Research Article

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

J. Anat Sci 17(3) Sept.

Submitted: August 22nd, 2026
Revised: September 8th, 2026
Accepted: September 17th, 2026

DOI: https://dx.doi.org/10.4314/jans.v17i3.13

Effects of *Phyllanthus amarus* intervention on ovarian Histomorphology and serum reproductive hormones in a rat model of polycystic ovarian syndrome

Kayode E. Adebisi, Aisha Abdulrauf, Faizah A. Tijani, Vanessa E. Osalohein, Temiloluwa O. Ogunnusi, Victor Olarinoye, Oluwole B. Akinola*

Neuroendocrinology Unit, Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria.

***Corresponding Author:** Email: akinolub@unilorin.edu.ng Tel**:** ORCID:

ABSTRACT

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder characterized by hyperandrogenism, metabolic disturbances, and ovarian dysfunction. This study investigated the effects of ethanolic leaf extract of *Phyllanthus amarus* on ovarian histomorphology and selected metabolic and reproductive hormonal parameters in female Wistar rats with dihydrotestosterone (DHT)-induced PCOS. Twenty-one female Wistar rats were randomly assigned to three groups (n = 7/group): control, DHT-induced PCOS, and DHT-induced PCOS treated with *P. amarus*. PCOS was induced by daily subcutaneous administration of DHT (0.075 mg/kg body weight) for 30 days, after which the treatment group received oral *P. amarus* extract (300 mg/kg body weight) for 14 days. At the end of the treatment, serum glucose, insulin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone were assayed, and HOMA-IR was calculated. Ovarian tissues were examined histologically using periodic acid-Schiff and Masson's trichrome staining. DHT administration was associated with significant increases in fasting serum glucose, fasting serum insulin, HOMA-IR, LH and testosterone, together with ovarian cystic follicular changes. Treatment with *P. amarus* significantly reduced fasting serum glucose, fasting serum insulin, HOMA-IR, LH and testosterone compared with the untreated PCOS group. Histological examination also showed attenuation of cystic follicular changes and improved ovarian tissue architecture following *P. amarus* treatment. These findings indicate that ethanolic *P. amarus* leaf extract ameliorates selected metabolic, endocrine and ovarian histomorphological abnormalities associated with DHT-induced PCOS in Wistar rats. Further studies are, however, required to establish the molecular mechanisms underlying these effects and how they translate into reproductive benefits in humans.

Keywords: Polycystic ovary syndrome; *Phyllanthus amarus*; dihydrotestosterone; insulin resistance; reproductive hormones

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common and clinically heterogeneous endocrine disorder of reproductive-age women. Its reported prevalence varies according to the population studied and the diagnostic criteria applied, with estimates ranging from approximately 5% to 20%.[1] The syndrome is characterized by a combination of reproductive, endocrine and metabolic abnormalities, including menstrual irregularity, anovulation, infertility, hyperandrogenic manifestations such as hirsutism and acne, and obesity.[2] The broad clinical spectrum of PCOS reflects the fact that the disorder is not confined to the ovary but involves interconnected disturbances in reproductive, metabolic and endocrine homeostasis.

The pathophysiology of PCOS is complex and multifactorial. Genetic susceptibility and epigenetic influences interact with environmental and lifestyle factors, oxidative stress, chronic low-grade inflammation, mitochondrial dysfunction and metabolic abnormalities to disturb normal ovarian function.[3-5] These interacting processes can affect follicular development, steroidogenesis and ovulation and may contribute to the persistence of the reproductive and metabolic phenotype. Insulin resistance is particularly important because it is frequently associated with PCOS and can aggravate hyperandrogenism.[4,5] Compensatory hyperinsulinemia may enhance androgen production and contribute to the metabolic and reproductive abnormalities that characterize the syndrome.

Endocrine dysregulation in PCOS is closely related to abnormal hypothalamic–pituitary–ovarian axis activity and altered gonadotropin secretion. Increased androgen exposure and disturbances in gonadotropin dynamics can interfere with normal follicular recruitment, maturation and ovulation.[4,5] At the same time, insulin resistance and associated metabolic dysfunction may reinforce ovarian androgen excess, creating a self-perpetuating interaction between metabolic and reproductive abnormalities. Consequently, experimental models that reproduce hyperandrogenism together with characteristic ovarian and metabolic changes are valuable for investigating the mechanisms of PCOS and for evaluating potential interventions.

Androgen-based experimental models have been used extensively to reproduce important features of PCOS in rodents. Dihydrotestosterone (DHT), a potent non-aromatizable androgen, has been used to induce a hyperandrogenic phenotype and ovarian abnormalities in rats and other experimental animals. Studies using DHT-based models have demonstrated ovarian and metabolic alterations resembling important components of PCOS, thereby providing a useful platform for investigating hyperandrogenism-associated pathology and potential therapeutic interventions.[11,12,19,25] In particular, the development of cystic follicular changes and associated endocrine or metabolic disturbances in these models provides an opportunity to evaluate whether candidate interventions can restore ovarian architecture and improve systemic abnormalities.

Because PCOS is a chronic disorder with reproductive and metabolic consequences, there is increasing interest in complementary and plant-derived therapeutic approaches. Phyllanthus amarus (*P. amarus*) is an ethnomedicinal plant that has attracted attention because of reported antioxidant, anti-inflammatory and antidiabetic activities.[6-9] Experimental evidence indicates that preparations of *P. amarus* may influence metabolic disturbances, oxidative stress and reproductive-related functions.[6,7,9] These properties are of particular interest in PCOS because oxidative stress, inflammation, insulin resistance and endocrine dysregulation are interconnected components of the disorder. However, the available evidence does not adequately define the effects of ethanolic *P. amarus* leaf extract on the ovarian microarchitecture and reproductive endocrine profile in a DHT-induced PCOS model, particularly in prepubertal Wistar rats.

The ovarian structural changes associated with PCOS are of particular importance because normal follicular development depends on coordinated interactions among the oocyte, granulosa cells, theca cells, ovarian stroma and vascular components. Persistent androgenic and metabolic disturbances may disrupt this microenvironment, resulting in abnormal follicular development, cystic follicle formation and stromal alterations. Evaluation of ovarian histomorphology alongside circulating metabolic and reproductive indices therefore provides a more comprehensive assessment of the response to a potential intervention. In this context, histological techniques can provide complementary information on ovarian tissue architecture and stromal changes.[13,14]

Against this background, the present study investigated the effects of ethanolic leaf extract of *P. amarus* on ovarian histomorphology and selected serological parameters in prepubertal Wistar rats with DHT-induced PCOS. Specifically, the study assessed fasting glucose, fasting insulin and HOMA-IR as indicators of metabolic status, together with serum luteinizing hormone, follicle-stimulating hormone and testosterone as indicators of reproductive endocrine function, and evaluated ovarian microanatomical alterations following *P. amarus* intervention. The study was designed to provide integrated preclinical evidence on the metabolic, endocrine and structural effects of *P. amarus* in an experimental model of PCOS.

MATERIALS AND METHODS

Chemicals

Dihydrotestosterone (DHT) was purchased from Central Research Laboratory, Tanke, Ilorin, Kwara State. *P. amarus* was collected locally. All other chemicals and reagents used were of analytical grade and were procured from local vendors.

Preparation of Ethanolic leaf extract of *P. amarus*

P. amarus was collected from Iseyin, Oyo State, identified and authenticated by Dr. Bolu Ajayi of the Herbarium Unit with the voucher number UILH/001/1051/2023. Extraction was done as reported by Bhat et al. [10]. Fresh leaves of *P. amarus* were air-dried, pulverized, and soaked in absolute ethanol for 72 hours, then sieved and left to evaporate in an open container at room temperature to ensure removal of ethanol. A gel-like extract was obtained. The extract was weighed using a weighing balance (SF-400 weighing balance). A total of 15 g of the extract was obtained

Animals

Twenty-one prepubertal female Wistar rats weighing an average of 40 g were procured from the Animal Holding Facility, Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, and bred in the College of Health Sciences Animal Holding Facility, using polycarbonate wire-topped cages (7 rats per cage), under suitable environmental conditions (at 23±1 ºC) throughout the duration of treatment. Animals were housed on a 12-hour light and 12-hour dark cycle and fed with a standard rat diet and tap water *ad libitum*, for one week before commencing PCOS induction. In line with global best practices, all protocols were approved by the

University Ethical Review Committee of the University of Ilorin. (approval number, UERC/ASN/2023/2515).

Study design

After one week of acclimatization, the animals were randomly assigned to one of three experimental groups (n=7 rats per group). The first group served as the control (Ctrl) and received oral normal saline and subcutaneous corn oil injection. The second group served as the DHT-induced PCOS group (PCOS). PCOS group received 0.075 mg/kg bw of DHT 11,12 via subcutaneous injection for 30 days, between 8.00 am and 9.00 am each morning. Corn oil served as vehicle. The third group served as the PCOS plus *P. amarus* (PCOS+*P. amarus*) group. This group was first administered DHT (0.075 mg/kg bw) for 30 days to induce PCOS, followed by oral treatment with 300 mg/kg body weight of *P. amarus* extract for 14 days. *P. amarus* was dissolved in normal saline.

Collection of blood and ovarian samples

Following the 14-day treatment regimen with *P. amarus* extract, rats from each group underwent anesthesia with intramuscular injection of ketamine. Blood was then collected into plain tubes via cardiac puncture, followed by successive intracardial perfusion with phosphate-buffered saline (PBS)and 4% paraformaldehyde solution. Ovarian tissue was excised through laparotomy and subsequently fixed in 4% paraformaldehyde solution in phosphate buffer. Fixed ovarian samples were subjected to histological processing by the Periodic acid-Schiff (PAS) and Masson's trichrome (MT) staining techniques.

Histological protocol

The staining protocols for PAS [13] and Masson's trichrome [14] were carried out at the Neuroendocrinology Lab, Department of Anatomy, College of Health Sciences, University of Ilorin. Ovarian tissue underwent dehydration using ascending ethanol concentrations through immersion, followed by xylene for clearing. Subsequently, the tissues were embedded in paraffin, cut into 5-µm-thick sections using a rotary microtome. Sections were selected using systematic random sampling by choosing a random starting section followed by every fifth serial section thereafter. Ovarian sections were then subjected to PAS and Masson's trichrome staining. These staining methods were employed to evaluate the extent of cysts, fibrosis, and study the structural characteristics of ovarian tissues.

Serum analysis for reproductive hormones

Blood samples were allowed to clot, and then centrifuged for 30 minutes at 10,000 rpm to obtain the serum. Hormonal and glucose assays were done using Enzyme-linked Immunosorbent Assays (ELISA) kits for follicle-stimulating hormone and luteinizing hormone (Abbott Lab, USA), Testosterone (Monobind Inc., USA), fasting serum insulin (R&D Systems, USA), and fasting serum glucose (Randox Lab, USA).

Calculation of homeostatic model assessment of insulin resistance

Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was determined using the formula: HOMA-IR = (fasting serum glucose in mg/dl) × (fasting serum insulin in mIU/L)/405 [15]. The interpretation of HOMA-IR values followed established cutoffs, where a range of 0.7-

1.9 indicates normal insulin sensitivity, while a value of 2.0 or higher indicates insulin resistance [16].

Statistical analysis

Data analysis was done by One-way Analysis of Variance (ANOVA) followed by Tukey's *post hoc* test using GraphPad Prism version 9.5.1 (GraphPad Software, San Diego, California, USA). Normality of data was tested by the Shapiro–Wilk test. $p < 0.05$ was considered significant.

RESULTS

Phyllanthus amarus treatment attenuates metabolic perturbation induced by PCOS in prepubertal Wistar rats

PCOS rats, with or without *P. amarus* intervention, exhibited notable alterations in their metabolic profiles (Fig. 1). Specifically, there were significant increases in fasting serum glucose (FSG) and fasting serum insulin (FSI) in the PCOS group compared with the controls. In contrast, the PCOS+*P. amarus* group revealed significant decreases in FSG, FSI, and HOMA-IR levels when compared with controls (hCON) and PCOS rats.

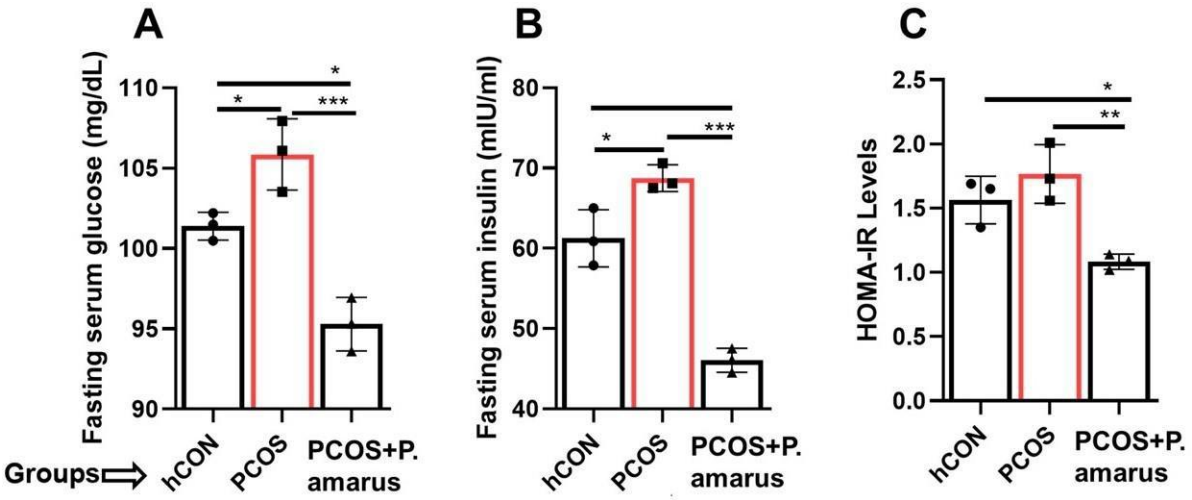

Figure 1: Fasting serum glucose levels (A), fasting serum insulin levels (B), and homeostatic model assessment of insulin resistance (HOMA-IR) (C). hCON (control group); PCOS (polycystic ovarian syndrome group). Data presented as Mean±SEM. *p<0.05 between groups, **p<0.05 between groups, ***p<0.05 between the groups.

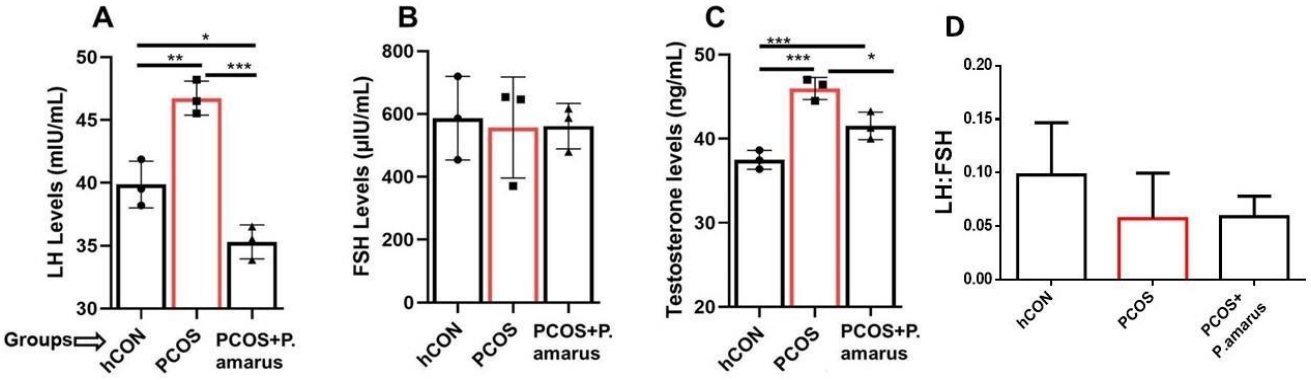

Figure 2: Serum luteinizing hormone levels (A), follicle-stimulating hormone levels (B), testosterone levels **(C)**, and LH: FSH ratio **(D).** hCON (control group); PCOS (polycystic ovarian syndrome group). Data presented as Mean±SEM. *p<0.05 between groups, **p<0.05 between groups, ***p<0.05 between the groups.

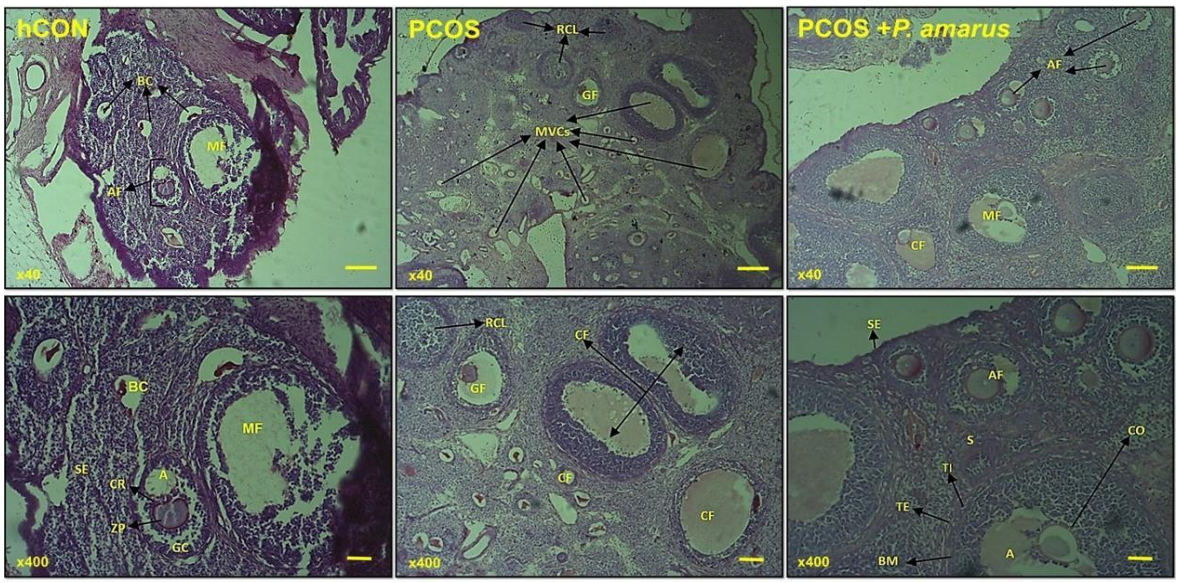

Figure 3: Ovarian histology of the controls, PCOS and *P. amarus*-treated groups**.** hCON (control); PCOS (polycystic ovarian syndrome); PCOS+*P. amarus (PCOS+Phyllanthus amarus)*. A: antrum, AF: antral follicle, BC: blood capillaries, BM: basement membrane, CF: cystic follicle, CO: cumulus oophorus, CR: corona radiata, GC: granulosa cells, GF: Graafian follicle, MF: mature follicle, multiple variable cysts, S: stroma, SE: surface epithelium, TE: theca externa, TI: theca interna, ZP: zona pellucida. Periodic acid-Schiff stain (PAS) 40x (Upper images) and 400x (Lower images).

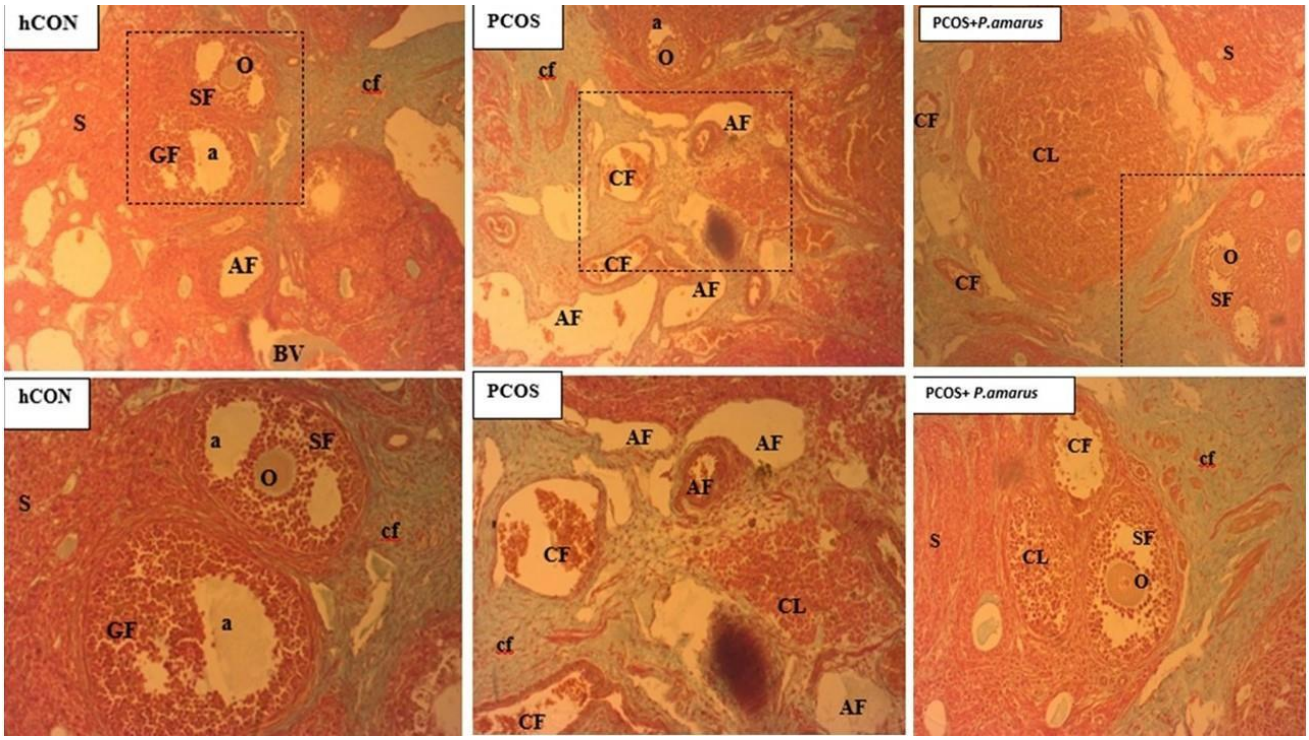

Figure 4: Ovarian histology of the controls, PCOS and *P. amarus*-treated groups**.** hCON: control; PCOS: Polycystic ovarian syndrome; PCOS+*P. amarus: PCOS+Phyllanthus amarus*; A: antrum; AF: antra follicle; BC: blood capillaries; BM: basement membrane; CF: cystic follicle; CO: cumulus oophorus; CR: corona radiate; GC: granulosa cells; GF: Graafian follicle; MF: mature follicle; MCFs: multiple variable cysts: S: stroma; SE: surface epithelium; TE: theca externa; TI: theca interna; ZP: zona pellucida; O: oocyte. Trichrome stain. 40x (Upper images) and 400x (Lower images).

Phyllanthus amarus attenuates endocrine dysfunction induced by PCOS in prepubertal Wistar rats

PCOS rats with or without *P. amarus* treatment exhibited significant changes in their hormonal profiles. Specifically, there were significant increases in serum luteinizing hormone (LH) and testosterone (T) levels following administration of DHT to induce PCOS (Fig. 2). Notably, these changes were observed without significant difference in serum follicle-stimulating hormone (FSH) levels and LH: FSH ratio. In contrast, *P. amarus* oral intervention reversed the perturbed serum levels of testosterone and LH.

***Phyllanthus amarus* ameliorates ovarian histomorphological alterations provoked by DHT-induced PCOS in prepubertal Wistar rats**

The ovarian morphology observed in the healthy control (hCON) group illustrates typical ovarian histological features, presenting follicles characterized by a discernible antrum, oocyte, surface epithelium, granulosa cells, and a distinct stroma, as depicted in Figures 3 and 4. In contrast, ovarian morphology of the PCOS group was characterized by the prevalence of cystic follicles of varying sizes, as illustrated in Figures 3 and 4. However, the photomicrograph depicting ovaries from rats treated with PCOS+*P. amarus* showed normal histological features with mature follicles similar to the hCON group.

DISCUSSION

The present study demonstrates that ethanolic leaf extract of Phyllanthus amarus (*P. amarus*) produced measurable metabolic, endocrine, and ovarian morphological improvements in female Wistar rats with dihydrotestosterone (DHT)-induced polycystic ovarian syndrome (PCOS). The untreated PCOS group showed increased fasting serum glucose, fasting serum insulin, HOMA-IR, luteinizing hormone (LH), and testosterone concentrations, together with cystic follicular changes in the ovary. In contrast, administration of *P. amarus* after PCOS induction was associated with lower fasting glucose, fasting insulin, HOMA-IR, LH and testosterone, as well as attenuation of cystic follicular formation that is comparable to the controls. Taken together, these findings suggest that *P. amarus* may exert a broad ameliorative effect on the metabolic, endocrine and structural manifestations produced by androgen exposure in this experimental model. However, the findings should be interpreted as evidence of an ameliorative effect in the rat model rather than proof of therapeutic efficacy in human PCOS.

The metabolic findings are consistent with the recognized association between PCOS and insulin resistance. Insulin resistance and compensatory hyperinsulinemia are important metabolic features of PCOS and may interact with androgen excess to amplify reproductive and metabolic abnormalities[5,21]. In the present study, DHT exposure was associated with increased fasting serum glucose and insulin, resulting in a higher HOMA-IR value than that observed in the control animals. These findings are broadly consistent with reports that androgen-based rodent models can reproduce important aspects of the metabolic phenotype of PCOS, including impaired insulin sensitivity and altered glucose homeostasis[11,24]. The reduction in fasting glucose, fasting insulin and HOMA-IR following *P. amarus* treatment is therefore noteworthy and suggests an improvement in metabolic homeostasis. Previous experimental work has also reported beneficial effects of *P. amarus* against diet-induced insulin resistance and hepatic oxidative stress, providing some support for the metabolic effects observed in the present study[6].

The mechanism responsible for the metabolic improvement observed after *P. amarus* administration cannot, however, be established from the present data. Although reduced hepatic gluconeogenesis or enhanced peripheral glucose uptake could contribute to the lower fasting glucose and insulin concentrations, neither hepatic glucose production nor tissue-specific insulin signaling or glucose transporter activity was measured in this study. Such mechanisms should therefore be regarded as hypotheses for future investigation rather than explanations established by the present experiment. Further studies assessing hepatic gluconeogenic enzymes, skeletal-muscle and adipose-tissue glucose uptake, GLUT4 expression, and insulin-receptor substrate/phosphoinositide 3-kinase signaling would help determine how *P. amarus* influences glucose homeostasis.

The endocrine findings provide additional evidence of an ameliorative effect of *P. amarus*. DHT is a non-aromatizable androgen that acts predominantly through the androgen receptor and is widely used to generate PCOS-like reproductive and metabolic abnormalities in rodents[11,24]. In the present study, DHT administration was associated with increased serum LH and testosterone, whereas FSH and the LH: FSH ratio did not differ significantly among groups. The increase in LH is compatible with disruption of the hypothalamic-pituitary-ovarian axis, which has an important role in the neuroendocrine abnormalities associated with PCOS[19,20]. Following *P. amarus* treatment, LH and testosterone were reduced toward control values. This finding suggests a possible modulatory effect on the endocrine disturbances associated with hyperandrogenism, although the study did not directly examine hypothalamic or pituitary signaling, ovarian steroidogenic enzymes, androgen-receptor activity, or gonadotropin-releasing hormone pulsatility. Consequently, the precise endocrine mechanism of the observed improvement remains to be determined.

The ovarian histological findings are particularly relevant because disruption of follicular development is a central reproductive manifestation of PCOS. The control ovaries showed recognizable follicular structures, whereas the ovaries of DHT-treated animals exhibited numerous cystic follicles and altered stromal morphology. Similar ovarian abnormalities have been reported in DHT-induced PCOS models, in which androgen exposure produces cystic and atretic follicles together with other disturbances of ovarian function[11,24,25]. Following *P. amarus* administration, the ovarian sections showed improved morphology characterized by the presence of antral follicles as seen in the controls. The presence of antral follicles in the *P. amarus*-treated rats may suggest improved folliculogenesis. However, follicle counts, follicular diameter, atretic follicle number, ovulation rate and estrous cyclicity were not quantitatively assessed in the present study and should therefore be considered in future investigation.

The apparent reduction in stromal fibrotic changes following *P. amarus* treatment is also of interest. The ovarian stroma provides structural and biochemical support for follicular development and steroidogenic activity, and alterations in the stromal microenvironment may adversely affect ovarian function[26]. In the present study, Masson's trichrome staining suggests greater ovarian stromal connective-tissue deposition in the PCOS group and a degree of attenuation of fibrosis following *P. amarus* intervention. This observation raises the possibility that *P.* amarus may influence the ovarian tissue remodeling processes associated with androgen-induced ovarian dysfunction. However, the study did not perform quantitative morphometric assessment of collagen deposition or determine the molecular mediators of fibrosis. Thus, the histological findings should be viewed as evidence of histomorphological improvement in the ovary, rather than definitive evidence of an anti-fibrotic mechanism. Future studies incorporating quantitative collagen measurement and assessment of fibrosis-related pathways would be useful.

The improved ovarian morphology observed after *P. amarus* intervention is biologically plausible given the reported antioxidant and metabolic activities of the *Phyllanthus* species. Experimental evidence indicates that *P. amarus* does attenuate insulin resistance and oxidative stress in animal models, in addition to the reported amelioration of ovarian, hormonal and metabolic abnormalities in experimental PCOS treated with extracts of various *Phyllanthus* species.[6,7] However, the present study did not measure oxidative-stress markers, inflammatory mediators, ovarian steroidogenic pathways or the phytochemical constituents of the extract. It is therefore premature to attribute the observed effects specifically to antioxidant, anti-inflammatory or insulin-sensitizing mechanisms of the extract. The beneficial effects may instead reflect the combined beneficial property of several phytoconstituents acting on interconnected metabolic, endocrine and ovarian pathways. Phytochemical characterization and mechanistic experiments will be necessary to clarify these possibilities.

An important consideration in interpreting the present findings is the nature and duration of the experimental model. DHT-induced PCOS models reproduce several reproductive and metabolic features of the syndrome but do not reproduce the full heterogeneity of human PCOS[11,24]. In particular, the present experiment used prepubertal rats and a relatively short treatment period with *P. amarus* extract following PCOS induction. The study therefore provides evidence of short-term morphological, metabolic and endocrine changes rather than long-term restoration of reproductive competence. These limitations should be considered when extrapolating the findings to other experimental models or to women with PCOS. Nonetheless, the concordant improvement in metabolic indices, selected reproductive hormones and ovarian morphology provides a useful basis for further investigation of *P. amarus* in experimental PCOS.

Overall, the present findings indicate that ethanolic leaf extract of *P. amarus* ameliorates several DHT-associated metabolic, endocrine and ovarian morphological abnormalities in Wistar rats. The reduction in fasting glucose, fasting insulin, HOMA-IR, LH and testosterone, together with the apparent improvement in ovarian morphology, support the potential of *P. amarus* as a candidate for further experimental investigation. Future studies may consider much larger sample sizes, estrous-cycle assessment, and quantitative follicular and stromal morphometry, in addition to oxidative parameters, inflammatory biomarkers, and metabolic signaling pathways, with a view to establishing the mechanisms of the observed reproductive effects of *P. amarus* in the murine model of PCOS.

CONCLUSION

The present study shows that ethanolic leaf extract of *Phyllanthus amarus* attenuates metabolic, endocrine and ovarian morphological perturbations associated with DHT-induced PCOS in female rats. These findings provide preclinical evidence of the ameliorative potential of *P. amarus* in experimental PCOS; however, further studies are warranted to further elucidate the underlying molecular mechanisms and evaluate its effects on follicular development, estrous cyclicity and ovulatory function.

Conflict of interest

The authors declare no competing interests.

Authors' contributions: Conceptualization**,** OBA**;** Data collection and analysis**,** KEA, AA, FAT, EOV, TOO, VO, JIE; draft of manuscript, KEA, JIE; Approval of manuscript, OBA.

Ethical approval

The study was approved by the Ethical Review Committee of the University of Ilorin (approval number: UERC/ASN/2023/2515).

REFERENCES

1. Forslund M, Melin J, Alesi S, Piltonen T, Romualdi D, Tay CT, *et al*. Different kinds of oral contraceptive pills in polycystic ovary syndrome: a systematic review and meta-analysis. Eur J Endocrinol. 2023;189(1):S1-S16. doi:10.1093/ejendo/lvad082.
2. Singh S, Pal N, Shubham S, Sarma DK, Verma V, Marotta F, *et al*. Polycystic ovary syndrome: etiology, current management, and future therapeutics. J Clin Med. 2023;12(4):1454. doi:10.3390/jcm12041454.
3. Sulaiman MA, Al-Farsi YM, Al-Khaduri MM, Saleh J, Waly MI. Polycystic ovarian syndrome is linked to increased oxidative stress in Omani women. Int J Womens Health. 2018;10:763-771. doi:10.2147/IJWH.S166461.
4. Cowan S, Lim S, Alycia C, Pirotta S, Thomson R, Gibson-Helm M, *et al*. Lifestyle management in polycystic ovary syndrome—beyond diet and physical activity. BMC Endocr Disord. 2023;23(1):14. doi:10.1186/s12902-022-01208-y.
5. Zhao H, Zhang J, Cheng X, Nie X, He B. Insulin resistance in polycystic ovary syndrome across various tissues: an updated review of pathogenesis, evaluation, and treatment. J Ovarian Res. 2023;16(1):9. doi:10.1186/s13048-022-01091-0.6.
6. Putakala M, Gujjala S, Nukala S, Desireddy S. Beneficial effects of Phyllanthus amarus against high fructose diet induced insulin resistance and hepatic oxidative stress in male Wistar rats. Appl Biochem Biotechnol. 2017;183:744-764. doi:10.1007/s12010-017-2470-9.
7. Ndeingang EC, Defo Deeh PB, Watcho P, Kamanyi A. Phyllanthus muellerianus (Euphorbiaceae) restores ovarian functions in letrozole-induced polycystic ovarian syndrome in rats. Evid Based Complement Alternat Med. 2019;2019:2965821. doi:10.1155/2019/2965821.
8. Le TM, Nguyen CD, Ha AC. Combination of Phyllanthus amarus Schum. & Thonn. and Gymnema sylvestre R. Br. for treatment of diabetes and its long-term complications. Fine Chem Technol. 2021;16(3):232-240. doi:10.32362/2410-6593-2021-16-3-232-240.
9. Iteire KA, Adenodi CA, Olatuyi OM, Uwejigho RE, Adeniyi TD. Phyllanthus amarus protects against potassium-dichromate pituitary toxicity via the oxidative pathway and improves the gonadotropins in male Wistar rats. J Med Sci. 2023;92(2):e834. doi:10.20883/medical.e834.
10. Bhat SS, Hegde KS, Chandrashekhar S, Rao SN, Manikkoth S. Preclinical screening of Phyllanthus amarus ethanolic extract for its analgesic and antimicrobial activity. Pharmacogn Res. 2015;7(4):378-384. doi:10.4103/0974-8490.159577.
11. Manneråas L, Cajander S, Holmäng A, Seleskovic Z, Lystig T, Lönn M, *et al*. A new rat model exhibiting both ovarian and metabolic characteristics of polycystic ovary syndrome. Endocrinology. 2007;148(8):3781-3791. doi:10.1210/en.2007-0168.
12. Masszi G, Buday A, Novak A, Horvath EM, Tarszabo R, Sara L, *et al*. Altered insulin-induced relaxation of aortic rings in a dihydrotestosterone-induced rodent model of polycystic ovary syndrome. Fertil Steril. 2013;99(2):573-578. doi:10.1016/j.fertnstert.2012.09.024.
13. Shedge S, Roy P, Shedge A, Doshi MA. Periodic acid Schiff (PAS) staining: a useful technique for demonstration of carbohydrates. Medico-Legal Update. 2020;20(2):353-357. doi:10.37506/mlu.v20i2.1129.
14. Van De Vlekkert D, Machado E, d'Azzo A. Analysis of generalized fibrosis in mouse tissue sections with Masson's trichrome staining. Bio Protoc. 2020;10(10):e3629. doi:10.21769/BioProtoc.3629.
15. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-419. doi:10.1007/BF00280883.
16. Pather R, Khathi A, Ngubane P. The effects of obesity on thyroid function in a metabolically healthy high-fat, high-carbohydrate diet-induced obese rat model. Front Endocrinol (Lausanne). 2025;16:1538627. doi:10.3389/fendo.2025.1538627.
17. Igbo EJ, Okoliko U, Aminu I, Kopada A, Olorunnado S, Akinola OB. Structural changes in the medial prefrontal cortex and anterior cingulate cortex of dehydroepiandrosterone-induced Wistar rat model of polycystic ovarian syndrome. Basic

Clin Neurosci. 2022;13(5):695-708. doi:10.32598/bcn.2022.3564.1.
18. Rocha AL, Oliveira FR, Azevedo RC, Silva VA, Peres TM, Candido AL, *et al*. Recent advances in the understanding and management of polycystic ovary syndrome. F1000Res. 2019;8:565. doi:10.12688/f1000research.15318.1.
19. Liao B, Qiao J, Pang Y. Central regulation of PCOS: abnormal neuronal-reproductive-metabolic circuits in PCOS pathophysiology. Front Endocrinol (Lausanne). 2021;12:667422. doi:10.3389/fendo.2021.667422.
20. Ding H, Zhang J, Zhang F, Zhang S, Chen X, Liang W, *et al*. Resistance to insulin and elevated level of androgen: a major cause of polycystic ovary syndrome. Front Endocrinol (Lausanne). 2021;12:741764. doi:10.3389/fendo.2021.741764.
21. Sanchez-Garrido MA, Tena-Sempere M. Metabolic dysfunction in polycystic ovary syndrome: pathogenic role of androgen excess and potential therapeutic strategies. Mol Metab. 2020;35:100937. doi:10.1016/j.molmet.2020.01.001.
22. Naik AQ, Zafar T, Shrivastava VK. Physiological Impact of the Non-Nutritive Artificial Sweetener, Aspartame, and the Therapeutic Potential of Aqueous Extract of Phyllanthus niruri. J Med Food. 2023;26(7):500-510. doi: 10.1089/jmf.2022.K.0136.
23. Han JC, Weiss R. Obesity, metabolic syndrome and disorders of energy balance. In: Sperling Pediatric Endocrinology. Elsevier; 2021. p.939-1003. doi:10.1016/B978-0-323-62520-3.00024-5. (please confirm edition year — 2020 vs. 2021 — against your copy)
24. Sun LF, Yang YL, Xiao TX, Li MX, Zhang JV. Removal of DHT can relieve polycystic ovarian but not metabolic abnormalities in DHT-induced hyperandrogenism in mice. Reprod Fertil Dev. 2019;31(10):1597-1606. doi:10.1071/RD18459.
25. Krishnan A, Muthusami S, Periyasamy L, Stanley JA, Gopalakrishnan V, Ramachandran I. Effect of DHT-induced hyperandrogenism on the pro-inflammatory cytokines in a rat model of polycystic ovary morphology. Medicina (Kaunas). 2020;56(3):100. doi:10.3390/medicina56030100.
26. Shen L, Liu J, Luo A, Wang S. The stromal microenvironment and ovarian aging: mechanisms and therapeutic opportunities. J Ovarian Res. 2023;16(1):237. doi:10.1186/s13048-023-01300-4.