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ANTI-DIABETIC EFFECT OF AQUEOUS EXTRACTS OF *Vernonia amygdalina* AND *Dacryodes edulis* LEAVES AND THEIR COMBINATION IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

In this study, the antidiabetic activities of the aqueous extracts of *Veronia amygdalina* (VA) and *Dacryodes edulis* (DE) leaves and their combined extracts (VA+DE) were examined. Thirty-six adult male Wistar rats were used in the study with 6 rats in each group. Specifically, group 1 (normal control) rats were received only the vehicle. The remaining groups (groups 2 to 6) were administered alloxan (150 mg/kg; i.p.) to induce diabetes and thereafter treated as follows: Group 2 animals were left diabetic but received only the vehicle (negative control), Group 3were treated with metformin (positive control) at 200 mg/kg body weight while groups 4, 5 and 6 received (p.o.) 300mg/kg of DE, VA and VA+DE, respectively for two weeks. Phenolic acids, tannins, saponins, fixed fat and oil, and flavonoids were more visible in the VA extract while the DE extract showed more alkaloids, proteins, phlobatannins. Administration of the extracts and their combination or metformin to the diabetic rats resulted in significant ($p< 0.05$) reductions in blood glucose, total cholesterol, triacylglycerol, low density lipoprotein cholesterol and increase in high density lipoprotein cholesterol levels compared to the negative control. Significant ($p< 0.05$) increase in serum total protein and marked reduction in liver and kidney function indices were recorded in the extracts (VA, DE, VA+DE) and the metformin treated diabetic rats in comparison to negative control. The results of this study revealed that *V. amygdalina* and *D. edulis* contained some biologically active compounds that may be efficacious in the management of diabetes mellitus.

KEYWORDS: Antidiabetic potential, phytochemical screening**,** *Vernonia amygdalina*, *Dacryodes edulis, Metformin*

INTRODUCTION

Diabetes mellitus (DM) is defined as a disorder of carbohydrate metabolism characterized by impaired ability of the body to produce or respond to insulin and thereby maintain proper levels of sugar (glucose) in the blood. It is a chronic metabolic disease of carbohydrate metabolism which has been classed into three main types. Type 1 or insulin dependent diabetes, Type II (which is predominantly insulin resistance) and gestational diabetes (Kerner *et al*., 2014). In another definition, diabetes mellitus (DM) is regarded as a cluster of metabolic disorder associated with derangement in the metabolism of carbohydrates, fats and proteins resulting from insulin insufficiency, insulin action or both (Kazeem *et al*., 2013). It is a disease with worldwide prevalence, with figures for 2021 showing 537million adults and 1.2million children and adolescents living with diabetes and projections expected to rise to 643million by 2030(IDF,2022). This picture is even grimmer in Sub-Saharan Africa with 24million persons (1:22) living with the disease whilst it is predicted to increase by 129% to 55 million by 2045(IDF,2022). The morbidity and mortality picture is worsened by the fact that 54% of those with DM are undiagnosed (IDF,2022).

The occurrence of type 2 diabetes mellitus in various population groups is attributed to the global rise in the prevalence of obesity caused by unhealthy life-styles resulting in insulin resistance in some cases. Peripheral insulin resistance inhibits the expression of glucose transporters (GLUTs) on insulin dependent plasma membrane such as striated muscles and adipocytes resulting in hyperglycemia. Studies have also implicated oxidative stress in the pathogenesis and complications of diabetes mellitus (Alfadda and Sallam, 2012). In the condition of hyperglycemia, stress sensitive pathways such as polyol (sorbitol), hexosamine, advance end glycation products and NF-kB pathways are activated. These events result in overwhelming depletion of endogenous antioxidant system, culminating in oxidative damage to proteins, cellular machinery as well as insulin resistance (Maritim *et al*., 2013).

Due to the evolving epidemic of obesity and DM in Sub-Saharan Africa, it is imperative that indigenous medications with local acceptance be developed to combat this disease. Different plants species have been found to be effective in the management of diabetes mellitus. Paloma *et al*. (2012) documented and published 800 plant species reported to possess varying degree of anti-diabetic potential. Studies on phyto-constituents and hypoglycemic properties of plants indicated that polyphenols, glycosides, alkaloids, terpenoids, and guanidine inhibit alpha amylase and reverse hyperglycemia in diabetic rats (Mentreddy, 2017).

The common bitter leaf plant (*Vernoniaamygdalina*), a shrub predominantly grown and eaten in tropical Africa has been documented to possess abundant medicinal properties. The hypoglycemic and alpha amylase inhibitory activities of various extracts of *V. amygdalina* have been reported and validated, hence could be used in the management of diabetes mellitus

(Akah and Okafor, 2017). Another African plant is the native pear or butter fruit *Dacryodes edulis*. It is native to Africa and a fruit tree of about 18-40 meters high which belongs to the family Burseraceae (Jecinta*et al.,* 2015). Several studies have demonstrated the hypoglycemic potential of different parts of the plant (Johns, 2017; Agbor *et al.,* 2017; Koudou *et al*., 2018; Chimaobi *et al.,* 2019). These plants are ubiquitous across Sub-Saharan Africa and already enjoy some acceptance as food sources being relatively cheap and abundant.

Healthcare expenditure for diabetes was $966billion in 2021 with 3 in 4 of diabetics living in low- or medium-income countries and Africa being over represented. (IDF, 2022). With nearly 1 in 2persons living below the poverty level in Africa (World Bank Poverty Data, 2022) and healthcare expenditure being out of reach to most persons, exploring the medicinal properties of alternative medicines in prevention and management of DM would prove to be a game changer in turning the grim picture of DM across Africa. Identifying plants with promising antidiabetic profile has the potentials to expand the therapeutic interventional options against the pathogenesis and progression of the disease and its complications. This study therefore evaluated and compared the anti-diabetic potential of the aqueous leaf extracts of *Vernonia amygdalina* and *Dacryodes edulis*.

MATERIALS AND METHODS

Collection and preparation of Vernoniaamygdalina and Dacryodes edulis Leaf Extracts

The method of Akah *et al*. (2018) was used in the preparation of the leaf extracts of *V. amygdalina* and *D. edulis* which were collected from Ukana Ikot Akpabin and Ikpe Annang in Essien Udim L.G.A of Akwa Ibom State, Nigeria. The fresh leaves of each plant were cleaned by using distilled water. The leaves were air-dried under the shade and hand crushed to powder. The crushed powder of *V. amygdalina* and *D. edulis* were separately macerated in distilled water for 24 hours. The filtrates obtained were filtered using doubled muslin cloth and then Whatman No. 1 filter paper until a clear filtrate was obtained, which was evaporated and concentrated using rotary evaporator. The semi-solid residues obtained (78 g of *V. amygdalina* and 65 g of *D. edulis*) were kept in a sealed plastic container refrigerated at 4^{o}C.

Phytochemical Screening

The presence of phytochemicals was detected by chemical reactions for alkaloids, phenolic compounds, flavonoids, tannins, saponins, phytosterol, fixed fat and oil, protein, gums and mucilage as well as phytobatannins. The method was based on that described by Ogundipe *et al.* (2000).

Induction of Diabetes Mellitus and Animal Grouping

Sets of male albino rats of the Wistar strain were obtained from the Animal house, Biochemistry Department, University of Benin, Benin City, Nigeria. Alloxan (150 mg/kg) was used to induce diabetes in these animals by administering a single dose of the drug via an intraperitoneal (i.p.) route. The alloxan was dissolved in 0.1mL fresh cold citrate buffer at the pH 4.5. Three

days after alloxan injection, fasting plasma glucose (FPG) was assessed using blood samples from the tail vein of the rats with Accu-Chek ® digital glucometer. Only rats with a minimum of 200 mg/dL of blood glucose concentration were used in the study (Osinubi, 2006). The diabetic rats were randomly distributed into six equal groups and treated for 14 consecutive days as follows:

Group 1: Normal control non-diabetic rats received only the standard rat chow.
Group 2: Negative control – Alloxan (150mg/kg; i.p.) diabetic rats without treatment
Group 3: Positive control – Alloxan (150mg/kg; i.p.) + Metformin (200 mg/kg; MF; p.o.)
Group 4: Alloxan (150mg/kg; i.p.) + *D. edulis* (DE; 300 mg/kg; p.o.) extract
Group 5: Alloxan (150mg/kg; i.p.) + *V. amygdalin* (VA; 300 mg/kg; p.o.) extract
Group 6: Alloxan (150mg/kg; i.p.) + *D. edulis* (DE; 300 mg/kg; p.o.) +*V. amygdalina* (VA; 300 mg/kg; p.o.) extracts.

Three days after the injection of alloxan, blood was collected from the tail vein, and fasting blood glucose was measured every week, with the aid of Accu-Chek® digital glucometer to monitor dynamic changes in all the rats. General characteristics of the rats such as mental status, eye colour, water and food intake as well as changes in body weight were observed across the groups. Body weight was measured at four days interval. After 14 days of treatment, the rats were fasted overnight for 12 hours. Thereafter, they were sacrificed and the blood samples were collected for biochemical analysis.

Collection and Preparation of Samples

At the end of treatment, all the rats were sacrificed after exposure to anesthesia. The thoracic region was cut open and blood was collected by cardiac puncture. The blood samples obtained were placed in ice-cold heparinized tubes and were further subjected to centrifugation at 2500 rpm for 15 min using Spinette – Damon / IEC bench top centrifuge. The sera obtained were subjected to various biochemical assays including fasting blood glucose, triacylglycerol (TAG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), bilirubin, total protein, creatinine, urea, and electrolytes were assessed using commercially available kits. All biochemical kits were purchased from Pars Azmun, Iran.

Analysis of Lipid Profile

Triacylglycerol, total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) were estimated using standard biochemical assays, including spectrophotometry and enzyme coupled reactions.

Estimation of Serum Total Bilirubin

Serum total bilirubin was estimated using spectrophotometric method

Estimation of Serum ALT, AST and ALP

Serum alanine amino transferase (ALT) and aspartate amino transferase (AST) were estimated by spectrophotometric method (Reitman,

and Frankel, 1957; Kind and King, 1994).

Estimation of Serum Total Protein

Total protein estimation was carried out according to the method of Teitz (1995).

Estimation of Fasting Blood Glucose Level

Digital Accu-Check ® glucometer was used in the measurement of fasting blood glucose.

Determination of Kidney Function

Flame photometer was used in the analysis of serum Na^+ and K^+ while colorimetric method was used in the estimation of creatinine and urea. Cl and HCO_3 were determined by titration (Kolhathar and Ochei, 2000).

Data Analysis

Results were expressed as mean± SEM. One way analysis of variance (ANOVA) was used to perform the statistical analysis using Minitab and Microsoft Excel statistical packages. Fisher pairwise comparison test at 95% significance level was used to determine significant differences between the mean values ($p = 0.05$).

RESULTS

Phytochemical Screening

The result of the preliminary phytochemical screening of leaf extracts of *V. amygdalina* and *D. edulis* is presented in Table 1. Flavonoids, phenolic compounds, tannins, saponins and fixed fats and oils are present in higher concentrations in *V. amygdalina* compared to *D. edulis*. Alkaloids, proteins and phlobatannins are present in high concentrations in *D. edulis* compared to *V. amygdalina*.

Table 1: Phytochemical composition of *V.amygdalina and C. edulis*

Phytochemical group	*V. amygdalina*	*D. edulis*
Alkaloids	+	++
Phenolic compound	+++	+
Tannins	+++	+
Saponins	+++	++
phytosterols	++	++
Fixed fat / oil	++	-
Proteins	-	++
Gums and Mucilage	+	-
Flavonoids	++	+
Phlobatannins	+	++

Key: += slightly present, + += moderately present, +++ =Highly present

Effects of D. edulis and V. amygdalina extracts on body weight changes in Alloxan-induced diabetic rats

As shown in Table 2, significant ($p < 0.05$) loss in weight was observed in the untreated diabetic rats which progressed from day 0 (180.17±6.72) to day 14 (119.83±0.31) of the experiment relative to the normal control. However, significant ($p < 0.05$) improvement in weight gain was noticed in the diabetic rats following the administration of the extracts [*V. amygdalina* (VA), *D. edulis* (DE) and the combined (VA+DE)] in contrast to the alloxan induced diabetic rats. A similar trend was observed in the metformin treated group.

Table 2: Effects of aqueous extracts of *D. edulis* and *V. amygdalina* and their infusion on body weight of alloxan induced diabetes in rats

Group	Duration (days)			
	0	4	8	14
Normal control	181.00±1.41	180.17±3.66[b]	185.6±4.52[b]	188.67±4.71[b]
Negative control (150 mg/kg)	180.17±2.72	152.33±3.68[c]	128.67±2.35[c]	119.83±0.31[b]
Positive control (200 mg/kg)	182.17±3.75	194.33±4.10[b]	202.83±5.17[a]	211.17±4.91[a]
VA (300 mg/kg)	180.50±2.45	198.50±8.92[b]	207.17±7.90[a]	220.00±5.17[a]
DE (300 mg/kg)	183.50±3.99	202.67±7.11[a]	201.83±3.89[a]	205.33±3.67[a]
DE + VA (300 mg/kg)	181.20±2.39	186.20±5.70[b]	203.00±4.35[a]	218.00±6.76[a]

Results are expressed as mean ± SEM. $^{*}p < 0.05$ significantly different compared to negative control, $^{\#}p < 0.05$ significantly different compared to normal control. n = 6. NC = Normal control; Negative control = Alloxan induced diabetic rats; Positive control = Metformin (200mg/kg) treated diabetic rats; DE = Diabetic rats treated with *Dacryodes edulis*; VA = Diabetic rats treated with *Vernonia amygdalina;* DE + VA = Diabetic rats treated with combined extracts of *Dacryodes edulis* and *Vernonia amygdalina.*

Effects of D. edulis and V. amygdalina extracts on some biochemical parameters in alloxan induced-diabetic Rats

Tables 3, 4, 5 and 6 show the effects of the aqueous leaf extracts of *V. amygdalina*, *D. edulis* and their combination as well as metformin were compared on fasting blood glucose (FBG), kidney function, liver function and lipid profile parameters of normal and alloxan-induced diabetic rats. In Table 3, fasting blood glucose concentration increased significantly ($p < 0.05$) in the alloxan induced diabetic rats that were left untreated (negative control) from day when compared to the normal control rats. However, significant ($p < 0.05$) reduction in glucose levels from day 0 to day 14 when the diabetic rats were treated with either the separate extracts, *V. amygdalina* and *D. edulis* or their combination, VA+DE in contrast to the negative control. Similar reduction in glucose concentration was observed in the antidiabetic drug, metformin treated (positive control) rats.

Table 4 shows the significant decrease in the levels of Na, K, Cl and HCO_3while creatinine and urea levels were increase in the untreated diabetic rats compared with the normal control. Treatment with either the extracts and their combination or metformin caused a reversal in these parameters. The total protein was significantly low in the serum of the diabetic group and administration of the extracts / metformin resulted in normal levels of proteins in these groups when compared to the normal control (Table 5). Liver function enzymes (including ALT, AST and ALP) activities and total bilirubin level were significantly high for the untreated diabetic rats. The levels of these parameters returned to normal or close to normal levels as a result of administration of either the extracts (VA, DE, VA+DE) or the antidiabetic drug, metformin (Table 5). The effects of the extracts on lipid profile indices are shown in Table 6. The extracts treated groups as well as the metformin group showed significant reductions in total cholesterol, triacylglycerol, LDL

cholesterol levels while the HDL cholesterol levels increased significantly in contrast to the diabetic control rat values.

Table 3: Effects of Metformin, Aqueous Leaf Extracts of *D. edulis* and *V. amygdalina* on Fasting Blood glucose levels (mg/dL) in plasma of alloxan-induced diabetic rats

Group	Duration (Days)		
	0	7	14
NC	77.83±1.20	79.00 ± 0.68^{d}	77.50 ±1.05^{d}
Negative control (150 mg/kg)	76.17 ± 0.74	98.17± 0.95^{a}	115.85 ± 0.45^{a}
Positive control (200 mg/kg)	77.67 ±2.16	72 .00 ± 0.47^{e}	75.00 ± 0.93^{d}
VA (300 mg/kg)	76.67 ± 1.02	88.00 ±1.71^{c}	83.00 ±1.79^{b}
DE (300 mg/kg)	77.33± 0.40	89.33 ± 0.04^{c}	82.83 ± 0.32^{b}
DE + VA (300 mg/kg)	77.17± 0.58	92.92 ±1.79^{b}	80.00 ± 0.61^{c}

Results are expressed as Mean ± SEM. Means that do not share the same letter in a column are considered significantly different ($p < 0.05$). n = 6. DE = Diabetic rats treated with *Dacryodes edulis*, VA = Diabetic rats treated with *Vernonia amygdalina,* DE + VA = Diabetic rats treated with combined *Dacryodes edulis* and *Vernonia amygdalina.* Glucose (mg/dL)

Table 4: Effects of *D. edulis* and *V. amygdalina* extracts on kidney function indices of alloxan-induced diabetic rats

Parameters	NC	Negative control	Positive control (200 mg/kg)	DE (300 mg/kg)	VA(300 mg/ kg)	DE +VA(30 0 mg/kg)
Creatinine	1.02±0.06	1.98±0.07$^{\#}$	1.06±0.09*	0.93±0.11*	0.90±0.08*	0.76±0.12*
Urea	4.78±0.42	15.4±0.51$^{\#}$	6.1±0.37	5.8±0.54*	4.7±0.49*	4.1±0.53*
Na	145.6±1.90	101.8±4.94$^{\#}$	135.6±2.16*	134.8±2.40*	139.4±0.93*	142.4±2*
K	4.7±0.23	2.58±0.2$^{\#}$	4.10±0.18*	4.02±0.25*	4.1±0.29*	4.76±0.32*
Cl	101.4±1.50	76.6±2.71$^{\#}$	97.4±0.93*	99.80±0.58 *	99.8±0.58*	100.6±0.51*
HCO_3	24.2±0.73	18.0±0.84$^{\#}$	22.8±0.49*	22.20±0.73*	23.6±0.51*	24.0±0.55*

Results are expressed as mean ± SEM. *$p < 0.05$ significantly different compared to negative control, $^{\#}p < 0.05$ significantly different compared to normal control. n = 6. NC = Normal control; Negative control = Alloxan induced diabetic rats; Positive control = Metformin (200mg/kg) treated diabetic rats; DE = Diabetic rats treated with *Dacryodes edulis*; VA = Diabetic rats treated with *Vernonia amygdalina;* DE + VA = Diabetic rats treated with combined extracts of *Dacryodes edulis* and *Vernonia amygdalina.*

Table 5: Effects of *D. edulis* and *V. amygdalina* extracts on liver function indices of alloxan-induced diabetic rats

Parameter	NC	Negative control	Positive control (200mg/kg)	DE (300 mg/kg)	VA (300 mg/kg)	DE +VA (300 mg/kg)
TP (U/L)	76.17±1.96^{a}	46.67±1.30^{c}	65.33±1.36^{b}	63.25±1.06^{b}	65.00±1.59^{b}	76.20±1.10^{a}
ALT(U/L)	9.88±1.20^{c}	46.33±1.90^{a}	16.75±1.58^{b}	18.17±1.08^{b}	18.00±0.97^{b}	9.89±1.51^{c}
AST(U/L)	19.17±1.35^{d}	49.17±1.60^{a}	22.67±1.02^{a}	23.83±1.22^{b}	21.67±0.99^{c}	21.5±1.23^{c}
ALP(U/L)	63.00±3.80^{d}	98.80±2.00^{a}	74.17±4.07^{c}	82.00±2.86^{b}	77.33±3.94^{c}	64.33±4.81^{d}
T.BIL.(U/L)	1.40±0.08^{c}	4.00±0.29^{a}	1.47±0.06^{b}	1.47±0.04^{b}	1.46±0.08^{b}	1.45±0.07^{c}

Results are expressed as mean ± SEM. $^{*}p < 0.05$ significantly different compared to negative control, $^{\#}p < 0.05$ significantly different compared to normal control. n = 6. NC = Normal control; Negative control = Alloxan induced diabetic rats; Positive control = Metformin (200mg/kg) treated diabetic rats; DE = Diabetic rats treated with *Dacryodes edulis*; VA = Diabetic rats treated with *Vernonia amygdalina;* DE + VA = Diabetic rats treated with combined extracts of *Dacryodes edulis* and *Vernonia amygdalina;* TP = Total protein; ALT = Alanine amino transferase; AST = Aspartate amino transferase; ALP = Alkaline phosphatase; Tot.B = Total bilirubin

Table 6: Effects of *D. edulis* and *V. amygdalina* extracts on lipid profile of alloxan-induced diabetic rats

Parameters Group / dose of extract	TC	TAG	HDL-C	LDL-C
NC	119.20 ± 2.22^{b}	85.60 ±1.60^{c}	57.60 ± 1.10^{a}	43.67 ± 2.35^{c}
Negative control (150 mg/kg)	202.20 ± 2.52^{a}	181.00±3.67^{a}	32.80 ± 0.86^{d}	188.50 ± 3.31^{a}
Positive control (200 mg/kg)	120.40 ± 1.63^{b}	87.20± 2.99^{ab}	47.80 ± 2.01^{b}	56.50 ± 3.04^{b}
VA (300 mg/kg)	117.00± 3.00^{c}	81.20 ± 1.71^{d}	46.60 ± 0.66^{b}	55.00 ± 2.14^{b}
DE (300 mg/kg)	120.00 ± 1.14^{ab}	90.40 ± 4.26^{b}	41.80 ± 0.66^{c}	63.67 ± 3.31^{ab}
DE + VA (300 mg/kg)	119.30± 3.360^{b}	84.89± 1.87^{c}	57.80 ± 1.91^{a}	43.17 ± 2.23^{c}

Results are expressed as mean ± SEM. Means that do not share the same letter are significantly different from normal control. $P < 0.05$, (n = 6). NC = Normal control, DE = Diabetic rats treated with *Dacryodes edulis*, VA = Diabetic rats treated with *Vernonia amygdalina,* DE + VA = Diabetic rats treated with combined *Dacryodes edulis* and *Vernonia amygdalina;* TC = Total cholesterol; TAG = Triacylglycerol; HDL-C = High density lipoprotein cholesterol; LDL-C = Low density lipoprotein cholesterol

DISCUSSION

The antidiabetic properties of plants have been linked to their phytochemical constituents (Patel *et al*., 2012). In the present study, the aqueous leaves extracts of *Vernonia amygdalina* (VA) and *Dacryodes edulis* (DE) were subjected to preliminary phytochemical screening,which detected flavonoids, phenols, tannins, saponins, steroids, phytosteroids, alkaloids, phlobatannins, gums and mucilages, as well as fixed oil and fats. Phenolic acids, tannins and saponins were found to be high in *Vernonia amygdalina*. Earlier studies have demonstrated that *Vernonia amygdalina* and *Dacryodes edulis* are rich in alkaloids, phenols, flavonoids, tannins, saponins and steroids, which are in consonance with the findings of

the present study (Omoregie and Okugbo, 2014; Olasunkanmi and Adeniyi, 2017; Ukpabi *et al.,* 2018). Phenols have also been demonstrated to possess varying antidiabetic properties including inhibition of α-amylase as well as the action of sodium glucose transporter 1 (S-GLUT-1) located on the intestinal brush border.

Kinetically, phenols and flavonoids bind covalently to α-amylase and change its activity due to the ability to form quinones or lactones that react with nucleophilic groups on the enzyme molecule (Sim *et al*., 2010). This may contribute immensely to their anti-diabetic potentials. Similarly, saponins, isoflavones and tannins have similar effect on S-GLUT-1 (Tiwari and Rao, 2020). These findings corroborate the capacity of saponins in regulating and maintaining tight glycemic control as earlier reported many decades ago (Chatopadhya, 1998; Yuan *et al.,* 1998). Weight loss is one of the characteristic features of diabetes mellitus. This is attributed to high depletion of body adiposity occasioned by hypoinsulinemia (Arit *et al*., 2007). In this study, the observed reduction in body weight seen in the untreated diabetic rats could be attributed to excessive breakdown of fatty acids and protein due to insufficient or lack of glucose in the cell. Similarly, glycosuria, one of the symptoms of diabetes mellitus results in excessive excretion (loss) of glucose resulting in weight loss in spite of concomitant increase in appetite. On the other hand, the improvement in body weight noticed in the diabetic rats following treatment with the individual extracts (VA, DE) and their combination (VA+DE) as well as the standard anti-diabetic drug, metformin, agrees with previous work of Akpaso *et al.* (2011).

Diabetes mellitus is characterized by extracellular hyperglycemia and intracellular hypoglycemia. In this study, the treatment of the diabetic rats with the extracts or metformin showed varying degree of normoglycemic effects on the treated rats. Extracts of *Vernonia amygdalina, Dacryodes edulis* and their combination restored blood glucose level to normal levels with the combined extract having the highest effect ($p < 0.05$). Similar trend was observed in the metformin treated rats. These results support previous reports by Ukpabi *et al* (2018), Chimaobi *et al* (2019) and Meng *et al* (2017) who found that *Vernonia amygdalina, Dacryodes edulis* and metformin decrease blood glucose level in alloxan and streptozotocin induced diabetic rats, respectively. Metformin is one of the recommended drugs for the treatment of type 2 diabetes because it not only lowers blood glucose level but also result in a decline in plasma fasting insulin level thereby averting hyperinsulinemia (Volle*et al*., 2012). Research shows that metformin also exerts antidiabetic effect by inhibiting complex 1 of the mitochondrial respiratory chain (Owen *et al.,* 2020). On the other hand, *Vernonia amygdalina* has been shown to avert hyperglycemia by suppressing hepatic production of glucose via gluconeogenesis and potentiates glucose oxidation via pentose phosphate pathway by increasing the expression of fructose 1-6 bisphosphate, phosphoenol pyruvate carboxykinase and glucose 6-phosphatase as well as

glucose 6- phosphate dehydrogenase in the liver (Item *et al*., 2014).

Another key characteristic of diabetes mellitus is the underlying dyslipidemia (Oluwole *et al.,* 2013) caused by excessive mobilization of free fatty acids from the adipocytes resulting in elevated levels of triacylglycerol, LDL-C, VLDL-C which are linked to insulin resistance and cardiovascular diseases (Bonama *et al.,* 2017; Nikkila, 2014). In this study, hyperglycemia was accompanied by concomitant increase in TAG, LDL-C and a reduction in HDL-C. Upon treatment with the anti-diabetic drug, metformin and aqueous leaves extracts of *Vernonia amygdalina* and *Dacryodes edulis* ,total cholesterol, TAG and LDL-C levels significantly ($p < 0.05$) reduced while HDL-C level increased. Extracts of *Vernonia amygdalina, Dacryodes edulis* and metformin have been shown to normalize hyperlipidemia (Meng *et al.,* 2017). Ukpabi *et al.* (2018) also reported the hypolipidemic activity of *Vernonia amygdalina.*

Liver adiposity and non-alcoholic liver diseases are implicated in the development of type 2 diabetes mellitus (Xie *et al.,* 2016). Non-alcoholic fatty liver disease (NAFLD) is a pathological condition that is characterized by fat accumulation in the hepatocytes. There is a correlation between plasma angiopoietin like protein- 8 (ANGPTL-8), hepatic fat content, insulin resistance and liver injury. These findings indicate the fact that fat accumulation in the liver may cause liver injury which was similarly observed in the untreated diabetic control in our study. Furthermore, the significantly ($p < 0.05$) reduced level of total protein in diabetic rats could as well be attributed to the high level of glycation of non-enzymatic proteins due to the hyperglycemia state. Equally the levels of ALT, ALP, AST and bilirubin have been reported to significantly increase in alloxan and streptozotocin induced diabetic rats (Akah *et al.,* 2009; Akinola *et al*., 2010). In the present study, metformin, *Vernonia amygdalina* and *Dacryodes edulis* as well as their combined extracts significantly ($p < 0.05$) reduced the once elevated levels of ALT, AST, ALP, total bilirubin as well as increase total protein in the diabetic treated rats ($p < 0.05$).

Elevated urea and creatinine as well as electrolytes imbalance are other key features of chronic hyperglycemia. Excessive break down of proteins to compensate for low cellular energy results in elevated concentrations of urea in diabetic patients. Likewise, in diabetes mellitus there is excessive mobilization of free fatty acids in the muscle, thus leading to elevated synthesis of phosphocreatine and subsequent excessive generation of creatinine. (Allen, 2012). In this study, elevated levels of creatinine and urea observed in the untreated diabetic rats were brought to normal following treatment with metformin and the plant extracts ($p < 0.05$). This agrees with the work of Akah *et al*. (2009). Osmotic diuresis in diabetes mellitus results in delusional hyponatremia, hypochlorinemia and subsequent loss of water and electrolytes due to glycosuria (Briggs *et al*., 2014). The concentrations of these electrolytes, as noticed in this study, were normalized upon treatment with metformin and aqueous crude extracts of *Vernonia*

amygdalina and *Dacryodes edulis* compared to the untreated group ($p < 0.05$).

CONCLUSION

In line with the findings of the present study, *Vernonia amygdalina* and *Dacryodes edulis* may be considered as important component of preventive therapy in the management of diabetes mellitus and its chronic complications. The present study was able to substantiate and compare the efficacy of these plants and the combined extracts with regard to their phytochemical constituents, hypoglycemic, hypolipidemic, renal and hepatoprotective properties against the damaging effect of high circulating plasma glucose and free fatty acids (FFAs) induced by alloxan. Altogether, it's notable that *Vernonia amygdalina* and the combination of *V. amygdalina* and *D. edulis* treatments caused more significant ameliorative changes due to the damaging effect of alloxan in comparison to *D. edulis* treatment.

REFERENCES

Agbor, A., Kuate, D. and Oben, J.E. (2017). Medicinal Plants can be Good Source of Antioxidants: Case study in Cameroon. *Pakistan Journal of Biological Science*, 10: 537-541,

Akah, P., Alemji, J. and Salawu, O. (2018). Effects of *Vernonia amygdalina* on Biochemical and Haematological Parameters in Diabetic Rats. *Asian Journal of Medical Science.*1(3):108-113.

Akah, P. (2009). *Vernonia amygdalina* and Glucose Metabolism in Experimental animals. *Plant Journal of Clinical Biochemistry*, 1: 45-51.

Akah, P. and Okafor, C. (2017). Hypoglycaemic Effect of *Vernonia amygdalina* (Del) in Experimental Rabbits. *Plant Medical Research*, 1: 6-10.

Akinola, G., Rush, J. and Dyck, D. (2010). AMPK Expression and Phosphorylation are Increased in Rodent Muscle After Chronic Leptin Treatment. *American Journal of Physiology, Endocrinology and Metabolism,*284: E648-654.

Akpaso, M., Hoogwerf, B., Gorshow, S., Boyle, P. and Wales D. (2011). Managing Type 2 Diabetes: Going Beyond Glycemic Control. *Journal of Managed Care and Specialty Pharmacy*, 4:12-19.

Alfadda, AA. andSallam, RM. (2019). Reactive Oxygen Species in Health and Disease. *Journal of Biomedicine and Biotechnology*, 4: 1-14.

Allen, P. (2012). Creatine Metabolism and Psychiatric Disorders: Does Creatine Supplementation Have Therapeutic Values? *Review of Neurological; Science*, 36(5): 1442-1462.

Arit, E., Olorunfemi, AE., Amaku, OI. andEdoho, JE. (2007). Studies of some Effects of *Vernonia amygdalina* on rats. *Asian Journal of Biochemistry,* 2: 193-197.

Benzie, IFF. and Strain, JJ. (1996). The Ferric Reducing Ability of Plasma as a Measure of Antioxidant Power; the FRAP Assay. Analytical Biochemistry, 239: 70-76.

Bonama, K., Kanna, G., Sushma, R., Balaraman, S. and Rathod, S. (2017). Antidiabetic and Antihyperlipidemic Effects of Neem Seed Kernel Powder on Alloxan Diabetic Rabbits. *International Journal of Pharmacology*, 29: 162-167

Briggs, J., Singh, I. and Sawaya, B. (2014). Disturbance of Salt Balance, 12th Edition, W. B. Saunders. Philadelphia, **P**p. 3-62.

Chatopadhya, R. (1998). Insulin Resistance and Type 2 Diabetes. *Diabetes,* 61(4): 778-779

Chimaobi, J., Ononamadu, A., Alhassan, K., Cimanga, R., Tona, L., Mesia, K., Musuamba, C., De Bruyne, T., Apers, S., Hernan, N., Miert, V., Pieters, L., Totte, J. and Vlietink, A. (2014). *In vitro* antiplasmodia acivity of extracts and fractions of seven medicinal plants used in the Democratic Republic of Congo. *Journal of Ethnopharmacology,* **93:**27-32.

International Diabetes Federation (IDF) (2017). Diabetes Atlas. Eighth edition

Item, J.A., Godwin, EE., Mariam, A., Mun, FA. andMohd, ZA. (2014). Antioxidant Versus Anti-Diabetic Properties of Leaf from *Vernonia amygdalina* Del. growing in Malaysia. *Food Chemistry,***141**: 3428–3434.

Jecinta, N., Anyam, T., Amom, T. and John, O. (2015). Studies on *Dacryodes edulis* 1: Phytochemical and Medicinal Principles of Raw Seeds. *Journal of Natural Product and Plant Resources*, 5(2):13-19.

Johns, T. (2017). Dietary, Diversity, Global Change and Human Health. Proceedings of the *Symposium Managing Biodiversity in Agricultural Ecosystems*, Nov 8–10, Montreal, Canada, 1–11.

Kazeem, M., Adamson, J. and Ogunwande, I. (2013). Mode of Action of Alpha-amylase and Alpha-glucosidase by aqueous extract of Morinda lucida Benth leaf. *BioMedicalResearch International,* 4: 45-51

Kind, P. and King, EJ. (1954). Colorimetric Method for Determination of Serum Alkaline Phosphatise. *Journal of Clinical Pathology,* 7: 322.

Kolhathar, A. and Ochei, J. (2000). Medical Laboratory Science; Theory and Practice.

Koudou, P., Edou, P., Obame, H., Bassole,. H. and Figueredo, G. (2018). Volatile Components, Antioxidant and Antimicrobial Properties of the Essential Oil of *Dacryodes edulis* G. Don from Gabon. *Journal of Applied Science*, **8:**3532-3535.

Malloy, HT. and Evelyn, KA. (1937). The Determination of Bilirubin with the Photoelectric Colorimetric Method. *Journal of Biology and Chemistry,* 119: 481-490

Maritim, A., Sanders, R. and Watkins, J. (2013). Diabetes, Oxidative Stress, and Antioxidants. *Journal of Biochemistry and Molecular Toxicology*, **17**(1):24-38.

Miguel, L., Mouankie, J., Okemy, N., Mokondjimobe, E., Diatewa, M. and Abena, A. (2017). Effets Psychopharmacologiques de l'huileEssentielle des Fruits de *Dacryodes edulis* (G. Don) H.J Lam. *Annales de I'UniversiteMarienNgouabi*, **15**(5):34-40.

Mitra A., Bhattacharya D, and Roy S. (2017). Dietary Influence on Type 2 Diabetes (NIDDM). *Journal of Human Ecology,* **21:**139-145.

Meng, X., Ma, X., Tian, Y., Jiang, Q., Wang, L., Shi, R., Ding, L. and Pang, G. (2017). Metformin Improves The Glucose Metabolism Via Influencing The Level Of Serum Total Bile Acids in Rats with Streptozotocin Induced Type 2 Diabetes Mellitus. *European Review of Medical and Pharmacological Sciences*, 21: 2232-2237.

Mentreddy, S. (2017). Medicinal Plant Species with Potential Antidiabetic Properties. *Journal of the Science of Food and Agriculture*, 87:743-750.

Nikkila, E. (2014). Plasma Lipid and Lipoprotein Abnormalities in Diabetes In: Diabetes and Heart Diseases R. F. Jarret, (Ed) Elsevier Science Publishers B. V. Amsterdam. The Netherlands, 134-167.

Ogundipe, O., Moody, J., Fakeye, T. and Ladipo, O.B. (2000). Antimicrobial Activity of *Mallotus oppositifolium* extracts. *Africa Journal of Medical Science*, 29:281-283.

Olasunkanmi O. and Adeniyi PO. (2017). Antibacterial and Antioxidant Activities of *Dacryodes edulis* Methanolic Leaf Extract. *Journal of Advances in Medical and Pharmaceutical Sciences*, 14(1): 1-11.

Oluwole, F., Falode, A. and Ogundipe, O. (2013). Antinflammatory Effect of Some Common Nigerian Vegetables. *Nigerian Journal of Physiological Science*, 18(1): 35-38.

Omoregie, S. and Okugbo, OT. (2014). *In vitro* Antioxidant Activity and Phytochemical Screening of Methanol Extracts of *Ficus capensis* and *Dacryodes edulis* Leaf *Journal of Pharmacy and Bioresources*11(2): 66-75.

Osinubi, AAA. (2006). Effects of and Chlorpropamide on Blood Glucose. *Science*, 16: 115-119.

Owen, M., Doran, E. and Halestrap, A. (2020). Evidence that Metformin Exerts Its Antidiabetic Effects Through Through Inhibition of Complex 1 of the Mitochondrial Respiratory Chain. *Biochemistry Journal*, 348: 607-614.

Paloma, M., Paula, M., Luiz-Alberto, S., Pérola, M. and Dâmaris, S. (2012). *Journal of Pharmacology and Pharmaceutical Science*, 15(1):141-183.

Patel, DK., Kumar, R., Laloo, D. and Hemalathan, S. (2012). Diabetes Mellitus: an Overview on Its Pharmacological Aspects and Reported Medicinal Plants Having Antidiabetic Activity. *Asian Journal Science*, 2: 411-420.

Sim, M., Sudheer, AR. and Menon, VP. (2010). Ferulic Acid: Therapeutic Potential Through Its Antioxidant

Property. *Journal of Clinical Biochemistry and Nutrition*, 40:92–100.

Titz, N. (1995). Principles of Clinical Chemistry. 230-240.

Tiwari, AK. and Rao, JM. (2020). Diabetes Mellitus and Multiple Therapeutic Approachs of Phytochemicals; Present Status and Future Prospects. *Current science*, 83 (1): 30-37.

Ukpabi, C., Nwankwo, J., Eshie, T. and Isu, J. (2018). Effect of Aqueous Extract of *Vernonia amygdalina* on Blood Glucose and Lipid Profile of Alloxan-Induced Diabetic Rats. *Joiurnal of Pharmacy and Biological Sciences,*13(5): 13-17.

Vollet, B., Gugas, B., Garcia, N., Leclerc, J., Foretz, M. and Andreelli, F. (2012). Cellular and Molecular Mechanism of Metformin: An Overview. *Clinicla Science*, 122: 253-270

Yuan, R. Rashid, C., Breitmaier, E. and Radloff, MA. (1998). Alloxantin dehydrate: Organic Syntheses. *Biochemistry*, 4: 25-33.

Xie, JH., Liu. Q., Yang, Y., Liu, Zl., Hu, SH. and Zhou, XR. (2016). Correlation of Liver Enzymes with Diabetes and Pre diabetes in Middle Age Rural Population in China. *Journal of Science*, **36**(1): 53-58.