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INVESTIGATING THE EFFECTS OF CHASTEBERRY AND GREEN TEA EXTRACT ON POLYCYSTIC OVARIAN SYNDROME RAT MODELS

ABBAS A. KHUDHAIR[1]; HAIDER N. JABBER[1] AND MOHAMMED A. QATHEE[2]

[1] Department of Clinical Laboratory Science, University of Basrah, College of Pharmacy, Iraq.
[2] Department of Animal Production Techniques, Technical Agricultural College of Mosul, Northern Technical University, Iraq.

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ABSTRACT

Polycystic ovarian syndrome is a gynaecological disorder associated with diverse health issues affecting numerous body systems. Therefore, this study was conducted to investigate the possible effect of chasteberry and green tea extract in treating polycystic ovary disorder. Twenty-four female rats were divided into four groups. Group I (control) was treated with distilled water. Group II positive control (polycystic ovary) treated with 1mg/kg letrozole orally for 4 weeks. Group III was treated with 1 mg/kg of letrozole administered orally, along with 750 mg/kg of chasteberry extract, for 4 weeks. Group IV was treated with 1 mg/kg of letrozole orally, combined with 250 mg/kg of green tea extract, for 4 weeks. At the end of the study, all animals were sacrificed for hormonal analysis and histopathological evaluation. Hormonal pictures of both extracts show a significant ($P<0.05$) increase in serum follicle-stimulating hormone along with a significant ($P<0.05$) reduction in serum prolactin hormone and luteinizing hormone, compared to the positive control group. Microscopically, there is a reduction in size and number of ovarian cystic follicles and restoration of normal ovary structure, presence of Graafian follicles with their corpora lutea and restoration of ovulation. These findings support the therapeutic potential of chasteberry extract and green tea extract in controlling and managing induced cystic ovaries in rat models.

Keywords: Polycystic ovary; Chasteberry; Green tea extract; Letrozole.

INTRODUCTION

Cystic ovarian syndrome is a prevalent, complicated and diverse hormonal disorder in women that is influenced by both environmental and genetic factors (Abdallah *et al*., 2023). This condition primarily impacts women in their formative years, particularly those in the early to late reproductive phase, which spans from 15-35 years. According to a recent study, globally every 10 women there is one with PCOS (Zeng *et al*., 2022). Polycystic ovary syndrome underscores the value of animal models in investigating the molecular mechanisms associated with genetic predisposition and developmental anomalies related to the disorder. Extensive research using a variety of animal species, including nonhuman primates such as rhesus and cynomolgus monkeys, rodents like mice and rats, ruminants such as sheep

Corresponding author: Abbas A. Khudhair
E-mail address: Abbas.razzaq@uobasrah.edu.iq
Present address: Department of Clinical Laboratory Science, University of Basrah, College of Pharmacy, Iraq.

and cattle, and zebrafish—has provided valuable insights into the multifaceted etiologies of PCOS (Liu *et al*.,2024).

Polycystic ovary syndrome is primarily identified through elevated levels of androgens (Abdel-Rhman *et al*., 2023) especially testosterone hormone and the presence of numerous cystic ovarian structures (over 10) that lead to irregular menstrual cycles, anovulation and infertility. Additionally, PCOS is associated with various endocrine and metabolic disorders including impaired glucose regulation, obesity, insulin resistance, diabetes, acne and hirsutism (Abdulmalek and Balbaa, 2019). The cause of PCOS is multifaceted, resulting from lifestyle and environmental factors, which highlights the need for a comprehensive treatment approach (Awonuga *et al*., 2023). Typical management of PCOS involves a combination of hormone therapy, lifestyle modifications and cosmetic treatments (Kshama, 2024).

The dried fruits of chasteberry or vitex have been employed for over 2000 years primarily for medicinal administration, including the treatment of various gynaeco-logical issues (Kakadia *et al*., 2019). Chasteberry is used to treat certain reproductive disorders including cyclical mastalgia, premenstrual syndrome and irregular menstrual cycle. In Europe over the past six decades, chasteberry was used in the treatment of insufficient corpus luteum, irregular menstrual bleeding and some menopausal symptoms (Puglia *et al*., 2023). The most important characteristic of chasteberry is its ability to reduce ovarian cyst production and regulate hormonal imbalance thereby alleviating PCOS condition (Feyzollahi *et al*., 2021).

Camellia sinensis is the plant from which green tea is produced and is considered the most popular beverage consumed globally. This type of tea contains a higher quantity of antioxidants compared to other teas and also boasts a particularly high concentration of polyphenolic substances known as catechins (Hazimeh *et al*., 2023). Green tea reduces the severity of symptoms associated with uterine fibroids and endometriosis by employing anti-fibrotic, antiangiogenic and apoptotic properties. Furthermore, it can decrease uterine muscle contractions and alleviate the widespread pain sensitivity linked to menstrual cramps and adenomyosis (Chairunnisa, 2022).

Letrozole is a selective and reversible aromatase inhibitor that effectively blocks the conversion of androstenedione and testosterone to estrogen; induced suppression of estrogen production also results in the production of endogenous hyperandrogenism, which has made it a useful tool for developing PCOS models in rodents. These animal models replicate key reproductive and metabolic characteristics of PCOS (Liu *et al*., 2024). Therefore, this study was designed to explore the ameliorative effect of both chasteberry extract and green tea extract in PCOS rats treated with letrozole.

MATERIAL AND METHODS

Materials:

Letrozole drug supplied from Denk Pharma Company (Germany). Chasteberry or Vitex extract and Green tea extract were purchased from a local pharmacy\USA pharmaceutical Company.

Animals:

Twenty-four female rats weighing (185-195gm) were used in this study. These animals are provided with water *ad libtum*, a temperature of 22℃, a light cycle every 12 hours and standard humidity. This study was done according to the Ethical Committee of the College of Pharmacy, University of Basrah, No (EC65) and these rats were kept for adaptation before the beginning of the study for 2 weeks. The estrus cycle was monitored for 10 days before the beginning of the study by making

a vaginal smear to maintain a normal cycle state. Administration of letrozole to rats for 28 days resulted in disturbance of the normal estrous cycle through domination of the diestrous stage, compared to the control group.

Study design:
The induction of PCOS via administration of Letrozole (1mg/kg) daily for 4 weeks through oral route. Then, the animals were classified into four groups;

Group I (Negative Control) (n=6): treated with distilled water orally.

Group II (Positive control) (n=6): induced PCOS group, treated with **Letrozole** 1mg/kg, orally for 4 weeks (Khodarahmi *et al*., 2023).
Group III (induced PCOS) (n=6): treated with **Chasteberry extract** 750 mg\kg, orally for 4 weeks (Schellenberg *et al*., 2012).

Group IV (induced PCOS) (n=6): treated with **Green tea extract** 250 mg/kg, orally for 4 weeks (Khodarahmi *et al*., 2023).
Thereafter, all groups were sacrificed for evaluation.

Hormonal analysis:
Blood samples were collected from vena cava and centrifuged at 3000 rpm for 15min. To collect serum and analysis of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and prolactin hormone (PRL) levels through using FSH, LH and PRL finecare^{TM} rapid quantitative test of (Guangzhou Wondfo Biotech company, China) respectively (Ghafurniyan *et al*., 2015)

Histopathological assay:
The formalin (10%) was used to preserve ovary tissues obtained from animals. Tissue dehydrated through ascending alcohol, cleared with xylene and infiltrated with paraffin to make paraffin blocks. Thin sections of 5µm-thick to be stain with hematoxylin and eosin. Then slides were examined with a Euromex microscope (Al Moziel *et al*.,2024).

Statistical analysis:
SPSS version 23.0 was used to analyse data that offered as mean ± standard deviation (M ± SD). The P value considered significant between groups less than 0.05. Analysis of variance is also used to assess the differences among groups.

RESULTS

Hormonal analysis
Results in Table (1) showed that PCOS rats present with a significant *(P<0.05)* increase in serum level of both PRL and LH hormone (3.43±0.09, 6.89±0.17), respectively, compared to all study groups. On the other hand, the PCOS group reveals a significant *(P<0.05)* decrease in serum FSH (3.45±0.22), compared to the control (4.52± 0.15) and treated groups (4.22±0.06, 4.18±0.06) respectively. Furthermore, both the chasteberry group and green tea-treated group increased serum levels of FSH hormone (4.22±0.06, 4.18±0.06) significantly *(P<0.05)* along with a significant *(P<0.05)* decrement in PRL (2.61±0.08, 2.65±0.07) and LH (3.22±0.31, 3.12±0.26) hormonal levels respectively. Although, they do not display any significant changes between them for the measured parameters.

Table 1: Effect of Chasteberry and Green tea on serum hormonal level.

Parameter/ Group	PRL (ng/ml)	FSH (ng/ml)	LH (ng/ml)
Control	2.81±0.52 b	4.52±0.15 a	3.52±0.14 b
PCOS	3.43±0.09 a	3.45±0.22 c	6.89±0.17 a
PCOS + Chasteberry	2.61±0.08 b	4.22±0.06 b	3.22±0.31 b
PCOS + Green tea	2.65±0.07 b	4.18±0.06 b	3.12±0.26 b

a,b,c, represent significant changes; (P<0.05).

Histopathological results

The normal ovarian histological structure was observed in the control group associated with the presence of Graafian follicles, large corpora luteum and secondary follicles (figure 1A). Histopathological evaluation of PCOS showed different-sized cystic follicles that were encapsulated with thickened fibrous tissue capsules, degenerated ovarian tissue and the absence of Graafian follicles (figure 1B). However, Chasteberry group (Figure 1C) and green tea group (Figure 1D), revealed a decrement in cystic follicle size, and number along with their disappearance and restoring normal architecture of the ovary through the formation of follicles with their corpora lutea.

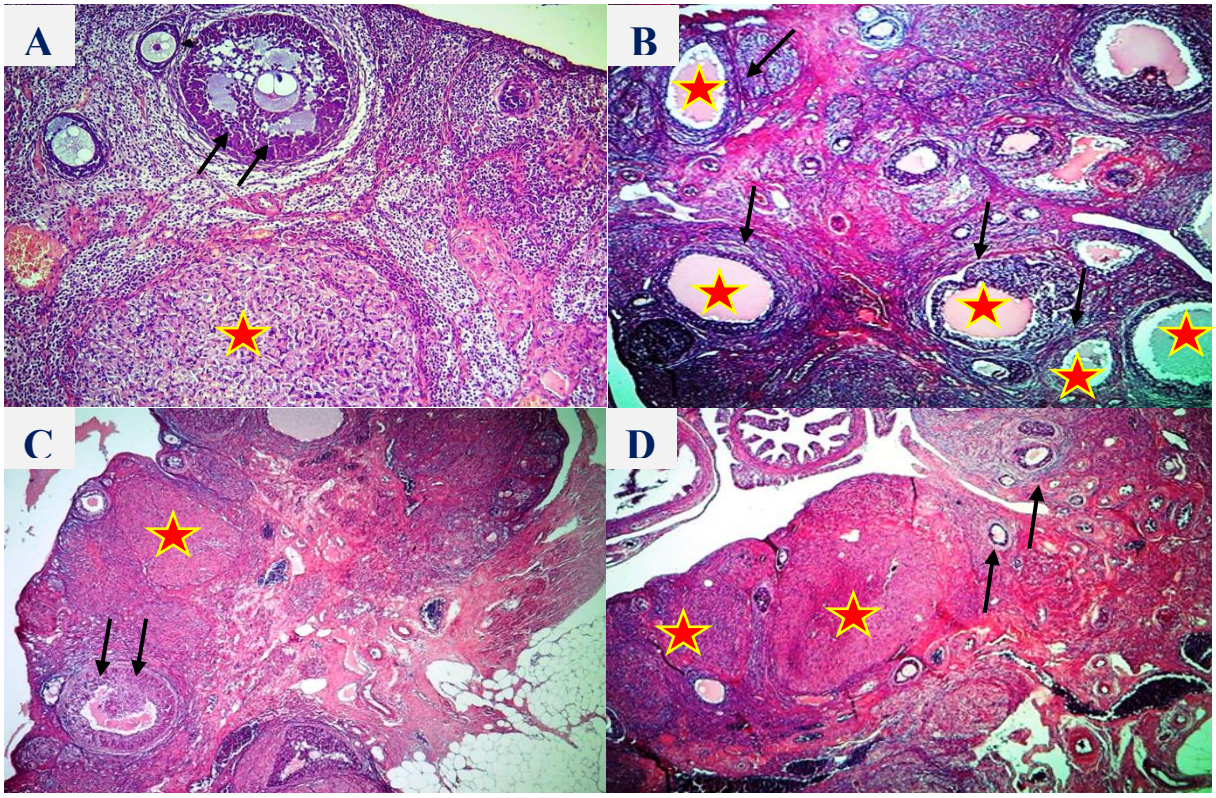

Figure (1): micrograph of ovary tissue (H&E stain X200). A) The control group shows follicle, corpus luteum (red star) and Graafian follicle (black arrow). B) PCOS ovary reveals different-sized cystic follicles (red stars) surrounded with fibrous capsules (black arrow) and the absence of corpora lutea. C) The Chasteberry group appears with new corpora lutea (red star), and the absence of cystic ovary while Graafian follicles (black arrow) were noted. D) Green tea group showed a decreased size of the cystic follicle and the presence of corpora lutea (red star) with follicle (black arrow).

DISCUSSION

Numerous complications associated with PCOS such as infertility, metabolic disturbances, cardiovascular issues and chronic health problems may persist throughout life. Although synthetic medications have proven effective in managing PCOS, their long-term use is often accompanied by adverse drug reactions. As a result, there is growing interest in herbal therapy as an alternative treatment approach, offering improved recovery rates and greater acceptance among patients. Moreover, the potential effect of chasteberry extract and green tea extract in managing the gynaecological condition, thus this study intended to determine their ameliorative effect in management of PCOS.

Our result reveals a hormonal picture representing a significant increase in serum PRL and LH. In contrast, serum FSH concentration is significantly decreased in PCOS group, this result is similar to (Moolhuijsen and Visser, 2020; Shehab *et al*., 2023). This hormonal disturbance is attributed to the letrozole effect for PCOS induction.

Ovarian cystic follicles usually develop through granulosa cell necrosis and the excess of androgen produced from theca cell, this occurs via the effect of letrozole with gonadotropin-releasing hormone (Jelodar *et al*., 2018). Previous studies mentioned that letrozole causes elevation of LH by preventing the conversion of excess androgen to estradiol and sex hormone disturbance; ultimately ending with follicular ovarian cyst formation (Paixao *et al*., 2017; Ryu *et al*., 2019; Younas *et al*., 2022).

The gynaecological disorder is often associated with hyperprolactinaemia because chasteberry plays an essential role in controlling and management of increased prolactin levels through its action on dopaminergic and estrogen receptors therefore, this extract administered to manage PCOS (Puglia *et al*., 2023).

Chasteberry extract efficacy is attributed to the presence of casticin flavonoids, which is a well-known phytotherapeutic agent that helps restore normal prolactin hormone levels, thereby improving PCOS symptoms and fertility (Lakshmi *et al*., 2023; Puglia *et al*., 2023).

Obesity and elevated insulin levels are considered contributing factors for PCOS development; excessive body fat is associated with androgen increment and insulin resistance which result in aggravation of follicular cyst formation and infertility. Therefore, several attempts for PCOS treatment with medicinal products to control insulin level, regulation of sex hormone disturbance and restoration normal ovulatory condition (Pavone *et al*., 2018).

Ghafurniyan and his colleagues reported that insulin resistance was greatly reduced with green tea extract by stimulating the differentiated adipocyte to secrete adiponectin (Ghafurniyan *et al*., 2015). This effect is mediated through the binding of its catechin to peroxisome proliferator activated receptors. Additionally, the thickness of the theca cell layer has been found reduced with green tea extract by initiating lipolysis and inhibiting cell proliferation, thereby decreasing thecal androgen secretion (Chairunnisa, 2022; Yavangi *et al*., 2024).

The therapeutic intervention of chasteberry extract and green tea extract helps mitigate hyperandrogenism, reduce excessive LH and improve insulin resistance (Yavangi *et al*., 2024). Additionally, it reinforces the effect of FSH on follicle formation, growth and maturation, ultimately improving symptoms of cystic ovary disorder as noted by (Pellatt *et al*., 2007). These findings are consistent with previous studies (Ghafurniyan *et al*., 2015; Abdallah *et al*., 2023).

PCOS from the histopathological field is characterized by several key features, including the presence of multiple cystic follicles in the subcapsular area with thickened capsule, corpora lutea absence and degeneration of granulosa cell accompanied by theca cells hyperplasia (Karateke *et al*., 2019; Ibrahim *et al*., 2022; Abdallah *et al*., 2023). These findings are consistent with the results of the current study. This outcome is attributed mainly to oxidative stress induced by letrozole (Seghinsara *et al*., 2019; Awonuga *et al*., 2023). Letrozole has been shown to reduce levels of antioxidant enzymes such as glutathione, catalase and superoxide dismutase (Jahan *et al*., 2018).

On the other hand, the green tea group and chasteberry group showed a marked reduction in the size and number of ovarian cysts along with the formation of new follicles and development of corpora lutea. This result is possibly due to the ability of chasteberry extract to restore hormonal homeostasis and the antioxidant effect produced by green tea components, thus manage of PCOS (Hazimeh *et al*., 2023; Khodarahmi *et al*., 2023).

CONCLUSION

According to the present finding, we found that both chasteberry extract and green tea extract have potential therapeutic approaches for the treatment of PCOS through their ability to control the hormonal imbalance produced with PCOS.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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دراسة تأثير مستخلص الشاي الأخضر وعشبة العفة في نماذج الجرذان المصابة بمتلازمة تكيس المبايض

عباس عبد الرزاق خضير ، حيدر ناصر جابر ، محمد عزيز قاضي

Email: Abbas.razzaq@uobasrah.edu.iq Assiut University web-site: www.aun.edu.eg

متلازمة تكيس المبايض هي اضطراب انثوي يرتبط بمشكلة صحية متنوعة تؤثر على العديد من أنظمة الجسم. لذلك، أجريت هذه الدراسة للتحقيق في التأثير المحتمل لمستخلص الشاي الأخضروعشبة العفة في علاج متلازمة تكيس المبايض. تم تصنيف أربع وعشرين أنثى جرذ إلى أربع مجموعات. المجموعة الأولى الضابطة عولجت بالماء المقطر. المجموعة الثانية الضابطة الإيجابية (متلازمة تكيس المبايض) عولجت بجرعة ١ مجم/كجم من الليتروزول عن طريق الفم لمدة ٤ أسابيع. المجموعة الثالثة عولجت بجرعة ١ مجم/كجم من الليتروزول عن طريق الفم مع ٧٥٠ مجم/كجم من مستخلص شاي العفة لمدة ٤ أسابيع. المجموعة الرابعة عولجت بجرعة ١ مجم/كجم من الليتروزول عن طريق الفم مع ٢٥٠ مجم/كجم من مستخلص الشاي الأخضر لمدة ٤ أسابيع. في نهاية فترات الدراسة ، تم التضحية بجميع الحيوانات للتحليل الهرموني والتقييم النسيجي المرضي. اظهرت الصور الهرمونية لكلا المستخلصين زيادة كبيرة ($P<0.05$) في FSH في مصل الدم مع انخفاض كبير ($P<0.05$) في PRL و LH في المصل بالمقارنة بمجموعة متلازمة تكيس المبايض. على المستوى المجهري، هناك انخفاض في حجم وعدد الجريبات الكيسية واستعادة البنية الطبيعية للمبيض، ووجود الجريبات مع أجسامها الصفراء واستعادة التبويض. تدعم هذه النتائج الإمكانات العلاجية لمستخلص فيتكس ومستخلص الشاي الأخضر في السيطرة وإدارة متلازمة تكيس المبايض المستحثة في نموذج الجرذان.

الكلمات المفتاحية: متلازمة تكيس المبايض؛ شاي العفة؛ مستخلص الشاي الأخضر؛ الليتروزول.