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ORIGINAL ARTICLE

Co-administration of probiotics and vitamin D3 ameliorates letrozole-induced polycystic ovarian syndrome in rats

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is the most challenging endocrinopathy in reproductive-age women owing to the continuous necessity for treatment modifications to satisfy the patient's needs. Gut dysbiosis and vitamin D deficiency have been found in women with PCOS, and their modulation is a target for medical research. So, the study was done to estimate the potential effects of both vitamin D3, probiotics, and co- administration in a letrozole-induced PCOS model in female albino rats.

Methods: Forty-five female albino rats were separated into five equal groups: control, supplemented with vehicle orally daily till the end of the study, PCOS; given letrozole 1mg/kg BW orally daily for 21 days for PCOS induction then given the vehicle daily for 28 days, probiotics-treated PCOS; supplemented with the probiotic (Linex Probio-Tec AB Blend 64) 210 mg/kg/day for 28 days orally, D3-treated PCOS; supplemented with vitamin D3 (1000 IU/kg/day) for 28 days orally and D3+Probiotics-treated PCOS; supplemented with both vitamin D3 and probiotics for 28 days. All treatments started after induction of PCOS by letrozole for 21 days. Blood samples and ovarian tissues were taken at the end of the study for biochemical and histopathological investigations.

Results: Treatment with either probiotics or vitamin D3 improved the ovarian polycystic morphological and hormonal changes and enhanced the serum adiponectin, lipid profile, glycemic, inflammatory, and oxidative changes compared with PCOS rats. The combined-treated PCOS group showed a remarkable synergistic effect on ameliorating all the studied parameters compared with either treated group alone.

Conclusions: Both probiotics and vitamin D3 had beneficial therapeutic effects in the letrozole-induced rat model of PCOS. Co-administration of probiotics and vitamin D3 had synergistic effects on ameliorating the polycystic ovarian changes and provided a promising therapeutic line for treating the PCOS.

Keywords: PCOS, Adiponectin, Probiotics, Vitamin D3.

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is an intricate endocrine disorder that impacts fertile females **[1]**. Menstrual cycle irregularities, hyperandrogenism (HA), and sonographic polycystic ovarian morphology were considered the hallmark characteristics of this condition **[2]**.

Most women who suffer from PCOS were reported to have multiple features, including obesity, hyperlipidemia, and hyper-inflammatory state. Even though implantation of the fertilized egg may occur in some clinical cases of PCOS, there are high possibilities of abortion, premature birth, gestational diabetes, pre-eclampsia, and other medical issues **[3]**.

Vitamin D was reported to play a complex role in PCOS via decreasing endometrial thickness, insulin resistance (IR), androgens, and anti-Müllerian hormone **[4]**. Also, PCOS patients reported having lower serum vitamin D concentrations accompanied with HA and IR **[5]**. Additionally, the expression of vitamin D receptors in granulosa cells and its content in follicular fluid was decreased in PCOS, so it was suggested that vitamin D administration might help in the treatment of PCOS-related infertility **[6]**.

As previously mentioned, the gut microbiome has been linked with the female reproductive functions. Women with PCOS were reported to have HA that may be linked to alterations in the gut microbiome **[7]**. Although probiotics supplementation was reported to affect the metabolic status and IR but, these effects are still controversial. Therefore, it was proposed that altering the gut microbiome could be a useful treatment for PCOS **[8]**. So, our study was performed to estimate the potential effects of probiotics, vitamin D3, and co-administration of both on structural, functional, and biochemical changes in the letrozole-induced model of PCOS in female albino rats.

METHODS

2.1. Animals and the design of the study

Forty-five, 8-9-week-old female albino rats weighing 150-200 g were purchased from Zagazig Faculty of Veterinary Medicine. 4-5 rats/ steel wire cages (40 x 28 x18 Cm, 4-5 rats/cage) were kept under hygienic conditions with unrestricted access to standard chow and water and at room temperature with a normal cycle of light/dark in the Physiology Department Animal House, Zagazig Faculty of Medicine. The experiment design was approved by Zagazig University Institutional Animal Care and Use Committee (**ZU- IACUC/3/F/452/2022).**

The rats were adjusted to laboratory conditions for one week then divided equally into five groups (all drugs and vehicles are taken via oral gavage): control group, in which rats were supplemented with 1% aqueous solution of carboxymethylcellulose (CMC) once daily till the end of the study, **PCOS group**; in which PCOS was brought about by letrozole once daily for 21 days then supplemented with 1% CMC once daily for 28 days, **probiotics-treated PCOS group**; in which PCOS was brought about then supplemented with the probiotic (Linex Probio-Tec AB Blend 64; 210 mg/kg/day) dissolved in 1% CMC once daily for 28 days **[9]**, **D3-treated PCOS group**; in which PCOS was brought about then supplemented with vitamin D3 (1000 IU/kg/day) once daily for 28 days **[10]**, **D3+Probiotics-treated PCOS group**; in which PCOS was brought about then supplemented with both vitamin D3 and probiotics in the same former doses once daily for 28 days.

2.2. Induction of PCOS

PCOS was brought about by letrozole (inhibits aromatase enzyme) at a dose of 1mg/kg dissolved in CMC 1%, taken orally once daily for 21 days. Then, we examined the vaginal smears to find out the changes in the estrus cycle. Persistent estrus was characterized by cornified cells for at least two consecutive 4-day estrous cycles, pointing to the formation of follicular cysts **[11]**.

2.3. Biochemical studies

At the end of the experiment on day 50 and after the overnight fasting, the retro-orbital plexus was punctured to get blood samples under light halothane anesthesia, and the serum was extracted through 15 minutes of centrifugation at 3000 rpm. Bioassays for 1- **sex hormones**: LH, FSH, testosterone and estradiol using (Shanghai Sunred biological technology, China) ELISA kits and progesterone using (Sigma Aldrich, USA) ELISA kits. **2- Adiponectin and lipid profile parameters:** adiponectin using (Cusabio Biotech Co., Wuhan, China) ELISA kits, HDL, total cholesterol (TC) and triglycerides (TG) using (Shanghai Sunred biological technology, China) ELISA kits. **3- Glycemic parameters:** glucose using (GOD-PAP- Liquizyme kits, Biotechnology, Egypt) and insulin using (BioVendor-Laboratorní medicína, Czech Republic) ELISA kits. **4- Inflammatory parameters:** interleukin-6 (IL-6) using (Bio diagnostic-Egypt) ELISA kits, tumor necrosis factor- α (TNF-α) using (BioSource Inc., California, USA) ELISA kits, and c-reactive protein (CRP) using (BD Biosciences, USA) ELISA kits. **5- Oxidative parameters:** malondialdehyde (MDA) using (Sigma Aldrich, USA) ELISA kits and superoxide dismutase (SOD) using (Cusabio Biotech Co., Wuhan, China) ELISA kits. All were measured following the manufacturer's instructions. LDL was measured as: =TC-HDL-TG/5 **[12]** and HOMA-IR as: = serum insulin (µIU/mL) x serum glucose (mg/dl)/405 **[13]**.

2.4. Tissue sampling and histopathological studies

The ovaries were removed and preserved in 10% formalin solution shortly after removal. The samples underwent dehydration, cleaning, and paraffin embedding. 5 µm-thick serial slices were prepared, stained with H&E, and inspected under a light microscope.

2.5. Histomorphometric studies

Leica Qwin 500 Image Analyzer Computer System (England) was used for histomorphometric analysis in the Human Anatomy and Embryology Department's image analysis unit. Utilizing the menu for measuring fields in ovarian H&E slides, the number of cystic follicles, corpora lutea, and graafian follicles underwent x40 power field counting. For each animal, five serial portions from their slides were used to calculate a mean of fifteen readings. Using the image analyzer (the Image J software plugin), the thickness of the granulosa cell layer and theca cell layers were measured for each animal in each group at x400 magnification. Five serial parts from the animals' slides were utilized to compute a mean of fifteen values.

STATISTICAL ANALYSIS

For statistical analysis, the SPSS program was used (version 18 for Windows) (SPSS Inc. Chicago, IL, USA). Testing of normality was done by the Kolmogorov-Smirnov test. The Statistics were presented as mean ± SD. One-way ANOVA, then LSD. A post hoc test was applied to compare statistical disparities between all groups. A statistically significant P-value was defined as <0.05.

RESULTS

3.1. Sex hormones

As mentioned in **Table 1**, serum levels of LH, LH/FSH ratio, and testosterone were significantly escalated, estradiol and progesterone serum levels were significantly reduced, while no significant change was found in the level of FSH in the PCOS group compared with the control group. Treatment with either probiotics, D3, or D3+probiotics led to a significant diminishing in LH, testosterone, and LH/FSH ratio while leading to a significant rise in progesterone and estradiol serum concentrations compared with the PCOS group.

3.2. Adiponectin and lipid profile

As noticed in **Table 2**, serum concentrations of adiponectin and HDL were significantly reduced, and serum concentrations of LDL, TG, and TC were significantly elevated in the PCOS group compared with the control group. Treatment with either probiotics, D3, or D3+probiotics led to a significant rise in HDL and adiponectin while leading to a significant decline in TG, TC, and LDL as compared with the PCOS group. A significant elevation in HDL and adiponectin, while a significant descent in the levels of LDL, TG, and TC were found in the D3+probiotics-treated PCOS group when compared with either probiotics or D3- treated groups.

3.3. Glycemic parameters

As represented in **Table 2**, serum concentrations of glucose, insulin, and HOMA-IR were significantly elevated in the PCOS group contrasted with the control group. Treatment with either probiotics, D3, or D3+probiotics led to a significant decrease in the levels of the above-mentioned parameters compared with the PCOS group. A significant decrease in the levels of the former parameters was found in the D3+probiotics-treated PCOS group contrasted with either probiotics or D3-treated groups.

3.4. Inflammatory and oxidative parameters

As demonstrated in **Table 3**, serum concentrations of CRP, TNF-α, IL-6, and MDA were significantly elevated. In contrast, serum concentration of SOD was significantly reduced in the PCOS group compared with the control group. A significant decrease in the concentrations of all mentioned parameters except SOD which was significantly increased in probiotics, D3, and D3+probiotics-treated PCOS groups contrasted with the PCOS group. MDA, TNF-α, IL-6, and CRP significantly declined, while SOD was significantly raised in the D3+probiotics-treated group when compared with either probiotics or D3-treated groups.

3.5. Histopathological results

The ovarian tissues from the control group revealed variable stages of ovarian follicles surrounded by normal stroma **(Fig. 1. a-f-k)**. The ovarian tissues from the PCOS group showed the presence of multiple follicular cysts filled with fluid and a marked diminishing in the number of corpora lutea **(Fig. 1. b-g-l)**. The ovarian tissues from the probiotics-treated PCOS group **(Fig. 1. c-h-m)**, D3-treated PCOS group **(Fig. 1. d-i-n),** and D3+probiotics-treated PCOS group **(Fig. 1. e-j-o)** revealed improvement of the ovarian architecture, elevation in the number of corpora lutea with diminishing in the number of follicular cysts.

3.6. Histomorphometric results

As noticed in **Table 4**, the number of corpora lutea and graafian follicles and the thickness of theca and granulosa cells were significantly diminished, while the number of the follicular cysts was significantly elevated in the PCOS group contrasted with the control group. Treatment with either probiotics, D3 or D3+probiotics led to a significant rise in the number of corpora lutea and graafian follicles and the thickness of granulosa and theca cells, while a significant decline in the number of follicular cysts compared with PCOS group.

Table 1: Serum levels of sex hormones in all studied groups

	Control	PCOS	PCOS + Probiotics	PCOS + D3	PCOS + D3 + probiotics
LH (IU/ml)	2.2± 0.48	7.21± 0.63[a]	5.71± 0.26[ab]	5.43± 0.31[ab]	3.71± 0.27[abcd]
FSH (IU/ml)	3.42± 0.37	3.16± 0.16	3.24± 0.3	3.3± 0.23	3.5± 0.31[b]
LH/FSH ratio	0.67± 0.17	2.29± 0.22[a]	1.77± 0.19[ab]	1.66± 0.186[ab]	1.07± 0.11[abcd]
Testosterone (pg/ml)	76.12± 3.5	122.94± 3.74[a]	104.59± 3.08[ab]	110.48± 3.86[abc]	99.07± 3.21[abcd]
Estradiol (pg/ml)	90.7± 0.64	71.99± 3.06[a]	82.74± 1.74[ab]	77.16± 1.52[abc]	87.89± 1.8[abcd]
Progesterone (pg/ml)	8.37± 0.34	4.77± 0.30[a]	6.60± 0.28[ab]	6.32± 0.26[ab]	7.29± 0.29[abcd]

Data are represented as mean ± SD, a: significant vs control, b: significant vs PCOS, c: significant vs **PCOS + Probiotics,** d: significant vs **PCOS + D3.**

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Table 2: Serum levels of adiponectin, lipid profile, and glycemic parameters in all studied groups

	Control	PCOS	PCOS + Probiotics	PCOS + D3	PCOS + D3 + probiotics
Adiponectin (ng/ml)	3.2± 0.26	0.43± 0.07[a]	1.16± 0.14[ab]	1.9± 0.13[abc]	2.15± 0.2[abcd]
HDL (mg/dl)	48.36± 5.11	28.28± 3.90[a]	31.44± 0.96[ab]	34.57± 1.14[abc]	37.77± 1.44[abcd]
TC (mg/dl)	69.26± 15.29	176.59± 8.28[a]	111.5± 7.6[ab]	127.5± 6.66[abc]	92.06± 3.58[abcd]
TG (mg/dl)	73.8± 8.09	143.12± 9.36[a]	114.62± 3.24[ab]	122.8± 4.22[abc]	103.33± 5.75[abcd]
LDL (mg/dl)	75.37± 5.62	107.55± 7.97[a]	89.88± 2.04[ab]	96.44± 2.47[abc]	85± 2.86[abcd]
Glucose (mg/dl)	82.44± 10.07	189.83± 4.15[a]	134.58± 2.7[ab]	142.78± 2.23[abc]	125.21± 3.21[abcd]
Insulin (µIU/ml)	7.17± 0.79	28.9± 1.24[a]	15.98± 1.32[ab]	18.46± 1.13[abc]	11.48± 0.98[abcd]
HOMA-IR	1.46± 0.26	13.55± 0.66[a]	5.31± 0.48[ab]	6.5± 0.36[abc]	3.55± 0.35[abcd]

Data are represented as mean ± SD, a: significant vs control, b: significant vs PCOS, c: significant vs **PCOS + Probiotics,** d: significant vs **PCOS + D3**

Table 3: Serum levels of inflammatory and oxidative parameters in all studied groups

	Control	PCOS	PCOS + Probiotics	PCOS + D3	PCOS + D3 + probiotics
IL-6 (pg/ml)	46.21± 2.49	70.82± 3.69[a]	54.84± 1.44[ab]	59.23± 2.12[abc]	49.39± 1.49[abcd]
TNF-α (pg/ml)	13.26± 1.46	84.46± 8.56[a]	46.16± 2.57[ab]	53.22± 1.48[abc]	39.62± 1.79[abcd]
CRP (ng/ml)	11.24± 2.48	112.68± 13.52[a]	52.68± 3.65[ab]	60.5± 4.72[abc]	25.1± 5.64[abcd]
MDA (nmol/ml)	0.53± 0.23	11.29± 1.62[a]	4.07± 0.71[ab]	6.29± 0.86[abc]	1.14± 0.17[bcd]
SOD (U/ml)	214.14± 4.8	37.19± 5.07[a]	143.97± 4.97[ab]	83.6± 3.18[abc]	197.23± 2.7[abcd]

Data are represented as mean ± SD, a: significant vs control, b: significant vs PCOS, c: significant vs **PCOS + Probiotics,** d: significant vs **PCOS + D3**

Table 4: Changes in the mean number of follicles per ovary and the mean thickness of granulosa and theca cells (µm) in all studied groups

	Control	PCOS	PCOS + Probiotics	PCOS + D3	PCOS + D3 + probiotics
No. of cystic follicles/ovary	0.44± 0.53	8.56± 1.42[a]	3.1± 0.93[ab]	4.3± 1.22[abc]	1.67± 0.5[abcd]
No. of corpora leutea / ovary	10.67± 1.8	2.78± 0.83[a]	8.44± 1.13[ab]	6.4± 0.73[abc]	9± 0.87[abd]
No. of graffian follicles/ovary	4.89± 0.78	1± 0.71[a]	2.3± 1[ab]	1.89± 0.78[ab]	3.33± 0.71[abcd]
Thickness of granulosa cells	90.61± 6.17	46.8± 3.16[a]	79.58± 4.01[ab]	73.08± 7.66[abc]	87.14± 4.6[bcd]
Thickness of theca cells	19.2± 2.51	10.31± 2.26[a]	15.54± 2.27[ab]	15.47± 1.81[ab]	15.81± 2.69[ab]

Data are represented as mean ± SD, a: significant vs. control, b: significant vs. PCOS, c: significant vs. **PCOS + Probiotics,** d: significant vs. **PCOS + D3.**

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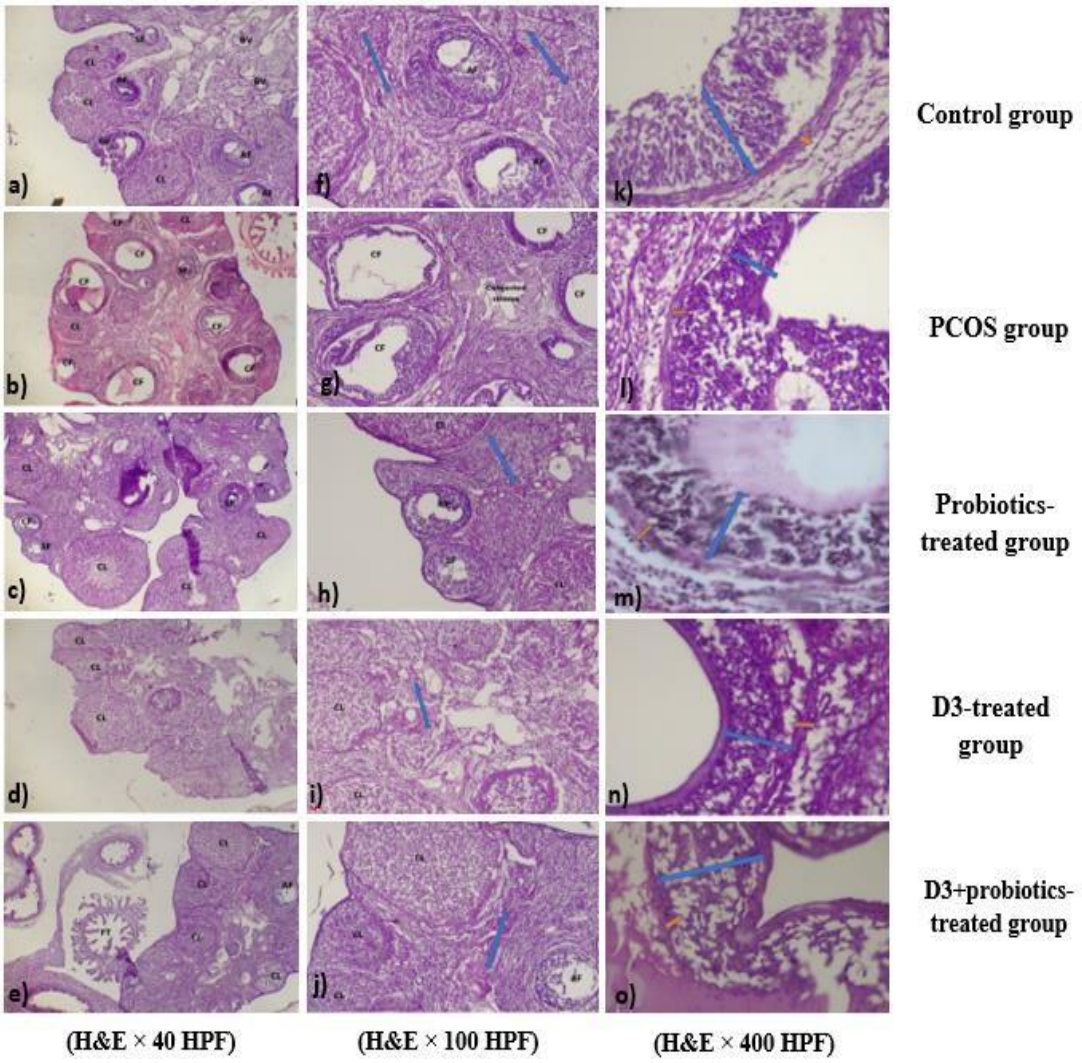

Figure 1: photomicrographs of ovarian tissues from all groups (H&E × 40, 100, 400 HPF). The control group showed **(a)** variable stages of ovarian follicles, **(f)** normal ovarian stroma (blue arrows), **(k)** normal thickness of granulosa cell layer (blue arrow), and no spacing between theca cells (orange arrow). PCOS group showed **(b)** multiple cystic follicles with a diminishing number of corpora lutea, **(g)** marked congested stroma, **(l)** marked descent in the thickness of granulosa cell layer (blue arrow) and detached, dark stained nuclei theca cells (orange arrow). The probiotics-treated PCOS group revealed **(c)** a reduction in follicular cysts and an increase in the number of corpora lutea, **(h)** a less congested stroma (blue arrow), **(m)** an escalation in the thickness of the granulosa cell layer (blue arrow) with improvement in theca cells (orange arrow). D3-treated PCOS group revealed **(d)** a descent in follicular cysts and a rise in the number of corpora lutea, **(i)** a less congested stroma (blue arrow), **(n)** an elevation in the thickness of the granulosa cell layer (blue arrow) with improvement in theca cells (orange arrow). D3+Probiotics treated PCOS group showed **(e)** a marked diminishing in follicular cysts and more escalation in the number of corpora lutea, **(j)** a marked descent in the congestion of the stroma (blue arrow), **(o)** a marked elevation in the thickness of granulosa cell layer (blue arrow) and improvement in theca cells (orange arrow). AF: antral follicle, BV: blood vessel, CL: corpus luteum, GF: graafian follicle, PF: primary follicle, SF: secondary follicle, CF: cystic follicle, FT: fallopian tube.

DISCUSSION

In the present study, PCOS was induced in female albino rats using letrozole, which was documented to block cytochrome P450 aromatase that is needed for the conversion of testosterone into estradiol, resulting in HA and ovarian polycystic

changes **[14]**. The successful induction of PCOS in our study was proved by significant elevation of serum concentrations of LH and testosterone and LH/FSH ratio associated with a significant reduction in serum concentrations of progesterone and estradiol, in addition to the persistent estrus and the histopathological polycystic ovarian features in comparison to control rats. These findings were supported by previous studies **[11,15]**. Also, it was found that PCOS rats induced by letrozole exhibited high levels of LH compared with normal rats **[16]**. Compared to healthy women, the high activity of GnRH neurons in the hypothalamus accounted for the higher LH pulse frequencies in PCOS women. Furthermore, it has been proposed that PCOS patients have high androgen levels and poor steroid negative feedback **[17].**

The present study found an insignificant change in serum FSH in the PCOS group contrasted with control rats. Consistent with our findings, other studies revealed an insignificant difference in FSH serum levels between PCOS patients compared with healthy women **[18]**. Conversely, other researchers found significantly diminishing serum FSH concentrations in PCOS rats **[19]**. Also, we found a significant elevation in the LH/FSH ratio in the PCOS group compared with control rats. In agreement with that, PCOS women were reported to have a significant elevation in LH/FSH ratio compared to controls **[20]**. Conversely, other researchers did not find any significant change in the LH/FSH ratio in PCOS patients **[21]**.

Our results revealed a significant rise in serum testosterone concentration, while a significant decline in progesterone and estradiol concentrations was found in the PCOS group compared with control rats. This was in line with **Liu et al. [22]**, who stated that PCOS women had higher total testosterone levels than controls, and **Rani et al. [23]**, who stated that PCOS rats had low estradiol and progesterone concentrations compared with controls. In contrast, others found an insignificant difference in estradiol levels in PCOS females compared with controls **[24]**. Adiponectin was suggested to inhibit LH and androgen secretion and promote ovulation, while low adiponectin concentrations in PCOS might adversely raise LH secretion **[25]**. In harmony with these suggestions, our study found a significant diminishing in serum adiponectin concentration in the PCOS group contrasted with control rats. Similarly, PCOS mice were reported to have lower concentrations of adiponectin **[26]**.

Dyslipidemia, one of the most prevalent metabolic conditions found in PCOS, was attributed to hyperinsulinemia and HA, which allow adipocytes to undergo increasing lipolysis **[27]**. In the same line, the current study found a significant escalation in TG, TC, and LDL, while there was a significant diminishing in HDL in the PCOS group compared with control rats. These findings came in parallel with **Behmanesh et al. [28].** Contrariwise, other researchers found that lean PCOS rats had insignificant changes in serum lipid profile parameters compared with controls **[29]**.

The current study revealed a significant escalation in serum insulin and glucose concentrations and HOMA-IR in the PCOS group contrasted with controls. In concordance with our finding, higher plasma insulin and glucose concentrations were reported in PCOS contrasted with controls **[30]**. The inflammatory cytokines might induce IR either by directly affecting insulin signaling or by activating the hypothalamic-pituitary-adrenal axis to cause central obesity **[31].** The present study found a significant rise in TNF-α, IL-6, and CRP concentrations in the PCOS group contrasted with controls. Similarly, several inflammatory biomarkers were stated to be elevated in PCOS patients **[32]**. Moreover, our study found a significant rise in serum concentration of MDA, while a significant decrease in serum concentration of SOD was found in the PCOS group compared with control rats. This came in harmony with the previous report of Uçkan et al. **[33]**.

The current research investigated the effect of probiotic administration in PCOS rats induced by letrozole. The ovarian histopathological studies revealed a significant improvement in the follicles' number compared with the PCOS group. Also, there was a significant diminishing in serum LH concentration and LH/FSH ratio, but an insignificant change in serum FSH concentration was found compared with PCOS rats. This finding came in harmony with Rahman et al. **[19]**. Also, a significant diminishing in LH/FSH ratio was found in probiotics-treated PCOS women **[34]**. In contrast, another study found that a 10-week probiotics treatment led to insignificant differences in LH levels **[35]**.

Our results revealed a significant decline in serum testosterone levels and a significant increase in

serum progesterone and estradiol concentrations in the probiotics-treated PCOS group contrasted with PCOS rats. This came in parallel with Moslehi et al. **[36]**, who reported that probiotics supplements diminished serum concentrations of testosterone, and Li et al. **[37],** who reported that probiotics in yogurt up-regulated serum concentrations of progesterone and estradiol in PCOS rats. On the other side, Shamasbi et al. **[38]** reported that probiotics did not lead to any improvement in serum testosterone in PCOS women. This can be explained by the short-term use of probiotics besides the difference in the species.

The ongoing study found a significant rise in serum adiponectin concentration in the probiotics-treated group as opposed to PCOS rats. This came in agreement with Fabersani et al. **[39].** In contrast, a meta-analysis found an insignificant difference in adiponectin levels between the probiotics-treated and control groups **[40].** Also, the ongoing study found a significant improvement in lipid profile parameters in the probiotics-treated PCOS group as opposed to PCOS rats. This improvement came in line with Bernini et al. **[41]**.

Moreover, our study found a significant improvement in glycemic parameters in the probiotics-treated PCOS group compared with PCOS rats. Likewise, it was found that probiotic supplementation improved glycemic control by reducing inflammatory cytokines **[42].** On the contrary, probiotic administration in PCOS patients was reported to have an insignificant effect on HOMA-IR and fasting blood glucose **[43]**.

The present study found a significant reduction in IL-6, TNF-α, MDA, and CRP serum concentrations and a significant elevation in SOD serum concentrations in the probiotics-treated group as opposed to PCOS rat; this was corroborated by a Previous report about the significant effect of probiotics on oxidative stress markers and inflammatory parameters **[44]**. This may be due to the ability of the probiotics to attenuate the inflammation via generating antioxidant metabolites, controlling inflammatory signaling pathways, chelating metal ions, and down- regulating reactive oxygen species **[45]**. Unlike our results, other researchers did not find any significant change in CRP levels among patients with PCOS receiving probiotics **[46]**.

The current study investigated the effect of vitamin D3 administration in PCOS rats induced by letrozole and found a significant improvement in the follicles' number in the D3-treated PCOS group compared with PCOS rats. Also, a significant decline in serum LH concentration and LH/FSH ratio but insignificant change in FSH serum concentrations were found in the D3-treated PCOS group as opposed to PCOS rats. This came in line with Behmanesh et al. **[28]**. At the same time, others reported that vitamin D did not have any effect on LH/FSH concentrations in PCOS women **[47]**.

The present study found a significant decrease in testosterone serum concentrations and a significant elevation in progesterone and estradiol serum levels in the D3-treated PCOS group compared with PCOS rats. Consistent with our results, Zhang et al. **[34]** found that vitamin D supplementation reduced the serum testosterone level. Moreover, Behmanesh et al. **[28]** reported that vitamin D administration in PCOS rats caused a significant increase in serum estradiol and progesterone concentrations. However, Moslehi et al. **[36]** reported that administration of vitamin D in PCOS patients did not lead to a significant change in androgen concentrations, and others reported that vitamin D did not cause any significant effect on serum estradiol levels or the other endocrine parameters **[48].**

The current study also revealed a significant escalation in serum adiponectin levels in the D3-treated PCOS group as opposed to PCOS rats. This was in harmony with the study done by Seyyed Abootorabi et al. **[49].** Additionally, our results showed a significant improvement in lipid profile in the D3-treated group compared with PCOS rats. Similarly, significant beneficial effects on lipid profile parameters were reported in vitamin D-treated rats **[28]**.

Our results noticed a significant improvement in glycemic parameters in D3-treated PCOS group as opposed to PCOS rats. Similar findings were reported in previous research **[28]**. However, another study reported insignificant changes in HOMA-IR and fasting blood glucose after vitamin D supplementations in PCOS females **[36]**.

The present study found a significant diminishing in serum concentrations of TNF-α, IL-6, CRP, and MDA and a significant rise in SOD serum concentrations in the D3-treated PCOS group as opposed to PCOS rats. This came in line with a previous meta-analysis done by Zhang et al. **[34]**.

On the contrary, other researchers found insignificant improvements in inflammation markers and oxidative stress markers in PCOS women receiving vitamin D supplementation **[36]**. The ongoing study investigated the impact of the combined administration of probiotics and vitamin D3 in PCOS rats induced by letrozole and found that the co-administration caused beneficial synergistic effects on all studied parameters and markedly ameliorated the pathological polycystic ovarian morphological and hormonal changes. This finding provides a promising therapeutic line for treating PCOS.

CONCLUSION

Both probiotics and vitamin D3 had beneficial therapeutic effects in the letrozole-induced rat model of PCOS. Co-administration of probiotics and vitamin D3 had synergistic therapeutic effects on ameliorating the polycystic ovarian structural and hormonal changes and modulating their associated metabolic, inflammatory, and oxidative pathological mechanisms.

Conflict of interest: None

Financial Disclosure: None

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Citation

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