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(RESEARCH ARTICLE)

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Evaluating alloxan-induced diabetic rats

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Abstract

The increasing incidence of diabetes and the adverse effects of chemical medications underscore the importance of exploring alternative non-pharmacological treatments. Vernonia amygdalina, a widely utilized medicinal herb, shows promising therapeutic potential. This study investigated the impact of aqueous leaf extracts of Vernonia amygdalina on various biochemical parameters in alloxan-induced diabetic rats.

Diabetes was induced in male Wistar rats through intraperitoneal injection of alloxan (150 mg/kg). The rats were then randomly divided into four groups: Group 1 (normal control), Group 2 (diabetic control), Group 3 (diabetic rats treated with Vernonia amygdalina at 80 mg/kg), and Group 4 (diabetic rats treated with glibenclamide at 5 mg/kg). The treatments were administered orally for 28 days.

Results showed that Vernonia amygdalina significantly reduced blood glucose and glycated hemoglobin levels in diabetic rats compared to untreated diabetic controls ($P < 0.001$). After 28 days of treatment, the extracts also notably improved altered biochemical parameters in diabetic rats versus the untreated controls ($P < 0.05$). Specifically, Vernonia amygdalina reduced elevated levels of alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) ($P < 0.05$). Additionally, the extract demonstrated hepatoprotective and nephroprotective properties, indicated by reductions in liver enzyme levels and improvements in kidney function markers.

In summary, the aqueous leaf extract of Vernonia amygdalina showed beneficial effects on selected biochemical markers in alloxan-induced diabetic rats, suggesting its potential role in diabetes management.

Keywords: Vernonia amygdalina; Alloxan; Glibenclamide; Kidney profile; Glycated hemoglobin

1. Introduction

Diabetes mellitus, a chronic metabolic condition characterized by impaired glucose regulation, has emerged as a major global health concern. This disorder is caused by either insufficient insulin production in the pancreas or by insulin resistance, where body cells lose their responsiveness to insulin. These issues lead to elevated blood glucose levels, which, if unmanaged, may result in serious complications (American Diabetes Association, 2018).

Vernonia amygdalina, commonly referred to as "bitter leaf," is a popular medicinal plant in Africa, deeply rooted in traditional medicine. This plant contains a diverse range of phytochemicals, including sesquiterpenes, flavonoids, alkaloids, and saponins (Omoregie & Pal, 2018). Research has demonstrated the potential of Vernonia amygdalina in managing diabetes and its complications. For example, extracts from this plant can influence glucose metabolism,

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improve insulin sensitivity, and possibly serve as adjunctive therapy for diabetes management (Izevbuwa et al., 2021; Ogbolu et al., 2018).

2. Material and methods

2.1. Animals

Twenty-four (24) male Wistar rats, weighing between 100g and 150g, were purchased and housed in suitable plastic cages in a well-ventilated animal facility at the Department of Pharmacology, Rivers State University, Port Harcourt. The rats had unrestricted access to standard rat pellets and water and were maintained on a 12-hour light/dark cycle. Before the experiment, the animals were acclimatized for 10 days, ethical clearance was granted by the Rivers State University.

2.2. Chemicals, Reagents, and Kits

The chemicals and reagents used in the study included hydrochloric acid, Ellman's reagent (DTNB), hydrogen peroxide, potassium chloride, and Tris buffer. Kits for biochemical assays (AST, ALT, urea, creatinine, glucose, and lipid profile) were procured from Randox Laboratories, UK. All reagents were of analytical grade.

2.3. Plant Materials

Fresh leaves of Vernonia amygdalina were obtained from the Port Harcourt fruit market in Rivers State, Nigeria, and verified by a botanist from the Department of Plant Science and Biotechnology, University of Port Harcourt. The leaves were air-dried to a constant weight and then ground into a fine powder.

2.4. Preparation of Aqueous Extracts

The powdered leaves (500 g) were macerated in distilled water (1 L) for 48 hours at room temperature with occasional stirring. The mixtures were then filtered through Whatman filter paper No. 1, and the filtrate was concentrated using a rotary evaporator under reduced pressure at 40°C, yielding the crude extract of Vernonia amygdalina, which was stored at 4°C until use.

2.5. Induction of Experimental Diabetes

Experimental diabetes was induced via a single intraperitoneal injection of freshly prepared alloxan monohydrate (150 mg/kg body weight) dissolved in normal saline. Blood samples were collected 48 hours post-injection via tail puncture, and fasting blood glucose levels were measured. Rats with fasting blood glucose levels above 200 mg/dL were classified as diabetic.

2.6. Experimental Design

Twenty-four male Wistar rats were randomly divided into four groups (n=6) as follows:

- **Group 1 (Normal Control)**: Non-diabetic rats receiving distilled water.
- **Group 2 (Diabetic Control)**: Diabetic rats receiving distilled water.
- **Group 3 (Test Group)**: Diabetic rats treated with Vernonia amygdalina (80 mg/kg) for 28 days.
- **Group 4 (Standard Control)**: Diabetic rats treated with glibenclamide (5 mg/kg) for 28 days.

3. Results and discussion

3.1. Effects of Selected Herbal Extracts on Body Weight of Treated Rats

The results revealed that inducing diabetes caused a significant ($\mathbf{p < 0.05}$) reduction in the body weight of diabetic rats when compared to normal, non-diabetic rats. This reduction is attributable to the metabolic disturbances associated with diabetes.

Upon treatment, the final body weight of diabetic rats showed variations among the groups:

- **Group treated with Vernonia amygdalina**: A slight decrease in body weight was observed. Although the extract improved glucose metabolism, its effects on restoring weight were limited during the treatment period.

- **Group treated with the standard drug (Glibenclamide)**: This group demonstrated an increase in body weight compared to the diabetic untreated control, suggesting better overall metabolic recovery.

Table Effects Of Selected Herbal Extracts On Body Weight Of Treated Rats

Table 1 The effect of different herbal extracts on body weight of treated rats for 4 weeks (28 days).

GROUPS	INITIAL (g)	WEEK 1	WEEK 2	WEEK 3	WEEK 4
GROUP 1	138.0 ± 6.25^{bc}	133.4 ± 7.29^{bc}	123.0 ± 29.00	128.2 ± 7.08^{b}	138.6 ± 6.04
GROUP 2	124.0 ± 5.19^{b}	121.6 ± 6.22^{ab}	94.2 ± 25.08	67.6 ± 28.37^{ab}	65.8 ± 27.97
GROUP 3	120.0 ± 2.41^{a}	116.4 ± 2.40^{a}	104.2 ± 2.69	61.0 ± 24.94^{a}	62.2 ± 25.43
GROUP 4	141.6 ± 3.37^{c}	138.8 ± 3.15^{c}	110.6 ± 27.81	72.0 ± 29.94^{ab}	71.2 ± 29.52

* Values are expressed as Mean ± SD (n=6), *P<0.05 versus control. Statistical analysis was carried out using one-way analysis of variance (ANOVA) and Duncan post hoc test.* **Group 1** = Normal Control, **Group 2** = Diabetes Control, **Group 3** = *V. amygdalina*, **Group 4** = Glibenclamide.

3.2. Effects of Selected Herbal Extracts on Organ Weight Of Treated Rats

Table 2 The effect of different herbal extracts on organ weight of treated rats.

GROUPS	PANCREAS	KIDNEY
GROUP 1	2.90 ± 0.32	1.29 ± 0.12
GROUP 2	0.27 ± 0.14	0.75 ± 0.67
GROUP 3	0.83 ± 0.35	0.37 ± 0.41
GROUP 4	0.58 ± 0.25	0.33 ± 0.13

* Values are expressed as Mean ± SD (n=8), *P<0.05 versus control. Statistical analysis was carried out using one-way analysis of variance (ANOVA) and Duncan post hoc test.* **Group 1** = Normal Control, **Group 2** = Diabetes Control, **Group 3** = *V. amygdalina*, **Group 4** = Glibenclamide.

3.3. Effects of Selected Herbal Extracts on Blood Glucose Level Of Treated Rats

Table 3 The effect of different herbal extracts on blood glucose levels of treated rats for 4 weeks (28 days).

GROUPS	INITIAL (mg/dl)	WEEK 1	WEEK 2	WEEK 3	WEEK 4
GROUP 1	98.6 ± 3.83	115.0 ± 4.82	98.6 ± 3.82	80.4 ± 4.04	108.2 ± 5.17
GROUP 2	579.0 ± 11.02	425.4 ± 60.55^{b}	578.0 ± 10.02	312.4 ± 72.26	268.8 ± 62.88
GROUP 3	222.8 ± 55.90	242.4 ± 87.56^{b}	162.3 ± 52.38	87.2 ± 33.96	74.4 ± 26.66
GROUP 4	288.0 ± 78.73	264.4 ± 115.19^{b}	189.0 ± 78.73	176.4 ± 82.51	125.4 ± 50.34

Values are expressed as Mean ± SD (n=6), *P<0.05 versus control. Statistical analysis was carried out using one-way analysis of variance (ANOVA) and Duncan post hoc test.* **Group 1** = Normal Control, **Group 2** = Diabetes Control, **Group 3** = *V. amygdalina*, **Group 4** = Glibenclamide.

3.4. Effects Of Selected Herbal Extracts On Liver Function Biomarkers In Treated Rats

Table 4 The effect of different herbal extracts on liver function biomarkers of treated rats.

GROUPS	AST	ALT	ALP	TP	ALB
GROUP 1	35.00 ± 2.00	13.50 ± 0.50	53.50 ± 2.50	70.30 ± 0.45	50.00 ± 0.20
GROUP 2	54.50 ± 4.50	60.00 ± 2.00	112.50 ± 12.50	54.45 ± 0.10	34.30 ± 1.20
GROUP 3	30.00 ± 2.00	11.75 ± 0.45	32.00 ± 1.00	74.50 ± 1.50	43.50 ± 1.50^{c}
GROUP 4	22.50 ± 1.50	11.45 ± 0.35	36.50 ± 1.50	68.50 ± 1.50	41.40 ± 0.50^{c}

* Values are expressed as Mean ± SD (n=6), *P<0.05 versus control. Statistical analysis was carried out using one-way analysis of variance (ANOVA) and Duncan post hoc test.* **Group 1** = Normal Control, **Group 2** = Diabetes Control, **Group 3** = *V. amygdalina*, **Group 4** = Glibenclamide.

3.5. Effects of Selected Herbal Extracts On Kidney Function Biomarkers In Treated Rats

Table 5 The effect of different herbal extracts on kidney function biomarkers of treated rats.

GROUPS	CREATININE (65–120 µmol)	UREA (1.9–8.4 mmol/l)
GROUP 1	92.95 ± 7.05	4.85 ± 0.05
GROUP 2	236.00 ± 6.00	17.35 ± 0.45
GROUP 3	135.00 ± 3.00	7.25 ± 0.15
GROUP 4	133.00 ± 2.00	5.75 ± 0.05

* Values are expressed as Mean ± SD (n=6), *P<0.05 versus control. Statistical analysis was carried out using one-way analysis of variance (ANOVA) and Duncan post hoc test.* **Group 1** = Normal Control, **Group 2** = Diabetes Control, **Group 3** = *V. amygdalina*, **Group 4** = Glibenclamide.

3.6. Effects of Selected Herbal Extracts On Lipid Profile Of Treated Rats

Table 6 The effect of different herbal extracts on lipid profile of treated rats.

GROUPS	TC	TG	HDL	LDL	VLDL
GROUP 1	4.35 ± 0.55	1.50 ± 0.10^{a}	1.65 ± 0.15^{a}	1.50 ± 0.10^{b}	0.45 ± 0.02
GROUP 2	7.30 ± 0.20	3.55 ± 0.15^{b}	0.50 ± 0.10^{a}	5.25 ± 0.50	2.27 ± 0.01
GROUP 3	2.50 ± 0.10	0.95 ± 0.03^{b}	1.34 ± 0.02^{a}	1.49 ± 0.13^{b}	0.44 ± 0.02
GROUP 4	2.85 ± 0.05	1.63 ± 0.03	1.69 ± 0.03	1.80 ± 0.04^{b}	0.74 ± 0.01

* Values are expressed as Mean ± SD (n=8), *P<0.05 versus control. Statistical analysis was carried out using one-way analysis of variance (ANOVA) and Duncan post hoc test.* **Group 1** = Normal Control, **Group 2** = Diabetes Control, **Group 3** = *V. amygdalina*, **Group 4** = Glibenclamide.

3.7. Histology of Sacrificed animal from each group showing their Pancreas and Kidney

The histological examination in Figure 1 to 8 provides valuable insights into the pancreatic and renal tissue morphology in the different experimental groups

3.7.1. Group 1 normal rats

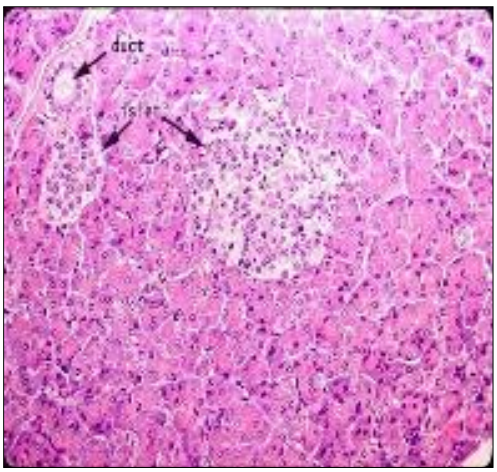

Figure 1 A Microphotograph-of-pancreas-from-normal-rat-group-1 no distortion of beta- cells, normal beta cells

3.7.2. Group 1 kidney normal rats

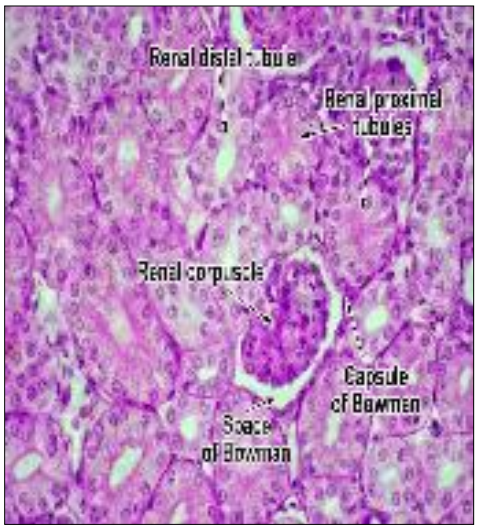

Figure 2 A Microphotograph-of-kidney-from-normal-rat-group-1 showing normal section of glomeruli

3.7.3. Group 2 negative control without treatment.

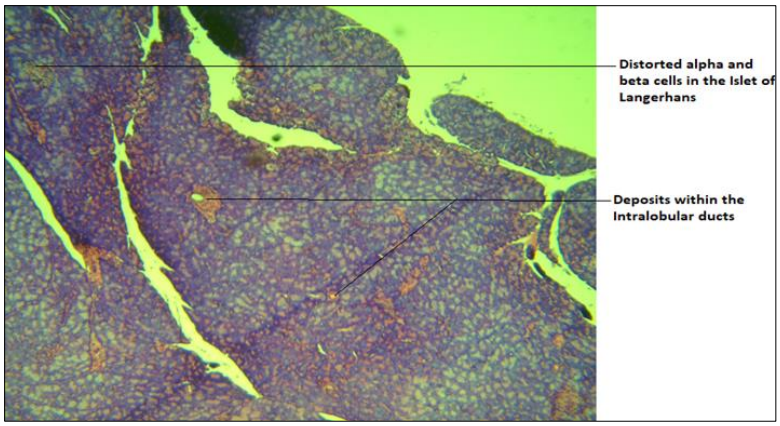

Figure 3 G2 Photomicrograph of pancreas showing distorted Islet tissues.

Deposits within the intralobular duct of the pancreas are observed. Numerous serous acini containing deposits are observed.

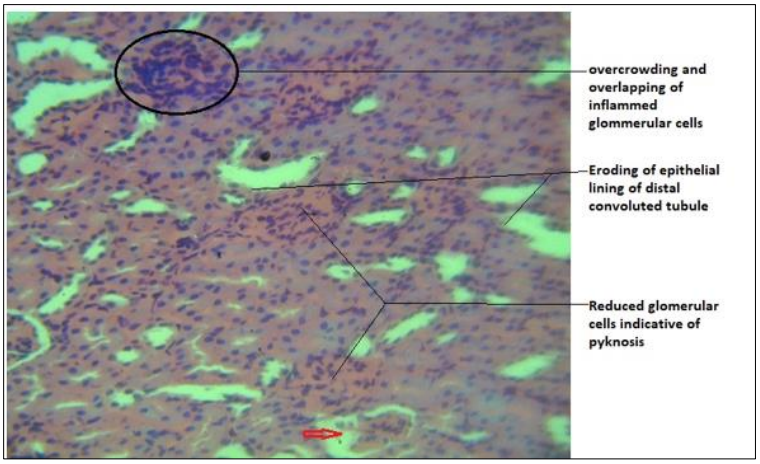

Figure 4 Group 2: photomicrograph showing inflamed glomerular cells overlapping

Bowman capsule also shows eroded glomerulus with large space (vacuolaton) as indicated by the red arrow. Also observed is the distortion of the epithelial lining the lumen of the distal convoluted tubule. H&E, X400

3.7.4. Group 3 treated with Vernomia amagydalina

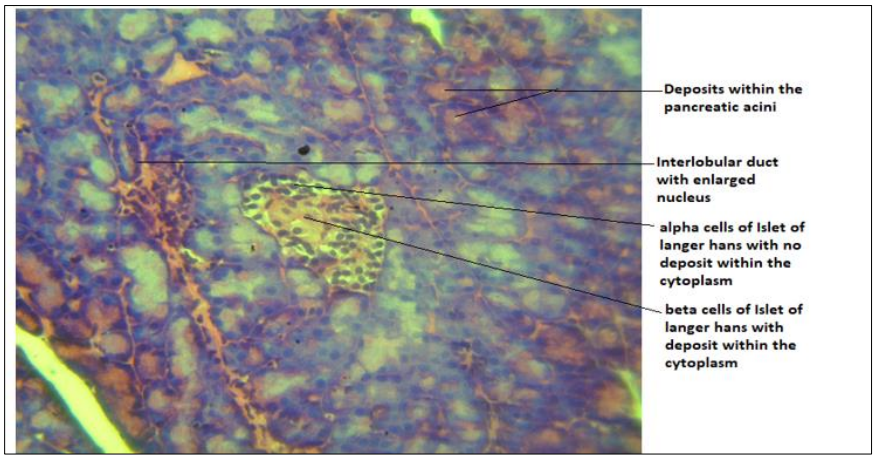

Figure 5 G3: Photomicrograph of pancreas showing numerous alpha and beta cells with enlarged nuclei

The interlobular duct is surrounded with inflamed cells with overlapping appearance. Some pancreatic serous acini contain deposits whose internal epithelial lining are eroded.

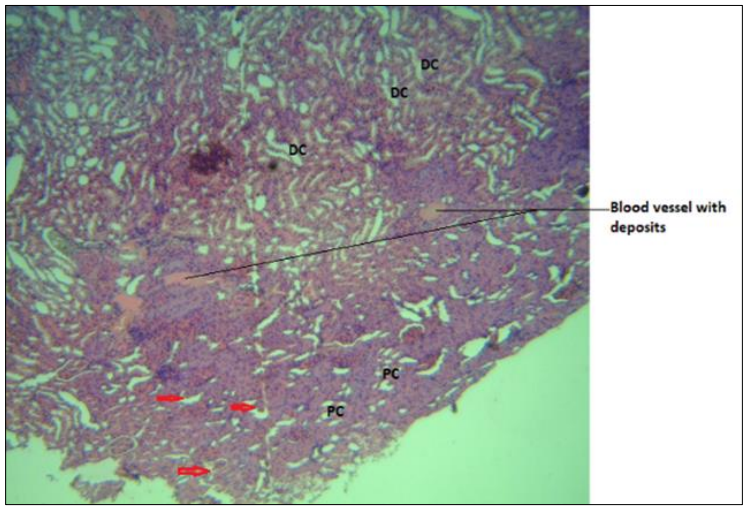

Figure 6 Group 4: photomicrograph showing several distal convoluted (DC) and Proximal convoluted (PC) tubules with no clear pathology

However, there are large spaces observed in the Bowman's capsule with clear destruction of glomerular capillaries (red)

3.7.5. Group 4 Treated WithGlibenclamide

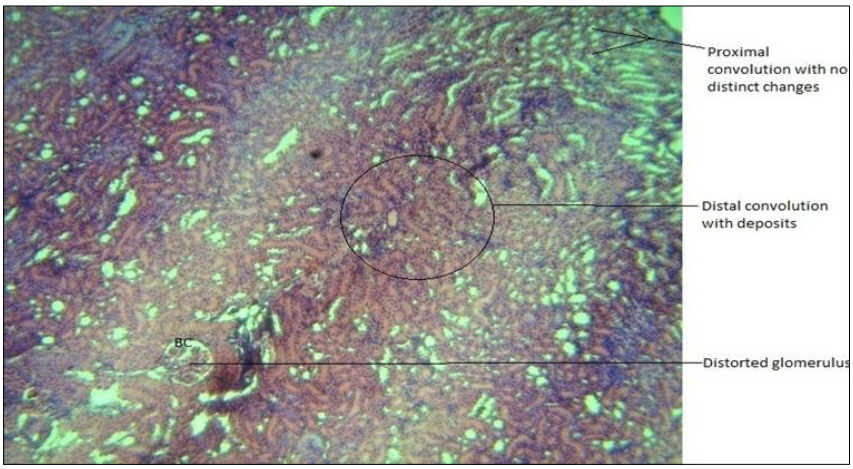

Figure 7 G5: Photomicrograph showing distorted glomerulus with vacuolation

However, the Bowman's capsule space is maintaine. Deposits are observed within the surrounding distal convoluted tubules.

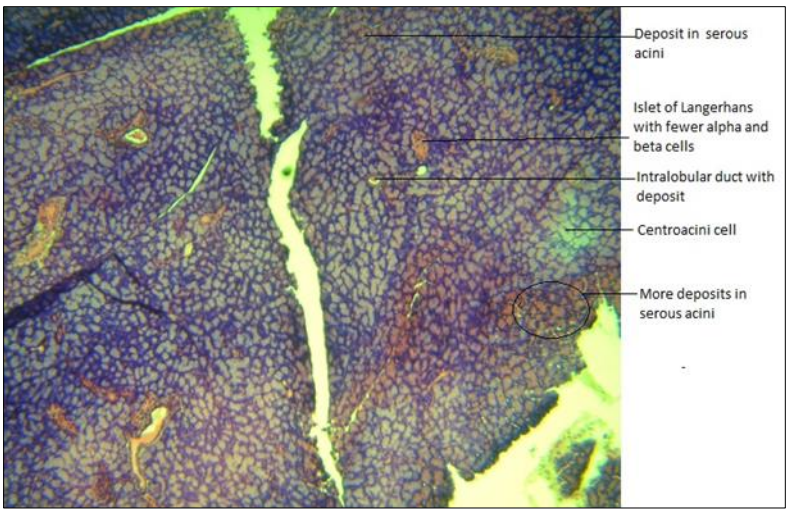

Figure 8 G6 : Photomicrograph showing intralobular duct with deposits, serous acini with deposits.

The Islet of Langerhans with few alpha and beta cells (amyloidosis of the pancreatic islet tissue). H&E, X100

4. Discussion

The findings from this study highlight the therapeutic potential of *Vernonia amygdalina* (bitter leaf) in ameliorating biochemical imbalances and protecting organ health in alloxan-induced diabetic rats. The study provides evidence supporting the extract's benefits in managing diabetes-related complications, particularly regarding body weight, blood glucose levels, liver and kidney function, and lipid metabolism.

4.1. Body and Organ Weights

The significant weight loss observed in the diabetic control group (Group 2) compared to the normal control group (Group 1) reflects typical diabetic conditions, often attributed to insulin deficiency and excessive blood glucose levels that induce a catabolic state (Leong & Wilding, 1999). Treatment with *Vernonia amygdalina* (Group 3) partially stabilized body weight, though not as effectively as glibenclamide (Group 4). Glibenclamide, known for stimulating insulin release and enhancing glucose utilization, effectively reduced catabolic effects, resulting in better weight preservation (Garg et al., 2015).

Additionally, the preservation of organ weights in the pancreas and kidneys of rats treated with *Vernonia amygdalina* suggests that the extract may offer protective effects against hyperglycemia-induced organ damage. These findings were corroborated by histological analyses, which showed reduced pathological changes in treated groups compared to untreated diabetic controls.

4.2. Blood Glucose Levels

The significant reduction in blood glucose levels observed in the *Vernonia amygdalina*-treated group demonstrates the plant's hypoglycemic potential. The extract's effects were comparable to those of glibenclamide over the four-week treatment period. This aligns with previous studies suggesting that *Vernonia amygdalina* enhances insulin sensitivity and promotes efficient glucose metabolism (Izevbuwa et al., 2021; Ogbolu et al., 2018). The glucose-lowering effects could be attributed to the phytochemicals present in the extract, including flavonoids and sesquiterpenes, which are known for their anti-diabetic properties.

4.3. Liver and Kidney Function

Diabetic conditions often lead to elevated liver enzyme levels, indicating hepatic stress and damage. In this study, untreated diabetic rats exhibited significantly increased levels of alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP). These elevations were significantly reduced in rats treated with *Vernonia amygdalina*, suggesting hepatoprotective effects. The observed improvement may be linked to the antioxidant properties of the plant, which help mitigate oxidative stress-induced liver damage.

Kidney function, assessed through markers like serum creatinine and urea, also showed notable improvements in the *Vernonia amygdalina*-treated group compared to the diabetic controls. This nephroprotective effect underscores the plant's role in preventing hyperglycemia-induced renal damage.

4.4. Lipid Profile

Diabetes often disrupts lipid metabolism, leading to dyslipidemia characterized by elevated cholesterol, triglycerides, and low-density lipoprotein (LDL) levels, alongside reduced high-density lipoprotein (HDL) levels. Treatment with *Vernonia amygdalina* helped restore lipid balance by reducing cholesterol and triglyceride levels and improving HDL levels, thereby reducing the risk of cardiovascular complications associated with diabetes.

5. Conclusion

This study demonstrates the potential of *Vernonia amygdalina* as an effective natural intervention for diabetes management. By significantly improving glycemic control, liver and kidney function, and lipid profiles, the extract offers a promising alternative or adjunct to conventional anti-diabetic therapies. Further studies are recommended to explore the molecular mechanisms underlying its therapeutic effects and to evaluate its safety and efficacy in human populations.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclsoed.

Statement of ethical approval

If studies involve use of animal/human subject, authors must give appropriate statement of ethical approval. If not applicable then mention 'The present research work does not contain any studies performed on animals/humans subjects by any of the authors'. Ethical Statement on the Use of Animals in Research

This study was conducted in accordance with ethical principles for the humane use of animals in research. Approval for the research protocol was obtained from the Rivers State University Animal Care and Use Committee (ACUC). All procedures adhered to national and international guidelines, including the Nigerian Animal Welfare Act and principles outlined by the World Organization for Animal Health (OIE).

The 3Rs principles of animal research—Replacement, Reduction, and Refinement—were rigorously applied:

- **Replacement**: Alternative methods were considered, and animals were used only when no viable alternatives existed.
- **Reduction**: The study minimized the number of animals used while ensuring statistical reliability.
- **Refinement**: Procedures were designed to minimize distress and enhance animal welfare.

All personnel involved in the study were trained in animal handling and ethical research practices. The welfare of the animals was monitored closely throughout the study, ensuring compliance with the highest standards of care.

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