Effect of Aqueous Extract of *Citrullus lanatus* (Water Melon) Seeds on Alloxan Induced - Diabetic Wistar Rats

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**Authors' contributions**

This work was carried out in collaboration among all authors. Authors HEO, DEP and MM designed the study, author HEO performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author HEO managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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**ABSTRACT**

This study was designed to evaluate the effect of aqueous extract of *Citrullus lanatus* (water melon) seeds on alloxan induced-diabetic wistar rats. The Wistar rats were grouped into 11 of 3 rats in each. Groups 2-11 received single dose of 120 mg/kg b.wt of alloxan monohydrate intraperitoneally. Groups 4 and 5 orally received a dose of 100 mg/kg b.wt of metformin for 7 and 21 days respectively. Groups 6, 8 and 10 orally received 200 mg/kg b.wt, 400 mg/kg b.wt, and 600 mg/kg b.wt. of the extract respectively for 7 days while groups 7, 9 and 11 orally received 200 mg/kg b.wt., 400 mg/kg b.wt and 600 mg/kg b.wt of the extract respectively for 21 days. Blood samples were collected 24 hours after 7 and 21 days of treatment and selected biochemical parameters were determined; glucose determination, pancreatic α- amylase activities and lipid profile, while histopathology examination of the pancreas was also examined. Qualitative phytochemical result showed the presence of flavonoids, alkaloids, saponin, tannin, oxalate and phytate. Flavonoids (176.87 ug/g) were most abundant and phytate was the least occurring anti nutrient (6.75±0.06). *Citrullus lanatus* seeds extract significantly (p≤0.05) decreased the plasma glucose levels.
pancreatic α-amylase activities, total cholesterol, triglycerides and lipoproteins while there was a significant (p≤0.05) increase in high density lipoprotein levels on 7 and 21 days in all the extract treated groups. The histopathological examination of the pancreas showed regeneration of pancreatic cells in all the extract treated groups. The aqueous extract of *Citrullus lanatus* (watermelon) seeds is rich in phytochemicals with antioxidant properties hence could serve as novel herbal antidiabetic agent.

**Keywords:** Citrullus lanatus; phytochemicals; diabetes; lipid profile; histopathology.

### 1. INTRODUCTION

Diabetes is a metabolic disease characterized by chronic hyperglycemia with carbohydrate, fat and protein metabolic disturbances. It can be a general in-born disorder and can also be described as a group of metabolic disorder in which the person has elevated blood glucose probably because production of insulin is inadequate or the body's cells do not act in response to insulin property or both. The patient will typically suffer polyuria, polydipsia and polyphagia and if left untreated can result to cardiovascular disease, stroke, kidney failure, foot ulcer and damage to the eyes [1,2]. In Nigeria, WHO has revealed that more than 171 million citizens above 15 years develop diabetes, 7000 children under 15 years develop type-1 diabetes every year and if nothing is done, the figure will rise to about 484 million worldwide by 2030 [3].

Plants derived drugs make up a significant part of natural product based pharmaceuticals. It is very imperative that the nutrients found in many foods, fruits, seeds and vegetables are responsible for the well documented health benefits. Many plants produce secondary compounds that are useful in the management of human diseases and have scavenging ability, cholesterol lowering ability, anti-inflammatory, antidiabetic, antimicrobial, antiviral, anticancer and antiparasitic activities [4]. Water melon is a popular member of the cucurbitaceae family. According to Jayaprakasha et al. [5] the seeds of some fruits such as *Citrullus lanatus* contains higher nutrients than other parts including the pulps. The seed has been reported to possess neuromodulatory, cardioprotective and hepatoprotective properties as a result of its predominant phytochemicals such as terpenoids, flavonoids, and cardioglycosides [6]. Based on these proposed effects of *C. lanatus* seeds, it was pertinent to provide scientific basis to its reported cardioprotective and metabolic effects.

### 2. MATERIALS AND METHODS

#### 2.1 Materials

**2.1.1 Drugs, chemicals and reagents**

Metformin (SKG-PHARMA, NAFDAC NO. A4-6597,0-7-18 to 0-7-23), Chloroform (Riedel-de Haen, England), Acetone (LobaChemie, India), Xylene (LobaChemie, India), Ether (LobaChemie, India), Normal saline(Kermel, China), Alcohol, Haematoxylin-eosin, 100% formalin (JHD China), Alloxan monohydrate (sigma-aldrich, Germany), ALP kit (Randox, united Kingdom), ALT kit (Randox, united Kingdom), AST KIT (Randox, united Kingdom), Cholesterol kits (Randox, united Kingdom), Triglycerides kit (Randox, united Kingdom), Pancreatic Amylase Elisa Kit was obtained from Abnova GmbH (Germany). All other reagents were of analytical grades.

**2.1.2 Experimental plants**

Water melon fruits (*Citrullus lanatus*) were purchased from Choba Market, Choba, Port Harcourt, Rivers State and Dr. E. Chimeze of Plant Science and Biotechnology (PSB) Department, University of Port Harcourt, Rivers State, identified and authenticated the plant seeds, with herbarium number UPH/P/183 and the specimen deposited in the herbarium.

**2.1.3 Experimental animals**

All experimental protocol aligned with stipulations of World Medical Association Declaration of Helsinki regarding ethical conduct of research involving animals, and appropriated by the Department of Biochemistry, Imo State University Ethics Committee (IMSU/BCM/ETS/20190405).

Thirty-three Wistar rats weighing 125-200 g of three months old bred in Animal House in Biochemistry Department, University of Port Harcourt were used for this research. The
animals were randomly selected, weighed and distributed into eleven groups and the females were separated from the male rats. These animals were put in plastic cages and left under suitable laboratory conditions for two weeks for them to adapt to the new environment before commencing the experiment. The cages were cleaned daily and the animals were given growers mash and water *ad libitum*. The animals’ body weights were recorded at the beginning and before the commencement of treatment.

### 2.2 Methods

#### 2.2.1 Aqueous extraction of *Citrullus lanatus* seeds

Ripe water melon fruits were collected and cut open; the seeds were washed, dried and blended in a warring blender. Four hundred grams of *Citrullus lanatus* seeds sample was macerated in 4000 mL of distilled water for one day in a macerating jar. Then the impurities were removed from the sample using Whatman No. 1 sieving paper. The filtrate was finally dried in a thermostat water bath at 60°C for use.

#### 2.2.2 Phytochemical screening

Test for various phytochemical constituents were carried out on *Citrullus lanatus* seed powder [7-9].

##### 2.2.2.1 Determination of saponin content

Saponin determination was done using the method of AOAC [10]. Saponin extraction was done using two different solvents. The first solvent, acetone, was used to extract crude lipid from the samples while the second solvent (methanol) was used for the extraction of the saponins proper. Two grams of the sample was folded and inserted into a thimble for easy extraction and put in a soxhlet extraction and a reflux condenser fitted on top. Extraction was done with acetone in a 250 mL capacity round bottomed flask for 3 hours, after which the apparatus was dismantled and another 150 mL capacity round bottomed flask containing 100 mL of methanol was fitted to the extractor and extraction was carried on for another 3 hours. The weight of the flask was taken before and after the second extraction in order to make the change in weight. At the end of the second extraction, the methanol was recovered by distillation and the flask was oven-dried to remove any remaining solvent in the flask. The flask was then allowed to cool and the weight of the flask taken. The saponin content of the sample was calculated thus:

\[
\% \text{ Saponin} = \frac{\text{Weight of saponin}}{\text{Weight of sample}} \times 100
\]

##### 2.2.2.2 Determination of alkaloid content

The gravimetric method of Harborne [7] was used in the determination of total alkaloid contents of the seeds. Five gram quantity of the sample was dispersed into 50 mL of 10% acetic acid solution in ethanol. The mixture was well shaken and allowed to stand for 4 hours before filtering. It was evaporated to one quarter of its original volume and drop wise concentration of ammonium hydroxide was added to precipitate the alkaloids. The precipitate was filtered off with a pre-weighed filter paper and washed with 1% ammonium hydroxide solution. The precipitate was ovened at 60°C for 30 mins and reweighed. The alkaloid contents of the sample were determined by using equation:

\[
\% \text{ Alkaloid} = \frac{W_2 - W_1 \times 100}{W}
\]

Where \(W\) = weight of sample; \(W_1\) = weight of empty filter paper; \(W_2\) = weight of paper + precipitate.

##### 2.2.2.3 Determination of flavonoid content

The flavonoid content was determined by the method of Milongo-Kone et al. [9].

Ten grams of the sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed. The flavonoid content of the sample was calculated thus:

\[
\% \text{ Flavonoid} = \frac{\text{Weight of flavonoid}}{\text{Weight of sample}} \times 100
\]

##### 2.2.2.4 Determination of tannin content

Analysis of the total phenolic compounds was determined by the method of Schofield et al. [8]. The extract was diluted with distilled water to a known concentration in order to obtain the readings within the standard curve range of 0.0 to 600 µg of tannin acid/mL. 250 µL of diluted extract or tannic acid solution was mixed with 1
mL of distilled water in a test tube followed by the addition of 250 µL of Folin-Ciocalteu reagent. The tube content was mixed well and then allowed to stand for 5 min at room temperature in order to allow complete reaction with the Folin-Ciocalteu reagent. Thereafter, 2.5 mL of 7% sodium carbonate aqueous solution was added and the final volume was made up to 6.0 mL with distilled water. The absorbance of the resulting blue color solution was measured spectrophotometrically at a wave length of 760 nm after incubating the sample for 90 min.

The determination of tannins content in the tested extracts was performed using Makkar et al., [11] method. Polyvinyl poly-pyrrolidone (PVPP) of 100 mg was weighed, and then 1.0 mL of distilled water was followed by 1.0 mL of the tannin containing extract was added. This mixture was stirred for 60 minutes, afterwards centrifuged for 10 min and finally the supernatant was collected.

The supernatant had only simple phenolic compounds other than tannins (the tannins would have been precipitated along with the PVPP. The phenolic content of the supernatant was then measured by the Folin-Ciocalteu reaction and this was accepted as the non-tannin phenols (NTP) [12]. The total tannins content was calculated as the difference between total phenol and non-tannin phenols. The content of non-tannin phenols was expressed on a dry plant basis (y%) and the percentage of tannins was calculated as tannin acid equivalent, estimated as grams per 100 grams dried plant as follows:

\[
\text{Tannin percentage} = (x\%) - (y\%)
\]

Where \(x\)% is the percentage of the total phenolic compounds (g/100g dried plant), \(y\)% is the content of the non-tannin phenols (g per 100 grams dried plant).

2.2.2.6 Determination of cyanogenic glycoside

Cyanogenic glycoside concentration was determined using alkaline picrate. An aliquot of the ground sample (5.0 g) was dissolved in 50 mL distilled water. The set up was allowed to stand overnight and then filtered [14]).

2.2.2.7 Preparation of cyanide standard curve

Different concentrations of KCN solution containing 0.1 mg/mL to 1.0 mg/mL cyanide were prepared. To 1 mL of the sample filtrate and standard cyanide solution in test tubes, 4 mL of alkaline picrate solution (1 g of picrate and 5 g of Na₂CO₃ in 200 mL distilled water) was added and incubated in water bath for 15 min. After color development, the absorbance was read in spectrophotometer at wavelength of 490 nm against blank containing only 1 mL distilled water and 4 mL alkaline picrate solution. The cyanide content was extrapolated from the cyanide standard curve.

Calculation:

\[
\text{Cyanogenic glycoside (mg/100 g)} = \frac{C \times (\text{mg}) \times 10}{\text{Weight of sample}}
\]

2.2.3 Acute toxicity of Citrullus lanatus seeds extract

According to the report of Varghese et al. [15], acute toxicity study showed zero mortality at a maximum dose level of 2000 mg/kg body weight of the extract after administration orally.

2.2.4 Induction of diabetes mellitus in wistar rats

This was done using alloxan monohydrate which was prepared by dissolving 0.9 g of alloxan monohydrate in 6 mL of distilled water to form a solution. Then 120 mg/kgb.w. of the alloxan monohydrate solution was administered to the rats intra peritoneally after the rats were made to fast overnight and diabetes was confirmed using the accu check active glucometer 3 days after alloxan administration [16,17].
2.2.5 Experimental design

The rats were divided into 11 groups of 3 rats in each and were treated as shown below.

Group 1: Normal rats which received distilled water for 21 days
Group 2: Untreated diabetic rats for 7 days
Group 3: Untreated diabetic rats for 21 days
Group 4: Diabetic rats treated with 100 mg/kg b.wt of metformin for 7 days
Group 5: Diabetic rats treated with 100 mg/kg b.wt of metformin for 21 days
Group 6: Diabetic rats treated with 200 mg/kg b.wt of the extract for 7 days
Group 7: Diabetic rats treated with 200 mg/kg b.wt of the extract for 21 days
Group 8: Diabetic rats treated with 400 mg/kg b.wt of the extract for 7 days
Group 9: Diabetic rats treated with 400 mg/kg b.wt of the extract for 21 days
Group 10: Diabetic rats treated with 600 mg/kg b.wt of the extract for 7 days
Group 11: Diabetic rats treated with 600 mg/kg b.wt of the extract for 21 days

Note: All treatments were done orally and alloxan administration was through intraperitoneal route.

2.2.6 Determination of biochemical parameters

2.2.6.1 Determination of the plasma glucose concentration

The plasma glucose was assayed using the multi Carein™ glucose strips and glucometer (Accu check, Roche, Germany) according to the procedures stipulated by Rheney and Kirk [18].

2.2.6.2 Determination of plasma pancreatic α-amylase activities

Alpha-amylase concentration was determined using Abnova Assay Kits (Abnova GmbH, Heidelberg, Germany). The immunoassay technique with a quantitative sand-wich enzyme was adopted from the protocol of Zakariya et al. [19].

2.2.6.3 Determination of plasma triglyceride concentration

Plasma triglyceride (Tg) concentration was assayed using the method of Roeschlaw [20].

2.2.6.4 Determination of total plasma cholesterol concentration

Total plasma cholesterol concentration was assayed using the method of Allain et al., [21].

2.2.6.5 Determination of plasma HDL-cholesterol concentration

Plasma HDL-concentration was assayed using the method of Lopes-Virella, [22].

2.2.6.6 Determination of plasma LDL-cholesterol concentration

Plasma LDL-cholesterol (LDL-C) concentration was calculated using the Friedewald equation [23] as follows:

\[(\text{LDL cholesterol}) \ (\text{mg/dL}) = (\text{Total cholesterol}) - (\text{HDL cholesterol}) - \frac{1}{5} (\text{Triglyceride})\]

2.2.7 Histology

The heart of a rat from each group after being excised were fixed in 5°C formaline for 2 hours after which the organs were dehydrated in graded alcohol (20%, 30%, 50%, 70%, 95%), 5 min each, cleared in xylene and embedded in paraffin. The resulting blocks were completely sectioned and randomized. The selected sections were stained in haemotoxylin and eosin and the slides were then examined at magnification of ×400 under optical microscope.

2.2.8 Statistical analysis

Data were expressed as Mean ± Standard error of Mean (M ± SEM) of triplicate values. Statistical analysis was done using statistical package for social science (SPSS) for window version (24) USA. Data were analysed using One Way Analysis of Variance (ANOVA), and multiple comparisons were done by post Hoc Tukey HSD at p ≤ 0.05 confidence level.

3. RESULTS

Tables 1a and 1b showed the phytochemical composition of Citrullus lanatus seeds sample. Qualitative analysis shown the presence of
flavonoids, alkaloids, tannin, saponin, oxalate and phytate with flavonoids having the highest composition \((\mu g)\) of 157.22 ± 0.43 followed by alkaloids \((34.87 \pm 0.46)\), saponin \((9.15 \pm 0.11)\), tannin \((8.95 \pm 0.09)\), oxalate \((6.75 \pm 0.06)\) and phytate \((1.45 \pm 0.09)\) which has the least concentration. Quantitative analysis showed the presence of different flavonoids with catechin having the highest value \((70.88 \pm 0.21)\) and anthocyanidines being the lowest \((1.91 \pm 0.02)\). Alkaloids shown the presence of spartein, lunamarin and quinine with lunamarin having the highest value \((13.23 \pm 0.15)\) followed by spartein \((12.98 \pm 0.05)\) and quinine \((8.66 \pm 0.26)\) having the least value.

Tables 2 and 3 showed the plasma glucose and pancreatic amylase concentration of the rats in the diabetic control groups (groups 2 and 3) were significantly \((P \leq 0.05)\) increased when compared to those in the normal control group (group 1). In the groups treated with the extract (groups 6, 7, 8, 9, 10 and 11) there was a significant \((p \leq 0.05)\) decrease when compared to groups 2 and 3.

Tables 4, 5, 6 and 7 showed the plasma T.C, T.G, HDL and LDL concentrations of the rats. The levels in the diabetic control groups (groups 2 and 3) significantly \((p \leq 0.05)\) increased while in the HDL there was a significant \((p \leq 0.05)\) decrease in the concentration of HDL when compared to those in the normal control group (group 1) and in the groups treated with the extract (group 6, 7, 8, 9, 10 and 11). Also, there was a significant \((p \leq 0.05)\) decrease in T.C, T.G and LDL while there was a significant \((p \leq 0.05)\) increase in HDL levels when compared to group 2 and 3.

### Table 1a. Qualitative phytochemical composition of *Citrullus lanatus* seeds sample

| Parameters  | Observation | Remark            |
|-------------|-------------|-------------------|
| Flavonoids  | + + +       | Very high          |
| Alkaloids   | + +         | High concentration |
| Saponin     | + +         | High concentration |
| Tannin      | +           | Low concentration  |
| Oxalate     | +           | Low concentration  |
| Phytate     | +           | Low concentration  |

### Table 1b. Quantitative phytochemical composition of *Citrullus lanatus* seeds sample

| Parameters  | Composition \((\mu g/g)\) |
|-------------|---------------------------|
| Flavonoids  | 176.87 ± 1.56             |
| Alkaloids   | 34.87 ± 0.46              |
| Saponin     | 1.45 ± 0.09               |
| Tannin      | 8.95 ± 0.09               |
| Oxalate     | 9.15 ± 0.11               |
| Phytate     | 6.75 ± 0.06               |

Values represent Mean ± SEM of triplicate sample

### Table 2. Effect of varying concentrations of aqueous extract of *Citrullus lanatus* seeds on glucose concentration in alloxan induced-diabetic wistar rats treated for 7 & 21 days

| Group | Treatment | Glucose level \((\text{mg/dl})\) |
|-------|-----------|---------------------------------|
| 1     | Normal control | 89.00 ± 2.31<sup>b</sup>     |
| 2     | Alloxan treated only | 212.00 ± 2.31<sup>a</sup>    |
| 3     | Alloxan treated only | 250.00 ± 2.34<sup>abc</sup>  |
| 4     | Alloxan (120 mg/kg b.w) + metformin (100 mg/kg b.w) | 207.00 ± 0.58<sup>a</sup>   |
| 5     | Alloxan (120 mg/kg b.w) + metformin (100 mg/kg b.w) | 194.00 ± 0.58<sup>abc</sup> |
| 6     | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (200 mg/kg b.w) | 195.00 ± 0.58<sup>abc</sup> |
| 7     | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (200 mg/kg b.w) | 171.00 ± 0.63<sup>abc</sup> |
| 8     | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (400 mg/kg b.w) | 181.00 ± 0.58<sup>abc</sup> |
| 9     | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (400 mg/kg b.w) | 130.67 ± 5.49<sup>abc</sup> |
| 10    | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (600 mg/kg b.w) | 182.00 ± 0.58<sup>abc</sup> |
| 11    | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (600 mg/kg b.w) | 98.00 ± 4.62<sup>abc</sup>  |

Values are represented as Mean ± Standard error of mean \((M±SEM)\); \(n=3\) per group. Figures in the same column with superscript \((a, b, c)\) are significantly different at \(p \leq 0.05\).

**Superscript (a)** represent significant difference when group 1 is compared to other groups at \(p \leq 0.05\).

**Superscript (b)** represent significant difference when group 3 is compared to other groups at \(p \leq 0.05\).

**Superscript (c)** represent significant difference when group 5 is compared to other groups at \(p \leq 0.05\).

Figures that does not have superscript shown no significant difference when group 1, 3 and 5 was compared with the remaining groups at \(p \leq 0.05\).
### Table 3. Effect of varying concentrations of aqueous extract of *Citrullus lanatus* seeds on α-amylase activities in alloxan induced-diabetic wistar rats treated for 7 & 21 days

| Group | Treatment | α - Amylase (µ/l) |
|-------|-----------|------------------|
| 1     | Normal control | 17.00 ± 0.58<sup>b</sup> |
| 2     | Alloxan treated only | 29.00 ± 0.58<sup>c</sup> |
| 3     | Alloxan treated only | 29.00 ± 1.73<sup>c</sup> |
| 4     | Alloxan (120 mg/kg b.w) + metformin (100 mg/kg b.w) | 21.00 ± 0.58<sup>bc</sup> |
| 5     | Alloxan (120 mg/kg b.w) + metformin (100 mg/kg b.w) | 19.00 ± 0.58<sup>bc</sup> |
| 6     | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (200 mg/kg b.w) | 20.67 ± 0.88<sup>abc</sup> |
| 7     | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (200 mg/kg b.w) | 18.67 ± 1.45<sup>abc</sup> |
| 8     | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (400 mg/kg b.w) | 20.00 ± 0.58<sup>bc</sup> |
| 9     | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (400 mg/kg b.w) | 17.67 ± 1.45<sup>bc</sup> |
| 10    | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (600 mg/kg b.w) | 21.67 ± 0.88<sup>abc</sup> |
| 11    | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (600 mg/kg b.w) | 14.67 ± 1.45<sup>abc</sup> |

Values are represented as Mean ± Standard error of mean (M ± SEM); n =3 per group. Figures in the same column with superscript (a, b, c) are significantly different at p ≤ 0.05. Superscript (a) represent significant difference when group 1 is compared to other groups at p ≤ 0.05. Superscript (b) represent significant difference when group 3 is compared to other groups at p ≤ 0.05. Superscript (c) represent significant difference when group 5 is compared to other groups at p ≤ 0.05. Figures that does not have superscript shown no significant difference when group 1, 3 and 5 was compared with the remaining groups at p ≤ 0.05.

### Table 4. Effect of varying concentrations of aqueous extract of *Citrullus lanatus* seeds on total cholesterol in alloxan induced-diabetic wistar rats treated for 7 & 21 days

| Group | Treatment | T.C (mg/dl) |
|-------|-----------|-------------|
| 1     | Normal control | 1.77 ± 0.03<sup>b</sup> |
| 2     | Alloxan treated only | 2.97 ± 0.15<sup>ac</sup> |
| 3     | Alloxan treated only | 3.17 ± 0.20<sup>ac</sup> |
| 4     | Alloxan (120 mg/kg b.w) + metformin (100 mg/kg b.w) | 2.04 ± 0.08<sup>b</sup> |
| 5     | Alloxan (120 mg/kg b.w) + metformin (100 mg/kg b.w) | 3.17 ± 0.20<sup>ac</sup> |
| 6     | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (200 mg/kg b.w) | 1.91 ± 0.02<sup>b</sup> |
| 7     | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (200 mg/kg b.w) | 1.87 ± 0.09<sup>b</sup> |
| 8     | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (400 mg/kg b.w) | 2.10 ± 0.12<sup>b</sup> |
| 9     | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (400 mg/kg b.w) | 1.90 ± 0.05<sup>b</sup> |
| 10    | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (600 mg/kg b.w) | 1.80 ± 0.06<sup>b</sup> |
| 11    | Alloxan (120 mg/kg b.w) + *C.l* seeds extract (600 mg/kg b.w) | 1.80 ± 0.06<sup>b</sup> |

Values are represented as Mean ± Standard error of mean (M ± SEM); n =3 per group. Figures in the same column with superscript (a, b, c) are significantly different at p ≤ 0.05. Superscript (a) represent significant difference when group 1 is compared to other groups at p ≤ 0.05. Superscript (b) represent significant difference when group 3 is compared to other groups at p ≤ 0.05. Superscript (c) represent significant difference when group 5 is compared to other groups at p ≤ 0.05. Figures that does not have superscript shown no significant difference when group 1, 3 and 5 was compared with the remaining groups at p ≤ 0.05.
Table 5. Effect of varying concentrations of aqueous extract of *Citrullus lanatus* seeds on triglycerides in alloxan induced-diabetic wistar rats treated for 7 & 21 days

| Group | Treatment | T.G (mg/dl) |
|-------|-----------|-------------|
| 1     | Normal control | 0.90 ± 0.04 |
| 2     | Alloxan treated only | 1.25 ± 0.06ac |
| 3     | Alloxan treated only | 1.28 ± 0.05bc |
| 4     | Alloxan (120 mg/kg b.w) + metformin (100 mg/kg b.w) | 0.99 ± 0.08 |
| 5     | Alloxan (120 mg/kg b.w) + metformin (100 mg/kg b.w) | 0.99 ± 0.06 |
| 6     | Alloxan (120 mg/kg b.w) + *C.* seeds extract (200 mg/kg b.w) | 0.96 ± 0.01 |
| 7     | Alloxan (120 mg/kg b.w) + *C.* seeds extract (200 mg/kg b.w) | 0.99 ± 0.00 |
| 8     | Alloxan (120 mg/kg b.w) + *C.* seeds extract (400 mg/kg b.w) | 0.95 ± 0.02 |
| 9     | Alloxan (120 mg/kg b.w) + *C.* seeds extract (400 mg/kg b.w) | 0.95 ± 0.06 |
| 10    | Alloxan (120 mg/kg b.w) + *C.* seeds extract (600 mg/kg b.w) | 0.92 ± 0.03c |
| 11    | Alloxan (120 mg/kg b.w) + *C.* seeds extract (600 mg/kg b.w) | 0.94 ± 0.09 |

Values are represented as Mean ± Standard error of mean (M ± SEM); n =3 per group. Figures in the same column with superscript (a, b, c) are significantly different at p ≤ 0.05. Superscript (a) represents significant difference when group 1 is compared to other groups at p ≤ 0.05. Superscript (b) represents significant difference when group 3 is compared to other groups at p ≤ 0.05. Superscript (c) represents significant difference when group 5 is compared to other groups at p ≤ 0.05. Figures that does not have superscript shown no significant difference when group 1, 3 and 5 was compared with the remaining groups at p ≤ 0.05.

Table 6. Effect of varying concentrations of aqueous extract of *Citrullus lanatus* seeds on high density lipoprotein in alloxan induced-diabetic wistar rats treated for 7 & 21 days

| Group | Treatment | HDL (mg/dl) |
|-------|-----------|-------------|
| 1     | Normal control | 0.85 ± 0.02ac |
| 2     | Alloxan treated only | 0.23 ± 0.04a |
| 3     | Alloxan treated only | 0.26 ± 0.02a |
| 4     | Alloxan (120 mg/kg b.w) + metformin (100mg/kg b.w) | 0.34 ± 0.05a |
| 5     | Alloxan (120 mg/kg b.w) + metformin (100 mg/kg b.w) | 0.41 ± 0.00a |
| 6     | Alloxan (120 mg/kg b.w) + *C.* seeds extract (200 mg/kg b.w) | 0.42 ± 0.17a |
| 7     | Alloxan (120 mg/kg b.w) + *C.* seeds extract (200 mg/kg b.w) | 0.56 ± 0.14a |
| 8     | Alloxan (120 mg/kg b.w) + *C.* seeds extract (400 mg/kg b.w) | 0.49 ± 0.00a |
| 9     | Alloxan (120 mg/kg b.w) + *C.* seeds extract (400 mg/kg b.w) | 0.78 ± 0.06a |
| 10    | Alloxan (120 mg/kg b.w) + *C.* seeds extract (600 mg/kg b.w) | 0.68 ± 0.07a |
| 11    | Alloxan (120 mg/kg b.w) + *C.* seeds extract (600 mg/kg b.w) | 0.70 ± 0.02a |

Values are represented as Mean ± Standard error of mean (M ± SEM); n =3 per group. Figures in the same column with superscript (a, b, c) are significantly different at p ≤ 0.05. Superscript (a) represent significant difference when group 1 is compared to other groups at p ≤ 0.05. Superscript (b) represent significant difference when group 3 is compared to other groups at p ≤ 0.05. Superscript (c) represent significant difference when group 5 is compared to other groups at p ≤ 0.05. Figures that does not have superscript shown no significant difference when group 1, 3 and 5 was compared with the remaining groups at p ≤ 0.05.
Table 7. Effect of varying concentrations of aqueous extract of \textit{Citrullus lanatus} seeds on low density lipoprotein in alloxan induced-diabetic wistar rats treated for 7 & 21 days

| Group | Treatment                                                                 | LDL (mg/dl)       |
|-------|---------------------------------------------------------------------------|-------------------|
| 1     | Normal control                                                             | 0.76 ± 0.00\textsuperscript{a} |
| 2     | Alloxan treated only                                                       | 1.09 ± 0.00       |
| 3     | Alloxan treated only                                                       | 1.10 ± 0.00       |
| 4     | Alloxan (120 mg/kg b.w) + metformin (100 mg/kg b.w)                        | 0.95 ± 0.090      |
| 5     | Alloxan (120 mg/kg b.w) + metformin (100 mg/kg b.w)                        | 0.83 ± 0.02       |
| 6     | Alloxan (120 mg/kg b.w) + \textit{C.l} seeds extract (200 mg/kg b.w)       | 0.99 ± 0.02\textsuperscript{b} |
| 7     | Alloxan (120 mg/kg b.w) + \textit{C.l} seeds extract (200 mg/kg b.w)       | 0.93 ± 0.00\textsuperscript{b} |
| 8     | Alloxan (120 mg/kg b.w) + \textit{C.l} seeds extract (400 mg/kg b.w)       | 0.89 ± 0.02\textsuperscript{b} |
| 9     | Alloxan (120 mg/kg b.w) + \textit{C.l} seeds extract (400 mg/kg b.w)       | 0.82 ± 0.02\textsuperscript{b} |
| 10    | Alloxan (120 mg/kg b.w) + \textit{C.l} seeds extract (600 mg/kg b.w)       | 0.87 ± 0.05       |
| 11    | Alloxan (120 mg/kg b.w) + \textit{C.l} seeds extract (600 mg/kg b.w)       | 0.80 ± 0.04       |

Values are represented as Mean ± Standard error of mean (M±SEM); n =3 per group. Figures in the same column with superscript (a, b, c) are significantly different at \( p \leq 0.05 \)

Superscript (a) represent significant difference when group 1 is compared to other groups at \( p \leq 0.05 \)

Superscript (b) represent significant difference when group 3 is compared to other groups at \( p \leq 0.05 \)

Superscript (c) represent significant difference when group 5 is compared to other groups at \( p \leq 0.05 \)

Figures that does not have superscript shown no significant difference when group 1, 3 and 5 was compared with the remaining groups at \( p \leq 0.05 \)
HISTOPATHOLOGY OF THE PANCREAS

Fig. 1. Photomicrograph of Pancreatic tissue given distilled water for 21 days (group 1- control) Mag. × 400 (H ×E staining)

Fig. 2. Photomicrograph of Pancreatic tissue administered 120 mg/kg b.w of alloxan only for 21 days (group 3-negative control) Mag. × 400 (H ×E staining)

Fig. 3. Photomicrograph of Pancreatic tissue of alloxan-induced diabetic rats treated with 100 mg/kg b.w of metformin for 21 days (group 5-positive control) Mag. × 400 (H ×E staining)
Fig. 4. Photomicrograph of Pancreatic tissue of alloxan-induced diabetic rats treated with 200 mg/kg b.w of aqueous extract of *C. lanatus* seeds for 7 days (group 6) Mag. × 400 (H ×E staining)

Fig. 5. Photomicrograph of Pancreatic tissue of alloxan-induced diabetic rats treated with 200 mg/kg b.w of aqueous extract of *C. lanatus* seeds for 21 days (group 7) Mag. × 400 (H ×E staining)

Fig. 6. Photomicrograph of Pancreatic tissue of alloxan-induced diabetic rats treated with 400 mg/kg b.w of aqueous extract of *C. lanatus* seeds for 7 days (group 8) Mag. × 400 (H ×E staining)
Fig. 7. Photomicrograph of Pancreatic tissue of alloxan-induced diabetic rats treated with 400mg/kg b.w of aqueous extract of *C. lanatus* seeds for 21 days (group 9) Mag. × 400 (H ×E staining)

Fig. 8. Photomicrograph of Pancreatic tissue of alloxan-induced diabetic rats treated with 600mg/kg b.w of aqueous extract of *C. lanatus* seeds for 7 days (group 10) Mag. × 400 (H ×E staining)

Fig. 9. Photomicrograph of pancreatic tissue of alloxan-induced diabetic rats treated with 600 mg/kg b.w of aqueous extract of *C. lanatus* seeds for 21 days (group 11) Mag. × 400 (H ×E staining)
4. DISCUSSION

On induction of diabetes, insulin was deficient leading to increased blood glucose level and entry of glucose into cells will be inefficient and hence cells are starved of glucose [24]. Treatment with aqueous extract of *Citrullus lanatus* seeds showed a significant (p≤0.05) decrease in the plasma glucose levels in all the treated groups in a time and dose dependent manner. The extract may be acting through the release of insulin for insulin facilitates the membrane transport of glucose. This findings correlates with the reports that insulin lowers blood glucose level by promoting utilization and storage of glucose in the liver [24,25].

Alpha-amylase activities of rats in the diabetic control groups increased significantly (p≤0.05) when compared to those in the normal group which is consistent with the reports of Date et al., [26] showing that induction of diabetes caused damage on the pancreas leading to the release of α-amylase in pancreatic juice thereby metabolizing carbohydrate (maltose) to glucose resulting to rise in blood glucose level. Treatment with aqueous extract of *Citrullus lanatus* seeds significantly (p≤0.05) reduced α-amylase activities in all the treated groups. This findings correlate with the reports that inhibitory effect of α-amylase limits the process of carbohydrate metabolism and absorption in the intestine leading to a decreased in blood glucose level [27,28].

There were significant increases in levels of triglycerides, cholesterol, low density lipoprotein-cholesterol and significant (p≤0.05) decreased in level of high density lipoprotein-cholesterol in the diabetic negative control groups when compared to the normal control group in this study. This is in line with reports of Nwanjo, [29] and Aynila et al., [30] showing that rise in glucose on induction of diabetes resulted to a corresponding increased in plasma lipids. Hyperlipidemia a risk factor for cardiovascular disease is one of the complications of diabetes mellitus characterized by elevated levels of cholesterol, triglycerides, phospholipids and other lipoproteins [31]. The aqueous extract of *citrullus lanatus* exhibited antilipidemic effect by reducing cholesterol, triglycerides and low density lipoprotein-cholesterol while HDL-cholesterol levels were increased in all the extract treated groups for 7 and 21 days. The observed anti-hyperlipidemia and hyperglycemia effects of the aqueous extract of *Citrullus lanatus* could be linked to the high concentration of flavonoids, alkaloids and tannins which are strong antioxidants that can act against free radical scavenging which destroys the β-cells of pancreas and enhance insulin secretion [32,33]. This implies that the free radical scavenging properties of the *C. lanatus* mitigates the diabetogenic effects of alloxan. Phytochemicals also help to protect organs from oxidative damage [33] lending credence to the amelioration of the insulin secreting cells. The antioxidant activity of flavonoids depends on their ability to donate protons and electrons to resist the effect of energetic oxidants such as free radicals [34]. The results of the histological examination clearly showed that *C. lanatus* potentiated the amelioration of the pancreas and could suffice as an antidiabetic regimen.

5. CONCLUSION

The result of this study shows that aqueous extract of *Citrullus lanatus* seeds exhibited antidiabetic effect against alloxan-induced diabetic mellitus in wistar rats. Hence *Citrullus lanatus* seeds could be a possible antidiabetic agent in the management of diabetes mellitus.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

All experimental protocol aligned with stipulations of World Medical Association Declaration of Helsinki regarding ethical conduct of research involving animals, and appropriated by the Department of Biochemistry, Imo State University Ethics Committee (IMSU/BCM/ETS/20190405).

COMPETING INTERESTS

Authors have declared that no competing interests exist.
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