



## Antidiabetic and Pancreatoprotective Effects of Ethanolic Leaf Extract of *Cnidoscopus Aconitifolius* in Alloxan-Induced Diabetic Female Wistar Rats

<sup>1</sup>Okoroigbo Franklin Chiedozie, <sup>2</sup>Ohanme Eugene Ohams, <sup>3</sup>Anyichie Nonye Eucharía, <sup>4</sup>Lotanna Somtoo Akudu, <sup>5</sup>Ezinwanne Emmanuel Enyinnaya, <sup>6</sup>Akunna Ujunwa Lynda, <sup>7</sup>Malize Chinwe Grace, <sup>8</sup>Akwuba Chinenye Francisca and <sup>9</sup>N. Nwakelu Benjamin

<sup>1</sup>Department of Anatomy, Faculty of Basic Medical Sciences, Rhema University, Aba, Abia State Nigeria

<sup>2,9</sup>Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Medicine, Alex Ekwueme Federal Ndufu-Alike Ikwo, Ebonyi State Nigeria

<sup>3</sup>Department Of Community Health, Anambra State College Of Health Technology Obosi Anambra State

<sup>4,8</sup>Department of Anatomy, Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University Uli Campus, Anambra State

<sup>5</sup>College of Community Health, University on the Niger Teaching Hospital, Iyi-enu Ogidi, Anambra State

<sup>6</sup>Department Of Community Health, Lasbery College of Health Technology, Ogidi Anambra State

<sup>7</sup>Anambra State College of Health Technology Obosi, Anambra State Nigeria

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#### Key Words

*Cnidoscopus aconitifolius*, antidiabetic activity, pancreatoprotection, alloxan-induced diabetes, wistar rats, flavonoids

#### Corresponding Author

Ohanme Eugene Ohams,  
Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Medicine, Alex Ekwueme Federal Ndufu-Alike Ikwo, Ebonyi State Nigeria

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#### ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to insulin deficiency or resistance. The increasing global prevalence of diabetes, particularly in developing nations, underscores the need for affordable, plant-based therapies. *Cnidoscopus aconitifolius* (Chaya), a nutrient-rich leafy plant, has been traditionally used in diabetes management. This study evaluated the antidiabetic and pancreatoprotective effects of the ethanolic leaf extract of *C. aconitifolius* in alloxan-induced diabetic female Wistar rats. Forty-one female Wistar rats (150–250 g) were divided into four groups: normal control, diabetic control, 100 mg/kg extract-treated, and 500 mg/kg extract-treated. Diabetes was induced using alloxan monohydrate (150 mg/kg, i.p.). The ethanolic extract of *C. aconitifolius* was administered orally for 14 days. Blood glucose levels were measured every two days. Phytochemical screening and acute toxicity (LD50) tests were performed using standard protocols. Histopathological examination of pancreatic tissues was carried out after treatment. Phytochemical analysis revealed the presence of flavonoids, tannins, saponins, alkaloids, steroids, and cardiac glycosides. The extract showed no acute toxicity up to 9000 mg/kg. A significant dose-dependent reduction ( $p < 0.001$ ) in blood glucose levels was observed in treated groups compared to diabetic controls. Rats receiving 500 mg/kg extract showed the highest hypoglycemic effect. Histological studies indicated marked regeneration of pancreatic  $\beta$ -cells and restoration of acinar structures in extract-treated rats, confirming the extract's antioxidative and tissue-protective effects. The ethanolic leaf extract of *Cnidoscopus aconitifolius* demonstrated significant antidiabetic and pancreatoprotective activities in alloxan-induced diabetic rats. These effects are likely mediated by its flavonoid and saponin content, which enhance insulin secretion and protect  $\beta$ -cells from oxidative injury. The findings support the therapeutic potential of *C. aconitifolius* as a natural remedy for diabetes mellitus. Further studies are recommended to isolate active compounds and establish clinical efficacy and safety.

## INTRODUCTION

Physiologically, glucose is the principal substrate used for synthesizing energy (ATP) needed to fuel metabolic activities. Proper glucose homeostasis is essential because organs like the brain and testes depend heavily on constant glucose supply. Any imbalance can result in metabolic derangements that either raise or lower blood glucose to disease levels. A persistent fasting glucose above 6.9 mmol/L is termed hyperglycaemia, medically known as diabetes mellitus. Diabetes is a significant global health problem<sup>[1]</sup>. Unfortunately, the incidence of diabetes is rising beyond earlier forecasts<sup>[2]</sup>. Data suggests that new cases are increasingly originating from Asia and Africa, mostly due to lifestyle transitions<sup>[3]</sup>.

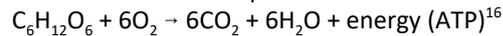
Diabetes mellitus (DM) develops due to a combination of genetic predisposition and environmental influences. It is marked by chronic hyperglycemia caused by inadequate insulin release, abnormal insulin function or a combination of both. Current global reports show that diabetes has reached 366 million cases-exceeding earlier WHO projections-with over 4 million recorded deaths attributed to the disease<sup>[4]</sup>. In 2010 alone, global diabetes care cost approximately \$320 billion<sup>5</sup>. The sharpest increase is seen among ages 40-59 in low- and middle-income populations. There is therefore an urgent need for preventive and therapeutic strategies<sup>[6]</sup>. Treatment must focus on more than blood glucose regulation<sup>[7]</sup>. Although several herbal plants demonstrate antihyperglycaemic potential, there is limited documentation on *Cnidioscolus aconitifolius*<sup>[8]</sup>.

**Conceptual Framework:** Environmental factors have major influence on body regulatory balance. Disruption of control mechanisms may trigger metabolic illnesses including hyperglycemia. Diabetes rates continue to climb in developing nations<sup>[9]</sup>. WHO estimates that about 437 million individuals currently live with diabetes, and mortality is projected to double between 2005 and 2030. Type 1 DM arises from absolute lack of insulin; Type 2 DM (which accounts for 90% of cases) is linked to insulin resistance<sup>[10]</sup>. Physical inactivity and obesity are major risk contributors<sup>[11]</sup>. The IFIH1 gene has been implicated in Type 1 DM<sup>[12]</sup>. In Type 2 DM,  $\beta$ -cell impairment, exaggerated hepatic glucose output, reduced insulin response, altered intestinal insulin function, and increased renal glucose reabsorption are key mechanisms<sup>[13]</sup>. Due to cost constraints and inadequate access to treatment, complementary therapy becomes necessary. WHO therefore supports scientific investigation of antidiabetic herbs<sup>[14]</sup>. Evaluation of *C. aconitifolius* phytochemicals is therefore justified. Investigating alloxan-induced

hyperglycemia also helps illustrate mechanisms of pathological glucose rise<sup>[15]</sup>.

### Empirical Review:

**Carbohydrate Metabolism:** Glucose is the most essential carbohydrate. Carbohydrates are divided into simple and complex groups. They serve as quick energy sources, support insulin regulation, and can be stored as glycogen or converted to fat. The general composition is  $C_nH_{2n}O_n$ ; glucose is  $C_6H_{12}O_6$ . The aerobic breakdown equation is:



**Metabolic Processes:** Core metabolic pathways include photosynthesis, glycolysis, the Krebs cycle, pentose phosphate pathway, glycogenesis, glycogenolysis and gluconeogenesis<sup>[17]</sup>.

**Regulation of Blood Glucose:** Insulin supports glucose transport, glycogen formation, and metabolic control. Glucagon increases glycogen breakdown. Several other hormones also regulate the process<sup>[18]</sup>. Dietary carbohydrates are converted to glucose in the liver. Glucose crosses cell membranes via facilitated diffusion; insulin increases cellular uptake<sup>[18]</sup>.

**Phosphorylation and Fate of Glucose:** Glucose is phosphorylated to glucose phosphate by either glucokinase or hexokinase. Glycogen is mostly stored in liver and muscle tissues, while surplus glucose is converted into fat<sup>[19]</sup>.

**Blood Glucose Concentration:** Normal blood glucose falls between 4-6 mmol/L. Regulation depends on both anabolic and catabolic hormones. Plasma glucose is calculated as whole blood glucose  $\times 1.15$ <sup>[20]</sup>.

**Glycaemic Index:** The glycemic index (GI) assesses how carbohydrate sources influence glucose levels. Low-GI foods assist blood glucose control<sup>[20]</sup>.

**Monitoring of Blood Glucose:** Common assessments include FBS, 2-hour post-prandial, RBS, OGTT, IVGTT, and HbA1c<sup>21</sup>. Low glucose levels cause confusion and coma; long-standing high glucose results in chronic complications<sup>[21]</sup>.

### Unit Conversion:

$\text{mg/dL} \div 18 = \text{mmol/L}$ ;  $\text{mmol/L} \times 18 = \text{mg/dL}$ <sup>[22]</sup>.

**Blood and Haematologic Parameters:** Full blood count (FBC) evaluates blood constituents. Haematologic markers give insight into health status<sup>[22]</sup>.

**Embryology of Blood and Haemopoiesis:** Blood initially develops from yolk-sac mesoderm and subsequently shifts to bone marrow for haemopoiesis<sup>[23]</sup>.

**Management of Diabetes:** Diabetes has no absolute cure; management consists of diet modification, exercise, and insulin/oral antidiabetics. Lifestyle changes remain vital. Metformin continues to be the drug of first choice. Aspirin is not routinely indicated in people with uncomplicated DM. Telemedicine is useful in diabetes management<sup>[24]</sup>.

**Oxidative Stress and Antioxidants:** Reactive oxygen species (ROS) induce injury through lipid peroxidation and DNA damage. Oxidative stress develops when ROS overwhelm antioxidant defenses. Antioxidants include flavonoids, phenolics, etc<sup>[25]</sup>.

**Medicinal Plants and Antidiabetic Potential:** Bioactive plant compounds possess antidiabetic activities. Saponins and flavonoids may stimulate insulin secretion. Diabetes numbers are increasing. Plants rich in antioxidants protect against ROS. Multiple plant preparations show hypoglycemic activity. The limited African-based data restricts wider application<sup>[26]</sup>.

**Phytomedicine for Diabetes:** Numerous herbs demonstrate glucose-lowering effects in both human and animal studies; they contain alkaloids, terpenoids, glycosides, flavonoids and saponins. WHO promotes herbal research. Flavonoids possess strong antioxidant properties.

**Cnidoscolus Aconitifolius (Chaya):** This plant has traditional antidiabetic usage. Boiling for at least 20 minutes eliminates cyanogenic glycosides. It is locally known as efo iyana ipaja/oguru obala/ Hospital- too-far<sup>[27,28]</sup>.

**Health Importance of Cnidoscolus aconitifolius (CA):** *Cnidoscolus aconitifolius* (Chaya) is a highly nutrient-dense leafy vegetable. It contains abundant protein, vitamins, iron, calcium and antioxidants. Research shows its nutrient content may be two to three times higher than many conventional leafy vegetables. It also contains high fibre and shows strong antibacterial activity<sup>[29]</sup>.

Qin *et al.* (2022) recorded marked antidiabetic activity in *C. aconitifolius* leaf extract using type 2 diabetic mice<sup>[30]</sup>. Usende *et al.* (2018) also noted improvement of anemia and reduced osmotic fragility resulting from protein-energy malnutrition. Altogether, available evidence supports the nutritional and pharmacological relevance of Chaya as both food and medicinal plant<sup>[31]</sup>.

Scientific Classification of Cnidoscolus aconitifolius

Taxonomic Rank	Classification
Kingdom	Plantae
Division	Magnoliophyta

Class	Magnoliopsida
Order	Euphorbiales
Family	Euphorbiaceae
Genus	Cnidoscolus
Species	Cnidoscolus aconitifolius (Mill.) I.M. Johnst.

Nutritional Composition of *Cnidoscolus aconitifolius*



Diagram Showing leaves of *Cnidoscolus aconitifolius*

These values align with earlier literature [59], indicating that Chaya leaves possess superior nutrient density compared to other conventional leafy vegetables such as spinach (6.4%), amaranth (11.3%), Chinese cabbage (7.0%), and lettuce (5.4%)<sup>[32]</sup>.

Chaya leaves contain abundant essential minerals relevant to human physiology. Potassium plays a vital role in blood pressure control and stroke prevention, calcium is central to bone mineralisation, while iron is required for red blood cell formation. Foods with substantial vitamin C content (including Chaya) also improve the absorption of non-haem iron, further enhancing its micronutrient potential<sup>[33]</sup>.

**Alloxan:** Alloxan (2,4,5,6-pyrimidinetrone) is a pyrimidine derivative that forms alloxan hydrate in aqueous solutions. It was originally isolated in 1818 by Brugnatelli, and Wöhler and Liebig later named it in 1838 from the combination of “allantoin” and “oxalic acid<sup>[34]</sup>.”

**Biological Actions of Alloxan:** Alloxan and streptozotocin are cytotoxic glucose mimics that preferentially destroy pancreatic  $\beta$ -cells, resulting in insulin-dependent diabetes in laboratory animals. This pathogenesis closely resembles Type 1 diabetes in humans. In the presence of cellular thiols, alloxan undergoes redox cycling with dialuric acid and generates reactive oxygen species (ROS). These ROS initiate the  $\beta$ -cell necrotic effect<sup>[35]</sup>.

**Beta Cell Toxicity:** Alloxan-induced diabetes occurs because alloxan is taken up by GLUT2 transporters due to its glucose-like structure, leading to irreversible oxidative destruction of  $\beta$ -cells and subsequent hyperglycaemia in experimental models<sup>[36]</sup>.

Given the current rise in metabolic diseases as a result of poor preventive habits, nutritional transition from indigenous diets to western fast foods, socioeconomic constraints, and adverse reactions associated with many synthetic drugs, there is a growing incidence of organ dysfunction and chronic illnesses<sup>[36]</sup>. Because phytomedicinal agents have shown protective and antihyperglycaemic effects across multiple studies, there is an increasing interest in their utilisation as complementary treatment options. Many plant-derived remedies possess antioxidant components, are less toxic, are cheaper and easier to access, and will therefore continue to gain attention as alternative therapeutic agents<sup>[37]</sup>.

### MATERIALS AND METHODS

The following materials were used: beakers, measuring cylinders, dissecting set, anaesthetics (chloroform), wooden cages with wire gauze, weighing balance, glucometers, glucose test strips, surgical gloves, syringes, normal saline, distilled water, EDTA sample bottles, electric grinder, Soxhlet extractor (Model No. 3587, Austria), rotary evaporator (Gallenkamp, UK), alloxan and ethanolic leaf extract of *Cnidioscolus aconitifolius*.

**Source and Identification of Plant Material:** Fresh leaves of *Cnidioscolus aconitifolius* were collected from Owerri and Isiala Mbano, Imo State, Nigeria. Authentication and botanical identification were performed by Mr. Finian Iroka, Department of Botany, Nnamdi Azikiwe University, Awka, with herbarium voucher number NAUH-321c.

**Preparation and Storage of Leaf Extract:** Extraction was carried out using the method of Okigbo *et al.* (2005). The fresh leaves were air-dried and milled into powder. Ethanol extraction was performed using a Soxhlet extractor (Model No. 3587, Austria). The extract was filtered using Whatman No. 1 paper, and the filtrate was evaporated under reduced pressure at 45°C using a Gallenkamp rotary evaporator (UK). The concentrated extract was then kept refrigerated at 4°C until required.

**Ethical Approval:** Ethical clearance was secured from the University Ethics Committee, Faculty of Basic Medical Sciences, Abia State University, Uturu, Nigeria.

**Source and Maintenance of Experimental Animals:** Healthy adult female albino Wistar rats were purchased from Amaka Farm, Uturu, and housed in the Animal House Unit of the Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State.

The rats were allowed 2 weeks acclimatization under standard conditions, fed with vital livestock grower mash (Grand Cereals Ltd., Jos) and had access to water *ad libitum*. Bedding (sawdust) was replaced every 3 days. Experimental rats weighed 150-250 g.

**Experimental Design:** Forty-one Wistar rats were used and randomly grouped after acclimatization:

Group	Description
Group 1 (Positive Control)	Non-diabetic; received distilled water only
Group 2 (Negative Control)	Alloxan-induced but untreated
Group 3 (Treatment A)	Alloxan-induced + 100 mg/kg <i>C. aconitifolius</i> ethanolic extract
Group 4 (Treatment B)	Alloxan-induced + 500 mg/kg <i>C. aconitifolius</i> ethanolic extract

Blood glucose was monitored every two days for 14 days using a glucometer prior to feeding.

**Feeding and Husbandry:** All rats received vital grower mash with free access to water. Feeding tools were cleaned daily and the environment was kept well ventilated at room temperature.

**Phytochemical Screening:** Phytochemical analysis was carried out at Springboard Research Laboratory, Awka, using the ethanol (1:4 v/v) extract of *C. aconitifolius* leaves. Standard techniques of Harborne (1973) and Pearson (1974) were used to detect tannins, saponins, alkaloids, steroids, cardiac glycosides and flavonoids.

**Acute Toxicity Study (LD<sub>50</sub>):** Acute toxicity of the ethanolic extract was evaluated in the Department of Human Anatomy, College of Health Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli, following Lorke's method (1983).

**Phase I:** Nine rats were divided into 3 groups (3 per group): 50 mg/kg, 500 mg/kg, 5000 mg/kg. Animals were observed for 24 hours for toxic signs and mortality.

**Phase II:** Four rats were used (one per dose): 6000, 7000, 8000 and 9000 mg/kg. Observation continued for 24 hours to determine toxicity and death if any.

### RESULTS AND DISCUSSIONS

The present study investigated the antidiabetic and pancreatoprotective effects of the ethanolic leaf

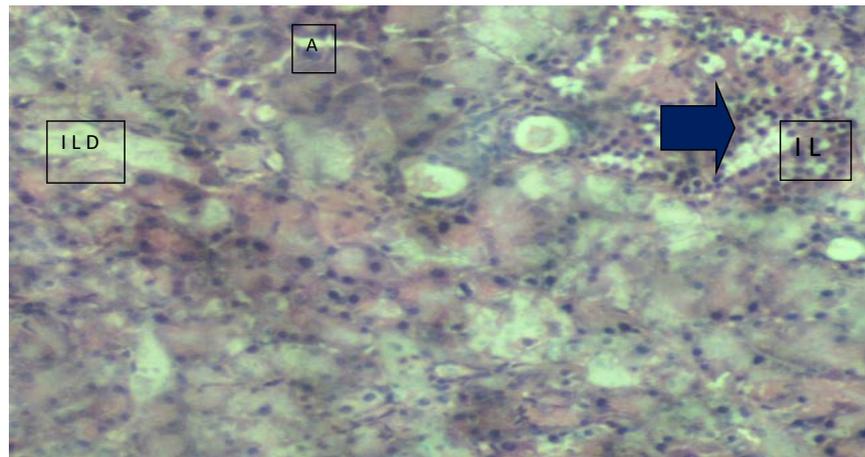


Fig. 1: Photomicrograph of the pancreas on the positive (non diabetics group) an h and e stained tissue section showing the normal structures of acini (a), interlobular duct (ild) and islets of langerhans (il).x400

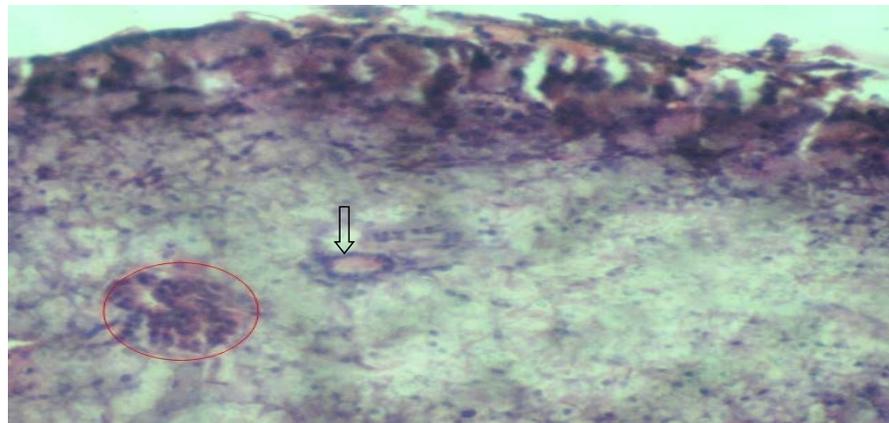


Fig. 2: Photomicrograph of the pancreas on the negative (control diabetics group): an h and e stained tissue section showing a focal inflammation (circle), beta cells necrosis (arrow). x400

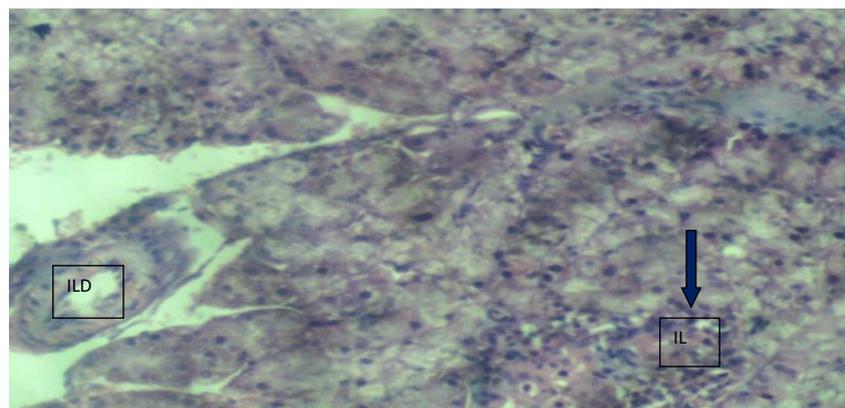


Fig. 3: Photomicrograph of the pancreas treated with 100mg/kg of ca an h and e stained tissue section showing a relatively mild regeneration of structures of acini (a), interlobular duct (ild) and islets of langerhans (il).x400

extract of *Cnidocolus aconitifolius* (CA) in alloxan-induced diabetic female Wistar rats. The results demonstrated that the plant possesses significant hypoglycaemic, hepatoprotective, nephroprotective, antihyperlipidaemic, and mild reproductive hormonal effects, largely attributable to its rich phytochemical constituents.

**Phytochemical Constituents and Their Implications:** Phytochemical analysis revealed the presence of tannins, saponins, alkaloids, flavonoids, steroids, and cardiac glycosides in the ethanolic extract of *Cnidocolus aconitifolius*. The high concentration of tannins (8.8%) and alkaloids (6.2%) suggests strong medicinal potential. These results align with the

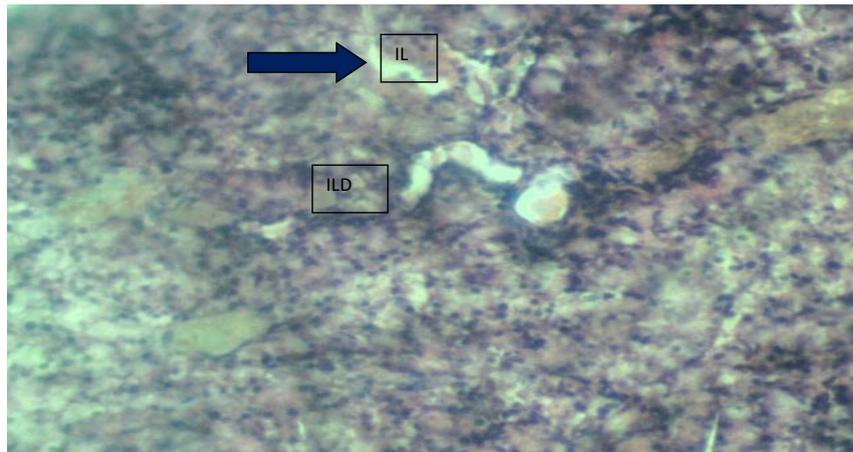


Fig. 4: Photomicrograph of the pancreas treated with 500mg/kg of ca: an h&e stained tissue section showing a relatively restorations of cellular structures of pancreas; acini (a), interlobular duct (ild) and islets of langerhans (il), few fat cell and few necrosis of a normal beta cell.x400 corpus albicans (ca) preserved. x400

Table 1: Nutritional Profile of *Cnidoscolus aconitifolius*

S/N	Nutrient Component	Percentage (%)
1	Water	85.3
2	Protein	5.7
3	Fat	0.4
4	Crude Fibre	1.9
5	Calcium	1.99
6	Phosphorus	0.39
7	Potassium	2.17
8	Iron	0.11
9	Vitamin C	1.65
10	Carotenoids	0.085

Table 2: Showing the hypoglycaemic effect of levels of Glucose from Day 0 to Day 12 on various groups of female abino wistar rats

DAYS	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12
	MEAN+SEM						
Positive Control	92.2+0.60	90.40+0.92	87.25+0.60	94.50+0.58	92.28+0.45	90.45+0.75	89.52+0.05
Negative Control	297.25+0.25	275.45+0.52	283.45+0.25	321.42+0.62	301.30+0.58	301.25+0.06	304.62+0.58
100mg/kg Group	298.46+1.95	287.25+0.92	287.40+0.82	272.45+0.72	220.24+0.54	304.48+0.05	189.45+0.09
500mg/kg Group	292.05+0.91	284.48+0.85	272.45+0.35	271.58+0.62	192.45+0.65	201.52+0.08	162.48+0.51
f- value	2285.212	2974.718	3916.378	2982.518	3462.416	2485.313	1256.524
p- value	0.000	0.000	0.000	0.000	0.000	0.000	0.000

findings of Fagbohun *et al.* (2012) and Faley *et al.* (2012), who also reported similar constituents, though differing slightly from Ndhlala *et al.* (2013) who found flavonoids absent in some extracts<sup>[38-40]</sup>.

The differences in reported phytochemical profiles may be due to variations in extraction solvents and environmental conditions of the plant source. The detected compounds are known to possess biological activities such as antioxidant, anti-inflammatory, antimicrobial, and antihypertensive properties. For instance, alkaloids have been associated with analgesic and antistress effects, while flavonoids are recognized for their potent antioxidant and antidiabetic actions through free radical scavenging and pancreatic  $\beta$ -cell protection<sup>[41]</sup>. The presence of saponins also implies the extract's potential in reducing serum cholesterol and improving glucose metabolism, supporting its traditional use in the management of diabetes and hypertension.

**Histological Effects:** istopathological observations revealed extensive cellular damage in the pancreas, of untreated diabetic rats, likely due to oxidative stress generated by alloxan. However, treatment with *Cnidoscolus aconitifolius* extract showed a dose-dependent restoration of tissue architecture, with reduced necrosis and improved cellular integrity, especially in groups administered 500 mg/kg.

This regenerative effect may be attributed to the antioxidative properties of flavonoids and tannins, which scavenge free radicals and inhibit lipid peroxidation<sup>[42]</sup>. These findings suggest that the extract protects against oxidative tissue injury and enhances the recovery of pancreatic  $\beta$ -cells, supporting its pancreatoprotective role.

**Hypoglycaemic Effects:** A significant dose-dependent reduction in blood glucose levels was observed in diabetic rats treated with CA extract. The

hypoglycaemic activity was more pronounced at 500 mg/kg and became statistically significant by the 14th day of treatment. This effect is consistent with previous reports by Mohammed *et al.* (2009), which linked the antidiabetic properties of *Cnidoscolus aconitifolius* to the presence of flavonoids and saponins<sup>[43]</sup>.

Flavonoids, such as quercetin, have been reported to stimulate insulin secretion, enhance glucose uptake, and regenerate pancreatic islet cells. The findings of this study therefore confirm that *C. aconitifolius* may act via insulinogenic and antioxidant mechanisms to regulate glucose homeostasis, offering therapeutic potential for diabetes mellitus<sup>[44]</sup>.

## CONCLUSION

The present study has shown that the ethanolic leaf extract of *Cnidoscolus aconitifolius* exhibits significant antidiabetic and pancreatoprotective activities in alloxan-induced diabetic female Wistar rats. Administration of the extract resulted in a marked, dose-dependent decrease in blood glucose levels, improvement in lipid profile, and restoration of hepatic and renal function biomarkers. Histological analyses revealed notable protection and regeneration of pancreatic  $\beta$ -cells, particularly at higher doses of the extract. These effects are attributed to the synergistic actions of the bioactive phytochemical constituents such as flavonoids, saponins, tannins, alkaloids, steroids, and cardiac glycosides, which collectively contribute to antioxidant defense, enhancement of insulin secretion, and protection against oxidative tissue damage. Overall, the findings affirm that *Cnidoscolus aconitifolius* ethanolic leaf extract holds substantial promise as a natural therapeutic agent for the management of diabetes mellitus and its associated complications. Nevertheless, its safety profile and mechanism of action warrant further detailed investigation.

## Recommendations:

- **Phytochemical Isolation and Characterization:** Future studies should focus on isolating and identifying the specific bioactive compounds responsible for the antidiabetic and pancreatoprotective effects observed.
- **Toxicological and Safety Evaluation:** Comprehensive acute and chronic toxicity studies are recommended to establish safe dosage ranges and rule out potential adverse effects, particularly on reproductive hormones and other organs.
- **Mechanistic Studies:** Molecular and biochemical investigations should be conducted to elucidate the exact mechanisms through which *C. aconitifolius* modulates glucose metabolism and promotes  $\beta$ -cell regeneration.

- **Clinical Trials:** Human clinical studies are necessary to confirm the efficacy, bioavailability, and safety of *C. aconitifolius* extract in diabetic patients.
- **Standardization of Extract Preparation:** It is important to standardize extraction methods to ensure reproducibility and potency of the plant extract in pharmacological formulations.
- **Public Health and Nutritional Integration:** Given its nutritional and medicinal potential, *Cnidoscolus aconitifolius* could be promoted as part of dietary interventions or nutraceutical formulations for diabetes prevention and management, particularly in low-resource settings.

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**Competing Interests:** The authors declare that they do not have any conflicts of interest.

**Ethics Approval:** Ethical clearance for this study was obtained from the Research and Ethics Committee of Abia State University Uturu, Abia State Nigeria

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## REFERENCES

1. B. Giri, Dey .S, Das .T, Sarkar .M, Banerjee J, Dash .S.K. Chronic hyperglycemia mediated physiological alteration and metabolic distortion leads to organ dysfunction, infection, cancer progression and other pathophysiological consequences: An update on glucose toxicity. *Biomedicine and Pharmacotherapy* 2018, 107:306-328.
2. T. Wu, Ding .L, Andoh .V, Zhang .J, Chen .L. The Mechanism of Hyperglycemia-Induced Renal Cell Injury in Diabetic Nephropathy Disease: An Update. *Life* 2023, 13:539.
3. A.M. Pisoschi, Pop .A, Iordache .F, Stanca .L, Predoi .G, Serban .A.I. Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status. *European Journal of Medicinal Chemistry* 2021, 209:112891.
4. C. Ghosh, Kundu .T, Pathak .T, Saini .S, Das .N, Saini .S, *et al.* Indian lychee honey ameliorates hepatic glucose uptake by regulating the ChREBP/Glut4 axis under insulin-resistant conditions. *Food Funct* 2025, 16:2031-2056.
5. Burden of 375 diseases and injuries, risk-attributable burden of 88 risk factors, and healthy life expectancy in 204 countries and

- territories, including 660 subnational locations, 1990-2023: a systematic analysis for the Global Burden of Disease Study 2023. *Lancet* 2025, 406:1873-1922.
6. Global age-sex-specific all-cause mortality and life expectancy estimates for 204 countries and territories and 660 subnational locations, 1950-2023: a demographic analysis for the Global Burden of Disease Study 2023. *Lancet* 2025, 406:1731-1810.
  7. M.R. Rickels, Ballou .C.M, Foster .N.C, Alejandro .R, Baidal .D.A, Bellin .M.D, *et al.* Islet Transplantation Versus Standard of Care for Type 1 Diabetes Complicated by Severe Hypoglycemia From the Collaborative Islet Transplant Registry and the T1D Exchange Registry. *Diabetes Care* 2025, 48:737-744.
  8. C.J. Hanick, Peterson .C.M, Davis .B.C, Sabaté .J, Kelly .J.H, Jr. A whole-food, plant-based intensive lifestyle intervention improves glycaemic control and reduces medications in individuals with type 2 diabetes: a randomised controlled trial. *Diabetologia* 2025, 68:308-319.
  9. H. Xu, Jia .Y, Sun .Z, Su .J, Liu .Q.S, Zhou .Q, *et al.* Environmental pollution, a hidden culprit for health issues. *Eco-Environment and Health* 2022, 1:31-45.
  10. P. Chandrasekaran, Weiskirchen .R. The Role of Obesity in Type 2 Diabetes Mellitus-An Overview. *Int J Mol Sci* 2024, 25.
  11. J. Mallaiyah, Leon .R, Jr., Williams .O, Allegrante .J.P. Cardiovascular Disease and Stroke-Focused Competency Assessment Tools for Community Health Workers in the United States: A Scoping Review. *Health Promot Pract* 2023;24:1183-1195.
  12. T. Yang, Liu .Y, Li .L, Zheng .Y, Wang .Y, Su .J, *et al.* Correlation between the triglyceride-to-high-density lipoprotein cholesterol ratio and other unconventional lipid parameters with the risk of prediabetes and Type 2 diabetes in patients with coronary heart disease: a RCSCD-TCM study in China. *Cardiovasc Diabetol* 2022, 21:93.
  13. D. Galiyeva, Gusmanov .A, Sakko .Y, Issanov .A, Atageldiyeva .K, Kadyrzhanuly .K, *et al.* Epidemiology of type 1 and type 2 diabetes mellitus in Kazakhstan: data from unified National Electronic Health System 2014-2019. *BMC Endocr Disord* 2022, 22:275.
  14. S. Sriraman, Sreejith .D, Andrew .E, Okello .I, Willcox .M. Use of herbal medicines for the management of type 2 diabetes: A systematic review of qualitative studies. *Complementary Therapies in Clinical Practice* 2023, 53:101808.
  15. E. Rutebemberwa, Lubega .M, Katureebe .S.K, Oundo .A, Kiweewa .F, Mukanga .D. Use of traditional medicine for the treatment of diabetes in Eastern Uganda: a qualitative exploration of reasons for choice. *BMC International Health and Human Rights* 2013, 13:1.
  16. M. Trius-Soler, Bramming .M, Jensen .M.K, Tolstrup .J.S, Guasch-Ferré .M. Types of dietary sugars and carbohydrates, cardiometabolic risk factors, and risk of diabetes: a cohort study from the general Danish population. *Nutrition Journal* 2025, 24:8.
  17. Clemente-Suárez .V.J, Mielgo-Ayuso .J, Martín-Rodríguez .A, Ramos-Campo .D.J, Redondo-Flórez .L, Tornero-Aguilera .J.F. The Burden of Carbohydrates in Health and Disease. *Nutrients* 2022;14:3809.
  18. N. Nirmalan, Nirmalan .M. Hormonal control of metabolism: regulation of plasma glucose. *Anaesthesia and Intensive Care Medicine* 2023;24:618-623.
  19. Y. Lin, Liu .J, Chen .J, Yao .C, Yang .Y, Wang .J, *et al.* FADD Phosphorylation Modulates Blood Glucose Levels by Decreasing the Expression of Insulin-Degrading Enzyme. *Molecules and Cells* 2020 , 43:373-383.
  20. V.Q. Mai, Meere .M. Modelling the Phosphorylation of Glucose by Human hexokinase I. *Mathematics* 2021, 9:2315.
  21. A.S. Bolla, Priefer .R. Blood glucose monitoring-an overview of current and future non-invasive devices. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews* 2020;14:739-751.
  22. E. Holmes-Truscott, Baptista .S, Ling .M, Collins .E, Ekinci .E.I, Furler .J, *et al.* The impact of structured self-monitoring of blood glucose on clinical, behavioral, and psychosocial outcomes among adults with non-insulin-treated type 2 diabetes: a systematic review and meta-analysis. *Frontiers in Clinical Diabetes and Healthcare* 2023;Volume 4 - 2023.
  23. Y. Xue, Thalmayer .A.S, Zeising .S, Fischer .G, Lübke M. Commercial and Scientific Solutions for Blood Glucose Monitoring-A Review. *Sensors* 2022;22:425.
  24. M. Tomkins, Lawless .S, Martin-Grace .J, Sherlock M, Thompson .C.J. Diagnosis and Management of Central Diabetes Insipidus in Adults. *J Clin Endocrinol Metab* 2022, 107:2701-2715.
  25. M. Christ-Crain, Winzeler .B, Refardt .J. Diagnosis and management of diabetes insipidus for the internist: an update. *J Intern Med* 2021, 290:73-87.
  26. S. Garima, Ajit Kumar .P, Marcy .D.M, Sakthivel .R, Bhim Pratap .S, Nachimuthu Senthil .K. Ethnobotanical survey of medicinal plants used in the management of cancer and diabetes. *J Tradit Chin Med* 2020, 40:1007-1017.
  27. C.G. Yedjou, Grigsby .J, Mbemi .A, Nelson .D, Mildort .B, Latinwo .L, *et al.* The Management of

- Diabetes Mellitus Using Medicinal Plants and Vitamins. *Int J Mol Sci* 2023;24.
28. A. Avila-Nava, Acevedo-Carabantes .J.A, Alamilla-Martinez .I, Tobón-Cornejo .S, Torre-Villalvazo .I, Tovar .A.R, *et al.* Chaya (*Cnidioscolus aconitifolius* (Mill.) I.M. Johnst) leaf extracts regulate mitochondrial bioenergetics and fatty acid oxidation in C2C12 myotubes and primary hepatocytes. *J Ethnopharmacol* 2023, 312:116522.
  29. R.N. Nurjanah, Hermana .W, Retnani .Y. Chaya Leaf Infusion (*Cnidioscolus aconitifolius*) as a Phytogenic for Productivity and Egg Quality of Japanese Quail (*Coturnix coturnix japonica*) of 17-20 Weeks of age. *Arch Razi Inst* 2024;79:234-239.
  30. Y. Qin, Li .M, Zhong .L, Wei .Q, Xiao .Y, Zhang .X, *et al.* The complete chloroplast genome sequence of chaya (*Cnidioscolus aconitifolius*) and phylogenetic analysis. *Mitochondrial DNA B Resour* 2022, 7:269-270.
  31. I.L. Usende, Olopade .J.O, Emikpe .B.O, Oyagbemi .A.A, Adedapo .A.A. Oxidative stress changes observed in selected organs of African giant rats (*Cricetomys gambianus*) exposed to sodium metavanadate. *International Journal of Veterinary Science and Medicine* 2018;6:80-89.
  32. Donkoh A, Kese AG, Atuahene CC. Chemical composition of chaya leaf meal (*Cnidioscolus aconitifolius* (Mill.) Johnston) and availability of its amino acids to chicks. *Animal Feed Science and Technology* 1990;30:155-162.
  33. Nascimento JBd, Viturino JJF, Ribeiro MAM, Costa JGMd. Nutritional Value, Ethnopharmacology, Chemistry, and Biological Activities of Species of the Genus *Cnidioscolus*: An Updated Review. *Foods* 2025;14:2092.
  34. Ighodaro OM, Adeosun AM, Akinloye OA. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina* 2017;53:365-374.
  35. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia* 2008;51:216-226.
  36. Weir GC, Butler PC, Bonner-Weir S. The  $\beta$ -cell glucose toxicity hypothesis: Attractive but difficult to prove. *Metabolism* 2021;124:154870.
  37. Wang RR, Qiu X, Pan R, Fu H, Zhang Z, Wang Q, *et al.* Dietary intervention preserves  $\beta$  cell function in mice through CTCF-mediated transcriptional reprogramming. *J Exp Med* 2022;219.
  38. Fagbohun E, Lawal O, Ore ME. The proximate, mineral and phytochemical analysis of the leaves of *Ocimum gratissimum* L., *Melanthera scandens* A. and *Leea guineensis* L. and their medicinal value. *International Journal of Applied Biology and Pharmaceutical Technology* 2012;3:15-22.
  39. Faley O. ? Fagbohun, E. D. and Faley, S. O. (2012). Effect of Storage on Chemical Composition and Mycoflora of Okra (*Abelmoschus esculentus*). *International Journal of Bioscience*. 2(7):83-89. *Internal Journal of Bioscience* 2012;2:83-89.
  40. Ndhala A, Aderogba M, Ncube B, van Staden J. Anti-Oxidative and Cholinesterase Inhibitory Effects of Leaf Extracts and Their Isolated Compounds from Two Closely Related Croton Species. *Molecules* 2013;18:1916-1932.
  41. Essiett UA, Ukpong U. Comparative Phytochemical, Nutrient and Anti-Nutrient of Stems of *Ipomoea Involucrata* Beauv, *Ipomoea Triloba* L. and *Ipomoea Batatas* Lam. *American Journal of Food and Nutrition* 2014;2:71-76.
  42. El Hage C, Rappeneau V, Etievant A, Morel A-L, Scarna H, Zimmer L, *et al.* Enhanced Anxiety Observed in Cocaine Withdrawn Rats Is Associated with Altered Reactivity of the Dorsomedial Prefrontal Cortex. *PLOS ONE* 2012;7:e43535.
  43. Mofarrah A, Li P, Ping J, Niu H, Husain T, Lee K. Risk-based decision-support modeling for offshore produced water management, 2009.
  44. Vessal M, Zal F, Vasei M. Effects of *Teucrium polium* on oral glucose tolerance test, regeneration of pancreatic islets and activity of hepatic glucokinase in diabetic rats. 2003;6.