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Original Research Article

Assessment of Pharmacodynamic Interactions and Toxicological Effects of *Vernonia amygdalina* –Metformin Co-Administration on Streptozotocin-Induced Diabetic Wistar Rats

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ABSTRACT

Studies have shown an increasing use of herbs concurrently with conventional drugs by patients with diabetes mellitus, with the attendant risks of herb-drug interactions. Hence, this study investigated the possible pharmacological interactions, and toxicological consequences of aqueous extracts of *Vernonia amygdalina* (VA) and metformin co-administered in streptozotocin (STZ)-induced diabetic Wistar rats. Model of type 2 diabetes was induced in Wistar rats by feeding them with high fat diet and a single dose of STZ (60 mg/kg, i.p.). Animals having blood glucose levels ≥ 200 mg/dl after 72 hours were classified as diabetic and were randomly assigned to one of eleven (11) groups (n=5 rats) to receive VA extract, metformin or combination at various doses. Blood sample was taken for biochemical and haematological tests while Haemoxylin and Eosin (HE) dye was used to assess histological changes. Results showed a non-additive decrease in blood glucose levels in the VA/metformin co-administration compared to VA and metformin alone. The reduction in blood glucose in VA 50 mg/kg, VA/MET 150 mg/kg and VA 100 mg/kg groups ware -51.20%, -53.52% and -59.68% respectively. There was significant improvement in urea and creatinine levels as well as liver enzymes at all the doses of VA and VA/metformin co-administration. There were no significant histological changes either in the extract or its combination with metformin on the kidney, liver or pancreas. Hence, VA showed a non-additive interaction with metformin to reduce blood glucose levels with no toxicological effects on major organs.

***Keywords*:** *Vernonia amygdalina* Extracts, Metformin, Streptozotocin, Hyperglycaemic, Wistar rats.

Introduction

Diabetes is a complex condition that is usually managed with both non-pharmacologic and pharmacologic modalities.[1] The non-pharmacologic modality includes lifestyle modifications such as dietary control, regular exercise, and weight control. Many conventional anti-diabetic medications are available including biguanide (metformin), sulphonylureas, meglitinides, dipeptidyl peptidase 4-inhibitors, glucagon-like peptide-1 (GLP-1) agonists, among others and insulin. However, the increase in the incidence of T2DM, especially in developing countries, together with the side effects associated with these drugs, has highlighted the need for more effective, safer and less costly pharmacological management options.[2] World Health Organization (WHO) has estimated that 80% of people worldwide rely on herbal medicines for some aspect of their primary healthcare[3] as they are frequently considered as being natural, cheap, readily accessible, less toxic and with lower side effects than the conventional orthodox drugs, majority of which are synthetic.

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It is reported that worldwide, up to 72.8% of individuals with diabetes used herbal medicine, dietary supplements and other complementary alternative medicine (CAM) therapies.[4] In Nigeria, more than 60% of individuals with diabetes are reported to be using medicinal plants including *Moringa oleifera*, *Aloe vera*, *Vernonia amygdylina*, *Momordica charantia* among others.[5] Furthermore, studies indicate that most people who use CAM therapies do so in addition to (i.e. as complementary), rather than in place of (alternative) conventional medicine.[6] The combination of anti-diabetic herbs and orthodox medications has raised safety issues since most of the biocactive substances contained in herbal products are not well characterized unlike in orthodox drugs.[7] The co-administration of herbs with pharmaceutical anti-diabetic medications may result in herbal-drug interactions (HDI) leading to enhancing effects (clinically desirable effects), decreased pharmacological effects (decreased therapeutic effects) or adverse drug events causing hypoglycaemia or toxicity to major organs in the body.[2] Studies reveals that *Vernonia amygdalina* is one of the most commonly used medicinal plants among individuals living with diabetes and it is usually co-administered with metformin[1]. The need to document scientific evidence on the efficacy, safety or toxicity induced from pharmacological interactions of *Vernonia amygdalina* extracts when co-administered with metformin in treating diabetes is important. Hence, this study was planned to evaluate toxicity potentials or otherwise due to pharmacodynamic interactions of *Vernonia amygdalina* extract (VAE) and metformin co-administration on Wistar rats following repeated doses on streptozotocin-induced diabetic rats.

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Materials and Methods

Drugs and Chemicals

Streptozotocin powder, metformin hydrochloride was purchased from Sigma-Aldrich Inc. USA. Kits for the assay of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) as well as triglycerides (TG) were obtained from Randox Laboratories Ltd. (Crumlin, UK). Glucometer (Accu Chek Active) of Roche Diagnostics, Germany was obtained from a reputable chemical store. All chemicals used were of analytical grade.

Plant Materials

Fresh leaves of *Vernonia amygdalina* were collected (10 February, 2021) from a garden in Osogbo, Osun State (7^0 46'15. 7^0 N $4^0$33'25. 1^0E), identified and authenticated at the Department of Pharmacognosy, Faculty of pharmacy, Obafemi Awolowo University (OAU), Ile-Ife, Nigeria. A Specimen with voucher No. FPI 2299 was deposited at the herbarium of the department for future reference. The leaves were cleaned with distilled water to remove adulterant, oven-dried at 40^0C to a constant weight and pulverized with a grinding machine. The powdered plant was macerated with 70% ethanol for 48 hours and filtered with Whatman filter paper (size No 1). The filtrate was evaporated to dryness over a rotatory evaporator maintained at 60˚C.

The percentage yield of the extract was calculated as:

$$\text{Percentage yield (\%)} = \frac{Weight\ of\ extract\ (g)}{Weight\ of\ pulverized\ leaves\ (g)} \times 100 =$$

141.2 g/1000 g X 100% = 14.12%

The value of the ethanol extract obtained was calculated to be 14.12% w/w

The*Vernonia amygydalina* extract (VAE) was then stored in a refrigerator until required for use.

Animals

Wistar rats weighing 180-220 g were procured from the Department of Pharmacology, OAU, Ile-Ife, Nigeria. They were acclimatized for 2 weeks, then fed on commercially prepared high fat diet to induce type 2 DM model. The animals were maintained and handled according to the recommendations of internationally accepted guidelines[12]. Permission to conduct the study was obtained from the Health Research Ethics Committee, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife with reference number OAU/HREC/1512.

Acute toxicity study

The oral median lethal dose (LD_{50}) of the extract was determined in rats according to the method previously described[6]. The study was done in two phases. In the first phase, nine rats were randomized into three groups of three rats which were given VAE orally at doses of 10, 100, and 1000 mg/kg. The rats were kept under similar conditions and were observed for signs of toxicity which included but were not limited to stretching, paw-licking, respiratory distress and mortality for 24 hours. Based on the results of the initial phase, three (3) other rats were administered 1600, 2900 and 5000 mg/kg VAE respectively. The rats were also monitored closely for the 24 hours after treatment for signs of toxicity and/or mortality. The results obtained in the second phase were used to calculate the LD_{50}. The LD_{50} was calculated as the geometric means of the maximum dose producing 0 % mortality (*a*) and the minimum dose that produced 100 % mortality (*b*) and mathematically expressed as:

$LD_{50} = \sqrt{ab}$

Induction of type-2 diabetes

After 6 weeks of feeding rats a commercially available high-fat diet (HFD) with the following composition (Table 1) to induce insulin resistance, the rats were fasted overnight and injected intraperitoneally (i.p) with freshly prepared STZ (dissolved in a citrate buffer pH 4.5, 0.1 M) at a single dose of 60 mg/kg. Following that, the animals were given free access to food and water. Plasma glucose was measured 72 hours after the injection using a glucometer (Accu-Chek® Active) following an 8-hour fast.

Table 1: Composition of the controlled and high fat diet

Diet components	Control diet	High fat diet (g)
Energy (Kcal/g)	3.00	6.4
Calorie percentage:		
Carbohydrate	60	30
Fat	15	65
Protein	25	5
Weight Percentage		
Carbohydrate	10	40
Fat	25	40
Protein	60	15
Materials	Standard chow diet	Maize (2.75), Wheat offal (0.25) Groundnut cake (6.75), Soya meal (10.25) Palm kernel (6.5) cake oil, Bone meal (0.25), Methionine (0.125), Lysine (0.125), Salt (0.03125), Finisher premix (0.03125), Cluppled (0.25)

On the glucometer, a single puncture on the tail tip of each rat (allowing only one drop to come out) was used to obtain a blood sample. The study used rats with a plasma glucose level of not less than 200 mg/dl.

Grouping of experimental animals

Animals were randomly divided into eleven groups of five animals each and were assigned to the following treatments;

Group I (Non-diabetic control): administered with 10 ml/kg of distil water daily

Groups II (Diabetic control): administered with 10 ml/kg of distil water daily.

Groups III, IV, and V (Diabetic): administered with doses of 50, 100 and 150 mg/kg respectively of VAE daily.

Groups VI and VII (Diabetic): administered with 100 mg/kg and 150 mg/kg of metformin respectively daily.

Groups VIII to XI (Diabetic): administered with a combination of metformin and VAE daily; V50/M100 mg/kg; V50/M150 mg/kg; V100/100 mg/kg and V100/150 mg/kg.

metformin hydrochloride solution was usually prepared fresh for each day's experiment to ensure stability

The extracts of *Vernonia amygdalina* leaves and metformin were administered orally throughout the study.

Alternate day fasting blood sugar (FBS) estimation

Fasting blood sugar (FBS) was estimated on day 0 (i.e. day 3 post induction for the hyperglycaemic sub-groups) and subsequently alternate day until next day 15. Blood samples were collected from the tail vein with a sterile scissors and tested using the digital glucometer and its test strips.

Blood analysis

On the 15th day of the experiment, all the rats were euthanized by Diethylether inhalation and blood samples were collected by cardiac puncture. The blood was collected into EDTA for haematological analysis such as estimation of complete blood counts and haemoglobin contents. Parameters such mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and haematocrit were evaluated.

Blood samples were also collected into plain serum tubes, allowed to clot and centrifuged at 3500 rpm for 15 minutes. The sera were separated, stored and used for evaluation of biochemical parameters, (aspartate transaminase (AST), alanine transaminase (ALT) levels, alkaline phosphatase (ALP) levels, total protein, albumin, total cholesterol, HDL-Cholesterol, LDL-Cholesterol, triglyceride, serum urea and creatinine levels.These were determined using a commercial

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assay kit and an automated chemistry analyser (Labmax Plenno, Labtest Co.Ltd., Lagoa Santa, Brazil).

Collection of organs
Some organs were collected for histopathological analysis. The pancreas of each rat was collected, washed with normal saline, wiped and weighed, thereafter put in buffered formalin (10% formaldehyde in phosphate-buffered saline) in order to preserve it. Liver and kidney were also collected into Touin buffer solution and stored as such.

Histopathological studies
The excised organs were fixed in 10% formal saline for about 48 hours. Tissue slices were cut and kept in the automatic tissue processor where they were fixed in 10% formal-saline saline solutions for 2 hours. They were then dehydrated for 2 hours in each of the following ascending grades of alcohol: 85%, 90%, and 100% v/v. The dehydrated tissues were then cleared in toluene for 2 hours, after which the tissue slices were embedded in paraffin wax and left to cool. The blocks were trimmed on the microtome at 5 microns. The ribbon of sections was dehydrated in xylene and rehydrated in the following grades of alcohol 100%, 90%, and 70% v/v. They were then stained in haemotoxylin for about 5 minutes, differentiated in 1% acid alcohol, blotted in scotch‟s tap water and stained in eosin for 3 minutes. They were later rinsed, dehydrated in ascending grades of alcohol: 70%, 90% and 100%. Finally, they were cleared in xylene and mounted. The slides were then examined microscopically for pathological lesions.
Statistical analysis
All data were expressed as mean ± standard error of mean (SEM) and statistical differences between means were determined by one-way ANOVA followed by Dunnett's *post –hoc* test for multiple comparison tests. Significance level is *P<0.01*.

Results and Discussion

Acute toxicity study
In both phases of the experiment, the ethanol extract of *Vernonia amygdalina* did not produce any sign of toxicity or mortality at the doses administered orally. The oral median lethal dose (LD_{50}) of the extract was, therefore, assumed to be greater than 5000 mg/kg body weight in rats.[8]

Effect of V. amygdalina extract, metformin and V. amygdalina/metformin co-administration on blood glucose levels in rats
The blood glucose levels (mg/dl) of Wistar rats after 14 days of treatment with extract, metformin and their various combinations are shown in Figure 1. While distilled water maintained the blood glucose level to almost same levels as day 1 of the experiment (101.0 ± 2.96 vs. 86.8 ± 2.95), there was a significant ($p<0.01$) increase in blood glucose levels upon diabetic induction before the commencement of treatment in all groups. With commencement of treatment, gradual, but varying degree of decrease in blood glucose level was observed among the VAE, metformin and VAE/metformin combination treated groups till the end of the experiment. While reduction in blood glucose in VAE 50 mg/kg group was by (-51.20%) and 50 mg/kg VAE/MET 150 mg/kg group was (-53.52% %), reduction in blood glucose was observed to be highest (-59.68%) in VAE 100 mg/kg group, Table 2 showed trends in the percentage of blood glucose changes over time.

Effect of Vernonia amygdalina extract, metformin and VAE/Metformin co-administration on serum lipid profile of streptozotocin-induced diabetic Wistar rats
Figure 2 summarizes the effect of the various concentrations of VAE, metformin and VAE-metformin combinations on the level of serum lipid in diabetic rats. The results showed that both total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) progressively declined significantly ($p<0.01$) with increased concentrations of VAE in the diabetic rats compared to normoglycaemic control rats. This decrease continued with co-administration of VAE-metformin in varying concentrations. Also, there was a significant ($p<0.01$) increase with increasing concentrations of VAE, metformin and VAE-metformin concentrations in the level of HDL-C in the diabetic groups compared to normoglycaemic control (Figure 2).

Effect of Vernonia amygdalina extract, metformin and VAE/metformin co-administrations on serum creatinine and urea of streptozotocin-induced diabetic Wistar rats
There was significant ($p<0.01$) elevation of both urea and creatinine in the diabetic-induced rats compared to normoglycaemic control rats. However, treatment with VAE (50 mg/kg), VAE (100 mg/kg) and MET (100 mg/kg) showed significant ($p<0.01$) reduction in urea and creatinine when compared to the diabetic control. Conversely, both VAE 150 mg/kg and MET (150 mg/kg) - treated groups showed significant ($p<0.01$) increase in urea and creatinine when compared with diabetic control.

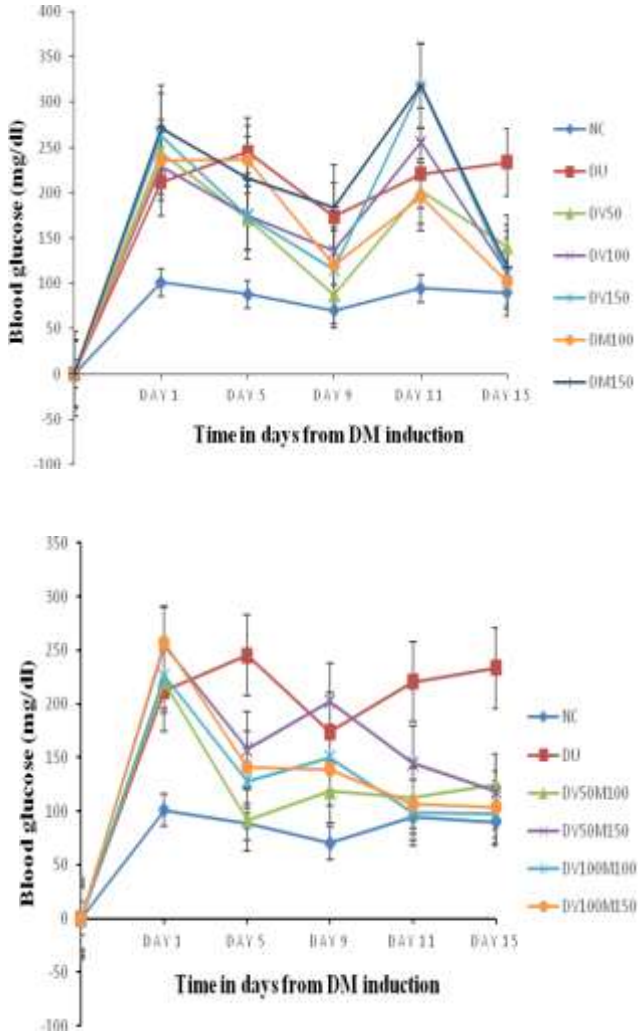

Figure 1(A&B): Effect of *V. amygdalina* extract, metformin and *V. amygdalina*/metformin co-administration on blood glucose levels in rats.
*=$p < 0.01$, i.e., statistically significant change in alternate day blood glucose levels by VAE, Metformin, & VAE/metformin co-administered groups. KEY: MET = Metformin, VAE = *Vernonia amygdalina* leaf extract; DV100 = Diabetic + VAE (100 mg/kg); DV150 = Diabetic + VAE (150 mg/kg) ; DM100 = Diabetic + MET (100 mg/kg); DM150 = Diabetic + MET (150 mg/kg); DV50M100 = Diabetic + VAE (50 mg/kg) +MET (100 mg/kg); NC Normoglycaemic control (Distilled water); DV50M150 = Diabetic + VAE (50 mg/kg) +MET150 mg/kg; DV50 = Diabetic + VAE (50mg/kg); DV100M150 = Diabetic + VAE (100 mg/kg) +MET (150 mg/kg).

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Table 2: Effects of blood glucose in rats after administration of *V. amygdalina* extract, metformin and *V. amygdalina*/metformin co-administration

Treatment groups	Percentage relative change of blood glucose (mg/dl)				Percentage (%) change in blood glucose
	Day 5	Day 9	Day 11	Day 15	
NC	-12.87 ± 9.10	-20.11 ± 5.12	34.42 ± 38.56	-4.97 ± 27.85	-11.09
DU	15.57 ± 11.01	-29.18 ± 31.64	27.09 ± 39.79	5.90 ± 14.98	+10.14
DV50	-16.14 ± 11.41	-49.27 ± 23.43	131.61 ± 127.90	-50.47 ± 128.75	-51.20
DV100	-37.06 ± 26.21	-22.53 ± 10.27	88.14 ± 78.26	-56.05 ± 101.96	-59.68
DV150	-33.92 ± 23.99	-33.83 ± 0.06	175.91 ± 148.31	-39.87 ± 152.58	-27.45
DM100	0.30 ± 0.21	-49.26 ± 35.04	62.92 ± 79.32	-48.08 ± 78.49	-56.96
DM150	-20.52 ± 14.51	-14.83 ± 4.02	73.29 ± 62.31	-62.86 ± 96.27	-56.43
DV50M100	-58.37 ± 41.27	28.87 ± 61.69	-5.07 ± 24.00	10.86 ± 11.26	-42.21
DV50M150	-38.11 ± 26.95	28.57 ± 47.15	-28.74 ± 40.52	-18.02 ± 7.58	-53.52
DV100M100	-43.36 ± 30.66	17.03 ± 42.70	-34.25 ± 36.26	-0.71 ± 23.72	-56.73
DV100M150	-45.14 ± 31.92	-1.42 ± 30.91	-23.53 ± 15.63	-2.35 ± 14.98	-59.61

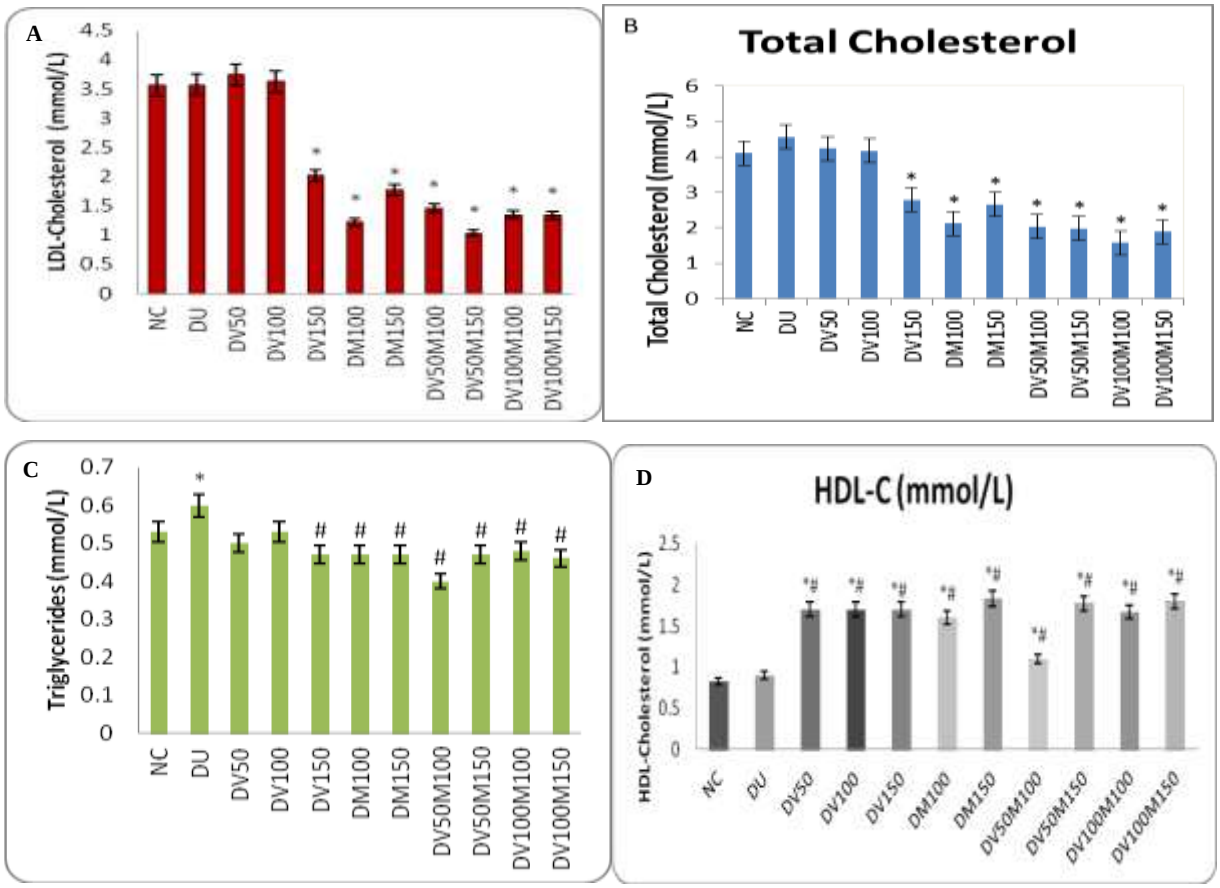

Figure 2 (A-D): Effect of Vernonia amygdalina extract, metformin and VAE/Metformin co-administration on serum lipid profile of streptozotocin-induced diabetic Wistar rats. Data represented as mean ± S.E.M of lipid profile, analysed by one-way ANOVA followed by Duncan's post – hoc test for multiple comparisons. *= $p < 0.01$ statistically significant compared with control, #=$p<0.01$ statistically significant compared with non-intervention group.
KEY: MET = Metformin, VAE = *Vernonia amygdalina* leaf extract
DV100 = Diabetic + VAE (100 mg/kg); DV150 = Diabetic + VAE (150 mg/kg)
DM100 = Diabetic + MET (100 mg/kg); DM150 = Diabetic + MET (150 mg/kg)
DV50M100 = Diabetic + VAE (50 mg/kg) +MET (100 mg/kg);
NC = Normoglycaemic control (Distilled water); DV50 = Diabetic + VAE (50mg/kg)
DV50M150 = Diabetic + VAE (50 mg/kg) +MET (150 mg/kg);
DV100M100 = Diabetic + VAE (100 mg/kg) +MET (100 mg/kg); DU = Diabetic untreated
DV100M150 = Diabetic + VAE (100 mg/kg) +MET (150 mg/kg)

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In the VAE-MET combination treated-groups, all showed significant ($p < 0.01$) reduction in the urea and creatinine levels while VAE (100 mg/kg)-MET (150 mg/kg) combination treated group demonstrated a significant ($p < 0.01$) elevation in both urea and creatinine levels, Figure 3.

Effect of Vernonia amygdalina extract, metformin and VAE/metformin co-administration on liver function enzymes of streptozotocin-induced diabetic wistar rats

There was a statistically significant ($p < 0.01$) increase in the serum levels of AST, ALT and ALP in the STZ-induced diabetic rats compared to non-diabetic control. In this study, treatment of the STZ-diabetic rats with *V.amygdalina* extract, metformin and various doses of extract/metformin co-administrations brought about decrease of the transaminases activity, except for MET 150 mg/kg, 100 mg/kg VAE+MET 100 mg/kg and 100 mg/kg VAE+ MET 150 mg/kg which were all associated with varying degrees of increase in transaminases activities. The rate of decrease occurred in a dose- dependent fashion with the VAE (150 mg/kg)-treated group showing the most reduction among the extract-alone treated groups.

However, metformin (150mg/kg) led to the highest increase in AST, ALT and ALP levels (possibly indicating toxicity at this dosage). Metformin (150mg/kg)-treated group was associated with significant ($p<0.001$) increase in AST, ALT and ALP compared to diabetic control, Figure 4.

Haematological parameters

It was observed that 14 days after the induction of diabetes, the values of haematocrit (HCT), haemoglobin (Hb), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were significantly lower in the diabetic untreated rats compared with normoglycaemic (non-diabetic) control rats. However, treatment with various combinations of VAE and metformin 100 mg/dl resulted in statistically significant ($p <0.05$) increase in HCT, Hb, MCHC and MCV compared with normoglycaemic control rats. There were also statistically significant ($p<0.05$) increases in the values of HCT, Hb, MCV and MCHC in the VAE-metformin combinations compared to either VAE or metformin alone among diabetic rats with the highest rise seen with VAE (100 mg/kg)-Metformin (100 mg/kg) combinations. In contrast however, there was significant reduction in the above blood morphologic parameters/indices (Hb, HCT, MCHC and MCV) with Metformin 150 mg/kg.

VAE, metformin and VAE-metformin combinations extract-treated rats showed a non-significant decrease in RBC values when compared with the diabetic control group (Figure 4.3), the lowest value of RBC was obtained with VAE (50 mg/kg)-metformin (100 mg/kg) combination.

Platelet count of both VAE-and metformin-treated diabetic rats significantly ($p < 0.05$) decreased when compared with the non-diabetic normoglycaemic rats. There was progressive reduction in the levels of the platelet counts as the concentrations of both VAE and metformin increased. This decrease further dropped with increase in the concentrations of both VAE and metformin in the combinations, (Figure 5).

Total white blood cells (WBC) and differentials were decreased in diabetic rats when compared with normoglycaemic control. Administration of VA resulted in a decrease in the total white blood cells (WBC) but a combination of VAE and metformin results in non-significant increase compared to VAE or metformin alone, (Figure 4.2).

Histopathology of organs of hyperglycaemic and normoglycaemic Wistar rats after 14 days of VAE, Metformin and VAE/Metformin oral administration

At the end of 14 days of drug administration, the effect of the VAE, metformin and VAE/metformin co-administration on the histological appearance of the selected organs (liver, kidney and pancreas) was microscopically examined following Haematoxylin and Eosin stain.

The histological features of liver, kidney and pancreas tissues of the animals administered VA extracts in all the groups are shown in Plates, 1-11 below.

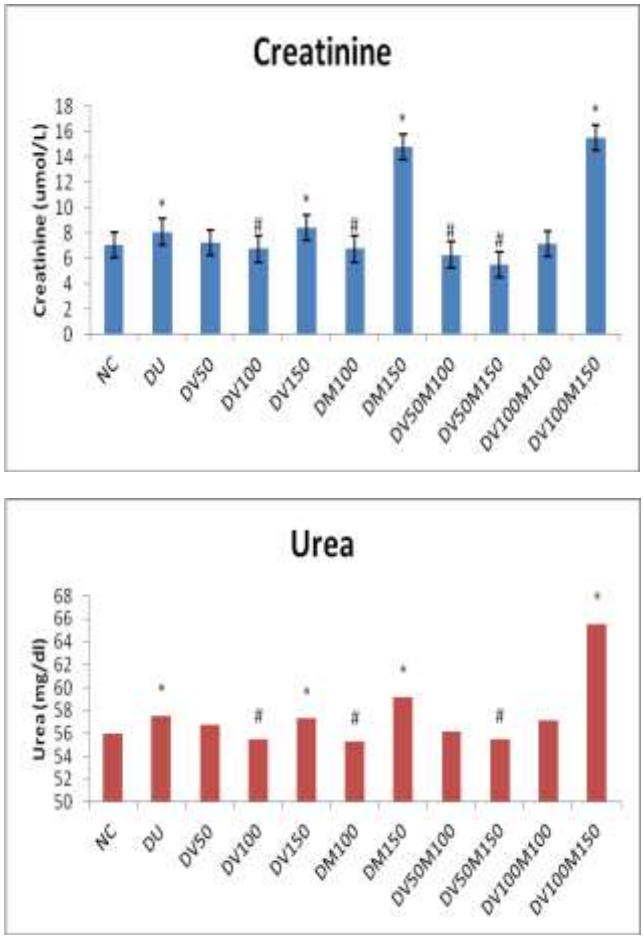

Figure 3: Effect of *Vernonia amygdalina* extract, metformin and VAE/metformin co-administrations on urea and creatinine of streptozotocin-induced diabetic Wistar rats.

Data represented as mean of serum creatinine and urea , analysed by one-way ANOVA followed by Duncan's post – hoc test for multiple comparisons. *= $p < 0.01$ statistically significant compared with control, #=$p<0.01$ statistically significant compared with non-intervention group.

KEY: MET = Metformin, VAE = *Vernonia amygdalina* leaf extract

DV100 = Diabetic + VAE (100 mg/kg); DV150 = Diabetic + VAE (150 mg/kg)

DM100 = Diabetic + MET (100 mg/kg); DM150 = Diabetic + MET (150 mg/kg)

DV50M100 = Diabetic + VAE (50 mg/kg) +MET (100 mg/kg);

NC = Normoglycaemic control (Distilled water); DV50 = Diabetic + VAE (50mg/kg)

DV50M150 = Diabetic + VAE (50 mg/kg) +MET (150 mg/kg);

DV100M100 = Diabetic + VAE (100 mg/kg) +MET (100 mg/kg); DU = Diabetic untreated

DV100M150 = Diabetic + VAE (100 mg/kg) +MET (150 mg/kg)

The liver of the rats in all the groups showed normal histological characteristics with preserved hepatic architecture and hepatocytes arranged in plates. The liver was devoid of vascular congestions, haemorrhage or fatty changes with scanty inflammatory infiltration. The kidney tissue showed no remarkable changes-the glomerulus, tubules and interstitium shows no abnormalities. The pancreatic tissues are divided into lobules by connective tissue with islet of Langerhans embedded within exocrine tissue. The anti-diabetic impact of VA extract was investigated in a high-fat-fed diet (HFD) STZ-treated rat (HFD-STZ) model of T2DM. The fortified or HFD-STZ Wistar rat model, which is one of the most extensively used rodent models of type 2 diabetes mellitus, has been demonstrated[7] to be a good T2DM model for screening anti-diabetic botanicals.

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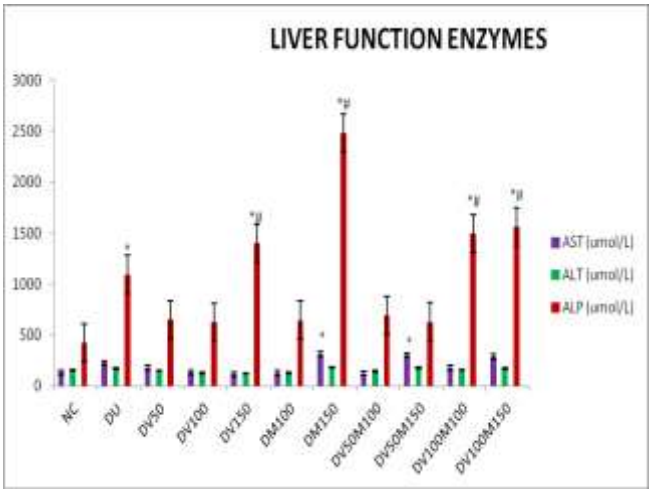

Figure 4: Effect of Vernonia amygdalina extract, metformin and VAE/metformin co-administrations on liver function enzymes of streptozotocin-induced diabetic Wistar rats.
Data represented as mean of liver function enzymes (umol/L), analysed by one-way ANOVA followed by Duncan's post – hoc test for multiple comparisons. *= $p < 0.01$ statistically significant compared with control, #=$p<0.01$ statistically significant compared with non-intervention group.
KEY: MET = Metformin, VAE = *Vernonia amygdalina* leaf extract
DV100 = Diabetic + VAE (100 mg/kg); DV150 = Diabetic + VAE (150 mg/kg)
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DV100M100 = Diabetic + VAE (100 mg/kg) +MET (100 mg/kg); DU = Diabetic untreated
DV100M150 = Diabetic + VAE (100 mg/kg) +MET (150mg/kg)

Frank hyperglycemia was observed in the HFD-STZ model, although there was no serious insulin shortage. This closely resembles the pattern and pathophysiologic process of T2DM in humans, which is characterized by hyperglycemia and relative insulin insufficiency.[10]
Streptozotocin was used to induce experimental diabetes. Streptozotocin induces diabetes by damaging the insulin secreting beta-cells of the islets of Langerhans resulting in reduced synthesis and release of endogenous insulin .[11] STZ reaches the beta cells of the islets of Langerhans through a glucose transporter (GLUT 2) mechanism where it induce diabetes by causing alkylation of DNA by liberating high levels of nitric oxide and nitrosourea, resulting into inhibition of aconitase.[12] STZ differ from alloxan whose cytotoxic action is mediated by reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration leading to a rapid total destruction of beta-cells producing a type 1-like diabetes mellitus .[13]
Although plants utilized in ethnomedicine are thought to be reasonably safe, the safety profile of such plants must always be rigorously validated when employed in official research.[14] The extract's oral LD50 value was found to be larger than 5000 mg/kg. After 14 days of oral dosing, none of the rats showed any toxic symptoms or mortality. This is in line with previous findings of [15-16] Many more researchers have confirmed that the leaf extract is not hazardous to the kidneys or liver [17-19]. As a result, the extract is relatively safe for oral use. Results obtained in this study showed that the ethanol extract of *Vernonia amygdalina* leaves produced significant reduction in blood glucose level in STZ-induced hyperglycaemic rats in a dose dependent manner. This is in agreement with those of other researchers, who previously demonstrated that extracts of *V. amygdalina* possess antidiabetic properties [17, 20-22]. This study demonstrated that *V. amydalina* at concentration of 100 mg/dl posses almost 100% potency of metformin 100 mg/kg.
Many mechanisms had been suggested for the hypoglycaemic effect of *V. amygdalina*. The activity of VA extract is attributable to the phytoconstituents which include flavonoid, saponin and glycoside fractions which have been found to lower blood glucose and ameliorate pancreatic damage [23,24]. It is also due to their antioxidant free radical scavenging activity which could counteract the generation of free radicals, one of the factors responsible for STZ induced diabetes [24]. Additionally, *V. amygdalina* extract is rich in non-starch polysaccharides and fibers which help to slow done rapid glucose excursion (when consumed in diet) hence reported to reduce postprandrial hyperglycaemia in humans [25]. Other findings have shown that fiber-rich food does not raise blood glucose [26,27] rather it enhances insulin sensitivity and may have role in the prevention and management of type 2 diabetes [28].
The anti-hyperglycaemic activity of the extract/metformin co-administration was not associated with significant increases in the antihyperglycaemic activity compared to the activity of the extract alone at doses of VAE 100 mg/dl and VAE 150 mg/dl administered. Similarly, there was no additional reduction in the antihyperglycaemic activity of the extract/metformin co-administration beyond the reduction achieved with comparative doses of MET 100 mg/dl and MET 150 mg/dl respectively. This suggests that the extract and metformin did not show any additive anti-hyperglycaemic activities especially at VAE 100 mg/dl and 150 mg/dl. The finding of non-additive effect of *V. amygdalina* extract/metformin co-administration as anti-hyperglycaemic agents is contrary the finding which reported possible additive action of both *V. amygdalina*/metformin co-administrations suggesting that both could be acting through the same mechanism. However, the said report also measured blood glucose reduction for 6 hours compared to this study which assessed longer term (14 days).
Dyslipidaemia is a condition that is characterized by abnormal levels and composition of plasma lipids, that is, increase in the levels of total cholesterol (TC), LDL-C, TG, VLDL-C and a decrease in the level of HDL-C. However, treatment with the extract brought about a reversal of the abnormalities in the plasma lipids. Treatment with the extract produced a dose-dependent decrease in the serum total cholesterol. The extract/metformin co-administration produced a reduction in serum total cholesterol which was comparable to the reduction produced by metformin alone. The observed reduction in serum cholesterol may be attributed to the levels of polyphenolic (flavonoids, tannin and saponins) compounds present in the extract [28]. Other studies have shown that soluble dietary fibres (SDF) in plants are known to bind to dietary cholesterol and prevent or reduce its absorption by the small intestine [29,30].
The extract produced a dose-dependent decrease in serum TG level. The extract/metformin co-administration also produced a decrease in serum TG level which was more than that of the extract at all the doses administered. However, the decrease produced by the extract/metformin co-administration was only seen in 50 mg/kg VAE combined with MET 100 mg/kg and 100mg/kg VAE/MET 100mg/kg combinations with highest reduction seen with the latter combination.
The toxicological assessment of the combinations did not show any deleterious effects in the haematological biochemical or histological parameters of major organ functions in the body. The absence of significant biochemical changes confirmed that there were pathological changes that could cause disruption in the filtration and concentrating ability of the kidney nor enzymatic disruption contained within the liver parenchyma. This study confirms similar findings.

Conclusion

In conclusion, ethanol extract of *V.amygdalina* leaves and metformin reduced the glucose levels and its attending disorders in diabetic rats with no additive or inhibiting interaction. This investigation has demonstrated that the co-use of ethanol extract of the leaves of *V.amygdalina* and metformin does not produce a significant or insignificant reduction in the glucose level although both appears to be therapeutically equivalent. The VAE also appears to be safe.

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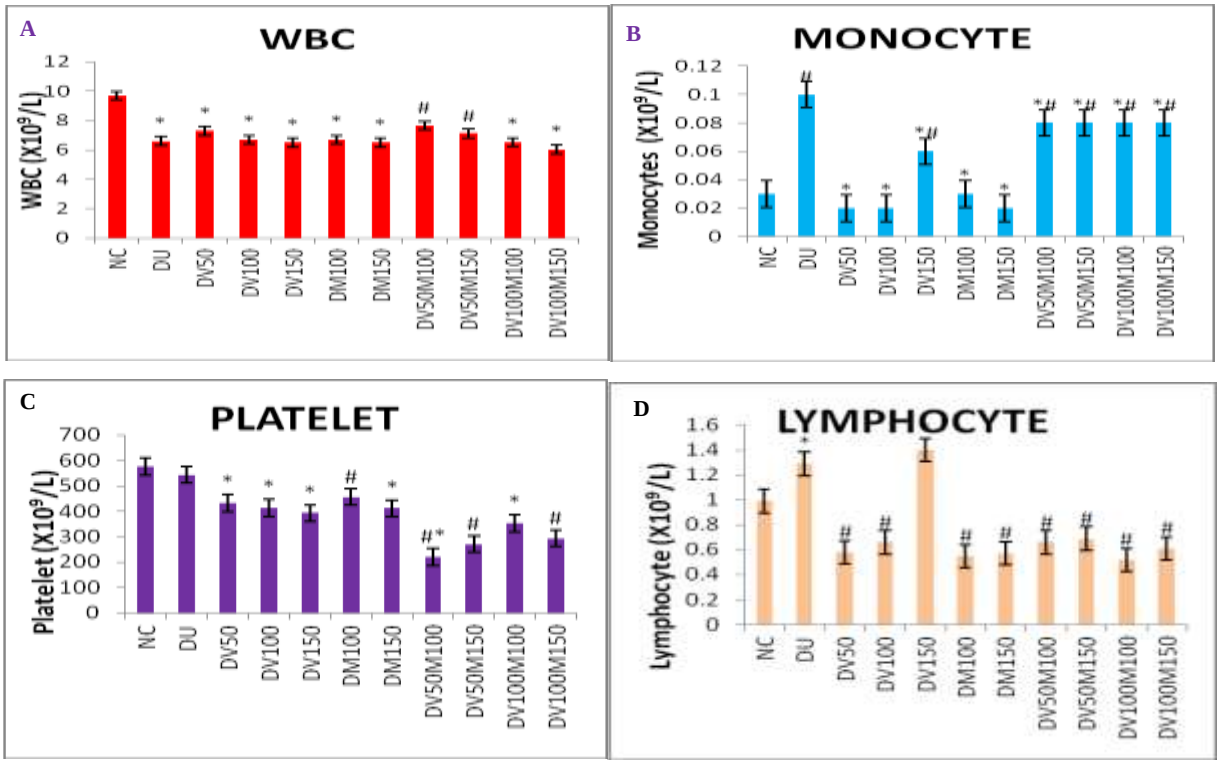
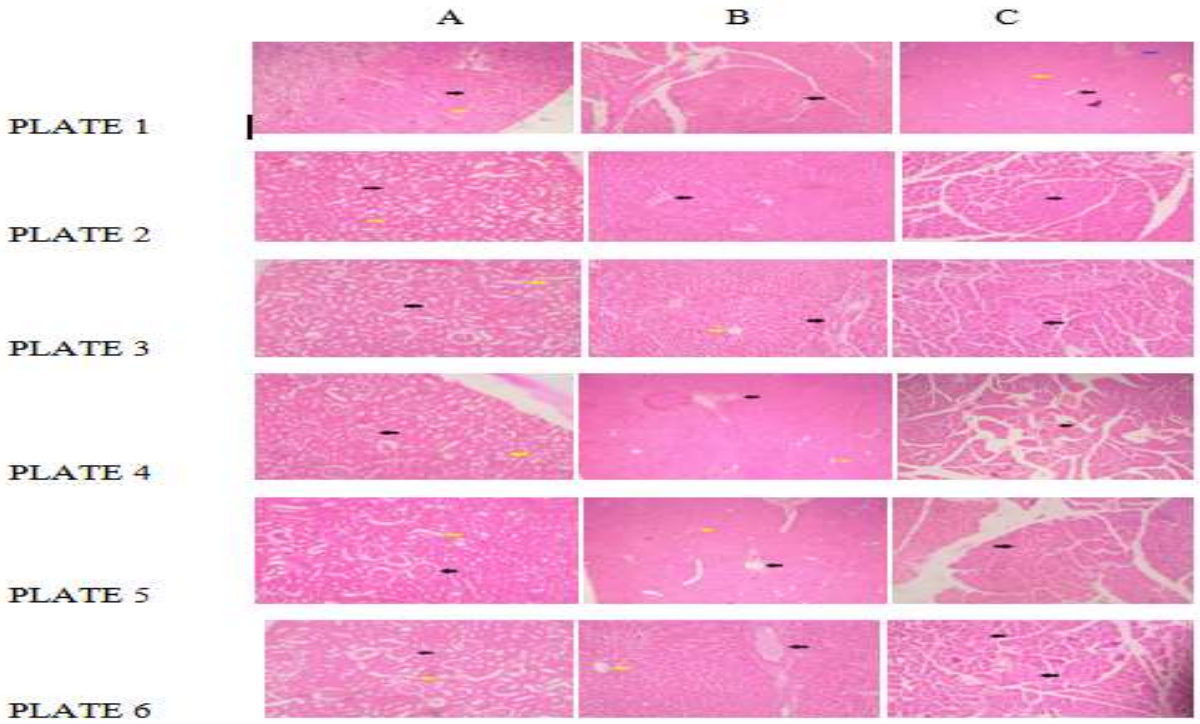


Figure 5 A-D: Figure showing the values of the haematologic parameters of the animals in the antidiabetic assays of VAE and MET.
Data represented as mean ± S.E.M of haematologic parameters, analysed by one-way ANOVA followed by Duncan's post – hoc test for multiple comparisons. *= $p < 0.01$ statistically significant compared with control, #=$p<0.01$ statistically significant compared with diabetic untreated group.
KEY: MET = Metformin, VAE = *Vernonia amygdalina* leaf extract
DV100 = Diabetic + VAE (100 mg/kg); DV150 = Diabetic + VAE (150 mg/kg)
DM100 = Diabetic + MET (100 mg/kg); DM150 = Diabetic + MET (150 mg/kg)
DV50M100 = Diabetic + VAE (50 mg/kg) +MET (100 mg/kg);
NC = Normoglycaemic control (Distilled water)
DV50M150 = Diabetic + VAE (50 mg/kg) +MET (150 mg/kg);
DV50 = Diabetic + VAE (50mg/kg)
DV100M100 = Diabetic + VAE (100 mg/kg) +MET (100 mg/kg); DU = Diabetic untreated
DV100M150 = Diabetic + VAE (100 mg/kg) +MET (150 mg/kg)



Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Fadare J, Olamoyegun M, Gbadegesin BA. Medication adherence and direct treatment cost among diabetes patients attending a tertiary healthcare facility in Ogbomosho, Nigeria. Malawi Med J. (2015); 27(2):65-70.
2. Gupta RC, Chang D, Nammi S. Bensoussan A, Bilinski K, Roufogalis BD. Interactions between antidiabetic drugs and herbs: an overview of mechanisms of action and clinical implications. *Diabetol Metab Syndr* (2017); 9:59.
3. World Health Organization Traditional Medicine Strategy 2002–2005, Geneva, Switzerland.
4. Chang HY, Wallis M, Tiralongo E. Use of complementary and alternative medicine among people living with diabetes: literature review. J Adv Nurs. 2007; 58(4):307–19.
5. Ogbera AO, Dada O, Adeyeye F, Jewo P. Complementary and alternative medicine use in diabetes mellitus. West Afri J Med. 2010; 29:158-62.
4. Ala OA, Ojo OA, Enikuomehin CA, Ajani GA, Olamoyegun MA, Akinlade AT. Complementary and alternative medicine (CAM) use among patients with type 2 diabetes in Southwestern, Nigeria. West Afr Med J 2020; 3:43-50.
5. Gupta RC, Chang D, Nammi S, Bensoussan A, Bilinski K, Roufogalis BD. Interactions between antidiabetic drugs and herbs: an overview of mechanisms of action and clinical implications. Diabetol metabolic syndrome. 2017; 9:59.
6. Lorke, D. A New Approach to Practical Acute Toxicity Testing. Arch of Toxicol. 1983; 53:275-287.
7. Okoduwa SI, Umar IA, James DB, Inuwa HM. Validation of the antidiabetic effects of *Vernonia amygdalina* delile leaf fractions in fortified diet-fed streptozotocin-treated rat model of type-2 diabetes. J Diabetol. 2017; 8:74-85.
8. WHO (2019): Classification of diabetes mellitus, brochure https://www.who.int/publications-detail/classification-of-diabetes-mellitus
9. Ghosh MN. *Fundamentals of Experimental Pharmacology*. 3rd Edition. Calcutta: Hilton and Company. 2005; 184pp.
10. Kulkarni CP, Bodhankar SL, Ghule AK, Mohan V, Thakurdesai PA. Antidiabetics activity of trigonella foenumgraecum I. seeds extract (ind01) in neonatal streptozotocin-induced (n-stz) rats. C*roatian* Med J (2012); 41:29-40.
11. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res. 2001; 50(6):537-46.
12. Asase A and Yohonu DT. Ethnobotanical study of herbal medicines for management of diabetes mellitus in dangme west district of Southern Ghana. Inter J Herb Med. 2016; 6:204-209.
13. Nwanjo HU. Efficacy of aqueous leaf extract of *Vernonia amygdalina* on plasma lipoprotein and oxidative status in diabetic rat models. Nigerian J Physiol Sci. 2005; 20 (1-2):39-42.
14. Adiukwu MU, Uzuegbu BD, Okoye CT, Uzor FP, Adibe MO, Amadi VB. Antidiabetic effects of combined aqueous leaf extract of *Vernonia amygdalina* and metformin in rats. The J Basic and Clin Physiol and Pharmacol. 2010;1(3):197–202.
15. Atangwho IJ, Ebong PE, Eyong EU, Eteng MU, Obi AU. Effects of Vernonia amygdalina Leaf on kidney function of diabetic rats. Inte J Pharm. 2007; 3:143-148.
16. Abdulazeez MA, Ibrahim K, Bulus K, Babvoshia HB, Abdullah Y. Effect of combined use of Ocimum gratissimum and *Vernonia amygdalina* extract on the activity of angiotensin converting enzyme, hypolipidaemic and antioxidant parameters in streptozotocin-induced diabetic rats. Afri J Biochem Res. 2013; 7:165-173.
17. Zakaria Y, Azlan NZ, Fakhuruddin N, Hassan N, Muhammad H. Phytochemicals and acute oral toxicity studies of the aqueous extract of Vernonia amygdalina from state of Malaysia. The J Med Plants Stud. 2016; 4:1-5.19.
18. Ebong PE, Atangwho IJ, Eyong EU, Egbung GE. The antidiabetic efficacy of combined extracts from two continental plants: *Azadirachta* alloxan-induced diabetic rats treated with ethanol extracts and fractions of *Naudea lafiloia* leaf'. Euro Sci J. 2008; 9(27):203–210.
19. Momoh J, Akoro SM, Godonu KG. Hypoglycemic and hepatoprotective effects of *Vernonia amygdalina* (Bitter Leaf) and its effect on some biochemical parameters in alloxan-induced diabetic male albino rats. Sci J Biotechnol. 2014; 194:1-7.
20. Adeoye AT, Oyagbemi AA, Adedapo AD, Omobowale TO, Ayodele AE, Adedapo AA. Antidiabetic and antioxidant activities of the methanol leaf extract of Vernonia amygdalina in alloxan-induced diabetes in Wistar rats. J Med Plants for Econ Develop. 2017; 1(1):30.
21. Vasudevan DM and Sreekumari S. Regulation of blood glucose, insulin and diabetes mellitus. Textbook of Biochemistry for Medical students.6th edn. New Delhi: Jaypee Brothers Medical Publishers (P) *Ltd.* 2011; 285-290pp.
22. Ugoanyanwu FO, Mgbeje BI, Igile GO, Ebong PE. The flavonoid rich fraction of Vernonia amygdalina Leaf extract reversed diabetes induced hyperglycaemia and pancreatic beta cell damage in albino Wistar rats. World J Pharm and Pharmaceut Sci. 2015; 4(10):1788-1802.
23. Monach C, Scalbert A, Morand C, Jimenez L. Polyphenols: food sources and bioavailabilty. American J Clin Nutr. 2004; 79:727-747.
24. Ylönen K, Saloranta C, *Kronberg*-Kippilä *C*, Groop L, Aro A, Virtanen SM. Associations of dietary fiber with glucose metabolism in non-diabetic relatives of subjects with type 2 diabetes. Diabetes care. 2003; 26:1979-1985.
25. Oli J, Ikeakor I, Onwuamaeze I. Blood glucose responses to common Nigerian foods. Trop and Geograph Med. (1982); 34:317-322.
26. Martin OW, Matthias M, Christof S, Ayman MA, Barbel O, Hannah V. Cereal Fibre Improves whole-body Insulin Sensitivity in Overweight and Obese women. American Diabetes Association. 2006; 29(4):775-780
27. Gerich JE. Hypoglycaemia and counter regulation in type 2 diabetes. *Lancet*. 2000;9;356 (9246):1946-7.
28. Chumark P, Khunawat P, Sanvarinda Y, Phornchirasilp S, Morales NP, Phivthongngam L, Ratanachamnong P, Srisawat S, Pongrapeeporn KS . The *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam Leaves. J Ethnopharmacol. 2008; 116:439-446.
29. Oloyede OB. *Carbohydrates* "All for the love of nutrient. The Seventy-Eight Inaugural Lecture. University of Ilorin. 2005; 14(31).
30. Gorinstein S, Caspi A, Libman I, Lerner H, Huang D, Feng S, Trakhtenberg S. Grapefruit positively influences serum triglycerides level in the patients suffering from coronary atherosclerosis: studies *in vitro* and in human. J Agri and Food Chem. 2006; 54(5):1887-1892.