



Effect of Combined Leaf Extracts of *Vernonia amygdalina* (Bitter Leaf) and *Gongronema latifolium* (Utazi) on the Pancreatic β -Cells of Streptozotocin-Induced Diabetic Rats

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Received 12th February 2011

Accepted 25th March 2011

Available online 28th April 2011

Research Article

ABSTRACT

The study evaluated the effect of combined extracts of *Vernonia amygdalina* (VA) and *Gongronema latifolium* (GL) on the pancreas of streptozotocin (STZ) induced diabetic Wistar rats. Thirty-two (32) albino rats were divided equally into 4 groups. Groups A and B which served as normal (NC) and diabetic (DC) controls respectively, received placebo treatment. The diabetic test groups C and D were respectively treated with combined extracts of VA and GL (200mg/kg b. w., p. o.) and insulin, (humulin 5 IU/kg, s.c.) for 28 days. Thereafter, the animals were sacrificed and blood and pancreas were collected for serum glucose and histological evaluation, respectively. Changes in animal weight were also measured within the period. From the results it was revealed that both the combined extracts and humulin significantly increased the animals' body weight ($p < 0.05$) from -10.5% reduction in the DC, to 7.6% and 8.9% respectively. In the same order, serum glucose significantly decreased ($p < 0.05$) by 12.49% and 14.96% after the 28-day treatment compared to DC. The extent of reversal of hyperglycemia in the extract treated animals compared well with the insulin treated group. The biochemical results were corroborated with results of histological evaluations: The pancreatic β -cells of DC animals which were distorted and degenerated with shrunken cell mass as against prominent islet cells with normal exocrine pancreas of NC animals became rapidly proliferated upon intervention with the combined extracts, suggesting a possible regeneration of the islet cells. On the otherhand,

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intervention with humulin did not produce observable differences in the cyto-architecture of the pancreatic islets compared to the diabetic control, confirming an extra-pancreatic mechanism of insulin.

Keywords: *Diabetes mellitus, V. amygdalina, G. latifolium, human insulin, pancreas β -cells regeneration;*

1. INTRODUCTION

Diabetes mellitus (DM) is a complex and chronic disease associated with a myriad of debilitating complications, causes of which are usually multi-factorial. The lesions in the patho-physiology of diabetes are multiple and therefore would require more than a single drug agent to reverse all or majority of the aspects of the disease. The effective therapeutic approach should be multimodal and in this light, several traditional medicinal herbs have been preferred given the plethora of active ingredients present in a single herb (Tiwari and Rao, 2002, Atangwho et al., 2009).

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and have remain relevant in both developing and the developed nations of the world for various chemotherapeutic purposes. The use of plant derived natural compounds as part of herbal preparations for alternate source of medicament continues to play major roles in chemotherapy especially in third world countries (Joy et al., 1998). Several studies carried out have shown that traditional medicines could provide better glycaemic control than currently used conventional drugs (Rates, 2001; Roja and Rao, 2000). Plants by means of secondary metabolism contain a variety of herbal and non-herbal ingredients that can ameliorate a disease condition by acting on a variety of targets (various modes and mechanisms) in the host organism. On the basis of the above, polyherbal therapy is considered the preferred therapeutic approach to management of diabetes mellitus given its multi-factorial pathogenicity (Tiwari and Rao, 2002; Ebong et al., 2008).

Polyherbal therapy which is the use of a combination of various agents from different plant sources for therapeutic purposes is a current pharmacological principle and has the advantage of producing maximum therapeutic efficacy with minimum side effects (Ebong et al., 2008). This enhanced efficacy is thought to derive from phytochemicals endowed traditional medicinal plants, since they present exciting opportunities for the development of new types of therapeutics for the management of diabetes mellitus. Such phytochemicals include tea polyphenols which suppress Post-Prandial Hyperglycaemia and glucose transport across the small intestine (Yoshikawa et al., 1999) and saponins which delay glucose transfer from the stomach to the small intestine (Yuan, 1998; Chatopadhy, 1998). Epicatechin has a restorative effect on pancreatic β -cells against alloxan damage (Chakravaty, 1982), and plant flavonoids which exert their antidiabetic activity via antioxidant properties (Bnouham et al., 2006). These reports have accelerated the global efforts to harness and harvest those medicinal plants that bear substantial amount of potential phytochemicals showing multiple beneficial effects in combating diabetes and diabetes-related complications (Tiwari and Rao, 2002).

In our laboratory, the antidiabetic activities of *Azadirachta indica* (AI), *Vernonia amygdalina* (VA) and *Gongronema latifolium* (GL) have been reported. In a recent report, the chemical components thought to exert the antidiabetic action were compared (Atangwho et al., 2009a). Although extracts from these plants have individually demonstrated antidiabetic action, recent evidence from our laboratory show that antidiabetic efficacy of the extracts is enhanced when given in combination (Ebong et al., 2008; Atangwho et al., 2009b; Atangwho et al., 2010b). It therefore became absolutely necessary to investigate the biochemical and histological mechanism(s) of the enhanced action of this combination of extracts. Accordingly, the present study was set up to investigate the effect of combined extracts of *V. amygdalina* and *G. latifolium* on blood glucose and the histology (morphology) of the pancreas, with a view to ascertaining whether or not the antidiabetic action is exerted via pancreatic (β -cell) action/modulation.

Vernonia amygdalina (VA) commonly known as bitter leaf is a shrub or small tree of 2 – 5 m belonging to the family *Asteraceae*. It has petiolate leaves of about 6 mm diameter and elliptic shape. The leaves are green with a characteristic odour and a bitter taste (Singha, 1966). In many parts of Nigeria, the plant has

been domesticated (Igile et al., 1994)). It is known as 'Ewuro' in Yoruba, 'Etidot' in Ibibio, 'Onugbu' in Igbo and 'Chusa-diki' in Hausa tribes in Nigeria (Egedigwe, 2010). VA grows under a range of ecological zones in Africa and produces large mass of forage and is drought tolerant; it is found in homes in villages as fence post and pot-herb (Bonsi et al., 1995). The leaves are used as soup condiment and washed before eating to get rid of the bitter taste. They are used as vegetable in meals to stimulate the digestive system, and as a treatment for fever. A wide array of phytochemicals has been shown to be present in VA. The presence of oxalates, phytates and tannins have been reported (Udensi et al., 2002; Ejoh et al., 2007; Eleyinmi et al., 2008), as well as flavonoids (Igile et al., 1994; Udensi et al., 2002; Tona et al., 2004). VA extracts have been shown to exhibit profound ethnomedical and pharmacological properties viz, anti-diabetic, antimalarial, antihelminthic and antibiotic properties (Farombi, 2003).

Gongronema latifolium is a tropical rainforest plant belonging to the Ascepiadaceae family (Chattopadhyay, 1998; 1999; Chattopadhyay et al., 1992). It is a shrub, with milky or less often, clear latex. The leaves are simple, opposite or occasionally whorled, very rarely alternate, usually without obvious stipules, and the margins are nearly always entire (Bingtao et al., 1977) *G. latifolium* is a perennial edible plant with soft and pliable stem. It is widely used in the West African sub region for a number of medicinal and nutritional purposes. In the South-Eastern States of Nigeria, the plant is known locally as "Utazi" and used primarily as a staple vegetable/ spice (Ugochukwu et al., 2003; Morebise et al., 2002; Ugochukwu and Babady, 2002). *G. latifolium* has a very bitter taste and its phytochemical composition indicates that it contains saponins (Morebise et al., 2002). In some African cultures it is used as a spice to support the pancreas (Okafor and Ham, 1999), and in the United States it is also a constituent of a DM Tea blend marketed for the maintenance of healthy blood glucose levels.

Consequently, the aim of this study is to investigate the efficacy of the extracts of *Vernonia amygdalina* and *Gongronema latifolium* in combination on the blood sugar and pancreas of streptozotocin induced diabetic Wistar rats.

2. MATERIALS AND METHODS

2.1 COLLECTION AND PREPARATION OF PLANT EXTRACTS

Four hundred grammes (400g) each of *V. amygdalina* and *G. latifolium* leaves harvested from the Endocrine Research Farm of the University of Calabar were separately homogenized in 80% ethanol (V/V) using an electric homogenizer (QL4-18L40). The homogenates were allowed in a refrigerator for 48 hours at 4°C after which, they were filtered using a cheese material and afterwards WhatMan No. 1 filter paper. The filtrates were separately concentrated at 37- 40°C under reduced pressure, using a rotary evaporator to one-tenth of their original volumes. These were then allowed in a water bath for evaporation to dryness yielding 49.1g (12.28%) and 26.7g (6.68%) for *V. amygdalina* and *G. latifolium*, respectively.

2.2 EXPERIMENTAL ANIMALS

Thirty-two (32) male albino rats weighing 140-180g were used for the work. The animals were obtained from the animal house of the Department of Pharmacology, University of Calabar and allowed to acclimatize in the animal house of the Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Calabar for about 3 weeks prior to experimentation. They were kept in properly ventilated cages, where bedding was replaced every two days, at a room temperature of about 27°C and 12 hour light/dark cycle. The animals were fed with growers' marsh and water obtained from tap *ad libitum*.

2.3 INDUCTION OF EXPERIMENTAL DIABETES

Diabetes was induced by intra-peritoneal injection of streptozotocin (STZ) (Sigma St. Louis, M.O. USA) (Batch No. U925) at a dose of 65mg/kg b.w., reconstituted in normal saline. Prior diabetes induction, the animals were fasted for 12 hours. Confirmation of diabetes was done seven days after STZ treatment (Fasting Blood Sugar), using One Touch glucometer (Lifescan, Inc 1995 Milpas, California, USA). Blood

sample for the FBS determination was obtained from tail puncture of the rats, and animals with FBS \geq 200mg/dl were considered diabetic and included in the study as diabetic animals.

2.4 EXPERIMENTAL DESIGN

Animal grouping and treatment schedule is as shown in Table 1. The dosages of plant extracts and insulin used were according to the methods of Ebong et al., (2006) and Sonia and Scrivivasan (1999) respectively. Plant extracts were administered twice daily via orogastric intubation for 28 days (at 6.00 am and 6.00pm) and insulin subcutaneously once a day post prandial. At the end of the 28 days, food was withdrawn from the rats and they were fasted overnight, but with free access to water. They were then euthanized under chloroform vapour and sacrificed. The pancreas was surgically removed, immediately blotted using filter paper to remove traces of blood, weighed with an analytical balance then fixed in 10% formal saline preparatory to histological processing. Whole blood was also collected via cardiac puncture using sterile syringes into plain tubes and allowed for about two hours to clot. This was centrifuged at 3000rpm for 10mins and serum separated from the clotted blood and used for glucose assay.

Table 1. Experimental design

Group	No. of rats	Treatments	Dosage
A: Normal control	8	Normal saline	0.2 ml
B: Diabetic control	8	Normal saline	0.2 ml
C: Diabetic extract treated group	8	<i>V. amygdalina</i> and <i>G. latifolium</i> leaf extracts	200 mg/kg b.w. (100mg/kg b.w. each)
D: Diabetic Standard control, insulin treated	8	Humulin	5 IU/kg b.w.

2.5 GLUCOSE ESTIMATION

Randox-assay kit (GOD-PAP) method based on Barham and Trinder (1972) was used. The principle involves the enzymatic oxidation of glucose in sample by the enzyme Glucose oxidase which generates hydrogen peroxide and gluconic acid. The concentration of H₂O₂ released is proportional to initial amount of glucose in the sample and it reacts under catalysis of peroxidase, with phenol and 4-amino phenazone to form a red violet quinoneimine dye whose colour intensity reflects the concentration of glucose in the sample.

2.6 HISTOPATHOLOGICAL STUDIES

The fixed pancreatic tissues were sectioned (5-micron thickness) and sections firstly stained with basic dyes, of Heamatoxylin and Eosin (H&E) according to Conn (Conn, 1946) procedure and later pancreatic sections were specifically stained for beta cells by the aldehyde fuschin procedure (Gomori aldehyde method) and photomicrographs (x 400) developed (see result section).

2.7 STATISTICAL ANALYSIS

Glucose and weight measurements are presented as mean \pm SE. One way Analysis of Variance (ANOVA) and the LSD post hoc test were used to analysis the data ($p < 0.05$).

3. RESULTS

3.1 MORPHOLOGICAL AND BIOCHEMICAL OBSERVATION

From tables 2 and 3 it can be said that, there was a significant variation ($p < 0.05$) in the weight and serum glucose concentration of the animals among the treatment groups. Body weight of diabetic control animals was decreased significantly by -10.5% ($p < 0.05$) when compared to the initial body weight, whereas the normal control and the treated groups showed a significant increases ($p < 0.05$) in the final body weight. The extent of increase in body weight of the extract treated animals (7.6%) was similar ($p > 0.05$) when compared to the Normal (6.8%) and Standard (8.9%) controls. As a converse to the effect of diabetes on weight, there was a significant increase, 53.23% ($p < 0.05$) in the serum glucose level of the untreated diabetic animals relative to the normal control. Decrease in serum glucose of animals treated with plant extracts (12.49%) was significant ($p < 0.05$) and it was observably similar to that of the standard control animals, humulin (14.96%). Combined extracts of VA and GL mimic insulin in these two respects.

Table 2. Variation in body weight of the animals in the various experimental/ treatment groups

Group	Initial (g)	Final (g)	% Increase
A	148.93±2.92	170.58±3.51 ^b	6.8
B	147.58±3.05	119.73±2.71 ^{a,c}	-10.5
C	146.98±3.07	171.49±2.28 ^b	7.6 ^d
D	147.23±2.23	176.20±6.02 ^b	8.9 ^d

Values are expressed as Mean ± SEM, n = 8

Table 3. Variation in serum glucose of the animals in the various experimental/ treatment groups

Group	Initial serum glucose	Final serum glucose	% Decrease
A	69.83±5.31	65.62± 2.47	3.11
B	74.65±4.02	244.62±8.64 ^{c,b}	-53.23
C	75.83±2.32	59.00±2.09 ^d	12.49
D	73.33±3.61	54.25±1.33 ^d	14.96

*Values are expressed as Mean ± SEM, n=8, a= significant decrease at $p < 0.05$ vs initial value
b= significant increase at $p < 0.05$ vs initial value, c= $p < 0.05$ vs NC, d= $p < 0.05$ vs DC.*

3.2 HISTOLOGICAL OBSERVATIONS

Effect of the combined extracts on the cyto-architecture of the pancreas in this work was studied using H/E staining technique and the Gomori Aldehyde Fuschin (GAF) staining method for pancreatic islet cells. Stained sections were examined using the light microscope.

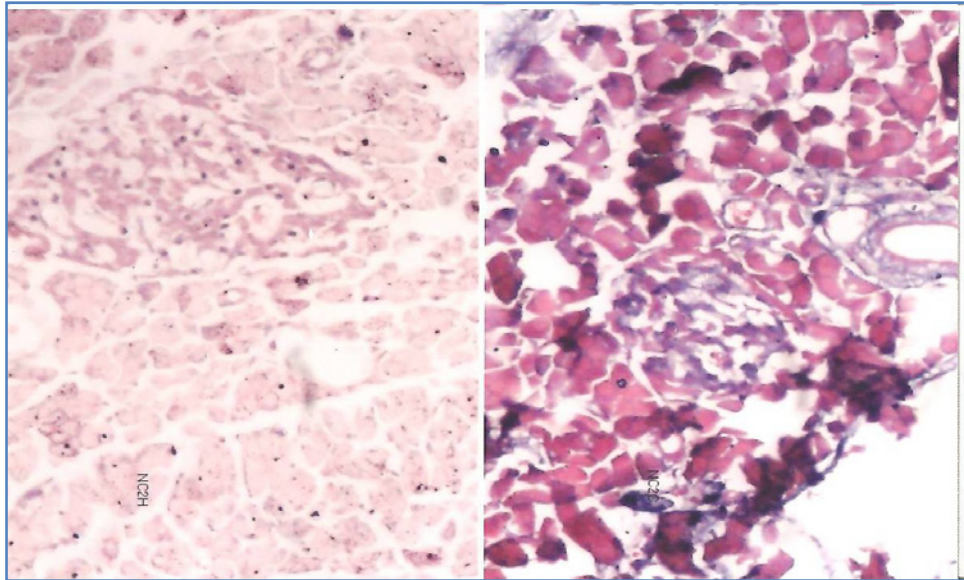
3.2.1 HAEMATOXYLIN EOSIN (H & E) STAIN

Normal Control (PLATE 1a): The pancreatic ducts are properly outlined; the islet cells are prominent and well circumscribed. Also, there are clearly defined secretory acini, centoracinar cells and excretory ducts.

Diabetic control (PLATE 2a): The secretory acini and centroacinar cells are present, the lobules are distorted and the Islet cells are necrotic and appeared degenerated.

Vernonia amygdalina and *Gongronema latifolium* treated (PLATE 3a): The pancreatic islet cells are clearly defined and prominent, the excretory duct is present, lobules are well defined and the acinar cells are present.

Humulin (PLATE 4a): Presence of vacuolations, with necrotic acinar cells and Islet cells which appeared degenerated.

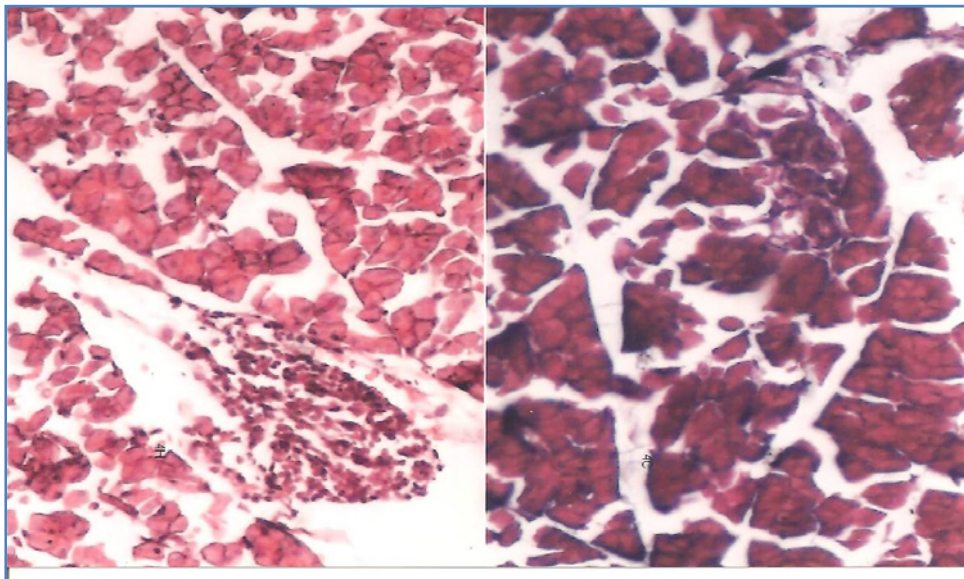


1a. H & E

1b. GAF

PLATE 1. Photomicrographs (x 400) of pancreas of Normal Control rats given placebo treatment

H&E= Haematoxylin and eosin, GAF=Gomori Aldehyde Fuschin stain



2a. H&E

2b. GAF

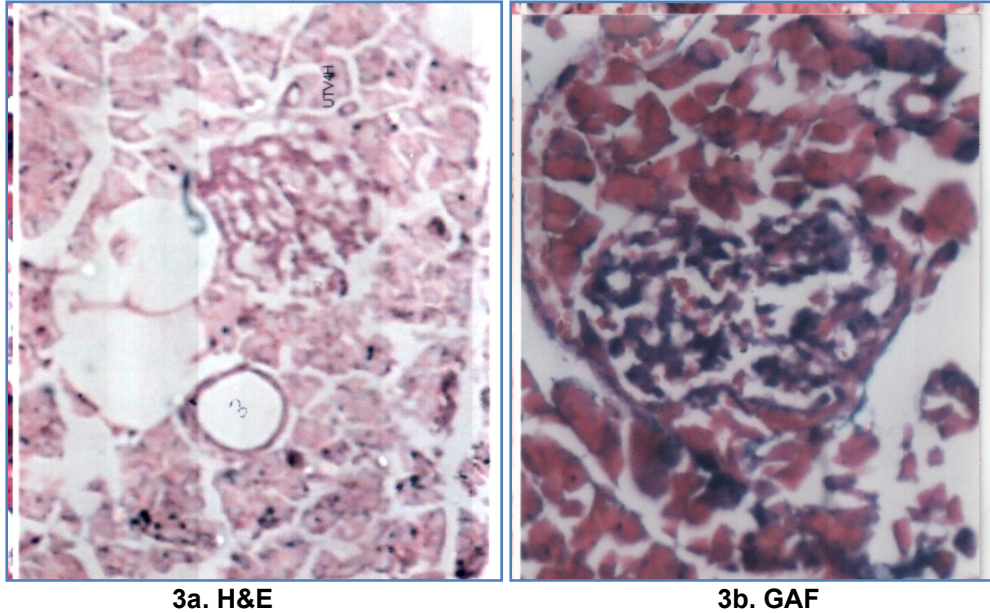
PLATE 2. Photomicrographs (x 400) of pancreas of Diabetic rats given placebo treatment

H&E= Haematoxylin and eosin, GAF=Gomori Aldehyde Fuschin stain

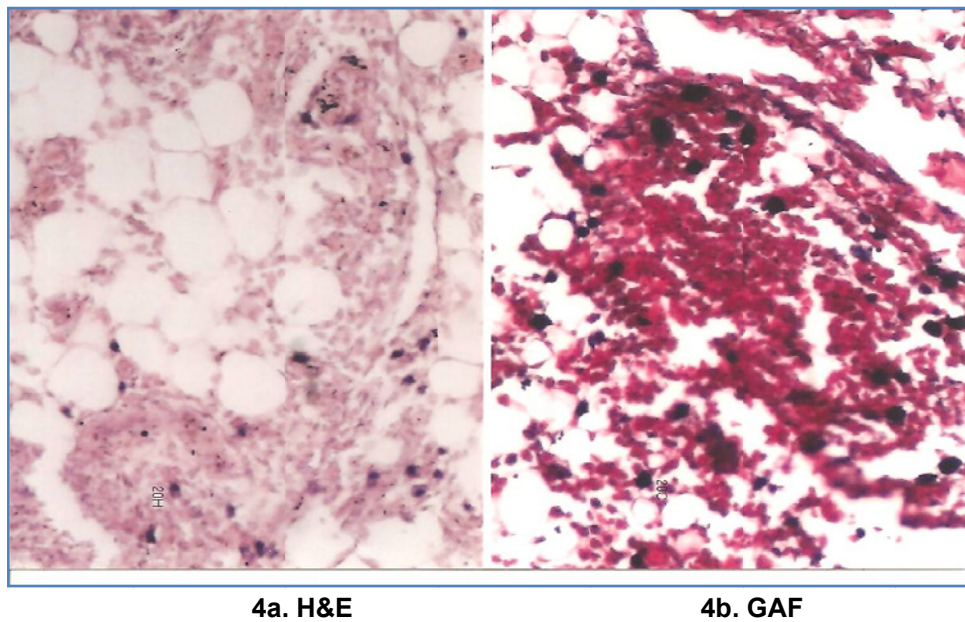
3.2.2 GOMORI ALDEHYDE FUSCHIN STAIN

Normal control (PLATE 1b): Islet cells are clearly defined, prominent and well circumscribed.

Diabetic control (PLATE 2b): Islet cells appear with distortion of the pancreatic cyto-architecture indicating cytotoxic action of streptozotocin.



**PLATE 3: Photomicrograph (x 400) of pancreas of Diabetic rats treated with combined extracts of *V. amygdalina* and *G. latifolium* 200mg/kg b.w. (100mg/kg b.w. of each extract).
H&E= Haematoxylin and eosin, GAF=Gomori Aldehyde Fuschin stain**



**PLATE 4. Photomicrograph (x400) of pancreas of Diabetic rats treated with Humulin (insulin) 5IU/kg b.w.
H&E= Haematoxylin and eosin, GAF=Gomori Aldehyde Fuschin stain**

Vernonia amygdalina and *Gongronema latifolium* treated (PLATE 3b): The islet cells are present, indicating regeneration of hitherto destroyed β -cells.

Humulin (PLATE 4b): The Islet cells are distorted, appearing degenerated with presence of vacuolations, and hence insulin may not have any effect on the pancreas.

4. DISCUSSION

The results from this study revealed significant loss of weight of untreated diabetic rats compared to non-diabetic animals. This may be due to the loss in muscle and adipose tissue resulting from excessive breakdown of tissue protein and fatty acids (Granner, 1996). Glycosuria is known to cause a significant loss of calories for every gram of glucose excreted and presumably this loss results in severe weight loss in spite of increased appetite, especially when it is coupled with loss of muscle and adipose tissue due to excessive breakdown of protein. Weight loss is one of the symptoms of diabetes mellitus occurring especially when glycaemic control is poor. Studies have equally reported significant weight reduction in untreated diabetic rats (Ahmed et al., 2005).

However, the animals treated with humulin tended to gain more weight than the extract treated group. This agrees with the report of Makimattila et al., (1999) that improved glycaemic control by insulin promotes weight gain by decreasing both metabolic rate and glycosuria. Such severe weight loss was prevented in the extract treated group probably due to interaction of several bioactives. However the extracts treated animals showed appreciable increase in weight compared to the diabetic control group. This appreciation in weight indicates that the treatment allowed the tissues to access the glucose both to supply energy and spared some to build tissue materials required for growth. Studies by Neminibio-audia (2003) showed that treatment with extracts of root VA resulted in appreciation in weight of the animals after 14 days.

The histology of the pancreas in the non-diabetic group showed normal pancreatic tissues with endocrine and exocrine parts. The endocrine portion showed normal islet cells. However, in the diabetic control animals, there was a marked distortion of the pancreatic cyto-architecture with necrotic and degenerated islet cells. This is in line with the report by Bolkent et al. (2000) and Noor et al. (2008) that streptozocin damages the pancreatic tissues especially the islet cells, resulting in a prototype of Type 1 diabetes. STZ induced pancreatic lesions that resulted in full blown diabetes (Szkudelski, 2001).

In the groups treated with the extracts, there was proliferation of the islet cells; the cyto-architecture of the pancreas was almost normal, with proliferation of the islet cells and other cells of the pancreas. This agrees with our earlier report on administration of extracts of VA alone (Atangwho et al., 2010a). However the recovery in this previous report was partial. The present observation in which the extract is co-administered with GL appears to be complete and holistic, suggesting a synergistic interaction of the abundant phytochemicals and antioxidants from the two different sources. Previous studies by Ebong et al. (2006) reported this possible synergistic action using the extracts of *Azadirachta indica* and *Vernonia amygdalina*. These observations on the effect of the extracts on the pancreatic lesions could also explain the antihyperglycaemic/ hypoglycaemic effects of these extracts as the regenerated islet cells could cause an increase in insulin production and secretion.

Vernonia amygdalina extract contains active ingredients such as vernoniosides, glucosides, (VA) flavonoids and antioxidants (Jisaka et al., 1993) which may be responsible for their potentials in reversing pancreatic damage caused by STZ in experimental animals. Several studies have demonstrated the antioxidant properties and components of VA and GL. VA is reported to be rich in antioxidants composition (Igile et al., 1995) whereas studies by Nwanjo et al. (2006) have demonstrated the antioxidant properties of *Gongronema latifolium*.

Diabetes mellitus is known to be associated with the generation of Reactive Oxygen Species (ROS) and this affects the antioxidant reaction catalysed by ROS scavenging enzymes, resulting in tissue damage. Studies by Kesavulu et al. (2000) demonstrated the defects in ROS scavenging enzymes in DM. A plant

extract that has the ability to ameliorate or reverse pancreatic lesions that results in hypoglycaemia would by one mechanism or the other address the ROS generation or process. This means that such plants must possess antioxidant properties which would reverse the cytotoxic cycle of STZ in the pancreas or mop up the ROS in circulation.

The pancreatic islet cells of the insulin treated animals appeared distorted and degenerated with vacuolations in the exocrine portions. There was indeed no observable difference in the pancreatic islet cyto-architecture compared to the diabetic control group. This is in accordance with earlier reports by Pirola et al., (2004) that insulin does not act directly on the pancreas to reduce blood sugar levels but on peripheral tissues, facilitating the uptake of glucose into the cells, thereby reducing blood glucose levels. The hyperglycaemia observed in the Diabetic Control Group suggests that the insulin producing cells of the pancreatic islets were destroyed. This agrees with reports by Rodrigues et al., (1999) that STZ selectively destroys insulin producing cells of the pancreas and it induces a prototype of Type 1 diabetes.

Extracts from the leaves of *V. amygdalina* and *G. latifolium* when combined produced reduction in glucose level similar in extent to insulin treatment insulin (Humulin). Plant extracts are known to contain phytochemicals including tannins, saponins, polyphenols and alkaloids and dietary fibre which are said to contribute to their blood sugar lowering effect (Tiwari and Rao, 2002). Micronutrients such as vanadium which have insulin-like activities are present in some plant extracts as well and this can equally account for their hypoglycaemic potency. Yeh et al. (2003) stated that majority of plant extracts exert their blood sugar lowering effects via insulin-like micronutrients present in the extracts. Besides the presence of phytochemicals and micronutrients in plant extracts which exert direct effect on blood sugar, the proliferation/regeneration of the pancreatic islet cells which was evident in this study could also account for the reduction in blood glucose of treated animals. Treatment with insulin (Humulin) significantly reduced the blood glucose to levels below normal control. This is in accordance with reports by Rother, (2007) that treatment with insulin is usually a common cause of hypoglycaemic attacks in diabetic subjects.

5. CONCLUSION

Combined extracts of VA and GL may exert their antidiabetic action via pancreatic islet regeneration. However, viability of the regenerated cells with respect to production and secretion of insulin is necessary to validate this observation.

REFERENCES

- Ahmed, S. M., Vrushabendra, S. B., Gopkumar, P., Dhanapal, R., Chandrashekara, J. (2005). Anti-diabetic activity of *Terminalia catappa* Linn. leaf extracts in alloxan-induced diabetic rats. Iranian Journal of Pharmacology and Therapeutics, 4(1), 38-39.
- Atangwho, I. J., Ebong, P. E., Eyong, E. U., Williams, I. O., Eteng, M. U., Egbung, G. E. (2009a). Comparative chemical composition of leaves of some antidiabetic medicinal plants: *Azadirachta indica*, *Vernonia amygdalina* and *Gongronema latifolium*. African Journal of Biotechnology, 8(18), 4685-4689.
- Atangwho, I. J., Ebong, P. E., Egbung, G. E., Ani, I. F. (2009b). Effect of co-administration of extracts of *Vernonia amygdalina* and *Azadirachta indica* on serum electrolyte profile of diabetic and non-diabetic rats. Australian Journal of Basic and Applied Sciences, 3, 2974-8.
- Atangwho, I. J., Ebong, P. E., Egbung, G. E., Obi, A. U. (2010a). Extract of *Vernonia amygdalina* Del. (African bitter leaf) can Reverse Pancreatic Cellular Lesion after Alloxan Damage in the Rat. Australian Journal of Basic and Applied Sciences, 4(5), 711-716.
- Atangwho, I. J., Ebong, P. E., Eyong, E. U., Eteng, M. U. (2010b). Combined administration of extracts of *Vernonia amygdalina* (Del) and *Azadirachta indica* (A.Juss) mimic insulin in time-course body weight and glucose regulation in diabetic and non-diabetic rats. Nigerian Journal of Biochemistry and Molecular Biology, 25(1), 44 – 49.
- Barham, D., Trinder, P. (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst, 97, 142-145.

- Bingtao L., Gilbert, M. G., Stevens, W. D. (1977). "Asclepiadaceae". in Flora of China Science Press (Beijing) and Missouri Botanical Garden Press., Vol. 16 Page 189.
- Bnouham, M., Ziyat, A., Melchfi, H., Yahri, A., Legssyen, A. (2006). Medicinal plants with potential antidiabetic activity– a review of 10 years of herbal medicine research (1990-2000). International Journal of Diabetes and Metabolism, 14, 1-25.
- Bolkent, R., Yanarda, A., Tabakolu-Ouz. O., Ozsoy, S. (2000). Effect of chard (*beta-Vulgaris L. Varcida*) Extract in pancreatic beta cells in streptozocin diabetic rats: Morphological and biochemical study. Journal of Ethnopharmacology, 73 (1-2), 251-259.
- Bonsi, M. L. K., Osuji, P. O., Tuah, A.K. (1995b). Effect of supplementing teff straw with different levels of leucaena or sesbania leaves on the degradabilities of teff straw, sesbania, leucaena, tagasaste and vernonia and on certain rumen and blood metabolites in Ethiopia Menz sheep. Animal Feed Science and Technology, 52, 101 - 129.
- Chakravathy, B. K., Gupta, S., Gode, K. D. (1982). Functional beta cell regeneration in the islets of the pancreas in alloxan induced Diabetic rats by (-) epicatechin. Life Sciences, 31(24), 2693-2697.
- Chattopadhyay, R. R. (1998). Possible mechanism of antihyperglycaemic effect of *Gymnens sylvestre* leaf extract, part I. General Pharmacology, 31, 495 - 496.
- Chattopadhyay, R. R. (1999). A comparative evaluation of some blood sugar lowering agents of plant origin. Journal of Ethnopharmacology, 67, 367-372.
- Conn, H. J. (1946). Biological Stains: A handbook on the nature and uses of the dyes employed in the biological laboratory. 5th edition, Geneva, N.Y., Biotech publication, pg 24-29.
- Ebong, P. E., Atangwho. I. J., Eyong, E. U., Egbung, G. E. (2008). The antidiabetic efficacy of combined extracts from two continental plants, *Azadirachta indica* and *Vernonia amygdalina* (Del). American Journal of Biochemistry and Biotechnology, 4(3), 239-244.
- Ebong, P. E., Atangwho, I. J., Eyong, E. U., Ukere, C., Obi, A. U. (2006). Pancreatic beta cell regeneration. A probable parallel mechanism of hypoglycaemic action of *Vernonia amygdalina* and *Azadirachta indica*. Proceedings of International Neem Conference, Kuming, China, Nov, 11-12.
- Egedigwe, C. A. (2010). Effect of dietary incorporation of *Vernonia amygdalina* and *Vernonia colorata* on blood lipid profile and relative organ weights in albino rats. M.Sc. Dissertation, Dept. of Biochemistry, Michael Okpara University of Agriculture, Umudike, Nigeria.
- Ejoh, R.A., Nkongha, D.V., Innocent, G., Moses, C. (2007). Nutritional components of some non-conventional leafy vegetables consumed in Cameroon. Pakistan Journal of Nutrition, 6, 712-7.
- Eleyinmi, A.F., Sporns, P., Bressler, D.C. (2008). Nutritional composition of *Gongronema latifolium* and *Vernonia amygdalina*. Nutrition and Food Sciences, 38, 99-100.
- Farombi, E. O. (2003). African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. African Journal of Biotechnology, 2, 662 – 7.
- Granner, D. K. (1996). Hormones of the pancreas and gastrointestinal tract. In D. K. Granner (Ed.), *Harper's biochemistry 25th ed.*, New York: McGraw Hill., 610-626
- Igile, G. O., Oleszk, W., Burda, S., Juryszta, M. (1995). Nutritional assessment of *V. amygdalina* leaves in growing mice. Journal of Agriculture and Food Chemistry, 93, 2162 - 2166.
- Igile, G. O., Olezek, W., Jurzysata, M., Burda, S., Fafunso, M., Fasanmade, A.A. (1994). Flavonoids from *Vernonia amygdalina* and their antioxidant activities. Journal of Agricultural and Food Chemistry (USA), 42 (11), 2445 –2448.
- Jisaka, M., Ohigashi, H., Takagawa, K., Nozaki H., Hiwta, M. Irie, R., Hoffman, M. A. and Koshimizu, K. (1993). Steroid glycosides from *vernonia amygdalina*. A possible chimpanzee medicinal plant. Phytochemistry, 34 (2), 409- 413.
- Joy, P. P., Thomas, J., Matthew S., Skaria, B. P. (1998). Medicinal Plants. Kerala Agricultural University, Kerala, India. pp 3-8.
- Kesavulu, M. M., Giri, R., Kameswara, R. B., Apparao, C. (2000). Lipid peroxidation and antioxidant enzyme levels in type 2 diabetics with microvascular complications. Diabetes metabolism, 26(5), 387-92.
- Makimattila, S., Nikkila, K., Yki –Jarvinen, H. (1999). Causes of weight gain during insulin therapy with and without metformin in patients with type II diabetes mellitus. Diabetologia, 42(4), 406-412.
- Morebise, Fafunso, M. A., Makinde, J. M., Olajide, O. A., Awe, E. O. (2002), Anti-inflammatory property of the leaves of *Gongronema latifolium*. Phytotherapy Research Supplement, 1, 75-77.

- Nimenibo-Uadia, R., Osagie, A. U. (2003). effect of *Vernonia amygdalina* (Del) in alloxan induced diabetic Albino rats. *Journal of Medical Laboratory Science*, 12(1), 25-31.
- Noor, A., Gunasekeran, S., Manicckan, A. S., Vijayalakshrin, M. A. (2008). Anti-diabetic activity of aloe vera and histology of organs in streptozocin induced diabetic rats. *Current Science*, 94(8), 1070-1076.
- Nwanjo, H. U. (2005). Efficacy of aqueous leaf extracts of *Gongronema latifolium* on plasma lipoprotein and oxidative stress in diabetic rat models. *Nigerian Journal of Physiological Science*, 20(1-2), 39-42.
- Okafor, J., Ham, R. (1999). Identification, utilization and conservation of medicinal plants in southeastern Nigeria. *Issues in African biodiversity*, (3), 1-8.
- Pirola, I., Johnston, A. M., Obberghen, E. V. (2004). Modification of insulin action. *Diabetologia*, 47, 170-184.
- Rates, S. M. (2001). Plant as source of drug. *Toxicon*, 39(5), 603-613.
- Rodrigues, B., Poucheret, P., Betell, M. L., Mc Neill, J. N. (1999). Streptozocin induced diabetes: Induction, mechanisms and dose dependency. In J. H. Mc Neill (Ed.), *Experimental models of diabetes* (3-7). Florida: CRC Press.
- Roja, G., Rao, P. S. (2000). Anti cancer compounds from tissue cultures of medicinal plants. *Journal of Herbs, Spices and Medicinal Plants*, 7, 71-102.
- Rother, K. I. (2007). Diabetes treatment - bridging the divide. *North England Journal of Medicine*, 365(15), 1499-1501.
- Sonia, B., Scrivinasan, B. D. (1999). Investigations into the anti-diabetic activity of *Azadirachta indica*. *Indian Journal of Pharmacology*, 31, 138-141.
- Singha, S.C. (1996). *Medicinal plants in Nigeria*. National Press Limited, Apapa, pp. 49.
- Szkudelski, T. (2001). The mechanism of alloxan and streptozocin action in Beta cells of the rat pancreas. *Physiology Research*, 50, 536-546.
- Tiwari, A. K., Rao, J. M. (2002). Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Current Science*, 83(1), 30-37.
- Tona L., Cimanga, R. K., Mesia, K., Musuamba, C. T., Bruyne, T., Apers, S., Hermans, N., Van Miret, S., Pieters, L., Totte, J., Vlietnk, A. J. (2004). In vitro antiplasmodial activity of extracts and fractions of seven medicinal plants used in Democratic Republic of Congo. *Journal of Ethnopharmacology*, 93, 27-32.
- Udensi, E. A., Ijeh, I. I., Ogbonna, U. (2002). Effect of traditional processing on the phytochemical and nutrient composition of some local Nigerian leafy vegetables. *Journal of Science and Technology*, 8, 37 - 40.
- Ugochukwu, N. H., Babady, N. E. (2002). Antioxidant effects of *Gongronema latifolium* in hepatocytes of rat models of non-insulin dependent diabetes mellitus. *Fitoterapia*, 73, 612-618.
- Yeh, G. Y., Eisenberg, D. M., Kaptcuk, T. J., Philips, R. S. (2003). System review of herbs and dietary supplements for glycaemic control in diabetes. *Diabetes Care*, 26(4), 1277-1294.
- Yoshikawa, M., Shimada, H., Morikawa, T. (1997). Medicinal food stuffs vii. On the saponin constituents with glucose and alcohol absorption in inhibitory activity from a food garnish 'Tonbur' the fruit of Japanese *Kachia scoparis* (L). *Chemical and Pharmaceutical Bulletin*, 45, 1300-1305.
- Yuan, Z., He, P., Ciu, J., Takeuchi, H. (1998). Hypoglycaemic effect of water soluble polysaccharide from *Auricularia auricular*- Judae Quel. on genetically diabetic KK-A^y mice. *Bioscience, Biotechnology and Biochemistry*, 62, 1898-1903.