (2). Polycystic ovary syndrome (PCOS) determined as a complex syndrom with endocrine, genetic, and metabolic components. The hyperandrogenic state typically presented clinically, throughout dermatologic manifestations involving hirsutism, acne, and seborrhea. Consequently, the anovulatory aspect manifested as menstrual irregularity (oligomenorrhea or amenorrhea) and it is frequently associated with subfertility or also, infertility (3). Moreover, neuroendocrine dysfunction is prominent feature in most women with PCOS, contributing significantly to its pathogenesis and related with long-term metabolic complexity. The genetic basis of PCOS has been recognized for decades, supporting its classification as a complex disorder which influenced by many hereditary factors (4).

The endocrine bothering characteristic of PCOS primarily include dysregulation of GnRH, which escaped normal ovarian steroid inhibition feedback. This can lead to elevated LH levels alongside suppressed secretion of FSH. The resultant deficiency of FSH impaired granulosa cell aromatase activity, that reducing the conversion of androgens to estrogens and disrupting follicular development and ovulation consequently. These endocrine

aberrations collectively contributed to significant reproductive dysfunctions, biochemical imbalances, as well as metabolic derangements (5). At the level of ovarian morphology, PCOS is marked by distinct ovarian alterations, involving cortical thickness, multiple follicular cysts, and stromal hyperplasia. These structural changes exacerbate folliculogenesis impairment, that creating vicious cycle of anovulation and hormonal dysfunction (6).

Vitex agnus-castus (VAC), is a medicinal plant which demonstrating therapeutic potential, contains numerous bioactive components including flavonoids, glycosides, progestins, alkaloids, volatile oils, and essential fatty acids. The pharmacological effects of this plants are mediated through multiple mechanisms: stimulation of LH production, promotion of ovulation, and suppression of FSH secretion. Additionally, VAC demonstrated dopaminergic activity that inhibited prolactin production (7). Clinical studies had documented VAC's efficiency in addressing different reproductive disorders, including corpus luteum insufficiency, menstrual irregularities associated with hyperprolactinemia, mastalgia, amenorrhea, and premenstrual disorders. These therapeutic properties were made VAC the promising candidate for managing hormonal imbalances characteristic of PCOS (8).

Vitex agnus-castus (VAC) had emerged as a valuable phyto-therapeutic agent particularly for PCOS management because of its ability to modulate pituitary gland function and restore balance of hormones. The herb demonstrated particular efficacy in improving the fertility outcomes in anovulatory cycles by normalizing estrogen and androgen dysregulation levels (9). The therapeutic effects of VAC are mediated by multiple ways. Its bioactive components influence the activity of pituitary gland directly, thereby regulating of gonadotropin secretion. Moreover, VAC fruit extracts exhibited binding affinity for opiate receptors, which could explain its efficacy in alleviating premenstrual syndrome symptoms that observed (10).

Women with PCOS significantly demonstrate elevated levels of interleukin-6 (IL-6) in both follicular fluid and serum compared to healthy controls (11). This proinflammatory state is further amplified through IL-18-mediated pathways, as this cytokine stimulates tumor necrosis factor- α (TNF- α) production, which subsequently enhances IL-6 synthesis. Notably, IL-18 serves not only as a predictor of cardiovascular mortality but also appears to contribute to PCOS pathogenesis through its involvement in anovulation and impaired folliculogenesis (12). The persistent inflammatory milieu in PCOS, characterized by elevated IL-6 levels, may contribute to the development of insulin resistance, suggesting its potential utility as a diagnostic biomarker for metabolic dysfunction in these patients (13). This research aimed to assess the efficacy of ethanol extract of *Vitex Agnus castus* in the management of Letrozole induced PCOS biochemically and physiologically.

MATERIALS AND METHODS

The research was conducted at the College of Veterinary Medicine/University of Karbala, from October 2024 to February 2025, involving 24 adult female rats aged between 8 to 10 weeks, with an average weight ranging from 200 to 250 grams. Following a 15-day acclimatization period in metal cages under controlled environmental conditions (temperature about 23 ± 2 °C, light/dark cycle conducted by 12 hours for each); with unrestricted access to food and water, the rats were randomly divided into three groups, each containing eight animals, as outlined below:

Experiments Design: -

The animals in current study were divided into three groups. Each group consists of 8 female rats used as the following:

1-Group I (negative control group): eight female rats were Received D.W daily for 30 days

2- Group II (positive Control Group): eight females were injected peritoneally with single dose of Letrozole 1mg/kg, to induce well-defined PCOS for 21 days (14)

3- Group III (VAC ethanol extract (150 mg/kg): eight Letrozole -induced PCOS rats will be administered 150 mg/kg bw (15), ethanol extract of VAC daily by oral gavage for 20 days.

Parameters Studied: After the end of the experiment blood serum was taken for the laboratory tests, according to the following:

Physiological parameters: -

1- Sexual Hormones (Luteinizing hormone (LH), Follicle-stimulating hormone (FSH) and Testosterone). Using ELISA and the specific kits provided by (Sunlog/China).

2- Immunological markers (Interleukin-6 (IL-6) and Interleukin-18 (IL-18)).

- Statistical Analysis: The statistical program Graph Pad Prism 8.0 the t-test was used, $P \leq 0.05$ was chosen as the standard of significance. The data points were shown as mean \pm SD.

Ethical Approval: The research adhering to ethical principles derived from the Declaration of Helsinki. The protocol of this study, subject information, and consent form underwent review and received approval from a local ethics committee under the reference number (UOK.VET.PH. 2024.102).

RESULT & DISCUSSION

In this study the curative role of VAC was investigated to treated PCOS in experimental female rats

As mentioned above, the last group used to detect that. The hormones of reproductive system (FSH, LH and testosterone) were studied and the results obtained, showed in the table (1).

Table 1. The effect of VAC on reproductive hormones (FSH, LH and Testosterone

Hormone	Group I	Group II (Letrozole-PCOS)	Group III (VAC)	Significance
FSH	7.42	3.52	7.47	Group II vs III (p=0.0001)
LH	5.93	9.94	5.70	Group II vs III (p<0.0001)
Testosterone	18.45	29.51	18.84	Group II vs III (p<0.0001)

P value ≤ 0.05 significant

Based on the results obtained, there were significant differences (p=0.0001) between GI and GII as well as between GII and GIII. The efficacy of management of Letrozole-induced PCOS group using ethanol extract of VAC, comparing it both biochemically and physiologically with a control group and the Letrozole-only group. The Interpretation was that, Letrozole significantly disrupted normal hormonal balance, by reducing FSH and elevating LH and testosterone, which is determined as hallmark features of PCOS. VAC treatment normalized hormone levels, bringing them back to near-control values, suggesting a restorative effect on ovarian function.

These results align with previous studies. A study done by Kar *et al.* (14) found that hydro-ethanolic extract of VAC effectively restored hormonal imbalances in Letrozole-induced PCOS rats, correcting the LH:FSH ratio and reducing testosterone levels. Another study reported that VAC supplementation led to decreased total and free testosterone levels and improved menstrual cycles in women with PCOS (16).

Table 2. The effect of VAC on the IL-6 and IL-8 comparing with the control groups.

Cytokine	Group I	Group II (Letrozole-PCOS)	Group III (VAC)	Significance
IL-6	75.60	169.60	99.56	Group II vs III (p<0.0001)
IL-18	35.04	46.62	40.31	Group II vs III (p=0.0113)

P value ≤ 0.05 significant

The result found that, there were significant differences (p=0.0001) between GI (control +ve) and GII (control -ve) as well as between GII and GIII (treatment group). Letrozole increased systemic inflammation, as shown because of elevated levels of both IL-6 and IL-18. While VAC reduced both markers significantly, indicating an anti-inflammatory effect, which is crucial since chronic low-grade inflammation is involved in PCOS pathogenesis. Thus, suggesting it had potential therapeutic value both physiologically and biochemically.

This study aligns with previous findings that *Vitex agnus-castus* can modulate the hypothalamic-pituitary-ovarian

axis and has anti-inflammatory and hormone-balancing properties, supporting its traditional use in managing menstrual and fertility disorders (17). Kakadia *et al.*, (18) observed that *Vitex negundo* extract reduced pro-inflammatory cytokines, including IL-6, in Letrozole-induced PCOS rats. Furthermore, the study focused on *Vitex agnus-castus*, similar therapeutic effects have been observed with *Vitex negundo* appear to modulate hormonal imbalances and reduce inflammation in PCOS models (19, 20, 21).

CONCLUSION

This research supports the therapeutic potential of *Vitex agnus-castus* in managing PCOS, aligning with previous studies that highlight its role in hormonal regulation and anti-inflammatory effects. These findings contribute to the growing body of evidence advocating for the use of herbal remedies in PCOS management, due to that, ethanol extract of VAC was effective in reversing Letrozole-induced PCOS symptoms in rats. It restored hormonal balance (normalized FSH, LH, testosterone), and reduced inflammation (lowered IL-6 and IL-18).

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