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# Assessment of Antihypergycemic Effect of Nut Fractions of *Vigna Subterranea* on Streptozotocin-Induced Diabetic Rats

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**ABSTRACT**

Bambaranut (Vigna subterranea) is a well-known staple food in Nigeria and belongs to the Fabaceae family, and it is known for its potential antidiabetic properties. This present study was conducted to determine which fraction of the nut (hexane, ethyl acetate, methanol, and aqueous) contains the most bioactive compounds with antihyperglycemic and antihyperlipidemic effects. The choice of solvent for fractionation was based on increasing polarity. Albino rats weighing between 170-200g were divided into seven groups, each consisting of five rats. Group A served as the negative control, Group B as the positive control, Group C was given the standard drug metformin 200mg, and Groups D to G were diabetic rats fed with four different fractions of the nut at a dosage of 200mg per body weight. Diabetes was induced in the albino rats through intraperitoneal administration of streptozotocin. Over a period of 28 days, blood glucose levels of the rat groups were measured using a glucometer at 7-day intervals. At the end of the 28-day study, biochemical and hematological assessments were conducted. A significant decrease in blood glucose levels was observed in the albino rat groups fed with the hexane, ethyl acetate, and methanolic fractions. However, the aqueous fraction did not show a significant reduction in blood glucose levels compared to the diabetic and normal control groups ($P \leq 0.005$).Furthermore, the groups that received the different fractions of the nut exhibited significantly lower fasting blood sugar levels and improved biochemical markers. At the end of this study, Serum enzyme markers, lipid profiles, and hematological parameters also showed statistically significant positive effects compared to the diabetic control group. Overall, the hexane fraction demonstrated the most pronounced antihyperglycemic and antihyperlipidemic properties, followed by the methanolic fraction, the presence of phytochemical compounds in the Bambaranut fractions are responsible for these effects.

Key word: antihyperglycemic, antihyperlipidemic and hematology

## 1.0 INTRODUCTION

Diabetes mellitus is a widespread metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both, which poses a severe threat to affected individuals and healthcare systems worldwide (1). Diabetes mellitus is a complex metabolic disorder that manifests itself through an elevation in blood glucose levels (2). It occurs due to a variety of factors, including defects in insulin secretion and/or action (3). This disease can have significant impacts on one's health, leading to severe complications if not managed properly. Therefore, it requires close monitoring and effective management strategies aimed at improving the body's ability to regulate glucose metabolism (4). The prevalence of diabetes mellitus is increasing at an alarming rate worldwide, according to the International Diabetes Federation (IDF) data in 2021, the IDF Africa Region had an estimated 24 million adults aged 20 to 79 living with diabetes in the year 2021. However, projections indicate a significant increase in the number of individuals affected by diabetes in the coming years. By 2030, it is estimated that the number of adults with diabetes in the IDF Africa Region will rise to 33 million. Looking further ahead, the figure is expected to reach 55 million by the year 2045 with severe consequences for affected individuals and healthcare systems (5). The limited effectiveness and potential adverse effects of current pharmacological therapies have led to an increased interest in alternative treatments for diabetes, such as the use of plant extracts (6). In line with this, the present study aimed to investigate the hyperglycemic effect of Nut fractions of *Vigna subterranea* in streptozotocin-induced diabetic rats. The study seeks to address the escalating global incidence of diabetes mellitus and subsequent need for effective alternative treatments by exploring the potential antidiabetic properties of Nut fractions of *Vigna subterraneane*. In addition, *Vigna subterranea* has been used to manage diabetes in Nigeria for decades, this study will provide more scientific information on the use of this Plant. The plant used in this study, *Vigna subterranea* (Bambaranut), was identified and authenticated at the Department of Plant Science and Biotechnology, Faculty of Natural Sciences, University of Jos, located in Jos, Nigeria. The plant was assigned a voucher number for reference: JUHN20000297.

**Plate 1: Images of *Vigna subterrenea* Plant and Nut**

Medicinal plant extracts have been gaining increasing attention as potential alternative treatments for diabetes due to their perceived safety and effectiveness (7). In line with this, researchers have been focusing on the medicinal properties of various plant species in order to uncover potential compounds that can be used for developing new diabetes treatments (8). These natural extracts, when compared to conventional drugs, are believed to possess fewer side effects and a lower risk of toxicity due to their organic origins (9). Additionally, many traditional medicines already incorporate plant-based ingredients as treatment options for diabetes management (10), therefore there is an abundance of evidence supporting this approach. With further research and development, these medicinal plant extracts may offer novel therapeutic solutions that could improve the lives of millions living with diabetes worldwide. The increasing use of traditional medicine represents a complex and ongoing trend in the healthcare industry (11). While it offers many benefits, it is important to continue to study and evaluate traditional medicine to ensure that it is used safely and effectively alongside modern medicine.

Streptozotocin-induced diabetics are a widely recognized animal model used to study the effects of antidiabetic interventions (12) and have been commonly utilized in previous research. This particular experimental setup has been extensively utilized throughout numerous studies, offering researchers an invaluable tool with which they can delve into the complexities associated with diabetes research (13). Streptozotocin (STZ), a compound derived from Streptomyces achromogenes and classified as a glucosamine-nitrosourea, is used as a chemotherapeutic agent for treating pancreatic β cell carcinoma. In experimental settings, STZ is used to induce diabetes by damaging pancreatic β cells, which leads to a decrease in insulin secretion and an increase in blood glucose levels: hyperglycemia (14). Streptozotocin (STZ) can cause diabetes through two different mechanisms, depending on the dosage used. At low doses, STZ targets β cells by binding to the glucose transporter receptor, which is mostly present in β cells, due to its structural similarity to glucose. This selectively accumulates STZ in β cells and can lead to an immune and inflammatory reaction, resulting in the destruction of β cells and the induction of hyperglycemia. In contrast, at high doses, STZ targets β cells through its alkylating properties similar to those of cytotoxic nitrosourea compounds, leading to the destruction of β cells and the development of hyperglycemia. However, STZ has adverse side effects, including hepatotoxicity and nephrotoxicity. These mechanisms have been well studied in mouse models. (13,15).

## 2.0 MATERIALS AND METHOD

### *2.1 Experimental Animals*

In this study, male albino rats were used as experimental subjects. The rats were obtained from the animal house at the University of Jos in Nigeria, which is known for its research and breeding facilities for laboratory animals.

The male albino rats were carefully selected based on their weight, with a range of 170-200g. This weight range ensures that the rats are mature enough for experimentation while minimizing variations in size that could affect the results.

To meet their nutritional needs, the rats were provided with unlimited access to standard pellet feed from Grand Cereal and Oil Mills Ltd, located in Jos, Nigeria. This feed is specifically formulated to provide the necessary nutrients required for the rats' growth and overall health.

In addition to the pellet feed, the rats were also given free access to water. Water is essential for hydration and plays a vital role in various physiological processes in the body.

By providing the rats with a standardized diet and ample access to water, confounding factors that could arise from differences in nutrition or hydration levels among the animals was minimized.

### *2.2 Preparation of V. subterrenea Plant Extracts*

After the *V. subterrenea* nut was harvested, it was washed to get rid of any sand particles and dried in a room for 2-3 days. Once fully dried, the nuts were crushed into a powdery form using a mortar and pestle. The resulting powder was then stored in an airtight container until it was needed for further use.

### *2.3 Fractionation of Nut Based on increasing Polarity of Solvent*

The process of fractionation was based on polarity and fractionation was done with the use of a non-polar solvent, which in this case was hexane. 500g of the *V. subterrenea* plant powder was soaked in the hexane solvent for 24 hours, during which it was frequently stirred. After soaking, the mixture was filtered using a sieve, and the filtrate was placed in an oven at 40ºC to allow for concentration. The concentrated hexane fraction was then preserved in an airtight container.

The residue that remained after the hexane extraction was evaporated until it became dry, and the same process was repeated using Ethylacetate, Methanol, and Water solvents, respectively. The result was four different fractions, which were named based on the solvents used for their extraction: Hexane fraction, Ethylacetate fraction, Methanolic fraction, and Aqueous fraction (16).

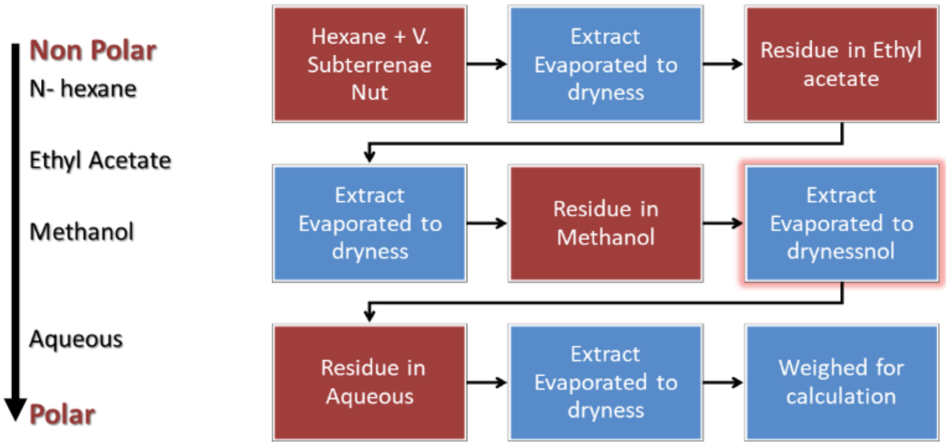


Fig. 1 : Overview of the fractionation process

### *2.4 Induction of Diabetes*

In this study, 35 male white albino rats weighing between 170g - 200g were utilized. To induce diabetes, the rats were given an intra-peritoneal injection of streptozotocin at a dose of 55mg/kg. The rats were left for 48 hours after which their blood glucose levels were measured using an on-call glucometer. The rats' weights were also measured weekly using a digital weighing balance.

### *2.5 Administration of the Plant Fractions*

The Nut fractions of n-Hexane, Ethylacetate, Methanol and Aqueous were administered through oral route at a dose of 200mg/kg, daily for 28 days. Metformin which was the standard drug was also was administered at 200mg.

### *2.6 Experimental Design*

The rats in this study were divided into different groups based on their treatment conditions. All groups, including the control group, were fed a normal diet and had unrestricted access to water for a duration of 28 days. Here is a summary of the different groups and their respective treatments:

Group A: Normal Control - This group served as the control group and did not receive any form of treatment. They were considered the baseline for comparison.

Group B: Diabetic Control - Diabetes was induced in this group, but they did not receive any treatment. They served as a comparison group to assess the effects of diabetes without intervention.

Group C: Diabetic, Treated with Metformin - This group consisted of diabetic rats that were treated with a standard drug called metformin. The dosage administered was 200 mg/kg body weight.

Group D: Diabetic, Treated with N-Hexane Fraction - The rats in this group were diabetic and received treatment with the n-hexane fraction at a dosage of 200 mg/kg body weight.

Group E: Diabetic, Treated with Ethyl Acetate Fraction - This group comprised diabetic rats that were treated with the ethyl acetate fraction at a dosage of 200 mg/kg body weight.

Group F: Diabetic, Treated with Methanol Fraction - The rats in this group were diabetic and received treatment with the methanolic fraction at a dosage of 200 mg/kg body weight.

Group G: Diabetic, Treated with Aqueous Fraction - This group consisted of diabetic rats that were treated with the aqueous fraction at a dosage of 200 mg/kg body weight.

The purpose of dividing the rats into these different groups and treating them accordingly was to investigate the effects of the different fractions and metformin on diabetic conditions. This experimental design allows for the comparison of the treated groups with both the normal control group and the diabetic control group, providing insights into the potential therapeutic effects of the treatments

### *2.7 Determination of Biochemical and Haematological Parameters*

The biochemical parameters assay include the following:

1. Phytochemical Screening of Fractions
2. Glucose, Protein, Albumin and Bilirubin
3. Lipid profile
4. Enzyme assay
5. Electrolyte determination
6. Kidney function test
7. Red Blood Cell analysis
8. White Blood Cell analysis

### *2.8 Data Analysis*

Result values are expressed as mean± standard deviation. Analysis of variance (ANOVA) was used for comparism. Differences were considered significant when values of $p \leq 0.05$. A Graph pad prism is used to carry out the above analysis (17).

## 3.0 DISCUSSION AND CONCLUSION

Diabetes is a chronic metabolic disorder characterized by elevated levels of blood glucose, either due to inadequate insulin production or the body's inability to effectively use insulin (18). It is a significant global health concern, affecting millions of people worldwide. Proper management of diabetes is crucial to prevent complications and maintain a good quality of life for individuals with the condition (19).

### *3.1 Percentage Yield*

There are many reasons why traditional medicine has become more and more popular recently, numerous civilizations have historically used traditional medicine (20), and this practice is still prevalent today since it is seen to be more effective and less expensive than modern treatment (21). Additionally, traditional medicine frequently combines cultural customs and beliefs, which increases its appeal among people in particular geographic areas (22). Below is the representation (Fig 2) of the percentage yield of the Nut fractions of *V. subterrenea* and it is seen that that Hexane fraction had the highest weight 173g (35%) after the extraction by fractionation and methanol had 115g (23%), followed by aqueous fraction (10.5%) and ethylacetate fraction was the least with 9.2g accounting for 1.9% .

To obtain the percentage yield, the following formular was used=

$$\frac{\text{Mass obtained from fraction}}{\text{Total mass of pounded Nut}} * 100$$

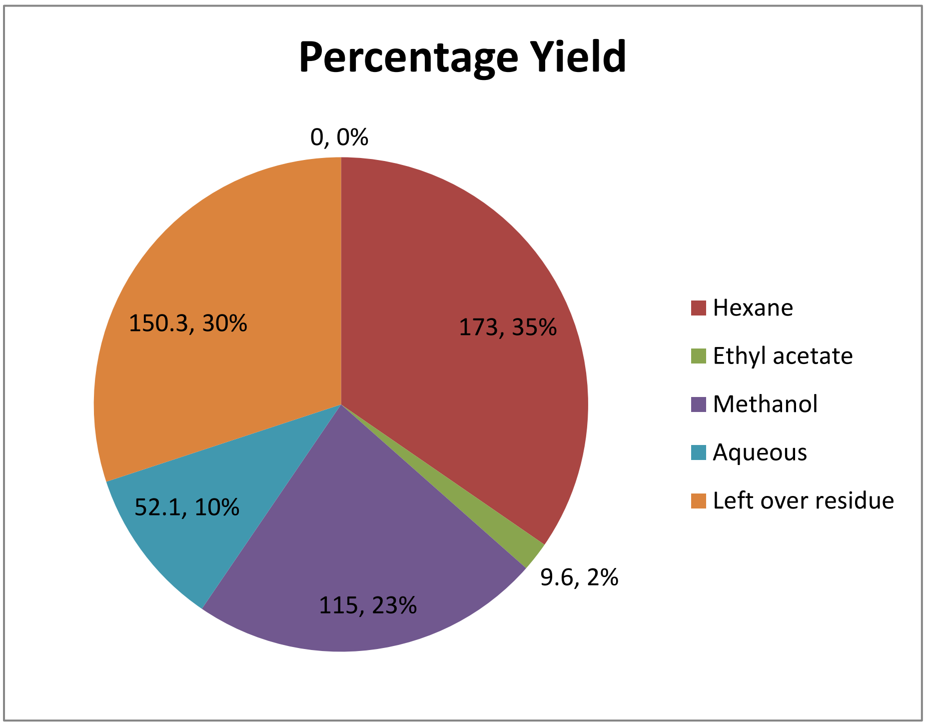


**Fig 2. Percentage yield of *V. subterrenae* Nut fractions**

### *3.2 Phytochemical Analysis*

Further, from table 1 below the fractionation of *V. subterrenae* Nut shows that the Methanol Fraction had more of the phytochemical from the phytochemistry analysis. Alkaloids, flavonoids, steroid, cardiac glycoside and carbohydrate were found present in the Methanol fraction. Aqueous fraction also had alkaloids, flavonoids and carbohydrate but lacked cardiac glycoside and steroid. Hexane and ethyl acetate fractions had the same phytochemical constituent. It could be deduced from the result obtained (Table 1) that methanolic fraction has more phytochemical constituent from *V. subterrenae* Nut, this might have occurred as a result of its polarity. Flavonoids act by lowering lipid peroxidation, preventing glucose absorption, and preventing the insulin-dependent activation of phosphoinositide 3-kinases (23). Additionally, flavonoids and their derivatives stimulate AMPK activation and glucose absorption in muscle cells, which adds to their overall therapeutic advantages in the treatment of diabetes (25). During food digestion, the breakdown of dietary polysaccharides by digestive enzymes increases blood glucose levels (26), and alkaloids have the ability of slowing down digestion which can reduce the level of blood glucose in circulation. Amylase is one such enzyme that is widely present in saliva and pancreatic juice. By hydrolyzing the α-1,4-glycosidic bonds, it catalyzes the degradation of starch, glycogen, and other oligosaccharides (27). The hydrolase class enzyme α-glucosidase, which is secreted by the brush border cells of the small intestine's epithelial lining, is another enzyme implicated in this process (28).The ability of alkaloids in inhibiting these digestive enzymes is a common strategy to lower postprandial blood glucose levels Glucose

**Table 1. Result of the Phytochemistry Screening of Nut Fraction**

| Constituents | Hexane | Ethylacetate | Methanol | $H_2O$ |
|---|---|---|---|---|
| **Alkaloids** | - | - | + | + |
| **Flavonoids** | - | - | + | + |
| **Tannis** | - | - | - | - |
| **Saponins** | - | - | - | - |
| **Steroids** | + | + | + | - |
| **Cardiac glycosides** | + | + | + | - |
| **Terponoids** | - | - | - | - |
| **Anthraquinone** | - | - | - | - |
| **Carbohydrate** | + | + | + | + |

Keys: + = Present - = Absent

### *3.3 Analysis of glucose, Protein and Bilirubin*

When the fasting plasma glucose level is continuously above 7 mmol/L (126 mg/dL), diabetes is considered to exist (29). The interaction between the only hormone that can lower blood glucose, insulin, and the hormones that spike glucose levels, such as glucagon, adrenaline, cortisol, and growth hormone, is what allows blood glucose levels to be precisely controlled (30). Since just one hormone may lower blood glucose while four hormones can raise it, this is a major contributing factor to the occurrence and prevalence of diabetes (31).There is evidence that *V. subterrenae* Nut plant has antidiabetic properties and it is capable of lowering blood glucose levels in diabetic situation from this study. The result (Table 2) shows that the hexane, ethyl acetate, methanol fractions showed a significant ($P \leq 0.005$) reduction in blood glucose level when compared with diabetic control. This reduction in blood glucose level could be as a result of phytochemocal constituent of *V. subterrenae* plant. However, hexane fraction showed more antihyperglycemic properties, there was a significant reduction $2.10 \pm 0.057^{ac}$ when compared to the diabetic control $22.90 \pm 2.836^{b}$ and normal control 5.80±0.057. From this fact, hexane fraction is more hypoglycemic.

**Table 2. Result of some Biochemical Parameters**

| Treatment | Glucose (Mmol/L) | TP (g/L) | ALB (g/L) | TB (Mmol/L) | CB (Mmol/L) |
|---|---|---|---|---|---|
| NC | 5.80±0.057 | 67.33±1.764 | 36.66±1.202 | 23.06±7.448 | 9.96±3.732 |
| DC | $22.90 \pm 2.836^{b}$ | $45.33 \pm 1.202^{a}$ | $24.66 \pm 3.528^{a}$ | $76.70 \pm 6.799^{b}$ | $44.70 \pm 6.389^{b}$ |
| Std Drug | $17.63 \pm 4.018^{bc}$ | $70.66 \pm 0.333^{bd}$ | $41.00 \pm 0.577^{bd}$ | $40.73 \pm 16.765^{bc}$ | $34.13 \pm 0.088^{bc}$ |
| Ethyl Acetate Extract | $8.23 \pm 0.504^{bc}$ | $65.66 \pm 1.764^{ad}$ | $38.66 \pm 0.881^{bd}$ | $14.53 \pm 2.379^{ac}$ | $2.80 \pm 1.210^{ac}$ |
| Methanol Extract | $7.93 \pm 1.224^{bc}$ | $68.00 \pm 1.155^{bd}$ | $39.66 \pm 0.666^{bd}$ | $29.33 \pm 9.404^{bc}$ | $8.60 \pm 0.264^{ac}$ |
| Hexane Extract | $2.10 \pm 0.057^{ac}$ | $67.00 \pm 1.000^{ad}$ | $36.33 \pm 0.333^{ad}$ | $37.70 \pm 9.560^{bc}$ | $15.86 \pm 2.999^{bc}$ |
| Aqueous Extract | $29.40 \pm 1.976^{bd}$ | $73.06 \pm 1.000^{bd}$ | $44.33 \pm 0.881^{bd}$ | $60.73 \pm 22.698^{bc}$ | $26.40 \pm 11.241^{bc}$ |
| p-values | <0.0001 | <0.0001 | <0.0001 | 0.0402 | 0.0005 |

**Values are expressed as mean ± SEM, n = 5.**

If p value is less than 0.05, there is significant difference in mean values

[a]Values are significantly low when compared with normal control ($p < 0.05$)

[b]Values are significantly high when compared with normal control ($p < 0.05$)

[c]Values are significantly low when compared with diabetic control ($p < 0.05$)

[d]Values are significantly high when compared with diabetic control ($p < 0.05$)

Diabetes is characterized by an increased breakdown of proteins, which can result in significant muscle loss. This muscle wasting plays a vital role in the decline of muscle mass and strength frequently observed in individuals with diabetes (32). Research have shown that insulin deficiency leads to reduction of liver proteins synthesis including albumin and serum protein (33) Further, the total protein and albumin (table 2) of diabetic rats respectively fed with 200mg of treatment groups showed no significant ($p<0.05$) reduction when compared with diabetic control. However, there was significant reduction in values of total serum bilirubin and conjugated bilirubin. All values were compared with normal control. This suggest that the nut fractions had some impact in replenishing and rejuvenating synthesis of protein and albumin in liver and serum. The development of diabetic complications is complex and involves not only high blood sugar levels (hyperglycemia), but also oxidative stress, inflammation, and an accelerated breakdown of proteins (34). Research has demonstrated that in diabetes, there are changes in the metabolism of both conjugated bilirubin and total bilirubin. As a result, individuals with diabetes may experience elevated levels of bilirubin, which could potentially increase the risk of developing complications associated with the disease (35). *V. subterrenea* fractions across all groups (table 2) were able to reduce serum bilirubin levels significantly when compared with the diabetic control.

### *3.4 Analysis on Lipid Profile*

Patients with diabetes frequently display a specific dyslipidemia pattern, which includes elevated triglyceride levels (36), low high density lipoprotein (HDL) levels, and a predominance of small, dense low-density lipoprotein (LDL) particles. Diabetes and problems with glucose metabolism may contribute to these changes in lipid profiles (37, 38). However, the table 3 shows that there was an overall reduction in the serum lipid profile across all fraction treatment groups. This is statistically significantly ($p \leq 0.05$) low when compared with the diabetic control and this suggest that *V. subterrenea* has hypolipidemic effect. Specifically, the hexane fraction showed more hypolipidemic properties when compared to diabetic control. There was significant reduction ($P \leq 005$) in serum LDL, TC, TG, and HDL when compared with diabetic control and normal control. To narrow it down to HDL ($0.89 \pm 0.005^{ac}$) analysis of hexane fraction, which was significantly lower than diabetic ($1.57 \pm 0.212^{b}$) and normal control (0.95±0.029).

**Table 3: Lipid profile Analysis**

| Treatment | HDL (Mmol/L) | TC (Mmol/L) | TG (Mmol/L) | LDL (Mmol/L) |
|---|---|---|---|---|
| NC | 0.95±0.029 | 2.16±0.120 | 0.50±0.057 | 1.16±0.102 |
| DC | $1.57 \pm 0.212^{b}$ | $3.23 \pm 0.260^{b}$ | $1.76 \pm 0.296^{b}$ | $1.10 \pm 0.109^{a}$ |
| Std Drug | $1.48 \pm 0.274^{bc}$ | $2.66 \pm 0.240^{bc}$ | $0.53 \pm 0.033^{bc}$ | $1.23 \pm 0.250^{bd}$ |

| | | | | |
|---|---|---|---|---|
| EthylAcetate Extract | 1.09±0.063[bc] | 2.63±0.088[bc] | 0.73±0.176[bc] | 1.09±0.074[ac] |
| Methanol Extract | 1.14±0.027[bc] | 2.40±0.057[bc] | 0.63±0.033[bc] | 0.90±0.064[ac] |
| Hexane Extract | 0.89±0.005[ac] | 1.93±0.088[ac] | 0.53±0.088[bc] | 1.04±0.030[ac] |
| Aqueous Extract | 1.10±0.113[bc] | 2.06±0.033[ac] | 1.16±0.260[bc] | 0.92±0.249[ac] |
| p-values | 0.0292 | 0.0005 | 0.0008 | 0.6837 |

**Values are expressed as mean ± SEM, n = 5.**

If p value is less than 0.05, there is significant difference in mean values

[a]Values are significantly low when compared with normal control ($p < 0.05$)

[b]Values are significantly high when compared with normal control ($p < 0.05$)

[c]Values are significantly low when compared with diabetic control ($p < 0.05$)

[d]Values are significantly high when compared with diabetic control ($p < 0.05$)

### *3.5 Analysis of Enzyme Biomarkers Test*

Individuals with diabetes mellitus have been found to have a higher prevalence of liver function test abnormalities compared to those without (39). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are liver enzymes that are present in abundance. ALT is predominantly found in the liver, while AST is also present in the heart, muscles, kidneys, brain, pancreas, and lungs. Alkaline phosphatase (ALP) is another enzyme that is found in various tissues, including the liver. In the liver, ALP plays a role in breaking down proteins in the body (40). From the table 4 the aspartate amino transferase (AST) and Alkaline Phosphatase (ALP) of rats treated with Nut fractions of *V. subterrenea* were significantly ($p \leq 0.05$) low when compared with the diabetic control. However, alanine amino transferase (ALT) was significantly ($p<0.05$) higher than diabetic control across the groups. The nut fractions were able to lower AST and ALP but had less impact on ALT

**Table 4. Result of Enzyme Biomarkers**

| **Treatment** | **AST (U/L)** | **ALT (U/L)** | **ALP (U/L)** |
|---|---|---|---|
| NC | 117.00±6.807 | 125.67±13.296 | 187.67±17.910 |
| DC | 197.67±40.989[b] | 246.00±56.536[b] | 274.33±53.336[b] |
| Std Drug | 82.33±25.432[ac] | 89.66±32.946[ad] | 203.00±2.083[bd] |
| Ethyl Acetate Extract | 74.33±2.603[ac] | 101.33±1.453[ad] | 105.67±1.764[ac] |
| Methanol Extract | 117.67±25.248[bc] | 139.00±19.399[bd] | 188.67±19.428[bc] |
| Hexane Extract | 122.33±10.483[bc] | 114.33±23.398[ad] | 176.67±18.622[bc] |
| Aqueous Extract | 158.67±33.348[bc] | 170.33±32.631[bd] | 199.00±38.527[bc] |
| p-values | 0.0421 | 0.0387 | 0.0350 |

**Values are expressed as mean ± SEM, n = 5.**

If p value is less than 0.05, there is significant difference in mean values

[a]Values are significantly low when compared with normal control ($p < 0.05$)

[b]Values are significantly high when compared with normal control ($p < 0.05$)

[c]Values are significantly low when compared with diabetic control ($p < 0.05$)

[d]Values are significantly high when compared with diabetic control ($p < 0.05$)

### *3.6 Serum Electrolyte Analysis*

Electrolytes play a vital role in various physiological processes, including the maintenance of acid-base balance, membrane potential, muscle contraction, nerve conduction, and regulation of body fluids. Disruptions in the balance of electrolytes can lead to physiological disorders. Studies have shown that insulin activates the $Na^+/K^+$-ATPase enzyme (41). Consequently, reduced serum insulin levels can result in decreased $Na^+/K^+$-ATPase activity, leading to impaired metabolism of sodium ($Na^+$) and potassium ($K^+$), hindered transport across biomembranes, and compromised uptake of monosaccharides by the intestinal epithelia (42). However, the table 5 shows that there was no significant reduction in serum electrolyte of diabetic rats treated with solvent fractions of *V. subterrenae* across all groups including standard drug (metfomin). This is statistically significantly ($p \leq 0.05$) high when compared with serum electrolytes of diabetic control. The values were also compared with normal control. This study suggest that the Nut fractions had little or no impact in the reduction of the concentration of serum electrolyte

**Table 5. Result on Serum Electrolyte**

| Treatment | $Na^+$**(Mmol/L)** | $K^+$ **(Mmol/L)** | $HCO_3^-$ **(Mmol/L)** | $Cl^-$ **(Mmol/L)** |
|---|---|---|---|---|
| NC | 141.33±0.666 | 5.36±0.328 | 22.00±0.577 | 102.00±1.528 |
| DC | 133.67±1.202 $^{a}$ | 4.43±0.318$^{a}$ | 23.00±0.577$^{a}$ | 97.33±0.881$^{a}$ |
| Std Drug | 141.00±0.577$^{ad}$ | 5.23±0.338$^{ad}$ | 18.00±0.577$^{ac}$ | 103.33±0.881$^{bd}$ |
| Ethyl Acetate Extract | 142.00±0.577$^{bd}$ | 4.20±0.305$^{ac}$ | 23.00±0.577$^{be}$ | 100.33±0.881$^{ad}$ |
| Methanol Extract | 141.00±0.577$^{ad}$ | 5.26±0.536$^{ad}$ | 23.66±1.453$^{bd}$ | 101.33±1.453$^{ad}$ |
| Hexane Extract | 140.67±0.666$^{ad}$ | 4.96±0.088$^{ad}$ | 21.00±2.083$^{ac}$ | 99.66±0.881$^{ad}$ |
| Aqueous Extract | 136.67±2.404$^{ad}$ | 6.56±0.497$^{bd}$ | 23.33±0.881$^{bd}$ | 98.66±1.453$^{ad}$ |
| p-values | 0.0017 | 0.0117 | 0.0337 | 0.0328 |

**Values are expressed as mean ± SEM, n = 5.**

If p value is less than 0.05, there is significant difference in mean values

[a]Values are significantly low when compared with normal control ($p < 0.05$)

[b]Values are significantly high when compared with normal control ($p < 0.05$)

[c]Values are significantly low when compared with diabetic control ($p < 0.05$)

[d]Values are significantly high when compared with diabetic control ($p < 0.05$)

[e]Valueis equal to diabetic control ($p < 0.05$)

### *3.7 Analysis of the Markers of Kidney Function*

Markers such as urea, uric acid and creatinine increases in diabetes as a result of renal impairment that may be caused by diabetes and this can lead to leakage of these biomarkers in the urine (43). Diabetes mellitus has a significant impact on the kidneys and is recognized as the primary cause of diabetic nephropathy. Alongside factors like oxidative stress and advanced glycation end-products (44), abnormal lipid metabolism and the accumulation of lipids in the kidneys have been suggested to contribute to the development of diabetic nephropathy. Various studies have observed lipid deposits in the kidneys of both diabetic individuals and animal models, indicating a potential role of these deposits in the pathogenesis of diabetic kidney disease (45). The diabetic rats treated with Nut solvent fractions of *V. subterrenea* showed (table 6) that there was no significant reduction ($P \leq 0.05$) in the serum urea, uric acid and creatinine when compared with the diabetic control. This values was compared with normal control. It can be deduce from this research that Nut fraction of *V. subterrenea* did not have high impact in reducing the concentration of serum biomarkers of renal functions

**Table 6: Result of the Markers of Kidney Function**

| Treatment | **Urea (Mmol/L)** | **Uric Acid (Umol/L)** | **Creatinine(Umol/L)** |
|---|---|---|---|
| NC | 3.03±0.120 | 39.66±0.333 | 265.00±22.898 |
| DC | 2.83±0.033$^{a}$ | 274.67±9.905$^{b}$ | 26.00±1.155$^{a}$ |
| Std Drug | 1.86±0.133$^{ac}$ | 275.33±9.838$^{bd}$ | 26.33±1.202$^{ad}$ |
| Ethyl Acetate Extract | 2.36±0.536$^{ac}$ | 280.00±11.547$^{bd}$ | 40.00±5.774$^{ad}$ |
| Methanol Extract | 3.36±0.318$^{bd}$ | 352.33±70.485$^{bc}$ | 36.33±9.062$^{ad}$ |
| Hexane Extract | 3.03±0.202$^{ed}$ | 265.00±4.359$^{bd}$ | 54.33±9.838$^{ad}$ |
| Aqueous Extract | 2.96±0.523$^{ad}$ | 291.67±15.836$^{bd}$ | 40.33±9.838$^{ad}$ |
| p-values | 0.0835 | <0.0001 | <0.0001 |

**Values are expressed as mean ± SEM, n = 5.**

If p value is less than 0.05, there is significant difference in mean values

[a]Values are significantly low when compared with normal control ($p < 0.05$)

[b]Values are significantly high when compared with normal control ($p < 0.05$)

[c]Values are significantly low when compared with diabetic control ($p < 0.05$)

[d]Values are significantly high when compared with diabetic control ($p < 0.05$)

[e]Valueis equal to diabetic control ($p < 0.05$)

### *3.8 Analysis of Red Blood Cell (RBC)*

The dangers that diabetic patients face include hyperglycemia, hyperosmolarity, oxidative stress, inflammation, and problems with lipid metabolism (45). These hazards can all have an impact on their red blood cells (46). These elements influence the aggregation, deformability, and fluidity of the erythrocyte membrane. In the end, these modifications disturb microcirculation and aid in the emergence of diabetic problems (47).Table 7 shows that there was significant reduction in the destruction of RBC, HGB, PLT of the diabetic rats treated with Nut fractions of *V. subterrenea*. These was statistically significantly ($p<0.05$) low when compared with diabetic control. These values were also compared with the diabetic control. The Nut fraction was able to maintain the erythrocytes of the diabetic wistars rats treatment groups. Hexane fraction of the Nut showed more.

**Table 7. Result of the RBC Assay**

| Treatment | RBC (L) | HGB (g/dL) | HCT(PCV) | PLT(L) | PCT |
|---|---|---|---|---|---|
| **NC** | 4.13±0.696 | 7.43±1.374 | 38.90±0.519 | 184.33±17.188 | 0.18±0.030 |
| **DC** | 7.61±0.173[b] | 14.53±0.589[b] | 47.46±1.369[b] | 574.33±121.02[b] | 0.45±0.088[b] |
| **Std Drug** | 5.84±0.586[bc] | 10.93±1.035[bc] | 31.60±3.166[ac] | 151.33±2.404[ac] | 0.12±0.015[ac] |
| **Methanol Extract** | 7.46±0.260[bc] | 13.26±0.480[bc] | 24.16±7.383[ac] | 88.66±42.451[ac] | 0.15±0.025[ac] |
| **Hexane Extract** | 3.83±1.644[ac] | 11.63±1.084[bc] | 30.50±4.010[ac] | 105.00±18.877[ac] | 0.11±0.019[ac] |
| **Ethyl Acetate Extract** | 5.88±0.978[bc] | 13.60±0.503[bc] | 37.73±0.384[ac] | 203.33±2.963[bc] | 0.15±0.009[ac] |
| **Aqueous Extract** | 3.47±0.616 | 7.36±0.523 | 19.00±3.044[a] | 312.00±68.223[bc] | 0.22±0.063[a] |
| | 0.0142 | 0.0001 | 0.0014 | 0.0002 | 0.0015 |

**Values are expressed as mean ± SEM, n = 5.**

If p value is less than 0.05, there is significant difference in mean values

[a]Values are significantly low when compared with normal control ($p < 0.05$)

[b]Values are significantly high when compared with normal control ($p < 0.05$)

[c]Values are significantly low when compared with diabetic control ($p < 0.05$)

[d]Values are significantly high when compared with diabetic control ($p < 0.05$)

**ABBREVIATION**: RBC, red blood cell; Hgb, hemoglobin; Hct, hematocrit;

PLT, platelet; PCV, Packed cell volum

### *3.9 Analysis of White Blood Cell (WBC)*

Changes in hematological parameters, including white blood cell (WBC) count and platelet function, have been detected in individuals with diabetes mellitus (48) In individuals with diabetes mellitus, both platelet and white blood cell (WBC) counts were found to be significantly higher compared to non-diabetic controls. The diabetic state can stimulate WBCs through various factors, such as advanced glycation end products (AGEs), oxidative stress, angiotensin II, and cytokines. Activated WBCs release cytokines and transcription factors that play a crucial role in inflammation (49). Moreover, activated leukocytes can generate superoxide radicals and proteases, contributing to oxidative stress. Chronic low-grade inflammatory responses, in combination with other risk factors, are believed to contribute to extensive vascular damage, endothelial dysfunction, increased oxidative stress, and ultimately play a role in the development of diabetic microvascular and macrovascular complications (50). Treatment with Nut solvent fractions of *V. subterrenea* had positive impact on the white cells of streptozotocin-induced diabetic rats. The table 8 showed that there was significant reduction in the white blood cells of the diabetic rats treated with each of the fraction. These was statistically significantly ($p<0.05$) low when compared with diabetic control. These values were also compared with the diabetic control

**Table 8. Result of WBC**

| Treatment | WBC (L) | NEU (L) | LYM (L) | MON (L) | EOS (L) | BAS |
|---|---|---|---|---|---|---|
| **NC** | 5.49±0.298 | 3.11±0.660 | 5.31±0.367 | 0.11±0.038 | 0.02±0.005 | **0.00±0.000** |
| **DC** | 7.29±1.236[b] | 45.03±14.851[b] | 37.90±16.317[b] | 1.13±0.348[b] | 2.60±0.251[b] | **0.93±0.433[b]** |
| **Std Drug** | 2.85±0.541[ac] | 0.71±0.216[ac] | 2.08±0.330[ac] | 0.09±0.043[ac] | 0.04±0.020[bc] | **0.01±0.003[bc]** |
| **Methanol Extract** | 3.04±1.007[ac] | 0.27±0.021[ac] | 0.82±0.064[ac] | 0.29±0.177[bd] | 0.02±0.014[ec] | **0.01±0.003[bc]** |
| **hexane Extract** | 2.82±0.770[ac] | 0.52±0.073[ac] | 1.76±0.690[ac] | 0.07±0.052[ac] | 0.04±0.023[bc] | **0.02±0.003[bc]** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Ethyl acetate Extract** | 3.58±0.741[ac] | 0.79±0.325[ac] | 0.99±0.228[ac] | 0.78±0.012[bc] | 0.04±0.017[bc] | **0.00±0.000[ec]** |
| **Aqueous Extract** | 4.30±0.467[ac] | 1.47±0.101[ac] | 1.96±0.739[ac] | 0.76±0.364[bc] | 0.21±0.086[bd] | **0.04±0.026[bc]** |
| **p-values** | 0.0093 | 0.0004 | 0.0072 | 0.0115 | <0.0001 | 0.0098 |

**Values are expressed as mean ± SEM, n = 5.**

If p value is less than 0.05, there is significant difference in mean values

[a]Values are significantly low when compared with normal control ($p < 0.05$)

[b]Values are significantly high when compared with normal control ($p < 0.05$)

[c]Values are significantly low when compared with diabetic control ($p < 0.05$)

[d]Values are significantly high when compared with diabetic control ($p < 0.05$)

[e]Valueis equal to diabetic control ($p < 0.05$)

ABBREVIATIONS: WBC, white blood cell; NEU, neutrophiles; BAS, basophiles, LYM, lymphocytes; MON monocytes; EOS, eosinophile

## 3.0 Conclusion

The study shows that the Nut fractions of *V. subterrenea* plant have some antihyperglycemic properties on the treatment groups as there was a significant decrease in blood glucose levels of albino rats groups fed with the hexane, ethyl acetate, and methanolic fractions. However, the aqueous fraction did not show a significant reduction in blood glucose levels compared to the diabetic and normal control groups. These fractions showed properties such as hypoglycemic, hypolipidemic and hypocholesterolamic effects but showed no positive impact on total protein and albumin metabolism on streptozotocin-induced diabetic rats. Serum enzyme markers and hematological parameters also showed statistically significant positive effects. Overall, the hexane fraction demonstrated the most prominent antihyperglycemic and antihyperlipidemic properties, followed by the methanolic fraction, this study suggest that the presence of phytochemical compounds in the Bambaranut fractions are responsible for these effects.

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