IJMS
Vol 48, No 2, March 2023

Original Article

Association of Oxidative Stress with Kidney Injury in a Hyperandrogenemic Female Rat Model

Nima Forghani[1], PhD; Zeinab Karimi[2], PhD; Mokhtar Mokhtari[1], PhD; Mehrdad Shariati[1], PhD; Fatemeh Masjedi[2], PhD

[1]Department of Biology, School of Sciences, Islamic Azad University, Kazerun Branch, Kazerun, Iran;
[2]Shiraz Nephro-Urology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Correspondence:
Zeinab, Karimi, PhD;
Shiraz Nephro-Urology Research Center, Khalili Ave., Postal code: 71936-35899, Shiraz, Iran
Tel/Fax: +98 71 36281563
Email: zkarimi@sums.ac.ir
Received: 22 November 2021
Revised: 03 March 2022
Accepted: 10 April 2022

Abstract

Background: Polycystic ovary syndrome (PCOS) is the most common reproductive dysfunction in premenopausal women. PCOS is associated with oxidative stress (OS), which is the main risk factor for renal diseases. This study aimed to investigate the mechanisms responsible for renal injury in a hyperandrogenemic female rat model.

Methods: This study was conducted from December 2019 to September 2021 at Shiraz Nephro-Urology Research Centre, Shiraz University of Medical Sciences (Shiraz, Iran). Thirty female Sprague-Dawley rats were randomly divided into three groups (n=10), namely control, sham, and dehydroepiandrosterone (DHEA). Plasma total testosterone, plasma creatinine (Cr), and blood urea nitrogen (BUN) levels were measured. In addition, total oxidant status (TOS), total antioxidant capacity (TAC), oxidative stress index (OSI), and histopathological changes in the ovaries and kidneys were determined. Data were analyzed using the GraphPad Prism software, and $P<0.05$ was considered statistically significant.

Results: Plasma total testosterone levels increased by nine-fold in DHEA-treated rats compared to controls ($P=0.0001$). Administration of DHEA increased Cr and BUN levels and caused severe renal tubular cell injury. In addition, plasma and tissue (kidney and ovary) TAC levels decreased significantly, but TOS levels and OSI values were significantly increased ($P=0.019$). Significant damage to both glomerular and tubular parts of the kidney and ovarian follicular structure was observed in the DHEA group.

Conclusion: Hyperandrogenemia caused systemic abnormalities through OS-related mechanisms and damaged renal and ovarian tissues. DHEA treatment in rat models is recommended to study the mechanisms that mediate PCOS-associated renal injury.

Please cite this article as: Forghani N, Karimi Z, Mokhtari M, Shariati M, Masjedi F. Association of Oxidative Stress with Kidney Injury in a Hyperandrogenemic Female Rat Model. Iran J Med Sci. 2023;48(2):187-197. doi:10.30476/IJMS.2022.93594.2497.

Keywords ● Polycystic ovary syndrome ● Oxidative stress ● Dehydroepiandrosterone ● Kidney disease

What's Known

- Oxidative stress (OS) and metabolic imbalance in women with polycystic ovary syndrome (PCOS) may affect kidney function.

What's New

- Hyperandrogenemia causes systemic abnormalities through OS-related mechanisms and damages renal and ovarian tissues.
- Dehydroepiandrosterone treatment in a rat model is recommended to study the mechanisms that mediate PCOS-associated renal injury.

Introduction

Polycystic ovary syndrome (PCOS) is among the most common endocrine conditions affecting 9-18% of women of reproductive age, depending on the diagnostic criteria used.[1, 2] PCOS is characterized by at least two of the following criteria, namely clinical and/or biochemical hyperandrogenism, oligo-ovulation or anovulation, and polycystic ovary morphology.[3] Clinically, these symptoms are associated with

decreased fertility due to follicular maturation dysfunction and subsequent anovulation and hyperandrogenism.[4] In women with PCOS, both chronic anovulation and androgen excess are associated with impaired folliculogenesis characterized by numerous cystic follicles (measuring 2 to 9 mm in diameter) and increased ovarian volume.[5]

Clinical studies showed that women with PCOS exhibit increased gonadotropin-releasing hormone (GnRH) pulse frequency, leading to excess luteinizing hormone (LH) secretion. This in turn induces premature acquisition of LH receptor expression resulting in ovarian follicles at very early stages, increased androgen production,[6,7] and arrest of antral follicle growth.[8] Ultrasound evaluation in women with PCOS indicated that follicular arrest leads to polycystic ovary morphology.[9] Furthermore, PCOS-affected ovaries exhibit increased preantral and antral follicles development, which results in antrum expansion, increased granulosa cell degeneration, and cystic follicles development with a thin layer of granulosa cells surrounded by a thick theca cell layer.[10] In addition to hormonal and metabolic derangements, oxidative stress (OS) is proactively involved in the etiology of PCOS.[11] OS is defined as an imbalance between pro-oxidants and antioxidant defenses. In fact, PCOS can be considered a condition due to OS, where the body's antioxidant defense is unable to offset the generation of excessive free radicals.[12, 13]

Several studies evaluated women with PCOS in terms of coronary health condition and hypertension.[14-16] Using a model of chronic hyperandrogenemia in female Sprague Dawley rats, a previous study reported that the animals exhibited high blood pressure, moderate proteinuria, and elevated glomerular filtration rate (GFR); most likely due to hyperglycemia related to insulin resistance.[16] Another study reported that chronic hyperandrogenemia leads to moderate hypertension and a 20% reduction in GFR with proteinuria.[17] Ratliff and colleagues found that the common link between all types of acute and chronic kidney injuries, irrespective of species, is enhanced generation of ROS and reactive nitrogen species (RNS) during injury/disease progression.[18] To date, the availability of literature on PCOS-associated renal complications is scarce, while it is well documented that OS-related mechanisms are common in the pathophysiology of kidney disease and PCOS. Hence, this study aimed to investigate the effect of hyperandrogenemia on renal injury and dysfunction through OS-related mechanisms.

Materials and Methods

This study was conducted from December 2019 to September 2021 at Shiraz Nephro-Urology Research Centre, Shiraz University of Medical Sciences (Shiraz, Iran). The study protocols complied with the *Guidelines for the Care and Use of Laboratory Animals* by the National Research Council[19] and were reviewed and approved by the Institutional Animal Care and Use Committee of the Shiraz University of Medical Sciences (IR.SUMS.REC.1398.173).

Animal Model

Thirty female Sprague-Dawley rats (200-220 g) were purchased from Shiraz University of Medical Sciences Animal Centre. All rats were housed in a cage and kept at a temperature of 25 °C with a 12h:12h light/dark cycle. The rats were fed with conventional feed pellets and given free access to food and water. In line with the sample size calculation in a previous study,[20] a sample size of 10 was used. The rats were randomly divided into three groups (n=10), namely the control, sham, and dehydroepiandrosterone (DHEA) groups. The control group was subcutaneously injected with normal saline for 28 consecutive days, the sham group with 0.2 mL sesame oil (Sigma-Aldrich, USA) for 28 days, and the DHEA group with 6 mg DHEA, dissolved in 0.2 mL sesame oil, per 100 g/day (Sigma-Aldrich, USA) for 28 days to induce hyperandrogenism.[21] At the end of the experiment, the rats were anesthetized with ketamine and xylazine (Alfasan Nederland B.V., The Netherlands) at 50 mg/kg and 10 mg/kg, respectively. Blood samples were taken from the tail vein of the animals, centrifuged to separate the plasma, and stored at -20 °C. These were then used to assess the total testosterone levels, oxidative status, and renal function indices. The rats were subsequently sacrificed, and the ovaries and kidneys were immediately removed. The left kidneys and ovaries were kept at -80 °C to assess the total oxidant status (TOS) and total antioxidant capacity (TAC). The right kidneys and ovaries were kept in 10% formalin for hematoxylin-eosin (H&E) staining and pathological evaluation.

Plasma Total Testosterone (PTT) Assay

The PTT assay was carried out using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (DiaMetra, Italy) with a sensitivity of 0.07 ng/mL, according to the manufacturer's instructions, and the Epoch 2™ microplate reader (BioTek Instruments, USA).

Renal Function Study

Plasma creatinine (Cr) levels were measured

based on the Jaffe and enzymatic colorimetric methods using commercially available kits (Pars Azmun, Iran) and the Epoch 2™ microplate reader at 500 nm wavelength. The assessment of blood urea nitrogen (BUN) levels was performed based on the urease method with glutamate dehydrogenase (GLDH) enzyme using commercially available kits (Pars Azmun) and the Epoch 2™ microplate reader at 340 nm wavelength.

Measurement of Oxidative Status Indicators

Total protein content in ovary and kidney tissue lysates was determined using the Pierce™ bicinchoninic acid protein assay kit (Thermo Fisher Scientific, USA). TOS and TAC values in ovary and kidney tissues were reported per mg of the total protein levels.

Total Oxidant Status Assay: Plasma and tissue (ovary and kidney) values of oxidants were measured using a commercially available kit (KiaZist, Iran) and the Epoch 2™ microplate reader at 560 nm wavelength. Briefly, oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to a ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. In an acidic medium, the ferric ion forms a colored complex with xylenol orange. The color intensity, which can be measured spectrophotometrically (560 nm), is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed as a nanomolar concentration of hydrogen peroxide equivalent per liter (nmol H_2O_2 Equiv./mL).

Total Antioxidant Capacity Assay: The capacity of antioxidants in plasma, ovary, and kidney tissues was measured using a commercially available kit (KiaZist, Iran) and the Epoch 2™ microplate reader at 490 nm wavelength. This assay, also known as the CUPRAC assay, is based on reducing Cu^{2+} to Cu^{1+} by the action of antioxidants present in the sample. The oxidant complex, consisting of Cu^{2+}-bathocuproinedisulfonic acid (Cu^{2+}-BCS), reacts with the antioxidants of the sample and is reduced to a Cu^{1+}-bathocuproinedisulfonic acid (Cu^{1+}-BCS), a stable complex which has a maximum absorbance at 490 nm. The antioxidant capacity of the sample is assumed to be equal to the extent of the complex Cu^{1+}-BCS formation. The results obtained from test samples were compared with a standard curve obtained with Trolox and expressed as nanomoles of Trolox equivalents per milliliter.

Oxidative Stress Index (OSI): The ratio of TOS to TAC yields the OSI, an indicator of the degree of OS. The OSI value was calculated according to the following formula:

OSI (arbitrary unit)=TOS (nmol H_2O_2 equiv./mL)/TAC (nmol Trolox equiv./mL)×100

Histopathological Analysis

Formalin-fixed hemisected kidneys and ovaries were embedded in paraffin and 5 µm thick serial sections were prepared and stained with H&E. For each renal slide, 10 randomly selected non-overlapping fields were assessed using a light microscope, and the degree of glomerular and tubular morphological changes was evaluated. The ovary tissue sections were observed under an optical microscope and different follicular groups were evaluated. To evaluate changes in ovarian tissue, ovarian structures were categorized into five groups based on morphology: primary follicles (PF), secondary follicles (SF), tertiary (Graafian) follicles (TF), cystic follicles (CF), and corpora lutea (CL), and their changes in the ovaries were examined. The number of follicles and CL per ovary section was counted for morphometric assessment. The average of different follicle numbers was calculated in 10 parts of ovarian parenchyma in each section (equivalent to 10 fields of 10× objective lens of the light microscope) and 20 fields in each group. The mean thickness of TF, theca, and granulosa cell layers was measured in 10 follicles in each section and 20 follicles in each group using ImageJ software, version 1.53q. (National Institutes of Health, Bethesda, Maryland, USA). Follicles were classified as PF when one or more granulosa cell layers around a primary oocyte were present, as SF based on the presence of antrum, and as TF when antrum development was completed and characterized by an oocyte surrounded by a cumulus oophorus. Those follicles containing four or five layers of granulosa cells surrounding a very large antrum or a large fluid-filled follicle with an attenuated granulosa cell layer, and thickened theca layer were classified as CF.

Statistical Analysis

Data were analyzed using GraphPad Prism software, version 8.0 (GraphPad Software Inc., La Jolla, California, USA) and expressed as mean±SEM. Comparison between the groups was performed using a one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* tests. $P<0.05$ was considered statistically significant.

Results

Plasma total testosterone levels: PTT levels

were measured to confirm hyperandrogenism in female rats. The results showed an approximately nine-fold increase in plasma total testosterone levels in hyperandrogenemic female rats (DHEA group) compared to untreated female rats (P=0.0001) (figure 1).

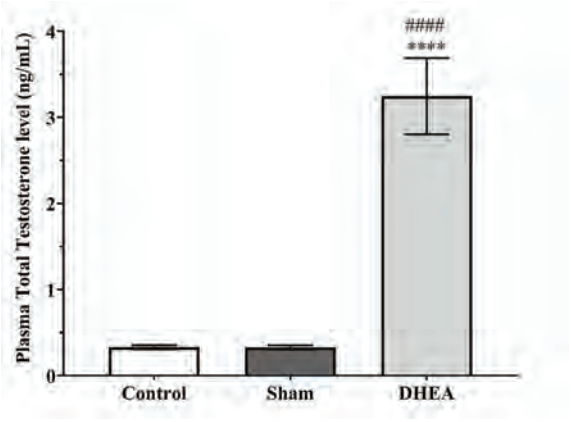

Figure 1: The figure shows the effect of 28 days of DHEA administration on the level of plasma total testosterone in the control, sham (received 0.2 mL sesame oil subcutaneously per day as a vehicle), and DHEA (subcutaneously injected with 6 mg DHEA per 100 g/day) groups (n=10). The data were analyzed using one-way analysis of variance (ANOVA) with Tukey's *post hoc* test and expressed as mean±SEM. **** and #### P<0.0001 represents significant differences between the control and sham groups, respectively. DHEA: Dehydroepiandrosterone.

***Effects of Hyperandrogenemia on Renal Function Parameters*:** Administration of DHEA (6 mg/100 g day, 1×subcutaneous injection) for 28 days caused a significant increase in plasma Cr (1.164±0.038 vs. 0.594±0.031 mg/dL) and BUN (17.50±1.341 vs. 9.398±0.830 mg/dL) levels in the DHEA group compared to the control group (P=0.0008) (figures 2A and 2B).

***Effects of Hyperandrogenemia on Plasma and Tissue TOS Levels*:** To demonstrate the potential role of the OS signaling pathway in hyperandrogenemia-associated kidney injury, plasma and tissue (kidney and ovary) TOS levels were measured at the end of the experiment. The results showed that rats in the DHEA group had higher levels of TOS in the plasma and tissue samples than the control and sham groups (P=0.003) (table 1).

***Effects of Hyperandrogenemia on the Plasma and Tissue TAC Levels*:** To confirm the involvement of antioxidant pathways and their possible protective mechanism in hyperandrogenemia-associated kidney injury, the plasma and tissue TAC levels were evaluated. As shown in table 1, the plasma and tissue (kidney and ovary) TAC levels in the DHEA group were significantly lower than in the control group (P=0.007). However, compared to the sham group, the decrease was only statistically significant for plasma TAC levels (P=0.002).

***Effects of Hyperandrogenemia on Plasma and Tissue OSI*:** Table 1 shows the distribution of plasma and tissue (kidney and ovary) OSI values in all groups. The results showed that similar to TOS levels, OSI values in the DHEA group were significantly higher than in other groups.

Histopathological Evaluation of Ovary and Kidney

***Effects of DHEA on Ovarian Histomorphology*:** Pathological assessment and light microscope photography of ovarian morphology are shown in figure 3. By analyzing the histological morphology of ovaries in the control and sham groups, different follicles were observed, including TF with a thick layer of granulosa cells and CL (figure 3A-D). A number of CF with a very thin layer of granulosa,

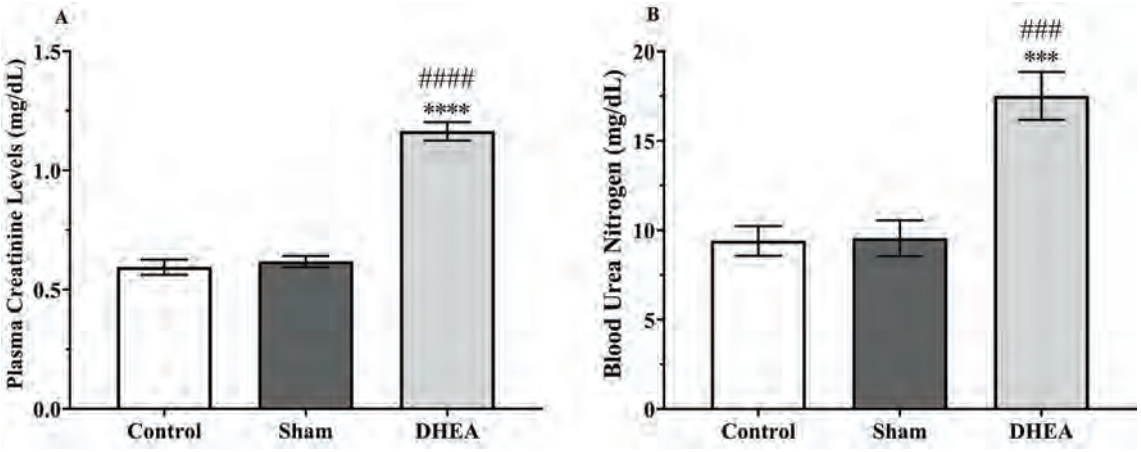

Figure 2: The figure shows the effects of 28 days of DHEA administration on the level of plasma creatinine (A) and blood urea nitrogen (BUN) (B) in the control, sham (received 0.2 mL sesame oil subcutaneously per day as a vehicle), and DHEA (subcutaneously injected with 6 mg DHEA per 100 g/day) groups (n=10). The data were analyzed using one-way analysis of variance (ANOVA) with Tukey's *post hoc* test and expressed as mean±SEM. ***P<0.001 and ****P<0.0001 represent significant differences with the control group, ###P<0.001 and ####P<0.0001 represent significant differences with the sham group. DHEA: Dehydroepiandrosterone.

Table 1: The effect of DHEA (6 mg/100 g day, 1× subcutaneous injection) on total oxidant status (TOS), total antioxidant capacity (TAC), and oxidative stress index (OSI) levels of plasma, kidney, and ovary in the experimental groups (mean±SEM, n=10)

Variables \ Groups		Control	Sham	DHEA	P value[a]	P value[b]
TOS (plasma levels: nmol/mL, tissue levels: nmol/mg of protein)	Plasma	3.057±0.326	3.720±0.511	5.824±0.648	0.003	0.042
	Kidney	2.495±0.159	2.746±0.253	5.053±0.216	0.0001	0.0001
	Ovary	4.182±0.752	4.000±0.271	6.561±0.524	0.017	0.019
TAC (plasma levels: nmol/mL, tissue levels: nmol/mg of protein)	Plasma	1451±75.6	1589±147.8	829.8±155.3	0.007	0.002
	Kidney	1682±83.7	1438±52.2	1199±72.9	0.0003	0.046
	Ovary	3639±425.4	2474±518.7	1657±198.5	0.001	0.048
OSI (arbitrary unit)	Plasma	0.225±0.016	0.233±0.042	1.190±0.350	0.027	0.046
	Kidney	0.175±0.016	0.183±0.031	0.430±0.030	0.0001	0.0001
	Ovary	0.125±0.016	0.217±0.040	0.433±0.093	0.011	0.044

[a]Significant difference with the control group, [b]Significant differences with the sham group, DHEA: Dehydroepiandrosterone. One-way analysis of variance (ANOVA) with Tukey's *post hoc* test was used to compare the groups.

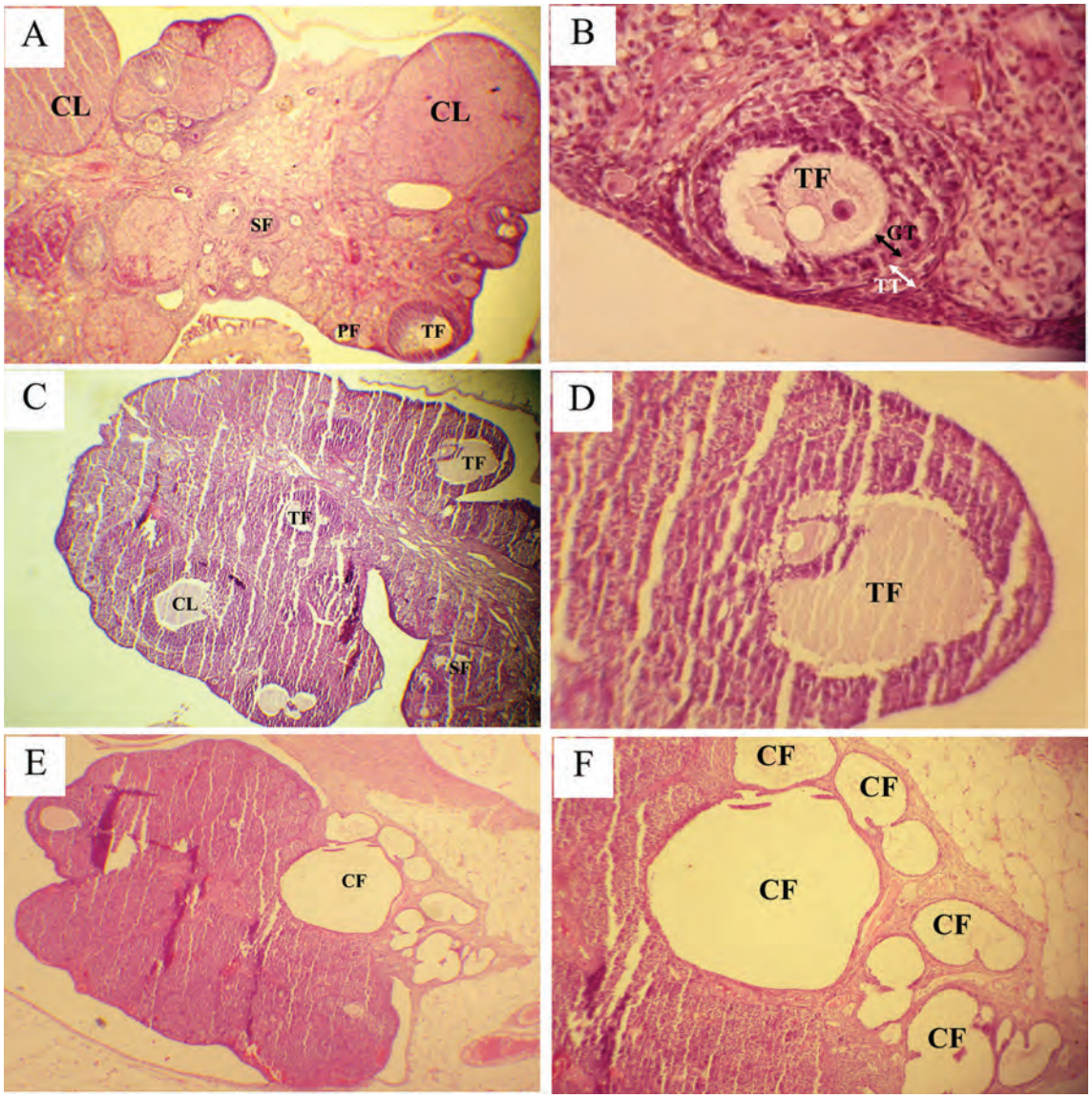

Figure 3: The figure shows the photomicrograph of ovarian tissue in the experimental groups (H&E staining). (A) The ovarian section of the control group with healthy growing follicles and corpora lutea and (B) a tertiary (Graafian) follicle. (C) The ovarian section of the sham group (received 0.2 mL sesame oil subcutaneously per day as a vehicle) with follicles at the different stages of development and corpora lutea and (D) a tertiary (Graafian) follicle. (E) The ovarian section of the DHEA group (subcutaneously injected with 6 mg DHEA per 100 g/day) with several cyst-like follicles and very few corpora lutea, and (F) cystic follicles. PF: Primary follicle; SF: Secondary follicle; TF: Tertiary (Graafian) follicle; CF: Cystic follicle; CL: Corpus luteum; TT: Theca layer thickness; GT: Granulosa layer thickness; DHEA: Dehydroepiandrosterone; Magnification: ×100 (A, C, E) and ×400 (B, D, F).

Table 2: The effect of DHEA (6 mg/100 g day^{-1}, subcutaneous injection) on the development of follicles in the experimental groups (mean±SEM, n=10)

Variables \ Groups	Control	Sham	DHEA	P value[a]	P value[b]
Primary follicle	10.23±4.08	9.42±2.17	15.14±2.62	0.632	0.842
Secondary follicle	3.40±0.92	3.20±1.31	4.4±0.36	0.657	0.712
Tertiary (Graafian) follicle	2.60±0.35	2.40±0.36	0.2±0.21	0.012	0.018
Cystic follicle	0.00	0.00	10.83±1.41	0.0001	0.0001
Corpus luteum	7.16±2.31	6.22±0.83	1.3±0.39	0.005	0.026

[a]Significant differences with the control group, [b]Significant differences with the sham group, DHEA: Dehydroepiandrosterone. One-way analysis of variance (ANOVA) with Tukey's *post hoc* test was used to compare the groups.

which is a characteristic of CF, were observed in the ovaries of the DHEA group. Only a few CL indicative of ovulation was observed in this group (figures 3E and 3F). Therefore, based on the observation of ovarian tissue, DHEA administration to female rats for 28 days resulted in the development of CF, decreased the number of TF and CL, and significantly reduced ovulation due to the reduction in the number of CL.

Effects of DHEA on Ovarian Histomorphometry: Histomorphometric analysis showed that, compared to the control group, the DHEA group had an increase in the number of CF (P=0.0001) and a decrease in the number of CL (P=0.029) (table 2). DHEA administration, to induce PCOS in the female rat model, reduced the number of TF and increased the number of CF compared to the control and sham groups (table 2). In terms of the diameter of follicles in the ovaries, significant differences were observed between the groups. A significant difference (P=0.0001) in the size of the TF due to cysts was observed between the control and DHEA groups (figure 4A). Theca layer measurements showed a significant increase (P=0.023) in the thickness in the DHEA group compared to the control and sham groups (figure 4B). In terms of the granulosa layer thickness, a significant decrease (P=0.036) was observed in the DHEA group compared to the control and sham groups (figure 4C).

Effects of DHEA on Renal Histomorphology: Histopathological renal changes in the control, sham, and DHEA groups are shown in figure 5. In the cortex and medulla regions, the renal tissue showed normal structure in the control and sham groups (figure 5A-D). Subcutaneous injection of DHEA for 28 days caused severe structural damage to the kidneys of female rats, i.e., complete shedding of the proximal tubules brush border, the hollow appearance of the proximal tubules, and enlarged Bowman's space (figure 5E). In addition, in the renal medulla, proximal tubular epithelial cells degenerated, and casts appeared inside the tubules (figure 5F). A quantitative histopathological analysis of the renal tissue was performed to score the extent of damage to the cortex and medulla. The results showed that administration of DHEA for 28 days caused severe damage to the nephrons (P=0.001) (table 3).

Discussion

In the current study, we sought to determine potential adverse renal effects in a hyperandrogenemic female rat model and characterized the renal consequences of increased androgens. The results showed an approximately nine-fold increase in total testosterone, a level consistent with androgen

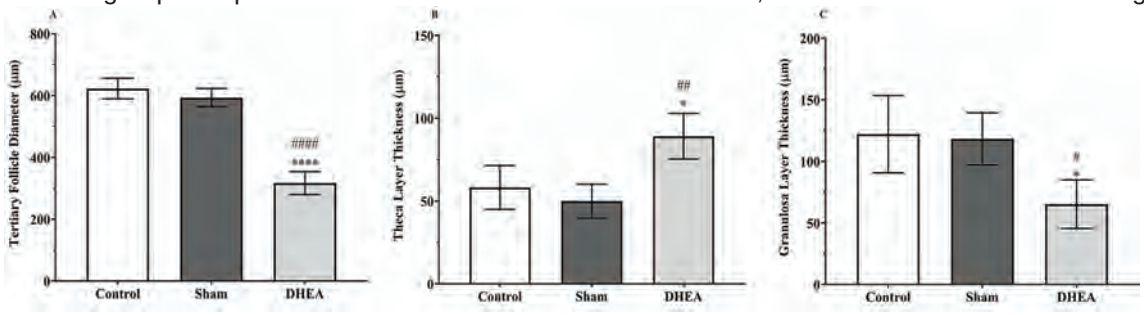

Figure 4: The figure shows the effect of 28 days of DHEA administration on (A) tertiary follicle diameter, (B) theca layer thickness, and (C) granulosa layer thickness of the ovary in the control, sham (received 0.2 mL sesame oil subcutaneously per day as a vehicle), and DHEA (subcutaneously injected with 6 mg DHEA per 100 g/day) groups (10 follicles in each section and a total of 20 follicles in each group). The data were analyzed using one-way analysis of variance (ANOVA) with Tukey's *post hoc* test and expressed as mean±SEM. *P<0.05 and ****P<0.0001 represent significant differences with the control group. #P<0.05, ##P<0.01 and ####P<0.0001 represent significant differences with the sham group. DHEA: Dehydroepiandrosterone.

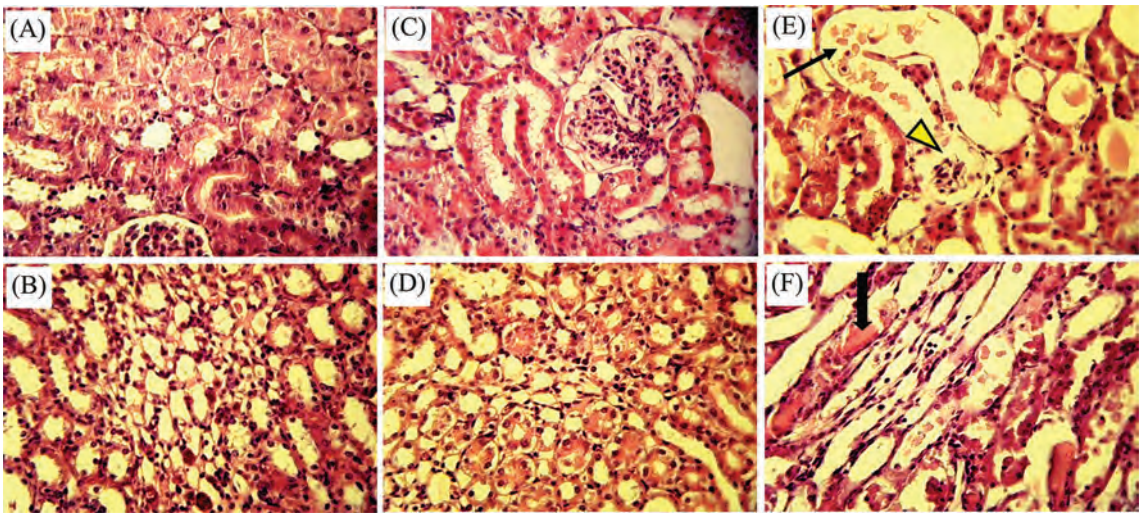

Figure 5: The figure shows the photomicrograph of renal tissue in the experimental groups (H&E staining). The renal section of the control group with a completely normal appearance in the (A) cortex and (B) medulla. The renal section of the sham group (received 0.2 mL sesame oil subcutaneously per day as a vehicle) with a normal condition in the (C) cortex and (D) medulla. The renal section of the DHEA group (subcutaneously injected with 6 mg DHEA per 100 g/day) with (E) epithelial cell injury and shedding of brush borders in the convoluted proximal tubules (thin black arrow) and bowman space enlargement (yellow arrowhead) in the cortex and (F) casts inside the tubules (thick black arrow) in the medulla. Magnification: ×400.

Table 3: The effect of DHEA (6 mg/100 g day^{-1}, subcutaneous injection) on the quantitative histopathological indicators of kidney damage in the experimental groups (mean±SEM, n=10)

Variables \ Groups	Control	Sham	DHEA	P value[a, b]
Proximal tubule injury	0.00	0.00	3.9±0.03	0.001
Bowman space enlargement	0.00	0.00	2.8±0.12	0.001
Intra tubular cast	0.00	0.00	1.8±0.06	0.001
Total histopathologic score	0.00	0.00	8.5±0.21	0.001

[a,b]Significant differences between the DHEA group and the control and sham groups, DHEA: Dehydroepiandrosterone. One-way analysis of variance (ANOVA) with Tukey's *post hoc* test was used to compare the groups.

levels in women with PCOS,[22] that caused significant renal damage. To identify possible mechanisms through which androgens may cause renal damage, we found that DHEA-treated female rats exhibited characteristics of oxidative imbalance.

Researchers tend to use animal models of PCOS instead of human experimentation because of the ethical and logistic restrictions involved. Widely used hormones in PCOS animal models include DHEA, dihydrotestosterone (DHT), estradiol valerate, and letrozole.[23] According to a previous study, the symptoms in DHEA-induced PCOS rat models are very similar to many PCOS symptoms in humans.[24] This animal model is shown to be representative of the early onset of PCOS in young adults.[25] A previous study in young females treated with DHT reported that their GFR increased slightly compared to the placebo controls, and renal morphology showed no glomerular or tubular damage.[16] Another study on postmenopausal hyperandrogenemic female rats reported an increase in proteinuria and kidney injury molecule-1 (KIM-1) excretion. This suggested that the rats had significant impairment of renal function and significantly lower GFR than aging controls.[17] Elevation of serum BUN and Cr levels is a sign of deterioration of glomerular function, which causes kidney failure over time.[26, 27] Our results showed that the administration of DHEA significantly increased plasma Cr and BUN levels, indicative of renal injury. Furthermore, proximal tubular and glomerular injuries were evident in renal tissues.

A study by Yanes and colleagues reported an increase in enzymes that control OS in the kidneys of young female rats treated with DHT.[16] Several other studies suggested that OS may play an important role in infertility and heterogeneous disorders in PCOS patients.[12, 13, 28, 29] On the other hand, PCOS is closely associated with the progression of metabolic abnormalities such as obesity, diabetes, and hypertension, which are the major causes of kidney disease.[30, 31] In this regard, Patel and colleagues reported that microalbuminuria in young women with PCOS was associated with metabolic syndrome and hypertension.[32] Solutes reabsorption is the kidney's most energy-demanding process,

therefore, renal tubular cells are rich in mitochondria. This, in turn, makes kidney cells prone to OS and damage.[33]

In the present study, we also investigated the association between PCOS and kidney injury through the OS signaling pathway. Kidney function was evaluated by measuring BUN and Cr levels in rats treated with DHEA compared to controls. We also assessed TOS, TAC, and OSI in plasma, ovary, and kidney. The results confirmed renal injury through OS-related mechanisms. Other studies investigated the role of testosterone in PCOS-associated kidney injury. The results of a previous study showed a significant positive correlation between serum testosterone and tubular proteinuria markers in PCOS patients.[34] This further demonstrates that testosterone plays an important role in the pathogenesis of PCOS-associated kidney injury, especially tubular cell injury. In this regard, hyperandrogenemia seems to contribute to OS in PCOS, but the underlying mechanisms of this association remain unclear. It is revealed that OS markers are positively correlated with androgen levels in PCOS patients.[35] Compared with controls, PCOS models induced by excess androgen exposure increased OS markers, such as malondialdehyde, glutathione, and superoxide dismutase.[36] Therefore, in our study, a high level of OS (probably induced by hyperandrogenemia in the ovarian tissue) could be related to significant ovarian histopathological changes. Duică and colleagues reported increased OS coupled with poor antioxidant status in women with PCOS.[28] Our results also revealed increased OS levels in female rats with DHEA-induced PCOS.

The results of the morphometric analysis showed that the number of TF and CL was reduced in the DHEA group. Moreover, antral follicles developed a cystic structure, their granulosa cells layer became thinner, and their theca cells layer thickened. Other studies using a PCOS rat model stated that these morphological changes result in impaired follicle development, irregular ovulation, and relatively increased atresia.[37, 38] Therefore, hyperandrogenism can induce OS resulting in follicles atresia and ovary apoptosis.[39] In line with a previous study, our results showed that it also accelerates early follicular growth leading to excess preantral follicles.[40] Overall, our findings indicate that the OS signaling pathway is involved in the adverse effects of androgens on ovarian tissue.

The main limitation of our study was the omission of measuring renal function indices, such as urinary albumin to creatinine ratio and KIM-1. It is recommended to include these in future studies. Another limitation was related to matching the reproductive cycle and ovarian age of rats in the animal model to those of women of childbearing age with PCOS. However, this does not undermine the advantages of the DHEA-induced rat model of PCOS in preclinical studies.[24]

Conclusion

Renal consequences of increased androgens in a hyperandrogenemic female rat model, which mimics many of the changes seen in women with PCOS, were characterized. The DHEA-treated female rats exhibited an increase in serum Cr and BUN levels as well as OS-associated factors such as increased TOS, decreased TAC, and subsequently higher OSI in the plasma, ovary, and kidney. Through OS-related mechanisms, hyperandrogenemia causes systemic abnormalities resulting in damage to kidney and ovary tissues. We trust that this female rat model will help explain potential mechanisms by which androgens cause kidney disease in women with PCOS.

Acknowledgment

The manuscript was extracted from the PhD dissertation of Nima Forghani, Department of Biology, Islamic Azad University, Kazerun Branch. The study was supported by a grant (number: 97-18903) from Shiraz University of Medical Sciences, Shiraz, Iran.

Authors' Contribution

N.F: Study design, acquisition of data, and drafting; Z.K: Study conception, analysis of data, and revising the manuscript; M.M and M.Sh: Study design, interpretation of data, and revising the manuscript; F.M: Study conception, acquisition, and analysis of data, and drafting the manuscript; All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

References

1 Azziz R, Carmina E, Chen Z, Dunaif A, Laven JS, Legro RS, et al. Polycystic ovary syndrome. Nat Rev Dis Primers. 2016;2:16057. doi: 10.1038/nrdp.2016.57. PubMed PMID:

27510637.
2 Chappell NR, Gibbons WE, Blesson CS. Pathology of hyperandrogenemia in the oocyte of polycystic ovary syndrome. Steroids. 2022;180:108989. doi: 10.1016/j.steroids.2022.108989. PubMed PMID: 35189133; PubMed Central PMCID: PMCPMC8920773.
3 Rotterdam EA-SPcwg. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19:41-7. doi: 10.1093/humrep/deh098. PubMed PMID: 14688154.
4 Bruni V, Capozzi A, Lello S. The Role of Genetics, Epigenetics and Lifestyle in Polycystic Ovary Syndrome Development: the State of the Art. Reprod Sci. 2022;29:668-79. doi: 10.1007/s43032-021-00515-4. PubMed PMID: 33709373.
5 Dewailly D, Lujan ME, Carmina E, Cedars MI, Laven J, Norman RJ, et al. Definition and significance of polycystic ovarian morphology: a task force report from the Androgen Excess and Polycystic Ovary Syndrome Society. Hum Reprod Update. 2014;20:334-52. doi: 10.1093/humupd/dmt061. PubMed PMID: 24345633.
6 Glintborg D. Endocrine and metabolic characteristics in polycystic ovary syndrome. Dan Med J. 2016;63. PubMed PMID: 27034186.
7 McCartney CR, Campbell RE, Marshall JC, Moenter SM. The role of gonadotropin-releasing hormone neurons in polycystic ovary syndrome. J Neuroendocrinol. 2022;34:e13093. doi: 10.1111/jne.13093. PubMed PMID: 35083794; PubMed Central PMCID: PMCPMC9232905.
8 Dewailly D, Robin G, Peigne M, Decanter C, Pigny P, Catteau-Jonard S. Interactions between androgens, FSH, anti-Mullerian hormone and estradiol during folliculogenesis in the human normal and polycystic ovary. Hum Reprod Update. 2016;22:709-24. doi: 10.1093/humupd/dmw027. PubMed PMID: 27566840.
9 Zhang J, Tang L, Kong L, Wu T, Xu L, Pan X, et al. Ultrasound-guided transvaginal ovarian needle drilling for clomiphene-resistant polycystic ovarian syndrome in subfertile women. Cochrane Database Syst Rev. 2021;11:CD008583. doi: 10.1002/14651858.CD008583.pub3. PubMed PMID: 34735019; PubMed Central PMCID: PMCPMC8567741.
10 Wang F, Yu B, Yang W, Liu J, Lu J, Xia X. Polycystic ovary syndrome resembling histopathological alterations in ovaries from prenatal androgenized female rats. J Ovarian Res. 2012;5:15. doi: 10.1186/1757-2215-5-15. PubMed PMID: 22607720; PubMed Central PMCID: PMCPMC3406938.
11 Papalou O, Victor VM, Diamanti-Kandarakis E. Oxidative Stress in Polycystic Ovary Syndrome. Curr Pharm Des. 2016;22:2709-22. doi: 10.2174/1381612822666160216151852. PubMed PMID: 26881435.
12 Masjedi F, Keshtgar S, Agah F, Karbalaei N. Association Between Sex Steroids and Oxidative Status with Vitamin D Levels in Follicular Fluid of Non-obese PCOS and Healthy Women. J Reprod Infertil. 2019;20:132-42. PubMed PMID: 31423416; PubMed Central PMCID: PMCPMC6670262.
13 Masjedi F, Keshtgar S, Zal F, Talaei-Khozani T, Sameti S, Fallahi S, et al. Effects of vitamin D on steroidogenesis, reactive oxygen species production, and enzymatic antioxidant defense in human granulosa cells of normal and polycystic ovaries. J Steroid Biochem Mol Biol. 2020;197:105521. doi: 10.1016/j.jsbmb.2019.105521. PubMed PMID: 31705961.
14 Caglar GS, Oztas E, Karadag D, Pabuccu R, Eren AA. The association of urinary albumin excretion and metabolic complications in polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol. 2011;154:57-61. doi: 10.1016/j.ejogrb.2010.08.024. PubMed PMID: 20888116.
15 Gozukara IO, Gozukara KH, Kucur SK, Karakilic EU, Keskin H, Akdeniz D, et al. Association of Glomerular Filtration Rate with Inflammation in Polycystic Ovary Syndrome. Int J Fertil Steril. 2015;9:176-82. doi: 10.22074/ijfs.2015.4238. PubMed PMID: 26246875; PubMed Central PMCID: PMCPMC4518485.
16 Yanes LL, Romero DG, Moulana M, Lima R, Davis DD, Zhang H, et al. Cardiovascular-renal and metabolic characterization of a rat model of polycystic ovary syndrome. Gend Med. 2011;8:103-15. doi: 10.1016/j.genm.2010.11.013. PubMed PMID: 21536229; PubMed Central PMCID: PMCPMC3093922.
17 Dalmasso C, Maranon R, Patil C, Bui E, Moulana M, Zhang H, et al. Cardiometabolic Effects of Chronic Hyperandrogenemia in a New Model of Postmenopausal Polycystic Ovary Syndrome. Endocrinology. 2016;157:2920-7. doi: 10.1210/en.2015-1617. PubMed PMID: 27145003; PubMed Central PMCID: PMCPMC4929551.
18 Ratliff BB, Abdulmahdi W, Pawar R, Wolin MS. Oxidant Mechanisms in Renal Injury and Disease. Antioxid Redox Signal. 2016;25:119-46. doi: 10.1089/ars.2016.6665. PubMed

PMID: 26906267; PubMed Central PMCID: PMCPMC4948213.
19 Council NR, Studies DEL, Research ILA, Animals CUGCUL. Guide for the Care and Use of Laboratory Animals. Eighth Edition. Washington: National Academies Press; 2011.
20 Charan J, Kantharia ND. How to calculate sample size in animal studies? J Pharmacol Pharmacother. 2013;4:303-6. doi: 10.4103/0976-500X.119726. PubMed PMID: 24250214; PubMed Central PMCID: PMCPMC3826013.
21 Neisy A, Zal F, Seghatoleslam A, Alaee S. Amelioration by quercetin of insulin resistance and uterine GLUT4 and ERalpha gene expression in rats with polycystic ovary syndrome (PCOS). Reprod Fertil Dev. 2019;31:315-23. doi: 10.1071/RD18222. PubMed PMID: 30103849.
22 Pinola P, Piltonen TT, Puurunen J, Vanky E, Sundstrom-Poromaa I, Stener-Victorin E, et al. Androgen Profile Through Life in Women With Polycystic Ovary Syndrome: A Nordic Multicenter Collaboration Study. J Clin Endocrinol Metab. 2015;100:3400-7. doi: 10.1210/jc.2015-2123. PubMed PMID: 26192874.
23 Mesbah F, Moslem M, Vojdani Z, Mirkhani H. Does metformin improve in vitro maturation and ultrastructure of oocytes retrieved from estradiol valerate polycystic ovary syndrome-induced rats. J Ovarian Res. 2015;8:74. doi: 10.1186/s13048-015-0203-x. PubMed PMID: 26577050; PubMed Central PMCID: PMCPMC4650318.
24 Walters KA, Allan CM, Handelsman DJ. Rodent models for human polycystic ovary syndrome. Biol Reprod. 2012;86:149. doi: 10.1095/biolreprod.111.097808. PubMed PMID: 22337333.
25 Kim EJ, Jang M, Choi JH, Park KS, Cho IH. An Improved Dehydroepiandrosterone-Induced Rat Model of Polycystic Ovary Syndrome (PCOS): Post-pubertal Improve PCOS's Features. Front Endocrinol (Lausanne). 2018;9:735. doi: 10.3389/fendo.2018.00735. PubMed PMID: 30564195; PubMed Central PMCID: PMCPMC6288467.
26 Pakfetrat Z, Janfeshan S, Masjedi F, Rafiei M, Karimi Z. Involvement of oxidative stress and toll-like receptor-4 signaling pathways in gentamicin-induced nephrotoxicity in male Sprague Dawley rats. Drug Chem Toxicol. 2022;45:2568-75. doi: 10.1080/01480545.2021.1977024. PubMed PMID: 34538191.
27 Gholampour F, Mohammadi Z, Karimi Z, Owji SM. Protective effect of genistein in a rat model of ischemic acute kidney injury. Gene. 2020;753:144789. doi: 10.1016/j.gene.2020.144789. PubMed PMID: 32442578.
28 Duica F, Danila CA, Boboc AE, Antoniadis P, Condrat CE, Onciul S, et al. Impact of Increased Oxidative Stress on Cardiovascular Diseases in Women With Polycystic Ovary Syndrome. Front Endocrinol (Lausanne). 2021;12:614679. doi: 10.3389/fendo.2021.614679. PubMed PMID: 33679617; PubMed Central PMCID: PMCPMC7930620.
29 Masjedi F, Gol A, Dabiri S, Javadi A. Investigating the Preventive Effect of Garlic on Blood Glucose Levels and Histopathology of Pancreas in Streptozotocin-induced Diabetic Rats. Physiology and Pharmacology. 2009;13:179-90.
30 Kidney Disease: Improving Global Outcomes Diabetes Work G. KDIGO 2020 Clinical Practice Guideline for Diabetes Management in Chronic Kidney Disease. Kidney Int. 2020;98:S1-S115. doi: 10.1016/j.kint.2020.06.019. PubMed PMID: 32998798.
31 Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Fertil Steril. 2018;110:364-79. doi: 10.1016/j.fertnstert.2018.05.004. PubMed PMID: 30033227; PubMed Central PMCID: PMCPMC6939856.
32 Patel AA, Bloomgarden ZT, Futterweit W. Premicroalbuminuria in women with polycystic ovary syndrome: a metabolic risk marker. Endocr Pract. 2008;14:193-200. doi: 10.4158/EP.14.2.193. PubMed PMID: 18308657.
33 Eirin A, Lerman A, Lerman LO. The Emerging Role of Mitochondrial Targeting in Kidney Disease. Handb Exp Pharmacol. 2017;240:229-50. doi: 10.1007/164_2016_6. PubMed PMID: 27316914; PubMed Central PMCID: PMCPMC5164968.
34 Song Y, Ye W, Ye H, Xie T, Shen W, Zhou L. Serum testosterone acts as a prognostic indicator in polycystic ovary syndrome-associated kidney injury. Physiol Rep. 2019;7:e14219. doi: 10.14814/phy2.14219. PubMed PMID: 31448581; PubMed Central PMCID: PMCPMC6709419.
35 Zhang J, Bao Y, Zhou X, Zheng L. Polycystic ovary syndrome and mitochondrial dysfunction. Reprod Biol Endocrinol. 2019;17:67. doi: 10.1186/s12958-019-0509-4. PubMed PMID: 31420039; PubMed Central PMCID:

PMCPMC6698037.
36 Zuo T, Zhu M, Xu W. Roles of Oxidative Stress in Polycystic Ovary Syndrome and Cancers. Oxid Med Cell Longev. 2016;2016:8589318. doi: 10.1155/2016/8589318. PubMed PMID: 26770659; PubMed Central PMCID: PMCPMC4684888.
37 Ahmadi M, Rostamzadeh A, Fathi F, Mohammadi M, Rezaie MJ. The effect of Melatonin on histological changes of ovary in induced polycystic ovary syndrome model in mice. Middle East Fertility Society Journal. 2017;22:255-9. doi: 10.1016/j.mefs.2017.03.009.
38 Forghani N, Karimi Z, Mokhtar M, Shariati M, Masjedi F. Functional and histopathological kidney injury in hyperandrogenic female rats-a polycystic ovary animal model. Medical Journal of Tabriz University of Medical Sciences. 2022;44:26-35. doi: 10.34172/mj.2022.011.
39 Rezvanfar MA, Shojaei Saadi HA, Gooshe M, Abdolghaffari AH, Baeeri M, Abdollahi M. Ovarian aging-like phenotype in the hyperandrogenism-induced murine model of polycystic ovary. Oxid Med Cell Longev. 2014;2014:948951. doi: 10.1155/2014/948951. PubMed PMID: 24693338; PubMed Central PMCID: PMCPMC3945218.
40 Diamanti-Kandarakis E. Polycystic ovarian syndrome: pathophysiology, molecular aspects and clinical implications. Expert Rev Mol Med. 2008;10:e3. doi: 10.1017/S1462399408000598. PubMed PMID: 18230193.