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Research Article

Ameliorative Effects of *Phyllanthus muellerianus* (Kuntze) Exell Roots Extracts on Hormonal Imbalances and Ovarian Histology in Letrozole-Induced Polycystic Ovary Syndrome in Rats

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Abstract

This study investigates the effects of *Phyllanthus muellerianus* roots extracts on hormonal levels and ovarian histology in a rat model of polycystic ovary syndrome (PCOS) induced by letrozole (LTZ). Female rats were treated with aqueous and ethanolic extracts of P. *muellerianus* at varying doses (30, 60, and 120 mg/kg bwt) for 21 days. Hormonal assays, including the measurement of serum estradiol, testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels, were conducted using enzyme-linked immunosorbent assay (ELISA) kits. Results revealed a significant increase in estradiol levels in all treated groups compared to the positive control, while testosterone levels significantly decreased in all treated rats, indicating a potential estrogenic effect and androgen reduction. However, LH and FSH levels remained unaffected by the treatment. Histological examination of ovaries showed significant improvements in follicular development and structural integrity in rats treated with P. muellerianus, particularly at 30 mg/kg bwt of the aqueous extract, which demonstrated nearly complete restoration of normal ovarian architecture. These findings suggest that *Phyllanthus muellerianus* possesses potential therapeutic properties in modulating reproductive hormones and improving ovarian histology in a PCOS model, supporting its traditional use in the management of reproductive health disorders.

Keywords: *Phyllanthus muellerianus*; Polycystic Ovary Syndrome (PCOS); Hormonal assays; Estradiol; Ovarian histology; Letrozole-induced model.

INTRODUCTION

Over the past 25 years, the diagnosis of polycystic ovarian syndrome has emerged from relative medical obscurity. Focused study efforts have only been made to understand this widespread but complex syndrome since the late 1980s. Since then, and especially in the last ten years, patients and professionals have begun to pay more attention to the disease that was earlier improperly diagnosed. As a result, more women are receiving the correct diagnosis, and research into successful therapies is being assessed [1].

In a previous report [2], different etiologies for polycystic ovarian syndrome (PCOS) were discussed because the aetiology of the condition is unknown. The phenotype of PCOS is influenced by both hereditary and environmental factors. Studies have examined a variety of potential causes, such as ovarian factors, insulin resistance, and aberrant gonadotropin production [1].

As a diverse illness, PCOS presents a number of challenges, one of which is encouraging patients to engage in self-care in order to lower their morbidities [1]. Making the diagnosis of PCOS is crucial in order to involve afflicted women in early preventative and treatment strategies. Numerous metabolic problems, such as early diabetes, obesity, high blood pressure, dyslipidemia, and fatty liver, have been discovered.

In women with PCOS, the normal ovarian function is disturbed mainly by hyperandrogenism and elevated levels of luteinizing hormone (LH) [3], thus resulting in multiple cysts [4], PCOS increases gonadotropin-releasing hormone (GnRH) pulse frequency, which favours LH production over follicle-stimulating hormone (FSH) [5]. This increase in LH concentration subsequently promotes androgen production in the theca cells, while the relative FSH deficiency reduces the ability of granulosa cells to convert androgen into estrogen and impairs follicle maturation and ovulation [6]. Previous reports have shown that letrozole efficiently establishes PCOS in rats [7,8]. These animals developed many human characteristics of PCOS, including abnormal follicles [7] and altered levels of reproductive hormones, including testosterone, estrogens, LH and FSH [7]. The treatment of PCOS involves the use of several drugs, such as metformin and clomiphene citrate, but they are commonly associated with serious side effects. Focus on the use of traditional folk medicine as an alternative source to solve some of the issues that arose from the commonly used drug has become an area of consideration. Hence, a new therapeutic approach with fewer side effects, easy availability and broad spectrum is necessary. *Phyllanthus muellerianus* is extensively used in some countries to treat digestive disorders, frequent menstruation and ovulation [9,10]. *P. muellerianus* has been demonstrated to possess antihyperglycemic [11], antihyperlipidemic [11], antioxidant [12], and aphrodisiac [13], properties. Therefore, this research aims to investigate the effects of aqueous and ethanolic roots extracts of *P. muellerianus* on estrus cyclicity, blood glucose level, sex hormones and ovarian histology in letrozole-induced PCOS rats after 14 days of treatment.

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MATERIALS AND METHODS

Materials

Plant Material and Extraction Process for Aqueous and Ethanolic Extracts: The plant material used for the study consisted of fresh roots

of *Phyllanthus muellerianus*, collected in early August 2022. Botanical authentication was carried out at the University Herbarium in Kaduna, and a voucher specimen number was assigned. The roots were cut into smaller pieces and air-dried under shade for seven days before being ground into a fine powder using an electric blender. For the aqueous extract, 250 g of the powdered material was infused in 1500 ml of boiling water for 15 minutes, followed by filtration. The filtrate was dried in an oven at 55°C for 48 hours, and the percentage yield of the extract was calculated. The ethanolic extract was prepared by macerating 2500 g of powdered root in 5000 ml of ethanol for 72 hours. The resulting mixture was filtered, and the filtrate was concentrated under reduced pressure using a rotary evaporator set to 75°C. The dry yield of the ethanolic extract was determined, and the extraction efficiency was expressed as a percentage.

Chemicals and Reagents: All chemicals and reagents used in this study were of analytical grade, ensuring high purity and reliability for experimental procedures. They were mostly products of BDH, Poole (England), and Randox (United Kingdom). The FSH, LH, estradiol, and testosterone ELISA assay kits were products of Monobind Inc. (USA). Letrozole was procured from a reputable pharmaceutical shop in Kaduna, Nigeria.

Methods

Experimental Animals: A total of forty-two adult female albino Wistar rats were sourced from the animal facility of the Animal Science and Environmental Biology Department, Ahmadu Bello University, Zaria. The animals were housed under standard laboratory conditions, maintained at a temperature of 27 ± 1°C with a 12-hour light-dark cycle. They were provided unrestricted access to pelletized rat chow and clean drinking water before and throughout the study. Only animals demonstrating at least three consecutive regular estrous cycles were selected for the experiment. Body weights were monitored twice weekly, and vaginal smears were examined microscopically to determine the stages of the estrous cycle. All experimental procedures adhered to the guidelines outlined by the National Institute of Health (NIH) for the Care and Use of Laboratory Animals (NIH publication no. 85-93, revised 1985).

Treatment of Animals and Induction of PCOS: The animals were acclimatized for one week before the start of the treatments. They were housed under standard laboratory conditions in well-ventilated wooden cages, maintained at 27 ± 1°C with a 12-hour light-dark cycle. Throughout the acclimatization and experimental periods, the animals were provided with unlimited access to standard pelletized rat chow and drinking water.

Female rats with regular estrus cycles, as previously described, were selected and divided into five groups. The control group (n=6) received an oral administration of vehicle (0.9% NaCl solution) once daily. The second group (n=36) was orally administered letrozole (1 mg/kg/day) dissolved in 0.9% NaCl for 21 days to induce PCOS. Vaginal smears were collected daily and examined microscopically to monitor the estrus stage.

At the end of the induction phase, six rats from the control group and six from the letrozole-treated group were randomly selected and sacrificed by cervical dislocation under anesthesia. Biochemical and histological analyses were conducted to confirm the induction of PCOS, which was characterized by key features such as hyperglycemia, hyperandrogenism, and the presence of multiple ovarian cysts, consistent with previous findings [12].

In addition, irregular estrus cyclicity (rats having a disturbed appearance of the four estrus stages) shall be the main criteria for selecting PCOS rats [12]. Subsequently, 36 PCOS rats were divided into 6 groups (6 rats per group) and treated orally for 14 days with distilled water (10 ml/kg/day), clomiphene citrate (2mg/kg/day), metformin (500mg/kg/day), and aqueous or methanolic roots extract of *P. muellerianus* (30, 60, and 120mg/kg/day). A healthy control group (n=6) was administered with distilled water (10ml/kg/day) for 14 days. The effective dose of letrozole (1mg/kg) and treatment period (14 days) were chosen from reference studies [7,15]. Doses of *P. muellerianus* (30, 60 and 120 mg/kg/day) were selected from a pilot study. During the treatment period, vaginal smears were collected daily for 14 days and examined microscopically for the identification of estrus stage. After 14 days of treatment, animals were sacrificed and organs excised (ovaries and uterus), weighed and subsequently homogenized in a pre-cooled mortar and pestle. The homogenates were stored at -20°C until required for biochemical assays while the ovaries were quickly excised and representative samples fixed in Bouin's solution for histology.

Estrus Cycle Motoring: Vaginal smears were collected daily in the morning to determine the reproductive cycle of each animal, as described in a previous study [16].

Animal Killing and Sample Collection: After 14 days of treatment, the rats were weighed and euthanized via cervical dislocation under halothane anesthesia. Blood was drawn from the abdominal artery into heparinized tubes, and plasma was separated by centrifugation at 1,000 x g for 15 minutes. The plasma was then stored at -20°C for subsequent hormonal analysis. Following blood collection, representative ovaries were carefully excised, weighed, and fixed in Bouin's solution for histological examination.

Reproductive Hormonal Assays: Plasma LH, FSH, estradiol and testosterone levels were determined using commercially available kits as previously described [17,18]. The level of these reproductive hormones was assayed in the plasma samples and estimated based on the standard principle of enzyme-linked immunosorbent assay (ELISA) as previously described in ELISA kit leaflets for LH, FSH, estradiol and testosterone, respectively.

Histopathological Evaluation: Ovaries were processed step by step through 10% neutral formalin fixation (24 h), paraffin embedding, and longitudinally and serially sectioned at 4 μm with a microtome. The samples were stained with hematoxylin and eosin and read under a microscope according to the methods described by [7]. Preantral, antral, atretic cystic follicles and corpus luteum were identified [19].

Statistical Analysis: All data were presented as mean ± SEM (standard error of the mean). Statistical analysis was performed using one-way ANOVA, followed by Tukey's post hoc multiple-range comparison test. Analyses were conducted with GraphPad Prism Software Version 6.0, and a significance level of $p < 0.05$ was considered statistically significant.

RESULTS

The result of the quantitative phytochemical screening of *P. muellerianus* is shown in Table 1. Estrus cyclicity and the hormonal levels in PCOS-treated rats are presented in Table 2 and Figure 1-2. The quantitative phytochemical screening of *Phyllanthus muellerianus* root extracts indicates the presence of key bioactive compounds, including flavonoids, tannins, phenols, alkaloids, and saponins. The results suggest that the plant root contains diverse phytochemicals, which may contribute to its therapeutic potential. The variations in the concentrations of these compounds between the aqueous and ethanolic extracts imply that different extraction methods may yield different phytochemical profiles, potentially influencing the plant's pharmacological activities.

From the table 1 below, the aqueous roots extract of *P. muellerianus* contains a higher quantity of flavonoid and alkaloid than the ethanolic extract, whereas the plant's ethanolic extract is richer in tannins, phenol, and saponins than the aqueous extract.

Estrus Cyclicity in PCOS treated Rats

Table 2 shows the results for average estrus cyclicity in PCOS-treated rats. The irregular estrus cycles observed in PCOS-induced rats were

Table 1: Quantitative phytochemical screening of *P. muellerianus*

S/N	PARAMETERS	AQUEOUS EXTRACT (mg/g)	ETHANOLIC EXTRACT (mg/g)
1	Flavonoid	0.420	0.013
2	Tannins	0.198	0.312
3	Phenol	0.016	0.661
4	Alkaloid	6.02%	2.47%
5	Saponins	4.13%	8.85%

Table 2: Estrus cyclicity in PCOS treated Rats

Group	Experimental Design (mg/kg bodyweight)	Estrus Cycle	Number of days for estrus cycle before treatment (days)	Number of days for estrus cycle after treatment (days)
I	Positive Control (Not Induced)	M D E P	5 - 6 3	1 5 5 3
II	Negative Control (Induced with Letrozole)	M D E P	- 12 1 1	3 4 4 3
III	Aqueous extract (30 mg/kg)	M D E P	4 8 1 1	2 3 6 3
IV	Aqueous extract (60 mg/kg)	M D E P	7 4 2 1	2 2 7 3
V	Aqueous extract (120 mg/kg)	M D E P	5 6 2 1	1 6 5 2
VI	Ethanolic Extract (30 mg/kg)	M D E P	5 7 - 2	1 6 7 -
VII	Ethanolic Extract (60 mg/kg)	M D E P	3 11 - -	1 5 7 1
VIII	Ethanolic Extract (120 mg/kg)	M D E P	4 9 - 1	1 8 3 2

***Note: M**, metestrus; D, diestrus; E, estrus; P, proestrus*

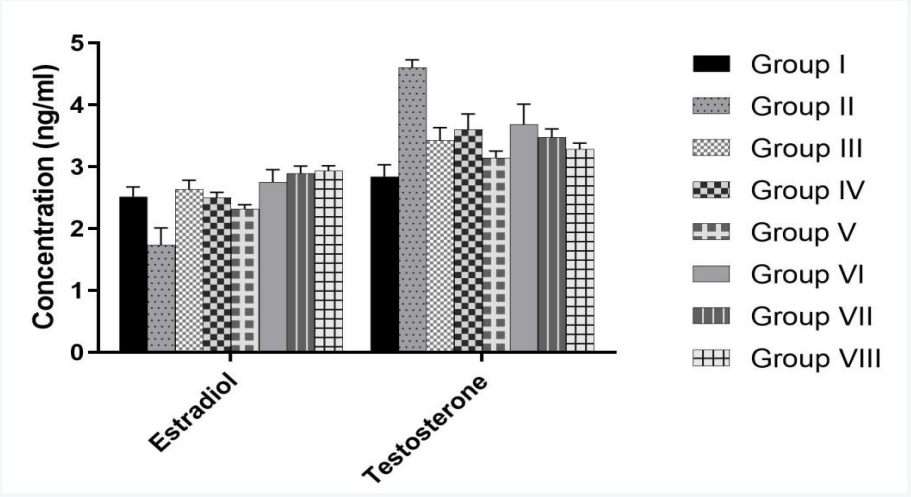

Figure 1: Serum levels of estradiol and testosterone in PCOS rats

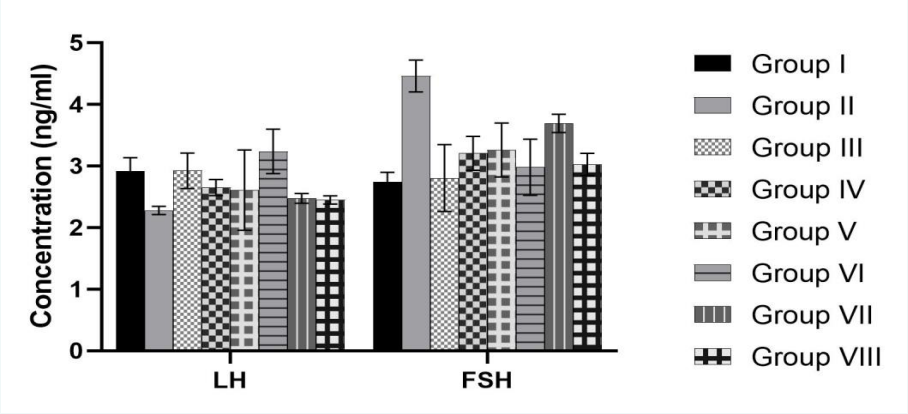

Figure 2: Serum levels of luteinizing hormone and follicle-stimulating hormone in PCOS rats.

normalized when rats were administered both aqueous and ethanolic roots extracts of *Phyllantus muellerianus*.

The results on estrus cyclicity in PCOS-treated rats show variations in the duration of the estrus cycle before and after treatment. Some rats improved their oestrous cycle, with more consistent and shorter cycle durations following treatment. However, other rats experienced disruptions in their cycle, including extended phases and irregular estrus patterns. These findings suggest that the treatments, including the plant roots extracts, had different impacts on the oestrous cycle in PCOS rats, with potential benefits in restoring normal cyclicity in some cases.

The effects of *P. muellerianus* roots extracts on reproductive hormones in LTZ-Induced PCOS rats

The results show that both aqueous and ethanolic roots extracts of *Phyllanthus muellerianus* significantly ($p<0.05$) increased estradiol levels in PCOS rats compared to the positive control but not the negative control. Additionally, testosterone levels were significantly reduced ($p<0.05$) in all treated groups compared to the positive control, though no significant difference was observed between the treated and negative control groups (Figure 1). LH and FSH levels remained unchanged across all treatment groups compared to the negative control. These findings suggest that *P. muellerianus* roots extracts affect estradiol and testosterone levels but not LH and FSH levels in LTZ-induced PCOS rats, as outlined in Figure 2.

Histology of the Ovary of Rat Induced with LTZ and Rats Administered with Aqueous and Ethanolic Roots Extract of *Phyllantus muellerianus*

The histological analysis of ovaries in LTZ-induced PCOS rats showed significant damage, with disrupted follicular structures and the absence of primary oocytes. Treatment with both aqueous and ethanolic roots extracts of *Phyllanthus muellerianus* resulted in varying degrees of recovery, with the aqueous extract showing more pronounced improvement.

Histology of Ovary of Rat not induced with LTZ

Examination of the photomicrograph (Plate 1) of an ovary obtained from a rat that was not induced with LTZ revealed a normal histoarchitecture. The ovary presents both the primary and secondary follicles at various stages of maturation, with normal histological architecture showing follicular antrum (FA), corona radiate (arrow), and zona granulosa (ZG).

Histology of Ovary of Rat Induced with LTZ

Examination of the photomicrograph (Plate 2), of the ovary obtained from rats that were induced with LTZ revealed several alterations involving follicular damage. The ovary presents primary and secondary follicles showing follicular antrum (FA), primary oocyte (OC), corona radiate (blue arrow) and zona granulosa (ZG). One of the Theca follicles (green arrow) appears without primary Oocytes.

Histology of Ovary of PCOS Rat Administered with 30 mg/kg bwt Ethanolic Roots Extract of *Phyllantus muellerianus*

Examination of the photomicrograph (Plate 3) of the ovary obtained from a PCOS rat that was administered with 30 mg/kg bwt revealed a progressive clearance of alterations caused by LTZ. The section presents a maturing ovary, both the primary and secondary follicles, with normal histological architecture, showing follicular antrium (FA), primary oocyte (OC), theca follicle (black arrow), corona radiate (blue arrow) and zona granulosa (ZG). Significant recovery, with normal maturing primary and secondary follicles, was noted.

Histology of Ovary of PCOS Rat Administered with 120 mg/kg bwt Ethanolic Roots Extract of *Phyllantus muellerianus*

Examination of the photomicrograph (Plate 4) of the ovary obtained from PCOS rat that was administered with 60 mg/kg bwt revealed apparent clearance of alterations caused by LTZ but with few damages. The ovary presents maturing primary and secondary follicles, with normal histological architecture showing follicular antrium (FA), the primary oocyte (OC) and zona granulosa (ZG). Partial recovery, with some remaining damage, was noted.

Histology of Ovary of PCOS Rat Administered with 120 mg/kg bwt Aqueous Roots Extract of *Phyllantus muellerianus*

Examination of the photomicrograph (Plate 5) of the ovary obtained from PCOS rat that was administered with 120 mg/kg bwt revealed partial clearance of alterations caused by LTZ but with few damages. The ovary presents features of maturing primary and secondary follicles. The Follicular antrium (FA) is smaller in size. The primary oocyte (OC) and zona granulosa (ZG) appear normal. Mild improvement, with smaller follicular antrium and normal primary oocytes, was noted.

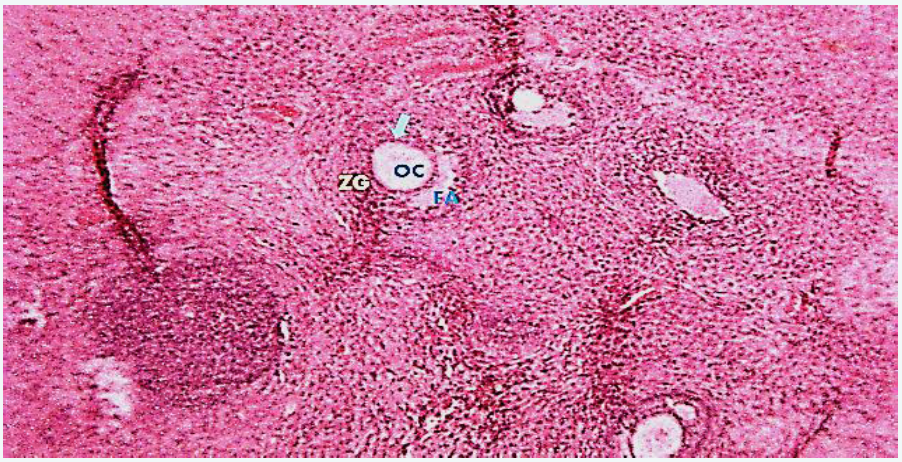

Plate 1: Photomicrograph of ovary from a rat that was not induced (Control) **(**H&E x100).

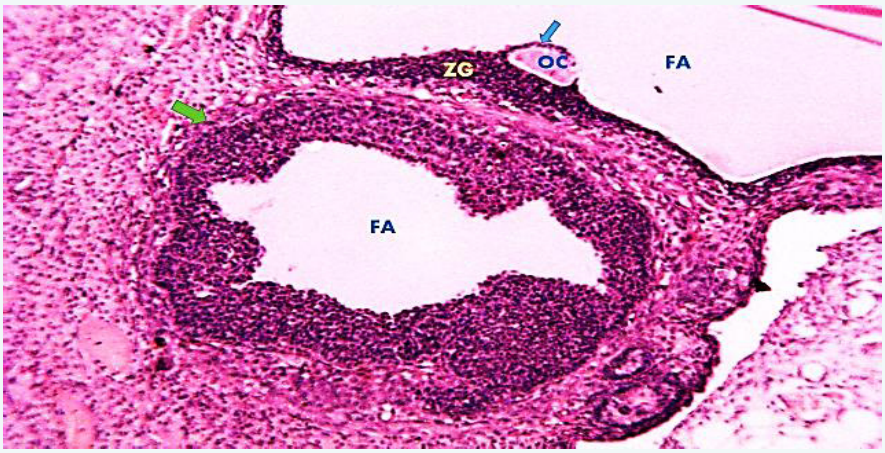

Plate 2: Photomicrograph of ovary from a rat administered with LTZ (H&E x100).

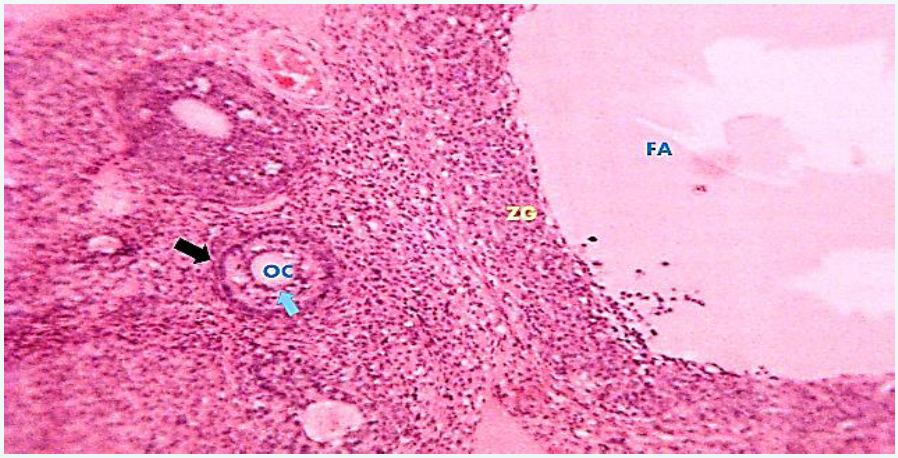

Plate 3: Photomicrograph of ovary from a rat administered 30 mg/kg bwt of aqueous extract of Phyllantus muellerianus (H&E x100).

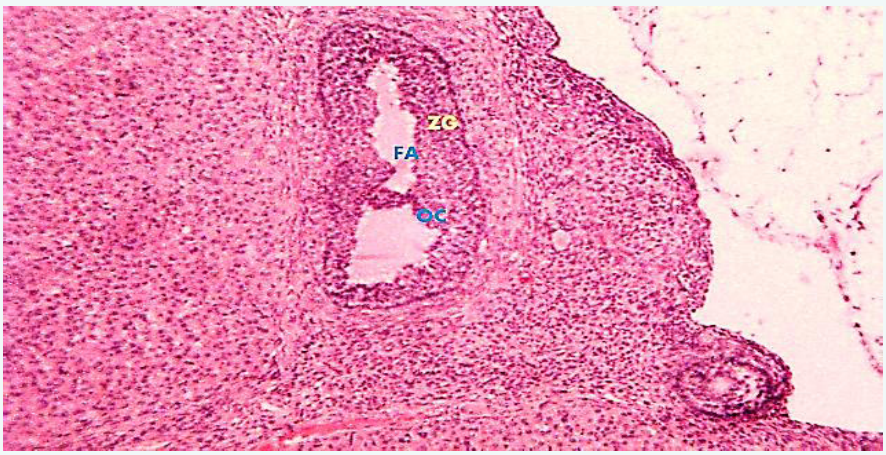

Plate 4: Photomicrograph of ovary from rat administered 60 mg/kg bwt of aqueous extract of Phyllantus muellerianus (H&E x100).

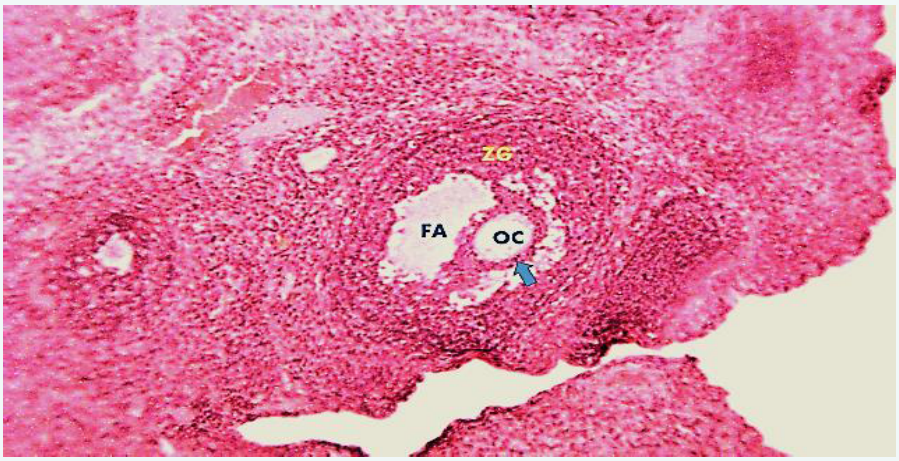

Plate 5: Photomicrograph of ovary from rat administered 120 mg/kg bwt of aqueous extract of Phyllantus muellerianus (H&E x100).

Histology of Ovary of PCOS Rat Administered with 30 mg/kg bwt Ethanolic Roots Extract of *Phyllantus muellerianus*

Examination of the photomicrograph (Plate 6) of the ovary obtained from a PCOS rat that was administered with 30 mg/kg bwt revealed mild clearance with features of the degenerated oocyte. The ovary presents features of degenerated primary oocyte (OC), corona radiate (blue arrow) and zona granulosa (ZG). Mild recovery, with degenerated oocytes and abnormal follicular structures, was seen.

Histology of Ovary of PCOS Rat Administered with 60 mg/kg bwt Ethanolic Roots Extract of *Phyllantus muellerianus*

Examination of the photomicrograph (Plate 7) of the ovary obtained from a PCOS rat that was administered with 60 mg/kg bwt revealed a progressive clearance with few damages. The ovary presents features of maturing primary and secondary follicles, with normal histological architecture, follicular antrium (FA), primary oocyte (OC), corona radiate (blue arrow), theca follicle (black arrow), zona granulosa (ZG). Note the hemorrhagic patches (green arrow). Progressive recovery, but with some hemorrhagic patches, was observed.

Histology of Ovary of PCOS Rat Administered with 120 mg/kg bwt Ethanolic Roots Extract of *Phyllantus muellerianus*

Examination of the photomicrograph (Plate 8) of the ovary obtained from a PCOS rat that was administered with 120 mg/kg bwt revealed a distinct clearance, though with few damages. The ovary presents features of maturing primary and secondary follicles. The follicular antrium (FA) primary oocyte (OC) and zona granulosa (ZG) appear underdeveloped. Distinct recovery, but with underdeveloped primary oocytes and zona granulosa, were noted. The results suggest that the aqueous roots extract of *P. muellerianus* is more effective in reversing LTZ-induced ovarian damage than the ethanolic roots extract.

DISCUSSION

The findings of this study provide insights into the effects of *Phyllanthus muellerianus* roots extracts on reproductive health in LTZ-induced PCOS rats, focusing on hormonal assays and histological analysis of ovarian tissues. The results suggest that both aqueous and ethanolic roots extracts of *P. muellerianus* have potential therapeutic effects in ameliorating the hormonal imbalances and histological alterations characteristic of PCOS.

The hormonal assay results showed significant increases in estradiol

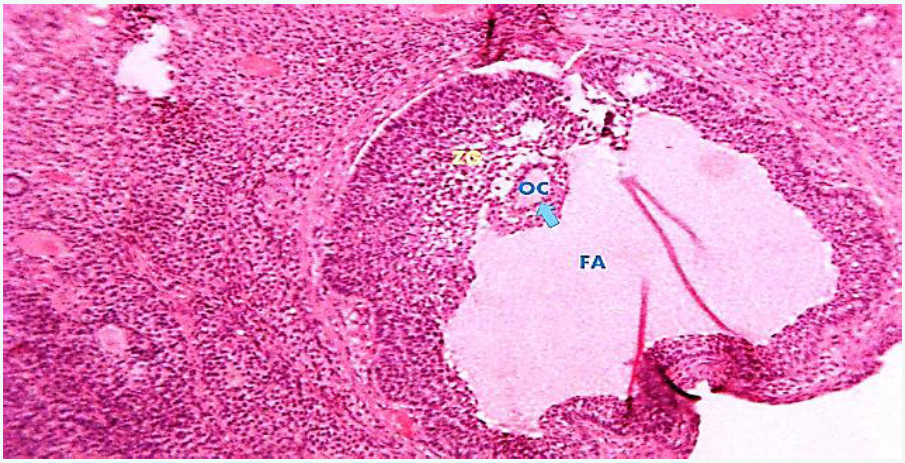

Plate 6: Photomicrograph of ovary from rat administered 30 mg/kg bwt of ethanolic extract of Phyllantus muellerianus (H&E x100).

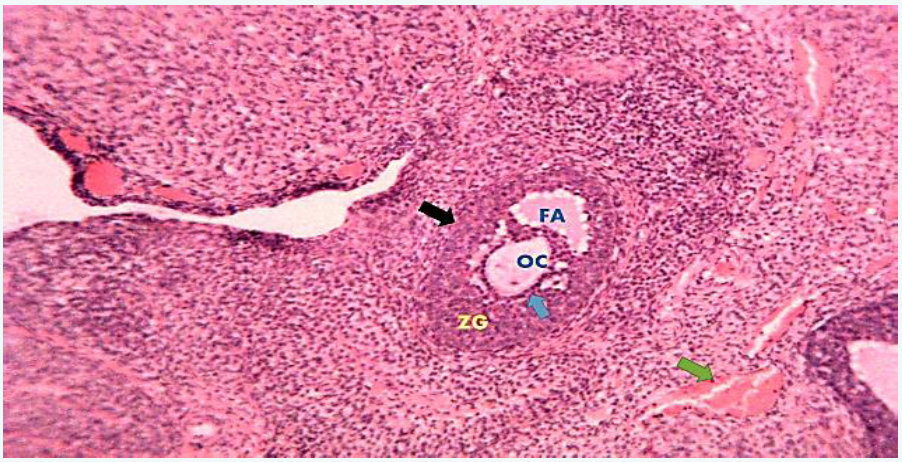

Plate 7: Photomicrograph of ovary from a rat administered 60 mg/kg bwt of ethanolic extract of Phyllantus muellerianus (H&E x100).

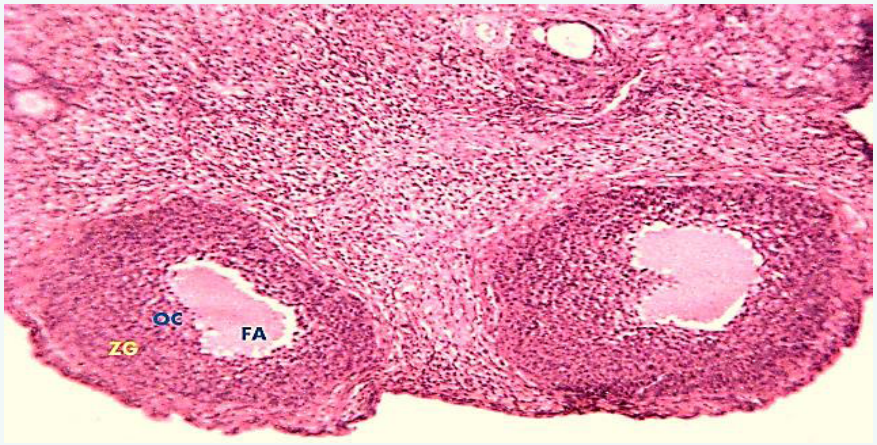

Plate 8: Photomicrograph of ovary from a rat administered 120 mg/kg bwt of ethanolic extract of Phyllantus muellerianus (H&E x100).

levels in rats treated with both aqueous and ethanolic roots extracts of *Phyllanthus muellerianus*, compared to the positive control group. The increase in estradiol indicates a potential regulatory effect of the plant extracts on ovarian function, as estradiol is a key hormone involved in follicular development and reproductive health [18]. However, the estradiol levels in the treated groups were not significantly different from the negative control group, suggesting that the plant roots extracts may help restore estradiol levels to a baseline similar to that of healthy animals.

On the other hand, the significant reduction in testosterone levels observed in all treated groups is notable. Elevated testosterone levels are a hallmark of PCOS and contribute to symptoms such as hirsutism and anovulation [19-21],. The decrease in testosterone after treatment with *P. muellerianus* extracts suggests that the plant may have anti-androgenic properties, helping to reduce the excess production of testosterone in PCOS. The lack of significant differences between the treated and negative control groups, however, suggests that the plant roots extracts may be effective in normalizing testosterone levels but may not fully normalize the hormonal balance to that of healthy, untreated rats.

Importantly, the levels of LH and FSH did not show significant changes across the treatment groups compared to the negative control. This indicates that the extracts may not directly impact the secretion of these gonadotropins. It is possible that *P. muellerianus* acts more specifically on ovarian steroidogenesis and androgen regulation rather than directly influencing the pituitary-gonadal axis [22].

Certain studies on polycystic ovary syndrome (PCOS) have revealed cases of hormonal imbalances and alterations in ovary histopathology [23]. Elevated levels of LH have been implicated as one of the alterations in reproductive hormones of women diagnosed with PCOS [3]. This observation is believed to result in multiple cysts [4]. Alteration in the levels of sex hormones such as testosterone, estrogens, LH and FSH has been linked to PCOS in humans [7]. PCOS-induced decrease in estradiol was significantly ameliorated in the presence of both the aqueous and ethanolic roots extract of *P. muellerianus*, though the reverse case was observed in the level of testosterone with no alterations in levels of LH and FSH. This observation suggests that *P. muellerianus* roots extract possesses some phytochemical principles, which may cause a surge in the production of estradiol. The aqueous roots extract of this plant has been demonstrated to be rich in alkaloids, flavonoids and saponin (Table 1).

The histological examination of ovaries further corroborates the hormonal findings, revealing significant structural alterations in the ovaries of LTZ-induced PCOS rats. The ovaries of untreated PCOS rats showed damage to follicular structures, including the absence of primary oocytes and alterations in the theca follicle (Plate 2). This histopathological evidence is consistent with the hormonal imbalances observed in PCOS, where follicular arrest and anovulation are common. This report was in consistent with the earlier report [23].

Treatment with *Phyllanthus muellerianus* roots extracts, particularly the aqueous roots extract, resulted in varying degrees of recovery in ovarian histology. The 30 mg/kg aqueous roots extract showed the most pronounced recovery, with the presence of normal primary and secondary follicles and minimal damage (Plate 3). This suggests that the aqueous roots extract may have the most significant regenerative effects on ovarian tissue, possibly by promoting follicular maturation and restoring normal ovarian function. The 60 mg/kg aqueous roots extract also showed partial recovery but with some remaining damage, indicating a dose-dependent response (Plate 4). Interestingly, the 120 mg/kg aqueous roots extract resulted in a mild recovery, with smaller follicular antrium and less pronounced ovarian changes, which may suggest a diminishing effect at higher doses (Plate 5).

The ethanolic roots extract demonstrated a more gradual recovery, with the 30 mg/kg dose showing mild clearance of ovarian damage (Plate 6) and the 60 mg/kg dose showing more pronounced restoration, though some hemorrhagic patches were observed (Plate 7). These results suggest that while the ethanolic roots extract may also provide therapeutic benefits, it may not be as effective as the aqueous roots extract in reversing ovarian damage. The 120 mg/kg ethanolic roots extract showed underdeveloped follicular structures (Plate 8), indicating that higher concentrations of the ethanolic roots extract may have a less favourable impact on ovarian tissue than lower doses.

More so, the estrus cyclicity in PCOS rats, which was marred with irregularities, was minimized in the presence of the extracts of *P. muellerianus*. These extracts were able to maintain estrus cyclicity balance in rats after treatment, as indicated by the increase in the number of days for estrus and, to some extent, in the number of days for proestrus (Table 2). The observed ameliorative properties of *P. muellerianus* can be corroborated by its ability to offer clearance against PCOS-induced histoarchitectural changes in the ovary of PCOS rats. Hence, this plant may be a promising drug candidate in the treatment and management of PCOS in infertile subjects.

CONCLUSION

In conclusion, it appears that *P. muellerianus* possesses some phytochemical principles which could be linked to its ameliorative properties in offering protection against PCOS-induced histoarchitectural and hormonal changes as evidenced in its ability to reverse the observed decreases in the level of estradiol-caused by PCOS induction. The results from both hormonal assays and histological analysis suggest that *Phyllanthus muellerianus* roots extracts, especially the aqueous extract, have therapeutic potential in the management of PCOS. The extracts appear to regulate hormonal imbalances by increasing estradiol and reducing testosterone levels, which are pivotal in the pathophysiology of PCOS. Moreover, the histological improvements in ovarian tissue further support the beneficial effects of *P. muellerianus* in restoring ovarian structure and function. These findings provide a basis for further investigation into the mechanisms underlying these effects and the potential of *P. muellerianus* as a natural therapeutic option for PCOS management.

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