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AMELIORATIVE ACTIVITIES OF *Vernonia amygdalina* Delile METHANOLIC LEAF EXTRACT IN ALLOXAN-INDUCED DIABETIC WISTAR RATS

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ABSTRACT

There is an increasing prevalence of diabetes mellitus (DM) in Africa. It is estimated that by 2030, people living with this condition would increase to 33 million in the African region alone. This study evaluated the ameliorative effects of *Vernonia amygdalina* on alloxan-induced diabetes mellitus in male rats. Thirty adult male rats were randomly assigned into five groups (n=6). Diabetes mellitus was induced in animals allotted to groups A-D. Groups A and B were treated with 100 and 200 mg/kg of *V. amygdalina* extract while C and D were treated with 5mg/kg glibenclamide and 10 mg/kg propylene glycol respectively. Group E (non-diabetic control) was treated with 10 mg/kg propylene glycol. All treatments were administered orally once daily for 21 days. Blood samples were obtained from the tail of the rats daily on days 1 – 7, days 14 and 21 for determination of fasting blood glucose using a glucometer. On day 21, five milliliters of blood was collected for haematology and serum biochemistry. Tissues were harvested for histopathology. There was a significant loss in weight in extract-treated groups. Similarly, blood glucose concentration was significantly lower ($p \leq 0.05$) in group B than in D. Haematology and protein profile values across the groups showed no significant difference ($p > 0.05$). Low-density lipoprotein was higher significantly ($p \leq 0.05$) in group B than in group C. The cytoarchitecture of pancreatic islets and kidneys was maintained in the extract-treated groups in contrast to group C. *V. amygdalina* leaves extract possess anti-diabetic potential.

Keyword: Diabetes, *Vernonia amygdalina*, Haematology, Histopathology, Ameliorative

DOI:

INTRODUCTION

Diabetes mellitus (DM) is fast becoming a threat to global health because of its increasingly high prevalence. According to WHO (2021), persons with DM rose from 108 million in 1980 to 422 million in 2014. In Africa, it is estimated that about 33 million persons would be diabetic by 2030 (International Diabetes Federation, 2021). Diabetes mellitus refers to hyperglycemia that results from deficiency of insulin hormone or impaired effectiveness of its action (Magliano *et al.,* 2015).

In Nigeria, the exact prevalence of DM is unknown, but estimates put it in the region of 8-10% which is an increase of more than 2.6 folds over the past 25 years (Ogbera and Ekpebegh, 2014; Uloko *et al.,* 2018). In Veterinary Medicine, DM is one of the most frequently diagnosed endocrinopathies in dogs and cats, with a reported hospital prevalence rate of 0.4-1.2% in the United States of America (Nelson and Reusch, 2014) and 0.22% in Nigeria (Gani and Ihedioha, 2015). Till date, the management of DM involves controlling blood glucose levels through adoption of dietary alterations, exercise, and the use of antidiabetic drugs such as intravenous insulin injection, sulfonylureas - tolbutamide, glibenclamide and the biguanides - metformin, phenformin (Nelson and Cox, 2005).

There are recommendations for the use of alternative therapy such as medicinal plants, especially in countries where access to conventional management procedures for DM is inadequate. Plant parts such as leaves, fruits, seeds, barks, roots and flowers have been used to cure various diseases of humans and animals (Phyllistin and James, 2000). It is believed that the consumption of green leafy vegetables (GLVs) plays a significant role in the prevention and management of certain degenerative health conditions, such as cancer and DM (Montonen *et al.,* 2004; Dasgupta and De, 2007). Since oxidative stress is known to play a vital role in the aetiology and/or progression of DM (Sarma *et al.,* 2010), the rich antioxidant components of GLVs can combat or mitigate the disease by reducing the generation of reactive oxygen species (ROS), scavenging the ROS or interfering with ROS-induced alterations (Yang *et al.,* 2004; Vassort and Turan, 2010).

Vernonia amygdalina Delile (Bitter leaf) (Family: Asteraceae), is a widely grown shrub plant in Africa, a GLV consumed in many households and has gained wide application in the treatment and management of various diseases (Oyeyemi *et al.,* 2017). Secondary metabolites in the plant confers on it some pharmacological activities [such as vernodalin (antiplasmodial), vemonioside B1 (antiplasmodial and antischistosomal) and luteolin (powerful antioxidant and anticancer)] (Muraina *et al.,* 2010). Although considerable research has been done to evaluate the hypoglycemic and hypolipidemic properties of *V. amygdalina* (Adeoye *et al.,* 2017; Okoduwa *et al.,* 2017, Katemo *et al.,* 2018), there is still a dearth of information on the ameliorative potential of the plant. This study, therefore seeks to examine the ameliorative effects of *V. amygdalina* methanolic leaf extract on the haematology, oxidative stress markers, serum biochemistry profiles, some organ functions and histology of the pancreas, kidney and liver of alloxan-induced diabetic rats.

MATERIALS AND METHODS

Ethical consideration

Ethical approval was obtained from the College of Veterinary Medicine Research Ethics Committee of the Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria. Approval number: FUNAAB/COLVET/CREC/2020/09/01.

Collection of plant sample

Fresh leaves of *V. amygdalina* were collected from the natural habitat in Abeokuta, Ogun State. Botanical identification was carried out at the Department of Botany, College of Biological Sciences, FUNAAB and voucher number FHA-3724 was assigned.

Preparation of plant extract

Vernonia amygdalina leaves were washed with distilled water to remove debris, dust particles and other contaminants. The leaves were thereafter air dried at room temperature and milled to coarse powder and extracted with methanol (90%) for 72 hours. The filtrate was poured through a muslin cloth into a beaker, heated in a water bath at 40^{O}C to evaporate the methanol, and the extract stored at 4^{o}C until use.

Experimental design

Thirty adult, male Wistar rats were used for this study. They were divided into five groups (A-E) consisting of six animals per group. Bodyweight and fasting blood glucose levels of all the rats were determined before the start of the experiment. Groups A-D were administered 158 mg/kg alloxan (KEM Light A05790B10) by intraperitoneal injection. Diabetes mellitus was confirmed in the rats by elevation of fasting blood glucose levels (above 180 mg/dl) 72 hours post -administration using a glucometer (Accu-Chek®, Germany). Groups A, B, C and D diabetic rats were administered 100 mg/kg *V. amygdalina,* 200 mg/kg *V. amygdalina,* 5 mg/kg glibenclamide and 10 mg/kg propylene glycol respectively, while Group E (Non -diabetic rats) were administered 10 mg/kg propylene glycol (Kao *et al.*, 2021). The administration of test samples was by oral intubation from 9:00 - 11:00 a.m. daily for 21 days.

Sample analyses

At the end of the experimental period, 5 ml of blood was collected from each rat via the medial canthus of the eye into Ethylenediamine tetraacetic acid (EDTA) and plain bottles for hematology and serum biochemistry respectively. Thereafter, rats were euthanized via cervical dislocation (Carborne *et al.,* 2012) and some tissue samples (pancreas, liver and kidney) were harvested for histopathology.

The packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC) count, mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), white blood cell (WBC) count and differential WBC counts were determined as described by Jain (1993).

Total protein, albumin, globulin, blood urea nitrogen (BUN), creatinine, total and conjugated bilirubin, alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), superoxide dismutase (SOD) and hydrogen peroxide (H_2O_2) were analyzed using commercial kits (Randox Laboratories Ltd, UK), following standard procedures as outlined by the manufacturer. Also, total cholesterol, triglycerides, phospholipids, high-density lipoproteins (HDL), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), were evaluated using standard procedures.

The pancreas, liver and kidney tissue samples were fixed in 10% buffered formalin for histopathology. The fixed tissues were dehydrated in graded levels of alcohol (40, 50, 70, 80 and 100%) and thereafter embedded in paraffin wax; sectioned with a microtome at 5μm and stained with Haematoxylin and Eosin stains. The sections were put in a medium to harden and produce a clear binder between the slide and cover slip, labeled and examined under a light microscope (Hawsley®, England) and viewed at ×400 magnification (Llewellyn, 2009).

Data analysis

Results were presented as Mean ± Standard error of mean (SEM). Data were subjected to statistical analyses using the Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp, 2011). Mean values were compared using a one-way analysis of variance (ANOVA). The post Hoc test was done using the Tukey HSD test. *P*-value ≤ 0.05 was considered to be statistically significant.

RESULTS

Effect of Vernonia amygdalina methanolic leaf extract on the body weights of alloxan-induced diabetic male Wistar rats

There was no significant difference between the means of the rats' initial and final weights in all the groups. However, the weight change for group C was significantly higher than that of the other groups (Table 1).

Table 1: Effect of Vernonia Amygdalina Methanolic Leaf Extract on Body Weights of Alloxan-induced Diabetic Male Rats

Group	Initial weight	Final weight	Weight Change
	Mean ± SEM (kg)		
A (100 mg/kg *Vernonia amygdalina*)	0.16±0.00	0.14±0.01	-0.02±0.01^{b}
B (200 mg/kg *Vernonia amygdalina*)	0.15±0.00	0.13±0.01	-0.02±0.01^{b}
C (5 mg/kg Glibenclamide)	0.14±0.00	0.15±0.01	0.01±0.01^{a}
D (Positive control)	0.18±0.01	0.15±0.01	-0.03±0.02^{b}
E (Non-diabetic control)	0.16±0.00	0.19±0.02	0.03±0.02^{a}

Mean values bearing different superscripts a,b are considered significantly ($p<0.05$) different

3.2 Effect of *Vernonia amygdalina* methanolic leaf extract on blood glucose of alloxan-induced diabetic male rats

Results from the extract treated groups A and B showed a decrease in blood glucose values on day 7 when compared with group D (Figure 1). There was a significant decline in the blood glucose of rats in groups A, B, and C in the first seven days of administration of the test samples. On day 21, an increase in blood glucose level was noticed in group A while group B rats had a continuous decrease. Group C maintained the level of blood glucose between day 7 and 14 followed with a slight increase by 21 (Figure 1).

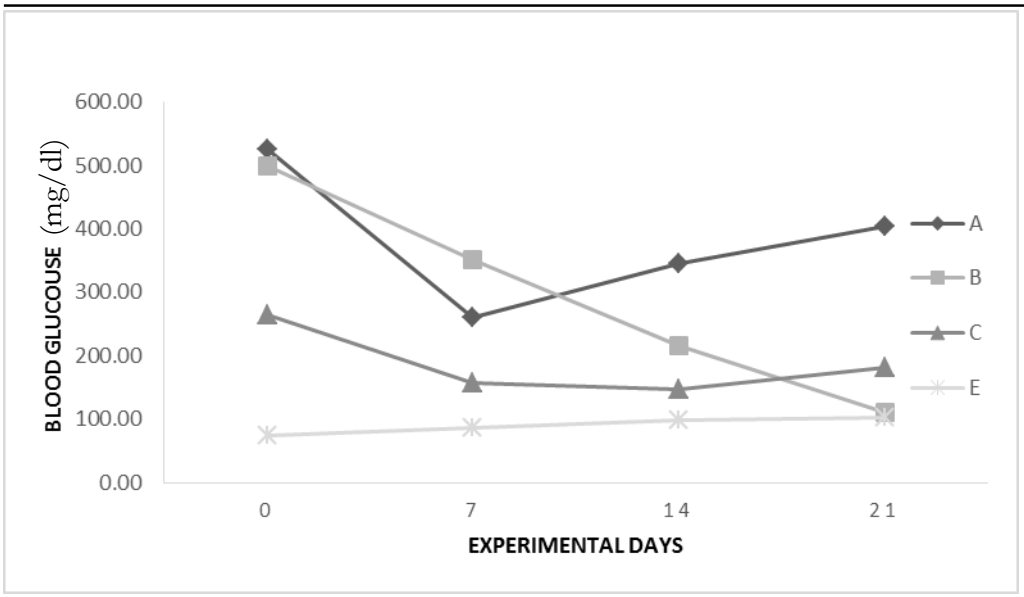

Figure 1: The effect of *Vernonia amygdalina* methanolic leaf extract on the blood glucose of alloxan-induced diabetic Wistar rats
A= 100 mg/kg *Vernonia amygdalina* methanolic leaf extract; B= 200 mg/kg *Vernonia amygdalina* methanolic leaf extract; C= 5 mg/kg glibenclamide; D= Positive control; E= Non diabetic control

Effect of Vernonia amygdalina methanolic leaf extract on the haematology, serum proteins and oxidative stress markers of alloxan-induced diabetic male rats

The PCV and Hb values of rats treated with 100 mg/kg *V. amygdalina* (Group A) were significantly lower than the other groups (Table 2). There was however no significant difference in the other haematological parameters.

Total protein values were significantly higher in Group D (8.50±0.28). However, there was no difference among the other Groups A, B and C (7.10±0.17, 6.77±0.67, and 6.30±0.26 respectively), while Group E was least (5.97±0.35). The differences in albumin values in Groups B, D and E were significantly different (3.63±0.33, 3.83±0.21 and 3.85±0.60 respectively). Globulin values were least in Groups C and E (2.90 ±0.26 and 2.27±0.31 respectively) - Table 3. For the serum enzymes (liver injury markers), the aspartate transaminase (AST) values were not statistically different between Groups B and D, although Group D had the highest value (106.33±5.61) while alanine transaminase (ALT) values were lower in Groups A, B, C and E but highest in group D (58.00 ±3.00). The alkaline phosphatase (ALP) value was least in Group C and E, although values were not significantly different (Table 3).

The blood urea nitrogen (BUN) values were higher in Group D (20.54±0.75), similar in Groups A and B (17.79±0.81 and 17.16±0.50 respectively) and between Groups C and E (13.24±1.31 and 13.25±1.21 respectively), total bilirubin values were higher in Group E (3.42±0.20)

when compared with values for Groups B and C, with Group D being the least (0.76±0.16). Values for direct bilirubin differed slightly, with Group B being higher (3.95±0.26). Creatinine values varied significantly, with value from Group E being statistically higher (0.70±0.66) from Group D (Table 4).

The Superoxide dismutase (SOD) and H_2O_2 values across the groups were not significant different. However, the SOD value was least from Group C (0.03±0.01) and highest from Group A (0.07±0.02). The H_2O_2 value amongst the group was highest from Group A (52.80± 3.10) and similar values were obtained from Groups B and C (42.47±3.64 and 42.10±2.87 respectively) -Table 5.

Table 2: Effect of *Vernonia amygdalina* methanolic leaf extract on the haematology of alloxan-induced diabetic male Wistar rats

Parameter	A (100mg/kg *Vernonia amygdalina*)	B (200mg/kg *Vernonia amygdalina*)	C (5mg/kg Glibenclamide) (Mean ± SEM)	D (Positive control)	E (Non-diabetic control)
PCV (%)	40.33±2.72[b]	50.00±2.44[a]	48.25±1.46[a]	48.50±1.19[a]	47.00±0.91[a]
Hb. (g/dL)	13.60±0.86[b]	16.95±0.86[a]	16.15±0.50[a]	16.30±0.29[a]	15.85±0.24[a]
RBC count ($\times10^{12}$/L)	6.73±0.48	8.40±0.43	8.15±0.27	8.08±0.19	7.95±0.21[a]
MCV (fl)	59.93±0.23	59.55±0.25	59.23±0.32	60.05±0.09	59.13±0.42
MCH(pg)	20.23±0.17	20.18±0.11	19.83±0.16	20.20±0.18	19.98±0.29
MCHC (g/dL)	33.73±0.17	33.90±0.26	33.48±0.16	33.63±0.27	33.78±0.33
WBC count ($\times10^{9}$/L)	11.47±0.84	11.18±0.53	10.80±0.57	11.43±0.59	10.05±0.15
Neutrophils	2.99±0.27	2.91±0.15	3.07±0.28	3.11±0.15	3.19±0.21
Lymphocytes	8.17±0.57	7.96±0.57	7.53±0.64	7.92±0.62	6.54±0.26
Eosinophils	0.11±0.06	0.11±0.07	0.02±0.02	0.20±0.07	0.13±0.05
Basophils	0.03±0.03	0.11±0.05	0.13±0.06	0.08±0.03	0.05±0.05
Monocytes	0.16±0.08	0.11±0.08	0.16±0.08	0.11±0.05	0.15±0.03

Mean values with different superscripts [a], [b] across the same row are significantly different ($p \leq 0.05$)

SEM = Standard error of mean; PCV= Packed cell volume; RBC= Red blood cell; Hb.= Hemoglobin concentration; MCV= Mean corpuscular volume; MCH= Mean corpuscular hemoglobin; MCHC= Mean corpuscular hemoglobin concentration; WBC= White blood cell

Table 3: Effect of Vernonia Amygdalina Methanolic Leaf Extract on some Serum Proteins and Markers of Hepatic Injury in Alloxan-induced Diabetic Male Rats

	A (100 mg/kg *Vernonia amygdalina*)	B (200 mg/kg *Vernonia amygdalina*)	C (5 mg/kg Glibenclamide)	D (Positive Control)	E (Non-diabetic control)
	Mean ± SEM				
Total protein (g/dl)	7.10±0.10[b]	6.77±0.38[b]	6.30±0.15[b]	8.50±0.20[a]	5.97±0.20[c]
Albumin (g/dl)	2.70±0.21[b]	3.63±0.17[a]	3.37±0.28[b]	3.83±0.12[a]	3.85±0.30[a]
Globulin (g/dl)	4.40±0.15[a]	3.73±0.55[a]	2.90±0.15[b]	4.20±0.59[a]	2.27±0.18[b]
AST (µ/L)	59.50±12.50[c]	104.33±7.17[a]	68.50±5.50[b]	106.33±5.61[a]	67.50±3.50[b]
ALT (µ/L)	31.50±4.50[b]	38.67±3.84[b]	28.00±3.00[b]	58.00±3.00[a]	33.33±4.26[b]
ALP (µ/L)	44.13±2.77[a]	42.30±2.44[a]	38.60±0.00[a]	42.30±4.60[a]	37.25±4.15[a]

Mean values bearing different superscripts [a], [b] are considered significantly different ($p<0.05$)

ALP: Alkaline phosphatase
ALT: Alanine transaminase
AST: Aspartate transaminase

Table 4: The Effect of Vernonia Amygdalina Methanolic Leaf Extract on some Kidney Function Parameters of Alloxan-induced Diabetic Male Rats

Parameter	A (100 mg/kg *Vernonia amygdalina*)	B (200 mg/kg *Vernonia amygdalina*)	C (5 mg/kg Glibenclamide)	D (Positive Control)	E (Non-diabetic control)
Mean ± SEM					
BUN (mg/dl)	17.79±0.81[b]	17.16±0.50[b]	13.24±1.31[c]	20.54±0.75[a]	13.25±1.21[c]
Total bilirubin (mg/dl)	1.37±0.14[b]	2.71±0.40[a]	1.67±0.37[b]	0.76±0.16[c]	3.42±0.20[a]
Direct bilirubin (mg/dl)	1.82±0.73[b]	3.95±0.26[a]	1.06±0.20[b]	3.22±0.58[a]	0.77±0.04[b]
Creatinine (mg/dl)	0.30±0.12[b]	0.30±0.06[b]	0.50±0.00[a]	0.67±0.09[a]	0.70±0.06[a]

Mean values bearing different superscripts [a], [b], [c] are considered significantly different ($p<0.05$)

Table 5: Effect of *Vernonia amygdalina* methanolic leaf extract on serum oxidative stress markers of alloxan-induced diabetic male rats

Parameter	A (100 mg/kg *Vernonia amygdalina*)	B (200 mg/kg *Vernonia amygdalina*)	C (5 mg/kg Glibenclamide)	D (Positive control)	E (Non-diabetic control)
			Mean ± SEM		
SOD (µ/L)	0.07±0.02	0.04±0.00	0.03±0.01	0.07±0.00	0.06±0.02
H_2O_2 (µM)	52.80±3.10	42.47±3.64	42.10±2.87	47.03±1.24	40.28±2.16

SEM : Standard error of mean; SOD: Superoxide dismustase; H_2O_2 :Hydrogen peroxide

Effect of Vernonia amygdalina methanolic leaf extract on the serum lipids of alloxan-induced diabetic male Wistar rats

Serum triglyceride values were significantly higher (p<0.05) in Groups A, B and C (70.97±4.27, 66.10±12.30, and 66.10±12.30 respectively) when compared with Group D (23.40±3.50) and E (10.13±1.03). Cholesterol values were significantly higher in Groups B and D (70.55±7.55 and 65.93±5.12 respectively) while Group E was lowest (25.88±2.98). Serum phospholipid level was significantly higher in Group B (150.33±0.88) when compared with Group C (119.13±2.86) - Table 6.

The high density lipoprotein (HDL) values were significantly higher in Group B (30.35±0.15) when compared with Group C (23.47±1.78), D (18.52±1.50) and E (15.30±3.29). However, the VLDL value was higher in Group D (20.00±4.30) when compared with Groups A, B, C and E (14.17±0.87, 18.47±1.43, 12.30±0.80 and 2.00±0.21 respectively) - Table 6.

Table 6: Effect of Vernonia Amygdalina Methanolic Leaf Extract on some Serum Lipids of Alloxan-induced Diabetic Male Rats

Parameter	A (100 mg/kg *Vernonia amygdalina*)	B (200 mg/kg *Vernonia amygdalina*)	C (5 mg/kg Glibenclamide)	D (Positive control)	E (Non-diabetic control)
			Mean ± SEM		
Triglycerides	70.97±4.27^a	66.10±12.30^a	49.90±11.74^a	19.30±0.60^b	10.13±1.03^b
Cholesterol	43.80±5.61^b	70.55±7.55^a	43.33±2.66^b	65.93±5.12^a	25.88±2.98^b
Phospholipids	125.41±3.87^b	150.33±0.88^a	119.13±2.86^c	134.60±2.12^b	108.89±2.18^d
HDL	15.70±4.10^b	30.35±0.15^a	23.47±1.78^a	24.97±1.30^a	15.30±3.29^b
LDL	9.97±0.27^c	27.47±3.01^a	10.19±0.60^c	18.52±1.50^b	9.47±0.95^c
VLDL	14.19±0.85^a	19.81±0.65^a	12.28±0.82^a	20.00±4.32^a	2.03±0.21^b

HDL: High density lipoprotein; LDL: Low density lipoprotein, VLDL= Very low density lipoprotein
Mean with different superscripts a,b,c,d across the row are statistically significantly different (p<0.05)

Effect of Vernonia amygdalina methanolic leaf extract on the histopathology of the pancreas, kidney and liver of alloxan-induced diabetic male rats

In rats treated with 100 mg/kg of *V. amygdalina* (Group A), there were areas of mild vacuolar degeneration of the islet of Langerhans of the pancreas, though maintaining their anatomical architecture (Plate 1). The kidneys showed areas with multiple foci of tubular dilatation, mild glomerular atrophy and mild vacuolar degeneration of the tubular epithelial cells (Plate 2). In contrast, rats in Group B showed areas with diffuse interstitial oedema of the islets of Langerhans with varying vacuolar degeneration of the cells (Plate 3). Histopathology of the kidneys revealed diffuse areas of tubular dilatation with the presence of protein cast in the lumen (Plate 4).

Kidneys of the rats in Group C showed areas with moderate diffuse vacuolar degeneration and necrosis of the tubular epithelial cell (Plate 5). There was also diffuse tubular dilatation with mild protein cast in the lumen. In the Group D rats, there were areas of dilated tubular lumen with vacuolar degeneration and necrosis. Across all groups, there were no hepatic pathologies seen morphologically (Plate 6).

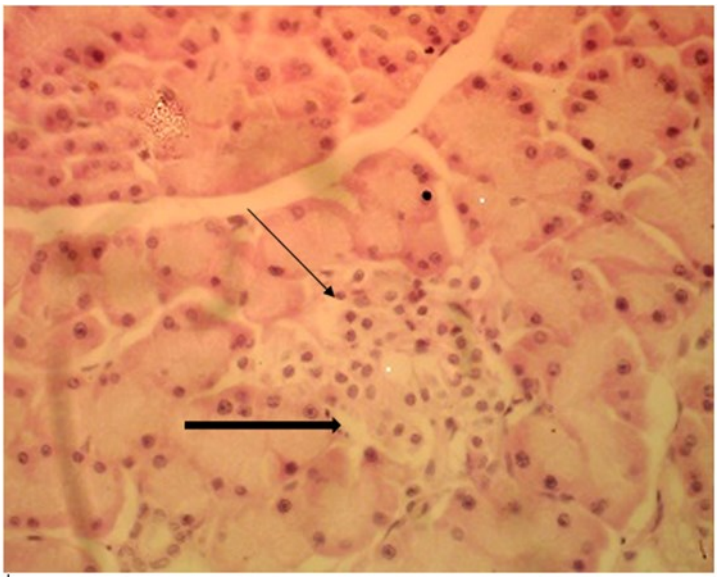

Plate 1: Histopathology of the pancreas of alloxan-induced diabetic male rat treated with 100 mg/kg Vernonia amygdalina methanolic leaf extract (Group A) showing very mild vacuolar degeneration (thick arrow) of the islet of Langerhans with the cells maintaining cellular architecture (thin arrow) (H&E stain ×400) scale bar: 25µM

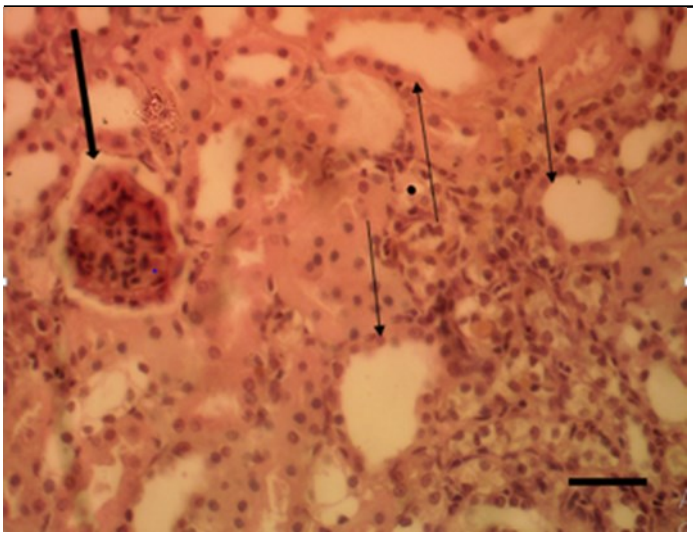

Plate 2: Histopathology of the kidney of alloxan-induced diabetic male rats treated with 100mg/kg V. amygdalina extract (Group A) with multiple foci of tubular dilatation (thin arrows), mild glomerular atrophy (thick arrow) and mild vacuolar degeneration of the tubular epithelial cells (H&E stain ×400) Scale bar: 20µM

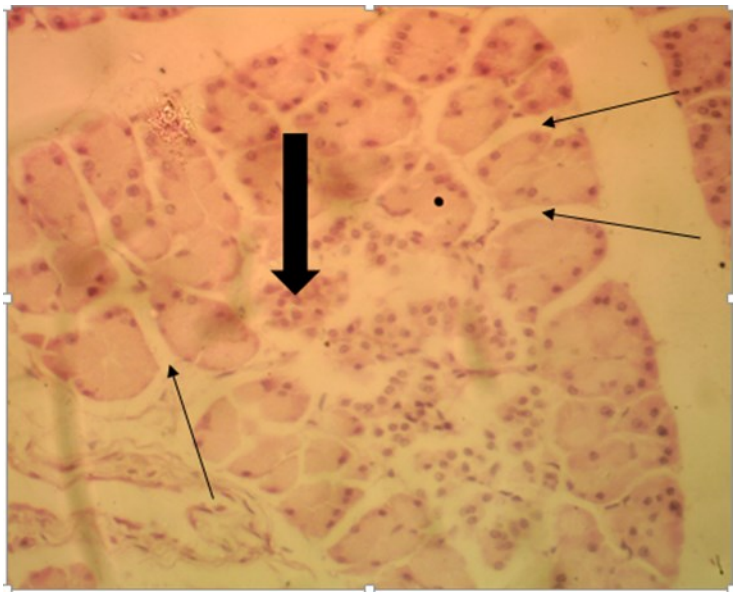

Plate 3: Histopathology of the pancreas showing areas of diffuse interstitial oedema of the islets of Langerhans (thin arrow) with varying vacuolar degeneration of the cells (thick arrow) in alloxan-induced diabetic male rats treated with 200 mg/kg Vernonia amygdalina methanolic leaf extract (Group B) (H&E ×400) Scale bar: 25µM

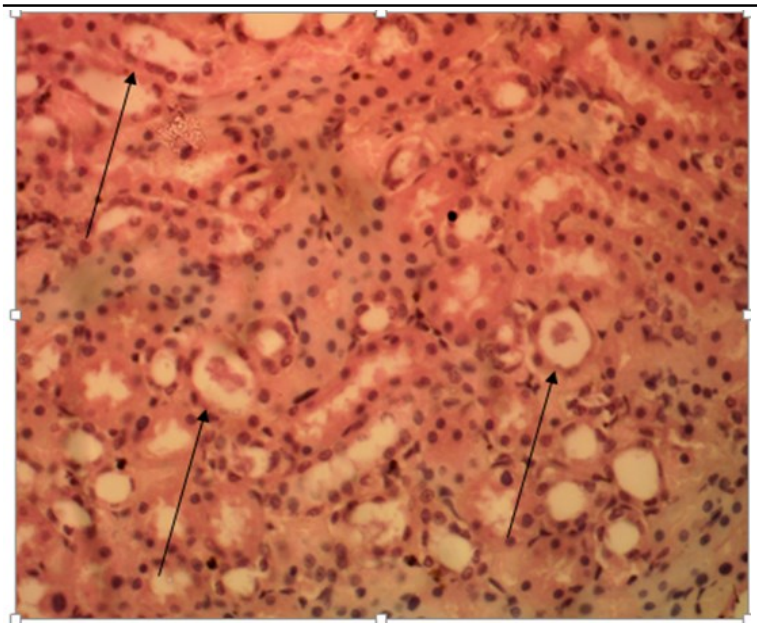

Plate 4: Histopathology of the kidney showing diffuse areas of tubular dilatation with protein cast in the lumen of alloxan-induced diabetic male Wistar rats treated with 200 mg/kg Vernonia amygdalina methanolic leaf extract (Group B) (H&E ×400) Scale bar: 20µM

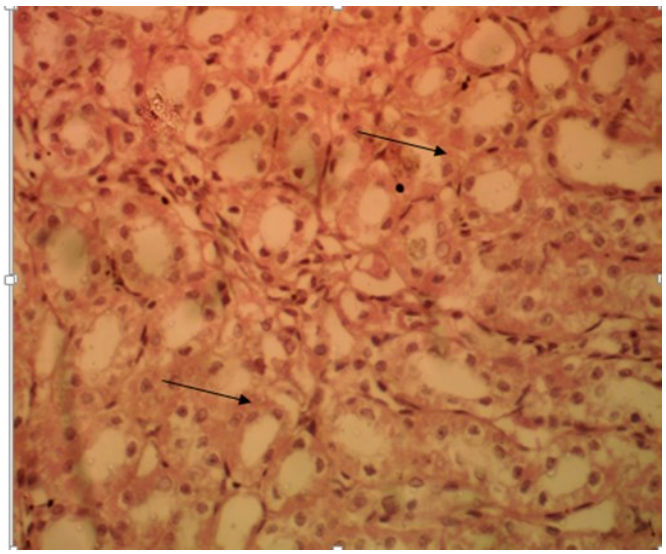

Plate 5: Histopathology of the kidney of alloxan-induced diabetic male Wistar rats treated with 5 mg/kg glibenclamide (Group C) showing areas with moderate diffuse vacuolar degeneration and necrosis of the tubular epithelial cell. There was also diffuse tubular dilatation with mild protein cast in the lumen (H&E ×400) Scale bar: 20µM

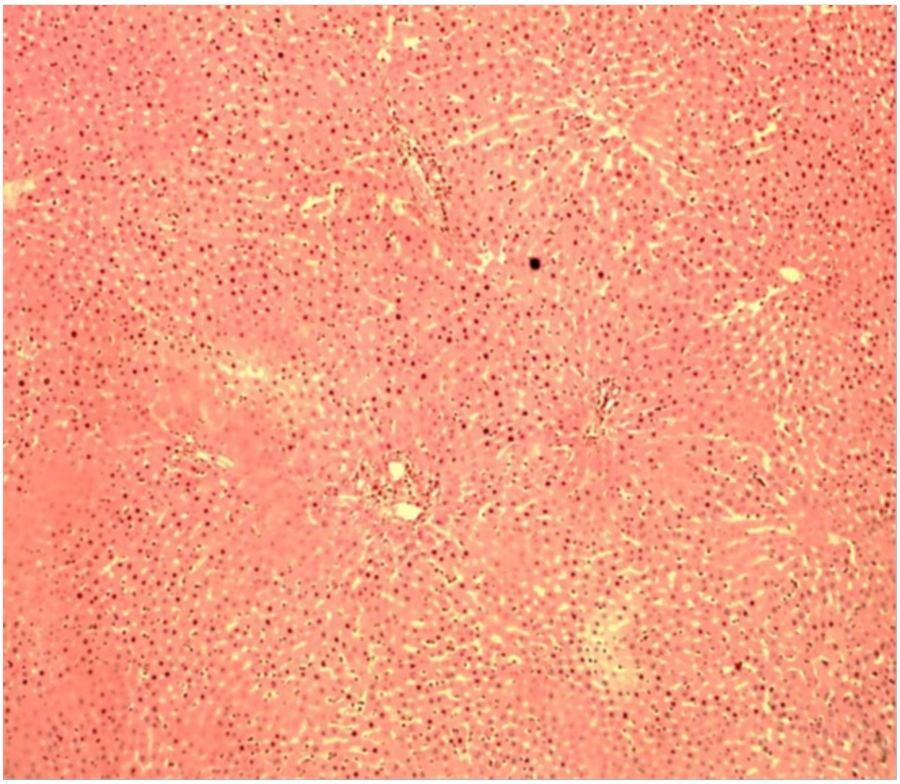

Plate 6: Photomicrograph of the liver in alloxan-induced diabetic male Wistar rats treated with 100mg/kg *Vernonia amygdalina* methanolic leaf extract (Group A) with no visible lesion observed (H&E stain ×400)

DISCUSSION

Diabetes mellitus is a debilitating health condition and is one of the leading causes of death, especially in medium and low income countries (WHO, 2022). There is global agreed target to halt the rise in diabetes and obesity by 2025 (WHO, 2022). This study was aimed at proffering solution to a global health disorder in human and pets.

The weight losses were recorded in the *V. amygdalina* extract-treated groups in this study. This aligns with the findings of Atangwho *et al.* (2012), demostrated that *V. amygdalina* leaves can induces weight loss, thereby suggesting that it may ameliorate the potential risk of glucose intolerance associated with diabetes mellitus.

Moreso, the hypoglycemic effect of *V. amygdalina* methanolic leaf extract in alloxan-induced diabetic male rats at dosages of 100 mg/kg and 200 mg/kg were outlined. At the 100mg/kg dosage, this extract demonstrated a probable biphasic hypoglycemic property. Other researchers have documented the hypoglycemic effects of the aqueous extract (Michael *et al.,* 2010), ethanolic extract (Effiong *et al.,* 2013), and chloroform fraction (Atangwho *et al.,* 2013; 2014) of *V. amygdalina* streptozotocin-induced diabetic rats. This suggests that the active blood sugar lowering principles in the plant may be extracted by both polar and non-polar solvents.

Uncontrolled hyperglycemia has been noted to affect certain haematological parameters such as glycated Hb levels, platelet volume, WBC counts (Biadgo *et al.,* 2016). These may serve as pointers to certain systemic events (markers) occurring in a body as seen in cases of diabetes mellitus. The present study showed increased PCV and Hb concentration in the 200 mg/kg *V. amygdalina* leaf extract-treated group when compared with the controls. The findings in this study agreed with the report of Asante *et al.* (2016) in Ghana, who observed that a dose-dependent increase in PCV values of Sprague Dawley rats treated with lower doses (10 and 30 mg/kg) of ethanolic extract of *V. amygdalina*. The results from this study however, differ from the findings of Chike *et al.* (2018) and Airaodion *et al.* (2019), whose studies revealed significantly lowered PCV and Hb concentrations after treatment with ethanolic extract of *V. amygdalina* leaves, and suggested that the extract of *V. amygdalina* had the potential of adversely affecting haematological indices.

Serum enzymes have been used to demonstrate hepatic and renal injury related to liver disease and hyperglycemia (Zafar and Navqi, 2010; Ajiboye *et al.,* 2016). Abnormal increases in aminotransferases specifically ALT generally reflect liver cell damage (hepatotoxicity), while that of ALP is more specific for cholestasis-hepatobiliary damage (Atangwho *et al.,* 2007). In this study, ALT values were decreased significantly in extract-treated groups, an indication that there was no hepatic injury resulting from the methanolic extract of *V. amygdalina*. This result could be due to the antioxidative activities of sequesterpene lactone in *V. amygdalina* (Arhoghro *et al.*, 2009). A review according to Kaur *et al.* (2019) documents the hepatoprotective effects of this plant which was demonstrated in this study. A study by Ekam and Udosen (2012), using the benzene, chloroform, ethyl acetate, butanol, and methanol fractions of *V. amygdalina*, on acetaminophen-induced hepatotoxicity also reported hepatic protection of this plant. Hepatoprotective effect of some other plants in related families such as the aqueous extracts of *Rosmarinus officinalis* and *Moringa oleifera* has also been reported (Ramadan *et al.,* 2013; Abakpa *et al.,* 2017). In addition, total bilirubin and creatinine values in the extract treated group were not significantly different from the non-diabetic control and can be inferred that there was no hepatobiliary damage as well as kidney damage relating to the use of the extract. Oxidative damage in various tissues may be controlled or prevented by enzymic and nonenzymic antioxidant defense systems such as SOD (Aksoy *et al.,* 2003). Studies have shown antioxidant enzymes activities are decreased in DM causing an increase in the level of H_2O_2 in serum (Shen *et al.*, 2009). The serious imbalance between reactive oxygen species (ROS) production and the decline of antioxidative ability leads to the enhancement of oxidative stress seen in DM. Several sesquiterpene lactones such as vernolide, vernodalol, vernolepin, vernodalin and hydroxyvernolide; flavonoids such as luteolin, luteolin 7-O-βglucoroniside and luteolin 7-O-β-glucoside, and stigmastane-type steroid saponins isolated from *V. amygdalina* leaves have been observed to possess antioxidant properties (Ong *et al.,* 2011; Adeoye *et al.,* 2018; Bashir *et al.,* 2020; Alara *et al.,* 2020; Zhao *et al.,* 2021). These phytochemicals have the ability to scavenge for free radicals which are increasingly produced in cells of diabetic subjects (Malik *et al.,* 2010). In this study however, serum antioxidant marker (SOD) was not significantly altered by DM but the decrease in H_2O_2 level in *V. amygdalina* treat-

ed group (200 mg/kg) could be due to the presence of radical scavenging compounds in leaves.

Dyslipidemia associated with DM is characterized by increase in total cholesterol, LDL, VLDL, triglycerides and a fall in HDL. In this study, the levels of total cholesterol and triglycerides were markedly increased in diabetic control following induction of DM with alloxan confirming that the dyslipidemia associated with DM can give useful information on lipid metabolism (Oyedemi *et al.,* 2010). There was also an increase in the good cholesterol (HDL) and bad cholesterol (LDL) in the *V. amygdalina* treated groups. Nwanjo (2005) observed that the aqueous extract of the plant reduced triacylglycerol levels and normalized cholesterol concentrations in the serum of diabetic rats. The ethanolic extracts of the plant have also been reported to keep the lipid profile of rats within the normal range (taken as that of the control rats) when doses of 100-1000 mg/kg body weight were administered (Atangwho *et al.,* 2012). The methanolic extracts of *V. amygdalina* have also been shown to have lipid-lowering effects in rats fed on a high cholesterol diet for nine weeks (Adaramoye *et al.,* 2008). These reports suggest that the plant may play very important roles in the future management of chronic diseases.

From the histopathology, *V. amygdalina* extract dosed at 100 mg/kg and 200 mg/kg caused an architectural maintenance of the pancreatic islets cells as well as in the kidneys, hence mitigating the effect of diabetes mellitus on the tissues in these groups. This is buttressed by Akinola *et al.* (2010), with the demonstration of the presence of viable cells of the pancreatic islets after treatment using *V. amygdalina* at 400mg/kg. In glibenclamide treatment, there was loss in the cellular architecture of the pancreatic islets as well as diffuse areas of necrotic lesions in the tubular epithelial cells and distention of the tubular lumen with presence of protein casts. The absence of morphological lesions in the livers of the rats across the groups could be due to the short duration of the study. According to Kini *et al.* (2016), steatosis, lobular inflammation, portal inflammation, nuclear glycogenation, and fibrosis are the characteristic histopathological changes seen in the liver due to DM.

In conclusion, this study evaluated the ameliorative effect of *V. amygdalina* on alloxan-induced diabetic male rats and showed favourable protective effect that was more pronounced in the 200 mg/kg treatment group. It is believed that the ameliorative signs associated with the extract is due to rich presence of flavonoids and polyphenols. Further studies should be focused on characterizing the active principle(s) responsible for this effect and the possible mechanism(s) of action.

Authors' contributions

OAA conducted the project and drafted the manuscript; OTA and JOO conceptualized designed the project; OLA read the histopathology slides; OAA did the statistical analysis; OTA and OJA revised the manuscript critically for important intellectual content. All authors read and approved the final version of the manuscript.

Conflict of interest

All authors declare that there is no conflict of interest.

REFERENCES

Abakpa, S.A.V., Akintunde, O.G., Adeleye, O.E., Okpara, E.O., Daramola,

O.O., Okandeji, M., Adeleye, A.I. 2017. Haematological and biochemical changes in alloxan-induced diabetic dogs treated with aqueous extract of *Moringa oleifera* leaves. *Journal of Medicine, Physiology and Biophysics* 33: 28-35.

Adaramoye, O.A., Akintayo, O., Achem, J., Fafunso, M.A. 2008. Lipid-lowering effects of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol diet. *Vascular Health and Risk Management* 4(1): 235-241.

Adeoye, A.T., Akinrinde, A.S., Oyagbemi, A.A., Omobowale, T.O., Adedapo, A.D.A., Ayodele, E.A., Yakubu, M.A., Adedapo, A.A. 2018. Phytochemical, analgesic, *in-vitro* anti-oxidant and GC-MS analysis of *Vernonia amygdalina* leaves. *African Journal of Biomedical Research* 21: 303- 312.

Adeoye, A.T., Oyagbemi, A.A., Adedapo, A.D., Omobowale, T.O., Ayodele, A.E., Adedapo, A.A. 2017. Antidiabetic and antioxidant activities of the methanol leaf extract of *Vernonia amygdalina* in alloxan -induced diabetes in Wistar rats. *Journal of Medicinal Plants for Economic Development* 1(1): 1-12.

Airaodion, A.I., Ogbuagu, E.O., Airaodion, E.O., Ekenjoku, J.A., Ogbuagu, U. 2019. Pharmacotherapeutic effect of methanolic extract of *Telfairia occidentalis* leaves on glycemic and lipidemic indexes of alloxan-induced diabetic rats. *International Journal of Bioscience and Biotechnology* 11(8): 1-17.

Ajiboye, B.O., Ojo, O.A., Adeyonu, O., Imiere, O., Olayide, I., Fadaka, A., Oyinloye, B.E. 2016. Inhibitory effect on key enzymes relevant to acute type-2 diabetes and antioxidative activity of ethanolic extract of *Artocarpus heterophyllus* stem bark. *Journal of Acute Disease* 5(5): 423-429.

Akinola, O.S., Caxton-Martins, E.A., Akinola, O.B. 2010. Ethanolic leaf extract of *Vernonia amygdalina* improves islet morphology and upregulates pancreatic G6PDH activity in streptozotocin-induced diabetic Wistar rats. *Pharmacology Online* 2: 932-942.

Aksoy, N., Vural, H., Sabuncu, T., Aksoy, S. 2003. Effects of melatonin on oxidative–antioxidative status of tissues in streptozotocin-induced diabetic rats. *Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or disease* 21(2): 121-125.

Alara, O.R., Abdurahman, N.H., Olalere, O.A. 2020. Ethanolic extraction of flavonoids, phenolics and antioxidants from *Vernonia amygdalina* leaf using two-level factorial design. *Journal of King Saud University-Science* 32(1): 7-16.

Arhoghro, E.M., Ekpo, K.E., Anosike, E.O., Ibeh, G.O. 2009. Effect of aqueous extract of bitter leaf (*Vernonia amygdalina* Del) on carbon tetrachloride (CCl4) induced liver damage in albino Wistar rats. *European Journal of Scientific Research* 26(1): 12-30.

Asante, D.B., Effah-Yeboah, E., Barnes, P., Abban, H.A., Ameyaw, E.O., Boampong, J.N., Dadzie, J.B. 2016. Antidiabetic effect of young and old ethanolic leaf extracts of *Vernonia amygdalina*: A comparative study. *Journal of Diabetes Research* 8252741.

Atangwho, I., Yin, K., Umar, I., Ahmad, M., Asmawi, Z. 2014. *Vernonia amygdalina* simultaneously suppresses gluconeogenesis and potentiates glucose oxidation via the

pentose phosphate pathway in streptozotocin-induced diabetic rats. *BMC Complementary and Alternative Medicine* 14(1): 426-436.

Atangwho, I.J., Ebong, P.E., Eyong, E.U., Eteng, M.U., Uboh, F.E. 2007. *Vernonia amygdalina* Del: A potential prophylactic anti-diabetic agent in lipid complication. *Global Journal of Pure and Applied Sciences* 13(1): 103-106.

Atangwho, I.J., Edet, E.E., Uti, D.E., Obi, A.U., Asmawi, M.Z., Ahmad, M. 2012. Biochemical and histological impact of *Vernonia amygdalina* supplemented diet in obese rats. *Saudi Journal of Biological Sciences* 19(3): 385-392.

Atangwho, I.J., Egbung, G.E., Ahmad, M., Yam, M.F., Asmawi, M.Z. 2013. Antioxidant versus Anti-diabetic properties of leaves from *Vernonia amygdalina* Del. growing in Malaysia. *Food Chemistry* 141(4): 3428–3434.

Bashir, R.A., Mukhtar, Y., Chimbekujwo, I.B., Aisha, D.M., Fatima, S.U., Salamatu, S.U. 2020. Phytochemical Screening and Fourier Transform Infrared Spectroscopy (FT-IR) Analysis of *Vernonia amygdalina* Del. (Bitter leaf) Methanol Leaf Extract. *FUTY Journal of the Environment* 14(2): 35-41.

Biadgo, B., Melku, M., Mekonnen, A.S., Abebe, M. 2016. Hematological indicies and their correlation with fasting blood glucose level and anthropometeric measurements in type 2 diabetes mellitus patients in Gondar, Northwest Ethiopia. *Diabetes, Metabolic syndrome and obesity: Targets and therapy* 9: 91-99.

Carbone, L., Carbone, E.T., Yi, E.M., Bauer, D.B., Lindstrom, K.A., Parker, J.M., Austin, J.A., Seo, Y., Gandhi, A.D., Wilkerson, J.D. 2012. Assessing cervical dislocation as a humane euthanasia method in mice. *Journal of the American Association for Laboratory Animal Science* 51(3): 352-356.

Chike, C.P.R., Njoku, B., Green, K., Akpojotor, P.I., Onyebuenyi, M.O., Numbara, D. 2018. Effect of ethanolic leaf extract of *Vernonia amygdalina* (Bitter Leaf) extract on some of the haematological parameters in wistar rats. *Journal of Complementary and Alternative Medical Research* 1-7. https://doi.org/10.9734/JOCAMR/2018/35690

Dasgupta, N., De, B. 2007. Antioxidant activity of some leafy vegetables of India: A comparative study. *Food Chemistry* 101(2): 471 -474.

Effiong, E.E., Igile, G.O., Mgbeje, B.I.A., Otu, E.A., Ebong, P.E. 2013. Hepatoprotective and anti-diabetic effect of combined extracts of *Moringa oleifera* and *Vernonia amygdalina* in streptozotocin-induced diabetic albino Wistar rats. *Journal of Diabetes and Endocrinology* 4(4): 45-50.

Ekam, V.S., Udosen, E.O. 2012. Total protein, albumin and globulin levels following the administration of activity directed fractions of *Vernonia amygdalina* during acetaminophen induced hepatotoxicity in Wistar albino rats. *Global Journal of Pure and Applied Sciences*18(1): 25-29.

Gani, E., Ihedioha, J. 2015. Evaluation of the prevalence of diabetes mellitus and impaired glucose homeostasis in dogs presented for Veterinary care in Warri, Delta State, Nigeria. Book of Abstracts, 6th AVA/53rd NVMA annual congress, Enugu, Veterinary Medical Association.

International Diabetes Federation, 2021. What is Diabetes? Facts and figures. Accessed online at https://idf.org/aboutdiabetes/what-is-diabetes/facts-figures.html

International diabetes Federation (IDF). 2021. What is Diabetes? Facts and Figures. https://idf.org/aboutdiabetes/what-is-diabetes/facts-figures.html#:~:text=In%202021%2C,low%2D%20and%20middle%2Dincome%20countries

Jain, N.C. 1993. Classification of myeloproliferative disorders in cats using criteria proposed by the Animal Leukaemia Study Group: A retrospective study of 181 cases (1969–1992). *Comparative Haematology International* 3(3): 125-134.

Kao, T.T., Wang, M.C., Chen, Y.H., Chung, Y.T., Hwang, P.A. 2021. Propylene glycol improves stability of the anti-inflammatory compounds in *Scutellaria baicalensis* extract. *Processes* 9(5): 894.

Katemo, M., Marini, D., Kadima, N. 2018. Antihyperglycemic activity of *Vernonia amygdalina* leaf extracts, *Hibiscus esculentus* fruit extract and *Garcinia kola* seed extract from Kisangani plants. *British Journal of Pharmaceutical Research* 21(5): 1-8.

Kaur, D., Kaur, N., Chopra, A. 2019. A comprehensive review on phytochemistry and pharmacological activities of Vernonia *amygdalina*. *Journal of Pharmacognosy and Phytochemistry* 8(3): 2629-2636.

Kini, S., Tripathi, P., Amarapurkar, A.D. 2016. Histopathology of Liver in Diabetes Mellitus-An Autopsy Study. *International Journal of Scientific Study* 4(5): 110-113.

Llewellyn, B.D. (2009). Nuclear staining with alum hematoxylin. *Biotechnic & Histochemistry* 84(4): 159-177.

Magliano, J., Zimmet, P. Shaw, E. 2015. Classification of Diabetes mellitus and other categories of glucose intolerance. *International Textbook of Diabetes Mellitus* 4th Ed.: pp 1-16.

Malik, V.S., Popkin, B.M., Bray, G.A., Després, J.P., Willett, W.C., Hu, F.B. 2010. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. *Diabetes care* 33(11): 2477-2483.

Michael, U.A., David, B.U., Theophine, C.O., Philip, F.U., Ogochukwu, A.M., Benson, V.A. 2010. Antidiabetic effect of combined aqueous leaf extract of *Vernonia amygdalina* and metformin in rats. *Journal of Basic and Clinical Pharmacy* 1(3): 197-203.

Montonen, J., Knekt, P., Jarvinen, R., Reunanen, A. 2004. Dietary antioxidant intake and risk of type 2 diabetes. *Diabetes Care* 27: 362-366.

Muraina, I., Adaudi, O., Mamman, M., Kazeem, H., Picard, J., Mcgaw, L., Eloff, J. 2010. Anti-mycoplasmal activity of some plant species from northern Nigeria compared to the currently used therapeutic agent. *Pharmaceutical Biology* 48: 1103-1107.

Nelson, L., Cox, M. 2005. Diabetes mellitus arises from defects in insulin production or action. In *Principles of Biochemistry* (4th Ed). Lehninger (ed). Freeman and Company: New York. pp 909-910.

Nelson, R., Reusch, C. 2014. Classification of diabetes in dogs and cats: A thematic review. *Journal of Endocrinology* 222: T1-T9.

Nwanjo, H.U. 2005. Efficacy of aqueous

leaf extract of *Vernonia amygdalina* on plasma lipoprotein and oxidative status in diabetic rat models. *Nigerian Journal of Physiological Sciences* 20(1): 39-42.

Ogbera, A.O., Ekpebegh, C. 2014. Diabetes mellitus in Nigeria: The past, present and future. *World Journal of Diabetes* 5(6): 905.

Okoduwa, S.I.R., Umar, I.A., James, D.B., Inuwa, H.M. 2017. Validation of the antidiabetic effects of *Vernonia amygdalina* Delile leaf fractions in fortified diet-fed streptozotocin-treated rat model of type-2 diabetes. *Journal of Diabetology* 8(3): 74-84.

Ong, K.W., Hsu, A., Song, L., Huang, D., Tan, B.K.H. 2011. Polyphenols-rich *Vernonia amygdalina* shows anti-diabetic effects in streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology* 133(2): 598-607.

Oyedemi, S.O., Yakubu, M.T., Afolayan, A.J. 2010. Effect of aqueous extract of *Leonotis leonurus* (L.) R. Br. leaves in male Wistar rats. *Human and Experimental Toxicology* 29(5): 377-384.

Oyeyemi, I.T., Akinlabi, A.A., Adewumi, A., Aleshinloye, A.O., Oyeyemi, O.T. 2017. *Vernonia amygdalina*: A folkloric herb with anthelminthic properties. *Beni-Suef University Journal of Basic and Applied Sciences* 7(1): 43-49.

Phyllistin, A., James, B. 2000. Tips for preventing food poisoning: Herbs. *American No.1 Guide Natural health* 3rd Ed. Publication Average 9: 383-386.

Ramadan, K.S., Khalil, O.A., Danial, E.N., Alnahdi, H.S., Ayaz, N.O. 2013. Hypoglycemic and hepatoprotective activity of *Rosmarinus officinalis* extract in diabetic rats. *Journal of Physiology and Biochemistry* 69(4): 779-783.

Sarma, D., Mallick, A., Ghosh, A. 2010. Free radicals and their role in different clinical conditions: An overview. *International Journal of Pharmaceutical Sciences and Research* 1(3): 185-192.

Shen, Y., Jin, L., Xiao, P., Lu, Y., Bao, J. 2009. Total phenolics, flavonoids, antioxidant capacity in rice grain and their relations to grain color, size and weight. *Journal of Cereal Science* 49(1):106-111.

Uloko, A.E., Musa, B.M., Ramalan, M.A., Gezawa, I.D., Puepet, F.H., Uloko, A.T., Sada, K.B. 2018. Prevalence and risk factors for diabetes mellitus in Nigeria: A Systematic Review and Meta-Analysis. *Diabetes Therapy: Research, Treatment and Education of Diabetes and Related Disorders* 9(3): 1307-1316.

Vassort, G., Turan, M. 2010. Protective role of antioxidants in diabetes-induced cardiac dysfunction. *Cardiovascular Toxicology* 10 (2): 73-86.

World Health Organization (WHO). 2021. Diabetes factsheet. https://www.who.int/news-room/fact-sheets/detail/diabetes

World Health Organization (WHO). 2022. Health topics: Diabetes. https://www.who.int/health-topics/diabetes

Yang, J., Meyers, K., van der Heide, J., Liu, R. 2004. Varietal differences in phenolic content, and antioxidant and antiproliferative activities of onions. *Journal of Agricultural and Food Chemistry* 52(21): 6787-6793.

Zafar, M., Naqvi, S.N. 2010. Altered liver morphology and enzyme in streptozotocin-induced diabetic rats. *International Journal of Morphology* 27: 719-725.

Zhao, M.L., Shan, S.J., Tao, R., Cui, L.T., Li, Q.R., Luo, J., Li, Y. 2021. Stigmastane-type steroid saponins from the leaves of *Vernonia amygdalina* Del. *Fitoterapia* 150: 104838.

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