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Histological studies of ethanolic extract of *Costusaferkergawl* leaves on the pancreas of alloxan-induced diabetes in Wistar rats.

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ABSTRACT
The hypoglycaemic property of ethanolic leaves extract of *Costusaferkergawl* on the pancreas of alloxan induced diabetic Wistar rats was evaluated to unravel the basis for its use in diabetes. Twenty-five male wistar rats weighing 180-200g were equally distributed into five groups of five rats designated A, B, C, D and E. Group A served as the normal control and did not receive any treatment, groups B, C, D and E were fasted overnight and diabetes was induced using single dose of 140mg/kg alloxan monohydrate (i.p). Group B served as the diabetic control and did not receive any other treatment while groups C, D and E received 100, 200 and 300mg/kg body weightof ethanolic leaf extract of*Costusaferkergawl* respectively for 14days. Blood was collected from tail vein for estimation of fasting blood glucose levelson the 0, 7 and 14thday of experiment. On day 15, the animals were anesthetized using 4mg/kg ketamine (i.p), the pancreas were harvested and processed for histological studies using H and E technique. Results of this study showed that ethanol extract of *Costus afer ker gawl* leaves ameliorated the diabetogenic effect of alloxan and decreased significantly the blood glucose level ($P < 0.05$) in treated rats when compared to the diabetic control (group B). Morphometric study of pancreas revealed that *Costus afer ker gawl* extract restored significantly the islets of Langerhans against alloxan-induced tissue changes. However, this restoration was dose dependant. In conclusion, ethanolic leaves extract of *Costus afer ker gawl* may have hypoglycaemic potentials.

Keywords: Diabetes, *Costus afer ke rgawl,* antidiabetes, pancreas, Wistar rats.

INTRODUCTION
In managing ofdiabetes mellitus, medicinal plants play important part mostly in developing countries where resources are not enough. Herbal plants of natural origin are safer, effective and have few side effects,unlike the compounds that are synthetic[1].Countries like the United States of America, China, Nigeria, and India have all made significantly great research investments in herbal medicines[2]. Antidiabetic activitiesof *Chysophyllum albidum*[3], *Sigesbeckia orientalis*[4] and several plants extracts have been reported.

Currently many plants are being studied, in orderto discover their medicinal abilities and *Costusaferker-gawl*has drawn attention as a result to its being consumed largely in rural communities[5]. It is a highly herbaceous leaf and a perennial plant, grows in clumps consisting of a number of strong unbranched stems. A network of rhizomes forms under the soil which sends up secondary root near the parent plant[6]. The plant is largely used as a medicinal plant in tropical Africa and pharmacological studies have confirmed these uses[7]. *Costusaferker-gawl* is a common medicinal plant used in South-East and South-West Nigeriain the management of diabetic, inflammatory and arthritic conditions[8]. The leaves are reputed to be an effective remedy for fever and malaria when boiled with leave of *carica papaya* (pawpaw), *citrus species* (orange) and bark of *Mangiferaindica* (Mango); the stem and juice has traditional use for treatment of cough, measles and malaria in Aluu community of Rivers State[9].*Costusaferker-gawl* is used as a remedy for cough, inflammation, arthritis, hepatic disorder, helminthic, miscarriages, epileptic attack and haemorrhoids, as laxative, aperient, diuretic, purgative, in rheumatism and treatment of several other diseases[10].The decoction of the plant is used to alleviate the clinical signs of diabetic patients in Ohafia community of Abia State Nigeria[11]

Diabetes Mellitus is a metabolic disease that have distinguished features such as abnormally elevated blood sugar level, which alters the tightly regulated metabolisms of carbohydrates, fats and proteins arising from malformation in the way insulin is secreted, the action of insulin or both[12]. It is an important health interest because it has become the third greatest "killer" after cardio-vascular diseases and cancer[13]. It is approximated that 5% of deaths in the world is as a result of diabetes, this number will increase by 50% in the next 10years[14]. At the time of clinical diagnosis, estimated 70% of the total beta cells mass is destroyed as a consequence of immune-mediated processes[15]. However, as there are no cures for diabetes, the goal in

the management of diabetes is the preservation and potential beta cells regeneration.

*Costusaferker-gawl*is known for its medicinal potential; published scientific information onits effect on the pancreas is lacking.The present study was designed to investigate the hypoglycemic effects of ethanolic leaves extracts of *Costusaferkergawl* on thehistological changes of thepancreas of alloxan induced diabetic rats.

MATERIALS AND METHODS

Preparation of Ethanolic Leaf Extract of *Costusaferkergawl:* Fresh leaves of *Costusaferkergawl* was collected from a farmland in Okija, Anambra State, Nigeria. They were thoroughly washed with distilled water to remove debris and contaminants: shade dried and then pulverised using laboratory mill to powered form.A total of 100g of the grounded leaves of *costusaferkergawl* was soaked in 500mls of absolute ethanol, sealed and allowed to stay for 48 hours inside a mechanical shaker. The mixture was sieved using a porcelain cloth and further filtered using filtering paper into a clean glass beaker. The filtrate was concentrated using Digital Rotary Evaporator (TT-53 Techmel and Techmel, USA) and was further dried using Thermostat Oven (DHG-9023A PEC medicals USA) to a jelly-like form and stored in a refrigerator until use.

Acute Toxicity Study: Using the Organization of Economic Cooperation and Development (OECD) guidelines 423, the acute toxicity study was done for *Costusaferkergawl* leaves in Wistar rats for 14 days. A total of 3 rats per groups were used for each phase as recommended by the guideline, while 3 rats were kept as control.First phase of acute toxicity study initial doses were (10, 100 and 1,000 mg/kg bw*Costusaferkergawl*) which was orally administered. Furthermore, in second phase doses (1,900, 2,600 and 5,000 mg/kg bw*Costusaferkergawl*) was orally administered.*Costusaferkergawl*leaves was found safeup to a dose of 5,000 mg/kg bw. After 72 hours of observation no mortality was recorded[16].

Experimental Animals: Healthy twenty-five male wistar rats, weighing 180- 200 g were used. The animals were fed standard pelletized diet and water *ad libitum* and kept in 12 hrs light/dark cycle. The rats were divided into five groups of five rats each: group A (control group); group B (diabetic control) group C (diabetic + 100 mg/kg bw), group D(diabetic + 200 mg/kg bw) and group E (diabetic + 300 mg/kg bw) orally of ethanolic leaves extract of *Costusaferkergawl* once every morning for 14 days using orogastric tube.

Induction of Diabetes in Rats: The rats were fasted overnight and diabetes was induced using single dose of freshly prepared alloxan monohydrate (Sigma Aldrich, Germany) 140 mg/kg body weight (intraperitoneal injection) in normal saline.However, dextrose solution (w/v) of 20% was administered after 6 hour to prevent alloxan-induced hypoglycemia as a result of the huge release of insulin from the pancreas. The rats were supported on 5% glucose in drinking water, in the next 24 hour. The development of hyperglycemia in rats was confirmed by fasting blood glucose estimation after 72 h of initial injection of the alloxanusing Accu-Check glucometer (Roche Diagnostics, Germany).The day of confirmation of diabetes was considered as day 0 of diabetes. The animals that had fasting blood glucose level above 250 mg/dl were considered diabetic and included for the studies[17].Blood was obtained from tail vein for estimation of fasting blood glucose on the 0, 7 and 14 days of experiment. On day 15, the animals were anesthetized using 4mg/kg ketamine (i.p.), the pancreas was excised and processed for histological studies using H and E technique.

Statistical Analysis: All data were analyzed using available statistics software package (SPSS for Windows, V. 20.0). One-way analysis of variance(Anova) was used, followed by multiple comparison using least significant differences (LSD) and results were considered significant at $P<0.05$.

RESULTS

Histological Results: Sections of the pancreas of control group showed prominent islet of Langerhans(IL) with numerous cells (FigureI). The diabetic group showed degeneratedislet of Langerhans (IL), devoid of cells compared with the control (Figure 2). The groups treated with 100 and 200mg/kg of *Costusaferkergawl* leaves extract showed mild regeneration of islet of Langerhans compared with diabetic group (Figure 3& 4 respectively). The group E(Figure 5) treated with 300 mg/kg of *costusaferkergawl* leaves extract showed prominentislet of Langerhans with regenerated cells indicating total restoration compared with the diabetic group.

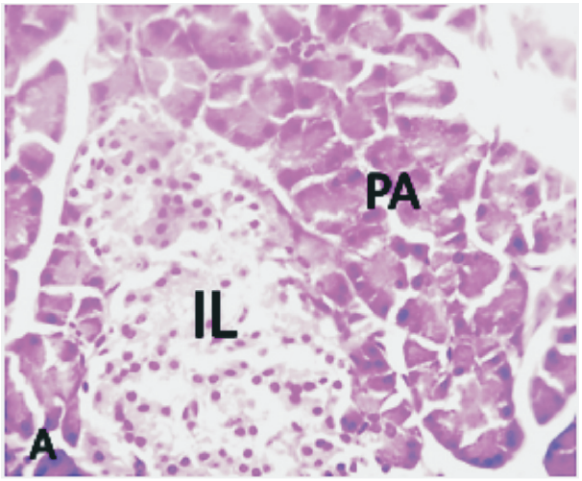

Figure 1: Photomicrograph of control pancreas showing prominent islet of Langerhans (IL)and pancreatic acini (PA). Hematoxylin and Eosin (H and E), magnification ×400.

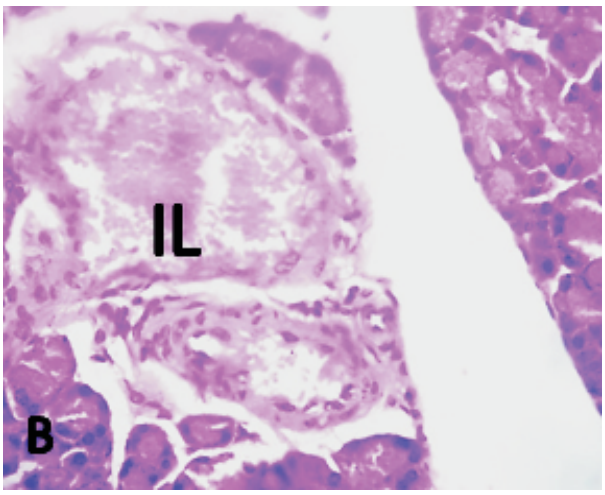

Figure 2: photomicrograph of diabetic pancreas showing degenerated islet of Langerhans (IL) with no cells. Hematoxylin and Eosin (H and E), magnification ×400

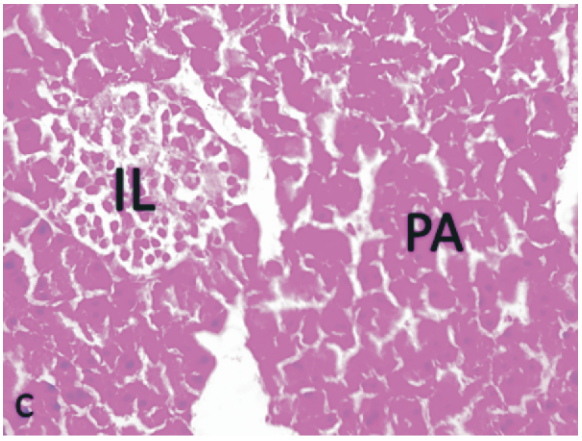

Figure 3: Photomicrograph of diabetic pancreas treated with 100mg/kg of ethanolic leaf extract of *Costusaferkergawl* showing mild regeneration of islet of Langerhans (IL)and pancreatic acini (PA).Hematoxylin and Eosin (H and E), magnification ×400

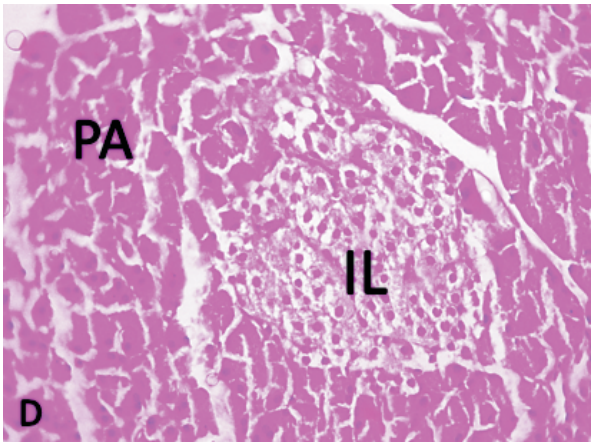

Figure 4: Photomicrograph of diabetic pancreas treated with 200mg/kg of ethanolic leaf extract of *Costus afer ker gawl* showing regenerating islets (IL)and pancreatic acini (PA). Hematoxylin and Eosin (H and E), magnification ×400

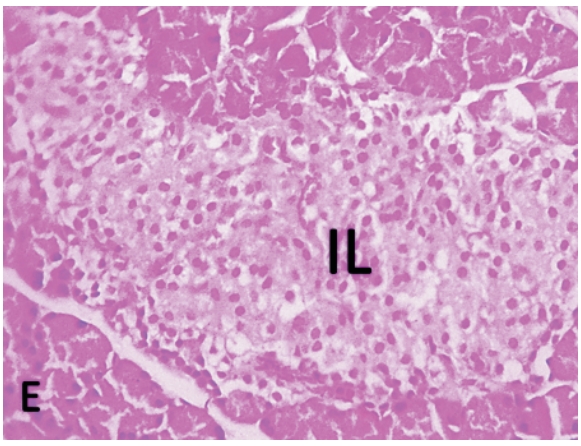

Figure 5: Photomicrograph of diabetic pancreas treated with 300mg/kg of ethanolic leaf extract of *Costusaferkergawl* showing prominent islet of Langarhans (IL) with numerous islet cells.Hematoxylin and Eosin (H and E), magnification ×400.

Table 1: showing the effect of Ethanolic leave extract of *Costusafer Ker gawl*on Blood glucose level on Day O, 7& 14 on Alloxan Induced diabetic wistar rats.

Blood Glucose (mg/dl)		MEAN ±SEM	P- VALUE	F-VALUE
Day O	Group A (-Ve Control)	76.80 ±19.25	0.001**	
	Group B (+Ve Control)	437.60±91. 58		10.090
	Group C (Low Dose)	411.80±97.64	0.780	
	Group D (Moderate Dose)	496. 50±11. 78	0.550	
	Group E (High Dose)	660.25 ±22.84	0.033*	
Day 7	Group A (-Ve Control)	78.20 ±17.85	0.000**	
	Group B (+Ve Control)	433.20 ±90.43		
	Group C (Low Dose)	384.00±41. 06	0.513	11.192
	Group D (Moderate Dose)	479.00 ±49.50	0.542	
	Group E (High Dose)	531.75 ±22.87	0.223	
Day 14	Group A (-Ve Control)	79.60 ±16.46	0.000**	
	Group B (+Ve Control)	387.80±76. 11		
	Group C (Low Dose)	123.75+9.47	0.001*	7.081
	Group D (Moderate Dose)	148.20 ±16.35	0.002*	
	Group E (High Dose)	143.50 ±130.50	0.010*	

All data were analyzed using One-way Anova, followed by multiple comparism using LSD, and data were considered significant at P<0.05. *P<0.05 shows that it was significant, **P<0.05 shows that it was more significant and P> 0.05 means not significant.

Result from the above (Table 1) showed that after induction of diabetes using alloxan, there was an increase in blood glucose level at Day O indiabetic group B (437.60±91.58),group C (411.80±97.64), group D(496.50±11.78) and group E (660.25±22.84) when compared to the control groupA(76.80 ±19.25), however, this increase was significant (P< 0.033*) in group E (300 mg/kg), while the groups C (100 mg/kg) and D (200 mg/kg) was not significant when compared to the diabetic control.

On day 7, there was a gradual blood glucose level decrease in groupC (384.00±22.87), group D (479.00±49.50) when compared todiabetic group B (433.20±90.43 and group E (531.75±22.87)) when compared the initial glucose level(660.25±22.84), however the decrease was not significant.
On day 14, there was significant (P<0.05) decrease in the blood glucose level in group C (123.75±9.47), group D (148.20±16.35) and group E (143.50±130.50) when compared to diabetic group B (387.80±76.11).

DISCUSSION

Throughout the world, the use of medicinal plants have become one of the basis for good health maintenance[18]. Some of these herbal preparations have been found to exert biological actions against diabetes mellitus and its complications[19].Many hypoglycemic plants have been used as non-prescription treatment for the management of diabetes[20].

This study showed significant increase in blood glucose levels after alloxan injection to the rats.One of the ways alloxan induces diabetes is by generating reactive oxygen species (ROS) that results in simultaneous massive increase in cytosolic calcium concentration, which will lead to rapid destruction of pancreatic beta cells and a concomitant reduction in the synthesis and release of insulin leading to hyperglycemia[21,22]. Furthermore, the hyperglycemia rapidly increase free radicals generations by glucose auto-oxidation[23].

Phytochemical analysis have reported the presence of alkaloids, tannins, cardiac glycosides, anthraquinones, flavonoids and phenols in ethanol extracts from the leaves of *Costus afer Kel gawl*[24]. This study revealed that the daily doses of *Costus afer Kel gawl* was able to cause significant reductions (P < 0.05) in mean blood glucose levels in the treated groups when compared to diabetic control and these decrease in blood glucose level is dose dependent, with the 300 mg/kg *Costus afer Kel gawl* dose bringing blood glucose levels down to normal ranges within 14 days of administration.Hence, this result could be as a result of the antioxidants present in the plant that have been observed in a previous study[24]. Antioxidants are known to beprotective agents that inactivate the reactive oxygen species (ROS) which cause cell damage[25].
The photomicrograph of pancreas of Group E animals

imply a restorative effect of leave extract of *Costus afer Kel gawl* as observed in significant regeneration of alloxan destroyed islets of Langerhans cells in the photomicrograph of animals in Group B. Flavonoids and phenols are abundant in the extract and these are potent antioxidants which prevent oxidative cell damage[26,27,24].

Pancreas of rats administered with *Costus afer Ker gawl,* mostly the higher dose showed reversal of damages caused by alloxan-induced hyperglycemia and resulted in normal pancreatic architecture which corresponded with the decrease in blood glucose levels. This showed that the extract of *Costus afer Kel gawl* was effective and this corresponds with earlier report[8].

CONCLUSION

In conclusion, this study showed that ethanolic leave extract of *Costus afer ker gawl* at the dose of 300 mg/kg has restorative potentials and possesses hypoglycemic properties. Hence, it may be useful in the management of diabetes. Further studies are needed to separate, characterize and to shed light upon the active components that are responsible for its hypoglycemic activities.

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