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Stereological Estimation of Volumes of Islet of Langerhans in Diabetic Wistar Rats Treated with *Tectonia Grandis* (Teak) Leaves.

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ABSTRACT

In this study we evaluated the changes in the pancreatic islet volume of diabetic rats following treatment with aqueous extract of *Tectona grandis* (teak) leaves. 25 apparently healthy young male Wistar rats divided into five (5) groups (normal control, diabetic control and three treatment groups) were used for the study. Diabetes was induced via high fat diet feeding for 10 weeks followed by streptozotocin injections (40mg/kg bwt). A normal control group was administered distilled water 1 ml/kg bwt. A diabetic group was administered normal saline 1ml/kg bwt, another group was treated with metformin 500mg/kg bwt. The last two groups were treated with high and low dose of the extract at 200mg/kg bwt and 100mg/kg bwt respectively. Treatment lasted for 35 days via oral route. Pancreatic tissue samples were collected after anesthesia/dissection and fixed in neutral buffered formal saline. Isotropic uniform random sections were obtained via the orientator method. Serial sections were cut with a rotatory microtome and stained with H & E. Pancreatic islet volumes were measured using the cavalieri estimator grid. The result showed increase in the volumes of pancreatic islets of the treated groups which was statistically significant when compared to the normal control. However, when compared to the diabetic and metformin control groups, the increases in the pancreatic islet volumes was seen not to be statististically significant.

Keywords: type 2 diabetes mellitus, volume estimation, stereology, pancreatic islets.

INTRODUCTION

Diabetes mellitus is a disease that has become a global burden and pancreatic variables such as pancreatic islet volumes, area, β-cell mass form fundamental variables in its research. The prevalence of this global disease is increasing despite the increase in advance conventional medications which are not without drawbacks [1,2] The disease is characterized by hyperglycaemia and insulin resistance which later lead to insulin insufficiency and action [3]. Hyperglycaemia is detrimental to cells and peripheral tissues because it leads to glucotoxicity that can pose a health challenge to the body. Such challenges are collectively referred to as diabetes-related complications and include cardiovascular neurological and urological complications [4].Herbal medicine is now offering the alternative in the search for anti-hyperglycaemic agents in plants because of the natural products contained in them that can offer better treatment with little or no drawbacks compared to the conventional drugs [5,6].In addition, herbal plants cost is relatively low and are easily available [7].

Teak is tropical hardwood tree. It's a specie in the flowering plant family Lamiaceae. It is native to South and Southeast Asia but it has over the years been naturalized and cultivated in some African and Caribbean countries. According to international journal of Ayurveda research Teak wood is acrid thus it's used as laxative and sedative. It is also useful in the treatment of piles, dysentery, headaches, antibacterial, anti-ulcer, anti-viral and anti-inflammatory [8]. The flowers and roots are useful in the treatment of bronchitis and urinary system related trouble. Oil from the wood of the plant has also been reported to alleviate liver related problems as well as possessing anti-helminthic and expectorant properties [9]. The methanolic extracts of the roots and bark of this plant have been reported to possess anti-hyperglycaemic tendencies [10, 11].

MATERIALS AND METHODS

Plant material: Fresh leaves of *Tectona grandis* were obtained from Jigawa state, identified and authenticated by a taxonomist in the herbarium unit of Biological Science department, Ahmadu Bello University Zaria. A voucher number (VN) 760 was assigned to it. Aqueous extraction of the leaves was done in the department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The leaves where first

mulched and air dried in the laboratory. Dried leaves were grinded into a fine powder and soaked in a litre of distilled water in a conical flask and allowed to stand for 24 hours. The mixture was stirred and filtered using muslin cloth followed by the whatman filter paper. The resultant filtrate was placed on a steam bath (50^{o}C) until all moisture evaporated.

Animal acquisition and handling: 25 apparently healthy Wister rats (*Rattus norvegicus*), age between 5-6 weeks, and weighing between 60-65g were bought from the department of human anatomy animal nursery and transferred to the laboratory animal facility of the same department on the same day and acclimatized for 2 weeks in plastic cages with mesh beddings under appropriate laboratory conditions prior to the commencement of the research work. Rats were fed with pelletized rat chow and water *ad libitum*.

High fat diet (HFD) feeding and weight measurement: High fat diet (100g of animal derived fat) from abattoir in Zango, Zaria, plus 100g of pelletized grower mash was fed to the rats for five (5) weeks twice a day. Body weights measurement of the rats was done weekly with a top-loading weighing scale (KERRO BL20001).

Type 2 diabetes mellitus induction: Streptozotocin (from Zayo sigma, Jos Nigeria), 40mg/kg bwt was injected intraperitoneally into the rats. Dextrose solution given orally for 12 hours to sustain the rats. Hyperglycemia was analyzed from blood collected from tail of rats after 1 week (fasting blood glucose) with an accu-check active glucometer. Rats with values $\geq$ 200 mg/dl blood glucose level were used for the research [12].

Table 1: Experimental design

GROUPS	DOSAGE
Group 1: Normal control	1ml/kg bwt distilled water
Group 2: Diabetic control	1ml/kg bwt normal saline
Group 3: Diabetic + metformin	500mg/kg bwt
Group 4: Diabetic + high dose extract	200mg/kg bwt
Group 5: Diabetic + low dose extract	100mg/kg bwt

Route: Oral gavage
Duration: 5 weeks

Sacrifice and Sample collection: Rats were fasted overnight and anaesthesized with ketamine hydrochloride (75mg/kg bwt). Harvesting of the samples was done after a midline incision along the rats' anterior abdominal wall. Blood was also obtained from the apex of the heart via a cardiac puncture technique and transferred into plain bottles and allowed to clot. The blood clot was centrifuged at 3000 rpm for 10 minutes to obtain the serum. Serum glucose levels of the rats in all the experimental groups and the normal control group were then measured with the aid of a glucose test kit (AGAPPE). Harvested pancreatic tissues were fixed in neutral buffered formalin for histological processing.

Isotropic uniform random Sampling (IURS) of tissues and histological processing: Appropriately preserved pancreatic tissue were selected and using systematic random sampling isotropic uniform random sections were taken as reported by [13].
At first the pancreas was placed at (a) center of the circle with equal division and a random number (2) was calculated and selected from the random number table and the sample was cut. Secondly (b) each part of the cut sample was again placed on a second circle with unequal divisions and another random number (6) was selected and the samples were cut here.

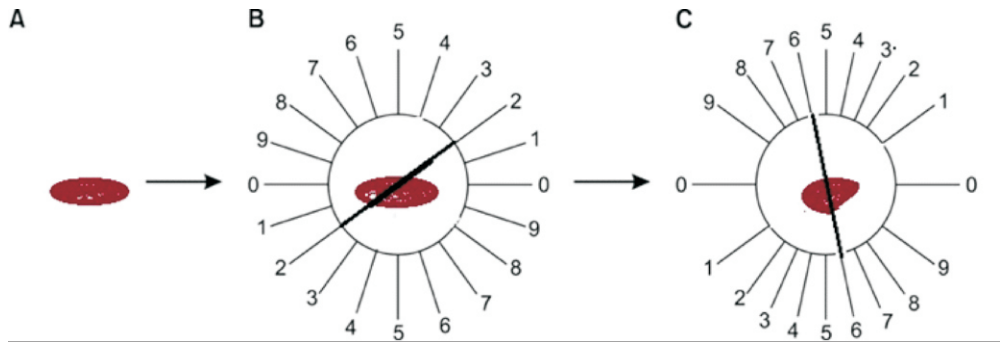

Figure 1: The Orientator Grid for IURS

After IURS, samples were processed using normal routine histological techniques [14]. Tissues were embedded in molten paraffin wax in cassettes. Sections were cut using a Rotatory Microtome (Leica) at 10µ. A random number 5 was calculated and selected from the random number table and 10 sections were systematically picked from the ribbon of cut sections. Floated out in a warm bath, mounted on charged slides, air-dried and stained with Haematoxylin and Eosin. Photomicrographs were taken with a microscope digital camera at 510 mega-pixel (DCM ScopePhoto® China) and a light microscope (Leitz Wetzlar, Germany) at ∓250 magnification.

Islet of Langerhans volume estimations: A test point counting grid (cavalieri estimator) was superimposed on the pancreatic tissue sections and single test points hitting the pancreatic islets were counted and summed. The volume changes of the pancreatic islets were calculated using the following imputations:

$V\ (mm3) = \bar{T} x\ APx\ \sum pi$ (where $\bar{T}$= distance from the 1st section to the 10th section; AP = area per point; $\sum pi$=sum of test points)

Noise due to errors in the sampling was calculated thus:

$noise = 0.0724 \times B / \sqrt{Ax} \sqrt{n} \times \sum pi$

Variations due to the systematic random sampling of the serial sections was calculated:

VARsurs=3(A-noise)-4(B+C)+C

Total variance (TVAR) = noise + VARsurs

Coefficient of error due to the entire sampling process (CE) was calculated: $CE = \sqrt{TVAR} / \sum pi$ [15]

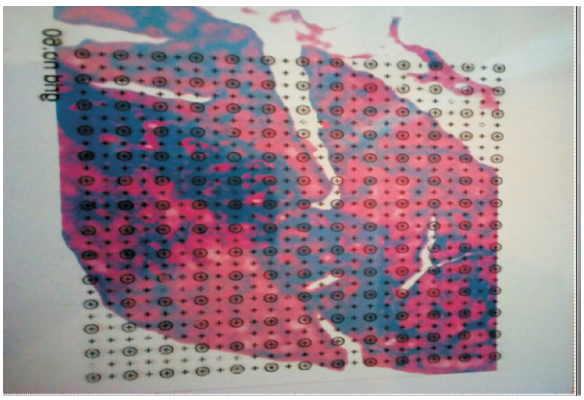

Plate 1: the Cavalieri volume estimator grid.

Statistical Analysis: Results were expressed as Mean±SEM. One way ANOVA was used to test for statistical significant difference at $p<0.05$. Tukey post-hoc test was used to determine where the difference lies. All statistics was done using SPSS (version 20).

RESULTS

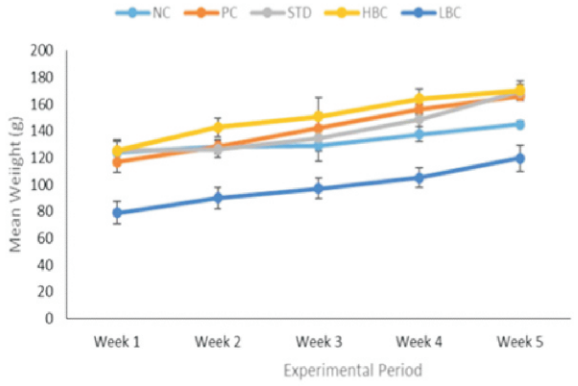

Figure 2: Means of body weights of control and experimental rats for five weeks period. Key: NC-Normal control, DC-diabetic control, STD-Standard drug (metformin), HDE-High dose of extract; LDE-Low dose of extract.

Table 2: Means of serum blood glucose (mg/dl) of normal control and experimental groups.

Parameter	Group 1 NC	Group 2 DC	Group 3 STD	Group 4 HDE	Group 5 LDE	p
FBG mg/dl	100.46±1.48	206±27.66*	93.38±5.06	117.48±30.23	107.28±5.75	0.02

*Indicates statistical significant difference when compared to normal control ($p<0.05$). Key: NC (normal control); DC-positive/diabetic control; STD-standard drug (metformin); HDE-high dose of extract; LDE-low dose of extract.

Table 3: means of volume estimation of pancreatic islets in normal control and treatment groups.

S/No.	Groups	Volume (mm^3)	CE	Mean±SEM	p
1	NC	2620	0.77	1.64±0.24	0.009
2	DC	1020	0.49	0.82±0.14*	
3	STD	2000	0.63	1.23±0.14	
4	HDE	1058	0.52	0.89±0.13	
5	LDE	1550	0.59	0.98±0.15	

*Indicates statistical significant difference when compared to normal control ($p<0.05$). Key: NC (normal control); DC-positive/diabetic control; STD-standard drug (metformin); HDE-high dose of extract; LDE-low dose of extract

Table 4:means of volume estimation of pancreatic islets in diabetic control and Treatment groups.

S/No.	Groups	Volume (mm^3)	CE	Mean±SEM	p
2	DC	1020	0.49	0.82±0.14	0.241
3	STD	2000	0.63	1.23±0.14	
4	HDE	1058	0.52	0.89±0.13	
5	LDE	1550	0.59	0.98±0.15	

None of the groups compared differed significantly to the diabetic control group.

Table 5:means of volume estimation of pancreatic islets in metformin group and extract treated groups

S/No.	Groups	Volume (mm^3)	CE	Mean±SEM	p
3	STD	2000	0.63	1.23±0.14	0.217
4	HDE	1058	0.52	0.89±0.13	
5	LDE	1550	0.59	0.98±0.15	

DISCUSSION

The present study described a stereological method for estimating volumes of pancreatic islets in diabetic wistar rats after treatment with aqueous extract of *Tectonia grandis* (teak) leaves through light microscopy and point counting routine with one grid points (cavalieri estimator grid). High fat diet feeding for a period of five weeks resulted in the induction of obesity an important marker in the pathogenesis of type 2 diabetes mellitus [16]. This caused a corresponding increase in the body weight of the rats for the periods of the feeding.

Hyperglycaemia is the hallmark of a poorly controlled diabetes. Streptozotocin injection at low dose resulted in the induction of type 2 diabetes mellitus in the rats, which may be due to selective destruction of beta cells of the pancreatic islets [17].

Earlier reports have shown that the methanolic extracts of the roots and bark of *Tectonia grandis* attenuated hyperglycaemic in diabetic rats [10, 11].

Likewise, in our present study we found the methanolic extract of the leaves of *Tectonia grandis* to equally attenuate hyperglycaemia in type 2 diabetic rats, this was

however shown to be better at the low dose of the extract.

Our result from the stereological volume estimations of the pancreatic islets similarly showed the aqueous extract of *Tectonia grandis* to cause an increase in the volumes of the pancreatic islets. The increase was more at the low dose of the extract. However, these increases were not significant when the extract treated groups were compared to the diabetic control group and the metformin treated groups. The increases experienced may suggest that the extract possibly contained biomolecules that could have encouraged glucose utilization by restoring delayed insulin response by recipient cells as shown in Table 2. [18] in a study had shown the reduced staining of insulin content in diabetic rats suggesting a loss in the number of viable pancreatic beta cells. Consequently, our volume estimation result supported this claim as shown in table 3. Treatment with the extract showed an increase in the pancreatic islet volumes which may mean restoration of lost β-cells at the pancreatic islets thus an increase in the amount (insulin content) which may further enhance the sensitivity of insulin to target tissues especially at the insulin receptors of these tissues with a subsequent amelioration of the diabetic condition in the rats.

CONCLUSION

In this study treatment of type 2 diabetes in rats showed the anti-diabetic potency contained in the extract of *Tectonia grandis* leaves via attenuation of hyperglycaemia by reversing glucose utilization and restoration of pancreatic islet volumes and thus a restoration in insulin content. However, while this anti-hyperglycaemic and stereological mediated effects in the rat models may reflect similar effects in humans, it may not necessarily mean clinical improvement on the overall. We are presently preparing studies in type 2 diabetes using pure defined preparations of this extract in-order to establish the precise mechanism of action of this plant.

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