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A STUDY OF THE EFFECT OF *VERNONIA AMYGDALINA* LEAF EXTRACT ON GLUCOSE AND HISTOLOGY OF THE PANCREAS IN ALLOXAN INDUCED ALBINO RATS

Brisibe Ngozi, Nwachuku Edna Ogechi and Elekima Ibioku*

Department of Medical Laboratory Science, Rivers State University, Port Harcourt.

***Corresponding Author: Dr. Elekima Ibioku**

Department of Medical Laboratory Science, Rivers State University, Port Harcourt.

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ABSTRACT

Diabetes mellitus is a chronic disease caused by insulin deficiency by pancreatic β cells or due to inability to respond to insulin produced leading to high blood glucose concentrations which may result in damage of some body organs including blood vessels and nerves. The increased prevalence of diabetes mellitus in Africa has necessitated the quest for natural remedies for its treatment and management. This work tested the efficacy of *Vernonia amygdalina* leaf extract (Bitter leaf) on alloxan induced diabetic rats as well as demonstrated the histological features of the pancreas when not induced or treated, induced and untreated then induced and treated. The study was carried out on 34 adult albino Wister rats which were divided into three (3) groups; A (neither induced nor treated), B (induced and untreated), and C (induced and treated). Comparisons between groups A (6.15±0.85) mmol/l and B (9.81±3.76) mmol/L showed fasting blood glucose significantly high and between A and C (induced and treated with 1.6g/kg bodyweight of *Vernonia amygdalina* extract), there was no significant change in the fasting blood glucose level (P=0.4545). Comparison between groups B and C (6.41±0.53) showed fasting blood glucose level significantly decreased t (P=0.0036). Furthermore, the research indicated histologically that *Vernonia amygdalina* extract ameliorated the damage caused by the diabetogen (alloxan) on islets β cells of Langerhans in the pancreas upon administration of the leaf extract for 21 days on daily basis.

KEYWORDS: Diabetes mellitus is a *Vernonia amygdalina* leaf extract for 21 days on daily basis.

1. INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders of carbohydrate, lipid and metabolism characterized by persistent hyperglycaemia in which there is a relative or absolute defect in insulin secretion, peripheral resistance to insulin action or both.[1,2,3] It is a condition in which glucose in the body is not properly utilized.[4,5,6] It is either that the pancreas no longer produces sufficient insulin or that the cells stop responding to the insulin produced.[4,7] As a result, glucose builds up in the blood instead of being absorbed by the cells in the body with resultant effect on the body cells being starved of energy despite high blood glucose levels.[2,8]

Globally, the prevalence of diabetes is rapidly increasing.[9] In 2014, 387 million people were estimated to have diabetes worldwide with type 2 diabetes accounting for 90% of the cases. Each year 1.5 to 4.9 million deaths were estimated from 2012 to 2014 as a result of diabetes.[5,10] Nigeria has the highest number of people living with diabetes with an estimated 3.9 million of the adult population aged 20 – 79 years old in Sub-Sahara Africa.[5,8,10] Diabetes is a major health concern due to its high prevalence and potential deleterious effects on the patient's health condition.[11] Management and control of diabetes mellitus focuses on maintaining normal glucose levels without causing hypoglycaemia.[11] This can be achieved by the use of dietary therapy, exercise and use of appropriate medication.[11]

In developing countries such as Nigeria, a significant population of diabetic patients find it increasingly difficult to manage hyperglycaemic conditions, the major cause of the complication of diabetes mellitus, not only because of the high cost of these antidiabetic drugs, but also for their deleterious side effects which may possibly lead to death.[12] Medicinal plants have formed the basis of healthcare throughout the world since the earliest days of humanity and are still widely used in the management and treatment of diseases.[13,14,15] Several plants have been explored for their antidiabetic activities and available literature indicates that more than 800 plant species have hypoglycaemic activities.[16]

In Nigeria, Phyto-pharmaceuticals have been developed with proven efficacy for the treatment of many ailments.[17] There has been increasing demand for the use of plant products with antidiabetic activity due to low

cost, easy availability and mild side effects.[17,18] Therefore, plant materials are always being scrutinized and explored for their effect as hypoglycaemic agents.[18]

Vernonia amygdalina is a perennial shrub of about 2-5m in height that is predominantly found in tropical Africa. It is popularly known as bitter leaf because of its bitter taste.[19,20] It belongs to the family known as Asteraceae. It is cultivated in Nigeria mainly for its nutritional value. Extracts from the plant have been used in traditional folk medicine as antidiabetic, hepatoprotective, nephro-protective, antioxidant, anticancer, antimalarial, anti-helminthic and antimicrobial.[14,21] These pharmacologic activities exhibited by *Vernonia amygdalina* are as a result of the presence of different phytochemicals found in it.[22,23,24,25] The quest to manage Diabetes Mellitus using natural remedies in dietary form necessitated the investigation on the hypoglycaemic efficacy of *Vernonia amygdalina* leaf extract in Alloxan induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Materials

Materials used in this study include: Albino rats, fluoride oxalate bottles, accu-check glucometer, centrifuge, spectrophotometer, gavage tube, 10% formal saline, bitter leaf (*Vernonia amygdalina)* purchased from Mile 3 market, Port Harcourt, H&E stain, alloxan monohydrate and glucose reagent purchased from Randox laboratories, United Kingdom.

2.2 Experimental Animals

Experimental animal used were adult male albino rats of about 12 weeks old. A total of 34 rats weighting approximately 125g was obtained from University of Port Harcourt, Rivers State. The experimental animals were housed in the Department Medical Laboratory Science animal house in well-ventilated cages and were acclimatized for 3 weeks with water and chicken finisher feds available *ad libitum*. More so, the animals were kept at room temperature of 12 hours light and dark cycle per day. The animals cared for with standard laboratory guidelines for handling animals.

2.3 Experimental Design

The animals were divided into these groups namely: Group A, Group B and Group C. Group A had a total of 8 rats and were neither induced nor treated, so served as control. Group B had a total of 13 rats of which diabetes was induced but not treated with *Vernonia amygdalina* leaf extract while Group C had a total of 13 that of which diabetes was induced and were also treated with aqueous leaf extract of Vernonia *amygdalina.*

2.4 Induction of Hyperglycaemia in Animals

A total of 26 rats were used in the induction of diabetes. However, before the induction, the blood glucose levels of these rats were checked using accu-check glucometer by collecting blood from the tail vein and were found to be within of 3.3 – 5.5mol/L. Thereafter, the rats were subjected to overnight fasting (12 hours) with exception to water followed injection of 0.59mg/kg bodyweight of diabetogen (alloxan monohydrate) that was freshly prepared by diluting 65mg of alloxan in 0.5ml of normal saline intraperitoneally. After 48 hours, their fasting blood glucose levels were determined with a glucometer again. To ascertain that diabetes was actually induced, the blood glucose level was checked again after 7 days and those with glucose level of 10-18.0mmol/L were used for the experiment and were treated with *V. amygdalina* leaf extract.

2.5 Plant (leaves) Preparation and Extraction

Fresh leaves of *V. amygdalina* were bought from mile 3 market in Port Harcourt and washed gently with water to eradicate debris. The washed leaves were then sun dried for 2 weeks and grinded into power which was filtered using 1.0mm sieve to obtain fine power. 150 grams of the fine power was weighed and placed into a beaker containing 800ml of distilled water. The content of the beaker was properly mixed and allow standing at room temperature for 24 hours before filtering with 0.1mm sieve. The filtrate was stored in a Winchester bottle at 4^{o}C prior to use.

2.6 Administration of *V. amygdalina* Leaf Extract

At the time of administration, the filtrate *V. amygdalina* in the Winchester bottle was properly mixed and aliquoted into in smaller test tube. The aqueous extract of *Vernonia amygdalina* was administered orally by gavage method. The group C rats were treated with 0.2g/ml (which is equivalent to 1.6g/kg bodyweight) of the extract daily for 21 days.

2.7 Collection and Preparation Specimens

Blood was collected directly from the heart by cardiac puncture using a 5ml syringe after the rats were anaesthetized using chloroform. The blood sample was transferred into a fluoride oxalate bottle. Thereafter, it was spun in a centrifuge for 10 minutes at 4000rpm to obtain plasma for the estimation of glucose concentration. Likewise, pancreatic tissues were also excised for histological evaluation. The tissues were washed in normal saline before fixation in 10% formol saline.

2.8 Evaluation of Glucose and Pancreatic Tissue

The laboratory assay of glucose concentration in the plasma samples were based on glucose oxidase method as described by Washko & Rice,[26] while the staining technique used for the examination of the pancreatic tissues was based on H&E staining method as described by Ehrlich.[27]

2.9 Statistical Analysis

GraphPad Prism version 5.03 was used for the statistical analysis. The values obtained were expressed as mean ±S.D. The statistical tool used was one-way ANOVA alongside Turkey's multiple comparative analysis (Post-Hoc) was done. Statistical significance was considered at $P<0.05$).

3. RESULT

3.1 Effect of *V. Amygdalina* Extract on Glucose of Alloxan Induced Albino Rats

Results obtained from this study showed that control rats had glucose values of 6.15±0.85 while alloxan induced rats had glucose value of 9.81±3.76. Rats induced with alloxan and later treated with bitter leaf ha glucose value of 6.41±0.53. the result obtained indicated significant increase in glucose value when control rats were compared with alloxan induced rats. Also, significantly lower values in glucose was observed when alloxan induced rasts were compared with bitter leaf treated rats after hyperglycaemia has been induced with alloxan. However, no significant difference was seen betwenn rats and bitter leaf treated rats after induction of hyperglycaemia using alloxan at $p<0.05$ (table 3.1).

Table 3.1: Comparison of Blood Glucose Level of group A, B, and C

Groups	Control	Group B	Group C	P value	T value	Remark
Glucose (mmol/L)	6.15 ± 0.85^{a}	9.81 ± 3.76^{bc}	6.41 ± 0.53^{ad}	0.001	3.304	S

Values in same row with different superscripts (a, b) differ significantly when control (Group A) was compared with Group B (alloxan treated rats) and group C (alloxan+bitterleaf treated rats). Values in same row with different superscripts (c, d) differ significantly when Group B (alloxan treated rats) was compared with Group C (alloxan+bitterleaf treated rats) at $p<0.05$.

Figure. 3.1. Graphical representation of the blood glucose levels of various groups (A, B and C).

3.2. Effect of *V. Amygdalina* Extract on Histology of the Pancreas of Alloxan Induced

Albino Rats

When the histological morphology of the pancreas of the various groups were considered, the photomicrograph of the untreated rats showed normal pancreatic Islet region (PI) with the alpha cells (AC) and beta cells (BC) of the PI region appearing distinct and normal. More so, the interlobular duct (ILD) and intralobular ducts (LD) appears normal without obstruction and were closely associated with the PI. The lumen (stars) of the serous acini (A) appears distinct and normal (Plate 3.1). However, in alloxan induced diabetic rats, clear cellular degeneration, necrosis, haemorrhage and possible occlusion of islet cells were observed. Loss of pancreatic islet (PI) cells (alpha & beta cells) were also seen with evidence of vacuolation. In addition, the lumen of the serous acini appears abnormally compressed and elongated in some area (Plate 3.2). Furthermore, the interlobular duct (ILD) and intralobular ducts appears occluded with parenchymal infiltration while serous acini (AC) appears degenerated and deeply stained. When the ameliorative ability of *Vernonia amygdalina* leaf extract on alloxan induced diabetic rats were considered (Plate 3.3), the photomicrograph showed regenerating pancreatic islet (PI) cells (alpha cells (AC) & beta cells (BC)), minute vacuolations (stars) with sclerotic condition in some lobules and islet cells. The lumen of the serous acini (circled area) appearing normal and distinct while the interlobular duct (ILD) and intralobular ducts (LD) appeared distinct without obstruction and parenchymal infiltration.

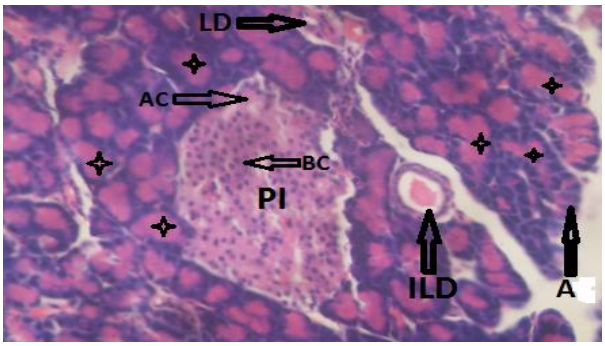

Plate. 3.1: H&E, Mag: x400, Pancreas, Treatment substance: None.

Observation: Photomicrograph of pancreas showing normal pancreatic Islet cells (PI). The Alpha cells (AC) and Beta cells (BC) of the PI region appears distinct and normal. The interlobular duct (ILD) and intralobular ducts (LD) appears normal without obstruction and are closely associated with the PI. The lumen (stars) of the serous acini (A) appears distinct and normal.

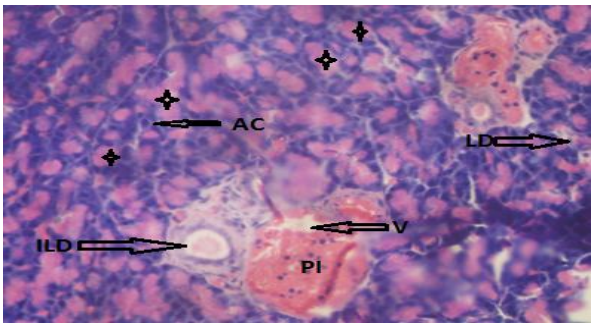

Plate. 3.2: H&E, Mag: x400, Pancreas, Treatment substance: 0.59mg/kg alloxan.

Observation: Photomicrograph of pancreas showing degeneration and loss of the pancreatic islet (PI) cells (alpha & Beta cells). There is evidence of loss of pancreatic islets cells due to induced vacuolation (V). The lumen of the serous acini appears abnormally compressed and elongated in some (stars). Also, serous acini (AC) appears degenerated and deeply stained. The interlobular duct (ILD) and intralobular ducts appears occluded with parenchymal infiltration.

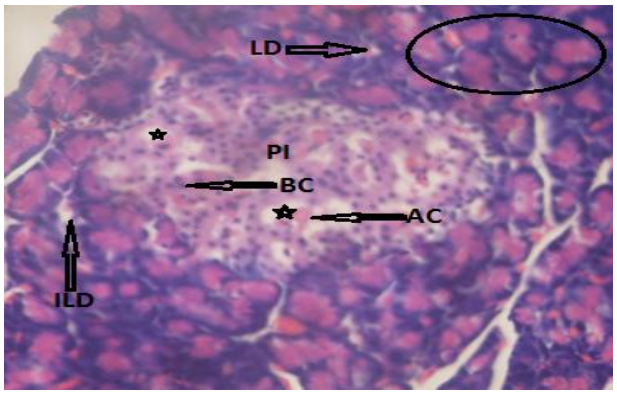

Plate. 3.3: H&E, Mag: x400, Pancreas, Treatment substance: 0.59mg/kg alloxan and 1.6g/kg of *Vernonia amygdalina* leaf extract for 21 days.

Observation: Photomicrograph of pancreas showing regenerating pancreatic islet (PI) cells: Alpha cells (AC) & Beta cells (BC) with minutes vacuolations (stars) with sclerotic condition in some lobules and islet cells. The lumen of the serous acini (circled area) appearing normal and distinct. The interlobular duct (ILD) and intralobular ducts (LD) appearing distinct without obstruction and parenchymal infiltration.

4. DISCUSSION

The effect of *Vernonia amygdalina* leaf extract on glucose and histology of the pancreas of alloxan induced albino rats was carried out in this study. Results obtained indicates that *V. amygdalina* leaf extract has high potency in lowering the glucose level of alloxan induced hyperglycaemic rats and also repair of the pancreatic islets cells.

The comparison of control group (group A) and diabetic induced untreated group (group B) showed significantly lower glucose concentration in the control group with glucose level of 6.15±0.85mmol/l against glucose level of 9.81±3.76mmol/l in the induced diabetic rats. More so, the comparison of control group (Group A) and diabetic induced treated group (group C) showed no significant difference in their glucose concentration. Furthermore, the comparative between diabetic induced untreated group (group B) and diabetic induced treated group (group C) also indicated significantly high level of glucose concentration in the untreated group (Group B) compare to the treated group (group C). The results obtained in this study supports the findings of.[23,24,25] Ijeh & Ejike[23], reported that *Vernonia amygdalina* induced hypoglycaemic effects in their study. Sunday et al.,[24], reported that *Vernonia amygdalina* revived atrophied islets cells in alloxan induced diabetic rats thereby lowering the glucose level in the diabetic rats. More so, Ong *et al*.,[25] also reported that polyphenol rich *Vernonia amygdalina* showed anti-diabetic effects in streptozotoxin induced diabetic rats.

More so, the histological examinations indicated normal features of the pancreas in the control untreated rats (Plate 3.1). However, in alloxan treated rats, clear cellular degeneration, disorientation, necrosis, haemorrhage and possible occlusion of islet cells by alloxan monohydrate were seen (Plate 3.2). Loss and atrophied of pancreatic islet (PI) cells (alpha & beta cells) were also seen with evidence of vacuolation. In addition, the lumen of the serous acini appears abnormally compressed and elongated in some cases (Plate 3.2). More so, the interlobular duct (ILD) and intralobular ducts appears occluded with parenchymal infiltration. Also, the serous acini (AC) appears degenerated and deeply stained. The results obtained in this study correlates with the report of.[28] Szkudelski,[28], reported that alloxan mediates pancreatic beta cell destruction through generation of reactive oxygen species (ROS) established by its decreased product in the cell dialuric acid.

When the effect of *Vernonia amygdalina* leaf extract on alloxan induced diabetic rats were considered (Plate 3.3), the photomicrograph of the pancreas showed regenerating pancreatic islet (PI) cells: alpha cells (AC) & beta cells (BC), minutes vacuolations (stars) with sclerotic condition in some lobules and islet cells. The lumen of the serous acini (circled area) appearing normal and distinct while the interlobular duct (ILD) and intralobular ducts (LD) appeared distinct without obstruction and parenchymal infiltration. The result obtained concur with the work carried out by.[24,29] Sunday *et al*.,[29], reported in their study that *Vernonia amygdalina* with abundant phytochemicals rich in anti-oxidative properties revived atrophied islets cells in alloxan induced diabetic rats.

The hyperglycaemia seen in the group B rats is obviously as a result of alloxan induced destruction of the islet of langerhans in the pancreas. However, the use of *V. amygdalina* extract at a dose of 1.6g/kg bodyweight for 21 days lead to the recovery of the islet cells of the pancreas which in turn restored the glucose concentration to almost normalcy as shown in table 3.1 and Plate 3.3. These results obtained in this present study therefore suggest hypoglycaemic efficacy of *Vernonia amygdalina* as a result of revived islets cells of the pancreas. This ability of *Vernonia amygdalina* leaf extract to ameliorate alloxan induced diabetes and damaged beta cells could be attributed to its antioxidative properties involved in mopping up the reactive oxygen species in circulation induced by alloxan. This finding is also in line with the report of.[19] Owen *et al*.,[19], reported that *V. amygdalina*, prevent lipid peroxidation of lipids in the hepatic cells

which in turn reduced the level of reacting oxygen species. The abundant presence of phytochemical with anti-oxidative properties such as alkaloids, saponins and flavonoids and so on in the aqueous extract of V. amygdalina has been reported to play beneficial role in oxidative induced derangements. Other phytochemical compounds in *V. amygdalina*, such as quinine, tannins, vernodaline, vernomyelin among others have also been reported to plays a renal and hepato-protective role by inhibiting oxidative stress associated diabetes mellitus and acetaminophen toxicity due to the presence of anti-oxidative agents like luteolin and β-gluconiside[21,22] Finally, our finding is also supportive of the reports of.[30] Igile,[30], reported that some of these antioxidants such as luteolin,7-0-betaglucoronoside, luelin and 7-0-betaglucosides isolated from *Vernonia amygdalina* may have caused the commencement in regeneration of beta cells that is accompanied by a gradual release of insulin to clear the excess glucose in circulation.

5. CONCLUSION

The findings from this present study revealed that the aqueous extract of *Vernonia amygdalina* has the ability to lower blood glucose level having been demonstrated in alloxan induced hyperglycaemic rats by regenerating islet cells of the pancreas.

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