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Therapeutic Potential of *Phyllanthus muellerianus* Root Extracts in Managing Lipid Imbalances and Metabolic Alterations in Letrozole-Induced Polycystic Ovary Syndrome Rats

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This study examined the impact of *Phyllanthus muellerianus* root extracts on lipid profiles in rats with letrozole-induced polycystic ovary syndrome (PCOS). Aqueous and ethanolic extracts of *P. muellerianus* were prepared using maceration and infusion methods. Forty-two adult female Wistar rats were divided into groups and treated for 21 days with letrozole to induce PCOS. After confirmation of PCOS, the rats were further treated for 14 days with distilled water, clomiphene citrate, metformin, or *P. muellerianus* root extracts at doses of 30, 60, and 120 mg/kg/day. Serum and ovarian lipid profiles, including total cholesterol (T-CHOL), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides (TRIG), were assessed. The results revealed that treatment with aqueous extracts of *P. muellerianus* significantly reduced T-CHOL levels in rats with PCOS, particularly at doses of 30, 60, and 120 mg/kg/day, compared to the positive control, while treatment with ethanolic extract at 30 mg/kg/day also showed a reduction in T-CHOL levels. However, no significant changes were observed in LDL and TRIG levels across the groups. The study demonstrates the therapeutic potential of *P. muellerianus* root extracts in addressing lipid imbalances in PCOS. The aqueous extract exhibited partial efficacy, particularly at certain doses, in improving lipid profiles, though results for oxidative stress biomarkers were inconclusive. These findings suggest that *P. muellerianus* may serve as a promising candidate for managing lipid-related metabolic dysfunctions in PCOS, with further research needed to clarify its broader effects.

Keywords: *Phyllanthus muellerianus*, polycystic ovary syndrome (PCOS), extract, lipid profile, antioxidant activity.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a prevalent hormonal disorder in women of reproductive age, marked by a multifaceted interaction of endocrine and metabolic abnormalities. It manifests with symptoms such as oxidative stress, dyslipidemia, obesity, irregular menstrual cycles, infertility, and hyperandrogenism (Teede et al., 2010; Swaroop et al., 2015). PCOS impacts approximately 5–10% of women in this age group, with infertility affecting 40% of those diagnosed. This makes PCOS the leading cause of ovulatory-related infertility (Hassanzadeh et al., 2013).

Oxidative stress (OS) is intimately linked to an increased risk of infertility in individuals with PCOS. Research has consistently demonstrated abnormalities in OS-related biochemical markers including malondialdehyde (MDA), catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx) in PCOS patients (Murri et al., 2013). In addition, OS is associated with excessive production of reactive oxygen species (ROS), resulting in damage of DNA and mutations in tumor suppressor genes. These alterations may contribute to uncontrolled proliferation of ovarian cells, the formation of multiple cysts, and subsequent infertility (Zuo et al., 2016).

Letrozole, an aromatase inhibitor usually prescribed for breast cancer treatment, is known to cause both metabolic and reproductive disturbances. By inhibiting aromatase activity, letrozole reduces the conversion of androgens to estrogens, leading to accumulation of androgen within the ovary (Garcia-Velasco et al., 2005). Studies have demonstrated that letrozole is capable of inducing PCOS in rats (Kafali et al., 2004; Lee et al., 2018).

These animal models exhibit several features of human PCOS, including disrupted follicular development (Kafali et al., 2004), elevated blood glucose levels (Zhu et al., 2013), and elevated oxidative stress (Zuo et al., 2016).

The management of PCOS often involves medications like metformin and clomiphene citrate; however, these drugs are frequently linked to significant 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, the need arises for a novel therapeutic approach that offers minimal side effects, is readily accessible, and has a broad spectrum of efficacy. Previous research has highlighted the effectiveness of various plants, including *Trigonella foenum-graecum* (Swaroop et al., 2015), *Ecklonia cava* (Yang et al., 2018), and *Allium fistulosum* (Lee et al., 2018), in improving ovarian function in PCOS-induced rats. *Phyllanthus muellerianus* (Euphorbiaceae) is extensively used in some countries for the management of digestive diseases, irregular menstruation, and ovulation (Burkill, 1995; Katsayal and Lamal, 2009). Phytochemical analysis of the bark and leaves of this plant has identified numerous bioactive compounds, including astragalin, caffeic acid, chlorogenic acid, corilagin, furosin, gallic acid, geraniin, isoquercitrin, methyl gallate, phaselic acid, rutin, and 3,5-*o*-dicaffeoylquinic acid (Agyare et al., 2011). In addition, *P. muellerianus* has demonstrated antihyperglycemic properties (Adeneye, 2012) and antihyperlipidemic effects (Mao et al., 2016), antioxidant properties (Boakye & Agyare, 2013), and aphrodisiac properties (Ben-Bala, 2008) in normal rats, while its effect in PCOS rats remains largely unexplored. Given its diverse pharmacological properties, *P. muellerianus* presents a promising potential for managing PCOS-related metabolic and reproductive complications. This study, hence, seeks to examine the impact of aqueous and ethanolic extracts of *P. muellerianus* on oxidative stress markers and lipid profile in letrozole-induced PCOS rats following a 14-day treatment regimen.

MATERIALS AND METHODS

MATERIALS

Plant Material and Preparation of Aqueous and Ethanolic Roots Extracts

The fresh roots of *P. muellerianus* were harvested in early August 2022, and botanical identification was performed at the University Herbarium in Kaduna, where a voucher specimen number was assigned. The roots were sliced, dried under shade for seven days, and then ground into a fine powder using an electric blender. 250 g of the powdered material was soaked in 1500 mL of boiling water for fifteen minutes and then filtered. The resulting filtrate was dried in an oven at 55°C for 48 hours. The final yield of the aqueous extract was determined, and the extraction efficiency was calculated as a percentage. For the ethanolic extract, 2500 g of the powder was macerated in 5000 mL of ethanol for 72 hours. The mixture was filtered, and under reduced pressure the solvent was removed using a rotary evaporator at 75°C. Following the evaporation process, the amount of ethanolic extract that was yielded was estimated, and the extraction yield was calculated in percentage.

Chemicals and Reagents

All chemicals and reagents used were of analytical quality. Most of the chemicals procured were BDH, Poole (England) and Randox (United Kingdom) products.

METHODS

Experimental Animals

Forty-two adult female albino rats (Wistar strain) were obtained from the Animal House of the Animal Science and Environmental Biology Department at Ahmadu Bello University, Zaria. The rats were housed under standard conditions with a controlled room temperature, a reversed natural light-dark cycle (12 hours of light and 12 hours of dark), and had unrestricted access to a standard rat chow and water throughout the study. Only rats that exhibited at least three consecutive regular estrus cycles were selected for the study. During the treatment period, the rats' weights were recorded two times in a week, and vaginal smears were examined under a microscope to monitor the estrus cycle. All experimental procedures followed the guidelines established by the National Institutes 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 allowed a one-week acclimatization period before the treatments began. They were housed under standard laboratory conditions in well-ventilated wooden cages, with a 12-hour light and 12-hour dark cycle, at a temperature of 27 ± 1°C. They were provided with ad libitum access to standard pelletized rat chow and drinking water throughout the study period.

Females showing regular estrus cycles, as described earlier, were chosen and divided into five groups. The first group (control, n=6) received a daily oral dose of vehicle (0.9% NaCl solution). To induce polycystic ovary syndrome (PCOS), the second group (n=36) was treated orally for 21 days with letrozole (1 mg/kg/day), dissolved in 0.9% NaCl. Vaginal smears were collected every day and microscopically examined to identify the estrus stage. Six rats from both the first and second groups were randomly selected and sacrificed by cervical dislocation under anesthesia after induction. Biochemical and histological assessments were conducted to ascertain the presence of PCOS, which manifested in characteristics including high blood sugar, hyperandrogenism, and several ovarian cysts, as previously made known (Ghafurniyan et al., 2015).

In addition, irregular estrus cyclicity, characterized by the disruption of the normal appearance of the four stages of estrus, was the primary criterion for selecting rats with PCOS (Ghafurniyan et al., 2015). Subsequently, a total of 36 PCOS rats were divided into six groups containing 6 rats each and orally treated for 14 days using distilled water (10 ml/kg/day), clomiphene citrate (2 mg/kg/day), metformin (500 mg/kg/day), and aqueous or ethanolic extracts of *P. muellerianus* (30, 60, and 120 mg/kg/day). A normal control group (n=6) received only distilled water (10 mL/kg/day) for this duration. The effective dose of letrozole (1 mg/kg) and the 14 days period of treatment were based on previous studies (Kafali et al., 2004; Rezvanfar et al., 2012). The selected doses of *P. muellerianus* used for this study

were determined from a pilot study. Vaginal smears were collected every day for 14 days in the course of the treatment and examined microscopically to determine the estrus stage. After 14 days, the rats were sacrificed, and their ovaries and uteri were excised, weighed, and homogenized using a moderately cold mortar and pestle. The homogenates were then stored at -20°C for subsequent biochemical analysis.

Oral Glucose Tolerance Test

The Oral Glucose Tolerance Test (OGTT) was carried out both before and after the induction of PCOS, as well as following treatments with the plant extracts and reference drugs. The rats were allowed to fast for 6 hours, and blood sugar levels were determined from a tail blood sample using a handheld glucometer at baseline (time 0), prior to a single oral glucose dose (2.5 g/kg). Subsequent measurements were measured at 30, 60, 90, and 120 minutes respectively following glucose administration (Fofie et al., 2018).

Euthanasia and Sample Collection

After 14 days of treatment, the rats were weighed and euthanized through 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 stored at -20°C until required for biochemical analysis. Ovaries were homogenized in ice-cold 0.9% saline (1:9 w/v) to prepare a 10% homogenate. The homogenate was then centrifuged for 15 minutes at 1,500 x g, while the supernatants were stored frozen at -20°C for later biochemical assays.

BIOCHEMICAL ASSAYS

Lipid Profile Assays

The lipid profile, which included total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and triglycerides (TG) in both ovaries and plasma, was determined using standard technique described in colorimetric kits (Randox, United Kingdom) as previously outlined (NCEP, 2001). The levels of LDL-C and VLDL-C were estimated using Friedewald's formula: LDL = TC – (TG/5) – HDL; VLDL = TG/5 (Friedewald et al., 1972).

Oxidative Stress Parameters Assays

The malondialdehyde (MDA) level was measured using the method outlined by Buege and Aust (1978), based on the quantity of thiobarbituric acid reactive substances (TBARS) formed which is an indicator of lipid peroxidation. The total peroxidase content was determined following the procedure described by Giustarini et al. (2014). The activity of superoxides dismutase (SOD) was determined as explained by Misra and Fridovich (1972), in which one unit represented the enzyme amount required to cause 50% inhibition of adrenaline during 1 minute, while the catalase (CAT) activity was evaluated following the procedure discussed by Cohen et al. (1970). The CAT quantification is based on measuring the rate of decomposition of hydrogen peroxide (H2O2) in addition to the substance containing the enzyme.

Statistical Analysis

The data were presented as mean ± standard error of the mean. A one-way analysis of variance was carried out, then comparisons were done using Tukey's multiple range test obtained from GraphPad Prism Software Version 6.0, with a significance threshold set at $p < 0.05$.

RESULTS

The results of the qualitative phytochemical analysis of *P. muellerianus* are presented in Table 1. The results showed that both extracts contained alkaloid, saponins, and tannins. However, the ethanolic extract contained all the phytochemicals screened except flavonoids and steroids.

Table 1 Qualitative phytochemical screening of *P. muellerianus*

S/N	Parameter	Aqueous	Ethanolic
1	Alkaloid	+	+
2	Flavonoid	+	-
3	Saponins	+	+
4	Phenol	-	+
5	Tannins	+	+
6	Steroids	+	-
7	Terpenoids	-	+
8	Cardiac Glycosides	-	+
9	Anthraquinone	-	+
10	Phlobatannins	-	+

Key: + Present; - Absent

The phytochemical screening of *P. muellerianus* showed the presence of alkaloids, saponins, and tannins in both aqueous and ethanolic extracts. The ethanolic extract demonstrated a broader range of phytochemicals, including phenols, terpenoids, cardiac glycosides, anthraquinones, and phlobatannins, but lacked flavonoids and steroids. Conversely, the aqueous extract contained flavonoids and steroids but lacked these additional compounds. These findings suggest distinct phytochemical profiles for the two extracts, potentially influencing their therapeutic properties.

Average Mean Weights of Rats Before and After Induction with LTZ

Table 2 shows the results for the mean weight before induction (after acclimatization) and the mean weight after treatment.

The results in Table 2 show that letrozole-induced PCOS led to significant weight loss in the negative control group compared to the positive control group. Treatment with aqueous and ethanolic extracts of *P. muellerianus* partially restored weight in LTZ-induced PCOS rats. However, no clear dose-dependent trend was observed. Rats treated with the ethanolic extract generally exhibited slightly lower weight gain compared to those treated with the aqueous extract. Overall, both extracts demonstrated potential in mitigating excessive weight gain associated with PCOS.

Table 2 Mean Weight of Rats before and after induction of PCOS

Group	Experimental Design (mg/kg body weight)	Mean Weight before treatment (g)	Mean Weight after treatment (g
I*	Positive Control (Not Induced)	151.6 ± 3.61^{a}	202.6 ± 7.41^{a}
II*	Negative Control (Induced with Letrozole)	121.5 ± 6.19^{b}	183.4 ± 6.34^{b}
III	Aqueous extract (30 mg/kg)	$118.5 \pm 4.16^{b,c}$	181.5 ± 3.62^{c}
IV	Aqueous extract (60 mg/kg)	$107.4 \pm 8.12^{b,c}$	168.4 ± 4.13^{c}
V	Aqueous extract (120 mg/kg)	$112.8 \pm 5.20^{b,c}$	178.2 ± 6.17^{c}
VI	Ethanolic Extract (30 mg/kg)	$106.7 \pm 5.32^{b,c}$	164.8 ± 5.86^{c}
VII	Ethanolic Extract (60 mg/kg)	$104.4 \pm 8.51^{b,c}$	167.6 ± 6.32^{c}
VIII	Ethanolic Extract (120 mg/kg)	$116.3 \pm 4.15^{b,c}$	170.4 ± 4.37^{c}

[a]Values with different alphanumeric superscripts in the same column are significantly different from each other ($p < 0.05$).

Table 3 Oral Glucose Levels in PCOS and Treated Rats

Group	Treatment (mg/kg)	Before Induction (mmol/L)	After Induction (mmol/L)	After Treatment (mmol/L)
I*	Control (Not Induced)	7.53 ± 0.16^{a}	8.60 ± 0.18^{a}	8.88 ± 0.38^{a}
II*	Control (Induced)	7.98 ± 0.19^{a}	8.73 ± 0.25^{a}	8.73 ± 0.59^{a}
III	Aq. Extract (30)	7.10 ± 0.44^{a}	7.74 ± 0.20^{a}	7.62 ± 0.26^{a}
IV	Aq. Extract (60)	6.83 ± 0.29^{a}	7.34 ± 0.31^{b}	8.64 ± 0.56^{a}
V	Aq. Extract (120)	8.03 ± 0.54^{a}	7.33 ± 0.39^{b}	8.04 ± 0.51^{a}
VI	Eth. Extract (30)	6.48 ± 0.15^{b}	8.56 ± 0.45^{a}	9.86 ± 0.39^{a}
VII	Eth. Extract (60)	7.13 ± 0.25^{a}	8.06 ± 0.25^{a}	8.90 ± 0.35^{a}
VIII	Eth. Extract (120)	7.48 ± 0.18^{a}	7.92 ± 0.21^{a}	9.08 ± 0.18^{a}

[a]Values with different superscripts in the same column differ significantly ($p < 0.05$).

Table 3 shows the results for the average OGTT of rats before induction (after acclimatization), after induction with PCOS, and after treatment following extract administration. There was a decrease in glucose level in rats administered with 60 mg/kg bwt and 120 mg/kg bwt of aqueous extract of *Phyllanthus muellerianus* roots following induction, but this was reversed after treatment. The significant drop in glucose levels observed in the 60 mg/kg and 120 mg/kg aqueous extract groups post-induction (7.34 ± 0.31^{b} and 7.33 ± 0.39^{b}, respectively) suggests that these doses of the aqueous extract of *Phyllanthus muellerianus* may have a modulatory effect on glucose metabolism in letrozole-induced PCOS rats. This could indicate an enhancement in glucose regulation, potentially through mechanisms such as improved insulin sensitivity, enhanced glucose uptake, or reduced gluconeogenesis.

The OGTT results show that letrozole-induced PCOS elevated glucose levels in the negative control group. Treatment with *P. muellerianus* root extracts showed varying effects. The aqueous extract effectively maintained glucose levels closer to pre-induction values, particularly at lower doses, while the ethanolic extract was less effective in glucose regulation, with higher glucose levels observed after treatment. This suggests that the aqueous extract may be more beneficial in modulating glucose levels in PCOS rats.

The Effects of P. muellerianus Root Extracts on Serum MDA and GSH Levels, Antioxidant Enzymes of LTZ-Induced PCOS Rats

The MDA and GSH levels in the serum of rats were not significantly different from those of the control ($p > 0.05$). Similarly, the SOD and CAT activities in serum were not significantly different ($p > 0.05$) from those of the control. The MDA and GSH levels, together with the antioxidant enzyme activities assayed, were not altered in rats administered with 30 mg/kg, 60 mg/kg, and 120 mg/kg of both aqueous and ethanolic extracts of *Phyllanthus muellerianus* root when compared to controls, as shown in Table 4.

The administration of *P. muellerianus* extracts had no significant effect on serum MDA level or the antioxidant enzymes (SOD and CAT) activities in letrozole-induced PCOS rats, whereas a significant reduction was observed in GSH level of rats treated at doses of 30 mg and 60 mg/kg aqueous root extracts of *P. muellerianus*. This indicates that the doses and duration of treatment may not have been sufficient to substantially influence lipid peroxidation or antioxidant enzyme activities in letrozole-induced PCOS rats, whereas the observed changes in GSH level suggest that *P. muellerianus* extracts may modulate the non-enzymatic antioxidant defense system by affecting GSH levels, which play a crucial role in neutralizing oxidative stress.

However, the ethanolic extracts across all doses (30 mg, 60 mg, and 120 mg/kg) maintained oxidative stress markers and

Table 4 The effects of *P. muellerianus* root extracts on serum MDA and GSH levels, SOD and CAT activities in LTZ-induced PCOS rats.

Group	MDA (nMols/mg protein)	GSH (μml)	SOD (U/ml)	CAT (U/mg protein)
I*	11.900 ± 0.601^{a}	35.023 ± 4.999^{a}	16.510 ± 0.500^{a}	46.190 ± 6.183^{a}
II*	12.807 ± 0.551^{a}	43.167 ± 2.638^{a}	19.003 ± 1.133^{a}	45.153 ± 2.469^{a}
III	14.987 ± 2.000^{a}	25.637 ± 2.967^{b}	20.407 ± 4.048^{a}	45.187 ± 5.936^{a}
IV	13.267 ± 2.017^{a}	25.943 ± 2.860^{b}	16.220 ± 0.203^{a}	46.160 ± 2.686^{a}
V	10.807 ± 0.205^{a}	39.097 ± 6.958^{a}	17.750 ± 1.685^{a}	46.030 ± 3.398^{a}
VI	13.160 ± 2.056^{a}	33.267 ± 6.362^{a}	17.687 ± 1.777^{a}	44.137 ± 2.287^{a}
VII	11.953 ± 0.462^{a}	35.377 ± 2.402^{a}	18.643 ± 1.960^{a}	40.137 ± 4.804^{a}
VIII	12.173 ± 0.494^{a}	37.850 ± 2.926^{a}	15.740 ± 0.433^{a}	42.630 ± 5.646^{a}

Note: Values with different alphanumeric superscripts in the same column are significantly different from each other ($p < 0.05$).

antioxidant enzyme activities similar to control groups, indicating no substantial impact on oxidative stress in the treated rats.

The Effects of P. muellerianus Roots Extracts on Serum Lipid Profile in LTZ-Induced PCOS Rats

The serum lipid profile of treated rats revealed a noticeable increase ($p < 0.05$) in the T-CHOL level of rats induced with letrozole (positive control). However, when compared to the positive control, there was a noticeable decrease ($p < 0.05$) in levels of T-CHOL in rats administered with 30 mg, 60 mg, and 120 mg/kg b.w of aqueous extract as well as 30 mg/kg b.w of ethanolic extract of *Phyllanthus muellerianus* root, but were not statistically different when compared to the negative control. Similarly, when compared to the positive control, there was a remarkable decrease ($p < 0.05$) in the serum HDL level of rats administered with 30 mg/kg b.w of aqueous extract of *P. muellerianus*. However, there was no remarkable difference ($p > 0.05$) in serum TRIG and LDL levels in all treated rats, as outlined in Table 5.

Treatment with *P. muellerianus* extracts reduced elevated T-CHOL levels in LTZ-induced PCOS rats, with aqueous extracts showing the most significant effect. Triglyceride and LDL levels were unaffected by treatment, while HDL levels decreased only in the group administered with 30 mg/kg of the aqueous extract. The decrease in HDL levels observed in the 30 mg/kg aqueous extract group may be due to an adverse effect, suboptimal dosing, or measurement error. Replicating the study and incorporating additional lipid-related biomarkers will help clarify the mechanism and ensure the reliability of the findings. The results suggest that *P. muellerianus* root extracts, particularly at higher doses, have a potential role in improving certain aspects of lipid metabolism, particularly T-CHOL and HDL, in PCOS-induced dyslipidemia.

DISCUSSION

The findings of this study offer important information on the impact of *P. muellerianus* extracts on lipid profile and oxidative stress markers in letrozole-induced PCOS rats. PCOS is often associated with dyslipidemia, characterized by elevated T-CHOL and LDL, as well as reduced HDL levels (Kim & Choi, 2013; Macut et al., 2013). The oxidative stress resulting from this disorder further exacerbates its metabolic effects (Macut et al., 2013; Uçkan et al., 2022). Therefore, investigating the potential of *P. muellerianus* to modulate these parameters offers therapeutic promise for managing PCOS-related dyslipidemia and oxidative stress.

The ability of the 60 mg/kg dose to show a significant reduction in glucose level compared to other groups may point to an optimal dose-response relationship for glucose regulation. Meanwhile, the 120 mg/kg dose, though also showing a reduction, might suggest that the effect plateaus beyond a certain threshold, indicating that higher doses may not necessarily translate to proportionally greater effects. These findings emphasize the potential antihyperglycemic properties of *P. muellerianus* at specific doses, aligning with its reported pharmacological activities in previous studies (Gnaléi et al., 2019; Ofokansi et al., 2023).

Several studies on PCOS have revealed cases of hormonal imbalances, excessive body weight, hyperandrogenism, irregular menstrual cycles, oxidative stress, and infertility in reproductive women (Teede et al., 2010; Swaroop et al., 2015; Hassanzadeh et al., 2013). The ameliorative effect of *P. muellerianus* aqueous and ethanolic extracts on PCOS-induced rats was studied. Due to side effects usually observed by certain drugs like metformin and clomiphene citrate in the treatment of PCOS, it is therefore imperative to look at some safer medicinal plants such as the root of *P. muellerianus*, which could serve as a novel candidate drug in the treatment of PCOS.

Our results suggest that *P. muellerianus* root extracts may have some antioxidant potential, as evidenced by changes in GSH, but the extract's impact on other oxidative stress markers like MDA, SOD, and CAT was limited under the conditions tested (Table 4, suggests no difference from controls). While MDA, SOD, and CAT remained unchanged, the observed differences in GSH suggest a potential dose-dependent effect, as some extract doses (e.g., aqueous extract at 30 mg/kg) appeared to maintain or improve GSH levels. This may indicate that the extract selectively influences specific components of the oxidative stress pathway. These biochemical parameters have been shown to be abnormal in patients with PCOS (Murri et al., 2013). This may be so; probably, there is less production of ROS, which could lead to DNA damage and genetic mutations, which may lead to uncontrolled proliferation of ovarian cells, multiple cyst development, and infertility (Zuo et al., 2016). While dyslipidemia is commonly associated with increased oxidative stress, the results of this study suggest that *P. muellerianus* extracts did not significantly alter serum levels of MDA nor did they affect the activities of antioxidant enzymes SOD and CAT but do lower the serum GSH level. These findings indicate that the

Table 5 The effects of *P. muellerianus* root extracts on serum lipid profile in LTZ-induced PCOS rats.

Group	T-CHOL (mg/dl)	TRIG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
I*	49.223 ± 0.781^{a}	87.153 ± 17.223^{a}	22.007 ± 1.139^{a}	9.767 ± 1.673^{a}
II*	69.667 ± 1.012^{b}	95.143 ± 1.504^{a}	38.923 ± 0.879^{b}	11.723 ± 1.130^{a}
III	$52.363 \pm 4.507^{a,c}$	96.367 ± 4.668^{a}	$23.470 \pm 4.457^{a,c}$	9.597 ± 4.809^{a}
IV	$57.973 \pm 1.371^{a,c}$	76.320 ± 3.919^{a}	27.523 ± 1.194^{a}	15.153 ± 1.949^{a}
V	$54.243 \pm 3.034^{a,c}$	93.410 ± 14.527^{a}	25.813 ± 2.743^{a}	9.730 ± 2.757^{a}
VI	$55.623 \pm 2.290^{a,c}$	78.623 ± 6.571^{a}	25.703 ± 3.035^{a}	14.173 ± 0.508^{a}
VII	63.333 ± 2.439^{b}	94.913 ± 2.328^{a}	31.803 ± 5.761^{a}	12.560 ± 5.361^{a}
VIII	$64.233 \pm 0.798^{b,d}$	95.097 ± 10.729^{a}	33.187 ± 2.548^{a}	11.997 ± 5.315^{a}

Note: Values with different superscript letters in the same column are significantly different from each other ($p < 0.05$).

extracts did not modulate most of the oxidative stress markers in a significant manner in this model except GSH. This could suggest that the observed effects on the lipid profile might not be mediated through antioxidant mechanisms in the context of PCOS. Alternatively, the treatment duration or dosage may not have been sufficient to produce a notable effect on oxidative stress parameters, as antioxidant effects are sometimes dose- and time-dependent (Hwang et al., 2016; Czékus et al., 2020).

In this study, the administration of *P. muellerianus* extracts (both aqueous and ethanolic) significantly reduced T-CHOL levels in PCOS rats. The reduction in T-CHOL was most notable in the aqueous extract-treated groups (30 mg/kg, 60 mg/kg, and 120 mg/kg) and the 30 mg/kg ethanolic extract group. This reduction suggests that *P. muellerianus* possesses potential hypocholesterolemic properties, which could be linked to the presence of bioactive components like saponins, alkaloids, and tannins, which are known for their lipid-lowering effects in other medicinal plants (Muoneke & Bayim, 2021). For instance, previous studies have shown that saponins can reduce cholesterol levels by inhibiting cholesterol absorption in the intestines and enhancing its excretion (Cao et al., 2024; Marrelli et al., 2016). This finding is consistent with reports on various plant extracts that modulate lipid metabolism by influencing hepatic cholesterol biosynthesis or enhancing lipoprotein clearance (Cao et al., 2024; Eilam et al., 2022; Li et al., 2020; Laka et al., 2022).

Interestingly, the reduction in T-CHOL levels in the treated rats was comparable to the negative control group (untreated rats), indicating that *P. muellerianus* extracts may help restore cholesterol levels to normal in PCOS, where hypercholesterolemia is often observed. However, despite the reduction in T-CHOL, no significant effects were observed on triglyceride (TRIG) and LDL levels. This suggests that the extract's action may be specific to cholesterol regulation rather than a broader effect on all lipid fractions.

The observed decrease in total cholesterol level in rats administered with either aqueous or ethanolic extract of *Phyllanthus muellerianus* root perhaps suggests that this plant possesses some hypocholesterolemic properties, which are responsible for its ameliorative properties against PCOS in rats. HDL level is also found to be lowered in rats administered with 30 mg/kg body weight of aqueous extract of *P. muellerianus*. This also suggests that this plant offers protection against PCOS-induced HDL in treated rats. This protection is assumed to antagonize any form of lipidemic disorders that may predispose to infertility, which occurs as a result of PCOS. Recently, *P. muellerianus* has been reported to possess antihyperlipidemic properties in normal rats (Mao et al., 2016). HDL is often considered a "good" cholesterol due to its role in reverse cholesterol transport. In this study, a remarkable reduction in HDL levels was noted in rats administered with 30 mg/kg aqueous extract of *P. muellerianus*, while other treatment doses and the ethanolic extract did not reveal noticeable changes in HDL levels. The decrease in HDL levels at 30 mg/kg suggests a possible adverse or suboptimal effect of this dosage on lipid metabolism. While the biological implications might be significant, the possibility of a study artifact cannot be entirely ruled out. The decrease in HDL could plausibly be a compensatory mechanism in response to the reduction in T-CHOL, reflecting an adaptive metabolic mechanism to maintain lipid homeostasis, although the clinical implications of this change require further investigation. Some plant extracts are known to influence lipid metabolism in complex ways (Marrelli et al., 2016), and while the reduction in HDL may seem concerning, it does not necessarily negate the positive effects on cholesterol regulation (Duan et al., 2022).

In this study, both the aqueous and ethanolic extracts of *P. muellerianus* have been shown to significantly reduce the extent of weight gain in the treated rats following LTZ induction, compared to the untreated PCOS-induced group. This observation highlights the potential of *P. muellerianus* extracts to regulate lipid metabolism and, by reducing the extent of weight gain, potentially prevent obesity. Diabetes or prediabetes has been listed as a risk factor in PCOS women (Lerchbaum et al., 2013); hence, there is a need to conduct an oral glucose tolerance test (OGTT) in this study. However, the OGTT revealed a decreased level of glucose following the rat's induction (group VI); the observed alteration was later restored back to normal after treatment with 60 mg/kg bwt aqueous extract of *P. muellerianus* as shown in Table 4. This observation demonstrates the plant's ability to protect against LTZ-induced changes in glucose levels. However, this observation suggests that *P. muellerianus* extract possesses some phytochemical principles which may be linked to this preventive property. The aqueous extract of this plant has been shown to contain alkaloids, flavonoids, steroids, and saponin (Table 1) .

CONCLUSION

In conclusion, this study highlights the potential of *P. muellerianus* root extracts in managing metabolic and oxidative disturbances associated with PCOS. The extracts improved

lipid profiles, stabilized weight, and partially restored glucose levels, likely due to their bioactive compounds such as alkaloids, flavonoids, and tannins. While oxidative stress biomarkers showed minimal changes, the reduction in GSH suggests an adaptive response. Overall, *P. muellerianus* demonstrates promise as a therapeutic agent for PCOS, though further research is needed to clarify its mechanisms and ensure safety.

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