Antihyperglycaemic and Hepatorenal Protective Effects of Combined Aqueous Extracts of *Piper guineense* and *Vernonia amygdalina* in Alloxan-Induced Diabetic Rat model

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Abstract

Introduction: Diabetes mellitus is a chronic metabolic disorder associated with hepatic and renal complications due to persistent hyperglycemia and oxidative stress. Given limitations of conventional drugs, plant-based therapies are being explored. This study evaluated the hepatoprotective and nephroprotective effects of mixed aqueous extracts of Piper guineense and Vernonia amygdalina in alloxan-induced diabetic rats.

Materials and Methods: Thirty male Wistar rats were randomized into five groups (n=6): Normal control, Diabetic control, Positive control [metformin 150 mg/kg], and two treatment groups receiving 200 mg/kg and 400 mg/kg of 1:1 mixed extract orally for 28 days. Fasting blood glucose [FBG] and body weight were measured at days 0, 14, and 28. Serum liver and kidney function markers were analyzed, and liver/kidney tissues were processed for histology. Data were analyzed using One-way ANOVA in GraphPad Prism 10.2.

Results: Diabetic controls had elevated FBG, reduced %BWD, and deranged liver and kidney markers. Treatment with the extract significantly reduced final FBG [P=0.002] and increased %BWD in a dose-dependent manner. Liver enzymes [AST][ALT][ALP][GGT] were significantly lowered [P=0.002] while total protein and albumin were restored [P=0.033]. BUN and creatinine were significantly reduced [P=0.002] and electrolyte imbalance corrected [P=0.033]. Histology showed preserved hepatic and renal architecture at 400 mg/kg, comparable to metformin.

Conclusion: Mixed aqueous extracts of P. guineense and V. amygdalina exhibited significant hepatorenal protective effects in diabetic rats, supporting their ethnomedicinal use and potential as adjunct therapy for diabetes-related organ damage.

Keywords: *Piper guineense*, *Vernonia amygdalina*, Hepatorenal protection, Diabetes mellitus, Medicinal plants.

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The global prevalence of diabetes mellitus has been escalating, posing significant health challenges worldwide.[1] Chronic hyperglycemia in diabetes is associated with long-

term damage and dysfunction of various organs, particularly the liver and kidneys, leading to complications such as diabetic hepatopathy and nephropathy.[2]

Oxidative stress and inflammation are key contributors to the pathogenesis of diabetic complications. Excessive production of reactive oxygen species (ROS) in hyperglycemic conditions leads to cellular damage in hepatic and renal tissues.[2] Consequently, there is growing interest in natural antioxidants as potential therapeutic agents to mitigate oxidative damage in diabetes.

Vernonia amygdalina, commonly known as bitter leaf, is a widely used medicinal plant in Africa, renowned for its antidiabetic, antioxidant, and hepatoprotective properties. Studies have demonstrated that extracts of *V. amygdalina* can significantly reduce elevated liver enzymes and improve histological architecture in diabetic models, indicating its potential in managing diabetic hepatopathy.[3,4] Additionally, *V. amygdalina* has shown nephroprotective effects by reducing serum creatinine and urea levels in diabetic rats.[5,6]

Piper guineense, known as West African black pepper, is another indigenous plant with reported antidiabetic and antioxidant activities. While its individual effects have been studied, the combined impact of *P. guineense* and *V. amygdalina* on hepatic and renal functions in diabetic conditions remains underexplored.

This study evaluated the hepatorenal protective effects of combined aqueous extracts of *Piper guineense* and *Vernonia amygdalina* in alloxan-induced diabetic rats. In addition to assessing liver and kidney function biomarkers, this research also monitored changes in fasting blood glucose (FBG) and percentage body weight difference (%BWD) to provide a comprehensive understanding of the therapeutic potential of the plant combination in mitigating metabolic and organ-related complications of diabetes.

MATERIALS AND METHODS

Plant Collection, Preparation, and Extraction

Fresh leaves of *Piper guineense* and *Vernonia amygdalina* with herbarium reference no: UPH/P/042 and RSUPb320 from University of Port Harcourt and Rivers State University for *Piper guineense* and *Vernonia amygdalina* respectively were obtained from Mile 3 Market in Port Harcourt, Rivers State, Nigeria. Botanical authentication was conducted by Professor Green, Department of Plant Science and Biotechnology, Rivers State University. The plant samples were thoroughly washed, air-dried for two weeks, and then pulverized using an electric blender. The powdered leaves were mixed in a 1:1 ratio and extracted using hot aqueous maceration, following the methods previously described.[7,8]

Experimental Animals and Design

Thirty (30) male Wistar albino rats weighing between 200–250g were procured and housed in the animal facility of the Department of Pharmacology, Rivers State University. The animals were allowed to acclimatize for 14 days under standard conditions (12-hour light/dark cycle), with free access to food and water. The study conformed to the university's ethical committee procedure; all procedures conformed to CPCSEA guidelines.

Rats were randomly divided into five groups (n = 6 per group):

- **Group 1**: Normal control (Feed + water only)
- **Group 2**: Negative control (Alloxan, 120 mg/kg)
- **Group 3**: Positive control (120mg/kg

Alloxan + Metformin 150 mg/kg)
- **Group 4**: 120mg/kg Alloxan + 200 mg/kg mixed extract
- **Group 5**: 120mg/kg Alloxan + 400 mg/kg mixed extract

Hyperglycemia was induced in Groups 2 to 5 via a single intraperitoneal injection of alloxan monohydrate (120 mg/kg) (OECD, 2001). Fasting blood glucose (FBG) levels were monitored at three specific time points: initial (before alloxan administration), intermediate (72 hours post-induction to confirm hyperglycemia), and final (on day 28, after treatment). Blood glucose measurements were taken using a glucometer via tail vein puncture.

The body weight of each rat was measured and recorded at weekly intervals, and the percentage body weight difference (%BWD) was calculated using the formula:

$$\%BWD = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

This was used to assess the effect of diabetes and treatment on body mass regulation. Treatment with metformin and plant extracts commenced after hyperglycemia was confirmed and continued daily for 21 days. This procedure was carried out as previously described.[9]

Blood Sample Collection and Biochemical Assays

At the end of the treatment period, animals were fasted overnight, anaesthetized, and sacrificed. Blood samples were collected via cardiac puncture into EDTA-containing and plain tubes for biochemical assays. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until analysis.

Liver function biomarkers—AST, ALT, ALP, GGT, total protein (TP), albumin (ALB), total bilirubin (TB), and conjugated bilirubin (CB)—and kidney function indices—blood urea nitrogen (BUN), creatinine, sodium (Na^+), potassium (K^+), chloride (Cl^-), bicarbonate (HCO_3^-), and calcium (Ca^{2+})—were analyzed using standardized colorimetric and enzymatic protocols, as described by Odinga et al. (2023).

Histopathological Examination of the tissues (Odinga et al., 2023).

The liver and kidney tissue biopsies of the rats were fixed in 10% formalin, dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin wax. The tissues were then cut into 2- to 3-mm-thick sections by a microtome, fixed on the slides and stained with hematoxylin-eosin. The slides were examined under a light microscope (Olympus CH; Olympus, Tokyo, Japan), and photomicrographs were taken with a Leica DM 750 camera at ×100 magnification.

Data Analysis

Data were analyzed using GraphPad Prism® version 10.2 (San Diego, CA, USA). Results were expressed as mean ± standard error of the mean (SEM). Statistical differences among groups were determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Values with $p < 0.05$ were considered statistically significant.

Results and Discussion

Effect of Mixed Aqueous Extracts of Piper guineense and Vernonia amygdalina on Percentage Body Weight Difference in Alloxan-Induced Diabetic Male Wistar Rats

Alloxan-induced diabetes led to a significant reduction in the percentage body weight difference (%BWD) in the negative control group compared to the normal control as seen in figure 1, indicating catabolic weight loss typically seen in uncontrolled diabetes. This weight reduction is likely due to insulin deficiency, increased muscle proteolysis, and lipid mobilization.[10]

Treatment with the combined extracts significantly improved %BWD, with the 400 mg/kg group showing a near-normal percentage gain comparable to the metformin group. This suggests a reversal of diabetic cachexia and improved metabolic status in treated animals.[11] Restoration of body weight in diabetic rats treated with *V. amygdalina* and *P. guineense* has been previously reported and attributed to improved insulin activity and nutrient utilization.[12,13]

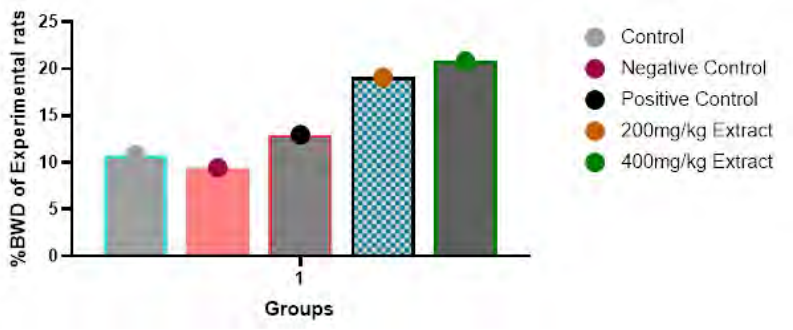


Figure 1: Effect of Mixed Aqueous Extracts of Piper guineense and Vernonia amygdalina on Percentage Body Weight Difference in Alloxan-Induced Diabetic Male Wistar Rats

Effect of Mixed Aqueous Extracts of Piper guineense and Vernonia amygdalina on Fasting Blood Glucose in Alloxan-Induced Diabetic Male Wistar Rats

Induction of diabetes with alloxan monohydrate resulted in a marked elevation in intermediate and final fasting blood glucose (FBG) levels in the diabetic control group compared to the normal control as shown in Figure 2. This hyperglycemic response reflects pancreatic β-cell destruction and insulin deficiency associated with alloxan toxicity. Treatment with the mixed aqueous extracts of *Piper guineense* and *Vernonia amygdalina* at both 200 mg/kg and 400 mg/kg significantly reduced final FBG levels compared to the diabetic control group ($p = 0.002$), producing results comparable to the metformin-treated group.

This hypoglycemic effect indicates the potential of the extract combination in improving glycemic control in diabetic conditions. The observed activity may be attributed to the phytochemicals in *V. amygdalina* and *P. guineense*, which have been reported to enhance insulin secretion, improve peripheral glucose uptake, and inhibit glucose absorption in the gut.[14,15] Previous studies have demonstrated that *V. amygdalina* can significantly lower blood glucose in alloxan and streptozotocin-induced diabetic models,[4,16] while *P. guineense* has shown similar effects through its rich content of alkaloids, flavonoids, and phenols.[17]

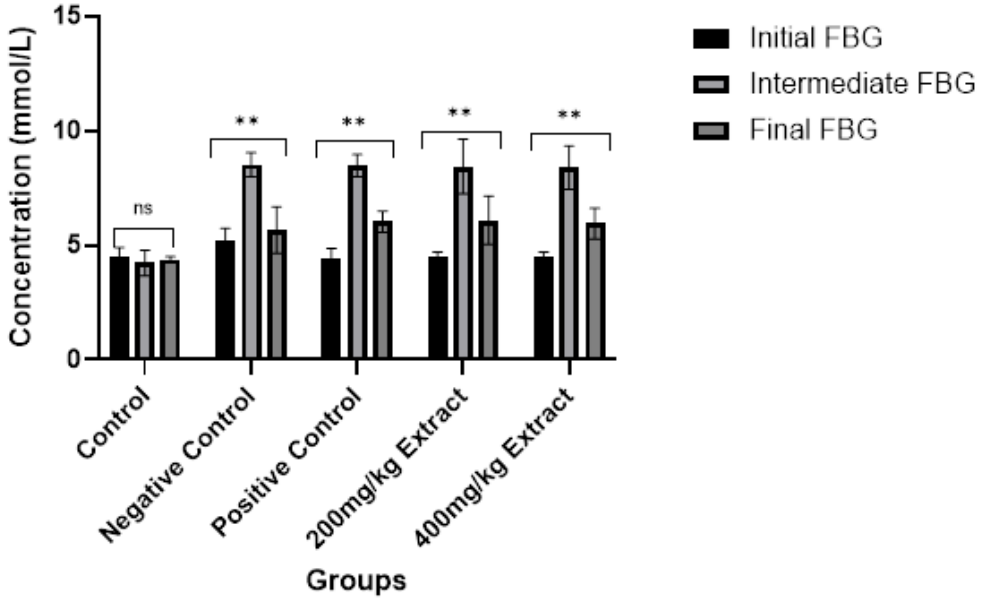


Figure 2: Effect of Mixed Aqueous Extracts of Piper guineense and Vernonia amygdalina on Fasting Blood Glucose in Alloxan-Induced Diabetic Male Wistar Rats

Key: 0.12(ns), 0.033(*), 0.002(**), <0.001(***), <0.0001 (***).

Effect of Mixed Aqueous Extracts of Piper guineense and Vernonia amygdalina on Liver Function Biomarkers in Alloxan-Induced Diabetic Male Wistar Rats.

The induction of diabetes using alloxan monohydrate significantly elevated liver enzymes—AST, ALT, ALP, and GGT—in the negative control group, indicating hepatic dysfunction. Treatment with mixed aqueous extracts of *Piper guineense* and *Vernonia amygdalina* at both 200 mg/kg and 400 mg/kg doses as shown in figure 3 resulted in a significant reduction in these enzyme levels ($p = 0.002$), comparable to the metformin-treated group. This finding suggests hepatoprotective effects and aligns with previous studies demonstrating the hepatoprotective and antioxidant activities of *V. amygdalina*, which mitigates hepatic damage through its antioxidant properties.[18]

Furthermore, total protein and albumin levels, which were decreased in the diabetic control group, were significantly restored in the extract-treated groups ($p = 0.033$), indicating improved hepatic synthetic function. Total and conjugated bilirubin levels, elevated in the diabetic control group, were significantly reduced in the extract-treated groups ($p = 0.033$), suggesting enhanced bilirubin clearance and liver function. These observations are consistent with the hepatoprotective effects of *V. amygdalina* and *P. guineense*, which have been reported to restore liver function markers in diabetic models.[19-21]

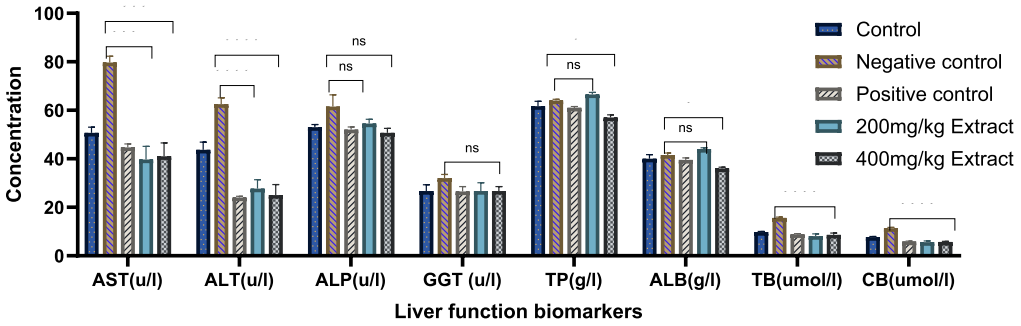


Figure 3: Effect of Mixed Aqueous Extracts of Piper guineense and Vernonia amygdalina on Liver Function Biomarkers in Alloxan-Induced Diabetic Male Wistar Rats.
Key: 0.12(ns), 0.033(*), 0.002(**), <0.001(***), <0.0001(****).

Effect of Mixed Aqueous Extracts of Piper guineense and Vernonia amygdalina on Kidney Function Biomarkers and Serum Electrolytes in Alloxan-Induced Diabetic Male Wistar Rats is as shown in Figure 4.

Alloxan-induced diabetes led to significant increases in renal biomarkers, with BUN and creatinine levels elevated in the negative control group as shown in figure 4. Treatment with the mixed extracts resulted in a dose-dependent decrease in these parameters ($p = 0.002$), comparable to the metformin group, indicating nephroprotective effects. These findings are supported by studies showing that *V. amygdalina* and *P. guineense* possess nephroprotective properties, reducing oxidative stress and improving renal function in diabetic models.[22,23]

Electrolyte imbalances observed in the diabetic control group, such as hyponatremia, hyperkalemia, and hypochloremia, were significantly corrected in the extract-treated groups ($p = 0.033$), indicating improved renal function and electrolyte balance. The ability of *V. amygdalina* and *P. guineense* to restore electrolyte balance has been documented in previous studies, highlighting their role in maintaining renal homeostasis in diabetic conditions.[21,22]

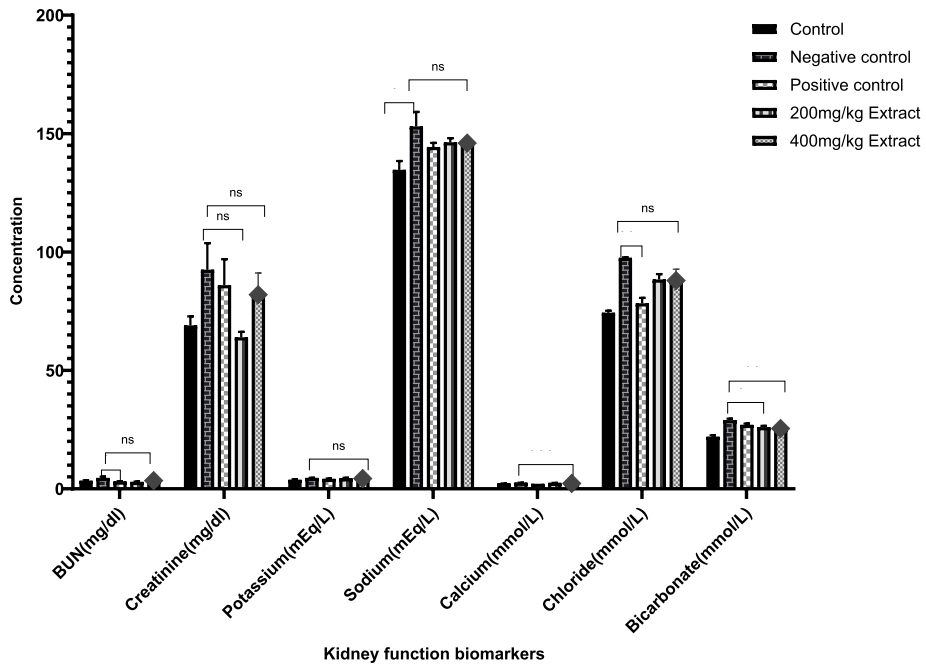
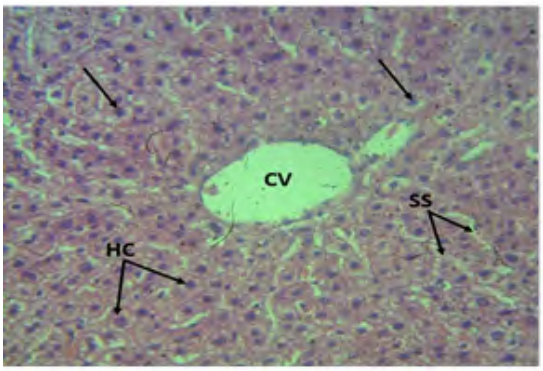


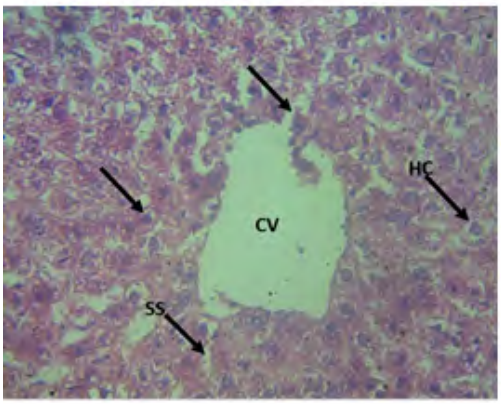
Figure 4: Effect of Mixed Aqueous Extracts of Piper guineense and Vernonia amygdalina on Kidney Function Biomarkers and Serum Electrolytes in Alloxan-Induced Diabetic Male Wistar Rats.
Key: 0.12(ns), 0.033(*), 0.002(**), <0.001(***), <0.0001(***).

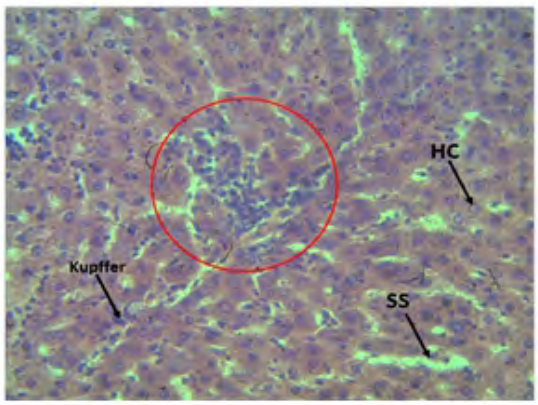
Histopathological Examination of the Liver and Kidneys of the Experimental rats

Photomicrograph 1 of the liver of the experimental rats showed a normal appearance of the liver tissues for the group 1 (Normal control) with normal central veins, adjacent sinusoids with hepatocytes. The negative control group photomicrographs (2a and 2b) revealed a disrupted morphology of the central venules and hepatocyte degeneration implying inflammation and second-degree distortion of the liver tissues. Previous studies have reported a distortion of the liver cells when exposed to hyperglycemic conditions without treatment.[24] The Positive control group treated with metformin showed an ameliorating state of the inflamed and distorted liver as shown in Photomicrograph 3a and 3b. The liver tissues showed mild distortion of the liver tissues while the groups treated with 200mg/kg and 400mg/kg extract revealed moderate distortion of the liver tissues as shown in photomicrographs 4 and 5. The amelioration of the inflamed liver tissues by the mixed extract of *Piper guineense* and *Vernonia amygdalina* is comparable to that of the metformin treated groups. The potency of the mixed extract could be associated with the phytochemicals present in the plants extract with greater efficiency when combined.[24] These phytochemicals have been associated with their ability to inhibit or modulate the MAPK and NF-kB pathways, thereby reducing inflammation of tissues.[26]



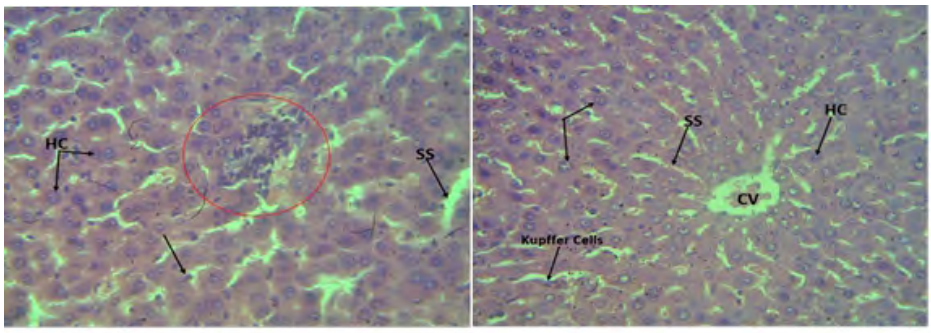
Photomicrograph 1: (H&E X400) of the liver outlining a well normal Central vein (CV) with adjacent sinusoids (SS) also with the hepatocytes (HC) (arrows). **Diagnosis:** Normal appearance of the liver tissue





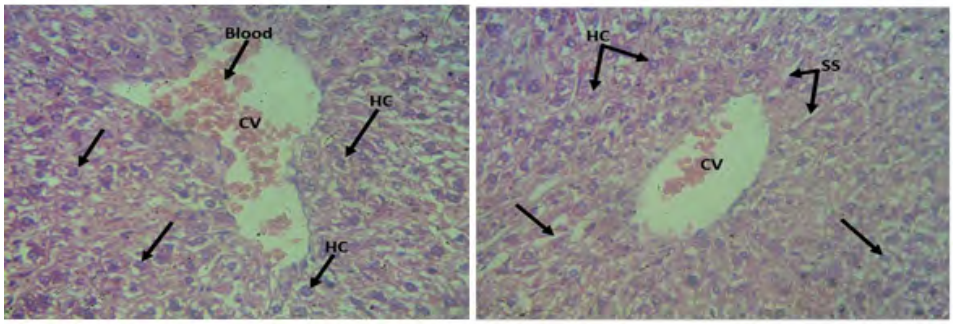
Photomicrograph 2a: (H&E X400) of the liver showing disrupted morphology of the central venules (CV); hepatocytes (HC), and sinusoids (SS) within zone 3 of the liver parenchymal (arrows). Diagnosis: Second degree distortion of the liver tissue

Photomicrograph 2b: (X400 H&E stain) of the liver showing lymphocytic activities (red circle) with hepatocytes degeneration (HC) and sinusoidal dilatation (arrows). **Diagnosis:** Severe Inflammation and distortion of the liver tissue.



Photomicrograph 3a: (X400 H&E stain) of the liver showing reduced lymphocytic infiltration (red circle). **Diagnosis:** Moderate Inflammation of the liver tissue

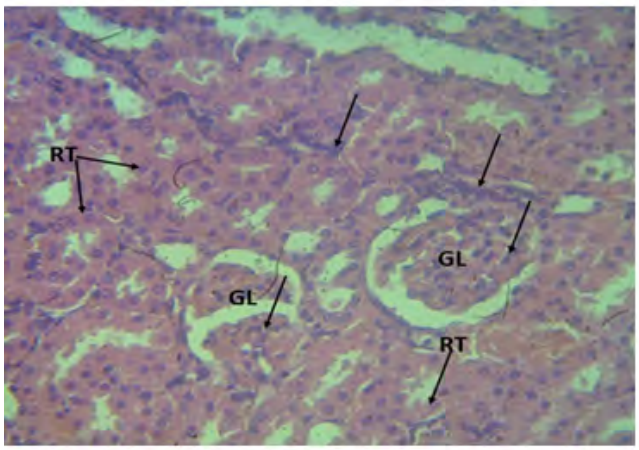
Photomicrograph 3b: (X400 H&E stain) of the liver showing the sinusoid (SS); congestion of the central vein (CV) (arrows). **Diagnosis:** Mild distortion of the liver tissue.



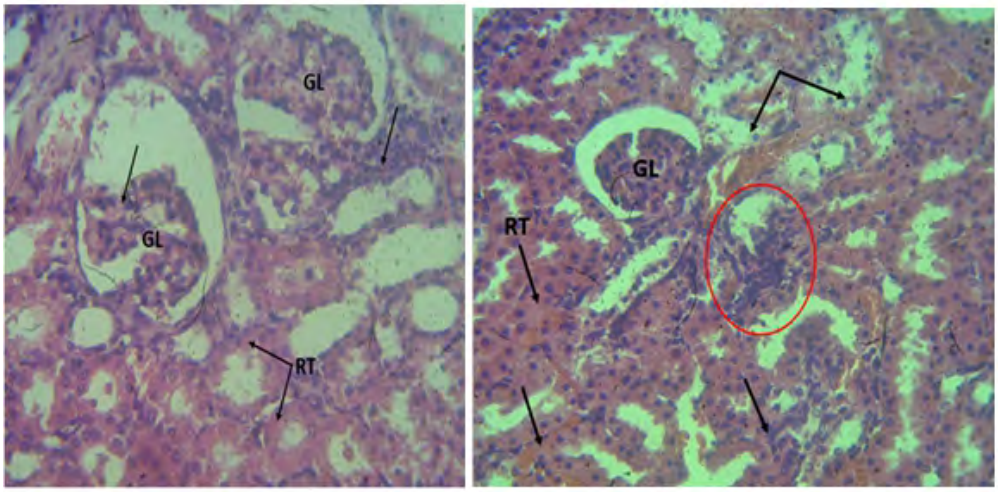
Photomicrograph 4: (H&E X400) of the liver showing hepatocyte hypertrophy and fatty degeneration (arrows). **Diagnosis:** Moderate Distortion of the liver tissue.
Photomicrograph 5: (H&E X400) of the liver showing reduced hepatocyte hypertrophy and fatty degeneration (arrows). **Diagnosis:** Moderate Distortion of the liver tissue.

Histopathological examination of the kidney tissues of experimental rats

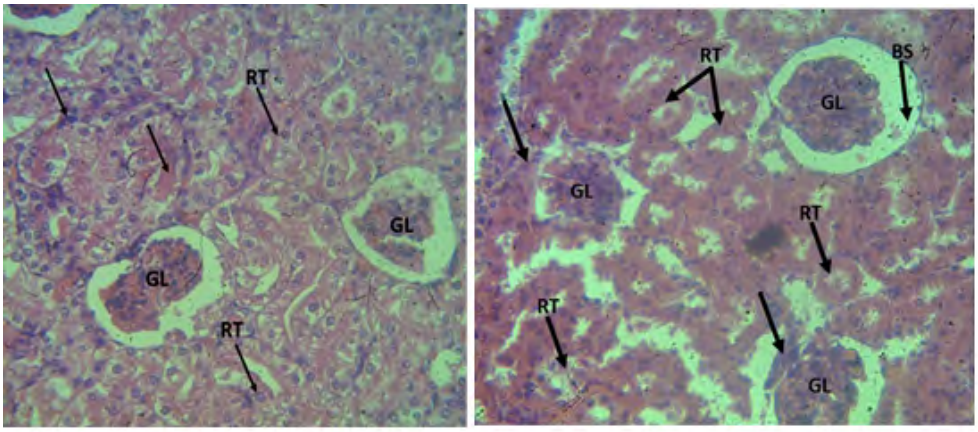
The kidney tissues of the experimental rats as shown in photomicrograph 1a for the normal control group had a normal appearance of the kidney tissue evidenced by the presence of the glomerulus, reduced podocytes and mild lymphoctytic activities around the renal tubules. Severe distortion, inflammation and degeneration of the kidney tissues were observed in the negative control group as shown in Photomicrographs 2a&b. These effects could be associated with the inflammation caused by the inducement of hyperglycemia in the experimental rats in this group using alloxan and were not subjected to any form of treatment.[2] Alloxan monohydrate in experimental rat models has been reported to cause inflammation and damage to the liver and kidney tissues.[27] Treatment with metformin and the mixed extracts of *Piper guineense* and *Vernonia amygdalina* at 200mg/kg and 400mg/kg caused a reduced inflammatory state of the kidney as seen in Photomicrographs 3a&b, 4a&b, 5a&b for metformin (Positive control), 200mg/kg and 400mg/kg mixed extract of *Piper guineense* and *Vernonia amygdalina* Respectively. The use of plant extract as therapeutics has been reported to be comparable to the conventional antidiabetic medications with even less side effects due to their role in the metabolism of inflammation markers such as TNF-alpha, IL-6, IL-1B, and mcp-1.[28]



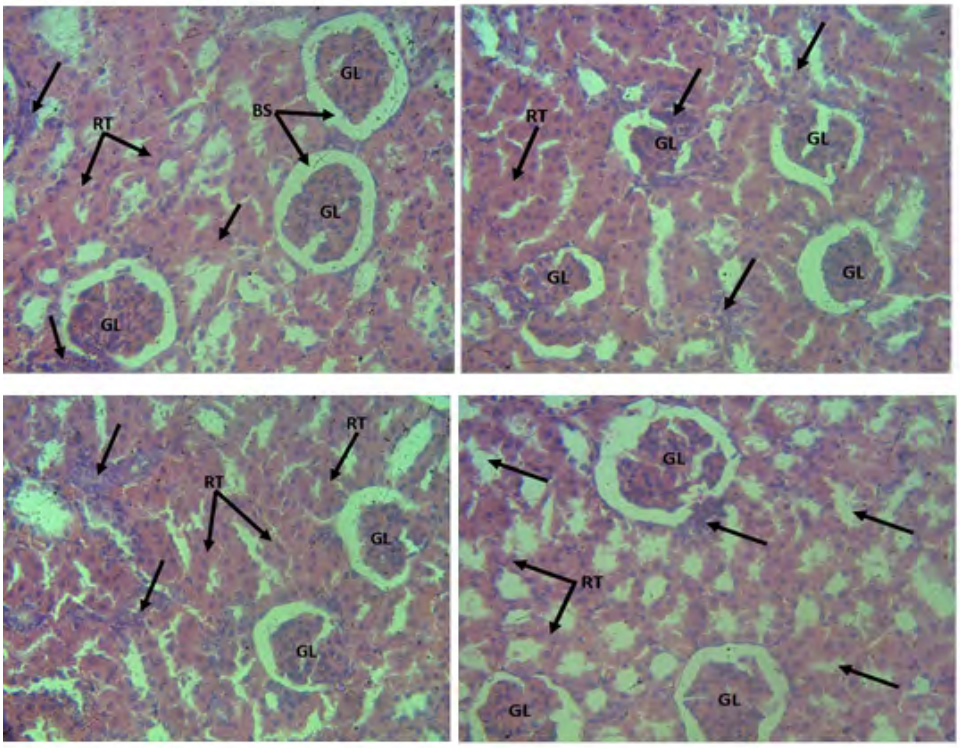
Photomicrograph 1a: (X400 H&E stain) of the kidney showing the glomerulus (GL) with reduced podocytes (red) and mild lymphoctytic activities surrounding the renal tubules (RT) (blue arrows) **Diagnosis:** Normal appearance of the kidney tissue.



Photomicrograph 2a. (X400 H&E stain) of the kidney showing focal lymphocytic activities; glomerular atrophy with reduced podocyte and interstitial oedema (arrows). **Diagnosis**: Severe distortion of the kidney tissue. **Photomicrograph 2b**. (X400 H&E stain) of the kidney showing lymphocytic infiltration (red circle) along the renal tubules and glomerular atrophy with reduced podocytes and hemosiderin deposit (arrow). **Diagnosis:** Inflammation and degeneration of the kidney tissue.



Photomicrograph 3a. (X400 H&E stain) of the kidney showing well delineated parietal epithelia cells lining the renal tubules; glomerular atrophy. **Diagnosis:** Mild glomerular distortion of the kidney tissue. **Photomicrograph 3b.** (H&E X400) of the Kidney showing renal tubular distention. **Diagnosis**: Moderate distortion of the kidney tissue.



Photomicrograph 4a. (H&E X400) of the Kidney showing reduced lymphocytic activities within the renal tubules with interstitial oedema(arrows). **Diagnosis**: Minimal Inflammation of the kidney tissue. **Photomicrograph 4b.** (H&E X400) of the Kidney showing reduced lymphocytic activities within the renal tubules (arrows). **Diagnosis**: mild Inflammation of the kidney tissue. **Photomicrograph 5a&b.** (H&E X400) of the Kidney showing renal tubules associated with interstitial oedema(arrows). **Diagnosis**: Mild Inflammation of the kidney tissue.

Conclusion

The present study demonstrates that mixed aqueous extracts of *Piper guineense* and *Vernonia amygdalina* exert significant antidiabetic, hepatoprotective, and nephroprotective effects in an alloxan-induced diabetic rat model. In addition to normalizing elevated liver enzymes and improving renal biomarkers and electrolyte balance, the extracts significantly reduced fasting blood glucose levels and improved percentage body weight difference. These findings indicate that the plant extracts not only mitigate diabetes-induced organ dysfunction but also support systemic metabolic recovery. The therapeutic effects are likely attributable to the phytochemical constituents with antioxidant, anti-inflammatory, insulin-modulating, and cytoprotective properties. Collectively, these results validate the traditional use of *P. guineense* and *V. amygdalina* in managing diabetes and its complications, and provide a scientific foundation for their further pharmacological development.

Recommendation

Further research is recommended to validate the efficacy and safety of *P. guineense* and *V. amygdalina* extracts through comprehensive toxicity studies, mechanistic evaluations, and clinical trials. Investigations into their synergistic mechanisms and formulation as standardized antidiabetic herbal products could support their integration into evidence-based complementary therapy for diabetes mellitus and its systemic complications.

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