

ORIGINAL CONTRIBUTION

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Comparative studies on the hypoglycemic and antioxidant activities of *Vernonia amygdalina* delile and *Baccharoides tenoreana* olive in alloxan-induced hyperglycemic rats

Samuel Chijioke Attama¹, Patrick Emeka Aba^{1*}, Chinwe Uchechukwu Asuzu² and Isaac Uzoma Asuzu¹

Abstract

Background: *Vernonia amygdalina* is a bitter culinary vegetable known to possess anti-diabetic principle. *Baccharoides tenoreana* belonging to the same species as *V. amygdalina*, is also used in cooking soup and it is comparatively non-bitter. However, its glycemic properties have not been studied. This study becomes imperative to find out if *B. tenoreana* possesses hypoglycemic and antioxidant properties and how it compares with *V. amygdalina*. Should *B. tenoreana* be better than *V. amygdalina*, it should be preferable since it is non-bitter and can comfortably be taken compared to *V. amygdalina*. This study investigated the comparative hypoglycemic and antioxidant potentials of *V. amygdalina* (VA) and *B. tenoreana* (BT).

Methods: Thirty male Albino wistar rats assigned into six groups of five rats per group were used for the study. Diabetes was induced in groups B-F rats by a single intraperitoneal injection of alloxan monohydrate at 160 mg/kg. Groups C-E rats were treated with VA (200 mg/kg), BT (200 mg/kg) and combination of VA & BT (100 mg/kg each) respectively. Group F rats were administered glibenclamide (2 mg/kg) whereas groups A and B rats were given distilled water. All treatments were through the oral administration, once daily for 21 consecutive days. Fasting blood glucose (FBG) levels were determined after 1 h, 3 h, 6 h, 24 h, 7 days, 14 days and 21 days while lipid profile, in vivo antioxidant and pancreatic histomorphology were assessed on day 21 post-treatment.

Results: The VA-treated rats recorded marginally reduced FBG, malondialdehyde and low-density lipoprotein levels when compared to the counterpart treated with BT. The high-density lipoprotein values were significantly higher in VA-treated rats than in BT-treated rats. The histomorphology of the pancreas of VA-treated rats expressed more islet cells compared to the counterpart treated with VT.

Conclusion: Both VA and BT exhibited hypoglycemic and antioxidant activities with varying potencies.

Keywords: Antioxidant, Comparative study, Hypoglycemia, *Vernonia amygdalina*, *Baccharoides tenoreana*

* Correspondence: patrick.aba@unn.edu.ng

¹Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Enugu state, Nigeria
Full list of author information is available at the end of the article

Introduction

V. amygdalina Del is popularly called bitter leaf in English language due to its bitter nature. The “sweet” variant of bitter leaf is called *B. tenoreana* Oliv and it is a native of Africa [1]. It is less bitter in taste when compared to *V. amygdalina* and can be chewed raw as vegetable or sliced for direct use in soup without prior squeeze washing. These culinary shrubs share a lot of botanical, nutritional and medicinal features in common and are distributed across tropical African countries where they are used in preparing delicious bitter leaf soup. They belong to the family Asteraceae or Compositae [2]. In Igbo, southeastern Nigeria, they are known as “onugbu” while Hausas, northern Nigerians call it “shuwakaa”. In Yoruba, western part of Nigeria, it is referred to as “Ewuro” [3].

Researchers have reported on various medicinal properties of *V. amygdalina*. It is used for treating gastrointestinal disorders, malaria and diabetes in folkloric medicine [4]. The hypolipidemic, hepatoprotective, hypoglycemic, and antioxidant activities of *V. amygdalina* have earlier been reported [5–7]. The antimicrobial activities of *B. tenoreana*, have been extensively studied [8]. However, the glycemic effect of *B. tenoreana* has not been fully investigated [9].

Diabetes mellitus is a metabolic and a debilitating endocrine disorder occasioned by hormonal dysregulation. Features of diabetes mellitus include hyperglycemia, dyslipidemia, polyuria and polydipsia consequent upon lack of insulin or non-sensitivity of cells to available insulin [10]. Based on aetiology, diabetes mellitus is traditionally classified as insulin-dependent diabetes mellitus (IDDM) or type 1 and Non-insulin dependent diabetes mellitus (NIDDM) or type 2 [11, 12]. Experimentally, diabetes is induced by the use of alloxan monohydrate or streptozotocin. These compounds generate free radicals which are known to destroy the beta cells of the islet of Langerhans that are responsible for insulin production. Lack of insulin production therefore occasions hyperglycemia [13]. Oxidative stress occurs when there is imbalance between the productions of free radicals and the body's ability to fight them off [14]. Too high levels of oxidative stress maybe a precursor to multiple diseases in which diabetes is one of them [15].

Conventionally, management of diabetes mellitus involves modifications of life style such as avoiding smoking, engaging in exercise and regulation of diet [16]. The uses of orthodox anti-diabetic drugs and medicinal hypoglycemic herbs have also been advocated as potent means of managing diabetes mellitus [17]. However, the complicated mode of intake and deleterious side effects associated with orthodox anti-diabetic drugs have discredited and discouraged their use [18]. The use of medicinal herbs or plants has been advocated due to their

wide safety margin [19]. Vegetables with hypoglycemic potentials are gaining attention by the day.

There is dearth of researched information on the comparative strengths of hypoglycemic and antioxidant activities of the two variants- *V. amygdalina* and *B. tenoreana*. This study, therefore aims to investigate and compare the hypoglycemic and antioxidant potentials of these two culinary vegetables and any beneficial therapeutic effect of combining both of them.

Materials and methods

Chemicals and reagents

Chemicals and reagents used in this study includes: Alloxan monohydrate (Sigma Aldrich, UK); Superoxide dismutase (SOD) kit (Merk KGaA Darmstadt, Germany); Catalase kit (Hardy diagnostics, Santa Maria, California); Malondialdehyde kit (Eagle Biosciences, Armherst, New Hampshire, Germany); Glutathione kit (Randox Laboratories, Crumlin, County Austrim, UK) and Glibenclamide (Hovid Hong Kong). All chemicals are of analytical grades.

Plant collection and extraction

V. amygdalina and *B. tenoreana* leaves were purchased during rainy season (July, 2020) from Ogige market, Nsukka and identified at Bioresources Development and Conservation Programme (BDPC), Aku road, Nsukka, Enugu state, Nigeria. The Voucher Specimens (*V. amygdalina*: INTERCEDD/041 *B. tenoreana*: INTERCEDD/2619) were kept at the herbarium. Nsukka is located between latitude 6° 5' and 6° 24' north and longitude 7° 23' and 7° 45' east in the south-east geopolitical zone [20]. The leaves were air-dried and pulverized into powder after removal of any foreign matter. Cold maceration method of extraction using distilled water was used. The pulverized materials were soaked in a distilled water for 48 h with intermittent shaking after every 2 h. Thereafter, they were filtered using NO1 Whiteman filter paper to obtain the filtrate (aqueous extract). The aqueous extracts were lyophilized and placed in airtight amber colored sample bottles and stored in refrigerator at 4 °C pending use.

The aqueous leave extracts of VA and BT gave a yield of 12.4 g (6.20% w/w) and 12.7 g (6.35% w/w) respectively.

Animals

Thirty (30) male Albino wistar rats aged between 7 and 9 weeks (160–190 g) were used for the study according to Aba and Edeh [15]. They were obtained from the animal house of the Department of Veterinary Physiology and Pharmacology, University of Nigeria Nsukka. The animals were acclimatized for two weeks during which they were kept in a stainless wire mesh cage and fed

with Vital® feed grower and clean water ad libitum. The experimental design used in this study was approved by the Faculty of Veterinary Medicine Institutional Animal Care and Use Committee with the approval number: FVM-UNN-IACUC-2020-0266.

Experimental design

The thirty (30) male Albino wistar rats were assigned into six (6) groups of five (5) rats per group. Diabetes was induced in rats assigned to groups B-F by intraperitoneal injection of 160 mg/kg of alloxan monohydrate following 16 h fasting while rats in group A served as normal control. Upon establishment of diabetes (FBG > 126 mg/dl), the rats were treated as follows (Table 1):

All treatments were made daily for 21 days through the oral route. The fasting blood glucose (FBG) levels were assessed every 1 h, 3 h, 6 h, 24 h, 7 days, 14 days and 21 days using Accu-Chek glucometer. At the end of 21 days duration of the study, samples were collected for analyses.

Sample collections

The whole blood sample for assessment of FBG was collected by tail snip while blood samples for serum biochemical (Total cholesterol, triacylglycerol, high density lipoprotein, superoxide dismutase, reduced glutathione, catalase and malondialdehyde) determinations were via the retrobulbar plexus of the medial canthus of the eye. The blood samples were centrifuged at 10,000 g for 10 min. Thereafter, the sera for determination of serum biochemical parameters were decanted. The rats were humanely euthanized using chloroform and the pancreas harvested for histopathology studies.

Determination of serum biochemical parameters

The serum cholesterol was determined by cholesterol oxidase-peroxidase method [16]. The serum high density lipoprotein (HDL) was determined by the dextran sulphate-magnesium (II) precipitation method [21]. The triacylglycerol (TAG) values were determined by phosphate oxidase enzymatic method [22]. The very low-density lipoprotein (VLDL) was calculated by dividing the triacylglycerol by 5 while Friedwald formula was

used to calculate the value of low-density lipoprotein (LDL) [23].

Superoxide dismutase activity was assayed by the method of Kakkar et al [24]. The activity of catalase was assayed by the method of Sinha [25]. The reduced glutathione level was determined by the method of Beutler and Kelley [26]. Lipid peroxidation was estimated by measuring spectrophotometrically, the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin et al [27].

The histopathology of the pancreas was processed according to Drury et al [28].

Data analysis

The data generated were analyzed with One-way Analysis of Variance (ANOVA) using SPSS version 20. The variant means were separated using Duncan's Multiple Range test. $P < 0.05$ was accepted as being significant. Results were expressed as Mean \pm Standard Error of the Mean (SEM) and presented in tables.

Results

Effect of aqueous leaf extract of *V. amygdalina* and *B. tenoreana* on the fasting blood glucose (FBG) levels of alloxan-induced diabetic rats

The fasting blood glucose (FBG) of rats treated with the aqueous extract of *V. amygdalina* (VA) was significantly ($p < 0.05$) lower than the diabetic untreated rats and compared very well with that of the normal control and those treated with glibenclamide, a known anti-diabetic drug on day 21 post treatment. The hypoglycemic activities of VA and *B. tenoreana* (BT) were comparable from 3 h post treatment (PT) up to 14 days PT but the FBG of the VA-treated rats were significantly ($p < 0.05$) lower than that of the VA & BT-treated rats (group E) on day 21 PT (Table 2).

Percentage reduction in FBG levels of alloxan-induced diabetic rats treated with aqueous leaf extract of *V. amygdalina* and *B. tenoreana*

Results also indicated consistent reductions in FBG levels of VA and BT-treated rats from 1 h to 21 days PT with VA, BT and glibenclamide-treated rats recording 65.80%, 65.10% and 66.54% respectively (Fig. 1).

Effect of the aqueous leaf extract of *V. amygdalina* and *B. tenoreana* on the lipid panel of alloxan-induced rats

The lipid panel results showed that the high-density lipoprotein (HDL) values of the VA and glibenclamide-treated rats were comparable and significantly ($p < 0.05$) higher than that of the diabetic untreated group. Significant ($p < 0.05$) decreases in low density lipoprotein (LDL) values of all the extract-treated groups when compared to the diabetic untreated group were recorded.

Table 1 Showing the treatment regimen for various groups

Group	Treatment
A	Non-diabetic + 10 ml/kg distilled water (Normal control)
B	Diabetic + 10 ml/kg distilled water (Negative control)
C	Diabetic + 200 mg/kg <i>V. amygdalina</i>
D	Diabetic + 200 mg/kg <i>B. tenoreana</i>
E	Diabetic + 100 mg/kg <i>V. amygdalina</i> & 100 mg/kg <i>B. tenoreana</i>
F	Diabetic + 2 mg/kg Glibenclamide (Standard control)

Table 2 Effect of aqueous leaf extract of *V. amygdalina* and *B. tenoreana* on the fasting blood glucose (FBG) levels of alloxan-induced diabetic rats

Group	Fasting blood glucose (FBG) levels (mg/kg)									
	PI	0 h PT	1 h PT	3 h PT	6 h PT	24 h PT	7 D PT	14 D PT	21 D PT	
A	75.60 ± 0.51 ^a	75.80 ± 0.37 ^a	76.00 ± 0.63 ^a	75.20 ± 0.97 ^a	75.20 ± 0.73 ^a	75.20 ± 0.97 ^a	75.60 ± 0.25 ^a	74.60 ± 0.68 ^a	74.40 ± 0.51 ^a	
B	79.80 ± 0.80 ^a	219.40 ± 1.08 ^d	219.60 ± 0.87 ^d	219.00 ± 0.45 ^d	216.40 ± 0.81 ^d	210.60 ± 1.08 ^d	209.80 ± 4.18 ^d	192.60 ± 2.36 ^d	180.00 ± 2.23 ^d	
C	75.80 ± 0.97 ^a	224.00 ± 1.58 ^d	199.80 ± 1.39 ^c	176.40 ± 1.03 ^c	159.40 ± 0.87 ^c	124.00 ± 1.48 ^c	118.20 ± 0.97 ^c	79.10 ± 1.07 ^{ab}	78.80 ± 1.59 ^{ab}	
D	81.00 ± 0.89 ^a	218.20 ± 1.88 ^d	201.40 ± 2.18 ^d	180.40 ± 1.03 ^c	161.20 ± 1.07 ^c	126.60 ± 1.81 ^c	121.00 ± 1.34 ^c	79.20 ± 0.81 ^{ab}	81.60 ± 2.01 ^{bc}	
E	77.80 ± 0.63 ^a	216.00 ± 1.58 ^d	200.80 ± 1.59 ^c	182.40 ± 1.17 ^c	161.00 ± 0.89 ^c	132.80 ± 1.16 ^c	124.20 ± 1.16 ^c	110.40 ± 0.75 ^c	85.60 ± 0.93 ^c	
F	81.80 ± 0.58 ^a	221.60 ± 0.93 ^d	125.00 ± 1.84 ^b	112.00 ± 0.95 ^b	106.00 ± 1.30 ^b	78.80 ± 1.24 ^{ab}	78.80 ± 2.01 ^{ab}	78.20 ± 0.58 ^{ab}	74.20 ± 0.58 ^a	

Different superscripts a, b, c and d along the same column (across groups) indicate significant difference at $P < 0.05$.

PI Pre-induction, PT Post-treatment, h hour (s), D Days

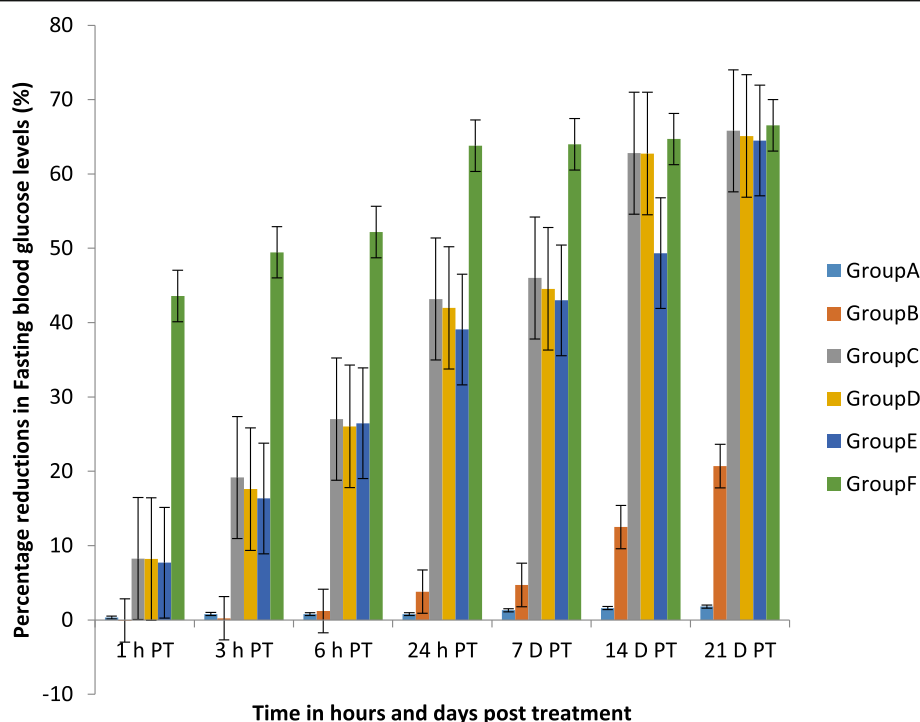


Fig. 1 Percentage reduction in FBG levels of alloxan-induced diabetic rats treated with aqueous leaf extract of *V. amygdalina* and *B. tenoreana*. PT Post-treatment, h hour (s), D Day

Triacylglycerol and total cholesterol levels for the extract-treated rats varied marginally ($p > 0.05$) when compared to that of the diabetic untreated group (Table 3).

Effect of the aqueous leaf extract of *V. amygdalina* and *B. tenoreana* on oxidative stress markers of alloxan-induced diabetic rats

The results of the effects of VA and BT aqueous extracts on oxidative stress markers indicated significant ($p < 0.05$) reductions in the malondialdehyde values of the rats treated with VA and glibenclamide compared to the diabetic untreated group. The catalase activities and the reduced glutathione values of all the extract and glibenclamide-treated groups were significantly

($p < 0.05$) higher than those of the diabetic untreated group (Table 4).

Photomicrograph of the pancreas of diabetic rats treated with aqueous leaf extracts of *V. amygdalina* and *B. tenoreana*

The pancreas photomicrograph showed that all the treated groups demonstrated more islet cells compared to the diabetic untreated group which expressed scanty islet cells. However, islet cells were more preponderant in the groups treated with VA and glibenclamide when compared to BT-treated rats. The islet cells in the glibenclamide-treated rats compared very well with those of the normal control group (Fig. 2).

Table 3 Effect of the aqueous leaf extract of *V. amygdalina* and *B. tenoreana* on the lipid panel of alloxan-induced rats

Group	CHOL (mmol/L)	TAG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)
A	4.93 ± 0.17 ^a	1.57 ± 0.06 ^a	2.91 ± 0.08 ^c	2.32 ± 0.84 ^a	0.31 ± 0.01 ^a
B	5.80 ± 0.10 ^b	1.96 ± 0.38 ^a	1.30 ± 0.1 ^a	2.91 ± 0.08 ^c	0.39 ± 0.08 ^a
C	5.47 ± 0.12 ^b	1.70 ± 0.16 ^a	2.65 ± 0.02 ^b	2.41 ± 0.04 ^a	0.34 ± 0.03 ^a
D	5.60 ± 0.15 ^b	1.73 ± 0.12 ^a	2.41 ± 0.04 ^a	2.65 ± 0.02 ^b	0.35 ± 0.02 ^a
E	5.53 ± 0.22 ^{ab}	1.95 ± 0.09 ^a	2.36 ± 0.04 ^a	2.68 ± 0.07 ^b	0.39 ± 0.02 ^a
F	4.97 ± 0.13 ^a	1.70 ± 0.16 ^a	2.68 ± 0.07 ^b	2.36 ± 0.04 ^a	0.34 ± 0.03 ^a

Superscripts a, b and c indicate significant difference at $P < 0.05$ down the columns (across the groups).

CHOL Cholesterol, TAG Triacylglycerol, HDL High Density Lipoproteins, LDL Low Density Lipoproteins

Table 4 Effect of the aqueous leaf extract of *V. amygdalina* and *B. tenoreana* on oxidative stress markers of alloxan-induced diabetic rats

Group	Catalase (μM)	SOD (μM)	MDA (mg/dl)	GSH (mg/dl)
A	8.89 \pm 0.20 ^c	0.11 \pm 0.01 ^a	0.43 \pm 0.16 ^a	0.23 \pm 0.07 ^b
B	4.67 \pm 0.24 ^a	0.09 \pm 0.01 ^a	1.78 \pm 0.15 ^b	0.03 \pm 0.00 ^a
C	6.37 \pm 0.30 ^b	0.10 \pm 0.01 ^a	0.85 \pm 0.18 ^a	0.14 \pm 0.01 ^b
D	6.04 \pm 0.46 ^b	0.10 \pm 0.01 ^a	1.55 \pm 0.21 ^b	0.14 \pm 0.23 ^b
E	6.04 \pm 0.46 ^b	0.09 \pm 0.00 ^a	1.69 \pm 0.22 ^b	0.13 \pm 0.01 ^b
F	8.34 \pm 0.27 ^c	0.11 \pm 0.00 ^a	0.73 \pm 0.02 ^a	0.20 \pm 0.07 ^b

Superscripts a, b and c indicate significant difference at $P < 0.05$ down the column (across the groups).

SOD Superoxide Dismutase, MDA Malondialdehyde, GSH Reduced Glutathione

Discussion

This study investigated the comparative strengths of the aqueous extracts of *V. amygdalina* (VA) Del and *B. tenoreana* (BT) Olive on their hypoglycemic and antioxidant potentials in alloxan-induced diabetic rats.

The fasting blood glucose (FBG) levels of the rats administered alloxan monohydrate increased significantly compared to the normal control rats.

Hyperglycemia is usually seen following administration of diabetogenic compounds such as alloxan monohydrate. Alloxan monohydrate is capable of generating free radicals which destroy beta cells of the islets of Langerhans with attendant reduction in insulin production; consequently, leading to a spike in blood glucose levels [29]. Rats treated with the extracts (VA and BT) showed significant reductions in their FBG values compared to the diabetic untreated counterpart from 1 h to 21 days post treatment. Previous studies had also demonstrated hypoglycemic potentials of VA [30]. However, the VA-treated rats showed a better hypoglycemic potential compared to VA & BT-treated rats on day 21 post treatment. This observation could be attributed to probably an anti-nutritive factor which has been reported in VA [31]. VA contains antinutritional factor such as alkaloids, saponins, tannins, steroids, glucosides (Vernoniosides), flavonoids, glycosides (Vernomin) and sterols which cause its bitterness [32, 33]. This anti-nutritive principle is capable of reducing the potency when both extracts are combined as seen in group E rats.

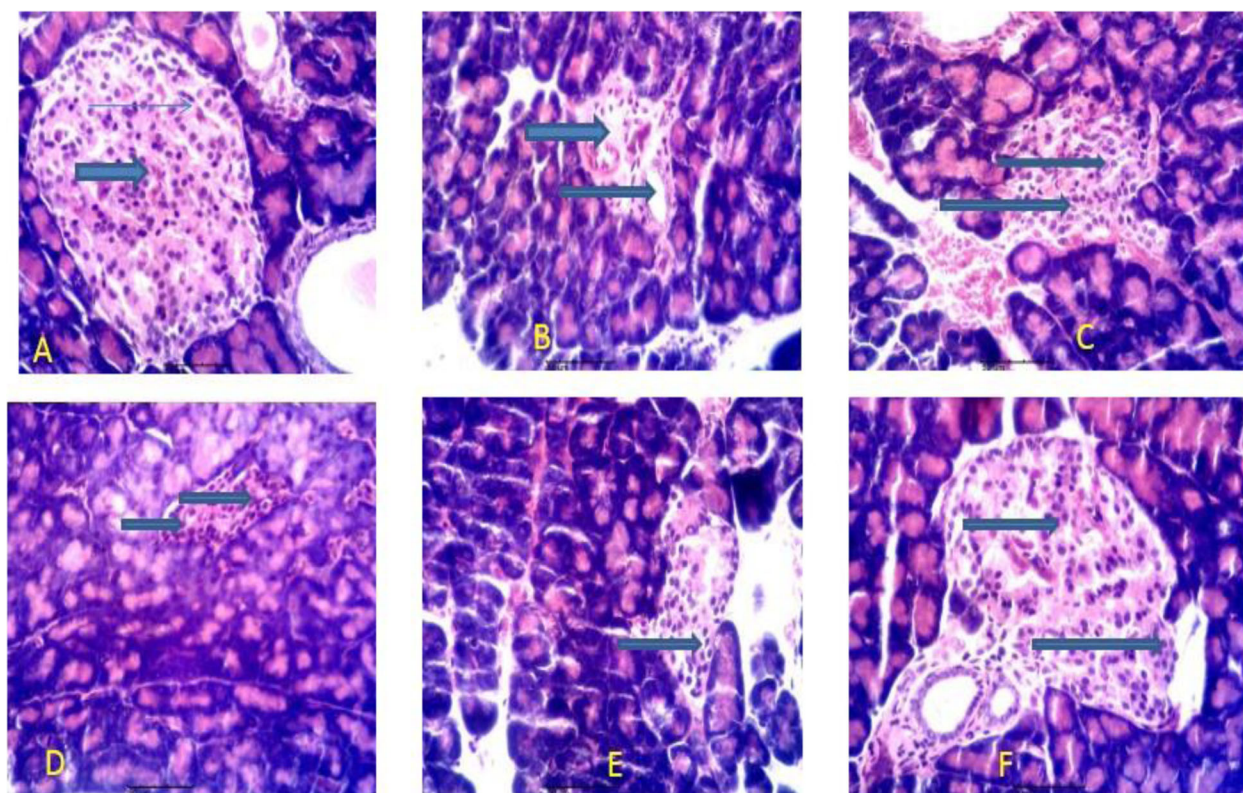


Fig. 2 Photomicrograph of pancreas of: A = Group A rats (Normal control) showing well populated islet cells (arrows); B = Group B rats (Diabetic untreated) showing severely depleted islet cells. Arrows show areas devoid of cells. C = Group C rats (Diabetic + 200 mg/kg of *Vernonia amygdalina*) showing evidence of adequately populated islet cells (arrows). D = Group D rats (Diabetic + 200 mg/kg of *Vernonia amygdalina*) showing relatively moderate population of islet (arrows). E = Group E rats (Diabetic + 200 mg/kg of *Vernonia amygdalina*) showing relatively moderate population of islet (arrows). F = Group F rats (Diabetic + 2 mg/kg glibenclamide) showing well populated islet cells (arrows). H&Ex 400

The results of the lipid panel indicate significant changes in the high-density lipoprotein (HDL) and low-density lipoprotein (LDL) only. Rats treated with VA demonstrated significantly higher HDL values and lower LDL values compared to the negative control group and the BT-treated rats. This again, demonstrates an advantageous activity of VA over BT in the management of diabetes. The HDL cholesterol is usually referred to as “good cholesterol” because of its anti-atherogenic potentials. It is also involved in the reverse transport of cholesterol where it carries cholesterol from the peripheral tissues to the liver for excretion into the bile [34]. Conversely, LDL cholesterol transport cholesterol from the liver to the peripheral tissues thereby predisposing to atherosclerosis. Increased LDL and decreased HDL are common features of diabetes mellitus [35]. The results of this study on amelioration of dyslipidemia by the extract of VA are in agreement with the earlier submissions of Adaramoye et al [5].

Significant increases in the catalase activities and glutathione levels of the extract-treated group compared to those of the diabetic untreated group bear eloquent testimony to the in vivo antioxidant potentials of both VA and BT aqueous extracts. Catalase is an in vivo antioxidant saddled with the responsibility of decomposing hydrogen peroxide into water and molecular oxygen [7]. Deficiency of catalase has been reported in diabetic conditions [36]. Glutathione, on the other hand is a master antioxidant that takes part in so many cellular reactions such as drug detoxification, antioxidant defense of cells and even in cellular signaling [37]. Diabetes is one of the pathological conditions in which reduced glutathione deficiency is a feature [37]. This observation of VA improving in vivo antioxidant activities in diabetics had earlier been reported [7]. From these results, VA-treated rats appeared to be marginally better than those of the BT-treated group.

The pancreatic islet cells of the diabetic untreated group appeared scanty indicating depletion of the cells. This, most probably is as a result of the effects of alloxan monohydrate on the pancreas. Several studies have reported that alloxan monohydrate generates free radicals that cause degeneration and subsequent necrosis of pancreatic beta cells of the islets of Langerhans [38, 39]. However, both the extract-treated and glibenclamide-treated rats demonstrated relatively more numbers of pancreatic islet cells compared to the diabetic untreated group. This could mean that the extracts and glibenclamide facilitated repopulation of these alloxanized pancreases, following treatment.

Conclusion

In conclusion, aqueous extracts of *V. amygdalina* and *B. tenoreana* have demonstrated potent hypoglycemic and antioxidant properties. *V. amygdalina* however, showed a better hypoglycemic effect when compared with *B. tenoreana* alone and when combined with *B. tenoreana*. It is therefore recommended that *V. amygdalina* or *B. tenoreana* be used alone but not in combination because combining them has no added advantage.

Mechanisms of action of the extracts as well as the active ingredients present in the extracts that produced hypoglycemic, antioxidant and antilipidemic effects observed in this study were not determined. Therefore, further studies to determine their mechanisms of action and active ingredients that produced these effects are necessary.

Abbreviations

VA: *Vernonia amygdalina*; BT: *Baccharoides tenoreana*; FBG: Fasting blood glucose; NIDDM: Non-insulin dependent diabetes mellitus; IDDM: Insulin dependent diabetes mellitus; BDCP: Bioresources development and conservation programme; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; TAG: triacylglycerol; Chol: Cholesterol; MDA: malondialdehyde; SOD: Superoxide dismutase; GSH: Reduced glutathione; PI: Post induction; PT: Post treatment; H: Hour; D: Day

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Authors' contributions

CU collected and extracted the plant material. SC carried out the experimentation while PE analyzed the data and drafted the manuscript. IU performed the literature review and supervised the entire process. The authors read and approved the manuscript.

Authors' information

¹Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Enugu state, Nigeria. ²Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu state, Nigeria.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval

All applicable international, national and or institutional guidelines for the care and use of animals were followed. The experimental design used in this study was approved by the Faculty of Veterinary Medicine Institutional Animal Care and Use Committee with the approval number: FVM-UNN-IACUC-2020-0266.

Consent for publication

Not applicable.

Competing interests

Authors declare no competing interest.

Author details

¹Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Enugu state, Nigeria. ²Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu state, Nigeria.

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