The Journal of Anatomical Sciences
Email: journalofanatomicalsciences@gmail.com

J. Anat Sci 15(2)

Submitted June 19th, 2024
Accepted September 5th, 2024
Published September 30th, 2024

Lycopersicon esculentum Attenuates Oxidative Stress and Hormonal Dysregulation in Letrozole-induced Polycystic Ovarian Syndrome Rat Model

Mary Oluwatobiloba Adedigba[1], Adeshina Oloruntoba Adekeye[1], Ephraim Samuel Jen[1], Kehinde S. Olaniyi[2], Oluwatosin Olalekan Ogedengbe[3]

[1]Department of Anatomy
[2]Department of Physiology, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti
[3]Department of Anatomy, College of Medicine, Federal University, Oye-Ekiti, Nigeria

Corresponding Author: Adekeye Adeshina Oloruntoba

Email**:** adekeyeao@abuad.edu.ng, Tel**:** +234(0)8038571625

ABSTRACT

Polycystic ovarian syndrome (PCOS) is the primary culprit behind anovulatory infertility and is one of the most prevalent endocrine disorders affecting women of childbearing age. The aim of the study was to explore the impact of *Lycopersicon esculentum* isolate on PCOS induced by letrozole in female Wistar rats. Twenty female Wistar rats weighing 120-150 g were divided into four groups; Group A (normal saline), Group B (5 mg of lycopene), Group C (1 mg of letrozole and 5 mg of lycopene) and Group D (1 mg of letrozole). Ovaries were processed for histological, immuno-histochemical and biochemical assays. The results revealed a significant increase in the final body and ovarian weight in the PCOS (LETZ) rats. Hematoxylin and Eosin staining showed attenuation of ovarian morphological disruption while periodic acidic Schiff staining revealed degeneration of the zona pellucida in the ovaries. However, Masson Trichrome staining revealed increased ovarian collagen fibers in Lycopene-treated PCOS rats compared with other groups. These significant increases in body and ovarian weight, malondialdehyde concentration, testosterone, luteinizing hormones, insulin and fasting gluocose level in PCOS treated rats was later decrease in PCOS (LETZ) rats treated with lycopene. The present results demonstrate that *Lycopersicon esculentum* may have antioxidant properties to attenuate metabolic and endocrine disorders underlying polycystic ovarian syndrome.

Keywords: endocrine, polycystic ovarian syndrome, lycopene, letrozole, antioxidant, oxidative stress

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is recognized as the foremost etiological factor contributing to anovulatory infertility and is classified among the most commonly encountered endocrine disorders impacting women in their reproductive years[1]. Individuals with PCOS typically exhibit multiple cysts in their ovaries, with approximately 70% of affected women experiencing infertility[2]. The global prevalence of PCOS varies between 4% and 21%, with rates among adolescents ranging from 9.1% to 36%. PCOS is a multifactorial endocrine disorder influenced by various genetic, environmental, and lifestyle factors and the syndrome are often correlated with metabolic anomalies, including insulin resistance and hyperinsulinemia. The likelihood of developing impaired glucose tolerance or type 2 diabetes mellitus prior to reaching the age of 26 years is observed in 40% of obese patients diagnosed with PCOS[3]. The precise mechanisms underlying this syndrome remain unclear. Nevertheless, insulin resistance is widely regarded as the principal factor contributing to the pathogenesis of this condition. There is limited research on the prevalence of PCOS in sub-Saharan Africa, with reported figures ranging from 16% to 32% across various center[4]. Additionally, the prevalence of PCOS among infertile women in Nigeria falls between 12.2% and 18.1%. Several studies have demonstrated various significantly elevated levels of circulatory oxidative markers in PCOS patients compared to those without the condition, suggesting their potential role as pathogenic factors in PCOS[5]. Oxidative stress has been linked to various adverse reproductive outcomes including impaired fertility, embryogenesis, miscarriage, birth defects (including autism), and childhood cancer[6].

Tomato (*Lycopersicon esculentum*) is a globally consumed fruit renowned for its rich array of bioactive nutrients. It serves as a crucial source of carotenoids

(such as lycopene, α-carotene, and β-carotene), phenolic compounds (including phenolic acids and flavonoids), vitamins (such as ascorbic acid and vitamin A), and glycoalkaloids like tomatine. These bioactive constituents within tomatoes exhibit antioxidant, anti-mutagenic, anti-proliferative, and anti-inflammatory properties. Lycopene, renowned for its antioxidant properties and ability to scavenge free radicals, is recognized as a significant dietary component contributing to human health[7]. Among carotenoids, lycopene derived from tomatoes is esteemed for its superior antioxidant activity[8], standing out as the most important carotenoid in human physiology with a half-life of approximately 2-3 days [9,10]. Additionally, lycopene has been associated with the management of cardiovascular diseases, prostate cancer, type II diabetes mellitus, and central nervous system disorders [11,12]. Thus, this study aimed to investigate the effects of lycopene on letrozole-induced PCOS in female Wistar rats.

MATERIALS AND METHODS

Twenty (20) female Wistar rats weighing between 120 and 150 g were sourced from the animal facility at Afe Babalola University, Ado-Ekiti, Nigeria, for this study. The research adhered to the guidelines outlined by the National Institutes of Health for the care and handling of laboratory animals, as well as the principles of the Declaration of Helsinki. Approval for the protocol (ABUADERC/23/2022) was granted by the Institutional Ethical Review Board of Afe Babalola University. Following a week of acclimatization, the animals were randomly assigned into 4 groups of five animals in each group, namely: Control (CTRL), Lycopene (Bulk Suppliments.com, Henderson, HV), Letrozole (Accord Healthcare, Durham, North Carolina) + Lycopene (LETZ+ LYP) and Letrozole (LETZ). The animals were housed in Animal Holdings of the Department of Anatomy AfeBabalola University, Ado-Ekiti, Nigeria. They were maintained on standard laboratory pellets which was purchased from Afe Babalola University, Ado Ekiti (ABUAD) feed mill, Ado Ekiti, Nigeria. They were subjected to natural day and night cycle and clean water were provided *adlibitum*. Normal Saline was the vehicle for the control group, Letrozole group received 1mg/kg orally, Letrozole + Lycopene group received 1mg/kg and 5mg/kg respectively[13] and Lycopene received 5mg/kg[14]. The administrations were continuously administered via oral gavage for a duration of 21 days. Both initial and final body weights were closely monitored, allowing for the estimation of body weight gain.

Measurement of Body Weight, Glucose Tolerance Test and Animal Sacrifice

Forty-eight hours prior to the animal sacrifice, one-hour post-load glucose levels were assessed. Following a 12-hour overnight fast, baseline blood glucose levels were measured, and the rats were administered a glucose load (2 g/kg via oral gavage). Blood glucose levels were monitored using a sensitive glucometer (Accu-Chek) at 30, 60, 90, and 120-minute intervals. Subsequently, the rats were euthanized by anesthetized with pentobarbital sodium (40 mg/kg i.p.)[15] and sacrificed by cervical dislocation and blood samples were obtained via cardiac puncture using a 2ml syringe. The blood samples were then centrifuged at 4000 rpm for 15 minutes to obtain serum, which was stored at 4°C until further biochemical analysis. The ovaries of the animals were carefully excised after careful removal of adhering adipose tissue of excised organs. Left ovaries were clean and fixed in 10 % neutral buffer formalin for further histological analysis. The right ovaries were homogenized in phosphate buffer (pH 6.9). The homogenate samples were centrifuged at 4,000 x g for 20 min to obtain a clear supernatant used for subsequent biochemical assay[16].

Estrus Cycle

The vaginal smear technique was used to verify each rat's estrus cycle prior to the start of treatment according to Quignon protocol for determination of puberty onset and oestrus stage[17]; generally, rats consist of four stages known as of 3 cell types. report the reproductive status of the female rats used revealed that estrous cycle into 4 stages (proestrous, estrus, metestrus/diestrus) all characterized by a different proportion of 3 cell types found in vaginal secretions. Observation of the rat vaginal opening and collection of vaginal smears for analysis of cytology. The majority of the rats were found to be in the diestrus stage and all of the rats were in the proestrus stage during the first week of treatment; however, between the second and third weeks of treatment, there were differences between the treated rats and the control.

Histological, Histochemical and Immunohistochemical Evaluation

For histo-morphological assessment employing hematoxylin and eosin (H & E), Periodic Schiff reagent Masson's Trichrome, and Ki67 staining techniques, an ovarian section was immersed in 10% formal saline overnight for fixation. Subsequently, it underwent dehydration, embedding in paraffin, and sectioning at a thickness of 5 µm. Following this, slides were prepared, scanned, and examined using Moticeasy Scan Pro 6.The cell counts, cell thickness

and collagen distribution in the subsequent histological analysis were obtained using Image J.

Biochemical Assay

Biochemical analyses were conducted to assess the levels of insulin, testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), and sex hormone binding globulin (SHBG). Blood samples were obtained from the heart by cardiac puncturevia the left ventricle using a 5mL syringe and transferred into non-heparinized sample bottles at room temperature. Serum was then obtained by centrifugation at 3000 revolutions per minute for 10 minutes. The resulting supernatant (serum) was utilized for hormone measurements. Insulin, testosterone, LH, FSH, and SHBG concentrations were determined usingenzyme-linked immunosorbent assay(ELISA) kits (Elabscience, USA) following the manufacturers' instructions. Additionally, levels of malondialdehyde (MDA), glutathione, and glutathione peroxidase were measured using ELISA kits (Elabscience, USA).

Estimation of Antioxidant Enzymes

The clear supernatant obtained from ovarian homogenate was aspirated to evaluate the oxidative stress markers using the following assay

Ovarian Malondialdehyde (MDA)

Malondialdehyde (MDA) a measure of lipid peroxidation in tissue homogenate, was determined chemically using spectrophotometry according to the method of Mihara and Uchiyama[18].Based on the reaction between MDA and thiobarbituric acid, the colored complexes were detected at 535nm.

Ovarian-reduced Glutathione (GSH)

The ovarian reduced form of glutathione was determined using Ell-man's reagent 5-5-dithiol-bis (2-nitrobenzoic acid) DTNB as a coloring reagent[19]. The absorbance was read at 412 nm by spectrophotometer.

Ovarian Glutathione Peroxidase (GPX)

The activity of the antioxidant enzyme glutathione peroxidase was determined using glutathione reductase and NADPH. This method is based on the oxidation of NADPH at 25uC, which is indicated by the decrease in absorbance at 340 nm[20]. Results are expressed in U/ mg protein.

Statistical Analysis

The data were expressed as Mean ± SD and visualized in bar charts using Graph Pad Prism Software 9.0. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by post hoc tests to compare different datasets. Statistical significance was defined as $p < 0.05$.

RESULTS

Estrous Cycle

Rats in the control group had a regular estrous cycle of 4-5 days throughout the experimental period. However, the estrous cycle was completely disrupted in all PCOS-induced rats and all of them remained mostly at the diestrous stage during the induction period. LYP and LETZ+LYP treatment animals displayed improvement in estrous cyclicity from days 6 to 30. Increase frequency of the estrus phase and less extended diestrus phase were found in comparison to the PCOS treated rats. Although, the PCOS +LYP treated rats revealed an improvement in the estrous cyclicity.

Body and Ovarian Weight

There was a significant increase of final body weight in week 3 in LETZ treated rats (PCOS-induced rats) compared to the control ($p<0.001$). However, there was a significant decrease in the final body weight and ovarian weight in the LYP and LETZ+LYP experimental rats in comparison to the LETZ (PCOS) treated rats.

In addition, the level of oral glucose tolerance (mg/dl) in each rat were carried out at 30, 60, 90 and 120 min in which the basal glucose level was checked before loading of the rats with glucose. There was a significant impairment in the oral glucose level in LETZ rats compare to other experimental rats.

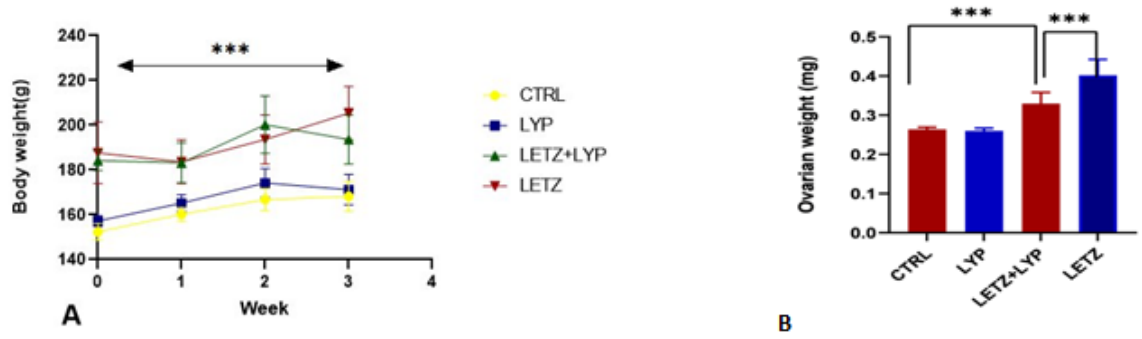

Figure 1: Effect of Lycopene on Animal Weight (A) Body Weight (B) Ovarian Weight.Data are presented as mean ± SD. *** indicates statistically significant differences ($p < 0.001$).

Hormonal Concentration

The PCOS rats (LETZ treated animals) exhibited a significant increase in testosterone, LH and FSH as compared with the control ($P<0.05$). However, post treatment with LYP shows a significant difference (reduction) in testosterone, FSH and LH when compared to that of the PCOS rats ($p<0.01$). There was an insignificant decrease in testosterone, LH and FSH in the LYP treated rats only compared with control but significantly decreased when compared to PCOS and PCOS+LYP treated rats ($P<0.05$).

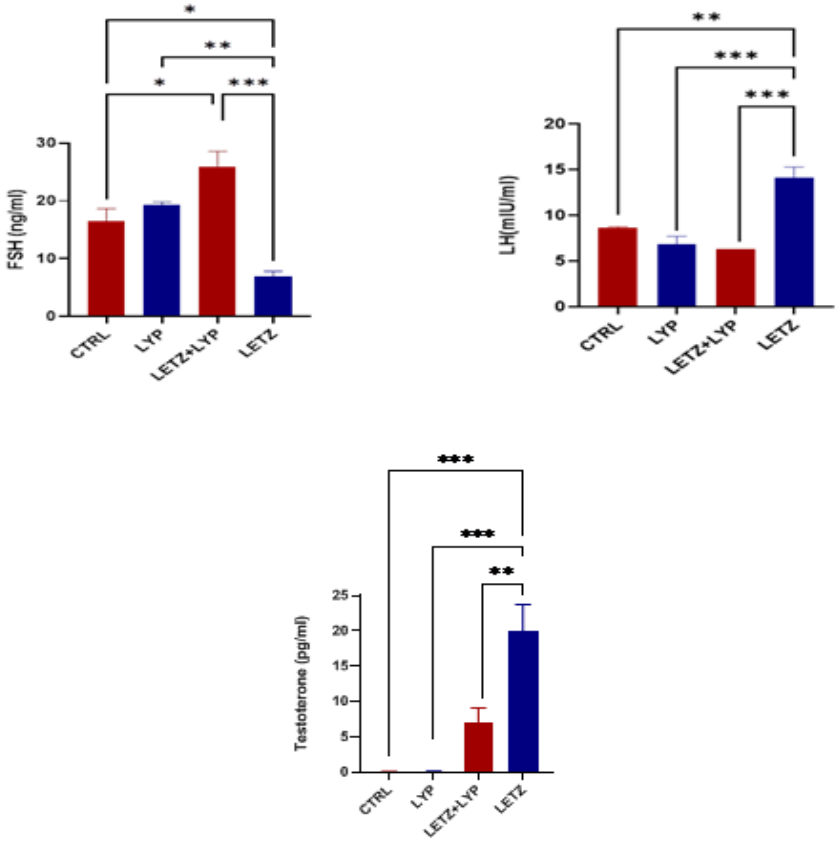

Figure 2: Levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone (CTRL, LYP, LETZ+LYP, and LETZ for the treatment). Data are presented as mean ± SD. ***, * and ** indicate statistically significant differences ($p < 0.001$, $p < 0.05$ and $p < 0.01$, respectively).

Fasting Insulin and Blood Glucose

The PCOS rats present a significant increase in fasting insulin and fasting blood glucose compared with the control. Meanwhile, treatment with LYP after induction of PCOS with LETZ significantly decreased the fasting insulin and fasting blood glucose and area under curve of blood insulin release as compared to the PCOS treated rats. Also, treatment with LYP alone revealed a significant decrease in fasting insulin and fasting blood glucose

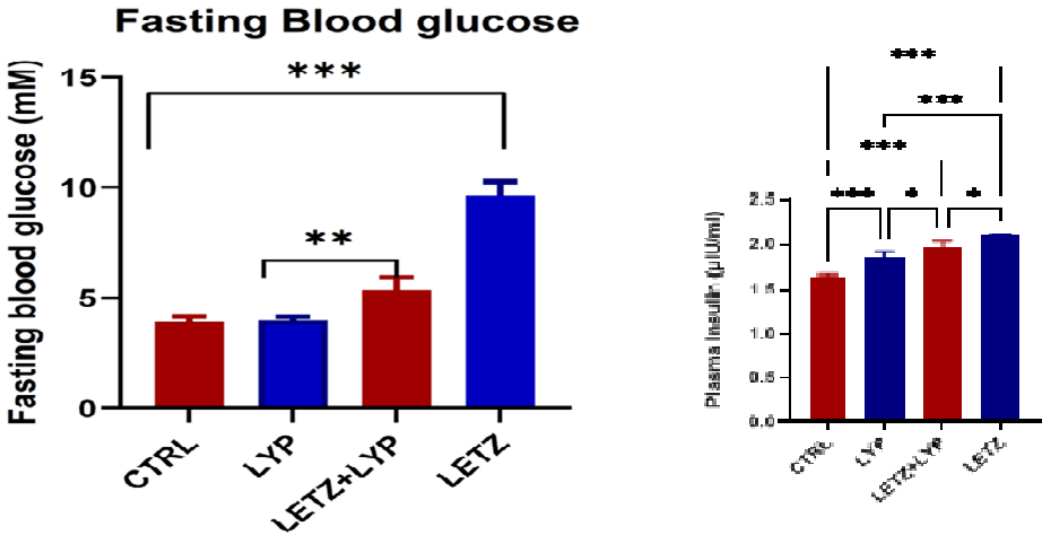

Figure 3: Level of fasting blood glucose and plasma insulin in treatment animals. (CTRL: Control; LYP, Lycopene, LETZ+LYP: Letrozole+Lycopene and LETZ: Letrozone). Data are presented as mean ± SD, number of animals per group = 5 ***, * and ** indicate statistically significant differences ($p < 0.001$, $p < 0.05$ and $p < 0.01$, respectively).

Ovarian Malondialdehyde (MDA)

In the serum, there was a significant increase of the malondialdehyde level in LETZ treated rats compared to other treatment ($p<0.01$), LYP and LETZ+LYP-treated rats showed a remarkable reduction in malondialdehyde level compared to LETZ-treated rats alone ($p<0.01$). In the ovarian homogenate, there was also a statistically significant increase in LETZ rats ($p<0.01$) when compared to other experimental rats, However, LYP and LETZ +LYP treated rats revealed a reduction in malondialdehyde level when compared with LETZ treated rats only ($p<0.01$). There was also a significant reduction in glutathione results of the LETZ treated rats compared to other treatment ($p<0.01$). In the homogenate tissue, there was also a significant reduction in the LETZ treated rats when compared with other groups ($p<0.001$). The glutathione peroxidase increased in the LYP and LETZ + LYP of the serum when compared with LETZ ($p<0.01$) while in the homogenate tissue, there was also a statistically significant increase in LYP and LETZ + LYP when compared with LETZ ($p<0.001$).

Sex Hormone Binding Globulin Level

There was a statistically significant reduction in LETZ rats compared to experimental groups ($p<0.0001$). However, LYP rats showed a remarkably level of SHBG higher than LETZ but similar to other experimental groups.

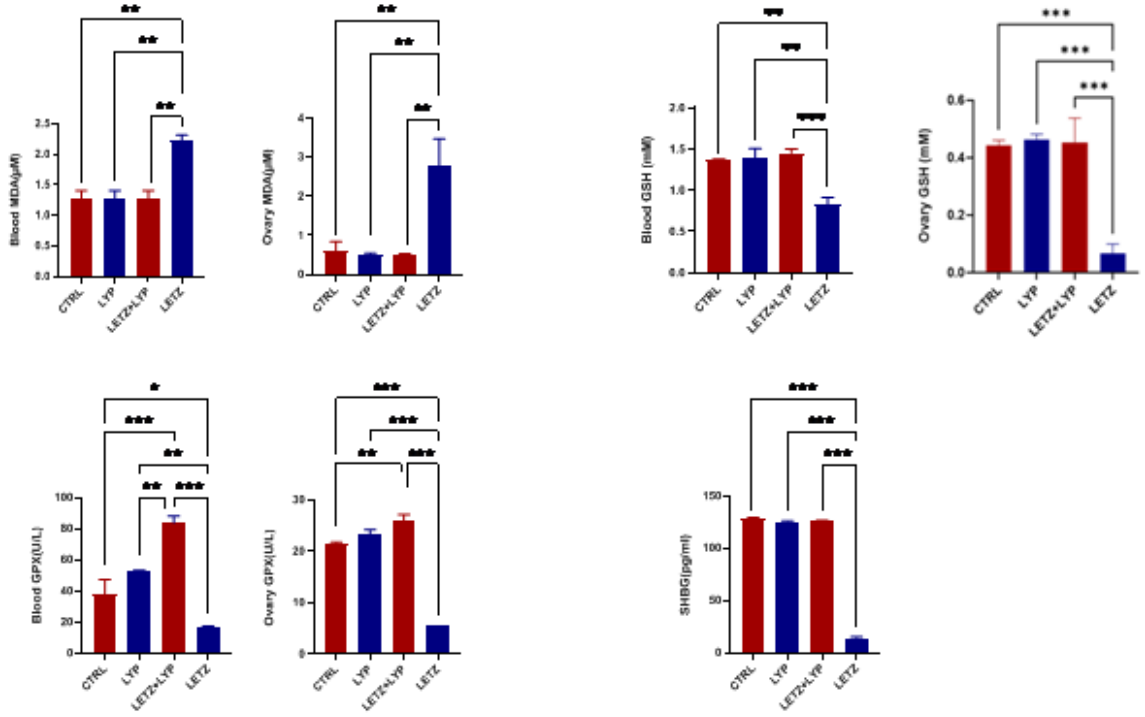

Figure 4: Levels of blood and ovarian malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GPX), and sex hormone-binding globulin (SHBG) in the different groups (CTRL: Control; LYP, Lycopene, LETZ+LYP: Letrozole+Lycopeneand LETZ: Letrozone). Data are presented as mean ± SD, **, * and ** indicate statistically significant differences ($p < 0.001$, $p < 0.05$ and $p < 0.01$, respectively).

Histological Observations

After collecting the photomicrographs, the digital images were imported into the program and calibrated to convert pixel measurements into micrometers. This application automatically identifies and tally individual cells and quantify the area of collagen distribution together with cell thickness which is the distance between the top and bottom surface of the cell using the line tool. The primordial follicle was slightly decreased in PCOS treated rats when compared to the control. However, treatment with LYP improved the number of the primordial follicles considerably compared to the LETZ (PCOS) rats. The mean number of primary, secondary, tertiary and graffian follicles was reduced in PCOS rats in comparison to the control. However, treatment with LYP and post treatment with LYP increased the number of follicles compared to the LETZ rats. Also, a significant increase in atretic and cystic follicle with a decrease in corpus luteum was observed in the PCOS rats compared to the control. Therefore, LYP post-treatment significantly decreased cystic and atretic follicle and increase corpus luteum when compared to the PCOS treated rats.

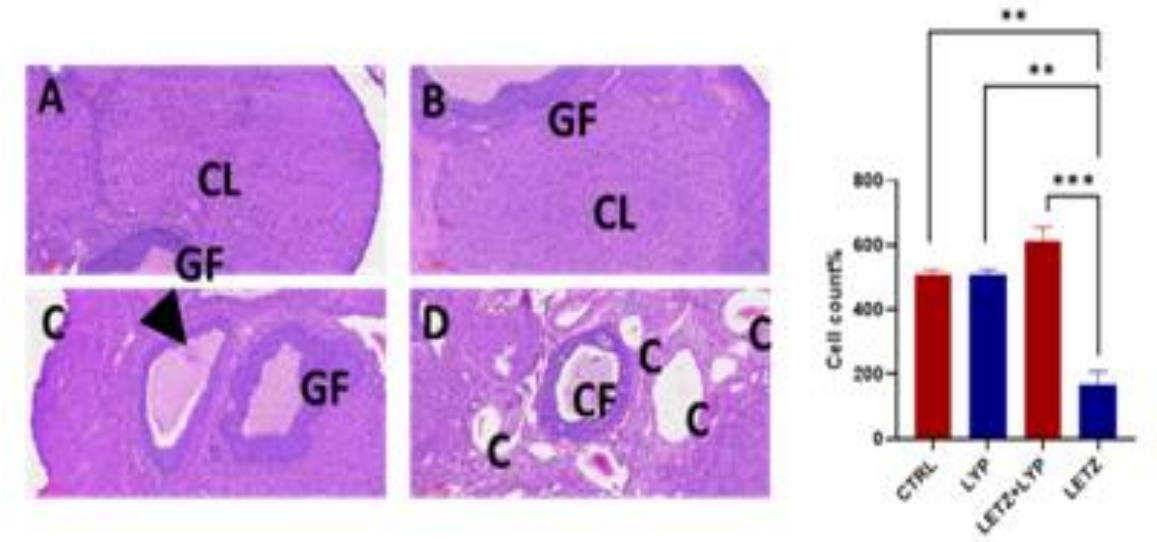

Figure 5: Photomicrographs (A-D) of ovaries of control, LYP, LETZ+LYP and LETZ-treated rats and its cell count charts. (A): cross section of Ovarian tissue of control (CTRL) rats showing a normal structure of ovarian follicles along with primary follicle also known as growing follicle (B): ovaries of LYP-treated rats. (C): ovaries of LETZ+LYP-treated rats C: Cycst; CF: Cystic follicle; DF: Degenerating follicle; F: Follicle; GF: Growing Follicle /Primary follicle, GL: Granulosa layer, GE: Glandular epithelium, CL: Corpora luteum, O:Oocytes and NF: Normal follicle. (D) Ovaries of LYP-treated rat. (H&E x 10 magnification).

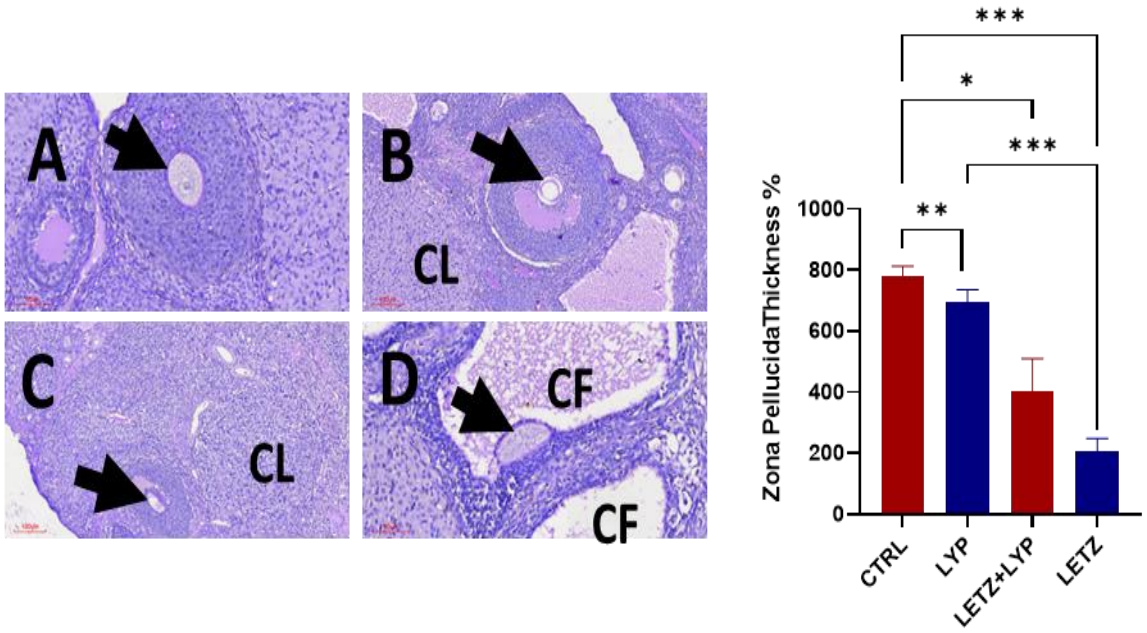

Figure 6: Photomicrographs (A-D) of ovarian sections of control, LYP, LETZ+LYP and LETZ-treated rats and its zona pellucida thickness. (A: CTRL, B: LYP, C: LETZ+LYP, D: LETZ), highlighting the zona pellucida (stained purple). (A): cross section of ovarian tissue of control (CTRL) rats and (B): LYP-treated rats display normal zona pellucida with intact structure. (C): LETZ+LYP-treated rats showing a partially restoration of zona pellucida thickness (D): LETZ-treated rats revealing a thinned and degenerated zona pellucida. The accompanying chart quantifies the zona pellucida thickness across groups, demonstrating a significant reduction in thickness in LETZ compared to LETZ+LYP. PASx20 Magnification

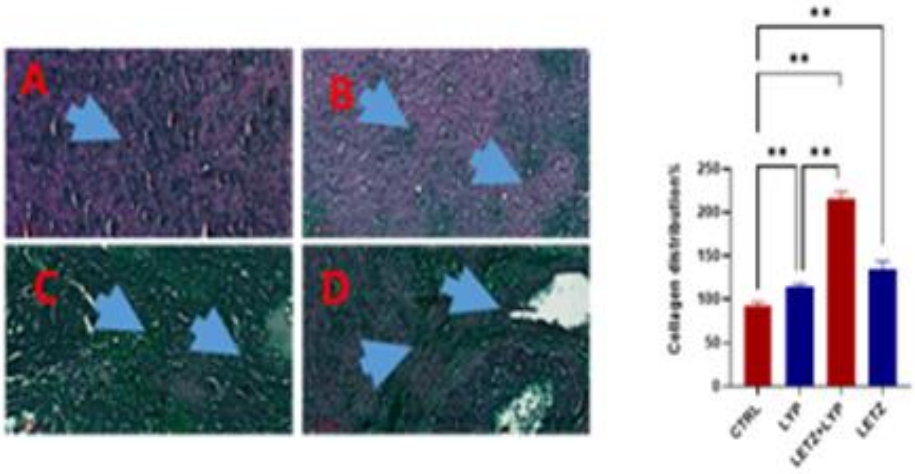

Figure 7: Photomicrographs (A-D) of ovarian sections and its collagen distribution of control, LYP, LETZ+LYP and LETZ-treated rats. (A): cross section of ovarian tissue of control (CTRL) rats exhibiting a minimal collagen presence with normal ovarian architecture; (B): ovaries of LYP-treated rats showing an increased collagen; (C): ovaries of LETZ+LYP-treated rats with an increased collagen deposition; (D): ovaries of LETZ-treated rats displaying an abatement collagen deposits. Masson Trichrome (MT) (A: CTRL, B: LYP, C: LETZ+LYP, D: LETZ) at x10 magnification, showing collagen distribution (stained green).The accompanying chart quantifies collagen distribution across groups, highlighting a significant increase in collagen in LETZ+LYP compared to LETZ. Data are presented as mean ± SD, ***, * and ** indicate statistically significant differences ($p < 0.001$, $p < 0.05$ and $p < 0.01$, respectively).

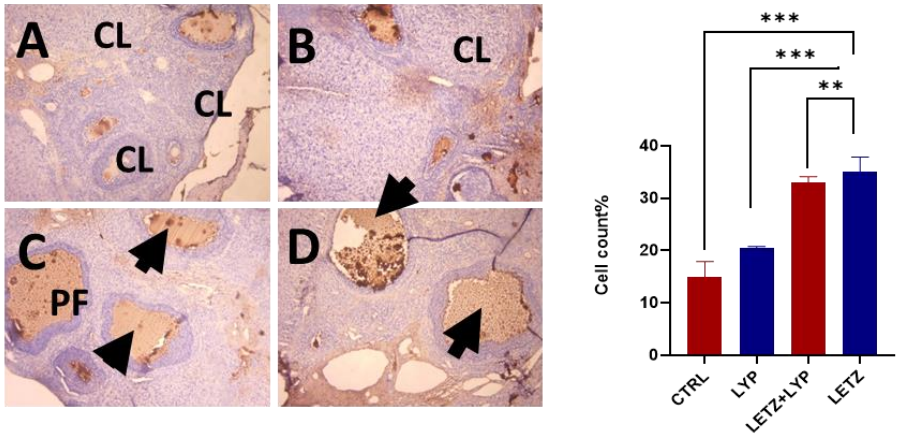

Figure 8: Cross section of ovaries and its cell count of control LYP, LETZ+LYP and LETZ treated rats (A-D). (A): Cross section of ovarian tissue of control (CTRL) rats showing ovarian follicles along with primary follicles; (B): ovarian tissue of LYP-treated rats showing normal cell growth; (C): ovarian tissues of LETZ+LYP-treated rats; and (D): LETZ group demonstrates Ki67 expression. Ki67-stained at x10 magnification. The accompanying chart on the right side quantifies the ovarian section cell count and indicates expression of cell proliferation across the groups. Data are presented as mean ± SD, with ***, * and ** indicate statistically significant differences ($p < 0.001$, $p < 0.05$ and $p < 0.01$, respectively).

DISCUSSION

Polycystic ovarian syndrome (PCOS) is a complex metabolic and endocrine disorder in reproductive women that causes impaired fertility or anovulatory infertility due to multiglandular pathophysiology[21,22]. This study assessed the beneficial effect of *Lycopersicon esculentum* on letrozole-induced PCOS rat model. Treatment with lycopene significantly reduced body weight which indicates the ability of

lycopene action as a potent antioxidant to modify gene associated with obesity. The increase in ovarian weight may be caused by the anabolic characteristics of Letrozole. These adverse effects are connected to the accumulation of ovarian fat and the development of multiple cysts[23]. The consequences of obesity on adipose tissue inflammation and insulin resistance are shielded in many animal models with reduced endogenous interleukin-1 activity [24]. In addition, the significant reduction in ovarian weight in PCOS-induced rats treated with LYP might be a result of actions related to the generation of normal follicle. PCOS rats presented with a significant increase in fasting insulin and fasting blood glucose level. Meanwhile, treatment with Lycopene after induction of PCOS significantly reduced the fasting insulin and fasting blood glucose levels compared to the PCOS-treated rats. Also treatment with Lycopene alone showed a remarkable decrease in fasting insulin and fasting blood glucose level in comparison to the PCOS treated rats. No significant difference in fasting insulin and fasting glucose levels was observed between control and Lycopene alone, PCOS + Lycopene-treated rats, highlighting its glucoregulatory effect. Routine examination of vaginal smears revealed that the PCOS rats lacked regular reproductive cycles and the clinical sign of ovarian cysts remained a remarkably chronic cornification[25]. The return of menstrual cyclicity following treatment may be attributable to the anti-inflammatory and antioxidant properties of LYP. Oxidative stress, inflammatory pathways and hyperglycemia are major factors that contribute to PCOS [27]. Oxidative stress has been found as one of the major pathogenesis–related contributors of PCOS [28]. Excessive oxidants level can alter steroidogenesis in the ovaries thereby increasing androgen output and leading to polycystic ovaries. According to Ozegowska *et al.*[29], the imbalance between reactive oxygen species and the physiologic antioxidant capacity of the body is referred to as oxidative stress[30,31]. Previous research has linked oxidative stress to the etiology of PCOS and shown that taking antioxidants improved symptoms remarkably [32]. The decreased activity of GSH and GPx in the ovary of PCOS rats demonstrates that the disturbance caused by ROS induces antioxidant enzyme imbalances. The antioxidant potential of *Lycopersicon esculentum* was demonstrated by improvements in GSH and GPx activity after treatment with LYP and this is in accordance to findings by Savic*et al* on GSH and GPx levels in PCOS rats[33]. Thiobarbituric acid reactive substances (TBARS) are lipid peroxidation markers [34]. Lipid peroxidation is described as the process of oxidative depletion of lipids which results in a free radical chain reaction in the membrane lipids. In people with PCOS, dyslipidemia is one of the leading cause of cardiovascular disease[35]. In the current study, there was a significant increase in the production of TBARS in the PCOS-treated rats, however, LYP treatment significantly reduced the level of the ovarian MDA, revealing the antioxidant efficacy of LYP and this result was in agreement with research done by Gong *et al* on oxidative stress and lipid peroxidation in PCOS animal model[36,37]. The progression of PCOS is influenced by chronic inflammation according to Yan *et al*.[38] Anovulation alters the feedback signaling of the ovarian sex hormones and this affect the generation of the gonadotropin-releasing hormone (GnRH) which interferes with the LH and FSH's normal release[39]. It was found that abnormal ovarian physiology is connected to a hyper-androgenized condition in Letrozole-induced PCOS rats. LH and testosterone levels rise in females when the hypothalamic-pituitary axis is compromised resulting in a pathological situation[40].The elevated level of LH in PCOS may be connected to inadequate estrogen feedback, which leads to the proliferation of theca cells[41]. In the current study, testosterone, FSH and LH levels increased in PCOS rats with a decreased level of sex hormone-binding globulin (SHBG). Sex hormone-binding globulin (SHBG), a sex hormone transporter, is produced by the liver and binds with circulating sex steroids with a high affinity to regulate the concentration of biologically active sex hormones in the blood, affecting their bioavailability. Therefore, SHBG can be used to assess the severity of hyperandrogenism and evaluate treatment efficacy[42]. Treatment with LYP reduced the levels of testosterone, LH, FSH, testosterone with a consequential increase in SHBG levels in PCOS rats, protecting the ovaries from excessive theca cell proliferation and aiding in the prevention of PCOS. The relative improvement in LH and FSH concentrations shown in PCOS rats treated with LYP is evidence of the apparent protection of the gonadotropin level by LYP. In normal women, testosterone is produced in part by the adrenal glands and in part by the ovaries[44]. However, in women with PCOS, the ovary is the exclusive source of testosterone[45,46]. Ovarian histological result revealed the extent of ovarian alterations with appearance of large multiple cysts, diminished oocyte, huge glandular degeneration, atrophy and increased atresia of follicles in PCOS treated rats in this study, which was consistent with a findings reported by Savic-Radojevic *et al.*[33] and Venegas *et al.*[47]. Notably, PCOS treated rats with the LYP displayed growing follicles, indicating the compound's positive impact on ovarian health. Periodic Acidic Schiff (PAS), Masson Trichrome (MT) and Ki67 activities revealed high intensity in the zona pellucida due to glycoprotein presence, differential collagen deposition and cell proliferation in PCOS treated rats respectively which was significantly reduced in LYP treated rats. This further corroborated the histological observation with the intact architecture of zona pellucida observed in the PCOS rats treated with LYP. However, the degeneration was less prominent in the Lycopene treated rats, consistent with findings by Archibong et

al.[48]. The corpus luteum plays an important role in the release of progesterone, which regulates reproductive cycles and prepares the uterus for conception[49]. Lycopene therapy, however, caused significant ovarian tissue regeneration in PCOS + LYP rats as evidenced by a decline in cyst development, luteinization regularity, and antral follicle growth. Lycopene may be an effective therapy for PCOS due to its capacity to restore ovarian function and its anti-androgenic qualities. For Lycopene to be employed as an adjuvant therapy or a treatment for PCOS, direct human trials are also required to assess its therapeutic potential [50].

Conclusion

The present results demonstrate that *lycopersicon esculentum* contains lycopene as an antioxidant agent and may be effective in attenuating metabolic and endocrine disorders underlying PCOS.

Acknowledgements

The authors wish to acknowledge the support of the staff, Department of Anatomy and Afe Babalola University Management for the support in terms of enabling environment to carry out this research

Authors' Contribution

AOA conceived the conceptual idea, MOA and ESJ designed and conducted the experiments, AOA and MOA analyzed and interpreted the data. MOA drafted the manuscript, KSO, OOO, and AOA reviewed and edited the manuscript. All authors gave the final approval for the manuscript to be published.

REFERENCES

1. Panti AA, Shehu CE, Saidu Y, Tunau KA, Nwobodo EI, Jimoh A, *et al*. Oxidative stress and outcome of antioxidant supplementation in patients with polycystic ovarian syndrome (PCOS). International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2018; 7(5):1667. https://doi.org/10.18203/2320-1770.ijrcog20181892
2. Zuo T, Zhu M, Xu W. Roles of Oxidative Stress in Polycystic Ovary Syndrome and Cancers. Oxidative Medicine and Cellular Longevity. 2015:8589318. https://doi.org/10.1155/2016/8589318
3. Chaudhary H, Patel J, Jain NK, Joshi R. The role of polymorphism in various potential genes on polycystic ovary syndrome susceptibility and pathogenesis. Journal of Ovarian Research. 2021;14(1):125. doi: 10.1186/s13048-021-00879-w.
4. Vaduneme K, Chidi O. Prevalence of polycystic ovary syndrome (PCOS) among infertile women attending Fertility Clinic at a University Teaching Hospital in Nigeria. J Gynecol Women's Health. 2019; 15(5): 555922. doi: 10.19080/JGWH.2019.15.555922
5. Abubakar P, Abubakar C, Shehu S, Yusuf T, Nwobodo E, Abdulgafar B, *et al.* Oxidative stress and antioxidants supplementation in patients with PCOS. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2018;10.18203/2320-1770.ijrcog20181892.
6. Archibong AE, Rideout ML, Harris KJ, Ramesh A. Oxidative stress in reproductive toxicology. Current Opinion in Toxicology. 2018; *7*:95–101. https://doi.org/10.1016/j.cotox.2017.10.004
7. Naveen k, Paruchuri, Elango P, Asmathulla S, Kavimani S. Effect of lycopene on antioxidant status and serum corticosterone in Wistar rats subjected to chronic mild stress. Journal of Clinical and Diagnostic Research. 2019; 13.10.7860/JCDR/2019/41674.13092
8. Suwanaruang T. Analyzing Lycopene Content in Fruits. Agriculture and Agricultural Science Procedia. 2016; 11:46-48. 10.1016/j.aaspro.2016.12.008.
9. Durairajanayagam D, Agarwal A, Ong C, Prashast P. Lycopene and male infertility. Asian Journal of Andrology. 2014; 16(3):420–425. https://doi.org/10.4103/1008-682X.126384
10. Pirayesh IJ, Mehrali H. Lycopene as a carotenoid provides radioprotectant and antioxidant effects by quenching radiation-induced free radical singlet oxygen: an overview. Cell Journal. 2015; 16(4):386–391. https://doi.org/10.22074/cellj.2015.485
11. Mirahmadi M, Azimi-Hashemi S, Saburi E, Kamali H, Pishbin M, Hadizadeh F. Potential inhibitory effect of lycopene on prostate cancer. Biomed Pharmacother. 2020; 129:110459. doi: 10.1016/j.biopha.2020.110459.
12. Leh HE, Lee LK. lycopene: A Potent Antioxidant for the Amelioration of Type II Diabetes Mellitus. Molecules. 2022; 27(7):2335. doi: 10.3390/molecules27072335.
13. Chen D, Huang C, Chen Z. A review for the pharmacological effect of lycopene in central nervous system disorders. Biomed Pharmacother.2019;111:791-801. doi: 10.1016/j.biopha.2018.12.151.
14. Olaniyi KS, Bashir AM, Areloegbe SE, Sabinari IW, Akintayo CO, Oniyide AA. Short chain fatty acid, acetate restores ovarian function in experimentally induced PCOS rat model. PloS One, 2022; 17(7) e0272124. https://doi.org/10.1371/journal.pone.0272124
15. Adelakun SA, Ogunlade B, Akintunde OW, Omilachi VO. Long-term exposure to

cimetidine induced gonadotoxicity in male rats: Modulating role of Ocimum gratissimum. Revista Internacional de Andrología. 2022;294:1–15.
16. Olorunnisola OS, Adegbola PI, Ajilore BS, Akintola OA, Fadahunsi OS. The role of poly-herbal extract in sodium chloride-induced oxidative stress and hyperlipidemia in male wistar rats. Medicines. 2021; 8(6):25.
17. Quignon, C. Collection and analysis of vaginal smear to assess reproductive stage in mice. Current Protocol.2023;3 e887.doi.10.1002/cpz/887.
18. Mihara M, Uchiyama M. Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. Anal Biochem. 1978;86(1):271–8.
19. Moron MS, Depierre JW, Mannervik B. Levels of GSH, GR, and GST activities in rat lung and liver. BBA. 1979;582:67–78.
20. Adelakun SA, Ogunlade B, Omotoso OD, Oyewo OO. Response of crude leaf extract of ageratum conyzoides on hormonal and biochemical assay in streptozotocin induced diabetic male Wistar rats. Indian Journal Physiol Pharmacol. 2018;62(4):413–23.
21. 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; 5558599. https://doi.org/10.1155/2022/5558599.
22. Patel S. Polycystic ovary syndrome (PCOS), an inflammatory, systemic, lifestyle endocrinopathy. J Steroid Biochem Mol Biol. 2018; 182:27-36. doi:10.1016/j.jsbmb.2018.04.008.
23. Nofal EA, El-Habeby MM, El-Kholy WB, El-Akabawy GF, Faried MA. Protective role of Broccoli extracts on estradiol valerate-induced polycystic ovary syndrome in female rats. European Journal of Anatomy. 2019;23(2):121–9.
24. Taghizadeh S, Izadi A, Shirazi S, Parizad M, Pourghassem GB. The effect of coenzyme Q10 supplementation on inflammatory and endothelial dysfunction markers in overweight/obese polycystic ovary syndrome patients. Gynecol Endocrinol. 2021;37(1):26–30.
25. Adelakun SA, Akintunde OW, Jeje SO, Alao OA. Ameliorating and protective potential of 1-isothiocyanate-4-methyl sulfonyl butane on cisplatin-induced oligozoospermia and testicular dysfunction via redox-inflammatory. Phytomedicine Plus. 2022:100268.
26. Ogunlade B, Adelakun SA, Iteire K. Sulforaphane response on aluminum-induced oxidative stress, alterations in sperm characterization and testicular histomorphometry in Wistar rats. International Journal of Reproductive BioMedicine. 2020;18:611–24.
27. Kheirollahi A, Teimouri M, Karimi M, Vatannejad A, Moradi N, Borumandnia N, et al. Evaluation of lipid ratios and triglyceride-glucose index as risk markers of insulin resistance in Iranian polycystic ovary syndrome women. Lipids Health Dis. 2020;19:235.
28. Caldwell AS, Middleton LJ, Jimenez M, Desai R, McMahon AC, Allan CM, et al. Characterization of reproductive, metabolic, and endocrine features of polycystic ovary syndrome in female hyperandrogenic mouse models. *Endocrinology*. 2014; *155*(8), 3146–3159. https://doi.org/10.1210/en.2014-1196
29. Ozegowska K, Korman M, Szmyt A, Pawelczyk L. Heterogeneity of endocrinologic and metabolic parameters in reproductive age Polycystic Ovary Syndrome (PCOS) women concerning the severity of hyperandrogenemia new insight on syndrome pathogenesis. Int J Environ Res Public Health. 2020;17:9291.
30. Adelakun SA, Ukwenya VO, Akingbade GT, Omotoso OD, Aniah JA. Interventions of aqueous extract of Solanummelongena fruits (garden eggs) on mercury chloride-induced testicular toxicity in adult male Wistar rats. Biomed J. 2020;43(2):174–82.
31. Fathi FH. Biomarkers of oxidative stress in polycystic ovary disorder. Ann Coll Med Mosul. 2020;41(2):112–6.
32. Caldwell AS, Middleton LJ, Jimenez M, Desai R, McMahon AC, Allan CM, *et al.* Characterization of reproductive, metabolic, and endocrine features of polycystic ovary syndrome in female hyperandrogenic mouse models. Endocrinology. 2014; *155*(8), 3146–3159. https://doi.org/10.1210/en.2014-1196
33. Savic-Radojevic A, Bozic-Antic I, Coric V, Bjekic-Macut J, Radic T, Zarkovic M, *et al.* Effect of hyperglycemia and hyperinsulinemia on glutathione peroxidase activity in non-obese women with polycystic ovary syndrome. Hormones (Athens, Greece). 2015;14(1), 101–108.https://doi.org/10.14310/horm.2002.1525
34. Adelakun SA, Ogunlade B, Siyanbade JA, Chukwunenye CC. Evaluation of the ameliorative potential of Nutritional Dietary supplementation of vitamin B12 following chronic tramadol hydrochloride exposure induced hyperlipidemia, atherogenic indices, and hepato-renal dysfunction by downregulating redox imbalance, NO/iNOS/NF-κB response, caspase-3- activation and DNA fragmentation in rat's model. Food Chemistry Advances. 2023;2:100286.

35. Luo X, Cai WY, Wu XK. Prevalence, pattern, and predictors for dyslipidemia of chinese women with polycystic ovary syndrome. Frontiers in Cardiovascular Medicine. 2021;8:790454.
36. Xing C, Zhang J, Zhao H, He B. Effect of Sex Hormone-Binding Globulin on Polycystic Ovary Syndrome: Mechanisms, Manifestations, Genetics, and Treatment. International Journal of Women's Health. 2022; *14*,91–105. https://doi.org/10.2147/IJWH.S344542
37. Kabiri N, Tabandeh MR, Tabatabaie SR. Beneficial effects of pioglitazone and metformin in murine model of polycystic ovaries via improvement of chemerin gene up-regulation. Daru: Journal of Faculty of Pharmacy, Tehran University of Medical Sciences. 2014; 22(1), 39 .https://doi.org/10.1186/2008-2231-22-39
38. Yang Y, Yang L, Qi C, Hu G, Wang L, Sun Z, *et al*. Cryptotanshinone alleviates polycystic ovary syndrome in rats by regulating the HMGB1/TLR4/NF–κB signaling pathway. Mol Med Rep. 2020;22(5):3851–61.
39. Casteel CO, Singh G. Gonadotropin-Releasing Hormone. Physiology, In: StatPearls [Internet]. Treasure Island (FL): Stat Pearls Publishing; https://www.ncbi.nlm.nih.gov/books/NBK558992/.
40. Allen MJ, Sharma S. Physiology, Adrenocorticotropic Hormone (ACTH). In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022.: https://www.ncbi.nlm.nih.gov/books/NBK500031/
41. Murugesan BM, Muralidharan P, Hari R. Effect of ethanolic seed extract of Caesalpiniabonducella on hormones in mifepristone induced PCOS rats. Journal of Applied Pharmaceutical Science 2020;10(2):72–6.
42. Xing C, Zhang J, Zhao H, He B. Effect of sex hormone-binding globulin on polycystic ovary syndrome: mechanisms, manifestations, genetics, and treatment. International Journal of Women's Health. 2022; *14*,91–105. https://doi.org/10.2147/IJWH.S344542
43. 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; 5558599. https://doi.org/10.1155/2022/5558599.
44. Khan SH, Chaudhry N. Beyond GnRH, LH, and FSH: The role of kisspeptin on hypothalamic-pituitary-gonadal (HPG) axis pathology and diagnostic consideration. J Pak Med Assoc. 2021;71:1862–9.
45. Shirooje S, Khaledi E, Dehpour AR, Noori T, Khazaei M, Sadeghi F, *et al.* The effect of dapsone in testosterone enanthate-induced polycystic ovary syndrome in the rat. J Steroid BiochemMolBiol.2021;214:105977.
46. Chandil N, Pande S, Sen SS, Gupta D. Comparison of metformin and N acetylcysteine on clinical, metabolic parameter and hormonal profile in women with the polycystic ovarian syndrome. J Obstet Gynaecol India. 2019;69(1):77–81.
47. Venegas B, De León Gordillo LY, Rosas G, Espinoza JA, Morán C, *et al.* In rats with estradiol valerate-induced polycystic ovary syndrome, the acute blockade of ovarian β-adrenoreceptors improve ovulation. Reproductive Biology and Endocrinology. 2019; 17(1): 95. https://doi.org/10.1186/s12958-019-0539-y
48. Archibong AE, Rideout ML, Harris KJ, Ramesh A. Oxidative stress in reproductive toxicology. Current Opinion in Toxicology. 2018; 7:95–101. https://doi.org/10.1016/j.cotox.2017.10.004
49. Jashni KH, Jahromi KH, Bagheri Z. The effect of palm pollen extract on polycystic ovary syndrome (POS) in rats. Int J Med Res Health Sci. 2016;5(5):317–21.
50. Ban Y, Ran H, Chen Y, Ma L. Lipidomics analysis of human follicular fluid form normal-weight patients with polycystic ovary syndrome: a pilot study. J Ovarian Res 2021;14:135.