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Anti-hyperglycemic Activity of Methanol Seed Extract of *Dioclear reflexa* in Alloxan-induced Diabetic Rats

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Abstract: Diabetes is a chronic disease characterized by high blood glucose level and abnormal metabolism of carbohydrates, protein and fat. This is either as a result of insufficient endogenous insulin production by the pancreatic beta cells or impaired insulin secretion. The anti- hyperglycaemic potential of methanol seed extract of *Dioclea reflexa* was investigated using alloxan induced diabetic rat model. The methanol seed extract of *Dioclea reflexa* at a concentration of 100 and 200 mg/kg body weight were orally administered to alloxan induced diabetic rats for a period of nine (9) days. The oral glucose tolerate test (OGTT) was also carried out using animal experimental procedure. Preliminary secondary metabolite screening of the extract revealed the presence of tannins, saponnins, terpenoids, flavonoid, phenols, and reducing sugar at a moderate concentration. The lethality dose of the plant extract was observed to be equal to 5000 mg/kg b. w. In the oral glucose tolerance test, the methanol extract exerted the highest response, similar to glibenclamide after 15 minutes and 30 minutes of administration compared to the control. The methanol extract recorded the highest blood glucose-lowering effects after day 9 of treatment ($p < 0.05$) compared with the diabetic rats that were administered normal saline and 0.3 mg/kg body weight of glibenclamide. Administration of the extract at 200 mg/kg body weight showed improved pancreas architecture compared with the pancreas of animals in other treatment groups. The plant extract possessed anti-diabetic activity like the reference drug glibenclamide, the findings in this study revealed, administration of 200 mg/kg b. w. of the extract caused regeneration of the beta cells of diabetic rats.

KEYWORDS: Hyperglycemia; Alloxan; Glibenclamide; Pancreas; *Dioclea reflexa*, Fabacea

1.0 Introduction

Glucose homeostasis in the organism is tightly regulated by insulin, a hormone that acts on the major glucose metabolic tissues such as muscle, liver and adipose tissue. Insulin's main effects include promoting glucose uptake, glycogen synthesis in the liver and muscles, triacylglyceride formation to be stored in adipocytes and protein synthesis (Vega-monroy and Fernandez-Meija, 2011). Insulin is secreted by the pancreatic beta cells, modulated by blood glucose concentration. The pancreatic β-cells dysfunction plays an important role in the pathogenesis of type-1 and type-2 diabetes.

Diabetes is a group of metabolic diseases characterized by hyperglycemia, caused by a defect in insulin production, insulin action or both (Vaga-monrey and Fernandez-Meija, 2011). Type 1 diabetes mellitus is due to an autoimmune destruction of the insulin producing pancreatic β-cells which usually leads to absolute insulin deficiency. During diabetes, persistent hyperglycemia causes increased production of free radicals for all tissues from glucose auto-oxidation and protein glycosylation (Robertson, 2004; Omoboyowa *et al.*, 2016;). Chemotherapy remains the kernel of diabetes control. These anti-diabetes drugs are not only expensive but also parade heterogeneous levels of toxicity and may invoke poor compliance in patients. Consequently, the diminished potency of anti-diabetes drugs has paved way for alternative drugs of plant origin. Hence, the choice of *Dioclea reflexa* seed for anti-diabetes study.

Dioclea reflexa is a woody climber, which provides flowers and pods on long rope-like stems that hang from the forest canopy. The

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seeds of *Dioclea reflexa* are found in Africa, Asia and some other part of the world (Adeneye *et al.*, 2006). The plant is called *Arin* or *Agbaarin* in the western Nigeria and *Ukpo* in the Eastern Nigeria. The anti-microbial activity of *Dioclea reflexa* leaf had been reported by Adeneye *et al.* (2006). This study was therefore designed to investigate the response of pancreatic β-cell of alloxan-induced diabetic rats to methanol seed extract and glibenclamide treatment.

2.0 Materials and Methods

2.1 Materials

2.1.1 Plant

The plant material for this study *Dioclea reflexa* (Fabacea) seeds were purchased from Owutu Market in Afikpo South Local Government Area, Ebonyi State. The seeds were identified and validated at the Department of Botany, University of Nigeria, Nsukka.

2.1.2 Glibenclamide

Glibenclamide used was manufactured by Emzor Pharm Ind. Ltd., Nigeria.

2.2 Methods

2.2.1 Preparation of Methanol Extract of Dioclea reflexa Seeds

The seeds were cracked to remove the pericarp and the seed coat. The cracked seeds were chopped with sharp, clean stainless knife before crushing and milling with a mechanical grinder to fine powder. Exactly 140 g of the ground sample was extracted in 400 ml of methanol for 72 hours with occasional stirring. The mixture was filtered with What-man No 4 filter paper, the filtrate was concentrated with rotary evaporator and the remaining solvent was allowed to evaporate at room temperature till the extract was obtained.

2.2.2 Screening of Secondary Metabolites

The qualitative phytochemical screening of the methanol extract of *Dioclea reflexa* seed was carried out using procedures outlined by Harborne (1984) and Pearson (1976).

2.2.3 Acute Toxicity and Lethality (LD_{50}) Test

The acute toxicity and lethality of methanol extract of *Dioclea reflexa* seed was determined using the modified method of Lorke (1983). The test was divided into two phases. In phase one, nine (9) randomly selected adult mice were divided into three groups. Three per group (n = 3) and received 10,100 and 1000 mg/kg body weight of methanol extract and the signs of toxicity and number of death for a period of 24 hours were recorded. After 24-hours observation, the doses for the second phase were determined based on the outcome of the first phase. Since there was no death, a fresh batch of animals were used following the same procedure in phase 1 but with higher dose ranges of 1900, 2600 and 5,000 mg/kg body weight of extract. The animals were also observed for 24 hours for signs of toxicity and possible number of death. The LD_{50} was calculated as the geometric mean of the high non-lethal dose and lowest lethal dose (Lorke, 1983).

2.2.4 Induction of Diabetes

The albino Wistar rats were injected with alloxan monohydrate, dissolved in normal saline at a dose of 130 mg/kg body weight intraperitoneally. On day 3, elevated blood glucose was observed; the animals were monitored for another day before diabetes was confirmed (Omoboyowa *et al.*, 2016).

2.2.5 Experimental Design

A total of twenty-five male albino rats weighing between 180-220 g were used for the study, the rats were obtained from the faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. They were acclimatized for seven days in the Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana animal house given

regular feed and water *ad libitum*. The rats were divided into five different groups with five animals per group (n=5):
Group 1: Non-diabetic control (not induced).
Group 2: Alloxan induced diabetic rats administered 0.3 ml of normal saline.
Group 3: Alloxan-induced diabetic rats administered 0.3 mg/kg body weight of glibenclamide
Group 4: Alloxan-induced diabetic rats administered 100 mg/kg body weight of methanol seed extract of *Dioclea reflexa*
Group 5: Alloxan-induced diabetic rats administered 200 mg/kg body weight of methanol seed extract *Dioclea reflexa.*

2.2.6 Glucose Profile Study

Glucose profile studies were conducted with alloxan-induced diabetic rats as described by Atangwho *et al.*, (2013) with modification.

2.2.7 Oral Glucose Tolerance Test (OGTT)

The procedure, dosage of extracts and the glibenclamide and animal groupings in this test were as described above. In addition to this protocol, the rats were orally administered glucose (2 g/kg body weight) 30 minutes after dosing, and blood samples were obtained through the tail at time zero (0) prior to glucose dosing and at 15, 30, 45, 60, 90 and 120 minutes after glucose administration to measure the glucose level.

2.2.8 Acute Anti-hyperglycemic Study

Glucose profile studies were conducted with diabetic rats, in this test, 4 groups of alloxan induced diabetic rats were treated as follows: group 2 received 0.3 ml normal saline, group 3 received 0.3 mg/kg body weight of glibenclamide, group 4 received 100 mg/kg body weight of *Dioclea reflexa* seed extract, group 5 received 200 mg/kg body weight of *Dioclea reflexa* seed extract and group 1 served as the normal control group. The normal saline and glibenclamide were administered in doses per day during the period of the study. Fasting blood glucose (FBG) was measured on day 0 (baseline), 3, 6 and 9. At the end of the study, the animals were euthanized, the pancreas were removed and preserved for histology. The ACCU-CHECK Glucometer was used to measure the glucose level with compatible strip.

2.2.9 Biochemical Assay

The concentration of glutathione was determined by the method of Habig *et al.* (1974), catalase activity was assayed spectrophotometrically according to the method described by Aebi, (1983). Superoxide dismutase activity was assayed by the method of Arthur and Boyne (1985). The concentration of malondialdehyde (MDA) was determined by the method of Wallin *et al.* (1993), The plasma vitamin C concentration was determined using the method of Tietz, (1983).

2.2.10 Histopathological Examination of the Pancreas

Dissected pancreas, from control, diabetic and treated rats were fixed in 10% formaldehyde, processed and used for histological analysis. Tissue processing was carried out using an autotechnicon and the prepared 5 µm thick sections were mounted on slides and stained with haematoxylin and eosin. The stained sections were morphologically evaluated (Ezejiofor *et al.*, 2013). The prepared slides were examined with a Motic™ compound light microscope using x4, x10 and x40 objective lenses. The photomicrographs were taken using a Motic™ 9.0 megapixels microscope camera at x100 and x400 magnifications.

2.2.11 Statistical Analysis

The data obtained were analyzed using One Way Analysis of Variance. The data were further subjected to LSD post hoc test for multiple comparisons and differences between Means regarded significant at $P < 0.05$. The results were expressed as Mean ± SD.

3.0 Results

The methanol extract of *D. reflexa* seed was concentrated in a rotatory evaporator and fixed

dried. The extract yield was observed to be 7.1g (5.07%).

In the experiment, there was no lethality or behavioural changes in the three groups of the mice that received 10, 100, 1000 mg/kg body weight of the extract at the end of the first experiment. Based on this result, further increased doses of 1900, 2600 and 5000 mg/kg body weight of the extract showed weakness and one death case was observed within 24 hours of administration. This result showed that the extract was relatively safe at dose below 5000 mg/kg body weight.

The qualitative phytochemical screening as observed in Table 1 showed moderate presence of compounds such as saponnins, terpeniods, carbohydrates, flavoniods, phenols, tannins and steriod while tannins and phenols were not detected in the sample.

The effect of methanol extract of *D. reflexa* seed and glibenclamide on oral glucose tolerance in non-diabetic rats is shown in Figure 1. The measured fasting blood glucose (FBG) reached its peak value 15 minutes after oral administration of glucose. Animals administered 2 g/kg body weight of glucose and 0.3 mg/kg body weight of glibenclamide had the highest significant ($p < 0.05$) reduction of FBG concentration and sustained throughout all the measured time compared to the glucose level of other treatment group. The animals administered 200 mg/kg body weight of glucose and 1 g/kg bodyweight of methanol seed extract of *D. reflexa* showed significant ($p < 0.05$) decrease in blood glucose level 30 minutes of treatment compared to glucose level after 15 minutes of treatment and also showed significant ($p<0.05$) reduction in glucose after 45, 60, 90 and 120 minutes respectively compared to glucose level after 30 minutes of treatment. The animals administered 2 g/kg body weight of glucose and 0.3ml of normal saline showed significant ($p<0.05$) decrease in glucose level after 30 minutes compared to glucose level after 15 minutes and showed significant ($p<0.05$) increase in glucose level after 60 minutes compared to glucose level after 45 minutes of treatment.

The effect of methanol seed extract of *D. reflexa* and glibenclamide on bodyweight of alloxan induced diabetic rats is shown in Figure 2. Animals induced and treated with 0.3 ml normal saline showed significant ($p < 0.05$) change in body weight on day 3 and 6 compared to normal control animals. Animals induced and treated with 100 mg/ kg body weight of *D. reflexa* seed extract showed significant ($p < 0.05$) increase in body weight on day 0, 6, and 9 respectively compared to animal treated with 0.3mg/ kg body weight glibenclamide. Animals induce and treated with 200 mg/kg body weight of methanol seed extract of *D. reflexa* showed significant ($p < 0.05$) reduction in body weight on day 0, 3, 6 and 9 respectively compared to animals treated with 100 mg/kg body weight of *D. reflexa* seed extract.

Effect of methanol seed extract of *Dioclea reflexa* and glibenclamide on selected organs (Spleen, Liver, Kidney and Heart) weight of alloxan induced diabetic rats is shown in Figure 3. Animals induced and treated with 0.3 ml of normal saline showed significant ($p < 0.05$) reduction in weight of the Spleen compared to normal control animals and showed significant ($p < 0.05$) increase in the weight of the Liver, Kidney and Heart compared to normal control animals. Animals induced and treated with 0.3 mg/kg body weight of glibenclamide showed significant ($p < 0.05$) reduction in weight of the Spleen, Liver, Kidney and the Heart respectively compared to animals induced and treated with 0.3 ml normal saline. Animals induced and treated with 100 mg/kg body weight of *D. reflexa* seed extract showed significant ($p < 0.05$) increase in weight of Spleen, Liver, Kidney and Heart compared with animals treated with 0.3mg/kg body weight of glibenclamide. Animals induced and treated with 200 mg/ kg body weight of *D. reflexa* seed extract showed significant ($p < 0.05$) reduction in weight of the Spleen, Liver, Kidney and Heart compared with animals treated with 100 mg/kg body weight of *D. reflexa* seed extract.

The effect of methanol seed extract of *D. reflexa* on glucose level of alloxan induced diabetic rats is shown in Figure 4. All animals induced with 150 mg/kg body weight of alloxan monohydrate showed significant ($p<0.05$) increase in blood glucose level on day 0. Animals induced and treated normal saline showed significant ($p<0.05$) reduction in blood glucose level on day 3, 6 and 9 respectively

compared to day 0. Animals induced and treated with 0.3 mg/kg body weight of glibenclamide showed significant ($p<0.05$) reduction in glucose level on day 3, 6 and 9 compared today 0. Animal treated 100 mg/kg body weight of *D. reflexa* seed extract showed significant ($p<0.05$) increase in blood glucose level on day 3 compared to day 0, 6 and 9 but showed significant ($p<0.05$) reduction in glucose level on day 6 and 9 compared to day 0 and 3. Animals treated with 200 mg/kg body weight of methanol seed extract of *D. reflexa* seed extract showed significant ($p<0.05$) increase in blood sugar level on day 6 and 9 compared to animals treated with 100 mg/kg body weight of the seed extract.

The animals induced with alloxan and administered 0.3 ml of normal saline showed non-significant ($P >0.05$) reduction in the MDA, glutathione, vitamin C concentration and significant ($P < 0.05$) reduction in catalase activity compared to the normal control animals. The alloxan induced diabetic rats treated with 0.3 mg/kg body weight of glibenclamide and 100 mg/kg body weight of *D. reflexa* seed extract showed non-significant ($P > 0.05$) reduction in plasma malondialdehyde concentration compared to the induced animals administered 0.2 ml of normal saline. The animals induced with alloxan and treated with 0.3 mg/kg body weight of glibenclamide and 100 mg/kg body weight of the extract showed significant ($P < 0.05$) increase in vitamin C concentration and catalase activity compared to the alloxan induced animals administered with 0.2 ml of normal saline. The glutathione concentration in the diabetic rats treated with 200 mg/kg body weight of the extract was significantly ($P < 0.05$) higher compared to the diabetic animals treated with 100 mg/kg body weight of *D. reflexa* seed extract (Table 2).

Discussion

Diabetes is heterogeneous condition that has β-cell dysfunction as its major consequences. Many metabolic disorders such as hyperglycemia, glucotoxicity, lipotoxicity, autoimmunity, oxidative stress etc emanating from diabetes influence the function of pancreatic β-cell. In the diabetic state, β-cells lose their mature identity and re-differentiate into neurogenin-3 positive and insulin-negative cells (Wang *et al.*, 2014).

The tolerance of non-diabetic rats to glucose administration in the absence of *D. reflexa* extract and glibenclamide as shown in Figure 1 showed that, the concentration of fasting blood glucose (FBG) was observed to reach its peak concentration at 15 minutes after glucose administration compared with glucose challenged rats administered the extract and standard drug. The methanol seed extract of *D. reflexa* caused significant ($P < 0.05$) reduction in FBG level and sustained the decrease in FBG throughout the entire time interval compared with the FBG of animals administered glucose and glibenclamide. This finding is consistent with report of Atangwho *et al.* (2013) on antidiabetic properties of leaves from *Vernonia amygdalina.*

The results shown in Figure 4 indicated that after day 6 and 9 of treatment, the extract at 100 mg/kg body weight significantly ($P < 0.05$) reduces the FBG level compared with rats treated with 200 mg/kg body weight. The alloxan-induced rats treated with glibenclamide and various doses of the extract after day 9 showed reduction in FBG compared with alloxan-induced rats administered normal saline. The data substantiate the use of *D. reflexa* for the management of diabetes and strongly support previous study in which *Chrysophylum albidum* seed cotyledon extract reduces the blood glucose level of alloxan induced diabetic rats (Omoboyowa *et al.*, 2016). The hypoglycemia effect of *D. reflexa* seed observed in this study could be as a result of the phytochemicals acting singly or synergism on the β-cells of the pancreas. The pharmacological potentials of these phytochemicals have been reported. Flavonoids have been reported to be active in many medicinal plants as antioxidants that protect organs toxicity due to agents such as alloxan (Ezejiofor *et al.*, 2013). Saponins possess anti-hyperglycemic activity by inhibiting liver glycogenesis and might have contributed to the anti-diabetic effect of *D. reflexa* extract.

The induced weight loss observed in diabetic untreated rats as a result of alloxan injection compared with the treated rats mimic the

Table 1: Qualitative phytochemical result of *D. reflexa* seed

Phytochemical compounds	Result
Steroids	+
Flavonoids	+
Tannins	++
Saponnins	++
Terpenoids	+
Reducing sugar	+
Phenol	+

KEY: + : Low Present; ++: moderate present.

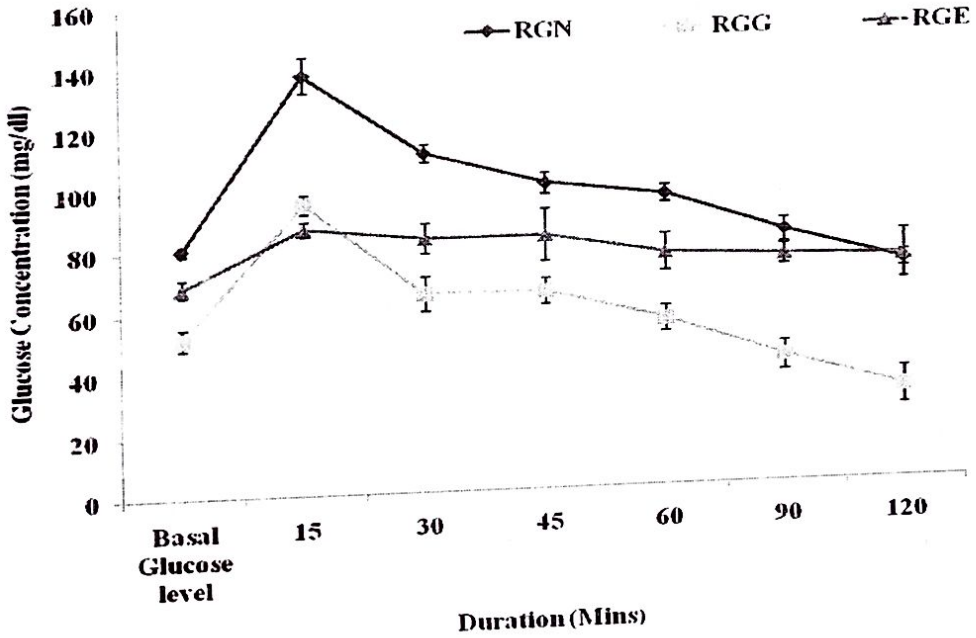

Figure 1: Effects of extract of *D. reflexa* and glibenclamide on oral glucose tolerance in non-diabetic rats

RGN: Rats administered 2 g/kg b. w of Glucose and 0.3 ml of normal saline; RGG: Rats administered 2 g/kg b. w of Glucose and 0.3 mg/kg b. w of glibenclamide; RGE: Rats administered 2 g/kg b. w of Glucose and 1 g/kg b. w of methanol seed extract of *D. reflexa*

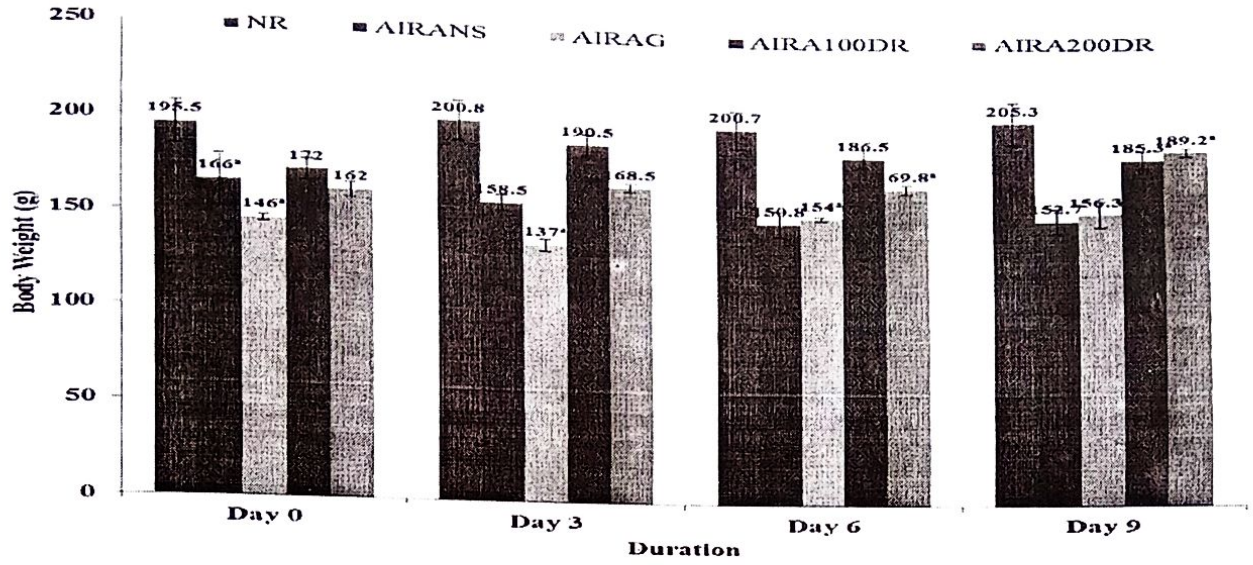

Figure 2: Effects of methanol extract of *D. reflexa* on body weight of alloxan induced diabetic rats

[a]P < 0.05: Significant compared with NR; NR: Normal control; AIRANS: Alloxan-induced rats administered 0.3 ml of normal saline; AIRAG: Alloxan-induced rats administered 0.3 mg/kg b. w of glibenclamide; AIRA100DR: Alloxan-induced rats administered 100 mg/kg b. w of methanol seed extract of *D. reflexa*; AIRA200DR: Alloxan-induced rats administered 200 mg/kg b. w of methanol seed extract of *D. reflexa*

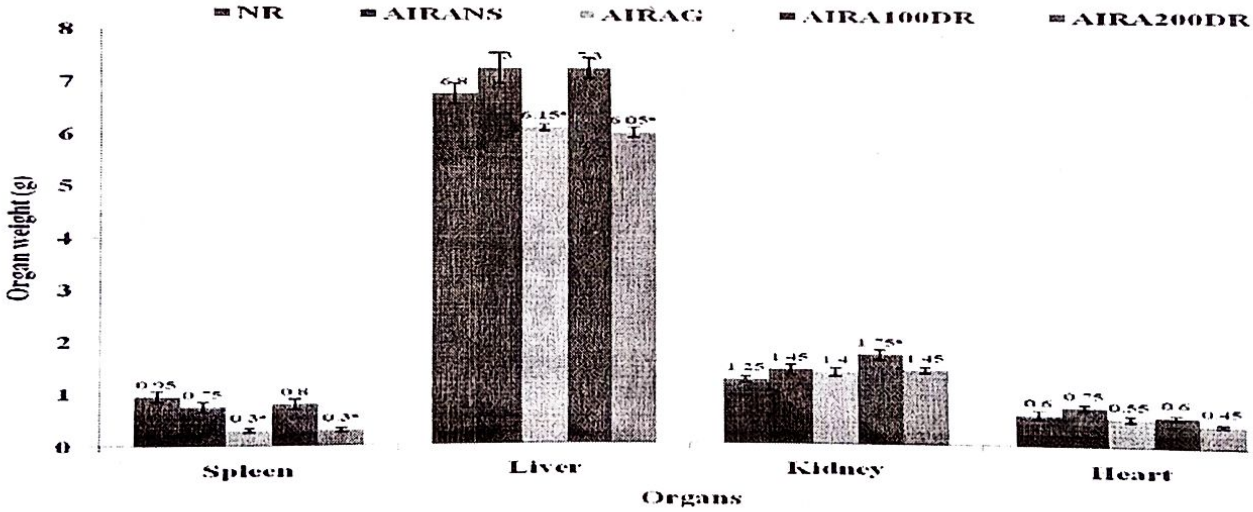

Figure 3: Effects of methanol extract of *D. reflexa* on selected organs weight of alloxan-induced diabetic rats

[a]P < 0.05: Significant compared with NR; NR: Normal control; AIRANS: Alloxan-induced rats administered 0.3 ml of normal saline; AIRAG: Alloxan induced Rats administered 0.3 mg/kg b. w of glibenclamide; AIRA100DR: Alloxan-induced rats administered 100 mg/kg b. w of methanol seed extract of *D. reflexa*; AIRA200DR: Alloxan-induced rats administered 200 mg/kg b. w of methanol seed extract of *D. reflexa*

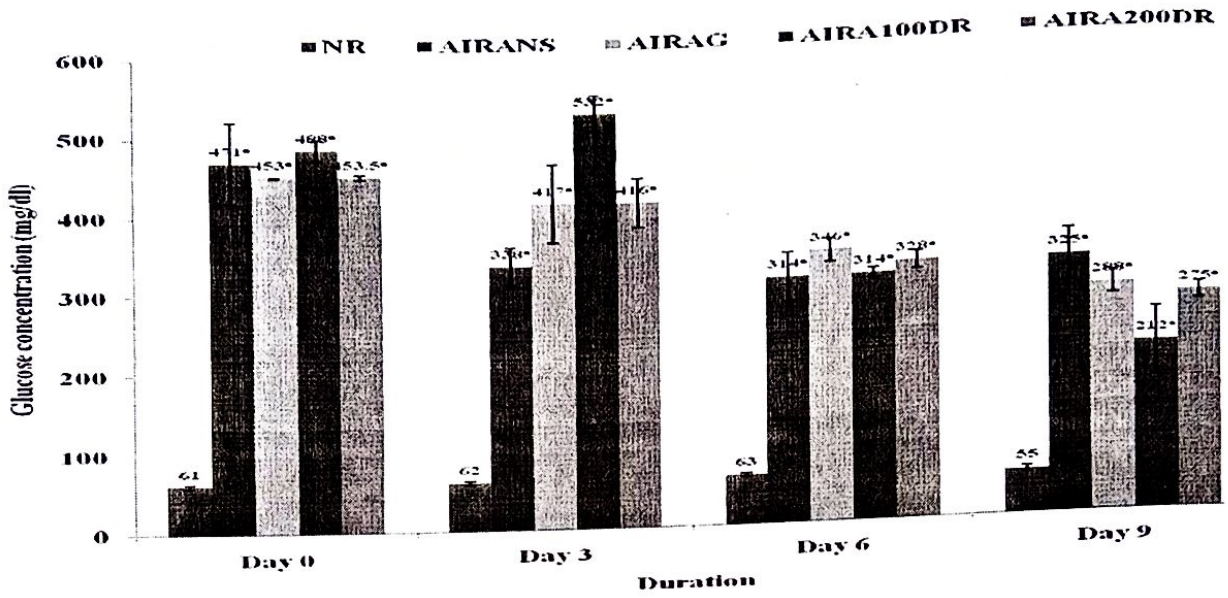

Figure 4: Effects of methanol extract of *D. reflexa* on glucose level of alloxan induced diabetic rats

[a]P < 0.05: Significant compared with NR; NR: Normal control; AIRANS: Alloxan-induced rats administered 0.3 ml of normal saline; AIRAG: Alloxan induced Rats administered 0.3 mg/kg b. w of glibenclamide; AIRA100DR: Alloxan induced Rats administered 100 mg/kg b. w of methanol seed extract of *D. reflexa*; AIRA200DR: Alloxan-induced rats administered 200 mg/kg body weight of methanol seed extract of *D. reflexa*

Table 2: Antioxidant status of alloxan-induced diabetic rats treated with methanol seed extract of *D. reflexa*

Treatments	Malondialdehyde (mg/ml)	Superoxide dismutase (µl)	glutathione (mg/dl)	Vitamin C (mg/dl)	Catalase (µl)
NR	2.80 ± 0.20	1.13 ± 0.002	4.20 ± 0.10	2.26 ± 0.05	6.45 ± 0.04
AIRANS	6.55 ± 0.25*	0.91 ± 0.01*	3.85 ± 0.15*	2.28 ± 0.03	10.02 ± 0.10*
AIRAG	4.80 ± 0.10*	1.11 ± 0.01	5.65 ± 0.05*	1.97 ± 0.13	5.57 ± 0.20*
AIRA100DR	6.20 ± 0.06*	1.10 ± 0.02	2.50 ± 0.08*	2.03 ± 0.10	4.49 ± 0.15*
AIRA200DR	5.65 ± 0.45*	1.09 ± 0.01	3.50 ± 0.10	2.12 ± 0.20	6.78 ± 0.03

All values were expressed as the Mean ± SD (n = 5); *P < 0.05 compared with normal control rats; NR: Normal control; AIRANS: Alloxan induced Rats administered 0.3 ml of normal saline; AIRAG: Alloxan induced Rats administered 0.3 mg/kg b. w of glibenclamide; AIRA100DR: Alloxan induced Rats administered 100 mg/kg b. w of methanol seed extract of *D. reflexa*; AIRA200DR: Alloxan induced Rats administered 200 mg/kg body weight of methanol seed extract of *D. reflexa*

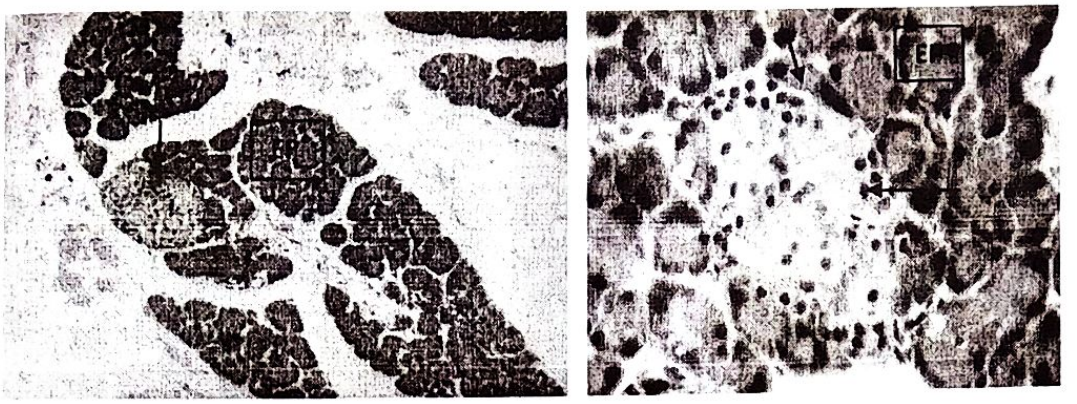

Plate 1: Sections of the pancreas collected from the normal control animals.

There was 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 eosinophillic cells with eccentrically located nuclei and abundant cytoplasm. Exocrine pancreas (EP). H&Ex100 and x400.

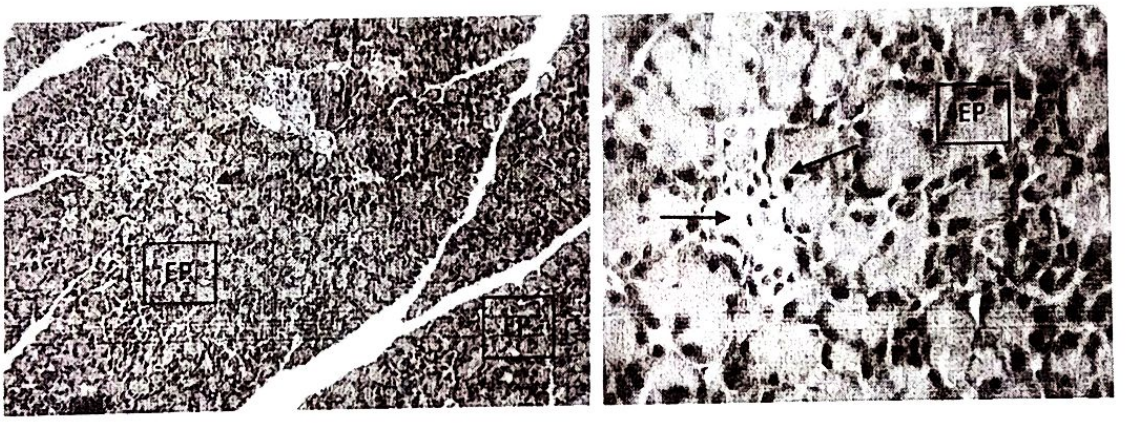

Plate 2: Sections of the pancreas of Alloxan induced rats administered 0.3 ml of distilled water

There was normal histo-architecture of the exocrine pancreas. The endocrine pancreas showed a decrease in the number and sizes of the pancreatic islets (arrow). The few observed pancreatic islets (arrow) were relatively inconspicuous. H &E x100 and x400.

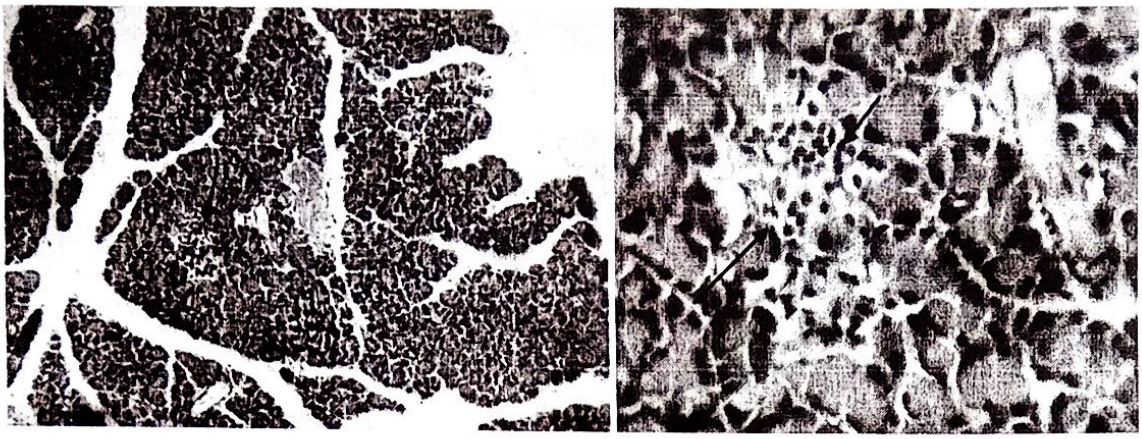

Plate 3: Sections of the pancreas of alloxan-induced rats administered 0.3 mg/kg b. w of Glibenclamide

The results showed normal histo-architecture of the exocrine pancreas. Just as observed in group 2 above, the endocrine pancreas showed a decrease in the number and sizes of the pancreatic islets (arrow). However, the few observed pancreatic islets (arrow) were relatively hypercellular (compared to group 2), comprising of numerous tiny cells with hyperchromatic nuclei and poorly demarcated cytoplasm. These cells does not have the characteristics of beta cells, thus they may be of the other cell populations in the islets.. H&Ex100 and x400.

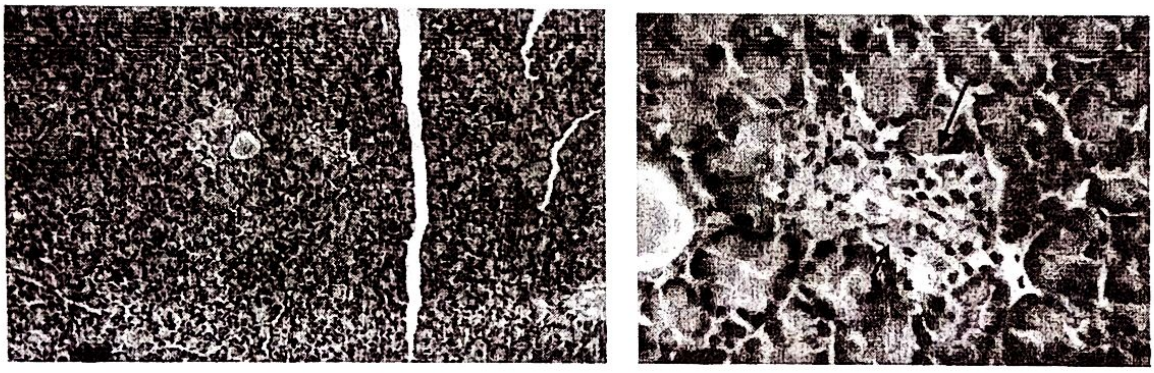

Plate 4: Sections of the pancreas of alloxan-induced rats administered 100 mg/kg b. w of *Dioclea reflexa*

The animals showed normal histo-architecture of the exocrine pancreas. Just as observed in group 2 above, the endocrine pancreas showed a decrease in the number and sizes of the pancreatic islets (arrow). H&Ex100 and x400.

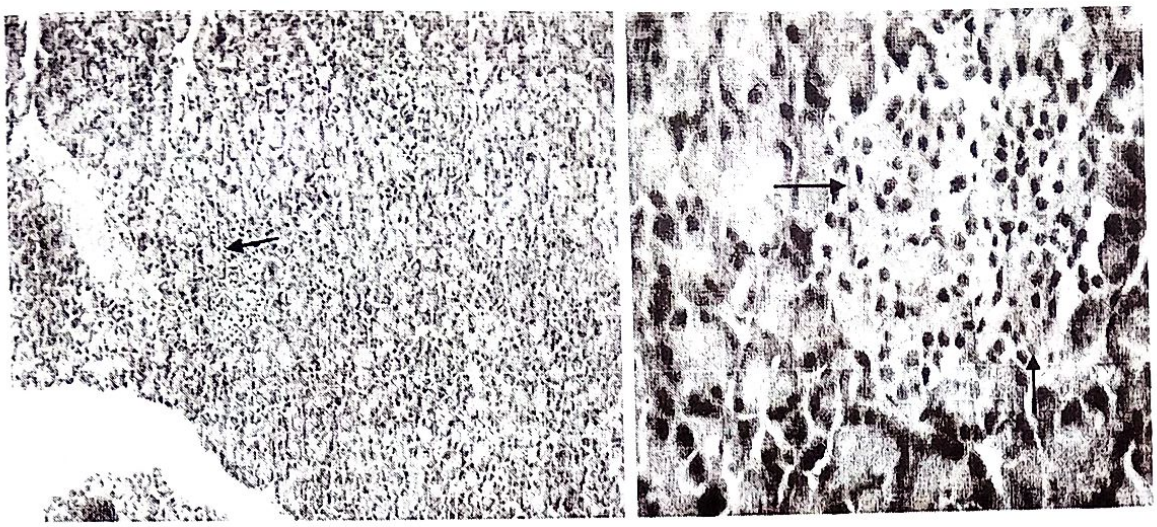

Plate 5: Sections of the pancreas of alloxan-induced rats administered 200 mg/kg b. w of *Dioclea reflexa*

There was normal histo-architecture of the exocrine pancreas. Just as observed in induced and untreated group, the endocrine pancreas showed a decrease in the number of the pancreatic islets (arrow). The sizes of the few observed islets appeared normal. H&Ex100 and x400.

common weight loss usually diagnose in clinical diabetic patients. During the 9 days of treatment, continuous reduction in the body weight of alloxan induced diabetic rats observed, whereas, a significant ($p<0.05$) gain in body weight was observed in *D. reflexa* treated rats. The treatment with *D. reflexa* extract ameliorates the loss in body weight and restored this level towards normal.

The potential of *D. reflexa* extract to correct the body weight might result from its anti-hyperglycemic ability by increasing the rate of glucose metabolism. Insufficient insulin secretion prevents the body from getting glucose from the blood into the body's cells to be metabolize as energy therefore, the body starts to mobilize fat and muscle for energy, causing a reduction in overall body weight. The ability of *D. reflexa* extract to reduce hyperglycemia and protective effect in controlling muscle wasting might increase the availability of glucose for energy production thereby, restored body weight.

Oxidative stress is caused by a relative overload of oxidants, this impairs cellular functions and contributes to the pathophysiology of many diseases (Bonnefort-Rousselot *et al.*, 2000), the complication of diabetes seem to be partially mediated by generation of reactive oxygen species (ROS). The ability of *D. reflexa* seed to generate antioxidants against free radicals in alloxan induced diabetic rats was studied. The result in table 2 showed significant ($P < 0.05$) reduction in catalase activity, vitamin C and glutathione level of alloxan induced diabetic rats treated with graded doses of the extract compared with alloxan induced diabetic rats administered normal saline. In hyperglycemia, glucose is preferentially utilized in polyol pathway which consumes NADPH necessary for oxidized glutathione regeneration by GSH reduction enzyme. Hyperglycemia is therefore an indirect cause of GSH reduction. GSH is an important free radical scavengers molecule, its depletion causes significant boost in oxidative stress. Reduced glutathione normally plays the role of an intracellular radical scavenger and is the substrate of many xenobiotic elimination reactions (Moussa, 2008).

The beta cells, which are the insulin secreting cells makes up over 80% of the total number of cells in the pancreatic islets. Alloxan causes necrosis of these beta cells of the pancreatic

islets. This might be the reason for the observed decrease in the number and sizes of the pancreatic islets of the alloxan induced diabetic rats administered normal saline and diabetic rats administered glibenclamide. However, the observed histopathology of the diabetic rats administered graded doses of the extract was quite interesting. The diabetic animals treated with 100 mg/kg b.w of *D. reflexa* extract showed decrease in size but hypercellular islets while the diabetic rats treated with 200 mg/kg b. w. showed relatively normal sized pancreatic islets. In literature, the beta cells are not known to regenerate in adult subjects but the findings in the high dose groups suggest that regeneration of the beta cells may have occurred.

Conclusion

Dioclear reflexa seed extract exerted a dose-dependent regenerative activity on the pancreas of alloxan induced diabetic rats. The data obtained from this study presented the pharmacological basis of the phytochemicals present in *D. reflexa* seed extract for the folklore use of the seed in the management of diabetes mellitus.

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