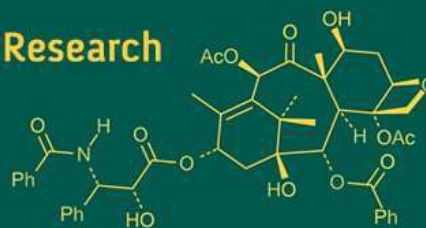


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Lipid-lowering and antioxidant potentials of mixed aqueous extracts of *Piper guineense* and *Vernonia amygdalina* in Alloxan-induced diabetic rat model

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Abstract

Background and Objective: Diabetes mellitus is a metabolic disorder with severe cardiovascular complications. Given the limitations of conventional treatments, alternative therapies are essential. This study evaluated the hypoglycemic, lipid-lowering and antioxidant effects of mixed aqueous extracts of *Piper guineense* and *Vernonia amygdalina* in alloxan-induced hyperglycemic rat models.

Methods: Thirty male Wistar rats were randomly divided into five groups (n = 6 per group): Control, Negative control, Positive control, 200 mg/kg and 400 mg/kg of the mixed extract for 21 days. The Fasting blood glucose levels were measured before induction, before treatment and before sacrifice of the rats. GraphPad prism 10.2 was used for the statistical analysis with significance at $p < 0.05$.

Results: Obtained revealed an elevation in the FBG levels after the induction of hyperglycemia with alloxan monohydrate and a concurrent decrease in the FBG levels after treatment with 200mg/kg and 400mg/kg mixed extracts of *Piper guineense* and *Vernonia amygdalina*. The lipid profile markers: TC, TG, LDL and VLDL showed a decrease in the groups treated with 200mg/kg and 400mg/kg bw of the mixed extract when compared with the negative group at $P = 0.033$. However, an increase in HDL was observed in all groups treated with varying concentrations of the mixed extract when compared with the negative control ($P = 0.033$). The values of the mixed extract groups were comparable with the positive control group which served as the standard drug group. An increase in the GSH ($P = 0.033$), GPx ($P = 0.002$), GST ($P = 0.033$), Catalase ($P = 0.002$) and SOD ($P = 0.033$) levels were observed in comparison to the negative control group. Malondialdehyde serum levels recorded a significant decrease at $P = 0.033$ when compared to the negative control group.

Conclusion: The mixed aqueous extracts of *Piper guineense* and *Vernonia amygdalina* significantly improved glycemic control, lowered the lipid profile, and improved the antioxidant status in the hyperglycemic rats, with the 400 mg/kg dose demonstrating the most pronounced effects. The study further highlights the enhanced potential of combined extracts as therapeutics for the management of hyperglycemic conditions and improvement of health, evidenced by the antioxidant status and lipid profile. This agrees with the WHO's advocate on the use of natural products for therapeutics. Further clinical trials are recommended.

Keywords: Mixed extracts of *Piper guineense*, *Vernonia amygdalina*, hyperglycemia, lipid profile, antioxidants, medicinal plants

Introduction

Diabetes mellitus, a multifactorial metabolic disorder characterized by chronic hyperglycemia, is a major global health concern with increasing prevalence and associated morbidity and mortality (American Diabetes Association, 2022) [2]. The disease is primarily linked to impaired insulin secretion, insulin resistance, or both, leading to disturbances in carbohydrate, lipid, and protein metabolism (Mensink, 2024) [19]. Hyperglycemia is often accompanied by dyslipidemia and oxidative stress, two critical factors that contribute to the pathogenesis of diabetic complications, including cardiovascular diseases, nephropathy, and neuropathy (Giacco & Brownlee 2010) [11].

Lipid profile disturbances such as elevated total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol

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(VLDL-C), alongside reduced high-density lipoprotein cholesterol (HDL-C), are frequently observed in diabetic patients (Szczepanek-Parulska *et al.*, 2022) [29]. Moreover, oxidative stress, resulting from excessive generation of reactive oxygen species (ROS) and weakened antioxidant defense systems, exacerbates lipid peroxidation and cellular damage (Birben *et al.*, 2012) [3]. Biomarkers such as malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GPx), glutathione-S-transferase (GST), catalase (CAT), and superoxide dismutase (SOD) are commonly evaluated to assess oxidative stress and antioxidant status in diabetic models (Wang *et al.*, 2020) [31].

Conventional antidiabetic therapies, although effective, are often limited by side effects, high costs, and poor accessibility in resource-limited settings. Consequently, the exploration of plant-based alternatives has gained significant scientific attention due to their ethnomedicinal relevance, safety profile, and bioactive compounds with antioxidant and lipid-lowering properties (Josa *et al.*, 2024) [14].

Piper guineense (Uziza) and *Vernonia amygdalina* (commonly known as bitter leaf) are two indigenous medicinal plants widely utilized in African traditional medicine. *Piper guineense* is rich in alkaloids, flavonoids, and essential oils, with reported antioxidants, anti-inflammatory, and hypolipidemic activities (Salaimon *et al.*, 2020) [28]. *Vernonia amygdalina*, on the other hand, contains bioactive phytochemicals such as sesquiterpene lactones, saponins, and phenolic compounds, and has been shown to exert antidiabetic, antihyperlipidemic, and antioxidant effects (Michel *et al.*, 2020) [20].

Although several studies have independently documented the pharmacological effects of *Piper guineense* and *Vernonia amygdalina*, there is a dearth of scientific data evaluating their combined effect on lipid metabolism and oxidative stress parameters, particularly in diabetic conditions. This research, therefore, seeks to bridge this knowledge gap by investigating the effect of mixed aqueous extracts of *Piper guineense* and *Vernonia amygdalina* on the lipid profile and antioxidant status in alloxan-induced hyperglycemic male Wistar rats.

The outcome of this study may provide empirical evidence supporting the synergistic therapeutic potential of these plants in mitigating hyperglycemia-induced dyslipidemia and oxidative stress, thereby contributing to the development of cost-effective, plant-based alternatives in diabetes management.

Materials and Methods

Plant Collection, Preparation, and Extraction

Fresh samples of *Piper guineense* and *Vernonia amygdalina* were obtained from Mile 3 Market in Port Harcourt, Rivers State, Nigeria. The plants were identified by Professor Green of the department of Plant Science and Biotechnology, Rivers State University. The plant materials were thoroughly washed to remove impurities and subsequently air-dried for two weeks. The dried leaves were pulverized into fine powder using an electric blender. They were prepared in 50:50 ratio for *Piper guineense* and *Vernonia amygdalina*. This extraction procedure follows the methods described by Frimpong *et al.* (2014) [9], Omeodu *et al.*, (2023) [24], Ahmad *et al.* (2023) [1].

Experimental Design

A total of 30 male Wistar rats (200-250g) were used for the study. The rats were procured and housed in the animal facility of the Department of Pharmacology, Rivers State University, where they were acclimatized for 14 days under standard environmental conditions (12-hour light/dark cycle) with unrestricted access to feed and water. Animal experiments and procedures ethical standards were in accordance with the guidelines provided by the CPCSEA via the Rivers State University ethical committee.

The animals were randomly divided into five groups (n = 6 per group), as outlined below:

- **Group 1:** Normal control (feed + water)
- **Group 2:** Negative control (feed + water + 120 mg/kg alloxan)
- **Group 3:** Positive control (feed + water + 120 mg/kg alloxan + 150 mg/kg metformin)
- **Group 4:** Feed + water + 120 mg/kg alloxan + 200 mg/kg plant extract
- **Group 5:** Feed + water + 120 mg/kg alloxan + 400 mg/kg plant extract

Body Weight Measurement

The experimental rats were weighed using an Atom electronic balance at weekly intervals to monitor changes in body weight. The percentage body weight gain or loss (%BWD) was calculated as:

$$\%BWD = \frac{(\text{Body weight on last day} - \text{Body weight on day one})}{\text{Body weight on day one}} \times 100\%$$

Hyperglycemia was induced in groups 2-5 via intraperitoneal administration of 120 mg/kg alloxan monohydrate. After three days, blood glucose levels were measured to confirm hyperglycemia before commencing treatment. Treatment lasted for 21 days, after which the animals were fasted overnight and sacrificed. Blood samples were collected in EDTA bottles for biochemical analysis of total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, and very-low-density lipoprotein were analyzed using enzymatic colorimetric methods as described by Chu *et al.* (2019) [6] for Lipid Profile status, while the antioxidant status of the experimental animals was assessed by determining malondialdehyde, reduced glutathione, catalase, superoxide dismutase, glutathione-S-transferase, and glutathione peroxidase levels using standard procedures as described by Gabriel-Brisibe *et al.*, (2020) [10] and Odinga-Israel *et al.*, (2024) [23].

Data analysis

GraphPad Prism® software 10.2 (San Diego, California, CA, USA) was used for the statistical analysis. The concentrations of the various markers were expressed as mean±SEM. One way ANOVA was used to compare the means among the groups and differences with a 95% confidence interval, resulting in $p < 0.05$ were considered significant using Tukey HSD test for multiple comparison.

Results and Discussions

This study investigated the effect of the mixed aqueous extract of *Piper guineense* and *Vernonia amygdalina* on the lipid profile and antioxidant status in alloxan-induced hyperglycemic male rats models. The results demonstrated a significant reduction in fasting blood glucose (FBS) and

improvement in lipid profiles, indicating the potential therapeutic value of these plants. The observed effects are likely due to the phytochemical constituents of the extracts, which exert hypoglycemic and lipid-regulating effects through multiple metabolic pathways. (Odinga and Nwaokezi, 2020) ^[10]. Several studies have reported findings on the hypoglycemic potential of *Vernonia amygdalina* and *Piper guineense* individually, however, there has not been any reported study on the potentials of the mixed extract on various conditions, thus this study is novel in this.

Effect of the mixed Extract of *Piper guineense* and *Vernonia amygdalina* on the Fasting Blood Glucose (FBG) levels of alloxan-induced hyperglycemic male Wistar rats

The induction of hyperglycemia with alloxan monohydrate has been reported by Kim, (2024) ^[15] as an effective experimental process. Figure 1 represents the FBG levels of the experimental rats before the inducement of hyperglycemia with Alloxan monohydrate, before treatment with Mixed extract of *Piper guineense* and *Vernonia amygdalina* and after treatment. The results revealed a

statistically significant increase ($P = 0.002$) in the FBG level after the induction of hyperglycemia using alloxan monohydrate in all the experimental groups (Intermediate FBG). A decrease in the FBG levels of the experimental rats in all the groups except the control was observed after the treatment with *Piper guineense* and *Vernonia amygdalina* at $P = 0.002$. These values are comparable to the metformin-treated group (Positive control), suggesting that the extracts exert similar glucose-lowering effects to standard antidiabetic medication. Demonstrated that *Vernonia amygdalina* enhanced insulin sensitivity by upregulating glucose transporter type 4 (GLUT4) expression in skeletal muscle, leading to improved glucose uptake. Similarly, Chukwuma *et al.* (2021) ^[7] reported that *Piper guineense* modulates glucose metabolism by inhibiting α -amylase and α -glucosidase, thereby reducing postprandial hyperglycemia. The presence of bioactive compounds such as flavonoids, alkaloids, and terpenoids in these plants has been implicated in their antidiabetic effects through the modulation of insulin signaling pathways, pancreatic β -cell protection, and inhibition of hepatic gluconeogenesis (Egbuna *et al.*, 2021; Roy *et al.*, 2024) ^[7, 26].

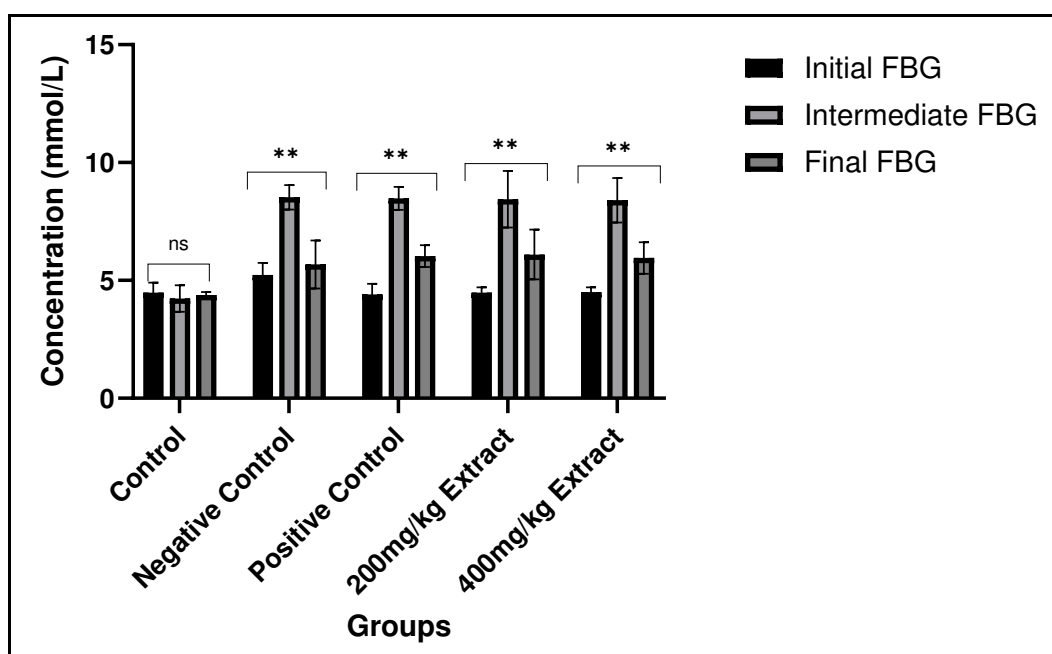


Fig 1: Fasting Blood Glucose (FBG) of Experimental rats Initial FBG = FBG before inducement with Alloxan, Intermediate FBG = FBG before treatment, Final FBG = FBG before Sacrifice. 0.12(ns), 0.033(*), 0.002(**), <0.001(***), <0.0001(****).

Body Weight Difference of alloxan-induced male Wistar rats.

The experimental rats as shown in Figure 2 had an increase in their %BWD. Weight loss is a hallmark of uncontrolled diabetes due to increased muscle catabolism and lipid oxidation (Gosmanov *et al.*, 2000) ^[12]. The negative control rats exhibited significant weight loss, while extract-treated groups maintained relatively stable body weights. Notably, the 400 mg/kg extract group experienced a 20.82%

reduction, slightly higher than the metformin group (8.95%). These findings align with the work of Erukainure *et al.* (2019) ^[8], who observed that *Vernonia amygdalina* improves energy metabolism by enhancing mitochondrial function and reducing oxidative stress in diabetic models. The slight increase in weight reduction in extract-treated groups may be attributed to increased basal metabolic rate and enhanced glucose utilization, as suggested by previous reports on plant-derived bioactive compounds.

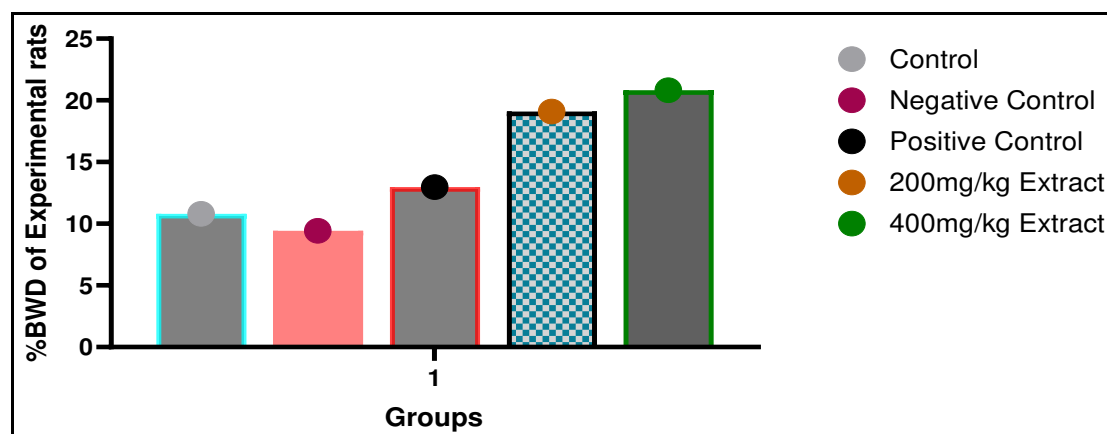


Fig 2: %Body Weight Difference of Experimental rats.

Effect of the mixed extract of *Piper guineense* and *Vernonia amygdalina* on the Lipid profile of alloxan-induced hyperglycemic male wistar rats.

Figure 3 shows the lipid profiles of alloxan-induced hyperglycemic male Wistar rats exposed to the mixed extract of *Piper guineense* and *Vernonia amygdalina*. Dyslipidemia is a major complication of diabetes, characterized by elevated total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), coupled with reduced high-density lipoprotein cholesterol (HDL-C) (Lokpo *et al.*, 2022) [18]. Total Cholesterol, and Triglycerides are two markers of lipid profile used in the assessment of heart disease and other conditions by measuring the levels of lipids in the blood (Lee *et al.*, 2023) [17]. TC gives information on the amount of cholesterol in the blood, TGs are fats used as energy in the body, however, an excess of TG in the serum has been associated with an increase in the risk of heart disease and stroke (Pappan *et al.*, 2025) [25]. Hyperglycemia has also been associated with heart diseases and stroke as its risk factor (Mosenzon *et al.*, 2023) [21]. Figure 3 revealed a decrease in the TC and TG in the experimental rat groups exposed to mixed extract of *Piper guineense* and *Vernonia amygdalina* at 400mg/kg extract when compared to the negative control group ($P = 0.033$). A statistical Increase ($P = 0.033$) in the HDL level and a corresponding decrease ($P = 0.033$) in the LDL level was observed in all the experimental hyperglycemic rats exposed to varying concentration of the mixed extract in comparison with the

Negative control group. LDL which is often referred to bad cholesterol, has been associated with an increase in the risk of developing cardiovascular diseases and its various risk factors. A decrease in the LDL level as reported by Odinga *et al.*, (2020) [10] has been associated with plants due to the phytochemicals potentials to inhibit cholesterol absorption in the intestines by inhibiting the activities of specific proteins involved in cholesterol uptake such as NPC1L1 and ACAT2, enhancing reverse cholesterol transport and reducing cholesterol synthesis in the liver (Islam *et al.*, 2021). An increase in HDL as seen in Figure 3 may be implicative of the potential of the mixed extract of study to lower risk of hyperglycemia and its accompanying disease such as heart disease, this may be due to the action of HDL to remove excess cholesterol from the blood and deliver it to the liver (Trimarco *et al.*, 2022) [30]. These effects were comparable to metformin, which suggests that the extracts possess lipid-lowering properties. Several studies have corroborated these findings. Reported that *Vernonia amygdalina* reduces hepatic cholesterol synthesis by downregulating HMG-CoA reductase, a key enzyme in cholesterol biosynthesis. Additionally, *Piper guineense* has been shown to enhance bile acid excretion, thereby reducing circulating lipid levels (Uhegbu *et al.*, 2015) [4]. The observed increase in HDL-C in extract-treated groups further supports their cardioprotective role, as elevated HDL-C is associated with improved lipid transport and reduced atherosclerosis risk.

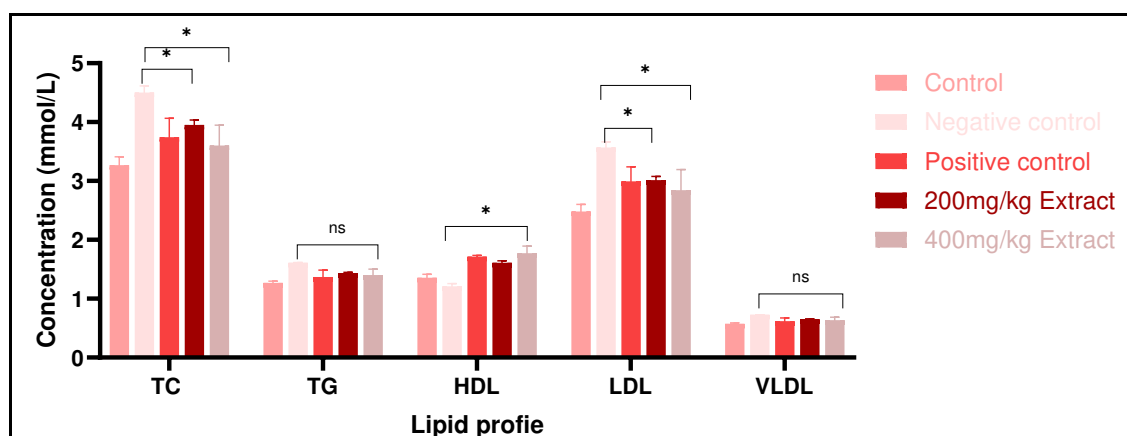


Fig 3: Effect of the mixed extract of *Piper guineense* and *Vernonia amygdalina* on the Lipid profile of alloxan-induced hyperglycemic male wistar rats.

Key: 0.12(ns), 0.033(*), 0.002(**), <0.001(***), <0.0001(****). TC = Total Cholesterol, TG = Triglycerides, HDL = High Density Lipoprotein, LDL = Low Density Lipoprotein, VLDL = Very Low-Density Lipoprotein

Effect of the mixed extract of *Piper guineense* and *Vernonia amygdalina* on the Antioxidant status of alloxan-induced hyperglycemic male wistar rats.

A significant increase in the GSH ($P = 0.033$), GPx ($P = 0.002$), GST ($P = 0.033$), Catalase ($P = 0.002$), and SOD ($P = 0.033$) were observed in the experimental groups exposed to 200mg/kg and 400mg/kg mixed extract of *Piper guineense* and *Vernonia amygdalina* Shown in Figure 4. These increases in the antioxidant marker levels were in comparison to the negative control hyperglycemic groups. MDA which is a product of lipid peroxidation was observed to have a decrease in the groups administered the mixed extract of *Piper guineense* and *Vernonia amygdalina* at varying concentrations in comparison to the control group ($P = 0.033$). Oxidative stress is a key driver of diabetes complications, leading to cellular damage, inflammation, and β -cell dysfunction (Caturano *et al.*, 2023) [5]. The assessment of antioxidant markers in this study revealed that the negative control group exhibited significantly reduced catalase activity compared to the control group, along with increased MDA levels, indicating heightened oxidative stress. This confirms previous findings that diabetes induces oxidative damage by overwhelming the endogenous antioxidant system (Kowluru & Mishra 2015) [16]. Treatment

with *Piper guineense* and *Vernonia amygdalina* extracts led to a notable improvement in antioxidant defenses. The results of the antioxidant activities indicate a possible enhancement of detoxification pathways. These findings indicate that while *Piper guineense* and *Vernonia amygdalina* exhibit antioxidant potential, their effects are selective, with more pronounced impacts on CATA, GST, and SOD rather than GPx. These results align with previous research highlighting the antioxidant potential of *Vernonia amygdalina* and its ability to upregulate nuclear factor erythroid 2-related factor 2 (Nrf2), a key transcription factor involved in cellular antioxidant defense. Similarly, *Piper guineense* has been reported to enhance SOD activity and reduce reactive oxygen species (ROS)-induced damage (Moukette *et al.*, 2015) [22]. The observed effects may be attributed to the polyphenols, flavonoids, and alkaloids present in these extracts, which act as free radical scavengers and cytoprotective agents (Saeed *et al.*, 2012) [27].

Overall, the findings suggest the enhanced effectiveness of the mixed extract of *Piper guineense* and *Vernonia amygdalina* extracts to oxidative stress reduction in diabetic conditions, though their effects on specific antioxidant enzymes vary.

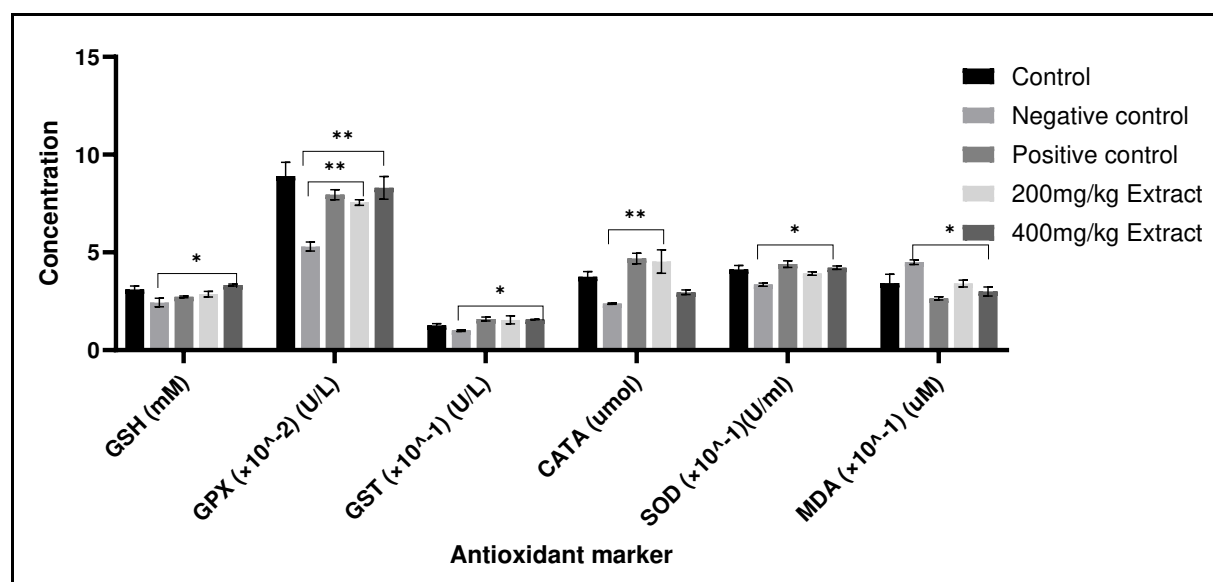


Fig 4: Effect of the mixed extract of *Piper guineense* and *Vernonia amygdalina* on the Antioxidant status of alloxan-induced hyperglycemic male wistar rats

Key: 0.12(ns), 0.033(*), 0.002(**), <0.001(***), <0.0001(****). GSH = Reduced Glutathione, GPx = Glutathione Peroxidase, GST = Glutathione-S-transferase, CATA = Catalase, SOD = Superoxide dismutase, MDA = Malondialdehyde

Conclusion

The findings of this study revealed that hyperglycemia was associated with significant dyslipidemia and oxidative stress, as observed in the negative control group. However, administration of the plant extracts at 200 mg/kg and 400 mg/kg led to an improvement in lipid profile parameters, with reductions in total cholesterol, triglycerides, LDL and VLDL alongside an increase in HDL. Additionally, the extracts enhanced antioxidant enzyme activities (SOD, CAT, GPx, GST, and GSH) while reducing lipid peroxidation, as evidenced by lower MDA levels. These results suggest the enhanced effectiveness of the mixed extract of *Piper guineense* and *Vernonia amygdalina* as it possess synergistic hypolipidemic and antioxidant properties that may contribute to mitigating hyperglycemia-induced

metabolic complications. Thus, the combined use of these plants could offer a promising, cost-effective, and accessible alternative for managing diabetes and its associated complications. Further research, including clinical trials, is recommended to explore their therapeutic potential in humans.

Recommendation

Further studies are required to elucidate the precise molecular mechanisms involved, particularly in relation to dose-dependent responses and long-term effects.

Conflict of Interest

The authors declare no conflict of interest

Authors Contributions

TO: Concept, design of study, data analysis, statistical analysis, manuscript editing.

PACA: Experimental studies, data analysis, manuscript preparation.

WN: Experimental studies, manuscript review

CCN: Manuscript preparation

OE: Manuscript editing, Manuscript review.

The manuscript has been read and approved by all the authors.

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