Australian Journal of Basic and Applied Sciences, 4(5): 711-716, 2010
ISSN 1991-8178

Extract of *Vernonia Amygdalina* Del. (African Bitter Leaf) Can Reverse Pancreatic Cellular Lesion after Alloxan Damage in the Rat

[1]Item Justin Atangwho, [1]Patrick Ekong Ebong, [1]Godwin Eneji Egbung and
[2]Augustine Uchechukwu Obi

[1]Departments of Biochemistry and [2]Human Anatomy, College of Medical Sciences, University of Calabar, P. M. B. 1115, Calabar, Nigeria.

Abstract: *V. amygdalina* Del. is used as a traditional treatment for diabetic. In this study, administration of the leaf extract (400mg/kg b.w.) via gastric intubation to alloxanised rats for 14days reduced significantly ($p<0.01$) blood and serum glucose respectively by 25.91% and 41.70% relative to the diabetic control. Serum alpha-amylase activity raised significantly ($p<0.01$) in untreated diabetic rats decreased significantly by about 15.38% after extract treatment. Histomorphological examination of pancreatic tissues showed evidence of cellular regeneration of the hitherto destroyed beta cells. Phytochemical screening of the plant parts revealed heavy presence of polyphenols and a moderate presence of alkaloids, flavoniods, saponins, and steroids.*V.amygdalina* extract may mediate its antihyperglycemic action via regeneration of pancreatic beta cells. Hence a potential source for discovery of orally active agent(s) for future diabetes therapy.

Key Words: *Vernonia amygdalina* Del., beta cell regeneration, blood and serum glucose, alpha-amylase activity, phytochemical screening.

INTRODUCTION

The plant, *Vernonia amygdalina* Del. (Compositae) is widely distributed in the west coast of Africa where it grows wild and as a domestic browse plant (Farombi, 2003). It is commonly known as "bitter leaf" in Nigeria because the leaves and the stem have an astringent bitter taste. In Nigeria, it is a major vegetable of the celebrated "bitter leaf soup" and has a long history of use in folk medicine Biser, (1998) particularly among the people of sub-Saharan Africa.

Among the people of Southern Nigeria, *V.amygdalina* has a high reputation for use in the traditional management of diabetes mellitus. In an ethno botanical survey which identified and documented 22 plants of the South Western Nigeria used by traditional healers in the management of diabetes mellitus, *V.amygdalina* came second only to *Cassia alata* as the most frequently used Abo and Adediwora, (2000). Scientific studies have also reported/confirmed it's antihyperglycemic (Akah *et al.,* 2004; Nimenbo-Uadia, 2003) and hypoglycemic (Gyang *et al.,* 2004) action in diabetic and non-diabetic rats respectively. The aqueous leaf extract have been shown to posses antihyperlipidemic and hypolipidemic effect respectively on diabetic and non-diabetic rats (Atangwho *et al.,* 2007a). Its protective role on the kidneys (Atangwho *et al.,* 2007b) and livers (Atangwho *et al.,* 2007c) of alloxan diabetic rats has additionally been investigated and results reported.

However, the actual mode and/or mechanism through which antihyperglycemic and hypoglycemic effect of the plant extract is mediated remains a mere speculation. In general, plants are known to exert their beneficial effect on diabetes via various mechanisms - manipulating carbohydrate/lipid metabolism in the liver(via key enzymes), influence on the beta-cell integrity and insulin releasing activity,aldose reductase activity and antioxidant defense system manipulation and glucose uptake and utilization Tiwari and Rao, (2002). Additionally, some plants posses phytochemicals that do not only interfere with carbohydrate digestion and absorption, but which also have insulin-like action and/or that may inhibit insulinase activity (Jelodar *et al.,* 2005).

The presence study was therefore undertaken to confirm the antidiabetic properties of *V.amygdalina* and to investigate at least in part, the mechanism responsible for the reported antihyperglycemic action. For the former, blood and serum glucose levels of diabetic rats after treatment with the plant extract were determined.

Corresponding Author: Item Justin Atangwho, Departments of Biochemistry and [2]Human Anatomy, College of Medical Sciences, University of Calabar, P. M. B. 1115, Calabar, Nigeria.

Whereas biochemical and histological markers of pancreatic cells integrity of the treated diabetic rats as well as the phytochemical composition of the plant extracts determined for the later purpose.

MATERIALS AND METHODS

Plant Materials:

Vernonia amygdalina Del. leaves were obtained from the Endocrine Research Farm (ERF) of the University of Calabar, Calabar. Specimen of the leaves was authenticated in Botany department of the same university and voucher specimen deposited in the herbarium.

Preparation of Extracts:

The leaves were rinsed with distilled water then spread under shade until they were dried. The dried leaves were pulverized into powder and a hundred grammes (100g) of it was soaked, and then agitated in 600ml of ethanol (98.67%OH) for 10 min with an electric blender. This suspension was stored in the cool (4^0C) for 24h after which it was filtered with a chess cloth and the filtrate concentrated *in vacuo* (rotary evaporator) to $1/10^{th}$ of the original volume at 40^0C. The concentrate was then allowed open in a water bath at 37^0C to evaporate to dryness forming the crude extract which was reconstituted prior to animal treatment.

Animals Used:

Twenty-one albino Wistar rats (120-160g) of both sexes obtained from the department of Anatomy, University of Calabar, Calabar were used for the study. The animals were acclimatized for one week in Biochemistry departmental animal house, after which they were weighed and housed in polycarbonated cages (North Kent,co.Ltd) under standard conditions of temperature (28 ± 2^0C) and relative humidity ($50 \pm 5\%$) and 12h light/dark cycle. The animal house was adequately ventilated and animals maintained on commercial rat chow and tap water *ad libitum*.

Experimental Design:

The animals were distributed into 3 groups of 7 rats each and treated thus:

Group 1-normal control, received 0.2ml distilled water (placebo)

Group 2-diabetic control, received 0.2ml distilled water (placebo)

Group 3-diabetic test group, received 400mg/kg BW of extract.

The extract was administered twice per day by gastric intubation in a 12h cycle (7.00am-7.00pm) for 14days.

Induction of Experimental Diabetes:

Diabetes mellitus was induced in groups 2 and 3 animals by intraperitoneal injection of 150mg/kg body weight of alloxan monohydrate (Sigma, St. Louis, MO, USA) suspended in distilled water. Five days later diabetes was confirmed using One Touch® Glucometer (Lifescan Inc., 1995 Milpitas California 95305, USA). Animals with blood glucose level $\geq$ 200mg/dl were considered diabetic and included in the study.

Collection of Samples:

After an overnight fast, the animals were euthanized under chloroform vapour and dissected. Blood obtained by cardiac puncture using sterile needles and syringes into non-heparinized tubes were allowed to clot for about 2h and thereafter centrifuged (4000g for 10min) to remove cells and recover serum which was then used for glucose and alpha-amylase assays. Pancreatic tissues were also removed surgically and preserved in a fixative (10% formaldehyde) preparatory to histopathological processing.

Biochemical Assays:

Analysis of serum glucose and alpha-amylase activity in serum were by use of assay kits obtained from DIALAB produktion, Gessellschaft m.b.H A-1160 Wien-panikengasse,Austria .The methods are respectively based on the principles of Trinder (1972) and Lorentz (1988).

Histopathological Studies:

The fixed pancreatic tissues were sectioned (5-micron thickness) and sections stained with basic dyes, Heamatoxilin and Eosin (H&E) according to Conn (1946) procedure and photomicrographs developed (x400).

Phytochemical Analyses:

Shade/air-dried leaves, stem and root of *Vernonia amygdalina* Del. were crushed into powdered form separately. The various plant parts were separately extracted in ethanol and water, and the extracts screened for the presence or absence of various secondary metabolites using standard phytochemical screening procedures described by Trease and Evans (1997) and Sofowora (2006)

Statistical Analysis:

Data on blood and serum glucose and alpha-amylase activity were expressed as Mean ±SD. Pair wise comparison between test and controls were done using the student t-test. Differences between groups were considered significant at $p<0.05$

RESULTS AND DISCUSSION

The results in table 2 above show changes in some biochemical indices determined in this study. Blood glucose which was significantly elevated ($p<0.01$), compared to both normal control and pre-alloxan treatment value, became decrease significantly too ($p<0.01$), after 14 days administration of the plant extract. This observation was replicated in serum glucose result obtained after sacrifice of animals. Determined alpha-amylase activity in serum showed significant increase in diabetic control rats ($p<0.01$) relative to non-diabetic control. However, this was decreased significantly by about 15.38% after 14days treatment with the *V.amygdalina* extract.

Table 1: Phytochemical components in aqueous and ethanolic extracts of leaves, stem and root of *Vernonia amygdalina* Del.

Chemical constituent	Remarks					
	Root extract		Stem extract		Leaf extract	
	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous
Alkaloids	++	++	+	+	+	++
Glycosides	+	+	+	+	+	+
Tannins	-	-	-	-	+	-
Saponins	+	++	-	+	-	+
Flavanoids	+	+	+	-	++	+
Reducing sugar	++	+	+	+	+	+
Polyphenols	+++	++	+++	++	++	+++
Phlobatanins	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-
Hydroxymethyl anthraquinones	-	-	-	-	+	-
Steroids	+	+	+	+	++	+

Key

+++	Abundantly present
++	Moderately present
+	Slightly present
-	Not detected

Table 2: Changes in blood glucose, serum glucose and alpha-amylase activity following a 14day administration of *V.amygdalina* Del. extract to alloxanised rats.

	Blood glucose (mg/dl)				
	Pre-alloxan treatment	5 days post alloxan treatment	Post extract treatment	Serum glucose (mg/dl)	a-amylase activity(U/L)
Normal control(NC) 0.2ml dist.water	62.00±11.85	58.60±6.73	57.00±11.38	73.51±18.98	215.56±8.92
Diabetic control(DC) 0.2ml dist.water	67.51±8.10	418.40±124.86[a]	448.60±104.14[a]	247.25±4.83[a]	274.96±10.11[a]
Diabetic test(DT) 400mg/kg b.w	75.45±7.00	477.33±125.64[a]	353.67±44.09[b,c]	144.14±25.83[b]	232.67±12.15[b]

Mean±SD, n=7, a $p<0.01$ vs. NC; b $p<0.01$ vs. DC and c $p<0.01$ vs. post alloxan

Histomorphology of Pancreatic Tissues:

Information obtained from the histology and architectural integrity of the pancreatic tissues of diabetic test rats relative to the control is revealing and striking: Whereas there was total and complete degeneration of (cellular lesion) islets and accini cells of the pancreas indicated by necrotic areas in the untreated diabetic animals (plate 2), zones of new islet cell regeneration were observed in plate preparation from the diabetic test animals that received 400mg/kg b.w. of the extract (see plate 3) .The nuclei and outline of the regenerated cells were fairly prominent, although the cells are proliferative (reduced in size and number) when compared to the non diabetic control. The non-diabetic control plate showed prominent and well stained islet (enmass) and accini cells devoid of any undue pathological changes (plate 1)

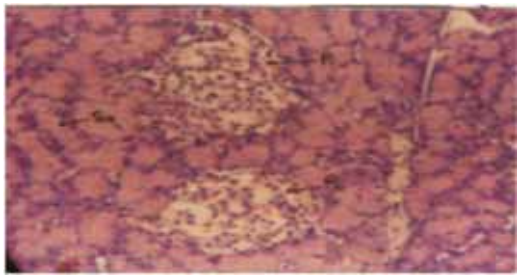

Plate 1: Phtomicrograph of pancreatic cells of non diabetic control rats stained with H&E (x400).Observe pancreatic islet (Pi), serous accini (Sa) and the close association of accini cells with intra and inter lobules

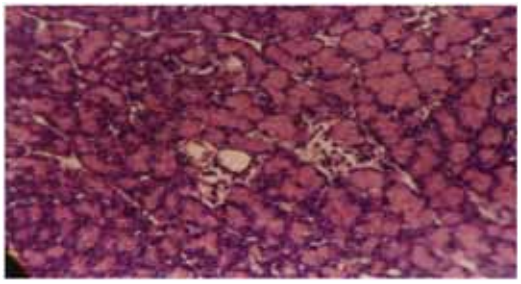

Plate 2: Photomicrograph of pancreatic cells of diabetic control rats stained with H&E (x400).Observe the degenerated pancreatic islet (Pi) and affected islet sclerosis (Is).The serous accini deeply stained and not evenly distributed may have beenreplaced by fatty deposits in some cases.

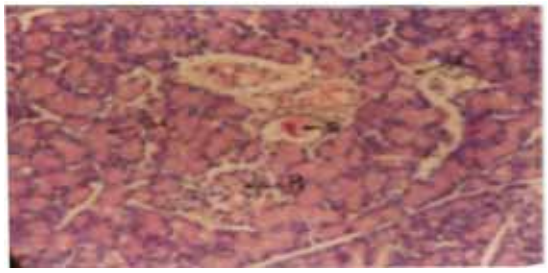

Plate 3: Photomicrograph of pancreatic cells of diabetic rats treated with extract from *V.amygdalina* Del. (400mg/kg b.w.) for 14 days, stained with H&E (x400).Observe well stained pancreatic islet (Pi) and its cells, indicating regeneration.The serous accini (Sa) cells are well stained and evenly distributed in the sectionAlso notice sclerotic condition in some lobules and islets.

Discussion:

The most routine and biochemical marker used in the diagnosis and progress monitoring during management of diabetes mellitus both in clinical and experimental settings is blood and/or serum glucose concentration (Mayfield, 1998). These where both measured in this study and results showed significant

reduction in diabetic rat following treatment with extract from leaves of *Vernonia amygdalina* Del. This agrees with our earlier reports (Atangwho *et al.,* 2007a; 2007b; 2007c) and those of other researchers (Akah *et al.,* 2004; Nimenbo-Uadia, 2003; Gyang *et al.,* 2004).

Two dominant pathogenic lesions are responsible for the complex diathesis called diabetes: one is a failure of the islet beta cells and the other a resistance of the action of insulin Arky, (1978). The former can be induced in experimental animals by selective destruction of beta cells using an acute dose of a diabetogenic agent, including alloxan used in this present study. Alloxan mediates a cytotoxic action which leads to an eventual destruction of the pancreatic cells. A detailed review of this action and mechanism has earlier been presented Szkudelski, (2001). In this study the histomorphology of the pancreas of untreated diabetic rats clearly indicated cellular degeneration and disorientation of islet cells. Soto *et al.,* (2004) have also in a study investigating the hypoglycemic action of silymarin, reported that 72h after alloxan administration, pancreatic tissues presented morphological abnormalities: islet shrinkage, necrotic areas, loss of cell organization, widespread lipoid deposit throughout the exocrine tissue and loss of beta cells. This entirely is in line with our present report. The necrotized pancreatic tissue became restored and serum and blood glucose significantly reduced after 14 days treatment with extract of *Vernonia amydgalina* Del. as was also observed in the silymarin treated diabetic animals Soto *et al.,* (2004). However, the restoration was only to a partial extent.

We hypothesized therefore, that extract from *V.amygdalina* Del. induces pancreatic function recovery by regenerating hitherto destroyed bitter cells. Alloxan, mediates pancreatic beta cell destruction via generation of reactive oxygen species (ROS) in a cycle established by its reduction product in the cell, dialuric acid Szkudelski, (2001). A plant with the ability to ameliorate alloxan induced diabetes would necessarily address in part or whole the ROS generation process and the ROS already in circulation. Such a plant extract must be endowed with powerful antioxidants which would reverse the cytotoxic action of alloxan and/or mop up the population of ROS in circulation. Igile *et al.,* (1994) have isolated and demonstrated the properties of some powerful antioxidants from *Vernonia amygdalina* to include luteolin, 7-0-betaglucoronoside and luelin, 7-0-betaglucosides. The action of these isolates may have caused a commencement in regeneration of the beta cells accompanied by a gradual release of insulin to clear excess glucose from circulation. The preposition here explains to a reasonable extent, why the reduction in serum glucose is not a total return to normoglycemia, as the beta cells may not have completely been regenerated as well as become matured in size and metabolic function. Corroborative and confirmatory studies are ongoing in our laboratory to ascertain by immunoreactive methods (immunohistochemical studies) the functionality and histomorphometric estimation of the regenerated cells.

We have shown also in this study the phytochemical composition of the various parts of the study plant. The leaves demonstrated the presence of flavoniods, polyphenols, steroids, moderately and the slight presence of tannins, glycosides, saponins and alkaloids amongst others. Secondary metabolites of plants such as these may posses some alpha-glycosidase inhibitors and competitively inhibit intestinal brush border enzymes with an eventual reduction in digestion and absorption of carbohydrates from the gut-postprandial hyperglycemia, hence an effective glucose control Tiwari *et al.,* (2002). The role of tannins has been discoursed in this light Nimenbo-Uadia, (2003). A positive correlation has als been indicated between the presence in plants, of flavoniods, glycosides and phytosterols with hypoglycemic and antihyperglycemic actions, respectively Winlenam,(1989). All of these substances have been detected in the ethanolic-leaf extract of the study plant. However this second mechanistic approach, on its own would be insufficient to establish an effective glucose control in an experimentally induced type 1 diabetes, requiring insulin. We opined therefore that this can only act in synergy with the regenerated beta cells for effective glucose homeostasis in diabetes, but could be strong enough to cause significant blood sugar reduction in non-diabetic situations (hypoglycemic action).

We conclude therefore that the antihyperglycemic action of *V.amygdalina* Del. is mediated via insulin production from the regenerated pancreatic beta cells in diabetic subjects; and that this action is achieved by more than just a single active constituent but a combination of agents in the plant extract. Hence, *Vernonia amygdalina* Del is a potential source for discovery of new orally active agent(s) for future diabetes therapy.

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