International Journal of Spectrum Research in Medical and Clinical Practice (IJSRMCP)
1(3), July-September, 2025, Pages 1-14

ISSN: 3092-975X

https://doi.org/10.5281/zenodo.16847592

# Antidiabetic Potential of Methanolic Extract of Newbouldia laevis (Ogirisi) Leaves in Hyperglycemic Wistar Rats

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## ABSTRACT

*Ideal management of prolonged hyperglycaemia presents both micro and macro vascular complications, a leading cause of morbidity and mortality in diabetic subjects. This study was under taken to give credence to the traditional use of Newbouldia laevis (Ogirisi) leaves in the treatment of diabetes mellitus (DM). Alloxan monohydrate (140 mg/kg) was administered to male wistar rats via the intra-peritoneal route. The oral $LD_{50}$ of the methanoic extract of N. laevis was >1200 mg/kg. The normal male rats were placed in Group A-Negative control group and the diabetic male wistar rats were then placed in 5 groups following stabilization of hyperglycemia. The diabetic groups include Group B–positive control was untreated, Group C received a standard drug, Gluformin (5 mg/kg) and the last three groups (Group D, E, F) received low dose 200 mg/kg, middle dose 400 mg/kg, high dose 800 mg/kg respectively of the methanolic N. Laevis extract. Treatment was via the oral route for 2 weeks and fasting blood sugar level was monitored over this period. Phytochemical screening revealed the presence of glycosides, phenolic, polyphenol and abundant flavonoids. Notably, at the 2nd week of treatment, the three doses (200 mg/kg, 400 mg/kg and 800 mg/kg) of the methanolic extract of N. laevis leaves administered significantly ($P<0.05$) lowered fasting blood glucose levels. Gluformin also significantly lowered fasting blood glucose ($P<0.05$). These findings suggest that methanolic extract of N. laevis leaves possesses antihyperglycaemic activities and can be used in folk medicine for the management of diabetes mellitus. Hence, it could be considered for further in vivo evaluation.*

**Keywords:** Antidiabetic, Methanolic Extract, *Newbouldia laevis*, Hyperglycemic Wistar Rats

## INTRODUCTION

Diabetes mellitus (DM) is taken from the Greek word "diabetes", meaning Siphon - to pass through and the Latin word "mellitus" meaning sweet (Medymology, 2023). DM is a group of metabolic disorders characterized by high blood sugar levels, which can lead to a variety of complications if left untreated or poorly managed (Amit & Priyanka, 2023). Diabetes mellitus is a chronic illness that develops when the body either cannot use the insulin that the pancreases produces properly or does not produce enough of it. Insulin is a hormone that regulates blood glucose. Hyperglycemia, commonly referred to as elevated blood glucose or elevated blood sugar, is a frequent consequence of uncontrolled diabetes mellitus that eventually causes major harm to numerous bodily systems, particularly the blood vessels and nerves (Amit & Priyanka, 2023). DM is classified into various subtypes, such as Type 1 Diabetes, Type 2 Diabetes, Gestational Diabetes, Steroid-induced Diabetes, Neonatal Diabetes, and Maturity-on set Diabetes of the young (MODY) (Mabel, Maclaren & Sperling, 2021)

In 2014, the prevalence of diabetes among adults aged 18years or older was 8.5%. 48% of all diabetes-related deaths occurred before the age of 70 in 2019. Diabetes mellitus was the direct cause

of 1.5million death in 2019. Diabetes contributed to an additional 460 000 deaths from kidney disease, and elevated blood glucose, accounts for 20% of death from cardiovascular disease (Institute for Health Metrics and Evaluation, 2020). Diabetes mellitus caused an increase in age- Standardized death rates of 3% between 2000 and 2019. In lower-middle-income countries, its mortality rates increased 13%. Efforts to curb the diabetes epidemic include global strategies to address obesity and sedentary lifestyles, improve healthcare access, and promote early diagnosis. Type 2 diabetes prevention remains crucial, focusing on public health measures such as promoting healthy diets, increasing physical activity, and controlling other risk factors (WHO, 2023).

Plant-based therapies have gained significant attention as alternatives approaches to conventional anti-diabetes treatments. This interest stems from their affordability, cultural acceptance, and potential for fewer side effects compared to synthetic drugs. Furthermore, herbal treatments and dietary interventions are generally more affordable than modern pharmaceuticals, making them accessible in low resources settings as well as they provide additional health benefits such as anti-inflammatory, anti-oxidant, or lipid lowering effect, which contribute to overall well-being (WHO, 2019). Traditional medicine has been an integral part of Nigeria culture for centuries, playing a vital role in the country's healthcare system, with a long history of use dating back to before, the advent of western medicine. Approximately, 80% of the population relies on traditional medicine for primary health care needs (WHO, 2019). In Nigeria, traditional medicine encompasses various remedy practices, including Herbal medicine - plant- based (Igoli, 2018).

*Newbouldia laevis* (*N.* laevis) is a medium size angiosperm which belongs to the Bignoniaceae family. The leaves, roots, root bark and stem bark treatment of *Newbouldia laevis* are widely used in treatment of diseases in traditional medicine in Africa. Its leaves contain bioactive compounds like alkaloids, flavnoids, tannins and Cardiac glycosides, contributing to their medicinal properties (Okoye, 2017). Extracts from the leaves and bark have shown a significant reduction in blood glucose levels in alloxan- and streptozotocin-induced diabetic models. These effects are comparable to standard antidiabetic drugs such as glibenclamide.

In mechanism, *N. laevis* facilitates insulin secretion, enhances glucose uptake, and improves pancreatic beta-cell function. This is particularly beneficial in reversing the damage caused by oxidative stress in diabetic states.Treatment with *N. laevis* extracts helps reduce serum cholesterol, triglycerides, and low-density lipoprotein (LDL) levels while increasing high-density lipoprotein (HDL) levels, reducing the risk of cardiovascular complications in diabetes (Igbokwe et al., 2018). Traditional Medicine provides clues about plants with overlapping activities. Example: Plants like *Curcuma longa* (turmeric) and *Zingiber officinale* (ginger), both containing curcuminoids, are widely used in traditional medicine for inflammation and digestive issues. Comparative bioassays often reveal shared activities. For instance, antioxidant or antimicrobial assays may show similar results for plants with analogous compounds.

Diabetes mellitus is a global health challenge, with an increasing prevalence that poses significant economic and social burdens. Current pharmacological treatments, while effective, often come with limitations such as high cost, limited accessibility, side effects, and suboptimal glycemic control for some patients. As a result, there is growing interest in exploring alternative or complementary therapies derived from medicinal plants, particularly those used in traditional medicine.

*N. laevis*, commonly referred to as "boundary tree," is widely used in African traditional medicine for various ailments, including diabetes. Despite its extensive use, the antidiabetic potential of this plant has not been adequately validated through rigorous scientific studies. The lack of comprehensive research on its efficacy, active phytochemical constituents, mechanisms of action, and safety profile creates a gap in knowledge that limits its potential development as a reliable and evidence-based treatment option. Addressing these gaps is critical to determining whether *N. laevis* can serve as an effective, safe, and affordable alternative or complement to existing antidiabetic therapies. This study seeks to scientifically evaluate the antidiabetic effect of methanolic extract of *N.*

*laevis* (Ogirisi) leaves in hyperglycemic wistar rats model to provide evidence for its potential therapeutic application.

The aim of this study is to evaluate the phytochemical constituent of methanolic extract of *N. laevis* leaves and it's antihyperglycaemic activity in diabetic induced wistar rats with specific objectives - identify and quantify the bioactive compounds present in the methanolic extract of *N. laevis* leaves using Gas Chromatography Flame Ionization Detection (GCFID; detect the major phytochemicals responsible for the antidiabetic activity; evaluate the antidiabetic potential of the methanolic extract of *N. laevis* leaves in hyperglycemic wistar rats; determine the effectiveness of the extract in reducing blood glucose levels and improving insulin sensitivity in diabetic rats.

## METHODOLOGY

### Collection and Preparation of plant materials

Mature fresh leaves of *N. laevis* was harvested from a farm at Ubommiri in Mbatiolu Local Government Area, Imo State, Nigeria. The plant sample was identified and authenticated by a Taxonomist Prof. F.N Mbagwu, in the Department of Plant and Biotechnology, Imo State University, Owerri. Its voucher number is IMSUH 453. The plant leaves were washed to remove contaminants, air-dried under shade and ground into powdered form. A known weight (40g) of the powder was extracted using 200ml of 80% methanolic and 20% of distilled water, shaken intermittently for 72 hours at room temperature. The mixture was filtered and the filtrate concentrated on steam bath until a semisolid residue of known weight was obtained. The weighed extract was stored in 150ml beaker, labelled and covered with foil paper and preserved in the refrigerator at 40°C for use in phytochemical screening, acute toxicity test, and anti-diabetic tests.

The extract was prepared by dissolving about 1.5g (Using high performance profile design compact weighing balance; CS200) of the extract in 10ml of distilled water, to give an effective concentration. The formula;

$$\text{Dosage} = \frac{\text{mg/kg} \times \text{Wt. Of animals(g)}}{1000 \text{ conc.(mg/ml)}}$$

was used to calculate the volume of the extract solution to be administered to each animal (Okipabhele & Aihiokhai, 2022). The solutions were prepared fresh daily before administration.

### Experimental Animals

A total of forty-five (45) Male Wistar rats (*Rattus norvegicus*) weighing 80-150g was housed in aluminum cages placed in a well-ventilated standard housing conditions (temperature 28-31°C; photoperiod 12hrs, humidity 50 -55%) allowed free access to standard commercial feed (SCF) (Bendel Feeds and Flour Mills Ltd., Ewu, Edo State, Nigeria) and tap water for 2weeks acclimatization period. The handling of the animals was in accordance with the standard principles of Laboratory Animal Care of the United States National Institute of Health (NIH, 1978).

### Experimental Designs and Methods

*Toxicity Study ($LD_{50}$) of the Samples*

The acute oral toxicity/lethality ($LD_{50}$) of the methanolic extract of *Newbouldia laevis* leaves(test sample) in rats was determined using OECD (Organization for Economic Cooperation and Development) Test No 425 method. This technique enables the estimation of an $LD_{50}$ with a confidence interval, and the outcome enables the classification of a material for acute toxicity in accordance with the Globally Harmonized system of classification and labelling of chemicals (OECD, 2022).

The test sample is administered generally in a single dose by gavage to adult male rats fasted prior to dosing. The test sample is administered orally to a series of animals at increasing or decreasing dose levels, depending on the outcome of the previous dose. The test uses a minimum number of

animals to determine the acute oral toxicity. The first 3 male wistar rats receive the initial dose. If the wistar rats survive, the next 3 male wistar rats will receive a higher dose. If the wistar rats die, the next 3 male wistar rats will receive a lower dose. The animals will be observed for 14days after dosing for signs of toxicity and mortality. The dose will be adjusted based on the outcome of the previous animals. The acute oral toxicity will be determined based on the dose that causes toxicity or mortality in 50% of the wistar male rats ($LD_{50}$).

As Osigwe, et al., (2015), concluded that the oral $LD_{50}$ of the methanolic extract of *Newbouldia laevis* is >5000 mg/kg dose was considered safe as no animal died at 5000 mg/kg. The intraperitoneal (i.p.) LD50 was calculated to be 3807.9 mg/kg. The maximum non-lethal dose was 2900 mg/kg and the minimum lethal dose was 5000 mg/kg. This was used as a dose guide, the OECD method was used giving a lower dose of 400mg/kg, no death of wistar rats and higher dose of 1200mg/kg which killed an average percentage of the wistar rats. Hence, a lower dose of the $LD_{50}$ was used as the doses for the research work.

**Determination of Blood Glucose and Induction of Hyperglycaemia**

After eight hours of fast, blood glucose levels of the animals were determined before induction of hyperglycaemia. An aliquot (0.6µl) of the blood sample was being placed on the test strip that had been inserted into the glucometer and the blood sugar level was read. Hyperglycaemia was then induced into the required animals following intra-peritoneal administration of 1ml corresponding to 150mg/kg body weight of alloxan monohydrate solution. (Prepared in sterile physiological saline). One hour after the administration of alloxan, the animals were to also take their feeds *ad libitum* and 5% dextrose solution to overcome the early hypoglycaemic phase (Hassanpour, Dehghani, Karami & Dehghani, 2018). The blood glucose was again being determined after 24hrs of alloxan monohydrate administration. Wistar rats with blood glucose level higher than 180mg/dl was declared hyperglycaemic and included in the antihyperglycaemic study (Kim, Choi, Lee & Kang, 2014).

**Grouping of Animals**

The thirty (30) male wistar rats (80-150g) were grouped into six groups (A-F). Five normal male wistar rats in group A and twenty-five male wistar rats completely randomized into the remaining 5 groups(B-F) as follows

**Group A:** Normal male wistar rats not induced with diabetes (Negative control)

**Group B:** Alloxan-induced hyperglycaemic male wistar rats not treated, to serve as positive control.

**Group C:** Alloxan-induced hyperglycaemic male wistar rats treated with0 standard diabetic drug (Gluformin) (standard).

**Group D:** Alloxan-induced hyperglycaemic male wistar rats treated with 200mg/kg body weight of extract. (Low dose)

**Group E:** Alloxan-induced hyperglycaemic male wistar rats treated with 400mg/kg body weight of extract. (Middle dose)

**Group F:** Alloxan-induced hyperglycaemic male wistar rats treated with 800mg/kg body weight of extract. (High dose)

Extract administration was done once daily for 14days after which blood glucose level was determined at interval of three days.

**Phytochemical Analysis**

**Qualitative phytochemical Analysis**

**The qualitative** phytochemical tests was carried out on the methanolic extract using standard protocols (Harbone, 1998) to qualitatively detect the presence, absence and relative amount of phytoconstituents.

**Quantitative phytochemical Analysis**

The quantitative phytochemical analysis of the methanolic extract was determined using GCFID for separation, identification and quantification of the extract mixture.

**Extraction of phytochemicals**

A known gram (1g) of sample was weighed and transferred in a test tube and 15ml ethanol and 10ml of 50%m/v potassium hydroxide was added. The test tube was allowed to react in a water bath at $60^0$c for 60mins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20ml of methanol, 10ml of cold water, 10 ml of hot water and 3ml of hexane, which was all transferred to the funnel. This extracts were combined and washed three times with 10ml of 10%v/v methanol aqueous solution. The solution as dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000ul of pyridine of which 200 ul was transferred to a vial for analysis (Kelly & Nelson, 2014).

**Quantification by Gas chromatography flame ionization detection GC-FID**

The analysis of phytochemical was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15m x 250um x 0.15um) was used. The injector temperature was $280^o$C with splitless injection of 2ul of sample and a linear velocity of 30cms$^{-1}$, Helium 5.0pa.s was the carrier gas with a flow rate of 40 mlmin$^{-1}$. The oven operated initially at $200^0$c, it was heated to $330^0$c at a rate of $3^0$c min$^{-1}$ and was kept at this temperature for 5min. the detector operated at a temperature of $320^0$c. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicalss express in ug/g (Kelly & Nelson, 2014).

**Proximate analysis**

The moisture, ash, crude fibre, crude fat and crude protein contents were determined following the method established by Association of Official Analytical Chemist, AOAC (1990) as cited in Aletan & Kwazo (2019) while the carbohydrate content was determined following the differential method established by AOAC (1990) as cited in Aletan & Kwazo, (2019).

**i.e Carbohydrate =** 100 – (%Protein + %Moisture + %Ash + %Fat + %Fibre)

**Blood and organ sample collection**

**Procedure**

After 14 days of treatment, the rats were sacrificed using anesthesia (e.g., ketamine and xylazine). The blood samples were collected from each rat via cardiac puncture or orbital sinus bleeding and the pancreas tissues were harvested from each rat.

**Tissue Fixation and Processing**

**Procedure**

The pancreas tissues were fixed in 10% neutral buffered formalin (NBF) for 24-48 hours. The tissues were dehydrated in a series of ethanol solutions (70%, 80%, 90%, and 100%) and were cleared

in xylene and embed them in paraffin wax. The tissues were sectioned into 5-µm thick slices using a microtome and was stained with hematoxylin and eosin (H&E) for histological examination.

**Histological Examination and Photomicrography**

**Procedure**

The H&E-stained tissue sections were examined under a light microscope and histological changes in the pancreas tissues was evaluated. Photomicrographs of representative tissue sections was captured using a microscope-mounted camera.

**Data analysis**

Statistical Package for Biological and Social Sciences (SPSS) Inc. 27.0 Software program was used. Mean values (M) ± SD was calculated and one-way analysis of variance (ANOVA) was performed for multiple comparison. Values (p) that was less than 0.05 ($p<0.05$) was considered statistically significant.

**Ethical approval**

All animals produced were conducted according to Natural Institutes of Health Guide for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of Imo State University under the ethical number (IMSU/FBS/2024/0016).

## RESULTS AND DISCUSSION

Table 1 shows quantitative phytochemical composition of methanolic extract of *Newboildia lead is. Results showed that the leaves extract of Newbouldia laevis* contained glycoside (Naringin and Lunamaria in Amount 0.21ppm and 0.46ppm respectively) phenolic (reveratral and vanillic in amount 6.25ppm and 0.15ppm respectively), polyphenol (Ellagic acid in amount 0.36ppm) and flavonoids in most abundant composition. (see Appendix for chromatograph).

**Table 1: Quantitative Phytochemical Composition of methanolic extract of *Newbouldia laevis* leaves.**

| S/N | Retention time (mins) | Area | Amount (ppm) | Phytochemical name | Phytochemical class |
|---|---|---|---|---|---|
| **1** | 2.62 | 210.53 | 29.60 | Kaempferol | Flavonoids |
| **2** | 3.06 | 129.45 | 19.19 | Catechin | Flavonoids |
| **3** | 4.57 | 5.96 | 0.82 | Quercetin | Flavonoids |
| **4** | 4.77 | 6.57 | 0.91 | Hesperidin | phenolic |
| **5** | 6.16 | 7.41 | 2.03 | Luteolin | Flavonoids |
| **6** | 6.38 | 37.42 | 6.25 | Resveratrol | Phenolic |
| **7** | 6.85 | 8.70 | 2.22 | Artemutin | Flavonoids |
| **8** | 7.07 | 4.36 | 0.60 | Myricetin | Flavonoids |
| **9** | 7.25 | 1.62 | 0.21 | Naringin | glycosides |
| **10** | 7.73 | 14.59 | 3.04 | Retusin | Isoflavones |
| **11** | 8.01 | 2.63 | 0.36 | Ellagic acid | Polyphenols |

| | | | | | |
|---|---|---|---|---|---|
| **12** | 8.52 | 1.13 | 0.15 | Vanillic | Phenolic |
| **13** | 8.88 | 9.34 | 1.30 | Isorhamnetin | Flavonoids |
| **14** | 9.11 | 2.83 | 0.39 | Naringenin | Flavonoids |
| **15** | 9.62 | 1.03 | 0.12 | Apigenin | Flavonoids |
| **16** | 9.90 | 130.07 | 18.28 | Maricetin | Flavonoids |
| **17** | 10.30 | 9.28 | 1.29 | Epicatechin | Flavonoids |
| **18** | 10.66 | 3.66 | 0.50 | Daidzein | Flavonoids |
| **19** | 11.17 | 4.50 | 0.61 | Genistein | Flavonoids |
| **20** | 11.77 | 2.89 | 0.40 | Apigenin | Flavonoids |
| **21** | 12.31 | 3.36 | 0.46 | Lunamarin | Glucosinolates |
| **22** | 12.80 | 3.23 | 0.44 | Gallocaechin | Flavonoids |
| **23** | 13.48 | 3.24 | 0.44 | Tangeretein | Flavonoids |
| **24** | 13.90 | 17.30 | 2.42 | Epicatechin | Flavonoid |
| **25** | 14.31 | 28.59 | 3.98 | Hesperidin | Flavonoid |

Table 2 shows the qualitative phytochemical composition of methanolic extract of *Newbouldia laevis* leaves. Results showed that leaves extract of *Newbouldia laevis* was abundant in Flavnoids followed by glycoside, phenolic, polyphenols. It further revealed that alkaloids, Lectins, Saponins, terpenoids, tannis and sterols were absent.

**Table 2: Qualitative Phytochemical Composition of methanolic extract of *Newbouldia laevis* leaves.**

| S/N | Phytochemical class | Results |
|---|---|---|
| **1** | Glycosides | ++ |
| **2** | Alkaloids | - |
| **3** | Flavonoids | ++++++++++++++++++ |
| **4** | Tannins | - |
| **5** | Lectins | - |
| **6** | Sapoins | - |
| **7** | Terpenoids | - |
| **8** | Sterols | - |
| **9** | Phenolic | ++ |
| **10** | Polyphenol | + |

Legend: + = present

- =absent

Table 3 shows the proximate analysis of methanolic extract of *Newbouldia laevis* leaves. Results showed that leaves extract of *Newbouldia laevis* has the highest percentage composition in carbohydrate (49.965%), moisture (16.077%) and protein (13.300%) and a lower percentage composition in fat (8.614%), fiber (6.802%) and ash (5.232%) respectively.

**Table 3: Proximate Analysis of methanolic extract of *Newbouldia laevis* Leaves.**

| S/no | Composition | Percentage % |
|---|---|---|
| **1** | Moisture | 16.08 |
| **2** | Fiber | 6.80 |
| **3** | Ash | 5.23 |
| **4** | Fat | 8.61 |
| **5** | Protein | 13.30 |
| **6** | Carbohydrate | 49.97 |

Table 4 shows the average weight of the experimental male wistar rats. The results revealed a high increase in average weight(grams) of the male wistar rats in groups E and F and a low increase in average weight(grams) of the male wistar rats in groups(A,B,C,D) over the time of the experiment exercise.

**Table 4: Average Weight of the Experimental Male Wistar Rats**

| Groups | Initial Weight before Treatment (g) | Final Weight after Treatment (g) |
|---|---|---|
| **A (Negative control)** | 80.50 | 100.00 |
| **B (Positive control)** | 80.50 | 100.50 |
| **C (Standard)** | 80.00 | 100.00 |
| **D (Low dose)** | 80.00 | 110.50 |
| **E(Middle dose)** | 80.50 | 135.00 |
| **F (High dose)** | 80.50 | 150.50 |

Table 5 shows the effect of methanolic extract of *Newbouldia laevis* leaves and Gluformin on the Blood glucose concentration of alloxan-induced male wistar rats. There was an observed significant difference ($p<0.05$) at the 1st week of treatment ($9.97\pm0.40^{c}$) and 2nd week of treatment ($6.08\pm0.40^{c}$) of Group C when compared to Group B (positive control) (9.80±0.57 and 8.02±0.72 respectively). There was equally a significant difference ($p<0.05$) in the blood glucose concentration of rats in groups (D, E

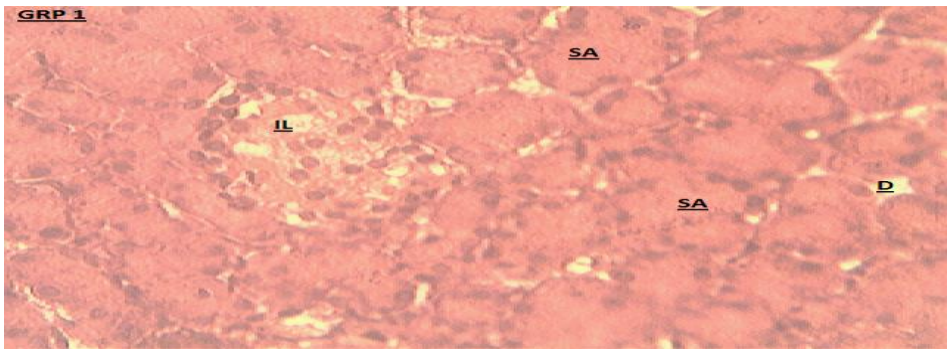
and F) at the 1st week of treatment (Group D 11.66±0.49,Group E 8.13±0.41, Group F 13.9±2.74) and 2nd week of treatment (Group D 9.13±0.43,Group E 4.55±0.70,Group F 5.92±0.42) when compared to Group B.

**Table 5: Effects of methanolic extract of *Newbouldia laevis* Leavis on the blood glucose concentration of male alloxan-induced wistar Rats**

| Groups | Initial blood glucose concentration (mmol/l) | Two days after alloxan administration (mmol/l) | 1st week after treatment (mmol/l) | 2nd week after treatment (mmol/l) |
|---|---|---|---|---|
| **A (Negative control )** | 5.49 ± 0.19[a] | - | - | - |
| **B (Positive control)** | 5.33 ± 0.21[a] | 13.38 ±1.10b | 9.80±0.57[b] | 8.02±0.72[b] |
| **C (Standard)(Gluformin)** | 5.27 ± 0.06[a] | 13.21 ± 1.08b | 9.97±0.40[c] | 6.08±0.40[c] |
| **D Alloxan induced + *Newbouldia laevis* leaves extract (low dose)** | 5.38 ± 0.09[a] | 13.38±0.74[b] | 11.66±0.49[c] | 9.13±0.43[c] |
| **E Alloxan-induced + Newbouidia laevis leaver etract (middle dost)** | 5.38 ± 0.21[a] | 12.54±0.34[d] | 8.13±0.41[c] | 4.55±0.70[d] |
| **F AlloxanilInduced + *Newbouldia laevis* leaves extract (high dose** | 5.44 ± 0.17[a] | 18.09±4.34 | 13.9±2.74[b] | 5.92±0.42[c] |

Values expressed as mean ± SD, n = 6 and means with different superscript (a to d) along the same column are significantly different (P<0.05).

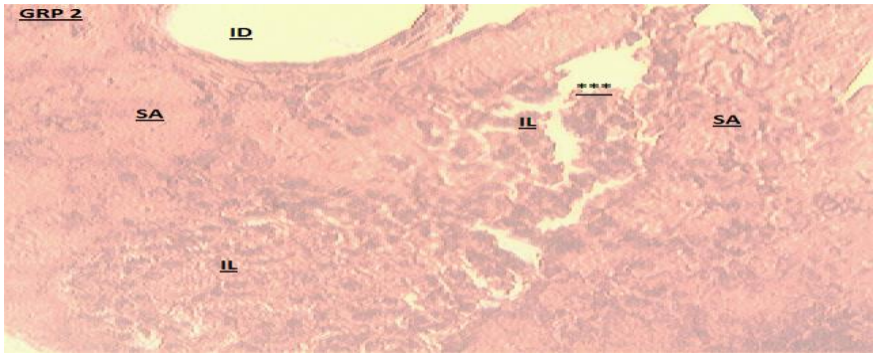
**Histopathological effect of methanolic extract of *Newbouldia laevis* leaves on the pancreatic Beta cells of experimental male wistar rats**




**Group A: Pancrease X 400**

**Plate 1: Representative Photomicrograph of Group A (Negative control) male wistar rats Pancrease ( H & E × 400 magnification)**

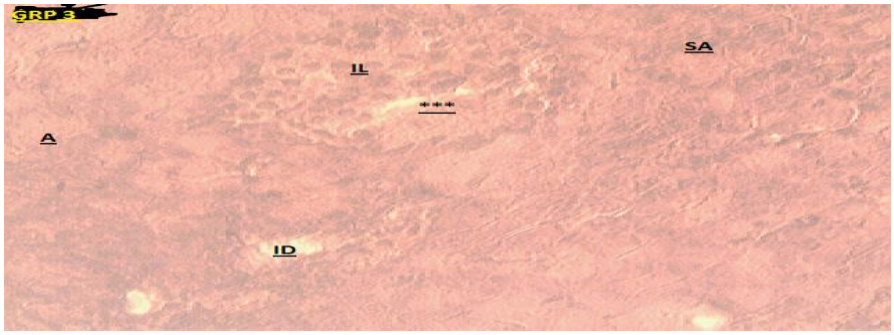
The photomicrograph of group A pancrease x 400 magnification stained in H and E shows a normal serous acini (SA) and islet of langerhan (IL), also seen is the pancreatic duct (D) (Normal pancrease).




**Group B: Pancrease x 400**

**Plate 2: Representative Photomicrograph of Group B( positive control) male wistar rats Pancrease ( H & E × 400 magnification)**

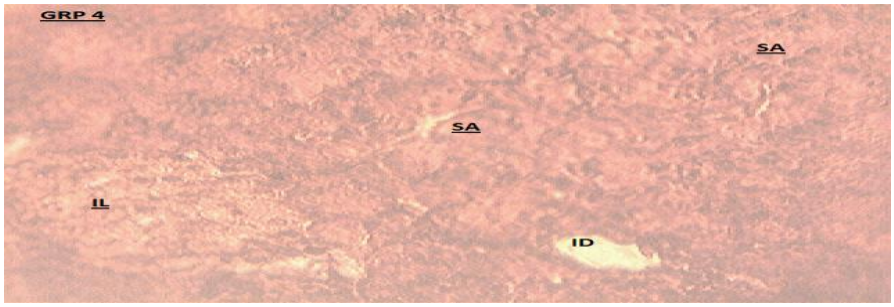
The cross section of Group B pancrease showing an enlarged and slightly distorted *** reaction islet of langerhan (IL), enlarged interlobular duct (ID), weakened serous acini cells (SA) (pathological change seen).




**GRP C: Pancrease x 400**

**Plate 3: Representative Photomicrograph of Group C( standard) male wistar rats Pancrease ( H & E × 400 magnification)**

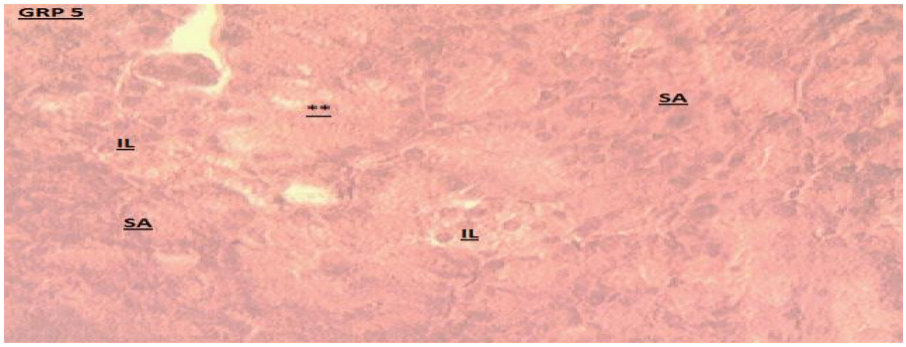
The photomicrograph of this group shows regeneration when compared with the group B as the islet of langarhan (IL) is recovering from its distortion. The serous acini (SA) and the interlobular duct (ID) are seen coming alive.




**Group D:Pancrease x 400**

**Plate 4 Representative Photomicrograph of Group D( Low dose) male wistar rats Pancrease ( H & E × 400 magnification)**

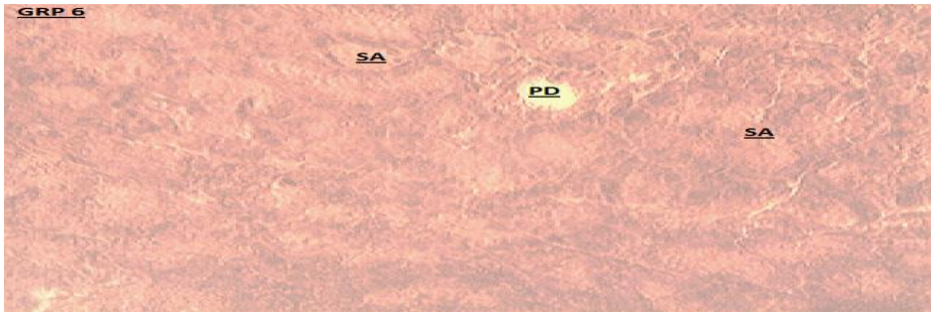
The photomicrograph of this group shows a serious hyperactivity going on as if there is a combat between the effect and the extract though there is a slight improvement seen when compared with group B.




**Group E: Pancrease x 400**

**Plate 5: Representative Photomicrograph of Group E( middle dose) male wistar rats Pancrease ( H & E × 400 magnification).**

The photomicrograph of this Group shows a more regenerated pancrease as the cells are looking closely lively when compared with Group A.




**Group F: Pancrease x 400 magnification**

**Plate 6: Representative Photomicrograph of Group F( High dose) male wistar rats Pancrease ( H & E × 400 magnification)**

The photomicrograph of this Group shows a transformed pancreatic cells, the pancreatic duct (PD) and the serous acini (SA) (are intact).

**Discussions**

Phytochemical are plant derived chemicals with bioactive properties. By implication, they are natural chemicals with specific effects on health. Plant extract often exert anti-diabetic effects through phytochemicals such as flavonoids, tannins, alkaloids and polyphenols (Adinortey et al., 2020). Similarly, Mamunn et al. (2014), proposed that the most common herbal active ingredients used in treating diabetes are flavonoids, tannins, phenolic and alkaloids. Hence, the existence of these compounds implies the importance of the anti-diabetic properties of these plants. In agreement, the Table 5 result shows that there is a significant difference ($P<0.05$) in the methanolic *N. laevis* leaves extract treated groups (D, E, F) in the 1st week treatment and 2nd week treatment when compared to the positive control group and this could be due to the phytochemical composition of the plant samples as seen in Tables 1 and 2. Okon et al., (2019) suggested that the presence of these biological active compounds in the plant could serve as potential sources of drugs and their secondary metabolites could exert some biological activities when taken by animals.

The plant extract is most abundant in Flavonoids, which have been shown to possess remarkable hypoglycaemic effect, linked to their capacity to avoid glucose absorption or improve glucose tolerance (Osigwe et al., 2015). It has been presented that flavonoids act as insulin secretagogues or insulin mimetics, attenuate diabetic complications, stimulate glucose uptake in peripheral tissues and regulate the activity and/or expression of the rate limiting enzymes involved in carbohydrate metabolism (Osigwe et al., 2015).

In addition, flavonoids have been reported to show potent inhibitory activity against a wide range of enzymes such as lipo-oxygenases, cyclo-oxygenases, and stop the generation or the action of

free radicals which cause tissue damage during inflammatory processes (EL-Abhar and Schaalam, 2014). However, they are therefore potent anti-oxidants and could modulate the activities of various enzymes involved in blood glucose responsible for the observed reduction of the blood glucose level by the plant extraction the alloxan-induced diabetic wistar rats. Also, polyphnolic compounds have been involved in the production of incretin hormone (glucagon-like peptide GLP-1) that seems to act through many mechanisms towards effective glucose disposal, including stimulating of insulin secretion, suppression of glucagon release, slowing of gastric emptying, improving insulin sensitivity, and reduction of food intake (El-Abhar and schaalam, 2014). Osigwe et al. (2015) documented evidence that polyphenols slow down glucose absorption by competitive inhibition of the intestinal brush border membrane sodium glucose co-transporter (SGLT1) as well as inhibition of disaccharidases. Furthermore, Khaled (2021) stated that the effect of plant sample to regenerate damaged pancreatic Beta-cells by alloxan in a diabetic model is linked to the bioactive compounds that enhance cell viability and repair mechanisms.

The ethnomedicinal use of *Newbouldia laevis* leaves in the treatment of diabetes mellitus can be supported with the findings of this research study. The presence of phytochemicals compounds like Glycosides, Phenolic, polyphenols and abundant Flavnoids in the methanolic extract of *N. laevis* leaves as seen in Tables 1 and 2 indicate the existence of these compounds, implies the importance of the antidiabtic properties of this plant. Hence, Mamunn et al. (2014) proposed that the most common herbal active ingredients used in treating diabetes are flavonoids, tannis, phenolic and alkaloids. Induction of diabetes using Alloxan monohydrate has been described as hopeful experimental model for studying the effect of hypoglycemic agents.

*Newbouldia laevis* leaves have been found to exhibit hypoglycemic or antidiabetic activity in various studies. The leaves extract has been shown to reduce blood glucose levels in diabetic rats, with the methanol fraction (Mf) being the most potent (Osigwe et al., 2015). Subsequently, Table 5 shows results of a dose-dependent lowering of fasting blood glucose level in diabetic rats treated with different doses of the methanol extract of *N. laevis leaves.* This dose-dependent effect compares well with Gluformin, the standard drug used. It is possible that the methanolic leaves extracts of the plant sample could have induced insulin secretion just like those treated with oral hypoglycemic drug (Gluformin). Likewise, Okon et al. (2019) stated that the extract of *N. laevis* also produced a similar reduction in blood sugar level as the standard hypoglyceamia drug (Glibenclamide). This may be due to its ability to enhance insulin secretion like standard drug. This positive result might be the reason behind its use by traditional medicine practitioners in folk medicine for the management of diabetes mellitus.

Induction of diabetes using alloxan has been deployed as acceptable experimental model for studying the effect of hypoglycemic agents. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide with a simultaneous massive increase in cytosolic calcium concentration, resulting in the destruction of pancreatic B-cells and diabetes (Okon, et al., 2019).

Histopathology screening test on the pancreatic Beta cells of the experimental wistar rats shown in plate 1 reveals that in group A (Negative control) has a normal pancreas, a pathological change on the pancreas of the positive control group (plate 2) and a regeneration of groups D, E and F treated with methanolic extract of *N. laevis* leaves (plates 4, 5 and 6). Groups D, E, F indicated a recovery state of the pancreas with group F (treated with highest dose of extract) showing the most improvement. This result proves a management effect of methanolic extract of *N. laevis* leaves on the reducing effect of diabetes mellitus.

## CONCLUSIONS

The results of this study suggest that the methanolic extract of *Newbouldia laevis* leaves possess antihyperglycaemic activity in Alloxan diabetic male rats. Glycosides, phenolic, polyphenols

and abundant flavnoids may be responsible for this activity. Hence, this study gave credence to the ethnomedicinal use of *N. laevis* leaves in the management of diabetes mellitus.

## RECOMMENDATIONS

Based on the findings arising from this study, the following recommendations are made for prospective researchers.

1. Further studies are suggested to isolate the phytochemical compounds responsible for the antihyperglycaemic activity.
2. Clinical trials should be conducted to evaluate the efficacy and safety of the methanolic extract of *Newbouldia laevis* in humans.

### Contributions to Knowledge

This study made the following contributions to knowledge

- The use of methanolic extract of *Newbouldia laevis* leaves for the management of diabetic mellitus due to its antihyperglycaemic activity it possesses.
- Also, treating alloxan-induced rats with methanolic extract of *N. laevis* leaves positively changes and reversed the pathological changes done on the pancreatic beta cells thereby enhancing insulin secretion and blood glucose transport.

### Limitations of the study

The major limitation in this study is failure to test specific doses of methanolic extract of *Newbouldia laevis* leaves, which may not be the optimal for antidiabetic effects.

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