Research Article

Effect of Vitex Agnus-Castus Ethanolic Extract and Ciprofene Citrate on Reproductive Hormones in Polycystic Ovary Syndrome in Female Rats

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Abstract | Polycystic ovary syndrome (PCOS) is a complex genetic, endocrine, and metabolic disorder which is characterized by chronic anovulation, polycystic ovaries, biochemical and clinical manifestations of hyperandrogenism. This study aims to evaluate the effects of the alcoholic extract Vitex agnus-castus on body weight gain and possible alteration of reproductive hormones in letrozole-induced PCOS induced by letrozole in adult female rats. The negative control received 1 ml of 0.5% carboxymethyl cellulose (CMC) whereas positive control animals received 1 mL of 0.5% CMC (carboxymethylcellulose). Three treatment groups were treated with either the VAC vitex agnus castus extract (150 mg/kg bw or 250 mg/kg bw or 1 mg/kg bw) orally. The result showed an increased in body weight gain in the control group and animals suffering from PCOS. Also, an increase in serum LH was observed in the PCO group and decreased in estrogen and progesterone was noticed. Additionally, an increased in the hormone testosterone and prolactin was noticed in the group of PCO. The ameliorative effect of extract of VAC was clearly observed at doses of 150 and 250 mg/kg bw on the release of estrogen and progesterone. Taken together, our findings illustrate that Vitex agnus-castus alcoholic extract impact the physiological parameters of female rats.

Keywords | PCOS, Letrozol, VAC, CC, BW

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex genetic, endocrine, and metabolic disorder, characterized by chronic anovulation, polycystic ovaries, biochemical and clinical manifestations of hyperandrogenism (Homburg, 2009). PCOS is found in approximately 4–10% of women of different reproductive age (Strowitzki et al., 2010). PCOS has a negative impact on the physiology and metabolism of the body, as it can evolve into a metabolic syndrome with insulin resistance, hyperinsulinemia, hypertension, cardiovascular disease, dyslipidemia, and abdominal obesity (Allahbadia and Merchant, 2011). PCOS is the most common gynecological endocrine disease, with a ranging from ~6% to ~20% (Escobar-Morreale, 2018). PCOS increases the risk factor for long-term metabolic disorder like type 2 diabetes mellitus (DM2) and atherosclerosis (Shi and Vine, 2012). Overweight and obesity exacerbate the reproductive and metabolic disorders of PCOS (Ching et al., 2007; Maliqueo et al., 2014). In women with PCOS, obesity is characterized mainly by accumulation of abdominal body fat. Hyperinsulinemia exists independently of obesity and therefore may be an independent factor in PCOS (Masszi et al., 2013; van Houten and Visser 2014).

However, the etiology of PCOS is still obscure. One hy-

pothesis is that PCOS is an inherited ovarian disease in which excess androgens in early life led to PCOS in adulthood. Prenatal testosterone-treated sheep and non-human primates show strikingly similar features to human PCOS (Padmanabhan and Veiga, 2013). However, these animal models are prohibitively expensive, difficult to house, and not subject to gene editing. Various androgen modeling strategies, such as dihydrotestosterone, dehydroepiandrosterone, and testosterone propionate have also been used to induce PCOS in rodents (Shi and Vine, 2012). However, in these rats, unlike in women with PCOS, the weight of the ovary is reduced and typical gonadotropin levels, lipid profiles and other metabolic characteristics are lacking (Maliqueo et al., 2013).

Letrozole is a highly specific non-steroidal aromatase inhibitor that prevents testosterone from converting to estradiol. It inhibits the aromatase enzyme by competitively binding to the heme of the cytochrome P450 subunit of the enzyme, resulting in a reduction of estrogen biosynthesis in all tissues, letrozole induces PCO changes similar to those of PCOS women, such as increased ovarian weight and size, a thickened theca interna cell layer, and anovulation Current studies suggest that letrozole may prompt the development of multiple follicles when used as a fertility regulating agent in women and animal models (Tulay et al., 2003). The main purpose of this study was to evaluate the effects of Vitex agnus-castus alcoholic extract, on body weight gain and some alteration of reproductive hormones in letrozole-induced PCOS induced by letrozole in adult female rats.

Experimental Animals

Clinically normal healthy adult rats with a normal estrus cycle will be used in the implementation of the research project after being subjected to adaptation at the animal house for two weeks under standard conditions of temperature and humidity while providing food and water ad libitum during the study period.

Preparation of plant extract

The ripened vitex agnus castus seed will be cleaned, dried in the sun, and ground. From the powder, the ethanol extract will be prepared with 70% ethanol solvent for 6 hours at 35 ° C using a magnetic stirrer. The mixture will be filtered through a Whatman No.1 filter paper. The resulting solution will be evaporated under vacuum and then dried at -50 ° C in a lyophilizer. After grinding, 500 g of plant was extracted in a Soxhlet apparatus with ethanol. Finally, the ethanol was evaporated with a rotary machine and the residual was dried to form a powder. The resulting extract was then reconstituted by solving it in saline before being administered orally to animals.

Experimental design:

Induced polycystic ovarian syndrome

First, the animals will be weighed, checked for two consecutive normal estrus cycles by vaginal smear examination, microscopically, and divided into two main groups. Group I: Ten animals in this group served as a negative control and received 1 mL of 0.5% CMC (carboxymethyl cellulose) for 21 days. Group II: - Forty animals will be treated with letrozole (1 mg/kg dissolved in 0.5% CMC) for 21 days for the induction of PCOS. From day 22 the experimentally PCOS induced rats' group will be divided into four subgroups each comprising 10 rats. All animals were subjected to vaginal smear analysis for a period of 21 days until the appearance of persistent vaginal cornification. After verifying the induction of PCOS, the PCOS group was divided into the following subgroups: Subgroup one: (negative control): received 1 ml of 0.5% CMC (carboxymethylcellulose) the next day for 30 days. Subgroup two: (positive control): PCOS animals received 1 mL of 0.5% CMC (carboxymethylcellulose) the next day of induction for 30 days. Subgroup three: PCOS induced rats treated with the VAC vitex agnus castus extract VAC (150mg/kg/bw) orally from the next day of induction for 30 days. Subgroup four: PCOS induced rats treated with the VAC vitex agnus castus extract VAC (250 mg / kg bw) orally the next day of induction for 30 days. Subgroup five: PCOS rats treated with clomiphene citrate (CC) at a dose of 1 mg / kg bw will be administered daily orally and continue for 30 days. At the end of the experimental period, twenty-four hours after the last treatment, the animals were sacrificed and serum was kept at -20 ° C for biochemical and hormonal tests.

RESULTS

A significant ($p \leq 0.05$) increase in body weight gain in the control group and animals suffering from PCOS compared with other treated groups which recorded a decrease in body weight gain (Table 1).

The means of serum hormones FSH, LH, Estr and Prog are presented in Table (2). A significantly ($p \leq 0.05$) increase ($p < 0.05$) was observed in the group of PCO compared to the control and other treated groups. The same table showed an improved effect of the groups suffering from PCOS treated with extract of VAC 150 and 250 mg/kg/bw extract and the drug CC 1 mg/kg.Bw ,also the table showed a significant decrease ($p < 0.05$) in hormone estrogen and progesterone in all treated groups compared to the control group.

Table 1: Effect of VAC extract and CC drug on body weight gain in PCO rats (Mean ± SE), n=10

Treatment	Initiate body weight(g)	Final body weight (g)	Weight gain (g)
Control	195.87 ± 2.41a	225.12 ± 5.08b	29.25 ± 2.14b
PCOS	203.50 ± 7.69a	241.62 ± 4.03a	38.12 ± 3.43a
PCOS + VAC 150 mg/kg.Bw	207.12 ± 5.51a	197.50 ± 6.35c	- 9.62 ± 1.64c
PCOS + VAC 250 mg/kg.Bw	198.12 ± 4.38a	191.00 ± 6.04c	-7.12 ± 1.86c
PCOS CC 1 mg/kg.Bw	204.62 ± 8.17a	198.87 ± 7.10c	-5.57 ± 1.30d

The different small letters refer to significant differences at p>0.05)

Table 2: Effect of VAC extract and CC drug on FSH, LH, Estr, and Prog hormones in PCO rats (Mean ± SE), n=10

Treatment	FSH (mIU/ml)	LH (mIU/ml)	Estro Pg/mL	Prog ng/ml
Control	11.85±0.79a	11.59±0.42b	113.59±2.06a	72.37±2.98a
PCOS	12.53±1.21a	25.94±0.72a	81.27±0.71c	56.41±3.13d
PCOS + VAC 150 mg/kg.Bw	10.92±0.92ab	11.66±0.74b	91.63±0.97b	63.12±1.40c
PCOS + VAC 250 mg/kg.Bw	10.14±0.77b	10.15±0.98c	94.90±1.09b	66.42±1.24b
PCOS +CC 1 mg/kg.Bw	10.01±0.97b	7.55±0.43d	86.06±0.96c	60.73±2.61c

The different small letters refer to significant differences at (p05)

Table 3: Effect of VAC extract and CC drug on Test. and prol hormones in PCO rats (Mean ± SE), n=10

Treatment	Test Pg/mL	Prol ng/Ml
Control	133.61 ± 2.13c	40.61 ± 2.01b
PCOS	167.54 ±3.29a	46.24 ± 1.04a
PCOS + VAC 150 mg/kg.Bw	139.73 ± 9.31c	40.08 ± 3.44b
PCOS + VAC 250 mg/kg.Bw	126.07 ± 4.42d	40.10 ± 1.82b
PCOS CC 1 mg/kg.Bw	151.92 ± 9.44b	40.77 ± 2.64b

The different small letters refer to significant differences at (p05)

Depending on the results clarified in Table (3) there was an increase in the hormone testosterone and prolactin in the group of PCO without treatments compared to the control and other treated groups. The animals treated with VAC 150 and 250 mg / kg Bw extract to PCOS showed an improvement effect compared to the control. The same table appeared to have an improvement effect on the hormone prolactin in all treatment groups compared to the PCO group.

DISCUSSION

The study results showed an increase in body weight in female rats treated with letrazol to induce PCOS compared to the control group, the increase in body weight is one of the most important indicators of PCOS induction. The results of this study agree with the study of (Amoura et al., 2015; Hamza, 2018; Bhoye et al., 2021), and this is due to the increase in adipose tissue in the abdominal cavity (Maharjan et al., 2010). However, high levels of androgens cause an increase in the enlargement of sebaceous cysts and their effect on the expression of enzymes and proteins involved in the carbohydrate and fat metabolism pathway (Gonzales et al., 2006).

The results of the study also agreed with the study by Ndeingang et al. (2019), they indicated a decrease in body weight in female rats after PCOS was induced by letrozole. Abdel Hakim and Saad (2019) also showed the effect of omega-3 at a concentration of 240mg/kg for a period of 3 weeks on the decrease in body weight of adult female rats after PCOS by reduce fat deposition in body tissues by suppressing fat-generating enzymes and increased beta-oxidation led to fat decomposition and body weight loss.

The decrease in body weight is due to the presence of effective compounds, peptides, steroids, and flavonoids found in herbs such as Vitex Agnus- Castus that have a role in reducing fats Sedighi et al. (2017), as allolytics are through flavonoids that reduce the activity of enzymes involved in cholesterol synthesis in cells. The liver is responsible for cholesterol esterification and storage (Matralis and Kourounakis, 2014). Flavonoids also contain antioxidant properties and therefore work to scavenge excess RO (OH) and prevent lipid peroxidation (Subash and Subramanian, 2008).

The results of the study showed that there were no significant differences in the concentration of follicle stimulating hormone between the PCOS group and the control group. The study showed a significant increase in the concentration of both testosterone and LH, and a significant decrease in the concentration of both estrogen and progesterone, the results agree with (Ndeingang et al., 2019), when they studied the induction of pcos in female rats. In the current study, Letrozole inhibited the non-steroidal aromatase enzyme, which is expressed by the CYP19 gene, which is responsible for converting androgens into estrogens in the ovaries, and the decrease of this enzyme leads to hormonal imbalance, especially hyperandrogenism, thus leading to an increase in male hormone testosterone and a decrease in estrogens, and this imbalance stimulates feeding. The retrograde effect increased LH production from the pituitary gland, and thus stimulated theca cells in the ovary to secrete testosterone (Shi and Vine, 2012). Several studies showed that the increase in androgen production results from a defect in steroids formation in PCOS, especially enzymes present in theca cells of the ovary, which participate in the formation of androgens (Ashraf et al., 2019). Androgen production is regulated in rat ovaries by the expression of several key enzymes involved in the process of manufacturing or forming steroids. The process of manufacturing steroids begins by transporting cholesterol from the outside to the inner mitochondrial membranes of the cell, after that, cholesterol is converted into Pregnenolone through the cytochrome P450 enzyme encoded by the CYP11 gene located on the inner side of the mitochondrial membrane Kashar-Miller et al. (2001), after that, Pregnenolone is transported to the smooth reticulum, and the conversion process continues to 17-hydroxypregnenolone, dehydroepiandrosterone, and progesterone to 17-hydroxyprogesteron and androstenedione by an enzyme with dual activity (17-alpha hydroxylase, 7,20-lyase) expressed by the CYP17 gene, which is the main enzyme that regulates the formation of Androgens, Rosenfield, (2020) indicated that the excessive expression of these enzymes, especially CYP17, in theca cells in the PCOS group explains the increase in androgen hyperemia, as confirmed by the study of Kafali et al. (2004) that oral letrozole dose causes a significant increase in the concentration of both the LH hormone testosterone in the serum of adult female rats, while the concentration of estrogen decreased, and this is consistent with the results of the current study. Maharjan (2015) also indicated that serum testosterone level increased significantly and estrogen level decreased in PCOS animals.

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On the other hand, the results of the current study showed that VAC extract has an improved effect on sexual hormone levels after dosing female rats induced with PCOS for a month, where FSH, LH, and testosterone decreased significantly, and both estrogen and progesterone increased significantly. These results agreed with the results of the study of Ndeingang et al. (2019) when they used the extract and it had an anti-androgen effect in female PCOS rats with letrozole by increasing the conversion of testosterone to estrogen and thus decreasing testosterone, abnormal increase in the hormone causes the occurrence of polycystic syndrome ovarian function and the level of estrogen and progesterone has also been observed. It also agreed with the results of the study by Yang et al. (2018) when using an extract (Ecklonia Cava), which caused an increase in the expression of CYP19, the gene encoding the aromatase enzyme, as it plays a major role in the progression of the estrous cycle of female rats in the PCOS group, and therefore an increase in the conversion of the androgen hormone to estrogens and the treatment of polycystic ovaries.

The study by Diab et al. (2015) confirmed the effect of the alcoholic extract of the VAC palm at a concentration of 0.225 mg/kg and two types of hormone replacement therapy on female sex hormone, the extract had a positive role in increasing estrogen and progesterone. Feyzallahi et al. (2021) demonstrated that VAC treatment caused hormonal changes in female rats induced with pcos through its effect in decreasing the expression of the Kiss-1 gene and causing an increase in progesterone and estrogen levels and a decrease in testosterone and LH.

Allahtavakoli et al. (2015) study, reported that VAC extract regulates the level of expression of α-estrogen receptors in ovariectomized rats. Other studies indicated the isolation of linoleic acid from VAC fruits, and it was shown that it mimics estrogen through its binding to the estrogen receptor and has an anti-androgenic effect. This indicates that VAC can increase the estrogen level in pcoc due to its estrogen-like activity (Gorkem et al., 2018).

The exact mechanism of action of VAC has not been fully known and it is believed that VAC regulates the levels of sex hormones through physiological mechanisms and the presence of active compounds in the extract, as indicated by Diab et al. (2015) that the flavonoids in the extract of VAC affect the level of hormones and organ functions and increase the fertility of reproductive organs in mice, as well as flavonols are considered reducing substances through donation of hydrogen atoms (H) and prevent the formation of free radicals, and at the same time they are antioxidants.

Additionally, Casticin, which was extracted from the VAC plant, was used as hormonal substitutes and therefore causes an increase in estrogen and progesterone (Ibrahim et al., 2007). The results showed in Table (3) a significant in-

crease in prolactin hormone in the PCOS-induced group compared to the control group and the other treated groups. The results agreed with the results of the study by (De-Leo et al., 2003; Hamza, 2018) they revealed that the levels of the hormone Prolactine increased significantly in PCOS, the reason for the increase in the hormone can be attributed to a decrease in the activity of the aromatase enzyme in the ovary, which causes disruption of the endocrine glands of the pituitary axis, which raises the level of LH, Prol, androgen hormones, which in turn hinders the maturation of the follicles and the lack of ovulation. Prolactin also inhibits the binding of FSH to its receptors on the surface of theca cells, thus inhibiting estrogen production (Porter et al., 2000).

Some experiments showed the benefits of VAC in the treatment of premenstrual and hyperprolactinemia disorders. VanDie et al. (2013), where the alcoholic extract contains dopamine, estrogen receptor phenols that regulate the level of hormones and reduce the level of Prolactin hormone (Chen et al., 2011). It was used by a group of women suffering from defects in the luteal phase due to hyperprolactinemia, which reduced prolactin secretion in the luteal phase and eliminated the deficit in progesterone (Jarry et al., 2003). Study of Liu et al. (2004). The extract of VAC acts as a dopamine stimulator, which reduces the expression of the prolactin hormone in the body.

The effect of clomiphene citrate effect on prolactin hormone decreased significantly compared to the PCOS group, and this agreed with the results of the study by Hamza (2018) using the drug metformin, which is used as an anti-androgen, lowers blood sugar and stimulates ovulation, and also agreed with the results of the study by Abouzaid et al. (2018) when using the drug Leuprolide, Carbergoline in female rats induced PCOS, and the results showed a decrease in prolactin hormone, from the hypothalamus by reactions directly or indirectly by other hormones, which indicates that the drug clomiphene citrate improves hormonal changes resulting from the induction of PCOS in female rats.

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CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS CONTRIBUTION

The author conceived of the presented idea, verified the analytical methods. also, investigate the findings of this work. Author contributed to the final manuscript.

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