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AMELIORATIVE EFFECT OF METHANOLIC EXTRACT OF *MANIHOT ESCULENTA* SEED ON ALLOXAN INDUCED DIABETIC PANCREATIC DAMAGE IN MALE WISTAR RATS

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ABSTRACT

Diabetes mellitus presents a significant risk factor for the development of pancreatic injuries and is associated with both acute and chronic pancreatitis. This study investigated the effects of methanolic seed extract of Manihot esculenta (MSME) on body weight and pancreatic tissue in diabetic rats induced with alloxan monohydrate (AMH). Eight groups of seven animals each were assigned as follows: Group A received only distilled water and laboratory chow, Groups B and C received 100 mg/kg and 1000 mg/kg of MSME respectively for 3 weeks, Group D received a single dose of 120 mg/kg of AMH intraperitoneally, Groups E and F received AMH followed by immediate treatment with 100 mg/kg and 1000 mg/kg of MSME respectively for 3 weeks, Group G received MSME for 2 weeks followed by AMH induction. Thereafter, the animals were sacrificed after mild anesthesia and pancreas was harvested for histopathological studies. Data was analyzed using SPSS version 25 and results were expressed in mean ± SEM. Body weight and relative organ weight were analyzed using paired Sample T-test and One-way ANOVA (post Hoc LSD) respectively. Values were considered significant at $P \leq 0.05$. Results indicated a significant increase in body weight in MSME-treated groups, particularly in Group F. Pancreatic tissue analysis revealed tissue regeneration and healing in MSME-treated groups, indicative of potential therapeutic effects on diabetes-related complications. These findings support the potential utility of MSME in managing diabetes and its associated complications. Further research is warranted to elucidate the underlying mechanisms and optimize treatment regimens.

KEYWORDS: *Manihot esculenta*, Diabetic pancreatic damage, Alloxan-induced diabetes, Pancreatic tissue regeneration, Diabetes management.

INTRODUCTION

Diabetes mellitus (DM) stands as one of the prevailing non-communicable diseases worldwide, according to the International Diabetes Federation (2015), Kaviarasan *et al.*, (2010) and Rawal *et al.*, 2017. Its impact is profound, ranking as the fifth leading cause of mortality in both developed and developing nations (Ezeigbo, 2010; Anjana *et al.*, 2018). Given its escalating prevalence, DM has been deemed a global crisis of the 21st century. In Africa, the burden is significant, with 14.2 million individuals diagnosed (prevalence of 3.8%) in 2015, a figure projected to soar to 34.2 million (prevalence of 4.2%) by 2040 (IDF, 2015; BelloOvosi *et al.*, 2018). DM manifests as a chronic metabolic disorder marked by persistent hyperglycemia and disruption of carbohydrate, fat, and protein metabolism due to deficiencies in insulin secretion and/or action. Its ramifications are far-reaching, causing enduring harm to vital organs such as the eyes, kidneys, nerves, heart, and vasculature (American Diabetes Association, 2014; Dilworth *et al.*, 2021). The classification of DM hinges on its etiology, dividing into Type-1 and Type-2. Type-1, also known as Insulin-Dependent Diabetes Mellitus (IDDM), stems from autoimmune destruction of insulin-producing β-cells within the pancreas (Bhat, 2010; Yau *et al.*, 2021). Conversely, Type-2 Diabetes Mellitus (T2DM) or Non-Insulin Dependent Diabetes Mellitus (NIDDM) is a complex, progressive syndrome accounting for over 90% of all diabetes cases. Its spectrum ranges from insulin resistance with relative insulin deficiency to impaired insulin secretion (American Diabetes Association, 2014; Biswas *et al.*, 2010; Galicia-Garcia *et al.*, 2020).

Alloxan, scientifically termed as 5, 5-dihydroxyl pyrimidine-2, 4, 6-trione, is an organic compound belonging to the urea derivative class, known for its carcinogenic nature and cytotoxic effects (Lenzen, 2008; AbdRashed and Rathi, 2021). Frequently utilized in experimental settings, alloxan serves as a common diabetogenic agent to evaluate the antidiabetic properties of both isolated compounds and botanical extracts (Osasenagaga *et al.*, 2017; Sierra-Campos *et al.*, 2020). Alloxan-induced diabetes represents a type of insulin-dependent diabetes mellitus, induced through the administration or injection of alloxan in animal subjects (Dunn and McLetchie, 1943; Gomori and Goldner, 1945; Osasenagaga *et al.*, 2017). Its diabetogenic mechanism primarily involves selective uptake by pancreatic beta cells, leading to their subsequent accumulation (Szkudelski, 2001; Osasenagaga *et al.*, 2017).

Cassava, scientifically known as *Manihot esculenta*, has its origins rooted in Latin America, where indigenous communities have cultivated it for over four millennia (Akinpelu *et al.*, 2011). Nigeria stands as the leading global producer of cassava, yielding an impressive 45 million metric tonnes, and boasts the most advanced cassava transformation industry on the African continent (FAO, 2008; Egesi *et al.*, 2006). The cassava plant bears small seeds within capsules, akin in appearance and size to those of Castor oil (*Ricinus communis*), which burst open upon maturity to release the minute seeds (FAO, 2008).

Despite the wealth of research focused on various aspects of *Manihot esculenta*, particularly its leaves and roots, there exists a notable gap in literature concerning its seeds. Nevertheless, within local Nigerian communities, cassava seed oil finds utility in addressing infections caused by opportunistic skin pathogens such as Staphylococcus aureus, Propionibacterium acnes, Piiyrosporum ovale, and Candida albicans (Popoola and Yangomodou, 2006). This study endeavors to explore the potential antidiabetic properties of *Manihot esculenta*, an aspect of the cassava plant that has received relatively little attention, thereby presenting a novel contribution to scientific inquiry.

MATERIALS AND METHOD

Procurement of Experimental Animals

A total of fifty – six (56) male Wistar rats weighing between 130-160g was obtained from the Animal House of the College of Health Sciences and Technology, Nnamdi Azikwe University and they were acclimatized for 2 weeks before the commencement of the study.

Procurement and Identification of Plant Material

Cassava seeds were procured from local cassava farms in Amesi, Anambra state, and was identified at Botany Department in Nnamdi Azikiwe University and the herbarium number was deposited in the herbarium catalogue. The hebarium number is NAUH-35.

Housing of Experimental Animals

They were housed in well-aerated laboratory cages in the Animal House of the Department of Anatomy Nnamdi Azikiwe University. They were fed with standard rat feed and distilled water. All experimental procedures were in tandem with the commendations provided in the "Guide for the care and use of laboratory Animals" prepared by The National Academy of Sciences and published by the National Institute of Health (1985).

PREPARATION OF PLANT EXTRACT

Fresh matured cassava seed was harvested from a local farm in Amesi, Aguata LGA of Anambra state. To get this seed, the mature seed pods on the cassava plant was harvested and shed dried. The pods were cracked and seeds retrieved. These seeds were subsequently shed dried. The dried cassava seed (*Manhihot esculenta*) was grounded using a local grinder into a coarse form. About 250gram of the *M. esculenta* powder was soaked in 1000mls of absolute methanol for 48 hours after which the mixture was sieved using a porcelain cloth and was further filtered using Whatman no 1 filter paper into a clean glass beaker. The filtrate was concentrated using a digital rotary evaporator (TT – 52Techmel and Techmel USA) and was further dried using a thermostat oven at 45^0c into a gel-like form and stored in a refrigerator for further usage. The extraction method was done with slight modification according to the method employed by Al-Attar and Abu Zeid (2013).

EXPERIMENTAL DESIGN

The animals were divided into eight groups of seven animals per group as follows

Group A received only distilled water and laboratory chow *ad libitum.*

Group B received 100 mg/kg of MSME (methanolic seed extract of *Manihot esculenta*) for 3-weeks.

Group C received 1000 mg/kg of MSME (methanolic seed extract of *Manihot esculenta*) for 3-weeks.

Group D received a single dose of 120 mg/kg of Alloxan monohydrate (AMH) intraperitoneally once.

Group E received a single dose of 120 mg/kg of Alloxan monohydrate (AMH) and treated immediately with 100 mg/kg of MSME (methanolic seed extract of *Manihot esculenta*) for 3-weeks

Group F received a single dose of 120 mg/kg of Alloxan monohydrate (AMH) and treated immediately with 1000 mg/kg of MSME for 3-weeks

Group G received 1000 mg/kg of MSME (methanolic seed extract of *Manihot esculenta*) for 2-weeks and induced with a single dose of 120mg/kg of Alloxan monohydrate (AMH)

Group H received a single dose of 120 mg/kg of Alloxan monohydrate AMH and treated immediately with 500 mg/kg of metformin (MET) for 3-weeks.

Administration of the MSME was done through oral route and alloxan monohydrate which was given once and done through intraperitoneal route.

INDUCTION OF DIABETES MELLITUS

Induction of diabetes was done intra peritoneally according to Farshid *et al.*, (2015) with slight modification. The animals were fasted for 12-16 hours prior to the experiment to prevent hypoglycemia. 120 mg/kg of alloxan monohydrate (Sigma-Aldrich, USA) was dissolved in 0.9% of 10 mls of normal saline to obtain a concentration of 100 mg/ml. The solution was then injected into the peritoneal cavity of rats using 1 Ml syringe. The injection was given slowly to avoid any injury to the organs. The animals were then monitored for any adverse reactions such as breathing difficulties, bleeding or swelling at the injection site, or changes in behavior. After injection, the animals were provided with food and water *ad libitum*. Blood glucose levels was measured after 24 hours, and animals with blood glucose levels greater than 250 mg/Dl was considered diabetic.

Termination of Experiment and Sample Collection

Administration of substrates lasted for 21 days. Twenty-four hours after the last administration, the animals were anesthetized using chloroform in an enclosed container. Tissue (pancreas) was immediately harvested and was fixed in 10% formal saline for histopathological studies.

Determination of Body Weight of Animals

The body weight of the experimental animals in all groups were measured twice weekly using the animal weighing balance and recorded in grams. Their final weight was done prior to sacrificing.

Determination of The Relative Organ Weight

The relative organ weight was estimated as described by Al-Quadi *et al.*, (2016), by expressing the absolute organ weight to animal's body weight multiply by 100.

$$Relative\ organ\ weight = \frac{Aboslute\ organ\ weight}{Animal\ body\ weight}\ X\ 100$$

Histopathological Examination

Tissue (pancreas) was fixed in 10% formal saline and were dehydrated in four (4) concentrations of Isopropyl alcohol, i.e., 70%, 80%, 90%, 100% for 1hour each and then cleared in xylene before embedding in molten paraffin wax to remove the isopropyl alcohol. Micro sections of 5micrometer using Leica RM 212 Rt. Rotary Microtome, tissues were stained using Haematoxylin and Eosin (H&E) to demonstrate general tissue structure. Tissues

sectioned was examined and interpreted using Leica DM 750 binocular microscope with photomicrographic facilities and then photomicrographed by a histopathologist (Ahmed, 2016). This was carried out in the department of Anatomy Histology Laboratory, Nnamdi Azikwe University. Nnewi Campus, Anambra state.

STATISTICAL ANALYSIS

Data was analyzed using SPSS version 25 and results were expressed in mean ± SEM. Body weight was analyzed using paired T-test relative organ weight was analyzed using ANOVA followed by post Hoc LSD (least square significant *difference*) multiple comparison to ascertain the level of significance between control and treated groups. Values were considered significant at $P \leq 0.05$.

RESULTS

Table 1.0: Effect of Methanolic seed extract of *Manihot esculenta* on body weight in alloxan diabetic rats.

	Initial weight (g)	Final weight (g)	P-value	T-value
	MEAN±SEM	MEAN±SEM		
Group A (control)	154.33±4.70	227.33±2.19	0.001*	-27.591
Group B (100 mg/kg of MSME)	156.00±7.64	195.67±9.13	0.002*	-21.373
Group C (1000 mg/kg of MSME)	159.67±4.70	205.33±3.93	0.024*	-6.286
Group D (120mg/kg of AMH)	159.67±4.70	137.33±1.45	0.005 *	3.716
Group E (120mg/kg of AMH + 100 mg/kg of MSME)	139.33±10.84	158.00±15.95	0.085 [a]	-3.212
Group F (120mg/kg of AMH + 1000 mg/kg of MSME)	148.00±4.04	165.67±6.33	0.049*	-4.357
Group G (1000 mg/kg of MSME + 120mg/kg of AMH)	151.00±7.00	204.00±9.5.29	0.044*	-4.596
Group H (120mg/kg of AMH + 500 mg/kg of MET)	153.67±4,48	175.00±7.00	0.015*	-8.194

Data was analysed using T-paired test and values were considered significant at $p \leq 0.05$. SEM: Standard error of mean, AMH: Alloxan monohydrate, MET: metformin, MSME: methanolic seed extract of *Manihot esculenta*, *: significant, [a]: not significant.

Table 1.0 result showed a significant increase in the body weight in groups A, B, C, F, G, and H and group D had a significant decrease when initial weight was compared to final weight while group E did not show statistical difference when the initial weight was compared to the final weight.

Table 2.0: Effect of Methanolic seed extract of *Manihot esculenta* on the relative kidney weight and relative pancreas weight in alloxan diabetic rats.

	Relative pancreas weight (g)
	MEAN±SEM
Group A (control)	0.44±0.52[b, c]
Group B (100 mg/kg of MSME)	0.39±0.10[a, b, c]
Group C (1000 mg/kg of MSME)	0.35±0.05[a, b, c]
Group D (120mg/kg of AMH)	0.54±0.05[a, c]
Group E (120mg/kg of AMH + 100 mg/kg of MSME)	0.51±0.14[a, b, c]
Group F (120mg/kg of AMH + 1000 mg/kg of MSME)	0.45±0.07[a, b, c]
Group G (1000 mg/kg of MSME + 120mg/kg of AMH)	0.48±0.16[a, b, c]
Group H (120mg/kg of AMH + 500 mg/kg of MET)	0.37±0.08[a, b]
F-Ratio	0.49

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at $p \leq 0.05$.

SEM: Standard error of mean, AMH: Alloxan monohydrate, MET: metformin, MSME: methanolic seed extract of *Manihot esculenta*, [*]: significant when compared with group A (control group), [a]: not significant when compared with group A(control group), [#]: significant when compared with group D(diabetic group), [b]: not significant when compared with group D(diabetic group), [@]: significant when compared with group H(metformin group), [c]: not significant when compared with group D(metformin group).

The mean relative pancreas weight of the study was shown on table 2.0

On Relative pancreas weight, analysis showed that there was no statistically significant difference when compared with groups A (control), D (diabetic), and H (metformin).

Histological Findings

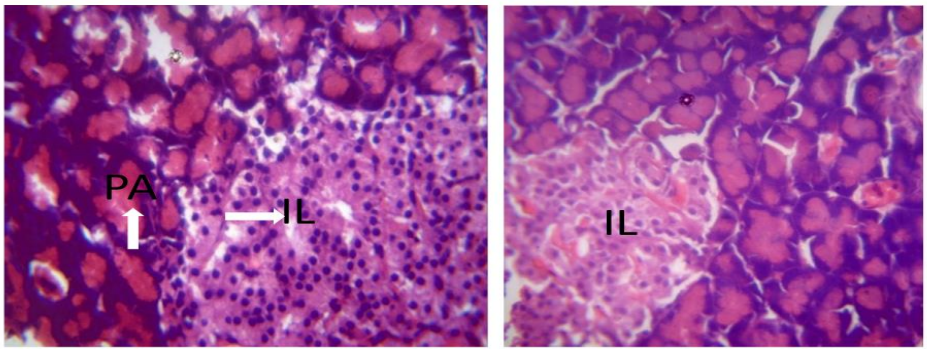

Plate 1.0 Photomicrograph of 1A and 1B control section of pancreas (x400) (H/E) shows normal pancreatic tissue with active islets of Langerhans (IL) and pancreatic acini (PA).

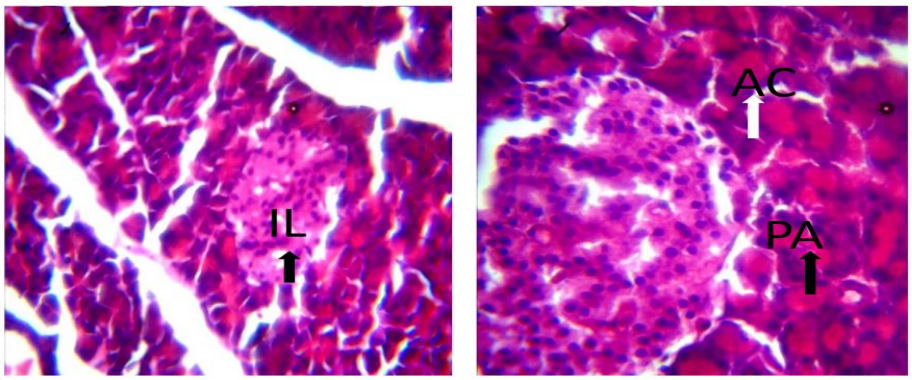

Plate 2.0 Photomicrograph of Group 2A and 2B section of pancreas administered with 100 mg/kg of MSME (x400)(H/E) shows active pancreatic tissue with islets of Langerhans (IL) surrounded by pancreatic acini (PA) and acini cell (AC).

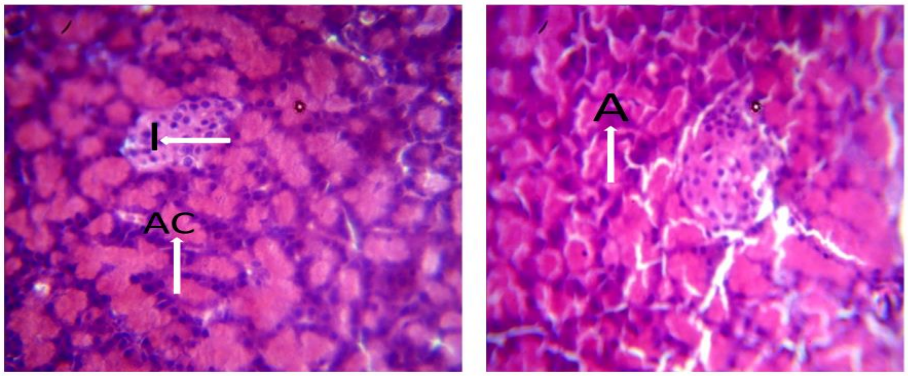

Plate 3.0 Photomicrograph of group 3A and 3B section of pancreas administered with 1000 mg/kg of MSME (x400) (H/E) shows pancreatic tissue with well perfused acini (A) and active islets (I) and acini cell (AC).

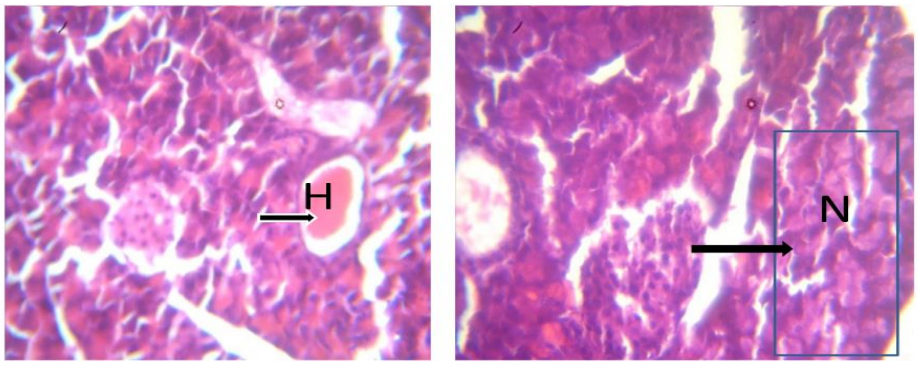

Plate 4.0 Photomicrograph of Group 4A and 4B section of pancreas induced with 120 mg/kg of AMH (x400)(H/E) shows moderate to severe degeneration with moderate area of hemorrhage (H) and severe necrotic acini and acini cell (N) in r2 with clumped islets of Langerhans.

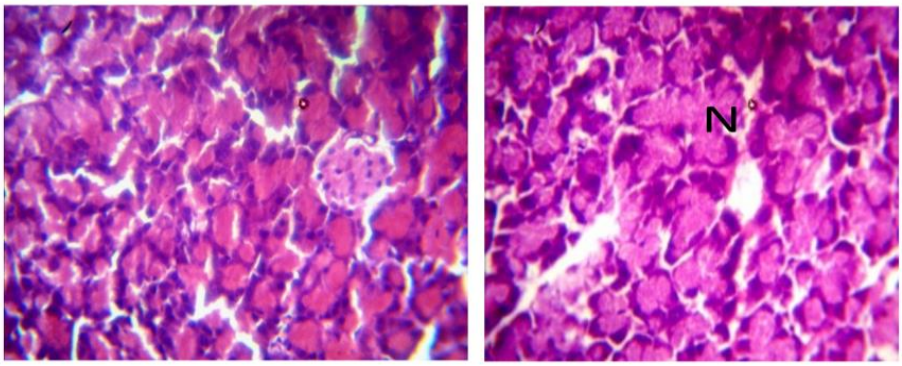

Plate 5.0 Photomicrograph of 5A and 5B section of pancreas induced with 120 mg/kg of AMH and treated with 100mg/kg of MSME (x400)(H/E) shows mild to moderate healing with moderate area of necrosis (N) in r2 and well outlined islet with non distinct pancreatic cell outline.

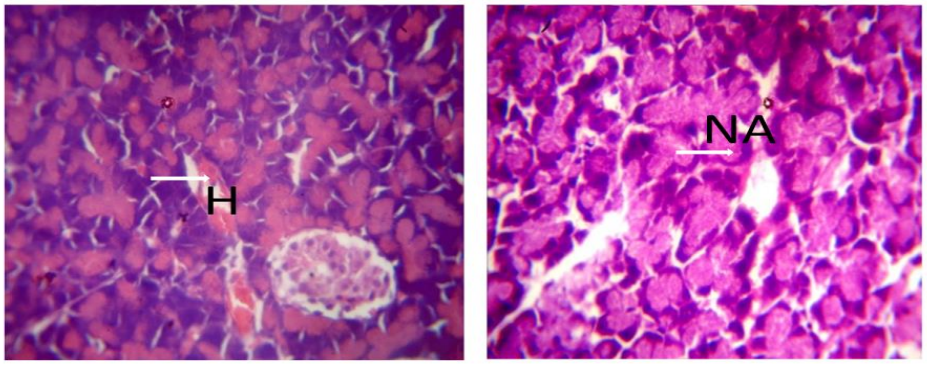

Plate 6.0 Photomicrograph of group 6A and 6B section of pancreas induced with 120 mg/kg of AMH and treated with 1000 mg/kg of MSME (x400)(H/E) shows moderate healing with mild area of hemorrhage (H) and necrotic acini (NA) in F2

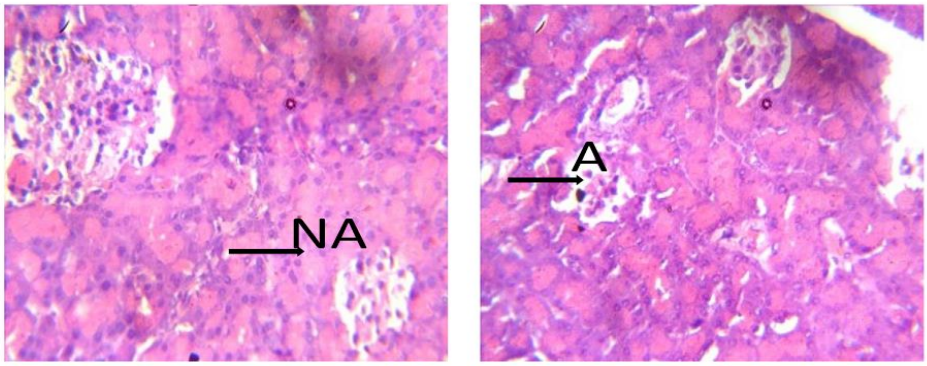

Plate 7.0 Photomicrograph of group 7A and 7B section of pancreas administered with 100 mg/kg of MSME and then received 120 mg/kg of AMH (x400)(H/E) shows moderate protection with mild area of necrotic acini (NA) and atrophy (A) of some islets cell.

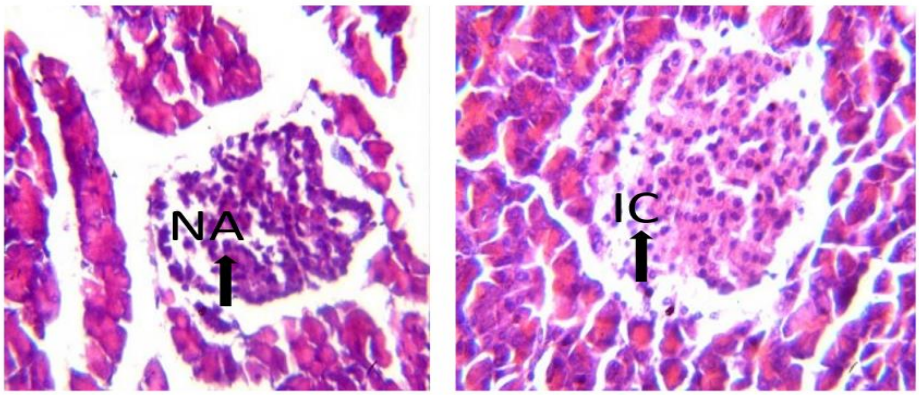

Plate 8.0 Photomicrograph of group 8A and 8B section of pancreas administered with 120mg/kg of AMH and treated with 500 mg/kg of Metformin (x400) (H/E) shows moderately protected with mild area of necrotic acini (NA) and atrophy (A) of pancreatic acini in H-1 and active islets cell (IC) in H-2.

DISCUSSION

Diabetes mellitus has shown to be linked to pancreatic cancer and it is a risk factor for the onset of acute and chronic cancer of the pancreas (Andersen *et al.*, 2017). Type II diabetes is caused by insulin resistance, resulting in impaired peripheral glucose utilization. However, the use of medicinal plants in the management of pancreatic function in diabetics have gained ground, resulting from the various secondary metabolites present (Solikhah *et al.*, 2023)

Findings from this study showed that alloxan monohydrate caused a decrease in the body weight gain as indicated in group D when initial was compared to final, while the intake of methanolic seed extract of *Manihot esculenta* alone at 100 and 1000 mg/kg caused a significant increase in the body weight as shown in groups B and C when initial was compared to final. Following the induction of diabetes and the subsequent treatment with 100 and 1000 mg/kg of methanolic seed extract of *Manihot esculenta* as shown in groups E and F, a significant increase was indicated in group F, and E had no significance when initial was compared to final. The intake of metformin demonstrated a significant increase in the body weight after diabetic induction, as shown in group H and group G revealed a significant increase in the body weight following intake of alloxan monohydrate after administration of 1000 mg/kg of methanolic seed extract of *Manihot esculenta*. The increase in weight gain (p 0.05) could be physiological.

In addition, the significant increase in the weight in the treatment groups in both diabetic and non-diabetic rats as indicated in groups B, C, F, and G could also be linked to the presence of

phenol and alkaloids, which plays a role in the alteration of body metabolism in a metabolic pathway although not fully elucidated. However, these secondary metabolites could help improve the energy homeostasis in the hypothalamus axis regulating feed intake and metabolism (Akapo *et al.,* 2014). The study is in agreement with the report of Akapo *et al.,* (2014) revealing a significant increase in the body weight gain following intake of *Manihot esculenta* root meal in birds. Palupi, Lubis, and Pratama, (2022) also demonstrated a significant body weight following the intake of *M. esculenta* root tuber in broiler, which is in line with the study outcome. Juan, (2023) in addition revealed a significant weight gain in the rabbit's following intake of 10% *Manihot esculenta* leaf extracts, which has consistency with the study findings. Also, the physiology linked to the increase body weight gain following metformin administration could be attributed to the regulation of brown adipose tissue, which is responsible for energy homeostasis (Naik *et al., 2022*). Naik *et al.,* (2022) reported a significant weight in diabetic rats administered with metformin, which is in line with the study report. Al Za'Abi *et al.,* (2021) reported a significant increase in the percentage gain weight following the administration of metformin in alloxan-diabetic rats, which is in line with the study findings. Li *et al.,* (2019) and Jiang *et al.,* (2017) reported a significant reduction in the body weight gain following metformin in diabetic rats, which contradict the study findings.

The loss of body weight seen in untreated diabetic group could be attributed to the decrease in the rate of metabolism following alloxan toxicity, which inhibits the neurotransmitters controlling feed intake and metabolism in the satiety centers in the hypothalamus. The study finding is in line with Opara *et al.,* (2021), Woldekidan *et al.,* (2021), Naik *et al.,* (2022). They reported a significant decline in the body weight following alloxan toxicity in diabetic rats compared to non-diabetic rats. Al-Qudah, Haddad, and El-Qudah, (2016) showed a decrease in percentage weight in diabetic rats following alloxan toxicity, which disagrees with the study findings.

The study findings showed that in diabetic rats, the pancreas indicates moderate to severe degeneration with moderate area of hemorrhage and severe necrotic acini and acini cell with clumped islets of Langerhans. The observed distortion of the pancreatic islet could be attributed to the effect of hydroxyl radicals generated from alloxan metabolism in the pancreatic beta-cells, which probably causes disruption of pancreatic tissue following oxidative stress(through the release of free oxygen from the hydroxyl radical combining with

pancreatic catalase enzyme (Osasenaga, Abiola and Oluseyi, 2017). The report of Ba and Ayuba, (2022), Prasetyo, Ghalib, Ahsani, and Fidianingsih (2023), Usman *et al.,* (2021) and Zhang *et al.,* (2003) showed similarity to the study findings as indicated in the pancreatic tissue following alloxan induction demonstrating pancreatic distortion. Following post-treatment with methanolic seed extract of *Manihot esculenta* after induction of diabetes, the pancreatic tissue showed tissue regeneration with mild to moderate healing in a dose dependent manner. However, methanolic seed extract of *Manihot esculenta* treatment alone at low and high dose indicated a well perfused acini cells and an active pancreatic tissue. The recuperating effect demonstrated by methanolic seed extract of *Manihot esculenta* following post-treatment in diabetes results from the presence of alkaloids and phenols, which have the tendency to combat ROS formation linked with increased lipid peroxidation. Prasetyo *et al.,* (2023) demonstrated that the ethanolic extract of the *M. esculenta* root showed recuperation of the pancreatic tissue following treatment win diabetic rats, which is similar to the study findings, which was linked to the high phenol content. Also, treatment with metformin indicated a significant recuperation of the pancreatic tissue after treatment in diabetic rats, which is linked to combating of ROS formation in the pancreas. The findings of Usman *et al.,* (2021), Moosavi *et al.,* (2020), Prasetyo *et al.,* (2023), Almuttairi, (2023) showed similarity to the study findings in diabetic rats. Yanti, Sucindra, and Jawi (2019) revealed that metformin had no effect on the pancreas histoarchitectural features, which disagree with the study findings.

CONCLUSION

In finding alternative ways to manage diabetes, this study delved into the effects of *Manihot esculenta*'s methanolic seed extract (MSME) on diabetic rats. In the study, some exciting findings that hint at the potential of MSME as a natural remedy for diabetes and its complications was uncovered. One standout discovery was the significant weight gain observed in rats treated with MSME, especially in those receiving higher doses. This suggests that MSME might play a role in regulating metabolism and energy balance in diabetic individuals, offering hope for improved health outcomes.

Moreover, when looked into the pancreatic tissue of these rats, we found signs of healing and regeneration. This hints at MSME's ability to protect the pancreas from damage caused by diabetes-related factors, potentially preserving its function and overall health. The findings

underscore the importance of exploring natural remedies like MSME in the fight against diabetes.

While there's still much to learn about how MSME works and its safety in humans, this study lays a solid foundation for future research and clinical trials. Ultimately, our hope is that MSME, with further investigation and validation, could emerge as a safe and effective option for individuals struggling with diabetes. By harnessing the power of nature, there may be new possibilities for managing this challenging condition and improving lives.

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