Series of Endocrinology, Diabetes and Metabolism

Open Access
Research Article
Vol 7 Iss 1

Evaluation of the Hypoglycemic Effect of *Vernonia amygdalina* Leaf Extract and Metformin on the Lipid Profile of Alloxan-Induced Diabetic Wistar Rats

Agada SA[1], Oyigeya FO[2], Onoja BA[3], Kasar A[4], Odama RI[4], Itodo SO[5] and Dennis A[*6]

[1]*Department of Medical Biochemistry, College of Medicine, University of Nigeria, Enugu Campus, Nigeria*
[2]*Department of Biochemistry, Benue State University, Makurdi, Nigeria*
[3]*Department of Anatomy, College of Medicine, University of Ilorin, Ilorin, Nigeria*
[4]*Department of Medical Biochemistry, College of Medicine, University of Nigeria, Enugu campus, Nigeria*
[5]*Department of Pharmacology and Therapeutics, College of Health Sciences, Benue State University Makurdi, Nigeria*
[6]*Department of Medical Biochemistry, College of Medicine, Veritas University, Abuja, Nigeria*

[*]**Correspondence:** Amaechi Dennis, Department of Medical Biochemistry, College of Medicine, Veritas University, Abuja, Nigeria

Received on 28 March 2025; Accepted on 16 April 2025; Published on 08 May 2025

Abstract

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It poses a significant global health burden. DM is one of the most common endocrine dysfunctions in the world, resulting from a defect in insulin dynamics, and has caused significant morbidity and mortality due to microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular complications with no known cure. Vernonia amygdalina is a valuable medicinal plant that is widespread in West Africa. The phytochemical studies of V. amygdalina reveal the presence of saponins, flavonoids, alkaloids, terpenes, steroids, coumarins, phenolic acids, lignans, xanthones, anthraquinones, edotides, and sesquiterpenes. This study evaluates the hypoglycemic effect of V. amygdalina leaf extract and metformin on the lipid profile of alloxan-induced type 2 diabetic adult Wistar rats. The study involved 5 groups of rats: the normal control, diabetic control, diabetic rat treated with V. amygdalina, diabetic rat treated with metformin, and diabetic rat treated with V. amygdalina and metformin. Blood glucose levels, lipid profile, and pancreatic histology were assessed. Results showed that treatment with V. amygdalina, metformin, and their combination significantly reduced blood glucose levels at $p < 0.05$ compared to the diabetic control group. V. amygdalina positively impacted lipid profiles, significantly at $p < 0.05$, reducing triacylglycerol (TAG) levels and displaying a dose-dependent hypolipidemic effect. Histopathological examination indicated cellular regeneration in pancreatic tissues treated with

Citation: Agada SA, Oyigeya FO, Onoja BA, et al. Evaluation of the hypoglycemic effect of *Vernonia amygdalina* leaf extract and metformin on the lipid profile of alloxan-induced diabetic wistar rats. Series Endo Diab Met. 2025;7(1):1-16.

metformin and the combination therapy. The study highlights the potential of V. amygdalina as an alternative to conventional diabetes treatment.

Keywords: histopathology, therapy, diabetes, medicinal plants, lipid profiles

Abbreviations: DM: diabetes mellitus; SEM: standard error of the mean; TAG: triacylglycerol; ANOVA: analysis of variance; H&E: hematoxylin and eosin; LSD: least significant difference; HDL: high-density lipoprotein; LDL: low-density lipoprotein

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It poses a significant global health burden. It is one of the most common endocrine dysfunctions in the world resulting from a defect in insulin dynamics and has caused significant morbidity and mortality due to microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, stroke, and peripheral vascular disease) complications with no known cure [1, 2]. DM affects millions of people worldwide, and its prevalence is increasing and is projected to reach 500 million by the year 2030 [3]. Reasons for this rise include an increase in sedentary lifestyle, consumption of energy-rich diet, obesity, higher life span, etc. Regions with the greatest potential are Asia and Africa; DM rates could rise to 2–3 times the present rates. DM is caused by the abnormality of carbohydrate metabolism, which is linked to low blood insulin levels or insensitivity of target organs to insulin [4]. Currently, available therapies for the management of DM include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides, metformin, alpha-glucosidase inhibitors, thiazolidinediones, and meglitinides. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, the search for newer drugs continues because the existing synthetic drugs have severe limitations [4]. Plants have always been an exemplary source of drugs, and many of the currently available drugs have been derived directly or indirectly from them. Treatment of illness and maintenance of health using herbal medicines is the oldest and most popular form of healthcare practice known to humanity that has been practiced by all cultures in all ages throughout the history of civilization [5]. WHO has recommended the evaluation of traditional plant treatments for diabetes because they are readily available, have low side effects, and are considered to be excellent candidates for oral therapy. In the developed countries, the rate of dependence on herbal medicine decreased based on the availability of synthetic drugs during the early part of the twentieth century. The resurgence of interest in antidiabetic medicinal plants in developed countries is believed to be motivated by several factors that include: adverse reactions, high secondary failure rates, and the cost of conventional synthetic antidiabetic remedies [6].

Many herbal medicines have been recommended for the treatment of diabetes; the ethnobotanical information reports about 800 plants that may possess antidiabetic potential. Most antidiabetic plants belong to the family Leguminoseae, Cucurbitaceae, Liliaceae, Lamiaceae, Asteraceae, Rosaceae, Euphorbiaceae, Moraceae, and Araliaceae [7]. The herbal drugs with antidiabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine. The biological effects of the plants or herbal products used as alternative medicines to treat diabetes are related to their chemical composition. Most plants containing phytochemicals like glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., are frequently implicated as having an antidiabetic effect. Many of such plants known to be used primitively to alleviate symptoms of illnesses have been screened to have medicinal importance, some of which include: *Azadirachta indica* , *Vernonia amygdalina*, *Allium sativum*, *Ocimum gratissimum*, *Zingiber officinale*, *Momordica charantia*, *Carica papaya*, *Aloe vera*, *Ocimum sanctum* [8]. *V. amygdalina* is one of the most popular antidiabetic traditional herbal remedies in Nigeria [9]. *V. amygdalina*, also known as ‘‘African bitter leaf’’, is a plant vegetable used for both food and traditional treatment of diseases, i.e., the leaves are macerated and used in cooking, while the extracts are used as a tonic for prevention of certain illnesses [10]. *V. amygdalina* is a valuable medicinal plant that is widespread in West Africa. It is known as bitter leaf due to its characteristic bitter taste and flavor, and can be used as an active anticancer,

antibacterial, antimalarial, antidiabetic, and antiparasitic agent [10]. *V. amygdalina* contains complex active components (phytochemicals) that are useful pharmacologically [10]. Phytochemicals are naturally occurring bioactive compounds known for their health benefits. They are mainly responsible for the color, flavor, and aroma of fruits and notably vegetables [11]. The phytochemical studies of *V. amygdalina* reveal the presence of saponins, flavonoids, alkaloids, terpenes, steroids, coumarins, phenolic acids, lignans, xanthones, anthraquinones, edotides, and sesquiterpenes [12]. Diabetes is a global health concern, and its prevalence is on the rise. Alloxan-induced diabetic Wistar rats are a widely accepted model for diabetes research. *V. amygdalina*, commonly known as bitter leaf in English, ewuro (Yoruba), onugbu (Igbo), chusar-doki or shuwaka (Hausa), oriwo (Edo), ityuna (Tiv), and afolo (Idoma), a plant with traditional use in various cultures, is believed to have antidiabetic properties. Metformin, a widely prescribed antidiabetic drug, is known to affect lipid profiles in addition to its hypoglycemic effects.

Understanding the impact of *V. amygdalina* and metformin on lipid profiles in a diabetic model will provide valuable insights into their potential as therapeutic agents. Natural products have gained attention as potential antidiabetic agents. *V. amygdalina*, a medicinal plant with antioxidant properties, is one such candidate. This research aims to evaluate the hypoglycemic effect of *V. amygdalina* extract and compare it with metformin, a known antidiabetic drug, on lipid profile in alloxan-induced diabetic adult Wistar rats. *V. amygdalina*, commonly known as bitter leaf, has been traditionally used in various parts of Africa for its potential antidiabetic and antihyperlipidemic properties. Alloxan-induced diabetic rats are an established animal model for studying diabetes and lipid profile. Metformin, a widely used oral hypoglycemic agent, is known to reduce blood glucose levels. Investigating the comparative effects of *V. amygdalina* and metformin on lipid profiles in diabetic rats will contribute to our understanding of potential alternative treatments for diabetes. It is clear that the relative importance of non-communicable diseases will increase [13]. This situation is a result of demographic change (populations with older age structures), increasing urbanization, and associated changes in risk-factor levels, such as tobacco smoking, obesity, and physical inactivity. Countries of Sub-Saharan Africa are in various stages of the epidemiological transition with a multiple burden of diseases. Type 1 diabetes is considerably rarer than type 2 diabetes, and large populations need to be surveyed. Also, to assess incidence, the population surveyed should be accurately known, and this is in itself difficult, as complete censuses in Africa are rare and migration in and out of study areas is common. Hyperlipidemia plays a role in the pathogenesis of diabetic complications. Natural products have gained attention as potential antidiabetic agents. *V. amygdalina*, a medicinal plant with antioxidant properties, is one such candidate.

Materials

Experimental animals

A total of 30 adult Wistar rats were purchased from the Animal House, College of Health Sciences, Benue State University, Makurdi, for this research. The animals were housed, acclimatized, treated, and fed with standard rat feed and water ad libitum in the animal house throughout the 28-day duration of this research. The rats were weighed before the commencement of the experiment, and at intervals of 7 days respectively till the end of the research and sacrifice on the 29th day after overnight fasting of the animals.

Experimental plant

V. amygdalina leaves were collected from a natural habitat in Makurdi. The botanical identity of the plant was confirmed by a plant taxonomist at the Plant Science and Biotechnology Laboratory in the Department of Biological Sciences, Benue State University, Makurdi, and was given herbarium index number-HBI-BLVA-001-BSU24.

Experimental drugs

The experimental drugs, alloxan injection and metformin were purchased from Mernax Pharmacy, opposite College of Health Sciences, Gboko Road, Makurdi. A solution of the metformin tablets is made and stored at optimum temperature in the refrigerator for the duration of this study.

Animal cages

A total of 5 plastic cages measuring 30 cm × 20 cm were obtained from the animal house, in which the experimental animals were housed, acclimatized, and fed throughout the duration of the experiment.

Other materials/chemical reagents

Other materials include: gloves, sterile bottles, syringes and needles, glucometer, centrifuge, distilled water, feeding plates, and water bottles.

Methods

Experimental drug solution

A 30 mg/ml concentration of metformin tablets in distilled water was prepared and stored at optimum temperature in the refrigerator for administration throughout the course of this research.

Experimental plant extract

The plant, *V. amygdalina* (bitter leaf), was washed, air-dried for 14 days, and the dried leaves pulverized into powder using a blender. Approximately 20 g of the powder was loaded into a thimble and continuously extracted with 99.9% ethanol in a Soxhlet extractor for 4 h. The solvent was distilled off in the rotary evaporator to obtain a solid residue. The concentrates were left open in a water bath (45°C) for complete dryness before the extract was transferred an airtight container and then stored in a refrigerator at 4°C.

Experimental design

The 30 adult Wistar rats were divided into 5 groups of 6 rats each, into 5 plastic cages and treated as follows:

Group 1: (normal control) 5 ml/kg body weight of normal saline.

Group 2: (hyperglycemic control) Induced (150 mg/kg alloxan injection) without treatment.

Group 3: 150 mg/kg alloxan + 800 mg/kg of *V. amygdalina*.

Group 4: 150 mg/kg alloxan + 30 mg/kg of metformin.

Group 5: 150 mg/kg alloxan + 800 mg/kg of *V. amygdalina* + 30 mg/kg of metformin.

Duration of study

The research was conducted over a period of 28 days as follows: acclimatization - 7 days, induction and confirmation - 7 days, and treatment - 14 days.

Diabetes induction and blood sugar measurement

The blood sugar level of the experimental animals was measured on day 1 before commencement of the experiment by taking blood samples from the tail of the animals. Diabetes was induced by administration of 150 mg/kg alloxan injection, and the blood sugar levels were measured again after 48 h of alloxan administration. Administration of the extract of *V. amygdalina* and metformin commenced on the seventh day of the experiment, and the blood sugar levels were measured at intervals of 3 days each throughout the duration of this experiment.

Animal sacrifice

At the end of the 28-day study period, all 30 Wistar rats were fasted overnight and then sacrificed by chloroform inhalation as anesthesia. The blood samples were collected by cardiac puncture into sterile bottles for biochemical

analysis, while the pancreas (tissue) was harvested and fixed in 10% formaldehyde in tissue sample bottles for histological processing and assessment.

Paraffin wax method of tissue processing

Tissues were harvested and fixed in 10% formalin for 3 days, cut into thin slices of 5 mm × 2 mm × 1 mm thick, and then processed in the following order using the SPIN tissue processor, STP 120 (Thermoscientific):

10% buffered formalin	
70% alcohol	2 h
80% alcohol	2 h
90% alcohol	2 h
95% alcohol	2 h
Absolute alcohol I	2 h
Absolute alcohol II	2 h
Absolute alcohol III	2 h
Xylene I	2 h
Xylene II	2 h
Paraffin wax oven I	2 h
Paraffin wax oven II	2 h

Embedding: Tissues were embedded in molten paraffin wax using embedding moulds. The tissues were embedded using embedding cassettes on a tissue Tek Embedding Centre (SLEE MPS/P2), and cooled rapidly on the cooling component.

Sectioning: Tissues were sectioned using a rotary microtome (MICROM HM 340E Thermo Scientific) set at 4 micromes, picked on slides, and ready for staining.

Hematoxylin and eosin staining techniques

Sections were dewaxed and hydrated by passing through two changes of xylene and through descending grades of alcohol (100%, 80%, 70%) for 3 min each and then into water, stained in Harris hematoxylin solution for 5 min, and washed in running water. They were differentiated in 1% acid-alcohol and then washed well in water, blued in Scott's tap water substitute for 5 min and rinsed briefly in distilled water, counterstained in 1% aqueous eosin for 2 min, washed well in water, dehydrated in descending grades of alcohol, cleared in xylene, and mounted in DPX (destrene, plasticiser, and xylene). Sections were then placed in slide carriers and placed in a 400°C oven to dry overnight. They were read microscopically.

Statistical analysis

Statistical data obtained in this research were analyzed using the Statistical Package for the Social Sciences (IBM SPSS) version 23. Mean and standard error of the mean (SEM) were calculated for all the values. Comparison between the control and treated groups was done using one-way analysis of variance (ANOVA) with least significant difference (LSD) multiple range tests. Differences were considered statistically significant at $p < 0.05$.

Results and Analysis

Physical observation: effect of *V. amygdalina* leaf extract and metformin on the body weight of alloxan-induced diabetic Wistar rats

The table shows the mean body weight changes for rats across different groups, compared using one-way ANOVA (Table 1). The normal control group (NC: 5 ml/kg NS) showed a statistically significant increase in body weight from

an initial 66.03 ± 6.29 g to 113.83 ± 12.76 g, with a weight difference of 47.80 ± 7.85 g, indicating normal growth without the impact of alloxan-induced diabetes.

The hyperglycemic control group (HC: 150 mg/kg ALX) experienced a significant increase in body weight from 106.00 ± 22.01 g to 136.35 ± 22.75 g, with a weight difference of 30.45 ± 14.82 g. However, this increase was less than that of the normal control group, demonstrating that alloxan-induced diabetes impairs weight gain. In the treated groups, Group 3 (150 mg/kg ALX + 800 mg/kg V. a) and Group 4 (150 mg/kg ALX + 30 mg/kg MTF), the body weight increases were 39.51 ± 8.70 g and 40.48 ± 18.49 g, respectively. These increases were significant compared to the hyperglycemic control group, suggesting that both treatments partially restored weight gain due to their hypoglycemic effects. However, these increases did not reach the levels observed in the normal control group. Group 5 (ALX + V. a + MTF) showed a body weight increase from 83.90 ± 15.74 g to 131.41 ± 23.81 g, with a weight difference of 47.51 ± 8.97 g, similar to that of the normal control group. This result underscores metformin's effectiveness in mitigating the effects of alloxan-induced diabetes, restoring the body weight to levels comparable to non-diabetic conditions. These findings indicate that both *V. amygdalina* and metformin can mitigate the body weight loss associated with alloxan-induced diabetes in rats. Metformin, in particular, shows a restoration of weight gain comparable to normal physiological conditions (Figure 1).

Diabetic state determination: effect of *V. amygdalina* leaf extract and metformin on the blood glucose level of alloxan-induced diabetic Wistar rats

The Tables show the blood glucose levels of rats across groups compared on one–way ANOVA to determine the effectiveness of alloxan to induce diabetes (Table 2) and the effectiveness of *V. amygdalina* to mitigate the diabetic effect of alloxan (Table 3). Initial measurements taken before and after alloxan induction confirmed successful induction of diabetes, with a significant increase in blood glucose levels observed in the hyperglycemic control group and all treatment groups compared to the normal control. The blood glucose levels of Group 1 remained stable and within the normal range throughout the study, validating the consistency in the non-diabetic model. The hyperglycemic control group (Group 2) showed persistently high glucose levels throughout the treatment period, confirming the successful induction of diabetes without any intervention. For Group 3 (150 mg/kg ALX + 800 mg/kg V. a), starting with high blood glucose levels, there was a noticeable gradual decrease over the treatment days. The group treated with 150 mg/kg ALX + 30 mg/kg MTF showed an even stronger hypoglycemic response than Group 3. Metformin, a well-established antidiabetic drug, also showed a potent reduction in glucose levels. The results in Tables 2 and 3 clearly illustrate that *V. amygdalina* treatments significantly reduced blood glucose levels in diabetic rats. *V. amygdalina* showed comparable efficacy to metformin, suggesting that *V. amygdalina* could be a potent natural alternative for managing hyperglycemia in diabetic conditions. Statistical analysis (one-way ANOVA) highlighted that all changes in the treatment groups were significant compared to the hyperglycemic control group, indicating the effectiveness of *V. amygdalina* and metformin in lowering blood glucose levels significantly (Figures 2, 3, and 4).

Groups (N)	Initial body weight (g)	Final body weight (g)	Body weight difference (g)
Group 1	$66.03 \pm 6.29^{+\#}$	$113.83 \pm 12.76^{+}$	$47.80 \pm 7.85^{+}$
Group 2	$106.00 \pm 22.01^{*\#}$	$136.35 \pm 22.75^{*}$	$30.45 \pm 14.82^{*\#}$
Group 3	$97.65 \pm 16.57^{*}$	$137.16 \pm 12.22^{*}$	39.51 ± 8.70
Group 4	$97.18 \pm 7.97^{*}$	$137.66 \pm 11.80^{*}$	40.48 ± 18.49
Group 5	$83.90 \pm 15.74^{*+}$	131.41 ± 23.81	$47.51 \pm 8.97^{+}$

Table 1: Showing the mean body weight changes across groups compared on one-way ANOVA. * = statistically significant difference in mean at $p < 0.05$ when compared to the normal control group; + = statistically significant difference in mean at $p < 0.05$ when compared to the hyperglycemic control group; # = statistically significant difference in mean at $p < 0.05$ when compared to the positive control group.

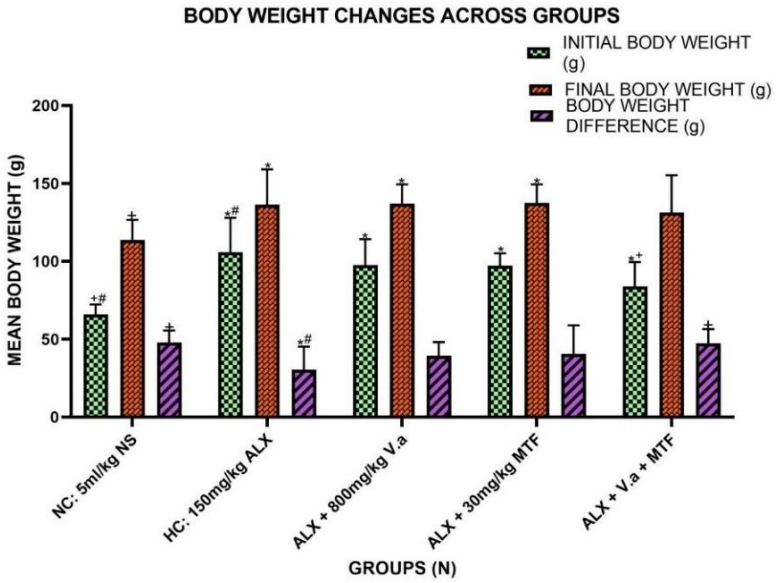

Figure 1: Simple bar chart showing the mean body weight changes across groups.

Groups (N)	Before induction with alloxan (mg/dl)	After induction with alloxan (mg/dl)
Group 1	3.98 ± 0.49	$4.03 \pm 0.49^{+\#}$
Group 2	4.21 ± 0.47	$10.68 \pm 4.36^{*}$
Group 3	4.10 ± 0.68	$10.21 \pm 2.95^{*}$
Group 4	3.93 ± 0.35	$8.93 \pm 0.90^{*}$
Group 5	3.83 ± 0.62	$8.70 \pm 0.81^{*}$

Table 2: Showing the mean blood glucose levels before and after diabetes induction with alloxan across groups compared on one-way ANOVA. * = statistically significant difference in mean at $p < 0.05$ when compared to the normal control group; + = statistically significant difference in mean at $p < 0.05$ when compared to the hyperglycemic control group; # = statistically significant difference in mean at $p < 0.05$ when compared to the positive control group.

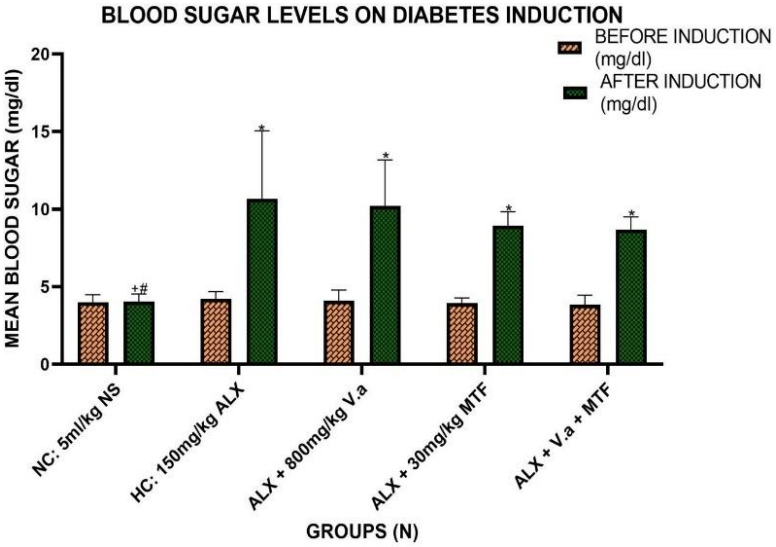

Figure 2: Simple bar chart showing the blood sugar levels before and after diabetes induction.

Groups (N)	Day 1	Day 2	Day 3	Day 4
Group 1	$3.73 \pm 0.39^{+\#}$	$4.05 \pm 0.51^{+\#}$	$4.03 \pm 0.60^{+}$	$4.35 \pm 0.66^{+}$
Group 2	$8.160 \pm 1.59^{*}$	$10.48 \pm 2.07^{*\#}$	$11.88 \pm 4.66^{*\#}$	$10.88 \pm 3.71^{*\#}$
Group 3	$9.50 \pm 2.78^{*\#}$	$7.25 \pm 0.55^{*+}$	$6.80 \pm 0.97^{*+}$	$5.91 \pm 0.84^{+}$
Group 4	$8.11 \pm 0.99^{*}$	$7.68 \pm 1.18^{*+}$	$6.73 \pm 0.85^{*+}$	$5.28 \pm 0.82^{+}$
Group 5	$7.55 \pm 1.01^{*}$	$7.13 \pm 0.89^{*+}$	$6.21 \pm 0.67^{+}$	$4.95 \pm 0.26^{+}$

Table 3: Showing the mean blood glucose levels during the treatment period across groups compared on one-way ANOVA. * = statistically significant difference in mean at $p < 0.05$ when compared to the normal control group; + = statistically significant difference in mean at $p < 0.05$ when compared to the hyperglycemic control group; # = statistically significant difference in mean at $p < 0.05$ when compared to the positive control group.

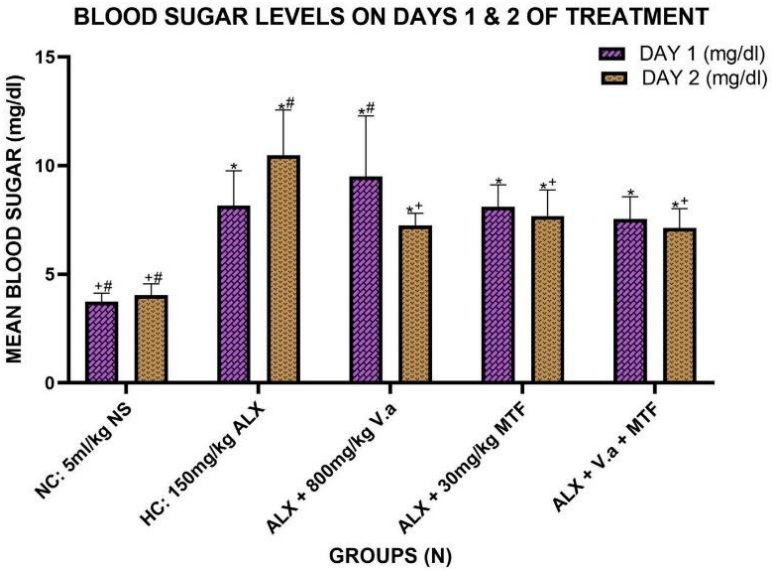

Figure 3: Simple bar chart showing the blood sugar levels on days 1 and 2 of treatment.

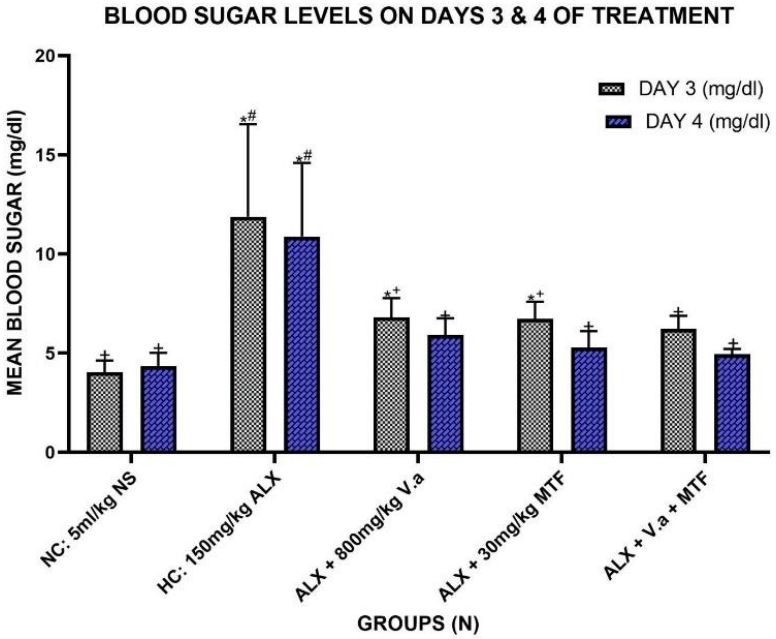

Figure 4: Simple bar chart showing the blood sugar levels on days 3 and 4 of treatment.

Biochemical analysis: effect of *V. amygdalina* leaf extract and metformin on the lipid profile of alloxan-induced diabetic Wistar rats

The table shows the lipid profile of the rats across groups (Table 4). A one-way ANOVA test was conducted to evaluate differences among the groups. For the cholesterol (CHOL) levels, the hyperglycemic control group displayed a decrease in cholesterol levels compared to the normal control, which was unexpected, as diabetes typically increases cholesterol levels. However, both the 150 mg/kg ALX + 800 mg/kg V. a group (Group 3) and Group 4 showed cholesterol levels similar to those of the hyperglycemic control group, indicating that *V. amygdalina* and metformin stabilize cholesterol levels. For triacylglycerol (TAG), there was a significant increase in TAG levels in Group 2 compared to Group 1. Interestingly, the 150 mg/kg ALX + 30 mg/kg MTF group (Group 4) showed a significant reduction in TAG levels, not only compared to Group 2 but also to Group 1, suggesting a dose-dependent effect of *V. amygdalina* in lowering TAG levels. High-density lipoprotein (HDL) levels did not show significant variations across most groups, indicating that neither the diabetic condition nor the treatments had a substantial effect on HDL levels in this experiment. For low-density lipoprotein (LDL), significant differences were observed in the LDL levels between groups. Group 3 showed a reduction in LDL levels compared to both Group 2 and Group 4, indicating that 800 mg/kg *V. amygdalina* could effectively lower LDL cholesterol. These results suggest that *V. amygdalina* leaf extract has beneficial effects in modulating lipid profiles in diabetic rats, akin to the effects of metformin. It was particularly effective in reducing LDL and TAG levels, which are crucial factors in the management of diabetes-associated dyslipidemia (Figures 5 and 6).

Groups (N)	CHOL (mg/dl)	TAG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group 1	$116.76 \pm 6.98^{+}$	$93.45 \pm 4.29^{+}$	60.64 ± 4.57	34.10 ± 4.32
Group 2	$94.98 \pm 3.13^{*}$	$115.90 \pm 16.79^{*\#}$	60.97 ± 1.71	$36.83 \pm 4.84^{\#}$
Group 3	$94.14 \pm 14.13^{*}$	101.87 ± 4.59	61.73 ± 1.70	$29.03 \pm 0.98^{+}$
Group 4	106.68 ± 15.38	$85.24 \pm 5.11^{+}$	59.80 ± 2.84	$39.47 \pm 3.24^{*\#}$
Group 5	109.20 ± 1.73	$93.52 \pm 5.96^{+}$	62.65 ± 0.33	27.85 ± 2.84

Table 4: Showing the mean lipid profile across groups compared on one-way ANOVA. * = statistically significant difference in mean at $p < 0.05$ when compared to the normal control group; + = statistically significant difference in mean at $p < 0.05$ when compared to the hyperglycemic control group; # = statistically significant difference in mean at $p < 0.05$ when compared to the positive control group. CHOL = cholesterol; TAG = triacylglycerol; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

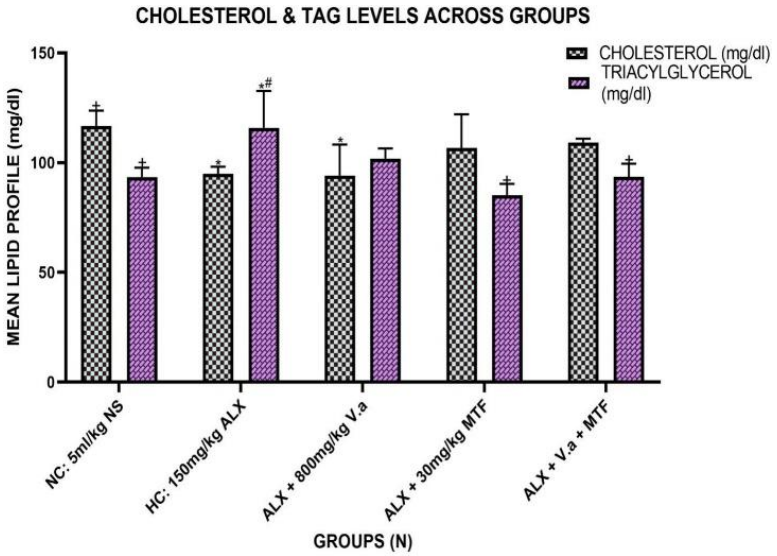

Figure 5: Simple bar chart showing the mean cholesterol and triacylglycerol levels across groups.

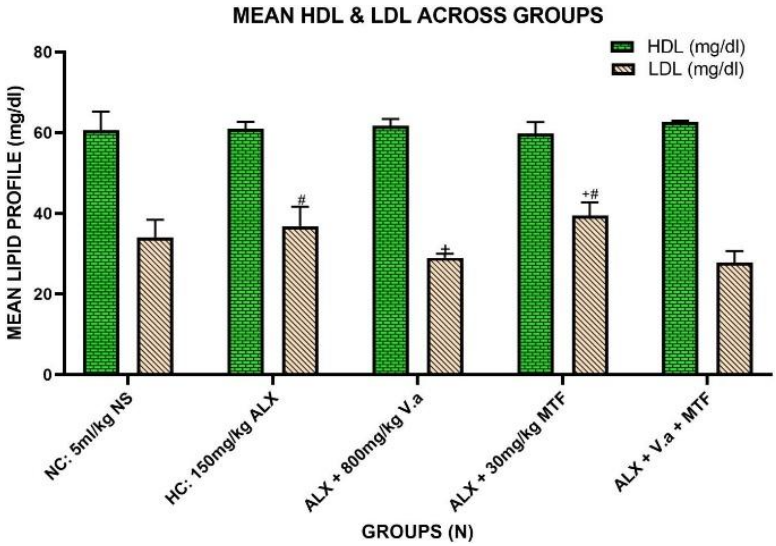

Figure 6: Simple bar chart showing the mean HDL & LDL levels across groups.

Test hypothesis

- Null hypothesis (H_O)

V. amygdalina leaf extract and metformin have no ameliorative effect on weight, glucose levels, lipid profile, and histological changes in the pancreas of alloxan-induced diabetic Wistar rats.

- Alternate hypothesis (H_A)

V. amygdalina leaf extract and metformin have ameliorative effects on weight, glucose levels, lipid profile, and histological changes in the pancreas of alloxan-induced diabetic Wistar rats.

On testing with one-way ANOVA, the average p-value for the lipid profile was $0.02 < 0.05$. Hence, we reject the null hypothesis and state that *V. amygdalina* leaf extract and metformin have an ameliorative effect on the lipid profile of alloxan-induced diabetic Wistar rats.

Histology profile

Hematoxylin and eosin (H&E) examination of the pancreatic tissue from Group 1 rats revealed normal pancreatic histoarchitecture, with centrally-placed centroacinar cells, and intact pancreatic islets. Sections from Group 2 showed much depleted pancreatic islet cells, peripherally-placed secretory acini, and the presence of inflammatory cells. Group 3 photomicrograph showed depleted pancreatic islets with the presence of inflammatory cells and secretory acini.

Group 4 pancreas sections showed similar normal pancreatic features with Group 1: centrally-placed centroacinar cells, and intact pancreatic islets. The photomicrograph of Group 5 rats also revealed normal pancreatic histoarchitecture, with centrally-placed centroacinar cells, and intact pancreatic islets (Plates 1–5).

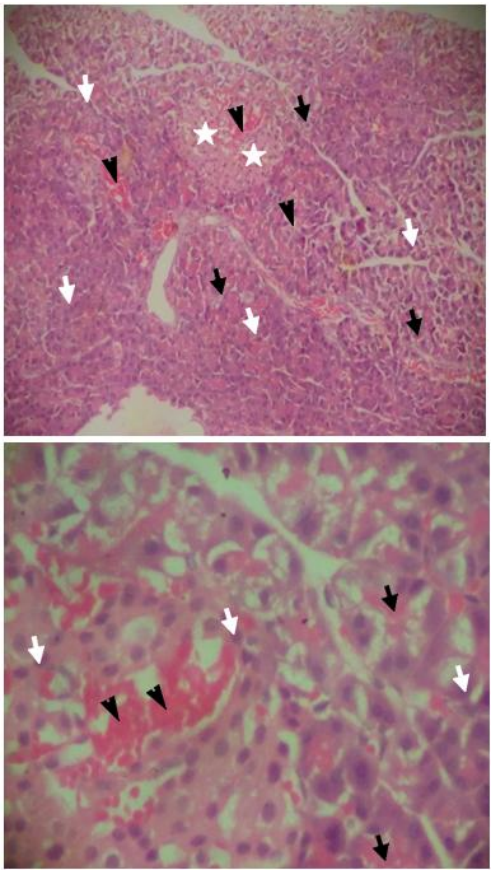

Plate 1 (Group 1): Pancreas of an albino rat exposed to normal atmospheric and nutritional conditions, showing normal morphology. The pancreas appears normal. Black arrows = secretary acini, white arrows = centroacinar cells, white stars = pancreatic islets, black arrow heads = red blood cells. H&E A: ×100, B: ×400.

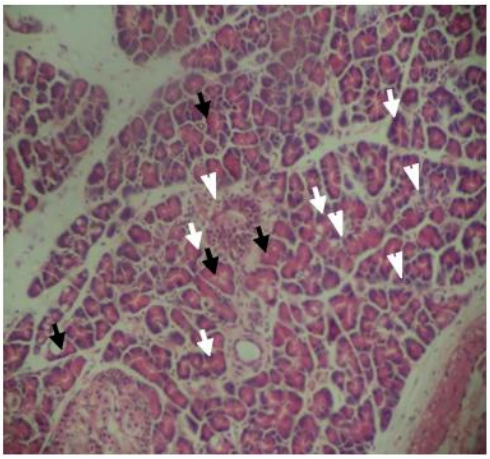

Plate 2 (Group 2): Pancreas of an albino rat administered alloxan, showing inflammation as seen by the presence of inflammatory cells (white arrow heads) within the tissue. The morphology remains intact. Black arrows = secretary acini, white arrows = centroacinar cells, with a massive decrease in pancreatic islets. H&E A: ×100, B: ×400.

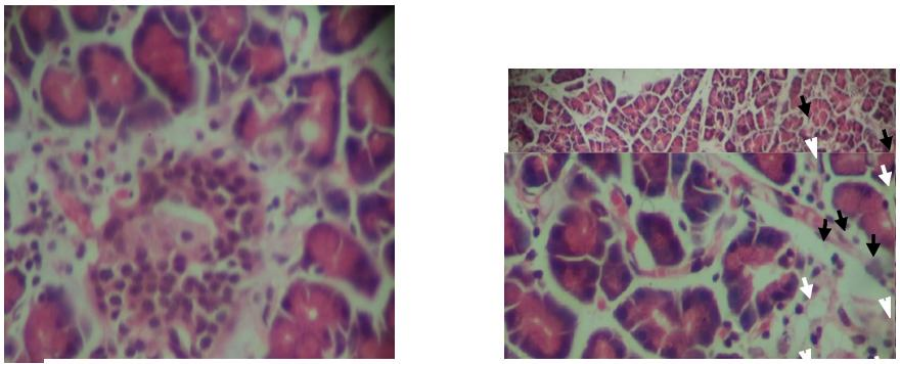

Plate 3 (Group 3): Pancreas of albino rat administered alloxan and treated with *V. amyglinda* extract, showing inflammation as seen by the presence of inflammatory cells (white arrow heads) within the tissue. The morphology remains intact. Black arrows = secretary acini, white arrows = centroacinar cells, with massive decrease in pancreatic islets. H&E A: ×100, B: ×400.

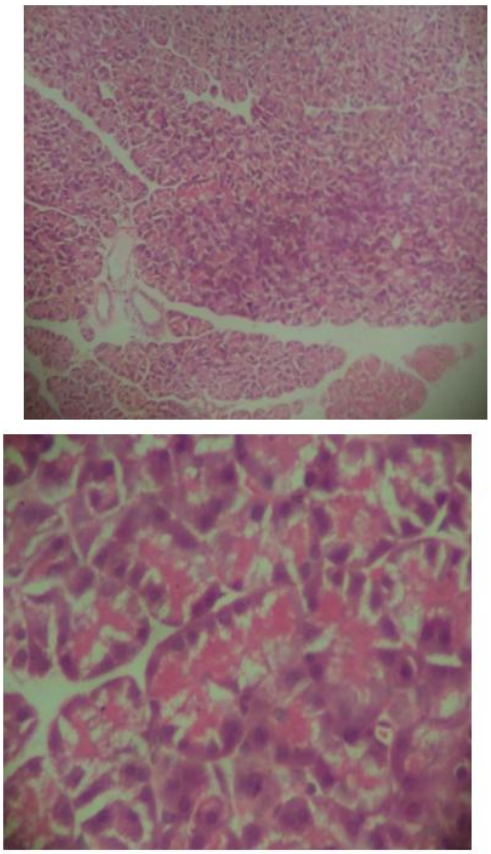

Plate 4 (Group 4): Pancreas of albino rat administered alloxan and treated with metformin showing normal morphology. Black arrows = secretary acini, white arrows = centroacinar cells. H&E A: ×100, B: ×400.

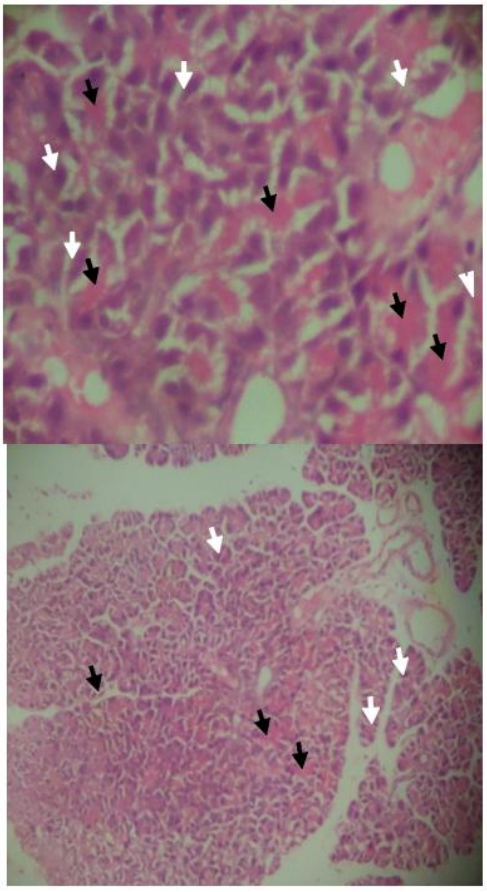

Plate 5 (Gourp 5): Pancrease of albino rat administered alloxan and treated with *V. amyglinda* extract and metformin showing normal morphology. Black arrows = secretary acini, white arrows = centroacinar cells. H&E A: ×100, B: ×400.

Discussion

The current study evaluated the impact of *V. amygdalina* leaf extract and metformin on body weight changes in alloxan-induced diabetic Wistar rats. Notably, alloxan induction typically leads to hyperglycemia by destroying insulin-producing pancreatic β-cells, which often results in weight loss or reduced weight gain due to catabolism of fats and proteins [14].

The normal control group exhibited significant weight gain, which is indicative of healthy growth and metabolic functioning in the absence of any diabetic induction. In contrast, the hyperglycemic control group showed a significantly lower weight gain, highlighting the detrimental metabolic effects of uncontrolled diabetes, consistent with the findings of Amaechi et al. [15].

V. amygdalina in the treated group led to better weight gain compared to the hyperglycemic control, but was not as effective as the normal control. This partial restoration of body weight can be interpreted as a beneficial effect of the leaf extract, potentially through its hypoglycemic properties. Prior studies have noted similar improvements in body weight with plant extracts, which they attributed to the stabilization of blood glucose levels and protection against β-cell damage [16].

The group treated with metformin and *V. amygdalina* showed weight gains almost equivalent to the normal control, illustrating the efficacy of metformin in not only controlling blood glucose but also normalizing other metabolic parameters, including body weight management. This aligns with established literature where metformin has been shown to effectively mitigate metabolic anomalies in diabetic conditions [17]. Studies have observed that natural

extracts like *V. amygdalina* possess both antidiabetic and antioxidant properties that might contribute to mitigating oxidative stress and aiding in weight stabilization [18]. For instance, Ekpe et al. [19] reported significant improvements in the antioxidant system of diabetic rats treated with *V. amygdalina*, which closely corresponds with the weight gain patterns observed in the current study. While *V. amygdalina* was slightly less effective than metformin, its significant improvement over untreated diabetic models suggests its potential as an alternative or complementary therapy in diabetes management, especially for patients who may prefer or require botanical remedies. The induction of diabetes using alloxan in the hyperglycemic control group (Group 2) was successful, as evidenced by the significant increase in blood glucose levels from baseline to post-induction. This result aligns with the mechanism of alloxan, which selectively destroys insulin-producing cells in the pancreas, thereby inducing hyperglycemia [20]. The administration of *V. amygdalina* produced a significant reduction in blood glucose levels in alloxan-induced diabetic rats, which supports the hypothesis of a hypoglycemic effect of the plant extract. In particular, Group 4 showed a more pronounced reduction in glucose levels, suggesting a dose-dependent hypoglycemic effect. This finding is consistent with previous studies that have reported the antidiabetic properties of V. amygdalina, attributing them to the presence of phytochemicals such as saponins, flavonoids, and terpenoids, which have been proposed to stimulate insulin secretion or mimic insulin action [17]. Comparison of the efficacy of *V. amygdalina* with that of metformin (Group 5), a standard antidiabetic drug, revealed that *V. amygdalina* treatment yielded glucose levels comparable to those achieved with metformin. This suggests that *V. amygdalina* might serve as an effective natural alternative for the management of diabetes, corroborating the findings of Amaechi et al. [11], who also observed significant hypoglycemic effects with herbal extracts in diabetic models. The consistency in the blood glucose levels of the normal control group across the study period confirms the stability of the non-diabetic model and the absence of external variables affecting the glucose metabolism in these rats. These results underscore the potential of *V. amygdalina* as an adjunct or alternative to conventional pharmacotherapy in the management of diabetes, especially considering its hypoglycemic effect and the advantage of having fewer side effects typically associated with synthetic drugs. Amaechi et al. [12] conducted a study that highlighted the antioxidant and hypoglycemic properties of V. amygdalina. The findings suggested that the improvement in glucose tolerance in diabetic rats might be attributed to enhanced insulin secretion and action. Amaechi et al. [13] compared the hypoglycemic effects of *V. amygdalina* with glibenclamide, a sulfonylurea, and observed that both treatments similarly improved glycemic control, but *V. amygdalina* also offered antioxidant benefits, reducing oxidative stress markers in diabetic rats. Amaechi et al. [14] noted the efficacy of herbal extracts, including *V. amygdalina*, in lowering blood glucose in diabetic rats, supporting the use of herbal medicines in traditional diabetes management practices. The present study also evaluated the hypoglycemic effect of *V. amygdalina* leaf extract and its impact on the lipid profiles of alloxan-induced diabetic Wistar rats, compared with the effects of metformin. The result obtained from this experiment provides significant insights into the potential therapeutic properties of *V. amygdalina*, especially in the management of dyslipidemia associated with diabetes. Unexpectedly, cholesterol levels were found to decrease in the hyperglycemic control group compared to the normal control. This is contrary to traditional expectations, as diabetes is generally associated with elevated cholesterol levels due to impaired lipid metabolism. Both the Group 3 and the positive control (metformin, PC) showed similar cholesterol levels to the hyperglycemic control group. This suggests that *V. amygdalina* at 800 mg/kg, as well as metformin, may help stabilize cholesterol levels in diabetic conditions, although the mechanism remains unclear and may differ from traditional lipid-lowering treatments. The TAG levels significantly increased in the hyperglycemic control group compared to the normal control, which aligns with known consequences of diabetes, where increased free fatty acid flux from insulin-resistant adipose tissue elevates TAG levels. Notably, the dose at 800 mg/kg of *V. amygdalina* group (HD) showed a significant reduction in TAG levels, suggesting a dose-dependent hypolipidemic effect of *V. amygdalina*. This reduction was even more pronounced when compared to the positive control group, indicating superior efficacy at higher doses for lowering TAG levels.

In terms of HDL levels, there were no significant changes observed across most groups. This indicates that neither the diabetic condition nor the treatment regimens significantly affected HDL levels in this study. This might imply a limitation in the ability of *V. amygdalina* and metformin to modulate HDL cholesterol, an important factor in cardiovascular risk, which often remains unaddressed in diabetic patients. LDL levels displayed a significant reduction

in the LD group compared to both the hyperglycemic control and positive control groups. This result is promising as lower LDL cholesterol is a crucial goal in managing the increased cardiovascular risk associated with diabetes. However, the HD group showed an increase in LDL compared to the positive control group, suggesting potential adverse effects or a less predictable action at a higher dose of 800 mg/kg of *V. amygdalina*.

The results of this study echo findings from similar research where *V. amygdalina* has been identified to have hypoglycemic and lipid-lowering effects. A study by Amaechi et al. [15] also noted that *V. amygdalina* extracts could effectively reduce blood glucose levels and improve lipid profiles in diabetic rat models, which supports the findings of significant TAG and LDL reduction in our study. Moreover, Amaechi et al. [11] provided evidence of the antioxidant properties of *V. amygdalina* that could contribute to its lipid-modulating effects, though this specific mechanism was not the focus of the current study.

These results suggest that *V. amygdalina*, especially at a dose of 800 mg/kg, may offer a complementary approach to conventional diabetes treatment regimens like metformin, particularly for managing associated lipid abnormalities.

Conclusion

This study highlights the potential of *V. amygdalina* as an adjunct or alternative to conventional diabetes treatment. The leaf extract of *V. amygdalina* not only demonstrates significant hypoglycemic effects but also shows hypolipidemic properties, which are crucial in managing the hyperlipidemia associated with diabetes. While it was slightly less effective than metformin in some aspects, its natural origin and lower likelihood of side effects render it a viable option for diabetes management, particularly for those who prefer botanical remedies. However, variability in its effects on lipid profiles, especially LDL cholesterol, suggests the need for cautious optimization of dosage and further investigation.

References

1. Abdirahman YA, Juma KK, Mukundi MJ, et al. In-Vivo Antidiabetic Activity and Safety of The Aqueous Stem Bark Extract of Kleinia squarrosa. J Diabetes Metab. 2015;6(9):100-108.

2. Patel DK, Prasad SK, Kumar R, et al. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed. 2012;2(4):320-30.

3. International Diabetes Foundation. (2014). DiabetesAtlas.Brussels.6thed.

4. Maiti R, Jana D, Das UK, et al. Antidiabetic effect of aqueous extract of seed of Tamarindus indica in streptozotocin-induced diabetic rats. J Ethnopharmacol. 2004;92(1):85-91.

5. Chikezie PC, Ojiako OA, Nwufo KC. Overview of Anti-Diabetic Medicinal Plants: The Nigerian Research Experience. J Diabetes Metab. 2015;6(6):546.

6. Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol Aspects Med. 2006;27(1):1-93.

7. Saravanamuttu S, Sudarsanam D. Antidiabetic plants and their active ingredients: A review. International journal of Pharmaceutical Sciences and Research. 2012;3(10):3639-650.

8. Udochukwu U, Omeje FI, Uloma IS, et al. Phytochemical analysis of Vernonia amygdalina and Ocimumgratissimum extract and their antibacterial activity on some drug resistant bacteria. Am J Res Commun. 2015;3(5):225-35.

9. Gbolade AA. Inventory of antidiabetic plants in selected districts of Lagos State, Nigeria. J Ethnopharmacol. 2009;121(1):135-39.

10. Farombi EO, Owoeye O. Antioxidative and chemopreventive properties of Vernonia amygdalina and Garcinia biflavonoid. Int J Environ Res Public Health. 2011;8(6):2533-555.

11. Amaechi D, Udosen EO, Edet EE, et al. Lipid profile status of streptozotocin induced diabetic rats treated with ethanolic leaf extract of Solenostemon monostachyus. J Med Plant Res. 2015;9(8):289-93.

12. Amaechi D, Ekpe IP, Yisa BN, et al. Phytochemical Profiling (GCMS) of the leaf extract of mangifera indica and its Hypolipidemic Effect on Serum Lipid profile in Streptozotocin induced diabetic Wistar rats. Asian Sci Bull. 2024;2(1):17-23.

13. Amaechi D, Mary AE, Ekpe IP, et al. Hypolipidaemic Effects of Ethanol Leaf Extract of Eucalyptus camadulensis in Wistar Rats. Asian J Biol Sci. 2024;17(4):846-51.

14. Amaechi D, Yisa BN, Ekpe IP, et al. Phytochemical Screening, Anti-obesity and Hepatoprotective Activities of Ethanol Leaf Extract of Jatropha tanjorensis in Wistar Rats. Asian J Appl Chem. 2022;12(4):20-26.

15. Amaechi D, Felix EA, Ekpe IP, et al. The Ameliorative Effects of Solanummelongena (Garden Egg), Solanumlycopersicum(Tomatoes) and Caucus carrots sub.sativus extracts on Lipid Profile against Lead Induced Toxicity in Wistar rats. Eur J Pharm Med Res. 2020;7(11):70-73.

16. Asanga EE, Eseyin OA, Ekanem AN, et al. Changes in Serum Zinc, Magnesium and Copper in Sickle Cell Patients: A Case Study in Jos, Nigeria. World Journal of Pharmaceutical Sciences. 2016;4(5):195-99.

17. Ekpe IP, Amaechi D, Yisa BN, et al. Evaluation of the Effects of Aqueous Fruit Extract of Tamarindus indica on Body Weight, Lipid Profile, Some Electrolytes and Urea of Wistar Rats. J Pharm Res Int. 2021;33(59A):444-51.

18. Ekpe IP, Udosen EO, Amaechi D, et al. Impact of Ethanolic Extract of Tecomastans and Costusafer Leaves on Lipid Profile Status of Streptozotocin Induced Diabetic Wistar Rats. International Journal of Sciences. 2018;7:16-20.

19. Ekpe IP, Apata DA, Amaechi D. Effects of methanol leaf extract of Corchorusolitorius on the liver function and lipid profile of albino Wistar rat. Eur J Pharm Med Res. 2021:8(11).13-19.

20. Amaechi D, Udosen EO, Ekam VS, et al. Effects of Methanolic Root Extract of Holarrhena floribunda on the Lipid Profile and Sex Hormones in Wistar Rats. Asian J Re. Biochem. 2018;3(3):1-7.

To access the full-text version of this article, please scan the QR code: