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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).

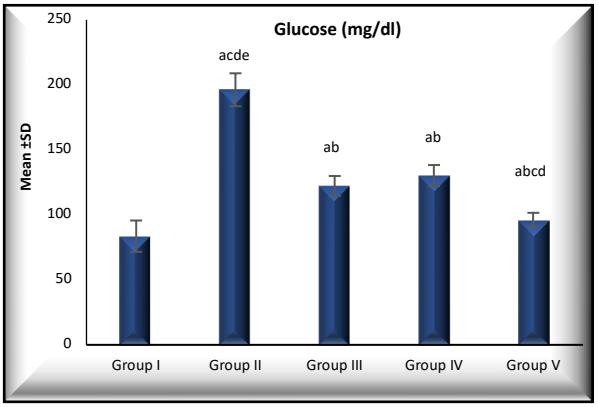

Fig. 1. The fasting plasma glucose levels in the study groups. [a]p< 0.05 significance versus control, [b]p< 0.05 significance versus PCOS, [c]p< 0.05 significance versus ZnONPs, [d]p< 0.05 significance versus metformin, [e]p< 0.05 significance versus ZnONPs+ metformin.

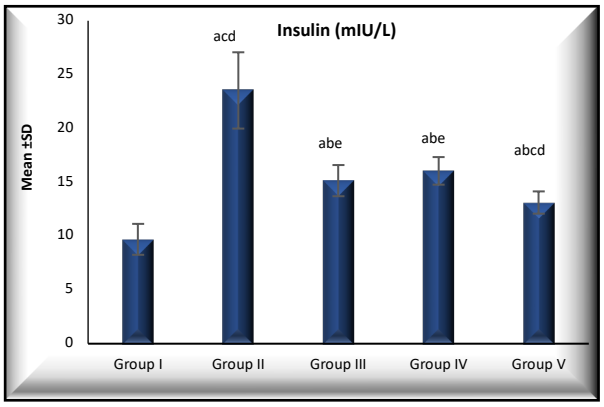

Fig. 2. The fasting serum insulin level in the study groups. [a]p< 0.05 significance versus control, [b]p< 0.05 significance versus PCOS, [c]p< 0.05 significance versus ZnONPs, [d]p< 0.05 significance versus metformin, [e]p< 0.05 significance versus ZnONPs+ metformin.

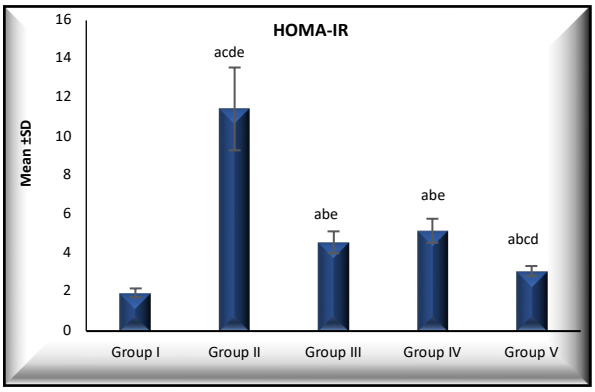

Fig. 3. The HOMA-IR in the study groups. [a]p< 0.05 significance versus control, [b]p< 0.05 significance versus PCOS, [c]p< 0.05 significance versus ZnONPs, [d]p< 0.05 significance versus metformin, [e]p< 0.05 significance versus ZnONPs+ metformin.

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).

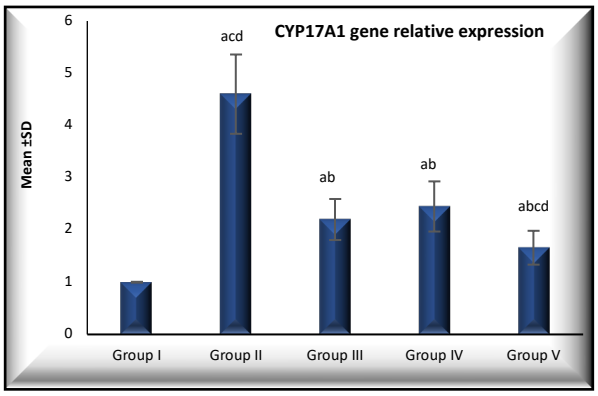

Fig. 4. The ovarian CYP17A1 gene relative expression in the study groups. [a]p< 0.05 significance versus control, [b]p< 0.05 significance versus PCOS, [c]p< 0.05 significance versus ZnONPs, [d]p< 0.05 significance versus metformin, [e]p< 0.05 significance versus ZnONPs+ metformin.

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].

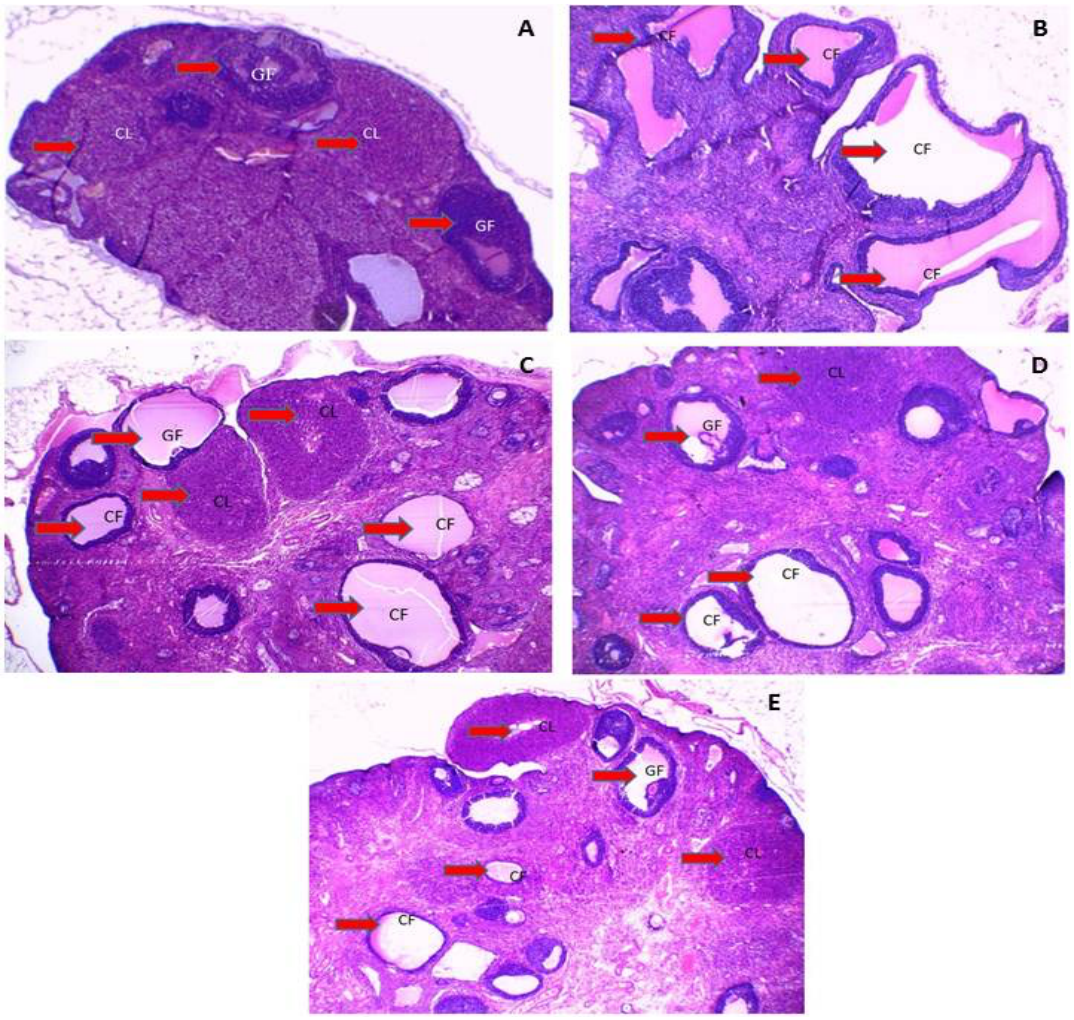

Fig. 5. Histopathological examination of ovary: A) control group. B) PCOS group. C) ZnONPs treated group. D) metformin treated group E) ZnONPs +metformin treated group.CL; corpus luteum, CF; cystic follicle, GF; graffian follicle. All slides are H&E stained. Magnification: ×100.

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.

References

[1] Dai X, Li J, Fu T, Long X, Li X, Weng R, et al. Sequential letrozole/ gonadotropin versus letrozole alone for ovulation induction in infertile women with polycystic ovary syndrome: A randomized controlled trial. Reproductive BioMedicine Online. 2023; 46(2):352-361.

[2] Rababa'h AM, Matani BR, Yehya A. An update of polycystic ovary syndrome: causes and therapeutics options. Heliyon. 2022; 8(10):1-8.

[3] Hsieh Y-C, Yang P-K, Chen M-J. Metabolic Syndrome in Polycystic Ovary Syndrome. Fertility & Reproduction. 2021; 3(04):125-35.

[4] Siddiqui S, Mateen S, Ahmad R, Moin S. A brief insight into the etiology, genetics, and immunology of polycystic ovarian syndrome (PCOS). Journal of Assisted Reproduction and Genetics. 2022; 39(11):2439-2473.

[5] Allahbadia GN, Merchant R. Polycystic ovary syndrome and impact on health. Middle East Fertility Society Journal. 2011; 16(1):19-37.

[6] Sudhakaran G, Guru A, Muthu BHD, Murugan R, Arshad A, Arockiaraj J. Evidence-based hormonal, mutational, and endocrine-disrupting chemical-induced zebrafish as an alternative model to study PCOS condition similar to mammalian PCOS model. Life Sciences 2022; 291:1-14. doi: 10.1016/j.lfs.2021.120276.

[7] Carreau AM, Battista MC, Baillargeon JP. Insulin Resistance and Lipotoxicity in PCOS: Causes and Consequences. In: Pal, L., Seifer, D.B. (eds) Polycystic Ovary Syndrome: Current and Emerging Concepts. Cham: Springer.; 2022. p. 133-54. doi.org/10.1007/978-3-030-92589-5_8.

[8] Wang J, Wu D, Guo H, Li M. Hyperandrogenemia and insulin resistance: The chief culprit of polycystic ovary syndrome. Life Sciences. 2019; 236:1-9. doi: 10.1016/j.lfs.2019.116940.

[9] Gjorgoska M, Rizner TL. Integration of androgen hormones in endometrial cancer biology. Trends in Endocrinology & Metabolism. 2022; 33(9):639-651.

[10] Mason HD, Dilaver N, Rice S. Ovarian Dysfunction in Polycystic Ovary Syndrome (PCOS). In: Pal, L., Seifer, D.B. (eds) Polycystic Ovary Syndrome: Current and Emerging Concepts. Cham: Springer.; 2022. p. 95-120. doi.org/10.1007/978-3-030-92589-5_6

[11] Kumar GS, Tirgar P, Dalal M. Development and evaluation of novel rodent model of PCOS mimicking clinical phenotype in human disease. Middle East Fertility Society Journal. 2022; 27(1):1-13.

[12] Shpakov AO. Improvement effect of metformin on female and male reproduction in endocrine pathologies and its mechanisms. Pharmaceuticals. 2021;14(1):1-45.

[13] Drzewoski J, Hanefeld M. The current and potential therapeutic use of metformin—the good old drug. Pharmaceuticals. 2021;14(2):1-33.

[14] Baynes K. Diabetes Mellitus, Obesity, Lipoprotein Disorders and other Metabolic Diseases. Medicine for Finals and Beyond: CRC Press. p. 429-70.e Book ISBN9781003193616.

[15] Patel JK, Patel A, Bhatia D. Introduction to nanomaterials and nanotechnology. In: Emerging Technologies for Nanoparticle Manufacturing: Cham: Springer International Publishing; 2021. p. 109-128.
[16] Lin CY, Adhikary P, Cheng K. Cellular protein markers, therapeutics, and drug delivery strategies in the treatment of diabetes-associated liver fibrosis. Advanced Drug Delivery Reviews.2021;174:127-39.doi: 10.1016/j.addr.2021.04.008.
[17] Aliyev U, Pehlivantürk-Kızılkan M, Düzçeker Y, Kanbur N, Aycan Z, Akgül S, et al. Is There Any Association Between Hirsutism and Serum Zinc Levels in Adolescents? Biological Trace Element Research. 2020; 198(2):403-9.
[18] Ramorobi LM, Matowane GR, Mashele SS, Bonnet SL, Noreljaleel AE, Swain SS, et al. Bioactive synergism between zinc mineral and p-coumaric acid: a multi-mode glycemic control and antioxidative study. Journal of Food Biochemistry. 2022; 46(10):1-17.
[19] Butt MA, Ullah A, Kiyani MM, Jahan S. Ameliorative effects of selenium nanoparticles on letrozole induced polycystic ovarian syndrome in adult rats. International Journal of Biomedical Nanoscience and Nanotechnology. 2020;4(1-2):49-69.
[20] Othman MS, Hafez MM, Abdel Moneim AE. The potential role of zinc oxide nanoparticles in MicroRNAs dysregulation in STZ-induced type 2 diabetes in rats. Biological trace element research. 2020;197(2):606-618.
[21] Shen X, Wang L, Zhou N, Gai S, Liu X, Zhang S. Beneficial effects of combination therapy of phloretin and metformin in streptozotocin-induced diabetic rats and improved insulin sensitivity in vitro. Food & Function. 2020;11(1):392-403.
[22] Duseja A, Thumburu KK, Das A, Dhiman RK, Chawla YK, Bhadada S, et al., Insulin tolerance test is comparable to homeostasis model assessment for insulin resistance in patients with nonalcoholic fatty liver disease. Indian Journal of Gastroenterology, 2007; 26(4):170-173.
[23] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry. 1972; 18(6):499-502.
[24] Nejabati HR, Samadi N, Shahnazi V, Mihanfar A, Fattahi A, Latifi Z, et al., Nicotinamide and its metabolite N1-Methylnicotinamide alleviate endocrine and metabolic abnormalities in adipose and ovarian tissues in rat model of Polycystic Ovary Syndrome. Chemico-Biological Interactions. 2020; 324:1-8. doi: 10.1016/j.cbi.2020.109093.
[25] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods, 2001; 25(4):402-408.
[26] Huang L, Huang Y, Pang Q. Molecular mechanism of Bushen Huatan Prescription in the treatment of Polycystic ovary syndrome based on network pharmacology and bioinformatics. Pharmacological Research-Modern Chinese Medicine. 2022; 5:1-8.Doi: 10.1016/j.prmcm.
[27] Xu Y, Zhu H, Li W, Chen D, Xu Y, Xu A, et al. Targeting adipokines in polycystic ovary syndrome and related metabolic disorders: from experimental insights to clinical studies. Pharmacology & Therapeutics. 2022; 240:1-17. doi: 10.1016/j.pharmthera.2022.108284.
[28] Feng J, Wang X, Ye X, Ares I, Lopez-Torres B, Martínez M, et al. Mitochondria as an important target of metformin: The mechanism of action, toxic and side effects, and new therapeutic applications. Pharmacological Research. 2022;177:1-18. doi: 10.1016/j.phrs.2022.106114.
[29] Vieira IH, Barros LM, Baptista CF, Rodrigues DM, Paiva IM. Recommendations for Practical Use of Metformin, a Central Pharmacological Therapy in Type 2 Diabetes. Clinical Diabetes. 2022; 40(1):97-107.
[30] Nowak-Jary J, Machnicka B. Pharmacokinetics of magnetic iron oxide nanoparticles for medical applications. Journal of Nanobiotechnology. 2022; 20(1):1-30.
[31] Sri D, YelamandaRao K, Basha S, Lakshmi M, Reddy LV, Mannarapu M, et al. Biosynthesis of zinc oxide nanoparticles using aqueous extract of Andrographis alata: characterization, optimization and assessment of their antibacterial, antioxidant, antidiabetic and anti-Alzheimer's properties. Journal of Molecular Structure. 2022;1273:1-15. doi.org/10.1016/j.molstruc.2022.134264.
[32] Deka B, Baruah C, Babu A, Kalita P. Biological and Non-Conventional Synthesis of Zinc Oxide Nanoparticles (ZnO-NPs): Their Potential Applications. Journal of Nanotechnology and Nanomaterials. 2022; 3(2):79-89.
[33] Zhou Y, Lan H, Dong Z, Li W, Qian B, Zeng Z, et al. Rhamnocitrin Attenuates Ovarian Fibrosis in Rats with Letrozole-Induced Experimental Polycystic Ovary Syndrome. Oxidative Medicine and Cellular Longevity. 2022; 1-18. doi: 10.1155/2022/5558599.
[34] Andrisse S, Feng M, Wang Z, Awe O, Yu L, Zhang H, et al. Androgen-induced insulin resistance is ameliorated by deletion of hepatic androgen receptor in females. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2021;35(10):1-27.
[35] Vachher M, Bansal S, Kumar B, Yadav S, Arora T, Wali NM, et al. Contribution of organokines in the development of NAFLD/NASH associated hepatocellular carcinoma. Journal of Cellular Biochemistry. 2022;123(10):1553-1584.
[36] Walters KA, Moreno-Asso A, Stepto NK, Pankhurst MW, Paris VR, Rodgers RJ. Key

signalling pathways underlying the aetiology of polycystic ovary syndrome. Journal of Endocrinology. 2022;255(1):1-26.
[37] Larsson SC, Spyrou N, Mantzoros CS. Body fatness associations with cancer from recent epidemiologic studies. Metabolism. 2022;137:1-12. doi: 10.1016/j.metabol.2022.155326.
[38] Hernández-Jiménez JL, Barrera D, Espinoza-Simón E, González J, Ortíz-Hernández R, Escobar L, et al. Polycystic ovarian syndrome: signs and feedback effects of hyperandrogenism and insulin resistance. Gynecological Endocrinology. 2022; 38(1):2-9.
[39] Dey A, Dhadhal S, Maharjan R, Nagar PS, Nampoothiri L. Partially purified non-polar phytocomponents from Aloe barbadensis Mill. gel restores metabolic and reproductive comorbidities in letrozole-induced polycystic ovary syndrome rodent model-an "in-vivo" study. Journal of Ethnopharmacology. 2022;291:1-17. doi: 10.1016/j.jep.2022.115161.
[40] Pervaz S, Ullah A, Adu-Gyamfi EA, Lamptey J, Sah SK, Wang MJ, Wang YX. Role of CPXM1 in impaired glucose metabolism and ovarian dysfunction in polycystic ovary syndrome. Reproductive Sciences. 2023;30(2):526-43. doi.org/10.1007/s43032-022-00987-y
[41] Sanchez-Garrido MA, Tena-Sempere MJ. Metabolic dysfunction in polycystic ovary syndrome: Pathogenic role of androgen excess and potential therapeutic strategies. Mol Metab. 2020; 35:1-16. doi.org/10.1016/j.molmet.2020.01.001
[42] Evans MC, Hill JW, Anderson GM. Role of insulin in the neuroendocrine control of reproduction. J Neuroendocrinol. 2021; 33(4):1-9
[43] Bakir MB, Abdel-Mageed SM, Mohamed EI. Etiology, Management, and Treatment of Polycystic Ovary Syndrome: A Systematic Review. Acta Scientific Women's Health. 2021; 3(3):1-18.
[44] Thongkittidilok C, Singh RP, Comizzoli P, Wildt D, Songsasen N, Insulin promotes preantral follicle growth and antrum formation through temporal expression of genes regulating steroidogenesis and water transport in the cat. Reprod Fertil Dev. 2018; 30(10):1369-1379.
[45] Jin Y, Zhang Q, Pan JX, Wang FF, Qu F. The effects of di (2-ethylhexyl) phthalate exposure in women with polycystic ovary syndrome undergoing in vitro fertilization. Journal of International Medical Research. 2019;47(12):6278-6293.
[46] Karam M, Najjar H, El Sabban M, Hamade A, Najjar F. Regenerative Medicine for Polycystic Ovary Syndrome: Stem Cell-Based Therapies and Brown Adipose Tissue Activation. Stem Cell Reviews and Reports. 2023;19(4):853-865.doi: 10.1007/s12015-023-10505-5
[47] Mićić B, Teofilović A, Djordjevic A, Veličković N, Macut D, Vojnović Milutinović D. AMPK Activation Is Important for the Preservation of Insulin Sensitivity in Visceral, but Not in Subcutaneous Adipose Tissue of Postnatally Overfed Rat Model of Polycystic Ovary Syndrome. International Journal of Molecular Sciences. 2022;23(16):1-17.
[48] Wu YX, Yang XY, Han B-S, Hu YY, An T, Lv BH, et al. Naringenin regulates gut microbiota and SIRT1/PGC-1α signaling pathway in rats with letrozole-induced polycystic ovary syndrome. Biomedicine & Pharmacotherapy. 2022; 153:1-13. doi: 10.1016/j.biopha.2022.113286.
[49] Bednarz K, Kowalczyk K, Cwynar M, Czapla D, Czarkowski W, Kmita D, et al. The Role of Glp-1 receptor agonists in insulin resistance with concomitant obesity treatment in polycystic ovary syndrome. International Journal of Molecular Sciences. 2022; 23(8):1-22.
[50] Zhou YX, Yuan X, Hu XF, Yang SS, Zhong SW, Yang TY, et al. Changes of oxidant-antioxidant parameters in small intestines from rabbits infected with E. intestinalis and E. magna. World Rabbit Science. 2022; 30(4):287-293.
[51] Fujii J, Homma T, Osaki T. Superoxide radicals in the execution of cell death. Antioxidants. 2022;11(3):1-30.
[52] Tamura Y, Thrombosis. The role of zinc homeostasis in the prevention of diabetes mellitus and cardiovascular diseases. Journal of Atherosclerosis and Thrombosis. 2021;28(11):1109-1122.
[53] Zhao T, Huang Q, Su Y, Sun W, Huang Q, Wei W. Zinc and its regulators in pancreas. Inflammopharmacology.2019;27(3):453-464.
[54] Choi S, Kang D, Kang J, Hong DK, Kang BS, Kho AR, et al. The Role of Zinc in Axon Formation via the mTORC1 Pathway. Molecular Neurobiology. 2022;59(5):3206-3217.
[55] Vivero A, Ruz M, Rivera M, Miranda K, Sacristán C, Espinosa A, et al. Zinc supplementation and strength exercise in rats with type 2 diabetes: Akt and PTP1B phosphorylation in nonalcoholic fatty liver. Biological trace element research. 2021;199(6):2215-2224.
[56] Morais JBS, Severo JS, Beserra JB, de Oiveira ARS, Cruz KJC, de Sousa Melo SR, et al. Association between cortisol, insulin resistance and zinc in obesity: a mini-review. Biological trace element research. 2019;191(2):323-30.
[57] Bala A, Roy S, Das D, Marturi V, Mondal C, Patra S, et al. Role of Glycogen synthase kinase-3 in the etiology of Type 2 Diabetes Mellitus: A review. Current Diabetes Reviews.2022;18(3):1-7.
[58] Guo S, Mangal R, Dandu C, Geng X, Ding YJA, Disease. Role of Forkhead Box Protein O1 (FoxO1) in Stroke: A Literature Review. 2022; 13(2):521-533.
[59] Abdulmalek S, Eldala A, Awad D, Balbaa M. Ameliorative effect of curcumin and zinc oxide nanoparticles on multiple mechanisms in obese rats

with induced type 2 diabetes. Scientific reports. 2021;11(1):1-22.

[60] El-Daly SM, Medhat D,ـA El-Bana M, Abdel-Latif Y, El-Naggar ME, Omara EA, et al. Stimulatory effect of docosahexaenoic acid alone or loaded in zinc oxide or silver nanoparticles on the expression of glucose transport pathway. Prostaglandins & Other Lipid Mediators. 2021; 155:1-9. DOI: 10.1016/j. .prostaglandins.2021.106566

[61] Jeyabharathi S, Naveenkumar S, Chandramohan S, Venkateshan N, Gawwad MRA, Elshikh MS, et al. Biological synthesis of zinc oxide nanoparticles from the plant extract, Wattakaka volubilis showed anti-microbial and anti-hyperglycemic effects. Journal of King Saud University-Science. 2022;34(3):1-8.

[62] Peng-Winkler Y, Wessels I, Rink L, Fischer HJ. Zinc Levels Affect the Metabolic Switch of T Cells by Modulating Glucose Uptake and Insulin Receptor Signaling. Molecular Nutrition & Food Research. 2022;66(9):1-10.

[63] Elseidy AM, Bashandy SA, Ibrahim FA, Abd El-Rahman SS, Farid O, Moussa S, et al. Zinc oxide nanoparticles characterization and therapeutic evaluation on high fat/sucrose diet induced-obesity. Egyptian Journal of Chemistry. 2022;65(9):1-3.

[64] Dogra S, Kar AK, Girdhar K, Daniel PV, Chatterjee S, Choubey A, et al. Zinc oxide nanoparticles attenuate hepatic steatosis development in high-fat-diet fed mice through activated AMPK signaling axis. Nanomedicine: Nanotechnology, Biology and Medicine. 2019; 17:210-222. doi: 10.1016/j.nano.2019.01.013.

[65] Sahin K, Orhan C, Kucuk O, Tuzcu M, Sahin N, Ozercan IH, et al. Effects of magnesium picolinate, zinc picolinate, and selenomethionine co-supplementation on reproductive hormones, and glucose and lipid metabolism-related protein expressions in male rats fed a high-fat diet. Food Chemistry: Molecular Science. 2022;4:1-9.doi: 10.1016/j.fochms.2022.100081.

[66] Bona S, Fernandes SA, Moreira ACJ, Rodrigues G, Schemitt EG, Di Naso FC, et al. Melatonin restores zinc levels, activates the Keap1/Nrf2 pathway, and modulates endoplasmic reticular stress and HSP in rats with chronic hepatotoxicity. World Journal of Gastrointestinal Pharmacology and Therapeutics. 2022;13(2):11-22.