Original Article

Evaluation of the Aqueous Extract of *Musa acuminata* Corm (Rhizome) for its Anti-diabetic Potential in Streptozotocin (STZ) Induced Diabetic Zebrafish (*Danio rerio*) Model

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ABSTRACT

Background Information: Diabetes Mellitus (DM) is spiking substantially in both emerging and developed countries and the use of a nutritional approach to diabetes control has recently attracted a lot of attention. The banana (*Musa* spp.) is ubiquitously favourite in the tropical areas of the world. The wild plant species *Musa acuminata*, also referred to as the Cavendish banana, is found in tropical and subtropical climates. The health advantages of *M. acuminata* have drawn a lot of attention in recent years. Every component of the plant has been used in traditional medicine to treat numerous diseases. While the anti-diabetic potential of various parts of *Musa acuminata* has been reported, the corm has not been extensively studied. **Objectives:** Considering the lack of data on the anti-diabetic potential of *M. acuminata* corm, we proposed to evaluate the same using a zebra fish model. **Materials and Methods:** Diabetes was induced in zebrafish by intraperitoneal administration of Streptozotocin (STZ). The fishes were maintained in 2% sucrose solution for 48 hr for induction of diabetes after which they were transferred to the respective treatment tanks containing the corm extract at 10, 20 or 30 µg/mL; on Day 8, they were all euthanized and used for biochemical and histopathological analysis. **Results:** *Musa acuminata* Corm Extract (MACE), at 10, 20 and 30 µg/mL caused significant glucose lowering action in diabetic zebra fish model. This is evident from the enzyme analysis. The histopathological analysis also revealed the enhanced growth of villi and increased number of goblet cells in the intestine of MACE treated group. **Conclusion:** The role of MACE in preventing diabetic complications like hypercholesteremia and hyperlipidaemia supports its claim that it can be used as an adjuvant or as an alternative to other diabetic medications. The precise mode of action of the antidiabetic potential of MACE identified in this study could not be completely deciphered at this point. Additional research is required to narrow down on the active phytoconstituents responsible for this effect and also the mechanism by which it exerts this effect.

Keywords: Diabetes mellitus, *Musa acuminata* Corm Extract (MACE), Anti-diabetic, Zebra fish.

INTRODUCTION

Diabetes Mellitus (DM) is spiking substantially in emerging and industrialised countries, impacting millions of adults, and is projected to affect 439 million individuals by 2030.[1] The use of a nutritional approach to diabetes control has recently attracted a lot of attention. Consuming plant-based antioxidants such as polyphenols can reduce the risk of oxidative stress, which is another factor in the pathophysiology of Type II diabetes and a significant risk factor for complications.[2,3]

Banana is highly consumed by the population in the tropical areas of the world.[4] Three banana cultivars, *Musa sapientum* (Latundan

banana), *Musa acuminata* (Cavendish banana) and *Musa acuminata* (Red Dacca), were identified by (Adedayo 2016).[5] It was noted that these species had enzyme inhibitory actions, low sugar, glucose index, amylose, and amylopectin concentrations, and low sugar content. The wild plant species *Musa acuminata*, also referred to as the Cavendish banana, is found in tropical and subtropical climates. The health advantages of *M. acuminata* have drawn a lot of attention in recent years. Each component of the plant has been utilized in traditional medicine to treat a variety of diseases. Historically, particularly in Asia and Africa, both infectious and noncommunicable diseases have been treated using *Musa acuminata*. Traditional medicine has used every part of the plant, which includes the fruits, peel, pseudostem, corm, blossoms, leaves, sap, and roots, to cure a variety of illnesses.[6]

M. acuminata leaves are rich in bioactive flavonoids with remarkable antioxidant, antidiabetic and anti-inflammatory activities.[7] *Musa acuminata* lowered the plasma glucose by using

Table 1: Study design.

Group	Treatment
Group 1	Untreated Control.
Group 2	350 mg/kg Streptozotocin i.p.
Group 3	350 mg/kg Streptozotocin i.p. + exposure to 10 μg/mL of MACE in water.
Group 4	350 mg/kg Streptozotocin i.p. + exposure to 20 μg/mL of MACE in water.
Group 5	350 mg/kg Streptozotocin i.p. + exposure to 30 μg/mL of MACE in water.

peripheral glucose and producing hepatic glycogen.[8] In a study with five hundred Type II diabetic patients, regular consumption of beans, *Brassica rapa, Abelmoschus*, and *Musa acuminata* has been found to dramatically lower the incidence of blindness or its primary causes in the African population with Type 2 diabetes.[9]

The corm is the vertical enlarged compact structure loosely covered with thin leaves. The ethanolic extract of corm reportedly contains sterols, flavonoids, terpernoids, glycosides, quinones and tannins. The *M. acuminata* corm ethanol extracts displayed potential antibacterial activity against eight clinically pathogenic isolates.[10] While the antidiabetic potential of various parts of *Musa acuminata* has been reported, the corm has not been extensively studied. Therefore, we proposed to evaluate the antidiabetic potential of the corm using a zebra fish model. The Zebrafish model was opted considering the remarkable analogies in glucose homeostasis between mammals and zebrafish.[11]

MATERIALS AND METHODS

Preparation of *Musa acuminata* corm extract

Musa acuminata corm was carefully obtained and thoroughly washed to get rid of adhering soil particles and debris. The corm was cut into tiny pieces, dried overnight at 65°C. A powder was made from the dried sample. To 10 g of this powder, 100 mL of sterile, distilled water was added; the mixture was heated to 60°C for 45 min while being continuously stirred, following which, the solution was filtered using Whatman No. 1 filter paper. The extract was labelled as MACE (*Musa acuminata* corm extract) and stored at 4°C for further use.

Zebrafish Maintenance

Adult zebra fishes were purchased from a local vendor. An acute toxicity test for determining the LC₅₀ was conducted according to OECD Test Guideline 203. Zebrafish were initially acclimatised to the testing environment and the test was conducted in static water. According to the guideline, the temperature, pH, salinity and dissolved oxygen levels were monitored on daily basis. The water was maintained at 27°C ± 2 and pH between 6.8-7.4. Zebra fish were fed with commercial fish feed twice a day. The fishes were reared in a 12 hr Light/12 hr Dark cycle. As recommended by the guideline, "Healthy adult zebra fishes devoid of any

malformations or infections" were used for the study. Fishes were grouped and split into designated tanks for toxicity assessments.

LC₅₀ determination for MACE

Fishes weighing between 0.5 g to 0.6 g were selected for the study and assigned in groups of twelve per tank. For determining the LC₅₀ of the MACE, various concentrations (20, 40, 60, 80 and 100 μg/mL) was introduced into the respective tanks. An untreated control group was maintained under the same environmental conditions. After an exposure period of 7 days, no toxicity was observed up to the highest dose selected (100 μg/mL).

Main experiment

For the main experiment, fishes weighing between 0.5 g to 0.6 g were selected and assigned into groups of ten fish each. Table 1 represents the study design:

Fishes in groups 2 to 5 were anesthetized by rapid cooling ice-cold method, followed by intraperitoneal administration of Streptozotocin (STZ) (10 μL). The fishes were maintained in 2% sucrose solution for 48 hr for induction of diabetes after which they were transferred to the respective treatment tanks. Fishes were exposed for 7 days to the extract at 10, 20 and 30 μg/mL and on Day 8, they were all euthanized and used for analysis.

Collection of blood sample

Fish were initially anesthetized in ice. The tail portion was cut right above the caudal fin. A drop of EDTA was put on the cut end, and the fish was placed in an Eppendorf tube with a collecting tube below it. A drop of EDTA was put in the collecting tube as well. The fish were centrifuged for 5 min at 3000 rpm. The collected blood was used for biochemical analysis.

Estimation of serum liver marker enzymes (AST, ALP and ALT)

Serum liver enzymes (Aspartate aminotransferase, Alkaline phosphatase and Alanine transferase) were estimated using commercially available kits (Biosystems Diagnostic, Cat. No: 11830, 11832 and 11592 respectively) and following manufacturer's instructions.

Estimation of Biochemical Blood Parameters

Blood Glucose, total cholesterol levels, and triglycerides levels were analysed in this study. Blood glucose levels was measured using a glucometer (SUGARCHEK®); an autoanalyzer was used to estimate total cholesterol and triglycerides in the sample.

Statistical Analysis

The experiments were triplicated and data were subjected to ANOVA utilizing GraphPad Prism software version 8.0. The comparative analysis examined the control group to the treated group applying Tukey's multiple comparison test. Data are

denoted as Mean±Standard deviation. $p<0.001$ regarded as significant.

RESULTS

Variations in serum liver marker enzymes of Zebra fish exposed to MACE are shown in Figures 1, 2 and 3, respectively.

Aspartate Aminotransferase (AST) Assay

Aspartate Aminotransferase converts aspartate and α-ketoglutarate to oxaloacetate and glutamate. This enzyme is predominantly found in the liver and skeletal muscle and also in other tissues except the bone. In this study, the activity of liver was estimated based on Aspartate Aminotransferase (AST) activity between control, induced and treated groups.

Figure 1 represents the effect of MACE on AST enzyme. Aspartate Aminotransferase (AST) activity in zebrafish treated with 10, 20 and 30 μg/mL MACE were significantly decreased when compared to the induced and control group. Results were subjected to One Way ANOVA (Tukey's multiple comparison test) and the values were statistically significant at (***$p<0.0001$, #$p<0.0001$ Induced vs Control). The highest concentration used showed the maximum activity, which was comparable to the control.

Alanine Aminotransferase (ALT) Assay

Alanine Aminotransferase (ALT) catalyzes amino group transfer from alanine to α-ketoglutarate, to form glutamate and pyruvate. This enzyme is primarily present in the liver and serum and at lower levels in other body tissues. Increase in ALT levels indicate hepatocellular injury and is measured in Units per Liter (U/L). Alanine Aminotransferase (ALT) activity between control, induced and treated groups were analyzed and activity of treated groups were found to be significantly decreased compared to

induced group (***$p< 0.0001$). Similar to AST activity, the group administered with higher concentration of extract (30 μg/mL) has decreased ALT activity, indicating that the injury to the liver has been reverted by the MACE (Figure 2).

Alkaline Phosphatase (ALP) Assay

The ALP works on various phosphate substrates through hydrolysis and transfer. When compared to the control, Alkaline Phosphatase (ALP) activity in treated groups greatly decreased. which was also statistically significant (Figure 3). Results were subjected to One Way ANOVA (Tukey's multiple comparison test) and the values were statistically significant at (***$p<0.0001$, #$p<0.0001$ Induced vs Control). Among the three, the highest concentration tested, 30 μg/mL, exhibited a clear decreasing ALP activity which was equivalent to control.

Blood Glucose Levels

Before STZ treatment, the initial blood glucose readings in all the five groups were between 99.56 mg/dL and higher. The animals in the normal control group did not experience a quick rise in blood glucose levels after STZ injection, but all other groups did. MACE @ 10, 20, and 30 g/mL was administered to the experimental group after diabetes induction. In zebrafish treated with *Musa acuminata* extract (10 μg/mL), blood glucose levels gradually dropped (Figure 4). The decrease was highly significant and rapid in zebrafish treated 30 μg/mL MACE, in comparison with diabetic control group. This reveals that MACE possesses anti-hyperglycemic activity.

Total Cholesterol Level

Total cholesterol and triglyceride levels in zebrafish were calculated as a biochemical diagnostic for diabetes. As expected, STZ raised total cholesterol and triglyceride levels in comparison to a healthy control. As shown in Figures 5(a) and 5(b), the level of
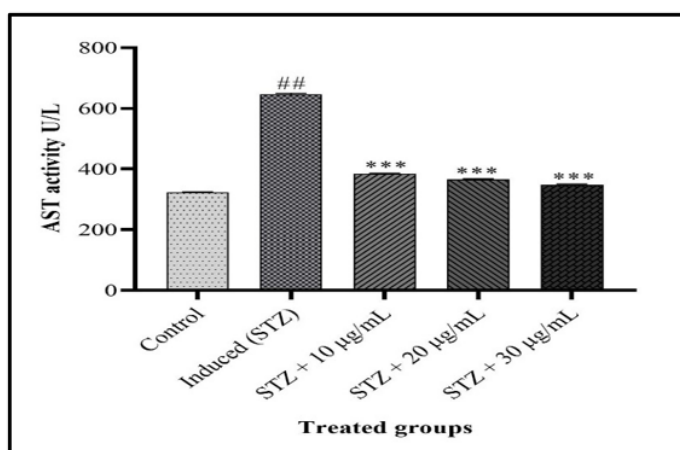

Figure 1: Effect of MACE on AST in Streptozotocin (STZ) induced Diabetic Zebrafish after 7 days of treatment. Values are denoted as Mean ± SD. Values are statistically significant at (***$p < 0.0001$, #$p <0.0001$ Induced vs Control).
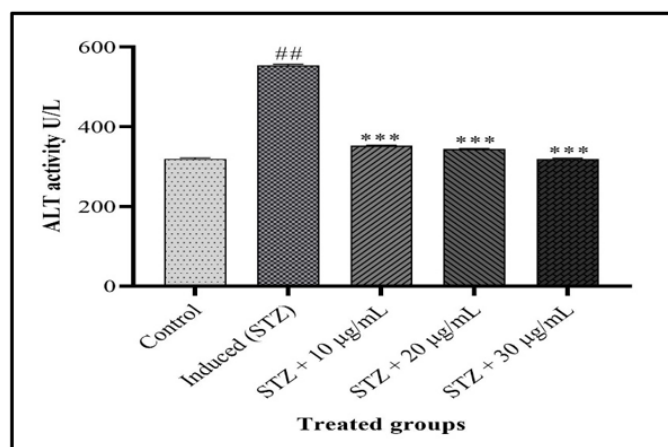
Figure 2: Effect of Corm on ALT in Streptozotocin (STZ) induced Diabetic Zebrafish after administration of MACE for 7 days. Values are denoted as Mean ± SD. Values are statistically significant at (***$p <0.0001$, #$p <0.0001$ Induced vs Control). Results were subjected to One Way ANOVA (Tukey's multiple comparison test).
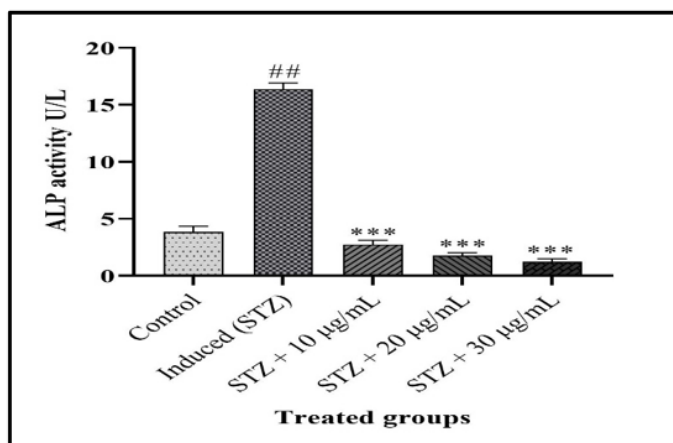
Figure 3: Effect of Corm on ALP in Streptozotocin (STZ) induced Diabetic Zebrafish after administration of MACE for 7 days. Values are denoted as Mean ± SD. Values are statistically significant at (***$p<0.0001$, #$p<0.0001$ Induced vs Control). Results were subjected to One Way ANOVA (Tukey's multiple comparison test).
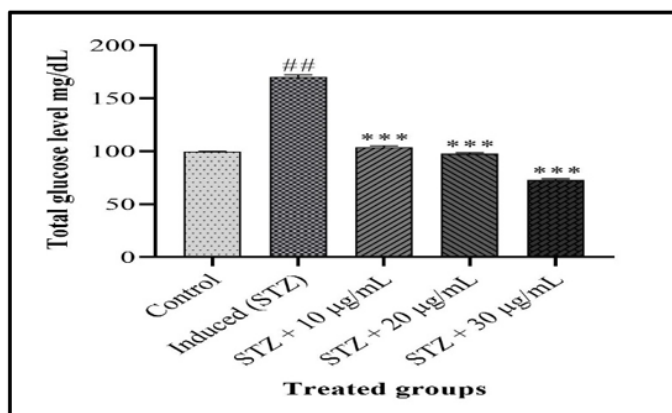
Figure 4: Blood glucose levels in zebrafish of different groups. The glucose level increased in the STZ treated group in comparison with the control and the MACE treated group. Values are denoted as Mean ± SD. One Way ANOVA (Tukey's multiple comparison test) was used to statistically compare the control and treated groups. MACE-treated groups showed significant difference in glucose reduction ($p<0.0001$).
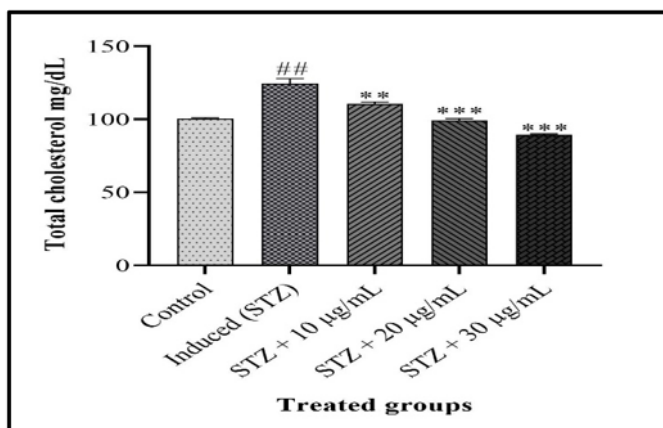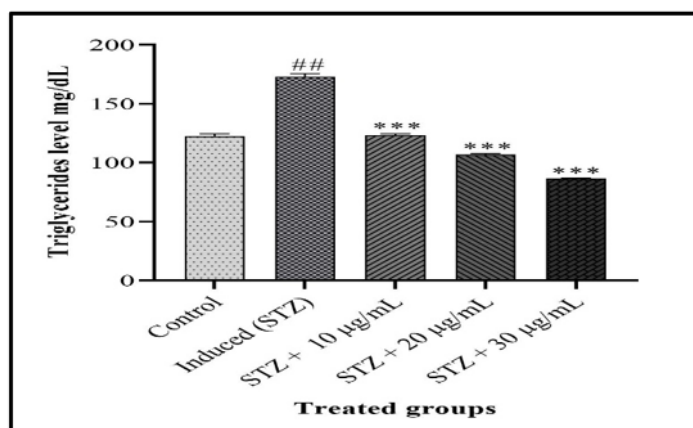

Figure 5 (a)

Figure 5 (b)

Figure 5: a) Effect of *Musa acuminata* extracts on cholesterol levels in zebrafish. Zebrafish were exposed to MACE at various concentrations for 7 days. Total cholesterol was measured. b) Effect of MACE on Triglyceride levels in zebrafish. Zebrafish were exposed to various concentration of MACE for 7 days. Values are denoted as Mean ± SD. One Way ANOVA (Tukey's multiple comparison test) was used to statistically compare the control and treated groups. MACE-treated groups exhibited remarkable difference in lowering cholesterol and triglyceride levels ($p<0.0001$).

total cholesterol and total triglyceride dramatically fell following treatment with the extract.

The mean total cholesterol levels of the healthy control group receiving conventional feeding are shown in Figure 5a. Based on the measurement results, the normal control group's mean total cholesterol level was 100.33 mg/dL. In the treatment groups (10, 20 and 30 g/mL), the mean total cholesterol level was 110.5, 99.1 and 89.5 mg/dL, respectively. Figure 5 depicts the impact of *Musa acuminata* extract on zebrafish total cholesterol levels. It is known from the one-way ANOVA results that all study groups, with the exception of the normal control group receiving treatment, showed a significant difference.

Figure 5b shows the triglycerides levels between control, induced and treated groups. Triglycerides levels in treated groups were substantially decreased in comparison to the induced group. However, the difference between the treated groups itself is not significant.

Histopathology of Intestine

Intestinal tissues were stained using the conventional Eosin and Hematoxylin stains. Microscopic images of intestinal tissues were captured using UIS optical system (Universal Infinity System, Olympus®, Japan).

The histopathology of intestine reveals that MACE treated groups showed rise in the height of villi which is similar to the control groups. When compared to 10 and 20 μg/mL, diabetic fish treated with 30 μg/mL corm aqueous extract exhibited considerable
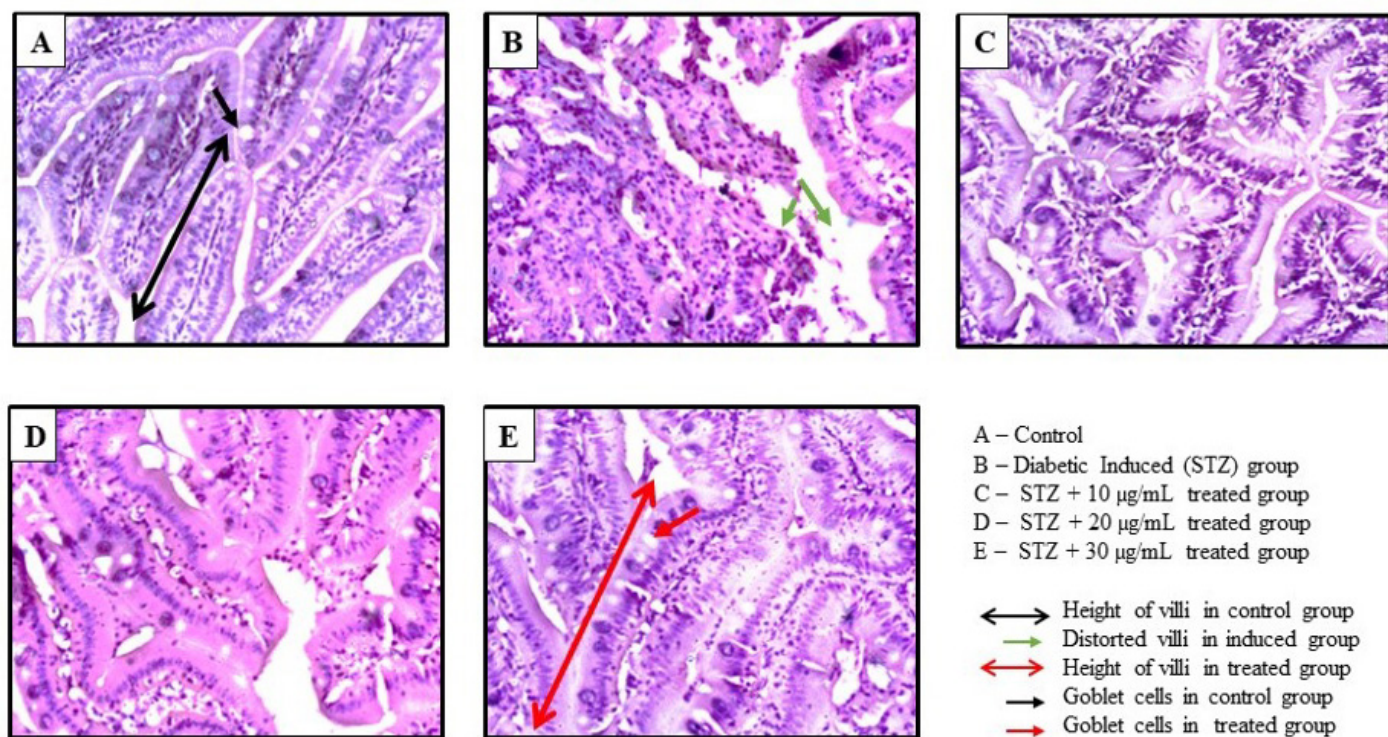
Figure 6: Histology of intestine in STZ-induced diabetic zebrafish after exposure to the MACE for 7 days. Microscopic images of the intestinal structures of zebrafish at 100X. A) Control group: Exhibited normal structure of intestine. B) Induced group: Distorted villi in the intestine (arrow). C, D and E) Treated group: Comparing to non-treated diabetic groups, the histological characteristics showed relative improvement.

improvement of height of the villi and number of goblet cells Figure 6.

DISCUSSION

Diabetes mellitus is primarily characterized by necrosis of the β-cells of the pancreas and resistance.[12] *Musa acuminata*, is a highly nutritious food with established therapeutic properties and identified to have anti-diabetic potential. By utilising peripheral glucose, creating hepatic glycogen, and further lowering protein catabolism, *Musa acuminata* is reported to enhance body weight while also reducing diabetes consequences like nephropathy, peripheral neuropathy, and dyslipidemia.[8] The corm has not yet been investigated for anti-diabetic and hypolipidemic activities despite the considerable presence of essential phytochemicals including betulinic acid, tricontane, lupeol, stigmasterol, and beta-sitosterol, among others. As a result, the current study examines the anti-diabetic potential of corm aqueous extract in a zebrafish (*Danio rerio*) model of diabetes induced by Streptozotocin (STZ).

Hepatic glucose synthesis, which processes the equivalent of 60–65% of the oral glucose load and contributes 79% of endogenous glucose generation in the fasted state, is essential for maintaining glucose homeostasis.[13-15] Some indicators of liver damage include Glutamyl-Transferase (GGT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), and Alanine Aminotransferase (ALT). These markers have been linked to insulin resistance[16] and the possibility of developing diabetes.[17] The present study's findings revealed that none of the extract-treated groups' serum levels of ALT, AST, or ALP differed notably ($p < 0.05$) from those of the healthy control group. These negligible modifications are consistent with the liver's healthy operation therein prove the reversal of diabetic state. Though ALT, AST, GGT and ALP showed considerable relation to the risk of diabetes[18,19] only a small number of studies have revealed the absence of a significant relationship between ALT and diabetes.[20,21]

When compared to induced zebrafish, Corm aqueous extract (*Musa acuminata*) at doses of 10, 20, and 30 μg/mL substantially lowered blood glucose levels. At the above mentioned three doses, Corm aqueous extract prevented blood glucose levels from rising by 103.6, 97.6, and 73 mg/dL, respectively and the extract at highest dose tested exhibited comparable anti-hyperglycemic activity as control suggesting existence of potential antihyperglycemic phytocompounds in *Musa acuminata*. The antihyperglycemic activity of corms may have been aided by the presence of betulinic acid, triacontane, lupeol, stigmasterol, beta-sitosterol, and its palmitate, according to available literature. A comparable investigation in rats has demonstrated that the presence of betulinic acid can have a hypoglycaemic impact.[22] Additionally, betulinic acid has been found to be a component of anti-diabetic plants that demonstrate alpha-glucosidase inhibitory activity.[23]

The therapeutic properties of betulinic acid and other pentacyclic triterpenoids in diabetes and related issues.[24] Similar

to the work of streptozotocin-induced diabetic rats using *A. marmelos* bark extract, lupeol another corm component, has been known as an active constituent and may have contributed to the hypoglycaemic and beta-cells regeneration effects.[25] The antihyperglycemic capability of corm may be due to beta-sitosterol in addition to betulinic acid and lupeol.[2]

The insulinogenic properties of MACE and fractions could also be a contributing factor in their potential antidiabetic effects. In DM, insulin deficiency results in a decline of the glycogen level of the liver and skeletal muscles since insulin is one that regulates glycogenesis in muscle and the liver.[26] According to the latest research, diabetic rats treated with corm extract had significantly lower blood glucose levels. It's possible that this results from increased hepatic glycogenolysis or decreased liver glycogen genesis.[27] The rise in the insulin response that speeds up the process by which inactive glycogen synthase converts into active form and, in turn, enhances the production of glycogen from blood glucose may be the cause of the increased glycogen level.

Lipoprotein lipase is normally stimulated by insulin and hydrolyses triglycerides and cholesterol to regulate plasma lipid levels. Diabetes is very commonly associated with hyperlipidaemia and increased lipoprotein lipase activity on deposits of fat as a result of insulin insufficiency. Diabetes-related hypertriglyceridemia and hypercholesterolaemia are risk factors for coronary heart disease development. Elevation of triglycerides, total cholesterol, low- and very low-density lipids, as well as decline in high-density lipids, are the hallmarks of hyperlipidaemia.[28,29] The abnormal serum lipid profile was reversed in diabetic fish treated with corm extract for 7 days. Significant lipid profile reduction was seen in corm extract and its fractions, which in turn reduced cardiac, atherogenic, and coronary artery parameters. *Musa acuminata*'s inner peels' ability to lower cholesterol could be attributed to their ability to prevent lipid absorption, as well as their ability to activate fatty acid synthase and block cholesterol esterase.[30,31]

In STZ induced groups, the altered intestinal morphology which includes distorted villi and goblet cells were observed, which might influence the rate of digestion and effectiveness of absorption of nutrients.[32] The corm extract of *Musa acuminata* plays a significant role in improving the height of villi in MACE treated groups. The increased growth of villi leads to effective functioning of digestive enzymes, causes more nutrients for transportation across the villus ultimately contributing to efficient nutrient absorption and consumption.[32] The MACE treated groups also elevated the count of sustained goblet cells in the parts of intestine. Thus, corm extract of *Musa acuminata* exhibited antihyperglycemic effect and it also improvises the nutrient absorption capability of intestine, thereby controlling the blood glucose levels, hence preventing the risk of diabetic condition.

CONCLUSION

Diabetes risk is indicated by elevated ALT, AST, and ALP levels. The current study's findings, MACE, at various concentrations caused significant glucose lowering action in diabetic zebra fish model. MACE's ability to avoid complications in diabetes, such as hypercholesteremia and hyperlipidaemia, supports its usage in conjunction with other diabetic treatments. The specific mechanism of action of the antidiabetic potential of MACE identified in this study is still undisclosed. Further research is needed to narrow down on the active phytoconstituents responsible for this anti-diabetic effect and also the mechanism by which it exerts the anti-diabetic activity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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