

Acalypha wilkesiana Exhibits Antihyperglycemic Potentials and Ameliorates Damages to Pancreas and Spleen of Diabetic Rat Model

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Abstract

Background: The present study investigated the antihyperglycemic and ameliorative effect of aqueous leaves extract of *Acalypha wilkesiana* on alloxan-induced diabetic male rats. The experimental rats were divided into six groups. Rats of the first group served as normal controls. Rats of the second group were negative control as they were induced with diabetes without treatment. The third group was treated with glibenclamide after induction of diabetes. The fourth, fifth and sixth groups were diabetic rats, treated with aqueous leaves extract of *Acalypha wilkesiana* at low dose (100mg/kg body weight), medium dose (200mg/kg body weight) and high dose (400mg/kg body weight) respectively. The blood glucose levels of the animals in each group were checked on day 5, 10 and 15 to examine the antihyperglycemic effect of the aqueous leaves extract of *Acalypha wilkesiana*. Also, on day 5, 10 and 15, the pancreas and spleen of experimental animals from each group were harvested for histological examination. **Result:** The result obtained showed that the graded doses of the extract significantly ($p < 0.05$) reduced the blood glucose levels of the diabetic animals in a dose-dependent manner. Also, the histological examination of the pancreas and spleen of the experimental animals treated with aqueous leaves extract of *Acalypha wilkesiana* showed normal pancreatic islet (PI) filled with islet cells (Granular appearance) and pancreatic acini (PA), while normal spleen with white pulp containing periarteriolar lymphatic sheath (PALS) and central arteriole (CA), splenic cord (SC) and sinusoids (SS) were seen in the same animals treated with the extract. The effect of the extract was similar in action to the standard drug glibenclamide. **Conclusion:** The aqueous leaves extract of *Acalypha wilkesiana* possesses antihyperglycemic potentials and ameliorates the pancreas and spleen of diabetic albino rats.

Keywords: *Acalypha wilkesiana*, Cooper leaf, Antihyperglycemia, Pancreas, Spleen Histology, Alloxan, Diabetes.

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INTRODUCTION

Diabetes prevalence is increasing worldwide; with 500 million expected by 2025 (King *et al.*, 1998; Mohan *et al.*, 2013). Diabetes mellitus is a metabolic disease with no known cure. High blood glucose levels are caused by impaired insulin secretion, insulin action in the body, or both. Insulin deficiency leads to hyperglycemia with impaired carbohydrate, fat, and protein metabolism (American Diabetes Association, 2009; Galicia-Garcia *et al.*, 2020). Chronic hyperglycemia in diabetes can lead to damage, dysfunction, and failure of various organs, including the eyes, kidneys, nerves, heart, and blood vessels (American Diabetes Association, 2009). World Health Organization (WHO) estimates that 180 million people worldwide have diabetes, and this number is expected

to double by 2030 (Kharroubi and Darwish, 2015; Wu *et al.*, 2014).

Diabetes and its complications remain a major health problem, despite the availability of anti-diabetic agents (Salehi *et al.*, 2019). Traditional diabetes medications have drawbacks, such as strict dose requirements, expensive costs, and unanticipated side effects (Nathan *et al.*, 2009; Anderson *et al.*, 2014). The effectiveness, low frequency of adverse effects, and low cost of natural plants used in traditional medicine make them appealing for screening for new anti-diabetic agents. This has sparked interest in the use of medicinal plants as complements to current therapies and as potential sources of hypoglycemic compounds in the treatment of diabetes.

Acylpha wilkesiana is a member of the Euphorbiaceae family native to the South Pacific Islands (Omage *et al.*, 2013, Akinde, 1986). It is known to contain steroid-like substances, alkaloids, phytates, anthraquinones, oxalates, saponins, tannins, and glycosides, making it a potential source of useful drugs (Omage *et al.*, 2013, Oladunmoye, 2006; Omage and Azeke, 2014). Tropical and subtropical nations cultivate it extensively.

Acylpha wilkesiana is a plant used therapeutically in traditional medicine, either alone or in combination with other herbs. It is utilized in traditional medicine to treat a wide range of illnesses (Egwaikhide and Gimba, 2007). It is used locally in Nigeria and other parts of West Africa to treat malaria, dermatological infections, and gastrointestinal infections, and has antibacterial and antifungal properties (Alade and Irobi, 1993; Akinde and Odeyemi, 1987). There is limited study on the effect of extract of *Acylpha wilkesiana* on the pancreas and spleen histology in diabetic rat models. Hence, this study investigated the anti-hyperglycemic potentials and the ameliorative effect of aqueous leaf extract of *Acylpha wilkesiana* on the pancreas and spleen histopathology.

METHODS

Experimental Animals

Animals used were two to three months old male Albino rats. Fifty-four (54) healthy male adult Albino rats with normal glucose level, weighing 180-200g were used in this experiment. All animals were acclimatized for two weeks before the commencement of the experiment. The animals were housed in a well-ventilated clean cages maintained under a 12-12hours light-dark cycle at a temperature of $23\pm 3^{\circ}\text{C}$ throughout the experimental period. Drinking water and food were provided ad libitum to the animals, but the food was withdrawn 2hours before and 2hours after administration of the drugs to rule out the effect of food on the absorption of the drugs. All animals received human care according to the criteria outlined in the guide for the care and use of laboratory animals prepared by the National Academy of Science and published by the National Institute of Health (1996). The experimental study was conducted between 9am and 5pm.

Chemicals and Plant Materials

Fresh leave of *Acalypha wilkesiana* were sourced from the University environment. The plant was identified and authenticated in the department of Plant Science and Biotechnology, Faculty of Science of the University. The drugs alloxan and glibenclamide were purchased from Gold Sparkle Pharmaco Nigeria Limited located at 5, Aggrey Road, Port Harcourt, Rivers State of Nigeria. Distilled water was used as a vehicle for the preparation of the alloxan for intraperitoneal administration.

Plant Extraction

Plant tissue homogenization method as described by Amita and Shalini (2014) was used in the extraction of the fresh plant part in distilled water. Wet, fresh plant parts were grinded in a blender to fine particles and a measured volume of distilled water was added to it and was shaken vigorously for 5 to 10 minutes after which the extract was filtered. The filtrate was then centrifuged for clarification of the extract (Das *et al.*, 2010). This was re-dissolved in the solvent to determine the concentration during administration process.

Experimental Design

Induction of Diabetes

In this study Albino rats were fasted overnight and were treated with 160mg/kg of alloxan by intraperitoneal injection to induce diabetes (Das *et al.*, 2012). After 96 hours of alloxan administration, animals with blood glucose values of 11.1mmol/L and above were considered diabetic.

Grouping of Experimental Animals

Animals in group 1 received feed and water *ad libitum* without alloxan and served as control; the alloxan-induced diabetic albino rats with blood glucose values of 11.1mmol/L and above were randomly divided into 5 groups of 9 animals (n=9) each. Those in group 2 were given no treatment following the induction and those in group 3 received 5mg/70kg or 0.07mg/kg of glibenclamide. Animals in groups 4, 5 and 6 received the aqueous leaf extract of *Acalypha wilkesiana* at doses of 100mg/kg, 200mg/kg and 400mg/kg following the induction.

Table 1: Experimental design for the antidiabetic screening

Group	Identification	Treatment
Group 1	Normal Control	Distilled Water
Group 2	Negative Control	Alloxan only
Group 3	Glibenclamide	Alloxan + Glibenclamide (5mg/70kg body weight)
Group 4	Low Dose of Extract	Alloxan + <i>Acalypha wilkesiana</i> Extract (100mg/kg body weight)
Group 5	Medium Dose of Extract	Alloxan + <i>Acalypha wilkesiana</i> Extract (200mg/kg body weight)
Group 6	High Dose of Extract	Alloxan + <i>Acalypha wilkesiana</i> Extract (400mg/kg body weight)

The plant extract was administered orally and the dosage of the extract was determined from preliminary studies in the laboratory. Three animals from each group were anaesthetized on day 5, 10 and 15 respectively with diethyl ether and pancreas and spleen samples were collected and transferred into 10% formalin solution for the histological studies.

Blood glucose determination

Blood glucose of the animals were monitored and documented in order to assess the effect of *Acalypha wilkesiana* leaf extract on alloxan-induced diabetic rats. The blood glucose level was monitored using a hand-held glucometer (Accu CHEK® Active GB) to determine the blood glucose level of the animals in each group from the tail vein using a tail clip. Blood glucose levels were checked on day 5, 10 and 15 before administering therapy.

Histopathology Examination

The animals were anaesthetized with diethyl ether, dissected aseptically to remove the spleen and pancreas which was then transferred into 10% chloroform and later trimmed to a size of 2mm to 4mm thickness, to allow the fixative to readily penetrate the tissue. The tissues were exposed to different stages of processing by standard methods as described by Baker (1945), including, fixation, dehydration, clearing, impregnation, embedding, sectioning and staining with hematoxylin and eosin (H&E) and finally mounting.

Ethics Approval

The study was carried out in adherence to ethical guidelines set by the National Institute of Health (NIH) for the ethical treatment of animals in research. The study was approved by the Research Ethics Committee of the University of Port Harcourt, Rivers State, Nigeria before

Statistical Analysis

The results are presented as Mean \pm Standard error of mean. Differences between means were assessed using Analysis of variance (ANOVA) using Dunnett method to assess any significant differences between the groups. Differences between groups at $p < 0.05$ were considered to be statistically significant (Mead and Curnow, 1982).

RESULTS

Effect of *Acalypha wilkesiana* on blood glucose level

Evaluation of the effect of *Acalypha wilkesiana* on blood glucose level of alloxan-induced albino rats as shown in Table 2 below revealed that there was a significant ($p < 0.05$) increase in blood glucose level in the untreated (negative control)

experimental animals (7.76 ± 0.11 mmol/L) when compared to the normal control animals (5.08 ± 0.29 mmol/L) on day 5. A progressive and significant ($p < 0.05$) increase was observed in this group on day 10 (7.79 ± 0.11 mmol/L) and day 15 (7.89 ± 0.07 mmol/L) when compared to the blood glucose levels of the normal control group animals on day 10 (5.08 ± 0.29 mmol/L) and day 15 (5.08 ± 0.29 mmol/L). Treatment with the standard drug (glibenclamide) significant ($p < 0.05$) caused a decrease in the blood glucose level on day 5 (5.01 ± 0.12 mmol/L) when compared to the negative control group (7.76 ± 0.11 mmol/L). A significant ($p < 0.05$) decrease in the blood glucose level was also observed on day 10 (5.01 ± 0.10 mmol/L) and day 15 (4.87 ± 0.07 mmol/L) when compared to negative control.

Treatment with graded doses of aqueous leaf extract of *Acalypha wilkesiana* showed that all doses (100mg/kg, 200mg/kg and 400mg/kg) of aqueous leaf extract of *Acalypha wilkesiana* caused no significant ($p < 0.05$) decrease in blood glucose levels in the experimental animals on day 5 when compared to the negative control but a significant ($p < 0.05$) increase in blood glucose level was observed in the animals treated with the graded doses of *Acalypha wilkesiana* extract on day 5 when compared to the normal control animals (See Figure 1 D1). A significant ($p < 0.05$) decrease in blood glucose level was observed in the animals treated with 200mg/kg aqueous leaf extract of *Acalypha wilkesiana* (6.34 ± 0.47 mmol/L) and animals treated with 400mg/kg aqueous leaf extract of *Acalypha wilkesiana* (5.58 ± 0.49 mmol/L) when compared to the negative control (7.79 ± 0.11 mmol/L) on day 10 (see Figure 1 D2). 100mg/kg of aqueous leaf extract of *Acalypha wilkesiana* did not cause any significant ($p < 0.05$) decrease in blood glucose level on day 10 when compared to negative control, but the decrease in blood glucose level was significant ($p < 0.05$) when compared to the normal control (5.08 ± 0.29 mmol/L) (see Figure 1 D2).

Similar decrease in the blood glucose level of animals treated with 200mg/kg and 400mg/kg aqueous leaf extract of *Acalypha wilkesiana* when compared to the negative control and decrease in blood glucose level with treatment with 100mg/kg of aqueous leaf extract of *Acalypha wilkesiana* when compared to the normal control on day 10 was also observed on day 15 (see Figure 1 D3). However, the effect of *Acalypha wilkesiana* leaf extract on blood glucose level of the experimental animals was dose dependent and the anti-glycemic effect of the extract is comparative to the standard drug (glibenclamide).

Table 2: Effect of *Acalypha wilkesiana* on blood glucose level of alloxan-induced albino rats

Group	Blood Glucose Level (mmol/L)		
	Day 5	Day 10	Day 15
Normal Control	5.08±0.29 ^b	5.08±0.29 ^b	5.08±0.29 ^b
Negative control	7.76±0.11 ^a	7.79±0.11 ^a	7.89±0.07 ^a
0.07mg/kg Glibenclamide	5.01±0.12 ^b	5.01±0.10 ^b	4.87±0.07 ^b
100mg/kg AWLE	7.59±0.31 ^a	7.39±0.37 ^a	6.92±0.18 ^a
200mg/kg AWLE	7.27±0.28 ^a	6.34±0.47 ^b	5.07±0.08 ^b
400mg/kg AWLE	7.21±0.27 ^a	5.58±0.49 ^b	5.30±0.50 ^b

Values are expressed as Mean ± Standard Error of Mean (SEM), n=5.

^aThe mean difference is significant at the p<0.05 when compare to Normal Control.

^bThe mean difference is significant at the p<0.05 when compare to Negative Control.

AWLE: *Acalypha wilkesiana* Leaf Extract

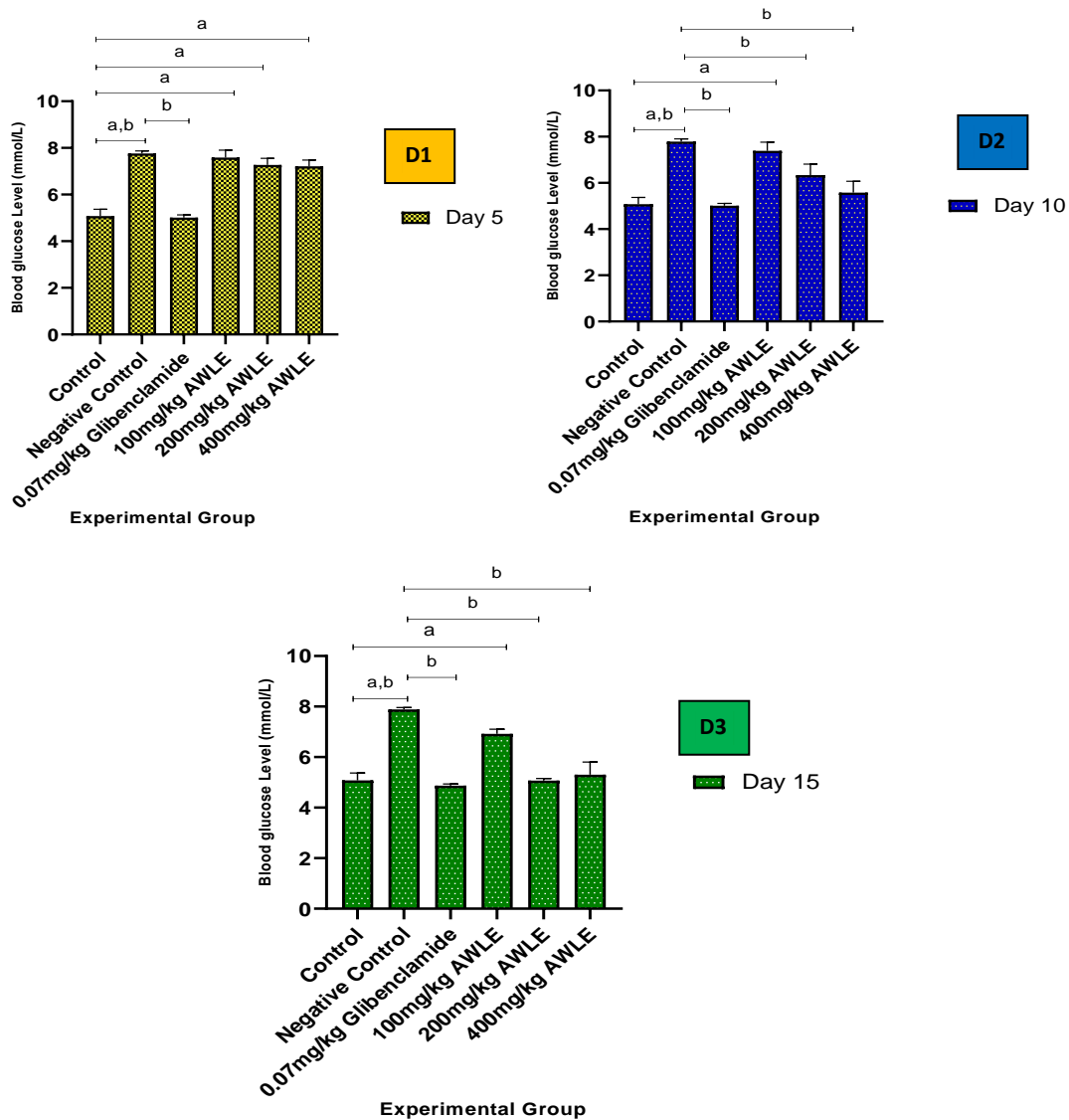


Figure 1: (D1-D3) Effect of *Acalypha wilkesiana* on blood glucose level of alloxan-induced diabetic albino rats on day 5, 10 and 15. Data are expressed as mean ± SEM. ^aThe mean difference is significant at the p<0.05 when compare to Normal Control. ^bThe mean difference is significant at the p<0.05 when compare to Negative Control. AWLE: *Acalypha wilkesiana* Leaf Extract

Effect of *Acalypha wilkesiana* leaf extract on the Pancreas Histopathology

Figure 2A1, 3B1 and 4C1 presents the pancreas histo-morphology from group one (normal

control group), 5, 10 and 15 days after the start of the experiment showing histologically normal pancreas with normal pancreatic islet (PI); large size, containing numerous pancreatic islet cells and pancreatic acini

(PA). Figure 2A2, 3B2 and 4C2 presents the histo-morphology of the pancreas from group two animals (exposed to alloxan without treatment), showing histologically distorted pancreas, with shrunken pancreatic islet (PI), having few islet cells pancreatic acini (PA). Figure 2A3, 3B3 and 4C3 present the histo-morphology of the pancreas from group two (animals exposed to alloxan and treated with 0.07mg/kg of glibenclamide for 5, 10 and 15 days) respectively, showing histologically normal pancreas, with normal pancreatic islet (PT) and pancreatic acini (PA). Figure 2A4 presents the histological cross section of the pancreas from group four (animals exposed to alloxan and treated with (100mg/kg) of *Acalypha wilkesiana* for five days), showing histologically distorted pancreas with pancreatic islet (PI), reduced concentration of islet cells and pancreatic acini (PA), while Figure 3B4 and 4C4 presents the pancreas histology from group four (animals exposed to alloxan and treated with 100mg/kg of *Acalypha wilkesiana* for 10 and 15 days), showing histologically distorted pancreas with shrunken pancreatic islets and pancreatic islets (PA) containing very few islet cells and pancreatic acini (PA).

Figure 2A5 presents the histo-morphology of pancreas from group five (animals exposed to alloxan and treated with 200mg/kg of *Acalypha wilkesiana* for five days), showing histologically distorted pancreas, with shrunken pancreatic islet (PI) with paucity of islet cells and pancreatic acini (PA), while Figure 3B5 and 4C5 present the histo-morphology of the pancreas from group five animals exposed to alloxan and treated with 200mg/kg of *Acalypha wilkesiana* for 10 and 15 days, showing a mildly distorted pancreas and shrunken pancreatic islet (PT) with almost no islet cells and a normal pancreas with normal pancreatic islet (PI) and pancreatic acini (PA) respectively. Figure 2A6, 3B6 and 4C6 present the pancreas histo-morphology from group five animals exposed to alloxan and treated with 400mg/kg of *Acalypha wilkesiana* for 5, 10 and 15 days, showing histologically distorted pancreas with shrunken pancreatic islet (PI) and pancreatic acini (PA); a mildly distorted pancreas with a slightly reduced size of pancreatic islet (PT) containing islet cells, and histologically normal pancreas with normal pancreatic islet (PI) filled with islet cells (granular appearance) and pancreatic acini (PA) respectively.

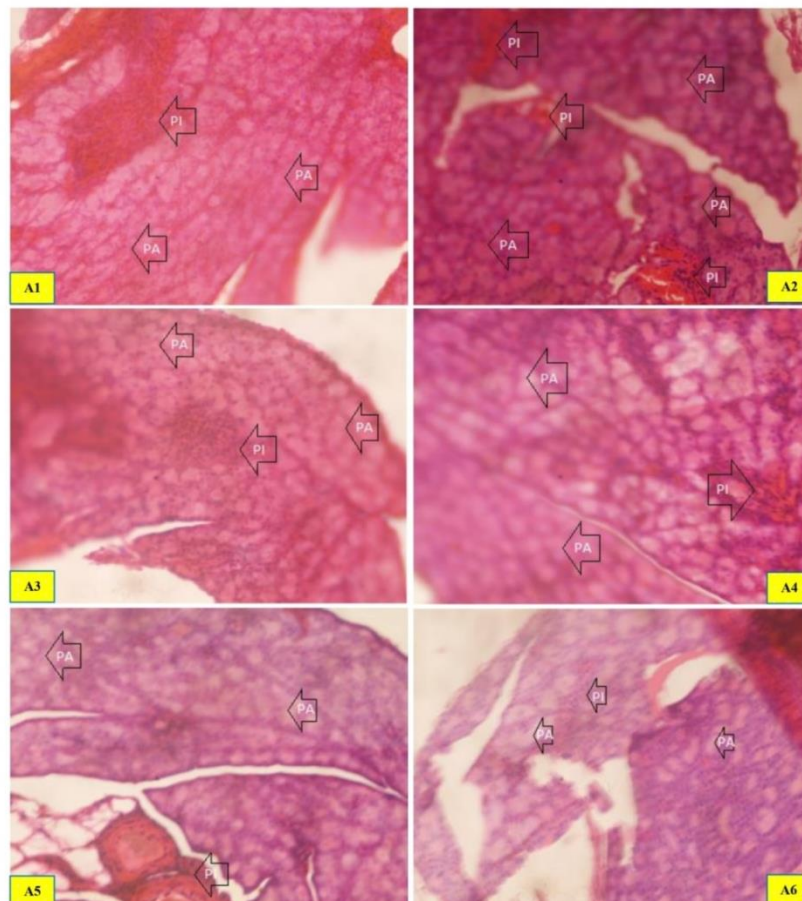


Figure 2: (A1–A6) Photomicrographs of pancreas sections in each group on day 5. (A1) Normal Control, (A2) Negative Control (Alloxan), (A3) 0.07mg/kg Glibenclamide, (A4) 100mg/kg *Acalypha wilkesiana* leaf extract, (A5) 200mg/kg *Acalypha wilkesiana* leaf extract, (A6) 400mg/kg *Acalypha wilkesiana* leaf extract. Magnification: X400

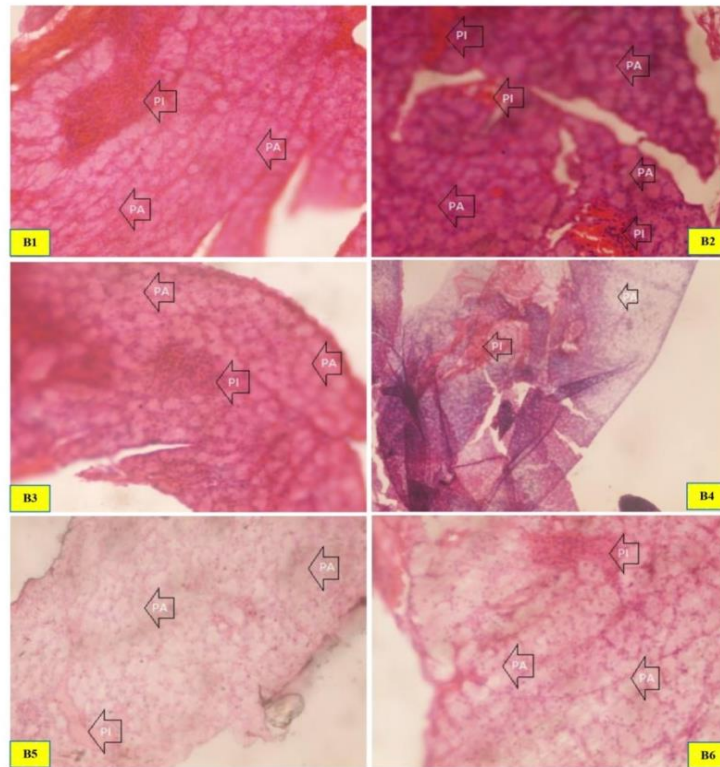


Figure 3: (B1–B6) Photomicrographs of pancreas sections in each group on day 10. (B1) Normal Control, (B2) Negative Control (Alloxan), (B3) 0.07mg/kg Glibenclamide, (B4) 100mg/kg *Acalypha wilkesiana* leaf extract, (B5) 200mg/kg *Acalypha wilkesiana* leaf extract, (B6) 400mg/kg *Acalypha wilkesiana* leaf extract. Magnification: X400

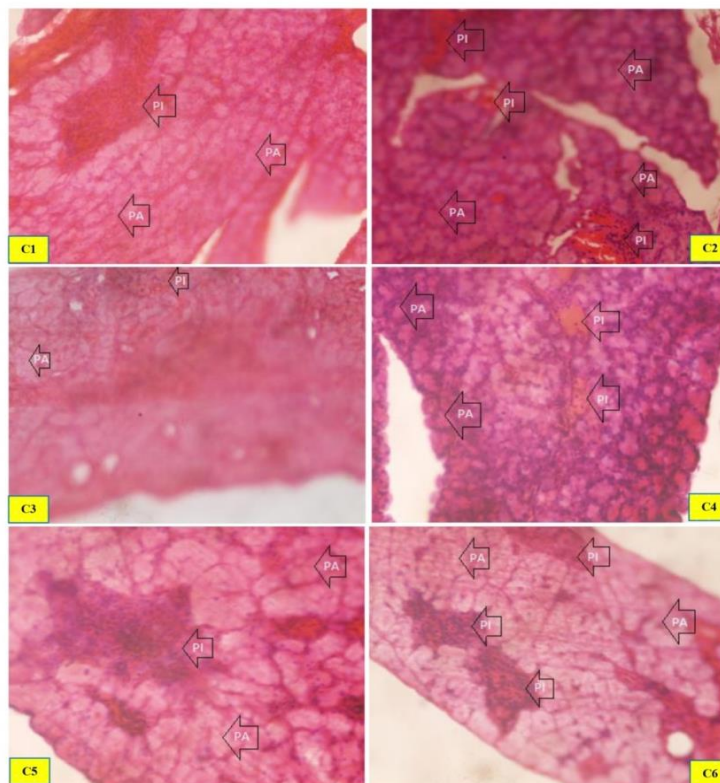


Figure 4: (C1–C6) Photomicrographs of pancreas sections in each group on day 15. (C1) Normal Control, (C2) Negative Control (Alloxan), (C3) 0.07mg/kg Glibenclamide, (C4) 100mg/kg *Acalypha wilkesiana* leaf extract, (C5) 200mg/kg *Acalypha wilkesiana* leaf extract, (C6) 400mg/kg *Acalypha wilkesiana* leaf extract. Magnification: X400

Effect of *Acalypha wilkesiana* leaf extract on the Spleen Histopathology

Figure 5X1, 6Y1 and 7Z1 presents the spleen histo-morphology from group one (normal control group), 5, 10 and 15 days after the start of the experiment showing histologically normal spleen histology with white pulp containing periarteriolar lymphatic sheath (PALS), splenic cord (SC) and sinusoids (SS). Figure 5X2, 6Y2 and 7Z2 presents the histo-morphology of the spleen from group two animals (exposed to alloxan without treatment), showing histologically distorted spleen indicated with white pulp with central arteriole (CA) and periarteriolar lymphatic sheaths (PALS), red pulp containing splenic cords (SC) and sinusoids (SS). Both PALS & SC were reduced in concentration. Figure 5X3, 6Y3 and 7Z3 present the histo-morphology of the spleen from group two (animals exposed to alloxan and treated with 0.07mg/kg of glibenclamide for day 5, 10 and 15) respectively, showing histologically normal spleen with white pulp containing periarteriolar lymphatic sheath (PALS), splenic cord (SC) and sinusoids (SS).

Figure 5X4 presents the histological cross section of the spleen from group four (animals exposed

to alloxan and treated with (100mg/kg) of *Acalypha wilkesiana* for five days), showing histologically normal spleen with white pulp containing periarteriolar lymphatic sheath (PALS), splenic cord (SS) and sinusoids (SS); while Figure 6Y4 and 7Z4 presents the spleen histology from group four (animals exposed to alloxan and treated with 100mg/kg of *Acalypha wilkesiana* for 10 and 15 days), showing histologically normal spleen with white pulp containing periarteriolar lymphatic sheath (PALS), splenic cord (SC) and sinusoids (SS). Figure 5X5, 6Y5 and 7Z5 presents the histo-morphology of spleen from group five (animals exposed to alloxan and treated with 200mg/kg of *Acalypha wilkesiana* for day 5, 10 and 15 respectively), showing histologically normal spleen with white pulp containing periarteriolar lymphatic sheath (PALS), splenic cord (SC) and sinusoids (SS). Figure 5X6, 6Y6 and 7Z6 present the spleen histo-morphology from group five animals exposed to alloxan and treated with 400mg/kg of *Acalypha wilkesiana* for 5, 10 and 15 days, showing histologically normal spleen with white pulp containing periarteriolar lymphatic sheath (PALS), central arteriole (CA), splenic cord (SC) and sinusoids (SS).

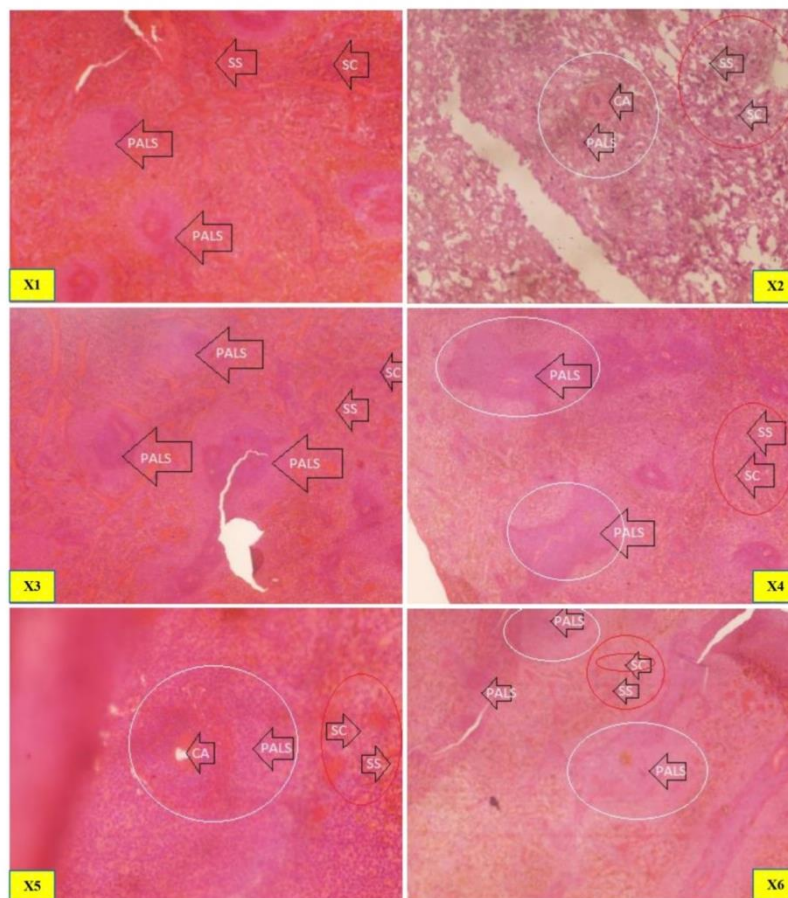


Figure 5: (X1–X6) Photomicrographs of spleen sections in each group on day 5. (X1) Normal Control, (X2) Negative Control (Alloxan), (X3) 0.07mg/kg Glibenclamide, (X4) 100mg/kg *Acalypha wilkesiana* leaf extract, (X5) 200mg/kg *Acalypha wilkesiana* leaf extract, (X6) 400mg/kg *Acalypha wilkesiana* leaf extract. Magnification: X200

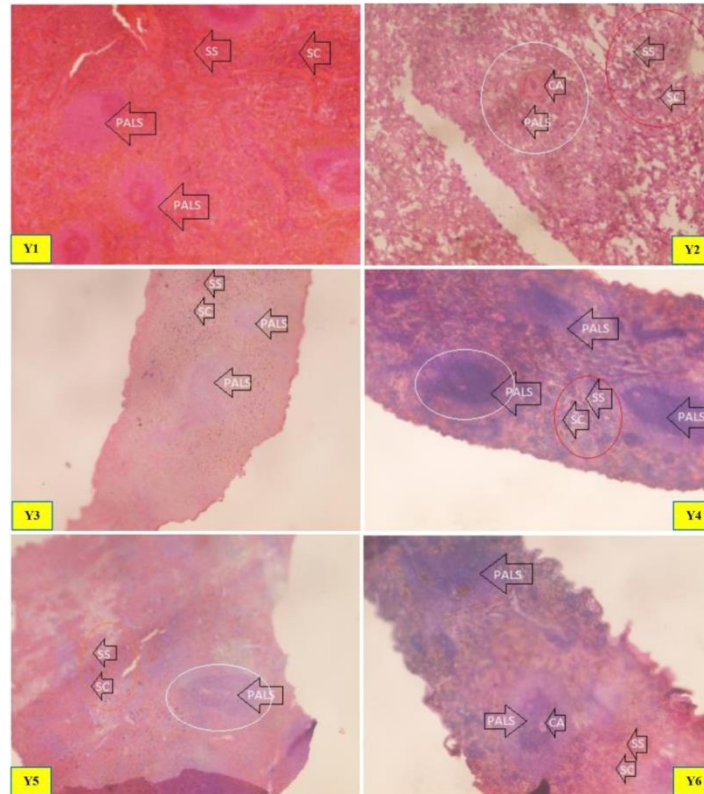


Figure 6: (Y1–Y6) Photomicrographs of spleen sections in each group on day 10. (Y1) Normal Control, (Y2) Negative Control (Alloxan), (Y3) 0.07mg/kg Glibenclamide, (Y4) 100mg/kg *Acalypha wilkesiana* leaf extract, (Y5) 200mg/kg *Acalypha wilkesiana* leaf extract, (Y6) 400mg/kg *Acalypha wilkesiana* leaf extract. Magnification: X200

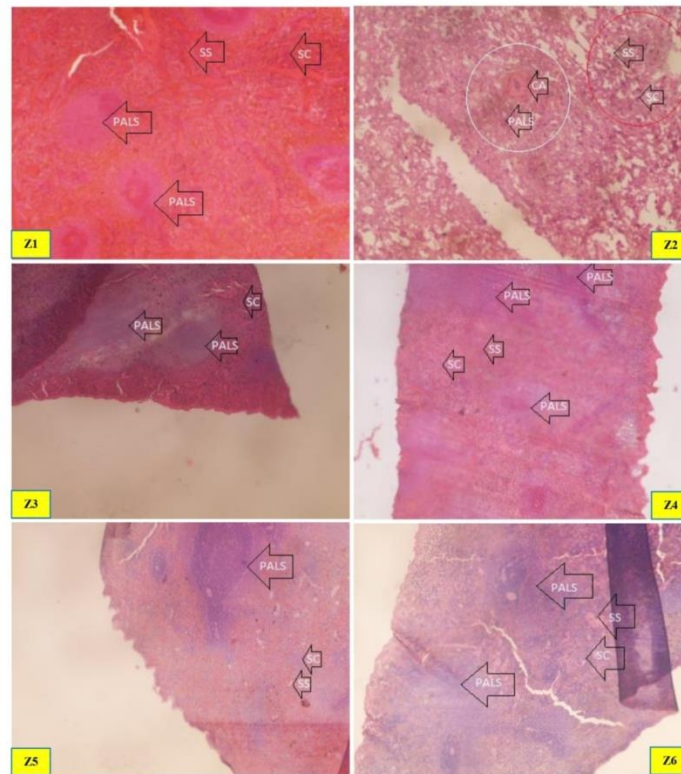


Figure 7: (Z1–Z6) Photomicrographs of spleen sections in each group on day 15. (Z1) Normal Control, (Z2) Negative Control (Alloxan), (Z3) 0.07mg/kg Glibenclamide, (Z4) 100mg/kg *Acalypha wilkesiana* leaf extract, (Z5) 200mg/kg *Acalypha wilkesiana* leaf extract, (Z6) 400mg/kg *Acalypha wilkesiana* leaf extract. Magnification: X200

DISCUSSION

The use of plant parts and extracts in herbal or traditional medicines is not an uncommon practice in Nigeria as well as in several countries, particularly Africa and Asia (Shapiro and Gong, 2002; Chacko, 2003). Considering the current prevalence and health risk of several diseases, particularly diabetes, there is no doubt that several medicinal plants have been tried and given some beneficial claims even if not yet scientifically proven. There is documentation of several drawbacks of currently available drug regimens for diabetes management and thus the need for safer and effective anti-diabetic drugs (Grover *et al.*, 2002; Rajagopal and Sasikala, 2008).

The present study investigated the anti-hyperglycemic and ameliorative effects of aqueous leaf extract of *Acalypha wilkesiana* on the pancreas and spleen histology of alloxan-induced diabetes in albino rats. The aqueous leaf extract of *Acalypha wilkesiana* significantly reduced the blood glucose level of alloxan induced hyperglycemia. The lowering effect of blood glucose level was observed to be in a dose-dependent manner. This finding suggests that the aqueous leaf extract of *Acalypha wilkesiana* exert this antihyperglycemic effect by insulin-like effect on peripheral tissues either by promoting glucose metabolism and uptake, stimulation of insulin secretion from pancreatic beta (β) cells and insulin like activity, or by conversion of pro-insulin to insulin, or alternatively, by inhibition of hepatic gluconeogenesis (Al-Attar, 2010; Ikewuchi *et al.*, 2011; Odoh *et al.*, 2014). This also supports the use of the plant in the management of diabetes (Omaga and Azeke, 2014; Seebaluck *et al.*, 2015).

In support of the antihyperglycemic effect of aqueous leaf extract of *Acalypha wilkesiana* from this study, Iyamu *et al.* (2021) reported similar finding with ethanolic leaf extract of *Acalypha wilkesiana*; Odoh *et al.* (2014) also reported a hypoglycemic effect of methanolic root extract of *Acalypha wilkesiana*, while Fonkoua *et al.* (2017) reported similar findings with hydro-ethanolic extract of *Acalypha wilkesiana* but reported no effect with aqueous extract. Ikewuchi *et al.* (2011) study reported a similar antihyperglycemic activity of the leaves extract of *Acalypha wilkesiana* but opined that the presence of phytochemicals such as tannins and flavonoids present in the leaf of the plant is attributed to the antihyperglycemic activity of the plant extract. In the phytochemical and elemental analysis by Madziga *et al.* (2010), they reported *Acalypha wilkesiana* to contain high levels of tannins and flavonoid.

Treatment with the standard drug, glibenclamide, significantly caused a reduction in the blood glucose level of the experimental animals. This is similar to reports by other researchers who used other sulfonylureas such as glimepiride, glipizide and

glibenclamide and reported a glucose lowering effect of these drugs in a diabetic model (Iyamu *et al.*, 2020; Iyamu *et al.*, 2021; Oboh *et al.*, 2014). The glucose lowering effect of glibenclamide is by binding to pancreatic β -cell receptors and stimulates insulin secretion (Malek and Davis, 2016). Thus, the observed suppression of glucose level after glucose loading and reduction in blood glucose level by the aqueous leaves extract of *Acalypha wilkesiana* in diabetic rats is an indication that the extract possesses glucose reducing properties and as such anti-diabetic properties. Hence, the plant may have similar mechanism of action to the insulin secretagogue, glibenclamide with respect to blood glucose lowering effect.

Previous studies have only focused more on the antihyperglycemic effect of *Acalypha wilkesiana* and also its protective effect on the liver, kidney and other biochemical markers. No study has reported the effect of *Acalypha wilkesiana* on the histology of the pancreas and spleen of diabetic rat model. Hence, this study is novel in bridging this research gap. The aqueous leaves extract of *Acalypha wilkesiana* reversed the damage to the pancreas and spleen of alloxan-induced damage to the pancreas and spleen. This was evident by a normal pancreatic islet (PI) filled with islet cells (granular appearance) and pancreatic acini (PA) in the pancreas and a normal spleen with white pulp containing periarteriolar lymphatic sheath (PALS), splenic cord (SC) and sinusoids (SS). The extract ameliorative effect of the pancreas and spleen of alloxan-induced experimental rats was progressive from day 5 to day 15 of the experiment to restore histologically distorted pancreas, with shrunken pancreatic islet (PI), having few islet cells pancreatic acini (PA) and the histologically distorted spleen indicated with white pulp with central arteriole (CA) and periarteriolar lymphatic sheaths (PALS), red pulp containing splenic cords (SC) and sinusoids (SS).

Alloxan is a diabetogenic chemical and a cytotoxic glucose analog used in diabetes research. It has two distinct pathological effects; first, by specifically inhibiting glucokinase, the β -cells glucose sensor and inhibits glucose-induced insulin secretion and secondly, by causing the formation of reactive oxygen species (ROS), which causes the selective necrosis of β -cell, leading to insulin dependence in diabetic condition. Both of these effects can be attributed to the particular chemical properties of alloxan, with the selective uptake and accumulation of alloxan by β -cells serving as a common denominator (Akoko *et al.*, 2022). This leads to pancreatic dysfunction and destruction of most islets of Langerhans cells. Experimental animals with untreated diabetes generally lost weight and their blood glucose levels increased due to the pancreatic dysfunction caused by the induced diabetes. Alloxan action results in death by necrosis of β -cells (Szkudelski, 2001; Akoko *et al.*, 2022).

The mechanism of alloxan diabetes has been the subject of many investigations and it is now generally accepted that free radicals are selectively involved in the initiation of the damage that ultimately leads to β -cells damage and eventual death (Minami *et al.*, 1999; Vanco *et al.*, 2004). Therefore, the pancreas is especially susceptible to the action of alloxan-induced free-radical damage. Many medicinal plants have been reported to ameliorate the diabetogenicity of alloxan in diabetic rat models which acts by reacting with free radicals formed from alloxan during its interaction with the β -cells, or prevent free radical formation (Jorns *et al.*, 1999; Akoko *et al.*, 2022; Omeodu *et al.*, 2022). Recently, it was reported that *Acalypha wilkesiana* exhibits significant radical scavenging activity (Anokwuru *et al.*, 2011; Omotayo *et al.*, 2017) and thus antioxidant activity (Wardah *et al.*, 2013; Oladimeji *et al.*, 2016; Anokwuru *et al.*, 2015). The present study indicates that the administration of *Acalypha wilkesiana* confirms the possibility that the major function of the extract is on the protection of vital tissues including the pancreas and spleen, thereby reducing the causation of diabetes in experimental animals. Therefore, the regenerative properties of *Acalypha wilkesiana* extract on the pancreas and spleen of alloxan-induced diabetic rats could be attributed to free radical scavenging activity and antioxidants present in the plant extract.

CONCLUSION

Overall, the present study showed that aqueous leaves extract of *Acalypha wilkesiana* possesses antidiabetic potentials in a dose-dependent manner. Its antidiabetic activity is comparative to that of glibenclamide and hence acts through similar mechanism to cause hypoglycemia in alloxan-induced diabetic albino rats. Also, the aqueous leaves extract of *Acalypha wilkesiana* regenerated alloxan-induced damage to the pancreas and spleen of albino rats. This is an indication that the aqueous leaves extract of *Acalypha wilkesiana* possesses protective and ameliorative potentials against pancreas and spleen damage. This supports its traditional use in the management of diabetes mellitus.

Availability of data and materials

All data generated or analyzed during this study are included in the manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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Authors' contributions

JCI conceived, designed, analyzed the data; POU performed the experiments and drafted the

manuscript; JCI critically revised the manuscript. Both authors read and approved the final manuscript.

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