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Hypoglycemic Activity Of The Aqueous Root-Back Extract Of Olax Mannii On Diabetic Induced Albino Rats

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Abstract: ***The abundance of medicinal plants in our environment and the realization that they posse's active ingredients with therapeutic values has made the need for their study imperative. In this study, we screened the root-bark of Olax mannii for the presence of some active ingredient and to study the effects of the aqueous root- bark extract of Olax mannii on fasting blood glucose of streptozotocin induced diabetic male rats. Adults male wistar strain albino rats were given graded doses (500, 1000 and 1500mg/kg) of the aqueous root- bark extract of Olax mannii for a period of 2, 4 and 6 weeks. The statistical analysis was carried out using one way ANOVA followed by post hoc LSD multiple comparison on SPSS 19. Olax mannii revealed a significant hypoglycemic effect ($p<0.05$) comparable with the glucose level of the normal control rats. The histological evaluation of the pancreas was carried out after 6 weeks of treatment. The result of this study makes the use of Olax mannii acceptable as a hypoglycemic plant in herbal medicine. Olax mannii may be important in the management of diabetes.***

Keywords: Diabetes mellitus, Olax mannii, Hypoglycemic, Streptoxotocin, Insulin

I. INTRODUCTION

Diabetes mellitus, simply referred to as diabetes, is a group of metabolic diseases in which a person has high blood glucose because the cell does not produce enough insulin, does not respond to the insulin that is produced or both. This high blood glucose produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Diabetes mellitus, is initially characterized by loss of glucose homeostasis resulting from defect in insulin secretion, insulin action or both resulting in impaired metabolism of glucose and other energy yielding fuels such as lipids and proteins (Sheen, 1990). It is also worthy to note that insulin action may be altered by an abnormally high amount of glucagon and other insulin antagonist like growth hormones and corticosteroids by causing diabetes (White and Campbell, 1992).

When blood glucose is poorly controlled over long periods in diabetes mellitus, blood vessels throughout the body begin to function abnormally and undergo structural changes that result to inadequate blood supply to the tissue. This in turn leads to increased risk of heart attack, stroke, kidney disease, retinopathy and blindness and gangrene of the limbs. In diabetic conditions there are significant changes in lipid metabolism and structure (Sochar *et al.*, 1985). These structural changes are clearly oxidative in nature and are associated with the development of vascular disease (Morel and Clusolm, 1989), and it gives rise to the complication associated with diabetes mellitus. Oxidative stress resulting from oxidation reaction is produced under diabetic conditions and possibly causes various forms of tissue damage in patients with diabetes.

About 143 million people worldwide are suffering from diabetes, almost five times more than the estimate ten years ago. This number may probably double by the year 2030.

Therefore, the global human population appears to be in the midst of an epidemic of diabetes. Reports from world health organization (WHO) indicate that diabetes mellitus is one of the major killers of our time with people in Southeast Asia and western pacific being at risk.

Olax mannii is a plant species in the family of Olacaceae that is widely distributed in the tropics especially Nigeria, Ghana and Sierra Leone. It is a climbing shrub that grows up to 2 meters high. The leaves are usually lanceolate to ovate or elliptic up to 6×3 inches with 5 – 6 pairs at lateral looped nerves. The flowers are greenish white in axillary racemes. The fruits are orange when ripe and about ½ - 3/4cm. the plant is called "Ngborogwu arua" in Ibo and "Tsada biri" in Hausa. Decoction of the leaves and roots of the plant is used for the treatment of fever, yellow fever and snake bite (Burkill, 1997). The foamy water that results when the root bark of *Olax mannii* is soaked in water and shaken thoroughly serves as a potent anti-convulsant agent in Ohafia in Abia State, Nigeria.

Generally, the phytochemical examination of the various crude extracts of the leaves, fruits and root bark of the plant showed the presence of coumarins, steroid/triterpenes, saponins, fatty acids and tannins in all parts of the plants; while volatile oils and flavonoids are present in the fruits and leaves. Alkaloids are absent in all parts of the plant (Sule *et al.,* 2011). The isolation of (E)-3-methyl-5-phenyl-2-pentenoic acid from the petroleum ether extract of the leaves has also been reported (Sule *et al.,* 2011). Olacaceae families have been reported to contain fatty acids (Galliard and Mercer, 1975).

Two triterpenoids glutinol and rhorptelenol were reportedly isolated from the acetone extract of the leaves of *Olax mannii* (Sule *et al.,* 2011).

This study is designed to evaluate the antihyperglycemic potentials of the aqueous root bark extract of *Olax mannii* on streptozotocin induced diabetic male rats.

II. MATERIAL AND METHOD

A. COLLECTION AND IDENTIFICATION OF PLANT SAMPLES

The roots of *Olax mannii* were collected in March and April 2013, in Ohafia, Nigeria. The plant was identified at the Department of Plant Science and Biotechnology, University of Port Harcourt Rivers State, where a voucher sample had been deposited in the herbarium. The plant root was thoroughly washed and air dried for 8 days at room temperature. The bark was scrapped off the root using a scrapper. The root barks were shade-dried again for 20days and then pulverized in a hammer mill and stored in an air tight container.

B. PROCUREMENT OF ANIMALS

Adult male wistar albino rats weighing 150 – 200g were obtained from the animal house of the Department of Biochemistry, University of Port Harcourt; Rivers State, Nigeria. A total of 72 rats were used in this research. All animals were kept in an environmentally controlled room with a 12 hours light and dark cycle. They were housed in separate cages within room temperature (25±2^{o}C, relative humidity 60 – 70%) and divided into 6 groups of 6 animals each.

C. PREPARATION OF PLANT (ROOT BARK) EXTRACT

The root-bark of *Olax mannii* was cut into pieces and shade-dried for 20 days at room temperature. The dried samples were ground into powder in a hammer mill and stored in an air tight container. 500mg, 1000mg and 1500mg of the resultant powder was soaked in hot water for 24 hours and the temperature of the water was maintained throughout the period of the extraction after which the resultant mixture was filtered and the filtrate (aqueous extract) was stored for subsequent use. 20ml of this extract was evaporated to dryness and the weight of the residue used to determine the concentration of the filtrate which was in turn used to determine the dose of administration of the extract to the test animals.

D. HYPOGLYCEMIC STUDY/ EXTRACT ADMINISTRATION

The test rats were divided into seven groups with each group containing six rats. The animals were housed in a plastic cage and fed ad libitum with deionised water and standard rat chow (Pfizer pharmaceuticals Plc, Ikeja Nigeria). To acclimatize the animals to the test condition, they were brought to the laboratory a week before the experiment. The animals were fasted overnight and diabetes was induced by intraperitoneal injection of freshly prepared solution of streptozotocin (160 mg/kg body weight) in distilled water. Whereas the normal control rats (NCR) were injected with distilled water alone. Seven days after the administration of streptozotocin, the animals were fasted again and their blood collected via tail cutting, for their fasting glucose levels. The rats were then kept for 3 days to stabilize the diabetic condition, before initiating treatment, which lasted for 6 weeks.

The first and second groups normal control rats (NCR) and diabetic control rats (DCR) received appropriate volume of distilled water using a gavage via intubation. The third group received 1.6mg/kg of glibenclamide (daonil) which serve as the reference drug. The fourth, fifth and sixth groups received 500 mg/kg, 1000 mg/kg and 1500 mg/kg body weight of the aqueous root-bark extract via the same route.

The animals were well fed at the end of each treatment period i.e., 2 weeks, 4 weeks, 6 weeks, the rats were weighed, fasted overnight and their fasting blood glucose level estimated. They were anaesthetized by exposure to chloroform. While under anaesthesia, they were painlessly sacrificed and blood collected from each rat into heparin anti-coagulated blood samples were centrifuged at 1000 × g for 10 minutes, after which their plasma was collected and stored for subsequent analysis.

E. EXPERIMENT DESIGN

The rats were divided into seven groups of six rats each after the induction of streptozotocin as follows

Group I - Normal control rats
Group II - Diabetic control rats
Group III - Diabetic rats received 1.5 mg/kg Glibenclamide (daonils)
Group IV - Diabetic rats received 500 mg/kg of the aqueous root-bark extract
Group V - Diabetic rats received 1000 mg/kg of the aqueous root-bark extract
Group VI - Diabetic rats received 1500 mg/kg of the aqueous root-bark extract

F. HISTOPATHOLOGY OF PANCREAS OF STZ INDUCED DIABETIC RATS

On the last week of the study period, i.e. week 6, the animals were sacrificed and quickly dissected and the pancreas samples were fixed in 10% formalin and used for histopathological studies.

G. STATISTICAL ANALYSIS OF DATA

The Data for pharmacological screening were analyzed for statistical differences between treatment groups, by means of one-way ANOVA and post hoc LSD, on SPSS 19. In all, $p<0.05$ was considered significant. Data are presented as mean±s.d (standard deviation).

III. RESULTS

Table 3.1 below shows the results of the effect of aqueous extract of the root-bark of *Olax mannii* on plasma glucose level of normal rats on streptozotocin induced diabetic male rats.

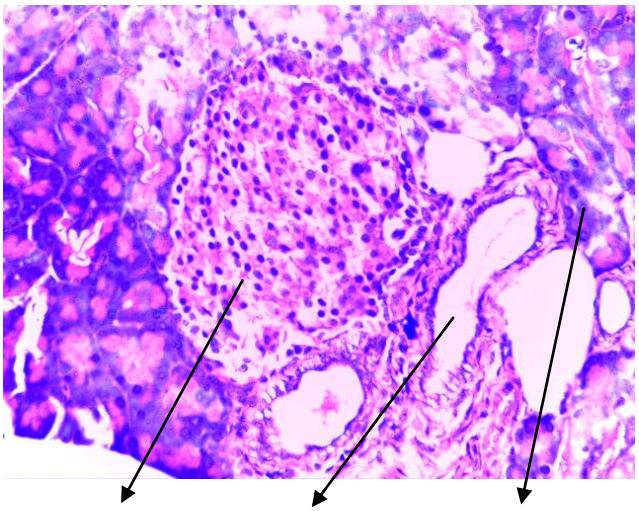
Groups	Treatment	Plasma glucose level (mg/dl)			
		0 weeks	2 weeks	4 weeks	6 weeks
I	Normal Control Rats (NCR)	**79.83±1.47**	**80.33±2.42**	**79.67±1.63**	**81.50±2.74**
II	Diabetic Control Rats (DCR)	**180.17±4.96**	**200.33±7.20**	**236.50±6.44**	**206.33±5.39**
III	DRC on Glibenclamide (1.5mg/kg)	**180.50±4.2**	**147.50±8.89**	**103.33±4.32***	**78.17±2.48***
IV	DRC on Aqueous Extract (500mg/kg)	**179.83±6.85**	**166.33±4.27**	**124.17±3.87**	**102.00±2.48***
V	DRC on Aqueous Extract (100mg/kg)	**176.50±8.12**	**155.17±10.01**	**117.50±3.45**	**96.33±3.06***
VI	DRC on Aqueous Extract (1500mg/kg)	**178.00±7.07**	**149.00±8.53**	**106.33±2.94***	**79.83±1.17***

*Values are mean ± standard deviation of triplicate determination. Values on same column having * are not significantly different (p >0.05) from the control*

Table 3.1: Effect of aqueous extract of the root-bark of Olax mannii on plasma glucose level of normal rats on streptozotocin induced diabetic male rats

PHOTOMICROGRAPH OF RAT PANCREAS

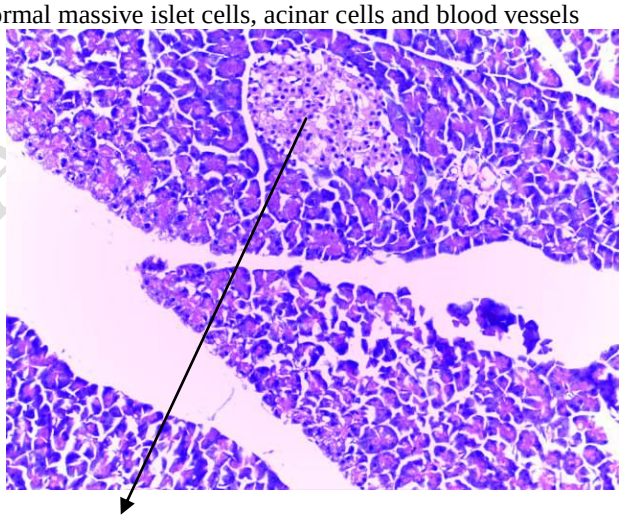
AFTER 6 WEEKS OF TREATMENT



ISLET CELL MASS BLOOD VESSEL ACINAR CELLS

Plate 1: Normal control rats after 6 weeks of treatment

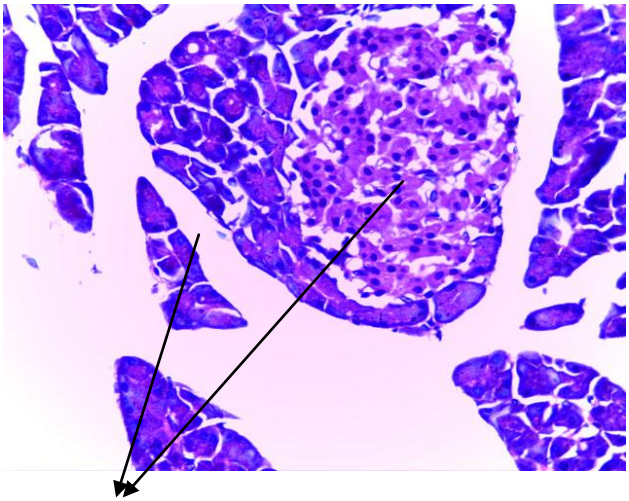
Treatment plate shows no histological change with a normal massive islet cells, acinar cells and blood vessels



REDUCED ISLET CELL MASS

Plate 2: Diabetic Control Rats after 6 weeks of treatment

Plate shows a reduced islet cell mass



INFLAMMED ISLET CELL MASS

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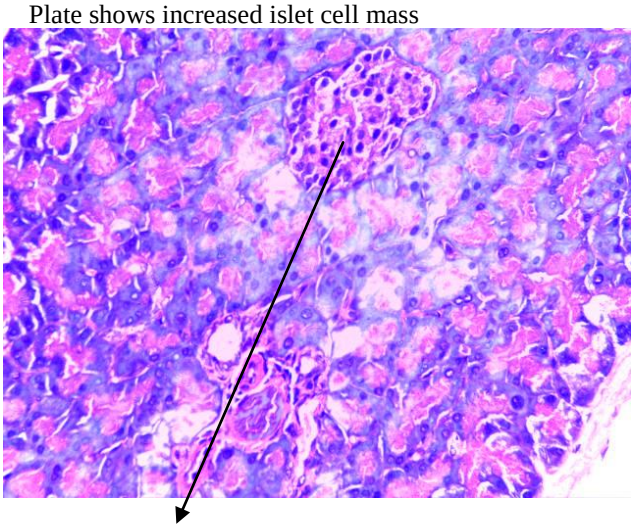
Plate 3: Diabetic Control Rats after 6 weeks of treatment with glibenclamide

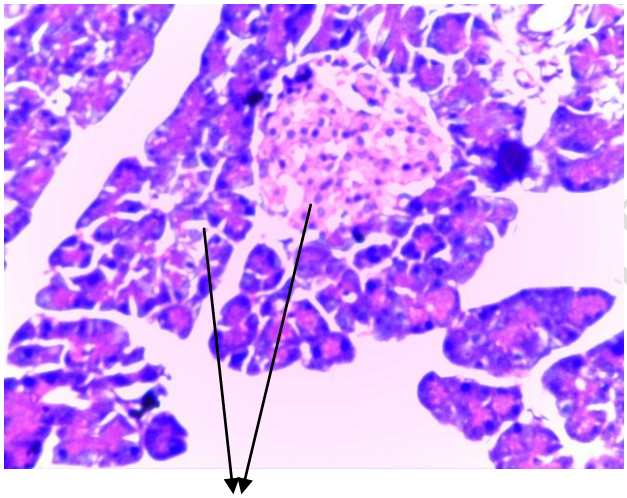
Plate shows increased islet cell mass



REDUCED ISLET CELL MASS

Plate 4: DRC treated with aqueous root- bark extract 500mg/kg for 6 weeks

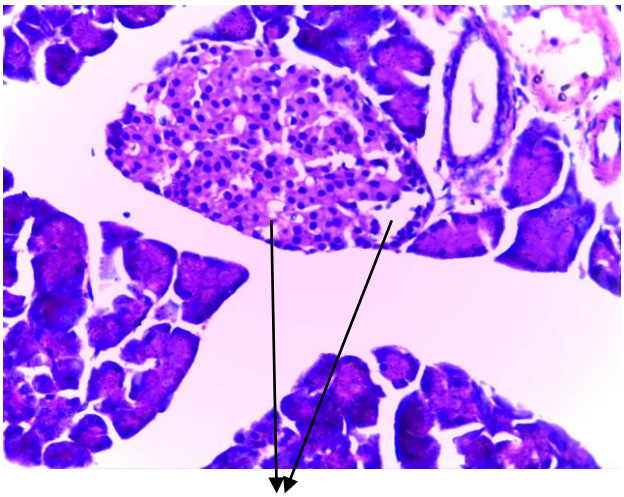
Plate shows a reduced islet mass



INFLAMMED ISLET CELLS

Plate 5: DRC treated with aqueous root- bark extract 1000mg/kg for 6 weeks

Plate shows increased islet cells



INFLAMMED ISLET CELL MASS

Plate 6: DRC treated with aqueous root- bark extract 1500mg/kg for 6 weeks

Plate shows increased islet cell mass

IV. DISCUSSION

The result obtained from this study indicate that the aqueous extract of the root-bark of *Olax mannii* has significant hypoglycemic activity at all doses examined although in a dose dependent manner. The success obtained in the use of streptozotocin (STZ) for the induction of diabetes mellitus through the administration of 160 mg/kg body weight can be attributed to the work of Nwauche *et al.,* 2014. This achievement was confirmed by the evaluation of fasting blood glucose level.

There was no significant increase in the glucose level of the normal control rats throughout the study period with the values 80.33±2.42, 79.67±1.63, 81.50±2.74 for 2, 4 and 6 weeks of treatment respectively. However, there was a significant increase (p<0.05) in the level of glucose concentration for the diabetic control rats approaching a hyperglycemic level of 200.33±7.20, 236.50±6.44, 206.33±5.39 for 2, 4 and 6 weeks of treatment respectively (see table 3.1).

This result affirms the development of hyperglycemia as a result of streptozotocin injection (Nwauche *et al.,* 2014). Streptozotocin is a glucosamine-nitrosourea compound. As with other alkylating agents in the nitrosourea class, it is toxic to cells by causing damage to the DNA; though other mechanisms may also contribute. DNA damage induces activation of poly ADP-ribosylation, which is likely more important for diabetes induction than DNA damage itself (Szkudelski, 2001). Streptozotocin is similar enough to glucose to be transported into the cell by the transport protein GLUT 2, but is not recognized by the other glucose transporters. This explains its relative toxicity to beta cells, since these cells have relatively high levels of GLUT 2 (Wang, 1998 and Schnedl *et al.,* 1994).

On administration of aqueous root-bark extract of *Olax mannii* at different doses of 500mg/kg, 1000mg/kg and 1500mg/kg body weight, i.e. groups IV, V and VI, the blood glucose level decreased significantly (p<0.05) on the 2nd , 4th and 6th week of treatment when compared to the diabetic control group (group II).

The administration of glibenclamide (group III) also revealed a worth mentioning decrease of blood glucose level for the same period of treatment when compared with the diabetic control group (group II).

From the above study, it is undoubtedly clear that the administration of the extract at different doses to the diabetic rats resulted in a significant (p<0.05) reduction in the plasma glucose level. Although the decline was dose dependent, the extract might posses an active ingredient which has an insulin-like effect on the peripheral tissues either by facilitating glucose uptake by cells or by promoting glycogenesis.

Histological evaluation of the pancreas, liver and kidney (fig. 3.1) showed a remarkable reduction in the islet cell mass of langerhan of the streptozotocin induced diabetic rats throughout the period of treatment. They were treated with

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different doses of the plant extract (*olax mannii*) and glibenclamide. The islet cells were preserved throughout the period of treatment.

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