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Hypoglycemic and Hypolipidemic Effects of Anthocleista vogelii Methanol Leaf Extract in Phenylhydrazine-Induced Anaemic Rats

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ABSTRACT

Anaemia is often associated with disturbances in glucose and lipid metabolism, increasing the risk of metabolic and cardiovascular complications. This study investigated the effects of methanol leaf extract of Anthocleista vogelii on glucose concentrations and lipid profile in phenylhydrazine-induced anaemic Wistar rats. Forty-five male Wistar rats were divided into five groups (n=9). Anaemia was induced with phenylhydrazine (40 mg/kg) administered intraperitoneally on two consecutive days. The rats were treated with Anthocleista vogelii extract at doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg body weight for 21 days. Results showed that anaemia significantly elevated blood glucose concentrations and disrupted lipid profiles, marked by increased total cholesterol (4.0 ± 0.20 mmol/L), triglycerides (1.15 ± 0.05 mmol/L), and LDL (2.961 ± 0.203 mmol/L), alongside reduced HDL (0.517 ± 0.029 mmol/L) compared to the normal control. Treatment with Anthocleista vogelii extract significantly reduced blood glucose concentrations to 4.160 ± 0.040 mmol/L in the 600 mg/kg group by day 21. Additionally, total cholesterol, triglycerides, and LDL concentrations were significantly decreased, while HDL concentrations improved in treated groups, particularly at the 600 mg/kg dose. These changes were observed progressively over the 7th, 14th, and 21st days of treatment. The hypoglycemic and hypolipidemic effects observed suggest that Anthocleista vogelii may offer therapeutic benefits in mitigating metabolic dysfunctions commonly associated with anaemia, including hyperglycemia and dyslipidemia. This study provides scientific support for the traditional medicinal use of Anthocleista vogelii in managing metabolic disorders.

Keywords: Anthocleista vogelii, phenylhydrazine-induced anaemia, glucose metabolism, lipid profile, phytotherapy

Running Title: Anthocleista vogelii Improves Glucose and Lipid Profiles in Anaemia

INTRODUCTION

Anaemia remains a major global health burden, particularly in developing countries where nutritional inadequacies, parasitic infections, chronic diseases, and limited access to healthcare services are prevalent (Safiri et al., 2021). It is clinically defined by a reduction in haemoglobin concentration or a decline in red blood cell mass, which leads to impaired oxygen delivery to tissues and organs (Gardner et al., 2023). While the classical symptoms of anaemia, such as fatigue, dizziness, and pallor, are well recognized(World Health Organization, 2025), recent studies have uncovered that anaemia can also trigger systemic metabolic dysfunctions that significantly compromise glucose and lipid homeostasis (Soliman et al., 2017; Agarwal et al., 2024; Fillebeen et al., 2020).

Among the various forms of anaemia, haemolytic anaemia presents with particularly severe metabolic consequences. It is characterized by the accelerated destruction of red blood cells, which releases large quantities of free haem and iron into the bloodstream (Palmer & Seviar, 2022). This process is known to generate substantial oxidative stress, which can disrupt the normal physiological balance and lead to insulin resistance. Several researchers have demonstrated that oxidative stress from haemolytic anaemia can impair insulin receptor signaling and pancreatic β-cell function, contributing to elevated blood glucose concentrations (Rains & Jain, 2011; Tangvarasittichai, 2015). Additionally, haemolytic anaemia often coincides with dyslipidemia(González et al., 2023; Hilton et al., 2023), which includes elevated serum total cholesterol, triglycerides, and low-density lipoprotein (LDL), coupled with reduced high-density lipoprotein (HDL) levels. This lipid imbalance poses significant cardiovascular risks and may exacerbate the clinical complications associated with anaemia.

Phenylhydrazine (PHZ) is commonly used to induce haemolytic anaemia in experimental studies due to its ability to generate reactive oxygen species (ROS), leading to oxidative damage and subsequent erythrocyte destruction(Berger, 2007). The PHZ-induced anaemia model closely mimics the pathophysiological events of haemolytic anaemia in humans, making it an ideal platform for exploring therapeutic interventions aimed at reversing both haematological and metabolic abnormalities.

There is growing interest in the use of plant-derived natural products for the management of metabolic and haematological disorders. Plants are rich sources of bioactive compounds that often possess antioxidant, anti-inflammatory, antidiabetic, and lipid-modulating properties. One such plant is Anthocleista vogelii, a species widely distributed in West Africa and traditionally used for the treatment of various ailments including diabetes mellitus, malaria, hypertension, obesity, and microbial infections(Soladoye et al., 2012; Jiofack et al., 2010; Igoli et al., 2005; Gbolade, 2012; Anyanwu et al., 2013; Musa et al., 2010; Omobuwajo et al., 2008). The therapeutic versatility of Anthocleista vogelii is believed to stem from its diverse phytochemical components such as flavonoids, phenolic acids, alkaloids, saponins, and omega-3 fatty acids.

Although Anthocleista vogelii has been ethnomedically recommended for diabetes and cardiovascular conditions(Haruna et al., 2024; Olubomehin et al., 2022), scientific validation of its efficacy in correcting metabolic abnormalities associated with anaemia remains scarce. Specifically, there is a lack of research addressing its potential to normalize blood glucose levels and improve lipid profiles in haemolytic anaemia. Understanding whether Anthocleista vogelii can mitigate these metabolic disruptions is critical to substantiating its traditional use and expanding its therapeutic applications.

The present study was therefore designed to evaluate the effects of methanol leaf extract of Anthocleista vogelii on glucose metabolism and lipid profile parameters in Wistar rats with phenylhydrazine-induced anaemia. By systematically assessing changes in blood glucose, total cholesterol, triglycerides, LDL, and HDL concentrations over a 21-day treatment period, this study aims to determine whether Anthocleista vogelii can effectively modulate the metabolic disturbances associated with haemolytic anaemia. The findings are expected to contribute to the scientific basis for the plant’s potential use in managing anaemia-induced metabolic dysfunctions and to offer insight into alternative, plant-based therapeutic strategies.

MATERIALS AND METHODS

Plant Collection and Extract Preparation

Fresh leaves of Anthocleista vogelii were collected from Delta State University, Abraka, Nigeria. The plant was identified and authenticated by a botanist at the herbarium of the Department of Botany, Delta State University, where a voucher specimen was deposited (Voucher No: UBH-A258). The collected leaves were thoroughly washed, air-dried at room temperature for three weeks, and ground into coarse powder using a mechanical grinder.

A total of 100 grams of the powdered leaves were extracted using cold maceration in 500 mL of methanol for 48 hours with occasional shaking. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated at 50°C using a rotary evaporator. The resulting extract was further dried in a water bath to obtain a dark brown residue. The dried extract was stored in an airtight container at 4°C until required for use.

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Experimental Animals and Grouping

Forty-five healthy male Wistar rats weighing between 150 g and 180 g were obtained and acclimatized under standard laboratory conditions (12-hour light/dark cycle, temperature of 25 ± 2°C) for one week. The rats were fed with standard rat chow and had free access to clean drinking water throughout the experimental period.

The rats were randomly assigned into five experimental groups (n = 9 per group) as follows:

- **Group 1 (Normal Control):** Received distilled water and standard rat chow.
- **Group 2 (Negative Control – PHZ):** Received phenylhydrazine to induce anaemia and no treatment.
- **Group 3 (PHZ + 200 mg/kg A.V.):** Anaemic rats treated with 200 mg/kg body weight of Anthocleista vogelii extract.
- **Group 4 (PHZ + 400 mg/kg A.V.):** Anaemic rats treated with 400 mg/kg body weight of Anthocleista vogelii extract.
- **Group 5 (PHZ + 600 mg/kg A.V.):** Anaemic rats treated with 600 mg/kg body weight of Anthocleista vogelii extract.

Induction of Anaemia

Anaemia was induced by intraperitoneal injection of phenylhydrazine (PHZ) at a dose of 40 mg/kg body weight on two consecutive days, following the method described by Iwalewa et al. (2009). Anaemia was confirmed 24 hours after the second administration by measuring haemoglobin levels in the experimental animals.

Extract Administration

After anaemia was confirmed, rats in the treatment groups (Groups 3, 4, and 5) received their respective doses of Anthocleista vogelii extract orally once daily for 21 consecutive days. The control groups (Groups 1 and 2) received equivalent volumes of distilled water.

Sample Collection

At the end of the 21-day treatment period, all animals were fasted overnight and euthanized under light anaesthesia. Blood samples were collected via cardiac puncture into plain tubes and allowed to clot. The samples were centrifuged at 3000 rpm for 15 minutes to separate the serum, which was stored at -20°C for subsequent biochemical analyses.

Biochemical Analyses

Serum Glucose Determination

Serum glucose concentration was determined using the Glucose Oxidase-Peroxidase (GOD-PAP) method as described by Ekun et al. (2018). The absorbance was read at 546 nm, and glucose concentrations were calculated using the standard reference formula provided in the assay kit.

Serum Lipid Profile Determination

Serum total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol concentrations were determined using Randox diagnostic kits according to the manufacturer's instructions. Low-density lipoprotein (LDL) cholesterol was calculated using Friedewald's formula(Friedewald et al., 1972):

$$\text{LDL-cholesterol (mmol/L)} = \text{total cholesterol} - \left(\frac{\text{trigylcerides}}{2.2} + \text{HDL-C}\right)$$

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Statistical Analysis

All results were expressed as mean ± standard deviation (SD). Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Statistical significance was set at P = .05.

RESULTS

Table 3.1: Effect of Plant Extract on Anaemic Wistar Rat Glucose (mmol/L)

DAY	Normal Control	Negative (AN) Control	AN + 200 mg A.V.	AN +400 mg A.V.	AN + 600 mg A.V.
7	3.970 ± 0.061^{a}	4.683 ± 0.091^{b}	4.397 ± 0.119^{c}	4.327 ± 0.064^{cd}	4.160 ± 0.056^{ad}
14	4.053 ± 0.064^{a}	4.680 ± 0.053^{b}	4.383 ± 0.076^{c}	4.317 ± 0.042^{c}	4.113 ± 0.103^{d}
21	3.967 ± 0.208^{a}	4.647 ± 0.136^{b}	4.377 ± 0.025^{bc}	4.283 ± 0.091^{ac}	4.160 ± 0.040^{ac}

Keys: Data reported as mean ± standard deviations of nine determinations, values bearing different superscripts on the same row differ significantly (p<0.05), while ones with the same superscripts indicate no significant difference (p>0.05) using ANOVA.

Table 3.2: Effect of Plant Extract on Anaemic Wistar Rat Lipid Profile

DAY		Normal Control	Negative (AN) Control	AN + 200 mg A.V.	AN +400 mg A.V.	AN + 600 mg A.V.
7	CHOL (mmol/L)	2.633 ± 0.306^{a}	4.0 ± 0.20^{b}	3.133 ± 0.153^{a}	2.80 ± 0.361^{a}	2.767 ± 0.252^{a}
	TG (mmol/L)	0.533 ± 0.058^{a}	1.15 ± 0.05^{b}	0.813 ± 0.032^{c}	0.633 ± 0.058^{a}	0.5 ± 0.1^{a}
	HDL (mmol/L)	0.967 ± 0.058^{a}	0.517 ± 0.029^{b}	0.5267 ± 0.025^{b}	0.633 ± 0.058^{bc}	0.733 ± 0.058^{c}
	LDL (mmol/L)	1.42 ± 0.250^{a}	2.961 ± 0.203^{b}	2.237 ± 0.138^{c}	1.879 ± 0.389^{ac}	1.945 ± 0.103^{ac}
14	CHOL (mmol/L)	2.267 ± 0.252^{a}	3.9 ± 0.10^{b}	3.40 ± 0.20^{bc}	3.033 ± 0.351^{cd}	2.567 ± 0.208^{ad}
	TG (mmol/L)	0.50 ± 0.10^{a}	1.317 ± 0.076^{b}	0.803 ± 0.100^{c}	0.70 ± 0.10^{ac}	0.60 ± 0.10^{ac}
	HDL (mmol/L)	1.067 ± 0.153^{a}	0.50 ± 0.10^{b}	0.533 ± 0.058^{b}	0.667 ± 0.058^{b}	0.767 ± 0.058^{c}
	LDL (mmol/L)	0.973 ± 0.129^{a}	2.802 ± 0.141^{b}	2.502 ± 0.206^{bc}	2.048 ± 0.306^{cd}	1.537 ± 0.182^{d}
21	CHOL (mmol/L)	2.333 ± 0.153^{a}	3.967 ± 0.153^{b}	3.383 ± 0.161^{cde}	3.533 ± 0.231^{bd}	2.967 ± 0.153^{e}

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	TG (mmol/L)	0.567 ± 0.153[a]	1.233 ± 0.208[b]	0.857 ± 0.051[a]	0.767 ± 0.058[a]	0.633 ± 0.058[a]
	HDL (mmol/L)	1.033 ± 0.058[a]	0.50 ± 0.1[b]	0.667 ± 0.058[bc]	0.767 ± 0.058[cd]	0.933 ± 0.058[d]
	LDL (mmol/L)	1.042 ± 0.189[a]	2.906 ± 0.180[b]	2.327 ± 0.20[bc]	2.418 ± 0.276[b]	1.745 ± 0.233[c]

Keys: Data reported as mean ± standard deviations of triplicate determinations, values bearing different superscripts on the same row differ significantly ($p<0.05$), while ones with the same superscripts indicate no significant difference ($p>0.05$) using ANOVA.

DISCUSSION

The present study investigated the effects of Anthocleista vogelii methanol leaf extract on glucose levels and lipid profiles in phenylhydrazine (PHZ)-induced anaemic Wistar rats. The findings provide compelling evidence that A. vogelii extract possesses significant hypoglycemic and hypolipidemic properties, which may be beneficial in the management of anaemia-associated metabolic disturbances.

Anaemia, especially when haemolytic in nature, has been shown to disrupt glucose homeostasis and lipid metabolism. The significant elevation in blood glucose levels observed in the PHZ-induced anaemic rats in this study aligns with earlier reports that haemolytic anaemia is associated with insulin resistance and impaired glucose utilization (Okorie et al., 2021; Darenskaya et al., 2021). The oxidative stress generated by phenylhydrazine-induced haemolysis may contribute to the dysfunction of insulin receptors and pancreatic β-cell impairment, ultimately leading to hyperglycemia(Rains & Jain, 2011; Tangvarasittichai, 2015).

Interestingly, administration of A. vogelii extract at all tested doses resulted in a dose-dependent reduction in blood glucose concentrations. This glucose-lowering effect supports the ethnomedicinal use of A. vogelii in managing diabetes mellitus. The observed hypoglycemic effect is consistent with previous studies that reported the anti-diabetic potential of A. vogelii, possibly through mechanisms such as α-amylase inhibition, enhancement of insulin sensitivity, or protection of pancreatic β-cells (Olubomehin et al., 2022).

Furthermore, the dyslipidemia seen in the anaemic untreated group, characterized by elevated total cholesterol, triglycerides, and LDL-cholesterol alongside reduced HDL-cholesterol, is a known metabolic complication of anaemia-induced oxidative stress. The oxidative breakdown of erythrocytes releases cellular contents that can influence lipid peroxidation and disrupt lipid transport and metabolism.

Treatment with A. vogelii extract significantly improved lipid profile parameters in a dose-dependent manner. The extract reduced total cholesterol, triglycerides, and LDL-cholesterol concentrations while promoting an increase in HDL-cholesterol levels, especially at the highest dose (600 mg/kg). These findings suggest that the extract contains bioactive compounds capable of modulating lipid metabolism and preventing oxidative damage to lipoproteins.

The lipid-lowering effect observed in this study may be attributed to the phytochemicals present in A. vogelii, particularly flavonoids, phenolic acids, and omega-3 fatty acids, which have been shown to possess cholesterol-lowering and triglyceride-reducing effects(Ekweogu et al., 2020; Cambiaggi et al., 2023). One of the key phytoconstituents, 9,12,15-octadecatrienoic acid (an omega-3 fatty acid), is known to reduce triglyceride concentrations, enhance HDL levels, and improve the LDL/HDL ratio, thereby contributing to cardiovascular protection(Olivia et al., 2021)

The anti-inflammatory and antioxidant properties of the bioactive components of A. vogelii may also play a crucial role in mitigating phenylhydrazine-induced oxidative damage. By scavenging free radicals and reducing oxidative stress, the extract could help restore insulin sensitivity, improve glucose uptake, and normalize lipid transport mechanisms.

Additionally, the progressive improvement in metabolic parameters observed over the 21-day treatment period suggests that continuous administration of the extract may be necessary to achieve significant therapeutic outcomes. This observation highlights the potential of A. vogelii for long-term management of metabolic disturbances associated with anaemia.

The findings of this study align with those of Ezeigwe et al. (2019), who demonstrated that plant-based therapies could effectively modulate lipid profiles in anaemic rats, suggesting a possible shared mechanism involving antioxidant-mediated protection and lipid regulation.

CONCLUSION

The ability of A. vogelii to simultaneously correct glucose and lipid abnormalities in anaemic rats suggests its potential as a multifunctional therapeutic agent. Its dual role as a hypoglycemic and hypolipidemic agent, combined with its antioxidant properties, makes it a promising candidate for the management of anaemia-associated metabolic syndrome and possibly the prevention of cardiovascular complications.

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