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Research Article

Effect of hydromethanolic extract of *Rauvolfia vomitoria* leaf on blood glucose, plasma insulin and histomorphology of the pancreas of streptozotocin-induced diabetic male wistar rats

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Keywords:
Rauvolfia vomitoria, anti-diabetic, blood glucose, plasma insulin, histomorphology

ABSTRACT

Background: Diabetes mellitus is one of the most dreaded health issues worldwide due to its exponential increasing rate and the limitations of synthetic drugs in providing complete cure for it, hence, the call by health stakeholders, for more researches on medicinal plants due to their promising results in providing solutions to some health issues with little or no side effect. In response to this call, this study investigates the effects of *Rauvolfia vomitoria* leaf extract on blood glucose, plasma insulin and histomorphology of the pancreas of diabetic rats in an attempt to evaluate its anti-diabetic potentials. **Methods:** Diabetes was induced via intraperitoneal injection of 55mg/kg b. wt. streptozotocin and treated with hydromethanolic extract of *Rauvolfia vomitoria* leaf (250mg/kg and 500mg/kg b. wt. doses) and glyburide (5mg/kg b. wt.) for 28 days. Blood glucose levels were measured after every 4 days of treatment during treatment period. Plasma insulin levels were estimated after 14 and 28 days of treatment via immunoassay; while the histomorphological study was done using hematoxylin and eosin staining technique. **Results:** Results from this study show dose dependent significant ($P<0.05$) decrease in blood glucose levels and a dose dependent significant increase in plasma insulin of the extract treated groups which were comparable to those of glyburide treated group. The histological study of the pancreas showed an improvement on the deranged pancreas of diabetic rats following 28-days treatment with *Rauvolfia vomitoria* leaf extract. This possibly explains the increase in plasma insulin levels and a corresponding decrease in blood glucose. **Conclusion:** From the results of this study, it is therefore concluded that *Rauvolfia vomitoria* has strong anti-diabetic property. The results show that *Rauvolfia vomitoria* leaf extract effect its anti-diabetic action by enhancing the regeneration of the pancreatic islets thereby increasing insulin secretion and plasma insulin level.

INTRODUCTION

The prevalence of diabetes mellitus, a chronic metabolic disorder caused by faulty management of blood glucose resulting from either a deficiency in insulin secretion or the body's insensitivity to insulin (Govindappa *et al.*, 2014; Ezuruike and Prieto, 2014; N'doua *et al*., 2016) has made it one of the most dreaded health issues worldwide. According to the International Diabetes Federation, there are more than 415 million people affected by diabetes mellitus worldwide and this figure is projected to rise to over 642 million or more by 2040 ((International Diabetes Federation, 2015). Allopathic drugs are having limitations in providing the needed solutions to the problems of diabetes mellitus and are often associated with side effects (Moller, 2001; Halimi *et al*., 2008; Sunil *et al*., 2009). This has necessitated the call by stakeholders including the recommendation by WHO expert committee on diabetes (WHO Expert Committee, 1980), for more researches on medicinal plants due to their promising results in providing solutions to health issues with little or no side effect (Govindappa *et al.*, 2014; Chikezie *et al*., 2015).

Rauvolfia vomitoria is an ever green perennial shrub belonging to the apocynaceae family. It is widely distributed all over the world especially in the tropical forest of Africa, South America and Asia (Amole, 2003; Ogbe *et al.*, 2009). It is commonly known as

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swizzle stick, Africa snake root and poison devil's pepper (Okereke et al, 2015). Its local names in Nigeria include "asofeyeje" by the Yorubas (Orwa *et al*., 2009), "ira" by the Igbos and "Wadda" by the Hausas (Ojo *et al*., 2012). The Oredo people of Edo state call it "akata" (Ogbe *et al.,* 2009) and the Urhobo ethnic group of Delta state call it "ogburukpe". It has been associated with various medicinal actions including anti-hypertensive (Amole, 2003), hepato-protective (Ezejindu *et al.,* 2013), anticonvulsant (Olatokunboh *et al*., 2009), anticancer (Yu *et al.,* 2013), anti-psychotic (Bisong *et al*., 2010), anti-diabetic (Campbell-Tofte *et al.,* 2011), etc. On its anti-diabetic potentials, quite a handful of literature have been made available on its blood glucose lowering action by the efforts of various researchers (N'doua *et al*., 2016; Campbell-Tofte *et al.,* 2011; N'doua *et al*., 2015), but very little is known of the mechanism of action, the phytoconstituent(s) responsible for its anti-diabetic property and its effect on plasma insulin and glucagon (primary regulator of blood glucose).

Hence this study was designed to investigate the effects of *Rauvolfia vomitoria* on blood glucose, plasma insulin and histomorphology of the pancreas of streptozotocin-induced diabetic male wistar rats.

MATERIALS AND METHODS

Materials, Drugs, and chemicals.

Insulin ELISA kit (Calbiotech, Inc. 1935 Cordell Ct., CA, USA) and Streptozotocin (St Louis, MO, USA) were purchased from BioRapid Diagnostic Nigeria Limited, Abuja. Accu-Chek Active blood glucose meter and Accu-Chek Active test strips (Roche Diabetes Care GmbH Sandhofer Strasse 116 68305 Mannheim, German) and glyburide were purchased from Greenhouse Group Pharmacy, University of Port Harcourt, Nigeria.

Ethical Approval

Ethical approval for this study was obtained from the Research Ethics Committee, College of Medical Sciences, University of Benin, Benin City, Nigeria, with approval number: CMS/REC/2021/159. Animal experiments were conducted in compliance with NIH guidelines for Care and Use of Laboratory Animals as contained in Guide for the Care and Use of Laboratory Animals 8th edition (NRC, 2011).

Collection and Identification of Plant Samples

The leaves of *Rauvolfia vomitoria* (a medicinal plant authenticated by Dr. Edwin Nwosu at the Herbarium Unit of the Department of Plant Science and Biotechnology of the University of Port Harcourt, with Ecoland Herbarium identification number EH – P – 051) were harvested from the farmland in Orhuwhorun community in Udu LGA of Delta State.

Preparation and Extraction of the Plant Samples

The harvested leaves were rid of dirt. Thereafter, air-dried at room temperature for 6 days and then milled to fine powder using manual engine grinder (Model Corene, A.5 lander YCIA S.A). The milled sample of the plant was subjected to hydromethanolic (ratio of water to methanol is 1:4) maceration extraction following the extraction method of Akpojotor and Kagbo (2016). 500 grams of the milled sample of the plant was then mixed with 5 litres of the extraction solvent (hydromethanol). The mixture was stirred occasionally for 48 hours at room temperature, thereafter filtered with Whatman No. 1 filter paper to separate the filtrate from the residue. The filtrate was then concentrated under reduced pressure in a vacuum at 40°C using a rotary evaporator (Searl Instruments Ltd. England) and activated silica gel was used to remove moisture, thereby producing the hydromethanolic extract which was used for the study.

Induction of Diabetes

The method of Rossini *et al.* (1977) was followed. Experimental animals were fasted overnight. Streptozotocin was dissolved in citrate buffer, pH 4.5 and injected intraperitoneally within 20 minutes of dissolution for the induction of diabetes in group 2-5. Dose of 55 mg/kg body weight Streptozotocin (according to the dose used by Rajasekaran *et al*., 2005; Davidson *et al*., 2011) was used. Thereafter, blood glucose level of animals was measured 72 hours (3 days) after streptozotocin administration for confirmation of diabetes, via collection of blood from the tip of the tail and using a blood glucometer (Accu-Chek active, Germany). Animals with blood glucose level equal to or more than 280 mg/dl were taken as diabetic as used in other studies.

Animals and Grouping

Experimental animals (male wistar albino rats) were purchased from the Animal House, Department of Pharmacology, University of Port Harcourt.

Fifty male wistar rats were divided into five groups of ten rats each (namely groups 1-5). The groups are as follows:

Group 1 (Normal control) - Diabetes was not induced and they were not treated.

Group 2 (Diabetic control) - Diabetes was induced but they were not treated.

Group 3- Diabetes was induced and they received extract (250mg/kg. b.wt).
Group 4- Diabetes was induced and they were treated with extract (500mg/kg. b.wt).
Group 5- Diabetes was induced and they were treated with 5mk/kg dose of glyburide (standard anti-diabetic drug).
The animals were kept in a spacious and well ventilated cage (2feet length by 1foot width by 1foot height) for each group, under room temperature (28 ± 2°C) on a 12 h light/dark cycle. They were allowed free access to water and food (Top Feeds, Broiler finisher Product of Eastern premier feed mills Ltd.) The study was approved by the Ethics Committee, School of Post-Graduate Studies, University of Port Harcourt, Choba, Rivers State, Nigeria.
All animal experiments were conducted in compliance with NIH guidelines for Care and Use of Laboratory Animals as contained in Guide for the Care and Use of Laboratory Animals 8th edition (National Research Council 2011).

Administration of Plant Extract and Standard Anti-Diabetic Drug (Treatment)
Administration (treatment) commenced immediately diabetes was confirmed (3 days after induction of diabetes). Daily administrations were via oral route using the gavage technique of Diehl *et al*., (2001).
The choice of extract doses for this study are based on the doses for which medicinal activities have been reported on *Rauvolfia vomitoria* leaf (Olatokunboh *et al.,* 2009; Ezejindu *et al.,* 2014).

Measurement of Blood Glucose Levels
'Measurement of blood glucose levels of animals were done following the method of Akpojotor and Ebomoyi (2021). In brief, the tip of the tail of the animal was pierced with a lancet and a drop from the blood that oozes out was applied to test strip inserted into the Accu-Chek blood glucose meter. The glucose concentration in the blood was displayed on the screen of the Accu-Chek blood glucose meter. This procedure was performed on all the animals.
Blood glucose levels of all groups were measured just before commencement of treatment and thereafter, after every four days of treatment.

Collection of Blood Samples and Organ
Five rats from each group were sacrificed after 14 and 28 days of treatment. During sacrifice, a portion of blood was collected by cardiac puncture into plain sample bottles for hormonal assay of insulin, while the pancreas of each rat was collected and transferred into plain bottles containing formalin for histological study.

Biochemical Assay of Insulin
The collected blood samples were subjected to immunoassay screening for plasma insulin and glucagon concentrations ELISA immunoassay Kits. In brief, 50µL of standard working solution of different concentration are added to the first two wells. Serum from the collected blood samples are added to the other wells and 50µL of biotinylated detection Ab working solution is added to all the wells and then covered with sealer and incubated for 45 minutes at room temperature. After incubation, the samples and standard working solution are decanted from the wells and the wells washed using wash buffer. After washing, 100µL of HRP Conjugate working solution is added to each well. The wells are then covered with sealer and incubated for 30 minutes at room temperature. After incubation, the wells are decanted washed using the wash buffer. 90µL of substrate reagent is added each well and incubated for 15 minutes at 37°C. At the end of the 15 minutes of incubation, 50µL of stop solution is added to each well. The optical density (OD) value of each well was immediately determined using a micro-plate reader set at 450nm.

Histomorphological Study
Histopathological examination was done following the method of Akpojotor and Kagbo (2016). Harvested organ (pancreas) from each animal was cut and fixed in buffered neutral formalin (10%). The tissue was then dehydrated in ascending grades of ethanol (70%, 80%, 90%, 95% and 100%), cleared in 2 changes of Xylene, impregnated with 2 changes of molten paraffin wax, and finally embedded in wax. Tissue sections of 4–5 µm in thickness was cut with a microtome and stained with hematoxylin and eosin.

Statistical Analysis
Results were presented as mean ± SEM and were analysed using one-way analysis of variance (ANOVA). Differences between means were analysed by applying Scheffe's t-test for comparison with control groups at 95% ($p<0.05$) confidence level. The data were statistically analysed by SPSS VERSION 23.

RESULTS
Table 1 shows the effect of low (250mg/kg) and high (500mg/kg) doses of hydromethanolic extract of *Rauvolfia vomitoria* leaf on streptozotocin induced

Table 1: Effect of treatment with hydromethanolic (HM) leaf extract of *Rauvolfia vomitoria (Rv)* on blood glucose level (mg/dl) of Streptozotocin-induced diabetic male wistar rats

Description	Before induction	72 hrs Post Induction	Treatment Post Induction (Days)							
			2	6	10	14	18	22	26	28
Normal control	82.80±3.34	81.60±3.61	85.60±2.71	80.40±2.84	82.80±3.95	82.00±3.08	85.00±3.16	85.00±3.63	89.00±2.10	88.20±1.62
Diabetic control	89.80±2.92	339.00±6.38	326.60±7.81	315.00±7.56	302.80±4.19	299.60±7.88	303.40±1.94	299.80±2.48	304.00±2.19	300.00±0.71
Diabetes plus 250mg/kg of extract	91.00±3.81	328.40±8.10	326.80±9.55	304.00±7.21	268.40±14.08	223.40±2.56	201.20±3.44*	146.00±10.32*	128.40±6.95*	115.00±8.76*
Diabetes plus 500mg/kg of extract	86.60±2.23	324.80±10.72	280.40±11.10	241.60±4.24	185.40±6.45*	127.20±4.25*	106.20±5.45*	94.20±2.22*	89.40±1.54*	87.80±3.31*
Diabetes plus glyburide (5mg/kg)	86.60±2.23	318.00±6.74	279.00±6.72	244.20±3.07	179.40±5.97*	130.80±3.77*	110.20±4.80*	100.60±2.25*	96.20±1.83*	81.00±4.23*

Values are expressed as Mean ± SEM; n=5; *=Significant at p<0.05

Table 2: Effect of treatment with hydromethanolic (HM) leaf extract of *Rauvolfia vomitoria (Rv)* on plasma insulin level of Streptozotocin-induced diabetic male wistar rats

S/N	Description	Insulin (µU/ml) After 14 days of treatment	Insulin (µU/ml) After 28days of treatment
1	Normal control	13.18±1.55	13.38±0.61
2	Diabetic control	6.99±0.78	7.29±0.72
4	Diabetes plus HM extract of *Rv* (250mg/kg)	9.49±0.92	11.19±0.87*
5	Diabetes plus HM extract of *Rv* (500mg/kg)	11.80±1.10*	13.25±0.72*
8	Diabetes plus glyburide (5mg/kg)	10.64±0.98*	13.98±0.45*

Values are expressed as Mean±SEM; n=5; *=Significant at p<0.05

diabetic rats. The results showed that, ten (10) days daily treatment with 500mg/kg dose of *Rauvolfia vomitoria* leaf extract caused significant (P<0.05) decrease (58.55%) in the blood glucose levels of diabetic rats, while it took fourteen-days treatment for the 250mg/kg dose to caused significant (P<0.05) decrease (53.42%) in the blood glucose levels of treated diabetic rats. The standard anti-diabetic drug, glyburide (5mg/kg B.W) record a significant (P<0.05) decrease (59.60%) after ten-days daily treatment. After twenty-eight (28) days treatment, the low and high doses of *Rauvolfia vomitoria* and the standard anti-diabetic drug caused further reduction (89.63%, 99.54% and 101.91% respectively) in the blood glucose levels of the diabetic rats.

Table 2 showed that 14-days treatment of streptozotocin-induced diabetic rats with 500mg/kg dose of *Rauvolfia vomitoria* leaf extract caused significant increase (P<0.05) in the plasma insulin levels (11.80±1.10µU/ml) which is comparable to that of the group treated with a standard anti-diabetic drug, glyburide (10.64±0.98 µU/ml), while treatment with 250mg/kg of the same extract had a non-significant effect on the plasma insulin levels (9.49±0.92µU/ml) of treated rats. It took 28-days of treatment for the 250mg/kg dose to cause a significant increase in the plasma insulin levels (11.19±0.87µU/ml). 28-days treatment with 500mg/kg dose of the extract and glyburide caused further increase in the plasma insulin levels to 13.25±0.72µU/ml and 13.98±0.45µU/ml respectively. The mean plasma insulin level of healthy untreated group was 13.38±0.61 µU/ml.

Figure 1 shows histologically normal pancreas from normal control group with normal islet of Langerhans and pancreatic architecture. Figure 2 (a) shows effect of 14-days untreated diabetes.

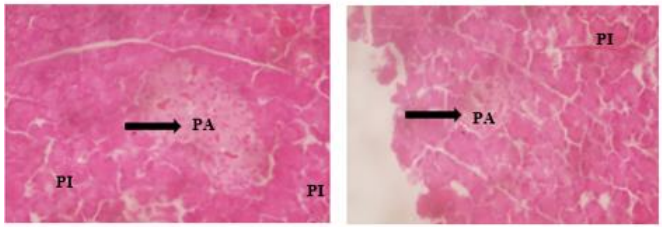

Fig. 1a (left)**:** Pancreas of wistar rat from normal control group after 14-days of experiment.
Fig. 1b (Right)**:** Pancreas of wistar rat from normal control group after 28-days of experiment

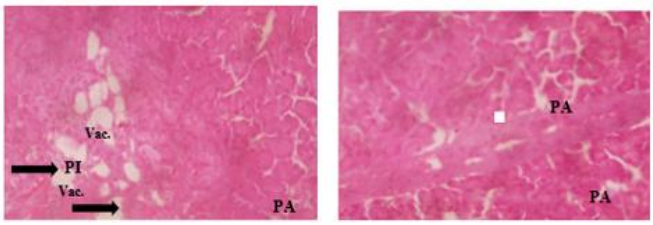

Fig. 2a*:* Effect of 14 days streptozotocin-induced diabetes on rat without treatment.
Fig. 2b: Effect of 14 days streptozotocin-induced diabetes on rat without treatment.

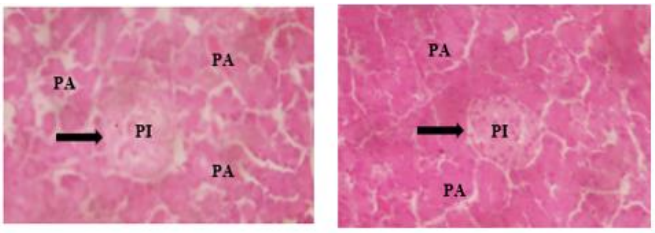

Fig. 3a: Effect of 14-days treatment with 250mg/kg b.wt. extract on streptozotocin-induced diabetic rat.
Fig. 3b*:* Effect of 28-days treatment with 250mg/kg b.wt. extract on streptozotocin-induced diabetic rat.

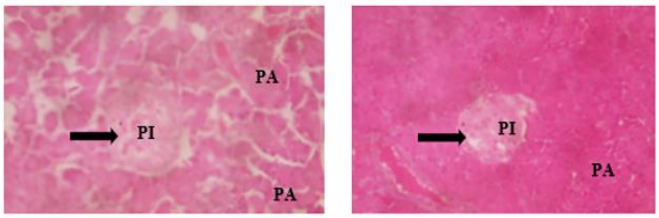

Fig. 4a: Effect of 14-days treatment with 500mg/kg b.wt. extract on streptozotocin-induced diabetic rat
Fig. 4b: Effect of 28-days treatment with 500mg/kg b.wt. extract on streptozotocin-induced diabetic rat.

The architecture of the pancreas islet has been compromised and greatly vacuolized (arrowed). Figure 2 (b) shows effect of 28-days untreated diabetes. The pancreatic islet is almost completely destroyed. Figure 3 (a) shows effect of 14-days treatment with extract (250mg/kg b.wt.) of hydromethanolic extract of *Rauvolfia vomitoria* leaf on streptozotocin-induced diabetic rat. The pancreatic islet is shrinked (arrowed), pancreatic acini not conspicuous. Figure 3 (b) shows effect of 28-days treatment with the same extract dose; pancreatic islets slightly in size compared to 14-days treatment, pancreas regains its architectural integrity.

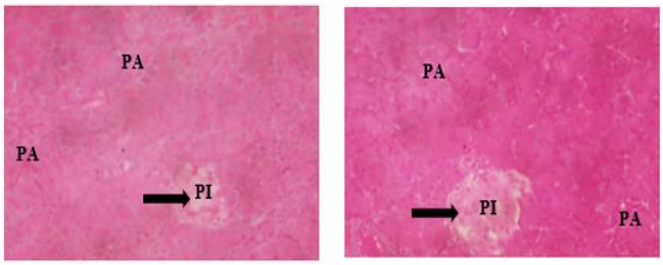

Fig. 5a: Effect of 14-days treatment with standard anti-diabetic drug (glyburide 5mg/kg b.wt.) on streptozotocin-induced diabetic rat.
Fig. 5b*:* Effect of 28-days treatment with standard anti-diabetic drug (glyburide 5mg/kg b.wt.) on streptozotocin-induced diabetic rat

Figure 4 (a) shows effect of 14-days treatment with extract (500mg/kg b.wt.) on streptozotocin-induced diabetic rat. The pancreatic islet is shrinked (arrowed) compared to that of normal control (figure 1), pancreatic architecture is still compromised and pancreatic acini not conspicuous. 4 (b) shows effect of 28-days treatment with the same extract dose; pancreatic islets slightly larger in size compared to 14-days treatment (figure 4 a) but smaller than that of normal control (figure 1), pancreas regains its architectural integrity. Figure 5 (a) shows effect of 14-days treatment with 5mg/kg b.wt. dose of glyburide on streptozotocin-induced diabetic rat. The integrity of the pancreatic islets is compromised (arrowed), pancreatic architecture had been compromised and pancreatic acini not conspicuous. 4 (b) shows effect of 28-days treatment with the same extract dose; pancreatic islet has regained its normal size; pancreas regains its architectural integrity.

DISCUSSION

The primary focus of diabetic treatment is to return the chronically elevated blood glucose level to the normal physiological range or as close as possible. Hence, the first criterion that qualifies a substance, drug or plant to be considered as an anti-diabetic agent, is its ability to cause a decrease in the blood glucose level of a system with an elevated blood glucose. The result of this study

(table 1) shows that *Rauvolfia vomitoria* leaf has anti-diabetic potentials. Our result from this study is in line with the reports of Campbell-Tofte *et al*. (2011) and N'doua *et al*. (2016) which associated *Rauvolfia vomitoria* leaf with blood glucose lowering (hypoglycemic) activity.

The relationship between the pancreatic hormones, (especially insulin) and blood glucose levels have long been established (Malherbe *et al*., 1969; Chen *et al*., 1988; Xu *et al*., 2003). The pancreas is the main organ which regulates blood glucose level, keeping it within the normal physiologic range via the secretion of insulin from its β-cell. Blood glucose level is inversely proportional to plasma insulin level.

One of the ways through which streptozotocin induces diabetes is by free radical generation and oxidative stress (Nakhaee *et al*., 2009; Busineni *et al*., 2015). The free radicals are selectively accumulated in the beta cells of the pancreas thereby causing oxidative stress which destroys the beta cells resulting in deficiency of plasma insulin (Nakatsuka *et al*., 1998; Raza *et al*., 2011). The most popular approach in the treatment of type 2 diabetes is by increasing plasma insulin. Results obtained from this study (table 4) showed that *Rauvolfia vomitoria* leaf was able to restore the insulin deficiency caused by streptozotocin administration.

The photomicrographs of treated groups showed that *Rauvolfia vomitoria* leaf extracts helped to ameliorate the degeneration and destruction of the pancreas caused by streptozotocin in a manner similar to glyburide. This is strongly believed to contribute to the improvement recorded in the blood glucose and plasma insulin levels of the treated groups. This finding corroborate the report of Ezejindu *et al*. (2013) which associated *Rauvolfia vomitoria* leaves with preventive and curative actions against the inflammatory and destructive effects of carbon tetrachloride (a toxic compound) on the liver. Ezejindu *et al*. (2013) suggested that the antioxidant properties of *Rauwolfia vomitoria* leaves was responsible for its anti-diabetic actions.

CONCLUSION

Findings from this study in addition to validating the anti-diabetic potentials of *Rauvolfia vomitoria* plant as reported by previous studies, has demonstrated for the first time, the effect of *Rauvolfia vomitoria* leaf on plasma insulin as well as its effect on the histomorphology of the pancreas. From the results of these investigations, it is therefore concluded that *Rauvolfia vomitoria* has strong anti-diabetic property. The results show that *Rauvolfia vomitoria* leaf extract effect its anti-diabetic action by enhancing the regeneration of the pancreatic islets thereby increasing insulin secretion and plasma insulin level.

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