Effects of Aqueous Leaf Extract of *Adansoni adigitata* (Baobab) on Alloxan-Induced Diabetes Mellitus on endocrine Pancreas of Adult Male Wistar Rats

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Abstract:

Adansonia digitata (Ad) also known as the baobab tree is very characteristic of the Sahelian region and belongs to the Malvaceae family. Baobab contains nutritionally significant levels of essential nutrients including fiber, proteins and minerals. As one of the main threats to human health in the 21st century diabetes mellitus (Dm) is a metabolic disorder that arises from a reduction of insulin available for the normal function of many cells in the body resulting from progressive destruction of Beta cells of the islets of Langerhans. Dm is associated with long-term complications that affect almost every part of the body; often leading to kidney failure, blindness, heart and blood vessel disease, stroke, amputations and nerve damage. Hence our study aims to evaluate the possible therapeutic potential of *Adansoniadigitata* leaf extract in experimentally alloxan-induced diabetes in Wistar rats with respect to the pancreas and its hormonal secretion. Thirty-six Wistar rats were randomly divided into 9 groups of four rats per group at the end of two weeks accalamization. Group(g) 1(control group) received 0.1ml/kg/bw normal saline/14days, g2 (Diabetes negative control) received alloxan (150mg/kg)/2days, g3 (Low dose) received 150mg/kg/bwAlloxan + 200mg/kg/bw Ad extract g4 (Medium dose) received 150mg/kg/bwAlloxan +400mg/kg/bw Ad extract, g5 (High dose) received 150mg/kg/bwAlloxan +600mg/kg/bw Ad extract, g6 (Standard) received 150mg/kg/bwAlloxan + 150mg/kg/bw Metformin, g7 (Low dose) received 200mg/kg/bw Ad extract only, gp8 (Medium dose) received 400mg/kg/bw Ad extract only and g9 (High dose) received 600mg/kg/bw Ad extract only. The animals were humanely sacrificed after 2 weeks of experiment under ketamine anesthetic. Blood was collected via cardiac puncture and as send to the lab for assay

of hormones, a midline laparotomy was performed and the pancreas was harvested and fixed for histological studies. Our study indicates that administration of Adextracts to alloxan-induced diabetic rats reduced the weight of the animals in a dose-dependent manner temporarily reduced blood glucose, increased glucagon and insulin concentrations, but was unable to restore the diabetic pancreas to its normal state. Although the mechanism of this effect remains to be experimentally proven, it possibly or apparently is related to the high carbohydrate content of the extract and absence of phytonutrients like tannin. Together our findings were able to show that the leaf extract of Ad could be a potential and effective therapeutic target for short term blood sugar regulation.

Keywords: *Adansonia digitata,* alloxan, diabetes mellitus, pancreas and pancreatic hormonal

INTRODUCTION

Medicinal plants on one hand for thousands of years, have been used as traditional treatment for numerous diseases (Mohamed *et al*, 2015). They are commonly used in treating and preventing specific ailments and diseases and are generally considered to play a beneficial role in healthcare (Sofowara, 1986). *Adansonia digitata*(L.) called the baobab tree in both English and French is very characteristic of the Sahelian region (Savannahdrylands of sub-Saharan Africa) and belongs to the Malvaceae family (De Caluwe*et al*, 2010). With vast medincinal and non-medicinal benefits local communities mainly utilize the leaves, pulp and seeds as a source of food and for income generation. It contains nutritionally significant levels of essential nutrients including fiber, proteins and minerals (Muthai *et al*, 2017 andVanWyk*et al*, 2000). The fruit pulp is conventionally used against small pox, measles, diarrhea, scurvy, cough and dysentery while the bark is used for fever treatment (Brendler *et al*, 2013).The extracts of seed and fruit pulp of *Adansonia digitata* have been demonstrated to have anti-tumor action, as well as restore and modulate tumor markers levels (Elsaid, 2013). The fruit pulp extract also has a hepatoprotective influence on the liver (Mohamed *et al*, 2015). With its various part been of utmost importance (Owen, 1970), Singh et al in his 2013 review summarized it posses anti-oxidant, antipyretic, analgesic, antimicrobial, antiviral; as well as antidiabetic properties.

Diabetes mellitus on the other hand is a metabolic disorder that results from a reduction of insulin available for normal function of many cells in the body (Bennett and Knowler, 2005). This results from progressive destruction of Beta cells of the islets of Langerhans (Eisenbarth *et al*, 1987). It is considered one of the main threats to human health in the 21st century, and is associated with long-term complications that affect almost every part of the body; often leading to kidney failure, blindness, heart and blood vessel disease, stroke, amputations and

nerve damage (Sournya and Srilatha, 2011). Although a chronic and progressive disease, initial lesions can be seen in very early stages, as also corroborated by previous studies where initial lesions were seen to occur after three days of diabetes mellitus induction in rats (Haligur, 2012; Ozmen, 2007). Clinically, the diagnosis of diabetes is based on a combination of hyperglycemia, variable loss of C-peptide secretion and dependence on exogenous insulin for survival, as well as destruction of pancreatic beta cells in autoimmune type 1 diabetes (Chitale, 2014).

The pancreatic islets secrete hormones of vital importance, especially in the regulation of glycaemia, and blood glucose concentration. The main cell types of the pancreas are: the alpha cells (α-cells), the beta cells (β-cells) and the delta cells (δ-cells), but only the alpha and beta cells would be explained in few detail for the purpose of our study. The α-cells secretes glucagon between meals, when glucose level falls below 100mg/dL, and in response to rising amino acid levels in the blood after a high-protein meal. The β-cells secretes insulin during and immediately after a meal when blood nutrient levels are rising. Its principal targets or insulin responsive tissues are the liver, skeletal muscle and adipose tissue (Saladin, 2018). The activities of certain enzymes controlling glycogen synthesis and glycolysis are stimulated by insulin. Thus, insulin stores excess glucose as glycogen, and also promotes the storage of fats and the synthesis of proteins (Flatt and Bailey, 1991). According to Saladin (2018), insulin insufficiency or inaction is well known as the cause of diabetes mellitus.

Alloxanhowever is a toxic glucose analogue that preferentially accumulates in pancreatic beta cells, generating harmful hydroxyl radicals, as well as inhibiting beta cell glucose sensor or glucokinase (Lenzen, 2007). It is known to causes damage to the β-cells (general necrosis), leaving islets composed almost entirely of α-cells at approximately ninety-six hours after alloxan treatment (Cagle, 1950). Hence, alloxan-induced diabetes is one of the most widely used and validated experimental model of chemical and drug-induced diabetic model for preclinical studies.

This present study aims to evaluate the possible therapeutic potential of *Adansoniadigitata* leaf extract in experimentally alloxan-induced diabetes in wistarrats with specific respect to the pancreas and its hormonal secretion effect compared with the standard Metformin.

Here we indicated that administration of Adextracts to alloxan-induced diabetic rats, reduced the weight of the animals in a dose-dependent manner, temporarily reduced blood glucose, increased glucagon and insulin concentrations (insulin-increasing effect of the extract was dose-dependent, and more potent than that of metformin), but was unable to restore or induce regeneration of the diabetic pancreas to its normal state as metformin. Altogether, our

findings were able to show that the leaf extract of Ad could be a potential and effective therapeutic target for short term blood sugar regulation.

MATERIALS AND METHODS

Collection, authentication and preparation of plant materials

Fresh leaves of A. digitata were procured from a local dealer in Kaduna state. It was identified by a botanist in the Department of Plant Sciences and Biotechnology, University of Nigeria, Nsukka. The extraction method described by Bello and Nwoso (2015) was used. 500g of the powdered plant material was soaked in 1500ml of distilled water for 72 hours. Separation of the plant extract was carried out using Whatman no. 4 filter paper. The resulting extract was then concentrated using rotatory evaporator, and stored at 4oC before use.

Experimental animals and ethical approval

A total of 36 adult male wistar rats were purchased from the animal house of the University of Nigeria, Enugu Campus. The animals were bred,housed in netted iron cages and provided easy access to food (Grower's mesh, New market Enugu) and water ad libitum; maintained under standard laboratory conditions (temperature 20^{o}C to 24^{o}C, with relative humidity of 60-70% under 12 hours light and day cycles) and allowed to acclimatize for two weeks prior to the experimentin the animal facility of Enugu State University of Science and Technology College of Medicine, Enugu. At the end of acclimatization, the animals were randomly divided into nine (9) groups of four (4) animals each. The body weights of the animals were obtained before and after acclimatization, and also at weekly intervals during the experiment. The protocol for conducting the in vivo study in wistar rats was approved by the Institutional Animal Ethical Committee (IAEC), Enugu State University of Science and Technology.

Induction of Diabetes using alloxan

Alloxan Stock solution was prepared by dissolving alloxan monohydrate (Sigma-Aldrich, USA) (0.9g) in distilled water (6cm^{3}), and diabetes was induced by single intraperitoneal injection of alloxan monohydrate (150mg/kg). The volume of the solution containing 150mg/kg given to each rat was determined by its weight. After a period of two days, the rats with blood glucose level greater than 200mg/dl was considered diabetic and used for the research work. The method of Mohammad and Hauwa'u (2013) was adopted in the study with slight modification.

Group 1(control group)- 0.1ml/kg/bw normal saline/14days

Group 2 (Diabetes negative control) - Alloxan (150mg/kg)/2days

Group3 (Low dose)-150mg/kg/bwAlloxan + 200mg/kg/bwAdansoniadigitata extract Group4 (Medium dose)-150mg/kg/bwAlloxan +400mg/kg/bwAdansoniadigitata extract
Group5 (High dose)-150mg/kg/bwAlloxan +600mg/kg/bwAdansoniadigitata extract
Group6 (Standard)-150mg/kg/bwAlloxan + 150mg/kg/bw Metformin
Group7 (Low dose)-200mg/kg/bwAdansoniadigitata extract only
Group8 (Medium dose)-400mg/kg/bwAdansoniadigitata extract only
Group9 (High dose)-600mg/kg/bwAdansoniadigitata extract only

Determination of Blood Glucose Level

Blood samples of rats were collected by cutting the tail tip of the rats for glucose determination before administration of extract, after administration of the extract for 7 days and after administration of the extract for 14days. Determination of the blood glucose level was carried out using a glucometer (BG Check) and results were reported in mg/dl.

Hormonal assay

At the end of the experiment, blood samples were collected via cardiac puncture from all rats. Serum was then separated and stored at -20^{o}C until the hormonal assay. Serum levels of total insulin and glucagon concentrations were determined byELISA (Enzyme-linked Immune-sorbent Assay) by Micallef*et al.* (1995)**.** The desired number of coated wells was properly securedin the holder and 100µl of standards, specimens, and controls was dispenseinto appropriate wells. It was thoroughly mix for 30seconds and incubated at room temperature (18-25^{0}C) for 60 minutes. The incubated mix was removed by flicking plate contents into a waste container. The microtiter well was rinse and flick 5 times with distilled or deionized water. the wells was strokedsharply onto absorbent paper or towels to remove all residual water droplets, then 100µl of TMB reagent was dispense into each well andmixed gently for 10 seconds. The reaction was finally stoped by the addition of 100µl of stop solution to each well and mixed gently for another 30seconds. The absorbance was then read at 450nm with a microtiter well reader within 15 minutes

Histopathology studies

At the end of the study, the animals were humanely sacrificed under anasthesia (ketamine hydrochloride), twenty-four hours after the last treatment. Sections of the pancreas were collected for histopathological examination. The samples were fixed in 10% phosphate buffered formalin for a minimum of 48 hours. The tissues were subsequently trimmed, dehydrated in 4 grades of alcohol (70%, 80%, 90% and absolute alcohol), cleared in 3 grades

of xylene and embedded in molten wax. On solidifying, the blocks were sectioned, 5µm thick with a rotary microtome, floated in water bath and incubated at 60˚C for 30 minutes. The 5µm thick sectioned tissues were subsequently cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90%, 80% and 70%).The sections were then stained with Hematoxylin for 15 minutes. Blueing was done with ammonium chloride. Differentiation was done with 1% acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant; DPX.

The prepared slides were examined with a Motic™ compound light microscope (Motic BA410E Elite Research Compound Microscope; Motic Asia, Hong Kong) using x4, x10 and x40 objective lenses. The photomicrographs were taken using a Motic™ 5.0 megapixels microscope camera at x160 and x400 magnification

Statistical analysis

Data obtained was expressed as the mean standard deviation. They were analyzed using Statistical Package for Social Sciences (SPSS version 21; IBM SPSS, Chicago, Illinois, USA) software package. Values were analyzed as Mean± SD using one way ANOVA with Tukey post hoc test. The level of significance was set to $p<0.05$.

RESULTS

Phytochemical analysis

The phytochemical analysis result (table 1) shows that the aqueous leaf extract of *Adansoniadigitata* used in the experiment contains alkaloids, flavonoids, glycosides, saponin and terpenoids. The carbohydrate content of the extract was very high, followed by protein, lipids and crude fibre respectively.

Table 1: Proximate Analysis of *Adansoniadigitata*leaves extract

Parameters	Values in %
Moisture	0.81
Protein	14.35
Crude fibre	7.22
Ash	1.43
Fats and Oil	10.00

Carbohydrate	66.19

Table 1: The phytochemical analysis result shows that the aqueous leaf extract of *Adansonia digitata* Moisture and ash content are negligible.

Body weight

From table 2A below our results (values without superscripts) showed that there was no significant difference in weight among and between groups from day one to day 15 (after accamatization). As revealed in table 2B, there was an increase (19.2%) in body weight of group 1 at the end of the experiment. In contrast, group 2 animals that served as the positive control (untreated diabetic group) showed a 10.7% decrease in body weight at the end of the experiment. Among the diabetes-treated groups (3, 4, 5 and 6) which were given low, medium, high doses of the aqueous leaf extract of *Adansoniadigitata* and Metformin respectively after alloxan induction, only group 3 showed an increase in body weight (27.5%) while groups 4, 5 and 6 showed a decrease (8.24%, 10.62% and 3.97% respectively) in their body weight at the end of the experiment. Group 7 and 8 showed a 4.98% and 5.48% decrease in their body weight respectively while group 9 also had a 5.26% decrease in body weight at the end of the experiment.

Table 2A: Results of Mean± SD of the body weights in gram of all the experimental animals

Groups	Day1	Day 8	Day 15
1	120.0±9.13	127.5±5.00	143.1±13.07
2	141.7±2.87	139.2±13.21	126.5±10.62*
3	152.5±6.46	174.7±16.07	194.5±15.98*
4	178.3±14.43*	139.8±9.20	$163.6\pm4.01^{\beta}$
5	171.3±27.80*	178.2±73.57	$153.1\pm10.57^{\beta c}$
6	173.8±15.48*	154.9±29.48	$166.9\pm29.30^{\beta}$
7	168.6±17.05*	161.2±14.61	$160.2\pm15.83^{\beta}$
8	153.2±10.19	144.9±11.30	$144.8\pm11.95^{\beta c}$
9	140.7±16.0	143.5±12.69	$148.1\pm13.71^{\beta c}$

* = $p<0.05$ compared with the control group 1; $^{\beta}$ =$p<0.05$ compared with the control group 2; ^{CDEFGHI}=$p<0.05$ compared with the control group 3,4,5,6,7,8,9.

Table 2B: Weekly and percentage changes in body weights (g)

Groups	Week 1 Changes (d8-d1)	Week 2 Changes (d15-d1)	% changes (d1-d8)	% changes (d8-d15)	% total changes (d15-d1)
1	7.5±9.13	23.1±3.94	6.5	12.24	19.2
2	2.5±10.34	15.2±7.75	1.7	1.26	10.7
3	22.2±9.61	42.0±9.52	14.56	11.33	27.5
4	38.5±5.23	14.7±10.42	21.59	17.02	8.24
5	7.2±5.23	18.2±17.23	4.2	14.09	10.62
6	18.9±14.0	6.9±13.82	10.87	7.75	3.97
7	7.4±2.44	8.4±1.22	4.39	0.65	4.98
8	8.3±1.11	8.4±1.1	5.42	0.06	5.48
9	2.8±3.31	7.4±3.31	6.5	12.24	5.26

Data analyzed at Mean± SD and significance at $p<0.05$

Fasting blood glucose

This table shows the effect of the extract on fasting blood glucose levels at weekly intervals. On Day 1, there is a statistically significant ($p<0.05$) increase in group 2 glucose level (422.7 ± 135.9 mg/dl) and a significant ($p<0.05$) decrease in group 7 when compared to the normal control (group 1), as well as statistically significant decreases in groups 3,5, 7, 8 and 9, compared to the diabetic control group (group 2). At the end of the first week of extract administration (Day 8), there shows a decrease in fasting blood glucose levels among the diabetes induced groups (groups 3, 4 and 6) compared to day 1. Also, there are increases in glucose level among groups 1, 2, 5, 7, 8 and 9 with the diabetic group (group 2) showing the highest level of glucose concentration (439.2 ± 13.21 mg/dl) compared to day 1. However, none of these are statistically significant difference ($p>0.05$) except groups 6 and 7 which shows a significant decrease ($p<0.05$) when compared to group 2.By the second week of the experiment, there is an increase in fasting blood glucose levels among all the groups when compared to the previous week (Day 8), but a decrease in the group 6 compared to Day 1. Group 2 shows the highest concentration (466.5 ± 110.62 mg/dl) followed by group 6 (309.8 ± 111.0 mg/dl) which was statistically significant ($p<0.05$) when compared to group 1. There were also statistically significant differences ($p<0.05$) among groups 3-6 showing decrease in glucose concentration compared to group 2, and also among groups 7-9 when compared to

group 6. The change in glucose concentration appears prominently in group 5 (69.9 %) and least in group 2 (10.4 %) at the end of the experimental period of two weeks.

Table 3: Results of the effects of aqueous extract of *Adansoniadigitata* leaf on fasting blood glucose level (mg/dl)

Group	Day 1	Day 8	Day15	Changes (D15-D1)	% Changes
1	85.5±3.7	95.2±6.8	110.0±4.32	24.5±0.62	28.7
2	422.7±135.9*	439.2±13.21	466.5±110.62*	43.8±25.3	10.4
3	181.8±71.3$^{\beta}$	153.8±92.1	223.5±68.4$^{\beta}$	41.8±2.9	22.9
4	191.3±134.1	120.33±69.2	217.3±142.6$^{\beta}$	26.0±8.0	13.6
5	149.8±64.3$^{\beta}$	199.0±200.95	254.5±117.89$^{\beta}$	104.8±53.6	69.9
6	389.5±185.2	198.8±77.5$^{\beta}$	309.8±111.0*$^{\beta}$	63.8±74.2	17.1
7	76.3±4.3*$^{\beta}$	84.3±5.50$^{\beta}$	108.3±47.5^{f}	37.5±43.2	49.4
8	80.5±5.5$^{\beta}$	92.5±13.8	113.8±78.9^{f}	33.3±73.5	41.4
9	76.5±1.3$^{\beta}$	82.0±2.5	120.0±30.0^{f}	43.5±28.7	63.4

*= $p<0.05$ compared with the control group 1; $^{\beta}$= $p<0.05$ compared with the control group 2; ^{CDEFGHI}= $p<0.05$ compared with the control group 3,4,5,6,7,8,9.

Hormonal analysis

From the table, there were significant increases in all the groups compared to the negative control (group 1), with the exception of group 2 (untreated diabetic group) which has the least insulin concentration at the end of the experiment (9.86 ± 0.87 mIU/mL). The increase in insulin concentration was more pronounced in group 5 (22.12 ±3.94 mIU/mL), followed by groups 6, 4 and 7 respectively. The differences in groups 3-9 were statistically significant ($p<0.05$) when compared to group 2. Likewise, there are statistically significant differences ($p<0.05$) between groups 2, 4, 5 and 6 when compared to the control group. The glucagon concentrations decreased significantly ($p<0.05$) at the end of the experiment among groups 2-9 when compared with the negative control group (group 1), while group 2 has the lowest glucagon concentration (25.44 ±1.36 pg/ml). There are also significant increases in glucagon among the groups, with group 4 having the highest glucagon concentration (63.86 ± 18.41 pg/ml), followed by group 5, 8, 9 and 7 respectively. The differences in groups 4 and 5 were statistically significant ($p<0.05$) compared to group 2.

Table 4: Results of the effect of aqueous leaf extract of*Adansoniadigitata*on hormonal (insulin & glucagon) level in the wistar rats

Groups	Insulin concentration (mIU/mL)	Glucagon concentration (pg/ml)
1	15.86±2.19	107.08±6.53
2	9.86±0.87*^{cdefgh1}	25.44±1.36*
3	20.66±1.13$^{\beta}$	48.65±27.99*
4	21.39±2.24*$^{\beta}$	63.86±18.41*$^{\beta}$
5	22.21±3.94*$^{\beta}$	55.38±7.09*$^{\beta}$
6	21.76±2.05*$^{\beta}$	39.33±4.49*
7	20.84±1.35$^{\beta}$	50.41±6.11*
8	17.41±2.64$^{\beta}$	51.34±2.75*
9	20.19±1.01$^{\beta}$	50.49±2.06*

*=p<0.05 compared with the control group 1; $^{\beta}$ =p<0.05 compared with the control group 2; ^{CDEFGHI}=p<0.05 compared with the control group 3,4,5,6,7,8,9.

Histological findings

The endocrine pancreas showed normal histoarchitecture in group 1 (negative control) while group 2 (positive control) showed decreased number of islets, confirming diabetes. Groups 3, 4 and 5 (extract-treated diabetic groups) showed decreased islet numbers and sizes while group 6 (metformin-treated diabetic group) showed regeneration by increased number of islets. Groups 7, 8 and 9 (extract-treated non-diabetic groups) showed normal histoarchitecture of the endocrine pancreas. These effects of *Adansoniadigitata*aqueous leaf extract on the histology of the endocrine pancreas are presented in the photomicrographs below.

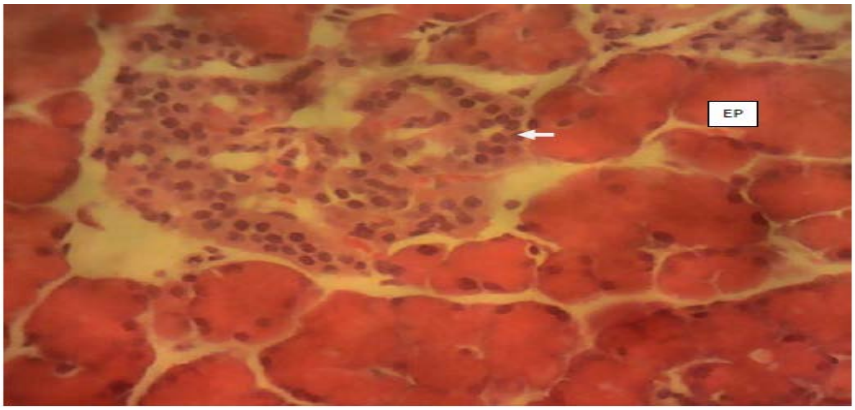

Plate 1: Sections of the pancreas collected from the animals in this group (group 1, normal saline) showed the normal histo-architecture of both the exocrine and endocrine pancreas. The pancreatic lobules contained normal sized pancreatic islets (arrow) which are composed of a cluster of pale eosinophilic cells with eccentrically located nuclei and abundant cytoplasm. Exocrine pancreas (EP).H&Ex160 and x400.

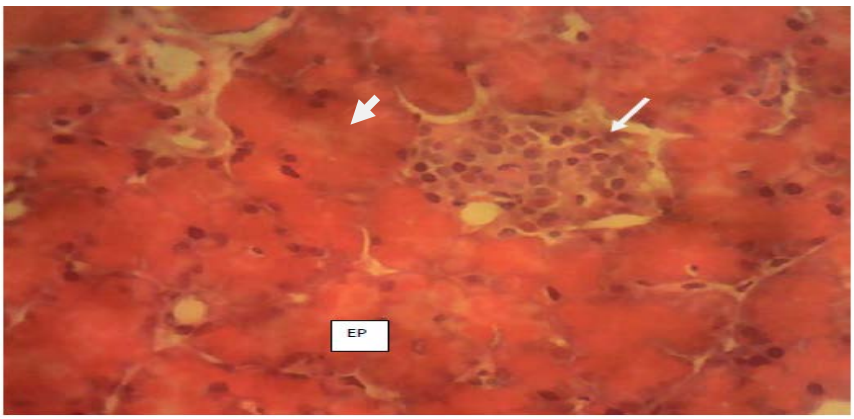

Plate 2: Sections of the pancreas collected from the animals in this group (group 2, alloxan monohydrate 150mg/kg) showed normal histo-architecture of the exocrine pancreas. However, the endocrine pancreas showed a marked decrease in the number and sizes of the pancreatic islets. The few observed pancreatic islets (arrow) were scanty, composed of tightly packed islet cells and are relatively inconspicuous. Exocrine pancreas (EP). H&Ex160 and x400

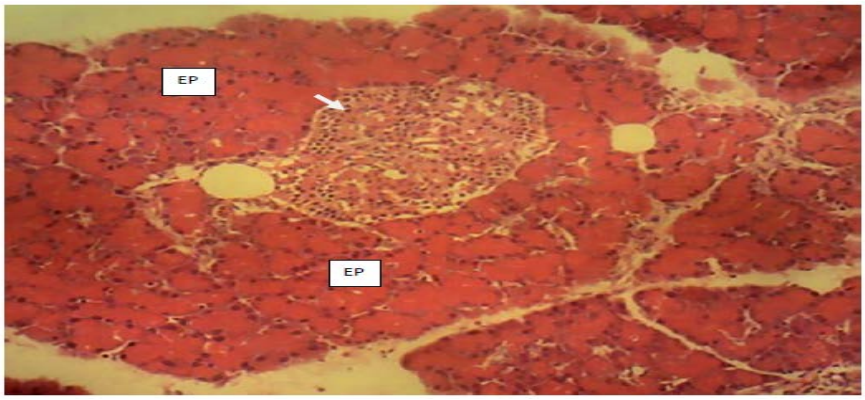

Plate 3: sections of the pancreas collected from the animals in this group (group 3, alloxan monohydrate 150mg/kg + *adansoniadigitata*leaf extract200mg/kg) showed normal histo-architecture of the exocrine pancreas. the endocrine pancreas showed a marked decrease in the number and sizes of the pancreatic islets (arrow). the few observed pancreatic islets (arrow) were relatively inconspicuous. exocrine pancreas (ep); blood vessel (bv); pancreatic duct (pd). h&ex160

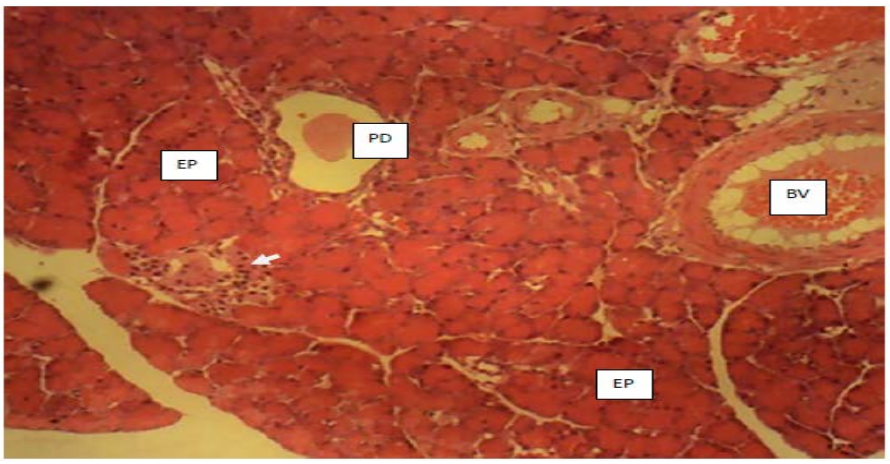

Plate 4: sections of the pancreas collected from the animals in this group (group 4, alloxan monohydrate 150mg/kg + *Adansoniadigitata*leaf extract 400mg/kg) showed normal histo-architecture of the exocrine pancreas. The endocrine pancreas showed a marked decrease in the number and sizes of the pancreatic islets (arrow). The few observed pancreatic islets (arrow) were relatively inconspicuous. Exocrine pancreas (EP); Pancreatic duct (PD); Blood vessel (BV). H&Ex160

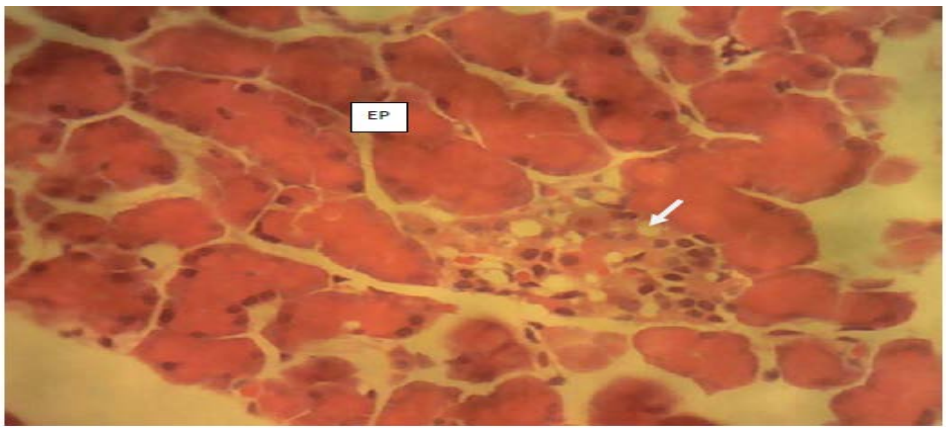

Plate 5: Sections of the pancreas collected from the animals in this group (group 5, alloxan monohydrate 150mg/kg + *Adansoniadigitata*leaf extract 600mg/kg) showed normal histo-architecture of the exocrine pancreas. The endocrine pancreas showed a marked decrease in the number and sizes of the pancreatic islets (arrow). The few observed pancreatic islets (arrow) were relatively inconspicuous. H&Ex400

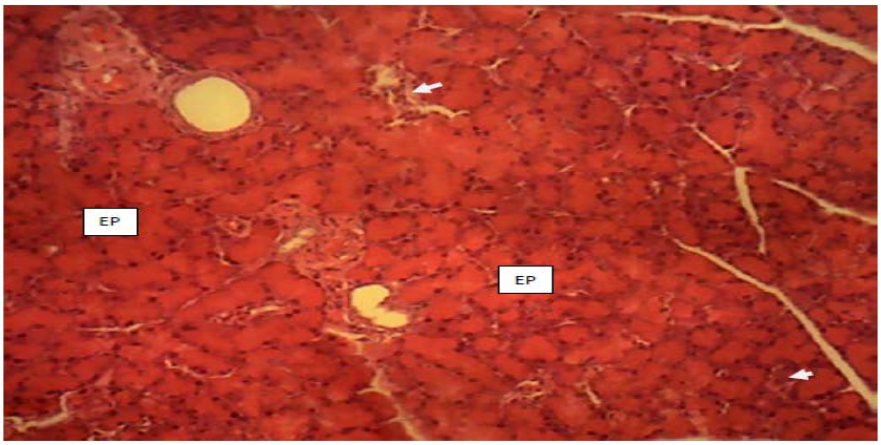

Plate 6: sections of the pancreas presented in this group (group 6, alloxan monohydrate 150mg/kg + metformin 150mg/kg) showed a marked decrease in the number of the pancreatic islets. A few of the pancreatic islets (arrow) showed marked increase in the number and size of the pancreatic islet cell. This is highly suggestive of pancreatic islet regeneration. Exocrine pancreas (EP).H&E x160.

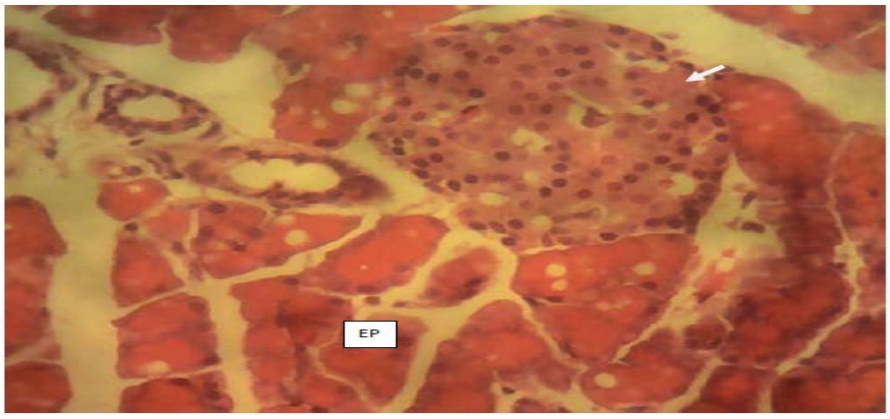

Plate 7: sections of the pancreas collected from the animals in this group (group 7, *Adansoniadigitata*leaf extract 200mg/kg) showed the normal histo-architecture of both the exocrine and endocrine pancreas. The pancreatic lobules contained normal sized pancreatic islets (arrow) which are composed of a cluster of pale eosinophilic cells with eccentrically located nuclei and abundant cytoplasm. Exocrine pancreas (EP).H&E x400; x160.

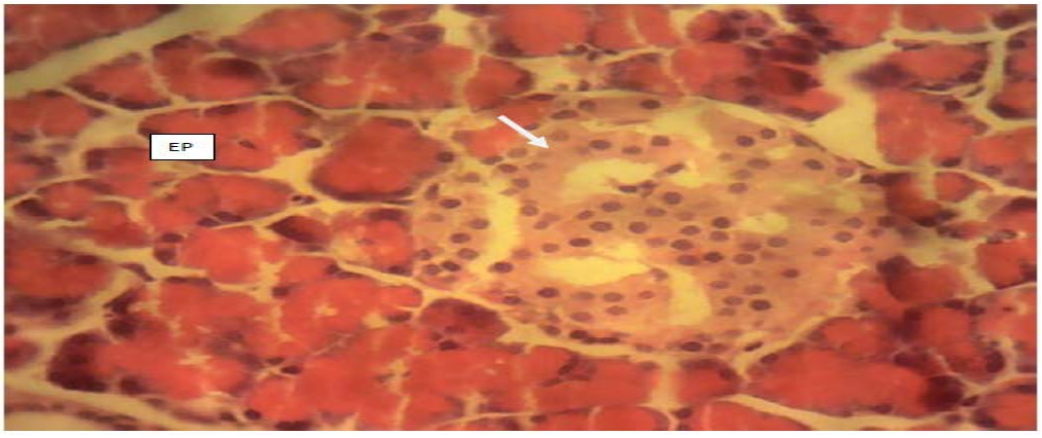

Plate 8:sections of the pancreas collected from the animals in this group (group 8, *Adansoniadigitata*leaf extract 400mg/kg) showed the normal histo-architecture of both the exocrine and endocrine pancreas. The pancreatic lobules contained normal sized pancreatic

islets (arrow) which are composed of a cluster of pale eosinophilic cells with eccentrically located nuclei and abundant cytoplasm. Exocrine pancreas (EP).H&Ex400; x160.

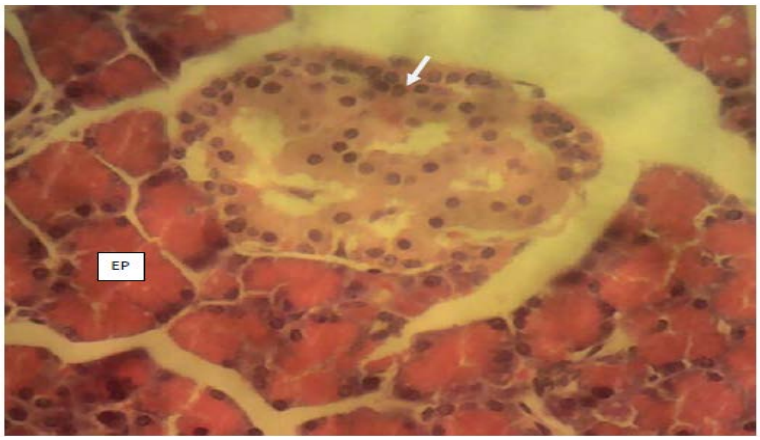

Plate 9: sections of the pancreas collected from the animals in this group (group 9, *Adansonia digitata* leaf extract 600mg/kg) showed the normal histo-architecture of both the exocrine and endocrine pancreas. The pancreatic lobules contained normal sized pancreatic islets (arrow) which are composed of a cluster of pale eosinophilic cells with eccentrically located nuclei and abundant cytoplasm. Exocrine pancreas (EP).H&Ex160 and x400.

DISCUSSION

In this study, the use of the leaf extract of *Adansoniadigitata* on alloxan-induced diabetic rats was to investigate the relationship between *Adansoniadigitata* leaves extract and some of the diabetic symptoms with an aim to establishing its usefulness in the treatment of diabetes. As stated by Melmed *et al.*(2010), blood plasma glucose concentration is one of the criteria for assessing for diabetes. Diabetes mellitus is marked by increased blood glucose sequel to decreased insulin secretion by β-cells of the pancreas (Rang *et al*, 2012), as observed in the untreated diabetic control (group 2). Hyperglycemia therefore, is a primary factor in the development of diabetes complications, and decreases in average blood glucose have a profound effect to prevent complications in diabetes mellitus (Diabetes Control and Complications Trial Research Group, 1993). The fasting blood glucose profile shows that *Adansoniadigitata*leaves extract was capable of lowering plasma glucose levels, as seen in the diabetic groups treated with low and medium doses of the extract (200mg/kg and 400mg/kg respectively) after the first week of the experiment. This supports previous studies on other parts of the *Adansoniadigitata*plant which were shown to possess antidiabetic potential (Tanko*et al*, 2008; Saravanaraj*et* al, 2017; Mohammad and Hauwa'u, 2013). The blood glucose levels of the diabetic group treated with a high dose of the extract (600mg/kg), as well as the normal groups treated with different doses of the extract (groups 7-9), on the other hand, were increased after administration. However, at the end of the second week of the experiment, the blood glucose levels increased in all the diabetic and non-diabetic groups

treated with the extract. This increase in blood glucose levelspossibly could be as a result of the absence of tannins in the leaf extract, which was otherwise detected in the fruit pulp (Mohammad and Hauwa'u, 2013) and stem bark (Tanko*et al*, 2008) extracts of *Adansoniadigitata*. Tannins is however fascinatedly known for inducing regeneration of β-cells (Anderson and Polansky, 2002) and also as an anti-hyperglycemic agents in diabetic rats (Pinent*et al*, 2004). Moreso, this increase in blood glucose might also have arised owing to the high carbohydrate content of the extract, as Eizirik and Cnop (2010)reported that carbohydrate consumption increases demand on the β-cell for insulin secretion, which may lead to endoplasmic reticulum stress, as well as oxidative stress (Robertson *et al*, 2004) both of which can result in β-cell damage after a long period of time. It is also possible that a high-carbohydrate/lower-fat diet such as contained in the extract, on a prolonged period of time could increase insulin sensitivity and lower fasting glucose levels, as reported by Gower, *et al* (2012). The increase in blood glucose levels after a decrease in the previous week could also have resulted from an increase in glucagon and insulin in the regulation of homeostasis, released in a pulsatile manner to regulate glucose production and metabolism (Farhy and McCall, 2009), in which glucagon promotes glycogenolysis and gluconeogenesis to increase endogenous blood glucose levels during prolonged fasting (Freyche*et al*, 1988). It was also observed, however, that the blood glucose levels of the diabetic group treated with the extract were significantly lower than that of the untreated diabetic group as well as the group treated with the standard drug (metformin). This suggests that the extract has an antagonistic effect between its high carbohydrate content and its hypoglycemic property via unclear mechanisms.

From the results, the insulin concentrations of all the groups were significantly elevated except in the diabetic control group which has the least concentration of insulin when compared with the negative control (group 1). This reduction in the insulin concentration is due to the selective destruction or necrosis of the insulin-producing β-cells of the pancreatic islets; thus causing diabetes. Insulin secretion is therefore inhibited due to the β-cell toxicity (Rohilla and Ali, 2012). As noted by Szkudelski (2001), the mechanism of cytotoxic action of alloxan on β-cells involve oxidation of essential sulphydryl (-SH) group, inhibition of glucokinase, generation of toxic free radicals and disturbance in intracellular homeostasis with the resulting damage resulting in decreased insulin release and attendant hyperglycemia. Conversely, the increase in insulin was most pronounced in the diabetic rats treated with high dose of the extract (group 5), followed by the diabetic rats treated with metformin (group 6). This suggests that the insulin-increasing effect of the extract was dose-dependent, and more

potent than that of metformin. The significant increase in insulin secretion can be attributed to a stimulation of the β-cells by flavonoids present in the extract (Benhabyles*et al*, 2015).

Insulin is known to regulate glucagon, as well as various β-cell signals that provide an inhibitory stimulus to the α-cells and suppress glucagon (Gromeda*et al*, 2007; Ishihara *et al*, 2003; Rorsman*et al*, 1989). Therefore, an increase in insulin secretion suppresses glucagon secretion and a decrease in insulin, in concert with a low glucose concentration, stimulates glucagon secretion (Cooperberg and Cryer, 2010). The findings of our study showed a significant decrease in the glucagon concentrations in all the groups compared to the negative control (group 1), as well as a significant increase in the diabetic rats treated with medium and high doses of the extract (groups 4 and 5) compared to the positive control (group 2). This also confirms that the extract has a dose-dependent effect on pancreatic hormonal concentrations.

The histopathological observations in the present work on one hand showed that the β-cells in the untreated diabetic control group were significantly reduced in size and number, thus confirming diabetes. The decrease in islet size and β-cell number, as well as the histoarchitecture of the islets, is in agreement with El-Esawy*et al* (2016) and Elkotby*et al* (2018). Therefore, the serum insulin level was decreased and the glucose concentrations were increased as shown in tables 3 and 4 The diabetic groups treated with varying doses of the extract (groups 3-5) all show marked decrease in size and number of β-cells, though this is more prominent in those treated with medium and high doses of the extract. These tally with the increased blood glucose levels in the groups. Thus, we can agree Karadimos*et al*.(2012) that the quantity of remaining β-cells was not sufficient to control blood glucose. On the other hand, the metformin treated diabetic group (group 6) shows the same symptoms as groups 3-5, but with a few of the islets showing an increase in the β-cells. This confirms metformin's ability to regenerate cells (El-Soud*et al*, 2016). The non-diabetic rats treated with different doses of the extract (groups 7-9) shows normal histoarchitecture of the pancreatic islets. This suggests that, while the high carbohydrate content may cause an increase in glucose concentrations, it is not enough to cause hyperglycemia (>200mg/dl) which is a characteristic of diabetes. Therefore, the extract has no adverse side effects on normal rats, and could lower blood glucose levels but on prolonged administration, might cause hyperglycemia in medium and high doses more than low doses, and subsequently lead to the onset of diabetes. In comparison, metformin increased the blood glucose levels even more than that of the extract, but also shows its curative potential in the regeneration of β-cells. The reasons for inducing hyperglycemia in diabetes treatment by the extract; after showing its hypoglycemic potential

is currently unknown. This could be due to paucity of data as *Adansoniadigitata*leaves have been poorly investigated (Irondi*et al*, 2017; Sokeng*et al*, 2019), in relation to diabetes.

The percentage decrease in body weight noticed in the diabetic group may be due to the adverse effects of diabetes to the body. This is in agreement with Lau *et al*., (2003) who stated that diabetes is often associated with a characteristic loss of body weight, partially due to increased muscle wasting. Groups 7, 8 and 9 showed decreases in their body weights at the end of the experiment. This suggests that the extract does not cause weight gain irrespective of the dose of the extract given, thus it is not dose-dependent. However, comparing the percentage changes in body weight of animals in group 7, 8 and 9 to group 1, it can be suggested that the aqueous leaf extract of *Adansoniadigitata*had no benefiting effect on the body weight of the animals. These observations agree with previous studies of Oyetunji, *et al* (2015) as well as Eghoi and Paul (2016). Among the diabetes-treated groups (3, 4, 5 and 6) which were given low, medium, high doses of the aqueous leaf extract of *Adansoniadigitata* and metformin respectively after alloxan induction, only group 3 showed an increase in body weight while groups 4, 5 and 6 showed decreases in their body weight at the end of the experiment. This reduction in weight may be due to the presence of alkaloids in the extract (Burkill, 1994). Since the animals in this group (4, 5 and 6) also had increased blood glucose levels (13.6%, 69.9% and 17.1% respectively) as seen in table 3, it may also be suggested that they were also experiencing the weight-decreasing effect of diabetes as also noticed in the group 2. This also agrees to the idea that the aqueous leaf extract of *Adansoniadigitata* was not able to maintain reduced blood glucose levels at the end of the experiment even when there was a decrease in the blood glucose levels in these groups during the first week (Table 4.5). Loss of body weight is a major consequence of diabetes in rats (Ramachandran*et al*, 2012). This could be due to dehydration and catabolism of fats and proteins (Rajkumar*et al*, 1991). The prevention of loss in body weight by the low extract dosage may be due to increasing glucose uptake in peripheral tissues or inhibition of catabolism by good glycemic control (Ambika*et al*, 2013).

To the best of our kwowlegde*Adansoniadigitata* leaf extracttherefore causes weight loss which may be due to the presence of alkaloids in the extract or simply as a result of the side effects of diabetes. There was a high increase in blood sugar levels in the diabetes-treated groups as well as a decrease in the pancreatic islets, which may be due to the high carbohydrate content of the extract. In contrast, the histoarchitecture of the non-diabetic groups treated with the extract remained normal while the blood sugar levels were minimally increased. This suggests that the extract has no adverse side effects on normal rats and so can

possibly be effective as a temporal (not on prolonged basis) therapeutic measure againt DM. The hormonal profiles which showed an increase in insulin concentration in all the groups may be due to the presence of flavonoids in the extract. Alternatively, the general decrease in glucagon concentrations may be as a result of the increase in insulin secretion.

CONCLUSION

In conclusion, the present study indicates that administration of *Adansonia digitata* extracts to rats with alloxan-induced diabetes, reduced the weight of the animals in a dose-dependent manner, temporarily reduced blood glucose, increased glucagon and insulin concentrations, but was unable to restore the diabetic pancreas to its normal state. The mechanism of this effect is yet unknown but is apparently related to the high carbohydrate content of the extract and absence of phytonutrients like tannin.

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