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Ethyl Acetate Fraction of Total Phenols from *Zanthoxylum zanthoxyloides* Leaves Treated Alloxan-Induced Diabetic Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Ethyl acetate fraction of total phenols from *Zanthoxylum zanthoxyloides* leaves was evaluated for its effects in alloxan-induced diabetic male albino rats.
Methodology: Diabetes was induced in rats by administrating alloxan monohydrate at a dose of 130 mg/kg body weight. The ethyl acetate fraction was administered to rats at 100, 200 and 400 mg/kg b.w. daily for 15 days. Blood glucose, lipid profile and pancreatic histopathology were monitored using standard methods.
Results: Results indicated that two weeks after treatment with *Z. zanthoxyloides*, significant ($p<0.05$) reductions in hyperglycemic blood glucose, total cholesterol and low-density lipoproteins (LDL) were recorded; with lowest values recorded in the group treated with the highest concentration of the extract. Histopathological examination of pancreatic tissues supported the protective effect of *Z. zanthoxyloides* phenol extract against diabetic damage.
Conclusion: In conclusion, total phenols of *Z. zanthoxyloides* exerted antidiabetic effects in alloxan-induced diabetic rats and protected pancreatic tissues from diabetic damage.

Keywords: Albino rats; alloxan monohydrate; diabetes mellitus; ethyl acetate fraction; lipid profile; total phenols; Zanthoxylum zanthoxyloides.

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1. INTRODUCTION

Diabetes mellitus is a major metabolic syndrome characterized by derangement in carbohydrate metabolism associated with defect in insulin production, secretion or action. It is associated with chronic hyperglycaemia which causes damage to eyes, kidneys, nerves, heart and blood vessels [1]. Diabetes is a major risk factor for the development of cardiovascular disease. More than 70% of deaths in diabetic patients are due to vascular disease. One of the greatest factors in the development and progression of the complications of *diabetes mellitus* is hyperglycaemia [2]. Treatment of diabetes involves use of drugs that reduce glucose levels, including insulin and oral antihyperglycaemic drugs. Although there is treatment for *diabetes mellitus*, most drugs in current use are seriously constrained by both their side effects and cost of treatment. Due to these challenges, populations mainly in Sub-Saharan Africa have resorted to cheaper and readily available alternative sources of treatment, such as use of medicinal plants or traditional medicines [3].

Nigerian *Zanthoxylum* is a common plant found in the rain forest vegetation of southern Nigeria, and is represented by eleven species [4]. *Zanthoxylum zanthoxyloides* contains a high diversity of essential oils and alkaloids, as well as several aliphatic and aromatic amides [4]. The mineral composition of the fruit pericarp per 100 g is: Ca 90 mg, P 41 mg, Fe 2 mg, Na 10 mg, K 46 mg, Mg 52 mg and Cu 55 mg. Throughout West Africa the aromatic roots, stem bark and leaves are commonly used in traditional medicine. They are considered antiseptic, analgesic and diaphoretic. Root or stem bark macerations, decoctions or infusions are widely taken to treat malaria, fever, sickle cell anaemia, tuberculosis, paralysis, oedema and general body weakness [5]. They are also widely taken to treat intestinal problems, including colic, dysentery, intestinal worms, gonorrhoea and urethritis, also as an emmenagogue, stimulant and to treat pain during childbirth, migraine and neuralgia [5]. The roots are externally applied to ulcers, swellings, haemorrhoids, abscesses, snake bites, yaws, wounds, leprosy, and syphilitic sores as well as rheumatic and arthritic pain, and hernia. Different plant extracts, however, showed low toxicity in laboratory tests [5]. The plant was chosen for this research considering its availability, edibility and other medicinal values. It is one of the medicinal plants in the South-East part of Nigeria. Many works have been reported about the crude extract of the plant; showing its non-toxicity as an edible plant, as well as its content of antioxidant phytochemicals like phenols and flavonoids. The plant however is readily available in the wild and as such, is not a commonly/domestically available plant.

The number of people with diabetes today has been growing and causing increasing concerns in medical community and the public. Thus, phenolic compounds that are found in beverages, vegetables, galenical pears and berries, may facilitate fitness by decreasing the risk of metabolic syndrome and relevant complications of type 2 diabetes [6]. Diabetics are at high risk for dyslipidaemia which is characterized by reduction in blood high-density lipoprotein (HDL) levels and increase in blood low-density lipoprotein (LDL), triacylglycerol (TAG) and total cholesterol (TC) levels; thus, pre-disposing diabetics to cardiovascular diseases [7].

The study reports the antidiabetic properties of the ethyl acetate fraction of total phenols of *Z. zanthoxyloides* leaves in alloxan-induced diabetic albino rats.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant materials

Fresh leaves of *Z. zanthoxyloides* were collected from Abakiliki, Ebonyi State, Nigeria. The plants were identified and authenticated by a taxonomist in the Department of Biological Sciences, Federal University of Technology, Owerri. They were deposited in the herbarium with voucher number FUTHB 048. The plant was chosen for this work considering its availability, edibility and other medicinal values it has. It is one of the most medicinal plants in the South-East part of Nigeria. Many works have been reported about the crude extract of the plant; showing its non-toxicity as an edible plant, as well as its content of antioxidant phytochemicals like phenols and flavonoids. The plant however is readily available in the wild and as such, is not a commonly/domestically available plant.

2.1.2 Chemicals, reagents and kits

Alloxan monohydrate,ethyl acetate and methanol were procured from Sigma, Germany. All assays

kits were from Randox® Laboratories Ltd., Antrim, UK. Other chemicals and reagents used were of analytical grade unless otherwise stated.

2.1.3 Animals

Thirty adult male Wistar rats weighing between 100.00-195.00 g were purchased from the Department of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. The animals were housed in standard animal cages, handled humanely in accordance with the NIH [8] regulations, fed with commercial feed and water, *ad libitum,* and acclimatized for 7 days.

2.2 Methods

2.2.1 Preparation of plant extract

One thousand grammes (1000 g) of dried and pulverized *Z. zanthoxyloides* leaves were macerated twice in 4.0 L of absolute methanol for 72 h. Filtration was done using cheese cloth and afterwards, with a Whatman No. 1 filter paper. Combined filtrates were evaporated using rotary evaporator at 40^{o}C. The crude extract obtained was dissolved in ethyl acetate (the choice of solvent was based on scope of research) and water in a ratio of 2:1, respectively; water being denser was filtered off first using a thistle funnel followed by the ethyl acetate fraction where the phenolic compounds were dissolved in. The filtrate was evaporated again using rotary evaporator at 40^{o}C which was then stored in the refrigerator and used for the experiments.

2.2.2 Determination of phenolic compounds

2.2.2.1 Determination of total phenolics

Total phenolic content was determined using the method of Wettashinghe and Shahidi [9]. Briefly, 50mg of methanol extracts were dissolved in 100% methanol to obtain a concentration of 0.5 mg/ml (Solution A). Folin-Denis reagent was added at a volume of 0.5 ml to centrifuge tubes containing 0.5 ml of solution A. Tubes were shaken and 1ml of a saturated sodium carbonate solution added. The volume was then adjusted to 10ml by the addition of 8 ml of deionised water and the content was mixed vigorously. Tubes were allowed to stand at ambient temperature for 25 min and later centrifuged for 5 min at 4000 g. Absorbance of the supernatants was measured at 725 nm. A blank sample (treated in the same way but without the extract) for each extract was used for background subtraction. Content of total phenolics in each extract was determined using a standard curve prepared for tannic acid. Total extracted phenolic compound was expressed as mgtannic acid equivalent/g extract.

2.2.2.2 Determination of flavonoid contents

The total flavonoid content of plant extract was determined colorimetrically as described by Zou, Lu, and Wei [10]. In brief, 0.5 ml of sample solution (A) above was mixed with 2.0 ml of distilled water and subsequently with 0.15 ml of 5% $NaNO_2$ solution. After 6 min of incubation, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, followed by addition of 2ml of 4% NaOH solution to the mixture. Immediately, water was added to the sample to bring the final volume to 5.0 ml. The mixture was thoroughly mixed and allowed to stand for another 15 min. The absorbance was read at 510 nm. Content of total flavonoids in each extract was determined using a standard curve prepared for Quercetin. Total extracted flavonoid was expressed as mg Quercetin equivalent/g extract.

2.2.3 Bioassays

The research employed *in vivo* assays in carrying out the biochemical parameters and *in vitro* assays in carrying out the histological studies. The adult male albino rats were housed in clean cages. They were exposed to 12 h each of natural daylight and darkness and given rat chow and water *ad libitum.* The rats were divided into six groups on body weight basis with each group differing from the other with ± 5 g. Group A served as the normal control which was not induced with diabetes, group B was the diabetic negative control which induced with diabetes but not treated,while groups C, D and E (diabetic test groups) were induced with diabetes and treated daily with 100mg/kg, 200mg/kg and 400mg/kg body weight *Z. zanthoxyloides* total phenolic extracts, respectively, while group F was treated with 5mg/kg body weight of a standard diabetic drug (glibenclamide). This choice of treatment concentrations was based on the significantly low toxicity (except for some parts like the root bark which was slightly toxic only at the highest dosage of 5000mg/kg b.w [11]) of the plant as well as the reports of Okpuzor, Ogbunugafor & Kareem [7] who worked with the ethyl acetate fraction of another plant at equivalent concentrations.

The thirty rats were weighed and their base line blood sugar levels recorded using a glucometer (Accucheck Active). A single dose intraperitoneal injection of alloxan monohydrate (2, 4, 5, 6 tetraoxypyridine 5, 6-dioxyuracil) in 5 percent normal saline solution, at a dose of 130 mg/kg body weight was given to twenty-five rats, whereas five rats served as the normal control group. Three days after alloxan administration, the blood sugar levels of the rats were recorded and the diabetic rats (with blood sugar levels ≥ 250.0 mg/dl) were divided into five groups of five animals each according to their body weights. See Appendix.

Three test groups received daily treatment with the phenolic extract of *Z. zanthoxyloides* leaves at low, medium and high doses of 100.0, 200.0 and 400.0 mg/kg body weight, respectively [12]; whereas the remaining animals served as the untreated diabetic (negative control) group. The reference group was treated with glibenclamide (0.5 mg/kg body) and the normal controlreceived only the vehicle (olive oil) with no plant extract treatment. Extracts and drugs were solubilized in olive oil [13] and the duration of treatment was fourteen (14) days. All administrations were done orally. The glycaemic levels of the rats were measured at five days intervals using the glucose oxidase method with the use of a glucometer. The animals were sacrificed on the last day byanaesthetizingthemby exposure to dichloromethane vapour, incisions made into their thoracic cavitiesand blood collected by cardiac puncture. The choice of anaesthetic was due to several regulations on the use of several commercially available anaesthetics which are carcinogenic, example is chloroform [14,15]. The blood was transferred to plain sample bottles, allowed to clot by leaving it undisturbed at room temperature for over 30 min. The clotted blood was centrifuged at 2,000 x g for 10 min and sera(supernatant) extracted from the pellet using Pasteur pipettes and transferred to another plain sample bottles for further biochemical analyses.

2.2.4 Determination of lipid profile

2.2.4.1 Total Cholesterol

Serum cholesterol content was determined in mg/dl according to the method [16] by adapting the Liebermann-Burchard reaction where the cholesterol is determined after enzymatic hydrolysis and oxidation using the indicator quinoneimine which is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

2.2.4.2 High-density lipoprotein

Serum high-density lipoprotein (HDL) levels were determined in mg/dl using the colorimetric method [17] where low-density lipoproteins (LDLs) and very low-density lipoproteins (VLDLs) in the sample precipitate with phosphotungstate and magnesium ions; the supernatant contains high-density lipoprotein (HDL), which was spectrophotometrically measured.

2.2.4.3 Low-density lipoprotein

Serum low-density lipoprotein levels were determined in mg/dl using the colorimetric method where low-density lipoproteins in the sample precipitate with polyvinyl sulphate and their concentrations were calculated from the difference between the serum total cholesterol and the cholesterol in the supernatant after centrifugation [18].

2.2.4.4 Triacylglycerol

Serum triacylglycerol levels were determined in mg/dl using the spectrophotometric method where triacylglycerol in the sample originates by means of coupled reactions leading to the development of a coloured complex which can be measured by spectrophotometry [19].

2.2.5 Histological study of pancreatic tissues

The histological study of the pancreatic tissue was carried out using standard methods [20]. The pancreas of the animals were excised and fixed in a 10% solution of formaldehyde. The tissues were dehydrated because the reagents used at a later stage were immiscible with water. Varying concentrations of isopropyl alcohol; that is, 70%, 80%, 90%, 96%, and 100%, were used for the dehydration. The minimum time for dehydration between two different concentrations was 1h.The fixed tissues were then cleared in xylene and embedded in paraffin wax. The sections (5µm) from each of the tissues were examined using a light microscope (×40) after staining with hematoxylin and eosin dye.

2.2.6 Statistical analysis

Data were evaluated using the SPSS/10.00 software and comparison between groups was done using ANOVA. Values were considered significantly different at 95% confidence level ($P<0.05$).

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Total phenolics and flavonoid contents

The results of the total phenolic and flavonoid contents of the plant extract are presented in Table 1. The results showed that the total phenolics in the plant extract were 7.69±1.45mg tannic acid equivalent/g extract while the total flavonoids were 39.76±1.33mg quercetin equivalent/g extract.

3.1.2 Glucose levels

The results of the glucose levels of animal samples treated within intervals of 5 days for over 15 days are presented in Table 2. There was no significant difference ($p>0.05$) in fasting blood glucose in diabetic control group on day 0 (346.00±14.00mg/dL), day 5 (279.00±23.00 mg/dL), day 10 (349.33±13.50 mg/dL), and day 15 (341.33±5.50 mg/dL). However, on administration of 100mg/kg b.w of *Z. zanthoxyloides*, no significant difference was found on day 0 (232.0±12.16 mg/dL) and day 5 (202.66±15.30 mg/dL), but there were significant differences ($P<0.05$) on day 10 (159.33±2.86 mg/dL) and day 15 (119.33±8.38 mg/dL). All the rats treated with 200 mg/kg b.w of *Z. zanthoxyloides* showed no significant difference ($p>0.05$) on day 0 (228.8±13.00 mg/dL) while there were significant differences on day 5 (144.66±12.89 mg/dL), day 10 (80.33±8.14 mg/dL), and day 15 (97.0±4.35 mg/dL). Administration of 400 mg/kg b.w of *Z. zanthoxyloides* show no significant reduction on day 0 (250±21.40 mg/dL) and day 5 (225.8±22.10 mg/dL) while significant reductions occurred on day 10 (172±15.80 mg/dL) and day 15 (98.2±9.75 mg/dL). There were also significant reductions of blood sugar on days 5 (171.0±2.73 mg/dL), 10 (96.6±8.38 mg/dL) and 15 (86.2±7.01 mg/dL) on administration of the standard anti-diabetic drug. It therefore becomes important to mention that there were significant differences ($p<0.05$) in fasting blood glucose between the diabetic groups treated with the extract and the untreated diabetic group.

3.1.3 Lipid profile

3.1.3.1 Total Cholesterol

The total cholesterol level was significantly increased in diabetic control group (175.79±5.69mg/dL) when compared to the normal control group (91.25±1.34 mg/dL). The cholesterol levels of 100mg/kg b.w, 200mg/kg b.w, and 400 mg/kg b.w were significantly reduced to 144.25±2.84 mg/dL, 150.44±4.28 mg/dL and 128.23±3.80 mg/dL, respectively, when compared to the diabetic control group while that of the standard drug reduced to 158.52±3.8 mg/dL.

Table 1. Total Phenolicsand Flavonoid Contents of Samples*

Parameter	Value
Total Phenolics(mg Tannic Acid Equivalent/g Extract)	7.69±1.45[a]
Total Flavonoids (mg QuercetinEquivalent/g Extract)	39.76±1.33[b]

**Values are mean ± SD of triplicate determination. Values on the same column bearing different superscript letters are significantly different (p<0.05)*

Table 2. Glucose Levels of Animal Samples*

	Day 0 (mg/dL)	Day 5 (mg/dL)	Day 10 (mg/dL)	Day 15 (mg/dL)
Control	68.2 ± 2.68[a]	67.6 ± 6.80[a]	75.6 ± 6.42[ab]	74.0 ± 6.44[ab]
Diabetic Control	346.0 ± 14.0 [c]	279.0 ± 23.0 [c]	349 ± 13.1 [c]	341.33 ± 5.6 [c]
Diabetic + 100mg/kg bw*Z. Zanthoxyloides*	232.0 ± 12.5 [c]	202.66 ± 15.53 [c]	159.33 ± 2.78 [b]	119.33 ± 8.02[ab]
Diabetic + 200mg/kg bw*Z. Zanthoxyloides*	228.8 ± 13.4 [c]	144.66 ± 12.89 [b]	80.33 ± 8.14 [a]	97.0 ± 4.35 [a]
Diabetic + 400mg/kg bw*Z. Zanthoxyloides*	250 ± 21.0[c]	225.8 ± 22.3 [c]	172.2 ± 15.8 [b]	98.2 ± 9.6 [a]
Diabetic + 5mg/kg bwGlibenclamide	267.4 ± 26.19 [c]	171.0 ± 2.73 [b]	96.6 ± 8.32 [a]	86.2 ± 7.04[a]

**Values are mean ± SD of triplicate determination. Values on the same column bearing different superscript letters are significantly different (p<0.05).*

3.1.3.2 High-density lipoproteins

In this study, there were significant ($p<0.05$) increases in the serum HDL levels of experimental groups of 200mg/kg b.w (97.72±1.47 mg/dL), 400mg/kg b.w (100.58±3.71 mg/dL) and glibenclamide (102.92±6.04 mg/dL) when compared with the diabetic control (62.90±1.09 mg/dL).

3.1.3.3 Low-density lipoproteins

In this study there were significant ($p<0.05$) decreases in the serum LDL levels of experimental groups of 100mg/kg b.w (76.04±4.22 mg/dL), 200mg/kg b.w (47.28±3.24 mg/dL), 400 mg/kgb.w (22.57±3.04 mg/dL) and glibenclamide (54.57±8.81 mg/dL) when compared with the diabetic control (101.82±3.87 mg/dL).

3.1.3.4 Very low-density lipoproteins

There were significant ($p<0.05$) decreases in the serum VLDL levels of experimental groups of 100mg/kg b.w (9.03±0.71 mg/dL), 200mg/kg b.w (5.43±0.43 mg/dL), 400mg/kg b.w (5.07±0.53 mg/dL) and glibenclamide (1.02±0.16 mg/dL) when compared with the diabetic control (11.06±0.71 mg/dL).

3.1.3.5 Triacylglycerol

Serum triacylglycerol (TAG) level was increased in diabetic groups (55.32±3.58 mg/dL) in comparison with the normal control group (38.91±3.56 mg/dL). The serum TAG levels were decreased significantly in the diabetic groups treated with 100mg/kg b.w, 200 mg/kg b.w, 400mg/kg b.w and glibenclamide (45.17±3.58 mg/dL, 27.17±2.17 mg/dL, 25.38±2.69 mg/dL and 5.12±0.83 mg/dL, respectively) when compared to the diabetic control group.

3.1.4 Histopathological Studies

Results of the histological study of pancreatic tissues are presented in Plates 1 to 6.

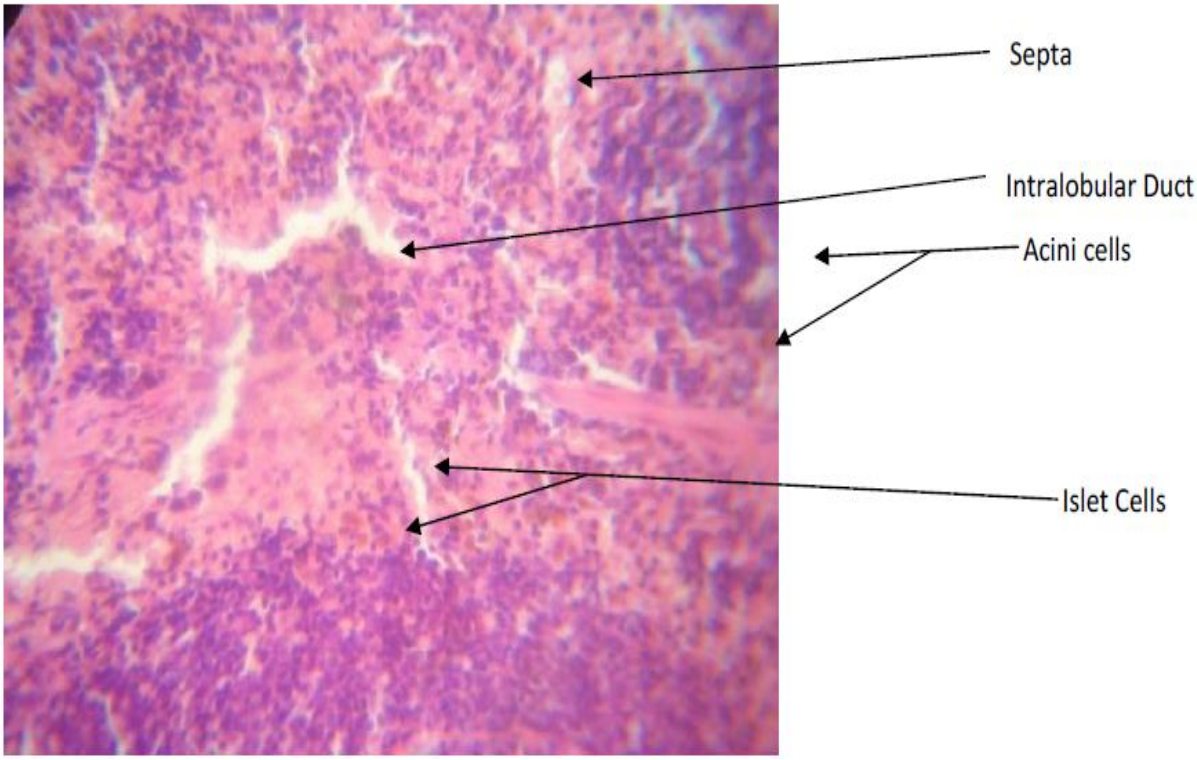

Plate 1. Transverse section of pancreas (control) H & E stain x 400

Plate 1 shows a section typical of the pancreas. Septa extend from the capsule into the gland and divide it into lobules. There is presence of intralobular and interlobular ducts. The gland is made of serous acini. The cells of the acini are highly basophilic (bluish staining); some are pale staining (centroacinar cells). In this normal photomicrograph, at some areas the acini are separated by aggregations cells that form the pancreatic islets (pale staining cells arranged as a group)

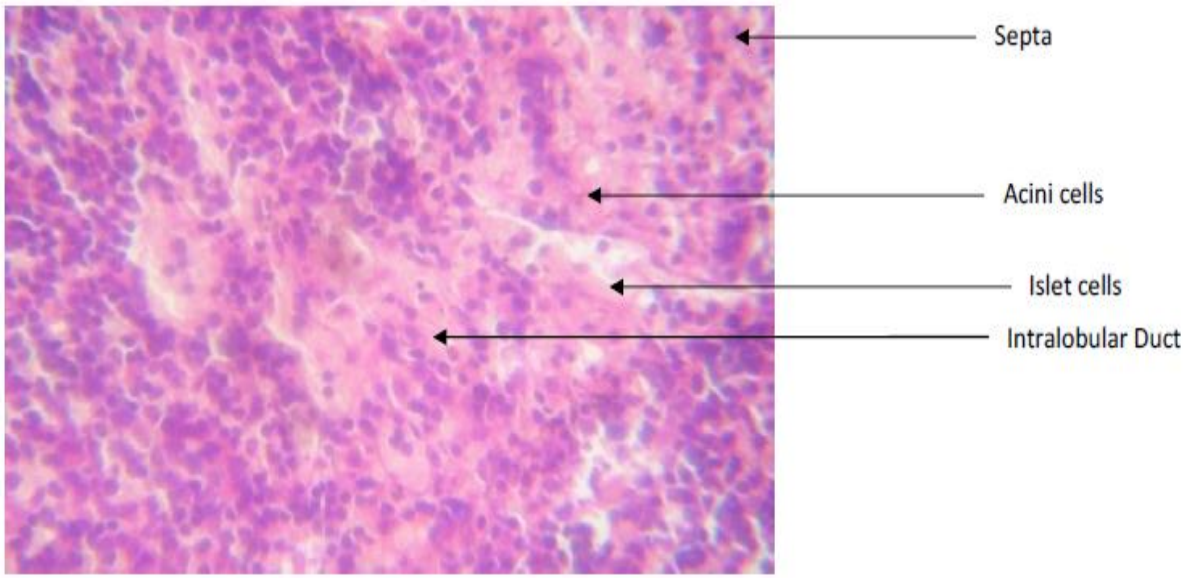

Plate 2. Transverse section of pancreas (diabetic control) H & E stain x 400

Plate 2 shows a section of pancreas that was exposed to diabetic agent. There is distortion of both the acini cells and islets cells. The distortion affected most of the islets cells and many have undergone necrosis. There is scanty of islets cells. The acini cells are numerous and stained basophyilic. Lobulation is more prominent.

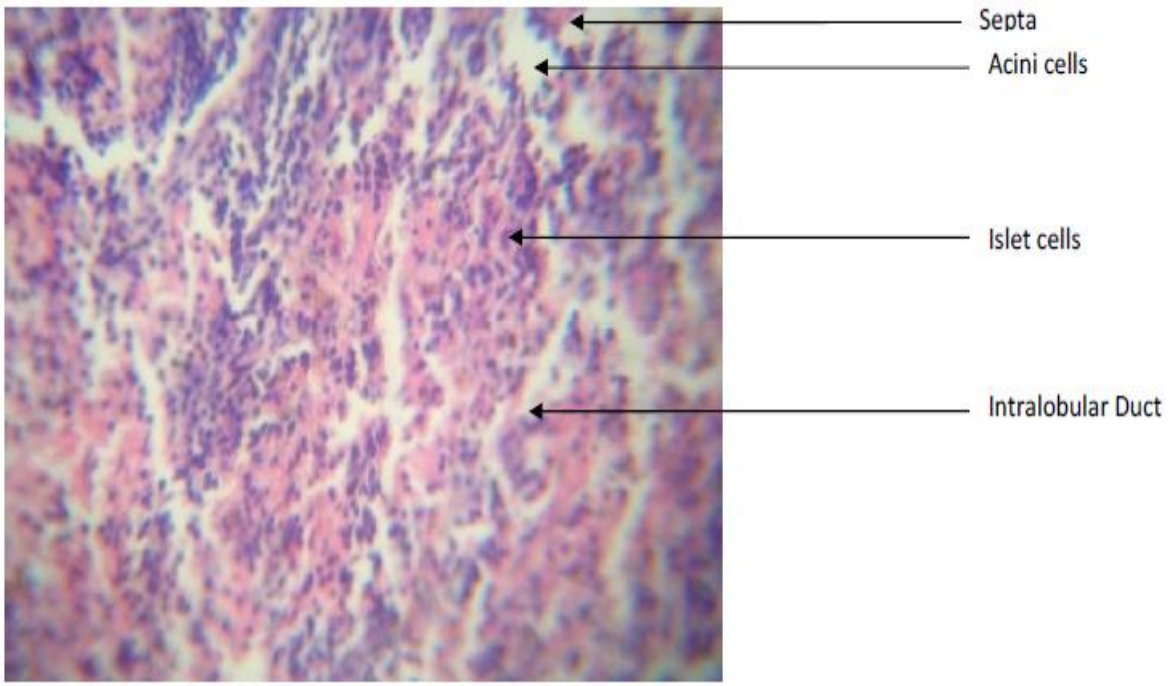

Plate 3. Transverse section of pancreas (diabetic with 100mg/kg bwZ.z.) H & E stain x 400

Plate 3 shows a section of pancreas that received 100mg of the extract. Regeneration of islet cells was very minimal and necrosis was more evident. The acini cells are appeared basophyilic while the few islets cell appeared pale.

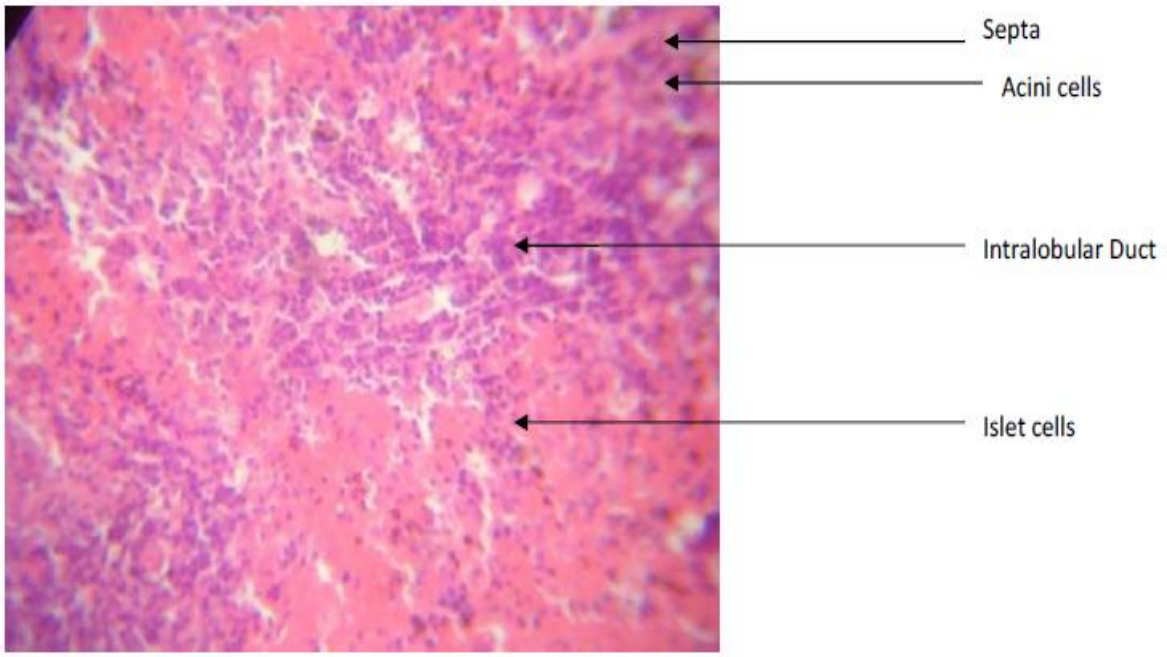

Plate 4. Transverse section of pancreas (diabetic with 200mg/kg b/w Z.z.) H & E stain x 400
Plate 4 shows a section of pancreas that received 200mg of extract. There was a slight increase in the regeneration of islet cells when compared to rats that received 100g of the extract. The acini cells are appeared basophyilic

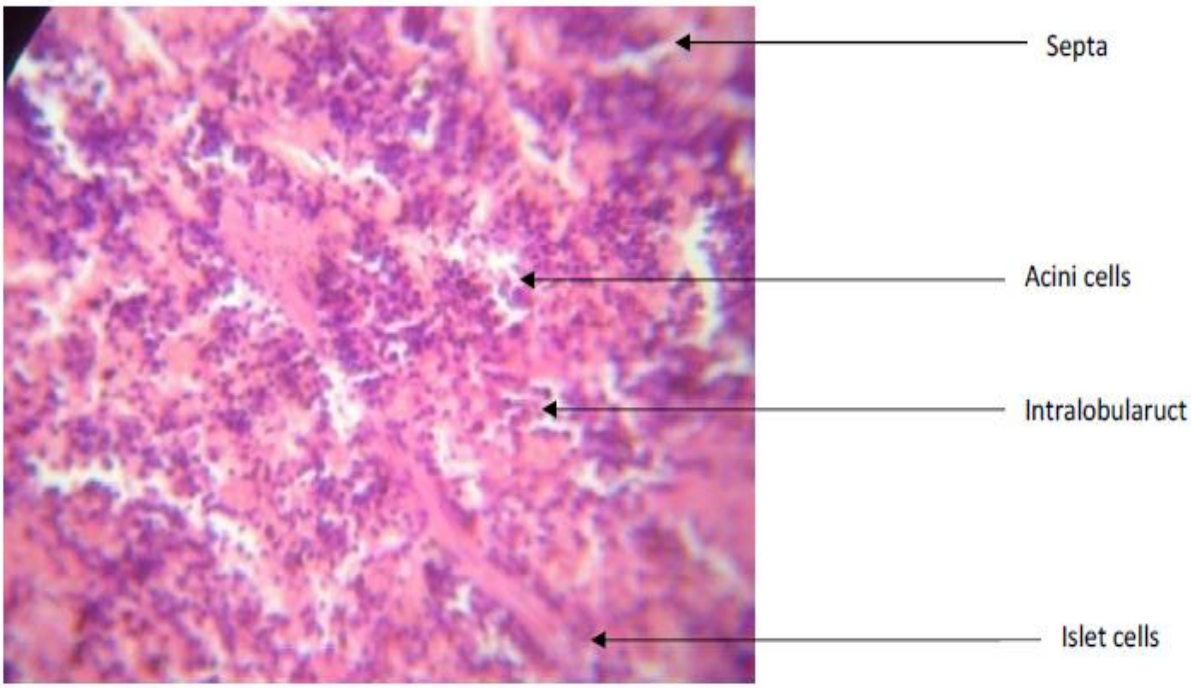

Plate 5. Transverse section of pancreas (diabetic with 400mg/kg b/wZ.z.) H & E stain x 400
Plate 5 shows a section of pancreas that received 400mg of the extract. There was more increase in islet cells regeneration when compared to photomicrograph of rats that received 100g and 200mg of the extracts. The acini cells appeared basophyilic while islets cells appeared pale.

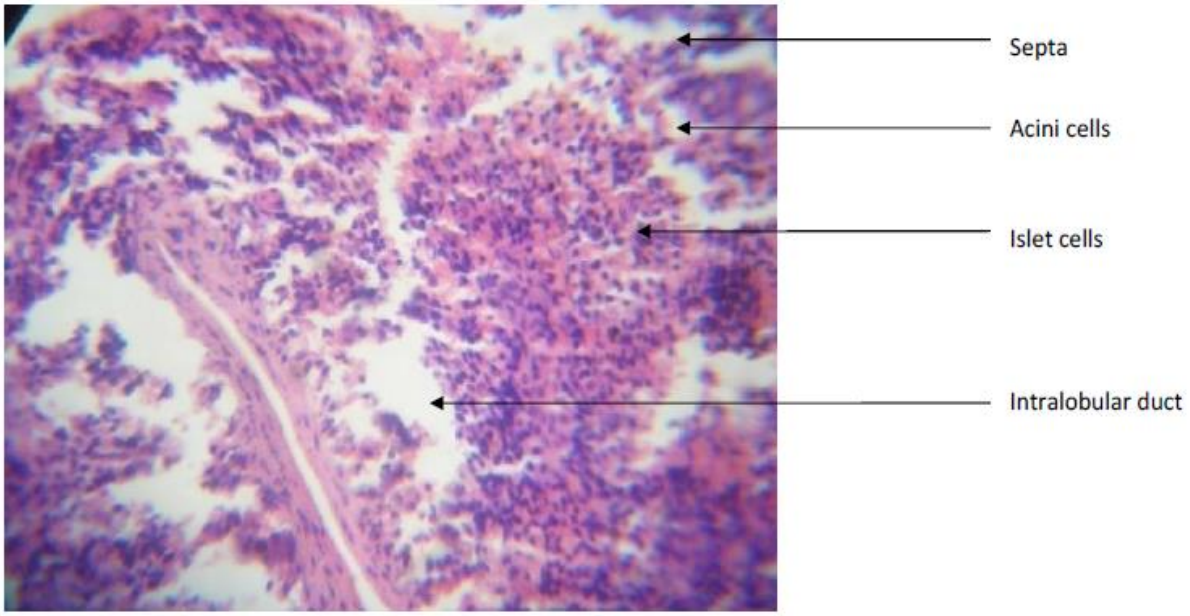

Plate 6. Transverse section of pancreas (diabetic with 5mg/kg b/w S.D.) H & E stain x 400

Plate 6 shows a section of pancreas that received standard drug treatment. The acini cells appeared to be basophyilic and numerous. Areas assumed to be occupied by islets cells appeared empty. Thus was necrosis of the islet cell and regeneration was not evident.

3.2 Discussion

The phytochemical analyses of the total phenol extracts of *Z. zanthoxyloides* leaves reported the presence of phenols and flavonoids.

Diabetes mellitus causes disturbances in the uptake of glucose by cells as well as glucose metabolism. Thus, alloxan-induced hyperglycaemia is a very useful experimental way of studying and demonstrating the activity of new hypoglycaemic agents [21]. Oral glucose tolerance tests were used to analyze blood glucose levels taken at different regular intervals after repeated treatments with *Z. zanthoxyloides* total phenol extract. Results show that using the total phenol extract of *Z. zanthoxyloides* at 400 mg/kg b.w, caused decreases in blood glucose levels of the alloxan-induced diabetic albino rats. This suggested that the total phenol extracts of *Z. zanthoxyloides* leaves enhanced glucose cellular uptake and utilization, thus improving glucose tolerance in diabetic rats.

In addition to the anti-diabetic effect, *Z. zanthoxyloides* leaves showed improvements in serum lipids in the diabetic rats treated with the total phenol extract of the leaves of *Z. zanthoxyloides* in comparison to the diabetic control rats. Interesting to note was the fact that the treated diabetic rats showed elevations in serum HDL-cholesterol levels; suggesting that the plant may protect against cardiovascular diseases. This actually supported a study carried out by Aloke et al. [12], where the rat samples were treated by orally ingesting the *Z. zanthoxyloides* leaves at different percentages. It is well known that in uncontrolled diabetes, the resultant increases in LDL, triglyceride and total cholesterol are associated with increased morbidity and mortality from coronary artery disease [22].

The presence of phenolic compounds in the extract may have been responsible for the hypoglycemic effects. Several authors have suggested that such compounds have anti-diabetic activity [23]. Flavonoids have also been known to regenerate the damaged beta cells in alloxan-induced diabetic rats and act as insulin secretagogues [24]. Thus, the hypoglycaemic activity of the total phenolic extracts of *Z. zanthoxyloides* leaves may be due to the presence of hypoglycaemic flavonoids and phenolics. These have been suggested to act through a free radical scavenging mechanism [25], [26]. *Diabetes mellitus* is a redox disease [27] and as such any drug with free radical scavenging potential would be of immense help. The antihyperglycaemic effect of *Z. zanthoxyloides* may also be attributed to the potentiation of insulin from existing β-cells of the

islets of Langerhans. The blood glucose lowering effect of the total phenol extract of *Z. zanthoxyloides* leaves was compared with that of glibenclamide, a standard drug which has been in use for many years for the treatment of diabetes and acts by stimulating insulin secretion from pancreatic β-cells [28].

The induction of diabetes using alloxan resulted in severe damage of β-cells of the islets of Langerhans (Plate 2). However, after repeated treatment with *Z. zanthoxyloides* especially at 400 mg/kg b.w for 15 days, there was regeneration of the central β-cells (Plate 5). There was also a notable increase in the number of secretive β-cells which are epithelial cells with ability to regenerate. The *Z. zanthoxyloides* total phenol extract appeared to stimulate the regeneration of β-cells of the islets of Langerhans. Albeit the still low number of the β-cells, the animals were able to maintain glucose levels close to normal. This therefore seemed to suggest that the total phenol extract of *Z. zanthoxyloides* leaves also increased the sensitivity of the insulin receptors to insulin. Previous reports indicated that medicinal plants that possessed hypoglycaemic activity acted through various mechanisms including improvement in the sensitivity of target cells to the effects of insulin, augmenting glucose-dependent insulin secretion and stimulating the regeneration of β-cells of islets of Langerhans in pancreas of alloxan-induced diabetic rats [29]. Some other medicinal plants seem to regulate enzymes of glycolysis, gluconeogenesis, and other pathways [30].

4. CONCLUSION

The ethyl acetate fraction of the total phenol of *Zanthoxylum zanthoxyloides* leaves exhibited anti-diabetic effects by lowering hyperglycemic blood sugar, serum total cholesterol and low-density lipoprotein in alloxan-induced diabetic albino rats. These it achieved by protecting the pancreatic cells from free radical damage, causing the regenerating of the β-cells of islets of Langerhans and increasing the sensitivity of the insulin receptors to insulin.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

INITIAL BODY WEIGHT, BLOOD GLUCOSE LEVELS, AND FINAL ORGAN WEIGHT OF EXPERIMENTAL ANIMALS

GROUP		WEIGHT	BLOOD GLUCOSE (ml/dl)				ORGAN WEIGHT (g)		
			0	5	10	15	Livers	Pancreas	Kidneys
Control	H	130	66	74	84	73	6.2	1.2	1.3
N		129	72	74	70	72	6.1	1.2	1.3
B		133	67	60	69	68	6.9	1.4	1.6
T		134	70	69	80	72	6.1	1.2	1.3
P		117	66	61	75	85	5.2	0.9	1.0
Diabetic Control	H	183	332	256	436	166	8.3	1.7	1.4
N		177	360	303	350	335	7.7	1.3	1.3
B		150	-	-	-	-	-	-	-
T		178	347	277	361	343	7.1	1.5	1.2
P		195	-	-	-	-	-	-	-
Diabetic + 100mg/ bw*Z. zanthoxyloides*	H	158	238	198	155	111	7.3	1.3	0.83
	N	150	-	-	-	-	-	-	-
B		150	218	190	161	120	7.25	1.3	0.9
T		148	-	-	-	-	-	-	-
P		144	241	220	162	127	7.2	1.3	0.75
Diabetic + 200mg/ bw*Z. zanthoxyloides*	H	140	217	141	71	95	8.01	1.69	1.33
	N	141	243	159	84	102	8.5	1.65	1.41
B		142	-	-	-	-	-	-	-
T		140	224	134	86	94	9.08	1.60	1.49
P		140	-	-	-	-	-	-	-
Diabetic + 400mg/ bw*Z. zanthoxyloides*	H	165	243	203	153	86	8.42	0.92	1.30
	N	153	237	224	162	90	8.75	1.4	1.60
B		128	226	184	234	107	8.53	1.16	1.67
T		160	274	297	234	102	6.75	0.85	1.65
P		158	270	248	194	106	11.33	2.70	1.81
Diabetic + 5mg/bw standard drug	H	173	287	170	90	89	10.5	1.15	2.01
	N	155	281	173	93	95	9.87	1.20	1.75
B		165	278	174	107	89	9.39	1.15	1.60
T		120	222	171	89	78	8.25	1.40	1.55
P		160	269	167	104	80	11.34	1.11	1.87

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