



Biochemical evaluation of antihyperglycemic and hypolipidemic effects of methanolic tepal extract of *Musa paradisiaca* studied in STZ-induced diabetic mice

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ABSTRACT

The use of medicinal plants as source of remedies for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition. In the present study, methanolic tepal extract (MTE) of *Musa paradisiaca* was evaluated for phytochemical screening, and *in vivo* antidiabetic therapeutic efficacy in streptozotocin (STZ)-induced diabetic mice. Diabetic mice were administered 500 mg/kg per day of MTE orally for one month. The mice were sacrificed and blood collected for key biochemical parameters such as, blood glucose, insulin, hemoglobin and glycosylated hemaoglin (HbA_{1c}), creatinine, urea, uric acid, total protein, lipid profile, aminotransferases and alkaline phosphatase. The histopathological studies of pancreas, liver and kidney was also performed. Preliminary phytochemical screening reveals the presence of phenolics, flavonoids, glycosides, terpenoids, tannins and alkaloids. Elevated blood glucose, HbA_{1c}, creatinine, urea and uric acid and decreased levels of plasma insulin and hemoglobin were significantly ($p < 0.05$) reverted back to near normal in STZ-induced diabetic mice after oral administration of MTE. Plasma protein, lipid profile, transaminases and alkaline phosphatase were also significantly normalized ($p < 0.05$) after the treatment. Histopathological analysis indicated tissue damages in the diabetic untreated mice. MTE treated groups shows the tissue protection (of pancreas and liver) against peroxidation damage, thus signifying tissue integrity maintenance of MTE. It can be inferred that their *in vivo* antidiabetic therapeutic efficacy of tepals of *Musa paradisiaca* may be attributed to the presence of phytochemicals such as phenolics, flavonoids, alkaloids etc.

Keywords: Streptozotocin, Antidiabetic, Histopathological, Transaminases.

INTRODUCTION

Diabetes mellitus (DM) poses a major health problem on both clinical and social plans, not simply for the high number of patients, but likewise for the onset of serious invaliding complications that often appear [1]. It is a prototypical, growing, costly chronic non-contagious disease causing and increasing morbidity and mortality worldwide, often disproportionately hurting the poor and young subpopulations in developing countries [2].

As per World Health Organization, DM is a chronic metabolic disorder characterized by common features of chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism [3]. There are numerous pathogenic processes involved in the development of diabetes. This includes autoimmune destruction of the β -cells of the pancreas, which leads to consequent insulin deficiency and abnormalities that result in resistance to insulin action. Deficiency of insulin in target tissues causes abnormalities in carbohydrate, fat, and protein metabolism. It may be due to inadequate insulin secretion and/or diminished tissue responses to insulin [4]. The International Diabetes Federation has predicted a worldwide increase from 8.3% to 9.9% by the year 2030, with China and India projected to have the largest number of diabetic cases [5].

At present, the treatment mainly involves a sustained reduction in hyperglycemia using oral hypoglycemic agents besides injectable insulin. However, prominent side-effects of such drugs are the main reason for an increasing number of people seeking alternative therapies that may have less severe or no side-effects, hence the demand has risen for using a more benign drug. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. This indigenous knowledge, passed down from generation to generation in various part of the world, has significantly contributed to the development of different traditional systems of medicine as well as helps in the exploration of different medicinal plants to find the scientific basis of their traditional use. About 80% of the individuals from developed countries used traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency [6]. In the last few years, a number of studies have been conducted in different countries to prove such efficiency [7,8]. Pharmacological mechanisms described for herbal extracts so far include stimulation of an insulin signaling pathway [9], enhancement of insulin secretion and insulin utilization, inhibition of corticosteroid concentration or endogenous glucose production [10].

Musa paradisiaca grows in humid lowland to upland tropical areas comprising banana and plantain, it is among the world's leading fruit crops, which are large perennial herbs growing from asymodial rhizome [11]. Various parts of *M. paradisiaca* have been used for various medicinal purposes. It has traditionally been used for antidepressant, antibacterial, antihypertensive, antiulcerogenic [12] urolithiasis, laxatives and antihelminthics [13]. This exploration of biologically active natural products has played an important role in finding new chemical entities (NCEs) for example, approximately 28% of NCEs between 1981 and 2002 were natural products or natural product-derived [14]. In addition many traditional uses of banana have been well documented, for example, the leaf and stem are used to treat diarrhoea; the stem is good for asthenia and wounds, and the leaf for the treatment of inflammation, headache and rheumatism [15]. Previous studies reported that *M. paradisiaca* had antimicrobial and healing activities. Nevertheless, only a few studies have reported on the efficacy of this plant against nematodes.

Therefore the aim of this study is to determine the *in vivo* antidiabetic activity of methanolic tepal extract of *Musa paradisiaca* as a possible source of antihyperglycemic agent in the management of diabetes.

EXPERIMENTAL SECTION

Plant materials

Fresh *Musa paradisiaca* tepals, were purchased in one of the local markets (kampung banggol cempedau Kuala Terengganu) here in Terengganu state, Malaysia.

Preparation of plant extract

The tepals were selectively removed from the bracts, oven dried for one week at 40°C. They were then crushed, grinded to a fine powder using a grinder. The tepal was soaked in methanol for 3 days. It was later filtered using Whatmann's No. 1 filter paper. The filtrate was then concentrated at 42°C to yield a dark brown semi solid using a rotary evaporator (N-1100, Shanghai, Eyela. Co. Ltd, Tokyo China). Dried extracts were weighed and dissolved in 10% dimethylsulphoxide (DMSO) to yield a stock solution from which lower concentrations were prepared.

Preliminary phytochemical screening.

The phytochemical screening of the extract was done on the extracts using standard procedures described by Trease and Evans [16]. The sample extract was screened for the presence of phenols, flavonoids, alkaloids, tannins, saponins, and terpenoids.

Animals

Male albino Wistar mice weighing (30-35 g) were procured from Penang, Animal Research And Services Centre (ARASC) Universiti Sains Malaysia (USM). They were stored in polypropylene cages lined with husk. The mice were fed with commercial pelleted rat chow and had free access to water *ad libitum*. The experimental mice were maintained in a controlled air conditioned environment (12:12 h light/dark cycle and temperature (30 ± 2°C)). The experimental design was conducted in accordance with the current ethical norms approved by UniSZA Animal Committee guidelines [UNISZA/AEC/14/007]. The mice were acclimatized for at least 14 days before starting the experiments.

Dosage fixation study

Acute toxicity study was performed in accordance with the organization for economic cooperation and development (OECD) guidelines for animal welfare in normal mice. Graded doses (200, 300, 500, 700, and 1000 mg/kg b.w) of MTE were administered orally at different time periods to the control groups of mice. The dosage was adjusted

every week, according to the change in body weight to maintain similar dose per kg of body weight of mice over the entire period of study for each group. Body weight, food intake, morphological and behavioral changes were monitored periodically to assess the signs of toxicity of MTE if any, in addition, fasting blood glucose was measured at timely interval. At the end of the experimental period, the animals were sacrificed and the blood was collected with and without anti-coagulant.

Induction of experimental diabetes mellitus

Experimental diabetes was induced in overnight fasted mice by single high intraperitoneal injection of streptozotocin (STZ) (200 mg/kg) dissolved in 0.9% of cold saline [17]. Since, STZ is capable of inducing fatal hypoglycemia as a result of the huge pancreatic insulin release, the animals were allowed to drink 10 % glucose solution after 6 h for the next 24 h to overcome the drug-induced hypoglycemia [18]. After one week of STZ injection their fasting blood glucose levels were measured by drawing blood from the tail vein puncture using a glucometer (Accu-Chek Advantage II USA) and the mice that exhibited blood glucose level above 180 mg/dl (10 mmol/L) were considered as diabetic.

Experimental design

The normal and diabetic mice were divided into four groups, comprising a minimum of six mice in each group as follows:

Group 1 - Normal control mice.

Group 2 - STZ induced diabetic mice.

Group 3 - Diabetic mice treated with MTE, (500 mg/kg b.w/mice/day) dissolved in aqueous solution orally for 30 days.

Group 4 - Diabetic mice treated with gliclazide (5 mg /Kg b.w/mice/day) in aqueous solution orally for 30 days.

During the experimental period, body weight, blood glucose, chow and water consumption and physical examinations were determined at regular intervals. At the end of the treatment period, the rats were fasted overnight, anaesthetized and sacrificed by cervical decapitation. The blood was collected with or without EDTA for plasma or serum separation, respectively.

Biochemical parameters

Fasting blood glucose (FBG) was estimated according to the method of [19]. Plasma was separated and used for insulin assay using radioimmunoassay (RIA) kit for rats (Linco Research, Inc., USA). Levels of hemoglobin and glycosylated hemoglobin were estimated according to methods of Drabkin and Austin [20] and Nayak and Pattabiraman [21] respectively. Alanine aminotransaminase (ALT), aspartate aminotransaminase (AST) and serum alkaline phosphatase (ALP) activities were assayed [22,23]. Plasma protein, [24] creatinine, [25], urea [26] and uric acid [27] assays were carried out. Lipid profile (TC, HDL-C, LDL-C and TG) levels in serum were determined according to the instructions of the manufacturer (Merck, Mumbai, India). low density lipoprotein cholesterol was calculated by Friedewald's formula:

LDL-C: Triglycerides/5 [28].

Histological studies

A slice of pancreas, liver and kidney tissues were fixed in 10% formalin for 1 week at room temperature. The specimens were dissected in a graded series of ethanol, cleared in xylene and embedded in paraffin wax. Tissue blocks were sectioned into 3 μ m thicknesses using a rotary microtome. Sections of pancreas, liver and kidney tissues were stained with hematoxylin and eosin, (H & E stain) [29]. Histological changes in the stained sections were viewed under the light microscope by a qualified pathologist without prior knowledge of the groups.

Statistical analysis

The values were expressed as mean \pm S.D. For six mice in each group. All data were analyzed with SPSS/20.0 student software. Hypothesis testing method included one way analysis of variance (ANOVA) followed by post hoc testing performed with least significant difference (LSD) test. Values of $p < 0.05$, $p < 0.01$ and $p < 0.001$, were considered as significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Preliminary phytochemical screening shows the presence of phenols, glycosides, flavonoids, alkaloids, tannins and terpenoids in MTE (Table 1). The extract was tested negative for saponins.

Effect of MTE on the body weight of the control and experimental groups of mice

The body weights of the mice in all the four groups were recorded on the first day followed by a weekly interval for a period of one month. There was an increase in the final body weight of the control group from the initial weight. The mean values of the initial and final body weights of the control group were found to be 35.5 ± 2.6 and 38.5 ± 2.6 g, respectively. There was a significant decrease ($p < 0.05$) in the body weight of diabetic group, where the mean values of initial and final body weight were 33.8 ± 3.9 and 26.1 ± 4.1 g respectively. There were no significant ($p > 0.05$) increase in the body weight of the extract and gliclazide-treated groups when compared to the diabetic group (Figure 1).

Table 1. Preliminary phytochemical screening of MTE of *Musa paradisiacal*

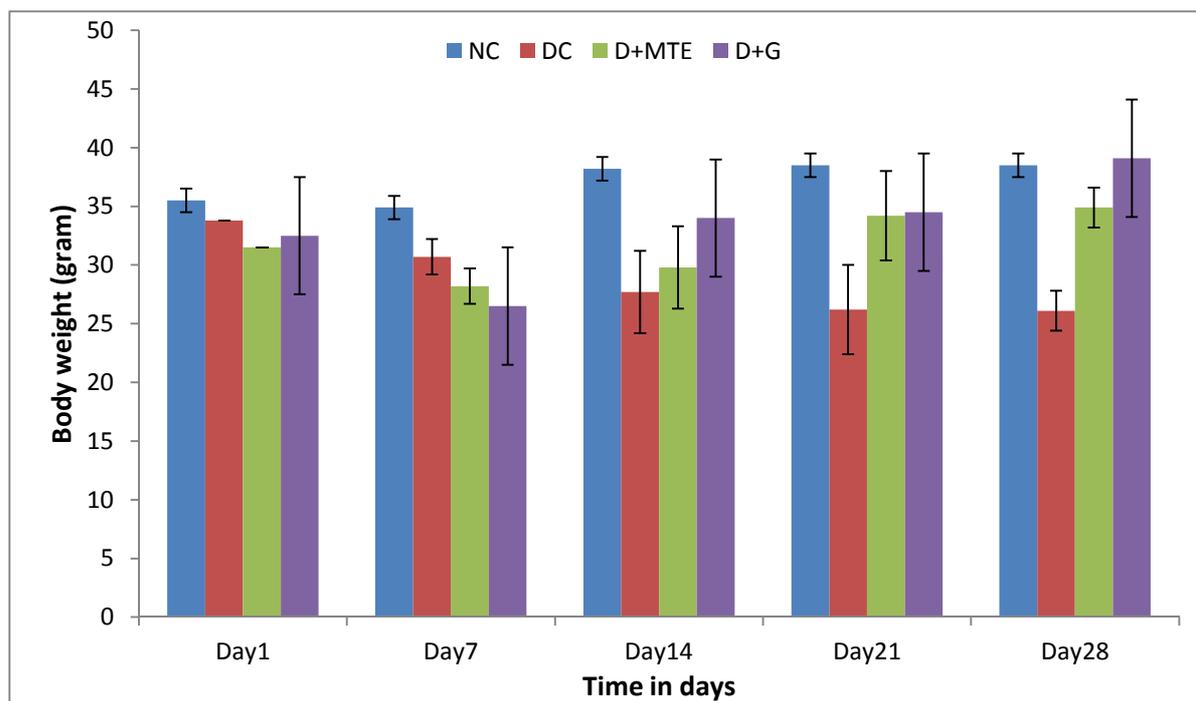
S.N.	Test	T (MeOH)
1	Saponin	-
2	Phenols	+++
3	Glycosides	++
4	Flavonoids	+
5	Alkaloids	+
6	Tannins	++
7	Terpenoids	+

Key: plus (+) indicates the presence and minus (-) signifies absence.

Table 2: Effect of MTE on Fasting blood glucose, plasma insulin, hemoglobin and glycosylated hemoglobin (HbA_{1c}) in control and experimental groups of mice

Groups	Blood Glucose	Insulin	hemoglobin	HbA _{1c}
Control	80.95 ± 7.76	16.30 ± 0.73	13.55 ± 0.95	5.62 ± 1.14
Diabetic control	296.10 ± 17.72^a	5.52 ± 1.23^a	8.22 ± 0.63^a	12.24 ± 0.89^a
Diabetic + MTE	110.38 ± 21.27^b	10.29 ± 0.84^b	11.22 ± 1.12^b	7.58 ± 1.21^b
Diabetic + gliclazide	102.88 ± 12.76^c	11.38 ± 1.29^b	11.51 ± 1.32^b	7.43 ± 0.98^b

Units mg/dl for glucose, ng/ml for plasma insulin, g/dl for haemoglobin and % haemoglobin for HbA_{1c}. Results are expressed as mean \pm SD [$n=6$]. One way ANOVA followed to Post Hoc test LSD. $p < 0.05$. the results were compared with control and diabetic mice. ^a. Contol mice. ^b. Diabetic mice.

**Figure 1: Effect of MTE on the body weight of control and experimental groups of mice**

Values are given as mean \pm SD for groups of six mice in each

Effect of MTE on liver markers, kidney markers, and lipid profile

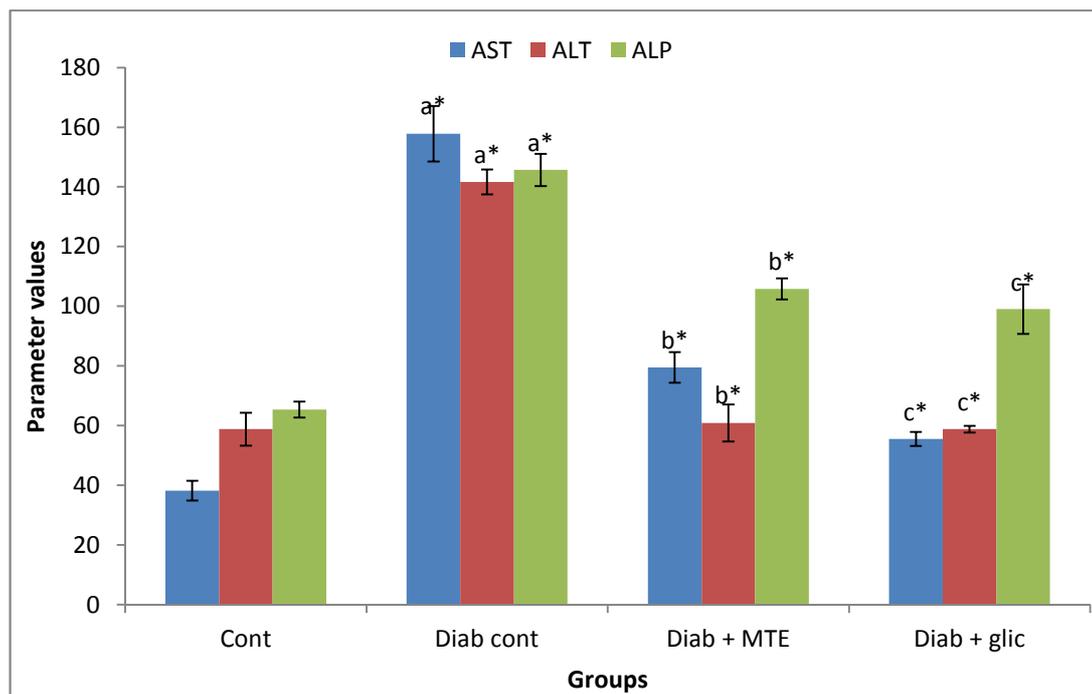
Dosage fixation studies using MTE revealed no signs and symptoms such as restlessness, respiratory distress, diarrhea, convulsions, and coma (data not shown). The levels of blood glucose, insulin, haemoglobin and glycated haemoglobin are shown in (table 2). There was an abnormal level of these parameters in the serum of STZ-induced diabetic mice. Oral administration of MTE after 30 days, reverted back these levels to near normal.

Table 3.Effect of MTE on the levels of serum lipid profile of control and experimental groups of mice

Groups	Total Cholesterol	HDL-C	LDL-C	Tiglyceride
Control	70.65±19.67	27.04±5.3	33.30±17.95	40.50±10.60
Diabetic control	84.86±11.59 ^a	14.88±0.86 ^a	44.42±8.87	67.96±23.54 ^a
Diabetic + MTE	67.80±8.43 ^b	24.54±4.81 ^b	35.40±6.83	44.75±12.51 ^b
Diabetic + gliclazide	63.6±10.66 ^b	22.03±7.57 ^b	31.20±3.42	41.55±13.29 ^b

Units: mg/dl for TC, TG, HDL-C and LDL-C. Results are expressed as mean±SD [n=6]. One way ANOVA followed to Post Hoc test LSD. $p < 0.05$. the results were compared with control and diabetic mice. ^a. Contol mice. ^b. Diabetic mice.

The activities of AST, ALT and ALP in the serum of control and experimental groups were presented in figure 2. A significant ($P < 0.05$) elevation in the levels of AST, ALT, and ALP were noted in serum of STZ-induced diabetic mice. Oral administration of MTE brought down the activity of AST, ALT and ALP to near normal in serum of diabetic mice (Figure 2).

**Figure 2:** Effect of MTE on hepatocellular markers of control and experimental groups of mice

Units, IU/L for AST, ALT and ALP. Results are expressed as mean ± SD [n = 6]. One-way ANOVA followed by Post-Hoc test LSD. Statistical significance was compared within the groups as follows: ^aDiabetic rats compared with control rats; ^bMTE-treated diabetic rats compared with diabetic rats; ^cGliclazide-treated diabetic rats compared with diabetic control rats.

Total protein, creatinine, urea and uric acid levels were shown in figure 3, their levels were also low in diabetic control compared to normal control (Figure 3). After 4 weeks of treatment with MTE, there was a significant increase ($p < 0.05$) in protein, creatinine, urea and uric acid when compared to the normal control. In the same fashion, gliclazide treated group also significantly increases ($p < 0.05$) their level to near normal.

Table 3 depicts the serum lipid profile in diabetic mice with chronic hyperglycemia. In comparison with the normal control, diabetic mice have shown a significant ($p < 0.05$) increase in triglyceride, total cholesterol, LDL-C, and a corresponding decrease in HDL-C. Daily administration of MTE at a dose of 500 mg/kg body weight significantly decrease ($p < 0.05$) the TG, TC, LDL-C compared to the diabetic control. HDL-C was also significantly increased in comparison to the diabetic control. Gliclazide treated group also shows an appreciable improvement when compared to the diabetic control.

Effect of MTE on the changes in histopathology of pancreas, liver and Kidney of control and experimental groups of mice

The changes in the pancreas histology of the different groups are presented in Figure 4. We observed focal necrosis, congestion in central vein and infiltration of lymphocytes in the pancreas of STZ induced diabetic mice (Fig. 4B). Such lesions were considerably diminished by MTE and gliclazide (Figure 4C and 4D). Further, β -cell structure of the MTE mice appeared normal.

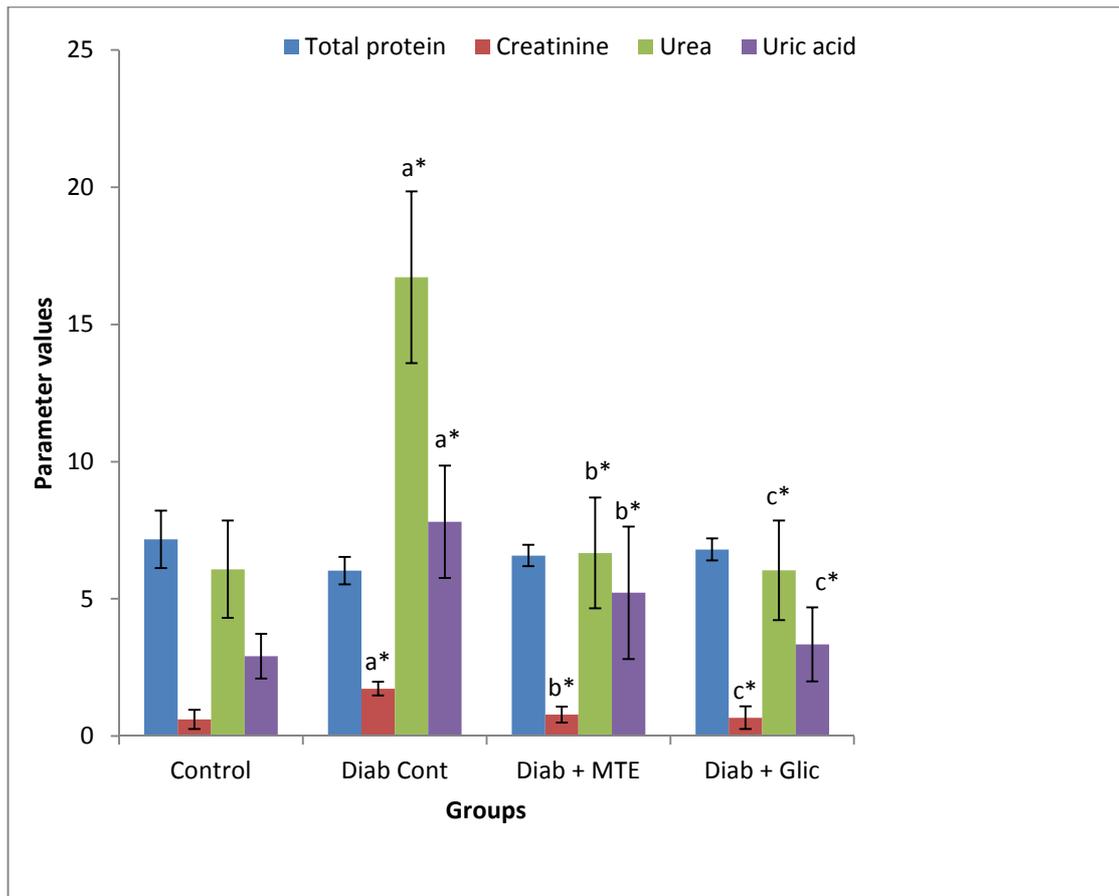
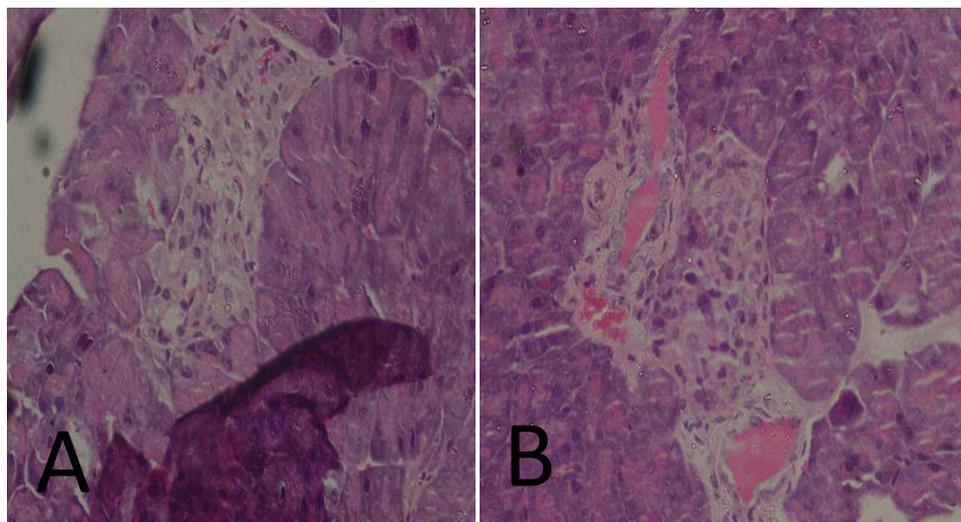


Figure 3: Effect of MTE on renal marker profile of control and experimental groups of mice
 Units: g/dL for plasma protein, mg/dL for blood urea, serum uric acid and serum creatinine. Results are expressed as mean±S.D [n = 6]. One-way ANOVA followed by Post-Hoc test LSD. *Statistically significant at p<0.05. Statistical significance was compared within the groups as follows: ^aDiabetic rats compared with control rats; ^bMTE-treated diabetic rats compared with diabetic rats; ^cGliclazide-treated diabetic rats compared with diabetic control rats.



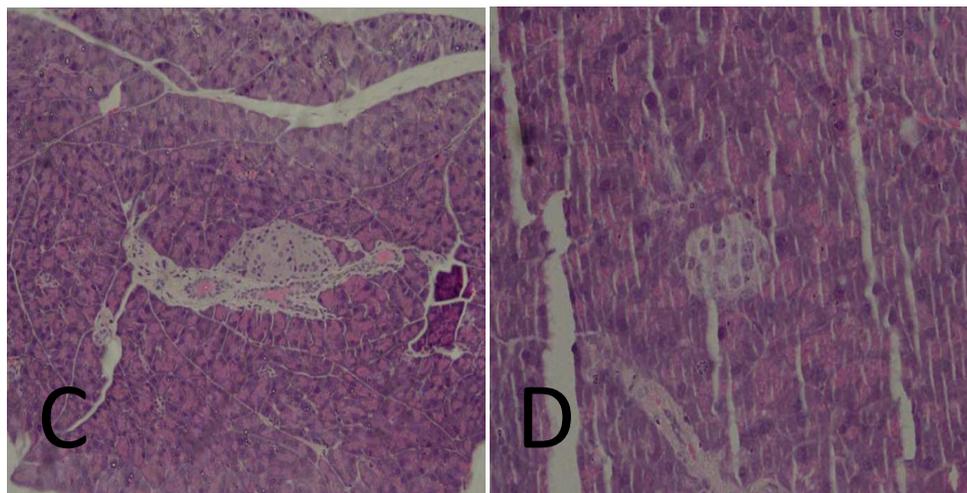


Figure 4: Photomicrograph of pancreatic tissues of control (A); Diabetic induced (B); Diabetic + MTE (C); and Diabetic + gliclazide (D) treated mice showed at 100X

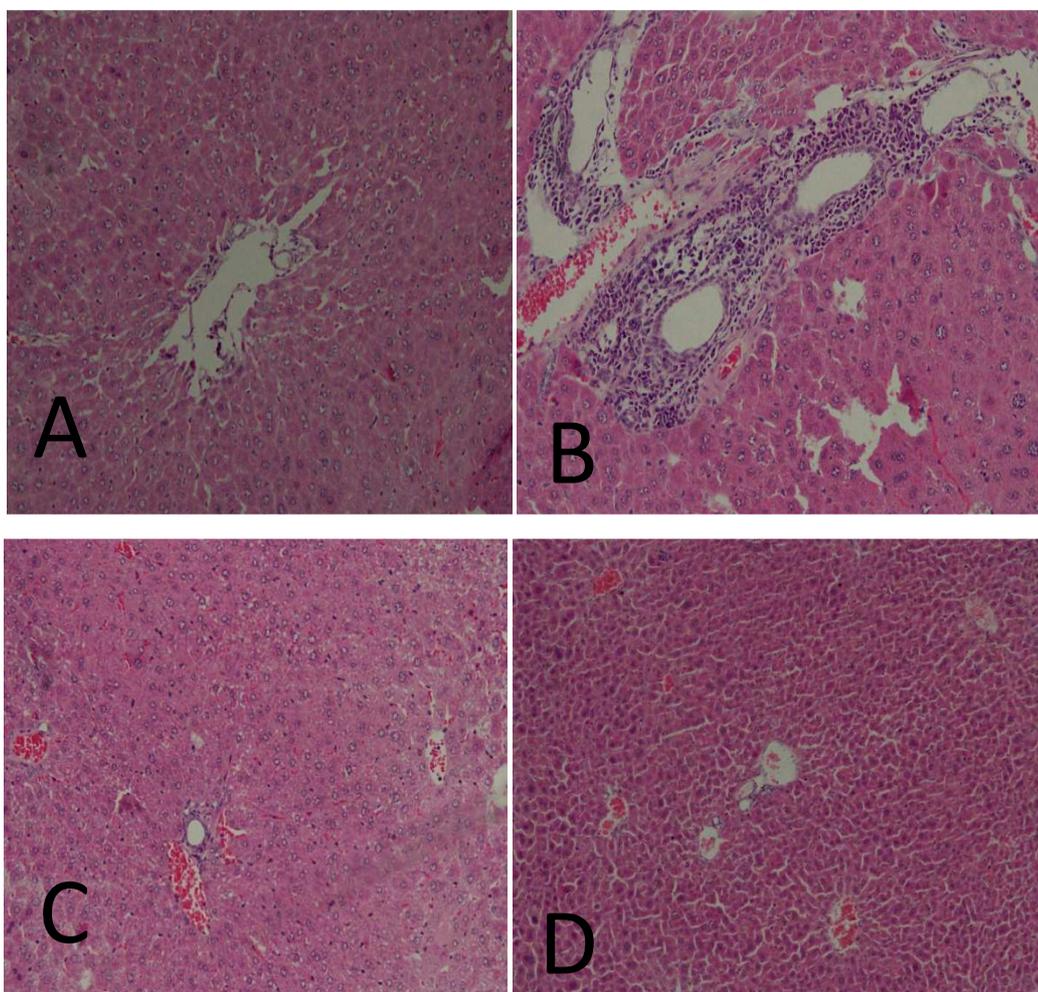


Figure 5: Photomicrograph of hepatic tissues of control (A); Diabetic untreated (B); Diabetic + MTE (C); and Diabetic + gliclazide (D) treated mice showed at 100X

The liver histopathological examination using hematoxylin and eosin (H&E) staining is shown in Figure 5. The liver of control mice (Figure 5A) shows the normal architecture. Figure 5B represents the section of diabetic liver showing inflammatory infiltration filling over the sinusoidal vacuolation of the hepatocyte nuclei, thickening of blood vessels and loss of hepatocytes around the central vein. The pathomorphological changes observed in STZ

induced diabetes becomes apparently normal architecture with the concentric arrangement of the hepatocytes around the central vein after treatment with MTE(5C) and gliclazide (5D)

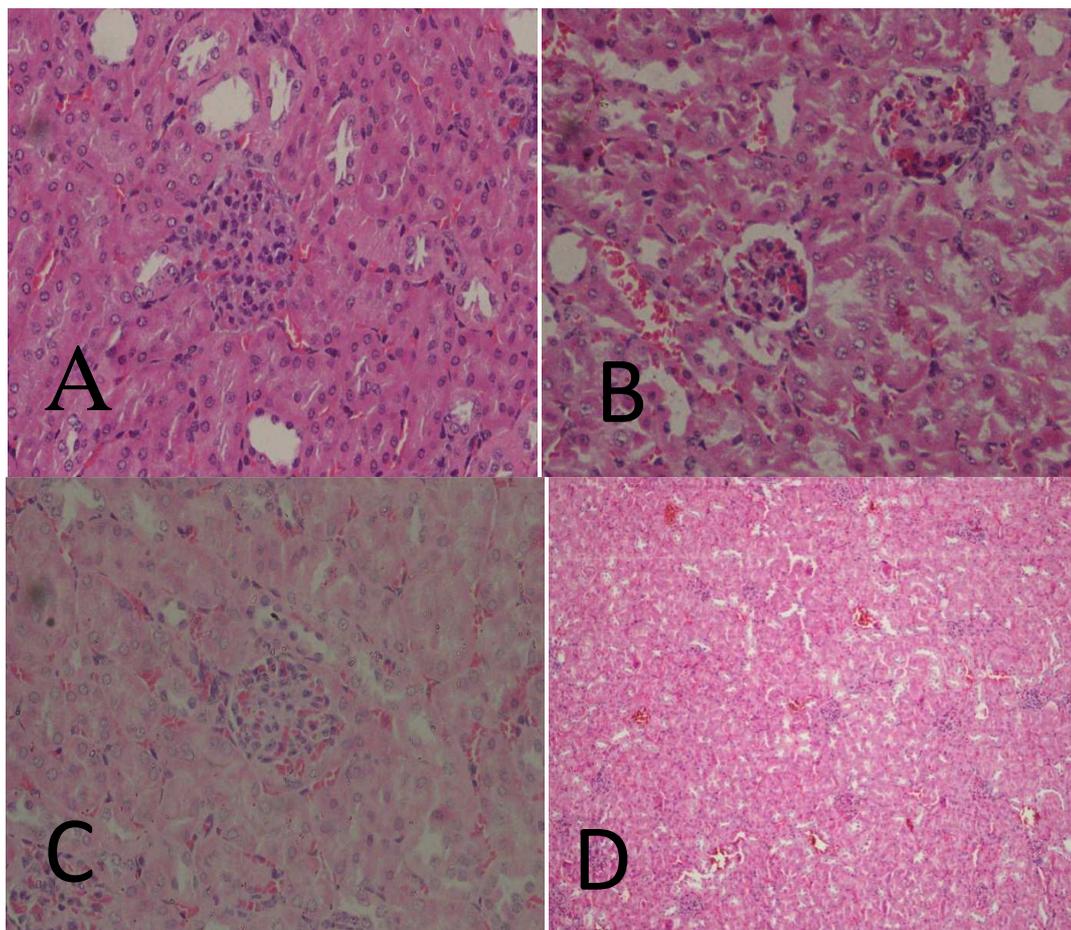


Figure 6: Photomicrograph of renal tissues of control (A); diabetic untreated (B); diabetic + MTE (C); and Diabetic + gliclazide (D); treated mice showed at 100X

The kidney of control mice (Figure 6A) shows normal glomeruli and tubules. Figure 6B represents the section of diabetic kidney showing thickening of vesicles, glomeruli shows some cellular proliferation with fibrosis and thickening of capillary walls. MTE (Figure 6C) however shows more cellular proliferation of the glomeruli, a sign of glomerulonephritis. This may be due to the mild toxic nature of the extract, STZ toxicity, or both. Additionally, the period of the experiment is not wide enough to ascertain how effective is the extracts in kidneys, as plant extracts exert their therapeutic effect slowly overtime. Gliclazide (Figure 3D) treated diabetic mice, however, shows a normal appearance of glomeruli and mild dilated tubules when compared to diabetic mice.

DISCUSSION

Blood glucose is the first index for diagnosis and prognosis of DM. During diabetes, the blood glucose levels are drastically increased which results from reduced glucose utilization by various tissues, which is a typical condition of insulinopenic, STZ-induced diabetes causes a notable reduction in insulin release by the destruction of the pancreatic β -cells, this defective insulin level ultimately results in inadequate oxidation of glucose leading to a pathological condition termed as hyperglycemia[30]. Hyperglycemia and glycosuria are the most critical abnormalities in diabetes. Therefore, the hypoglycemic effect and consequent decrease in urine sugar excretion have been treated as one of the essential characteristics of anti-diabetic agents [31]. DM is characterized by hyperglycemia, which results from reduced glucose utilization by various tissues. The estimation of the blood glucose and HbA1c are useful in the management and prognosis of the disease. STZ-induced diabetes causes a notable reduction in insulin release by the destruction of the pancreas β -cells. In our present study, the administration of MTE, significantly reverts the blood glucose level to near normal when compared to control, a similar trend was seen in gliclazide treated diabetic mice. The HbA1c concentration reflects the patient's average plasma glucose over the previous several weeks making it useful in assessing diabetic control [32]. During

diabetes, the excess of glucose present in the blood reacts irreversibly with amino groups of lysine residues in Hb to form HbA1c [33]. Oral administration of MTE decreased the levels of glucose and glycosylated haemoglobin indicating the beneficial role of MTE in maintaining glucose homeostasis.

Transaminases such as ALT and AST are the intracellular enzymes that have leaked into the circulation and serve as a marker of tissue injury chiefly hepatocyte as well as renal injury. ALP acts as a marker of biliary function and cholestasis. It is hypothesized that elevation in the levels of serum ALT, AST and ALP are considered as predictors of diabetes [34]. The cytosolic enzymes AST, ALT and membrane bound ALP are the physiological markers normally present in low levels in serum and their activities elevated during tissue damage. A rise in ALT activity indicates the hepatocellular damage followed by cardiac tissue damage and is usually accompanied by a rise in AST activity [30]. Further, ALP is a marker of biliary function and cholestasis. The observed increase in the activities of these enzymes in the serum of diabetic mice may be due to the leakage of these enzymes from the liver cytosol into the blood stream as a consequence of the hepatic tissue damage [35]. The reversal of AST, ALT and ALP activities in MTE treated diabetic mice towards near normalcy indicate the hepatoprotective nature of MTE.

In diabetic condition, occurrence in protein loss, and albumin may be attributed to proteinuria, albuminuria or even a marked augmentation of protein catabolism due to the shift from glucose metabolism, this in turn are clinical signs of diabetic nephropathy [36]. The protein level was reduced significantly in the diabetic control mice, and after the administration of MTE as well as gliclazide, the level was restored to near normal. This action may possibly be due to increase in the insulin mediated amino acid uptake, enhancement of protein synthesis, and / or inhibition of protein degradation. Additionally, elevated levels of serum urea, uric acid, and creatinine were observed, this may also be due to renal damage caused by abnormal glucose regulation and glycosylated protein levels during diabetes [37]. The increase in the synthesis of urea in diabetic mice may be due to the enhanced catabolism of both liver and plasma proteins. Oral administration of MTE to diabetic mice significantly inhibits proteolysis caused by insulin deficiency and thus increases the level of plasma proteins to near normal levels.

Dysregulation in lipid metabolism is an important determinant of the course and status of the disease [38]. Diabetic dyslipidemia is featured with elevated TG, TC, LDL-C and decreased HDL-C. These changes impose an increased risk for coronary heart disease in patients with DM [39,40]. It was shown that LDL-C positively and HDL-C negatively correlates with risk of cardiovascular disease [41]. HDL-C possesses antioxidant and anti-inflammatory actions and promotes the efflux of cholesterol from the peripheral tissues to the liver [42]. These lipids are known to be elevated during severe diabetes and have been implicated in the development of atherosclerosis [43]. The serum lipid levels of the extract treated diabetic mice were significantly reduced after 4 weeks of treatment as against that in the untreated diabetic mice in this study.

CONCLUSION

It is therefore hope that the above study will be recognized as a novel breakthrough and emphasizing the antidiabetic medicinal importance of tepals of *Musa paradisiaca*. This therapeutic effect portrayed by tepals, may be attributed to the presence of the phytochemicals such as phenols, flavonoids, glycosides, terpenoids etc.

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