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Original Research Article

Ameliorative Potentials of *Vernonia amygdalina* in High-fat Diet and Letrozole-induced Polycystic Ovary Syndrome in Female Wistar Rats

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a metabolic and reproductive disorder that disrupts the female reproductive cycle, resulting in infertility. There is a need for safer and more effective options for managing this disorder. Therefore, this study explored the effects of *Vernonia amygdalina* (VA) extract administration on insulin levels, inflammation markers, oxidative stress, and reproductive hormones in PCOS rats. Twenty-five female Wistar rats were randomly divided into 5 groups (n = 5) namely: PCOS untreated, normal control, treatment groups (400 mg/kg and 200 mg/kg of VA extract, respectively), and standard group (metformin and clomiphene citrate). PCOS was induced with 1 mg/kg letrozole coupled with a high-fat diet for 28 days. The treatment groups were orally administered with VA extract for 2 weeks. Blood samples were obtained for insulin, oxidative stress markers, lipid profile, inflammatory markers, and reproductive hormone level analysis, and data were analyzed. Extracts of VA significantly reduced insulin and luteinizing hormone levels; increased follicle-stimulating hormone and progesterone levels in comparison with the PCOS untreated group. Serum IL-6, CRP, and TNF-α levels were lower in the treatment groups compared to the PCOS untreated group. A significant reduction in malondialdehyde level was observed, with no significant difference in catalase and superoxide dismutase levels in comparison to the PCOS untreated group. Groups administered VA had significant reductions in triglycerides and LDL-C levels, while HDL-C levels was significantly increased in comparison to the PCOS untreated group. Extracts of *Vernonia amygdalina* could be therapeutic in PCOS management.

Keywords: Polycystic ovary syndrome, *Vernonia amygdalina*, letrozole, insulin, antioxidants

Introduction

Polycystic ovarian syndrome (PCOS), which is prevalent among women of childbearing age, is a hormonal and metabolic condition characterized by hyperandrogenism, dyslipidemia, anovulation, oxidative stress, hyperinsulinemia, inflammation, polycystic ovaries, and infertility.[1,2] It is considered the most common cause of anovulatory infertility affecting 5-10% of reproductive females.[3] Reduced high-density lipoprotein (HDL) levels with increased levels of total cholesterol, triglycerides, and low-density lipoprotein (LDL) are observed in PCOS women.[4] Imbalance in luteinizing hormone (LH), testosterone, estrogen, progesterone, and follicle-stimulating hormone (FSH) are also observed.[5] The increase in LH/FSH ratio has been suggested to result from the increase in amplitude and pulse frequency of gonadotropin-releasing hormone (GnRH) which induces LH production over FSH. The precise cause of this disorder is still unknown but a relationship between PCOS, type 2 diabetes, obesity, and insulin resistance has been established.[6] According to some studies, insulin resistance is implicated in the onset and long-term complications of PCOS.[7] Therefore, PCOS being a multifactorial disorder requires a multidisciplinary mode of managing the related symptoms.[8]

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Treatments for PCOS involve the reproductive and metabolic features related to the disorder, and they include lifestyle adjustments, the use of metformin, oral contraceptives, and anti-androgen medications.[9] However, these medications have severe side effects such as increased body weight, gastrointestinal disorders, irregular menstruation, and elevated insulin resistance, therefore there is a need for alternative modes of treatment with fewer or no adverse effects and more efficacy. Medicinal plants might be useful for PCOS treatment due to the presence of various phytochemicals, which have both therapeutic and pharmacological potentials.[10,11] Some medicinal plants have been proven to improve ovulation, insulin sensitivity and reduce the level of androgens.[8]

Vernonia amygdalina (VA) also known as bitter leaf is an angiosperm belonging to the Asteraceae family. In West Africa, notably Nigeria, it is widely available and utilized as a food supplement, vegetable, and soup.[12] The name 'bitter leaf' is due to its bitter taste which is ascribed to its nutritious composition. It has been shown to have anti-diabetic, antioxidant, antimicrobial, hepatoprotective, neuroprotective, anti-inflammatory, anti-allergy, anti-malaria, anticancer, and immunomodulatory potentials.[13-16] According to an ethnobotanical survey by Ogunlakin & Sonibare[17] traditionally, VA is used in treating menstrual disorders but there is no scientific justification for its use. Hence, this current study was designed to investigate the effects of *Vernonia amygdalina* in high-fat diet and letrozole PCOS-IR rat model.

Materials and Methods

Plant Collection and Identification

Vernonia amygdalina was collected in Ilishan-Remo, Ogun State, Nigeria in January 2022. The plant was identified and a voucher specimen (Specimen Identification Number: DPHU10669) was deposited at the Department of Pharmacognosy Herbarium, University of Ibadan (DPHUI), Ibadan. The plant materials (leaves) were air-dried

and ground to powder. After macerating 1 kg of the pulverized plant materials in methanol (10 L) at room temperature for 72 hours with intermittent shaking, the mixture was filtered and evaporated *in vacuo*. Methanolic extract was stored under refrigerated conditions until use.

Study Design

The Babcock University Health Research Ethics Committee (BUHREC) authorized the experimental protocols (BUHREC 920/21). Twenty-five female adult Wistar rats (mean body weight, 180 g) were randomly distributed into 5 groups (n=5/group) with one control group and four experimental groups. Animals in group I (normal control group) were orally administered 0.5% carboxymethylcellulose (CMC) as a vehicle. Animals in groups II to V (PCOS untreated, 400 mg/kg treatment, 200 mg/kg treatment, standard drug groups) were orally administered 1 mg/kg letrozole dissolved in 0.5% CMC and high-fat diet (20% protein, 20% carbohydrate, 60% fat) for 28 days for induction of PCOS-IR.[18] Group II received no treatment, while groups III and IV were orally administered 400 mg/kg and 200 mg/kg methanolic extract of *Vernonia amygdalina* extract, and group V was administered metformin (500 mg/kg) and clomiphene citrate (2 mg/kg). The duration of the treatment with *Vernonia amygdalina* was 14 days.

Analysis of Samples

Serum testosterone, LH, FSH, progesterone, estradiol, and insulin levels were determined using enzyme-linked immunosorbent assay (ELISA) based kits (Monobind Inc. Lake Forest, CA 92630, USA). Serum C-reactive protein (CRP), interleukin-6 (IL-6), and TNF-α levels were determined using enzyme-linked immunosorbent assay (ELISA) based kits (Elabscience Biotechnology Inc., 14780 Memorial Drive, Suite 216, Houston, Texas). Triglycerides (TG), High-density lipid cholesterol (HDL), Low-density lipid cholesterol (LDL), and Total-cholesterol (TC) levels were assessed using standard assay kits (Randox Laboratory Limited, United Kingdom). Catalase (CAT), superoxide dismutase activity (SOD), and malondialdehyde (MDA) levels were assessed using standard assay kits (Elabscience Biotechnology Inc. 14780 Memorial Drive, Suite 216, Houston, Texas).

Statistical Analysis

The results were expressed as mean ± standard error of mean (SEM). Graph Pad Prism version 9.0 software was used in analyzing the data and one-way ANOVA was used to compare the significance of differences between the groups followed by Dunnett's multiple comparison test. P values less than 0.05 were considered statistically significant.

Results and Discussion

Administration of letrozole and high-fat diet (HFD) has been used to mimic the PCOS-insulin resistance in animal models[18], hence the use of this model in this study. Letrozole, an aromatase inhibitor, decreases androgen conversion to estrogens, resulting in the elevated level of androgens observed after letrozole treatment in the rats.

In this study, there was a significant increase in body and ovary weights in the PCOS untreated group compared to the normal control group. There was a reduction in weight gain in the 200 mg/kg VA-treated group while the ovary weight was significantly reduced in the 400 mg/kg VA-treated group in comparison to the PCOS-untreated group (Table 1). High-fat diet increases adiposity, which is associated with insulin resistance. Insulin resistance significantly promotes lipolysis and modifies hepatic lipase and lipoprotein lipase expression.[19] In the PCOS untreated group, insulin levels significantly increased compared to the normal control group. Insulin levels were significantly reduced after treatment with 400 mg/kg and 200 mg/kg in comparison with the PCOS untreated group (Figure 1). Excess androgen stimulates increased secretion of insulin from the β-cells. Hyperinsulinemia has been reported to induce weight gain. This could be due to adenosine monophosphate-activated protein kinase (AMPK) inhibition, resulting in improved activity of acetyl CoA carboxylase, thereby promoting fatty acid biosynthesis.[20] This accumulated fat might be the cause of the weight gain and ovary weight in the PCOS untreated group. The administration of VA significantly reduced insulin levels, thereby improving insulin sensitivity. *Vernonia amygdalina* has previously been proven to possess antidiabetic potential due to the rich flavonoid component of the plant. Some flavonoids can change the ability of the pancreatic β-cell to produce insulin.[4] A reduction in insulin level could result in LH reduction, which was observed in this study.

After PCOS induction, the level of triglycerides was increased with no significant change in the levels of HDL, LDL, and total cholesterol. After treatment with 400 mg/kg VA, there was a significant reduction in LDL, triglycerides, and total cholesterol levels with no significant difference in HDL level in comparison with PCOS untreated group. Similarly, there was a significant reduction in triglycerides, total cholesterol levels, and LDL with a significant increase in HDL level after administration with 200 mg/kg of *Vernonia amygdalina* (Table 2). This reduction may be a result of the hypolipidemic effect of VA due to tannins, saponins, triterpenoids, and flavonoids present in the plant, which is well documented in literature.[21,22] This shows that VA extract significantly reduced lipid levels in PCOS-IR model. In a study by Olamoyegun *et al.*,[23] VA had a positive impact on lipid profile in diabetic rats. This finding is also consistent with studies that administered *Phyllanthus muellerianus*, curcumin, and quercetin to PCOS animal models.[24,25,26]

Oxidative stress is one of the pathophysiologies of PCOS and is associated with insulin resistance, hyperandrogenism, and obesity.[27] Malondialdehyde level in the PCOS untreated group was significantly increased compared to the normal control group. There was a significant reduction in MDA level following administration of 400 mg/kg VA compared with the PCOS untreated group. In all the groups, there was no significant difference in the levels of CAT and SOD except in the group treated with metformin and clomiphene citrate (Table 3). According to Adesanoye & Farombi[28] and Adewole *et al*,.[29] saponins, flavonoids, and tannins found in VA could be responsible for the plant's antioxidant properties.

Table 1: Effect of *Vernonia amygdalina* on body and ovary weights in high-fat diet and letrozole-induced polycystic ovary syndrome in female Wistar rats

Groups	Weight gain (g)	Ovary Weight (g)
PCOS untreated	39.75 ± 5.88*	0.489 ± 0.056*
Normal control	23.50 ± 3.97	0.275 ± 0.047
PCOS + VA (400 mg/kg)	32.25 ± 5.45	0.275 ± 0.048*
PCOS + VA (200 mg/kg)	27.00 ± 9.83	0.350 ± 0.05
PCOS +Met (500 mg/kg) + CC (2 mg/kg)	19.75 ± 6.52*	0.250 ± 0.05*

Values are presented as mean ± SEM. *p<0.05

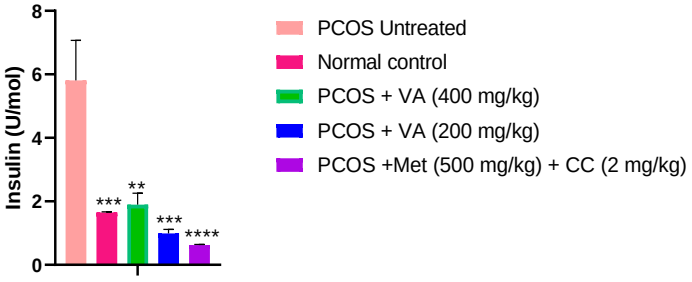

Figure 1: Effect of *Vernonia amygdalina* on insulin levels in high-fat diet and letrozole-induced polycystic ovary syndrome in female Wistar rats. Values are represented as Mean ± SEM. **p < 0.01, ***p < 0.001, ****p < 0.0001

Elevated testosterone and LH levels, reduced FSH, progesterone, and estradiol concentrations are biochemical biomarkers for diagnosing PCOS.[30] In this study, in comparison with the normal control group, LH and testosterone levels were significantly elevated, while FSH level was significantly reduced, with no significant change in the concentrations of progesterone and estradiol in the PCOS untreated group. The increased LH, testosterone, and reduced FSH concentrations are consistent with other findings.[24,31,32] Negative feedback due to the increased androgen levels acts on the anterior pituitary, resulting in decreased FSH and increased LH production, thereby promoting excess GnRH secretion by the hypothalamic neurons,[33] which was observed in this study. Also, excess androgen stimulates increased secretion of insulin, which induces the overproduction of glutamate, an excitatory neurotransmitter known to stimulate LH and GnRH production.[32] Administration of 400 mg/kg VA significantly reduced LH levels, increased FSH and testosterone levels, but had no statistical difference in progesterone and estradiol concentration. Similarly, treatment with 200 mg/kg of VA significantly reduced LH levels, while FSH, progesterone, estradiol, and testosterone concentrations were unchanged statistically (Table 4). The reduction in LH could be mediated by estrogen receptor 1 (ESR1), which is involved in the negative feedback of estrogen on the production of LH.[34] This ability of VA to increase FSH concentration is consistent with a study by Oladele *et al.*,[35] where different concentrations of VA increased FSH activities, and this is attributed to the bioactive compounds and phytochemicals present in the plant.

There has been a link between hyperandrogenism and chronic low-grade inflammation in PCOS.[36] The level of IL-6 significantly increased with no significant change in TNF-α and CRP levels in the PCOS untreated group in comparison with the normal control. Treatment with VA had no effect on IL-6 and TNF-α, but there was a significant decrease in CRP level in comparison with the PCOS untreated group (Table 5). The anti-inflammatory potentials of VA are well documented in literature, which could be a result of the activities of some bioactive compounds like vernoniosides.[37]

Inhibin A is a marker of follicular number, and it increases with FSH stimulation because it acts as an endocrine modulator of the production of FSH from the anterior pituitary.[38] There was a decrease in the concentration of Inhibin A in the PCOS untreated group in comparison with the normal control group. An increased concentration of Inhibin A was observed in VA-treated PCOS rats in comparison with the PCOS untreated group (Figure 2). The decrease in inhibin A level corresponds with the decrease in FSH concentration observed in this study.

Conclusion

The study shows that the methanolic extract of *Vernonia amygdalina* may ameliorate insulin resistance in the PCOS -IR model through a reduction in lipid levels and a reduction of pro-inflammatory markers, which play a role in ovarian cyst formation.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

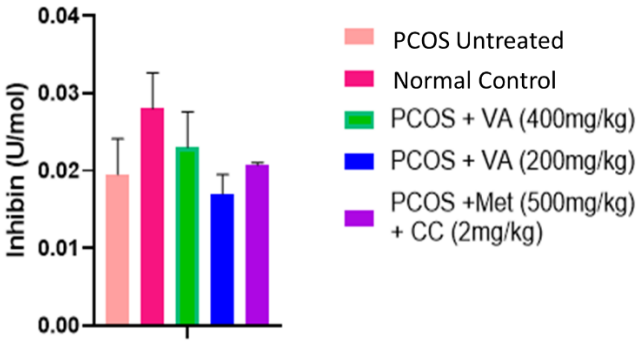

Figure 2: Effect of *Vernonia amygdalina* on Inhibin-A levels in high-fat diet and letrozole-induced polycystic ovary syndrome in female Wistar rats. Values are presented as mean ± SEM. $p < 0.05$

Table 2: Effect of *Vernonia amygdalina* on lipid profile in high-fat diet and letrozole-induced polycystic ovary syndrome in female Wistar rats

Groups	Triglycerides (mg/dl)	Total Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
PCOS untreated	76.42 ± 2.027*	90.63 ± 7.692	20.86 ± 0.909	62.05 ± 0.665
Normal control	53.10 ± 6.343	80.58 ± 10.240	27.11 ± 6.692	53.50 ± 3.522
PCOS +VA (400 mg/kg)	42.25 ± 2.076**	69.16 ± 6.777*	22.47 ± 3.988	46.70 ± 6.669*
PCOS + VA (200 mg/kg)	30.68 ± 5.167****	64.83 ± 5.824*	48.14 ± 8.798**	10.35 ± 1.184****
PCOS +Met (500 mg/kg) + CC (2 mg/kg)	21.40 ± 7.775****	85.97 ± 0.851	33.21 ± 0.814	53.48 ± 0.281

Values are represented as Mean ± SEM. *p < 0.05, **p < 0.01, ****p < 0.0001

Table 3: Effect of *Vernonia amygdalina* on oxidative stress parameters in high-fat diet and letrozole-induced polycystic ovary syndrome in female Wistar rats

Groups	CAT (U/mol)	MDA (U/mol)	SOD (U/mol)
PCOS untreated	1.64 ± 0.049	0.45 ± 0.004	1.64 ± 0.049
Normal control	1.60 ± 0.011	0.02 ± 0.002*	1.60 ± 0.012
PCOS + VA (400 mg/kg)	1.61 ± 0.061	0.02 ± 0.002*	1.60 ± 0.039
PCOS + VA (200 mg/kg)	1.62 ± 0.061	0.36 ± 0.011	1.42 ± 0.214
PCOS +Met (500 mg/kg) + CC (2 mg/kg)	0.69 ± 0.043*	0.02 ± 0.005*	0.62 ± 0.086*

Values are represented as Mean ± SEM. *p < 0.05

Table 4: Effect of *Vernonia amygdalina* on reproductive hormones in high-fat diet and letrozole-induced polycystic ovary syndrome in female Wistar rats

Groups	Progesterone (ng/ml)	FSH (mlu/ml)	Testosterone (ng/ml)	E2(ng/ml)	LH (mlu/ml)
PCOS untreated	2.83 ± 0.084	2.92 ± 0.029****	15.04 ± 0.958***	20.29 ± 0.035	14.26 ± 0.482****
Normal Control	2.37 ± 0.222	98.51 ± 4.215	7.97 ± 0.139	20.48 ± 0.162	2.11 ± 0.018
PCOS + VA (400 mg/kg)	3.46 ± 0.266	19.80 ± 2.142****	20.58 ± 0.355*	20.05 ± 0.667	2.30 ± 0.120****
PCOS + VA (200 mg/kg)	2.39 ± 0.199	2.43 ± 0.196	18.42 ± 1.023	19.85 ± 0.613	2.31 ± 0.133****
PCOS +Met (500 mg/kg) + CC (2 mg/kg)	2.95 ± 0.026	2.19 ± 0.023	15.31 ± 1.438	21.40 ± 0.442	2.17 ± 0.104****

Values are presented as mean ± SEM. *p < 0.05, ****p < 0.0001

Table 5: Effect of *Vernonia amygdalina* on inflammatory markers in high-fat diet and letrozole-induced polycystic ovary syndrome in female Wistar rats

GROUPS	TNF-*α* (U/mol)	IL-6 (U/mol)	CRP (U/mol)
PCOS untreated	2.36 ± 0.889	0.58 ± 0.154	0.45 ± 0.005
Normal control	0.79 ± 0.008	0.11 ± 0.001*	0.37 ± 0.049
PCOS + VA (400 mg/kg)	0.82 ± 0.042	0.41 ± 0.214	0.33 ± 0.021
PCOS + VA (200 mg/kg)	1.29 ± 0.255	0.55 ± 0.026	0.32 ± 0.020*
PCOS +Met (500 mg/kg) + CC (2 mg/kg)	0.64 ± 0.161*	0.48 ± 0.040	0.30 ± 0.009*

Values are represented as Mean ± SEM. *p < 0.05

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