Open Access

OBM Genetics

LIDSEN Publishing Inc.

Original Research

Artocarpus Communis Seed Regulates P53, IRS, HsD17β2, FTO, and CYP11a Genes in Polycystic Ovarian Syndrome Rats

Akingbolabo Daniel Ogunlakin [1, 2, *], Oluwafemi Adeleke Ojo [1, 2], Chimzi David Onu-Boms [2], Oluwafemi Samson Afolayan [2], Peluola Olujide Ayeni [2], Idayat Adeola Akinwumi [3], Opeyemi Josephine Akinmurele [3], Great Oluwamayokun Adebodun [4], Damilare Iyinkristi Ayokunle [5], Owoola Azeezat Ambali [6], Omolola Adenike Ajayi-Odoko [7], Oluwaseun Abigael Ogunlakin [8], Mubo Adeola Sonibare [9]

1. Bowen University Good Health and Wellbeing Research Cluster (SDG 03), Iwo, 232101, Nigeria; E-Mails: gbolaogunlakin@gmail.com; oluwafemiadeleke08@gmail.com;
2. Phytomedicine, Molecular Toxicology, and Computational Biochemistry Research Laboratory (PMTCB-RL), Department of Biochemistry, Bowen University, Iwo, 232101, Nigeria; E-Mails: chimziboms@gmail.com; oluwafemiafolayan7@gmail.com; peluola.ayeni@bowen.edu.ng;
3. Department of Pharmacognosy, Faculty of Pharmacy, Lead City University, Ibadan. Nigeria; E-Mails: doyetoro@yahoo.com; opeyemiakinmurele@gmail.com;
4. Department of Physiology, Babcock University, Ilisan-Remo, Nigeria; E-Mail: alabig@babcock.edu.ng
5. Department of Pure and Applied Biology, Bowen University, Iwo, 232101, Nigeria; E-Mail: opeoluwa02@gmail.com
6. School of Medicine and Allied Health Sciences, University of the Gambia, Banjul Gambia; E-Mail: uklabby@gmail.com
7. Microbiology programme, Bowen University, Iwo, 232101, Nigeria; E-Mail: omolola.ajayi@bowen.edu.ng
8. Agriculture programme, Bowen University, Iwo, 232101, Nigeria; E-Mail: oluwaseun0202@gmail.com
9. Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan. Nigeria; E-Mail: sonibaredeola@gmail.com

* **Correspondence:** Akingbolabo Daniel Ogunlakin; E-Mail: gbolaogunlakin@gmail.com

Academic Editor: Paola Ungaro

OBM Genetics
2024, volume 8, issue 1

Received: November 20, 2023
Accepted: February 14, 2024

doi:10.21926/obm.genet.2401213 **Published:** February 20, 2024

Abstract

Polycystic ovarian syndrome (PCOS) is a prevalent endocrine illness that affects 5-10% of reproductive women globally. It is a multifaceted hormonal disorder characterized by the involvement of numerous molecular mechanisms that contribute to its development. This study investigates the effect of *Artocarpus communis* seed on the hormonal imbalance and P53, IRS, HsD17β2, FTO, and CYP11a genes expression in the ovaries of letrozole-induced polycystic ovarian syndrome rats. To induce PCOS in 30 female Wistar rats, letrozole was administered at a dosage of 1 mg/kg. For 12 days, *Artocarpus communis* seed aqueous extract (100 and 250 mg/kg body weight) and Clomiphene citrate (1 mg/kg body weight), a standard medication, were given. ELISA assessed luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol levels. The levels of P53, IRS, HsD17β2, FTO, and CYP11a gene expression in the ovaries were assessed. The aqueous extract reduced LH and increased FSH levels in Letrozole-induced PCOS rats. Additionally, seed aqueous extract (250 mg/kg bw) regulated the expression of P53, type 2 17-HSD (17-HSD), fat mass and obesity-associated (FTO), 11a-hydroxylase/17,20-desmolase (CYP11a), and insulin receptor substrate (IRS) genes in the ovaries of PCOS rats. Therefore, *Artocarpus communis* seed might have multifaceted effects on molecular pathways associated with PCOS, potentially normalizing androgen metabolism, hormonal imbalance, and ovarian function.

Keywords

Polycystic ovarian syndrome; hormonal imbalance; gene expression; *Artocarpus communis* seed

1. Introduction

Polycystic ovarian syndrome (PCOS) is a prevalent endocrine illness that affects women, particularly those who are of reproductive age. Estimates suggest that 5-10% of people globally have PCOS [1]. Infertility, acne, hirsutism, insulin resistance, obesity, hyperandrogenism, amenorrhea or oligomenorrhea, and polycystic ovaries by ultrasonography can all be signs of PCOS [2]. Belief holds that 40% of female infertility is caused by PCOS, which exhibits a well-established correlation with infertility [3, 4]. The metabolic and reproductive effects of PCOS are well-documented at all life stages, and the syndrome is a lifelong condition. Insulin resistance is aggravated in women with PCOS because they are often obese, with a noticeable central or abdominal fat distribution. In their later years, these women are more likely to experience dyslipidemia, cardiovascular disease, hypertension, impaired glucose tolerance (IGT), type 2 diabetes, and metabolic syndrome [5].

PCOS is a multifaceted hormonal disorder characterized by the involvement of numerous molecular mechanisms that contribute to its development. Several ovarian signaling pathways may be perturbed, thereby contributing to the distinctive features of PCOS [6]. The most often altered gene in human malignancies is the tumor suppressor gene P53 [7, 8]. There is a close correlation between the development of tumors and the malfunctions brought on by P53 mutations [9, 10].

The p53 protein is a transcription factor that controls many processes, including cell cycle arrest, DNA repair, cell apoptosis, autophagy, and metabolism [11-13]. The tumor suppressor p53 is an additional crucial metabolic regulator. Consequently, deregulation of p53 metabolic activities leads to PCOS [14, 15]. According to research, P53 may influence ovarian function and follicular development, which are fundamental aspects of polycystic ovary syndrome [16]. The cytochrome P450 (CYP) protein family contains significant enzymes that preserve lipid homeostasis [17, 18]. One crucial factor in PCOS is the suppressed expression of the cytochrome P450 (CYP) gene [19]. As CYP11A1 collaborates with other enzymes to facilitate androgen synthesis, modifications in its activity could potentially influence the overabundance of androgens observed in polycystic ovary syndrome. Some research indicates that steroidogenesis enzymes such as CYP11A1 may be associated with insulin resistance, a prevalent symptom of PCOS. Insulin resistance can exacerbate hormonal imbalances, increasing androgen secretion in the ovaries and adrenal glands [20-26]. HSD17B4 is a bifunctional enzyme implicated in the peroxisomal beta-oxidation pathway for fatty acids, catalyzing the synthesis of 3-ketoacyl-CoA. Although there is limited research examining the precise involvement of 17-HSD in polycystic ovary syndrome (PCOS), changes in androgen metabolism have been linked to the development of PCOS, and 17-HSD enzymes may potentially contribute to PCOS [27]. FTO is widely expressed in human skeletal muscles and adipose tissues, with its highest expression observed in the arcuate nucleus of the hypothalamus. This area regulates energy balance and may be crucial in controlling energy metabolism and appetite [28]. According to Chella et al. [29], an increase in fat mass and the obesity-related (FTO) gene showed a reciprocal association with the risk of obesity in PCOS patients, particularly in European and Asian ethnic groups. Current genome-wide association studies have connected genetic variants in the FTO gene to human obesity and metabolic disorders [30].

Research has shown that using medicinal plants as an alternative therapy is safe and beneficial in treating several illnesses [31-34]. The ability of herbs to enhance the immune system and regulate the menstrual cycle without altering hormone levels is thought to be the reason behind their effectiveness in treating PCOS [35]. *Artocarpus communis* (Parkinson) Fosberg (Family Moraceae) is a perennial, evergreen, terrestrial, single-stemmed, erect flowering plant, commonly known in English as the “breadfruit” tree because of the “bread-like texture” of its edible fruits. The root bark of *A. communis* is traditionally used in Nigeria to treat and manage a range of human illnesses, such as diabetes mellitus, diarrhea, and dysentery [36]. Recently, compounds identified in the seed were found to stabilize proteins by binding to the p53 core, FTO protein, and cytochrome P450 CYP11A1 domains, suggesting their beneficial effect on PCOS management [37]. In the present study, we aimed to investigate the effect of *Artocarpus communis* on the expression of P53, IRS, HsD17β2, FTO, and CYP11a genes in letrozole-induced polycystic ovarian syndrome rats. To the best of our knowledge, the present study is the first to explore this scope of research.

2. Materials and Methods

2.1 Plant Collection and Extraction

Artocarpus communis seeds were obtained in December 2022 in Oluponna, Osun State, Nigeria, and were identified and authenticated in the Bowen University Herbarium with the voucher number BUH: 034. The seeds were air-dried for 3 weeks and then ground into a coarse powder using an electric grinder. 1.55 kg of the powdered material was macerated in 5.4 L of distilled water for 72

hours at room temperature, occasionally swirled and shaken [10, 38]. The filtrate was subsequently passed through Whatman (Number 1) filter paper and a fresh cotton plug, followed by freeze-drying using a freeze-drier (Gunman, Germany). The percentage yield was then determined. The percentage yield was determined.

2.2 In Vivo Studies

2.2.1 Experimental Animals

Wistar female laboratory-bred rats with normal estrous cycles and weights between (150 and 220 g) were used. At 20-25°C, the animals were kept in cages with a 12-12-hour light-dark cycle. Before beginning the experimental treatments, animals underwent at least one week of acclimatization. The experiment followed the guidelines outlined in the Guide for the Care and Use of Laboratory Animals. Softwood shavings were used as bedding in cages to absorb animal feces and were replaced frequently. Throughout this trial, they had unlimited access to water and food pellets provided by Breedwell Feeds Ltd. This study was conducted with prior approval of the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/19/0051).

2.2.2 Induction of PCOS and Administration of Test Samples

Twenty-five female rats, weighing between 150 and 220 g and displaying a typical estrous cycle, were randomly divided into four groups, each containing five rats. Twenty female rats were given oral letrozole at a dose of 1 mg/kg daily for 21 days to induce PCOS, using 0.5% carboxymethyl cellulose (CMC) as a carrier [10]. These rats were divided into five groups of five rats, each at random. The control group is the first group, while the untreated group is group II. These groups were administered two milliliters of distilled water with 5% w/v CMC. One milligram of clomiphene citrate (Colid, Pfizer Pharmaceuticals, USA) per kilogram of body weight was administered to the third group (Group III) [10]. Groups IV and V received 100 and 250 mg/kg of *Artocarpus communis seed* aqueous extract, respectively.

2.2.3 Determination of the Estrus Cycle Pattern

Vaginal cytology was utilized to assess the stages of the estrous cycle. Vaginal lavage was collected more easily by gently inserting a Pasteur pipette containing 0.1 mL of normal saline (0.9% NaCl) into the rat's vagina. The recovered fluid was placed on a glass slide, and the distribution of cells was immediately examined under a microscope using a 10× objective lens. For the length of the investigation, this procedure was carried out every day between 7 and 9 am [38].

2.2.4 Blood and Organs Collection

The animals had 12-day therapy, and 24 hours after the administration of ketamine (50 mg/kg), they were anesthetized for a laparotomy [39]. The animals were anesthetized between 9 and 11 am to reduce the effects of diurnal fluctuation, and their ovaries were taken out for molecular research. Based on a 2 mL blood sample, the amounts of testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, and other hormones in each group were calculated [10].

2.2.5 Hormonal Analysis

The rat blood samples were subjected to the Enzyme-Linked Immunosorbent Assay (ELISA) method to assess the serum levels of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), testosterone, and estrogen [10]. Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) were assessed in the serum samples using the microwell kits manufactured by Fortress Diagnostics Limited, United Kingdom. Simultaneously, testosterone and estradiol levels were measured using microwell kits manufactured by Dialab, Austria. The samples and the test reagents were equilibrated at room temperature before the test. Then, 0.05 mL of calibrators and rat samples were pipetted inside the wells, followed by adding 0.1 mL of dilute enzyme conjugate to each well, excluding the blank well. The mixtures were left to rest for 60 minutes at room temperature. The mixtures in the microwells were thrown out, and the wells were cleaned with 0.2 mL of distilled water. The process of eliminating water from the well was repeated twice. A volume of 0.1 mL of the substrate solution was pipetted into each microwell in the same order and interval as for the enzyme conjugate. A blank well was included, and the plate was incubated in the dark for 20 minutes at room temperature. Stop solution (0.1 mL) was added into each microwell using the same order and timing as for the reaction of the substrate solution. The absorbance of each microwell was read at 450 nm against blank using a microplate reader. The developed color stabilized for at least 30 minutes, and the optical densities were read during this period.

2.3 Molecular Study

2.3.1 Isolation of Total RNA from the Ovaries

Total RNA was isolated from the ovaries with Quick-RNA MiniPrep™ Kit (Zymo Research). The DNA contaminant was removed following DNase I (NEB, Cat: M0303S) treatment. The RNA was quantified at 260 nm, and the purity was confirmed at 260 and 280 nm using an A&E Spectrophotometer (A&E Lab. UK).

2.3.2 cDNA Conversion

One (1 µg) of DNA-free RNA was converted to cDNA by reverse transcriptase reaction with the aid of a cDNA synthesis kit based on ProtoScript II first-strand technology (New England BioLabs) in a condition of 3-step reaction: 65°C for 5 min, 42°C for 1 h, and 80°C for 5 min [40].

2.3.3 PCR Amplification and Agarose Gel Electrophoresis

A polymerase chain reaction (PCR) to amplify the genes of interest was carried out with OneTaqR2X Master Mix (NEB) using the primers from Inqaba Biotec, Hatfield, South Africa. PCR amplification was conducted in a 25 µL volume reaction mixture containing cDNA, forward and reverse primers, and Ready Mix Taq PCR master mix. The processes are as follows: Initial denaturation at 95°C for 5 min, followed by 30 cycles of amplification (denaturation at 95°C for 30 s, annealing for 30 s, and extension at 72°C for 60 s) and ending with a final extension at 72°C for 10 min. The amplicons were separated on a 1.0% agarose gel. The GAPDH gene served as a normalization control for each gene's relative expression level, and band intensity was quantified using "ImageJ" software [41]. The primer sequences are provided below.

FTO:	5’-TTTCTTTAGCGGGCAGTGGT-3’	5’-GGTTGACACCACCAGTCAGT-3’
CYP11A:	5’-GTGGCCTATCACCAGTATTACC-3’	5’-GGCCATCACCTCTTGGTTTA-3’
IRS:	5’-CCTCACCAACCCTTAGGCAG-3’	5’-GTCTTTCATTCTGCCTGTGACG-3’
P53:	5’-ACATGACTGAGGTCGTGAGA-3’	5’-GATTTCCTTCCACCCGGATAAG-3’
HSD17B2:	5’-GACCGCCGATGAGTTTGT-3’	5’-TTTGGGTGGTGCTGCTGT-3’
GAPDH:	5’GCAAGGATACTGAGAGCAAGAG-3’	5’-CATCTCCCTCACAATTCCATCC-3’

3. Statistical Analysis

The outcomes of all tests were presented as the mean value ± the standard error of the mean. The experiments were conducted three times in order to ensure accuracy. The collected data were analyzed using Graph Pad software (Version 9.0). To examine the statistical variances between the groups at a significance level of $p < 0.05$, a one-way ANOVA followed by Dunnett’s Multiple Comparison Test was utilized.

4. Results

Despite their acyclic circumstances, administration of Artocarpus communis seed aqueous extract improved the estrous cycle in all treated rats. Figure 1 shows the increase in the frequency of the estrous and proestrus phases. Additionally, as seen in Table 1, the extract affected hormone levels, causing a drop in LH and increased FSH levels. Comparing the circulating LH level at 100 mg/kg to the untreated group (24.22 ± 0.06 mIU/mL), it is 12.97 ± 0.05 and 12.10 ± 1.44 mIU/mL for 100 mg/kg bw, respectively. Notably, rats given an aqueous extract of Artocarpus communis seed had the lowest level of LH. The FSH level was higher in the group treated with Artocarpus communis seed aqueous extract (100 mg/kg bw) (4.52 ± 0.05 mIU/mL) than in the untreated group (4.31 ± 0.05 mIU/mL). However, compared to the untreated group, which had testosterone levels of 3.41 ± 0.05 ng/mL, the rats’ testosterone levels decreased after receiving the Artocarpus communis seed aqueous extract. The level of LH in the group treated with Artocarpus communis (250 mg/kg bw) fruit aqueous extract was 12.10 ± 1.44 mIU/mL. In the group treated with 250 mg/kg bw Artocarpus communis seed aqueous extract, the level of testosterone was 2.59 ± 0.22 ng/mL, while the level of oestradiol was 209.29 ± 0.50 pg/mL. Table 1 illustrates that the Artocarpus communis seed aqueous extract-treated group had elevated estradiol levels compared to the untreated group. Additionally, the extract (250 mg/kg bw) regulated the expression of P53, type 2 17-HSD (17-HSD), fat mass and obesity-associated (FTO), 11a-hydroxylase/17,20-desmolase (CYP11a), and insulin receptor substrate (IRS) genes in the ovaries of PCOS rats, as shown in Figure 2.

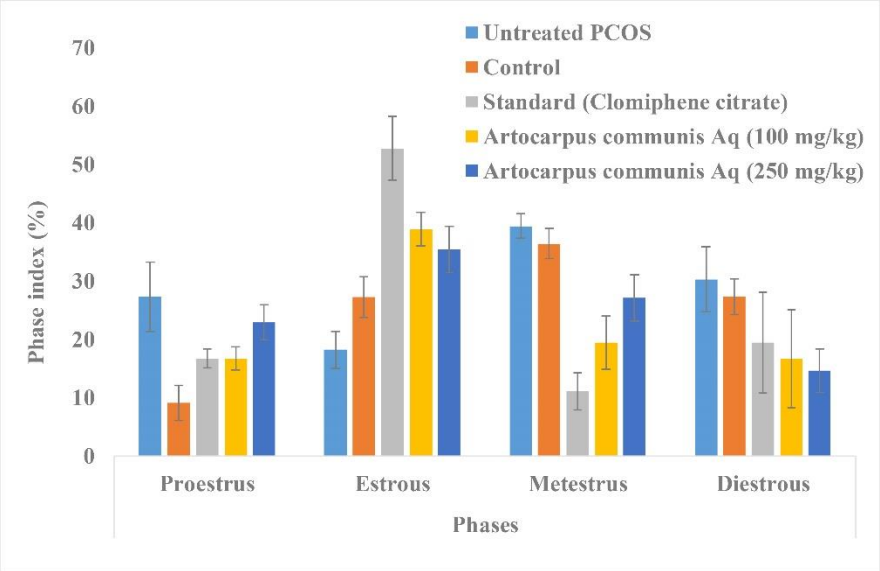

Figure 1 Index of estrous cycle phases of albino rats after twelve (12) days of treatment with clomiphene citrate and *Artocarpus communis seed aqueous* extract.

Table 1 Effect of *Artocarpus communis seed aqueous* extract on the PCOS groups' LH, FSH, and testosterone levels.

Parameters	Group I	Group II	Group III	Group IV	Group V
LH (mIU/mL)	12.97 ± 0.05	24.22 ± 0.06	21.15 ± 0.05^{b}	12.97 ± 0.05^{c}	12.10 ± 1.44^{b}
FSH (mIU/mL	4.52 ± 0.05	4.31 ± 0.05^{a}	5.95 ± 0.06^{ab}	4.52 ± 0.05	4.37 ± 0.16^{ab}
Testosterone (ng/mL)	2.78 ± 0.03	3.41 ± 0.05^{a}	2.75 ± 0.03^{ab}	2.78 ± 0.03^{b}	2.59 ± 0.22^{ab}
Estradiol (pg/mL)	199.06 ± 0.30	137.98 ± 0.50^{a}	209.29 ± 0.50	199.06 ± 0.30^{b}	209.29 ± 0.50

Group I - Control group, Group II - Untreated group, Group III - Standard group, Group IV - Dose group (100 mg/kg of *Artocarpus communis seed aqueous* extract), Group V - Dose group (250 mg/kg of *Artocarpus communis seed aqueous* extract). Data represented as mean ± SEM (n = 5). Values are statistically different at aP < 0.05 vs. control, bP < 0.05 vs. untreated PCOS, and cP < 0.05 vs. standard.

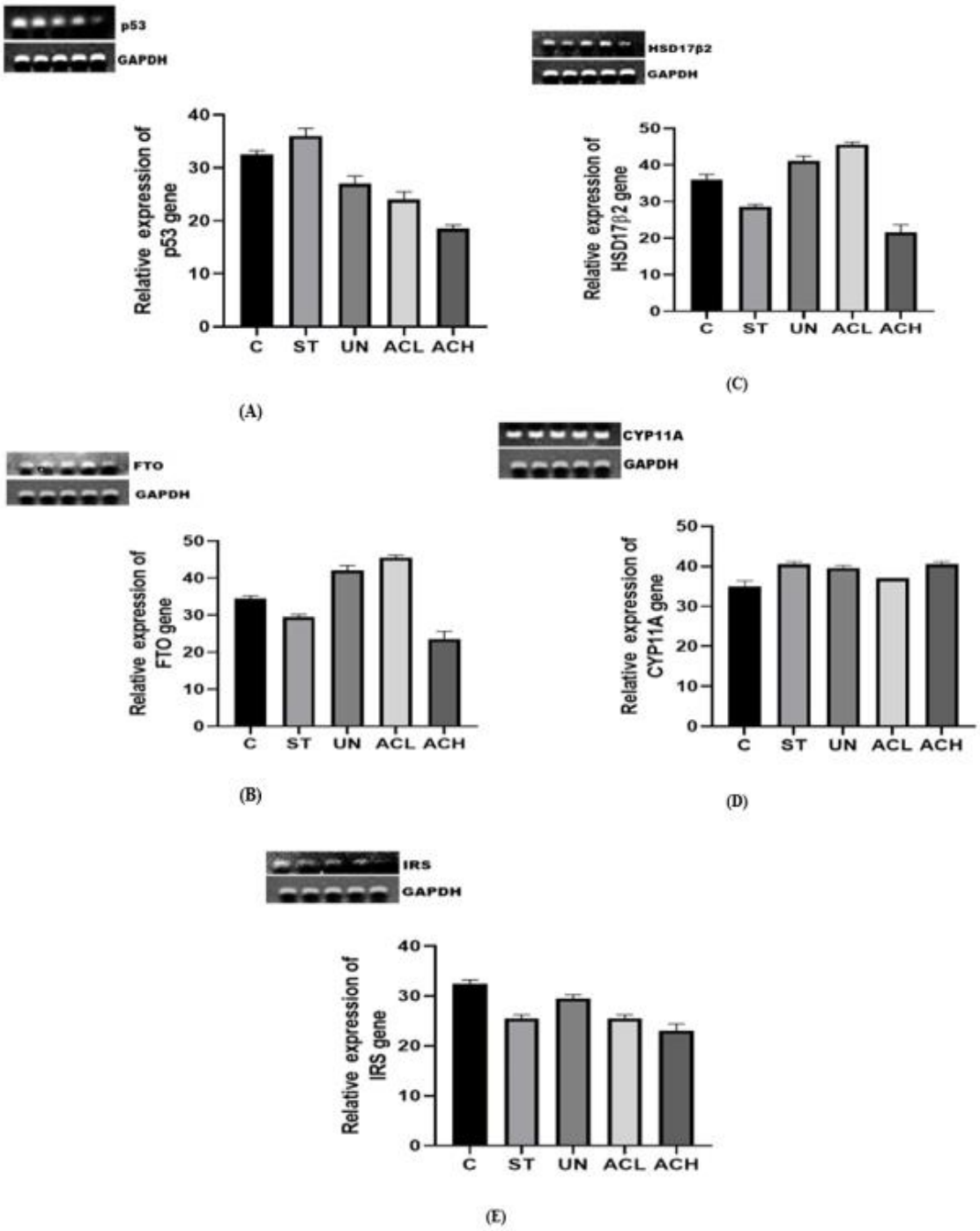

Figure 2 Effect of *Artocarpus communis* seed extract on P53 (A), FTO (B), HsD17β2 (C), CYP11a (D), and IRS (E) genes in ovaries of PCOS rats. The data indicate the mean ± SEM for the animals, with n = 5. PCOS - polycystic ovarian syndrome; *Artocarpus communis* Aq - *Artocarpus communis seed aqueous* extract. GAPDH and cyclophilin were used as loading control for cervix P53, IRS, HsD17β2, FTO, and CYP11a. Data are expressed as mean ± SD (n = 5). C: Control group; UN: Untreated group (PCOS rats); ST: Standard group (clomiphene citrate); ACL: PCOS + *Artocarpus communis seed aqueous* extract (100 mg/kg b.w.); ACH: PCOS + *Artocarpus communis seed aqueous* extract (250 mg/kg b.w.).

5. Discussion

Letrozole is an aromatase inhibitor used in PCOS induction. It prevents androgen conversion to estrogens, leading to hyperandrogenism, reduced follicle count, muscular hypertrophy of the uterus, and increased body and reproductive organ weights [42]. Following PCOS induction with letrozole, there was the presence of predominant leukocytes in the vaginal smear, indicating a disruption in the estrous cycle due to the prolonged diestrous phase and short estrous phase [43, 44]. In this study, letrozole-treated rats exhibited abnormal estrous cyclicity compared to the control group. The irregularity of the estrous cycle following letrozole administration could be due to changes in FSH and LH levels, hyperandrogenism, and altered steroid hormones [45]. However, 100 and 250 mg/kg *of Artocarpus communis seed aqueous* extract restored the imbalance by reducing the diestrous phase and increasing the estrous phase. This could be due to the reduced level of LH and improved estradiol concentration, indicating that *Artocarpus communis seed* aqueous extract can effectively treat menstrual irregularity. Increased testosterone and LH levels, low progesterone, FSH, and estradiol concentrations are hormonal hallmarks of PCOS diagnosis [46]. In this study, the letrozole-induced PCOS group showed significantly elevated LH and testosterone levels but decreased FSH and estradiol concentration in comparison to the control group. This finding is consistent with other studies [47-49]. Increased LH and reduced FSH levels could be due to excessive androgen production, resulting in more pulsatile secretion of GnRH [50]. The administration of 100 and 250 mg/kg *Artocarpus communis seed aqueous* extract significantly decreased LH and testosterone levels while increasing estradiol and FSH levels. The 100 and 250 mg/kg had a dose-dependent effect on hormonal concentration. For instance, 250 mg/kg in the estradiol level had a more pronounced effect than the 100 mg/kg extract-treated group. The restored hormonal balance could enhance folliculogenesis and induce ovulation.

The p53 gene controls insulin resistance, diabetes, and steroid hormones [51, 52]. Furthermore, P53 potentially exerts indirect influences on hormonal regulation by modulating many signaling pathways implicated in the synthesis and reaction to hormones. PCOS is characterized by hormonal imbalances and exclusively elevated androgen levels. Moreover, P53 is associated with metabolic pathways that govern glucose metabolism and insulin sensitivity. Given the frequent association between insulin resistance and polycystic ovary syndrome (PCOS), any potential impact of P53 on these signaling pathways may have implications for the development or progression of PCOS. In this study, we found a significant decrease in the relative expression of P53 compared to the untreated and the control group. P53 is pivotal in regulating apoptosis, DNA repair, and cell cycle progression. A reduction in its expression could indicate that cellular processes within the ovarian tissue impacted by polycystic ovaries may be altered. This modification can potentially impact ovarian cells' survival, differentiation, or proliferation. Furthermore, it is worth noting that a reduction in P53 expression may affect ovarian cell apoptosis, thereby potentially influencing ovarian follicle development, maintenance, or turnover. Potentially exacerbating the formation or persistence of ovarian cysts observed in polycystic ovaries, a reduction in P53 could modify the dynamics of follicular maturation and growth. Moreover, modifications in its expression could potentially affect the synthesis or responsiveness of hormones in the ovaries, thereby potentially exacerbating the hormonal imbalances that are distinctive of PCOS [53-56]. Recent studies [57, 58] have found abnormal p53 tumor suppressor genes in the endometrium of PCOS patients. This study demonstrates that, in comparison to controls, the ovaries of PCOS rats consistently contain the P53

enzyme; the two doses of aqueous extract decreased the P53 gene expression in the ovaries of the treated PCOS rats. This finding validates the findings of Ogunlakin et al. [15] and suggests that any medicinal plant with anti-PCOS activity should be able to suppress the P53 gene production.

According to recent studies, people with PCOS appear to be more susceptible to the effects of FTO gene variations on obesity, glucose intolerance, and insulin resistance [59, 60]. The Fat Mass and Obesity-Associated gene (FTO) has been linked to various aspects of metabolism and obesity, making it a subject of research interest regarding its involvement in polycystic ovary syndrome (PCOS). Still, relatively little research has looked at the relationship between PCOS symptoms and the susceptibility of the FTO enzymes [61, 62]. It is recognized that the FTO gene impacts body mass index (BMI) and correlates with obesity [60]. Research has indicated a possible association between FTO variants and the risk of polycystic ovary syndrome, explicitly concerning the typical metabolic disruptions and insulin resistance observed in PCOS [63]. Additionally, obesity and an elevated body mass index (BMI) have been linked to FTO variants, both of which are prevalent among women with PCOS. Elevated BMI is associated with heightened insulin resistance and hormonal disturbances, both of which contribute to the onset and worsening of symptoms associated with polycystic ovary syndrome. FTO variants may exacerbate a critical factor in PCOS insulin resistance. Elevated insulin levels can stimulate ovaries to generate surplus androgens, impeding ovulation and potentially contributing to the hormonal imbalances in polycystic ovary syndrome [63-65]. Our research's findings, however, indicated that compared to PCOS rats, rats treated with 250 mg/kg bw of aqueous extract had reduced expression of the FTO gene.

Although there is limited research examining the precise involvement of 17-HSD in polycystic ovary syndrome (PCOS), changes in androgen metabolism have been linked to the development of PCOS, and 17-HSD enzymes may potentially contribute to this [27]. PCOS is often characterized by an imbalance of reproductive hormones, particularly elevated androgen levels. In peripheral tissues such as the ovaries, feeble androgens (such as androstenedione) are converted to more potent androgens (such as testosterone) by 17-HSD enzymes, specifically 17-HSD type 5. Potentially, dysregulation or overactivity of these enzymes may be a factor in the elevated androgen levels observed in polycystic ovary syndrome. Fluctuations in the metabolism of androgens within the ovaries can disrupt regular ovulation processes and impede follicular development. Androgen excess can disrupt ovulation and follicle maturation, resulting in the development of ovarian cysts, a hallmark feature of polycystic ovary syndrome [27, 66-68]. A prevalent complication of PCOS, insulin resistance may contribute to an increase in androgen secretion. Variations in the activity of 17-HSD could potentially affect insulin sensitivity, consequently influencing androgen levels. Hay and Hodgins [69] and Oliveira et al. [70] have linked the type 2 17-HSD gene to maintaining androgen homeostasis in hirsute women. Type 2 17-HSD (17-HSD) gene expression in the ovaries of PCOS rats was reduced by 250 mg/kg of aqueous extract of A. communis seed in this study. Rats administered with 100 mg/kg of the extract showed elevated levels of type 2 17-HSD gene, consistent with the group's observed high levels of LH and testosterone.

Particularly in the adrenal glands and gonads, the enzyme 11β-hydroxylase/17,20-desmolase, encoded by the CYP11A1 gene, is involved in steroidogenesis, the process by which steroid hormones are synthesized. In the early stages of steroid hormone synthesis, CYP11A1 converts cholesterol to pregnenolone, an essential precursor to an assortment of steroid hormones, including androgens. Alterations or disruptions in this enzymatic activity may impact the synthesis of androgens, including testosterone, which is frequently increased in individuals with polycystic

ovary syndrome [21, 26]. PCOS is characterized by elevated androgen levels, which can interfere with ovarian function and result in symptoms such as irregular ovulation and follicular follicles. As CYP11A1 collaborates with other enzymes to facilitate androgen synthesis, modifications in its activity could potentially influence the overabundance of androgens observed in polycystic ovary syndrome [20, 71]. Thus, a deficit of the CYP11a gene that leads to a gain in function or a loss of repression may cause an increase in P450scc activity and probably hyperandrogenism. By administering 250 mg/kg of the aqueous extract to PCOS-treated rats, the ovaries showed enhanced expression of the CYP11a gene. Several studies have linked allelic variations in IRS genes IRS-1 Gly972Arg and IRS-2 Gly1057Asp to PCOS [72, 73]. Insulin-stimulated translocation of the glucose transporter GLUT4 to the cell surface caused a fourfold reduction in the sensitivity of the dose-response curve when an antisense ribozyme directed against rat IRS-1 was expressed in isolated rat adipocytes [74]. Reducing the IRS gene may, therefore, lower metabolic abnormalities, insulin resistance, and lipid metabolism, lowering the underlying risk of diabetes in women with PCOS [75, 76]. In the ovaries of PCOS rats, the expression of the IRS gene was decreased by the two doses of *A. communis* seed aqueous extract.

6. Conclusion

This study examined the beneficial effects of an aqueous extract of the seed of *Artocarpus communis* on letrozole-induced PCOS rats. Treatment with Artocarpus communis seed aqueous extract normalizes hormonal imbalance and causes notable alterations in the expression of FTO, CYP11A, IRS, P53, and HSD17B2 genes in the ovaries of PCOS rats. These findings collectively suggest that *Artocarpus communis* seed might have multifaceted effects on molecular pathways associated with PCOS, potentially normalizing androgen metabolism, hormonal imbalance, and ovarian function.

Acknowledgments

We appreciate Bowen University via Bowen University Research Grant (BURG) (Grant No: BURG/2023/001) for supporting this research.

Author Contributions

Conceptualization: Akingbolabo Daniel Ogunlakin and Oluwafemi Adeleke Ojo. Methodology: Akingbolabo Daniel Ogunlakin, Oluwafemi Adeleke Ojo, and Peluola Olujide Ayeni. Validation: All authors. Formal analysis: Akingbolabo Daniel Ogunlakin, Oluwafemi Adeleke Ojo. Investigation: All authors. Writing of the original draft: Akingbolabo Daniel Ogunlakin, Oluwafemi Samson Afolayan, and Chimzi David Onu-Boms. Writing, review and editing: All authours. Resources: Akingbolabo Daniel Ogunlakin, Oluwafemi Adeleke Ojo, Damilare Iyinkristi Ayokunle, and Peluola Olujide Ayeni.

Funding

This research was funded by Bowen University via Bowen University Research Grant (BURG) (Grant No: BURG/2023/001) for 2022-2023.

Competing Interests

The authors declare no conflicts of interest.

Additional Materials

The following additional materials are uploaded at the page of this paper.

1. Gel agarose images.

References

1. Kovanci E, Buster JE. Polycystic ovary syndrome. In: Clinical Gynecology. Cambridge, UK: Cambridge University Press; 2015. pp. 1024-1046.
2. Hardiman P, Pillay OS, Atiomo W. Polycystic ovary syndrome and endometrial carcinoma. Lancet. 2003; 361: 1810-1812.
3. Krysiak R, Okopieñ B, Gdula-Dymek A, Herman ZS. Update on the management of polycystic ovary syndrome. Pharmacol Rep. 2006; 58: 614-625.
4. Khan MJ, Ullah A, Basit S. Genetic basis of polycystic ovary syndrome (PCOS): Current perspectives. Appl Clin Genet. 2019; 12: 249-260.
5. Teede H, Deeks A, Moran L. Polycystic ovary syndrome: A complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. BMC Med. 2010; 8: 41.
6. Scarfò G, Daniele S, Fusi J, Gesi M, Martini C, Franzoni F, et al. Metabolic and molecular mechanisms of diet and physical exercise in the management of polycystic ovarian syndrome. Biomedicines. 2022; 10: 1305.
7. Levine AJ. P53: 800 million years of evolution and 40 years of discovery. Nat Rev Cancer. 2020; 20: 471-480.
8. Wang H, Guo M, Wei H, Chen Y. Targeting p53 pathways: Mechanisms, structures, and advances in therapy. Signal Transduct Target Ther. 2023; 8: 92.
9. Zhou X, Hao Q, Lu H. Mutant p53 in cancer therapy-the barrier or the path. J Mol Cell Biol. 2019; 11: 293-305.
10. Ogunlakin AD, Sonibare MA. Ameliorative effect of kigelia africana (Lam.) benth. fruit methanol extract in letrozole-induced polycystic ovarian syndrome rat. Kuwait J Sci. 2023; 50: 622-626.
11. Al-Salam A, Irwin DM. Evolution of the vertebrate insulin receptor substrate (Irs) gene family. BMC Evol Biol. 2017; 17: 148.
12. Bykov VJ, Eriksson SE, Bianchi J, Wiman KG. Targeting mutant p53 for efficient cancer therapy. Nat Rev Cancer. 2018; 18: 89-102.
13. Sullivan KD, Galbraith MD, Andrysik Z, Espinosa JM. Mechanisms of transcriptional regulation by p53. Cell Death Differ. 2018; 25: 133-143.
14. Lacroix M, Riscal R, Arena G, Linares LK, Le Cam L. Metabolic functions of the tumor suppressor p53: Implications in normal physiology, metabolic disorders, and cancer. Mol Metab. 2020; 33: 2-22.
15. Ogunlakin AD, Sonibare MA, Yeye OE, Gyebi GA, Ayokunle DI, Arigbede OE, et al. Isolation and characterization of novel hydroxyflavone from kigelia africana (Lam.) benth. fruit ethyl acetate

fraction against CHO 1 and heLa cancer cell lines: In vitro and in silico studies. J Mol Struct. 2023; 1282: 135180.
16. Biglari-Zadeh G, Sargazi S, Mohammadi M, Ghasemi M, Majidpour M, Saravani R, et al. Relationship between genetic polymorphisms in cell cycle regulatory gene TP53 and polycystic ovarian syndrome: A case-control study and in silico analyses. Biochem Genet. 2023; 61: 1827-1849.
17. Simpson AECM. The cytochrome P450 4 (CYP4) family. Gen Pharmacol. 1997; 28: 351-359.
18. Gao H, Cao Y, Xia H, Zhu X, Jin Y. CYP4A11 is involved in the development of nonalcoholic fatty liver disease via ROS-induced lipid peroxidation and inflammation. Int J Mol Med. 2020; 45: 1121-1129.
19. Tabur S, Oztuzcu S, Oguz E, Demiryürek S, Dagli H, Alasehirli B, et al. CYP gene expressions in obesity-associated metabolic syndrome. Obes Res Clin Pract. 2016; 10: 719-23.
20. Gharani N, Waterworth DM, Batty S, White D, Gilling-Smith C, Conway GS, et al. Association of the steroid synthesis gene CYP11a with polycystic ovary syndrome and hyperandrogenism. Hum Mol Genet. 1997; 6: 397-402.
21. Pusalkar M, Meherji P, Gokral J, Chinnaraj S, Maitra A. CYP11A1 and CYP17 promoter polymorphisms associate with hyperandrogenemia in polycystic ovary syndrome. Fertil Steril. 2009; 92: 653-659.
22. Wickenheisser JK, Biegler JM, Nelson-DeGrave VL, Legro RS, Strauss III JF, McAllister JM. Cholesterol side-chain cleavage gene expression in theca cells: Augmented transcriptional regulation and mRNA stability in polycystic ovary syndrome. PLoS One. 2012; 7: e48963.
23. Zhang CW, Zhang XL, Xia YJ, Cao YX, Wang WJ, Xu P, et al. Association between polymorphisms of the CYP11A1 gene and polycystic ovary syndrome in Chinese women. Mol Biol Rep. 2012; 39: 8379-8385.
24. Emami N, Moini A, Yaghmaei P, Akbarinejad V, Shahhoseini M, Alizadeh A. Differences in expression of genes related to steroidgenesis in abdominal subcutaneous adipose tissue of pregnant women with and without PCOS; a case control study. BMC Pregnancy Childbirth. 2021; 21: 490.
25. Kaur R, Kaur T, Sudhir N, Kaur A. Association analysis of CYP11A1 variants with polycystic ovary syndrome: A case-control study from North India. Reprod Sci. 2021; 28: 2951-2960.
26. Heidarzadehpilehrood R, Pirhoushiaran M, Abdollahzadeh R, Binti Osman M, Sakinah M, Nordin N, et al. A review on CYP11A1, CYP17A1, and CYP19A1 polymorphism studies: Candidate susceptibility genes for polycystic ovary syndrome (PCOS) and infertility. Genes. 2022; 13: 302.
27. Koeva Y, Atanassova N. Hydrohysteroid dehydrogenases-biological role and clinical importance-review. In: Dehydrogenases. London: IntechOpen; 2012. doi: 10.5772/54149.
28. Lan N, Lu Y, Zhang Y, Pu S, Xi H, Nie X, et al. FTO-a common genetic basis for obesity and cancer. Front Genet. 2020; 11: 559138.
29. Chella Krishnan K, Vergnes L, Acín-Pérez R, Stiles L, Shum M, Ma L, et al. Sex-specific genetic regulation of adipose mitochondria and metabolic syndrome by Ndufv2. Nat Metab. 2021; 3: 1552-1568.
30. Zhao X, Yang Y, Sun BF, Zhao YL, Yang YG. FTO and obesity: Mechanisms of association. Curr Diab Rep. 2014; 14: 486.

31. Ghosh S, Bishal A, Ghosh SK, Jana K, Gayen B, Sahu S, et al. Herbal medicines: A potent approach to human diseases, their chief compounds, formulations, present status, and future aspects. Int J. 2023; 10: 442-464.
32. Jamal A. Embracing nature's therapeutic potential: Herbal medicine. Int J Multidiscip Sci Arts. 2023; 2: 117-126.
33. Moiketsi BN, Makale KP, Rantong G, Rahube TO, Makhzoum A. Potential of selected African medicinal plants as alternative therapeutics against multi-drug-resistant bacteria. Biomedicines. 2023; 11: 2605.
34. Larit F, León F. Therapeutics to treat psychiatric and neurological disorders: A promising perspective from algerian traditional medicine. Plants. 2023; 12: 3860.
35. Manouchehri A, Abbaszadeh S, Ahmadi M, Nejad FK, Bahmani M, Dastyar N. Polycystic ovaries and herbal remedies: A systematic review. JBRA Assist Reprod. 2023; 27: 85-91.
36. Adewole SO, Ojewole JA. Artocarpus communis forst. root-bark aqueous extract-and streptozotocin-induced ultrastructural and metabolic changes in hepatic tissues of wistar rats. Afr J Tradit Complement Altern Med. 2007; 4: 397-410.
37. Ogunlakin AD, Ojo OA, Gyebi GA, Akinwumi IA, Adebodun GO, Ayokunle DI, et al. Elemental evaluation, nutritional analysis, GC-MS analysis and ameliorative effects of artocarpus communis JR Forst. & G. forst. seeds' phytoconstituents on metabolic syndrome via in silico approach. J Biomol Struct Dyn. 2023. doi: 10.1080/07391102.2023.2293271.
38. Ogunlakin AD, Sonibare MA, Jabeen A, Shaheen F, Shah SF. Antiproliferative and ameliorative effects of tetracera potatoria and its constituent. Adv Trad Med. 2021; 21: 815-824.
39. Rakic D, Joksimovic Jovic J, Jakovljevic V, Zivkovic V, Nikolic M, Sretenovic J, et al. High fat diet exaggerate metabolic and reproductive PCOS features by promoting oxidative stress: An improved EV model in eats. Medicina. 2023; 59: 1104.
40. Elekofehinti OO, Lawal AO, Ejelonu OC, Molehin OR, Famusiwa CD. Involvement of fat mass and obesity gene (FTO) in the antiobesity action of annona muricata annonaceae: In silico and in vivo studies. J Diabetes Metab Disord. 2020; 19: 197-204.
41. Olumegbon LT, Lawal AO, Oluyede DM, Adebimpe MO, Elekofehinti OO, Umar HI. Hesperetin protects against diesel exhaust particles-induced cardiovascular oxidative stress and inflammation in Wistar rats. Environ Sci Pollut Res. 2022; 29: 52574-52589.
42. Rosenfield RL, Ehrmann DA. The pathogenesis of polycystic ovary syndrome (PCOS): The hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocr Rev. 2016; 37: 467-520.
43. Khaled N, El-Bahy AA, Radwan R, Handoussa H, AbdelMaksoud S. Ocimum kilimandscharicum L. restores ovarian functions in letrozole-induced polycystic ovary syndrome (PCOS) in rats: Comparison with metformin. Life Sci. 2019; 232: 116640.
44. Bu N, Jamil A, Hussain L, Alshammari A, Albekairi TH, Alharbi M, et al. Phytochemical-based study of ethanolic extract of saraca asoca in letrozole-induced polycystic ovarian syndrome in female adult rats. ACS Omega. 2023; 8: 42586-42597.
45. Tanruean K, Poolprasert P, Kumla J, Suwannarach N, Lumyong S. Bioactive compounds content and their biological properties of acetone extract of cuscuta reflexa roxb. grown on various host plants. Nat Prod Res. 2019; 33: 544-547.

46. Khosrowpour Z, Fahimi S, Jafari F, Tansaz M, Sahranavard S, Faizi M. Beneficial effects of teucrium polium hydroalcoholic extract on letrozole-induced polycystic ovary syndrome in rat model. Obstet Gynecol Sci. 2022; 66: 107-117.
47. Bednarska S, Siejka A. The pathogenesis and treatment of polycystic ovary syndrome: What's new? Adv Clin Exp Med. 2017; 26: 359-367.
48. Younas A, Hussain L, Shabbir A, Asif M, Hussain M, Manzoor F. Effects of fagonia indica on letrozole-induced polycystic ovarian syndrome (PCOS) in young adult female rats. Evid Based Complement Alternat Med. 2022; 2022: 1397060.
49. Pachiappan S, Ramalingam K, Balasubramanian A. Evaluation of gymnema sylvestre R. Br. against letrozole induced polycystic ovarian syndrome in rats. Res J Pharm Technol. 2023; 16: 385-390.
50. Shende A, Joshi S, Koli PG, Joshi SS. Evaluation of the effects of caesalpinia crista on letrozole-induced models of polycystic ovarian syndrome. Cureus. 2023; 15: e34215.
51. Charni-Natan M, Aloni-Grinstein R, Osher E, Rotter V. Liver and steroid hormones-can a touch of p53 make a difference? Front Endocrinol. 2019; 10: 374.
52. Kung C-P, Murphy ME. The role of the p53 tumor suppressor in metabolism and diabetes. J Endocrinol. 2016; 231: R61-R75.
53. Siddamalla S, Reddy TV, Govatati S, Guruvaiah P, Deenadayal M, Shivaji S, et al. Influence of tumour suppressor gene (TP53, BRCA1 and BRCA2) polymorphisms on polycystic ovary syndrome in South Indian women. Eur J Obstet Gynecol Reprod Biol. 2018; 227: 13-18.
54. Tong C, Wu Y, Zhang L, Yu Y. Insulin resistance, autophagy and apoptosis in patients with polycystic ovary syndrome: Association with PI3K signaling pathway. Front Endocrinol. 2022; 13: 1091147.
55. Yang X, Wang K, Lang J, Guo D, Gao H, Qiu Y, et al. Up-regulation of miR-133a-3p promotes ovary insulin resistance on granulosa cells of obese PCOS patients via inhibiting PI3K/AKT signaling. BMC Womens Health. 2022; 22: 412.
56. Zanjirband M, Hodayi R, Safaeinejad Z, Nasr-Esfahani M, Ghaedi-Heydari R. Evaluation of the p53 pathway in polycystic ovarian syndrome pathogenesis and apoptosis enhancement in human granulosa cells through transcriptome data analysis. Sci Rep. 2023; 13: 11648.
57. Shafiee MN, Malik DA, Yunos RIM, Atiomo W, Omar MH, Ghani NAA, et al. The effect of metformin on endometrial tumor-regulatory genes and systemic metabolic parameters in polycystic ovarian syndrome-a proof-of-concept study. Gynecol Endocrinol. 2015; 31: 286-290.
58. Gadducci A, Biglia N, Tana R, Cosio S, Gallo M. Metformin use and gynecological cancers: A novel treatment option emerging from drug repositioning. Crit Rev Oncol Hematol. 2016; 105: 73-83.
59. Attaoua R, El Mkadem SA, Radian S, Fica S, Hanzu F, Albu A, et al. FTO gene associates to metabolic syndrome in women with polycystic ovary syndrome. Biochem Biophys Res Commun. 2008; 373: 230-234.
60. Kowalska I, Malecki M, Straczkowski M, Skupien J, Karczewska-Kupczewska M, Nikolajuk A, et al. The FTO gene modifies weight, fat mass and insulin sensitivity in women with polycystic ovary syndrome, where its role may be larger than in other phenotypes. Diabetes Metab. 2009; 35: 328-331.
61. Barber T, Bennett A, Groves C, Sovio U, Ruokonen A, Martikainen H, et al. Association of variants in the fat mass and obesity associated (FTO) gene with polycystic ovary syndrome. Diabetologia. 2008; 51: 1153-1158.

62. Yan Q, Hong J, Gu W, Zhang Y, Liu Q, Su Y, et al. Association of the common rs9939609 variant of FTO gene with polycystic ovary syndrome in Chinese women. Endocrine. 2009; 36: 377-382.
63. Tan S, Scherag A, Janssen OE, Hahn S, Lahner H, Dietz T, et al. Large effects on body mass index and insulin resistance of fat mass and obesity associated gene (FTO) variants in patients with polycystic ovary syndrome (PCOS). BMC Med Genet. 2010; 11: 12.
64. Liu Ying LY, Chen YongXia CY. Fat mass and obesity associated gene polymorphism and the risk of polycystic ovary syndrome: A meta-analysis. Iran J Public Health. 2017; 46: 4-11.
65. Chaudhary H, Patel J, Jain NK, Panchal S, Laddha N, Joshi R. Association of FTO gene variant rs9939609 with polycystic ovary syndrome from Gujarat, India. BMC Med Genom. 2023; 16: 216.
66. Schiffer L, Arlt W, O'Reilly MW. 33-49: Understanding the role of androgen action in female adipose tissue. In: Frontiers of hormone research. Basel, Switzerland: S. Karger AG; 2019. pp. 33-49.
67. Yu L, Liu M, Wang Z, Liu T, Liu S, Wang B, et al. Correlation between steroid levels in follicular fluid and hormone synthesis related substances in its exosomes and embryo quality in patients with polycystic ovary syndrome. Reprod Biol Endocrinol. 2021; 19: 74.
68. Storbeck K-H, O'Reilly MW. The clinical and biochemical significance of 11-oxygenated androgens in human health and disease. Eur J Endocrinol. 2023; 188: R98-R109.
69. Hay J, Hodgins M. Distribution of androgen metabolizing enzymes in isolated tissues of human forehead and axillary skin. J Endocrinol. 1978; 79: 29-39.
70. Oliveira IO, Lhullier C, Brum IS, Spritzer PM. Gene expression of type 2 17β hydroxysteroid dehydrogenase in scalp hairs of hirsute women. Steroids. 2003; 68: 641-649.
71. Urbanek M, Legro RS, Driscoll DA, Azziz R, Ehrmann DA, Norman RJ, et al. Thirty-seven candidate genes for polycystic ovary syndrome: Strongest evidence for linkage is with follistatin. Proc Natl Acad Sci. 1999; 96: 8573-8578.
72. El Mkadem SA, Lautier C, Macari F, Molinari N, Lefebvre P, Renard E, et al. Role of allelic variants Gly972Arg of IRS-1 and Gly1057Asp of IRS-2 in moderate-to-severe insulin resistance of women with polycystic ovary syndrome. Diabetes. 2001; 50: 2164-2168.
73. Villuendas G, Botella-Carretero JI, Roldán B, Sancho J, Escobar-Morreale HF, Millán JLS. Polymorphisms in the insulin receptor substrate-1 (IRS-1) gene and the insulin receptor substrate-2 (IRS-2) gene influence glucose homeostasis and body mass index in women with polycystic ovary syndrome and non-hyperandrogenic controls. Hum Reprod. 2005; 20: 3184-3191.
74. Quon MJ, Butte AJ, Zarnowski MJ, Sesti G, Cushman SW, Taylor SI. Insulin receptor substrate 1 mediates the stimulatory effect of insulin on GLUT4 translocation in transfected rat adipose cells. J Biol Chem. 1994; 269: 27920-27924.
75. Guo S. Insulin signaling, resistance, and the metabolic syndrome: Insights from mouse models to disease mechanisms. J Endocrinol. 2014; 220: T1-T23.
76. Yaribeygi H, Farrokhi FR, Butler AE, Sahebkar A. Insulin resistance: Review of the underlying molecular mechanisms. J Cell Physiol. 2019; 234: 8152-8161.