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Cytoprotective Effects of *Garcinia kola* Stem Bark Extract on the Pancreas of Alloxan Induced Diabetic Wistar Rats

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Abstract

The prevalence of Diabetes mellitus, a multiorgan system metabolic disease is on the increase even in developing countries like Nigeria. This study therefore investigated the protective effects of stem bark extract of *Garcinia kola* on the histology of pancreas of alloxan induced diabetic whistar rats using histological and histochemical techniques. The animals used in this study were divided into 6 groups viz: Control group, Diabetic control group, *Garcinia kola* stem bark only group, Diabetic+Glibenclamide group, Diabetic+low Dose *Garcinia kola* group and Diabetic+High Dose *Garcinia kola* group. The duration of study was 56 days. Studies on the rats on days 7 and 14 were regarded as short and medium term effect study. Day 21 study was taken as long term effect study while day 56 study was for reversibility study as treatment had been discontinued. On day 7, 14, 21 and 56, 3 rats from each group were sacrificed under chloroform anaesthesia and pancreas harvested for histological assay. The data obtained were analysed using GraphPad Prism® software version 6.01 to determine the mean and standard error of mean, changes in body weight, insulin and glucose levels were represented in percentages while one-way ANOVA at 95% confidence interval ($p<0.05$) was used to show the level of significance. Results revealed that the histology of the pancreas was grossly distorted in the diabetic control group animals whereas histology of the glibenclamide and *Garcinia kola* stem bark treated groups showed grossly repaired pancreas. These results demonstrate a protective effect of the hydro-methanolic extract of G.kola stem bark on organ damage in Diabetic mellitus.

Keywords: Garcinia kola, Stem Bark Extract, Hydro-methanolic, Alloxan, Diabetic, Pancreas.

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INTRODUCTION

Amongst the basic food nutrients quite available to man, carbohydrate meal seems more common. In the present dispensation, there has been emergence of various debilitating as well as deleterious ailments with many older ailments still prevalent. This leaves one to query not just lifestyle but also the commitment to exploring our environment for solutions to these circumstances. In most African countries particularly in Nigeria, almost all meals are carbohydrate based and diabetes is therefore predominant. The ailment is believed to be a carbohydrate metabolic disease caused as a result of insufficient insulin production. Insulin which causes conversion of surplus glucose to glycogen for storage in the liver is manufactured by pancreatic islets cells under regulation by the pituitary gland. Recent estimates indicate there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030 [1]. Secondary plant metabolites previously with unknown pharmacological actions have been extensively investigated as sources of medicinal agents [2-4]. *Garcinia kola* is one of such plant of choice. The seed and leaves have shown evidence of causing hypoglycemic effects and thus have been implicated as a possible means of managing the disease. However, its seedling may not occur all year long. Little or no study has been documented on the likely effects of the stem bark.

Garcinia kola belongs to the family Guttiferae and it is a popular plant in Southern Nigeria and extensively used as food and herbal medicine. It is found in the tropical rain forest of West-Africa [5]. Several works done on Garcinia kola seeds have confirmed its hypolipidemic [6], antifungal [7], anti-histaminic [7], spermatogenic [8], aphrodisiac and anti-microbial [9], erythropoietic [8], antiviral [10] and anti-ulcerogenic effects [11]. Garcinia kola is reported to have a protective effect against variety of experimental hepatotoxins [12, 13]. Garcinia kola stem bark has been shown to contain a complex mixture of phenolic compounds such as bioflavonoids, xanthenes and

benzophenone [14], kola flavanone and garcinia flavanone [15]. Udenze *et al*., [16] studied the pharmacological effects of Garcinia kola seed powder on blood sugar, lipid profile, and atherogenic index of diabetic rats. This investigation showed that Garcinia kola powder is an anti-diabetic, anti-lipidemic, and anti-antherogenic agent with a tremendous potential to protect against coronary heart disease. The Garcinia kola seed having been shown to exert an influence on blood cholesterol concentration, blood glucose level among others and coupled with the fact it is generally prescribed by traditional medical practioners, studying its stem bark to understand its effects on glucose and body weight thus becomes necessary.

MATERIALS AND METHODS

Research Design

The research design for this study was experimental which investigated the effect of *G.kola* stem bark extract on body weight, lipid profile and glucose level of alloxan induced diabetic rats. Ethical clearance was obtained from the School of Graduate Studies' Research Ethics Committee, University of Port Harcourt. One hundred (100) adult male wistar rats were used for the study. A pilot study involving the use of twenty (28) animals was first done to confirm the effective dose to be used as stated in previous literature. The main study involved the use of seventy two (72) animals which were grouped into six (6) major groups as control, Diabetic, Garcinia kola only, Diabetic + Glybenclamide (a test drug used as control for diabetic studies), and the test groups divided into high dose (1/10 of the LD50) and low dose (1/20 of the LD50).

Animal Grouping

The animals were grouped as follows:

Group 1 (control group; negative control): normal animals which received water and food ad libitum.

Group 2 (positive control for diabetes): alloxan-diabetic animals which were similarly given water and food at will.

Group 3: given the plant extract at a high dose of 35.8mg/kg body weight to determine the effect of the drug on normal animals.

Group 4: induced with alloxan diabetes and given the experimental drug Glibenclamide at 5mg/kg body weight as standard check for the procedure.

Group 5- low dose of the extract on alloxan-induced diabetic rats (17.9mg /kg of the extract)

Group 6- high dose of the extract on alloxan-induced diabetic rats (35.8/kg of the extract)

The experimental period was for eight (8) weeks, which was divided into weeks 1, 2, 3, and 8 representing short, medium and long time effects. The 8th week study was to check for reversibility in the effect of the extract as no extract was administered after the 3rd week. At the end of each period the body weight was taken, blood collected for analysis of glucose level, insulin and organ function test (OFT) by analysis of serum enzymes and electrolytes.

Drug Treatment

Drug treatment was initiated immediately after it was found that the blood glucose level had become stable.

These treatment protocols were thus followed and performed daily until the end of the test period.

Sample Collection

Stem bark samples were collected from trees in a local farm in Egbeda community, Emohua Local Government Area of Rivers State, Nigeria with the aid of local farmers in November, 2014.

Sample Identification

The plant was identified and authenticated by the plant taxonomist in the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria.

Sample Processing and Extraction

After identification, the samples were washed with clean tap water to remove dirt on the stem bark. The samples were further sundried for 24 hours for the water to drain and dried with the hot air oven. The desiccated sample was pulverised using a manual blender. About 600g of the ground plant material was divided into 3 containers of 200g each then macerated in 500ml of distilled water, 500ml of methanol and in 500ml of hydromethanol extracting fluid (consisting of 350ml of methanol and 150ml of distilled water) respectively, placed in a stoppered container and allowed to stand for 48hours with constant agitation. After 48 hours, the mixture was strained, the marc was pressed and the liquid was filtered with Whatman No. 1 filter paper and concentrated. The filtrate was preserved in a refrigerator under a temperature of 4˚C until required for use.

Phytochemical Analysis of the Sample

Qualitative phytochemical analysis was carried out on the dried methanolic, hydro-methanolic and aqueous extract in other to identify the chemical constituents using the methods by Sofowara [17] and Edeoga *et al*., [18] and Trease and Evans [19].

Determination of LD_{50} and Dose

The LD_{50} as determined from previous works done by Kagbo and Ejebe[20] was 358mg/kg body weight of animals used for the study. The experimental doses used were derived from this published study after

investigation as 1/10 and 1/20 of this value. These values were taken as high and low doses respectively.

Diabetes induction using Alloxan, Body Weight and Glucose Level Monitoring

After the acclimatization period, the animals were prepared for induction. They had twelve (12) hours overnight fasting. Groups 2, 3, 5 and 6 were injected with 150mg/kg of alloxan monohydrate (manufactured by Qualikeins Fine Chemicals pvt. Ltd. New Delhi, India) intraperitoneally (IP) leaving groups 1 and 4 non-induced.

Thereafter, the body weight and blood glucose level were monitored on weekly basis using Analytical balance CPA 3245 (manufactured by Sartonus AG Grohingen, Germany) and Accuchec glucometer (manufactured by Roche Diagnostics GMBH, Sandohofer Strasse 11,668,305 Mannheim, Germany).

The body weights were measured using weighing balance to monitor the change in body weight.

Blood Sample Collection and Analysis

The blood samples of the rats were collected by cutting the animals' tail with a sterile sharp blade and read off on a glucometer for blood glucose level.

Statistical Analysis

The data generated were analysed using GraphPad Prism® software version 6.01 to determine the mean and standard error of mean, changes in body weight, insulin and glucose levels were represented in percentages while one-way ANOVA at 95% confidence interval ($p<0.05$) was used to show the level of significance.

RESULTS

Table-1: Changes in Body Weight (gm) in Experimental Animals Across Weeks and Percentage Weight Change in Weeks (% Change)

S/no		GROUPS					
		Control	Diabetic	*GKSB*	Glibenclamide	Low Dose GKSB (T1)	High Dose GKSB (T2)
1	Week 1 Initial Final %Change	195.99±1.56 198.33±7.31 (1.19)	191.01±2.33 172.00±6.43 (-9.95%)	187.66±0.39 188.00±4.37 (0.18%)	186.34±1.97 180.00±3.46 (-3.40%)	193.33±1.88 178.00±3.22 (-7.93%)	201.32±0.97 184.33±2.85 (-8.44%)
2	Week 2 Initial Final %Change	202.00±1.88 210.00±2.00 (3.96%)	207.68±0.96 184.00±2.08 (-11.40%)	198.00±0.88 201.33±2.03 (1.68%)	191.33±1.87 184.67±2.91 (-3.48%)	205.67±1.94 200.67±0.88 (-2.43%)	196.65±0.97 193.33±6.36 (-1.69%)
3	Week 3 Initial Final %Change	190.00±0.77 205.67±5.18 (8.25%)	200.33±1.35 171.00±0.58 (-14.64%)	192.68±0.97 202.00±4.16 (4.84%)	198.34±1.99 192.33±4.18 (-3.03%)	190.99±0.98 190.00±2.89 (-0.52%)	200.67±1.87 200.67±2.60 (0%)
4	Week 8 Initial Final %Change	196.01±0.45 208.00±3.51 (6.12%)	188.67±0.94 180.67±5.70 (-4.24%)	186.33±0.85 205.00±2.65 (10.02%)	190.00±0.84 188.67±8.01 (-0.70%)	201.34±1.07 203.67±2.96 (1.16%)	194.34±0.98 197.33±8.01 (1.54%)

Note: Values are given as mean ± SEM for each group (N=3). Percentage weight change is expressed in brackets with negative values representing reduction from initial values, T1 = test group one while T2 = test group 2

Table-2: Changes in Blood Glucose Level Across Groups in Weeks And Percentage Reduction (% Reduction)

S/no		GROUPS					
		Control	Diabetic	*GKSB*	Glibenclamide	Low Dose GKSB (T1)	High Dose GKSB (T2)
1	Week 1 Initial Final %Change	79.33±0.94 80.00±0.00 (0.84%)	321.67±0.88 343.67±34.67 (6.84%)	77.67±1.01 76.00±0.58 (-2.15%)	279.30±0.88 91.33±2.67 (-67.30%)	343.66±1.02 273.00±4.36 (-20.56%)	325.30±0.95 174.33±6.17 (-46.41%)
2	Week 2 Initial Final %Change	81.33±0.87 81.00±3.22 (-0.41%)	323.33±0.95 321.00±14.18 (-0.72%)	79.99±1.64 75.33±2.40 (-5.83%)	286.64±0.78 84.33±6.89 (-70.58%)	371.65±1.83 226.00±14.19 (-39.19%)	319.20±0.65 104.67±1.76 (-67.19%)
3	Week 3 Initial Final %Change	81.01±1.23 81.67±1.20 (0.82%)	315.66±0.92 251.33±21.67 (-20.38%)	77.99±0.85 72.33±1.45 (-7.26%)	285.37±1.92 84.67±4.26 (-70.33%)	307.29±0.76 94.00±6.11 (-69.41%)	325.70±0.44 98.33±8.67 (-69.81%)
4	Week 8 Initial Final %Change	81.67±0.75 81.00±2.08 (-0.82%)	321.67±0.55 82.67±4.06 (-74.30%)	80.00±0.21 72.00±1.73 (-10.00%)	304.73±0.77 76.67±5.84 (-74.84%)	311.00±0.25 84.00±0.58 (-72.99%)	298.36±1.12 85.33±2.91 (-71.40%)

Note: Values are given as mean ± SEM for each group (N=3). Percentage glucose level change is expressed in brackets with negative values representing reduction from initial values.

Table-3: Changes in Insulin Level Across the Groups In Weeks (mg/dL) and Percentage Change (% Change)

S/no		GROUPS					
		Control	Diabetic	*GKSB*	Glibenclamide	Low Dose GKSB (T1)	High Dose GKSB (T2)
1	Week 1 Initial Final %Change	16.54±0.63 17.87±1.00 (8.06%)	8.30±0.44 8.33±0.07 (0.40%)	15.50±0.23 15.60±0.31 (0.65%)	7.87±0.53 11.77±0.09 (49.58%)	8.04±0.72 9.07±0.24 (12.86%)	8.20±0.53 9.27±0.35 (13.01%)
2	Week 2 Initial Final %Change	16.46±0.44 17.03±0.67 (3.44%)	7.90±0.15 7.73±0.27 (-2.11%)	15.00±0.31 15.70±0.55 (4.67%)	8.20±0.20 12.27±0.18 (49.59%)	7.93±0.45 10.50±0.51 (32.35%)	8.40±0.30 10.47±0.41 (24.60%)
3	Week 3 Initial Final %Change	18.27±0.43 18.30±0.51 (0.18%)	8.06±0.67 7.83±0.12 (-2.89%)	16.37±0.32 17.77±0.61 (8.55%)	7.50±0.43 12.73±0.26 (69.78%)	7.63±1.20 12.60±0.31 (65.07%)	8.14±0.68 13.37±0.72 (64.34%)
4	Week 8 Initial Final %Change	17.60±1.34 17.43±0.95 (-0.95%)	8.40±1.10 10.57±1.18 (25.79%)	16.13±0.18 16.43±0.19 (1.86%)	7.87±1.67 13.97±1.69 (77.54%)	7.67±1.34 14.63±0.27 (90.87%)	8.27±1.45 15.80±0.31 (91.13%)

Note: Values are given as mean ± SEM for each group (N=3). Percentage insulin level change is expressed in brackets with negative values representing reduction from initial values.

Histological analysis

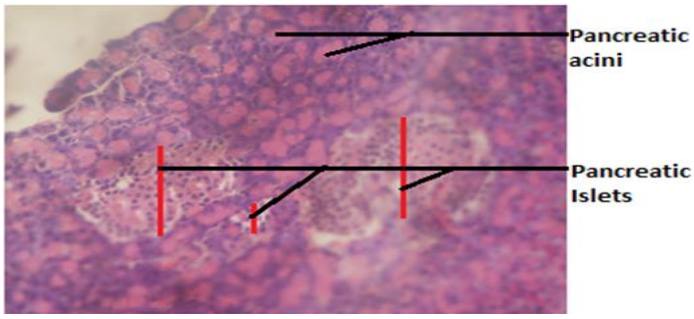

Fig-1: Control pancreatic tissue

Picture shows low magnification of the pancreatic tissue. The islet and acinar cells are labelled. H & E. M x100.

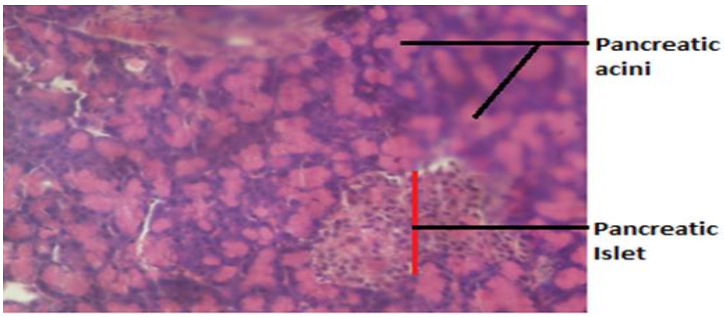

Fig-2: Garcinia kola stem bark administered tissue

There is still prevalence of pancreatic islets at the end of the third week of administration. Group 3, week 3. H & E. M x100.

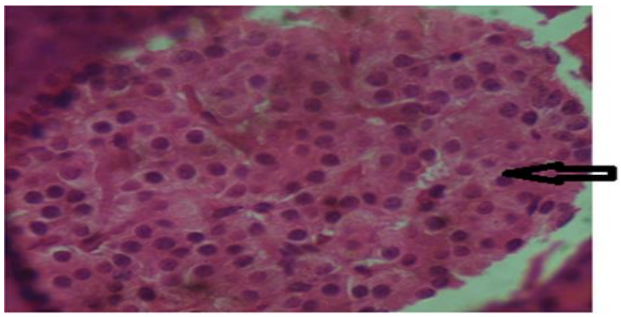

Fig-3: Control Pancreatic tissue

Photomicrograph of pancreas. A luxurious islet is in focus, all the cell types are clearly seen. Group 1. M x400 H&E.

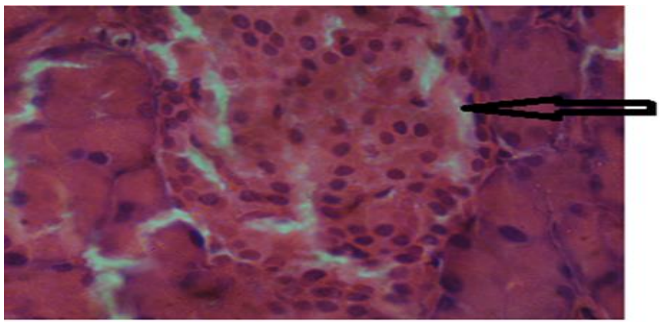

Fig-4: Diabetic pancreatic tissue

Photomicrograph of Pancreas. Picture shows early stage of the diabetes. There are vacuolations (arrow head) and reduction of the islet cells. Acinar cells are essentially normal at this stage. Group 2, week 1. M x400 H&E.

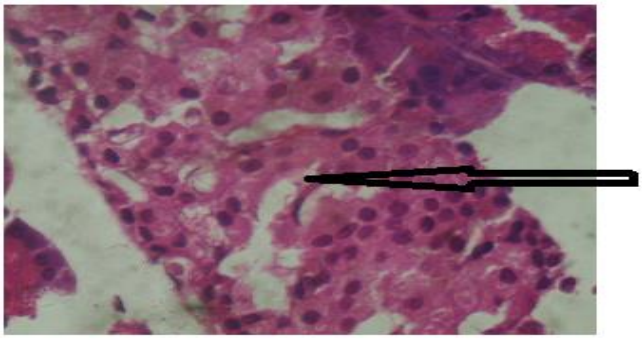

Fig-5: Diabetic Pancreatic tissue

Photomicrograph shows increased vacuolations and loss of islet cells (arrowed). Group 2, week 2. M x400. H & E.

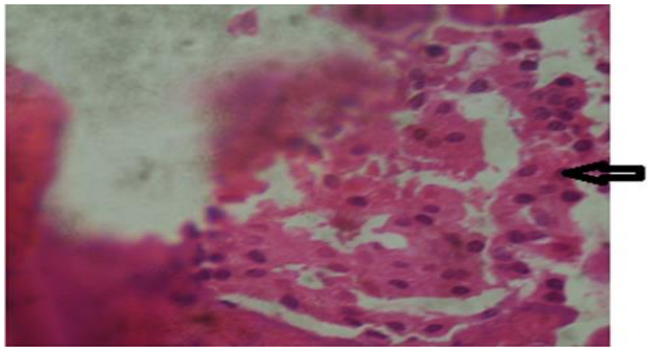

Fig-6: Diabetic Pancreatic tissue

Photomicroraph of pancreas. Picture shows drastic loss of islet tissue leading to indiscrete cells that are paler and thinner. There is also reduced concentration of β-cells and vacuolations. The cell outlines are also poorly defined with irregular outlines. Group 2, week 2. M. x400. H&E.

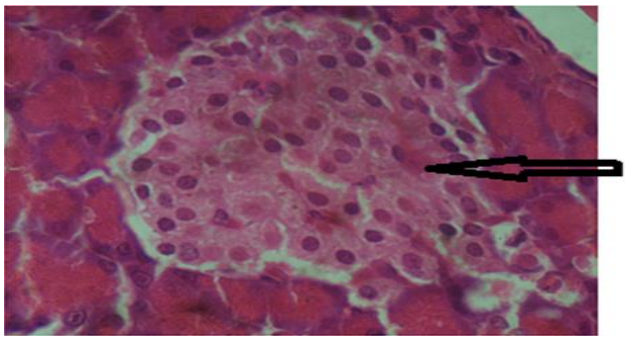

Fig-7: *Garcinia kola* stem bark administered pancreatic tissue

Photomicrograph of Pancreas. Showing islet arrowed (α-cells and β-cells are centrally located). Acinar cells are histologically normal. Group 3, week 3. M. x400 H&E.

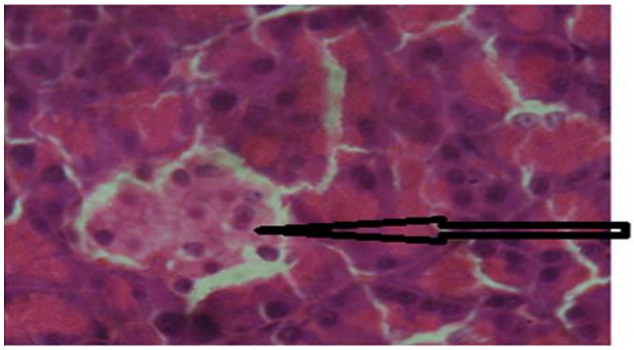

Fig-8: Glibenclamide treated diabetic tissue

Photomicrograph of pancreas. Showing Shrunken Islet which is surrounded by condensation of fibers (arrowed). Group 4, week. M. x400 H&E.

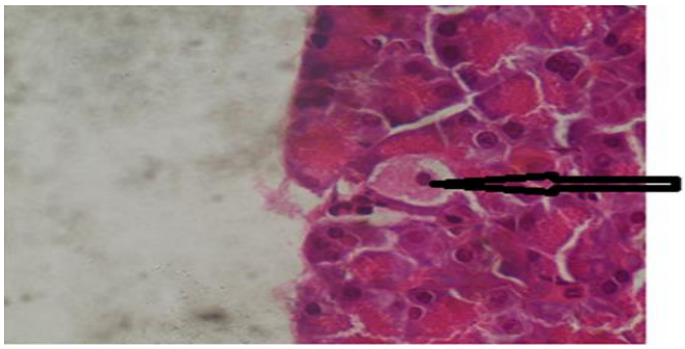

Fig-9: *Garcinia kola* stem bark extract treated diabetic tissue

Photomicrograph of Pancreas shows a sprouting islet (arrowed). The tissue is characterised by few sprouting islet cells while the acinar cells appear in good histologic condition. Group 5, week 2. M. x400 H&E.

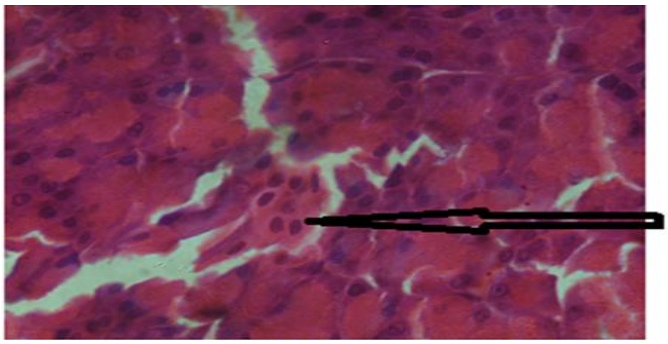

Fig-10: *Garcinia kola* stem bark extract treated diabetic tissue

Photomicrograph of Pancreas showing a sprouting islet (arrowed). The tissue is characterised by few sprouting islet cells while the acinar cells appear in good histologic condition. Group 5, week 2. M. x400 H&E.

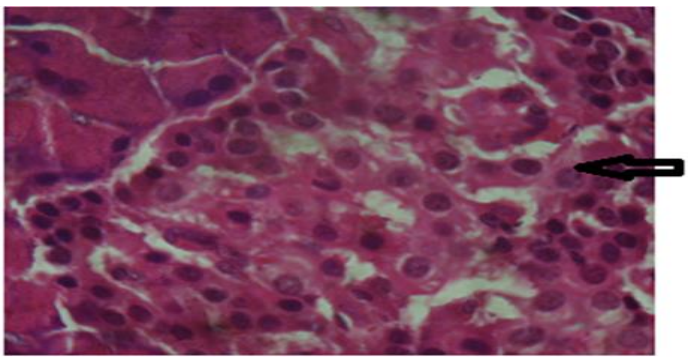

Fig-11: *Garcinia kola* stem bark extract treated diabetic tissue Photomicrograph of Pancreas.

Islet arrowed; showing features of regeneration of cells and vacuolations. Group 5, week 3. M x400. H & E

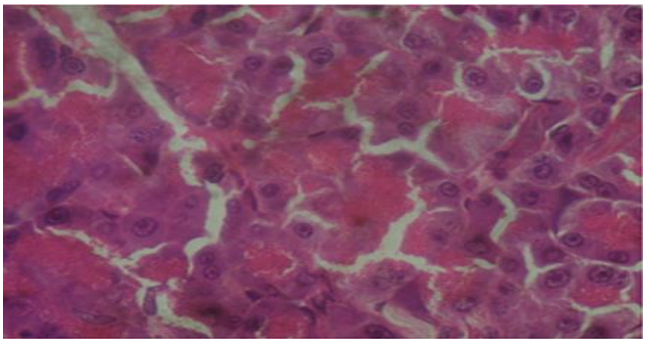

Fig-12: *Garcinia kola* stem bark extract treated diabetic tissue

Photomicrograph of pancreas showing normal acinar cells, Islets cannot be seen in this view. Group 5, week 3. M x400. H & E

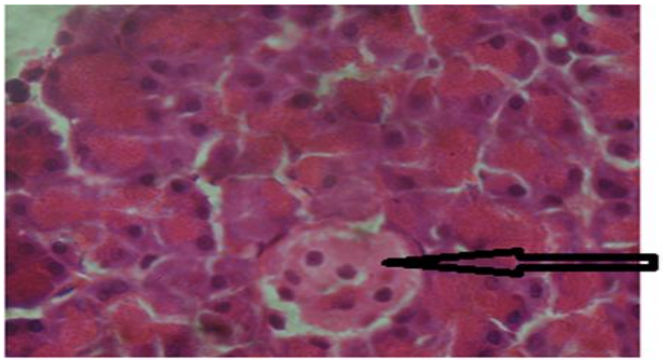

Fig-13: *Garcinia kola* stem bark extract treated diabetic tissue

Photomicrograph of Pancreas showing sprouting Islets (arrowed) amidst acinar cells. Group 6, week 2. M x400. H & E.

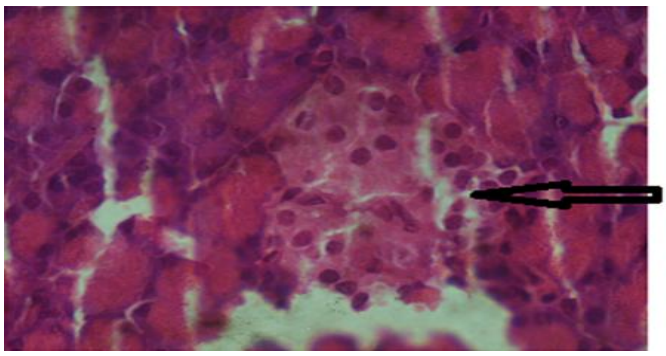

Fig-14: *Garcinia kola* stem bark extract treated diabetic tissue

Photomicrograph of Pancreas showing sprouting (regenerating) Islets from existing ones (arrowed). Acinar cells are essentially normal. Group 6, week 2. M x400. H & E

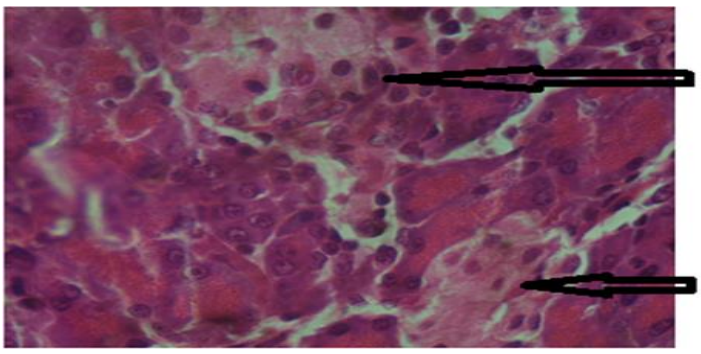

Fig-15: *Garcinia kola* stem bark extract treated diabetic tissue

Photomicrograph of Pancreas. Regenerating Islets arrowed; budding and increasing concentration of islet cells seen. Group 6, week 3. M x400. H & E

DISCUSSION

Alloxan brings about huge reduction in insulin release via the destruction of β-cells of the islets of langerhans, thereby inducing hyperglycaemia[21]. In the present study, administration of alloxan induced diabetes on analysis of the blood sample thereby producing tissue injury and distortion of the pancreas on histological examination. This could be attributed to the action of alloxan via induction of free radicals. On administration for certain duration, hydro-methanolic extract of Garcinia kola stem bark produced an antidiabetic effect on analysis of the blood. Histologically, the pancreas presented normal α and β-cells of the islets in the *Garcinia kola* stem bark treated group suggesting repairs.

The healing activity of the extract is attributed to presence of bioflavonoids in G. kola stem bark extract. Flavonoids are well known for their multi-directional biological activities including antidiabetic efficacy [22, 23]. Various studies have explored the potential role of flavonoids in the treatment of diabetes [24-26].

The phytochemical screening indicated that the stem bark of *Garcinia kola* contains the following constituents; flavonoid, steroids, fixed oils, carbohydrates, cardenolide, low tannins and high saponine, thereby contrasting the phytochemical analysis results by Kagbo and Ejebe [20] that used methanolic extract of the same plant part and Monago and Akhidue [27] who reported the occurrence of high tannins and devoid of saponins as well as on the phytochemistry of the seeds of G. kola respectively. This difference could be as a result of the dissimilarity in the extracting medium and the source of plant material. This argument is in agreement with Evans [19] who stated that various parts of a plant may grow well in different situations but fail to yield the same constituents, and Elujoba [28] who stated that there could be dissimilarity in the occurrence of bioactive compounds in different parts of the same plant or even in the same plant parts in plants found in different environments.

The phytochemical analysis shows promises of high anti-oxidant activity by the constituents. Tannins, saponins, kolavirons and steroids have all been isolated in different plant samples with antidiabetic and anticarcinogenic properties [29-33]. However, the bioactivity of drugs is based on its dosage. Kagbo and Ejebe [20] found the methanol extract of the stem bark of Garcinia kola to have an LD_{50} of 348mg/kg body weight. This places the plant extract at a high toxic range. This disagrees with the results in this study. The preliminary toxicity examination of this study did not record any animal death at this dose. Whereas samples utilized in the former research were gotten from Bori, in Ogoni land known for high environmental pollution, the samples used in this research were obtained from a sacred play ground in Egbeda community in Emohua. This seeks to validate the findings in both studies and conforms to the works by Elujoba [28] and Evans [19] and the works by Chakrabarti *et al*., [34] where they observed that same extracts from different locations may contain different active ingredients and may have varied degree of effects.

CONCLUSION

In conclusion, hydro-methanol extract of G. kola stem bark is an effective extract in the management of blood sugar level as experimental doses reduced the observed increase in the parameters on comparison with Control values. The effect of the extract is similar to that caused by treatment with daonil (glibenclamide) but does not show better outcomes in managing the seen toxicity. The ability of the extract of G. kola to induce significant reduction in the blood glucose level of alloxan induced diabetic rats as well as the repair or restoration of the histology of the pancreas indicates significant protective effects of this medicinal plant against multi-organ damages caused by Diabetes mellitus. It may therefore be appropriate to recommend that hydromethanol extract of G.kola stem bark could be effective in the prevention of multiorgan damages associated with Diabetes mellitus.

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