Effects of *Vernonia amygdalina* Leaf on Nutritional and Biochemical Parameters in Alloxan-Induced Diabetic Rats

Nuria Chinonyerem Amaechi[1], Philippa Chinyere Ojimelukwe[2] and Samuel Okwudili Onoja[3,*]

[1]*Department of Food Science and Technology, Abia State University, Umuahia Campus, Abia State Nigeria*

[2]*Department of Food Science and Technology, Michael Okpara University of Agriculture, PMB 7267, Umudike, Abia State Nigeria*

[3]*Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, PMB 7267, Umudike, Abia State Nigeria*

Abstract: The effects of *Vernonia amygdalina* leaf on the nutritional and biochemical parameters in alloxan-induced diabetic rat were investigated. *Vernonia* amygdalina (VA) leaf was squeeze-washed, dried, pulverized and mixed with standard feed at 2.5%, 5%, 10% and 20%. The proximate nutrient composition of the standard and prepared rations was determined. The Vernonia amygdalina incorporated rations and standard ration were fed to alloxan-induced diabetic rats for 70 consecutive days. Thereafter the nutritional and biochemical parameters as well as the histopathology of pancreas vital organs of the treated rats were determined. The *Vernonia Amygdalina* at 2.5% inclusion rate significantly ($p < 0.05$) reversed the nutritional indices and biochemical parameters which were compromised in diabetic rats fed with standard ration alone. The VA also reversed the degenerative changes in the pancreatic islet induced by alloxan. *Vernonia Amygdalina* has potent antidiabetic activity and its incorporation in excess of 5% in the diet should be avoided.

Keywords: Alpha amylase, apparent digestibility, biological value, cholesterol, gas chromatography, glycosylated haemoglobin.

INTRODUCTION

Diabetes is a chronic disorder of glucose metabolism resulting from dysfunction in pancreatic beta cells and insulin resistance [1]. It affects over 150 million people in the world today and the prevalence is increasing rapidly [2]. There are two main categories of this disease namely type 1 diabetes mellitus also called insulin-dependent diabetes mellitus (IDDM) and type 2 diabetes mellitus also called non-insulin dependent diabetes mellitus (NIDDM). NIDDM is far more common and results from a combination of defects in insulin secretion and action [3]. Nutrition is an integral part of diabetes care aimed at restoring carbohydrate metabolism to normal [4]. Recently, there has been a recurring interest in the herbal treatment of diabetes [5]. Hypoglycemic agents like acarbose, miglitol and voglibose are synthetic drugs and have their limitations; produce serious side effects [6]. Therefore, the apparent reversal of trend from Western to herbal medicine is partly due to the fact that synthetic drugs have always shown adverse reactions and other undesirable side effects [5]. Herbal treatment is recommended in the treatment of diabetes due to their reduced side effects [7].

*Address correspondence to this author at the Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, PMB 7267, Umudike, Abia State Nigeria; Tel: +2348030613032; E-mail: samonreal@yahoo.com, samuelonoja19@yahoo.com

Vernonia amygdalina is commonly called 'Bitter leaf' (English), 'Onugbu/Olugbu' (Igbo), 'Ewuro' (Yoruba), 'Shiwaka'/'Chusardoki' (Hausa), 'Gwara' (Amharic), 'Etidot' (Ibibio), 'Ityuna' (Tiv) [8]. It belongs to the *Asteraceae* family and is widely distributed in South America and African [8]. The leaf decoction is commonly used in traditional medicine in the management of malaria, diarrhea, dysentery, hepatitis, cough, fever and as fertility inducer [9]. The leaves are used for the treatment of diabetes while the root infusion is used as aphrodisiac [10]. This study investigated effect of *Vernonia amygdalina* on nutritional and biochemical parameters in diabetic rat.

MATERIALS AND METHODS

Plant Collection and Preparation

Fresh *Vernonia amygdalina* leaves were procured from Abakpa Market Abakaliki, Ebonyi state, Nigeria. It was picked, squeeze-washed and drained off water before air drying under a shade for 6 days at environmental temperature ($25 \pm 2\ ^0C$). The air dried leaves were pulverized and stored in clean, dried and labeled air tight container prior to use.

Determination of Alpha Amylase Inhibitory Activity (AAI)

The squeeze-washed *Vernonia amygdalina* leaves was extracted using different solvents namely water,

chloroform, ethylacetate, absolute ethanol and 70% ethanol using cold maceration method as described by Jeremy and Whiteman [11]. The extract was concentrated with rotary evaporator. The AAI of each extract was done by the modified dinitrosalicylic acid method of Bernfeld [12] to estimate maltose.

Gas Chromatography/Mass Spectrometry Analysis

The bioactive compounds in ethanol extracts of squeeze-washed *Vernonia amygdalina* leaf was analyzed using gas chromatography/mass spectrometry (QP 2010 Plus Shimadzu, Japan) equipped with a flame ionization detector (FID). Helium was used as carrier gas and at a flow rate of 3.0 mL/min. The column temperature was programmed from 70 to 280°C at the rate of 5°C/min. Injector and detector temperatures were set at 250 and 260°C, respectively. All quantifications were carried out using a built-in data-handling program provided by the manufacturer of the GC (Perkin Elmer, Norwalk, CT, USA). Interpretation of mass spectrum from gas chromatography-mass spectrum was conducted using the database National Institute Standard and Technology (NIST) version 2.0, 2009 library.

Formulation of Experimental Diet

A standard diet containing 83% corn starch, 10% casein, 2% vegetable oil, 3% rice bran and fibre and 2% vitamin and mineral mix was prepared. The dried pulverized squeeze-washed *Vernonia amygdalina* (VA) leaves were incorporated at different concentrations of 2.5%, 50%, 10%, 20% per 100g of standard diet.

Experimental Animals

Weanling albino Wistar rats (22-26 days old) of both sexes with average initial body weight of 40 $\pm$ 2 gram were used for the experiments. The rats were obtained from the Department of Veterinary Medicine, University of Nigeria, Nsukka. They were house in the animal housed of the Department of Food Science and Technology, Michael Okpara University of Agriculture Umudike. They were divided into groups of six rats each. They were housed in stainless steel metabolism cages and maintained at environmental temperature of 28 $\pm$ 2^0C and natural light-dark cycle. All the experimental animals were acclimatized for 14 days on the standard diet. The rats were maintained in accordance with the recommendation in the guide for the care and use of laboratory animals [13]. The experimental protocol was approved by the institutional ethics committee.

Proximate Analysis of Diet Samples

Proximate composition of the various diet samples were analyzed by the methods described by James [14].

Induction of Diabetes

Diabetes was induced by intraperitoneal administration of 160 mg/kg alloxan monohydrate to overnight (16 h) fasted rats that were not deprived of water. After 72 h, fasting blood glucose levels of the experimental rats were checked using a glucometer (Accu-check active, Germany).

Feeding Trials

The method described by Pellet and Young [15] was adopted. Eight (8) normoglycemic and 40 diabetic rats were used for the experiment. The normoglycemic rats were assigned group A and diabetic rats were randomly assigned to 5 groups (B-F) of 8 rats each. The rats were individually housed in metabolic cage. Groups A and B received standard feed while groups C – F received feed containing 2.5, 5, 10 and 20% VA leaves, respectively. The rats were fed *ad libitum* for 70 consecutive days. The daily feed and water intake was recorded, while the body weight was recorded at weekly interval. The daily fecal output was recorded, collected and air dried while the daily urine output was recorded, pooled and stored by addition of 1 ml of 0.1 N HCl in brown bottle nitrogen analysis. After the 70^{th} day of feeding the rats were fasted for 16 h and blood sample was collected via ocular puncture into EDTA and plain bottle for whole blood and serum preparation respectively. Thereafter, the rats were sacrificed by cervical dislocation, immediately laparotomised and pancreas; were excised and preserved in 10% formalin solution for histopathological study.

Evaluation of Nutritional Indices

Nitrogen content of both urine and feacal samples was analyzed using the kjeldahl method described by James [14]. Protein efficiency ratio (PER), feed efficiency ratio (FER), apparent digestibility (AD), true digestibility (TD), biological value (BV) and net protein utilization (NPU) were calculated.

Biochemical Analysis

Fasting blood glucose (FBG) was determined using a glucometer (Accu- check, Germany). Glycosylated

haemoglobin (HbA1c) was analyzed by the method of Trivelli *et al.* [16], using a glycohaemoglobin test kit (Teco Diagnostics, USA). The serum total proteins, albumin, creatinine, total cholesterol, triglycerides high density lipoprotein cholesterol (HDL-C) were analysed using a commercial available diagnostic test kit (Randox Laboratory, UK). Serum low density lipoprotein cholesterol (LDL-C) was calculated using Fridewald equation [17]:

LDL-C (mg/dl) = Total cholesterol – (Triglycerides/5) - HDL-C.

Histopathology

The Pancreas of the rats were excised and fixed in 10% formol saline for 24 h. They were washed in ascending grades of ethanol, cleared with xylene, embedded in paraffin wax, sectioned with a microtome, and stained with hematoxylin and eosin (H and E) and mounted on Canada balsam (Sigma-Aldrich, St. Louis, MO) [18]. All the sections were examined under a light microscope under different (100 and 400) magnifications. Photomicrographs of lesions were taken with an Olympus photo microscope (Olympus Scientific Equipment, Ashburn, VA) for observations and documentation of histopathological lesions.

Statistical Analysis

One way analysis of variance (ANOVA) followed by Duncan's Post hoc test was used to separate differences of data collected for the various parameters analyzed. Data obtained were expressed as mean $\pm$ standard deviation and differences in means were considered to be significantly different ($P < 0.05$). All statistical analysis was determined using SPSS statistical software version 20.

RESULTS

Alpha-Amylase Inhibitory Activity

The effects of different solvent extracts of squeezed washed VA on α-amylase activity are presented in Table **1**. The 99% ethanol, chloroform, water, ethyl acetate and 70% ethanol extracts caused 47.82%, 42.85%, 40.63% 33.35% and 26.77% inhibition of α-amylase activity.

Table 1: Percentage α-Amylase Inhibitory Activities of Squeeze Washed *Vernonia amygdalina* Leaves in Different Solvents

Solvents	Water	Ethylacetate	70% Ethanol	Chloroform	99% Ethanol
% inhibition	40.63±2.12	33.35± 1.42	26.77±0.43	42.85±0.26	47.82±0.34

Table 2: The Compounds Identified in the Ethanol Extract of Squeeze-Washed Vernonia Amygdalina Leaf

S/N of Peak	RT (Minutes)	Identity of Compound	Conc. (%)	Molecular Weight	Molecular Formular
1	20.64	Methyl Nonanoate	0.82	172	$C_{10}H_{20}O_2$
2	22.33	1-butyl-heptyl-benzene	0.35	232	$C_{17}H_{28}$
3	25.72	Methyl-hexadecanoate	6.76	270	$C_{17}H_{34}O_2$
4	26.31	Hexadecanoic acid	12.70	256	$C_{16}H_{32}O_2$
5	27.47	Methyl (E)-octadec-9-enoate	26.17	296	$C_{19}H_{36}O_2$
6	27.67	Methyl Octadecanoate	4.22	298	$C_{19}H_{38}O_2$
7	28.05	9-Octadecenoic acid (Oleic acid)	37.19	282	$C_{18}H_{34}O_2$
8	29.25	1,3-dihydroxypropan-2-yl pentadecanoate	2.01	316	$C_{18}H_{36}O_4$
9	30.35	9, 12-octadecadienoyl chloride (Linoleic acid chloride)	0.87	298	$C_{18}H_{31}ClO$
10	30.73	Cis 9- Hexadecenal	5.26	238	$C_{16}H_{30}O$
11	30.89	(2-hydroxy-3-octadecanoyloxypropyl) octadecanoate	1.77	624	$C_{39}H_{76}O_5$
12	31.14	1,3-dihydroxypropan-2-yl hexadecanoate	1.89	330	$C_{19}H_{38}O_4$

Legend: RT = Retention Time, conc. = concentration.

Gas Chromatography-Mass Spectrometry (GC-MS) Chromatogram of Ethanol Extract of Squeeze – Washed VA

The squeeze-washed leaf of VA contained twelve (12) compounds (Table **2**). Octadec-9-enoate (peak 7) with molecular weight and formular, 282 and $C_{18}H_{34}O_2$ respectively had the highest concentration (37.19%). It has retention time of 28.5 minutes. Another compound that occurred in high proportion (26.17%) was methyl (E)-octadec-9-enoate (peak 5) with retention time of 27.47 minutes and molecular weight and formular, 296 and $C_{19}H_{38}O_2$ respectively.

Proximate Composition of the Rations

The results of the proximate analysis of the rations are shown in Table **3**. The standard ration (SD) had significantly ($p < 0.05$) lower moisture, ash, crude fiber, fat and crude protein content and significantly ($p < 0.05$) higher carbohydrate (CHO) content when compared with other rations. Squeezed-washed *Vernonia amygdalina* leaf caused a significant ($p < 0.05$) concentration-dependent increase in the moisture, ash, crude fibre, fat and crude protein content of the ration. Also, VA caused a significant ($p < 0.05$) concentration-dependent decrease in the CHO content of the ration.

Effect of Vernonia Amygdalina (VA) on the Nutritional Qualities of Ration Fed to Normal and Diabetic Rats

Table **4** shows the nutritional qualities of rations fed to normal and diabetic experimental rats. The feed intake (FI) and protein intake (PI) of the diabetic control group was significantly ($p < 0.05$) higher than the normal control group. The VA caused a significant ($p < 0.05$) concentration dependent decrease in the FI and PI of the treated groups when compared with the diabetic control. The FI and PI of the 5%, 10% and 20% VA treated groups were significantly ($p < 0.05$) lower when compared with the normal control groups. The WG of the normal control group was not significant ($p > 0.05$) when compared with the diabetic control group. The WG of the 2.5% VA treated group was significantly ($P < 0.05$) higher when compared with the diabetic control group. The 5%, 10% and 20% VA treatment caused significant ($p < 0.05$) concentration-dependent decrease in WG of the treated group when compared with the diabetic control group. The PER and FER of the diabetic control, 2.5%, 5%, 10% and 20 % VA treated groups were significantly ($p < 0.05$) lower when compared with the value of the normal control group in concentration-dependent manner.

The nitrogen intake (NI) of the diabetic control and 2.5% VA treated groups were significantly ($p < 0.05$) increased when compared with the normal control. The urinary nitrogen (UN), endogenous faecal nitrogen (EFN) and endogenous urinary nitrogen (EUN) of the diabetic control group were significantly ($p < 0.05$) lower when compare with the normal control, while the UN, EFN and EUN of 2.5% VA treated group were significantly ($p < 0.05$) higher when compare with the normal and diabetic control groups. The NI, UN, EFN and EUN of the 5%, 10% and 20 % VA treated groups were significantly ($p < 0.05$) decreased in a concentration dependent manner when compared with the normal control group. The fecal nitrogen (FN) of the diabetic control, 2.5%, 5%, 10% and 20 % VA treated groups were significantly ($p < 0.05$) higher in a concentration dependent manner when compared with the normal control (Table **4**).

The apparent digestibility (AD), true digestibility (TD), biological value (BV), net protein utilization (NPU), nitrogen retention (NR) and percentage nitrogen retention (%NR) of the groups feed with 5%, 10% and 20% VA were significantly reduced when compared with normal and diabetic control groups.

Table 3: Proximate Composition of Standard Ration and Rations Containing VA at Varied Concentrations

Samples	SD	2.5% VA	5% VA	10% VA	20% VA
Moisture	6.52±0.18^{e}	7.13±0.25^{d}	8.22±0.07^{c}	10.19±0.02^{b}	12.59±0.13^{a}
Ash	2.58±0.13^{d}	2.60±0.16^{d}	2.81±0.05^{c}	3.10±0.07^{b}	3.24±0.06^{a}
Crude Fibre	2.60±0.07^{e}	3.30±0.11^{d}	4.40±0.12^{c}	5.18±0.09^{b}	6.29±0.11^{a}
Fat	2.68±0.04^{e}	3.69±0.10^{d}	4.95±0.19^{c}	5.26±0.17^{b}	6.21±0.03^{a}
Crude Protein	16.95±0.05^{e}	19.01±0.18^{d}	20.72±0.25^{c}	22.95±0.22^{b}	25.66±0.17^{a}
Carbohydrate	68.68±0.28^{a}	64.27±0.32^{b}	58.89±0.03^{c}	53.09±0.74^{d}	46.00±0.45^{e}

Values are means of triplicate determinations ± standard deviation. Means in the same row bearing different superscripts are significantly different ($p < 0.05$). SD = standard diet, VA = *Vernonia amygdalina*, CHO = carbohydrate.

Table 4: Effect of VA on the Nutritional Qualities of Ration Feds to Normal and Diabetic Rats

Group	Normal control	Diabetic control	2.5% VA	5% VA	10% VA	20% VA
FI (g)	282.38±38.77^{b}	330.68±25.85^{a}	311.05±11.09^{a}	235.52±12.50^{c}	188.72±8.12^{d}	147.22±5.07^{e}
PI (g)	47.84b±6.57^{b}	56.02±4.38^{a}	58.64±2.08^{a}	48.44±2.79^{b}	42.88±1.84^{c}	37.82±1.30^{d}
BMG (g)	87.57±10.40^{ab}	80.23±2.79^{b}	91.92±12.25^{a}	57.47±3.62^{c}	48.63±3.21^{d}	41.57±3.72^{d}
PER	1.85±0.07^{a}	1.44±0.09^{c}	1.56±0.16^{b}	1.19±0.03^{d}	1.13±0.06^{d}	1.10±0.06^{d}
FER	0.31±0.01^{a}	0.24±0.02^{c}	0.30±0.03^{ab}	0.20±0.01^{d}	0.21±0.03^{d}	0.28±0.02^{b}
NI (mg)	1297.76±178.21^{b}	1519.76±118.77^{a}	1590.81±56.54^{a}	1314.08b+75.57^{b}	1163.24±50.02^{c}	1012.39±32.28^{d}
FN (mg)	108.22±24.85d	155.64±16.48^{c}	151.55±8.89^{c}	157.15±12.32^{c}	181.82±7.44^{b}	243.94±11.68^{a}
UN (mg)	41.10±3.52^{b}	38.97±0.05^{c}	39.93±0.13^{b}	46.55±1.88^{a}	41.20±0.96^{b}	36.34±0.47^{c}
EFN (mg)	39.19±4.37^{b}	35.49±1.29^{bc}	45.58±5.54^{a}	31.78±1.67^{c}	30.78±1.50^{c}	29.48±1.65^{c}
EUN (mg)	11.93±1.75^{b}	10.45±0.52^{b}	14.46±2.19^{a}	8.96±0.67^{d}	8.56±0.59^{d}	8.04±0.66^{d}
AD	91.77±0.96^{a}	88.11±4.29^{b}	90.50±0.26^{a}	88.05±0.31^{b}	84.36±0.43^{c}	75.90±1.12^{d}
TD	88.74±0.66^{a}	87.43±0.30^{b}	87.64±0.57^{b}	85.65±0.28^{c}	81.92±0.45^{d}	72.98±1.21^{e}
BV	95.30±0.46^{b}	96.26±0.26^{a}	96.32±0.57^{a}	95.04±0.20^{bc}	94.76±0.23^{c}	94.20±0.33^{d}
NPU	84.56±0.37^{a}	84.17±0.29^{a}	84.22±0.55^{a}	81.40±0.19^{b}	77.43±0.55^{c}	68.95±1.03^{d}
NR	1148.78±153.22^{b}	1325.16±102.57^{a}	1499.66±48.66^{a}	1105.88±55.99^{b}	940.22±44.42^{c}	732.12±29.78^{d}
%NR	88.56±0.40^{a}	87.20±0.20^{b}	87.99±0.21^{ab}	84.19±0.87^{a}	80.82±0.52^{d}	72.31±1.16^{e}

Values are means ± standard deviation. Means in the same row bearing different superscripts are significantly different ($p < 0.05$). VA = *Vernonia amygdalina*, FI = Feed Intake, PI = Protein Intake, BMG = Body Mass Gain, PER = Protein Efficiency Ratio, FER = Feed Efficiency Ratio, NI = Nitrogen Intake, FN = Fecal Nitrogen, UN = Urinary Nitrogen, EFN = Endogenous Fecal Nitrogen, EUN = Endogenous Urinary Nitrogen, AD = Apparent Digestibility, TD = True Digestibility, BV = Biological Value, NPU = Net Protein Utilization, NR = Nitrogen Retention, %NR = Percentage Nitrogen Retention.

There was no significant ($p > 0.05$) difference in AD and NPU between the normal rats and the diabetic rats fed 2.5% VA. The AD, TD and %NR of the diabetic control group were significantly ($p < 0.05$) reduced when compared with the normal control group, while the BV and NR of the diabetic control group were significantly ($p < 0.05$) elevated when compared with the normal control group (Table **4**).

Effects of VA on Biochemical Parameters in Rats

The effects of VA on the biochemical parameters of rat are presented in Table **5**. The glycosylated haemoglobin (HbA1c), fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), and low density lipoprotein cholesterol (LDL-C) of the diabetic control group increased significantly ($p < 0.05$) when compared with the normal control. The VA treatment caused significant ($p < 0.05$) concentration-dependent decrease in the level of HbA1c, FBG, TC, TG and LDL-C levels in the treated rats when compared with diabetic control rats. The HDL-C level of the diabetic control group was significantly ($p < 0.05$) reduced when compared with the normal control group but the high density lipoprotein cholesterol (HDL-C) of the VA treated groups increased significantly ($p < 0.05$) in a concentration dependent manner when compared with the normal and diabetic control groups. Total protein (TP) of the VA treated groups increased significantly ($p < 0.05$) in a concentration-dependent manner when compared with the diabetic control group but were not significant ($p > 0.05$) when compared with the normal control. Serum creatinine (SC) levels of 5% and 10 % VA treated rats were significantly ($p < 0.05$) higher when compared with the normal and diabetic control groups, while the SC levels of diabetic control, 2.5% and 20% VA were not significant ($p > 0.05$) when compared with the normal control.

Histopathology

Photomicrograph sections of Pancreas of rats after 70 days feeding with VA are shown in Figure **1**. The diabetic control showed loss of islet cells and necrosis of the acini. The VA treatment caused concentration dependent regeneration of the islet and acini cell.

DISCUSSION

The extracts of squeeze-washed *Vernonia amygdalina* inhibited alpha-amylase activities, produced hypolipidemic and antidiabetic activities as well as reversed pancreatic damage in alloxan-induced

Table 5: Effects of VA on Biochemical Parameters in Diabetic Rats

Group	Normal control	Diabetic control	2.5% VA	5% VA	10% VA	20% VA
HbA1c (%)	4.05±0.10^{c}	10.65±1.99^{a}	7.23±1.12^{b}	5.65±0.93^{c}	4.73±0.43^{c}	4.45±0.06^{c}
FBG (mg/dl)	93.17±12.80^{e}	212.50±35.86^{a}	147.33±16.12^{b}	135.17±10.11^{b}	113.50±6.35^{c}	100.17±5.53^{c}
SA (g/l)	3.87±0.45^{d}	4.23±0.18^{abc}	3.93±0.18^{cd}	4.00±0.13^{cd}	4.24±0.36^{ab}	4.43±0.21^{a}
TP (g/l)	6.81±0.62^{ab}	6.40±0.74^{b}	7.28±0.56^{a}	7.45±0.70^{a}	7.42±0.31^{a}	7.53±0.30^{a}
SC (mg/dl)	0.67±0.08^{bc}	0.58±0.08^{c}	0.68±0.13^{bc}	0.87±0.05^{a}	0.87±0.05^{a}	0.73±0.20^{ab}
TC (mg/dl)	165.39±14.18^{cd}	251.28±13.47^{a}	194.35±85.68^{bc}	221.16±13.71^{ab}	182.31±13.32^{bcd}	136.67±8.66^{d}
TG (mg/dl)	117.82±6.70^{d}	195.77±5.88^{a}	155.51±6.18^{b}	131.73±10.62^{c}	110.19±12.78^{d}	91.54±17.36^{e}
HDL (mg/dl)	54.14±6.40^{b}	27.44±8.27^{d}	27.50±8.27^{d}	44.61±7.58^{c}	69.68±5.75^{a}	73.01±7.13^{a}
LDL (mg/dl)	86.44±11.99^{c}	184.69±14.24^{a}	170.25±22.89^{a}	150.71±12.94^{b}	91.92±13.01^{c}	45.35±10.16^{d}

Values are means ± standard deviation. Means in the same row bearing different superscripts are significantly different ($p < 0.05$). VA = *Vernonia amygdalina*, HbA1c = Glycosylated hemoglobin, FBG = Fasting blood glucose, SA = Serum albumin, TP = Total protein, SC = Serum creatinine, TC = Total cholesterol, TG = Triglycerides, HDL = High density lipoprotein, LDL = Low density lipoprotein.

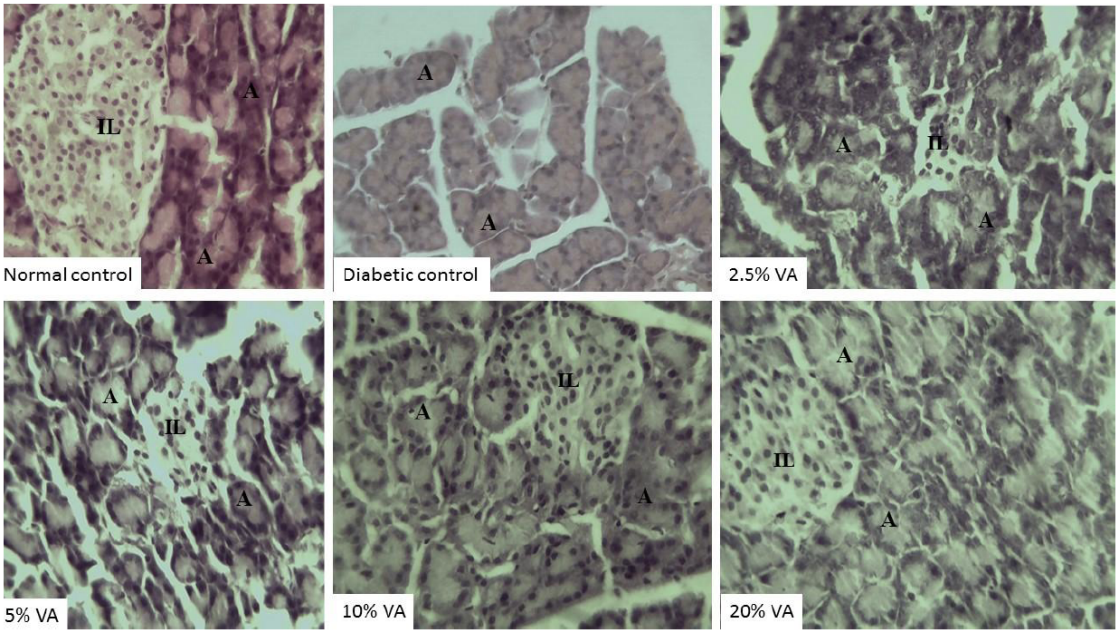

Figure 1: Photomicrograph sections (H and E ×400) of pancreas of normal and diabetic rats.

Legend: IL = pancreatic islet, A = acini cell.

diabetic rats. The squeezed-washed *Vernonia amygdalina* (VA) leaf was used for the animal experiment. The washing of the VA was to simulate the traditional method of preparation before its addition in human diet. The squeeze-washing of VA has been reported to reduce the saponins, tannins and flavonoids content which in most cases may lead to loss of its bitter taste [19].

The result of the alpha-amylase test suggests that VA contains alpha-amylase inhibitor soluble in both polar and non-polar solvents. The *in vitro* inhibition of alpha-amylase activities demonstrate that VA can delay carbohydrate digestion and absorption which will lead to reduced blood glucose level and hence, considered a therapeutic strategy for the treatment of diabetes mellitus [20, 21]. Some alpha-amylase inhibitors have been reported to be polyphenols and flavonoids [22].

The GC-MS analysis revealed the presence of predominantly Oleic acid, hexadecanoic acids and their respective derivatives. These compounds have been reported to possess anti-inflammatory, antioxidant and hypocholesterolemic activities [23]. Agents that have these activities are prescribed in diabetic condition for improved healthy living through the prevention of the development of diabetic complications [24]. The bioactive compounds reported in this study are at

variance with report of Farombi and Owoeye [25]. This is attributed to the differences in the place of collection and mode of extract preparation [26].

The supplementation of the ration with VA caused concentration dependent increase in ash, crude fibre, fat, and crude protein content, which is in agreement with the report of Nnamani [27]. He noted that vegetables are cheap source of vitamins, mineral and amino acids. The increased crude fibre content may enhance peristalsis by acting as bulk; which will reduce intestinal transit time and nutrient digestion and absorption [28].

Alloxan monohydrate is commonly used to induce experimental diabetes in animals [29]. It causes destruction of the β-cells of the pancreatic islet through the generation of reactive oxygen species (ROS) with the subsequent reduction in insulin production and attendant hyperglycemia and dyslipidemia [30]. Diabetes induced hyperglycemia is due to underutilization of glucose by body tissues while dyslipidemia is associated with the excessive mobilization of fat from adipose tissues as a result of reduced glucose uptake by the cells [31].

The higher feed intake of the diabetic control group when compared to the normal control was in agreement with the report of previous researchers. El-shobaki *et al.* [32], stated that the feed intake of diabetic rats were higher than normal rats due to poor glucose uptake by cell as a result of insulin resistance or deficiency which characterize diabetes mellitus. The decreased feed intake at 5% VA supplementation and beyond could be attributed to high fiber content which gives rise to satiation and satiety [33, 34] and/or poor palatability of the feed. VA contains bitter principle which confers bitter taste and poor palatability to the feed [35]. The decrease in the WG, FER and PER (Table **4**) of the diabetic control group is due to muscle wasting which characterize alloxan induced diabetic condition [36]. The increase in WG, FER and PER observed in 2.5% VA groups may be attributed the reported antidiabetic potential of VA [37]. The supplementation of the ration with 5% and above VA caused concentration dependent decrease in feed intake, protein intake, WG and generalized nutrient absorption and utilization. This is attributed to the high dietary fiber content of the supplemented feeds. Burton-Freeman [38] reported that dietary fiber reduces feed intake and WG through satiation and satiety effects. Also, Bawa *et al.* [39] reported that fiber reduces nutrient absorption through the reduction in gastric emptying, intestinal transit time and accessibility of the nutrient intestinal absorptive surface and chronic alteration of the secretion of digestive enzymes and physiological functions of the intestinal absorptive cells.

The incorporation of VA in the ration of the rats improved the clinical diabetes mellitus in the treated rats. This is evident by the reduction in the FBG, Hba1c, TC, TG, LDL-C and increase in HDL-C level (Table **5**). The improved clinical diabetes may be as a result of increase in insulin secretion and/or sensitivity [40]. The VA caused concentration dependent regeneration of the pancreatic islet cells damaged as a result of alloxan administration (Figure **1**). The regeneration of pancreatic islet cell may have caused increased insulin secretion. Insulin enhances glucose uptake by the cells and attendant decrease in the serum level of glucose and glycosylation of haemoglobin [41]. The feeding of the rats with VA incorporated feed caused the regeneration of pancreatic islet damaged by alloxan administration. The regeneration of this tissue may be linked to the antioxidant activity of VA [42]. The antioxidant effects of the constituents of VA might have stopped the spontaneous production of ROS and damage of cell membrane induced by the administered alloxan in the rats [43].

The addition of VA to the feed caused concentration dependent correction of dyslipidemia induced by alloxan in the rats. Dyslipidemia is characterized by hypercholesterolemia, hypertriglyceridemia and high level of LDL-C [44]. It is the major risk factor for cardiovascular disease; common complication of diabetes mellitus [45]. This suggests that VA can be used in the treatment and/or prevention of the development of complication in diabetes mellitus. The mechanism of the lipid lowering effects of VA is not known but could be through the stimulation of insulin production and activation of lipoprotein lipase [46]. The lipid lowering effects of VA incorporated feeds can also be attributed to the high dietary fiber content. Dietary fiber has been reported to lower feed intake, decrease cholesterol and triglyceride absorption and increase faecal bile acid and cholesterol excretion [47].

CONCLUSION

The extracts of squeeze-washed *Vernonia amygdalina* inhibited alpha-amylase activities, produced hypolipidemic and antidiabetic activities as well as reversed pancreatic damage in alloxan-induced diabetic rats. This study justifies the use of *Vernonia*

Amygdalina in the folkloric management of diabetic mellitus and suggests that its incorporation in excess of 5% in the diet should be avoided.

DECLARATION OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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