International Journal of Biochemistry Research & Review

Volume 32, Issue 3, Page 20-32, 2023; Article no.IJBCRR.100494
ISSN: 2231-086X, NLM ID: 101654445

Evaluation of Antidiabetic Effect of Crude Aqueous Extract of Nut, Leaf and Stem Parts of *Vigna subterranae* on Streptozotocin-Induced Diabetic Rats

Adugbe Abigail [a*], Vongdip Nanman Godwin [a], Obot Blessing Uduakobong [a] and Carol D. Luka [a]

[a] *Department of Biochemistry, College of Health Sciences, University of Jos, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2023/v32i3805

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/100494

Original Research Article

Received: 23/03/2023
Accepted: 26/05/2023
Published: 22/06/2023

ABSTRACT

Diabetes is a metabolic disorder characterized by abnormally high blood glucose level. One of the plants frequently used to manage diabetes is *Vigna subterrenea*, also known as the Bambaranut. The aim of this study is to verify the usefulness of this plant to mitigate the effects of diabetes. Thirty (30) Wistar rats weighing 170-200 g were used for this evaluation, the rats were randomly divided into six groups namely; group A: normal control, group B: diabetic control, group C: metformin (500 mg), group D: leaf extract (1000 mg), group E: stem extract (1000 mg), and group F: nut extract (1000 mg). Alkaloids, flavonoids, saponins, balsam carbohydrate, phenol, steroids, and cardiac glycosides were all identified during the phytochemical screening of aqueous extracts.

**Corresponding author: E-mail: abbynoble24@gmail.com;*

Intraperitoneal injections were used to cause diabetes using streptozotocin across groups and treatment commenced after 48 hours of induction upon confirmation of diabetes mellitus. The sacrifices of animals occurred after a 28-day session of treatment. For hematological and biochemical examination, blood, liver, kidney, and pancreas were obtained. Significantly lowering of fasting blood sugar in the extract treatment groups resulted in improved biochemical markers (P≤0.05). Serum enzyme markers showed a significant drop (P≤0.05) while High Density Lipoprotein (HDL) level increased considerably when compared with diabetic control. Also, when compared to the diabetic control group, the markers of kidney function all noticeably dropped, while electrolyte levels rose. Additionally, there was a significant positive impact on hematological parameters. In accordance with the results of this investigation, *V subterrenea* is a potent hypoglycemia and hypolipidemic agent on streptozotocin-induced diabetic rats.

Keywords: Antidiabetic effect; metabolic disorder; diabetic control.

1. INTRODUCTION

Vigna subterranea is a part of the Fabaceae family of plants, which is also known as Bambara nut, Bambara bean, gurjiya (Hausa), and okpa (Igbo). *Vigna subterranea* is cultivated for its edible seeds or pods. It is thought to have come from West Africa, where the Bambara ethnic group lives in nations including Burkina Faso, Guinea, Mali, and Senegal [1]. This plant also allows its pods to develop underground, just like peanuts do. After being dried, the pods can either be eaten boiled or used to make “moimoi”.

In sub-Saharan Africa, subsistence farmers frequently plant this particular variety of bean called *Vigna subterranean*. It has a reputation for producing respectable yields despite being exposed to challenging circumstances like drought and poor soil fertility. The plant produces tiny subterranean pods that are about 1.5 cm long. There are cream, dark brown, crimson, or a mixture of these colors as its seeds. The plant is removed from the ground and harvested to reveal the underground nuts [2].

The plant was identified and authenticated at department of Plant Science and Biotechnology, Faculty of Natural Sciences University of Jos, Jos, Nigeria. With voucher number: JUHN20000297.

Plants have been used for centuries in Africa for medicinal purposes and many modern medicines have been derived from them. The use of medicinal plants is a vital ingredient of healthcare worldwide, as they have been found to have therapeutic and curative properties [3]. Traditional medical systems have used medicinal plants for ages, and their uses go far beyond just curing illnesses; to enhance health and stave off sickness, many medicinal plants are employed.

Diabetes, commonly known as diabetes mellitus, is a group of metabolic illnesses that causes persistently high blood glucose levels due to the pancreas' inability to produce enough insulin or the body's cells' failure to react to insulin effectively, these are the primary factors that contribute to diabetes [4]. High blood sugar is characterized by excessive thirst, frequent urination, and ongoing hunger. Diabetic ketoacidosis, cardiovascular disease, stroke, chronic renal disease, foot ulcers, and eye impairment are just a few of the serious health problems that can develop from untreated diabetes [5]. The prevalence of Diabetes at 5.77% rate means that out of every 100 adults in Nigeria, approximately 5 or 6 have diabetes mellitus [6]. This may seem like a relatively small percentage, but when you consider the population of Nigeria, which is estimated to be around 206 million people, the number of adults affected by DM becomes quite significant. Around 11.2 million adults in Nigeria have DM [6]. This is a substantial number, and it highlights the importance of addressing the issue of diabetes in the country. If left unchecked, diabetes can lead to serious health complications such as cardiovascular disease, kidney damage, and nerve damage, among others.

Furthermore, the fact that one out of every 17 adults in Nigeria has DM is concerning because it indicates a high burden of the disease in the population. This underscores the need for increased awareness, screening, and access to appropriate medical care and treatment for those affected by DM in Nigeria [7].

According to [8], the glucosamine-nitrosourea family of chemicals includes the substance streptozotocin (STZ), which is generated from the fungus Streptomyces achromogenes. In clinical settings, it is frequently utilized as a

A: whole plant

B: Dark brown and Cream nut

Plate 1. Images of *Vigna subterrenea* plant and nut

chemotherapeutic treatment for pancreatic cell cancer by harming pancreatic beta-cells and resulting in hypoinsulinemia and high blood sugar levels which can result in diabetes. STZ can enter the pancreas with the help of the glucose transporter receptor, STZ can attach to this receptor because of how structurally similar it is to glucose. STZ specifically targets β-cells and builds up inside of them, inflame them until destruction. The most comprehensive understanding of its route of action comes from studies on mice.

2. MATERIALS AND METHODS

2.1 Experimental Animals

At the University of Jos in Nigeria's animal home, thirty (30) male Wistar, White albino rats weighing between 170-200g were purchased. Water and regular pellet meal (bought from Grand Cereal and Oil Mills Ltd, Jos Nigeria) have been rendered freely to the rats without limit.

2.2 Preparation of *V. subterrenea* Plant Extracts

The *V. subterrenea* Nut, Leaf and Stem was harvested washed to remove sand particles and allowed to air-dry in a room. The dried parts were reduced to powdery form using mortar and pestle. A container that was airtight was used to retain the powder of the individual parts until it was time to use them. *V. subterrenea* Nut (1000 g), Leaf (700 g) and Stem (700 g) was awash separately in one litre of distilled water respectively for 5 hours and stirred occasionally. After 5 hours, each part of the plant was individually filtered into a beaker using a mesh and the finest particle was sieved into the beaker respectively for 1 hour. The filtrate was concentrated in the oven at 40^{O}C for 3 days to dry up the solvent and to obtain a fine powder of each part of the plant.

2.3 Induction of Diabetes

This procedure was carried out using thirty (30) mature albino wistar rats weighing between 170 g and 200 g. streptozotocin was administered Intraperitoneally at 55 mg/kg to cause diabetes. STZ was dissolved in citrate buffer of 0.1M and pH 4.5 and delivered intraperitoneally at a dose of 55 mg/kg to the animals. The animals were then left alone for 48 hours before having the blood sugar level measured using an on-call glucometer. On days 1, 7, 14, 21, and 28, an electronic weighing balance was used to measure the animal weights.

Diabetes was determined based on the results of this test and observed polyuria, rats in both control and STZ-treated groups received unlimited amounts of food and drink. 48 hours after the injection of streptozotocin, it was noted that the blood glucose level was above 126 mg/dl. Every week, body weights were recorded.

2.4 Administration of the Plant Extracts

Oral administration of *Vigna subterrenea* crude aqueous extract of Nut, Leaf, and Stem were each given orally for 28 days at a dose of 1000 mg/kg. The typical medication, metformin, was given at a dosage of 500 mg.

2.5 Experimental Design

Group	Description	Treatment	Dosage
A	Normal control rats	None	None
B	Diabetic control rats	None	None
C	Diabetic treated	Standard drug (Metformin)	500 mg/kg b.w
D	Diabetic treated	Leaf extract	1000 mg/kg b.w
E	Diabetic treated	Stem extract	1000 mg/kg b.w
F	Diabetic treated	Nuts extract	1000mg/kg b.w

2.6 Determination of Biochemical Parameters

a. Phytochemical Screening of Crude Extract of *Vigna subterrenea* Nut, Leaf and Stem: The phytochemical analysis of the aqueous leaf extract was carried out using methods and procedure outlined by [9].

b. Determination of Serum Glucose by Glucose oxidase method.

c. Determination of Total Protein by biuret method and Albumin by Modified colorimetric method [10].

d. Determination of Serum Total Bilirubin by Colormetric method based on that described by [11].

e. Determination of HDL-Cholesterol, Total Cholesterol, Triglycerides, and LDL-Cholesterol was determined in serum by colorimetric method [12].

f. Determination of Alanine Aminotransferase and Aspartate Aminotransferase (AST) Determination by colorimetrically [13].

g. Determination of Alkaline Phosphatase by colorimetric method [14]

h. Determination of Serum Creatinine, Serum Urea and uric acid

i. Determination of Serum Bicarbonate (HCO_3^-), Sodium (Na^+), Potassium (K^+) and Chloride (Cl^-)

j. Determination of Packed Cell Volume (PCV%) Method: Microhaematocrit method [15]

k. Method of determination of Total White Blood Count (TWBC) and Red Blood Cell Count (RBC) Method: Haematocytometer method [16].

2.7 Data Analysis

Results are presented as mean and standard deviation values. ANOVA was utilized for comparison. When values of $p \leq 0.05$ were reached, differences were deemed significant. The aforementioned analysis is performed using a graphing calculator prism.

3. RESULTS

3.1 Yield *Vigna subterrenea* Plant Aqueous Extract

1000 g of powdered Nut, 700g of powdered Leaf and 700 g of Stem was dissolved in 1 litre of distilled water and allowed to stand for one hour and filtered respectively. The filtrate was evaporated in the oven at 40^{o}C to yield 377.4g of *Vigna subterrenea* Nut, 284.5 g of Leaf and 246.8 g of Stem of aqueous extract.

3.1.1 LD_{50} Toxicity Test Result of Crude Aqueous Extract of *Vigna subterrenea* Plant (Leaf, Stem and Nut)

Table 2 shows that there was no mortality in both phase one and two, after the acute toxicity studies. This is possible because the plant is edible.

3.2 Phytochemical Result of Aqueous Crude Extract

Flavonoids and saponins were found in all of the aqueous extracts of *Vigna subterrenea* after a phytochemical screening. Tannins, balsam, and phenol were lacking from the nut extract but present in the leaf and stem extracts. It's important to remember that alkaloids are only found in nut aqueous extracts (Table 3).

Table 1. Yield of crude aqueous extract of *Vigna subterrenea* nut, leaf and stem

	Plant powder/g	quantity of solvent/L	yield /g	% yield
Nut	1000	1	377.4	37.7
Leaf	700	1	284.5	28.5
Stem	700	1	246.8	24.7

Table 2. LD_{50} toxicity test result of crude aqueous extract of *Vigna subterrenea* plant (Leaf, stem and nut)

Group mg/kg b.w	No of rats	Mortality	LD50
10	3	0	≥10
100	3	0	≥100
1000	3	0	≥1000
1600	3	0	≥1600
2900	3	0	≥2900
5000	3	0	5000

Table 3. Results of phytochemical screening of crude aqueous extracts of *Vigna subterrenea* nut, leaf and stem

Phytochemical constituents	NUT	LEAF	STEM
Alkaloids	+	-	-
Flavonoids	+	+	+
Tannis	-	+	+
Saponins	+	+	+
Terpenes and steroids	-	-	-
Cardiac glycosides	-	-	-
Balsam	-	+	+
Carbohydrate	-	+	-
Phenol	-	+	+
Resin	-	-	-

KEY: + = Present, - =Absent

3.3 Effect of Crude Aqueous Extract of *V. subterrenea* Nut, Leaf and Stem on Serum Glucose, Total Protein, Serum Albumin, Total Bilirubin and Conjugated Bilirubin

From Table 4, it is seen that *Vigna subterrenea* Nut, leaf and stem glucose values were significantly (P≤0.05) low when compared with normal control and diabetic control, generally, from the statistical analysis, *V. subterrenea* plant extract was able to reduce the blood glucose level significantly when compared with diabetic and normal control.

The table also show that total protein (TP) level of the Nut, Leaf and Stem were significantly high (p≤0.05) when compared with the diabetic control and normal.

The table further illustrates that Albumin (ALB) is significantly high (p≤0.05) in standard drug metformin, Nut and stem when compared with diabetic control and significantly low when compared with normal control.

From Table 4, it is seen that there is significant decrease (p≤0.05) in total bilirubin and conjugated bilirubin across group when compared with both normal and diabetic control.

3.4 The Effect of Crude Aqueous Extract of *Vigna subterrenea* Nut, Leaf and Stem on Serum Lipid Profile Level of Streptozotocin Induced Diabetic Rats

Table 5 shows the lipid profile of the experimental rats. In general, it is seen that total cholesterol and triglyceride (TG) of rats fed with *V. subterrenea* Nut and Leaf extract is significantly low when compared with diabetic control. However, High density lipoprotein (HDL) is significantly high when compared with both normal and diabetic control.

3.5 Effect of *Vigna subterrenea* Nut, Leaf and Stem Crude Aqueous Extract on Serum Enzyme Level of Streptozotocin Induced Diabetic Rats

The Table 6 shows that the serum enzyme level of diabetic rats was significantly higher than normal control. Streptozotocin-induced diabetic rats fed with *V. subterrenea* Nut, Leaf and Stem crude extract showed reduced serum Aspartate Amino Transferase (AST), Alanine Amino

Transferase (ALT) and Alanine Amino Phosphatase (ALP) levels. This is statistically significant (p≤0.05) when compared with both normal and diabetic control rats.

Table 4. Results of the effect of crude aqueous extract of *Vigna subterrenea* nut, leaf, and stem on serum glucose, total protein, albumin, total bilirubin and conjugated bilirubin of streptozotocin induced diabetic rats

Group	Glucose (Mmol/L)	TP (g/L)	ALB (g/L)	TB (Mmol/L)	CB (Mmol/L)
NC	5.87±0.035	68.53±0.285	40.56±0.270	47.12±21.304	4.71±0.243
DC	23.47±2.755[b]	45.01±1.152[a]	24.03±3.457[a]	79.07±6.373[b]	45.06±6.375[b]
Metformin	5.85±0.295[ac]	67.53±0.859[ad]	39.54±0.286[ad]	11.33±0.644[ac]	5.58±0.263[bc]
Nut	5.32±1.692[ac]	69.55±0.289[bd]	39.57±0.309[ad]	19.29±0.495[ac]	14.29±0.112[bc]
Leaf	5.86±0.032[ac]	78.02±1.148[bd]	46.61±0.363[bd]	13.82±0.351[ac]	5.82±0.238[bc]
Stem	5.52±0.224[ac]	67.64±0.884[ad]	39.53±0.278[ad]	11.32±0.631[ac]	5.58±0.269[bc]
p-value	≤0.0001	≤0.0001	≤0.0001	0.0002	≤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)

Table 5. Result of the effect of crude aqueous extract of *Vigna subterrenea* nut, leaf and stem on serum lipid profile level of streptozotocin induced diabetic rats

Group	TC (Mmol/L)	TG (Mmol/L)	HDL (Mmol/L)	LDL (Mmol/L)
NC	2.32±0.063	0.96±0.034	1.68±0.025	0.79±0.063
DC	3.25±0.262[b]	1.71±0.285[b]	1.01±0.190[b]	1.73±0.116[b]
Standard drug	3.12±0.165[bd]	1.61±0.296[bd]	1.58±0.139[bd]	1.11±0.054[bc]
Nut	2.62±0.018[bc]	1.16±0.025[bc]	1.24±0.020[bd]	0.89±0.026[bc]
Leaf	2.66±0.141[bc]	1.54±0.287[bc]	1.35±0.168[bd]	1.42±0.298[bd]
Stem	3.13±0.152[bd]	1.63±0.296[bd]	1.58±0.159[bd]	1.26±0.029[bd]
p-value	0.0025	0.2132	0.0452	0.0452

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)

Table 6. The result of effect of *Vigna subterrenea* nut, leaf and stem crude aqueous extract on serum enzyme level of streptozotocin induced diabetic rats

Group	AST (U/L)	ALT (U/L)	ALP (U/L)
NC	56.60±2.049	68.62±2.583	95.09±1.198
DC	202.53±40.695[b]	258.53±55.141[a]	300.55±46.459[b]
Standard drug	50.03±2.325[ac]	63.57±2.617[ac]	79.52±5.472[ac]
Nut	84.10±2.915[bc]	112.08±4.583[bc]	127.15±2.917[bc]
Leaf	56.60±1.987[ac]	74.52±2.588[bc]	95.02±0.573[ac]
Stem	50.23±2.296[ac]	63.53±2.579[ac]	79.55±5.481[ac]
p-value	≤0.0001	≤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)

3.6 Effect of *Vigna subterennae* Nut, Leaf and Stem Crude Aqueous Extract on Serum Urea, Uric Acid and Creatinine of Streptozotocin Induced Diabetic Rat

From the Table 7, the serum urea, uric acid and creatinine of the diabetic control group increased, this is statistically significant (p≤0.05) when compared to normal control. The diabetic rats fed with *V. subterrenea* Nut and Leaf showed reduction in serum Urea, Uric Acid and Creatinine. This is statistically significant (p≤0.05) when compared with diabetic control.

However, the standard drug (Metformin 500 mg/kg) and the Stem showed no significant (p≤0.05) reduction in serum urea and Creatinine when compared with normal control and diabetic control.

3.7 The Effect of *Vigna subterrenea* Nut, Leaf and Stem Crude Aqueous Extract on Serum Electrolyte Level of Streptozotocin Induced Diabetic Rats

The Table 8 shows that there was a significant (p ≤0.05) increase in serum electrolyte of the diabetic control rats when compared with normal control.

Also, the table shows that there was reduction of the serum Sodium ion, Potassium ion and Bicarbonate of the Standard drug, Nut, Leaf and Stem which is significant (p ≤0.05) when compared with normal and diabetic control. However, the chloride ion of all the groups showed no reduction when compared with the diabetic control. The normal control was also compared with these groups.

Table 7. Result of the effect of *Vigna subterennae* nut, leaf and stem crude aqueous extract on serum urea, uric acid and creatinine

Group	Urea (Mmol/L)	Uric Acid (Mmol/L)	Creatinine (Mmol/L)
NC	1.02±0.123	312.57±31.471	13.07±1.176
DC	4.20±0.232[b]	505.13±34.591[b]	32.63±0.276[b]
Standard drug	2.81±0.011[bd]	347.12±30.015[bc]	21.74±11.208[bc]
Nut	2.06±0.205[bc]	377.25±15.608[bc]	21.56±2.608[bc]
Leaf	1.88±0.036[bc]	370.11±4.007[bc]	20.06±1.757[bc]
Stem	2.83±0.021[bd]	347.11±29.965[bc]	32.65±0.315[bd]
p-value	≤0.0001	0.0015	0.0629

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)

Table 8. Results of the effect of crude aqueous extract of *Vigna subterrenea* nut, leaf and stem on serum electrolyte level of streptozotocin induced diabetic rats

Group	Na^+(Mmol/L)	K^+(Mmol/L)	HCO_3^-(Mmol/L)	Cl^-(Mmol/L)
NC	145.58±0.306	4.86±0.140	25.07±0.599	103.56±0.897
DC	147.20±0.617[b]	27.41±13.052[b]	25.88±0.944[b]	105.16±1.182[b]
Standard drug	134.04±1.158[ac]	4.46±0.319[ac]	23.03±0.587[ac]	97.54±0.869[ad]
Nut	135.07±2.891[ac]	4.07±0.136[ac]	21.66±0.305[ac]	97.04±2.903[ad]
Leaf	136.06±0.036[ac]	5.53±0.019[bc]	25.73±0.450[bc]	98.07±0.052[ad]
Stem	134.17±1.100[ac]	4.52±0.336[ac]	22.67±0.462[ac]	97.57±0.872[ad]
p-value	≤0.0001	0.0369	0.0002	0.0016

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)

3.8 Haematology Analysis for Crude Aqueous Extract of *V. subterrenea* Nut Leaf and Stem on Streptozotocin Induced diabetic Rats

From the Table 9, the Leaf, Nut, Stem of *V. subterranae* crude extract on streptozotocin-induced diabetic rats showed that the red blood cells analysis are statistically significantly ($p \leq 0.05$) higher than diabetic control and normal control.

However, the haemoglobulin and blood platelet investigation of diabetic rats fed with *V. subterrenea* Nut, Leaf and Stem showed a decrement significantly when compared with diabetic control.

3.9 White Blood Cell Analysis for Crude Aqueous Extract Of *V. subterrenea* Nut, Leaf and Stem on Streptozotocin Induced Diabetic Rats

From the Table 10, the white blood cells of the Leaf, Nut and Stem of *V. subterranae* crude extract on streptozotocin-induced diabetic rats showed that the total white blood cells are statistically significantly ($p \leq 0.05$) higher than diabetic control and normal control. The individual white blood cells of diabetic rats fed with *V. subterrenea* Nut, Leaf and Stem showed a significant increment when compared with normal control and significantly low when compared with diabetic control.

Table 9. Results of haematology for crude aqueous extract of *V. subterrenea* nut leaf and stem on streptozotocin induced diabetic rats

Groups	RBC(L)	HGB(g/dL)	HCT(PCV)	PLT(L)	PCT
NC	4.13±0.696	7.10±1.464	38.90±0.519	207.67±34.720	0.14±0.037
DC	7.61 ± 0.173^{b}	14.53 ± 0.58^{b}	47.46 ± 1.36^{b}	594.33 ± 121.020^{b}	0.45 ± 0.088^{b}
MET	5.22 ± 1.228^{bc}	11.10 ± 1.87^{bc}	37.40 ± 7.81^{ac}	357.00 ± 97.709^{bc}	0.56 ± 0.079^{bd}
LEAF	7.71 ± 0.154^{bd}	14.36 ± 0.27^{bc}	50.43 ± 0.14^{bd}	357.67 ± 68.960^{bc}	0.44 ± 0.025^{bc}
STEM	7.88 ± 0.285^{bd}	14.26 ± 0.64^{bc}	48.70 ± 2.70^{bd}	379.33 ± 80.344^{bc}	0.30 ± 0.077^{bc}
NUT	7.53 ± 0.189^{bc}	14.03 ± 0.06^{bc}	45.46 ± 1.98^{bc}	132.33 ± 0.007^{ac}	0.13 ± 0.007^{ac}
p-values	0.0027	0.0015	0.1079	0.0210	0.0013

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)
ABBREVIATIONS: RBC, red blood cell; Hgb, hemoglobin; Hct, hematocrit; PLT, platelet; PCV, packed cell volume

Table 10. Result of white blood cell analysis for crude aqueous extract of *V. subterrenea* nut, leaf and stem on streptozotocin induced diabetic rats

Groups	WBC (L)	NEU (L)	LYM (L)	MON (L)	EOS (L)
NC	5.69±0.173	3.11±0.660	5.45±0.332	0.11±0.038	0.02±0.005
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}
MET	8.52 ± 0.386^{bd}	3.11 ± 0.660^{ec}	5.31 ± 0.367^{ac}	0.11 ± 0.039^{ec}	0.02 ± 0.005^{ec}
LEAF	12.83 ± 0.783^{bd}	5.42 ± 0.967^{bc}	6.66 ± 1.803^{bc}	0.10 ± 0.052^{ac}	0.28 ± 0.097^{bc}
STEM	18.37 ± 2.223^{bd}	7.65 ± 1.661^{bc}	10.31 ± 0.548^{bc}	0.11 ± 0.009^{ec}	0.05 ± 0.017^{bc}
NUT	12.88 ± 0.752^{bd}	5.79 ± 1.519^{bc}	6.46 ± 0.799^{bc}	0.07 ± 0.002^{ac}	0.14 ± 0.069^{bc}
p-values	≤0.0001	0.0026	0.0310	0.0013	≤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 normal control (p ≤ 0.05)

4. DISCUSSION

4.1 Phytochemistry of Extracts

Fruits, vegetables, Plant bark, flowers, leaves, and roots all contain phytochemicals, which are physiologically active substances that are naturally present in plants. They function as an anti-disease defense mechanism in concert with nutrients and fibre [17]. The bioactive phytochemical components of plants, which have particular physiological impacts on the human body, are thought to be the source of their therapeutic value and form the basis for creation of the contemporary prescription pharmaceuticals. Flavonoids and saponins were found in all of the aqueous extracts of *Vigna subterrenea* after a phytochemical screening (Table 3). Tannins, balsam, and phenol were lacking in nut extract but present in leaf and stem extract. It is noteworthy that only nut aqueous extracts contain alkaloids and alkaloids are defined as small compounds with nitrogen in the form of primary, secondary, or tertiary amines are known as alkaloids. Increased blood glucose levels result from digestive enzymes breaking down the complex carbs in our meals into simple sugars [18]. One of these enzymes, called - amylase, can be found in saliva and pancreatic juice, amylase can degrade starch, glycogen, and other compounds by rupturing their α-1,4-glycosidic linkages. The small intestine's epithelial cells also contain another enzyme called α-glucosidase, which converts oligosaccharides into readily absorbed monosaccharides and this raises blood sugar levels after meals. Inhibiting these digestive enzymes with secondary plant metabolites is a typical method to lower postprandial hyperglycemia. Enzyme activity can be decreased via alkaloids by binding to the active sites of digestive enzymes and preventing the formation of enzyme-substrate complexes [19]. According to [20] there is evidence that saponins may help in the management of hyperglycemia. The Leaf, Nut, and Stem of the *V. subterrenea* plant's aqueous extract are traditionally used to clean and purify blood due to the saponins present in the plant. Saponins have blood purifying effect, therefore, saponins are essential in conventional medicine [21]. Aqueous plant extracts of *V. subterrenea* protect the body from free radical harm by neutralizing it. This can be explained by the fact that the aqueous extracts of the *V. subterrenea* Nut plant contain flavonoids, which are known to have antioxidant properties. Free radicals, which damage the human body's cells every day, are highly unstable and reactive chemicals that antioxidants neutralize [22]. The harm caused by free radicals is regarded to be a contributing factor to many health conditions, including cancer, heart disease, diabetes, aging, and more. The presence of tannins (Table 3) also promotes wound healing [23].

4.2 Effect of Crude Aqueous Extract of *V. subterrenea* Leaf, Nut, Stem and Nut Fractions on Serum Biochemistry and Hematology

V. subterrenea Nut is used traditionally to manage diabetes mellitus in Nigeria. This research work has shed light on the antidiabetic effect of *V. subterrenea* Nut. From the result obtained from this work, *V. subterrenea* aqueous extracts have shown activity in serum chemistry and hematology parameters of streptozotocin-induced diabetic rats.

Medicinal plants are gaining more attention than ever, according to [17], because they have the potential to be extremely beneficial to society and, by extension, to all humankind. When rats fed an aqueous extract of *V. subterrenea* Nut, Leaf, and Stem were compared to diabetic control rats from Table 4, it was observed that the serum glucose level of the extract-fed animals was significantly lower ($P \leq 0.05$). This demonstrates *V. subterrenea's* antidiabetic abilities and its capacity to reduce blood sugar levels in diabetic conditions. The presence of phytochemical components in the *V. subterrenea* plant may be the cause of the drop in blood sugar levels.

Gluconeogenesis is increased when there is high level of blood glucose and this leads to high catabolism of protein and loss of nitrogen which can create negative balance of nitrogen. A decrease in the total serum protein level is frequently seen in diabetics, which can be attributed to oxidative phosphorylation inhibition, decreased protein synthesis, increased catabolic processes, and decreased protein absorption [24]. However, compared to the diabetic control group, the administration of *V. subterrenea* Nut Leaf and Stem aqueous extract significantly raised protein and albumin levels. This demonstrates that the treated groups' blood protein and plasma albumin levels were elevated by an aqueous extract of *V. subterrenea* Nut, Leaf, and Stem (Table 4).

Bilirubin is formed through the breakdown of heme and is eliminated from the body by the liver

[25]. It is a known fact that measuring bilirubin levels is an essential test for evaluating liver function since an elevated level of bilirubin in the blood may indicate problems with the liver's excretory function, excessive hemolysis, or obstruction in the biliary tract. Hyperglycemia can result in increased protein glycation. A high level of total bilirubin suggests an excess breakdown of hemoglobin or a malfunction in liver function in relation to hemoglobin [25]. From Table 4, the total bilirubin and conjugated bilirubin levels were significantly higher (P≤ 0.05) in the diabetic control rats, which may be related to decreased in liver uptake, this indicates that diabetes affects the aforementioned clinical conditions as compared to normal control on Table 4. However, after administration of an aqueous *V. subterrenea* Nut, Leaf, and Stem extract, there was a significant reduction in total bilirubin (P ≤0.05) and in conjugated bilirubin (Table 4).

Dysfunction in lipid and carbohydrate metabolism is one of many symptoms of diabetes [26]. Free radical overproduction is the main cause of the elevated lipid peroxidation seen in diabetes mellitus. Glycosylated proteins, auto-oxidation, decreased levels of the enzyme superoxide dismutase, ascorbic acid, shortage of reduced glutathione are other variables that cause oxidative stress [26,27]. Abnormally high amounts of cholesterol in the bloodstream define hypercholesterolemia. In this study, Table 5 shows the triglycerides and total cholesterol of the treatment group was experimentally significantly low when compared to diabetic control rats. However, high density lipoprotein (HDL) as seen in Table 5 is significantly high when compared with both normal and diabetic control. Elevated levels of cholesterol in the blood, specifically higher concentrations of LDL-cholesterol and lower concentrations of HDL-cholesterol, are strongly associated with cardiovascular diseases since they encourage the development of atheroma in arteries, leading to atherosclerosis [27]. The study also shows that *V.subterrenea* aqueous plant extracts was able to significantly (P≤0.05) increase HDL in rats across groups when compared with diabetic control. The LDL (Table 5) showed a significant reduction (P≤0.05) in the nut aqueous extract and metformin when compared with diabetic control but leaf and Stem did not show a reduction in serum LDL as compared to the nut.

Alkaline phosphatase (ALP) is an enzyme that is vital for various physiological processes, including liver function and bone development. Conversely, significant deviations in ALP levels can indicate an underlying medical condition, usually related to the liver, bones, or gallbladder [28]. In order to diagnose a condition and track a patient's progress during therapy, it is possible to measure the activity of the enzymes alanine and aspartate aminotransferase in blood serum. This measurement can also be used to analyze the degree of harm and toxicity a chemical compound has on organs or tissues [29]. In the case of diabetes, the concentration of these enzymes in the blood is often increased, the diabetes control in Table 6 revealed that AST and ALP were elevated, and this finding is consistent with other investigations. When compared to the untreated streptozotocin-induced diabetic rat, the serum levels of ALT, AST, and ALP were considerably (P≤0.05) decreased by the aqueous extract of *V. subterreanea* as obvious in the treatment group.

Renal function indicators such serum creatinine, urea, and uric acid are higher in diabetes patients [30]. A kidney function test (Table 7) revealed that the untreated diabetic rats' urea, creatinine, and uric acid levels were significantly (P≤0.05) high, affecting kidney functions. When plant extracts was received, the levels of urea, creatine, and uric acid significantly decreased (P≤ 0.05), easing the impaired effect. The accelerated breakdown of liver and plasma proteins that occurs with gluconeogenesis associated with hyperglycemia may be to blame for the rise in urea levels seen in diabetes [31]. According to reports, poorly controlled diabetes mellitus may be to responsibility for the substantial muscle breakdown that results in increased levels of creatinine in diabetics [29]. The indices of renal function were significantly (P ≤0.05) decreased by the aqueous extract of the *V. subterrenea* plant.

Deficiency in insulin as seen in hyperglycemia, and hyperketonemia may all contribute to subjects with diabetes having an electrolyte and water imbalance [29]. Electrolytes, which are necessary for numerous body processes including controlling fluid levels, pH balance, nerve conduction, blood clotting, and muscle contraction, might become unbalanced as a result of diabetes. Electrolyte imbalances can be caused by kidney disease, dehydration, a high temperature, vomiting, and other conditions. They can also worsen the symptoms of diabetes and other endocrine problems because of the increased excretion of metabolites through the kidneys in diabetes [29]. The current study

demonstrates that oral administration of *V. subterrenea* extracts significantly (P≤0.05) reduced the level of serum electrolytes (Na+, K+, and HCO3-) Table 8. On the other hand, the diabetic untreated rats had significant increment when compared with the normal control. The result obtained from this work demonstrates that it the plant extracts exhibits potent anti-diabetic activity; however, there was significantly elevated chloride in the diabetic rats treated with *V. subterrenea* aqueous extract.

Studies have also shown that patients with diabetes exhibit changes in their hematological indices, Patients with diabetes experience persistent high blood sugar levels, which can lead to the glycation of various proteins involved in clotting mechanisms, such as hemoglobin, prothrombin, and fibrinogen, in addition to red blood cells [31]. This alteration in hematology indices is a consequence of the glycation process [32]. High levels of hematocrit (Hct) and mean corpuscular volume (MCV) in diabetic patients may be caused by various changes in the shape and size of red blood cells (RBCs) as well as alterations in the plasma composition [33]. In this study (Table 9), RBC indices had shown significant (p≤0.05) increment in diabetic untreated rats as compared to normal control group, also, the Leaf and Stem treatment group showed increment in RBC indices. However, administration of *Vigna subterrenea* Nut and Metformin extract was able reduce RBC indices significantly when compared with diabetic control. This disparity may be caused by non-enzymatic glycosylation of RBC membrane proteins brought on by persistent hyperglycemia, which accelerates the aging of RBCs. This research also compared the platelet indices between the extracts group and the diabetic control groups; the latter revealed a substantial rise in platelet indices when compared with normal control, demonstrating that DM can raise platelet indices. According to Table 9, there was a significant difference (P≤0.05) in the decline in platelet count between the Nut, Leaf, and Stem groups and the diabetes control groups. Additionally, a statistically significant increase in MPV and PDW was discovered in the diabetic control, which is consistent with other studies that have demonstrated an increase in the number of large circulating platelets compared to controls [34].

Research indicates that there may be a link between inflammation and the development of diabetes, and studies have also shown a correlation between higher white blood cell (WBC) counts and diabetes [35]. When compared to the normal control group, WBC indices considerably rose in the diabetic control group. However, there was a significant reduction in WBC indices of rats fed with *V. subterrenea* extracts when compared with diabetic control as observed in Table 10.

5. CONCLUSION

The study shows that the aqueous extracts (Nut, Leaf and Stem) of *V. subterrenea* plant were effective in reducing the negative impact of diabetes mellitus on the treatment groups. These extracts showed properties such as hypoglycemic, hypolipidemic and hypocholesterolamic effects. The serum enzyme activities were also lowered, and there was enhanced protection against renal dysfunction. Furthermore, the extract had a positive effect on red blood cells, platelets, and white blood cells. The presence of several phytochemicals in the extract could be the cause of the observed effects.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Agricultural Research Service. Vigna subterranea. Germplasm Resources Information Network (GRIN), United States Department of Agriculture (USDA); 2017. Retrieved 15 December 2017.
2. FAO. Traditional Crops. FAO.org; 2019
3. Rafieian-Kopaei Mahmoud. Medicinal plants and the human needs, J Herb Med Pharma Col. 2012;1(1):1–2.
4. Abo KA, Fred-Jaiyesimi AA, Jaiyesimi AEA. Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. Journal of Ethnopharmacology. 2008;115(2008): 67–71.
5. American Diabetes Association [ADA]. Standards of medical care in diabetes.

Diabetes Care. 2011;34(Suppl. 1):S11–S61.

6. Uloko AE, Musa BM, Ramalan MA, Gezawa ID, Puepet FH, Uloko AT, et al. Prevalence and Risk Factors for Diabetes Mellitus in Nigeria: A Systematic Review and Meta-Analysis. Diabetes Therapy. 2018;9(3):1307-1316.
Available:https://doi.org/10.1007/s13300-018-0441-1
7. Gezawa ID, Puepet FH, Mubi BM, Uloko AE, Haliru I. Prevalence of overweight and obesity in Maiduguri North-Eastern Nigeria. Nigerian J Med. 2013;22(3):171–174. [PubMed] [Google Scholar]
8. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008;51:216–226
9. Khalid S, Shahzad A, Basharat N, Abubakar M, Anwar P. Phytochemical Screening and Analysis of Selected Medicinal Plants in Gujrat. J Phytochemistry Biochem. 2018;2:108.
10. Sánchez Rojas F, Cano Pavón JM. In Encyclopedia of Analytical Science (Third Edition); 2019.
Roberto Macrelli MATH, Marcello Ceccarelli M, BIOL, Letizia Fiorucci, Journal of Herpetological Medicine and Surgery. 2013;23(1-2):20–24.
11. Jagroop Singh, Sukhraj Kaur, Manjinder Kaur, Manpreet Kaur. A Comparative Study of Detection of Serum Total and Direct Bilirubin by Manual and Automated Methods. International Journal of Scientific Research in Science and Technology. 2022;9(4):209-212.
12. Lipid Laboratory Johns Hopkins. Total Cholesterol, HDL-Cholesterol, Triglycerides, and LDL-Cholesterol: Laboratory Procedure Manual. 2004;1-23.
13. Collaborative Laboratory Services, L.L.C Alanine Amino Transferase (ALT) Laboratory Procedure Manual. 2007;1-10.
14. Akcakaya H, Aroymak A, Gokce S. A quantitative colorimetric method of measuring alkaline phosphatase activity in eukaryotic cell membranes. Cell Biol Int. 2007 Feb;31(2):186-90.
DOI: 10.1016/j.cellbi.2006.11.014
Epub 2006 Nov 28. PMID: 17207647.
15. Old J, Harteveld CL, Traeger: -Synodinos J, et al. Prevention of Thalassaemias and Other Haemoglobin Disorders: Laboratory Protocols [Internet]. 2nd edition. Nicosia (Cyprus): Thalassaemia International Federation;. Chapter 2, Haematological Methods. 2012;2.
Available:https://www.ncbi.nlm.nih.gov/books/NBK190583/
16. Blaxhall PC, Daisley KW. Routine haematological methods for use with fish blood. Journal of Fish Biology. 2006;5:771-781
17. Krishnaiah D, Devi T, Bono A, Sarbatly R. Studies on phytochemical constituents of six Malaysian medicinal plants. Journal of Medicinal Plants Research. 2009;3(2):067-072,
18. Akinmoladun C, Afolabi, Ibukun EO, Dan-Ologe. Phytochemical constituents and antioxidant properties of extracts from leaves of Chromolaena odorata. Scientific Reasearch and Essay. 2007;2(6):191-194.
19. Bikash Adhikari, Roles of Alkaloids from Medicinal Plants in the Management of Diabetes Mellitus, Journal of Chemistry. 2021;Article ID 2691525:10.
Available:https://doi.org/10.1155/2021/2691525
20. Ekwueme FN, Nwodo OFC, Joshua PE, Nkwocha C, Eluka PE. Qualitative and Quantitative Phytochemical Screening of the Aqueous Leaf Extract of *Senna mimosoides* Its Effect in *in vivo* Leukocyte mobilization induced by inflammatory stimulus, Int. J. Curr. Microbiol. App. Sci. 2015;4(5):1176-1188.
21. Jung M, Park M, Lee HC, Kang YH, Kang ES, Kim SK. Anti-diabetic agents from medicinal plants. Curr. Med. Chem. 2006;13:1203-1208.
22. Dai J, Mumper RJ. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010;15:7313-7352.
23. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants. African Journal of Biotechnology. 2006;5(4):357-361.
24. Murray RK, Granner DK, Mayes PA, Rodwell VW. Harpers's Illustrated Biochemistry. 25th Edn., Appleton and Lange, Philadelphia. 2000;:179-203, 621-630.
25. Nwogo Ajuka Obasi, Kalu Mong Kalu, Uchechukwu Okorie and Glory Otuchristian. Hypoglycemic effects of Aqueous and Methanolic Leaf Extracts of Vitex donianaon Alloxan Induced Diabetic Albino Rats. Journal of Medical Sciences. 2013;13:700-707.

26. Suryawanshi NP, Bhutey AK, Nagdeote AN, Jadhav AA, Manoorkar GS. Study of Lipid Peroxide and Lipid Profile In Diabetes Mellitus. Indian Journal of Clinical Biochemistry. 2006;21(1):126-130.
27. Lenka Jessica Lapshak, Luka Carol Domkat, Larfa Simon Nansah. The Effect of Aqueous Extract of Buchholzia coriacea Seeds on Some Biochemical Parameters in Normal and Alloxan-induced Diabetic Rats. International Journal of Biochemistry Research & Review. 2016;11(1):1-10. Article no.IJBCRR.24495.
28. Ohaeri OC. Effect of Garlic Oil on the Levels of Various Enzymes in the Serum and Tissue of Streptozotocin Diabetic Rats. Bioscience Reports. 2001;21(1).
29. Shenqi Wang, Xuhong Ho, Yu Liu, Huijuan Lu, Li Wei, Yuqian Bao, Weiping Jia. Serum electrolyte levels in relation to macrovascular complications in Chinese patients with diabetes mellitus. Cardiovascular Diabetology. 2013;12:14. Available:http://www.cardiab.com/content/12/1/146
30. Keith GT, Vivian S, Anthony D. Spectrum Of Liver Disease In Type 2 Diabetes And Management OF Patients With Type 2 Diabetes And Liver Disease. Disease Care, 2007;30(3):734-743
31. Dallatu MK, Anaja PO, Bilbis LS, Mojiminiyi FBO. Antioxidant micronutrient potentials in strengthening the antioxidant defense in alloxan-induced diabetic rats. Niger J Pharm Sci. 2010;8:89–94.
32. Selvin E, Steffes MW, Zhu H. Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. N Engl J Med. 2010;362:800–811.
33. Marcinkowska-Gapinska A, Kowal PA. Blood fluidity and thermography in patients with diabetes mellitus and coronary artery disease in comparison to the healthy subject. ClinHemorheol Microcir. 2006;35:473.
34. Belete Biadgo, Mulugeta Melku, Solomon Mekonnen Abebe, Molla Abebe. Hematological Indices and their Correlation With Fasting Blood Glucose Level And Anthropometric Measurements In Type 2 Diabetes Mellitus Patients In Gondar, Northwest Ethiopia. J Diabetes, Metabolic; 2016.
35. Karthikeyan VJ, Lip GY. White blood cell count and hypertension.J Hum Hypertens. 2006;20:310–312.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/100494