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Anti-diabetic effect of methanolic leaf extract of *Cissus cornifolia* on alloxan-induced hyperglycemic in wistar rats

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ABSTRACT

Cissus corniifolia commonly called splanch, has been allegedly used in the treatment of diabetes. The effect of methanolic leaf extract of this plant on glucose concentration and histopathology of the pancreas and liver were assessed. The preliminary phytochemical screening of methanolic extract of Cissus cornifolia revealed the presences of alkaloid, flavonoid, saponin, steroid, terpenoid and tannin. Also, the LD_{50} of the extract was found to be above 5000mg/kg orally. Rats were made diabetic by intraperitoneal injection of 150mg/kg body weight of Alloxan monohydride and divided into five (5) 1-5 with group 5 rats in each group. Groups 2-4 were treated with 50,100 and 200mg/kg body weight of the extract respectively while group 1 and 5 was served as negative and positive controls respectively. The fasting blood glucose levels were determined at intervals of 0, 1, 3, 5, 7, 9, 11 and 13 days. On day 0 there were no significant differences in the fasting blood glucose levels in all the groups when compared with the control. As regard to the dose of 100 mg/kg 7 days extract administration there was a significant decrease in the blood glucose levels when compared with control group. Glibenclamide as a positive control significantly reduce the fasting blood glucose level at 11 and 13 days respectively when compared to the negative control .The highest activity reside at the lowest dose of the extract administered 50mg/kg body weight. At the end of two weeks of the experiment, the animals were sacrificed and the pancreas and liver were section for histopathology. The histopathological studies of the pancreas of diabetic animals revealed the degeneration of pancreatic islet cells, but with the restoration after treatment with various doses of the plant extract. The liver shows no histopathological change. The implications of the result obtained in the present study provide the scientific rationale for the use of Cissus cornifolia as anti-diabetic agent in the management of diabetes.

Keywords: Blood glucose, hyperglycemia, *Cissus cornifolia*, diabetes mellitus

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INTRODUCTION

Diabetes mellitus is the chronic endocrine disorder characterized by a high blood glucose concentration caused by absolute or relative insulin deficiency, combined with insulin resistance [1] . Diabetes mellitus is a major cause of disability and hospitalization and it results in significant financial burden[2] . It has been projected by the year 2010 total number of people worldwide with diabetes mellitus will reach 239 million [3] and 324 million by 2025[4] .

Many traditional plant treatments for diabetes mellitus are used throughout the world [5]. Challenge within the medical system are management of diabetes without any side effect. This had led to an increasing demand for

natural products with antidiabeic activity and fever side effects [6] . The growing public interest and awareness of natural medicines have led to the pharmaceutical industry and academic researches to pay more attention to medicinal plants[7] .

Many herbs and plant products have been shown to have hypoglycemic action. *Cissus cornifolia* (bark) is a species of the genera *Cissus* that belongs to the family vitaceae. The plant is widely reputed in the African traditional medicine. It is locally called riigarbirri (rope of the monkey) in Hausa [8]..

Various morphological parts of this plant have been reported to be useful as effective remedies. African traditional medicine amongst which is being used by the Fulani of Northern Nigeria as a remedy for gonorrhea, while the leaf sap is used among the Tanganyika as a sedative in cases of menta lderangement, the roots-decotion is also used for malaria, septic tonsils and pharyngitis[8].

The present study was undertaken to evaluate the effects of methanolic extract of *Cissus cornifolia* on the blood glucose levels of alloxan-induced hyperglycemic wistar rats.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Cissus cornifolia* plants were collected from Kufena village, Zaria, Kaduna State, Nigeria in the month of November, 2010. It was identified and authenticated at the herbarium unit of Biological Sciences, Department, A.B.U. Zaria by Mallam A.U. Gallah. It was given a voucher specimen (No. 024) and deposited at the herbarium.

Animal

A total of 25 adult Wistar rats weighing (150-250g) bred in the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmaceutical Science, A.B.U. Zaria were used for the study. The animals were kept in well aerated laboratory cages in Human Physiology Department Animal House and were fed with grower and starter mash from Vital Feeds Company and water were provided during the stabilization period.

Plant preparation

The leaves were dried under the shade and ground into powder. The air-dried powdered plant (800g) material was extracted with 70% methanol and 30% aqueous using soxhlets apparatus; the solvent was removed in-vacuno and evaporated using rotatory evaporator to yield a residue of 150g of aqueous methanolic extract.

Phytochemical Screening of the Plant

Preliminary phytochemical screening of the crude extract of *Cissus cornifolia* was performed for the presence of its constituents using the following reagents and chemicals: Alkaloids – with Mayer's and Dragendorff's reagent[9,10] . Flavonoids with the use of mg and HCl [11].

Acute toxicity studies (LD_{50})

The median lethal doses (LD_{50}) of the plant extract was determined by method of [12] . using 12 rats. In the first phase, rats were divided into three (3) groups of 3 rats each and were treated with the extract at doses of 10, 100 and 1000mg/kg body weight orally and observed for 24 hours for sign of toxicity. In the second phase, three rats were divided into three groups of 1 rat each and were treated with the extract at doses of 1600, 2900 and 5000mg/kg body weight orally. The LD_{50} values were determined by calculating the geometric mean of the doses for which 0/3 and 0/1 were found.

Induction of experimental diabetes mellitus

The animals were fasted for 16-18 hours with free access to water prior to the induction of diabetes. Induction was carried out by single intraperitoneal injection of Alloxan monohydrate (Sigma St Lous, M.O., USA) dissolved in $0.9\%^{v/v}$ cold normal saline solution at a dose of 150mg/kg body weight[13] . Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution intraperitoneally after 6h.The rats were then kept for the next 24h on 5% glucose solution bottles in their cages to prevent hypoglycemia[14] . The diabetes was assessed in alloxan- induced rats by determining the blood glucose

concentration 72hours after injection of alloxan. The rats with blood glucose level above 200mg/dl were then selected for the study.

Experimental design for alloxan-induced hyperglycemic groups
Animal fasted overnight were randomly divided into 5 group of 5 rats (n = 5):
Group 1: As negative control and were treated with normal saline (0.2ml) orally.
Group 2: received 50mg/kg body weight of the *Cissus cornifolia* extract orally.
Group 3 : received 100mg/kg body weight of the *Cissus cornifolia* extract orally
Group 4: received 200mg/kg body weight of the *Cissus cornifolia* extract orally
Group 5: As a positive control group and were treated with glibenclamide 1mg/kg orally.

Determination of blood glucose level for normoglycemic group
The blood samples were collected by cutting the tail of the rats. Blood samples for blood glucose determination were collected from the tail at interval of 0, 1, 3, 5, 7, 9, 11 and 13 days respectively. Determination of the blood glucose level was divided by the glucose oxidase principle[15] . using the one touch glulometer strips and reported as mg/dl.

Statistical analysis
All the data were expressed as mean ± S.E.M. Statistical comparisons were performed by one way analysis of variance (ANOVA) using the Duncan's multiple range tests[16] . A value of $p<0.05$ was considered statistically significant. The data were analysed using SPSS vision 17.0.

RESULTS

Preliminary phytochemical screening
The results of the preliminary phytochemical analysis of the leaf extract of *Cissus cornifolia* revealed the presence of alkaloids, flavonoids, sapnonins, steroids and tannins.

Table 1: Preliminary phytochemicl constituents of *Cissus cornifolia*

Constituent/test	Inference
Alkaloids	+
Flavonoids	+
Saponins	+
Steroids	+
Tannins	+
Key:	*+ = present*

Acute toxicity studies
The LD_{50} of methanolic extract of *cissus cormfolia* in Wistar rats was found to be above 5000mg/kg body weight orally.

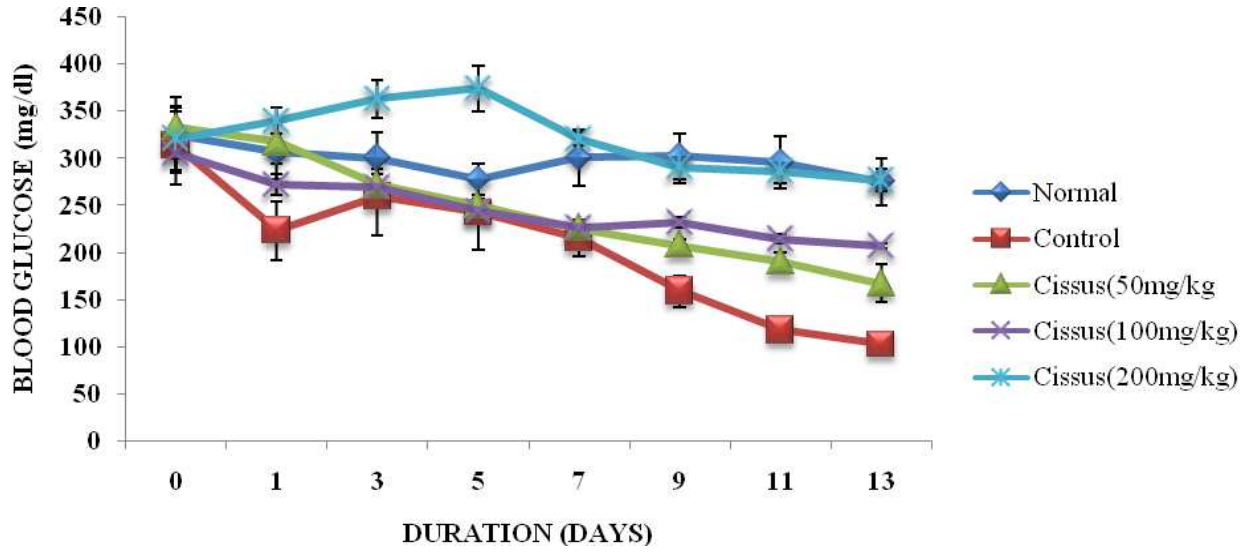

Figure 1: Effect of aqueous-methanolic leaf extract of *Cissus cornifolia* on blood glucose level of alloxan- induced wistar rats as compared with glibenclamide treated group and untreated control group Values are expressed as mean ± SEM; n = 5 Value considered statistically when compared with control group: a = p<0.05 significant and ns = not significant

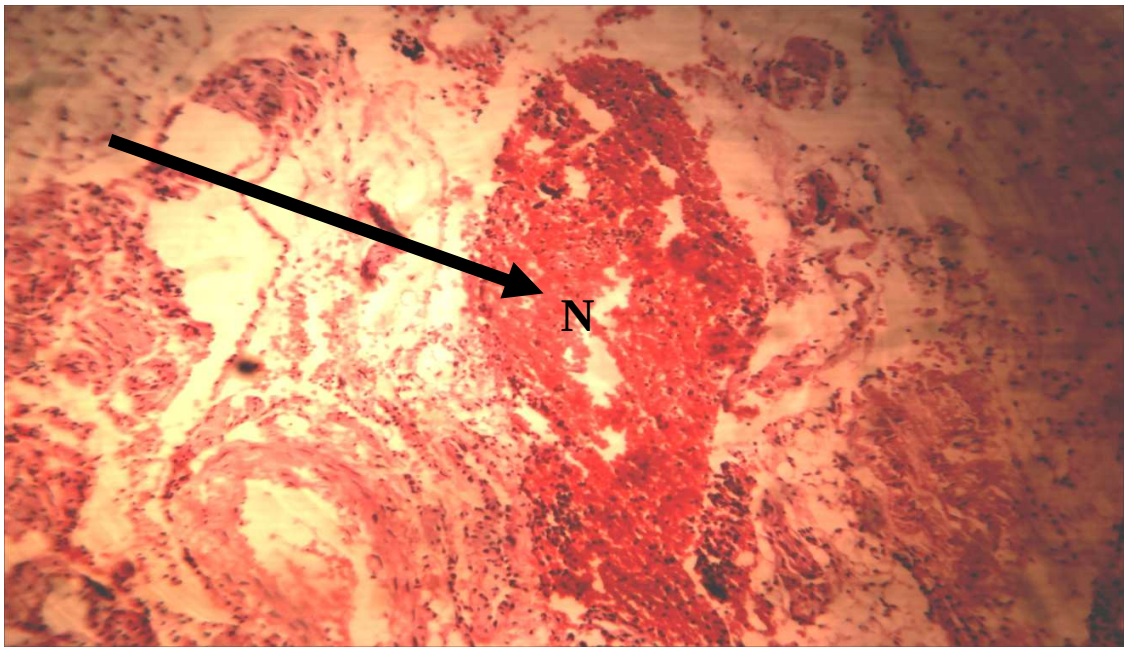

Plate 1a: Photomicrograph of a section of pancreas of Alloxan- induced diabetic Wistar rats administered with 0.2ml normal saline orally. Note: Areas of necrosis of pancreatic islet cells (N) H &E Stained X 250.

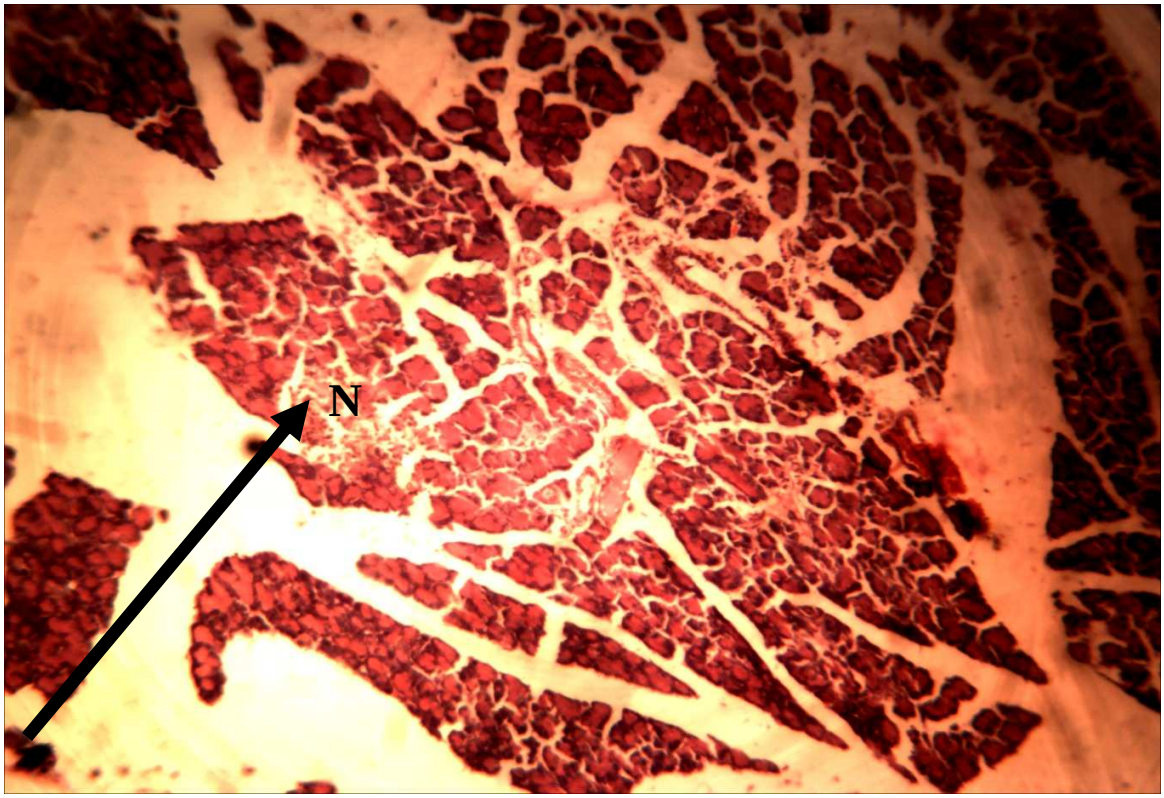

Plate 1b: Photomicrograph of a section of pancreas of Alloxan- induced diabetic Wistar rats administered with 50mg/kg bw methanolic leaf extract of *Cissus Cornifolia* orally. Note: Areas of partial restoration of pancreatic islet cells (N). H & E Stained X 250.

The effect of the different doses (50, 100 and 200mg/kg) of the extract of *Cissus cornifolia* and the control group (glibenclamide and normal saline treated groups) in alloxan-induced diabetes wistar rats as shown in (figure 1) above. The result shows a significant decrease ($p<0.05$) in the blood glucose level with 100mg/kg after 5days of the extract treatment when compared with the normal saline untreated group, while 50mg/kg shows reduction in blood glucose level but not statistically significant when compared to the normal saline group. 200mg/kg extract shows no significant decrease in blood glucose level when compared with the normal saline group.

The histopathological changes in the pancreas of diabetic rats indicates pancreatic damage as shown (in plate 1) above. This means that administration of alloxan cause partial destruction of the islets of Langerhan's. After administration of various doses of the extract after 14 days, there was partial restoration of the pancreatic islet cell. There was no histopathological changes in the liver of both treated and untreated diabetic rats.

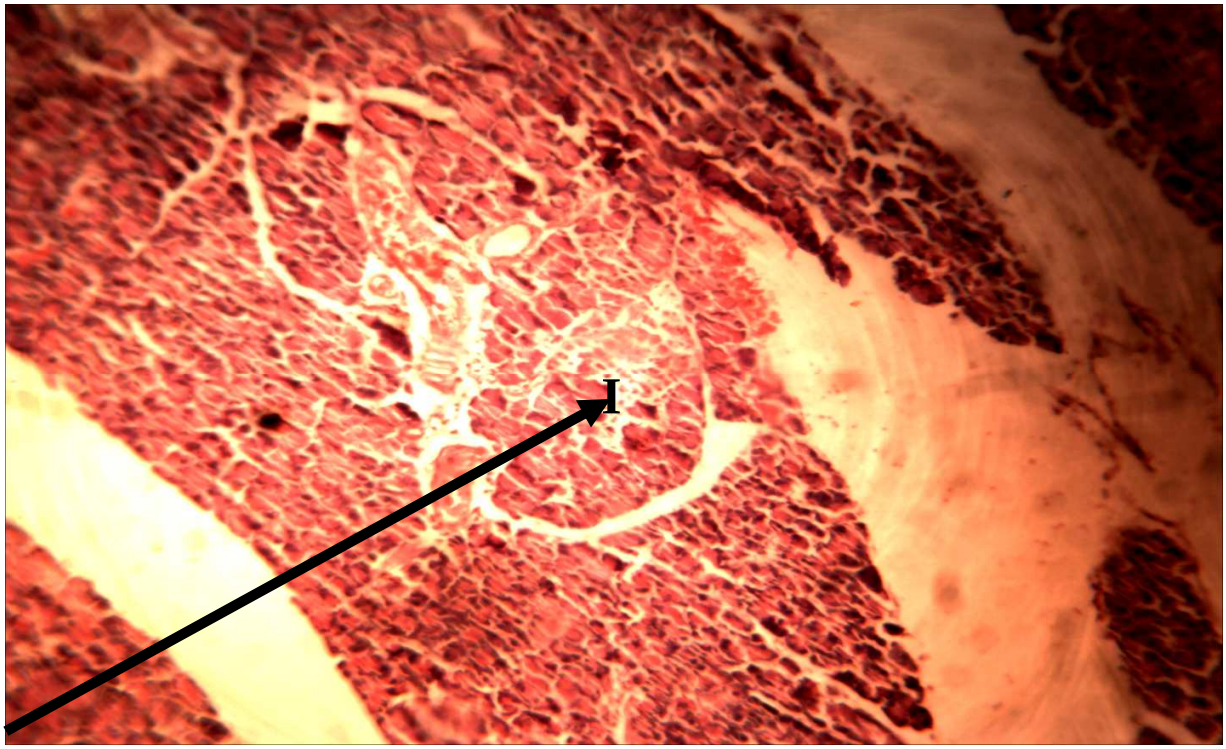

Plate 1c: Photomicrograph of a section of pancreas of Alloxan-induced diabetic Wistar rats administered with 1mg/kg body weight of glibenclamide orally. Note areas of restoration of pancreatic islet cells (I) H & E Stained X 250.

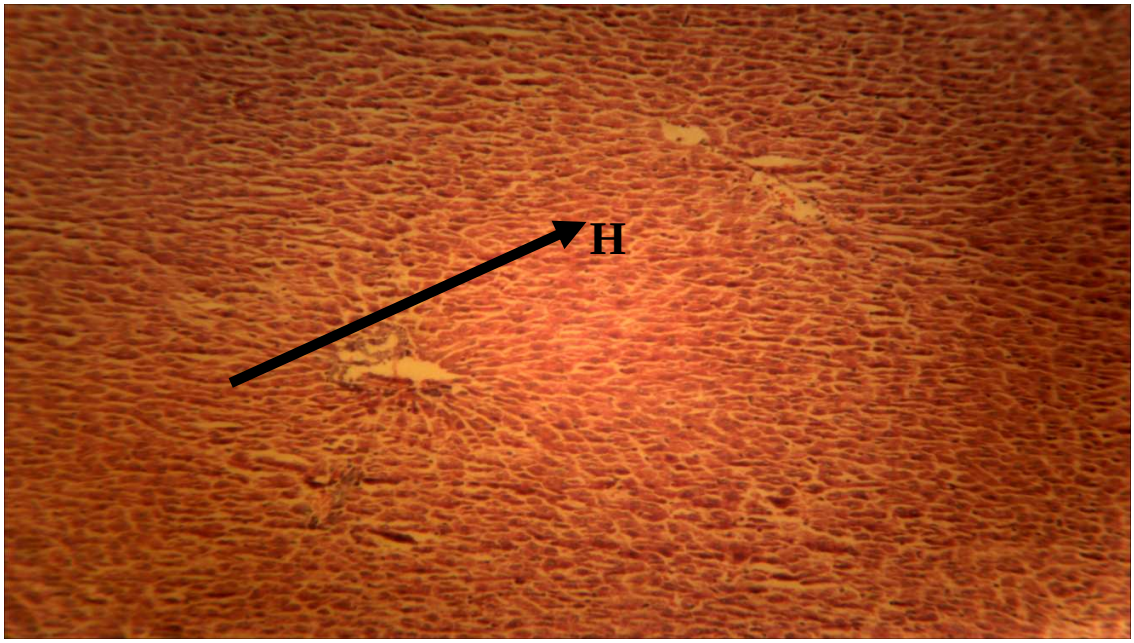

Plate 2a: Photomicrograph of a section of liver from Alloxan- induced diabetic Wistar rats administered with 0.2ml of normal saline orally. Note: There was no observable histopathology on the hepatocytes (H). H & E Stained X 250.

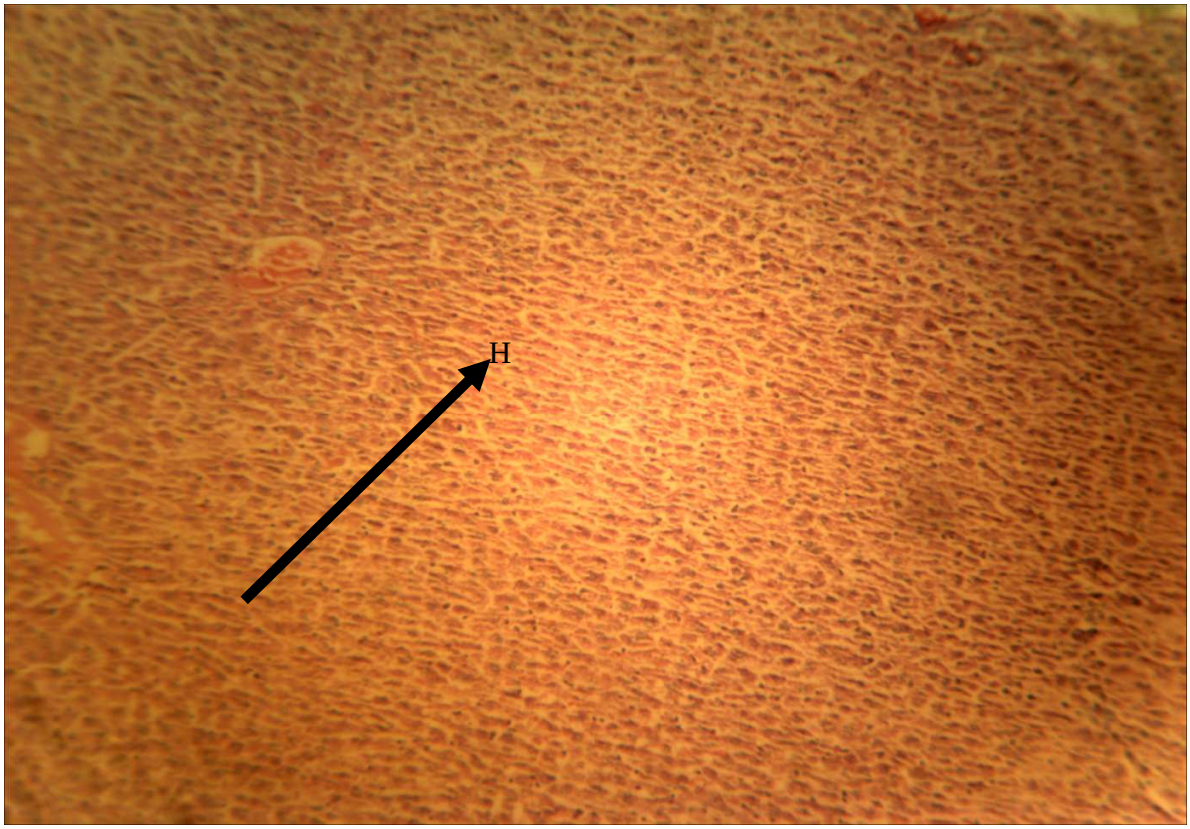

Plate 2b: Photomicrograph of a section of liver from Alloxan-induced diabetic Wistar rats administered with 50mg/kg bw of methanolic leaf extract of *Cissus Cornifolia* orally. Note: There was no observable histopathology on the hepatocytes (H). H & E Stained X 250.

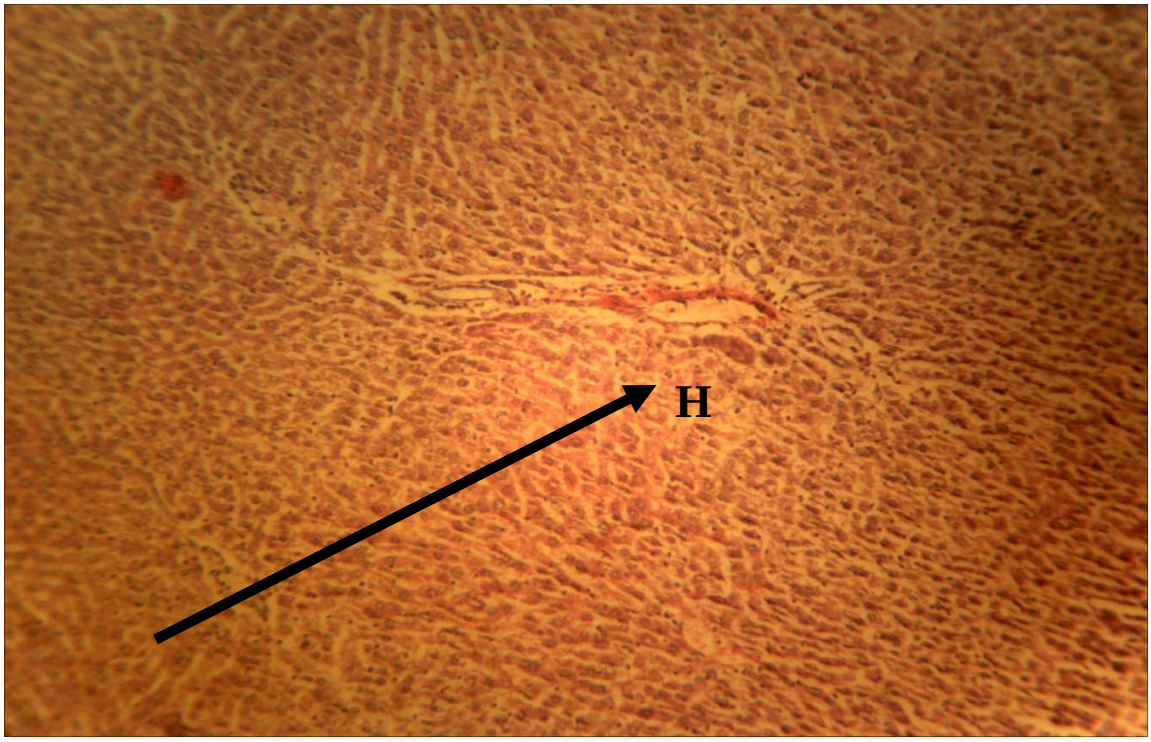

Plate 2c: Photomicrograph of a section of pancreas of Alloxan- induced diabetic Wistar rats administered with 1mg/kg body weight of glibenclamide orally. Note: There was no observable histopathology effect on the hepatocytes. H & E Stained X 250.

DISCUSSION AND CONCLUSION

Alloxan induces diabetes by destroying the beta-cells of the islets of langerhans in the pancreas leading to reduction in synthesis and release of insulin [17] . This model has been used to study the anti-diabetic effect of several plant products[18,19] .

The hypoglycemic properties of numerous medicinal plants have been studied and reported [20,21,2,22] . Oral administrations of methanolic extract of Cissus corniifolia cause a significant reduction in the blood glucose concentration of the diabetic rats. The effect of three doses (50mg/kg, 100mg/kg and 200mg/kg) of the extracts *Cissus cormifolia*, glibenclamide and normal saline group were evaluated. The dose of glibenclamide (1mg/kg) as a positive control showed a significant ($p<0.05$) decrease after 9 days of administration when compared to negative control (normal saline) group. However, after 1 day of treatment with extract, there was a reduction in blood glucose level in 50mg/kg and 100mg/kg when compared with negative control (normal saline). Also at 7, 11 and 13 days of extract administration, there was also a significant ($p<0.05$) decrease in (100mg/kg of extract) when compared with control.

200mg/kg of extract does not show a significant change in blood glucose level when compared to the negative control throughout the experimental period. The highest activity resides at the lowest dose of the extract administered. The effect of the extract was not dose-dependent. This could be due to antagonism. The extract contained many molecules, some of which could be antagonistic. At lower dose, the antagonistic molecules offered no hindrance to the hypoglyceamic causative substances due to decrease in concentration. Similar result was reported by [23] on the effect of bark extract of *pterocarpus santalinus* on blood glucose in streptozotocin-induced diabetic rats.

The partial restoration of the pancreatic islets cells after treatment with extract indicate that the possible mechanism by which the methanolic extracts of *Cissus cornifolia* reduced blood glucose concentration of the diabetic rats may be either by increasing the pancreatic secretion of insulin from the islets of langerhan's or its release from bound insulin. Similar result has been reported by [24].

The absence of histopathological changes in the liver indicate that chronic study needed to be carried out before any effect can be manifested and also it indicate that the extract of *Cissus cornifolia* does not possessed any toxic effect to the hepatolyte owing to the fact that the LD_{50} is above 5000mg/kg and thus, the extract is safe to use.

The LD_{50} of the extract was found to be 5000mg/kg body weight in rats. The preliminary phytochemical screening of the extract revealed the presence of alkaloids, steroids, tepensids, tannins, saponins and flavonsids. Generally, the biological effect of methanolic extracts of *Cissus cornifolia* are connected with their active principles including flavonoids, tannins and alkaloids which have been reported to have hypoglycaemic properties amongst others [25]. In conclusion, the results of the present study clearly indicates that the methanolic extract of *Cissus cornifolia* have glucose lowering effect on alloxan-induced diabetic rats. Further studies are in fact currently on the way to isolate the active principle and elucidate the exact mechanisms of action of *Cissus cornifolia*.

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