

Original article

Mitigating Diabetic Renal Damage: A Study on the Therapeutic Role of *Vernonia amygdalina* in Diet-STZ-Induced Rats

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Abstract

Background: Dipeptidyl phosphate-4 (DPP-4) inhibitors like vildagliptin (Galvus) are widely used in type 2 diabetes management but are associated with side effects including pancreatitis, hypersensitivity reactions, and rare life-threatening conditions. These concerns have driven interest in safer, plant-based alternatives. *Vernonia amygdalina*, known for its antioxidant and antidiabetic effects, may offer renal protection. This study evaluates its role in diabetic nephropathy. **Materials and Methods:** Fifty male Wistar rats (90–120 g) were randomly assigned to five groups: Control, Diabetic only, Diabetic + *V. amygdalina* (3,808 mg/kg), Normal + *V. amygdalina*, and Diabetic + Galvus (6 mg/kg). Diabetes was induced after 14 days of high-fat diet feeding with a single intraperitoneal dose of streptozotocin (40 mg/kg). Treatment was administered for two weeks, and the experiment lasted 32 days. Parameters assessed included blood glucose, body weight, kidney function markers (urea, creatinine, sodium), oxidative stress indicators (SOD, MDA, GSH and ROS), and renal histopathology. **Results:** *V. amygdalina* significantly reduced blood glucose, improved kidney function markers, and reduced oxidative stress close to those observed with Galvus treatment. Histological evaluation revealed that kidney damage in diabetic rats was markedly attenuated by *V. amygdalina* treatment, showing superior tissue preservation compared to the Galvus group. **Conclusion:** Given the safety concerns associated with synthetic DPP-4 inhibitors like Galvus, *Vernonia amygdalina* emerges as a promising natural alternative. Its strong antioxidant, anti-inflammatory, and renoprotective effects suggest it may serve as an effective and safer option for managing diabetic nephropathy.

Keywords: Diabetic, Galvus, Mitigating, Renal Damage, *Vernonia amygdalina*

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Introduction

Diabetes mellitus (DM) is a major global health concern and a leading cause of morbidity and mortality across all age groups. According to the World Health Organisation,³⁷ of the 1.6 million diabetes-related deaths in 2021, 47 per cent occurred in individuals under the age of 70. The global prevalence of DM has more than tripled from 151 million in 2000 to 537.5 million in 2022, with cases projected to exceed 783 million by 2045.²¹ This burden is particularly severe in low and middle income countries (LMICs), where over half of affected individuals remain undiagnosed or untreated.³⁸ In Nigeria, the country with the highest diabetes prevalence in Africa, approximately 73 percent of patients do not monitor their blood glucose regularly.¹⁰ DM is characterised by chronic hyperglycemia resulting from impaired insulin secretion (type 1) or insulin resistance (type 2), leading to widespread metabolic disturbances in carbohydrate, protein, and lipid metabolism.²² If poorly managed, persistent hyperglycemia can lead to long-term

complications affecting vital organs, with diabetic nephropathy (DN) being one of the most common microvascular complications. DN is a leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD), affecting nearly one-third of individuals with type 1 DM and almost half of those with type 2 DM.¹² Oxidative stress, inflammation, and fibrosis are key contributors to the pathogenesis of diabetic kidney injury.²⁶

While several conventional antidiabetic medications exist including sulfonylureas, biguanides, thiazolidinediones, DPP4 inhibitors, and SGLT2 inhibitors their use is often limited by side effects such as hypoglycemia, hepatotoxicity, and nephrotoxicity.²⁹ This highlights the need for safer, more effective, and affordable alternatives, especially from natural sources.²

Vernonia amygdalina (bitter leaf), a member of the Asteraceae family, is widely used in African traditional medicine due to its rich phytochemical content, including flavonoids and alkaloids. It has demonstrated antioxidant, anti-inflammatory, and antidiabetic properties.³² Emerging evidence suggests its potential in mitigating diabetic kidney damage through antioxidant and anti-inflammatory mechanisms.⁵

Table 2.1: Experimental design A= Control group; B = Diabetic group; C= Diabetic + Bitter leaf; D= Normal + Bitter leaf; and E= Diabetic + Galvus. Normalsaline (0.5 mLorally), High fat diet (100g/day), STZ (Single dose of 40mg/kg B.W Intraperitoneally), Galvus (6mg/kg B.W orally), Bitter leaf extract (3808mg/kg B.W orally)

	STZ 40 mg/kg	Normal saline 0.5 ml	Curcuma longa 100 mg/ kg	Galvus® (Vildagliptin) 6 mg/kg
Group	40 mg/kg	0.5 ml	100 mg/ kg	6 mg/kg
A	No	Yes	No	No
B	Yes	No	No	No
C	Yes	No	Yes	No
D	No	Yes	Yes	No
E	Yes	Yes	No	Yes

Preparation of High- fat diet

However, the extent of its nephroprotective effects in diabetic conditions remains underexplored. Moreover, its comparable efficacy to standard drugs like vildagliptin (Galvus) has not been established. This study investigates the renoprotective effect of *Vernonia amygdalina* in a

high-fat diet and streptozotocin-induced diabetic rat model. By comparing it to vildagliptin (Galvus), the study explores its potential as a safer alternative to DPP-4 inhibitors. The findings support its use as a cost-effective and accessible adjunct therapy, especially in resource-limited settings.

Ethical Consideration

All experimental protocols followed the guidelines set by the Department of Anatomy, Faculty of Basic Medical Sciences, FUOYE, Nigeria (FUOYE/BMS/0005), and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH, 1986).¹⁸

Animal Procurement and Caring

A total of fifty (50) healthy male Wistar rats, weighing from 90 - 120 grams, were obtained from Mctemmy Research Animal Concepts located in Ogbomoso, Oyo State. The rats were kept in well-ventilated cages under standard laboratory settings, which included regulated temperature, humidity, and lighting. They were allowed a two-week acclimation period and were given standard pellet feed along with clean water supplied through water troughs.

Experimental Design

Following two weeks of acclimatisation, the animal was randomly assigned into 5 groups and treated as follows;

The high-fat diet was formulated using maize, sucrose, fish meal, premix, oyster shell, dicalcium phosphate, soya meal, salt, soybeans, and butter, with butter constituting the highest proportion of the composition. The diet was prepared according to the methods described by Varady ³³.

Preparation of Streptozotocin

Immediately before injection, STZ was dissolved in pre-chilled 0.1 M citrate buffer (prepared from 0.1 M sodium citrate and citric acid, pH 4.5). The solution was protected from light by covering it with aluminum foil, and all injections were completed within 15 minutes of preparation.²

Induction of diabetes

The animals were fed a high-fat diet for 14 days. On day 15, following a 12 hour fast, a single intraperitoneal injection of streptozotocin (STZ) at 40 mg/kg body weight was administered to induce diabetes. Seventy-two hours post-injection, fasting blood glucose levels were measured. Rats with glucose levels ≥ 200 mg/dL were considered diabetic.

Collection and preparation of *V. amygdalina* extract

Fresh *V. amygdalina* leaves were collected from farms in Oye Local Government, Oye-Ekiti, Ekiti State, Nigeria. The fresh leaves of *Vernonia*

amygdalina were cleaned from extraneous materials, leaves attacked by microbes or insects were removed and the rest were air-dried at room temperature with good airflow to reduce any microbial attack as well as to facilitate grinding then it was ground into powder with a kitchen blender, in the histology Laboratory, Federal University Oye-Ekiti, Ekiti State, Nigeria. The powdered plant material was weighed (500g) and stored in an air-tight sack.

The extraction process was carried out in the Department of Anatomy, Faculty of Health Sciences, University of Osun, Osun State, Nigeria. It was done in with methanol and n-hexane as extraction solvents with the ratio of 1:10 (w/v). 100 g of plant leaves were weighed and soaked in 1000 mL of methanol, and 1000 mL of n-hexane as the extraction solvent for 72 h. The extract was filtered, and it was further subjected to a rotary evaporator to separate the solvents from the plant extracts. These methods were employed for the extraction of the plant stems, and the percentage yield of the extract was calculated.¹¹

Lethal dose of V. amygdalina extract

LD50 of the *V. amygdalina* was carried out in the Department of Pharmacology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State. The acute toxicity/Lethal dose tests were carried out according to Lorke's method, recently used by Aliyu⁷ and were found to be 3808mg/kg.

Administration of V. amygdalina

The 3808mg/kg B.W. was totally dissolved in normal saline and administered to each group taking the *V. Amygdalina* extract daily. The administration was done orally via oral gavage.

Administration of Galvus

Vildagliptin (Galvus® 50 mg tablets) was dissolved in normal saline and administered orally to the rats using oral gavage tubes at a dose of 6 mg/kg/day.¹⁴

Measurement of body weight and food intake

Record for animal daily consumption and weekly body weight evaluation was achieved using weighing balance (CS 5000).

Fasting blood sugar

Before the start of the study, the animals' baseline blood glucose levels were determined. During the course of the study, blood glucose levels were measured weekly. All blood samples were collected from the tail vein of the rats. Blood glucose levels were determined using the Fine Test™ glucometer.

Euthanasia and Tissue Collection

At the end of the experimental period (33rd day), the animals were sacrificed by decapitation between 7:00 and 10:00 AM. Blood was collected using 2 mL syringes and stored in lithium-

heparinised tubes for kidney function tests. For histological analysis, animals were transcardially perfused with phosphate-buffered saline (PBS, pH 7.4) to clear the blood vessels and prevent cell shrinkage, followed by perfusion with 10% neutral-buffered formalin (NBF) as a pre-fixative. The kidneys were excised, weighed, and fixed in 10% NBF. For biochemical studies, renal tissues were perfused, homogenised in PBS, and then frozen for further analysis.

Biochemical Analysis

Homogenised kidney tissue was centrifuged at 3,000 rpm for 10 minutes to separate the supernatant from the residue. The resulting supernatant was carefully pipetted into clean sample tubes and stored at -20°C for subsequent biochemical analyses. Levels of superoxide dismutase, glutathione, malondialdehyde, and reactive oxygen species were determined using enzyme-linked immunosorbent assay (ELISA) following the protocols specified in the manufacturer's kits in accordance to the methods by ^{31,26}.

Determination of Kidney Function Markers

Serum sodium (Na⁺) and chloride ion Cl⁻ were determined using the ion-selective electrode method employed by,⁴⁰ while serum urea was measured using the enzymatic colourimetric assay method employed by ³⁴. Blood samples were collected aseptically, centrifuged, and serum was stored at -20°C until analysis. Serum Creatine and Urea levels were assessed using the reverse reaction-based spectrophotometric method employed by ²⁷.

Histological Analyses

The fixed tissues of the kidney were subjected to dehydration using a graded series of alcohols, followed by clearing in xylene, embedding in paraffin, and sectioning at a thickness of 5 µm. Some of these sections were then stained with Haematoxylin and Eosin and some with Gordon and Sweet.

Statistical Analyses

A one-way ANOVA (analysis of variance) or a mixed model was employed, and differences between groups were assessed using a post hoc Tukey test with the assistance of GraphPad Prism Software 9.0.0 (121). The results of the statistical analysis were depicted using bar charts and graphs, with error bars indicating the mean ± SD (standard deviation). A significance threshold was established at p<0.05

Results

4.1 Body weight and blood glucose

4.1.1 Assessment of body weight following *V. amygdalina* treatment on Diabetic- induced Wistar rats

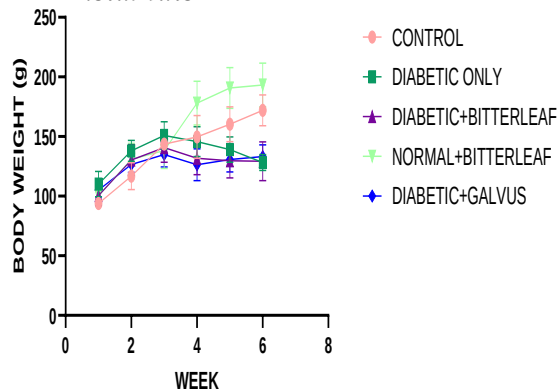


Figure 4.1A Assessment of body weight following *V. amygdalina* administration on hyperglycaemia toxicity. Data are expressed as mean $\pm$ SD; n=10

4.1.2 Assessment of blood glucose level following *V. amygdalina* administration on Diabetic-Induced Wistar rats

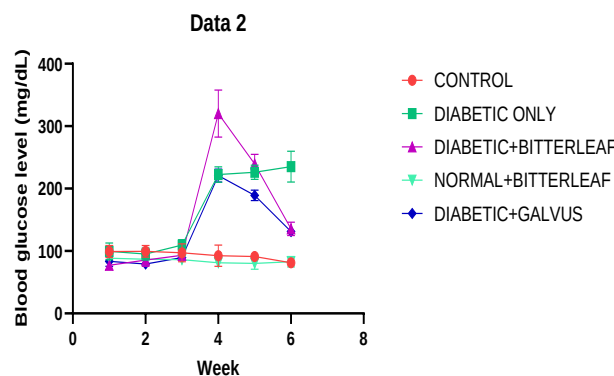


Fig. 4.1B Assessment of blood glucose level following *V. amygdalina* administration on hyperglycaemia toxicity: Data are expressed as mean $\pm$ SD; n=10

4.2 Effect of *V. amygdalina* on oxidative stress markers in diabetic Wistar rats

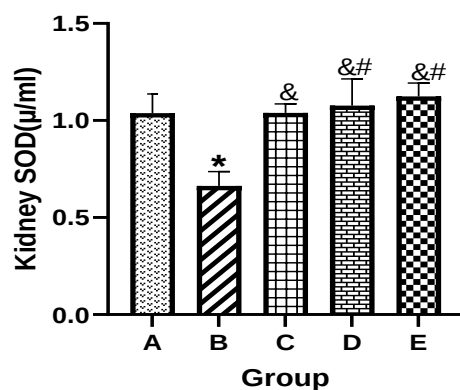


Fig.4.2A: Effect of *V. amygdalina* on kidney Superoxide dismutase (SOD) in diabetic Wistar rats.

Data are expressed as mean $\pm$ SD; n=10. Values are significantly different at * $p < 0.05$ compared to control, & $p < 0.05$ compared to Diabetic only and # $p < 0.05$ compared to Diabetic+ bitterleaf. A= Control; B= Diabetic only; C= Diabetic + Bitterleaf; D= Normal + Bitterleaf; E= Diabetic + Galvus.

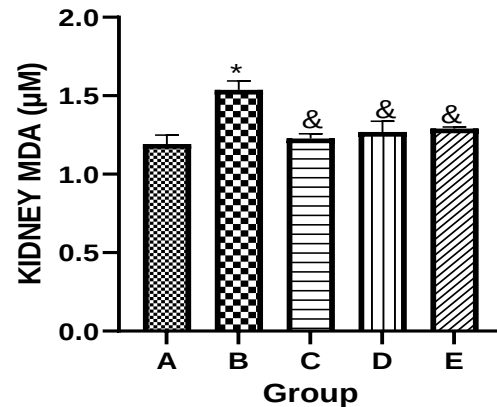


Figure 4.2B: Effect of *V. amygdalina* treatment on Kidney MDA on Diabetic Wistar rats

Data are expressed as mean $\pm$ SD; n=10. Values are significantly different at * $p < 0.05$ compared to control, & $p < 0.05$ compared to Diabetic only. A= Control; B= Diabetic only; C= Diabetic + Bitterleaf; D= Normal + Bitterleaf; E= Diabetic + Galvus.

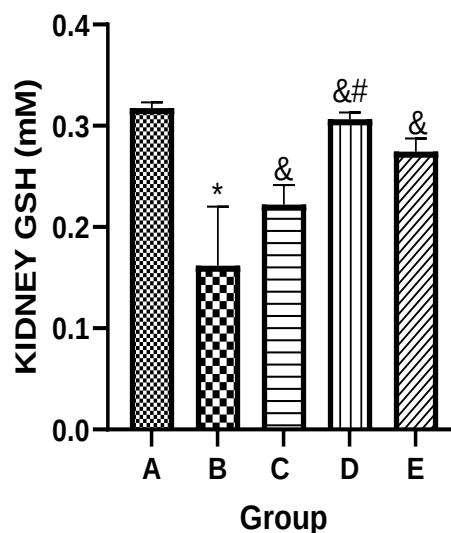


Figure 4.2C: Effect of *V. amygdalina* on kidney Glutathione (GSH) in diabetic Wistar rats.

Data are expressed as mean $\pm$ SD; n=10. Values are significantly different at * $p < 0.05$ compared to control, & $p < 0.05$ compared to Diabetic only and # $p < 0.05$ compared to Diabetic+ bitterleaf. A= Control; B= Diabetic only; C= Diabetic + Bitterleaf; D= Normal + Bitterleaf; E= Diabetic + Galvus.

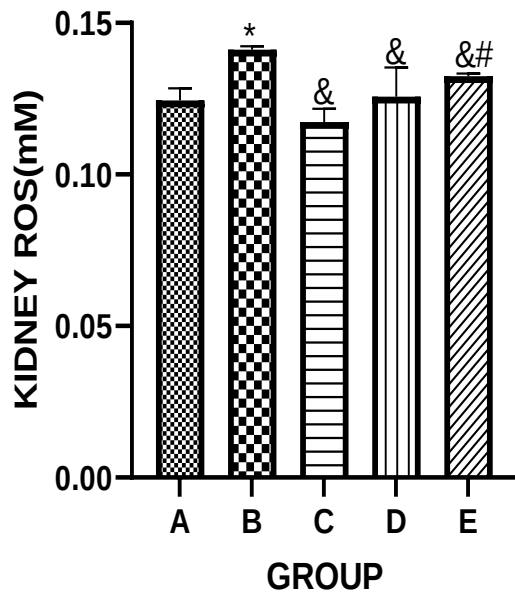


Figure 4.2C: Effect of *V. amygdalina* on kidney reactive oxygen species (ROS) in diabetic Wistar rats.

Data are expressed as mean $\pm$ SD; n=10. Values are significantly different at *p<0.05 compared to control, &p<0.05 compared to Diabetic only and #p<0.05 compared to Diabetic+ bitterleaf. A= Control; B= Diabetic only; C= Diabetic + Bitterleaf; D= Normal + Bitterleaf; E= Diabetic + Galvus.

Table 1: Data presented as Mean $\pm$ S.E.M., ^ap<0.05 significant difference from control. ^bp<0.05 significant differences from diabetic group only, (n=10). A-control group-Diabetic group, C-Diabetic+Bitterleaf, D-Normal+ bitterleafand E-Diabetic + Galvus

Group	Na ⁺ (mmol/L)	Cl ⁻ (mmol/L)	Urea (mmol/L)	Creatinine (μ mol/L)
A	132.5 $\pm$ 0.5774	94.00 $\pm$ 2.309	5.00 $\pm$ 1.039	47.50 $\pm$ 4.330
B	143.0 $\pm$ 0.2887 ^a	112.5 $\pm$ 4.330 ^a	13.85 $\pm$ 0.895 ^a	95.50 $\pm$ 2.598 ^a
C	137.0 $\pm$ 0.6774 ^{ab}	128.0 $\pm$ 6.309 ^{ab}	8.50 $\pm$ 0.723 ^{ab}	76.50 $\pm$ 2.598 ^{ab}
D	133.0 $\pm$ 0.6774 ^b	89.0 $\pm$ 2.887 ^b	3.18 $\pm$ 0.662 ^b	50.00 $\pm$ 0.754 ^b
E	139.0 $\pm$ 0.6774 ^{ab}	138.0 $\pm$ 7.889 ^{ab}	4.911 $\pm$ 1.02 ^b	74.50 $\pm$ 2.188 ^{ab}

4.4Histological findings

4.4A: Histological observation of the Kidney using Haematoxylin and Eosin stain (H&E)

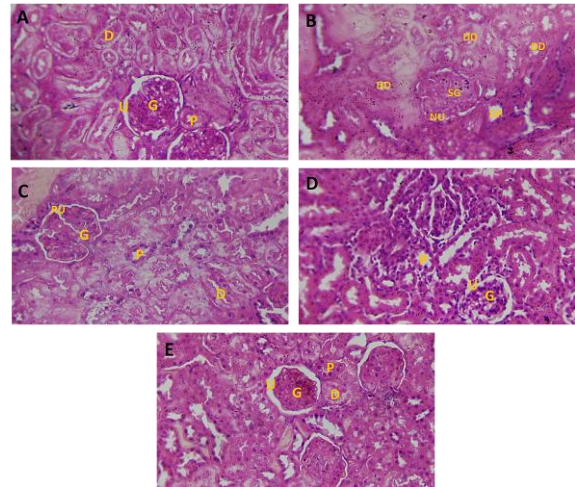


FIGURE 3A: Photomicrograph of the renal tissue (H&E) following *Vernonia amygdalina* in diabetic Wistar rats. D-distal convoluted tubules, P-proximal convoluted tubules, G-glomerulus and U-urinary space, DD- Degenerating tubules, NU- Narrowed Urinary space, SG- Sclerosed glomerulus, and RU- crescent formed urinary space. Mag $\times$ 400

4.4B: Histological observation of Kidney using Gordon and Sweet stain (G&S)

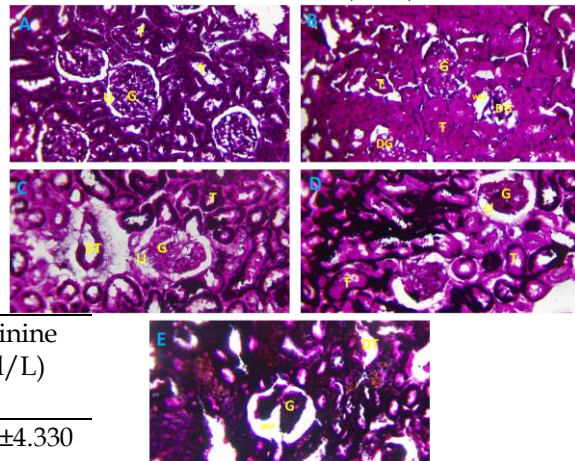


Figure 3B: Photomicrograph of the renal tissue (G&S) following *Vernonia amygdalina* in diabetic Wistar rats. T-tubules, G-glomerulus and U-urinary space, wU- widened urinary space, DT-dilated tubules, DG- degenerated glomerulus. Mag $\times$ 400

Discussion

Diabetic nephropathy (DN), a major cause of end-stage renal disease (ESRD), is one of the consequences that diabetes mellitus frequently causes. It is still a major global health concern.^{12,21} Chronic hyperglycemia is the main cause of DN

pathogenesis because it causes inflammation and oxidative stress, which harm kidney tissues and reduce renal filtration.³⁹ Elevated serum creatinine, blood urea nitrogen (BUN), and electrolyte imbalances which are all important markers of declining kidney function, frequently reflect these pathological alterations.³⁰

The need to explore novel therapeutic options for diabetic nephropathy is urgent, owing not only to the rising incidence of diabetes but also to growing concerns about the safety profile of existing pharmacological agents. Although DPP-4 inhibitors show therapeutic promise, their use is limited by risks such as pancreatic cancer, immune-mediated disorders, hypersensitivity reactions, and potential drug-drug interactions,^{19,41} highlighting the need for safer and more effective alternatives. The therapeutic potential of natural compounds, especially those with anti-inflammatory and antioxidant properties, is drawing attention.^{2,3,8} A popular plant in West Africa, *V. amygdalina* is known for its traditional medical uses, which include anti-inflammatory, anti-diabetic, and anti-malarial properties.⁵ In this work, a high-fat diet and low-dose STZ-induced Type 2 diabetes rat model are used to examine the preventive effects of *V. amygdalina* on diabetic nephropathy.

Changes in body weight are one early sign of the onset of diabetes. In line with earlier research by¹³, who observed that insulin resistance or shortage results in increased fat and protein catabolism and impaired glucose consumption, diabetic rats in this study showed a substantial decrease in body weight following hyperglycemia induction. Renal stress is exacerbated by these metabolic abnormalities, which are exacerbated by excessive urination and dehydration. Nonetheless, diabetic rats given *V. amygdalina* saw a minor increase in body weight, which is in line with the results of⁴, who found that *V. amygdalina* enhances metabolic balance and nutrition utilisation.

Initially, all rats maintained normal blood glucose levels. Following hyperglycemia induction, the diabetic groups exhibited significantly elevated glucose levels, confirming successful model establishment.³⁶ Treatment with *Vernonia amygdalina* significantly reduced blood glucose levels compared to untreated diabetic rats, reinforcing its antihyperglycemic potential and aligning with the findings of²⁴.

Oxidative stress biomarkers further supported the protective effects of *Vernonia amygdalina*. In the diabetic group, levels of superoxide dismutase (SOD) and glutathione (GSH) were significantly reduced, while malondialdehyde (MDA) and

reactive oxygen species (ROS) were markedly elevated, indicating that hyperglycemia compromises the antioxidant defence system.³⁹ Treatment with *V. amygdalina* significantly modulated these oxidative markers, demonstrating antioxidant effects comparable to, or even exceeding, those observed in the Galvus-treated diabetic group.^{6,25,42}

Assessments of renal and electrolyte function revealed notable alterations across experimental groups. Diabetic rats (Group B) showed significantly elevated sodium and chloride levels compared to the control group (Group A), likely due to osmotic diuresis-induced dehydration commonly associated with hyperglycemia.⁹ Treatment with *Vernonia amygdalina* (Group C) moderated these electrolyte imbalances, bringing sodium and chloride levels closer to normal values. Interestingly, rats in the normal + *V. amygdalina* group (Group D) also showed normalized electrolyte values, suggesting no adverse effects from the extract in healthy animals. Serum urea and creatinine levels were significantly higher in diabetic rats (Group B), indicating impaired renal function. This aligns with existing evidence of diabetes-induced nephropathy. Treatment with *V. amygdalina* (Group C) led to a marked reduction in these biomarkers, reflecting improved renal function and consistent with the plant's renoprotective properties.²⁸ Notably, *V. amygdalina* was comparably effective to Galvus (Group E) in restoring urea and creatinine levels, highlighting its therapeutic potential. The normal + *V. amygdalina* group (Group D) maintained biochemical parameters similar to the control group, reinforcing the extract's safety in non-diabetic conditions. These findings collectively indicate that *V. amygdalina* not only attenuates diabetes-induced renal and electrolyte disturbances but does so with a safety profile comparable to that of conventional antidiabetic treatment.

Hematoxylin and eosin (H&E) staining revealed classical diabetic nephropathy (DN) features in the diabetic group, including tubular dilation and destruction, shrunken and distorted glomerular architecture, narrowed Bowman's space, and overall loss of cellular integrity.^{35,43} In contrast, rats treated with *Vernonia amygdalina* exhibited notable histological recovery, with evidence of tubular regeneration and improved glomerular structure. The urinary space, previously disjoined in diabetic rats, appeared crescent-shaped and more unified, indicating structural restoration.^{9,15,18} These improvements closely mirrored those observed in the diabetic group treated with Galvus.

The Normal + V. amygdalina group showed no histological alterations compared to the control, confirming the safety of V. amygdalina in non-diabetic conditions.⁹ Gordon and Sweet's staining further demonstrated the restoration of reticulin fibre architecture in V. amygdalina-treated rats, compared to the reduced reticulin fiber intensity observed in the diabetic-only group, supporting extracellular matrix structural recovery.²³

Conclusion

Moving beyond the uncertainties and high cost associated with antidiabetic drugs like Galvus, this study highlights the phytotherapeutic potential of *Vernonia amygdalina* in regulating blood glucose, modulating key biochemical markers, and restoring renal cellular integrity. Its comparable efficacy to Galvus, coupled with the absence of known side effects and its natural availability, positions V. amygdalina as a promising and accessible alternative to conventional DPP-4 inhibitors.

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