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Original Research Article

Antioxidative Effect of Phenolic Extract of *Vitex doniana* Leaves on Alloxan-Induced Diabetic Stress and Histological Changes in the Pancreas of Wistar Rat

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ABSTRACT

Hyperglycemia is implicated in the generation and accumulation of reactive oxygen species (ROS), resulting in endothelial dysfunction and vascular damage. However, plant derived phenolics are primary antioxidants with potential to prevent chronic and oxidative stress-related disorders. The study determined the antioxidative stress capacity and restoration of pancreatic activities by *Vitex doniana* phenolic leaf extract (VDPE) in alloxan-induced diabetic Wistar rats. The ethylacetate leaf extracts is referred to as the phenolic extract. Thirty adult male Wistar rats used in the study were divided into six groups of five each. Diabetes was induced in rats of Groups II - VI with 150 mg/kg of Alloxan and subsequently subjected to the following treatment; Group II: received distilled water; Group III: received VDPE (100 mg/kg). Group IV: received VDPE (200 mg/kg). Group V: received VDPE (400 mg/kg) and Group VI: received glibenclamide (0.5 mg/kg). The rats were sacrificed after 14 days of treatment, blood samples were collected and pancreas excised for histopathological studies. Treatment of alloxan-induced diabetic rats with *V. doniana* phenolic leaf extract showed significant reduction in lipid peroxidation product-malondialdehyde and enhanced concentration of reduced glutathione and vitamin C. Microscopic examination of the pancreatic sections of diabetic rats treated with VDPE showed restoration of pancreatic architecture when compared to the untreated diabetic group. These results indicate *V. doniana* possesses potent antioxidant capacity and prospects of attenuating pancreatic β-cell damage.

***Keywords*:** *Vitex doniana*, Diabetes, Phenols, Antioxidants, Pancreas, Oxidative stress.

Introduction

The incidence of diabetes is on the rise despite increased research efforts on the subject.[1,2] The rate of increase has resulted to over 346 million people affected worldwide and with expected rise to 544 million people in the year 2030.[2,3] Prolonged diabetic condition leads to adverse changes in physiological processes, resulting to complications such as atherosclerosis, cardiovascular disease, stroke, blindness, kidney failure etc.[4,5,6] Type 2 diabetes which accounts for over 90% diabetic incidences worldwide is a heterogeneous disorder characterized by insulin resistance leading to the inability of β-cells of pancreas to compensate for insulin resistance. This *β*-cell dysfunction results to elevated blood glucose.[7] Chronic elevated blood glucose (hyperglycemia) leads to auto-oxidation of glucose and formation of advanced glycated end products (AGEs), usually involved in the generation of reactive oxygen species (ROS). The ROS formed are implicated in lipid peroxidation and processes leading to secondary complications of Type 2 Diabetes.[8,9] The ROS triggered by hyperglycemia, links diverse mechanisms for the pathogenesis of microvascular and macrovascular complications of diabetes.[10]

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Increased oxidative stress is a key factor in the development and progression of diabetes mellitus and its associated complications,[11] as well as the red blood cells damage manifested in diabetic patients. The forgoing indicates that antioxidants and antioxidants containing compounds could be exploited in the treatment of type 2 diabetes and prevention of its chronic vascular complications.[12]
Natural products represent more than 50% of all drugs in clinical use.[13] Many plants with antidiabetic potentials are endowed with enormous therapeutic potentials. The search for a more effective and safer hypoglycemic plant extract for protective and, or curative effects on diabetes and accompanying complications is very important. Traditional medicines have used the leaves of *Vitex doniana* plant for treatment of ailments due to its rich phytochemical content. The leaf extracts of *V. doniana* exhibit analgesic, antifungal and anti-inflammatory activities.[14,15] In developing countries majority of diabetic patients who cannot afford effective but expensive drugs, resort to herbal treatment, as an alternative therapy.[16,17] Extracts of plants can successfully manage diabetes, being always an exemplary source of drugs and derivatives of many currently available drugs directly or indirectly.[18] The present research was designed to determine the extent to which the ethylacetate leaf extract of *V. doniana* referred to *Vitex doniana* phenolic leaf extract (VDPE) attenuated diabetic-induced oxidative stress and adverse histological changes of the pancreas in Alloxan-induced diabetic Wistar rats.

Materials and Methods

Plant collection and preparation
The leaves of *Vitex doniana* were collected from Abakiliki, Ebonyi State, Nigeria, on the 27th day of March, 2016. The samples were

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identified and authenticated by a plant taxonomist, in the Department of Crop Science and Technology, Federal University of Technology, Owerri (FUTO). The leaf sample was deposited with a voucher number, IMSUH/467 at Imo State University Owerri, Nigeria. *Vitex doniana* leaves were air dried at room temperature, pulverized to powder with electric blender. The pulverized leaves were divided into three portions of 200 g. Each portion of the leaf powder was macerated in 800 ml absolute methanol, agitated for 72 hrs. The setup was filtered using cheese cloth and Whatman No. 1 filter paper. The filtrate of the three portions was pooled together and concentrated using rotary evaporator. The crude extract was dissolved in ethylacetate and water. This was partitioned and separated with separating funnel and concentrated with rotary evaporator. The extract was tested for the presence of phenol and then refrigerated pending use for the study. The extract is referred to as *Vitex doniana* phenol extract (VDPE).

Study design

Forty male Wistar rats (100 – 195 g) purchased from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, were used. They were housed and maintained in animal cages in the Department of Biochemistry, FUTO. The animals were kept on commercial animal pellets (Guinea Feeds Ltd, Delta State, Nigeria) and were allowed seven days for acclimatization within the laboratory environment. All the animals had access to both water and feed *ad libitum*. The study was approved (FUTO/BCH/EC/2016/05) by the Ethics committee of the Department of Biochemistry, Federal University of Technology Owerri, Nigeria. The experiment adhered to guidelines for protection of human subjects and animal welfare.[19]

After acclimatization, animals were weighed and their baseline blood glucose concentrations measured using One Touch Basic Glucometer (Code – 12). Group 1 was used as Normal control and diabetes mellitus was induced in the remaining animals with single intraperitoneal (i.p) injection of alloxan monohydrate at a dose of 150 mg/kg body weight. Three hours after alloxan administration, the test animals were kept on 50 % oral glucose solution to prevent hypoglycaemia usually caused by hyperactivity of the pancreas, induced by Alloxan two to three hours after its administration. Two days after alloxan injection, the animals were weighed again and their blood glucose concentrations recorded using the glucometer. Twenty-five diabetic rats (with blood glucose concentrations of 250 mg/dl and above) were selected and divided into five groups labeled group 2, 3, 4, 5 and 6 of five rats each according to their body weights. The diabetic animals in groups 3, 4 and 5 were treated with daily doses of 100 (D100), 200 (D200) and 400 (D400) mg/kg body weight (bw) of *Vitex doniana* phenol extract (VDPE), group 6 were treated with glibenclamide at 0.50 mg/70kg, whereas group 1 (Normal control) and group 2 (Diabetic control) rats received normal saline, 1.0 ml/kg bw. All the extracts and drug were dissolved in normal saline and were given orally. At the end of the study period all the Animals were anaesthetized with chloroform and blood samples collected by cardiac puncture.

Biochemical studies

Lipid peroxidation (MDA): The method as described by Usoh *et al*.[20] was used to determine the concentration of malondialdehyde (MDA), a lipid peroxidation product. Coagulated blood sample was spun in a centrifuge for 10 min at 3,000 rpm to obtain the serum which was taken up with a Pasteur pipette into labeled specimen tubes that were refrigerated until used. Serum aliquots (0.4 ml) were pipetted into the test tubes and mixed with 1.6 ml of 0.25N HCl, 0.5 ml of 15.0 % trichloroacetic acid (TCA) and 0.5 ml of 0.375 % of thiobarbituric acid (TBA). The reaction mixtures were placed in 100 ^{o}C boiling water for 15 min, cooled and centrifuged at 3,000 rpm for 10 min. The optical densities of the supernatants were recorded at 532 nm against a reagent blank which contained only distilled water.

*Glutathione concentration:*Glutathione (reduced) was determined according to the method as previously described.[21] Equal quantity of homogenate was mixed with 10 % trichloroacetic acid and centrifuged to separate the proteins. To 0.25 ml of this supernatant, 2.25 mL of 5, 5-dithio, bis (2-nitrobenzoic acid) in phosphate buffer (pH 8.4) was added. The mixture was vortexed and the absorbance read at 412 nm within 15 min. *Calculations:* Absorbance of glutathione was calculated from the standard calibration curve (y = Mx) prepared by plotting absorbance of standard glutathione concentrations against the standard concentrations when subjected to the same experimental conditions.

Determination of Vitamin C (Ascorbic acid)

The concentration of vitamin C was determined by the method described by Omaye *et al.*[22] Briefly: In a clean test tube containing 0.5 ml of plasma, 0.5 ml of water and 1 ml of TCA were delivered. This was thoroughly mixed and spurned in a centrifuge and the supernatant collected. To 1.0 ml of the supernatant, 0.2 ml of DTC reagent was added and the setup was incubated at 37°C for 3 hr. Furthermore, 1.5 ml of H_2SO_4 was added, properly mixed and the solution allowed to stand for 30 min at room temperature. The colour developed was read at 520 nm in a spectrophotometer.

Histological studies of tissues

The method described by Okoro[23] was used with minor modifications. Each tissue of the pancreas was fixed in 10% formal saline which covered the entire tissue. The setup was subjected to Dehydration; Clearing or dealcoholisation; Impregnation; Embedding and finally Trimming. Excess wax was carefully removed from the tissues at this point, attached to a holder and sectioned with a rotary microtome.

Furthermore, the section was subjected to twelve steps which include dewaxing and hydration and then stained. The setup was dehydrated in ascending grades of alcohol, cleared in xylene and mounted with dibutylpthalate, polystyrene, and xylene (DPX).The thin sections were examined under high resolution microscope with photographic facility and photomicrographs were taken.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using SPSS program (version 20 SPSS Inc., Chicago, IL, USA). The results were presented as Mean ± standard deviation (SD). P value less than 0.05 was considered as significant ($P< 0.05$).

Results and Discussion

Biochemical studies

The results of lipid peroxidation (Figure 1) showed significant increase in MDA concentration of the Diabetic control rats compared to Normal control group. This result agreed with reports of Aluwong *et al.*[24] and AlFaris *et al.*[25] that showed significant increase in MDA concentration among diabetic rats in comparison to the controls.

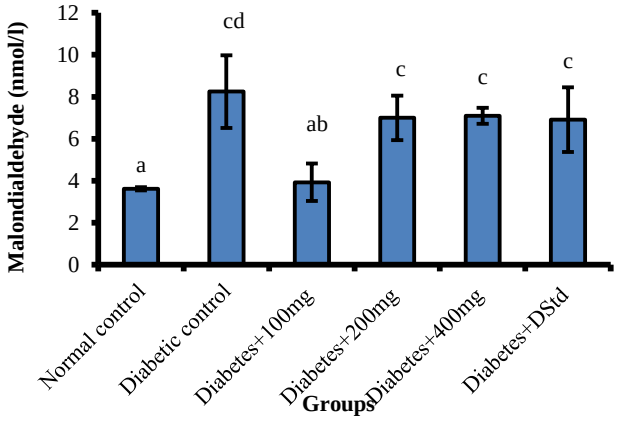

Figure 1: Concentration of MDA of alloxan-induced diabetic Wistar rats treated with VDPE. Bars represent mean ± standard deviation of triplicate determinations and bars with different alphabets indicate significant difference at $p < 0.05$.

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Similarly, Chevan and Melinker[26] observed similar results in a study population from Gujarat, while Masola *et. al*.[27] in the study to evaluate the potential benefits of *Centella asiatica* on diabetes-induced stress, reported that diabetes increased MDA concentration by 39%.

Furthermore, the result of this study showed that MDA reduced significantly in diabetic rats treated with D.100 VDPE and non-significantly in D.200, D.400 and glibenclamide when compared to Diabetic control rats. This reduction could be an indication of decreased oxidative stress supported by the antioxidants contents of VDPE and hence a reduction in the rate of progression of diabetic complications of the liver. The significant reduction in MDA of D100 strongly suggests attenuation of lipid peroxidation by the phenolic content of *V. doniana* leaf extract. Phenolic-rich extract of chestnut was reported to attenuate oxidative stress, through improving the natural antioxidant system and inhibiting lipid peroxidation.[28] Phenolic compounds found in human diets are classified as phenolic acids, flavonoids and tannins.[29,30] Plant phenolics are primary antioxidants with potential to prevent chronic and oxidative stress-related disorders.[31] Phenolic acids and flavonoids promote good health by reducing the risk of complications such as inflammation and dyslipidemia of type II diabetes mellitus.[32,33]

The result of the study shows significant reduction in GSH concentration in Diabetic control rats compared to Normal control and diabetic groups treated with D.200 and D.400 VDPE and glibenclimides (Figure 2). Glutathione is a thiol group containing molecules, acting as cofactor for glutathione peroxidase (GPx), by indirectly donating important electrons for the decomposition of H_2O_2, and directly scavenges hydroxyl radical and singlet oxygen.[34] Glutathione peroxidase detoxifies H_2O_2 and lipid peroxides using reduced glutathione (GSH) as substrate. The results suggest that VDPE extract may have contributed in the reduction of oxidative stress in diabetic rats as indicated by increased concentration of glutathione of D.200 and D.400. Therefore, *V. doniana* phenolic leaf-extract has reducing potential, possibly due to the content of phenolic compounds, with the ability to scavenge and neutralize Alloxan-induced oxidative stress, providing a significant recovery in altered enzyme defense mechanism of treated rat groups. This may be related to the presence of polyphenols of flavonoids, phenolic acids and tannins, that can inhibit α-glucosidase and *α*-amylase involved in carbohydrate metabolism, regulate lipid metabolism, inhibit hyperglycemia, dyslipidemia and insulin resistance and attenuate oxidative stress and inflammatory processes.[32,35]

Serum vitamin C content were significantly ($P \leq 0.05$) higher in the Normal control, *V. doniana* phenolic leaf-extract treated and standard drug groups when compared to Diabetic control rats (Figure 3). This result agrees with an earlier study that reported that administration of plant extract increased the concentration of vitamin C in diabetic rats.[36] Vitamin C is an important antioxidant with excellent free radical scavenging ability and can prevent or delay the oxidation of biomolecules such as DNA, lipids or other important cellular compounds. Vitamin C acts as a co-antioxidant by regenerating α-tocopherol radicals produced during scavenging of reactive oxygen molecules.[37,38] Vitamin C presents high concentration in many natural sources, especially fresh fruits (citrus showing the richest) and vegetables.[39] The high serum content of vitamin C recorded, therefore, was possibly contributed by the extract.

Histological studies

Histopathology of the pancreas in Alloxan induced diabetic rats

Histopathological sections of the pancreas are presented in plates A-F (Control, diabetic and diabetic+ *V. doniana* extract treated rats). Plate A (Normal control) shows a typical section of the pancreas, with the septa extending from the capsule into the gland and dividing into lobules and showing the presence of intralobular and interlobular ducts. The acini cells are highly basophilic (bluish staining), some are pale, separated by aggregated cells that form the pancreatic islets (pale staining cells arranged as a group). The pancreas of the Diabetic control group (Plate B) showed distortions of both acini cells and islets cells. These distortions may have affected most islets cells leading to increased necrosis. Plate C: presents pancreas section of D.100 treated group.

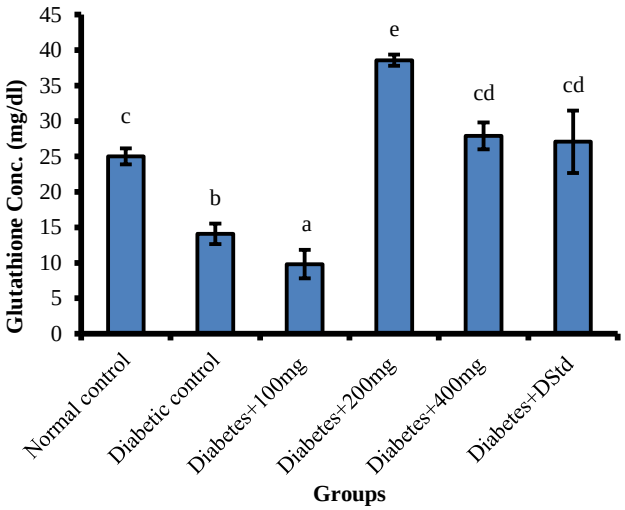

Figure 2: Concentration of glutathione of alloxan-induced diabetic Wistar rats treated with VDPE. Bars represent mean ± standard deviation of triplicate determinations and bars with different alphabets indicate significant difference at $p < 0.05$.

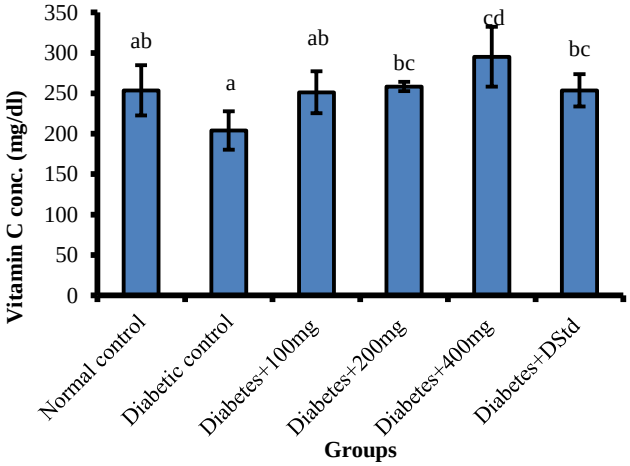

Figure 3: Concentration of Vitamin C of alloxan-induced diabetic Wistar rats treated with VDPE. Bars represent mean ± standard deviation of triplicate determinations and bars with different alphabets indicate significant difference at $p < 0.05$.

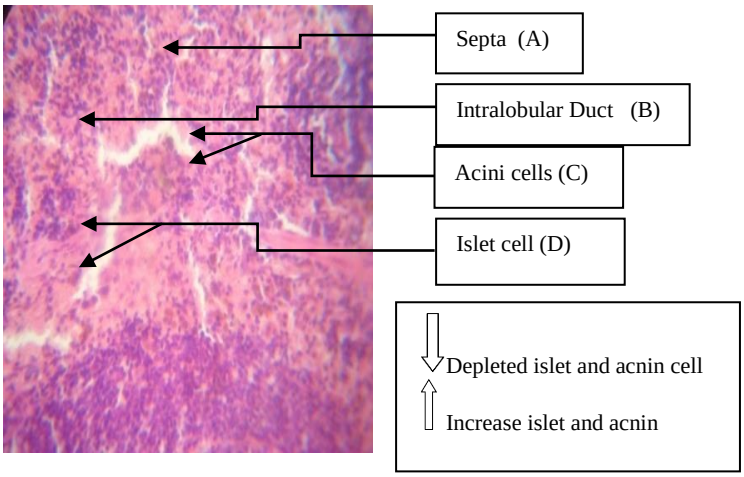

Plate A: Transverse section of pancreas (Normal control) H&E stain ×400

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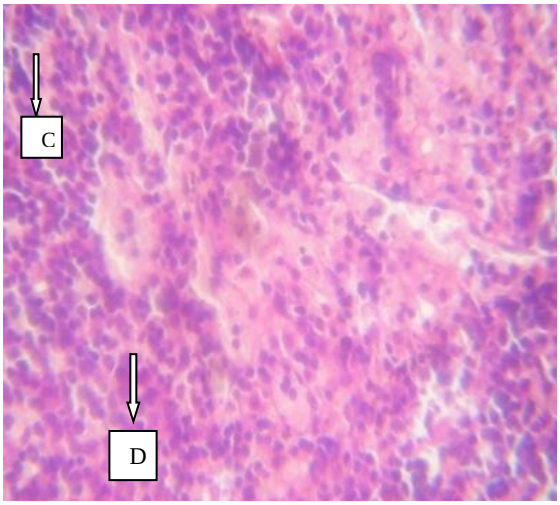

Plate B: Transverse section of pancreas (diabetic control) H&E stain ×400

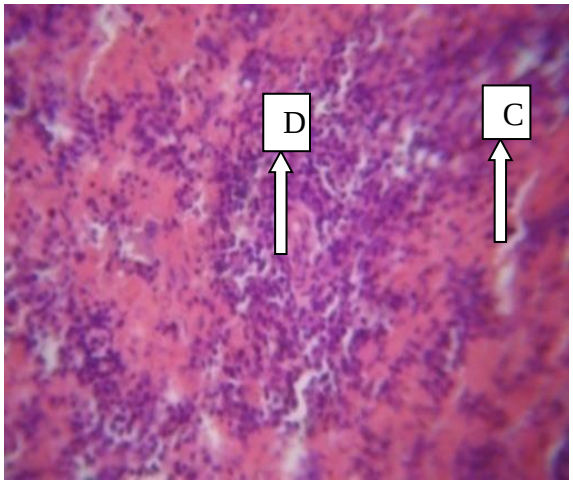

Plate C: Transverse section of pancreas (diabetic + 100mg/kg b/w Vd) H&E stain ×400

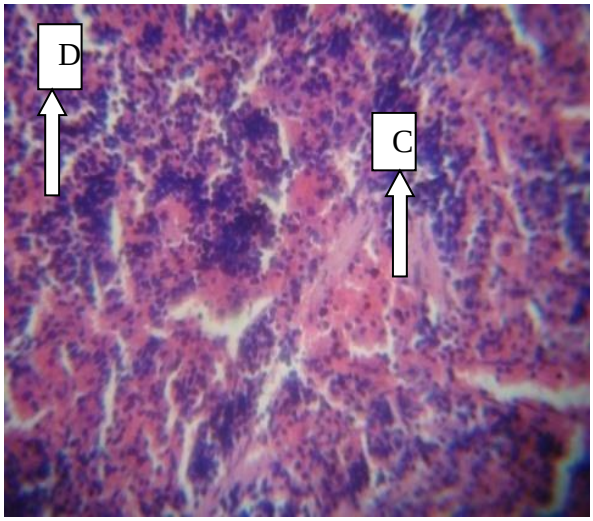

Plate D: Transverse section of pancreas (diabetic + 200mg/kg b/w Vd) H&E stain ×400

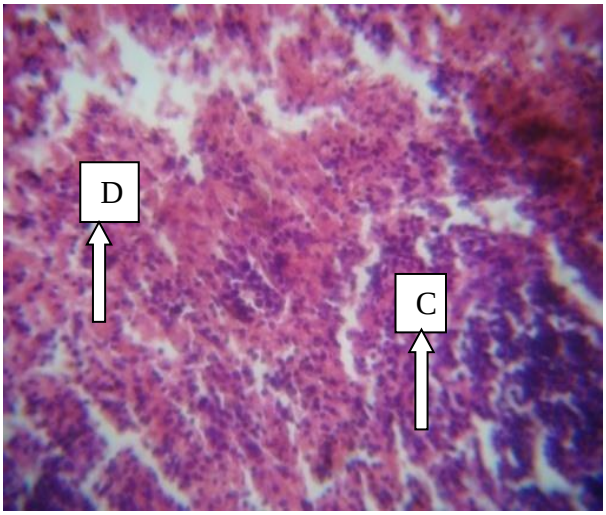

Plate E: Transverse section of pancreas (diabetic + 400mg/kg b/w Vd) H&E stain ×400

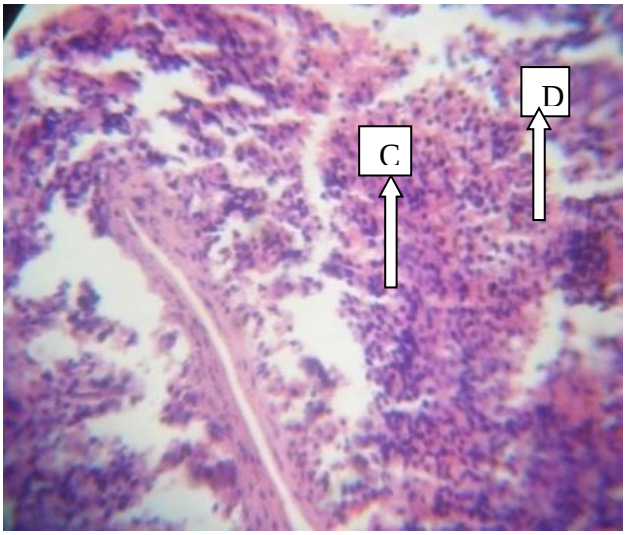

Plate F: Transverse section of pancreas (diabetic + Standard Drug) H&E stain ×400

This plate shows restored β-cells and increased regeneration of islet cells with moderate necrosis.

The acini cells appeared basophilic while a few islets cell appeared pale. Administration of 200 mg/kg of extract (Plate D) enhanced the restoration of β-cells as shown by more islet cells regeneration and minimal necrosis. The acini cells appeared basophilic, while a few islets cell appeared pale. The pancreas of 400 mg/kg group (Plate E) showed increased regeneration of islet cells, the acini cells appeared basophilic, while the islets cell appeared pale. In rats administered standard drug (Plate F), acini cells appeared basophilic and numerous. These areas assumed to be occupied by islet cells appeared empty, thus indicating necrosis of islet cell with poor regeneration.

The alloxan used in this study produced selective destruction of insulin-producing pancreatic β-islets.[40,41] The reduction in pancreatic β-cells can be as high as 50 % in diabetics,[42] as corroborated by the result presented by the diabetic control. Similarly, the present study revealed presence of damaged β-cell in the pancreas of diabetic rats. The histopathological results show positive prospect of β-cells repairs, and regeneration in diabetic-treated groups as indicated by increase in volume density of islets, percentage *β*-cells and size of islet in plates C to E. This regeneration of *β*-cells has been reported following consumption of plants extracts in diabetic-induced animals.[43]

Conclusion

The study has shown the capacity and quality of the *Vitex doniana* phenolic leaf extract in the management of diabetes mellitus. Phenolics of *V. doniana* have the potential to reduce the risk of type 2 diabetes; the associated damage to pancreas and increased risk of complications. Furthermore, in the context of phenolic antioxidants, those from *V. doniana* improved reduced glutathione and vitamin C content and decreased lipid peroxidation and oxidative stress and attenuated pancreatic β-cells damage.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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