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Assessment of Anti-diabetic Potentials of Methanol Extract of *Manihot dulcis* Tuber in Aloxan-Induced Diabetic Wistar Rats

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ABSTRACT

Though many plants and their active chemical compounds have been used in the management of diabetes mellitus, the increasing worldwide incidence of diabetes especially amongst young adults continues to constitute global public health burden and the possibility of a total cure in most cases still remains a challenge. This study assesses the anti-diabetic potentials of methanol extract of *Manihot dulcis* tuber in Aloxan-Induced Diabetic Wistar Rats. Forty two matured male Wistar rats were divided into six groups. DM was induced in group 2, 4, 5 and 6 rats using Aloxan monohydrate (150 mg/kg), after fifteen minutes interval of administration of *Nicotinamide* while rats in group 1 and group 3 were not induced. Group one and Group 2 rats serves as the non-diabetic and diabetic untreated control rats respectively. Group 3 and 4 rats received *M. Dulcis* tuber (350 mg/kg) respectively Group 5 rats received *M. Dulcis* tuber (700 mg/kg) body weight while Group 6 received metformin. Treatment lasted for 21 days before euthanizing and collection of Blood for biochemical examinations. Data was represented as Mean $\pm$ Standard Error of Mean (SEM) and analyzed using GraphPad prism version 8.0 (GraphPad Software, San Diego, CA, USA). Comparison of mean value differences between groups was performed using one-way analysis of variance (ANOVA), followed by Turkey post hoc test. p - Values ≤ 0.05 was considered statistically significant. Result obtain shows that *M. dulcis* methanol tuber extract has potent anti-diabetic properties through weight-restorative properties, increasing C-peptide level while decreasing blood glucose, HOMA-insulin resistance, *alpha-glucosidase* and *alpha-amylase* activities. However, comparative analysis with standard anti-diabetic drugs such as metformin is recommended to evaluate the relative efficacy of the extract *M. dulcis*.

Keyword: Alloxan, Blood glucose, Diabetes, Hypoglycemia, Manihotdulcis, Wistar rat.

1.0 INTRODUCTION

Diabetes Mellitus (DM) is a disease associated with severe derangement of carbohydrate, protein, and lipid metabolism (1, 2). It is one of the most severe chronic diseases associated with chronic hyperglycemia due to insulin secretion and/or insulin action defects. (3, 2). The increasing worldwide incidence of diabetes mellitus in adults continues to constitute a global public health burden, especially Type 2 diabetes mellitus (T2DM), which continues to be a major worldwide life-threatening problem (3, 4). The World Health Organization (WHO) reports estimated that over 422 million people worldwide had diabetes in 2014, and this is likely to double in the year 2030, with India, China, and the United States predicted to have the largest number of affected individuals (5).

Many plants and their active chemical compounds have been used in the management of DM and its complications (3, 6, 7). According to ethnobotanical discovery, more than 800 plants have been used as

traditional remedies in different forms for diabetes care (8, 9). The management of diabetes is always a challenge without side effects; hence, plants continue to play an important role in the discovery of new compounds for treatment (10). One method of managing diabetes is by reducing post-prandial hyperglycemia by delaying the activities of the enzymes α -amylase and α -glucosidase, which are responsible for the digestion of carbohydrates and absorption of glucose in the digestive tract, respectively (11). Drugs derived from natural products have played a major role in the development of pharmaceutical treatments for diabetes. Metformin, the single most prescribed agent for the treatment of diabetes, originated from herbal medicine 'galesine,' a plant-derived anti-diabetic agent which was isolated from *Galega officinalis*, and experimental and clinical evaluations provided the pharmacological and chemical basis for the subsequent discovery of metformin (10). 1- Deoxynojirimycin (DNJ), a potent inhibitor of α -glucosidase, has also been isolated from the water extract of mulberry leaves (*Morus Alba*) (10). Natural product-based agents are especially desirable because their side effects are minimal and therapies are well tolerated compared to other currently available oral hypoglycemic agents (12).

Plants are an important source of biologically active natural products, which present a wide variety of structures, physical/chemical, and biological properties. Within the plant sub-kingdom, *Tracheobionta*, represent a group of vascular plants that comprises over 23,600 species, of which are located in the tropical and sub-tropical regions of the world (13, 14). Few studies have reported the presence of secondary metabolites in *Euphorbiaceae juss* (Spurge family). The most commonly reported are flavonoid glycosides, mainly kaempferol, quercetin, luteolin, and apigenin derivatives, xanthones, spiropyranosyl derivatives, and triterpenoids (15, 16).

Manihot dulcis (Sweet cassava) is a brittle-stemmed, dichotomously branched shrub up to 5 m tall, with root tubers up to 50 cm long, smooth bark, and watery whitish latex (17). It is widely cultivated throughout tropical Africa for its tuberous roots, which serve as a staple food in many tropical regions as well as in native populations of the East tropical South America (18). The tubers of *Manihot dulcis* contain prussic acid, a potent poison, which is destroyed by cooking (17). It had been reported as a good source used in making sweet Tapioca Flour (19), and food plant of *Manihot dulcis* had been reported used to the management of hypertension, headache, and other pains, irritable bowel syndrome and fever (20), and the bitter variety leaves of *Manihot esculenta* which is a subfamily of *Manihot* plants family are also used to treat hypertension, headache, and pain (21). The leaves of *Manihot dulcis* are high in vitamins A and C, and iron, and have 2% protein by dry weight (17). While there is an opinion that *Manihot* family, especially the *M. esculenta*, could be a healthier choice in the management of diabetes (20), there is no scientific evidence in the literature on the use of *Manihot dulcis* in the management of Diabetes mellitus. The food plant *M. dulcis* is used medicinally to treat fever, irritable bowel syndrome, headaches, and other problems (8), while the bitter leaves of *M. esculenta* are also used to treat fever, headaches, and hypertension (7). *M. dulcis* leaves have 2% protein by dry weight and are rich in iron, vitamin A, and vitamin C (8). They can be substituted for spinach or lettuce (9). Prussic acid, a potent toxin found in the tubers, is destroyed by heating.



(a) (b) (c)

Figure 1: The pictures above are the (a) leaf, (b) root (c) stem of *Manihot dulcis* Field work

Taxonomy of *Manihot dulcis*

Rank	Scientific Name and Common Name
Kingdom	<i>Plantae</i> – Plants
Subkingdom	<i>Tracheobionta</i> - Vascular plants
Superdivision	<i>Spermatophyta</i> - Seed plants
Division	<i>Magnoliophyta</i> - Flowering plants
Class	<i>Magnoliopsida</i> – Dicotyledons
Subclass	<i>Rosidae</i>
Order	<i>Euphorbiales</i>
Family	<i>Euphorbiaceae</i> Juss. - Spurge family
Genus	<i>Manihot</i> Mill. – cassava
Species	<i>Manihot dulcis</i> – sweet cassava

(14).

Ranges and Distribution of *Manihot dulcis*

Manihot dulcis is widely cultivated throughout tropical Africa for the tuberous roots which form a staple food in many tropical regions as well as in native of East tropical South America (18). It is used to make sweet tapioca flour (19). It is also used as a food plant and in medicine to treat fever, irritable bowel syndrome, hypertension, headaches, and other pains (20). The bitter leaves of the *Manihot esculenta* variety are also used to treat fever, headaches, and hypertension (21). *Manihot dulcis* leaves contain 2% protein by dry weight and are rich in iron, vitamins A and C, and other nutrients (20). You can use young leaves in place of spinach or lettuce (17). Aside from having lower levels of methionine, lysine, and possibly isoleucine, the research currently available indicates that *Manihot* plant leaf protein has an amino acid profile that is comparable to that of milk, cheese, soy, fish, and eggs, making it a good source of protein (8). Nevertheless, nothing is known about the nutritional value of *manihot* plant leaf meal concentrates. In their investigation of the impact of *M. esculenta*, *crantz*, and *M. dulcis* extract on the biochemical profile of infected albino rats, Zekarias *et al.* (12) found that *M. dulcis* significantly decreased ($P<0.05$) liver enzymes, *ALT*, *ALP*, and *AST*, while rats that consumed *M. esculenta*, *crantz* significantly increased ($P<0.05$) these parameters when compared to the control group.

Though many plants and their active chemical compounds have been used in the management of diabetes mellitus, the increasing worldwide incidence of diabetes especially amongst young adults continues to constitute global public health burden and the possibility of a total cure in most cases still remains a challenge. This study therefore assesses the anti-diabetic potentials of methanol extract of *M. dulcis* tuber in Aloxan-Induced Diabetic Wistar Rats.

2.0 MATREIALS AND METHODS

Identification of Plant

Fresh tuber of *M. dulcis* tuber was harvested from its growing habitant in Ughoton, Okpe Local Government Area of Delta State and was scientifically identified at the Department of Plant Science and

Biotechnology, University of Port Harcourt with Herbarium Number (UPH/V/283) and then taken for extraction at the Department of Human Anatomy and Cell Biology, Delta State University, Abraka, Delta State, Nigeria.

The tuber was divided into three (3) parts, each with different processing method; *M. Dulcis* tuber (Air-dried), *M. Dulcis* tuber (Fried-Garri) and *M. Dulcis* tuber (Fermented). These extracts were transported to the advance research center of the Delta State University, Abraka for evaluation of cyanide level and glucose concentration. The extract with the least concentration of cyanide and glucose was used for this study.

Extraction of Plants

M. dulcis tuber (Air-dried) was washed, air-dried and grounded in powder form. The powder was measured with Mettler weighing balance and 1000g of the grounded powder was soaked in 4 litres of methanol/water (80%/20% of methanol and water respectively) for 72 hours. The mixture was filtered using Whatmans filter paper No. 1. The filtrate was condensed to dryness in vapour at 40°C using water bath, yielding 9g (18% w/w) of a dark green semi-solid extract. For the experiment, the extract was put in sealed container and stored in the refrigerator at 4°C until use.

Drug Procurement and Preparation

Sodium Citrates

The sodium citrates ($C_3H_4(OH)(COONa)_3 \cdot 2H_2O$) (BDH chemicals LTD Pools England, Batch No. 5425/18/58) was purchased from Uche Scientific Co. Ltd. 21, Head bridge Market Onitsha, Anambra State, Nigeria. Two grams (2g) of Sodium Citrate was dissolved in 100ml of water to yield 2% of citrate buffer

Alloxan Monohydrate

The Alloxan (monohydrate) LR, $C_4H_2N_2O_4 \cdot H_2O$, product number: Allo108, Batch number CE102AK01) was purchased from Uche Scientific Co. Ltd. 21, Head bridge Market Onitsha, Anambra State, Nigeria. Alloxan monohydrate of 0.6g was dissolved in 10ml of citrate buffer to yield 60mg of stock solution (Diabetic agent).

Metformin

Metformin (Glucophage) was purchased from Demek Pharmacy Ltd. Obairuku, Ukwuani Local Government Area, Delta State, Nigeria. 500mg of metformin (Glucophage) was dissolved in 10ml of distilled water, to give a solution of anti-diabetic drugs. Rat dose = (Human dosage / Average Body Weight) x 7.

Animal Use

Forty two (42) matured male Wistar rats weighing between 120-180 g were used for this study. The rats were procured from the animal house at the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. The animals were acclimatized for seven days in well aerated cages with the natural controlled system (room temperature and a natural 12 h-12 h light-dark cycle). The rats were allowed free access to water and feed ad libitum with standard commercial pelleted feed, a product of Top Feed in Sapele, Delta State.

Ethical consideration

The Research Ethics and Grants Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria, evaluate, examine and approved the protocol of the study with ref number

(RBC/FBMC/DELSU/26/1224). This study was carried out in compliance with the established ethical standards for animal care and usage as laid down by Helsinki, in 1964 and adopted by Ossai *et al.* (22).

Induction and Confirmation of Diabetes Mellitus

Forty two (42) matured male Wistar rats were divided into six groups of seven rats per group for the study. The animals were fasted for about 8 – 11 hours prior to the induction of diabetes. Type 2 Diabetes mellitus was induced in rats by a single intraperitoneal (I.P) injection of freshly prepared solution of alloxan at a dose of 150 mg/kg body weight (6), after a fifteen minutes oral administration of Nicotinamide (10 mg/kg) body weight. Diabetes was not induced in group one and three rats.

Confirmation of Diabetes Mellitus

A glucometer (Accu-check active, Germany) and glucometer strip was used to confirm diabetes mellitus by measuring blood glucose level after a 72 hours period of alloxan injection. Rats with elevated fasting serum glucose over 200 mg/dL, were considered diabetic.

Experimental Design:

The Wistar Rats was grouped and treated as follows;

Group 1 (n = 7): Non-diabetic Control Wistar rats received distil water (0.5 ml).

Group 2 (n = 7): Diabetic Control Wistar rats received distil water (0.5 ml).

Group 3 (n = 7): Non-diabetic Wistar rats received *Manihot dulcis* tuber (350 mg/kg) extract.

Group 4 (n = 7): Diabetic Wistar rats received *Manihot dulcis* tuber (350 mg/kg) extract.

Group 5 (n = 7): Diabetic Wistar rats received *Manihot dulcis* (700 mg/kg) extract.

Group 6 (n = 7): Diabetic Wistar Rats received metformin glucophage (100 mg/kg).

Euthanizing of Animals and Sample Collection

Rats were euthanized by cervical dislocation. Each rat was placed on its dorsal surface to perform laparotomy for expose of the internal organs. The small intestine was excised and blood was also collected by cardiac puncture, using 2ml syringes and 23G needles into blood sample containers for biochemical analysis.

Body Weight and Blood Glucose Determination

Determination of Body Weight

Weekly assessment of body weight was carried out using a digital electronic weighing balance (Model JA 2003). Values obtained was rerecorded and expressed in grams.

Percentage body weight change was calculated as follows;

$$\text{Percentage weight change (\%)} = \frac{\text{final-initial bodyweight (g)}}{\text{intial bodyweight (g)}} \times \frac{100}{1}$$

Determination of Blood Glucose Level

Weekly assessment of blood glucose level was carried out using an Accu check glucometer (Accu Check active German) and Accu Check active strip. Blood was collected from the tip of the animal's tail. Values obtained was rerecorded and expressed in mg/dl.

Biochemical Analysis

Determination of C-Peptide Using ELISA Method

The procedure for collection of blood sample and preparation of c-peptide assay were adopted from the study of Tietz, in 2015. Blood samples were collected via cardiac puncture into plain sample bottles and allowed to clot at room temperature. The samples were then centrifuged at 3000 rpm for 10–15 minutes to obtain serum, which was separated and stored at 2–8°C for short-term use for the analysis.

Principle of the Assay

Following the method by Donald *et al.* (1967) and adopted by Burtis and Bruns (24), C-peptide concentration was determined using the enzyme-linked immuno-sorbent assay (ELISA) based on the sandwich principle, where C-peptide in the sample binds to specific antibodies coated on the micro-plate wells, followed by binding of an enzyme-linked secondary antibody. The intensity of the color developed is directly proportional to the concentration of C-peptide present in the sample.

Estimation of Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)

Principle of the Method

The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) is an indirect method used to estimate insulin resistance from fasting blood glucose and fasting insulin levels (25). It is based on a mathematical model that describes the relationship between insulin secretion and glucose regulation under fasting conditions (26).

Sample Collection

Blood samples were collected from experimental animals after an overnight fast (8–12 hours). The blood was centrifuged at 3000 rpm for 10–15 minutes to obtain serum or plasma, which was stored at 2–8°C for immediate analysis (23).

Determination of Fasting Blood Glucose

Fasting blood glucose was measured using an accu check glucometer (Accu Check active German) and accu Check active strip.

Determination of Fasting Insulin

Fasting serum insulin concentration was determined using an enzyme-linked immunosorbent assay (ELISA) kit, following the manufacturer's instructions (24).

Calculation of HOMA-IR

The HOMA-IR value was calculated using the formula: (26)

$$\text{HOMA-IR} = \frac{\text{Insulin} \times \text{Glucose (mg/dL)}}{405}$$

Where, 405 is the adjusted-conversion-adjusted constant when glucose is measured in mg/dL instead of mmol/L.

Interpretation

1. Low HOMA-IR → high insulin sensitivity (normal condition)
2. High HOMA-IR → increased insulin resistance

A decrease in HOMA-IR after treatment indicates improved insulin sensitivity

α -amylase level assay

Principle of the Assay

The method by Payen and Jean-Francois (1833) and adopted by Ossai *et al.* (11), was used. The α -amylase assay is based on the enzyme's ability to hydrolyze starch into smaller sugars such as maltose. The amount of reducing sugars produced is then measured using 3,5-dinitrosalicylic acid (DNSA)

reagent, which reacts with reducing sugars to form a colored complex. The intensity of the color, measured spectrophotometrically, is proportional to α -amylase activity. In the presence of an inhibitor (e.g., plant extract), enzyme activity decreases, resulting in reduced color intensity

α -Glucosidase Activity Assay.

Principle of the Assay

Kim *et al.* (27) method as adopted by Apostolidis *et al.* (28) and Ossai *et al.* (11), was used for this assay. The α -glucosidase assay is based on the ability of the enzyme α -glucosidase to hydrolyze a synthetic substrate, p-nitrophenyl- α -D-glucopyranoside (pNPG), to release p-nitrophenol, a yellow-colored product. The intensity of the yellow color, measured spectrophotometrically, is proportional to enzyme activity. In the presence of an inhibitor (e.g., plant extract), enzyme activity is reduced, leading to decreased color intensity

Statistical analysis

Data was represented as Mean $\pm$ Standard Error of Mean (SEM) and analyzed using GraphPad prism version 8.0 (GraphPad Software, San Diego, CA, USA). Comparison of mean value differences between groups was performed using one-way analysis of variance (ANOVA), followed by Turkey post hoc test. p - values ≤ 0.05 was considered statistically significant.

3.0 RESULTS

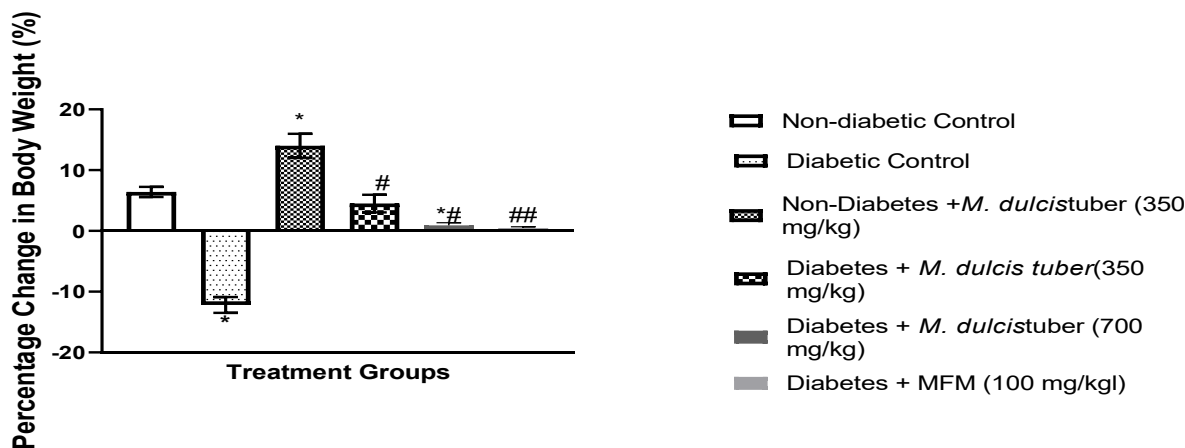


Figure 2: The graph above shows the effect of *M. dulcistuber* methanol extract on percentage changes in body weight of alloxan-induced diabetes in male Wistar rats

Bars represent Mean $\pm$ SEM (n = 7). One-way ANOVA followed by Tukey's post hoc test. *p < 0.05 vs non-diabetic control; #p < 0.05 vs diabetic control.

The graph shows the percentage change in body weight (%) across different treatment groups of alloxan-induced diabetic male Wistar rats. Non-diabetic control rats shows a moderate increase in body weight (~+5–7%) indicating normal growth. Diabetic control shows a significant decrease in body weight (~–12 to –15%) confirming that alloxan-induced diabetes causes weight loss. The Non-diabetic + *M. dulcis* (350 mg/kg) shows the highest increase (~+13–15%) suggesting that the extract may promote weight gain even in healthy rats. Diabetic + *M. dulcis* (350 mg/kg) shows a moderate increase (~+4–6%) indication of partial reversal of weight loss. The Diabetic + *M. dulcis* (700 mg/kg) shows near normal/slight increase (~0–2%) suggestive of improvement, but less than 350 mg/kg (dose-dependent effect may not be linear).

The Diabetic + Metformin (100 mg/kg) shows almost no change (~0%) suggestive of stabilization of body weight

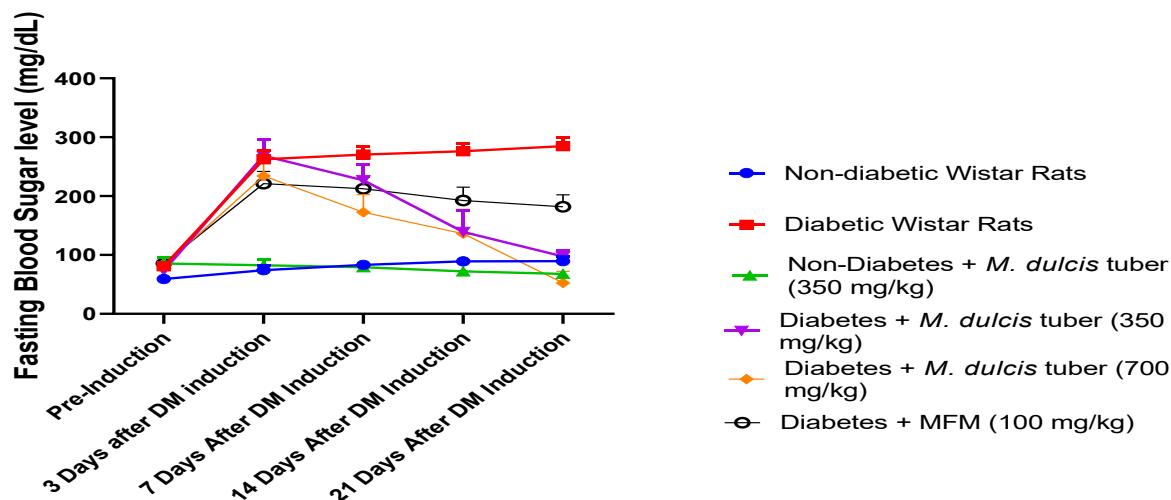


Figure 3: The graph above shows the effect of *M. dulcis* tuber methanol extract on blood sugar level in alloxan-induced diabetes in male Wistar rats

Values are Mean $\pm$ SEM (n = 7). *p < 0.05 vs non-diabetic control, #p < 0.05, ###p < 0.01, ####p < 0.001 vs diabetic control (One-way ANOVA followed by Tukey's post hoc test)

Administration of *M. dulcis* tuber methanol extract resulted in a significant reduction (p < 0.05) in fasting blood glucose levels in alloxan-induced diabetic rats in a dose- and time-dependent manner. The 700 mg/kg dose exhibited greater glycemic control compared to 350 mg/kg, although the effect was less pronounced than the standard drug-treated group.

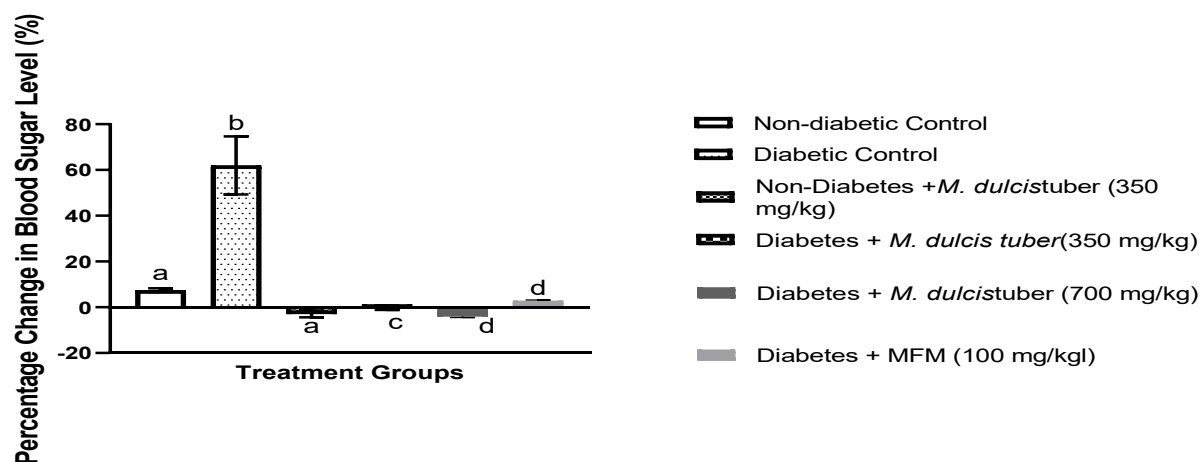


Figure 4: The graph above shows the effect of *M. dulcis* tuber methanol extract percentage change in the blood sugar level of alloxan-induced diabetes in male Wistar rats

Values are expressed as Mean $\pm$ SEM (n = 7). Bars with different superscript letters (a–d) are significantly different at p < 0.05 (One-way ANOVA followed by Tukey's post hoc test).

The diabetic control group exhibited a significant increase (p < 0.05) in percentage blood glucose levels compared to the non-diabetic control. Treatment with *M. dulcis* tuber extract resulted in a dose-dependent

decrease in blood glucose levels, with the 700 mg/kg group showing a more pronounced reduction compared to the 350 mg/kg group. The effect was comparable to the standard drug-treated group. No significant alteration was observed in non-diabetic rats treated with the extract.

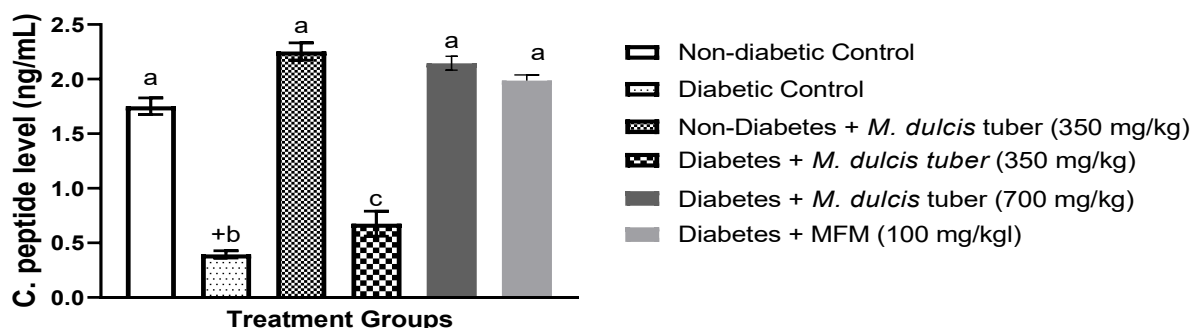


Figure 5: The graph above shows the effect of *M. dulcis* tuber methanol extract on C-peptide level in alloxan-induced diabetes in male Wistar rats

The statistical annotations (a, b, c) indicate significant differences among groups, where groups with different superscripts differ significantly at $p < 0.05$. The diabetic control group differed significantly from the treated groups, further supporting the efficacy of both the extract and standard drug in restoring C-peptide levels.

The diabetic control group showed a significant ($p < 0.05$) reduction in C-peptide levels compared to the non-diabetic control, indicating impaired β -cell function. Treatment with *M. dulcis* tuber extract resulted in a dose-dependent increase in C-peptide levels, with the 700 mg/kg group showing a marked improvement comparable to the standard drug-treated group. This suggests restoration of pancreatic β -cell function and enhanced insulin secretion.

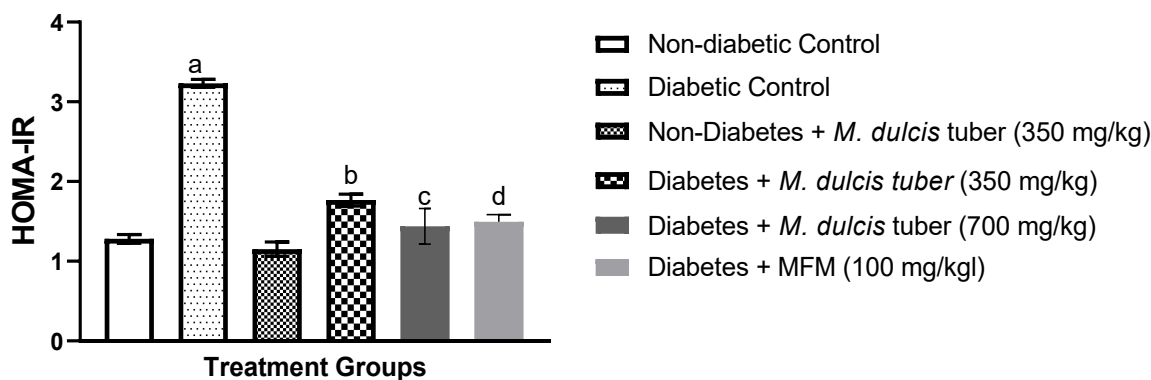


Figure 6: The graph above shows the effect of *M. dulcis* tuber methanol extract on HOMA-Insulin Resistance/Sensitivity in alloxan-induced diabetes in male Wistar rats

Values are expressed as mean $\pm$ SEM ($n = X$). Bars with different superscripts (a, b, c, d) are significantly different at $p < 0.05$. a = significantly different from non-diabetic control, b, c, d = significantly different from diabetic control

The diabetic control group exhibited a significant increase in HOMA-IR compared to the non-diabetic control group, indicating a high level of insulin resistance. This confirms that alloxan induction impaired insulin action and glucose utilization. Administration of *M. dulcis* tuber extract led to a reduction in HOMA-IR values in diabetic rats. The group treated with 350 mg/kg showed a moderate decrease, while the 700 mg/kg group showed a more pronounced reduction, indicating a dose-dependent improvement in insulin sensitivity. The standard drug group (metformin, 100 mg/kg) also showed a significant decrease in HOMA-IR compared to the diabetic control, consistent with its known insulin-sensitizing effect. However, the 700 mg/kg extract demonstrated a comparable or even greater reduction, suggesting strong antidiabetic potential. The non-diabetic group treated with the extract maintained HOMA-IR values similar to the normal control, indicating that the extract does not induce insulin resistance under normal physiological conditions.

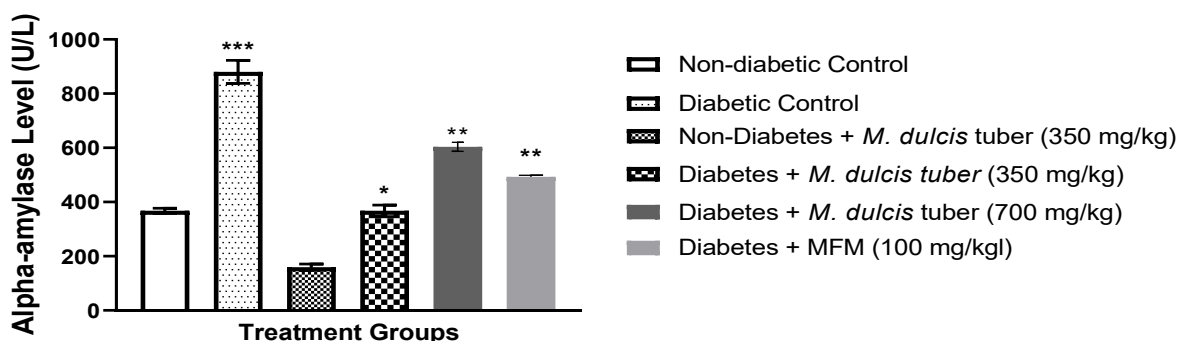


Figure 7: The graph above shows the effect of *M. dulcis* tuber methanol extract on alpha-amylase level in alloxan-induced diabetes in male Wistar rats

Bars represent Mean $\pm$ S.E.M. (n = 7) (One-way ANOVA followed by Turkish *post hoc* test). * $p < 0.05$, relative to controls; ** $p < 0.05$ relative to control, *** $p < 0.05$ relative to control.

The results show that the diabetic control group exhibited a marked and statistically significant increase ($p < 0.05$) in *alpha-amylase* activity compared to the non-diabetic control group. This elevation suggests enhanced carbohydrate digestion and glucose release, which is consistent with the hyperglycemic state observed in diabetes. In contrast, administration of *M. dulcis* tuber extract resulted in a significant reduction ($p < 0.05$) in *alpha-amylase* levels in diabetic treated groups when compared to the diabetic control. The effect appears dose-dependent, as the group treated with 700 mg/kg showed a greater reduction in enzyme activity than the 350 mg/kg group. This indicates that the extract may inhibit *alpha-amylase* activity, thereby reducing the rate of carbohydrate breakdown and subsequent glucose absorption. Interestingly, the non-diabetic group treated with 350 mg/kg extract showed a decrease in *alpha-amylase* activity compared to the normal control, suggesting that *M. dulcis* may exert inhibitory effects on carbohydrate-digesting enzymes even under normoglycemic conditions. Furthermore, the metformin-treated group also demonstrated a significant reduction ($p < 0.05$) in *alpha-amylase* levels compared to the diabetic control, with values comparable to those observed in the extract-treated groups. This suggests that the effect of *M. dulcis* may be similar to that of standard antidiabetic therapy in modulating carbohydrate metabolism.

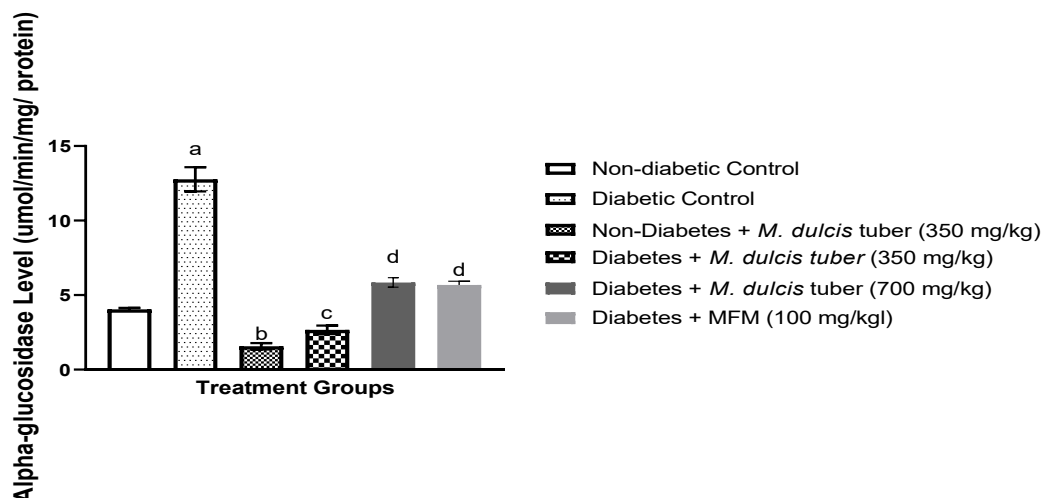


Figure 8: The graph above shows the effect of *M. dulcis* tuber methanol extract on alpha-glucosidase level in alloxan-induced diabetes in male Wistar rats

Values are expressed as mean $\pm$ SEM (n = 7). Statistical analysis was performed using one-way ANOVA followed by Tukey post hoc test. Bars with different superscripts (a–d) are significantly different ($p < 0.05$), a = significantly different from non-diabetic control, b, c, d = significantly different from diabetic control

The diabetic control group exhibited a significant increase ($p < 0.05$) in *alpha-glucosidase* activity compared to the non-diabetic control group. This elevation indicates enhanced breakdown of disaccharides into glucose, which contributes to increased intestinal glucose absorption and worsening hyperglycemia in diabetes. In contrast, treatment with *M. dulcis* tuber extract caused a significant reduction ($p < 0.05$) in alpha-glucosidase activity in diabetic rats when compared to the diabetic control. This suggests that the extract has inhibitory effects on *alpha-glucosidase*, thereby slowing carbohydrate digestion and reducing glucose release into the bloodstream. The effect was dose-dependent, as the group treated with 700 mg/kg showed a greater reduction in enzyme activity than the 350 mg/kg group. This indicates stronger enzyme inhibition at higher extract concentrations, likely due to increased availability of active phytochemicals. Additionally, the non-diabetic group treated with 350 mg/kg extract showed a reduction in *alpha-glucosidase* activity compared to the normal control, suggesting that *M. dulcis* may regulate carbohydrate metabolism even under normal physiological conditions. The metformin-treated group also showed a significant decrease ($p < 0.05$) in *alpha-glucosidase* activity relative to the diabetic control, with values comparable to the extract-treated groups, indicating that the extract exhibits effects similar to standard antidiabetic therapy.

4.0 DISCUSSION

The present study evaluated the effect of *M. dulcis* tuber methanol extract on percentage changes in body weight in alloxan-induced diabetic male Wistar rats. The findings demonstrate significant alterations in body weight across experimental groups, reflecting both the pathological effects of diabetes and the therapeutic potential of the extract. The diabetic control group showed a marked decrease in body weight compared to the non-diabetic control. This observation is consistent with the established pathophysiology of alloxan-induced diabetes. Alloxan selectively destroys pancreatic β -cells through the generation of reactive oxygen species, resulting in insulin deficiency (29). The lack of insulin impairs glucose utilization by peripheral tissues, forcing the body to rely on fat and protein catabolism for energy, which ultimately leads to weight loss (30). Additionally, persistent hyperglycemia promotes dehydration and increased metabolic demand, further contributing to the observed reduction in body weight (31). Similar

findings have been reported in previous studies, where untreated diabetic animals exhibit progressive weight loss due to metabolic imbalance and insulin deficiency (32). In contrast, the non-diabetic rats treated with *M. dulcis* extract (350 mg/kg) showed an increase in body weight compared to the normal control group. This suggests that the extract may possess nutritive or metabolic-enhancing properties that promote growth and improve nutrient utilization. The presence of bioactive compounds such as flavonoids, alkaloids, and phenolic constituents may contribute to improved digestion, enhanced appetite, and stimulation of anabolic processes (31). More importantly, diabetic rats treated with *M. dulcis* extract exhibited improvement in body weight in a dose-dependent manner. The group treated with 350 mg/kg showed moderate recovery, while the higher dose (700 mg/kg) demonstrated a more pronounced increase in body weight. This suggests that the extract is capable of attenuating the catabolic effects of diabetes. The improvement in body weight may be attributed to enhanced glycemic control and possible restoration of insulin activity. Previous studies have shown that agents capable of improving insulin secretion or protecting pancreatic β -cells can significantly reduce diabetes-induced weight loss (32, 29). The standard drug-treated group (metformin, 100 mg/kg) also showed improvement in body weight compared to the diabetic control group. This aligns with the known pharmacological action of metformin, which enhances insulin sensitivity and promotes glucose uptake in peripheral tissues while reducing hepatic glucose production (33). The similarity between the effects of the extract and metformin suggests that *M. dulcis* may possess comparable antidiabetic properties. The partial restoration of body weight observed in treated diabetic groups may be explained by several mechanisms. These include improved glycemic control, which reduces excessive breakdown of fats and proteins; possible stimulation of insulin secretion or preservation of pancreatic β -cell integrity; reduction in oxidative stress induced by alloxan; and enhanced nutrient absorption and metabolic efficiency (31). However, the inability of the extract to fully restore body weight to normal levels indicates that while it significantly ameliorates metabolic dysfunction, complete recovery may require prolonged treatment or higher doses. Overall, the findings of this study indicate that *M. dulcis* tuber methanol extract exerts a protective effect against diabetes-induced weight loss, likely through its antihyperglycemic and antioxidative properties. This supports its potential role as a therapeutic agent in the management of diabetes and its associated metabolic complications.

The findings from this study demonstrate that induction of diabetes using Alloxan resulted in a marked elevation in fasting blood glucose levels in the untreated diabetic group, confirming successful establishment of experimental diabetes. This is consistent with the known mechanism of alloxan, which selectively destroys pancreatic β -cells through the generation of reactive oxygen species, leading to insulin deficiency and hyperglycemia (2). Administration of *M. dulcis* tuber extract produced a progressive reduction in fasting blood glucose levels across the treatment period. This effect was both dose-dependent and time-dependent, with the 700 mg/kg dose showing a greater glucose-lowering effect compared to the 350 mg/kg dose. However, the standard drug-treated group (100 mg/kg MFM) exhibited a more pronounced reduction, suggesting that while the plant extract is effective; its potency is comparatively lower than conventional pharmacotherapy. The observed antihyperglycemic effect of *M. dulcis* may be attributed to several possible mechanisms. First, the extract may enhance residual β -cell function or promote regeneration of pancreatic β -cells, thereby improving endogenous insulin secretion. Previous studies have shown that certain plant extracts possess β -cell regenerative potential, which contributes to restoration of glycemic control (34). Secondly, the hypoglycemic activity may be linked to improved peripheral glucose utilization and increased insulin sensitivity, similar to the mechanism of metformin, which enhances glucose uptake in tissues and reduces hepatic gluconeogenesis (33). In addition, the phytochemical constituents of *M. dulcis*, such as flavonoids, alkaloids, and phenolic compounds, may exert antioxidant effects, thereby mitigating oxidative stress induced by alloxan. Since oxidative stress plays a key role in β -cell destruction and the progression of diabetes, the antioxidant activity of the extract could help preserve pancreatic integrity and function (32). This aligns with reports that plant-derived antioxidants contribute significantly to glycemic control in experimental diabetes models. Interestingly, the non-diabetic rats treated with the extract maintained normal blood glucose levels throughout the study period, indicating that *M. dulcis* does not induce hypoglycemia in

normoglycemic conditions. This suggests a level of safety and glucose-dependent activity, which is a desirable property for antidiabetic agents.

C-peptide is widely recognized as a reliable indicator of endogenous insulin secretion and pancreatic β -cell function, since it is released in equimolar amounts with insulin from proinsulin cleavage (35). Therefore, its measurement provides important insight into the functional status of pancreatic β -cells. The findings from this study revealed that the diabetic control group showed a significant reduction ($p < 0.05$) in C-peptide levels compared to the non-diabetic control group. This observation is consistent with the known mechanism of alloxan-induced diabetes, where alloxan selectively destroys pancreatic β -cells through the generation of reactive oxygen species, leading to impaired insulin synthesis and secretion (32, 29). The reduced C-peptide level observed in this study therefore confirms β -cell dysfunction and successful induction of experimental diabetes. Administration of *M. dulcis* tuber extract resulted in a dose-dependent increase in C-peptide levels, suggesting a restorative effect on pancreatic function. The group treated with 350 mg/kg showed moderate improvement, while the 700 mg/kg group demonstrated a more pronounced increase, with values approaching those of the non-diabetic control. This indicates that the extract may promote β -cell regeneration or enhance residual insulin secretion. Similar findings have been reported for plant-derived extracts rich in phytochemicals such as flavonoids and phenolic compounds, which are known to exert antioxidant and β -cell protective effects (36, 37). Interestingly, the non-diabetic groups treated with *M. dulcis* extract maintained C-peptide levels comparable to the normal control group, indicating that the extract does not over stimulate insulin secretion under normal physiological conditions. This suggests a modulatory rather than excessive stimulatory effect, which is desirable in antidiabetic therapy (37). The standard drug-treated group (metformin, 100 mg/kg) also showed a significant increase in C-peptide levels compared to the diabetic control group. Although metformin primarily acts by improving insulin sensitivity and reducing hepatic glucose production, it has been reported to indirectly preserve β -cell function (33). Notably, the higher dose of *M. dulcis* extract produced an effect comparable to that of metformin, suggesting that the plant extract may possess clinically relevant antidiabetic potential. Overall, the results suggest that *M. dulcis* tuber extract exerts antidiabetic effects, likely through mechanisms involving antioxidant activity, protection of pancreatic β -cells, and enhancement of endogenous insulin secretion. These findings align with previous reports highlighting the therapeutic potential of medicinal plants in diabetes management (36, 37).

Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) is a well-established index for assessing insulin resistance and reflects the interaction between hepatic glucose production and insulin secretion (26). Findings from this study revealed that the diabetic control group exhibited a significant increase in HOMA-IR compared to the non-diabetic control group. This elevation indicates pronounced insulin resistance, a core feature of diabetes mellitus (29). This observation is consistent with earlier reports that alloxan-induced diabetes leads to pancreatic β -cell destruction, hyperglycemia, and impaired insulin signaling, thereby increasing insulin resistance (29, 38). Increased HOMA-IR values have also been strongly associated with metabolic dysfunction and poor glycemic control (26, 39). Treatment with *M. dulcis* tuber extract resulted in a significant reduction in HOMA-IR levels compared to the diabetic control group, suggesting an improvement in insulin sensitivity. This reduction appears dose-dependent, with the 700 mg/kg dose showing a more pronounced effect than the 350 mg/kg dose. Dose-dependent improvements in insulin sensitivity following plant extract administration have been widely reported and are often attributed to the bioactive phytochemicals present in such extracts (40, 41). The observed improvement in insulin sensitivity may be due to several mechanisms. Phytochemicals such as flavonoids and phenolic compounds present in medicinal plants are known to enhance insulin receptor activity, promote glucose uptake in peripheral tissues, and suppress hepatic gluconeogenesis (42, 43). Additionally, antioxidant properties of these compounds may reduce oxidative stress, which is a major contributor to insulin resistance in diabetes (44). Therefore, the reduction in HOMA-IR observed in the treated groups suggests that *M. dulcis* may exert its antidiabetic effect through both insulin-sensitizing and antioxidant mechanisms. Furthermore, the HOMA-IR values in the extract-treated groups approached

those of the non-diabetic control group, indicating partial restoration of normal metabolic function. This finding aligns with studies showing that improvement in insulin resistance is a critical therapeutic target in diabetes management and is associated with better glycemic outcomes (45). The effect of *M. dulcis* extract was also comparable to that of the standard drug-treated group (metformin), a well-known insulin sensitizer, suggesting that the extract may possess pharmacologically relevant antidiabetic properties (46). The statistical annotations (a, b, c, d) in the figure indicate that the differences observed among groups are significant ($p < 0.05$), particularly highlighting the disparity between the diabetic control and treated groups. This reinforces the reliability of the findings and supports the hypothesis that *M. dulcis* tuber extract ameliorates insulin resistance. Overall, the results of this study demonstrate that *M. dulcis* tuber methanol extract significantly reduces insulin resistance in alloxan-induced diabetic rats, as evidenced by decreased HOMA-IR values, thus supporting its potential as a therapeutic agent in the management of diabetes mellitus. The findings are in agreement with previous studies emphasizing the importance of improving insulin sensitivity as a strategy for controlling hyperglycemia and preventing diabetic complications (39, 45).

Alpha-amylase is a key enzyme involved in the hydrolysis of dietary starch into absorbable sugars, and its activity is directly linked to postprandial glucose levels (47, 48). Results from this study showed that the diabetic control group exhibited a significant increase ($p < 0.05$) in *alpha-amylase* activity compared to the non-diabetic control group. This elevation suggests enhanced carbohydrate digestion and increased glucose availability, which contributes to hyperglycemia. This finding is consistent with previous reports that diabetes is associated with increased digestive enzyme activity and impaired glycemic control (47, 49). Treatment with *M. dulcis* tuber extract resulted in a significant reduction ($p < 0.05$) in *alpha-amylase* activity compared to the diabetic control group. This indicates that the extract possesses inhibitory activity against *alpha-amylase*, thereby potentially reducing the rate of starch breakdown and glucose absorption. Inhibition of *alpha-amylase* is a well-established therapeutic approach in the management of diabetes, particularly for controlling postprandial hyperglycemia (48, 50). The reduction in *alpha-amylase* activity observed in this study was dose-dependent, with the higher dose (700 mg/kg) producing a greater inhibitory effect than the lower dose (350 mg/kg). This suggests that the bioactive constituents of *M. dulcis* exert stronger enzyme inhibitory effects at higher concentrations. Similar dose-dependent inhibitory effects have been reported for plant-derived compounds such as flavonoids and phenolics, which interact with the active site of *alpha-amylase* and reduce its catalytic activity (42, 51). Interestingly, the non-diabetic group treated with *M. dulcis* also showed a reduction in *alpha-amylase* activity compared to the normal control, suggesting that the extract may modulate carbohydrate metabolism even under normal physiological conditions. This supports earlier findings that plant extracts can regulate digestive enzyme activity irrespective of disease state (49). Furthermore, the metformin-treated group demonstrated a significant reduction ($p < 0.05$) in *alpha-amylase* activity compared to the diabetic control group, with values comparable to those of the extract-treated groups. Although metformin primarily reduces hepatic glucose production and improves insulin sensitivity, its comparable effect in this study suggests that *M. dulcis* may exert complementary antidiabetic actions through enzyme inhibition (46).

The present study demonstrated a significant increase in *alpha-glucosidase* activity in the diabetic control group compared to the non-diabetic control. Diabetes was induced using Alloxan, a compound known to selectively destroy pancreatic β -cells, resulting in insulin deficiency and persistent hyperglycemia (29). The elevated *alpha-glucosidase* activity observed suggests enhanced hydrolysis of complex carbohydrates into absorbable monosaccharides, thereby exacerbating postprandial hyperglycemia. This finding is consistent with previous reports that intestinal carbohydrate-digesting enzymes are often upregulated in Diabetes Mellitus, contributing to poor glycemic control (27, 12). Treatment with *Musa dulcis* tuber extract significantly reduced *alpha-glucosidase* activity in diabetic rats compared to the untreated diabetic group. This reduction indicates that the extract may possess inhibitory effects on *alpha-glucosidase*, thereby slowing carbohydrate digestion and glucose absorption. The observed effect may be attributed to the presence of bioactive phytochemicals such as flavonoids, tannins, and phenolic compounds. These

compounds are known to inhibit carbohydrate-hydrolyzing enzymes and improve glycemic control (52, 53). Furthermore, the reduction in enzyme activity was dose-dependent, with the 700 mg/kg dose showing greater efficacy than the 350 mg/kg dose. This suggests that higher concentrations of the extract provide increased bioactive compounds capable of exerting stronger enzyme inhibition. Similar dose-dependent inhibitory effects have been reported in plant-based antidiabetic studies (54). The standard drug group treated with Metformin showed a more pronounced reduction in *alpha-glucosidase* activity compared to the extract-treated groups. This aligns with the well-established efficacy of metformin in the management of type 2 diabetes. Although metformin primarily acts by reducing hepatic gluconeogenesis and improving insulin sensitivity, its overall effect in lowering blood glucose levels may indirectly reduce the activity of digestive enzymes, including *alpha-glucosidase* (33). The comparatively lower effect of *M. dulcis* extract suggests that while it has promising antidiabetic potential, it may be less potent than conventional pharmacological agents. However, its natural origin and possible lower side-effect profile make it a valuable candidate for complementary therapy.

Summary of findings

1. Diabetes causes significant weight loss and *M. dulcis* extract improves body weight in diabetic rats with the 350 mg/kg showing more effective than the 700 mg/kg
2. Study demonstrated a significant decreased blood sugar in diabetic rats treated with *M. dulcis* tuber methanol extract when compared to the diabetic untreated rats, and the effect is Dose-dependent (700 mg/kg > 350 mg/kg), and time-dependent (greater reduction over time)
3. The study also observed a significant increased C-peptide level in diabetic rats treated with *M. dulcis* tuber methanol when compared to diabetic untreated rats, and the increase was observed in a dose-dependent manner, indicating improved pancreatic β -cell function and enhanced endogenous insulin secretion.
4. result also indicate that *M. dulcis* tuber methanol extract significantly reduces HOMA-insulin resistance in diabetic rats in a dose-dependent manner, when compared to the diabetic untreated rats, thereby improving insulin sensitivity and supporting its potential use in the management of Type 2 Diabetes.
5. Alloxan significantly increases *alpha-amylase* and *alpha-glucosidase* activity in diabetic rats whereas, *M. dulcis* tuber extract effectively reduces the activities of these enzymes in a dose-dependent manner.

Contribution to Knowledge

1. This study also observed that *M. dulcis* tuber methanol extract possesses weight-restorative and anti-diabetic properties, increasing C-peptide level while decreasing HOMA-insulin resistance,
2. Findings also established that the decreased *alpha-amylase* and *alpha-glucosidase* activities observed in diabetic rats treated with *M. dulcis* tuber methanol extract may contribute to its antihyperglycemic action by decreasing carbohydrate digestion and glucose availability, thereby supporting its potential role in diabetes management.

Conclusion

This study concludes that *M. dulcis* methanol tuber extract has potent anti-diabetic properties through its ability to bring about weight-restorative properties, increasing C-peptide level while decreasing blood glucose, HOMA-insulin resistance, *alpha-glucosidase* and *alpha-amylase* activities

Suggestion for Further Studies

Additional research is required to isolate and characterize the specific bioactive compounds that may be responsible for the observed effects present in *M. dulcis* tuber.

Declaration

Not applicable

Ethical Approval

The Research and Ethics Committee of the Faculty of Basic Medical Sciences at Delta State University in Abraka, Nigeria approved the protocol for the experiments used in this work (REC/FBMS/DELSU/21/134). The ethical guidelines established by Helsinki in 1964 for the use and care of animals were followed in conducting this study

Conflict of Interest:

Author declares that there is no conflict of interest

Author's Contribution:

Author **JIW** designed, approved the research work and supervised the bench work, Author **SEE** also approved, reviewed the manuscripts and supervised the bench work in this research, Author **NRO** assisted during the bench work and cared for the experimental animals used and also carried out the Biochemical and statistical analysis, while Author **SUV** prepares the manuscript, carried out the bench work and beard the entire financial burden in this study. All Authors approved of the manuscript before been send for publication

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Availability of Data and Materials

Data will be made available on request through the corresponding Author

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