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Synergistic Ameliorative Effects of Zinc Oxide Nanoparticles and Metformin against Letrozole- Induced Polycystic Ovary Syndrome in Rats: Biochemical, Genetic, and Antioxidant Studies

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Abstract
Polycystic ovary syndrome (PCOS) presents itself as a multifaceted disorder affecting the reproductive, endocrine, and metabolic systems, with enduring complications. Although the intricate mechanisms behind the condition's pathology remain mostly obscure, the primary culprits seem to revolve around hyperandrogenism, insulin resistance, and oxidative stress. Within the realm of disease therapies, Zinc oxide nanoparticles (ZnONPs) hold significant potential for numerous applications, including insulin resistance. This study evaluated the role of ZnONPs alone and when combined with metformin in Letrozole- induced PCOS. Methods: PCOS was induced in rats using a 36 days-course of letrozole; ZnONPs or/and metformin were given from day 22 for 15 days. Results: PCOS group resulted in a significantly higher body weight, ovarian weight as well as elevated testosterone, insulin, glycemia and lipid profile levels. All of these effects were significantly reduced by ZnONPs. Besides, ZnONPs remarkably inhibited the letrozole induced oxidative stress in the ovaries by reducing the upraised malondialdhyde and increasing the suppressed superoxide dismutase and glutathione peroxidase activities. ZnONPs were able to reduce ovarian tissue CYP17A1 expression, the key in androgen biosynthesis that was significantly higher in PCOS group. Ovarian histopathological examination confirmed the biochemical findings. Conclusion: The outcomes of this study indicated that ZnONPs might hold great promise in addressing PCOS impairments through multiple mechanisms. By reducing insulin resistance, hyperandrogenism, and improving redox status. When combined with metformin, they could synergistically enhance control over the condition.

Keywords: ZnONPs; Polycystic ovary syndrome; CYP17A1.

1. Introduction

Polycystic ovary syndrome (PCOS) affects up to 20% of females in their reproductive years, according to the Rotterdam criteria, making it the most common endocrine disorder [1].The clinical presentation of PCOS typically exhibits considerable diversity, encompassing oligomenorrhea or amenorrhea, infertility, and hyperandrogenemia with associated symptoms like hirsutism and alopecia [2]. Furthermore, individuals affected by PCOS commonly display conditions like obesity, dyslipidemia, insulin resistance, type 2 diabetes, and an elevated susceptibility to cardiovascular disease [3].

The pathophysiology of PCOS seems to be primarily driven by insulin resistance and hyperandrogenemia[4]. As a result of insulin resistance and compensatory hyperinsulinemia, there is an observed increase in androgen secretion. The rise is linked to the reduction in hepatic insulin-like growth factor binding protein-1 (IGFBP-1). As a result, there is an increase in the free insulin-like growth factor-1 (IGF-1) level. Consequently, hyperthecosis occurs, resulting in higher androgen levels [5]. Furthermore, the liver's diminished production of sex hormone binding globulin (SHBG) is a contributing factor to increased levels of free androgen [6]. It is also noteworthy that insulin exerts similar effects to luteinizing hormone (LH) on theca cells, eventually leading to the development of hyperandrogenism [7]. Moreover, an overabundance of androgen leads to a decrease in glucose transporter 4 (GLUT4) sensitivity and expression, increases adipocyte mass with visceral adiposity, resulting in insulin resistance [8].

Cytochrome P450 17α-hydroxylase (CYP17A1), a crucial enzymatic player in androgen synthesis within the gonads and adrenal cortex, exhibits dual functionalities as a 17α-hydroxylase and a 17, 20-lyase [9]. The expression of CYP17A1 is significantly elevated in cases of PCOS [10].

Numerous animal models are employed to replicate PCOS in humans. Among these models is the

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utilization of letrozole, an aromatase inhibitor that hinders the conversion of androgens to estrogens. As a consequence, it leads to follicular atresia and gives rise to metabolic and reproductive irregularities characteristic of PCOS [11].
Metformin, an oral hypoglycemic agent belonging to the biguanide group, is commonly utilized to manage PCOS [12,13]. The predominant side effects associated with metformin are gastrointestinal, while there exists an escalated likehood of experiencing severe problems such as cardiovascular and thromboembolic issues, along with lactic acidosis [14].

The 21st century has witnessed an extraordinary advancement in science, and nanotechnology stands out as one of the most remarkable examples [15]. Zinc oxide nanoparticles (ZnONPs) have emerged as a pioneer for delivering zinc so addressing numerous disease therapies, including insulin resistance linked to PCOS [16]. Due to Zn involvement in insulin secretion, insulin action in peripheral tissues, as well as possessing antiandrogenic and antioxidant properties[17,18].

2. Materials and methods

Conforming to the National Institutes of Health (NIH) guidelines concerning laboratory animal care and use (NIH Publications No. 8023, revised 1978), the current investigation was conducted at the Faculty of Medicine's Medical Biochemistry Department, Tanta University. The primary objective was to ensure the utmost care and minimize any potential distress encountered by the animals during the study. Prior to commencing the research, the Ethical Committee of Medical Research, Faculty of Medicine, Tanta University, Egypt, granted appropriate approval under the code 34384/1/21. Additionally, this committee offered continuous guidance throughout the research process.

2.1. Chemicals

All chemicals and solvents utilized, unless specifically noted were purchased exclusively from Sigma Aldrich (Sigma, St. Louis, USA), All of high analytic grade. Standard diet was purchased from El-Gomhoria Company, Cairo, Egypt.

2.2. Experimental animals

Conducted on 75 female albino rats weighing around 120-150g, obtained from Tanta University experimental animal colony. Throughout the investigation, the rats were accommodated in wire mesh cages, provided by standard diet Kcal% (Fat 5% [corn oil 5%], carbohydrates 65% [corn starch 15% and sucrose 50%], proteins 20.3% [casein 20% and DL-Methionine 3%], fiber 5%, salt mixture 3.7%, and vitamin mixture 1%) and unrestricted water intake. The rats remained in a stable environment with consistent conditions, including a temperature of 25 ºC and a lighting schedule of alternating 12-hours darkness and 12-hours light.

2.3. Experimental design.

In this study, rats were categorized into five distinct groups, each consisting of 15 rats. (1)The control group, was administered 0.5 % w/v carboxymethyl cellulose (CMC) via oral gavage on a daily basis for a total of 36 days; (2) The PCOS induced group, rats received letrozole at a dose of 1 mg/kg, dissolved in 0.5 % CMC, through oral gavage for 36 consecutive days [19]; (3) The PCOS group treated with ZnONPs, rats received letrozole just like the PCOS group, with intraperitoneal injections of ZnONPs (50 nm) of a purity of 97% once daily, at a dose of 5 mg/kg, starting from day 22 until day 36 [19,20]; (4) The PCOS group treated with metformin ,rats received letrozole like the previous PCOS groups , metformin was given via oral gavage once daily at a dose of 300 mg/kg from day 22 to day 36 [21]; (5) The PCOS group treated with both ZnONPs +metformin, rats received letrozole as in PCOS group and treated with both ZnONPs and metformin, mirroring the procedures followed in group (3) and (4).

2.4. Blood sampling

Upon concluding the study, the rats that had undergone overnight fasting were weighed. Following this, they were anesthetized using isoflourane and subsequently sacrificed through decapitation. A dry, sterile centrifuge tube was employed for blood collection, followed by a clotting period of thirty minutes at room temperature. Afterwards, it underwent a 10-minutes centrifugation at 3000 rpm. The resulting sera were then separated and stored in individual portions at a temperature of −80°C until they were ready to be utilized for additional analysis. Plasma, on the other hand, was separated in sterile tubes containing EDTA, specifically for glucose measurement purposes.

2.5. Tissue sampling

The initial step involved making an incision into the abdomen. Following this, the ovaries were removed, and a cleansing procedure with ice cold saline was performed to eliminate any extraneous substances. Excess saline was then absorbed using gauze, after which the ovaries were chilled on ice and subsequently divided for future use. The left ovaries were immersed in a solution of 10% neutral buffered formalin for fixation, allowing for examination of morphological alterations through the utilization of hematoxylin and eosin (H&E) stained sections. As for the right ovaries, they were divided into sections and preserved at a temperature of -80ºC until required for further analysis. One portion from each ovary was homogenized in ice cold phosphate-buffered saline, and then subjected to centrifugation (10,000 rpm at 4 °C for 15 minutes) to separate the supernatant for subsequent assessment of oxidative stress markers. The remaining segment of ovarian tissue was employed for estimating CYP17A1 gene expression.

2.6. Measured biochemical parameters

2.6.1. Hormonal analysis

Using ELISA technique, the Sun Red Biotechnology Company from Shanghai, China, provided the necessary kits for quantitative determination of serum testosterone and estradiol levels. For testosterone, the ELISA Testosterone (Rat) kit with Catalogue number #:201-11-5126 was used, while the ELISA estradiol (Rat) kit with Catalogue number #:201-11-0175 was utilized to measure estradiol levels.

2.6.2. Fasting plasma glucose, insulin levels and calculated homeostatic model assessments (HOMA-IR) to assess insulin resistance (Glucose indices).

The fasting plasma glucose level was measured using the enzymatic colorimetric method and a commercial kit sourced from Biodiagnostic, Egypt. To determine fasting serum insulin levels, a quantitative ELISA technique was applied, utilizing a commercially available insulin (Rat) kit obtained from Sun Red Biotechnology Company, Shanghai, China (Catalogue number #:201-11-0708). Afterwards, the HOMA-IR was calculated.

HOMA-IR equation = (Fasting insulin in mIU/L x Fasting glucose in mg/dL) / 405 [22].

2.6.3. Lipid profile

Enzymatic colorimetric assays were employed to detect Total cholesterol (TC), HDL-cholesterol (HDL-C) and Triacylglycerol (TAG) levels using kits acquired from Biodiagnostic, Egypt. Additionally, the estimation of LDL-cholesterol (LDL-C) was conducted via applying the FriedeWald's equation [23].

2.6.4. Oxidative stress markers in the ovary

Malondialdehyde (MDA) level, superoxide dismutase (SOD), and glutathione peroxidase (GPx) enzyme activities were measured in ovarian tissue homogenates (10% W/V) through the utilization of kits acquired from Biodiagnostic, Egypt.

2.6.5. Quantitative analysis of CYP17A1 gene expression by real-time polymerase chain reaction.

a) Following the utilization of the PureLink® RNA Mini Kit (Life Technologies Corporation, USA), total RNA was isolated from frozen ovarian tissue samples. Subsequently, the NanoDrop Spectrophotometer (analyticajena model scandrop, Germany) was employed to determine the concentration, purity and quality of the extracted mRNA.

b) The Fast Gene 55-Scriptase Complementary DNA (cDNA) synthesis kit (Nippon genetics Europe, LS-61) was employed to accomplish the reverse transcription of the extracted RNA into cDNA.

c) The cDNA amplification was conducted with the utilization of the SensiFAST™ SYBR® Lo-ROX Kit, which is provided by Bioline Reagents Ltd, United Kingdom (BIO-94005). In order to assess the CYP17A1 mRNA transcripts, the internal control, Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was used.

d) The design of sequence-specific primers was carried out as follows: For Rat CYP17A1 (Gene Bank Accession No. "NM_012753.3), the forward primer was (5'-ACAGTGATCATCGGCCACTAT C -3'), and the reverse primer was (5'- AGCTACCA GCATCTGCAAAG -3'). For rat GAPDH (Gene Bank Accession No. NM_0017008.4), the forward primer was (5'- CAACAGCAACTC CCATTCTTCC-3'), and the reverse primer was (5'-TCCAGGGTTTCTT ACTCCTTGG-3') [24].

The cycling procedure consisted of several stages. First, an initial activation phase was conducted at a temperature of 95°C for 2 minutes. This was followed by 40 cycles, with each cycle involving 5-seconds denaturation at 95°C, 10-seconds annealing at 60°C, and 20-seconds extension at 72°C. To perform the analysis, the Step One plus real-time thermal cycler (manufactured by Applied Biosystems, Life Technology, USA) and its accompanying software were used. The results were obtained based on the cycle threshold (Ct) values of the target genes, which were later normalized using the Ct value of the housekeeping gene. Finally, the $2^{-\Delta\Delta Ct}$ method was employed to calculate the fold change [25].

2.7. Histopathological analysis

The ovarian tissue samples were conserved in 10% neutral buffered formalin, were subjected to paraffin fixation. Afterward, the specimens stained with hematoxylin and eosin (H&E). Then examined using the Olympus BX51 light microscope equipped with its associated camera (Olympus DP50; Olympus Optical Co. ltd. Tokyo, Japan).

2.8. Statistical analysis

Statistical analysis was conducted using the mean ± standard deviation (SD), and the data were processed using the Statistical Package for Social Sciences (SPSS), version 16.0 for Windows (SPSS, Chicago, IL). The data were then subjected to the One-way ANOVA test, followed by the post-hoc Tukey test.

3. Results

3.1. Anthropometric measurements

The final body weight and ovarian weight of the PCOS group exhibited a significant increase in comparison to the treated and the control groups, as indicated by statistical analysis. The groups treated with ZnONPs or/and metformin also demonstrated a substantial increase in comparison to the control group. Although no statistically significant distinction was detected between the ZnONPs and metformin-treated groups, but they both exhibited an increase when compared to the group received both ZnONPs and metformin (Table 1).

3.2. Serum sex hormonal levels

The Letrozole induced PCOS group exhibited a profound disruption in sex hormones, displaying a noteworthy rise in testosterone levels and a considerable decrease in estradiol levels, in comparison to the treated and the control groups. Conversely, the administration of ZnONPs or/and metformin to the affected groups substantially ameliorated this impairment. However, the treatment groups showed a considerable rise in testosterone levels and a noticeable decline in estradiol levels in comparison to the control group. Notably, no statistically significant difference was found between the groups treated with ZnONPs and metformin. Nevertheless, both groups exhibited a significant increase in testosterone levels and a noteworty decrease in estradiol levels, in comparison to the group that received a combined treatment of ZnONPs and metformin (Table 1).

3.3. Fasting plasma glucose, insulin levels and HOMA-IR (Glucose indices)

The fasting plasma glucose, insulin levels, and HOMA-IR demonstrated a significant increase in the PCOS group when compared to both the treated and the control groups. Conversely, even after treatment with ZnONPs or/and metformin, the groups still demonstrated a statistically significant increase compared to controls. While there was no statistically significant distinction between the ZnONPs and metformin-treated groups, both of these groups displayed a statistically significant increase in comparison to the group that received a combination of ZnONPs + metformin (Fig. 1,2,3).

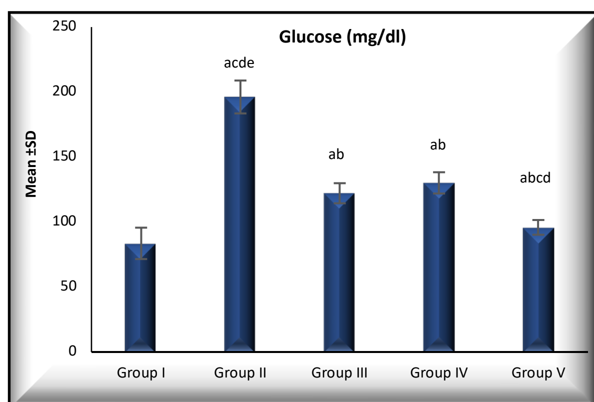

Fig. 1. The fasting plasma glucose levels in the study groups. [a]p< 0.05 significance versus control, [b]p< 0.05 significance versus PCOS, [c]p< 0.05 significance versus ZnONPs, [d]p< 0.05 significance versus metformin, [e]p< 0.05 significance versus ZnONPs+ metformin.

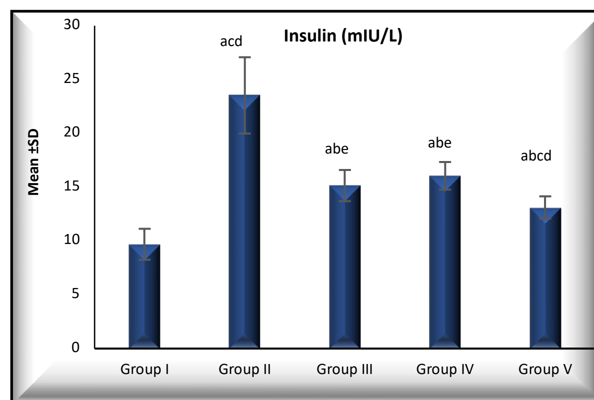

Fig. 2. The fasting serum insulin level in the study groups. [a]p< 0.05 significance versus control, [b]p< 0.05 significance versus PCOS, [c]p< 0.05 significance versus ZnONPs, [d]p< 0.05 significance versus metformin, [e]p< 0.05 significance versus ZnONPs+ metformin.

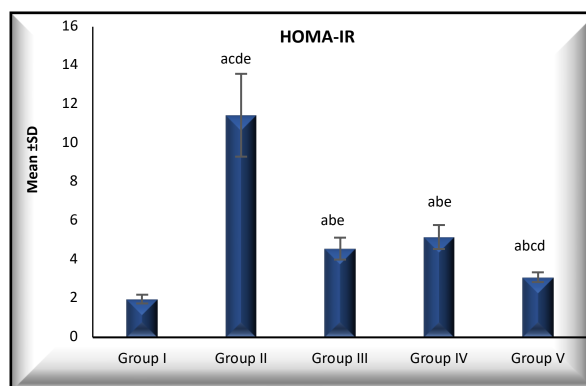

Fig. 3. The HOMA-IR in the study groups. [a]p< 0.05 significance versus control, [b]p< 0.05 significance versus PCOS, [c]p< 0.05 significance versus ZnONPs, [d]p< 0.05 significance versus metformin, [e]p< 0.05 significance versus ZnONPs+ metformin.

3.4. Lipid profile

The serum TAG, TC, and LDL-C levels significantly increased, while the serum HDL-C levels significantly decreased in the PCOS group in comparison to the treated and the control groups. Even after treatment with ZnONPs or/and metformin, the groups still showed a considerable increase in serum TAG, TC, and LDL-C levels, as well as a significant decrease in serum HDL-C levels in comparison to the control group. The comparison of the ZnONPs and metformin-treated groups did not reveal any statistically significant difference, yet they both displayed a notable increase in serum TAG, TC, and LDL-C levels, along with a significant reduction in serum HDL-C levels in comparison to the group that received both ZnONPs and metformin (Table 1).

3.5. Oxidative stress markers

The PCOS group demonstrated significant oxidative stress, evident from a significant rise in MDA levels and a decline in GPx and SOD enzyme activities, in comparison to both the control and treated groups. Despite receiving treatment with ZnONPs or/and metformin, the groups continued to show a noteworthy elevation in MDA levels and a considerable reduction in SOD and GPx enzyme activities compared to the

control group. Interestingly, the GPx and SOD enzyme activities in the ZnONPs treated group demonstrated a more significant increase when compared to the metformin treated group. Nevertheless, both groups exhibited a statistically significant increase in MDA levels and a statistically significant decline in SOD and GPx enzyme activities when compared to the group that received both ZnONPs and metformin (Table 1).

3.6. Quantitative analysis of ovarian CYP17A1 gene expression by real-time polymerase chain reaction (RT-PCR).

The relative gene expression of CYP17A1, the key enzyme in androgen biosynthesis, demonstrated a significant increase in the PCOS group in comparison to both the treated and the control groups. Nonetheless, the groups treated with ZnONPs or/and metformin still exhibited a noteworthy rise when compared to the control group. The difference between the ZnONPs and metformin-treated groups was not statistically significant; however, both groups showed a notable increase when compared to the group that received both ZnONPs + metformin (Fig. 4).

3.7. Results of the histopathological examination

Histological examination utilizing H&E staining revealed that in group I (the control group), the ovarian architecture and thickness were normal, exhibiting numerous corpus lutea and mature graffian follicles. Conversely, group II (the PCOS group) displayed various subcapsular cysts but lacked graffian follicles and corpus lutea. On the other hand, treated groups III, IV, and V (ZnoNPS, metformin, and combination) receiving treatment demonstrated minimal ovarian alterations, characterized by multiple corpus lutea and mature graffian follicles, along with a reduced presence of cysts (fig. 5).

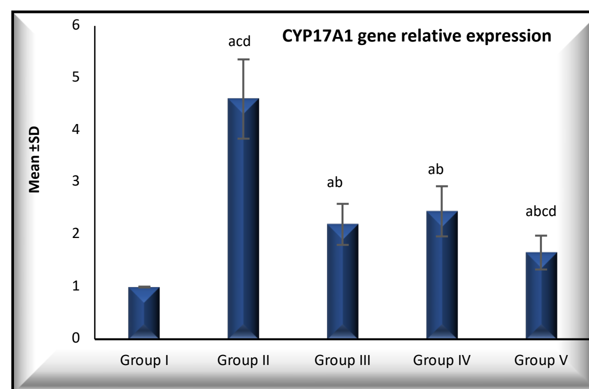

Fig. 4. The ovarian CYP17A1 gene relative expression in the study groups. [a]p< 0.05 significance versus control, [b]p< 0.05 significance versus PCOS, [c]p< 0.05 significance versus ZnONPs, [d]p< 0.05 significance versus metformin, [e]p< 0.05 significance versus ZnONPs+ metformin.

Table 1: Effect of different treatments on anthropometric measurements and biochemical parameters in the studied groups

	Control	PCOS	ZnONPs	Metformin	ZnONPs + metformin
Final Body weight (g)	180.360 ±12.982	231.340 ±10.150^{acde}	201.08 ±7.678^{abe}	202.247 ± 5.918^{abe}	191.553 ±7.010^{abcd}
Ovarian weight (mg)	61.786 ± 8.001	109.726 ± 9.045^{acde}	81.460 ± 6.925^{abe}	83.366 ±10.037^{abe}	72.600 ±6.563^{abcd}
TAG (mg/dl)	70.366 ± 7.678	155.053 ±12.278^{acde}	101.313 ±7.410^{abe}	106.525 ±6.363^{abe}	81.690 ±7.239^{abcd}
Total cholesterol (TC) (mg/dl)	84.960 ±10.020	190.866 ±20.705^{acde}	123.933 ±19.910^{abe}	129.366±26.639^{abe}	101.935±8.016^{abcd}
LDL-C (mg/dl)	30.368 ± 9.425	154.019 ±22.596^{acde}	76.409 ±1.550^{abe}	84.417 ±27.259^{abe}	50.790 ±8.320^{abcd}
HDL-C (mg/dl)	45.795 ±5.507	17.466±5.265^{acde}	34.860±3.231^{abe}	31.633±3.638^{abe}	40.933±3.188^{abcd}
Testosterone (ng/dl)	70.660±7.050	133.153±11.506^{acde}	87.752±6.998^{abe}	91.186±7.410^{abe}	79.400±6.045^{abcd}
Estradiol (pg/mL)	68.520 ±4.431	27.513±5.844^{acde}	51.360±7.361^{abe}	47.300±5.094^{abe}	59.080±5.470^{abcd}
MDA (nmol/g.tissue)	28.086 ±2.676	59.826±3.844^{acde}	36.567±3.065^{abe}	39.418±3.495^{abe}	31.696±3.043^{abcd}
SOD (U/mg protein)	59.720±2.208	23.107±1.830^{acde}	44.467±2.135^{abde}	39.835±2.696^{abce}	51.300±2.151^{abcd}
Gpx (U/mg protein)	161.853±2.566	72.680±6.193^{acde}	122.060±8.502^{abde}	109.747±3.833^{abce}	145.993±5.461^{abcd}

a-e: significant difference between the study groups at p<0. 05*. Data are mean + standard deviation (SD). The one way ANOVA test and Tukey's post hoc test were applied. [a]p< 0.05 significance versus control, [b]p< 0.05 significance versus PCOS, [c]p< 0.05 significance versus ZnONPs, [d]p< 0.05 significance versus metformin, [e]p< 0.05 significance versus ZnONPs+ metformin.

4. Discussion

The significant impact of PCOS on both health and socioeconomic aspects cannot be overlooked, and is believed to be a crucial cause in female infertility related to ovulation issues [26]. Moreover, PCOS is implicated in the pathogenesis of some disorders like type 2 diabetes, cardiovascular diseases, gynecological tumors and immune disorders [27].

Metformin, a conventional approach, proves its efficacy in PCOS treatment. Numerous studies support its role in enhancing insulin sensitivity, reinstating a typical hormonal profile in the ovaries, promoting ovulation, and ameliorating menstrual patterns. Additionally, it provides other metabolic advantages such as reducing weight and dyslipidemia [28,29].

Lately, there has been a significant surge in fascination surrounding nanomedicine-based approaches, owing to their potential for active / passive targeting, excellent solubility, high bioavailability, biocompatibility, and multifunctionality [30]. Among the various metal nanoparticles available, ZnONPs have garnered considerable interest for their use in addressing numerous medical conditions, such as cancer, insulin resistance, diabetes mellitus, and diabetic

complications, primarily due to their remarkable ability to efficiently deliver zinc ions [31,32].

In this study, the primary objective was to examine how ZnONPs, both alone and in conjunction with metformin, can modulate Letrozole-induced PCOS. The study focused on several aspects, including hyperandrogenism, insulin resistance, and oxidative stress, while also assessing the impact of ZnONPs on CYP17A1 gene expression.

The Letrozole-induced PCOS group exhibited elevated androgen, alongside diminished estrogen levels. These findings can be attributed to Letrozole's capacity to impede androgen-to-estrogen conversion, thereby elevating serum and intra-ovarian androgen levels, culminating in sex hormone imbalances and follicular atresia with multiple ovarian cysts formation, absence of mature graffian follicles and corpus lutea in histopathological examination with increased ovarian weight [33].

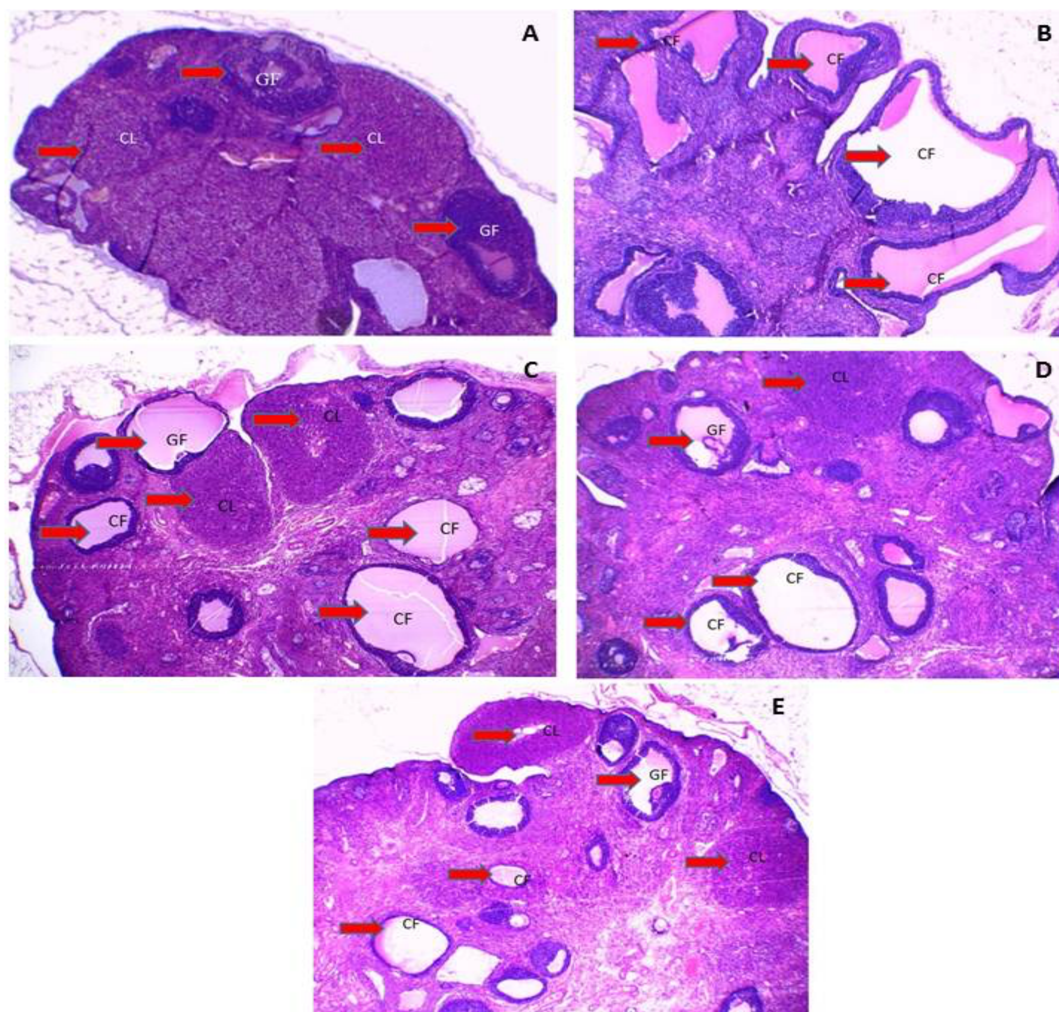

Fig. 5. Histopathological examination of ovary: A) control group. B) PCOS group. C) ZnONPs treated group. D) metformin treated group E) ZnONPs +metformin treated group.CL; corpus luteum, CF; cystic follicle, GF; graffian follicle. All slides are H&E stained. Magnification: ×100.

Furthermore, the PCOS group displayed fasting hyperglycemia and insulin resistance, which can be elucidated by the impact of heightened androgen levels on GLUT 4 expression and sensitivity with increased adipocyte mass and visceral adiposity, resulting in increased body weight, insulin resistance, dyslipidemia and oxidative stress [33,34]. Additionally, a decline in adiponectin, a vital adipocytokine responsible for regulating energy, insulin, glucose, and lipid metabolism through its insulin-sensitizing and anti-inflammatory properties, was observed in visceral adiposity, contributing to insulin resistance and hyperglycemia [35].

Furthermore, it was observed that a vicious cycle emerged, triggered by insulin resistance and resulting hyperinsulinemia. Insulin acts peripherally causing a decline in sex hormone-binding globulin (SHBG), subsequently increasing free androgen levels and centrally magnifying GnRH frequency favoring LH over FSH production [36,37]. Notably, insulin, and LH, act synergistically resulting in an overactive androgenic state through hyperthecosis[38]. The disturbed glucose metabolism observed in PCOS aligns with findings from prior PCOS model investigations [39,40].

Besides, the role of CYP17A1 as a contributing gene in the development of PCOS has been documented. The CYP17A1 gene is responsible for encoding 17-α-hydroxylase/17–20 lyase (P450 17α), a crucial regulatory enzyme that catalyzes a pivotal androgen biosynthesis process within the steroidogenic pathway [41].

In PCOS group, a significant rise in the relative expression of the CYP17A1 gene was noted. This rise can be linked to insulin's ability to heighten both the frequency and magnitude of GnRH, increasing LH pulse secretion resulting in the augmentation of the relative expression of the CYP17A1 gene [42]. Furthermore, the escalated insulin secretion appears to imitate the tropic effects of LH on ovarian theca cells, ultimately resulting in the upregulation of CYP17A1 gene expression [43,44]. The association between insulin and CYP17A1 has been substantiated by prior research studies [45,46].

The primary cause of the disturbed lipid profile in PCOS is primarily linked to elevated androgen levels and insulin resistance [47]. In the current investigation, the PCOS group displayed an altered lipid profile and dyslipidemia, which concurred with previous research on PCOS [48].

PCOS presents another prominent aspect known as oxidative stress, which exhibits a direct correlation with insulin resistance and testosterone levels. [49].Malondialdehyde (MDA) serves as an indicator of lipid peroxidation and indirectly reflects the extent of cellular and tissue harm [50]. Concurrently, glutathione peroxidase (GPx) and superoxide dismutase (SOD) are enzymes responsible for neutralizing superoxide radicals and hydrogen peroxide, consequently safeguarding the cells from damage caused by lipid peroxidation mediated by these molecules [51]. In PCOS group, they showed a significant rise in MDA level and a significant decline in the activities of antioxidant enzymes GPx and SOD.

Interestingly, the group treated with ZnONPs showed significant improvement in fasting plasma glucose level, insulin-related homeostasis. The reason behind the beneficial effect of ZnONPs in PCOS lies in their capacity to release zinc ions. This is crucial because zinc plays a fundamental role in the synthesis, secretion, and sensitivity of insulin. An essential aspect is that Zn has the ability to attach to and render protein tyrosine phosphatase (PTPase) 1β inactive [52], an enzyme responsible for dephosphorylating the β subunit of the insulin receptor. Consequently, Zn deactivates this enzyme, leading to an increase in insulin receptor phosphorylation [53].

Furthermore, it should be noted that zinc has the ability to hinder Phosphatase and tensin homolog (PTEN), an enzyme responsible for catalyzing the dephosphorylation of phosphatidylinositol 3,4,5-triphosphate (PIP3), inhibiting protein Akt activation [54]. As a result, Akt remains activated and facilitates the translocation of GLUT4, subsequently, improving the uptake of glucose by cells [55].

Zinc plays a fundamental role in triggering the phosphorylation process of two important proteins, forkhead box protein O1 (FoxO1) and glycogen synthase kinase 3 (GSK-3), so exhibit similar effects to insulin [56]. Consequently, the suppression of GSK-3 occurs, leading to a preference for the dephosphorylation and activation of glycogen synthase, the critical enzyme in glycogen synthesis [57]. Concurrently, the phosphorylation of FoxO1 induces its translocation from the nucleus to the cytoplasm, thus inhibiting its capacity to activate gluconeogenic genes [58].

Previous studies conducted on high fat diet, obesity, and diabetes have proven the hypoglycemic impact of ZnONPs [59-61]. ZnONPs effectively disrupted the harmful cycle between insulin resistance and

hyperandrogenism, leading to an enhancement in PCOS-related hyperandrogenism [62]. Also, the reduction in CYP17A1 gene expression brought about by ZnONPs enhanced insulin sensitivity and improved insulin levels. In addition, through improving ovarian histopathological studies with less cysts and appearance of mature graffian follicles and corpus lutea with reduced ovarian weight. .Furthermore,the administration of ZnONPs ameliorated dyslipidemia as a result of improved insulin sensitivity and androgen level [63,64].

The current investigation observed that ZnONPs resulted in a significant enhancement of the redox status. This improvement can be attributed to the antioxidant properties of ZnONPs, as zinc acts as a cofactor for the antioxidant enzymes Cu-Zn-superoxide dismutase (SOD1) and glutathione peroxidase (GPx) [65]. Additionally, ZnONPs exert their influence on Nuclear factor erythroid 2-related factor 2 (Nrf2), a crucial transcription factor responsible for regulating the activity of the antioxidant system [66].Also, indirectly through improved insulin sensitivity and hyperandrogenism the main contributors for oxidative stress.

5. Conclusion

To conclude, the current investigation demonstrated the robust and promising capacity of ZnONPs in addressing PCOS deficiencies through a multitude of mechanisms. These include enhancements in insulin sensitivity, antiandrogenic effects, antioxidant properties, and their role as essential modulators of CYP17A1 gene expression, which encodes the key androgen-producing enzyme in PCOS. Notably, the combination of ZnONPs with metformin yielded even more significant results and synergistic effects. It is highly encouraged to conduct additional research to extensively investigate the therapeutic possibilities of ZnONPs in addressing the various manifestations of PCOS. Moreover, exploring its potential in combination with varied and tailored doses of metformin could be beneficial in mitigating the side effects associated with both treatments.

Conflict of interest

No potential conflicts of interest were reported by the authors.

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