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Effect of Vitex Agnus-Castus Ethanolic Extract and Clomiphene Citrate Oxidative Stress and Immunological Parameters in Female Rats with Polycystic Ovary Syndrome Induced by Letrozole

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Abstract

Introduction. Polycystic ovary syndrome (PCOS) is the most predominant hormonal disorders among women of reproductive age lead to irregular menstrual cycles, excessive body weight, dyslipidemia, hyperandrogenism, oxidative stress and infertility.
Purposes. The present study was conducted to evaluated oxidant and antioxidant and immunological parameters in polycystic ovary syndrome in female rats induced by letrozole (1 mg/kg b.w.).
Methods. We used fifty female rats divided randomly to five subgroups (ten in each), subgroup one considered (negative control) received 1 mL of 0.5% CMC, subgroup two (positive control): PCOS animals received 1 mL of 0.5% CMC (carboxymethyl cellulose), subgroup three PCOS induced rats treated with vitex agnus-castus extract VAC (150 mg/kg b.w.) orally, subgroup four PCOS induced rats treated with vitex agnus-castus extract VAC (250 mg/kg b.w.) orally, subgroup five PCOS rats treated with clomiphene citrate (CC) (1 mg/kg b.w.). At the end of the experimental period, twenty-four hours after the last treatment, animals were euthanized and the serum was kept at –20 °C for biochemical and hormonal assays.
Results. The result show increase MDA enzyme and decreased in SOD, GSH and CAT animals suffering from PCOS, increase in serum TNF-α, IL-6 and CRP was observed in group of PCOS. The ameliorative effect of extract of VAC 150 and 250 mg/kg b.w. and I mg/kg b.w., showed in all of above parameters when compared with control group.
Conclusion. Letrozole could be used for PCOS induction and vitex agnus-castus ethanolic extract and clomiphene citrate could be used for treating PCOS symptoms.
Keywords: polycystic ovary syndrome, letrozol, vitex agnus-castus extract, clomiphene citrate, carboxymethyl cellulose

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Влияние этанольного экстракта Vitex Agnus-Castus и цитрата кломифена на окислительный стресс и иммунологические показатели у самок крыс с синдромом поликистозных яичников, индуцированным летрозолом

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Резюме

Введение. Синдром поликистозных яичников (СПКЯ) – наиболее распространенное гормональное нарушение среди женщин репродуктивного возраста, приводящее к нерегулярным менструальным циклам, избыточной массе тела, дислипидемии, гиперандрогении, окислительному стрессу и бесплодию.
Цель. Настоящее исследование проведено с целью оценки оксидантных, антиоксидантных и иммунологических показателей при СПКЯ у самок крыс, индуцированном летрозолом (1 мг/кг массы тела).
Методы. Мы использовали пятьдесят самок крыс, разделенных случайным образом на пять подгрупп (по десять в каждой). Первая рассматриваемая подгруппа (отрицательный контроль) получала 1 мл 0,5% карбоксиметилцеллюлозы (КМЦ); вторая подгруппа (положительный контроль), животные с СПКЯ, – 1 мл 0,5% КМЦ; крысы подгруппы три, индуцированные СПКЯ, получали экстракт Vitex agnus-castus VAC (150 мг/кг массы тела) перорально; крысы четвертой подгруппы, индуцированные СПКЯ, получали экстракт Vitex agnus-castus VAC (250 мг/кг массы тела) перорально; крысы подгруппы пять с СПКЯ обрабатывались кломифенцитратом (CC) (1 мг/кг массы тела). В конце экспериментального периода, через двадцать четыре часа после последней обработки, животных подвергали эвтаназии и хранили сыворотку при –20 °C для биохимических и гормональных анализов.
Результаты. Полученные результаты показывают увеличение фермента MDA и снижение уровня SOD, GSH и CAT, а также увеличение сывороточных TNF-α, IL-6 и CRP в группе СПКЯ.
Заключение. Летрозол можно использовать для индукции СПКЯ, а этаноловый экстракт Vitex agnus-castus и цитрат кломифена – для лечения симптомов СПКЯ.
Ключевые слова: синдром поликистозных яичников, летрозол, экстракт vitex agnus-castus, кломифенцитрат, карбоксиметилцеллюлоза

INTRODUCTION

Polycystic ovary syndrome (PCOS), is the most predominant hormonal disorders among animals and women of reproductive age. It is endocrine and metabolic disorder, lead to irregular menstrual cycles, excessive body weight, dyslipidemia, hyperandrogenism, oxidative stress and infertility [1, 2]. PCOS caused disturbed in normal ovarian function mainly by hyperandrogenism and increased in level of luteinizing hormone (LH), thus resulting in multiple cysts [3]. PCOS increased gonadotropin-releasing hormone (GnRH) pulse frequency, which caused LH production over follicle stimulating hormone (FSH). The increase in LH concentration promotes androgens production in the theca cells, while the FSH deficiency reduces the ability of granulosa cells to convert androgen into estrogen and impairs follicle maturation and ovulation [4].

Oxidative stress (OS) is referred as the imbalance between oxidants and antioxidants and the generation of excessive amounts of reactive oxygen species (ROS). Many studies have revealed that oxidative circulating markers are significantly increased in patients with PCOS compared with the normal and recognized to play a central role in the pathophysiology of many different disorders, including PCOS [5]. Oxidative stress (OS) is also linked to a higher risk of infertility in patients with PCOS. Many studies have shown that OS-related biochemical parameters such as glutathione peroxidase (GPx), superoxide dismutase (SOD) catalase, and malondialdehyde (MDA), and are abnormal in patients with PCOS [5]. Also, OS is associated with overproduction of reactive oxygen species (ROS), which could cause DNA damage and mutations in tumor suppressor genes, leading to uncontrolled ovarian cells proliferation, development of multiple cysts, and infertility [6].

The main purpose of this study was to evaluate the effects of vitex agnus-castus alcoholic extract, on oxidant and antioxidant stress and immunological parameters alteration in PCOS induced by letrozol in adult female rats.

METHODS

Ethical approval

This work approved by Department of Biology, College of Education for pure Sciences, University of Basrah. The experiment was conducted in accordance with the ethical principle for animal experiments of the University of Basrah (No. 1099).

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 in the animal house for two weeks under standard conditions of temperature and humidity while providing food and water with ad libitum during the study period.

Preparation of plant extract

The ripened vitex agnus-castus seed will be cleaned, dried under sunlight, 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, ethanol was evaporated with a rotary machine and the residual was

dried to form a powder. The resultant extract was then reconstituted by solving it in saline before being orally administered 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 will be divided into two main groups.

Group I: Ten animals of this group served as negative control and received 1 mL of 0.5% CMC (carboxymethyl cellulose) for 21 days.

Groups II: Forty animals will be treated with letrozole (1 mg/kg dissolved in 0.5% CMC) for 21 days for induction of PCOS.

From the 22nd day the experimentally PCOS induced rats' group will be divided into four subgroups each comprise 10 rats.

All animals were under 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 (carboxymethyl cellulose) from the next day for 30 days.

Subgroup two: (positive control): PCOS animals received 1 mL of 0.5% CMC (carboxymethyl cellulose) from the next day of induction for 30 days.

Subgroup three: PCOS induced rats treated with vitex agnus-castus extract VAC (150 mg/kg b.w.) orally from the next day of induction for 30 days

Subgroup four: PCOS induced rats treated with vitex agnus-castus extract VAC (250 mg/kg b.w.) orally from the next day of induction for 30 days.

Subgroup five: PCOS rats treated with clomiphene citrate (CC) in the dose of 1 mg/kg b.w. will be administered daily orally and continued for 30 days.

At the end of the experimental period, twenty-four hours after the last treatment, animals were euthanized and the serum was kept at –20 °C for biochemical and hormonal assays.

Statistics

The data were analyzed using SPSS 22.0 statistics software. Categorical data are reported as numbers and percentages and quantitative data are reported as mean and SD. One way ANOVA was used to compared between groups. The $p \leq 0.05$ was consider to be statistical significant.

■ RESULTS

Table 1 show a significant ($p \leq 0.05$) increase MDA enzyme in group of animals suffering from PCOS without treatment compared with control and other treated groups, the table showed ameliorative effect of MDA in group suffering PCOS treated with extract of VAC 150 and 250 mg/kg b.w. and drug CC 1 mg/kg b.w. compared with control especially in CC group. The same table appeared decreased a significant ($p \leq 0.05$) in SOD, GSH and CAT enzyme in group PCOS compared with control and other treated groups, treated with extract of VAC tow dose and CC drug showed ameliorative effect.

Depending on the results clarified in table 2 there was increase significantly ($p \leq 0.05$) in the immunological parameters TNF-α, IL-6 and CRP in group of PCOS without treatments

Table 1
Effect of VAC extract and CC drug MDA.SOD, GSH and CAT in PCOS rats (Mean ± SE), n=10

Treatment	MDA, ng/Ml	SOD, ng/Ml	GSH-Px, µmol/L	CAT, U/ml
Control	4.74±0.73 b	4.76±0.71 b	64.15±2.72 c	5.72±0.45 b
PCOS	7.30±0.96 a	2.06±0.56 c	22.50±1.33 e	1.69±0.48 d
PCOS + VAC 150 mg/kg b.w.	4.65±0.83 b	5.33±0.62 b	61.27±2.52 d	3.75±0.63 c
PCOS + VAC 250 mg/kg b.w.	4.32±0.89 b	5.07±3.90 b	71.53±3.90 b	5.56 ±0.74 b
PCOS +CC 1 mg/kg b.w.	2.75±0.67 c	6.75±0.77 a	85.68±1.66 a	7.00±0.42 a

Note: the different small letters refer to significant differences at (p≤05).

Table 2
Effect of VAC extract and CC drug on TNF-α, IL-6 and CRP in PCOS rats (Mean ± SE), n=10

Treatment	TNF-α, Pg/ml	IL-6, Pg/mL	CRP, mg/dl
Control	78.55±14.10 b	4.14±0.68 b	0.01±0.01 d
PCOS	185.78±51.58 a	6.23±1.00 a	0.21±0.07 a
PCOS + VAC 150 mg/kg b.w.	88.31±1.60 b	4.04±0.61 b	0.12±0.01 b
PCOS + VAC 250 mg/kg b.w.	80.64±1.16 b	4.04±0.38 b	0.07±0.02 c
PCOS +CC 1 mg/kg b.w.	97.39±1.70 b	4.48±0.55 b	0.16±0.03 b

Note: the different small letters refer to significant differences at (p≤05).

compared with control and other treated groups. The treated with extract of VAC 150 and 250 mg/kg b.w. and CC drug 1mg/Kg b.w. to PCOS animals showed ameliorative effect compared with control group.

■ DISCUSSION

The results of the present study showed a significant increase in the level of MDA enzyme compared with the control group and the rest of the other treated groups accompanied by a significant decrease in the level of each of CAT, SOD and GSH in the PCOS group. These results agreed with the results of each of Jafarsani et al., 2016 [7], Ndeingang et al., 2019 [8] and Alahmadi et al., 2020 [9] in their studies on female rats induced with PCOS. The high level of MDA and the low level of antioxidants in this study is evidence of the presence of oxidative stress associated with PCOS. induced by letrazole and this was confirmed by the study of Jahan et al. [10], indicating that letrazole, which causes PCOS in female rats, is associated with oxidative stress in the ovaries, which leads to abnormal levels of antioxidants.

Oxidation occurs from reactive oxygen species (ROS) and active nitrogen species (RNS), and these are eliminated by antioxidants that prevent or remove oxidative damage. Liu and Zhang [11], it is noted that the imbalance in the oxidants and antioxidants in PCOS rats can it damages biological molecules and the cell membrane and leads to an increase in the level of MDA and thus the failure of antioxidant systems and the occurrence of oxidative stress due to the excessive production of certain molecules, including types of ROS that arise from nitric oxide (NO) and MDA that damage all components of the cell, including proteins and lipids and DNA [12].

On the other hand, the results of the study showed the extract of VAC 150 and 250 mg/kg b.w. Increased in group of MDA and decreased in the levels of antioxidants.

The results agree with study of Hamza et al. [13] they confirmed that the treatment of PCOS induced in female rats treated with VAC as an alcoholic extract significantly reversed the level of oxidative stress compared with the PCOS group, and this is due to the presence of flavonoids in plant that affects androgen levels.

Vijayababu et al. [14] showed that flavonoids in many plants have the ability to inhibit the oxidation and thus the action of the extract on the increase in the level of antioxidants and this is consistent with the results of the current study. As the study by Saul [15] indicated that phytochemicals such as flavonoids, carotenoids and other phenolic compounds provide antioxidant activity to VAC and thus is considered a natural source of antioxidants.

As for the effect of CC drug at a concentration of 1 mg/kg on the level of MDA and antioxidants (CAT, SOD, and GSH), it showed a better and improved role in reducing MDA levels and significantly increasing antioxidant levels, compared with the PCOS group and other treatments, and this is consistent with the results of the study of Ndeingang et al. [8] and Kamal et al. [16], when they used the drug CC in the treatment of induced PCOS in female rats, as the drug showed an improvement in the levels of antioxidants due to its effect on the levels of the hormone Test, and LH, thus reducing oxidative stress and enhancing physiological hormonal processes.

The results of the current study showed a significant increase in the levels of (TNF-α, IL-6, CRP) in the PCOS group compared with the control group and other treated groups, and this is consistent with the results of the study of Mohammadi et al., 2017 [17], Wu et al., 2020 [18] and Safaeian et al., 2022 [19] in their study on PCOS-induced female rats. Several studies indicated that low-grade chronic inflammation and oxidative stress play an important role in the physiology of PCOS [20, 21], where inflammation is characterized by an increase in anti-inflammatory cytokines that are associated with insulin resistance [22].

In PCOS rats blood mononuclear cells migrate to adipose tissue, and macrophages begin to secrete cytokines represented by TNF-α. In addition, smooth muscle cells and fat cells increase CRP in PCOS, and this was confirmed by the study of Karczewski et al. [23], that more increases in body weight and adipose tissue lead to increased levels of cytokines resulting from lipid peroxidation, which damage ovarian and uterine tissues. Several in vitro studies showed the ability of pro-inflammatory cytokines to stimulate the steroid enzyme responsible for androgen production in the theca cells of the ovaries, causing an elevation of androgens [24].

On the other hand, the results of the study showed a significant decrease in the levels of TNF-α, IL-6, and CRP when treated with the alcoholic extract of VAC at a concentration of 150,250 mg/kg, as it had an effective and improved role in reducing inflammatory cytokines, and this may be attributed to its inhibitory effect on the expression of TNF-α, IL-6 and CRP, which led to a decrease in the levels of inflammatory cytokines, and this is consistent with the results of the study by Mohammadi et al. [17], while the study of Yu et al. [25] showed that the extract of eucalyptus leaves, isorhamnetin significantly reduced the levels of inflammatory factors TNF-α, IL-6 and CRP in female rats induced PCOS through its effect on suppressing TNF-α, IL-6 and CRP receptors, thus decreasing their levels and preventing apoptosis in the ovaries.

On the other hand, the results of the study shown a significant decrease in the levels of TNF-α, IL-6 and CRP when treated with the drug CC compared to the PCOS group and

this is consistent with the results of the study of Ibrahim et al. [26] reducing the levels of inflammatory cytokines.

■ CONCLUSION

Letrozole could be used for PCOS induction and vitex agnus-castus Ethanolic Extract and clomiphene citrate could be used for treating PCOS symptoms.

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