European Review for Medical and Pharmacological Sciences 2022; 26: 8893-8902

The effect of Nateglinide and Octreotide on follicular morphology and free radical scavenging system in letrazole-induced rat model of PCOS

P. KIRICI[1], S. KAPLAN[2], E. ANNAC[3], E.S. TANRIVERDI[4], F.T. CAGIRAN[5], Z. KALI[6], N. MAVRAL[7], M.C. TASKAPAN[8]

[1]Department of Obstetric and Gynecology, Faculty of Medicine, Adıyaman University, Adıyaman, Turkey
[2]Gözde Academy Hospital Obstetrics and Gynecology, Adıyaman, Turkey
[3]Department of Histology and Embryology, Faculty of Medicine, Adiyaman University, Adıyaman, Turkey
[4]Department of Microbiology, Faculty of Medicine, İnönü University, Malatya, Turkey
[5]Private Clinic Obstetrics and Gynecology, Diyarbakır, Turkey
[6]Gözde Academy Hospital Obstetrics and Gynecology, Malatya, Turkey
[7]LOTUS Hospital Obstetrics and Gynecology, Şanlıurfa, Turkey
[8]Department of Biochemistry, Faculty of Medicine, İnönü University, Malatya, Turkey

Abstract. – ***OBJECTIVE:*** **To investigate the effects of octreotide and nateglinide on ovarian follicle count, ovarian tissue damage, biochemical parameters and free radical scavenging system in letrazole-induced rat model of PCOS.**

MATERIALS AND METHODS: **Forty-two female Sprague-Dawley rats were divided into six groups. Group 1 (Control Group): after localizing the ovaries and the uterine horns, the abdominal wall was closed without any surgical procedure. Group 2 (PCOS Group): PCOS was induced by administrating Letrozole orally for 21 successive days. At the end of 21 days, rats underwent ovarian biopsies. The experimental PCOS model was considered successful in the presence of atretic follicles without granulosa cell stratification. Group 3 (PCOS + Nateglinide Group): Nateglinide was administered by oral dropper for 30 days to the rats in which PCOS model was created. Group 4 (Nateglinid only Group): 30 days of NG was applied to the rats without PCOS. Group 5 (PCOS+Octreotide Group): 0.1 mg/kg/day Octreotide was given intraperitoneally for 4 weeks to the rats in which PCOS model was created. Group 6 (Octreotide only Group): animals without PCOS were given 0.1 mg/kg/day Octreotide. Bilateral oophorectomy was performed and blood samples were collected from all groups at the end of the treatment. Ovarian tissue was stained immunohistochemically with TLR-4 in addition to conventional staining. In addition to follicle classification, ovarian damage was graded. Serum insulin, FSH and LH, TNF-α, IL-6, SHBG, SOD, IGF-1, MDA and GSH levels were also measured.**

RESULTS: **The cystic and degenerated follicle density of PCOS group was high compared with the other groups. Both cystic and degenerated follicles were significantly reduced in PCOS+NG and PCOS+OC groups compared to PCOS group. There was no difference between the groups in terms of serum LH, FSH and insulin levels ($p>0.05$). Serum testosterone level was significantly higher in the PCOS group compared to the other groups ($p<0.01$). Adding OC or NG to PCOS groups did not cause significant changes in testosterone levels. TNF-α and IL-6 levels were high in PCOS group ($p<0.03$). IGF-1 and MDA levels were higher in PCOS than in other groups ($p<0.03$, $p<0.01$ respectively). Adding OC or NG to the treatment normalized IGF-1 and MDA levels. Serum GSH levels were significantly lower in the PCOS group ($p<0.05$). Adding NG to the treatment increased GSH levels.**

CONCLUSIONS: **Both NG and OCT reverses atretic and degenerate follicle damage due to PCOS through TLR-4, antioxidant and anti-inflammatory pathways.**

Key Words:
PCOS, Letrazol, NG, OCT, Follicle, Oxidation, Inflammation.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age, characterized by hyperandrogenism, anovulation, and polycystic ovaries findings and presenting with subfertility[1]. Although the etiology of the disease is not known exactly, excess androgen production determines the clinical pic-

Corresponding Author: Pınar Kırıcı, MD; e-mail: pinarkiricidr@hotmail.com

ture[2,3]. Although there are many human studies on the treatment of PCOS, it is not ethically possible to test the efficacy of every drug in humans. It is only possible to test the effect of many drugs used to treat non-PCOS diseases in PCOS patients in experimental PCOS models. Genetic PCOS models as well as long-term androgen use have been used for the PCOS model. One of the most widely used methods to generate experimental PCOS is the chronic use of the aromatase inhibitor letrazole. Long-term exposure of the animal to this drug increases testosterone and luteinizing hormone levels, leading to anovulation, and obesity, largely mimicking the morphological, laboratory, and clinical manifestations of PCOS[4,5].

Endocrine parameters, synthesis and secretion of which are impaired due to PCOS, make the treatment of metabolic findings very difficult. Elevated androgen levels and insulin resistance increase the long-term tendency to glucose intolerance and subsequently to type 2 diabetes[2]. Vitamin D and myoinositol are two different molecules that come to the fore in the treatment of metabolic disorders related to PCOS. Myo-inositol and D-chiro-inositol are two isomers that play a critical role in follicle development[6]. Both inositol derivatives regulate the aromatase enzyme, leading to androgen increase and estrogen synthesis. While long-term treatment with inositol causes an increase in androgen, short-term administration of inositol induces ovulation[7,8]. Vitamin D leads to an improvement in insulin sensitivity and endometrial receptivity. However, high doses of vitamin D negatively affect oocyte development[9]. Since most of the drugs developed for the treatment of the metabolic picture do not provide a definitive cure, the search for new treatments continues and many different drugs are tried. Nateglinide and octreotide are two different drugs that control serum glucose levels through different mechanisms, and there is no study on their effectiveness in PCOS patients. Nateglinide is a D-phenylalanine derivative drug and regulates insulin release *via* beta cells. When nateglinide is used alone, it both corrects hyperglycemia and provides glycemic control by reducing glycosylated hemoglobin[10]. Octreotide is a somatostatin analogue drug that blocks the exocrine and endocrine functions of the pancreas and is mainly used in the treatment of acromegaly[11,12]. We investigated the efficacy of these two drugs in the PCOS model we created with letrazole to investigate its effectiveness in PCOS patients. This study was designed to investigate the effects of octreotide and nateglinide use on ovarian follicle count, ovarian tissue damage, biochemical parameters and free radical scavenging system in letrazole-induced rat model of PCOS.

Materials and Methods

The experiments were conducted in accordance with the guidelines for animal research of the National Institutes of Health and were approved by Animal Research and Ethics Committee (Date: 27.05.2021, Approval No.: 2021/010).

Groups and Experimental Protocol

Forty-two female Sprague-Dawley rats aged 10-12 weeks and weighing 340-360 g were divided into six groups, with seven animals in each group. No intervention was performed for 7 days to ensure adaptation of the animals placed in cages. The reproductive cycles of the animals were checked by vaginal smears. Rats were in their estrus phase were included in the study. The experimental and control groups were determined as follows:

a) Group 1 (Control Group) (n = 7): After localizing the ovaries and the right and left uterine horns, the abdominal wall was closed with 4-0 nylon sutures and no other surgical or medical procedure was performed throughout the experiment (sham).
b) Group 2 (PCSO Group) (n = 7): Letrozole induction was used to establish an experimental PCOS model. PCOS was induced by administrating Letrozole orally for 21 successive days. Letrazole was given orally at 1 mg/kg/day after dissolving in 1% CMC and 0.9% saline. At the end of 21 days, intraperitoneal ketamine (50 mg/kg) and xylazine (10 mg/kg) anesthesia was applied to the rats and biopsies were taken from both ovaries. In the histopathological examination of the biopsy specimens, the experimental PCOS model was considered successful in the presence of atretic follicles without granulosa cell stratification. After a four-week recovery period, the rats were reoperated and bilateral oophorectomy was performed and blood samples were collected[13].
c) Group 3 (PCOS + Nateglinide Group) (n = 7): Nateglinide was administered by oral dropper for 30 days to animals in which PCOS model was established, similar to Group 2. At the end of the treatment, bilateral oophorectomy was performed and blood samples were collected.

d) Group 4 (Nateglinid only Group) (n = 7): Bilateral oophorectomy and intraoperative blood sampling were performed on animals given Nateglinide by oral dropper for 30 days.
e) Group 5 (PCOS+Octreotide Group) (n = 7): As in Group 2, 0.1 mg/kg/day Octreotide was given intraperitoneally for 4 weeks to the rats in which PCOS model was created. At the end of the treatment, the rats underwent bilateral oophorectomy and blood sampling[14].
f) Group 6 (Octreotide only Group) (n = 7): Animals given 0.1 mg/kg/day Octreotide intraperitoneally for 30 days underwent bilateral oophorectomy and blood sampling at the end of treatment.

Histopathological Evaluation

Ovarian tissue samples were fixed in 10% formaldehyde, embedded in paraffin blocks, and cut into 7 micrometer-thick sections with a microtome device (Thermo Fisher Scientific, Cheshire, UK). The obtained sections were stained with Hemotoxylin Eosin, Masson-Trichrome and Toluidin blue for histopathological examination. In addition, immunohistochemistry staining was performed with Tool-like receptor 4 antibody. Stained preparations were examined and illustrated with a microscope with a digital camera attachment (Carl Zeiss Microscop, ERc5 model, GmbH 07745 Jena, Germany).

Immunohistochemical Analysis

The avidin-biotin-peroxidase (ABC) complex for TLR-4 was applied to the ovarian tissue. 5 µm thick sections from paraffin blocks were deparaffinized and treated with TLR-4 antibody diluted 1/50 (sc-293072; Santa Cruz Biotechnology, Inc.). The staining degree was determined as 0: no staining, +0.5: very little, +1: little, +2: moderate, +3: severe staining[14].

Ovarian Follicle Count

Serial sections of 5 µm thickness and 100 µm intervals were taken from the ovarian tissue at 5 different levels. Image J software program (Image Processing and Analysis in Java) was used for follicle counting. The presence of oocyte surrounded by more than two granulosa cell layers was considered as primary follicle, while fluid accumulation between granulosa cells was recorded as secondary follicle. While the presence of the antrum filled with a large amount of fluid was accepted as a graff follicle, ruptured follicle corpus luteum, follicles with morphological integrity at any stage of development, atretic (degenerative) and follicles with a fluid-filled sac and cell mass were considered as cystic follicles. The results of follicle typing are shown in Table I.

Histopathological Scoring for Ovarian Damage

In the evaluation of ovarian damage, follicular cell degeneration, edema, vascular congestion, fibrosis, hemorrhage and inflammation were taken as criteria. Findings were graded separately for each parameter. Grade 0: no damage to the ovary; Grade 1: <33% damage; Grade 2: 33-66% damage; and Grade 3: >66% damage. The distribution of ovarian damage indicators is shown in Table II.

Biochemical Analysis

Approximately 2 ml of blood was taken into vacuum tubes with gel. Afterwards, the plasmas were separated and stored in a refrigerated centrifuge (Hettic Zentrifugen Universal 320, Germany) at 4 °C at 14000 rpm. Serum FSH, LH, insulin, testosterone were studied by electrochemiluminescence method in cobas e 601 autoanalyzer (Roche Diagnostics, Mannheim, Germany). Serum TNF-α, IL-6, SHBG, SOD, IGF-1, MDA and GSH levels were studied using the ELISA kit (Bioassay Technology Lab., Shanghai, China) in accordance with the manufacturer's instructions. All ELISA procedures were performed on DS2 automated ELISA instruments (Dynex Technologies Inc, Chantilly, Va, USA). Absorbance values were obtained and total levels were calculated with controls consisting of known serum concentrations according to the kit instructions. Tumor necrosis factor-alpha (TNF-a), interleukin-6 (IL-6), sex hormone-binding globulin (SHBG), insulin-like growthfactor 1 (IGF-1), superoxide dismutase (SOD), malondialdehyde (MDA), and reductglutathione (GSH) levels were measured using a commercially available kit. Kits were run on the DS2 automated ELISA instrument (Dynex Technologies Inc, Chantilly, Va, USA) according to the manufacturer's instructions (MyBioSource, San Diego, CA, USA).

Statistical Analysis

Variables were analyzed with descriptive statistics such as frequency, percentage, arithmetic mean, standard deviation, confidence intervals and graphs. Normality of data was determined by Kolmogorov-Smirnov and Shapiro-Wilk test. Oneway ANOVA, a parametric test, was used for normally distributed data, and Kruskalwallis, a

Table I. Showing the distribution of follicles within groups.

Groups	Cystic luteum	Corpus luteum	Atretic follicle	Primary follicle	Secondary follicle	Graff follicle
Control	0.88 ± 1.00	6.68 ± 0.09	3.00 ± 0.96	2.95 ± 1.45	1.36 ± 0.98	1.05 ± 1.11
PCOS	10.46 ± 2.69*	6.42 ± 1.51	3.42 ± 0.78*	2.44 ± 0.95	1.14± 0.89	1.71 ±0.75
OC	1.14 ± 1.21	7.97 ± 3.10	1.85 ± 1.34	2.38 ± 1.25	1.57 ± 1.13	0.85 ± 0.69
NG	1.05 ± 0.89	10.15 ± 2.76	2.42 ± 0.98	3.10 ± 1.00	1.28± 0.90	0.57 ± 0.53
PCOS+OC	1.42 ± 0.97	10.42 ± 3.95	2.15 ± 0.86	2.08 ± 1.29	1.45 ± 1.11	0.80 ± 0.92
PCOS+NG	1.00 ± 0.81	11.05 ± 1.52	1.95 ± 1.64	3.00 ± 1.29	1.81 ± 1.11	1.24 ± 1.18

* It expresses the significant difference between PCOS and other groups ($p < 0.05$).

nonparametric test, was used for data that did not show normal distribution. Comparisons between groups were made using Tamhane and Tukey tests. The upper limit of significance level was determined as $p<0.05$. It was analyzed with SPSS 25.0 for Windows package program.

Results

Histopathological Findings

In the examination of the ovarian sections of the control, NG and OK groups, it was observed that the histological structures evaluated were similar. Follicles with normal morphological appearance and at different stages of development were detected. Few atretic and degenerated follicles were observed. Vascular structure had similar appearance in control and NG groups. Connective tissue mast cell density was normal. Fibrosis, edema, inflammation and hemorrhage were not observed. (Figures 1, 2 and 3). When the PCOS group was examined, the cystic follicle density was high. Compared with the other groups, an increase in the number of degenerated follicles was detected. No different appearance was observed in the other groups in the vascular structuring. Mast cell density in the connective tissue was normal. Fibrosis, edema, inflammation and hemorrhagic findings were not observed (Figure 4). It was determined that cystic follicles were significantly reduced in PCOS+NG and PCOS+OC groups compared to PCOS group. No different findings were observed in the vascular structure compared to the other groups. Mast cell density in the connective tissue increased compared to the other groups. Fibrosis, edema, inflammation, and hemorrhagic findings were not observed (Figures 5, 6).

Immunohistochemical Findings

While very little TLR uptake was observed in the control and PCOS groups (+0.5), less uptake was detected in the NG and OC groups (+1). Severe TLR uptake was observed (+3) in PCOS+NG and PCOS+OC groups (Figure 1F, 2F, 3F, 4F, 5F and 6F).

Follicle Distributions

The number of cystic follicles in the PCOS group was significantly increased compared to the other groups ($p<0.05$). There was no statistical difference between the groups in terms of the number of corpus luteum, atretic follicle, primary follicle, secondary follicle and graff follicle ($p>0.05$). The statistical results of the groups are summarized in Table I.

Histopathological Scoring of Ovarian Samples

Follicle degeneration in the PCOS group

Table II. Comparison of histopathological scoring of ovarian samples.

Groups	Degenerated follicles	Edema	Vascularr congestion	Fibrosis	Hemorrhage	Inflammation
Control	0.91 ± 0.88	0.57 ± 0.53	0.71 ± 0.75	0.42 ± 0.53	0.24 ± 0.55	0.56 ±1.05
PCOS	2.14 ± 0.69*	0.57 ± 0.78	0.71 ± 1.11	0.71 ± 0.75	0.42 ± 0.53	0.57 ± 0.55
OC	0.71 ± 0.69	0.85 ± 0.89	0.42 ± 0.53	0.57 ± 0.78	0.67 ± 1.01	0.88 ± 0.92
NG	0.85 ± 0.69	0.71 ± 0.48	0.57 ± 0.53	0.85 ± 0.69	0.98 ± 0.69	0.91 ± 0.25
PCOS+OC	0.71 ± 0.75	0.71 ± 0.75	0.57 ± 0.78	0.66 ± 0.95	0.85 ± 0.69	0.28 ± 0.95
PCOS+NG	0.85 ± 0.69	0.85 ± 0.69	0.85 ± 0.48	0.75 ± 0.69	0.59 ± 1.00	0.42 ± 0.53

* It expresses the significant difference between PCOS and other groups ($p < 0.05$).

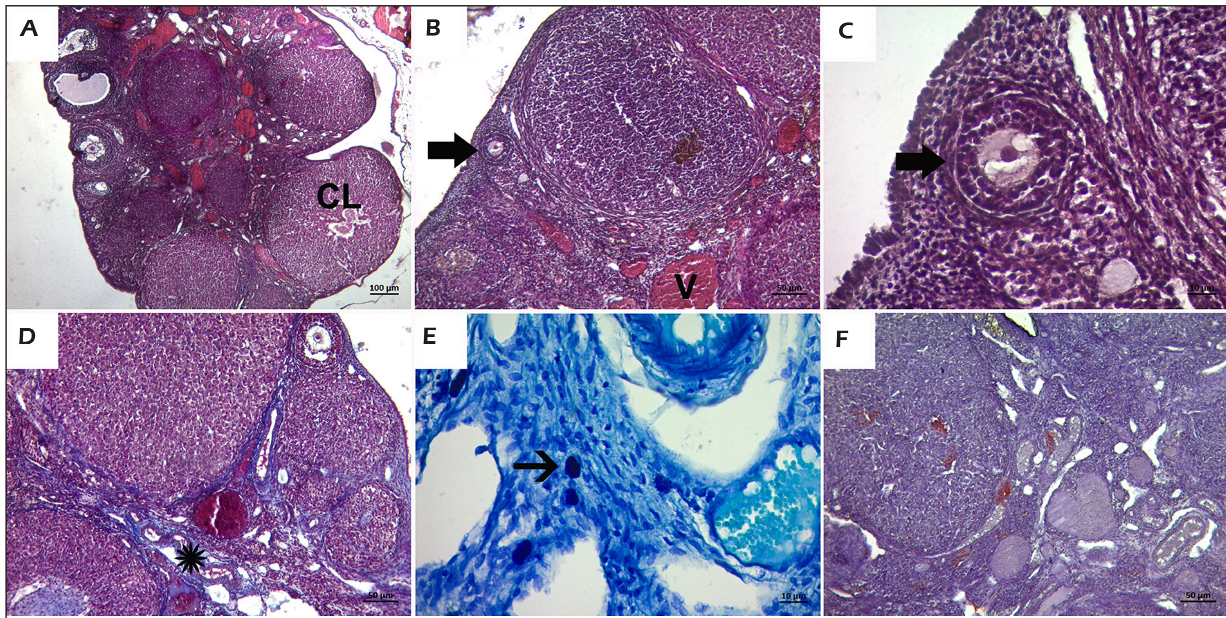

Figure 1. Light microscopy image of the ovaries of the control group. **A**, Image of X4 objective magnification (Hematoxylin-Eosin Staining). **B**, Image of X10 objective magnification (Hematoxylin-Eosin Staining). **C**, X40 objective magnification (Hematoxylin- Eosin Staining). **D**, X10 objective magnification image (Masson staining). **E**, X40 objective magnification image (Toluidine Blue Staining). **F**, X10 objective magnification image (TLR 4 staining), CL; Corpus Luteum, V; Vein, Bold Arrow; Normal follicle, Asterix; Connective tissue, Thin arrow; Mast cell.

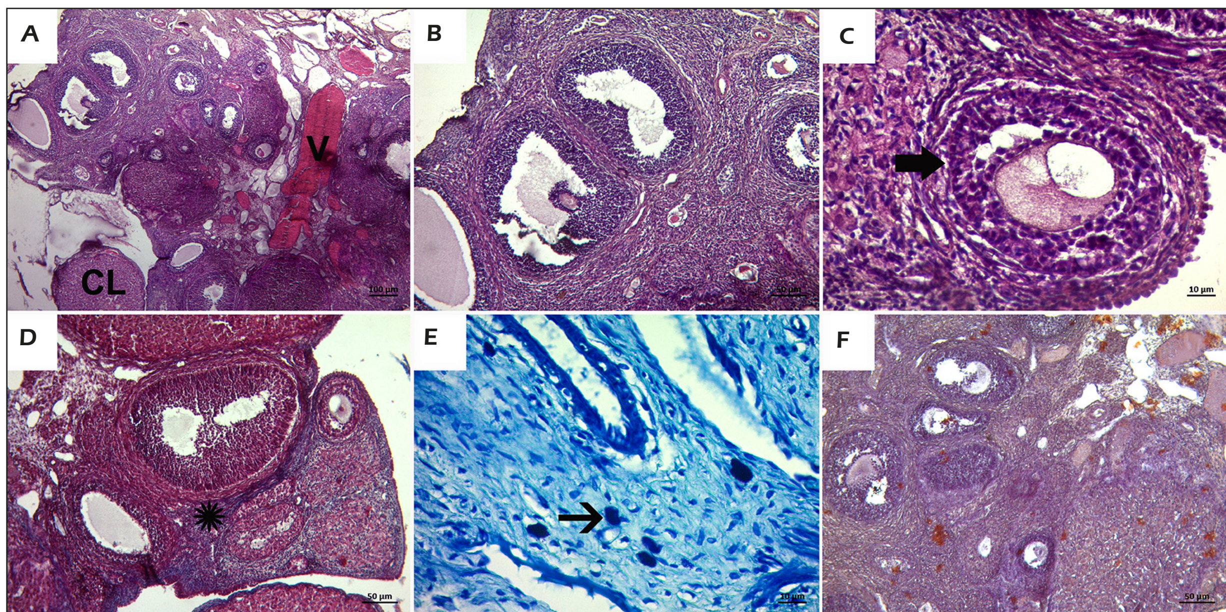

Figure 2. Light microscopy image of the ovary of the NG group. **A**, Image of X4 objective magnification (Hematoxylin-Eosin Staining). **B**, Image of X10 objective magnification (Hematoxylin-Eosin Staining). **C**, X40 objective magnification (Hematoxylin- Eosin Staining). **D**, X10 objective magnification image (Masson staining). **E**, X40 objective magnification image (Toluidine Blue Staining). **F**, X10 objective magnification image (TLR 4 staining), CL; Corpus Luteum, V; Vein, Bold Arrow; Normal follicle, Asterix; Connective tissue, Thin arrow; Mast cell.

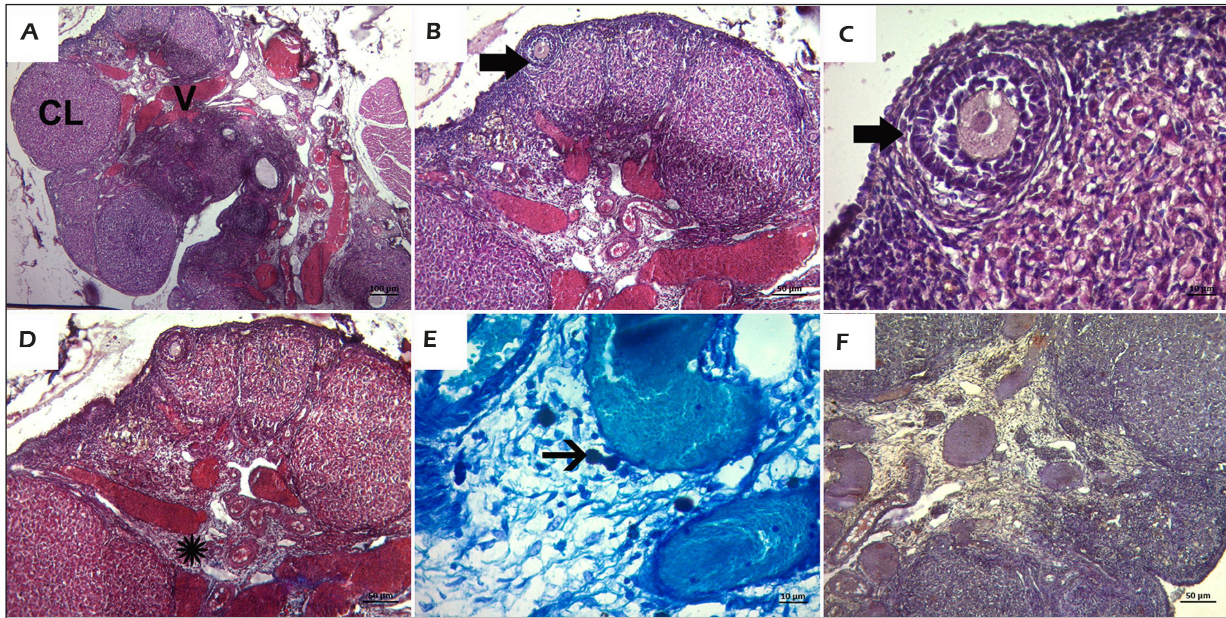

Figure 3. Light microscope image of the ovaries of the OK group. **A**, Image of X4 objective magnification (Hematoxylin-Eosin Staining). **B**, Image of X10 objective magnification (Hematoxylin-Eosin Staining). **C**, X40 objective magnification (Hematoxylin- Eosin Staining). **D**, X10 objective magnification image (Masson staining). **E**, X40 objective magnification image (Toluidine Blue Staining). **F**, X10 objective magnification image (TLR 4 staining), CL; Corpus Luteum, V; Vein, Bold Arrow; Normal follicle, Asterix; Connective tissue, Thin arrow; Mast cell.

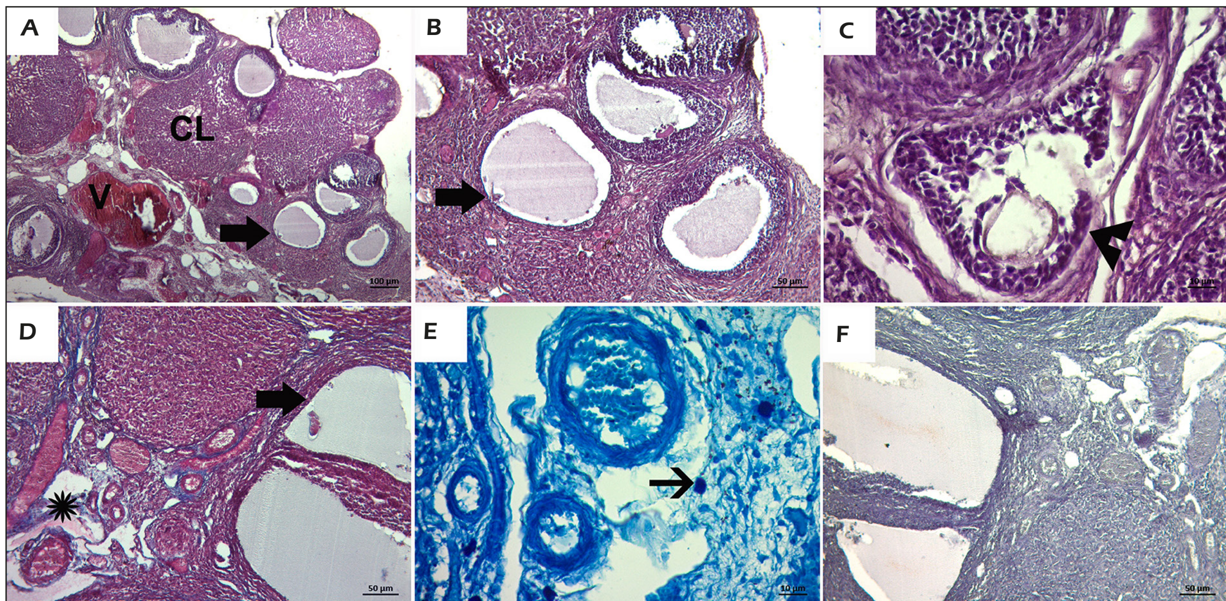

Figure 4. Light microscope image of the ovaries of the PCOS group. **A**, Image of X4 objective magnification (Hematoxylin-Eosin Staining). **B**, Image of X10 objective magnification (Hematoxylin-Eosin Staining). **C**, X40 objective magnification (Hematoxylin- Eosin Staining). **D**, X10 objective magnification image (Masson staining). **E**, X40 objective magnification image (Toluidine Blue Staining). **F**, X10 objective magnification image (TLR 4 staining), CL; Corpus Luteum, V; Vein, Bold Arrow; Normal follicle, Asterix; Connective tissue, Thin arrow; Mast cell.

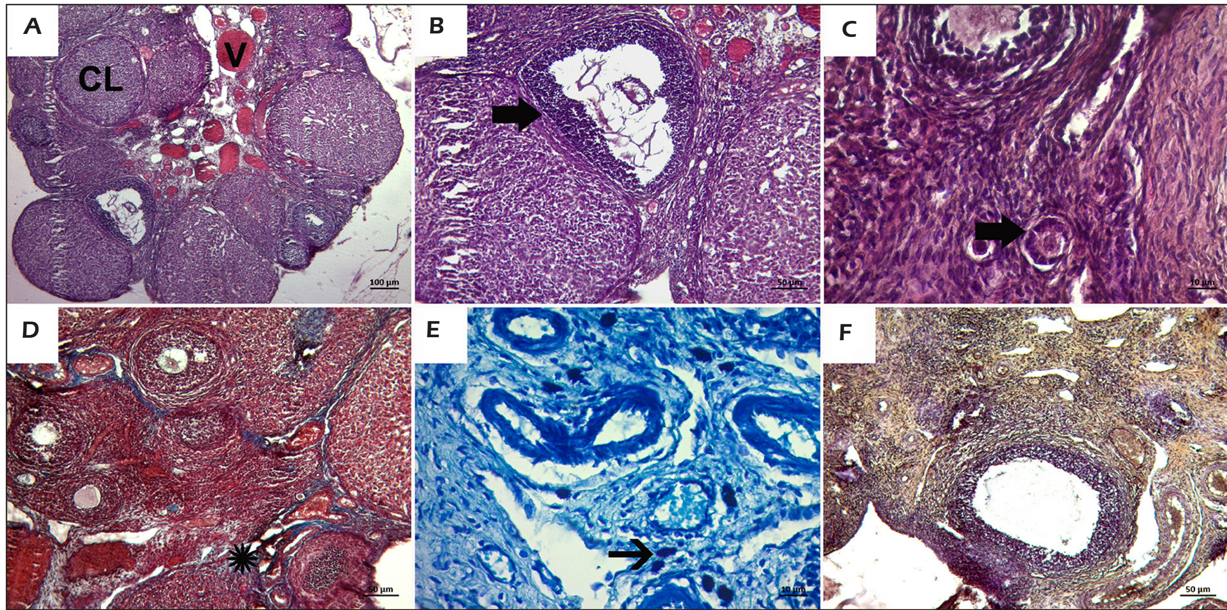

Figure 5. Light microscope image of the ovaries of the PCOS+NG group. **A**, Image of X4 objective magnification (Hematoxylin-Eosin Staining). **B**, Image of X10 objective magnification (Hematoxylin-Eosin Staining). **C**, X40 objective magnification (Hematoxylin- Eosin Staining). **D**, X10 objective magnification image (Masson staining). **E**, X40 objective magnification image (Toluidine Blue Staining). **F**, X10 objective magnification image (TLR 4 staining), CL; Corpus Luteum, V; Vein, Bold Arrow; Normal follicle, Asterix; Connective tissue, Thin arrow; Mast cell.

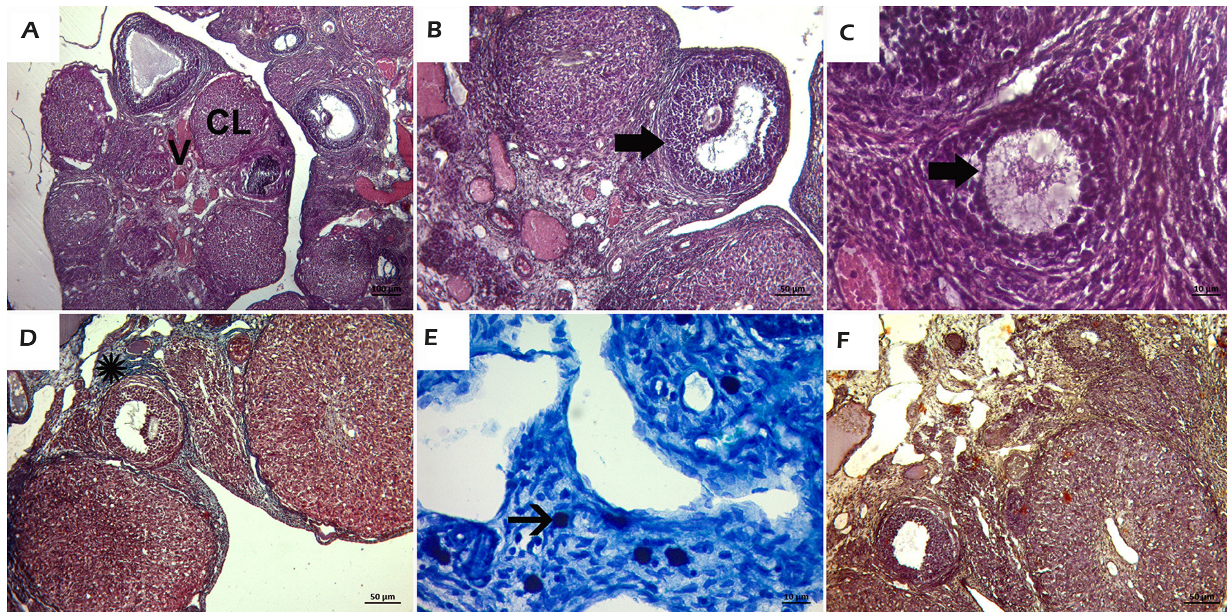

Figure 6. Light microscope image of the ovaries of the PCOS+OK group. **A**, Image of X4 objective magnification (Hematoxylin-Eosin Staining). **B**, Image of X10 objective magnification (Hematoxylin-Eosin Staining). **C**, X40 objective magnification (Hematoxylin- Eosin Staining). **D**, X10 objective magnification image (Masson staining). **E**, X40 objective magnification image (Toluidine Blue Staining). **F**, X10 objective magnification image (TLR 4 staining), CL; Corpus Luteum, V; Vein, Bold Arrow; Normal follicle, Asterix; Connective tissue, Thin arrow; Mast cell.

showed a statistically significant increase compared to the other groups ($p<0.05$). There was no statistical difference between the groups in terms of edema, vascular congestion, fibrosis, hemorrhage and inflammation ($p> 0.05$). Histological scores of the groups are shown in Table II.

Biochemical Results

There was no difference between the groups in terms of serum LH, FSH and insulin levels ($p>0.05$). Serum testosterone level was significantly higher in the PCOS group compared to the other groups ($p<0.01$). Adding OC or NG to PCOS groups did not cause significant changes in testosterone levels. TNF-α and IL-6 levels were high in PCOS group ($p<0.03$), while SHBG levels were low ($p<0.04$). There was no significant difference between PCOS and other groups in terms of SOD levels ($p<0.08$). IGF-1 and MDA levels were higher in PCOS than in other groups ($p<0.03$, $p<0.01$ respectively). Adding OC or NG to the treatment normalized IGF-1 and MDA levels (Table III). Serum GSH levels were significantly lower in the PCOS group ($p<0.05$). Adding NG to the treatment increased GSH levels. Adding OC to the treatment did not significantly change GSH levels ($p<0.55$).

Discussion

Octreotide is a somtaostatin analog that stimulates free radical scavenging and blocks the release of superoxide anions[15]. This drug, which is primarily used in the treatment of acromegaly, has subsequently been found to have different exocrine and endocrine effects[7]. Its anti-inflammatory and antioxidant effect protects against pancreatic damage[15]. OCT prevents pancreatic damage by binding to somatostatin receptors in pancreatic cells[12]. Nateglinide stimulates insulin secretion by blocking ATP-dependent potassium channels with a mechanism of action similar to sulfonylureas. We show that the letrazole-treated female rat showed multiple PCOS phenotypes, including increased testosterone, LH, and polycystic ovarian morphology. Letrasol exposure also resulted in increased body weight and increased adiposity. When we tested the endocrine, antioxidant and anti-inflammatory effects of these drugs in the experimental PCOS model, we found that they had no effect on insulin secretion. The antiandrogen effects of both drugs are weak, and the testosterone-lowering effect of OCT is more pronounced than that of NG. Similarly, both drugs did not show a significant effect on LH secretion.

Elevated serum TNF-α and IL-6 suggest that inflammatory pathways are activated in PCOS. Both NG and OC normalized the increase in TNF-α. NG normalized the increase in IL-6, while OC had no effect. OC and NG reversed both IGF-1 and MDA increase. The decrease in GSH was reversed by OC, but NG did not show a significant effect. The increase in serum MDA levels is evidence that PCOS induces oxidative stress. Since both OC and NG reduce serum MDA levels, we can say that both drugs are effective in free radical scavenging. The normalization of IGF-1 levels with both OC and NG suggests that these drugs have a positive effect on follicle growth. In the light of these data, we can say that OC exhibits an antioxidant feature in PCOS. We thought that NG could exhibit antioxidant properties due to its free radical scavenging effect. However, the antioxidant effect of NG was less pronounced than that of OC. Our study presents the first data showing that OC exhibits antioxidant properties in PCOS similar to other diseases. Antiandrogen effects can be negligible as neither drug significantly reduces elevated androgen levels. Since both drugs reduce TNF-α levels, we can think that their anti-inflammatory properties are similar.

Antioxidant and anti-inflammatory effects of OCT have been demonstrated in most of the studies to date[15,16]. Consistent with these studies, we demonstrated that OCT exerts similar effects in the PCOS model. We found that NG in PCOS also exhibits similar effects, albeit weakly, compared to OCT. The antioxidant and anti-inflammatory effects of these drugs can be used in the relief of chronic inflammation due to PCOS and in the treatment of follicle loss due to oxidative damage. The effects of both drugs on ovarian tissue and follicle development were evaluated for the first time in this study. The number of cystic and degenerated follicles in the PCOS group was significantly higher than the other groups. Since adding NG or OCT to the treatment leads to a significant decrease in the levels of both follicles, we can think that these two drugs positively affect the development of the follicle. Since both NG and OCT normalize IGF-1 levels, we can say that they treat follicular damage due to PCOS through this pathway. Another reason for the decrease in follicle damage may be due to the positive effects of both drugs on the TLR-4 pathway. It may be that OCT and NG exert their anti-inflammatory effects through toll-like receptor-4[17,18]. The fact that adding NG and OCT

Table III. Comparison of biochemical parameters of all groups.

	Control	PCOS	PCOS+NG	NG	PCOS+OC	OC
FSH (mlU/ml)	<0.10	<0.10	<0.10	<0.10	0.225	<0.10
Insülin (iU/ml)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
lh (mlU/ml)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Testosterone (ng/dl)	3.78	8.59	8.86	4.45	6.58	3.72
TNF-α (pg/ml)	6.4±4.7	14.77±8.5	6.9±5.2	7.24±3.5	11.7±6	5.25±4
IL-6 (pg/ml)	10.7±0.30	19.6±0.31	15.4±1.0	11.5±0.2	18.2±0.31	10.4±0.3
SHBG (nmol/l)	40.54±2.1	14.29±6.3	20.0±4.3	35.1±4.7	15.7±3.1	38.4±7.2
SOD (U/mg)	1.13±0.1	0.85±0.1	0.77±0.05	1.4±0.2	1.07±0.1	1.31±0.1
IGF-1 (ng/ml)	197.2±45.5	268.5±60.2	220.4±57	195.0±78	192.25±29	180.6±77.1
MDA (nmol/mg)	10.7±1.0	42.4±10.8	22.3±7.7	18.1±4.2	12.0±9.87	10.3±2.5
GSH (nmol/mg)	2.01±0.1	0.9±0.03	0.9±0.5	1.62±0.1	1.7±0.05	2.09±0.05

increases intrafollicular TLR-4 uptake in immunohistochemical analysis supports this idea.

Conclusions

In summary, while both OC and NG exhibit anti-inflammatory and antioxidant properties at different intensities, they do not have significant effects on insulin resistance. Since both drugs do not significantly alter serum insulin levels, we cannot comment on their use in the prevention of diabetes or insulin resistance. Similarly, both drugs failed to normalize hyperandrogenemia. Considering the short duration of use of drugs, it is difficult to make a definitive interpretation of the antiandrogen and anti-insulin effects. The fact that they did not significantly change LH and FSH levels strongly suggests that drugs do not have an effect on GnRH pulsations. In this context, we can say that both OCT and NG have no effect on treating ovulatory dysfunction due to PCOS. However, since both drugs treat degenerated and cystic ovarian morphology, they may contribute to the development of a healthy follicle pool. They may be treating impaired ovarian morphology due to PCOS, as both drugs normalize the increase in rates of degenerated and cystic follicles *via* TLR-4 and IGF-1.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' Contributions

All authors contributed to the study conception and design. Material preparation was performed by P. Kirici. Data were collected by P. Kirici, F.T. Cagiran, Z. Kali. Statistical analysis was performed by S. Kaplan, E. Annac, E.S. Tanriverdi. The first draft was written by P. Kirici. The article was edited and finalized by N. Mavral and M.C. Taskapan. All authors approved the final version of the manuscript.

Funding

None.

Ethics Approval

The experiments were conducted in accordance with the guidelines for animal research of the National Institutes of Health and were approved by Animal Research and Ethics Committee (Date: 27.05.2021, Approve No: 2021/010).

References

1) Celik N, Aydin S, Ugur K, Yardim M, Acet M, Yavuzkir S, Sahin İ, Celik O. Patatin-like phospholipase domain containing 3-gene (adiponutrin), preptin, kisspeptin and amylin regulates oocyte developmental capacity in PCOS. Cell Mol Biol 2018; 64: 7-12
2) Celik O, Aydin S, Celik N, Yilmaz M. Peptides: Basic determinants of reproductive functions. Peptides 2015; 72: 34-43.
3) Gulbay G, Yesilada E, Celik O, Yologlu S. The Investigation of Polymorphisms in DNA Repair Genes (XRCC1, APE1 and XPD) in Women with Polycystic Ovary Syndrome. Asian Pac J Cancer Prev 2017; 18: 1219-1223.
4) Kauffman AS, Thackray VG, Ryan GE, Tolson KP, Glidewell-Kenney CA, Semaan SJ, Poling MC, Iwata N, Breen KM, Duleba AJ, Stener-Victorin E, Shimasaki S, Webster NJ, Mellon PL. A novel letrozole model recapitulates both the reproductive and metabolic phenotypes of polycystic ovary syndrome in female mice. Biol Reprod 2015; 93: 69.
5) Maliqueo M, Sun M, Johansson J, Benrick A, Labrie F, Svensson H, Lönn M, Duleba AJ, Stener-Victorin E. Continuous administration of a P450 aromatase inhibitor induces polycystic ovary syndrome with a metabolic and endocrine phenotype in female rats at adult age. Endocrinology 2013; 154: 434-445.
6) Gullo G, Carlomagno G, Unfer V, D'Anna R. Myo-inositol: from induction of ovulation to menopausal disorder management. Minerva Ginecol 2015; 67: 485-486.

7) Unfer V, Orru B, Monastra G. Inositols: From physiology to rational therapy in gynecological clinical practice. Expert Opin Drug Metab Toxicol 2016; 12: 1129-1131.
8) Bezerra Espinola MS, Laganà AS, Bilotta G, Gullo G, Aragona C, Unfer V. D-chiro-inositol Induces Ovulation in Non-Polycystic Ovary Syndrome (PCOS), Non-Insulin-Resistant Young Women, Likely by Modulating Aromatase Expression: A Report of 2 Cases. Am J Case Rep 2021; 22: e932722.
9) Menichini D, Forte G, Orrù B, Gullo G, Unfer V, Facchinetti F. The role of vitamin D in metabolic and reproductive disturbances of polycystic ovary syndrome: A narrative mini-review. Int J Vitam Nutr Res 2022; 92: 126-133.
10) Tentolouris N, Voulgari C, Katsilambros N. A review of nateglinide in the management of patients with type 2 diabetes. Vasc Health Risk Manag 2007; 3: 797-807.
11) Anthony L, Freda PU. From somatostatin to octreotide LAR: evolution of a somatostatin analogue. Curr Med Res Opin 2009; 25: 2989-2999.
12) Lembcke B, Creutzfeldt W, Schleser S, Ebert R, Shaw C, Koop I. Effect of the somatostatin analogue sandostatin (SMS 201-995) on gastrointestinal, pancreatic and biliary function and hormone release in normal men. Digestion 1987; 36: 108-124.
13) Saad MA, Eltarzy MA, Abdel Salam RM, Ahmed MAE. Liraglutide mends cognitive impairment by averting Notch signaling pathway over expression in a rat model of polycystic ovary syndrome. Life Sci 2021; 265: 118731.
14) Celik O, Celik E, Turkcuoglu I, Yilmaz E, Ulas M, Simsek Y, Karaer A, Celik N, Aydin NE, Ozerol I, Unlu C. Surgical removal of endometrioma decreases the NF-kB1 (p50/105) and NF-kB p65 (Rel A) expression in the eutopic endometrium during the implantation window. Reprod Sci 2013; 20: 762-770.
15) Gao Y, Hou L, Wang Y, Guo S, Yuan D, Jiang Y, Duan G, Zhang Y, Xu Z, Che L, Sun C, Li S, Zhang S, Sun T, Li Y. Octreotide alleviates pancreatic damage caused by paraquat in rats by reducing inflammatory responses and oxidative stress. Environ Toxicol Pharmacol 2020; 80: 103456.
16) Yang B, Zhou Y, Tian L, Lu Y. Octreotide Reduces Pancreatic Islet Apoptosis and Improves Islet Transplantation Efficiency In Vitro and In Vivo. J Biomed Nanotechnol 2020; 16: 1082-1101.
17) González F, Considine RV, Abdelhadi OA, Acton AJ. Saturated Fat Ingestion Promotes Lipopolysaccharide-Mediated Inflammation and Insulin Resistance in Polycystic Ovary Syndrome. J Clin Endocrinol Metab 2019; 104: 934-946.
18) Xu XH, Shah PK, Faure E, Equils O, Thomas L, Fishbein MC, Luthringer D, Xu XP, Rajavashisth TB, Yano J, Kaul S, Arditi M. Toll-like receptor-4 is expressed by macrophages in murine and human lipid-rich atherosclerotic plaques and upregulated by oxidized LDL. Circulation 2001; 104: 3103-3108.