

Proximate Analysis and Effect of Aqueous Leaf Extract of *Luffa aegyptiaca* on Some Biochemical, Hematological Parameters and Histology of The Liver and Kidney of Alloxan-Induced Type 1 Diabetes in Rats

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Abstract

This research investigated the nutritional components of leaves of *Luffa aegyptiaca* (sponge gourd) and the effects of its aqueous leaf extract on serum lipid profile, serum liver markers, serum renal indices, blood glucose level, some hematological indices and histology of the liver and kidney of male wistar rats induced with alloxan at 150mg/kg. The result of the proximate composition revealed a high value of carbohydrate (47.89%). A total of 45 rats with weight range of 80-155g were used for this study and were randomly divided into six groups where group 1 served as normal control (those not induced and not treated), group 2 served as diabetic control (those induced but not treated), group 3,4 and 5 were administered 100, 200 and 300mg/kg b.w aqueous leaf extract of *Luffa aegyptiaca* extract respectively (those induced and treated with plant extract) and group 6 were treated with 100mg/kg b.w. of metformin (those induced and treated with the standard diabetic drug). The result revealed that there was a significant ($p<0.05$) decrease in AST and ALT activities in extract treated groups when compared with the diabetic control. However, there was a significant ($p<0.05$) increase in total protein concentration in the extract treated groups when compared with the diabetic control. Furthermore, there was a significant ($p<0.05$) decrease in urea and creatinine concentrations in all extract treated groups when compared with the diabetic control. Similarly, bicarbonate levels were significantly ($p<0.05$) decreased in all extract treated groups when compared with the diabetic control. More so, total cholesterol and high density lipoprotein levels showed a significant ($p<0.05$) decrease in all extract treated groups when compared with the diabetic control. Similarly, low density lipoprotein levels were significantly ($p<0.05$) decreased in all extract treated groups when compared with the diabetic control. Furthermore, the results of the Packed cell volume and Hemoglobin levels showed a significant ($p<0.05$) increase in all extract treated groups when compared with the diabetic control. However, mean cell volume levels were significantly ($p<0.05$) decreased in extract treated groups when compared with the diabetic control. Moreover, there was a significant ($p<0.05$) decrease in the blood glucose level in all extract treated group when compared to the diabetic control. Furthermore, the histology of the liver and kidney of treated groups showed that aqueous leaf extract of *Luffa aegyptiaca* ameliorated the damage caused by alloxan on the organs. In conclusion, the administration of aqueous leaf extract of *Luffa aegyptiaca* mitigated the level of damage induced by alloxan in male wistar rats.

Keywords: *Luffa aegyptiaca*, sponge gourd, diabetes, glycemic control, antioxidant, phytochemical composition.

1. Introduction

From time immemorial, plants have always been a component of human life; mostly for therapeutic purposes (traditional herbal medicines). Indigenous medicine systems all around the world use medicinal plants as a key component. Traditional practitioners and their arsenal of medicinal herbs are relied on by a considerable proportion of the population in many developing nations to address health care needs. Regardless of the fact that modern medicine coexists alongside such ancient practices, herbal remedies have remained popular for historical and cultural reasons (Hosseinzadeh et al., 2015).

Diabetes mellitus is a chronic disease distinguished by high blood glucose levels in the fasting and after meal periods due to insulin resistance and relative insulin insufficiency. Diabetes patients will eventually develop nephropathy, neuropathy, retinopathy, diabetic foot ulcers, ketoacidosis and even high risk of cardiovascular diseases like hypertension etc. (Tiwari et al., 2014). In high-income countries, type 2 diabetes accounts for 85-95% of all diabetes, with a higher prevalence in low-income (developing) countries. The inappropriate use of insulin by target cells and tissues is closely linked to this surge. Diabetes is currently a widespread and serious health issue all over the world (Piero et al., 2014).

Modern medicine has made a significant step forward by focusing on “Alternative Medicine” which accentuates the utilization of plants for medicinal purposes. Medicinal herbs are commonly utilized as source materials for extracting active components needed for drug production. In addition, unlike chemically synthesized medications, the constituents in plants interact simultaneously, allowing their applications to complement or disrupt others while also neutralizing their potential bad effects (Hassan, 2012).

Diabetes mellitus, formerly considered a small concern to global health, has now risen to become one of the most serious threats to human health in the 21st century (Tripathi & Srivastava, 2006). Various organ failures as well as progressive metabolic problems such as retinopathy, nephropathy, and/or neuropathy are all severe outcomes of diabetes mellitus. Diabetics are more likely to develop cardiovascular, peripheral vascular and cerebrovascular disorders (Piero et al., 2014). Diabetes mellitus is treated with a combination of diet, exercise and medication. Insulin and hypoglycemic medicines are currently the most common and efficient treatments for diabetes; nevertheless, these medications come with a myriad of negative side effects. Considering these aforementioned factors, it's imperative to research on medicinal herbs having antidiabetic effects for diabetes treatment (Moradi et al., 2018).

The work is aimed at evaluating the effect of oral administration of aqueous leaf extract of *Luffa aegyptiaca* on alloxan-induced type 1 diabetes in male wistar rats. *Luffa aegyptiaca* is a plant from the cucumber family grown for its multipurpose fruit in many tropical countries. The leaves are being used locally for the treatment of diabetes by the Urhobos in Delta State. Therefore, this study will authenticate or refute the claims of *Luffa aegyptiaca* possessing antidiabetic properties. This study is limited to the effect of aqueous leaf extract of *Luffa aegyptiaca* on the serum liver and kidney marker enzymes, lipid profile, blood glucose level, hematological indices and the histopathology of the liver and kidney of alloxan-induced male wistar rats.

2. Methodology

2.1. Chemicals and Reagents

Alloxan Monohydrate	Loba Chemie Mumbai, India
Sodium Chloride	JHD Shantou, Guangdong, China
Gluformin (Metformin hydrochloride 500mg)	Nigerian-German Chemicals Plc
Sodium hydroxide	Titan Biotech Ltd, Bhiwadi-Rajasthan, India
Acetone	Loba Chemie Mumbai, India

Sulphuric acid	Loba Chemie Mumbai, India
Hydrochloric acid	Loba Chemie Mumbai, India
Chloroform	M&B laboratory reagent May & Baker Ltd Dagenham England.

2.2. Equipment

Rotary Evaporator RH – HB	(Buchi labortechnik AG Switzerland CH-9230).
Water bath – Super fit digital	(Superfit continental (p) Ltd Mumbai 343).
Glucometer (ACCU-CHEK Active)	(Roche Diagnostics, Mannheim, Germany).
Laboratory Oven	(TT-9023A Techmel & Techmel USA).
Electronic Weighing Balance	(Life Assistance Scientific Co. UK).
Furnace	(Vecstar Ltd. Chesterfield U.K).

2.3. Collection of plant material and identification

Luffa aegyptiaca leaves were harvested from an open space field at 11 Omoteko Anikori Street, Okuokoko, Delta State, Nigeria in the Month of July, 2021. The specimen was identified and authenticated by a staff of the Department of Plant Biology and Biotechnology, University of Benin, Benin City Edo State, Nigeria.

2.4. Animals

Forty-five healthy male Wistar rats weighing between 80 and 155g were obtained from the Animal house of the Department of Biochemistry, University of Port Harcourt. They were acclimatized for one week. The rats were maintained on standard pellets of growers mash (Top feed) and water ad libitum.

2.5. Extract preparation

The Fresh leaves of *Luffa aegyptiaca* were washed with distilled water, debris were removed and air dried for four weeks and then reduced to coarse powder using a manual grinder. Then, 100 grams of coarsely powdered leaves were extracted with 400 ml of distilled water using cold maceration for 24 hours (A total of 368.853 grams of powdered form of the plant was used for the study). The extract was filtered through a cheese cloth with fine pore, and the filtrate was filtered for the second time using Whatman filter paper to have a pure aqueous crude extract. The resulting extract was then concentrated at 50oC in a rotary evaporator for a period of 2 hours and water bath at 40oC to yield a dark brown mass. The obtained extract was put in a glass container and stored at 4oC until when required for use. The percentage yield of the extract was calculated using the formula

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of plant material}} \times \frac{100}{1} \quad (1)$$

The extraction process was carried out using the extraction method as reported by Ogunka-Nnoka et al., (2019).

2.6. Acute toxicity study

The analysis of toxic, cytotoxic and genotoxic damage generated by plant compounds is of crucial importance in curbing the possible dangers of these agents, particularly when they are part of long-term treatment. A genotoxicity and antigenotoxicity study of aqueous and hydro-methanol extracts of *Spondias mombin*, *Nymphaea lotus* and *Luffa cylindrica* (*Luffa aegyptiaca*) using animal bioassays carried out by Oyeyemi et al., 2015 indicated that the extracts, each at a dose of 5000mg/kg, did not result in mortality or any clinical sign of toxicity. This experiment led to the conclusion that the lethal dose (LD50) of the tested extracts in mice is higher than 5000mg/kg. Therefore, these extracts can be considered non-toxic (Oyeyemi et al., 2015).

2.7. Proximate Analysis

The moisture content, ash, crude fibre, crude fat, crude protein and total carbohydrate values were determined using standard analytical protocol (AOAC, 1990).

2.7.1. Determination of Moisture Content

A glass Petri-dish was washed and dried in an oven, the weight of the dried petri dish (W_1) was taken. Then, 3 grams of the sample was weighed and put into the petri-dish (W_2) and oven dried at 105°C for 2 hours, then the petri-dish was removed and allowed to cool. The petri-dish containing a dried sample was weighed (W_3).

$$\% \text{Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad (2)$$

where,

W_1 = initial weight of empty petri-dish

W_2 = weight of petri-dish + sample before drying

W_3 = final weight of petri-dish + sample after drying

2.7.2. Determination of Ash Content

A crucible was washed and dried in an oven, the weight of the dried crucible (W_1) was recorded. Then, 3 grams of the sample was weighed into the crucible (W_2). The sample was turned to ash in a furnace at 600–700°C for 2 hours. The crucible was removed from the furnace and allowed to cool in a desiccator and weighed (W_3).

$$\% \text{Ash (dry basis)} = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \quad (3)$$

where,

W_1 = weight of empty crucible

W_2 = weight of crucible + sample before ashing

W_3 = weight of crucible + ash

2.7.3. Determination of Crude Fibre

Three grams of sample was weighed into a filter paper (W_1). To it 150 ml of 1.25% of H_2SO_4 (Sulphuric acid) was added. A beaker was then placed on digestion apparatus with a pre-adjusted heater and boiled for 30 minutes. The beaker was rotated from time to time to keep solids from clinging to the sides. The beaker was removed and its content was filtered with a separating funnel. The beaker was rinsed with boiling water and washed thoroughly through the funnel. The residue was returned to the beaker by blowing through the funnel and 150 ml of boiling 1.25% of NaOH (Sodium hydroxide) solution was added. It was boiled for 30 minutes, after which the beaker was removed and filtration was done for a second time. The residue was dried at $130^{\circ}C$ for 2 hours, and was then cooled in a desiccator and weighed (W_2). It was then incinerated at $600^{\circ}C$ to constant weight for about 30 minutes, after which it was cooled in desiccator and weighed (W_3).

$$\% \text{ Crude fibre} = \frac{W_2 - W_3}{W_1} \times 100 \quad (4)$$

2.7.4. Determination of Crude Fat

Filter paper was folded into the shape of a thimble and weighed, then its weight was zeroed. After that, three grams of the sample was weighed into the thimble. The thimble was then slipped into a thimble holder. 250 ml of acetone was measured into the extraction flask and was connected to the condenser and heated on hot plate, followed by extraction for a minimum of 4 hours on a high setting (that has condensation rate of 5-6 drops per second). After extraction, the heater and water tap were turned off, and the acetone (with the fat extract) was transferred into a beaker of known weight (W_1); the thimble was rinsed with more acetone. The beaker was taken into an oven at $70^{\circ}C$ for about 30 minutes. It was then allowed to cool and the acetone was drained out. The weight of the beaker and the fat it contains was weighed (W_2)

$$\% \text{ Crude Fat} = \frac{W_2 - W_1}{\text{Weight of sample (3g)}} \times 100 \quad (4)$$

where,

W_1 = weight of beaker

W_2 = weight of beaker (g) + fat extract (g)

2.7.5. Determination of Crude Protein

Two grams of sample was weighed into the digestion tube. Then, 15 ml of H_2SO_4 and 5g of kjeldahl catalyst mixture was added, the tube was swirled gently until the sample and acid were properly mixed. The solution was heated intensely for 2 hours until it became clear. The solution was allowed to cool and then transferred to a 100 ml volumetric flask and diluted to full volume with distilled water and mixed thoroughly, bringing an end to the digestion process. After this, the digest was subjected to distillation. 10 ml of 2% of boric acid was measured into a 100 ml conical flask after which 1-2 drops of mixed indicator was added. 10ml aliquot of the digest was transferred into a distillation apparatus. 15 ml of 40% of NaOH was added to the mixture. The nitrogen distilled into the boric acid/indicator flask for at least 10-15 minutes after which the condenser tip was rinsed with distilled water. The distillate was then titrated with 0.025N H_2SO_4 to give a pink end point and the burette reading was taken.

$$\%N = \frac{\frac{0.014(\text{MeN})}{100} \times \text{Titre Value} \times \text{digest volume (100 ml)} \times \text{normality of acid (0.025)}}{\text{Weight of sample (2 grams)} \times \text{Volume of aliquot used (10 ml)}} \times 100\% \quad (5)$$

So, Total crude protein = %N x 6.25.

Where,

0.014= Milli equivalent weight of Nitrogen (MeN).

2.7.6. Determination of Total Carbohydrate

Total carbohydrate as a Nitrogen Free Extract (NFE) was calculated by difference (The addition of values of Crude protein, crude fibre, moisture, ash and crude fat then subtracting them from 100).

Carbohydrate (NFE) = 100 – (% Protein + % Fibre + % Moisture + % Ash + % Fat)

2.8. Experimental Induction of Diabetes Mellitus

Diabetes Mellitus was induced by single intraperitoneal injection of alloxan monohydrate at a dose of 150mg/kg body weight (BW) dissolved in 0.9% normal saline solution. The rats were fasted for 12 hours before administration of alloxan monohydrate. After 72 hours, diabetes was confirmed in the alloxan induced rats whose fasting blood glucose level was 200mg/dl and above. Blood was obtained from the tail vein of the rats, and placed on a glucometer to determine the fasting blood glucose level (FBGL) (Iweala *et al.*, 2013).

2.9. Experimental Design

In the experiment, a total of forty-five (45) male Wistar rats were used. They were randomly divided into 6 groups. Group 1 contained 5 rats and the remaining groups containing 8 rats each. The blood glucose level was monitored every four days for sixteen days.

- Group 1: Normal rats that received distilled water orally (Normal control).
- Group 2: Alloxan induced rats that received distilled water orally (Diabetic control).
- Group 3: Alloxan induced rats that received 100mg/kg body weight of aqueous leaf extract of *Luffa aegyptiaca* orally.
- Group 4: Alloxan induced rats that received 200mg/kg body weight of aqueous leaf extract of *Luffa aegyptiaca* orally.
- Group 5: Alloxan induced rats that received 300mg/kg body weight of aqueous leaf extract of *Luffa aegyptiaca* orally.
- Group 6: Alloxan induced rats that received 100mg/kg body weight of Standard drug, Metformin orally.

2.10. Collection and preparation of sera samples and tissue for histology

The study lasted for two (2) weeks after which the rats were fasted for 12 hours before they were sacrificed. A desiccator containing chloroform soaked cotton wool at the base (bottom of the desiccator) was prepared, the experimental animal was then placed inside the desiccator and the lid was covered tightly. The rats remain in the desiccator for about a minute to inhale the chloroform. The effect of the chloroform led to dizziness and suffocation which caused the rats to pass out. With properly gloved hands, a sharp surgical blade was used to cut open the body cavity of the animal; then with the aid of a syringe, blood is collected. Blood samples were collected into plain EDTA tubes that have been labelled accordingly. Tissue organelles of the liver and kidney were also collected for histopathological studies. The blood samples and tissue organelles were taken to the laboratory for analysis.

2.11. Determination of biochemical parameters

2.11.1. Determination of Aspartate transaminase (Reitman and Frankel, 1975)

Principle

α -Oxoglutarate + L-aspartate $\xrightarrow{\text{GOT/AST}}$ Oxaloacetate + L-Glutamate.

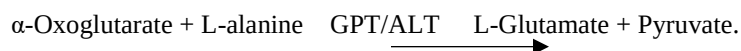
AST is measured by monitoring the concentration of oxaloacetate hydrazones formed with 2,4-Dinitrophenylhydrazine.

Procedure

The test tubes were labeled as sample blank and sample. 0.5 ml of reagent 1 (R1) was pipetted into all the test tubes, after which 0.1 ml of the sample was added to the tube labeled sample. The content was homogenized and incubated for exactly 30mins at 37°C. 0.5 ml of reagent 2 (R2) was pipetted to all the test tubes and 0.1 ml of the sample is added to the tube labeled sample blank. The content was homogenized and allowed to stand for exactly 20mins at 25°C. 5.0 ml of NaOH was then pipetted into all the test tubes, mixed and the absorbance of the sample was read against the sample blank after 5mins at 540nm. The value was expressed in U/L.

2.11.2. Determination of Alanine transaminase (Reitman and Frankel, 1975)

Principle



ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-Dinitrophenylhydrazine.

Procedure

The test tubes were labeled as sample blank and sample. 0.5 ml of reagent 1 (R1) was pipetted into all the test tubes, after which 0.1 ml of the sample was added to the tube labeled sample. The content was homogenized and incubated for exactly 30mins at 37°C. 0.5 ml of reagent 2 (R2) was pipetted to all the test tubes and 0.1ml of the sample was added to the tube labeled sample blank. The content was homogenized and allowed to stand for exactly 20mins at 25°C. 5.0 ml of NaOH was then pipetted into all the test tubes, mixed and the absorbance of the sample was then read against the sample blank after 5mins at 540nm. The value was expressed in U/L.

2.11.3. Determination of Total protein (Biuret Method) (Tietz, 1995)

Principle

The copper atoms of the Biuret solution react with the peptide bonds of the proteins in an alkaline medium to form a violet coloured complex. The intensity of the violet colour is proportional to the amount of protein present in the specimen. The sodium potassium tartrate contained in the biuret reagent complexes Cu^{2+} and maintains their solubility in alkaline solution.



Procedure

The tubes were labelled as test, standard and blank. 1.0 ml of the reagent was pipetted into all the tubes, then 10µl of the standard, samples and distilled water were added into appropriate tubes. The content was mixed and incubated for 10mins at 25°C. The absorbance was then read and recorded at 540nm. The value was expressed in g/dL.

2.11.4. Determination of Total Cholesterol (Enzymatic end-point method) (Parekh & Jung, 1970)

Principle

Total cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator Quinoneimine is formed from H_2O_2 (hydrogen peroxide) and 4-Aminoantipyrine in the presence of phenol and peroxidase.

Procedure

The tubes were labelled as test, standard and blank. 1.0 ml of the reagent was pipetted into all the tubes, then 10µl of the standard, samples and distilled water were added into appropriate tubes. The content was mixed and incubated for 10mins at 25°C. The absorbance was read and recorded at 540nm. Unit for expression of values is mmol/L.

2.11.5. Determination of High Density Lipoprotein– Cholesterol (Precipitant Method) (Castelli et al., 1977)

Principle

Low density lipoprotein, VLDL and Chylomicron fractions are precipitated by the addition of Phosphotungstate acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction which remains in the supernatant is determined.

Procedure

The tubes were labelled as standard, blank and test. 0.7 ml of the reagent was pipetted into all the tubes. Thereafter, 40µl of the standard, sample and distilled water were added into the respective tubes. The content was then mixed and centrifuged for 10mins at 4000rpm. Into the various test tubes labelled: test, standard and blank, 1.0 ml of Cholesterol (reagent) was pipetted. After that, 0.1ml of the supernatant was added into appropriate tubes. The content was mixed and incubated for 10mins at 25°C. The absorbance was read and recorded at 540nm (Unit mmol/L).

2.11.6. Determination of Low-Density Lipoprotein– Cholesterol (Friedewald et al., 1972)

Low Density Lipoprotein was determined using Friedewald equation

$$LDL = TC - HDL - TG/2.2$$

2.11.7. Determination of Urea (Urease-Berthelot Method) (Weatherburn, 1967)

Principle

Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction.

Procedure

The test tubes were labelled as test, standard and blank. Into them was pipetted 0.1 ml of the reagent (R1). Also, 10µl of the samples, standard and distilled water were added into the appropriate tubes. The content was mixed and incubated at 37°C for 10mins. After that, 2.5 ml of Reagent 2 & Reagent 3 were pipetted into all the tubes. The mixture was homogenized and incubated at 25°C for 15mins. The absorbance was read and recorded at 546nm. The value was expressed in mmol/L.

2.11.8. Determination of Creatinine (Direct End-Point Method) (Bartels & Bohmer, 1972)

Principle

Creatinine reacts with picric acid in alkaline solution to form a coloured complex. The amount of complex formed is directly proportional to the creatinine concentration.

Procedure

The tubes were labelled: test, standard and blank respectively. Into all the test tubes, 2.0 ml of reagent was pipetted. Added also into the respective tubes was 0.1 ml of the sample, standard and distilled water. The content was then

mixed and after 30seconds, the absorbance of the standard and sample were read. Two minutes later, the absorbance of the standard and sample were read again. The unit was expressed in $\mu\text{mol/L}$.

Therefore,

A2 of standard and sample. $A_1 - A_2 = A$.

2.11.9. Determination of Bicarbonate (HCO_3) (Back Titration Method) (Tietz, 1995)

Principle

Serum HCO_3 is reacted with excess standard HCL. The remaining HCL is back titrated with standard NaOH using phenol red as indicator.

Procedure

To a 50ml conical flask, 250 μl of CO_2 -free distilled water, 200 μl of sample and 1 ml of 0.01N HCl were added and mixed properly. Also added to the mixture was 3 drops of phenol red. The flask was then whirled to release the CO_2 . Thereafter, the resultant solution was titrated with 0.01N NaOH until the initial light yellow colour faded to give a light purple as the end-point.

Result

The remaining NaOH that did not take part in the reaction was read. The reading obtained was then divided by two; this gave the concentration of HCO_3 in the sample. Its unit is expressed in mmol/L .

2.12. Hematological parameters

2.12.1. Determination of Packed Cell Volume/Hematocrit (PCV/HCT %)

Principle

The packed cell volume is that proportion of whole blood which is occupied by red cells and it is expressed as a volume fraction. Unit = L/L.

Materials

Micro-hematocrit, centrifuge, capillary tubes, sealant, micro-hematocrit reader.

Procedure

A capillary tube is filled up to $\frac{3}{4}$ with well-mixed EDTA blood, the unfilled end is sealed and the tube is placed in micro-hematocrit rotor, and centrifuged for 5 minutes. Immediately after centrifuging, the PCV is read using the micro-hematocrit reader.

2.12.2. Determination of Hemoglobin

To get the value for Hemoglobin, the PCV value was divided by 3. Hb unit: g/dl.

$$\text{Hb} = \frac{\text{PCV (\%)}}{3}$$

2.12.3. Determination of Mean Cell Volume (MCV)

The Mean Cell Volume provides information on the size of the red blood cells. The unit of measurement is Femtoliters (fl).

$$\text{MCV (fl)} = \frac{\text{PCV (\%)} \times 10}{\text{RBC}}$$

2.12.4. Determination of Blood Glucose (Glucose-oxidase method) (Trinder, 1969)

Principle

Glucose is oxidized by glucose oxidase (GOD) forming gluconic acid and H_2O_2 (hydrogen peroxide). Addition of peroxidase (POD) breaks down H_2O_2 into H_2O and O_2 . As an oxygen acceptor, 4-Aminoantipyrine takes up the O_2 and together with phenol forms a red coloured complex. The amount of colour produced is directly proportional to the glucose content in the sample.

Glucose $\xrightarrow{\text{glucose-oxidase}}$ Gluconic acid + H_2O_2

$\text{H}_2\text{O}_2 \xrightarrow{\text{peroxidase}} \text{H}_2\text{O} + \text{O}_2$

$2\text{H}_2\text{O}_2 + 4\text{-Aminoantipyrine} + \text{phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine dye} + 4\text{H}_2\text{O}$

Procedure

The tubes were labelled as test, standard and blank. 1.0 ml of the glucose reagent was pipetted into all the tubes, then 10 μl of the glucose standard, samples and distilled water were added into appropriate tubes. The content was mixed and incubated for 10mins at 25°C. The absorbance was then read and recorded at 540nm. Values were expressed in mg/dL.

2.13. Histological procedure

2.13.1. Manual processing of tissues

The tissues were grossed and placed into tissue cassettes that had been labeled, and then fixed in 10% formal saline. Tissues were dehydrated in increasing grades of alcohol concentration for 2hr in each grade i.e. 70%, 90%, and 100% and then were cleared in xylene for 2hr. After clearing, the cassettes containing the tissues were infiltrated in paraffin wax for 2hr each with stirring every 10 min with the wax placed in an oven at a regulated temperature of 55°C to keep the wax in a molten state.

The casting and embedding were done with the help of metallic moulds. Metallic base moulds were placed on a metallic plate, which acted as a base of the mould and molten wax was poured into it. The tissues were placed in the mould filled with wax and left to solidify and cool in a refrigerator. After solidification the blocks of the wax were removed and properly labeled for microtomy.

2.13.2. Sectioning and mounting

The blocks of tissues were sectioned using a rotary microtome at 3-5 μ . The section(s) was flattened in a water bath for removal of greases and wrinkles present in the sections. The slides were used to pick the ribbons, drained and then heated on the histological hot plate at 60°C. The prepared 3-5 μ thickness sections were stained with haematoxylin and eosin (H&E). The slides were cleaned beyond the area of tissue implantation, and then mounted on Distrene (a polystyrene), a plasticizer (tricresyl phosphate) and Xylene (DPX) treated slides. The DPX acted as adhesive for the sections on the slides and then the sections were allowed to dry and examined first under the low power and then high power magnification of the light microscope (Carlton, 1967).

2.13.3. Staining

Haematoxylin & eosin techniques:

De-waxed sections of the slides were immersed in xylene (for 2 minutes) and were hydrated in descending grade of alcohol (absolute, 90%, 70%, 70% for 2 minutes each). The slides were washed in distilled water (for 3 minutes) and

stained in haematoxylin for 15 minutes. After which they were rinsed in tap water for 5 minutes. The slides were then differentiated in 1% acid alcohol (99 ml of 70% alcohol in 1ml of HCl) for 2 minutes and then washed in running tap water for 5 minutes. The slides were stained in eosin for 3 minutes and rinsed in water for 3 minutes. The slides were then dehydrated in ascending grades of alcohol (70%, 90%, and absolute). After which the slides were cleared in xylene and mounted in DPX (Distrene, Polystyrene and Xylene) (Carlton, 1967).

2.13.4. Photomicrography

The stained tissue images were captured using a digital microscopic eyepiece, 'SCOPETEK DCM 500, 8.0 MEGA PIXEL' connected to the USB port of a computer.

2.13.5. Analysis

The stained slides were interpreted with the aid of a light microscope and analyzed for both histological and morphological changes across the groups.

2.13.6. Statistical Analysis

All data were subjected to statistical analysis. Values were reported as Mean $\pm$ Standard deviation while one-way ANOVA was used to test for differences between treatment groups. The results were considered significant at p-values of less than 0.05, that is, at 95% confidence level ($p < 0.05$).

3. Results and Discussion

3.1. Proximate composition of the leaves of *Luffa aegyptiaca*.

The results of the proximate composition of *Luffa aegyptiaca* leaves are presented in Table 1. Total carbohydrate (47.89%), crude protein (3.80%), crude fat (7.21%), Fibre (15.3%), Moisture (8.34%) and Ash (17.5%).

Table 1: Proximate composition of *Luffa aegyptiaca* leaves.

Parameters	Composition (%)
Total carbohydrate	47.89 $\pm$ 0.10
Crude protein	3.80 $\pm$ 0.14
Crude fat	7.21 $\pm$ 0.08
Fibre	15.3 $\pm$ 0.07
Moisture	8.34 $\pm$ 0.31
Ash	17.5 $\pm$ 0.13
Energy content (Kcal/100g sample)	235

3.2. Effect of aqueous leaf extract of *Luffa aegyptiaca* on serum liver marker enzymes of alloxan-induced diabetic male wistar rats.

The findings of the effect of aqueous leaf extract of *Luffa aegyptiaca* on serum liver marker enzymes of alloxan induced male wistar albino rats are presented in Table 2. The result revealed that there was a significant ($p < 0.05$) decrease in the serum levels of aspartate transaminase and alanine transaminase in all extract treated groups when

compared to diabetic control (alloxan induced only). In comparison with the standard drug (metformin) it was observed that the group treated with 300mg/kg b.w. of the aqueous extract of *L. aegyptiaca* recorded a significant ($p<0.05$) decrease in aspartate transaminase and alanine transaminase serum levels. Total protein levels were significantly ($p<0.05$) increased in extracts treated groups of 200 and 300mg/kg b.w. when compared to the diabetic control.

3.3. Effect of aqueous leaf extract of *Luffa aegyptiaca* on serum renal indices of alloxan-induced diabetic male wistar rats.

The outcome of the effect of aqueous leaf extract of *Luffa aegyptiaca* on serum renal indices of alloxan induced male wistar albino rats are shown in Table 3. The result revealed that there was a significant ($p<0.05$) decrease in the serum levels of urea and creatinine in all extract treated groups in comparison to the diabetic control (alloxan induced only). In comparison with the standard drug (metformin), it was observed that the group treated with 300mg/kg b.w. of the aqueous extract of *Luffa aegyptiaca* recorded a significant ($p<0.05$) decrease. Bicarbonate levels were significantly ($p<0.05$) decreased in all extract treated groups when compared to the diabetic control.

Table 2: Effects of aqueous leaf extract of *Luffa aegyptiaca* on serum liver marker enzymes of alloxan-induced diabetic male wistar rats.

Groups	AST (U/L)	ALT (U/L)	Total Protein (g/dL)
I	15.0±1.00 ^a	7.30±1.42 ^a	71.0±4.58 ^a
II	35.0±1.00 ^b	17.3±2.40 ^b	54.5±1.50 ^b
III	29.5±0.50 ^c	14.2±0.80 ^c	54.3±6.03 ^b
IV	25.0±1.00 ^d	11.0±0.70 ^d	67.0±8.54 ^a
V	11.3±1.53 ^e	6.60±1.90 ^a	75.0±5.57 ^a
VI	21.3±1.53 ^f	10.6±0.60 ^d	73.0±7.00 ^a

Values are means $\pm$ standard deviations of triplicate determinations. Values not sharing a common superscript on the same column differ significantly ($p<0.05$). AST = Aspartate transaminase, ALT = Alanine transaminase Group I = Normal control, group II = Diabetic control, Group III – V = Extract treated group of 100, 200 and 300mg/kg b.w. respectively, Group VI = Standard drug (metformin 100mg/kg b.w.).

Table 3: Effects of aqueous leaf extract of *Luffa aegyptiaca* on serum renal indices of alloxan-induced diabetic male wistar rats.

Groups	Urea (mmol/L)	Creatinine (μ mol/L)	Bicarbonate (mmol/L)
I	5.40±0.66 ^a	109±12.8 ^a	22.7±2.08 ^a
II	11.9±1.36 ^b	194±2.52 ^b	30.0±2.00 ^b
III	9.20±0.70 ^c	183±1.53 ^b	26.3±1.53 ^c

IV	7.90±0.20 ^c	154±7.51 ^c	25.7±1.53 ^a
V	5.66±1.15 ^a	81.0±11.0 ^d	22.7±2.08 ^a
VI	7.10±0.10 ^{cd}	142±2.52 ^c	24.3±2.08 ^{ac}

Values are means $\pm$ standard deviations of triplicate determinations. Values not sharing a common superscript on the same column differ significantly ($p < 0.05$). UR = Urea, CR = Creatinine, HCO_3 = Bicarbonate. Group I = Normal control, group II = Diabetic control, Group III – V = Extract treated group of 100, 200 and 300mg/kg b.w. respectively, Group VI = Standard drug (metformin 100mg/kg b.w.).

3.4. Effect of aqueous leaf extract of *Luffa aegyptiaca* on serum lipid profile of alloxan-induced diabetic male wistar rats.

The result of the effect of aqueous leaf extract of *Luffa aegyptiaca* on serum lipid profile of alloxan-induced diabetic male wistar rats are presented in Table 4. The result revealed that there was a significant ($p < 0.05$) decrease in the serum levels of total cholesterol and High density lipoprotein in all extract treated groups in comparison to diabetic control (alloxan induced only). In comparison with the standard drug (metformin) it was observed that the groups treated with 200mg/kg b.w. and 300mg/kg b.w. of the aqueous extract of *L. aegyptiaca* recorded significant ($p < 0.05$) decrease. Low density lipoprotein levels were significantly ($p < 0.05$) decreased in all extract treated groups when compared to the diabetic control.

3.5. Effect of aqueous leaf extract of *Luffa aegyptiaca* on hematological indices of alloxan-induced diabetic male wistar rats.

The findings of the effect of aqueous leaf extract of *Luffa aegyptiaca* on hematological indices of alloxan induced male wistar albino rats are shown in Table 5. The result revealed that there was a significant ($p < 0.05$) increase in the Packed cell volume and Hemoglobin levels in all extract treated groups when compared to the diabetic control (alloxan induced only). Mean cell volume levels were significantly ($p < 0.05$) decreased in all extract treated groups in comparison to the diabetic control.

3.6. Effect of aqueous leaf extract of *Luffa aegyptiaca* on blood glucose of alloxan-induced diabetic male wistar rats.

The outcome of the effect of aqueous leaf extract of *Luffa aegyptiaca* on blood glucose of alloxan induced male wistar albino rats are shown in Table 6. The result revealed that there was a significant ($p < 0.05$) decrease in the blood glucose levels of the experimental animals in all extract treated group in comparison to the diabetic control.

Table 4: Effects of aqueous leaf extract of *Luffa aegyptiaca* on serum lipid profile of alloxan-induced diabetic male wistar rats.

Groups	Total cholesterol (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
I	2.63±0.25 ^a	0.96±0.23 ^a	0.80±0.18 ^a
II	4.23±0.21 ^b	2.34±0.11 ^b	1.97±0.13 ^b
III	2.80±0.10 ^a	1.29±0.05 ^c	1.37±0.19 ^c
IV	2.66±0.45 ^a	0.85±0.23 ^a	0.97±0.23 ^a

V	2.40±0.10 ^{ac}	0.87±0.04 ^a	0.86±0.11 ^a
VI	2.90±0.26 ^{ad}	1.49±0.08 ^c	0.86±0.16 ^a

Values are means $\pm$ standard deviations of triplicate determinations. Values not sharing a common superscript on the same column differ significantly ($p < 0.05$). Total Cholesterol, HDL = High Density Lipoprotein, LDL = Low Density Lipoprotein. Group I = Normal control, group II = Diabetic control, Group III – V = Extract treated group of 100, 200 and 300mg/kg b.w. respectively, Group VI = Standard drug (metformin 100mg/kg b.w.).

Table 5: Effects of aqueous leaf extract of *Luffa aegyptiaca* on hematological indices of alloxan-induced diabetic male wistar rats.

Groups	PCV (L/L)	Hb (g/dl)	MCV (fl)
I	40.0±1.00 ^a	13.3±0.35 ^a	66.7±1.53 ^a
II	30.3±2.52 ^b	10.2±0.68 ^b	73.3±1.53 ^b
III	36.0±0.00 ^c	12.0±0.00 ^c	69.3±1.15 ^c
IV	38.0±1.00 ^{ac}	12.7±0.35 ^{ac}	70.0±1.00 ^{cd}
V	40.0±1.00 ^a	13.3±0.35 ^a	67.0±1.00 ^{ce}
VI	37.3±1.53 ^{ac}	12.2±0.15 ^c	69.7±1.53 ^{cd}

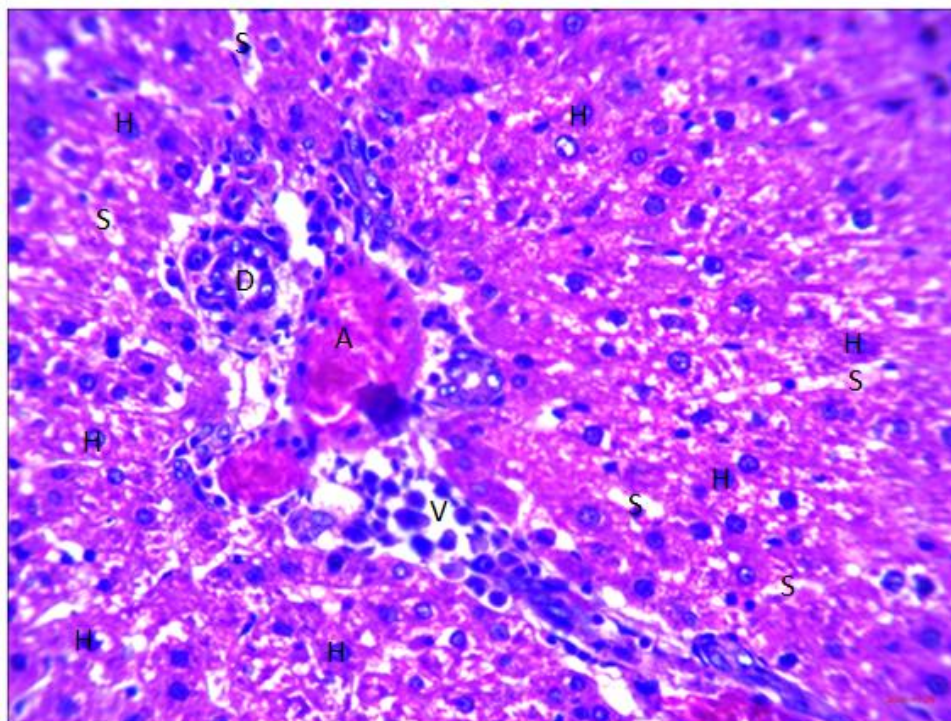
Values are means $\pm$ standard deviations of triplicate determinations. Values not sharing a common superscript on the same column differ significantly ($p < 0.05$). PCV = Packed Cell Volume, Hb = Hemoglobin, MCV = Mean Cell Volume. Group I = Normal control, group II = Diabetic control, Group III – V = Extract treated group of 100, 200 and 300mg/kg b.w. respectively, Group VI = Standard drug (metformin 100mg/kg b.w.).

Table 6: Effects of aqueous leaf extract of *Luffa aegyptiaca* on blood glucose (mg/dL) of alloxan-induced diabetic male wistar rats.

Days	Normal	Diabetic	100mg/kg	200mg/kg	300mg/kg	Metformin
1	99.3±5.03 ^a	416±39.0 ^a	317±10.9 ^a	406±22.9 ^a	346±19.7 ^a	404±5.03 ^a
4	99.3±5.03 ^a	418±5.57 ^a	279±5.69 ^b	356±21.3 ^b	202±9.17 ^b	356±21.3 ^b
8	99.3±5.03 ^a	307±3.00 ^b	231±7.55 ^c	214±11.9 ^c	115±8.72 ^c	214±11.7 ^c
12	94.0±6.01 ^a	268±4.51 ^{bc}	190±8.89 ^d	168±4.00 ^d	102±4.58 ^c	168±4.00 ^d
16	93.7±10.5 ^a	231±7.55 ^c	140±3.51 ^e	125±5.03 ^e	96.0±2.00 ^c	125±5.00 ^e

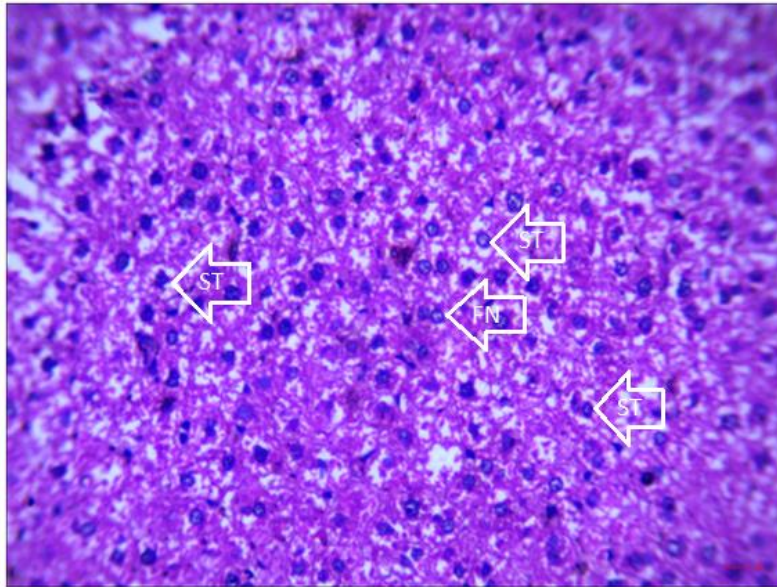
3.7. Histological findings

The histological findings from the effects of aqueous leaf extract of *Luffa aegyptiaca* on alloxan-induced diabetic male wistar rats are shown below. Plate 1 showed the micrograph of the liver of the normal control group with normal hepatocytes. Plate 2 showed the micrograph of the liver of the diabetic control group (alloxan induced only) with signs of a histologically distorted liver. Plate 3 showed the micrograph of the liver of alloxan induced group that was administered 100mg/kg of aqueous leaf extract of *Luffa aegyptiaca* still with signs of a histologically distorted liver. Plate 4 showed the micrograph of the liver of alloxan induced group that was administered 200mg/kg of aqueous leaf extract of *Luffa aegyptiaca* with normal hepatocytes. Plate 5 showed the micrograph of the liver of alloxan induced group that was administered 300mg/kg of aqueous leaf extract of *Luffa aegyptiaca* still with normal hepatocytes. Plate 6 showed the micrograph of the liver of alloxan induced group that was administered 100mg/kg of the standard drug (metformin) with normal hepatocytes. Plate 7 showed the micrograph of the kidney of the normal control group with normal glomerular tuft. Plate 8 showed the micrograph of the kidney of the diabetic control group (alloxan induced only) with signs of a histologically distorted kidney. Plate 9 showed the micrograph of the kidney of alloxan induced group that was administered 100mg/kg of aqueous leaf extract of *Luffa aegyptiaca* still with signs of a histologically distorted kidney. Plate 10 showed the micrograph of the kidney of alloxan induced group that was administered 200mg/kg of aqueous leaf extract of *Luffa aegyptiaca* with signs of a histologically distorted kidney. Plate 11 showed the micrographs of the kidney of alloxan induced group that was administered 300mg/kg of aqueous leaf extract of *Luffa aegyptiaca* with signs of a histologically distorted kidney. Plate 12 showed the micrographs of the kidney of alloxan induced group that was administered 100mg/kg of the standard drug (metformin) still with signs of a histologically distorted kidney.



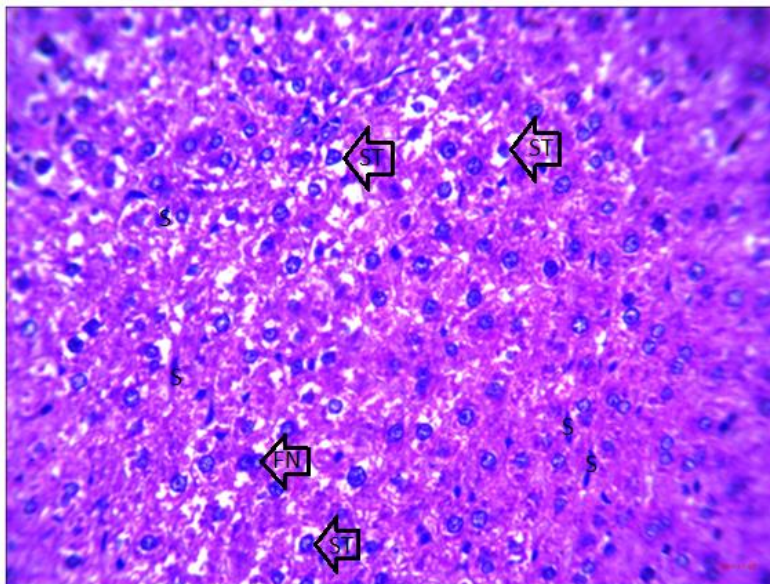
Control

Plate 1: Histology of the liver section of the normal control group showing normal liver histologic features. H&E x400. H = Intact hepatocytes, S = Sinusoid containing kupffer cells D = Bile duct, A = Congested hepatic artery, V = Portal vein.



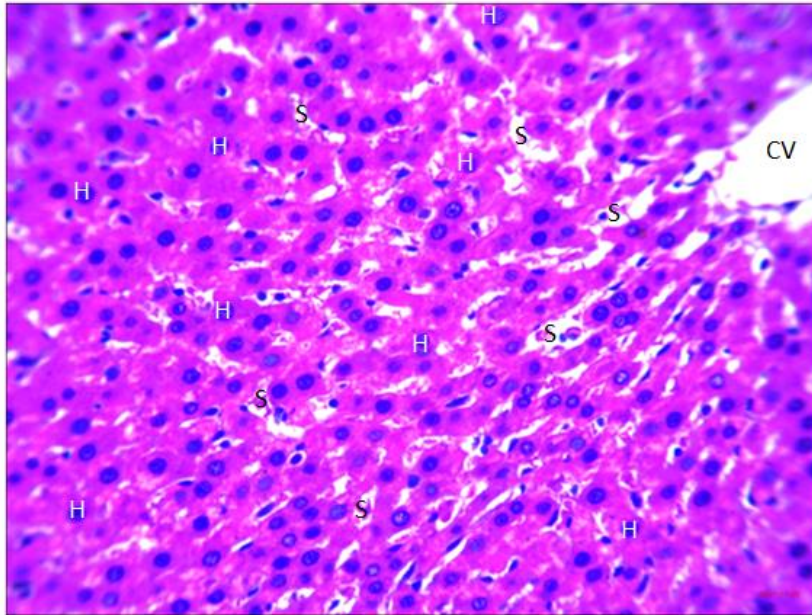
Alloxan-induced Diabetes

Plate 2: Histology of the liver section of the alloxan induced control group showing histological distorted liver. H&E x400. ST= hepatocytes with features of fatty changes (steatosis), FN = Fusion of nuclei.



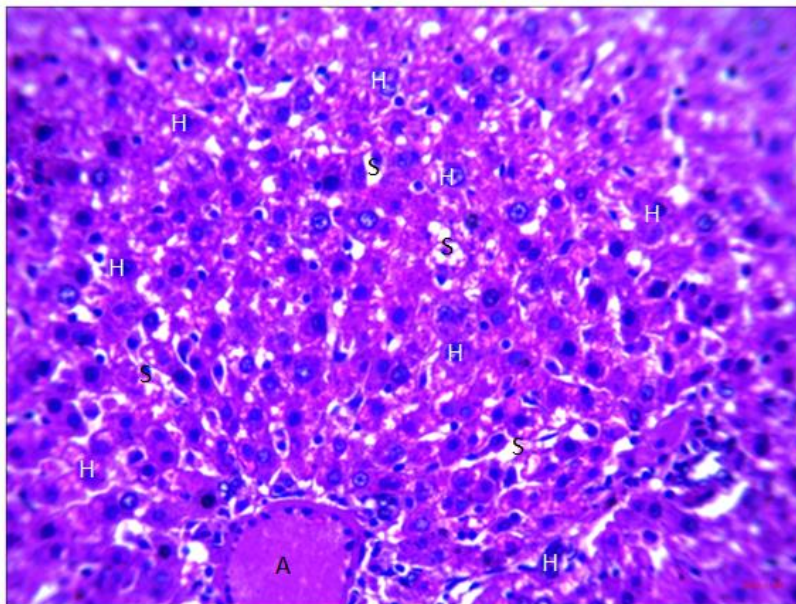
Diabetes treated with Extract (100ml)

Plate 3: Histology of the liver section administered with 100mg/kg of *Luffa aegyptiaca* extract showing a histological distorted liver. H&E x400. S = Sinusoid containing kupffer cells, ST= hepatocytic steatosis of different degrees, FN = Fusion of nuclei.



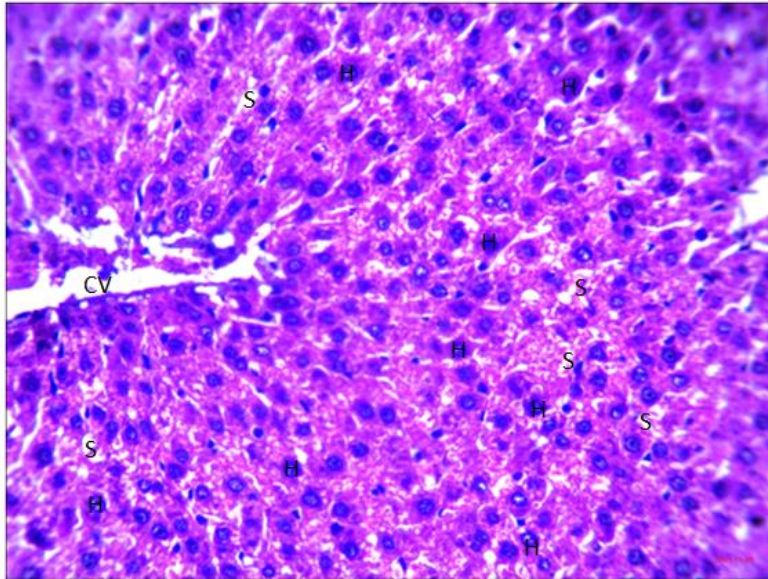
Diabetes treated with Extract (200ml)

Plate 4: Histology of the liver section administered with 200mg/kg of *Luffa aegyptiaca* extract showing a histological normal liver. H&E x400. H = Intact hepatocytes, S = Sinusoid containing capillaries and kupffer cells, CV = Patent central vein.



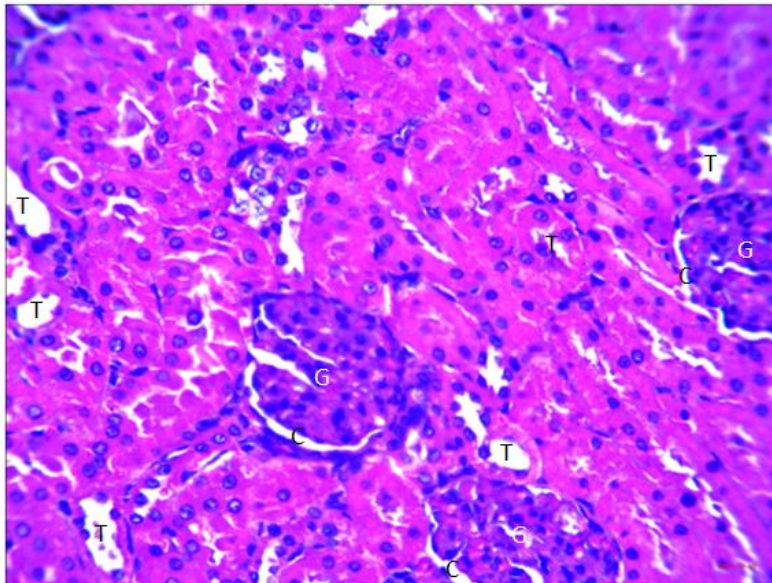
Diabetes treated with Extract (300ml)

Plate 5: Histology of the liver section administered with 300mg/kg of *Luffa aegyptiaca* extract showing a histological normal liver. H&E x400. H = Intact hepatocytes, S = Sinusoid containing kupffer cells, A = Congested hepatic artery.



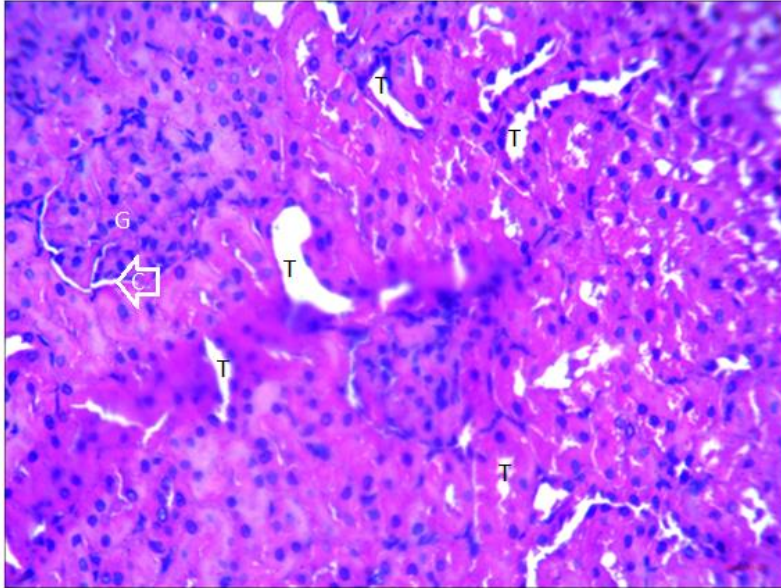
Diabetes treated with Metformin (100ml)

Plate 6: Histology of the liver section administered with 100mg/kg of metformin showing a histological normal liver. H&E x400. H = Intact hepatocytes, S = Sinusoid containing kupffer cells, CV = Patent central vein.



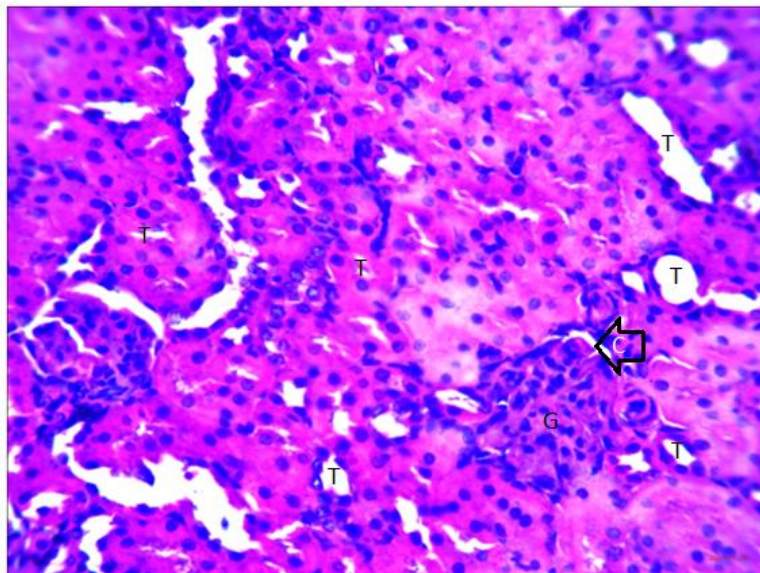
Control

Plate 7: Histology of the kidney section of the normal control group showing histological normal kidney. H&E x400. G = Glomerular tuft containing mesangial cells, mesangial matrix and capillaries, C= Patent Bowman's capsular space, T= Renal tubules lined with simple epithelial cells.



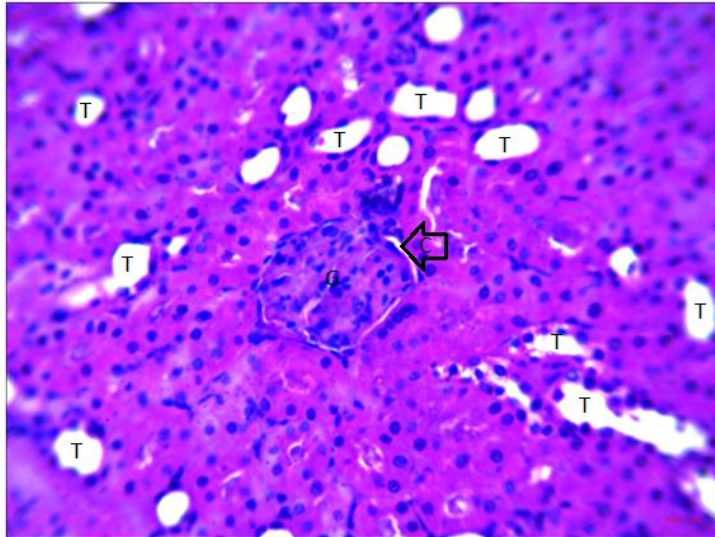
Alloxan-induced Diabetes

Plate 8: Histology of the kidney section of the alloxan induced control group showing histologically distorted kidney. H&E x400. G = Enlarged glomerular tuft with marked decrease in bowman's capsular space, C= Patent bowman's capsular space, T= Intact renal tubules.



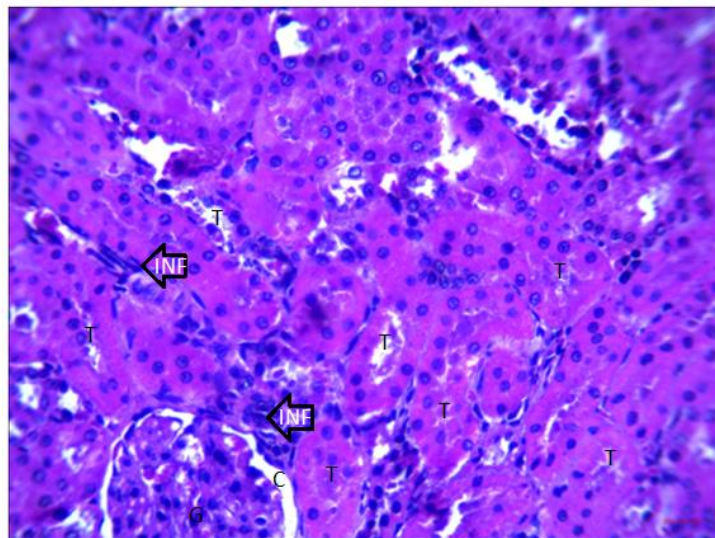
Diabetes treated with Extract (100ml)

Plate 9: Histology of the kidney section administered with 100mg/kg of *Luffa aegyptiaca* extract showing a histological distorted kidney. H&E x400. G = Enlarged glomerular tuft with marked decrease in bowman's capsular space, C= Patent bowman's capsular space, T= Renal tubules.



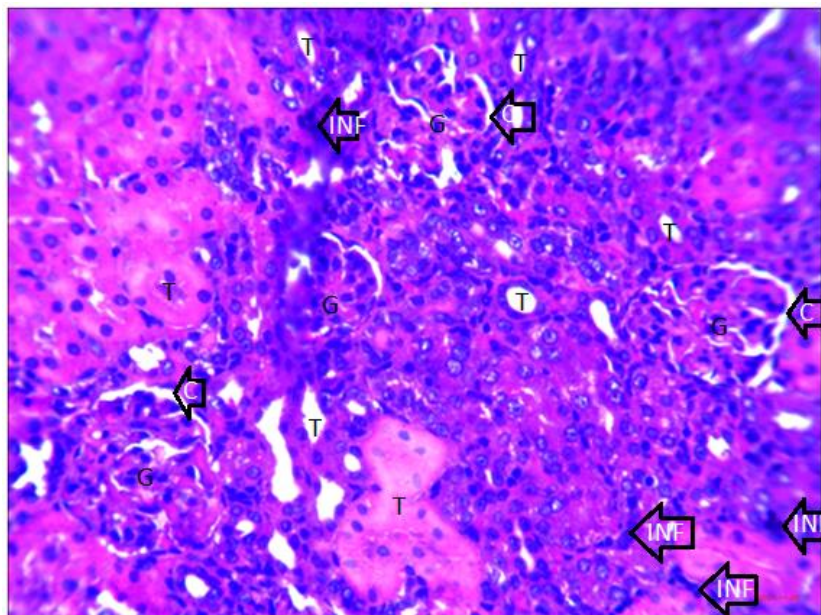
Diabetes treated with Extract (200ml)

Plate 10: Histology of the kidney section administered with 200mg/kg of *Luffa aegyptiaca* extract showing a histological distorted kidney. H&E x400. G = Intact glomerular tuft with marked decrease in bowman's capsular space, C= Patent bowman's capsular space, T= Renal tubules.



Diabetes treated with Extract (300ml)

Plate 11: Histology of the kidney section administered with 300mg/kg of *Luffa aegyptiaca* extract showing a histological distorted kidney. H&E x400. G = Intact glomerular tuft with patent bowman's capsular space, C= Patent bowman's capsular space, T= Renal tubules lined with simple epithelial cells, INF = Interstitial inflammation.



Diabetes treated with Metformin (100ml)

Plate 12: Histology of the kidney section administered with 100mg/kg of metformin showing a histological distorted kidney. H&E x400. G = Intact glomerular tuft, C= Patent bowman's capsular space, T= Renal tubules, INF = Interstitial inflammation.

Proximate analysis of *Luffa aegyptiaca* leaves showed that the plant is rich in carbohydrate (47.89%). Carbohydrates constitutes a major class of naturally occurring organic compounds that are essential for the maintenance and sustenance of life in plants and animals and also provide raw materials for many industries (Aborisade *et al.*, 2017). Proteins carry out an essential role in the transmission of information in the body, serving as neurotransmitter and genetic transmission of traits, tissues repair and general growth. Proteins are also a vital component of diet required for the survival of animals and humans; and the basic function in nutrition is to supply adequate amount of amino acids (Voet *et al.*, 2008; Ogunyemi *et al.*, 2020). Moisture content is a major quality factor in food stability and preservation. High values of moisture content result in short shelf life of food (Ogunyemi *et al.*, 2020; Shukla *et al.*, 2015). Dietary fibre in the body contributes to weight, bulk and softness of faecal matter, allowing it to move through the gastro intestinal tract with ease, thereby prevents constipation. It also reduces the occurrence of coronary heart diseases, while the level of ash content is an indication of mineral concentration in *Luffa aegyptiaca* leaves (Essiett *et al.*, 2019). Dietary fat increases the palatability of food by absorbing and retaining flavour (Aborisade *et al.*, 2017). Consequently, with a total carbohydrate content of 47.89%, crude protein content of 3.80%, dietary fibre of 15.3%, ash content of 17.5% and crude fat content of 7.21%, the leaves of *Luffa aegyptiaca* can serve as a food supplement and it could be of great nutritional benefits.

This study also examined the effect of aqueous leaf extract of *Luffa aegyptiaca* on liver markers, lipid profile, renal indicators and hematological indices using alloxan as the agent for inducing diabetes in male wistar albino rats. This

study revealed a significant increase ($p < 0.05$) in the activity of serum aspartate transaminase and alanine transaminase upon alloxan administration and a significant decrease ($p < 0.05$) in the level of total protein. Alloxan mediates the destruction of the beta-cells of pancreatic islet via the production of reactive oxygen species and subsequent compromise in the quality and quantity of insulin produced by these cells (Ighodaro *et al.*, 2017). Administration of aqueous leaf extract of *Luffa aegyptiaca* attenuated the increased activities of the serum hepatic enzymes aspartate transaminase and alanine transaminase, produced by alloxan and caused a subsequent recovery towards normalization comparable to the normal control group. This study is in line with the reports of Shendge & Belemkar (2018). Total protein, its activity was increased in extract treated groups of 200 and 300mg/kg as shown in Table 2. The attenuation of the serum hepatic enzymes and total protein levels buttress the fact that the extract could have possibly enhances the synthesis of new cellular proteins and prevention of further protein degeneration (Anigboro *et al.*, 2018).

The renal indicator of urea, creatinine and bicarbonate recorded a significant increase ($p < 0.05$) prior to treatment depicting that the nephron has lost its metabolic function (Gowda *et al.*, 2010). However, administration of aqueous leaf extract of *Luffa aegyptiaca* caused decreased activities of the renal indices created by alloxan and initiating recovery that is comparable to the normal control group as is observed in table 3 could be attributed to the restoration of the integrity of the Bowman's capsule of the nephron in maintaining strict ultrafiltration process.

The presence of lipid in the body are essential in maintaining cellular homeostasis, cellular signaling and hormone production. Prior to treatment, there was a significant increase ($p < 0.05$) in the lipid profile of total cholesterol, high density lipoprotein and low density lipoprotein upon the administration of alloxan. Nonetheless, the administration of aqueous leaf extract of *Luffa aegyptiaca* led to decrease in lipid profile levels as compared to the normal control group this could be possibly achieved by the removal of cholesterol from circulation via the down regulation of glucose and metabolic intermediates towards the amino acid pool (Shendge & Belemkar, 2018).

The hematological indices of packed cell volume and hemoglobin recorded a significant decrease ($p < 0.05$) upon inducement of alloxan and a significant increase ($p < 0.05$) in mean cell volume, thereby indicating a trigger in immunomodulatory response. However, upon administration of aqueous leaf extract of *Luffa aegyptiaca* there was increase in the packed cell volume and hemoglobin levels. The mean cell volume experienced a decrease after administration of aqueous leaf extract of *Luffa aegyptiaca*. The restoration of the hematological indices could be inferred to the extract ability to mediate blockage/ inhibition of red blood cell lysis and maintaining the general wellbeing of the blood (Oyedemi *et al.*, 2011).

The result of the blood glucose showed a significant ($p < 0.05$) decrease in the blood glucose level of the treated animals in comparison to the untreated animals in the diabetic control. The ability of aqueous leaf extract of *Luffa aegyptiaca* to decrease the blood glucose level may be by enhancing the effect of insulin which involves increasing its secretion from beta cells of islets of Langerhans. This result agrees with the Antidiabetic activity of *Luffa aegyptiaca* (Mill) in alloxan induced diabetic rats by Sangeeta *et al.*, (2011).

The result of the histological studies revealed that aqueous leaf extract of *Luffa aegyptiaca* showed restoration to the damage caused by alloxan with increasing dose dependent manner. In the liver, noteworthy restoration was seen in the group administered with 200mg/kg and 300mg/kg of the extract and the metformin treated group. These findings also validate the result obtained from the biochemical studies.

4. Conclusion

From pre-historic times, the plant realm has contributed immensely to the wellbeing of humans when no idea of medical procedures existed. The active components of medicinal plants such as alkaloids, phenols, tannins, cryogenics, glycosides, terpenoids as well as their antibacterial, antifungal, anticancer, antidiuretic, anti-inflammatory

and anti-diabetic properties have made them more valuable for treatment of various diseases. Also, medicinal plants work in a favorable manner with practically no unfavorable side effects to the body in comparison with modern medicines (Sohani, 2021).

The proximate analysis, liver markers, lipid profile, renal indicators and hematological indices study against alloxan induced diabetes treated with aqueous leaf extract of *Luffa aegyptiaca* were investigated. The study showed that *Luffa aegyptiaca* leaves are rich in carbohydrate as shown in the result of the proximate analysis. Aqueous leaf extract of *Luffa aegyptiaca* reduced significantly serum levels of liver enzymes of AST and ALT and also increased Total protein levels. The aqueous leaf extract of *Luffa aegyptiaca* in addition ensued decreased activities of the renal indices of urea, creatinine and bicarbonate created by alloxan. The study further revealed that administration of aqueous leaf extract of *Luffa aegyptiaca* also led to decrease in lipid profile levels of total cholesterol, high density lipoprotein and low density lipoprotein. There was increased level of hematological indices of the packed cell volume and hemoglobin as well as decrease of the mean cell volume upon aqueous leaf extract of *Luffa aegyptiaca* administration. Aqueous leaf extract of *Luffa aegyptiaca* also significantly reduced the blood glucose level of the experimental animals upon administration. The histological studies revealed that aqueous leaf extract of *Luffa aegyptiaca* caused some restoration of the liver and kidney cells.

The study showed that the administration of aqueous leaf extract of *Luffa aegyptiaca* mitigated the damage induced by alloxan in male wistar rats which were confirmed by the biochemical and histological analyses. The leaves of *Luffa aegyptiaca* are rich in carbohydrates and dietary fibre showing that the plant can be used as a food supplement and could be of great nutritional advantage. It is recommended that:

- i. Chronic studies should be carried out to ascertain the effect of prolong administration of the aqueous leaf extract of *L. aegyptiaca* in alloxan-induced diabetic *in-vivo* model.
- ii. Isolation, purification and identification of the bioactive ingredients responsible for the anti-diabetic properties of the leaves of *L. aegyptiaca* should be conducted for proper understanding and clarity of the possible mechanism behind their action.

This study showed that the leaves of *Luffa aegyptiaca* could potentially be a good source of carbohydrate and dietary fibre. The aqueous leaf extract of *Luffa aegyptiaca* showed therapeutic effect in restoring the changes in the levels of serum hepatic markers, renal markers, lipid profile, blood glucose level and hematological indices.

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