



Antidiabetic and Regenerative Effects of Double stem cell aqueous extract (*Malus domestica*) in Alloxan-induced Diabetic Rats

*Adejumo IA¹, Fageyinbo MS², Obiekwe PS¹, Ibe-Uba FN¹, Oreagba IA¹ and Agbaje EO¹

¹Department of Pharmacology, Therapeutics, and Toxicology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Nigeria

²Department of Pharmacology and Therapeutics, Faculty of Clinical Sciences, University of Medical Sciences, Ondo City, Ondo State, Nigeria

Corresponding Author: ifedolapokayode@gmail.com

Abstract

Diabetes Mellitus (DM) cannot be divorced from consequent dire health challenges with a projected prediction of the afflicted population expected to rise to 783 million by 2045. This study evaluated the acclaimed antidiabetic effect of aqueous extract of Double Stem Cell® (DSC) in alloxan-induced diabetic rats. Adult male rats were randomly assigned into six groups of five animals. Group I served as non-diabetic control while groups II-VI were induced with diabetes using 150 mg/kg-1, *b.w*, *i.p* of alloxan. Once daily administration of *Malus domestica* (50, 100, or 200 mg/kg-1, *p.o.*), normal saline or glibenclamide was done for 21 days. Fasting blood glucose (FBG) was recorded on days 1 and 21 after establishing diabetes mellitus using the alloxan model, followed by an assessment of biochemical and haematological parameters. The liver and pancreas were harvested for histopathology and antioxidants. Alloxan administration significantly increased FBG, indicating hyperglycaemia. Post-treatment with all doses of the extract and glibenclamide resulted in a significant ($p < 0.001$; $p < 0.0001$) reduction in FBG. Attenuation of alloxan-induced elevated Malondialdehyde (MDA), enhanced catalase, superoxide dismutase activities and regeneration of the alloxan-induced cell damage was demonstrated. Double Stem Cell® extract achieved hypoglycaemia and interestingly, regeneration of cytoarchitecture following alloxan-induced damage was established.

Keywords: Alloxan; Diabetes Mellitus; Double stem cell®; *Malus domestica*; Regenerative medicine

Introduction

Diabetes Mellitus (DM) is a chronic metabolic disease that affects all ages, defined as persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both and if left unmanaged can eventually lead to debilitating complications (Prandi *et al.*, 2022). Currently, an estimated 537 million individuals live with diabetes, with projections to increase to 643 million by 2030 and 783 million by 2045 (Tariq *et al.*, 2022). Urban areas and high-income countries (10.8 %; 10.4 % respectively) have higher prevalence rates of diabetes compared to rural and low-income countries (7.2 %; and 4.0 % respectively) (Saeedi *et al.*, 2019). The increased prevalence of diabetes has inflicted a global menace on human health due to its debilitating and long-term complications, leading to high morbidity and mortality rates (Madan *et al.*, 2023). Within the United States of America, diabetes holds the position of being the eighth leading cause of death (Pearson-Stuttard *et al.*, 2021).

In Nigeria, it is noteworthy that the incidence of diabetes was placed at around 2.8 million in 2010 and a notable upsurge is projected to approximately 5.3 million by 2030 from the 2010 figure (Billa, 2023). Pancreatic beta cell damage resulting from an immune-mediated response is responsible for Type 1 DM (Roep *et al.*, 2021), while insulin resistance primarily causes Type 2 DM (Eizirik *et al.*, 2020). The acute clinical presentation varies, from symptoms such as polyphagia, polydipsia, and polyuria, to chronic complications affecting the kidneys, heart, and brain, which can occur over time due to the destruction of the nerves and blood vessels (Prabu, 2018). Diabetes management effectively entails tailoring personalized

goals and strategies to the unique needs of each individual as necessary, reflecting a patient-centred approach to healthcare (Catapan *et al.*, 2021).

However, the prospect of a lifetime commitment to conventional anti-diabetic agents can be daunting for individuals in Sub-Saharan Africa, where personal finances may not be sufficient for long-term sustainability (Iyun, 2022). The utilization of plant stem cell therapy, particularly through the application of stem cell extract from Double stem Cell® (or MD), such as the research by Ishola *et al* where the neuroprotective potential of DSC in Parkinson's disease was established (Ishola *et al.*, 2023). From the standpoint of moving from a reactive to a preventive and restorative approach, Double Stem Cell® possesses the capacity to significantly transform the realm of healthcare (Lorden *et al.*, 2015). The therapeutic outcome of diabetic treatment could have limitless possibilities. Implementing plant stem cell therapy as a less invasive and enduring treatment solution for diabetes can offer an advantage over traditional approaches (Ho *et al.*, 2023). Despite this, there is a reluctance to accept this unfamiliar turf among health practitioners due to the paucity of adequate information on the already available successful therapeutic outcomes in several aspects of regenerative medicine including diabetes (Ramzy *et al.*, 2023). The reason for the hesitation among health practitioners to embrace plant stem cell therapy is the limited information available about its applications and implications (Saha *et al.*, 2023). Suffice it to say, there remain various unanswered questions related to plant stem cell therapy, such as the syntax of healthy and damaged tissues and their interactions during the

reparative process, and the need to develop ethically acceptable clinical protocols. In this study, we have therefore investigated the possibly acclaimed antidiabetic and regenerative effect of Double Stem Cell® in alloxan-induced rats as a possible strategy for permanently treating diabetes now and in the future.

Materials and Methods

Materials

Double Stem Cell® (Phyto science, Kuala Lumpur, Malaysia), Ascorbic acid, Glibenclamide, D-glucose, and Alloxan monohydrate (1, 3-Diazinane - 2, 4, 5, 6 - tetrone) were products of (Sigma Aldrich, St Louis MO, United States of America), Glucometer (Accu-Chek Compact Plus Glucose Meter, Roche Diagnostic, Mannheim, Germany), Haematological parameters (Mindray BC-3200 Auto haematology analyser, Hollinger diagnostics, Germany), Biochemical parameters (XL 200 Auto- Clinical chemistry analyser, ERBA Diagnostics Mannheim, Germany). Light Microscope (Leica DM- 500, Leica Microsystems, Germany), Leica Tissue Processor (Microm STP125, Thermofisher, USA), Haematoxylin & Eosin (Haematoxylin Stain Sigma Aldrich, Germany), Coverslips and glass slides (Alltech microscope frosted slides), Rotary Microtome (Microm HM 325, Thermofisher, USA). Other chemicals and assay kits were of analytical grade.

Double Stem Cell® (PhytoCellTec TM) was acquired through a Phyto Science representative in Nigeria. It comes in powder form, is soluble in water without alcohol, and does not contain preservatives. This patented blend consists of stem cell extracts derived from Swiss Uttwiler spätlauber apples (*Malus domestica*) and Burgundy grapes (*Vitis vinifera*), intended for sublingual administration. An experiment with 0.2 grams of extract identified distilled water as the best solvent, yielding a uniform pinkish solution. Subsequently, a 1-gram extract solution with a concentration of 100 mg/ml in 10 millilitres was prepared for dosing based on body weight when needed. Double Stem Cell® (DSC) was administered orally at doses of 50, 100 and 200 mgkg⁻¹. The rationale for the dose selection was established with an LD50 of 5 g/kg, *p.o.* Freshly prepared aqueous extract (10 ml) was obtained by dissolving 1 g of the extract in 100 mg/ml of distilled water to achieve the required doses per body weight at room temperature.

Acute toxicity testing (LD₅₀ determination) of Double Stem Cell® aqueous extract (*Malus domestica*) in mice

In adherence to OECD Test Guideline 425 (2001), eight male albino mice (10 - 12 weeks; 140 - 200 g) were fasted overnight. They were subsequently used for the limit dose test (Emordi *et al.*, 2018). Subsequently, the experimental group of five mice received an immediate oral dose of 5 g/kg aqueous *Malus domestica* (MD) extract, and the control group of three mice was administered 10 ml/kg normal saline. Behavioral observations including hyperactivity, hyperventilation, diarrhea, urination, and tranquility were meticulously documented during the initial 2-hour period. Following this, mortality was assessed 24 hours post-treatment, and observations continued for 2 weeks to detect any delayed toxic reactions or mortality.

Experimental design

Type 1 diabetes was induced in male rats by administering 150 mgkg⁻¹ of alloxan monohydrate in cold normal saline via intraperitoneal injection, and

hyperglycaemia was confirmed after 72 hours. 30 out of 42 rats developed fasting blood sugar levels of ≥ 200 mgkg⁻¹ and were considered diabetic (excluding Group I) and were randomly assigned to the following treatment groups for 21 days (Emordi *et al.*, 2018).

The mice were dosed using the following regimen:

1. Group I non-diabetic: normal saline (10 mlkg⁻¹ body weight/day, *p.o.*)
2. Group II diabetic: alloxan (150 mgkg⁻¹, *i.p.*) + normal saline (10 mgkg⁻¹ body weight/day, *p.o.*)
3. Group III diabetic: alloxan (150 mgkg⁻¹, *i.p.*) + glibenclamide (5 mgkg⁻¹, *p.o.*)
4. Groups IV-VI diabetic: alloxan (150 mgkg⁻¹ /kg, *i.p.*) + *Malus domestica* (50-200 mgkg⁻¹, *p.o.*).

The effects of *Malus domestica* on fasting blood glucose (FBG) were determined on days 1 and 21.

Statistical Analysis

Data were presented as the mean $\pm$ standard error (SEM). To compare the mean values among multiple groups, a One-way analysis of variance (ANOVA) was performed followed by Tukey's post hoc test multiple-comparison tests using Graph-Pad Prism 6 (Graph-Pad Software Inc., California, United States of America). The threshold level for statistical significance was established at $p < 0.05$.

Results and Discussion

This study showed that Double Stem Cell® aqueous extract (or MD) achieved an anti-diabetic effect, increased haemoglobin and weight, improved oxidative parameters and preserved alloxan-induced liver and pancreas cytoarchitecture damage. Suffice it to say, these effects scientifically validate the use as a Type 1 antidiabetic drug. Alloxan-induced diabetes is commonly used as an experimental model to evaluate the antidiabetic properties of test compounds (Islam and Code, 2017). Alloxan acts as a diabetogenic agent by mimicking Type 1 Diabetes mellitus, inducing chemical diabetes via the partial degradation of pancreatic islet beta cells and resulting in cell necrosis (Moke *et al.*, 2023). In this study, compared to the healthy and MD-treated rats, weight loss was observed only in the alloxan-induced diabetic groups. Although glibenclamide has been reported to cause weight gain as reported in literature (Zulkifli *et al.*, 2022), our results showed no significant weight gain compared to the alloxan-induced diabetic group. However, following the post-treatment with 50 mgkg⁻¹, *p.o.* of the extract, a substantial increase in body weight ($p < 0.05$) was observed.

Glycaemic homeostasis is the process of balancing glucose utilization to prevent it from reaching harmful levels that could result in cellular dysfunction (Sarkodie, 2023). Impaired glucose utilization can result in complications that can negatively impact the lifespan and longevity of those affected (van Duinkerken and Ryan, 2020). Therefore, using Double stem cell therapy to alleviate the side effects of conventional antidiabetic medication, which is currently available, could be a potential therapeutic option. Our study found that all doses of MD-post-treated rats were effective in attenuating alloxan-induced hyperglycaemia, indicating the potential antidiabetic effects of the compound. The administration of 150 mgkg⁻¹ body weight of alloxan monohydrate induced diabetes in the animals within

72 hours, resulting in FBG levels ranging from 227.70 $\pm$ 36.6 to 600.00 $\pm$ 0.00 mg/dL (as presented in Table 1). This is consistent with previous studies by Adejumo *et al.* (2024), which demonstrated the glucose-lowering effects of *Malus domestica* in hyperglycemic but not normoglycemic rats, and Patocka *et al.* (2020) on the medicinal properties of *Malus domestica*.

In this research, all the MD-post-treatment groups effectively reduced FBG levels in the rats (Figure 1). However, the most significant decrease ($p < 0.0001$) was observed on day 21 in the 100 mg/kg body weight treatment group, *p.o.* doses of DSC extract. Glibenclamide, a sulfonylurea and conventional oral hypoglycemic drug, stimulates insulin secretion to lower blood glucose levels (Xu *et al.*, 2019).

cause anaemia according to literature surveys (Sinha and Ghosal, 2020). Importantly, our study showed that the glibenclamide groups exhibited a considerable downsurge ($p < 0.001$) in haemoglobin compared to the non-diabetic group (Figure 2). However, at a dose of MD-200 mg/kg *b.w.*, *p.o.*, there was a notable amelioration ($p < 0.05$) of alloxan-induced anaemia compared to the diabetic group. This suggests that the extract may have an advantage over the reference drug in terms of glibenclamide-induced anaemia. Our findings also suggest that the ability of the extract-treated alloxanised diabetic rats to increase haemoglobin levels was comparable to that of the untreated animals.

Liver injury or damage can lead to the release of liver enzymes such as SGPT, SGOT, and total bilirubin

Table 1: Fasting blood glucose level (mg/dl) post-alloxan administration

Treatment groups	Day 0	Day 1	Day 21
Non-diabetic rats (Control)	93.67 $\pm$ 5.75	100.00 $\pm$ 7.07	95.5 $\pm$ 4.51
Diabetic rats+ DW	97.17 $\pm$ 4.02	227.7 $\pm$ 36.66	189.0 $\pm$ 37.17
Diabetic rats+ Glibenclamide	100.00 $\pm$ 5.22	600.00 $\pm$ 0.00	126.3 $\pm$ 35.18
Diabetic rats+ MD 50 mg/kg body weight of extract	94.00 $\pm$ 2.53	273.00 $\pm$ 91.15	102.0 $\pm$ 6.36
Diabetic rats+ MD 100 mg/kg body weight of extract	94.5 $\pm$ 3.56	427.3 $\pm$ 93.08	63.33 $\pm$ 6.95
Diabetic rats+ MD 200 mg/kg body weight of extract	91.5 $\pm$ 5.01	496.7 $\pm$ 136.80	89.83 $\pm$ 9.30

Values are expressed in Mean $\pm$ SEM

In this research, treatment of alloxan-induced diabetic rats with glibenclamide also led to a significant decrease ($p < 0.0001$) in FBG levels as expected. Alloxan-induced diabetes is known to cause repression in haemoglobin levels, potentially leading to vascular diseases due to the glycosylation and stiffening of red blood cells (Oršolić *et al.*, 2021). In a case study by Xu *et al.* (2022), the use of glibenclamide resulted in decreased haemoglobin levels and glibenclamide-induced haemolytic anaemia was observed in some cases.

into the bloodstream, while alkaline phosphate (ALP) can be an indicator of an obstructive liver disorder. This indicates that the extract restored the normal architecture of the red blood cells to produce more haemoglobin and reach their target levels. In line with these findings, this study also demonstrated that the liver markers (SGPT, SGOT) in the treatment groups were not significantly increased in comparison with the diabetic groups (Assawarachan *et al.*, 2020). These enzymes are often used as indicators of hepatotoxicity (Swamy *et al.*, 2021). Alloxan-induced diabetes can cause liver damage and cytoarchitectural changes, leading to an increase in these markers (Lucchesi *et al.*, 2015). A study by Patel has shown that *Malus domestica* fruit extract possesses hepatoprotective properties in rats (Patel, 2018).

Additionally, the alloxan-induced increase in ALP was significantly reversed ($p < 0.01$) with the peak protective effect observed at a dose of MD-100 mg/kg, *p.o.* This suggests that Double stem cell aqueous extract could potentially be hepatoprotective as supported by findings in the literature (Zou *et al.*, 2020). In the alloxan-treated rats of this study, a noteworthy rise ($p < 0.05$) was observed in the total bilirubin level compared to the non-diabetic group. However, the treatment with glibenclamide led to a considerable decrease ($p < 0.0001$) in total bilirubin levels, in contrast to the other treatment groups. It is hypothesized that the positive correlation of the antioxidant effect of *Malus domestica* on oxidative stress is due to the presence of phytochemical constituents which may act alone or synergistically.

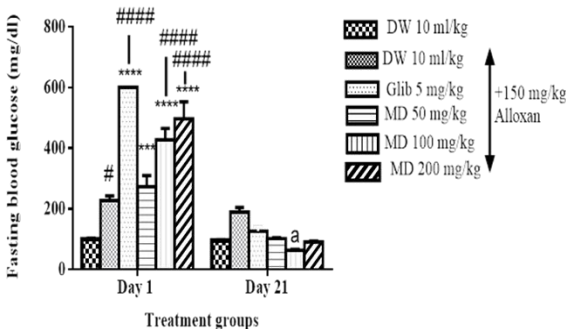


Figure 1: Effect of *Malus domestica* on FBG in alloxan-induced diabetic rats. Values are expressed as mean $\pm$ SEM. # $p < 0.05$ vs Non-diabetic control group, ### $p < 0.0001$ vs Non-diabetic control group, **** $p < 0.0001$ vs diabetic control group.

Furthermore, thiazolidinediones, another commonly used antidiabetic drug, have also been reported to

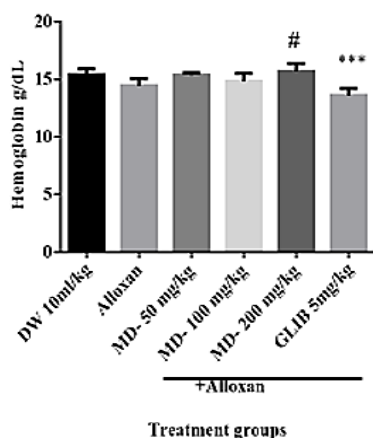


Figure 2: Effect of *Malus domestica* on Hb in alloxan-induced diabetic rats. Values are expressed as mean $\pm$ SEM (n=5). ***p<0.001 vs Non-diabetic group, #p<0.05 vs Diabetic group

Notably, the present study and a preliminary investigation by Adejumo *et al* (2024) suggested that the aqueous extract of *Malus domestica* possessed significant antioxidant activity both *in vitro* and *in vivo*. The imbalance of antioxidant mechanisms in diabetic patients and the generation of free radicals by hyperglycaemia are critical factors in the pathophysiology of diabetes (Bhatti *et al.*, 2022). To alleviate diseases associated with free radicals, including diabetes, the use of chemical compounds with high levels of antioxidant constituents has been proposed as an effective therapeutic approach (Akbari *et al.*, 2022). MDA is a well-known biomarker of oxidative stress and lipid damage induced by free radicals (Świdarska *et al.*, 2019). The heightened levels of MDA found in diabetic patients indicate a possible involvement of peroxidative damage in contributing to diabetic complications (Robinson *et al.*, 2020). This was demonstrated in the diabetic group (p<0.0003) which was elevated compared to the non-diabetic group. From our study, there was a substantial amelioration (p<0.0001) in liver MDA levels in all extract-treated groups as opposed to the alloxan group (figure 3).

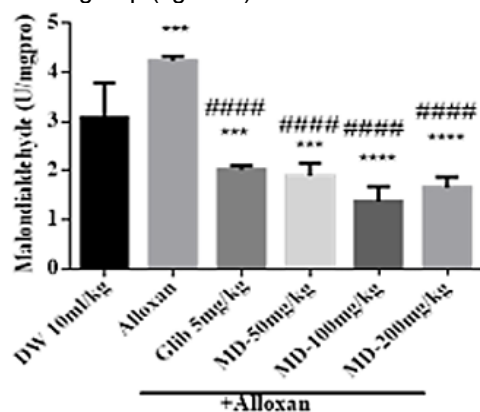


Figure 3: Effect of *Malus domestica* on the liver MDA of alloxan-induced diabetic rats. Values are expressed as Mean $\pm$ SEM, ***p<0.0003 vs non-diabetic groups, ****p<0.0001 vs non-diabetic groups, ####p<0.0001 vs diabetic group, ****p<0.0001 vs non-diabetic groups.

GSH is a biomarker of redox imbalance at the cellular level, and numerous studies have shown that its levels are reduced in diabetes (Bjørklund *et al.*, 2020). However, no statistically significant changes in liver GSH levels across all groups were observed

from our research.

Catalase plays a crucial role in regulating hydrogen peroxide metabolism, and its deficiency can lead to an increase in oxidative stress (Nandi *et al.*, 2019). This was consistently seen in our study as there was a notable upsurge (p<0.001) in catalase activity in the treatment groups; MD-50 mgkg⁻¹, *p.o.*, MD-100 mgkg⁻¹, *p.o.*, and MD-200 mgkg⁻¹, *p.o.* compared to the alloxan group (figure 4). Similarly, the diabetic group demonstrated a notable diminution (p<0.001) in the hepatic catalase effect when compared to the non-diabetic group. On the other hand, in line with previous studies, SOD is known for its protective roles against cellular and histological damage caused by reactive oxygen species (ROS), and a decline in its levels has been reported in diabetic tissue and blood (Altinoz *et al.*, 2015). Similarly, findings in our study revealed a decrease (p<0.0001) in SOD levels in the alloxan group compared to the non-diabetic group. However, post-treatment with MD extract at doses MD-50 mgkg⁻¹ *b.w.*, *p.o.* and MD-100 mgkg⁻¹ *b.w.* led to an increase in liver SOD levels (p<0.0001 and p<0.01, respectively) compared to the alloxan group (figure 5). Interestingly, the MD-50 mgkg⁻¹ *b.w.*, *p.o.* group showed the highest increase in liver SOD levels compared to the other doses. In the pancreas, only the glibenclamide group showed prominently lower GSH activity compared to diabetic and non-diabetic groups (p<0.05).

Conversely, the pancreatic MDA was markedly higher (p<0.05) in the MD-200 mgkg⁻¹, *p.o.* group than in the non-diabetic group. Moreover, the pancreas SOD levels in the alloxan group were markedly lower (p<0.001) than those in the non-diabetic group. The MD-50 and 200 mg/kg, *p.o.* groups displayed an augmentation in SOD levels (p<0.0001) in comparison to the diabetic groups (figure 6). Our study showed a statistically significant decrease (p<0.05) in the catalase level in the alloxan group when compared to the non-diabetic group, in tandem with previous findings observed in the liver. In addition, among the treatment groups, only the MD-200 mgkg⁻¹, *p.o.* group exhibited the highest pancreatic catalase levels, showing remarkably higher (p<0.0001) values than the others (figure 7).

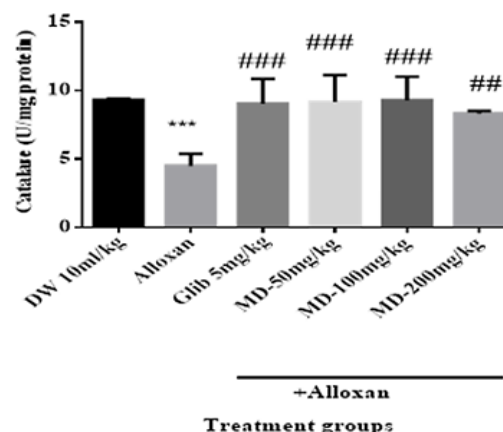


Figure 4: Effect of *Malus domestica* on the liver Catalase of alloxan-induced diabetic rats. Values are expressed as Mean $\pm$ SEM. Significant increase ***p<0.001 vs non-diabetic groups, ##p<0.01 vs diabetic group, ###p<0.001 vs diabetic group, ####p<0.0001 vs diabetic group.

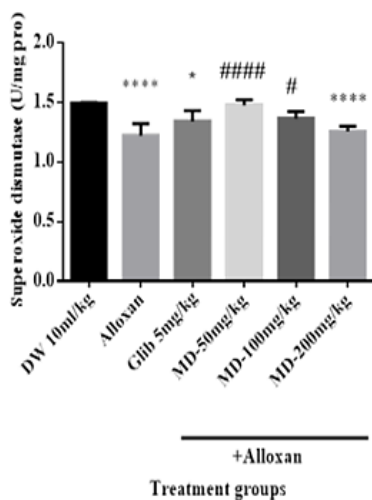


Figure 5: Effect of *Malus domestica* on the liver SOD of alloxan-induced diabetic rats. Values are expressed as Mean±SEM, *p<0.01 vs non-diabetic groups, ****p<0.0001 vs non-diabetic groups. #p<0.01 vs Alloxan, #####p<0.0001 vs Alloxan.

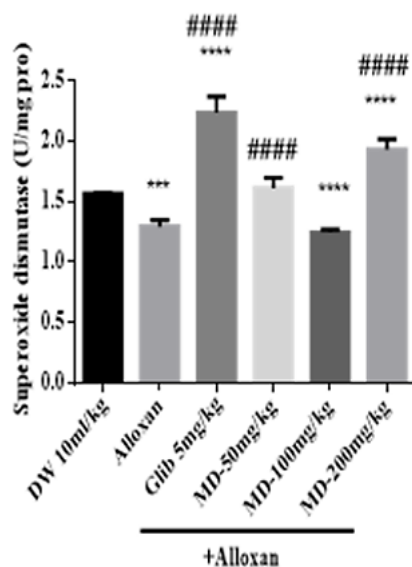


Figure 6: Effect of *Malus domestica* on the pancreatic catalase of Alloxan-induced diabetic rats. Values are expressed as Mean±SEM, Significant increase ***p<0.001 vs non-diabetic group, ****p<0.0001 vs non-diabetic groups. #####p<0.0001 vs diabetic group.

Histopathological analysis revealed that alloxan administration induced acute cytotoxic effects, as evidenced by chronic lymphocytic peri-portal inflammation, and central caseous necrosis surrounded by fibroblast and bile duct proliferation (figure 8). These pathological changes may explain the elevated total bilirubin levels observed in the alloxan treatment group. Our findings are consistent with other research on alloxan-induced histopathological anomalies, such as the study by Kanedi and Mumtazah (2021). However, post-treatment with *Malus domestica* demonstrated peak hepatoprotective effects at a dose of 200 mg/kg-1 *b.w*, *p.o*. In the pancreas, administration of 50 and 100 mg/kg-1 *b.w*, *p.o*. of the extract in alloxan-induced rats restored normal tissue morphology.

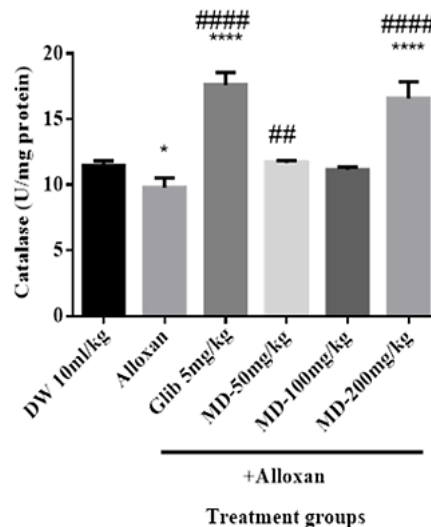


Figure 7: Effect of *Malus domestica* on the pancreatic catalase of Alloxan-induced diabetic rats. Values are expressed as Mean±SEM, Significant increase *p<0.01 vs non-diabetic group, ****p<0.0001 vs non-diabetic groups. ##p<0.01 vs diabetic group, #####p<0.0001 vs diabetic group.

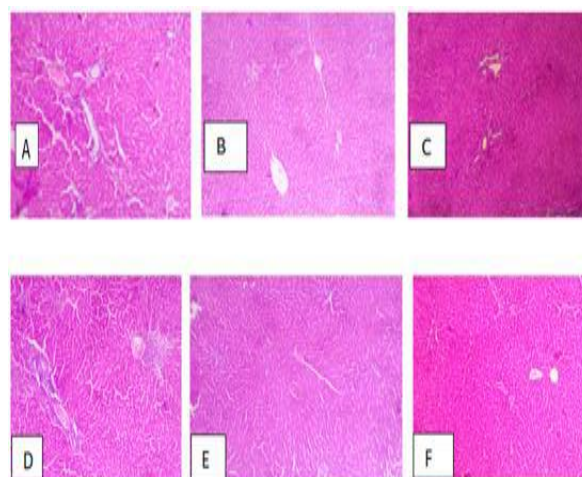


Figure 8: Histopathological effect of the aqueous extract of Double stem cell (*Malus domestica*) on the liver of rats (A) Diabetic Liver + Alloxan. 150 mg/kg, *p.o*. with chronic lymphocytic peri-portal inflammation, central caseous necrosis and surrounding fibroblast and bile duct proliferation (B) Non-diabetic Liver + Dist. water 10 ml/kg, *p.o*. with preserved architecture and hepatocyte (C) Diabetic Liver+ Glib. 5 mg/kg, *p.o*. with well-preserved architecture and hepatocyte (D) Diabetic Liver + MD-50 mg/kg, *p.o*. with moderate vascular congestion with oedema (E) Diabetic Liver+ MD-100 mg/kg, *p.o*. showing a perivascular inflammation (mild) mostly peri-portal, few congested blood vessels are also seen (F) Diabetic Liver+ MD-200 mg/kg, *p.o*. showed preserved architecture and hepatocyte (normal liver).

Conclusion

This study is a continuum of a preliminary study that established safety and efficacy in oral glucose tolerance tested rats and provided evidence that Double Stem Cell® (*Malus domestica*) can be an additional potential alternative for orthodox medication in treating alloxan-induced Diabetes mellitus.

Furthermore, the complex interplay between metabolic and electrogenic mechanisms regulating insulin release or resistance in a Type 2 Diabetes Mellitus experimental model is currently under research.

Declaration of interest

The authors have no relevant financial or non-financial interests to disclose.

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