Original Research Article

ADMINISTRATION OF COMBINED METHANOLIC LEAF EXTRACTS OF *VERNONIA AMYGDALINA* AND *GONGRONEMA LATIFOLIUM* ENHANCE GLUT 2 EXPRESSION IN THE PANCREAS AND DOWNREGULATES SERUM CASPASE 3 ACTIVITY OF STZ INDUCED DIABETIC WISTAR RATS.

ABSTRACT

Aim: The study evaluated the effects of the combined extracts of *Vernonia amygdalina* (VA) and *Gongronema latifolium* (GL) on pancreatic GLUT 2 expression and caspase 3 activity in streptozotocin (STZ, 45 mg/Kg)-induced diabetic rats.

Study design: 15 Albino rats were used for the study and were placed in 3 groups of 5 rats each, A- normal control, B – Diabetic control and C – experimental group

Place and duration of study: The study was carried out in the department of Anatomy, University of Calabar. **Duration:** 6 months

Methodology: Half of the diabetic rats were treated with VA+GL (400mg/kg, ratio 1:1, DE group) for 28 days, while the other half was untreated and served as diabetic control (DC). Normal control (NC) rats were untreated. After 28 days, the rats were sacrificed and their blood glucose, serum GLUT 2 and caspase 3 activity were measured. Histochemical evaluation of the pancreas was also carried out.

Results: Blood glucose concentrations for the 3 groups were 60.31±7.28, 257.00±4.43, and 116.60±10.11 mg/dl for NC, DC and DE respectively. This represented a 4-fold increase in the DC compared with NC and a significant amelioration in the extract-treated DE group compared with DC group. Serum GLUT 2 concentrations were 70 ng/ml in NC, dropped to 8 ng/ml ($p<0.05$) in the DC and recovered to 20ng/ml in DE ($p<0.05$). Serum caspase was 3.2 ng/ml for NC, increased to 8.5 ng/ml in DC ($p<0.05$) and reduced to 1.8ng/ml in DE ($p<0.05$). The histology of the pancreas showed distorted, degenerated and shrunken β-cells mass in DC compared with NC and DE groups. The DE group showed clear signs of regeneration of the islet cells which was corroborated by positive Feulgen's reaction compared with the DC group.

Conclusion: The data suggests that the combined VA+GL extract has the potential to effectively reverse pancreatic damage in diabetes.

Key words: Vernonia amygdalina; Gongronema latifolium; GLUT 2; Caspase 3; Blood glucose

INTRODUCTION

Diabetes mellitus is recognized as a group of metabolic disorders with the common element of hyperglycaemia due to either insulin deficiency or impaired effectiveness of insulin action which may be inherited or acquired (38). The hyperglycaemia is a manifestation of several historical anomalies and depicts a complete deterioration of the endocrine control rather than a pathogenic factor. Individual chemotherapeutic agents act only on part of the pathogenic process and only to a partial extent (26) hence cannot address the problem holistically. The morbidity and mortality from the disease continues

to rise despite the availability of huge number of pharmacologic agents for treatment. Thus a multimodal therapeutic approach is required (39).

GLUT 2 is a glucose transporter which facilitates the movement of glucose across cell membranes. It is the principal transporter of glucose in the pancreatic beta cell where it initiates the first step in glucose mediated insulin secretion. It functions as a glucose sensor in pancreatic β-cells of rodents (16) and is found primarily in cellular membranes of liver and the pancreatic beta cells (17). Caspase 3 on the other hand is a member of the cysteine – aspartic acid protease family, which plays a central role in the execution phase of cell apoptosis (11). β-cell apoptosis is one of the critical events that contribute to the pathogenesis of type 1 diabetes and apoptosis mediated by caspase 3 has been reported to be the primary mechanism through which β-cells are destroyed. Consequently, caspase 3 mediated β-cell apoptosis is said to be an important step in the development of diabetes (30). Studies have also shown that although type 1 diabetes is an autoimmune disease which occurs as a result of the selective destruction of insulin producing pancreatic cells by antigen- specific-T cells (27), apoptosis is a fundamental process involved in the destruction of the insulin producing cells (9;27). β-cell apoptosis is also reported to facilitate cross-presentation of islet cells antigens which is a step critical in the activation of cell specific T cells, a major requirement for the onset of diabetes mellitus (1,34,39)

Vernonia amygdalina Del (African bitter leaf), Azadirachta indica A. Juss (Neem) andGongronema latifolium (locally known as Utazi in Eastern Nigeria) are some of the plants used traditionally in the management of diabetes in African and Asia (18). Generally, plants exert their beneficial effect on diabetes via various mechanisms such as: carbohydrate/lipid metabolism modulation in the liver (via induction of key enzymes), influence on β-cells integrity, aldose reductase activity, and antioxidant defence system regulation, and glucose uptake and utilization (37). Some plants possess phytochemicals that interfere with carbohydrate digestion and absorption, and may have insulin-like activity or able to inhibit insulinase activity (22).Thus plants offer exciting opportunities for the development of novel therapeutics.

Vernonia amygdalina Del belongs to the Compositae family and grows extensively in a range of ecological zones in tropical Africa (14). The antihyperglycemic action of the plant (7), hypoglycaemic effect (18); hypolipidemic and antihyperlipidemic action of the aqueous leaf extract (8) and its protective effect on kidneys of diabetic rats (8) have been reported. Gongronema latifolium on the other hand is a tropical rainforest plant belonging to the Ascepiadaceae family. (10). The hypoglycemic, hypolipidaemic (31) and anti-oxidant (29) properties of its leaves extracts have been articulated. (28) reported on its anti-inflammatory effects, (20) reported on its antioxidant properties whereas and we (3) reported on its regenerative properties on pancreatic b-cells. The combined extracts of VA and GL have been reported to improve sperm parameter and testicular damage in STZ induced diabetic rats (4), enhance insulin secretion and reproductive hormone level in diabetic state (5) and the hypoglycaemic potential of the combined extracts have been shown to compare favourably with that of metformin (6). Despite these reports, the molecular mechanism of the anti-diabetic actions of Vernonia amygdalina and Gongronema latifolium are still not well understood. In particular, the benefits of a combined therapy are not known.

In developed countries such as United States, it is estimated that plant–based drugs constitute as much as 25% of the total drugs available, whereas, in developing countries including China and India, the contribution is as much as 80% (23). In Africa, remedies made from indigenous plants play a crucial role in the health of millions, with growing number of people relying more and sometimes exclusively on plants for treatment of various illnesses and ailments (2).

2. MATERIALS AND METHOD

Fresh and matured Gongronema latifolia (P.E.S (BOT)/ HERB/UC/ 718) and Vernonia amygdalina (P.E.S (BOT)/ HERB/UC/188) leaves were bought from local market in Calabar municipality of Cross River State, Nigeria. The leaves were identical with previously deposited specimen in the herbarium unit, Department of Botany, University of Calabar, Calabar, Nigeria. The leaves were washed severally with clean tap water followed with distilled water and thereafter allowed to completely drain. The leaves were then air dried under shade and ambient temperature. The air dried leaves were homogenized using an electric blender into powder form. The powdered plant materials were respectively soaked in

plastic buckets and methanol added, the solvent to solute ratio being 2:1 for 48 hours with intermittent agitation. The solution was filtered using a chess cloth followed by the filtrate being filtered again through Whatman No1 filter paper of pore size 0.45micrometer. The filtrate was placed in beakers and allowed to concentrate in a water bath by evaporation at 40°C to total dryness producing 93g of crude extract each.

2.1 Induction of Diabetes

Diabetes was induced in overnight fasted experimental animals by a single dose of intraperitoneal injection of freshly prepared streptozocin (STZ) 45mg/kg body weight reconstituted in 0.1M sodium citrate buffer (pH4.5-5.0) as solvent. Diabetes was ratified in the STZ treated rats by checking their fasting blood sugar concentration 48hrs after STZ injection using a glucometer (on-call-plus) and rats having fasting blood sugar above 180mg/dl were regarded to be diabetic and were included in the study.

2.2 Experimental animals:

Fifteen (15) adult albino rats, weighing 80-140g, were used for this study. The animals were kept in properly ventilated cages and at a room temperature of about 27oC and 12 hour light/dark cycle.All experiments were conducted in accordance with international guidelines for the care and use of laboratory animals.

2.3 Study design

The animals were divided into 3 groups of 5 rats each. Group A was the Normal control group which was given tap water. Group B was the Diabetic control group. The animals in this group were induced for diabetes and were given normal feed and water. They received no treatment. Group C were induced for diabetes and were treated with the combined extracts of VA and GL (400mg/kg twice daily) administered through orogastric tube (Table 1).

Table 1

Experimental design

Group	Agent administered	Dose
A (Normal control)	Feed and water	Ad libitum
B (Diabetic Control)	STZ	45mg/kg single dose
C (Diabetic+Extract)	VA + GL .	400mg/kg (ratio1:1) twice daily for 28 days

After twenty eight days of treatment the animals were sacrificed using chloroform inhalation. During this process blood was collected by cardiac puncture and organs collected for biochemical and histological assessments.

2. 4 Determination of GLUT 2 and Caspase 3 expression

2.4.1 Sample collection and Preparation

Following euthanasia, the pancreas was collected, cleaned of excess blood and weighed. The tissue was then minced to small pieces and homogenized in PBS. The resulting suspension was subjected to ultra-sonication and centrifuged for 15 minutes. The supernatant was then collected and used for GLUT 2assay while the serum was collected for Caspase 3 assay.

2.4.2 Caspase 3 assay: CASPASE-3 ELISA kit applies the competitive enzyme immunoassay technique utilizing a monoclonal anti-CASPASE-3 antibody and a CASPASE-3-HRP conjugate. The assay sample and buffer were incubated together with CASPASE-3-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells were decanted and washed five times. Thereafter, the wells were incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution was added to stop the reaction, which turned the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the CASPASE-3 concentration since CASPASE-3 from samples and CASPASE-3-HRP conjugate compete for the anti-CASPASE-3 antibody binding site. Since the number of sites is limited, as more sites are occupied by CASPASE-3 from the sample, fewer sites are left to bind CASPASE-3-HRP conjugate. A standard curve is plotted relating the intensity of the color (O.D.) to the concentration of standards. The CASPASE-3 concentration in each sample was then interpolated from the standard curve.

2.4.3 Glut2Assay: This assay is based on the sandwich ELISA principle. Each well of the supplied microtiter plate was pre-coated with a target specific capture antibody. Samples were added to the wells which caused the target antigen to bind to the capture antibody. Unbound sample was washed away. A biotin-conjugated detection antibody was then added which bound to the captured antigen. Unbound biotinylated detection antibody was washed away. An Avidin-Horseradish Peroxidase (HRP) conjugate was then added which bound to the biotin. Unbound Avidin-HRP conjugate was washed away. A TMB substrate was thereafter added which reacted with the HRP enzyme resulting in color development. A sulfuric acid stop solution was added to terminate color development reaction and then the optical density (OD) of the well was measured at a wavelength of 450 nm ± 2 nm. An OD standard curve was generated using known antigen concentrations and the OD of the sample was compared to the standard curve in order to determine its antigen concentration. The investigations were carried out according to the manufacturer's instructions and the kits were obtained from LifeSpan BioSciences, (LSBio) Inc for Rat CASP3/Caspase 3 and Glut2 assays.

Ethical Approval was obtained from the Faculty Animal Research Committee of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria

3.0 RESULTS

3.1 Blood glucose concentrations: Table 2 shows the blood glucose concentrations for the experimental groups. There was a significant increase ($p<0.05$) in the blood glucose of the DC group compared to the NC group. The blood glucose level of the normal control group and the treatment group was significantly reduced ($p<0.05$) compared to the DC group at the end of the experiment.

TABLE 2. Blood glucose concentrations for the experimental groups

GROUPS	BLOOD GLUCOSE (mg/dl)
Normal control	60 ± 7.28
Diabetic control	257 ± 4.43a
Diabetic+Extract	116 ± 10.11*

Data are represented as Mean ± SEM, n=6

*Significantly different from DC at p<0.05

a= significantly different from NC at p<0.05

3.2 Serum Caspase 3: Fig 1 shows the serum concentration of caspase 3 in the pancreatic tissue of the different experimental groups. The level of the enzyme in the diabetic control group was significantly higher (8.5ng/ml) (p<0.05) compared to the normal control (3.2 ng/ml) and the treatment group (1.8ng/ml) while that of the treatment group was significantly reduced (p<0.05) compared to the diabetic control.

3.3 Pancreatic GLUT 2: Fig 2 shows the serum concentration of GLUT 2 in the various experimental groups. In the diabetic control group, there was a significant reduction in the serum level of the enzyme (8ng/ml) (p<0.05) compared to the normal control (70ng/ml). In the treatment group, there was a significant increase (20ng/ml) (p<0.05) in the serum concentration of the enzyme compared to the diabetic control

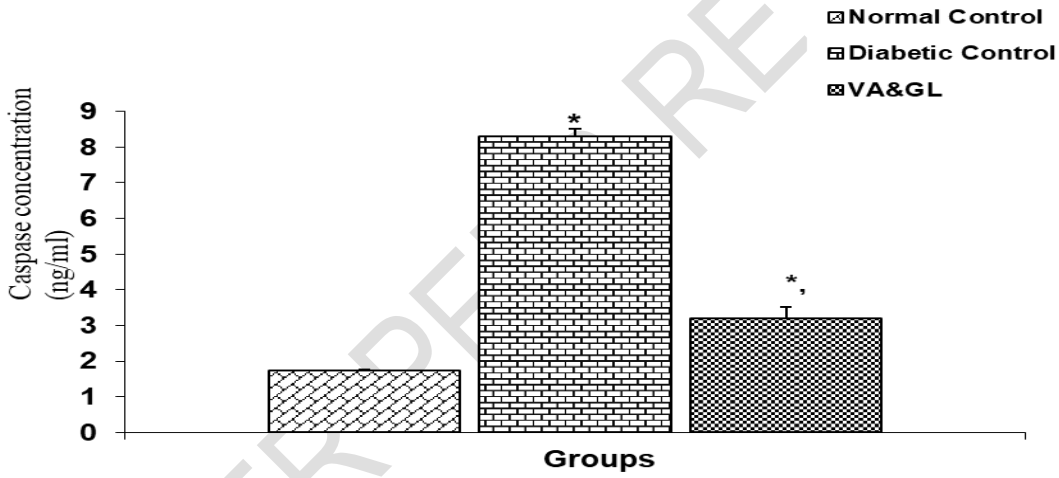

Figure 1: Comparison of Caspase 3 serum concentrations in the different experimental groups.
Values are expressed as mean ± SEM, n =5.
*significantly different from Normal Control at p<0.05;
a = significantly different from Diabetic control at

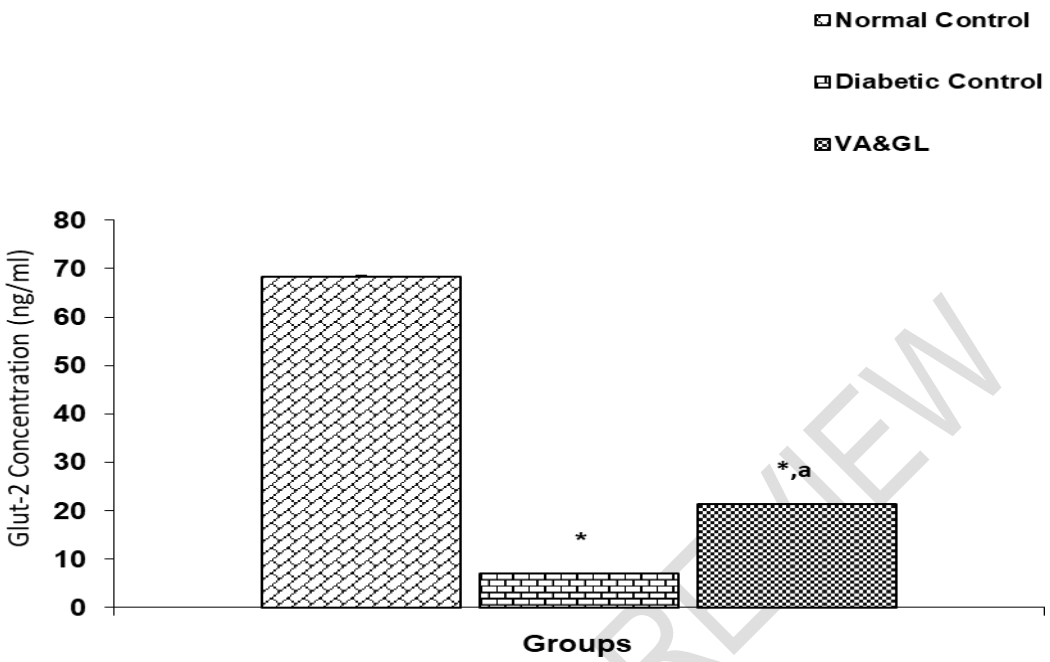

Figure 2: Comparison of GLUT-2 serum concentrations in the different experimental groups.
Values are expressed as mean + SEM, n = 5.
*significantly different from NC at $p<0.05$;
a = significantly different from DC at $p<0.05$.

3.4 **HAEMATOXYLIN AND EOSIN**

Staining of the pancreas from the normal control group revealed normal and prominent islet of Langerhans and acinar cells that were strongly positive to Feulgen's reaction. A pancreatic duct was also observed in the specimen (Plate 1). In the diabetic control group, the Islet of Langerhans appeared shrunken and necrotic as the islet cells showed no reaction to Feulgen's reaction which is indicative of necrosis that had occurred in the cells. However, the acinar cells were strongly positive to Feulgen's reaction. Macrophages (inflammatory cells) were observed in the islet, the cellularity of the islet cells was reduced and fibrous tissues were seen showing damage to the islet cells (Plate 2).

For the Diabetic+Extract group that received the combined extracts of VA and GL, the islet of Langerhans was prominent and appeared normal with cells that were strongly positive to Feulgen's reaction but compared to the normal control group, there was reduced cellularity in the islet. Pancreatic duct was observed and the acinar cells appeared normal (Plate 3)

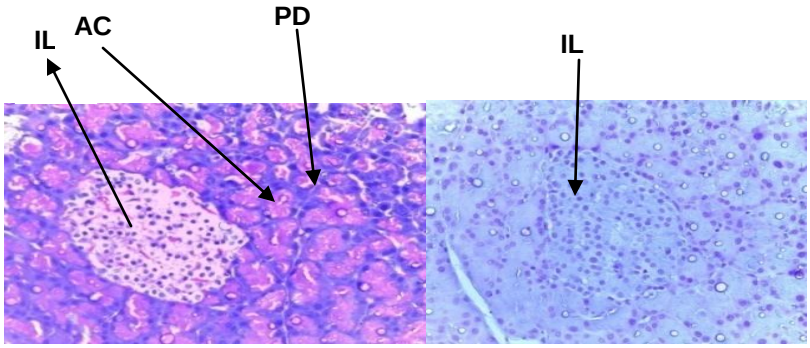

PLATE 1: Photomicrograph of pancreas of Normal Control animals, stained with H & E (A) and Feulgen's reaction (B) (X400)
The islet of langerhans and the cells are prominent
Acinar cells are present and there is a pancreatic duct observed in the specimen
AC – Acinar cell, IL – Islet of Langerhans, PD- Pancreatic duct

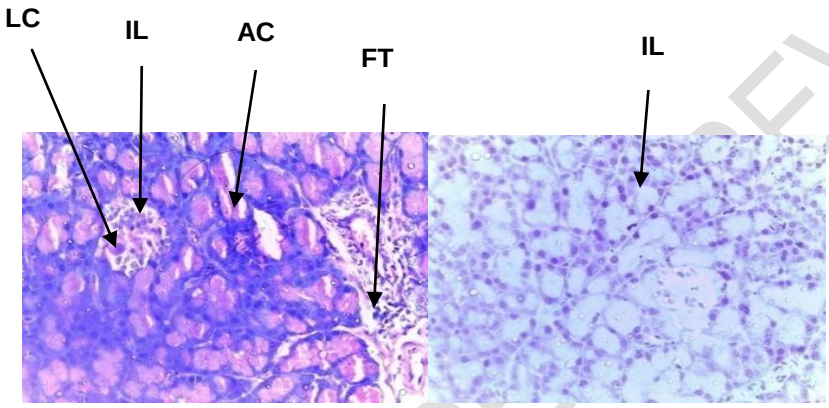

PLATE 2: Photomicrograph of pancreas of Diabetic Control animals, stained with H & E(A) and Feulgen's reaction (B) (X400)
The Islet of Langerhans appear shrunken and pyknotic
Lymphocytes (inflammatory cells) are present in the islet
Cellularity of the islet cells appear reduced and
Fibrous tissues are present in the islet
Acinar cells are present and appear normal
IL – Islet of Langerhans, **LC – Lymphocytes**
FT – Fibrous tissue, **AC- Acinar cells**

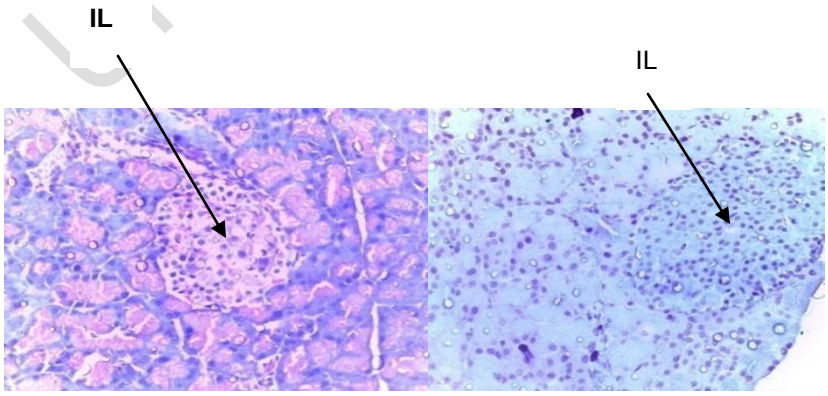

PLATE 3: Photomicrograph of Pancreas of Group D animals given 400mg/kg of combined extracts of *Vernonia amygdalina* and *Gongronema latifolium* (200mg/kg each) stained with H & E (A) and Feulgen's Reaction (B) (X400)

There is a prominent islet of Langerhans, Pancreatic duct and normal acinar cells.
The Islet with cells are strongly positive for Feulgen's Reaction.
IL- Pancreatic Islet

3.5 DISCUSSION

Diabetes was successfully induced with STZ (45 mg/Kg) as evidenced by the sustained increase in blood glucose concentration in the diabetic control group compared with the normal control group. In addition, GLUT 2 was significantly under-expressed in the diabetic group compared with the normal control, consistent with previous studies (32), and clearly demonstrating successful STZ diabetes. Moreover most of the islet cells from the diabetic control group were negative for the Feulgen's reaction, consistent with damage to the DNA, which is a known consequence of STZ toxicity (35). STZ selectively damages the pancreatic beta cells because it shares with glucose the same transporter GLUT 2, which is abundantly expressed in these cells (12,25). The resulting hyperglycaemia can induce chromatin remodelling and further DNA damage (15), and excessive production of free radicals (36). STZ also triggers destructive immune and inflammatory reactions within the pancreatic islets by causing the release of glutamic acid decarboxylase autoantigens (33). In this study inflammatory cells (lymphocytes) were located within the pancreatic islets harvested from the STZ treated group consistent with this possibility. The cytoarchitecture of the pancreatic Islets were markedly distorted by STZ treatment, with the tissues shrunken and degenerated.

Treatment with the combined extracts VA+GL substantially reversed the above changes; The Diabetic+Extract group had a much smaller increase in blood glucose, normal islet cytoarchitecture, positive Feulgen's reaction and increased GLUT 2 expression. VA+GL appeared to offer some protection against STZ induced diabetes and damage to the pancreatic beta cells. Although the molecular mechanism of this protection is not clear, these extracts have been shown to have the potential to stimulate the regeneration of the pancreatic beta cells (3). The cytoarchitecture of the Islet from the Diabetic+Extract group was less distorted; the cells had histological features similar to those of the normal control group. The beta cells also showed strong positive reaction to Feulgen's test, indicating possible extract-induced regeneration and recovery from the diabetic insults. This observation is in agreement with previous reports of the potentials of these extracts to cause a regeneration of pancreatic beta cells in STZ induced diabetic rats (3,13). That way VA+GL could ensure sustained insulin release and lowered blood glucose. The VA+GL treated group also expressed a slightly higher but significant level of GLUT 2 compared with the untreated diabetic group, although the level remained lower compared with normal control. This again may be related to the potential of the extracts to reverse STZ-induced pancreatic beta cell damage.

Caspase 3 was over expressed in the diabetic control group compared with the normal control group, consistent with previous reports (19) and suggests an increase in apoptotic index. In a study by (24), it was also found out that Caspase-3 level was significantly elevated in diabetic rat pancreas while treatment with curcumin resulted in a significant reduction of the enzyme. Curcumin is a plant derivative known to have potent antioxidant properties (21). Thus, increased Reactive Oxygen Species generation and the simultaneous decrease in antioxidant defence mechanism in diabetic patients appear to contribute to organ damage associated with the disease (36). Vernonia amygdalina, which is part of the combined extracts used in the current study has a rich antioxidant property capable of ameliorating the damage caused by free radicals to tissues including the pancreas (13). Thus the decrease in caspase 3 activity in the Diabetic+Extract group is consistent with the ability of these extracts to counteract the free radicals and thereby prevent apoptosis.

CONCLUSION

Combined methanolic leaf extracts of Vernonia amygdalina and Gongronema latifolium contain phytochemicals which have antioxidant and other medicinal properties that have the potentials of reversing pancreatic damage and ameliorating diabetic insults in STZ treated animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist for this work

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the University of Calabar Faculty Animal Research Ethics Committee (FAREC-FBMS). Approval number: FAREC/PA/010BC31012.

REFERENCES

Albert, M. L., B. Sauter, and N. Bhardwaj. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. *Nature.*1998. 392**:**86-89

Ahmad, I., Agil, F & Owais, M. (Eds). *Modern phytomedicine: Turning medicinal plants into drugs*. West Sussex, England. John Wiley and Sons. 2006. pp 2-24

Akpaso, M. I., Atangwho, I. J., Akpantah, A., Fischer, V., A, Igiri, A.O & Ebong, P. E. Effect of combined leaf extracts of VA and GL on the pancreatic beta-cells of STZ induced Diabetic wistar rats. *British Journal of Medicine and medical research*. 2011. 1(1) 22-34

Akpaso, M. I., Igiri, A. O., Fischer, V. A., Fischer, C .E & Asuquo, O. R Combined methanolic leaf extracts of Vernonia amygdalina and Gongronema latifolium improves sperm parameter and testicular damage in STZ induced diabetic Wistar rats. *Journal of Biology, Agriculture and Healthcare.* The International Institute for Science, Technology and Education (IISTE). 2017. 7 (6)

Akpaso, M. I & Elot, K.E. Combined methanolic leaf extracts of Vernonia amygdalina and Gongronema latifolium enhances insulin secretion and Reproductive hormone level in Diabetic state.*International journal of health and Pharmaceutical Research*. 2017. 3(1)

Akpaso, M I, Igiri, A.O & Odey, P.A A comparative study on the effect of Combined methanolic leaf extracts of Vernonia amygdalina and Gongronema latifolium and Metformin on the Pancreatic beta cells of STZ induced Diabetic Wistar rats. *Asian journal of Pharmacy, Nursing and Medical sciences*. 2017, 5(2)

Akah, P., Njoku, O., Nwanguma, A & Akinyuli, D. Effect of aqueous leaf extract of Vernonia amygdalina on blood glucose and triglyceride level of alloxan-induced diabetic rats. *Animal Research International*, 2004. 1(2): 90-94

Atangwho, I. J., Ebong, P. E., Eteng, M. U., Eyong, E. U., & Obi, A. U. Effects of *Vernonia amygdalina* Del Leaf on kidney function of diabetic rats. *International Journal of pharmacology*. 2007. 3(2), 143-148

Bach, J. F., L. Chatenoud, A. Herbelin, J. M. Gombert, and C. Carnaud. Autoimmune diabetes: how many steps for one disease? Res. Immunol. 1997. 148**:**332-338.

Chattopadhyay, R. R. Possible mechanism of antihyperglycaemic effect of Gymnena Sylvestre leaf extract, part I. *General Pharmacology.* 1998. 31:495-496.

Creagh, E. M., H. Conroy, and S. J. Martin. Caspase-activation pathways in apoptosis and I mmunity. Immunol. Rev. 2003. 193:10-21.

Dufrane, D., Van Steenberghe, M., Guiot Y., Goebbels, R. M., Saliez, A & Gianello, P. STZ-induced diabetes in large animals (pigs, primates): role of GLUT 2 transporter and B- cell plasticity. *Transplantation*. 2006. 81: 36-45

Ebong, P. E., I. J Atangwho., E. U. Eyong., C. Ukere & A. U. Obi. 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, 2006. pp. 83-89,

Farombi, E. OA frican Indigenous plants with chemotherapeutic potentials and biotechnological plants with production of bioactive prophylactic agents. *African journal of Biotechnology,* 2003. 2(12) 662-671.

Ghiraldini, F. G., Crispim, A. C & Mello, M. Effects of hyperglycaemia and aging on nuclear sirtuins and DNA damage on mouse hepatocytes. *Molecular Biology of the Cell Journal*. 2013. 24 (15) 2476-76

Guillam M T, Hümmler E, Schaerer E, Yeh J I, Birnbaum M J, Beermann F, Schmidt A, Dériaz N, Thorens B, Wu J Y. "Early diabetes and abnormal postnatal pancreatic islet development in mice lacking Glut-2*". Nature Genetics. 1997.17 (3): 327–30.*

Gwyn W. Gould; Helen M. Thomas; Thomas J. Jess; Graeme I. Bell. "Expression of human glucose transporters in Xenopus oocytes: kinetic characterization and substrate specificities of the erythrocyte, liver, and brain isoforms". *Biochemistry*. 1991. 30 (21): 5139–5145

Gyang, S. S., David, D. N. & Elijah, N. S. Hypoglycaemic activities of vernonia amygdalina (chloroform extracts) in normoglycaemic and alloxan induced hyperglycaemic rats. *Journal of pharmacy and Bioresources.* 2004. 1(1): 61-66.

Hashish, H. A and Kamal, R. N. Effect of curcumin on the expression of caspase-3 and Bcl-2 in the spleen of diabetic rats. *Journal of Experimental and Clinical Anatomy*. 2015. 14 (1) 18- 23

Hernandez, N. E., Tereschuk, M. L & Abdola, L. R. Antimicrobial activity of flavonoids in medicinal plants from Tafi del valle. *Journal of Ethnopharmacology*. 2000. 73 (1-2): 317-322

Hsuuw, Y. D., Chang, C.K Chan W.H, and Yu, J. S. Curcumin prevents methylglyoxal- induced oxidative stress and apoptosis in mouse embryonic stem cells and blastocysts. Journal of cellular Physiology. 2005. 205 (3): 379-86

Jelodar, G., Khaksar, Z & Pourahmadi, M. Endocrine profile and testicular histomorphometry in adult rat offspring of diabetic mothers. *Journal of Physiological Sciences*. 2009. 59:377–382.

Joy, P. P., Thomas, J., Mathew, S & Skaria, B. P. In Bose T.K et al (eds) Medicinal plants. *Tropical Horticulture*. 1998. 2:449-632

Kamel, R., Hashim A and Ali, S. Palliative effect of curcumin on STZ induced diabetes in rats. Int J Pharm Pharm Sci. 2014. 1491 (6) Suppl 2, 558 – 63

Lenzen, S. The mechanism of alloxan and STZ induced diabetes. *Diabetologia. 2008.* 51: 216-226

Luna, B & Feinglos, M. N. Drug-induced hyperglycemia. *Journal of the American Medical Association.* 2001.286 (16): 1945–8.

Mathis, D., L. Vence, and C. Benoist. β-Cell death during progression to diabetes. Nature. 2001. 414**:**792-798

Morebise, O., Fafunso, M. A., Makinde, J. M., Olajide, A. O & Awe E. O (2002). Anti-inflamatory properties of leaves of G. latifolium. *Phytotherapy Research,*16 suppl. 1:S75-7

Morebise, O. A (2015) A review on Gongronema latifolium, an extremely useful plant with great properties.*European Journal of medicinal plants*. 2015. 10(1):1-9

Nicole Liadis, Kiichi Murakami, Mohamed Eweida, Alisha R. Elford, Laura Sheu, Herbert Y. Gaisano, Razqallah Hakem, Pamela S. Ohashi and Minna Woo. Caspase-3-Dependent β-Cell Apoptosis in the Initiation of Autoimmune Diabetes Mellitus. *Molecular and Cellular Biology*. 2005. 25 (9)

Ogundipe, O. O., Moody, J. O., Akinyemi, T. O & Raman, A. Hypoglycaemic potentials of methanolic extracts of selected plant foods in alloxanised mice. *Plant food and Human Nutrition.* 2003. 55(3): 1-7

Orci L, Unger RH, Ravazzola M. Reduced b-cell glucose transporter in new onset diabetic BB rats. J Clin Investig. 1990. 86:1615–1622

Paik, S. G., Fleischer, N & Shin, S. I. Insulin – dependent diabetes mellitus induced by subdiabetogenic doses of STZ: Obligatory role of cell mediated autoimmune processes. *Proceedings of the National Academy of Science*, USA. 1980. 77: 6129-6133

Rovere, P., C. Vallinoto, A. Bondanza, M. C. Crosti, M. Rescigno, P. Ricciardi-Castagnoli, C. Rugarli, and A. A. Manfredi. Bystander apoptosis triggers dendritic cell maturation and antigen-presenting function.J. Immunol. 1998. 161:4467-4471.

Szkudelski, T. The mechanism of alloxan and streptozocin action in Beta cells of the rat pancreas. *Physiology Research.* 2001. 50:536-546.

Sharma, B., Kumar, S., Siddiqui, S., Ram, G & Chaudhary, M. Ameliorative effects of aqueous leaf extracts of aloe arboresiens on antihyperglycaemia and antihyperlipidaemia alterations in alloxan-induced diabetic mice. *Journal of Investigational Biochemistry*, 2013. 2(2): 71-76

Tiwari, A. K & Rao J.M. Diabetes Mellitus and multiple therapeutic approaches of phytochemicals: present status and future prospects. *Current science.* 2002. 83(1) 30-37.

World Health Organisation (WHO). Diabetes mellitus fact sheet (2018). Acccessed online on 15/05/2019

Zhang, Y., B. O'Brien, J. Trudeau, R. Tan, P. Santamaria, and J. P. Dutz. In situ beta cell death promotes priming of diabetogenic CD8 T lymphocytes. J. Immunol. 2002. 168**:**1466-1472