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Anti-diabetic effects of methanol extract of *Dennettia tripetala* on diabetic mice

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Abstract
With recent advances in Diabetes research ranking it the eighth leading cause of death across the globe, it has become imperative to advance and harness the use of herbal medicines to better the lots of its sufferers. This study was hitherto designed to evaluate the anti-diabetic effects of methanol leaf extract of *Dennettia tripetala* on alloxan induced diabetic mice. A total of sixty five (65) mice rats with confirmed evidence of diabetes (following induction with alloxan monohydrate) were randomly grouped into thirteen (13) of five mice per group. Animals were then given various treatment as follows; Group 1 (normal control), group 2 (diabetic control), group 3 (received 5 mg/kg of Glibenclamide, a known anti-diabetic agent) and groups 4 -13 were given 250 and 500 mg/kg doses of Methanol Extract [ME], N-Hexane Fraction [NH], Ethyl Acetate Fraction [EAF], Butanol [BF] and Water [WF] Fractions respectively. After period of administration of test substances, Fasting blood glucose (FBG) levels were determined (before and after test periods) using a glucometer, and the lipid profile levels were assayed with the aid of spectrophotometer. Statistical comparisons of mean differences revealed that extract treated group [ME] had a statistically significant decrease ($p < 0.05$) in FBG levels within 10 hours of acute treatment and 14 days short term treatment. Also, inhibitory concentration (IC_{50}) of the methanol extract and fractions of *Dennettia tripetala* samples was observed to significantly increase in the following order: Ascorbic acid (3.00 ug/ml) < BF (29.13 ug/ml) < NF (30.03ug/ml) < EAF (30.05 ug/ml) < ME (32.03 ug/ml) and WF (40.16 ug/ml). Lastly, Histological examination showed a marked and mild rejuvenation of the pancreatic β cells of diabetic mice treated with methanol extract and fractions of *Dennettia tripetala*, suggestive that *Dennetia tripetala* could be anti-diabetic.

Keywords: anti-diabetes, glucose, *Dennettia tripetala*

Introduction
Diabetes mellitus is defined as an elevated blood glucose associated with absent or inadequate pancreatic insulin secretion, with or without concurrent impairment of insulin action [1]. It is usually due to a combination of hereditary and environmental causes, resulting in abnormal high blood sugar level (hyperglycaemia) [2]. It is one of the most common non-communicable diseases globally [3, 4]. It is either the fourth or fifth leading cause of death in developed and developing countries of the world. The disease is increasing rapidly and vast amounts of resources are spent in all countries [5]. Symptoms of diabetes mellitus include classical hyperglycaemia, polyuria, polydypsia, weight loss, sometimes with polyphagia, and blurred vision [6].
A 2012 report shows that about 1.5 million deaths worldwide are directly caused by diabetes [7]. It was the eighth leading cause of death among both sexes and the fifth leading cause of death in women in 2012 [7]. World Health Organization estimates globally that, 422 million adults over 18 years were living with diabetes in 2014. In 2015, an estimated 1.6 million deaths were directly caused by diabetes. Another 2.2 million deaths were attributable to high blood glucose in 2012. Almost half of all deaths attributable to high blood glucose occur before the age of 70 years. WHO projects that diabetes will be the seventh leading cause of death in 2030.
Currently, Three (3) forms of treatment for DM exist: (a) Change in diet and associated physical exercise (b) Insulin replacement therapy and (c) the use of oral hypoglycaemic agents (OHAs). It is expected that an 'ideal' anti-diabetic drug should reduce hyperglycaemia, improve glucose utilization and help reduce the onset and progression of diabetes-related pathologies and non-toxic, orally effective. However, synthetic hypoglycaemic agents cannot meet up this standard, because they produce some side effects including haematological, cutaneous and gastrointestinal reactions, hypoglycaemic coma and disturbances of the liver and kidney and they are expensive [8].
Also, it has been estimated that 80% of the people living in developing world including Nigeria rely mostly on herbal medicine as an integral part of primary health care and traditional medicine in the management of diabetes [9].
Drugs made of plants are therefore known to be less toxic and are considered to be free from several side effects than synthetic chemicals. Before the discovery of insulin as well as other chemical drugs for the treatment of diabetes and other known ailments, drugs made from plants have been used as traditional remedies for the treatment of various diseases including diabetes mellitus [7, 10]. More than 400 plants are being used in different forms for their hypoglycaemic effects in treating diabetes with *Dennettia tripetala* being one of them.

Aim of Study
The study was designed to evaluate the antidiabetic properties of

Dennettia tripetala extract on alloxan induced diabetic mice. Specifically, study examined the effect of different fractions of *Dennettia tripetala* on blood glucose level, determining also in any case, the effect of *Dennettia tripetala* on body weight, as well as on the histo-architecture of the pancreatic beta cells.

Materials and Method

Study Design

Table 1: Sixty-five (65) mice were procured and randomly grouped into thirteen (13) of five mice per group as follows;

Groups	Mice Condition	Treatments	Dose (Mg/Kg)
Group 1	Normal control	5% Tween 80	
Group 2	Negative Control (Diabetic)	5% Tween 80	
Group 3	Positive Control (Diabetic Treated)	Glibenclamide	5mg/kg
Group 4	Diabetic	Methanol Crude Extract	250mg/kg
Group 5	Diabetic	Methanol Crude Extract	500mg/kg
Group 6	Diabetic	Ethyl Acetate Fraction	250mg/kg
Group 7	Diabetic	Ethyl Acetate Fraction	500mg/kg
Group 8	Diabetic	N-hexane Fraction	250mg/kg
Group 9	Diabetic	N-hexane Fraction	500mg/kg
Group 10	Diabetic	Butanol Fraction	250mg/kg
Group 11	Diabetic	Butanol Fraction	500mg/kg
Group 12	Diabetic	Aqueous Fraction	250mg/kg
Group 13	Diabetic	Aqueous Fraction	500mg/kg

Animals

A total of 100 male (only males to avoid pregnancy) albino mice, (weighting between 18-35g) were purchased from the Laboratory Animal Facility of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka. They were housed in clean metal cages, supplied portable water and fed with commercial pelleted feed (Guniea Feed, Nigeria). The animals were acclimatized for two weeks. Animals were handled in compliance with the National Institute of Health Guidelines for care and use of laboratory animals.

Collection and Authentication of Plant Materials

Fresh leaves of *Denettia tripetala* were collected from Irri Town, Isoko South Local Government Area in Delta State. The plant was authenticated by a Taxonomist, Mr. Patrick Ugwuozo of the Department of Botany, Nnamdi Azikiwe University, Awka. A voucher number (PCG/UNIZIK/0631) was given to the plant after identification.

Preparation of Plant Extract

Fresh leaves of *D. tripetala* were washed in a running tap to remove dust and other debris, and air dried for two weeks. Dried leaves of *Dennettia tripetala* were pulverized with electrical blender and kept in clean air tight amber bottle. 750 g of the powdered material was cold macerated in 80% ethanol. The mixture was agitated continually for two days (48 hours). The filtrate was recovered and concentrated to dryness using water bath at 40^0C. The extract was stored in a refrigerator before use. The percentage yield of the extract was calculated using the following formula.

$$\% \text{ yield} = \frac{\text{Mass of Dry Extract}}{\text{Weight after extraction}} \times 100$$

Fractionation of methanol crude extract

Fractionation was carried out using N-hexane, Ethyl Acetate and Butanol following the method described by Ihekwereme *et al.*, (2016). One hundred grams of crude extractwas dispersed in 500 ml of distilled water then poured inside a separating funnel. N-hexane 500ml was added to funnel and shake thoroughly to mix. The mixture was allowed to separate into two distinct layers. The n-hexane portion, (upper layer) was separated and the other portion was subjected to fresh n-hexane until the n-haxane portion was clear completely. After the n-hexane phase, the other portion was subjected to ethyl acetate and butanol successively using the same process as described for n-hexane. The various fractions were filtered and concentrated to dryness using water bath set at 40^0C. The fractions were stored in a refrigerator before use [11].

LD_{50} Determination

Acute toxicity, LD_{50} test was carried out using the method of Lorke (1983) [12]. A total of 13 mice, weighing 28-30g were used in the two phases. In the first stage, the animals were divided into 3 groups of 3 mice each, and the extract was administered at three dose level (10, 100 and 1000 mg/kg) body weight. The animals were then monitored for 24 hours. Absence of deaths in the first phase led to the use of 2000, 3000, 4000 and 5000 mg/kg dose of extract for 4 groups of 1 animal each. Animals were examined again for another 24 hours. Lorke (1983). The number of death (s) was noted for each group and the LD50 was calculated as follows;

$LD_{50} = \sqrt{(D_0 \times D_{100})}$.

Where: D_0 = Highest dose that gave no mortality

D_{100} = Lowest dose that produced mortality.

Yields of extract and fractions

The methanol leaf extract of *D. tripetala* was dark green in colour

after concentration to dryness. Two hundred gram (26.67% w/w) of extract was recovered from powdered leaves of 750 g. Weight of fractions and their yields calculated from 100g of the crude extract are: n-hexane fraction (24 g, 25% w/w), ethyl acetate fraction (28 g, 30% w/w), butanol fraction (21 g, 21% w/w), water fraction (18 g, 19% w/w).

Induction of Diabetes

Alloxan monohydrate was used to induce experimental diabetes in mice using the method described by Kalbag *et al*, (2011). Animals were fasted for 24 hours, followed by injection of single dose of 150 mg/kg body weight of alloxan monohydrate intraperitoneally. The alloxanized mice were kept for 3 days with free access to feed and water for hyperglycaemia to develop. Baseline fasting blood glucose levels were determined using one Touch Glucometer (Lifescan, USA). Mice with glucose levels above 200 mg/dl were recruited for the study.

Dosage selection

Dosage of extract administered to animals was determined from $1/10^{th}$ and $1/20^{th}$ of the estimated LD_{50} as described by Neharkar and Galkwad (2011) [13]

Statistical Analysis

Data obtained from the study were analysed with the Statistical Package for Social Sciences (SPSS-20). Results were presented as Mean ± Standard error of mean (SEM) of sample replicates. Raw data were subjected to one-way analyses of variance (ANOVA) followed by post hoc Tukey's test. P-values < 0.05 was considered to be statistically significant.

Results

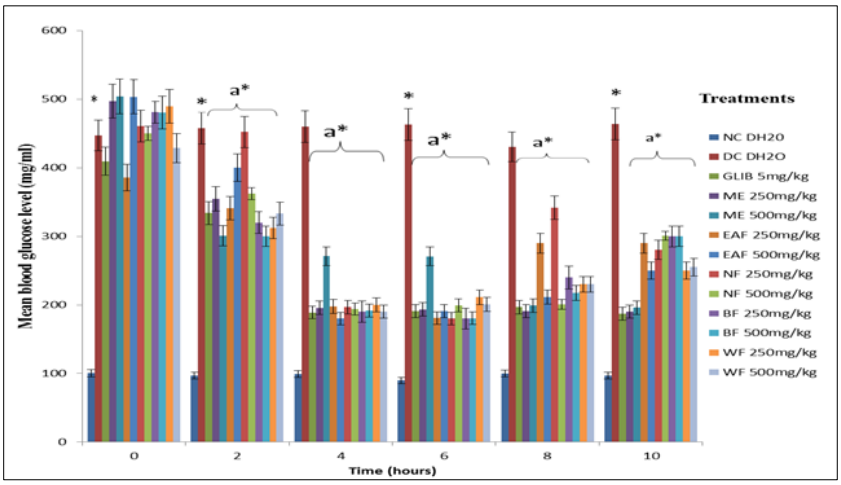

Fig 1: Comparative Effect of *Dennettia tripetala* on Blood glucose level

[2 hours and 10^{th} hour significantly reduced (a*p<0.05)] when compared to diabetic induced (*)

Figure 2: Effect of various treatments on blood glucose level (hourly study) NC: Normal control, DC: Diabetic control, GC: Glibenclamide, ME: methanol extract, EAF: Ethyl acetate fraction NF: n-Hexane fraction, BF: Butanol fraction, WF: Water fraction and $D.H_2O$ = Distilled water

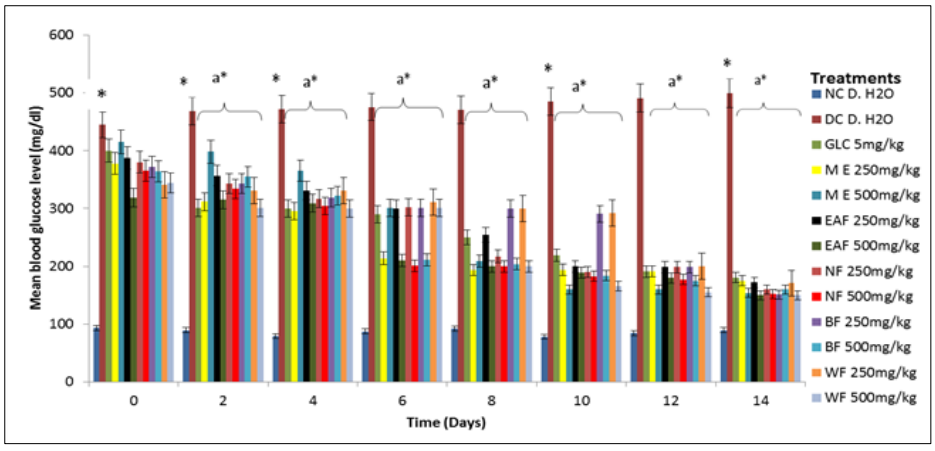

Fig 2: Effect of various treatments of *Dennettia tripetala* on blood glucose level (Daily study)

[Day 2 to the 14th day significantly reduced (a*p<0.05)] when compared to diabetic induced (*). NC: Normal control, DC: Diabetic control, GLC: Glibenclamide control ME: methanol extract, EAF: Ethyl acetate NF: N-Hexane fraction, BF: Butanol fraction, WF: Water fraction and D. H_2O: Distilled Water

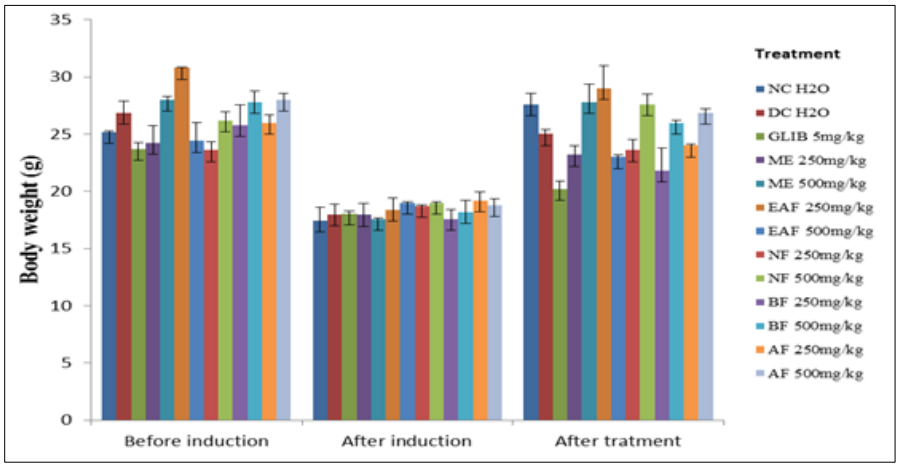

Fig 3: Effect of various treatments of *Dennettia tripetala* on Body Weights

Above figure shows the final Change in body weight of mice in different treatment groups. CBW= Change in body weight, NC= Normal control, Glib. = glibenclamide, ME=methanol, EAF=Ethyl acetate, BF=Butanol fraction, NF=N-hexane, WF= water fraction and D. H_2O: Distilled Water

Above figure shows the final Change in body weight of mice in different treatment groups. CBW= Change in body weight, NC= Normal control, Glib. = glibenclamide, ME=methanol, EAF=Ethyl acetate, BF=Butanol fraction, NF=N-hexane, WF= water fraction and D. H_2O: Distilled Water

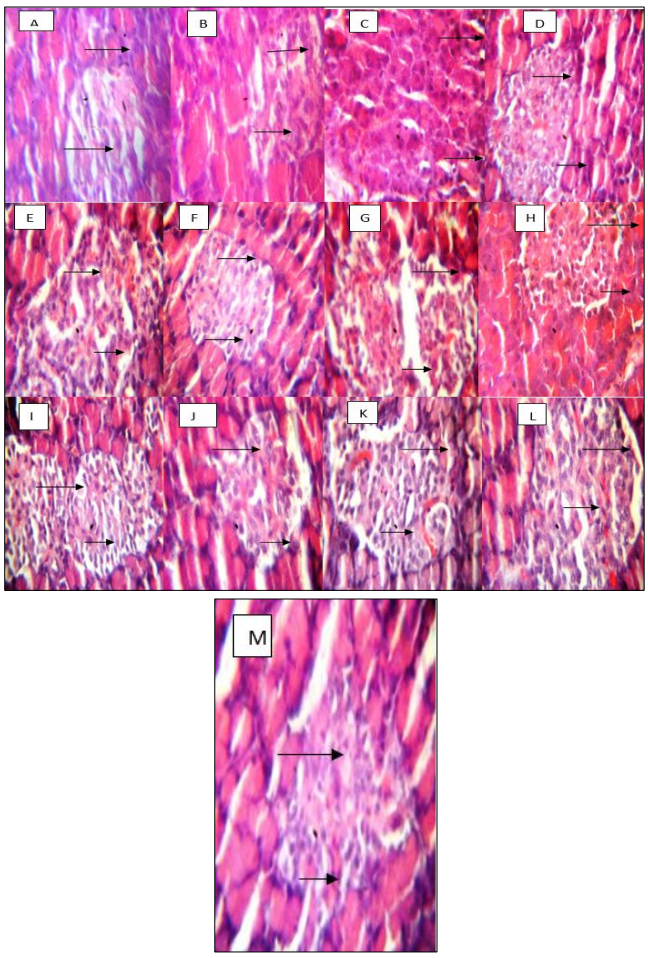

Fig 4: Showing photomicrograph of pancreas histology for groups 1-13 [Labelled A - M]

Discussion

In today's changing world, plants and many of their derivatives are being used as natural remedies and folk medicines for the treatment of diabetes [14]. In the past few years some of the new bioactive drugs isolated from hypoglycaemic plants showed antidiabetic activity with more efficacy than oral hypoglycaemic agents used in clinical therapy [15]. To this point, current study investigated the hypoglycaemic effect of *Dennettia tripetala* on alloxan induced diabetic mice.

Effect of *Dennettia tripetala* on blood glucose level (acute study)

Noticeable from our results (figure I), the extract and fractions significantly ($p < 0.05$) reduced the fasting blood glucose levels in alloxan induced diabetic mice. The two doses (250 and 500 mg/kg) of the methanol extract and 5mg/kg glibenclamide caused a reduction in the fasting blood glucose levels from the 2nd hour to the 10th hour of treatment. The doses of 250 and 500 mg/kg of fractions of methanol extract also showed a significant ($p < 0.05$) reduction in fasting blood glucose levels when compared to the untreated group after 2 h to 10th h respectively. The extract and fractions exhibited dose related and significant ($p < 0.05$) reduction in blood sugar. At 2 hours the reduction was marked, at 4 and 6 hours respectively the reduction was at maximum. Though at 8 and 10th hours the blood glucose started rising again. The methanol extract and fractions of *Dennettia tripetala* demonstrated a significant ($p < 0.05$) decrease in blood glucose level when compared to untreated control from 2 hour to the 10th hour. Though at 8 and 10th hours the blood glucose started rising. The non-consistent decrease with time per group may be due to bioavailability of the various treatments in the systemic circulation. There was no reduction in blood glucose level of the diabetic group, as they were not treated. In both hourly and daily studies the extract and its fractions are found to be effective when compared with the standard drug Glibenclamide. The non-consistent decrease with time per group may be as a result of bioavalability. The glucose lowering activity observed in the diabetic animals may be due to the stimulation of β - cells of pancreatic islets [16, 17]. Glibenclamide a sulphonylurea used as a reference drug caused a marked reduction in blood glucose level. Sulphonylureas are known to produce their hypoglycaemic effect primarily through increased release of insulin in pancreatic β cells. Thus any plant secondary metabolite or chemical constituent which is capable of affecting the insulin secretion from pancreatic β cells may be similar to sulphonylureas in action [18]. The result of this study are in line with previous results reported on some plants with similar phytoconstituents, which have been confirmed to have both in, *in-vivo* and *in-vitro* diabetic activity. These plants include: *Phyllanthus niruri* [15], *Raphia hookeri* [15], *Diosorea dumetorum* [16] *Azadirachta indica* [12] *Momordica charantia* [13], *Cola acuminate* [8], *Phyllanthus amarus* [11], *Croton macrostachyus* [15]. These plants mentioned above contains similar phytochemicals found in *D. tripetala* and were able to lower blood glucose level.

Effect of various daily treatment of *Dennettia tripetala* on blood glucose level

The two doses, (250 and 500 mg/kg) of the methanol extract of *Dennettia tripetala* as well as Glibenclamide produced significant ($p < 0.05$) reduction in the fasting blood glucose levels when compared with the untreated control from day 0 to 14th day of treatment. The fasting blood glucose reduction was dose dependent. The reduction was consistent, as the extracts and various fractions gradually reduced blood glucose level on daily bases.

The highest reduction was seen in the crude extract which is methanol 250mg/kg followed by methanol 500mg/kg, with percentage reduction of 61.76% and 61.09% respectively for the hourly studies. Ethyl acetate fraction (500mg/kg) was found to have the highest reduction rate among the three fractions, with percentage of 50.38. Water fraction 250mg/kg was next with a percentage of 48.96. The least was found to be water fraction 250mg/kg with percentage of 24.87. The order of reduction rate on hourly studies for the various treatment received is as followed: ME (250mg/kg; 61.76%) > ME (500mg/kg; 61.09) > EF (500mg/kg; 50.38) > WF (250mg/kg; 48.96) > NH (250mg/kg; 44.4%) > WF (500mg/kg; 40.50%) > BF(250mg/kg; 39.79%) > BF (500mg/kg; 37.55) > NH (500mg/kg; 33.33) > EF (250mg/kg; 24.87%). The methanol extract and fractions of *Dennettia tripetala* also demonstrated a significant ($P<0.05$) decrease in blood glucose level when compared to untreated control from day 1 to the 14th day of treatment. The highest decrease in blood glucose level was observed in 500mg/kg with a percentage of 62.89% followed by Butanol fraction 250mg/kg with a percentage of 59.40 and the least among the fractions was water or aqueous fraction 250mg/kg with percentage reduction of 50.4. The order of reduction rate on the dialy treatment is summarized as follows: ME (500mg/kg; 62.89%) > BF (250mg/kg; 59.40%) > NH (500mg/kg; 250mg/kg; 58.38%) > NH (500mg/kg; 57.89%) > WF (500mg/kg; 56.39%) > BF (250mg/kg; 56.04%) > EF (500mg/kg; 55.59%) > (250mg/kg; 53.99%) > EF (250; 52.99%) > WF(250mg/kg; 50.40%).

Effect of *Dennettia tripetala* treatment on Body Weight

Reduction in body weight of diabetic mice and a later increase after treatments was shown. Weight increase was more in normal control (36.74%) and the treated group when compared to diabetic control (6.25%). The weight, increased in the following order, (250mg/kg) dose: EAF (31.85%)> BF (20.59%) > NF (19.27%) > ME (19.9%) > WF (12.73%). (500mg/kg) dose: ME (19.19%) > NF (19.47%) > WF (16.81%) > (EAF=BF (14.41%). Alloxan could have caused the reduction of the body weight of the mice. While the extracts of methanol and fractions of *Dennrttia tripetala* from all indication reversed the effect [19], reported a significant ($P<0.05$) decrease in the body weight of a diabetic rats as a result of induction with alloxan monohydrate and a significant increase in body weight of albino rats administered ethanol extract of *Cajanus cajan* leaf. This appears to be consistent with the previous report of World Health Organization, that diabetes mellitus is often characterized by rapid and significant weight loss leading to fatigue which is not easily reversed [19].

The blood glucose ameliorative effect of *D. tripetala* could be attributed to the presence of heterogeneous phytoconstituents such as alkaloids, saponins, tannins, and flavanoids, associated with hypoglycemic activity [20]. The presence of flavonoids, and saponins had previously been reported in ethanolic fruit extracts of *L. camara* which demonstrated hypoglycemic activity in streptozotocin induced diabetic male wistar rats [21]. Flavonoids act on various molecular targets and regulate different signaling

pathways in pancreatic β-cells, hepatocytes, adipocytes and skeletal myofibers [22, 23]. Flavonoids quercetin and ferulic acid on pancreatic β-cells leads to their proliferation and secretion of more insulin [22] as the mechanism by which they reduced hyperglycaemia caused by streptozocin in diabetic rats. Flavonoid fraction from *Pterocarpus marsupium* has been shown to cause pancreatic beta cell regranulation. Epicatechin, its active principle, has been found to be insulinogenic thus enhancing insulin release and conversion of proinsulin to insulin *in vitro* [20, 24].

Photomicrograph of the pancreas (figure IV) showed atrophic pancreatic islet with vacuolations which indicates significant damaged islets of Langerhans in the beta cells. The regenerative effect of methanol extract and fraction of *D. tripetala* on the pancreatic cells indicate positive effects of *Dennettia tripetala* on the production of insulin. Onyegeme and Essien (2015) [23] had earlier reported that the ultrastructure of alloxan diabetic pancreas showed considerable reduction in the islet of langerhans as well as depleted islets. The regeneration of islet of Langerhans may be due to the effect of methanol extract and fractions of *Dennettia tripetala*on the pancreatic cells, which resulted in the production of insulin. Again, histological examinations revealed a marked and mild rejuvenation of the pancreatic β cells of diabetic mice after treatement with methanol extract of *Dennettia tripetala* and its fractions. Both the crude extract and fractions of *Dennettia tripetala* possessed antidiabetic properties.

Conclusion

The results of this study in a nut shell indicate that the methanol leaf extract of *Dennetia tripetala and* its fractions can be used to manage diabetes. This confirms the local use of *Dennetia tripetala* leaves for the treatment of diabetes mellitus by the indigenous people of Irri, Delta state. The methanol extract and fractions of *Dennettia tripetala* demonstrated a significant ($p < 0.05$) decrease in blood glucose level, as this was more pronounced in the high dose (500mg/kg) for both hourly and daily studies.

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