

EVALUATION OF THE ANTIDIABETIC POTENTIALS OF *MUSA ACUMINATA* LEAVES CRUDE EXTRACT IN ALLOXAN-INDUCED WISTAR RATS

ABSTRACT

Context: *Musa acuminata* is a plant of the tropical and subtropical regions. Over the past few decades, the health benefits of *Musa acuminata* have received much attention. All parts of the plant, including fruits, peel, pseudo stem, corm, flowers, leaves, sap, and roots, have found their use in treating many diseases in traditional medicine.

Aim: This study was conducted to appraise the protective effect of *Musa acuminata* extract on alloxan-induced Diabetics Mellitus in wistar rats.

Settings and Design: This investigation was carried out using 24 Wistar rats, both males and females. The experimental models were divided into six groups; 4 rats per group.

Materials and methods: The experimental models were divided into six groups; 4 rats per group. Alloxan was intraperitoneally injected at a dose of 150 mg/kg body weight for day 1 across all groups except the positive control which is Group A together with oral administration of the aqueous extract of *Musa acuminata* (100 mg/kg, 200mg/kg and 300mg/kg body weight for the treatment group) and Group F was administered a standard drug. The animals were sacrificed on the 11th day under deep anesthesia with chloroform. The blood was collected by cardiac puncture and kidneys were collected for the histological profile.

Statistical Analysis: Kidney oxidative stress marker Malondialdehyde (MDA) and antioxidant enzyme activities Creatinine and Urea, were determined.

Result: The alloxan injection indicated an increase in Malondialdehyde (MDA), with a decrease in Urea and Creatinine when compared with the control. A remarkable decrease in antioxidant enzymes was also observed. Oxidant/antioxidant imbalance, alloxan-induced diabetic mellitus, and histological changes in the kidneys were reduced almost to normal by the per-administration of *Musa acuminata*.

Conclusion: Based on the current findings, *Musa acuminata* leaves at low dose (100 mg/kg body weight) in the alloxan Induced Wistar rat has some anti diabetics potential than at 200 mg/kg body weight, 300 mg/kg body weight and standard drug (Metformin).

Keywords: Alloxan, *Musa acuminata*, Diabetics Mellitus, Kidney.

INTRODUCTION

According to Thomas D. et al., 2019, Herbal medications have been used to treat various ailments, and a vast number of the population in the world is entirely dependent on traditional medicines. 80% of the world's population relies on medicinal plants for primary health care (WHO 2022). “Herbal drugs are readily available, cheaper, time tested, and considered safer than most modern synthetic drugs. The World Health Organization(WHO) believes that the significant population of developing countries rely on traditional medicine for their primary healthcare needs” (WHO 2022). Therefore, there is an increased demand for medicinal plants in developing and developed countries.

“Musa spp (bananas) is a good source of carbohydrates, proteins, vitamins, and minerals. They contain different amino acids like threonine, tryptamine, and tryptophan, as well as flavonoid, dopamine, beta-carotene, and sterols” (Choudhury H. et al., 2018). *Musa acuminata* is a species of banana native to Southern Asia, its range comprising the Indian Subcontinent and Southeast Asia.

“Alloxan, chemically known as 5,5-dihydroxyl pyrimidine-2,4,6-trione, is an organic compound, urea derivative, carcinogen, and cytotoxic glucose analog. Alloxan is one of the common diabetic agents often used to assess the anti diabetic potential of pure compounds and plant extracts in studies involving diabetes. Oxidative stress is the principal mechanism of many diabetic complications because of its active role in cellular injury in neuronal and vascular cells” (Maiese K., 2015). A hyperglycemic state reduces antioxidant levels, consequently increasing free radical production.

“Diabetics Mellitus is a health crisis in modern society and affects 537 million people worldwide. This number is expected to be increased and become the 7th leading cause of death in the world in 2030” (Ramachandran et al., 2013). “Diabetes is a metabolic disorder of carbohydrate, fat, and protein, affecting a large population worldwide. The management of hyperglycemia is of utmost importance to limit the severe complications of Diabetics Mellitus” (Viigimaa M., and Sachinidis A., 2020). The conventional treatment of Diabetics Mellitus includes insulin injections and several anti diabetic drugs such as sulfonylureas (Juan J. and José M. 2016), metformin (Glossmann H.H., and Lutz O. M., 2019), glinides, biguanides, and acarbose (Ma R. 2014).

“Despite the success of these drugs in lowering and regulating blood glucose levels, most of these anti diabetic drugs have adverse side effects, including gastrointestinal disorders, anemia, renal failure, weight gain, and hypoglycemia” (Syed W. G et al, 2017). “Therefore, searching for new natural medications with more effective and safer properties is a priority for discovering new anti diabetic drugs” (Laurie E. et al., 2020, Dinda B. and Dinda M., 2022, Riaz Z. et al., 2020).

MATERIALS AND METHODS

MATERIALS:

Acquisition of Plant Materials:

Musa Acuminata were obtained from the modern market in Makurdi, Nigeria and was authenticated by a plant scientist in Nigeria.

Acquisition of Alloxan:

Alloxan was obtained from Unique Pharmacy and chemicals at Ibadan, manufactured by Oxford Lab Fine Chem LLP and authenticated by a University chemist in Nigeria.

Acquisition of Metformin:

Metformin was obtained from Unique Pharmacy at Ibadan, manufactured by Pharmatex industries limited and authenticated by a University chemist in Nigeria.

Extraction of Plant Materials:

The preparation of plant (*Musa acuminata* leaves) was washed and sun-dried for five days. The dried leaves were ground into fine flour using a laboratory mortar and pestle, then it was sieved using a laboratory test sieve. 500mg of the sieved *Musa acuminata* leaves was dissolved in 1L of distilled water and left to stand for 48hrs after which the sample obtained was kept in a container for administration.

Preparation of Alloxan:

1.6g of alloxan was pounded using a laboratory mortar and pestle into a fine powder after which it was dissolved in 50 ml of distilled water, and diluted. The concentration of alloxan obtained was 32mg/ml.

Preparation of Metformin:

700mg of Metformin was pounded using a laboratory mortar and pestle into a fine powder after which it was dissolved in 50 ml of distilled water, and diluted. The concentration of Metformin obtained was 14mg/ml.

Experimental Animal:

Twenty-four (24) Wistar rats were purchased from Animal House, College of health science, Benue State University Makurdi, Benue, Nigeria. They were housed in the Animal House of the Department of Human Anatomy and allowed to acclimatize for one week before the commencement of the experiments. All the animals were given food (rat chow) and water *ad libitum*. The experiment lasted for a period of ten days during which diabetics were induced in rats in group B to E and then administration of extract started after 48h of inducements and the blood glucose level was measured

using a glucometer before administration of extract in groups C to F after which experimental groups were administered aqueous extract of *Musa Acuminata* of various dosages. The experimental rats were weighed at the study's beginning and end.

METHOD

Experimental Design:

A total number of 24 Wistar rats (male and female) were distributed randomly into six groups (Six rats/group). The experiment lasted for ten days, during which alloxan was induced in rats in groups B to F, and after 48h of inducements and the blood glucose level was measured using a glucometer before administration of extract in groups C to F.

Group A (negative control group) received distilled water, 5mls/kg daily for ten days.

Group B (the positive control group) received 150mg/kg of alloxan intraperitoneally.

Group C received single dose of Alloxan 150mg/kg intraperitoneally on day 1 and 100mg/kg of extract orally from day 3 to 10 days.

Group D received single dose of Alloxan 150mg/kg intraperitoneally on day 1 and 200mg/kg of extract orally from day 3 to 10 days.

Group E received single dose of Alloxan 150mg/kg intraperitoneally on day 1 and 300mg/kg of extract orally from day 3 to 10 days.

Group F received single dose of Alloxan 150mg/kg intraperitoneally on day 1 and 14mg/kg of standard drug (metformin) orally from day 3 to 10 days.

ANIMAL SACRIFICE:

At the end of the experiment (day 11), all the animals were humanely sacrificed. Blood was collected through the animals' left ventricle of the heart in a heparinized centrifuge tube under deep anesthesia with chloroform. The blood collected was centrifuged using a centrifuge machine at 10,000 rpm (revolution per minute) for five minutes, and the serum collected was subjected to a kidney function test (Urea and Creatinine) and estimation of oxidative stress enzymes Malondialdehyde (MDA). The kidney tissue was harvested for histological examination.

BIOCHEMICAL ASSAY KIDNEY FUNCTION TEST:

The kidney enzymes analysis, Creatinine, and Blood Urea Nitrogen (BUN) were done using an auto-analyzer.

ESTIMATION OF OXIDATIVE STRESS ENZYMES:

Using the auto-analyzer, the kidney oxidative stress makers analysis was carried out for Malondialdehyde (MDA).

DATA ANALYSIS

Results obtained were analyzed using the statistical software Statistical Package for Social Scientist (SPSS version 18.0), and results were expressed as mean \pm SEM. Differences among means of the groups were determined using one-way ANOVA with LSD post hoc test. Paired sample t-test was also used as appropriate, and values were considered statistically significant when $p < 0.05$.

RESULTS

PHYSICAL OBSERVATION:

During administration, all the experimental animals (Wistar rats) were observed to have routine physical activity. Weight changes were observed in the experimental animals, **Day 1** before administration and **Day 11** after administration.

During the administration period, **Table 4.1** shows body weight changes in experimental animals using one-way ANOVA. Weight changes were observed in the groups. An increase in body weights of animals was observed in **Group B, C, D, E and F** when compared to **Group A**. However, results revealed a slight decrease in the body weight of **Group D** compared to **Group B**.

There was a significant increase ($p < 0.05$) in the body weight of **Group B, C, D, E and F** animals compared to **Group A**. However, results revealed a significant increase ($p < 0.05$) in the body weight of **Group C, E and F** when compared to the **Group B**.

Table 4.1: Showing the mean \pm standard deviation of body weight across all groups.

Groups	Initial body weight (g)	Final body weight (g)	Body weight difference (%)
Group A	88.70 \pm 12.44	134.82 \pm 30.88	46.12 \pm 27.30
Group B	112.50 \pm 15.90	151.84 \pm 23.26	39.34 \pm 9.13
Group C	113.10 \pm 12.81	154.08 \pm 16.90	40.98 \pm 11.94
Group D	108.30 \pm 19.17	136.04 \pm 23.99	27.74 \pm 13.93
Group E	117.70 \pm 7.54	157.18 \pm 18.23	39.48 \pm 17.31
Group F	115.65 \pm 14.65	156.98 \pm 17.45	31.33 \pm 15.86

BIOCHEMICAL ASSAY

KIDNEY ENZYMES

Creatinine and Blood Urea Nitrogen (BUN):

Table 4.2 revealed that the Alloxan-treated group (**Group B**) expressed decreased levels of kidney enzymes (Creatinine and Blood Urea Nitrogen (BUN)). There was a decrease in kidney enzyme (creatinine) levels in **Groups B, C, D, E and F** compared to **Group A** and an increase in **Group C, D, E and F** compared to **Group B**. However, in Urea, there was a decrease in **Group B, C, D, E and F** when compared to **Group A** and an increase in **Group C, D, E and F** when compared to **Group B**

However, there was a significant decrease in both creatinine and Urea in **Group C, D, E and F** when compared to **Group A**

Table 4.2: Showing the mean \pm standard deviation of kidney enzymes across all groups.

Groups	Creatinine (mg/dl)	Blood urea nitrogen (mg/dl)
Group A	2.02 \pm 0.08	24.12 \pm 2.87
Group B	0.77 \pm 0.19	18.36 \pm 1.52
Group C	0.91 \pm 0.27	20.56 \pm 2.06
Group D	0.99 \pm 0.53	18.81 \pm 2.51
Group E	0.97 \pm 0.26	20.81 \pm 2.59
Group F	0.95 \pm 0.37	19.87 \pm 2.34

OXIDATIVE STRESS INDICATOR

Estimation of lipid peroxidation malonaldehyde:

Results depicted in **Table 4.3** showed that the administration of *Musa acuminata* crude extract caused a significant increase in the levels of MDA compared to the low levels observed in the control group. However, there is also a substantial difference in the decrease of MDA in **Group C, D, E and F** compared to the **Group B**.

In the liver tissue, increased levels of Malondialdehyde were recorded in **Group C, D, E and F** rats.

Table 4.3: Showing the mean \pm standard deviation of lipid peroxidation malonaldehyde across all groups.

Groups	MDA (nmol/mg pro)
Group A	0.79 \pm 0.16
Group B	2.14 \pm 0.14
Group C	0.99 \pm 0.26
Group D	1.87 \pm 0.52
Group E	1.29 \pm 0.37
Group F	1.46 \pm 0.34

NOTE: *= $p > 0.05$, extract= *Musa acuminata*

HISTOLOGICAL STUDY OF THE KIDNEY

Histological Observations:

Microscopically the kidneys of **Group A** as seen in the histological test with surrounding surfaces being granular, and numerous cortical tissues were present with corticomedullary closure and devoid of vascular markings.

The photomicrograph of group A showed a normal kidney micro-structure which are: a spherical glomerulus that is composed of superficial endothelial cell capillaries, Bowman's capsules with simplified squamous cells, proximal convoluted tubules with a cuboidal cell with micro villi, distal contortus tubules with a cuboidal cell with no micro-villi, and the medulla consists of a loop of Henle with simple squamous cell.

Group B, C, D, E and F kidney sections appear contracted and have an agranular surface. The cut surface shows a general cortical tissue loss, corticomedullary differentiation, and vascular markings. Their pyramids are small but intact. The parenchyma showed much hemorrhage and vascular congestion. We also observed that there were degenerative changes in the swelling of the epithelium of the proximal.

When you compare **Group B** kidneys to **Group A**, there were varying degrees of relatively cellular interstitial nephritis. A characteristic feature is presence of atrophic tubules, rendering a granular appearance to the kidney surface. The histomorphology of **Groups C, D, E and F** when compared to **Group B** has a near normal appearance but were not completely normal.

Plate 1a and 1b: served as the control group and (sterile water and feed only),

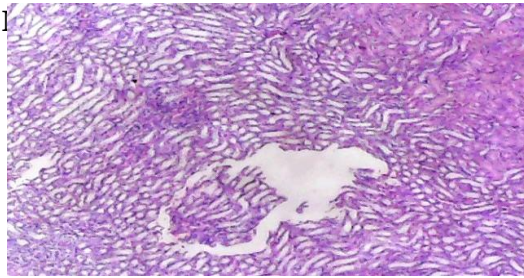
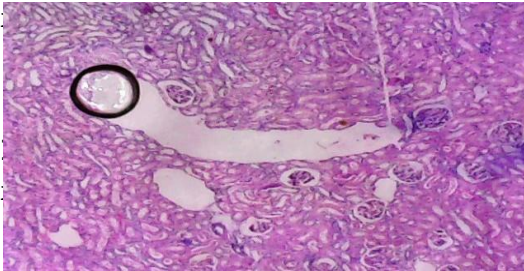
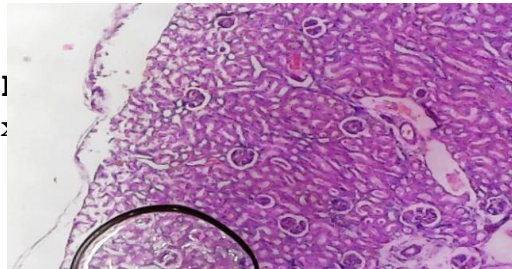


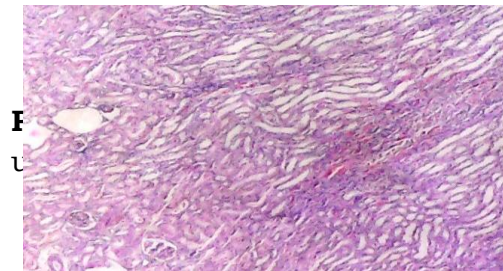
Plate 2a and 2b: served as the alloxan group (positive control), Magnification=



extract (low dose) and alloxan. Magnification=



extract (medium dose) and alloxan. Magnification=



F extract (high dose) and Alloxan, Magnification = x40

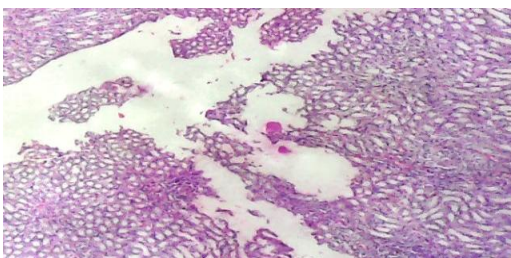
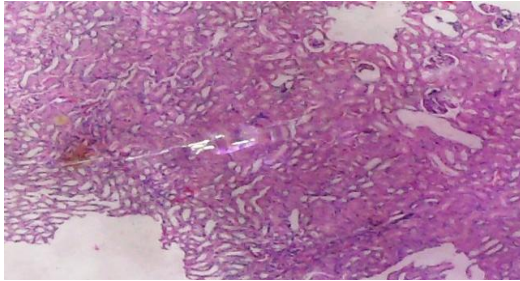


Plate 6a and 6b served as the metformin and Alloxan, Magnification = x40 using H and E



DISCUSSION

Glycemic homeostasis refers to glucose balance or control within circulation in living organisms. It usually is compromised mainly in diabetes. The ability of therapeutic compounds, including medicinal plants, to restore glycemic balance or homeostasis in hyperglycemic conditions is an index of their anti diabetic function and relevance. Diabetes was induced by alloxan which aggravates the destruction of beta cells of the pancreas caused by reactive oxygen species (ROS), simulating type-1 and 2 Diabetics Mellitus (Ali, 2018).

PHYSICAL OBSERVATION: The present study evaluated the changes in body weight in diabetes mellitus-induced and treated animals for the entire study period. A decrease in body weight is considered a marker for developing Diabetics Mellitus. Our results indicated a considerable change in body weight between alloxan-induced and treated Wistar rats. These results agree with the findings of Miaffo D et al., 2019.

Physical observation of the experimental animals (Wistar rats) revealed changes in the body weights with decrease in body weight observed in the alloxan-treated group (**Group B**), it could be due to the administration of alloxan. Changes in body weight have been used to indicate the adverse effects of drugs and chemicals (Miaffo D et al., 2019).

The observed increase in **Group C, D, E and F** body weights suggests that the extract might be partially potent against alloxan compared to Group B's weight (Umerah N.N et al., 2023).

KIDNEY ENZYMES: Our results showed that alloxanization caused a significant decrease in Creatinine and blood Urea nitrogen. Our results are consistent with those reported by others (Idonije B.O. et al., 2011), who showed that serum Urea and

Creatinine levels were decreased in diabetics mellitus in Wistar rats. The reduced concentrations of Urea and creatinine could be due to excessive lipolysis in severe diabetes mellitus leading to ketosis and later on to acidosis in **Groups B,C,D, E and F**, as per the studies of (Daisy and Kani, 2013)

A Remarkable decrease of Creatinine and Blood Urea Nitrogen (BUN) enzyme level observed with alloxan administration in **Group B** animals indicates diabetic mellitus. **Groups C, D, E and F** shows an increase in Urea and Creatinine compared to **Group B**. This was ascertained by a comparative analysis of the results obtained in rats pretreated with *Musa acuminata*. **Group F** (Metformin) shows a decrease in Urea and Creatinine which indicates protection effect on anti diabetic.

OXIDATIVE STRESS INDICATOR: Malondialdehyde (MDA) dependently act in the metabolic pathways that involve free radicals. Therefore changes in antioxidant enzyme activity and oxidative stress markers MDA are indicators of Diabetics Mellitus. Extensive Malondialdehyde leads to membrane disorganization by peroxidation of unsaturated fatty acids, which also alters the ratio of poly-unsaturated to other fatty acids. This would lead to a decrease in membrane fluidity and the death of cells. The increase in MDA is due to the toxic effect of alloxan (Joydeep D. et al., 2012).

The increased lipid Peroxidation leads to cellular infiltration and islet cell damage in Diabetics Mellitus (O. Sekiou et al., 2018). This study noticed elevated lipid peroxidation levels in alloxan-treated Wistar rats following Omar Sekiou et al. 2021 investigations. The antioxidant and free radical quenching nature of *Musa acuminata* may accomplish this normalization.

Increased antioxidant enzyme activity (MDA) observed in alloxan treated group (**Group C**) reflects treatment-related toxicity. This is in agreement with reports from alloxan-related toxicity studies; The increase in the level of MDA in this study toxic effect of alloxan on the kidney by Omar Sekiou et al., 2021.

From the results obtained (**table 4.3**), it can be concluded that the extract at a high dose (**Group E**) shows a high-risk case of diabetes mellitus when compared to the low dose (**Group C**) of the extract, as demonstrated in kidney enzyme level and oxidative stress indicators. Group F (Metformin) shows an increase in Malondialdehyde which indicates protection effect on anti diabetic but not better than **Group C**.

HISTOPATHOLOGY OF TREATED EXPERIMENTAL ANIMALS: Histological examination was used to show the severity of toxicity of alloxan-induced Diabetic mellitus. Microscopically the kidneys of Group A appeared to be expected, as seen in histological text. The surrounding surfaces were granular, and numerous cortical tissues were present with corticomedullary closure and devoid of vascular markings. **Group B, C, D, E and F** kidney sections appear contracted and have an agranular surface. The cut surface shows a general loss of cortical tissue, corticomedullary

differentiation, and vascular markings, which agrees with the findings of Mahmoud and Mahmoud, 2017, Al-Ankily et al., 2020; Elias 2020, Salem et al., 2021 .

“The recorded alterations could describe diabetes as enhancing parenchyma destruction leading to the induction of oxidative stresses” (Sadeghinezhad et al., 2016). There was coagulative necrosis (pyknosis) in the epithelium of Henle's loop and the presence of albuminous exudates in the proximal convoluted tubules. In the glomerulus, necrosis in endothelial cells caused a significant gap in the Bowman's space area, and the capsules were seen necrotic in simplex squamous cell wall structure, proximal contortus tubule, and distal contortus tubules were seen to be necrotic, simplex scaly cell wall structure was necrotic and appeared desquamated in its features. Even in the tubular sections, numerous mononuclear inflammatory cells were seen to be necrotic, following the studies of El-Ghazawy et al., 2020.

When you compare **Group B** kidneys to **Group A**, there were varying degrees of relatively cellular interstitial nephritis, areas of dilated tubules alternate with atrophic tubules, rendering a granular appearance to the kidney surface, a characteristic feature. The histomorphology of **Groups C, D, E and F (especially Group C)** seems to be better off than **Group B**, but these were not better than **Group A**.

CONCLUSION

Data from the present study indicate that the aqueous extract of *Musa acuminata* leaves at low dose (100 mg/kg body weight) in the alloxan Induced Wistar rat has some anti diabetics potential than at 200 mg/kg body weight, 300 mg/kg body weight and standard drug (Metformin). From this study, it can be concluded that the crude extract of *Musa acuminata* leaves protects against kidney oxidative damage at low dose (100mg/kg) and could be used as an effective protector against alloxan-induced diabetic mellitus.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

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