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Effect of vitamin D supplementation on the efficacy of anti-diabetic drugs in streptozotocin- induced albino Wistar rat model

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Abstract

Background: Diabetes mellitus (DM) is a major health challenge that has reached alarming levels worldwide; and due to limitations of existing therapies, the disease has remained an enduring public threat for several decades. Vitamin D deficiency has been shown to play an important role in the pathogenesis of diabetes mellitus as it can affect several key processes in the development of diabetes and its complications, such as pancreatic insulin secretion, peripheral insulin resistance, persistence of systemic inflammation, and immune activation. This study aims at assessing the suitability of vitamin D as a therapeutic adjuvant in the management of diabetes mellitus.

Methods: The effect of vitamin D supplementation was tested on three different drugs, including the typical type 1 DM Glibenclamide therapy, metformin for type 2 DM, and a category 3 Improved Traditional Medicine (coded as ITM-1) under investigation at the Medical Research and Applied Biochemistry Laboratory of the University of Buea. This research focused on acute hypoglycemic/anti-hyperglycemic effects in Oral Glucose Tolerance Test and sub-acute antidiabetic effects in streptozotocin-rat model. For the latter, fasting blood sugar and body weight were measured on days 0, 5, 10, 15, and 21. Other clinical markers included food and water intakes, and daily urine volume. The effects on selected vital organs were equally assessed, notably the liver (serum level of hepatic transaminases) and kidney (serum creatinine and urea).

Results: This study showed that vitamin D supplementation significantly enhanced the activity of all three anti-diabetic drugs through both their hypoglycemic and anti-hyperglycemic activity (post-prandial peak suppression) as well as the sub-acute anti-diabetic activity (a significant improvement on clinical markers, prevention of diabetic and metformin-induced nephropathy). The micronutrient showed an improvement in renal function which was evidenced by the significant decrease in creatinine and urea serum levels ($p<0.01$).

Conclusion: Findings from the present work suggested the potential of vitamin D as a therapeutic adjuvant for the management of Type 2 Diabetes Mellitus.

Keywords: Diabetes mellitus; vitamin D; Metformin; Glibenclamide; Improved Traditional Medicine.

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Background

Diabetes mellitus is a serious, chronic, and complex metabolic disorder of multiple causes, with profound consequences being both acute and chronic [1]; it is a major cause of blindness, kidney failure, heart attacks, stroke, and lower limb amputation [2]. In 2021, it is estimated that 537 million people have diabetes, and this number was projected to reach 643 million by 2030, and 783 million by 2045. The number of children and adolescents up to 19 years old living with diabetes also increases annually, with over 1.2 million cases of type 1 diabetes in 2021. DM is rapidly spreading in Africa at an exponential rate because of increased unhealthy lifestyles, poor dietary habits, lack of exercise, urbanization, and westernization (Francoise, 2011). Genetic and environmental factors contribute immensely to the development of diabetes [3]. Another cause for alarm is the consistently high percentage (45%) of people with undiagnosed diabetes, which is overwhelmingly type 2 [4].

Despite consistent efforts, there is still no effective cure for DM. Moreover, virtually all existing drugs are seriously limited with side effects that can range from simple diarrhea, abdominal discomfort, obesity, to anemia, pulmonary edema, and hypoglycemic coma [5]. Efforts have been devoted to finding innovative approaches for diabetes prevention and treatment, and a recent focus has been on vitamin D supplementation. In fact, observational studies have indicated an association between vitamin D deficiency and the onset and progression of diabetes. Vitamin D has been shown to play a vital role in glucose metabolism by stimulating insulin secretion via the vitamin D receptor on pancreatic beta cells and by reducing peripheral insulin resistance through vitamin D receptors in the muscles and liver [6-8]. Recent studies have suggested that low vitamin D levels were associated with diabetes complications such as dyslipidemia, diabetes nephropathy, and retinopathy [9, 10]. However, the relevance of vitamin D supplementation is yet to be set. This study thus aims to investigate the effect of vitamin D supplementation on the efficacy of antidiabetic drugs (Metformin, Glibenclamide, and a category 3 Improved Traditional Medicine - ITM-1, under development at the University of Buea) in streptozotocin-induced type-2 albino Wistar rat model.

Methods

Materials

Streptozotocin, glucose, chloroform, and other consumables were purchased from Sigma-Aldrich (Germany). The different kits for biochemical parameters alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea and glucose strips were bought from Biopharcam (Buea, Cameroon).

Targeted drugs for the study

Metformin is an oral hypoglycemic agent of the biguanide class that lowers blood glucose levels mainly by decreasing hepatic glucose production and improving the insulin sensitivity of the peripheral tissues by increasing peripheral glucose uptake and utilization. It is generally recommended as the first-line pharmacological agent in management guidelines for patients with type 2 diabetes mellitus (DM) because of its low cost. The generic metformin was bought from a local in Buea. Glibenclamide is an insulin production-stimulating anti-diabetic drug that belongs to the sulphonylurea class of anti-diabetics. The ITM-1 capsules were provided by the MRABL of the University of Buea. It is a product with established anti-diabetic properties, still under development at the Medical Research and Applied Biochemistry Laboratory of the University of Buea. The product was formulated using lyophilized aqueous extracts of *Mangifera indica*, *Persea americana,* and *Ageratum conyzoides*, following the proprietor's procedures.

Experimental animals

Male Wistar albino rats (70 in number) aged between 12-16 weeks, weighing 130 g – 300 g were used for this study animals were selected from animal breeding cages of the MRABL and acclimatized in metabolic cages in groups of three for a period of five days. Food and water were made available to the animals *ad libitum*.

Experimental Design

This study targeted two conventional drugs (metformin, glibenclamide) and one antidiabetic product under development at the University of Buea (coded as ITM-1). It was conducted in two phases: (i) The first phase made use of the Oral Glucose Tolerance Test (OGTT) assay to assess the effect of vitamin D supplementation on the ability of the different target drugs to stimulate regulations/reduction of blood sugar in non-diabetic male Wistar albino rats. In phase two, each of the drugs with and without vitamin D supplementation was then assessed for sub-acute antidiabetic activity in streptozotocin-induced diabetic male Wistar albino rats.

Dose of the various anti-diabetic drugs and Vitamin D

A dose of 10mg/kg of metformin, 5mg/kg of glibenclamide, and 500 IU of vitamin D were used in this experiment.

Hypoglycemic and anti-hyperglycemic activity of anti-diabetic drugs

The hypoglycemic and anti-hyperglycemic activities of the different anti-diabetic drugs were determined by carrying out the oral glucose tolerance test (OGTT). Blood glucose levels will rise after a meal, because of the absorption of glucose from the meal. This transitional hyperglycemia is reversed by regulatory mechanisms of the organism generally within 2-4 hours [11]. The main objective of the OGTT is to investigate the ability of the different drugs supplemented with vitamin D to stimulate regulations/reduction of blood sugar level, in non-diabetic subjects. A total of 35 male Wistar albino rats were divided into 7 groups of 5 animals each according to their fasting blood sugar.

The fasting blood sugar (FBS) level was first measured, and the time was recorded as t-30 mins. Immediately after FBS measurement, animals were randomized into different groups using a Microsoft Excel matrix design to ensure homogeneity in FBS. Afterward, the different groups were administered corresponding products (ITM-1, ITM-1 + Vitamin D, Glibenclamide, Glibenclamide + Vitamin D, Metformin, Metformin + vitamin D, and water -distilled water). Thirty minutes, the FBS levels of the animals were again recorded as t0 and all the animals were then given an oral dose of glucose (2 g/kg body weight). The fasting blood sugar levels of the animals were further measured at t30, t60, t90, t120, and t240.

Interpretation of hypoglycemic activity

The hypoglycemic activity was appreciated using the parameters:

(i) By comparing FBS to the values observed 30 mins upon administration of the given treatment
(ii) By deriving the glycemia restoration time: The term "glycemia restoration time (RT) is the time taken in each treatment group to reach back the initial glycemia (FBS at t-30). This was derived as the intersection of the variation curve with the x-axis after post-prandial.

Interpretation of anti-hyperglycemic activity

Anti-hyperglycemic activity is the ability of a given treatment to prevent a post-prandial peak of glucose. The peak suppression rate was calculated as follows:

%PS= (Glu (30)NC – Glu(30)x)*100/Glu (30)NC
Where Glu (30) NC is the glycemia of the negative control at t30; Glu (30)x is the glycemia of the different treatment groups at t30.

Effect in sub-acute anti-diabetic activity

Induction of Obesity in rats: The normal diet was prepared by mixing and pelleting 5 kg of corn, 1.5 kg of wheat bran, 1.5 kg of powdered soya beans, 1 kg of powdered fish, 1.5 kg of powdered bone. To 1000 g of the normal diet pellet, 550 g of butter and 10 g of vitamin were added to give the high-fat diet. Five (5g) of sugar were additionally included in 200ml of water and given to animals receiving high-fat diet for 30 days.

Streptozotocin diabetes induction of type 2 DM in obese rats: Animals with body weights within the range, 200-300 g, were considered for diabetic induction. Animals were submitted to 12 hr fasting and their blood sugar levels were measured using a CodeFree® glucometer to ascertain the status of the selected animals prior to diabetes induction. Streptozotocin at a dose of 40mg/kg was dissolved in 0.1M citrate buffer at pH 4.5 and administered to the animals intraperitoneally according to their body weights. After induction, animals were fasted for two hours after which they were supplied with feed and water *ad libitum*. Animals were observed and their urine volumes and color were closely monitored for a period of 2 days. On the second day after diabetes induction, animals were fasted overnight, and the fasting blood sugar levels were measured using a glucometer of mark Code Free. Animals with FBS ≥200 mg/dL were considered diabetic and used for the study.

Sub-acute anti-diabetic activity: Following testing on acute hypoglycemic and anti-hyperglycemic effects in healthy animals, the effect of Vitamin D supplementation was evaluated in the Streptozotocin-induced T2DM in Wistar albino rat model, targeting the same drugs (Metformin, Glibenclamide and ITM-1). Both clinical and biochemical markers were considered in comparing different groups for disease recovery and occurrence of liver and kidney complications of T2DM. A total of 35 diabetic rats were divided into 7 groups of 5 animals each, having statistically similar average fasting blood sugar levels on day 0 (using One-way ANOVA, Student Newman-Keuls test in SPSS [12]). The groups were assigned different treatments as follows: Group I (glibenclamide 5 mg/kg/day); Group II (glibenclamide 5 mg/kg/day + vitamin D 500U I/kg/day) , Group III (Metformin 10mg/kg/day), Group IV (Metformin 10 mg/kg/day + vitamin D 500 UI/kg/day), Group V (ITM-1 25 mg/kg/day), Group VI (ITM-1 25 mg/kg/day + Vitamin D 500 UI/kg/day) and Group VII, negative control (Distilled water 10 mL/kg/day).

The different groups received food and water *ad libitum* and the different treatments administered daily, for 21 consecutive days. Fasting blood sugar levels and body weight were measured on days 0, day 5, day 10, day 15, and day 21 to track changes in glucose levels. The amount of food and volume of water consumed, and urine eliminated were measured daily except on days when the animals were fasting. The animals in different experimental groups were also monitored for any behavioral changes. At the end of the treatment period, the rats were sacrificed, and blood samples were collected retro-orbitally, and through cardiac puncture into tubes, then centrifuged at 1500rpm for 10mins to obtain serum. The serum obtained was stored at -20ºC, then used to determine the markers of liver (ALT and AST activities) and kidney (creatinine, urea levels) functions.

Markers of liver and kidney functions

The liver amino-transferases (AST and ALT) and markers of the kidney function (serum Creatinine and Urea) were measured as previously described [13].

Ethical considerations
The proposal including the standard operating procedures used in this research was reviewed and approved by the University of Buea Institutional Animal Care and Use Committee (UB-IACUC) and ethical clearance was obtained from the committee with reference number UB-IUCUC Nº 05/2023.

Statistical analysis

From bench experiments, data was keyed into Microsoft Excel version 2020, and subsequently exported and analyzed using the Statistical Package for Social Sciences (SPSS) version 26. Secondary data was generated and interpreted for the acute hypoglycemic/anti-hyperglycemic activity (restoration time -tR, percentage post-prandial peak suppression rate - %PS), and for the sub-acute antidiabetic activity testing (Weekly food, water intake, and urine elimination; variation in glycemia and body weight; recovery rate; and relative organ weight for liver and kidney). All values were expressed as the mean ± Standard deviation, except for the percentage recovery rate. The different groups that received vitamin D supplements were compared against the same drugs alone, while all test groups were compared against the negative control for activity evaluation, and for assessing the effect of the supplementation. The independent sample t test and One-way ANOVA (Duncan test) were used at a 95% confidence interval.

Results

Effect of vitamin D supplementation on hypoglycemic and anti-hyperglycemic activity

The effect of a 500UI/kg Vitamin D supplementation on the acute hypoglycemic and anti-hyperglycemic activities of metformin, Glibenclamide, and ITM-1 in albino Wistar rats recorded from OGTT is illustrated below in Figure 1. The graph above shows that between t-30 and t0 there was a significant drop in glycemia in the group receiving Met + Vit D as opposed to the group receiving Met where an increase in glycemia was observed ($p<0.05$). The group

receiving Met+ Vit D had total peak suppression, whereas for the group receiving Met the restoration was at t 60 with no peak suppression. The negative control water had a very high post-prandial peak with a longer restoration time of t120. ITM-1 +Vit D group shows no peak suppression a shorter restoration time of t 90 compared to the ITM-1 group with restoration time at t120. The group receiving water had a very high post-prandial peak with a restoration time t120.

Between the time t-30 and t0 there was a decrease in glycemia in Glib + Vit D group and the groups receiving Glib but negative control group showed an increase. Also, the group receiving Glib + Vit D had a total peak suppression. The Glib group restored glycemia back to normal at t 60 and as well as peak suppression. The group receiving water had a very high post-prandial peak with a restoration time at t120.

Effect on Subacute anti-diabetic activity

The results obtained for the effect of vitamin D supplementation on the sub-acute anti-diabetic activity of the selected anti-diabetic drugs were presented based on clinical markers and markers of vital function.

Effect on clinical markers: The weekly food intake of the different experimental groups is summarized in Figure 2 below.

The amount of food consumed by the group receiving metformin + Vit D was lower as opposed to the group taking Met only. This showed a significant difference with $p<0.05$. The amount of food consumed by the group receiving ITM-1 + Vit D was lower as opposed to the group receiving ITM-1 only. This showed a significant difference with $p<0.05$. The amount of food consumed by the group receiving Glib + Vit D shows a great decrease as opposed to the group receiving Glib.

There was a significant increase in food intake in the group receiving water as compared to the other groups.

Effect on water intake: Polydipsia which is another major symptom of diabetes mellitus was assessed. The result for weekly water intake is illustrated in Figure 3. The group receiving met +Vit D showed a significant decrease in water intake all through as opposed to the group receiving met only which had a significant increase on day 20 ($p<0.05$). There was no significant difference in water intake between the group receiving ITM-1 + Vit D and those receiving ITM-1 only. The was a significant decrease in water intake in the group receiving Glib + Vit D as opposed to the group receiving Glib alone ($p<0.05$). There was a significant increase in water intake in the group receiving water as compared to the other groups.

Effect on Urine elimination: Figure 4 presents the status of urine elimination recorded in the different experimental groups of animals.

The group receiving met +Vit D had a significantly lower urine volume as opposed to the group receiving met only with a higher urine volume with p 0.01. There was no significant difference in urine volume between the group receiving ITM-1 + Vit D and those receiving ITM-1 only. There was no significant difference in urine volume between the group receiving Glib + Vit D and those receiving Glib only. There was a significant increase in Urine volume in the group receiving water as compared to the other groups.

Effect on body weight: The figures below illustrate variations in body weight among the groups. The group receiving Met + Vit D showed a decrease in body weight, but an increase was observed from day 15 to day 21 which was not statistically significant with $p>0.05$. On the contrary, the group receiving Met showed a decrease in body weight from day 0 to day 21. The highest decrease in body weight was observed in the group receiving water (Table 1). There was a decrease in the body in both the groups receiving Glib + Vit D and those receiving Glib. On day 21 there was an increase in body weight in the group receiving Glib + Vit D which was not statistically significant with $p>0.05$.

Effect of vitamin D supplementation on the fasting blood sugar

Findings on the evolution of Fasting Blood Sugar in the different experimental groups are summarized in Figure 5. There was a general decrease in FBS in the groups receiving Glib + Vit D and those receiving Glib with the highest decrease being observed in the group receiving Glib + Vit D which was not statistically significant with P. 0.05. The group receiving water showed the lowest decrease in glucose levels. The group receiving Met + Vit D had a higher decrease in Fasting blood sugar compared to the group receiving Met. This difference was not statistically significant. The negative control group showed the lowest reduction in glucose levels. There was a general reduction in glycemia among the groups, with the group receiving ITM-1 showing the highest decrease compared to the other groups.

Effect on the liver function

The amount of liver enzymes in the serum serves as an indicator of liver damage. The enzymes ALT, AST, and Liver weight of the liver was measured. The results are presented in Figure 6 below. From the bar chart above it is observed that: there is no significant difference in the liver weight between the group receiving Met and Vit D and the group receiving Met. Also, between the group receiving ITM-1 + Vit D and those receiving ITM-1 no significant difference was observed. The group receiving Glib +Vit D had the highest mean liver weight as opposed to that of the group receiving Glib. The group receiving ITM-1 + Vit D has a lower ALT level compared to the groups receiving ITM-1 only which was not statistically significant with $p>0.05$. The group receiving Glib + Vit D shows a lower level of ALT compared to the group receiving Glib only. This difference was not statistically significant $p=0.256$. The negative control group showed the highest ALT levels compared to the rest of the groups. The group receiving Met +Vit D had a higher AST level as compared to the group receiving Met only. The group receiving ITM-1 + Vit D has a lower AST level compared to the groups receiving ITM-1 only. The group receiving Glib + Vit D shows a lower level of AST compared to the group receiving Glib only. The negative control group showed a low AST level compared to the rest of the groups except for the group receiving Met which had the lowest level of AST.

Markers of Kidney function

The presence of creatinine and urea serve as indicators of kidney function. Their levels were measured, and relative kidney weights were measured, the results are presented in Figure 8 below. There is no significant difference between relative kidney weight between the group receiving Met +Vit D and those receiving Met Only. There is no significant difference between relative kidney weight between the group receiving Glib+Vit D and those receiving Glib Only. There is no significant difference between relative kidney weight between the group receiving ITM-1 +Vit D and those receiving ITM-1 Only. The group receiving water has the highest relative kidney weight.

The creatinine levels were significantly lower in animals receiving metformin supplemented with vitamin D, as compared to those under metformin alone ($p<0.01$). Similarly, the creatinine levels in the Glib group were greater than those in the glib +Vit D

group, though the difference was not statistically significant (p=0,08). Also, there was no significant difference between the group receiving vitamin D in supplementation to ITM and the group that took ITM only (p=0.356). All the groups receiving the vitamin D supplement showed very low urea levels as opposed to the groups not receiving the supplements which were statistically significant with P< 0.01 The Met group showed the highest level of Urea (above the normal range cut-off value of 25mmol/L), followed by the negative control water.

Discussion

Diabetes mellitus continues to pose a threat and remains a public health challenge despite consistent efforts and research work put in by scientists to mitigate and eradicate this disease. This study was aimed at assessing the suitability of vitamin D supplementation as a therapeutic adjuvant in the management of diabetes mellitus.

The main objectives of this study were to assess the efficacy of vitamin D supplementation of the efficacy of the anti-diabetic drugs Metformin, Glibenclamide, and ITM-1e in streptozotocin-induced albino Wistar rats by measuring some clinical markers as well as biochemical markers. The study was done in two phases: (i) Evaluating the effect of vitamin D supplementation on the hypoglycemic and antihyperglycemic activities of the anti-diabetic drugs (metformin, glibenclamide, and ITM-1) in healthy albino Wistar rats using the oral glucose tolerance test; (ii) Assessing the effect of vitamin D supplementation on sub-acute anti-diabetic activities of an insulin receptor stimulating drug (Glibenclamide), insulin stimulating drug (Metformin) and a new drug under development (ITM-1). Continuous hyperglycemia is the most important characteristic of diabetes. Other features include weight loss, excessive urination, excessive thirst, and extreme hunger. Findings from OGTT showed significantly lower peaks in the groups receiving the vitamin D supplement compared to the other groups. The negative control group showed the highest postprandial peak compared to the other groups. The vitamin D supplemented groups also showed a shorter restoration time with total peak suppression being observed in the groups receiving Met + Vit D and Glib + Vit D. This shows the positive effect of Vitamin D on the acute activity of the anti-diabetic drugs, this result is similar to findings of Patricio *et al.,* 2015 which showed that dietary vitamin D supplementation improved postprandial hyperglycemia in mice. This further proves that vitamin D enhances the ability of these drugs to stimulate a decrease in blood sugar by increasing glucose uptake and storage. Fasting blood sugar is one of the most important biomarkers that is considered when dealing with hyperglycemia in diabetes mellitus. The results obtained showed a significant decrease in the fasting blood sugar in all the treatment groups with no significant difference among the groups however vitamin D showed a slight enhancement in glycemic control in metformin. Although there was a general decrease in body weight across all the groups, but a slight increase was observed from day 15 to day 21 in the vitamin D supplemented groups. Weight loss is one of the symptoms of diabetes; hence the ability of vitamin D to improve body weight shows it has a positive impact on anti-diabetic drugs. Also, there was a significant reduction in urine volume across all the treatment groups compared to the negative control group. The groups receiving the vitamin supplement showed a greater decrease in urine volume which was more significant in the group receiving Met + vitamin D. Polyuria has been shown to develop in VDR-null mice as a result of VDR inactivation leading to an increase in the production of Angiotensin II, which plays the central role in the occurrence of polyuria [14]. Again, there was a significant decrease in water intake and food intake in all the treatment groups compared to the negative control; however, the decrease was more appreciated in the groups receiving the vitamin D supplement. These findings were similar to those of Juan *et al.* (2008) who showed that loss of vitamin D through its receptors causes polyuria by increasing thirst. Vitamin D increases the levels of serotonin a neurotransmitter that plays a major role in controlling appetite by increasing satiety and decreasing food intake [15].

Diabetes mellitus apart from its effect on blood glucose levels, this disease has been shown to impact several body vital functions such as the kidney and liver. The Liver marker (AST, ALT) and Kidney markers (Urea, Creatinine) as well as the weight of these organs were measured. Based on the result there was no significant difference in liver weight among all the groups.

Serum AST and ALT of the treatment groups were not significantly different from each other except for the Met and negative control which showed lower AST levels compared to the rest of the groups. Diabetes nephropathy is of the long-term complications of diabetes, urea and creatinine are important markers of the kidney and their levels in blood show the functioning of the kidney.

Serum creatinine levels for the treatment groups' Met+ vitamin D, Glib + vitamin D, ITM-1 + vitamin D, ITM-1, and Glib were lower compared to that of the negative control. These low creatinine levels were more prominent in the vitamin D supplemented groups. On the other hand, the metformin-treated group demonstrated significantly higher creatinine levels compared to the rest of the groups. Though metformin toxicity is thought to be rare with therapeutic dosing [16], the safety of metformin has always been questioned as the drug has long been said to be associated with an increased risk for lactic acidosis, particularly in people with high levels of creatinine [17,18]. It has been clearly demonstrated that metformin increased the incidence of lactic acidosis [19], and this could happen in type 2 diabetes patients without renal dysfunction. Metformin is excreted unchanged in the kidney, hence accumulation of metformin results in the buildup of lactic acid which affects renal functions [20]. Understanding the factors that increase the probability of developing MALA is crucial. Zheng *et al.* [21] investigated the effect of metformin on renal medullary interstitial cell (RMIC) survival in normal and T2DM C57BL/6 mice. It was observed that animals with diabetes treated with metformin developed renal medullary interstitial cell apoptosis. The authors suggested that metformin's action could be dependent on the activation of AMPK, which hypothesis was confirmed by 5-aminoimidazole-4-carboxyamide ribonucleoside (AICAR) treatment inducing a significant amount of RMIC apoptosis.

The vitamin D supplemented groups showed significantly lower serum urea levels compared to the other groups. The low creatinine and urea levels in the vitamin D supplemented groups in this study are similar to that of previous studies. Studies have shown that treating diabetic animals with vitamin D significantly improved the kidney functions such as blood urea and serum creatinine levels. Gembillo *et al.* [22] reported that Vitamin D supplementation has been shown to reduce proteinuria, improve glomerular filtration rate, and preserve the renal structure whereas Wang *et al.* [23] found that vitamin D supplementation significantly reduced blood urea but not serum creatinine in diabetic patients with chronic kidney diseases. From the present study, Vitamin D was observed to reverse metformin nephrotoxicity, suggesting its possible use as a therapeutic adjuvant in a metformin combined treatment for T2DM. Vitamin D has been successfully tested as an adjuvant for the management of other pathologies. The supplementation of 0.5 µg vitamin D3 per day in Chinese subjects

was reported to have beneficial effects on coronary artery disease, and the authors concluded that Vitamin D could be an adjuvant therapy for patients with coronary artery disease [24]. Similarly, from a meta-analysis of work carried out, Cintya *et al.* [25] reached the conclusion that Vitamin D3 supplementation may fasten wound healing and decrease the burden caused by diabetic foot ulcers.

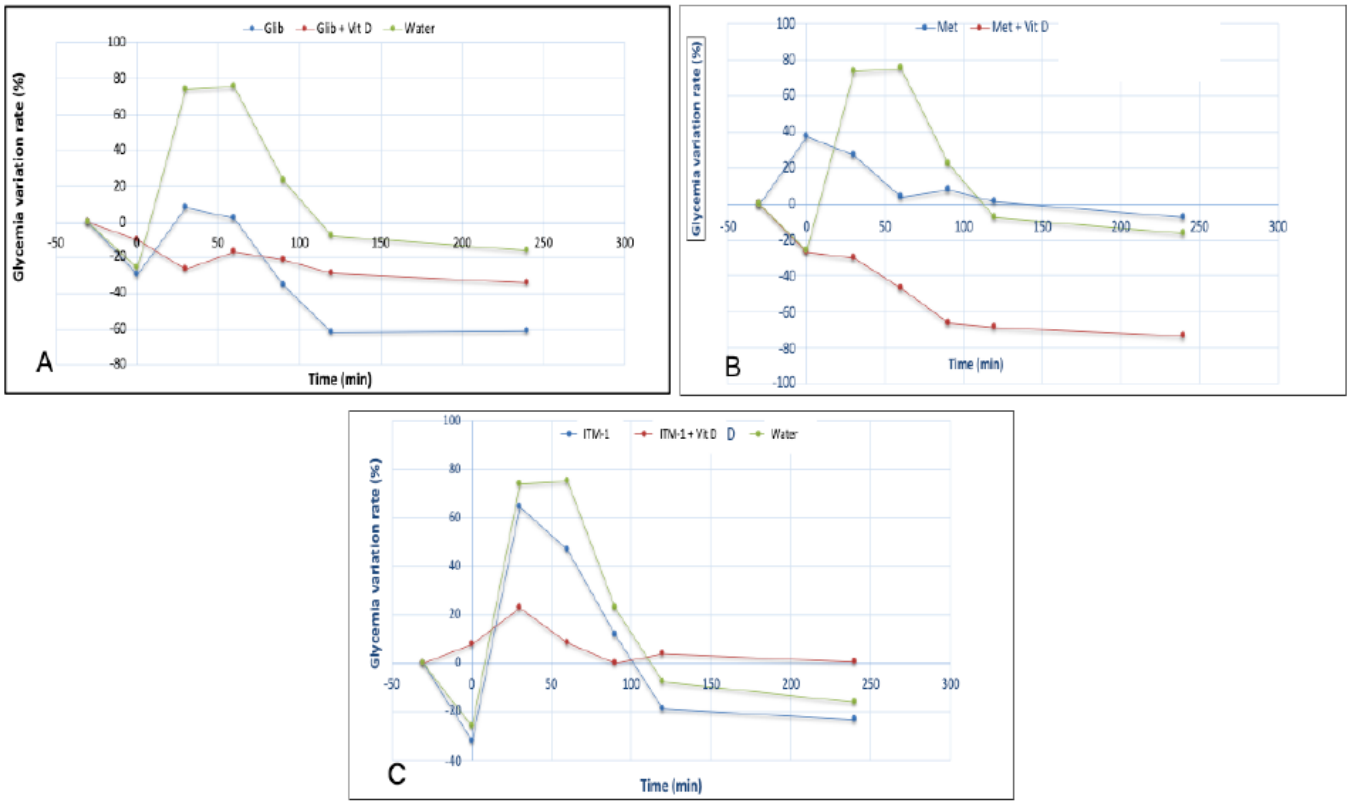

Figure 1. Effect of Vitamin D supplementation on acute hypoglycemic and antihyperglycemic activities of Metformin, Glibenclamide and ITM-1
A: Effect on metformin; B: Effect ITM-1; C: Effect on Glibenclamide; Met : Metformin (10 mg/kg); Met + Vit D : Metformin (10 mg/kg) + Vitamin D (500 UI/kg); Glib: Glibenclamide (5 mg/Kg); Glib + Vitamin D: Glibenclamide (5 mg/Kg) + Vitamin D (500 UI/kg; ITM-1: Capsule Improved Traditional Medicine from lyophilized aqueous extracts *of Mangifera indica*, *Persea americana* and *Ageratum conyzoides* leaves (25 mg/Kg); ITM-1+Vit D: ITM-1 (25 mg/Kg) + Vitamin D (500 UI/kg); Water: Negative control- distilled water (10 mL/Kg).

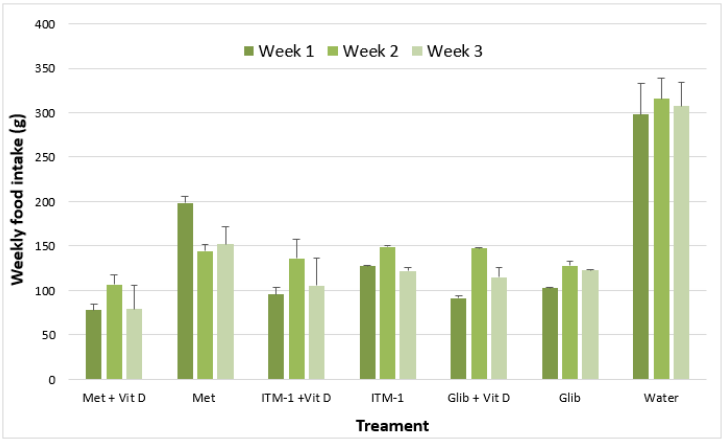

Figure 2. Effect of vitamin D on food intake in diabetic rats
ITM-1: Capsule Improved Traditional Medicine from lyophilized aqueous extracts of *Mangifera indica*, *Persea americana* and *Ageratum conyzoides* leaves (25 mg/Kg); ITM-1+Vit D: ITM-1 (25 mg/Kg) + Vitamin D (500 UI/kg); Glib: Glibenclamide (5 mg/Kg); Glib + Vitamin D: Glibenclamide (5 mg/Kg) + Vitamin D (500 UI/kg); Met : Metformin (10 mg/kg); Met + Vit D : Metformin (10 mg/kg) + Vitamin D (500 UI/kg); Water: distilled water (10 mL/Kg).

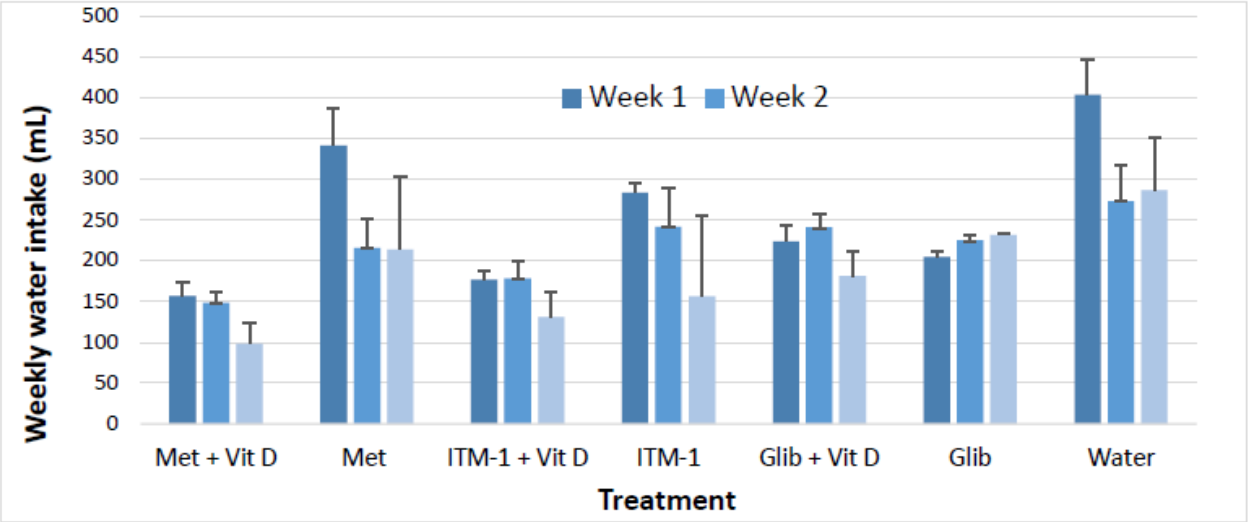

Figure 3. Effect of vitamin D on water intake in diabetic rats

ITM-1: Capsule Improved Traditional Medicine from lyophilized aqueous extracts of *Mangifera indica, Persea americana* and *Ageratum conyzoides* leaves (25 mg/Kg); ITM-1+Vit D: ITM-1 (25 mg/Kg) + Vitamin D (500 UI/kg); Glib: Glibenclamide (5 mg/Kg); Glib + Vitamin D: Glibenclamide (5 mg/Kg) + Vitamin D (500 UI/kg); Met: Metformin (10 mg/kg); Met + Vit D : Metformin (10 mg/kg) + Vitamin D (500 UI/kg); Water: distilled water (10 mL/Kg).

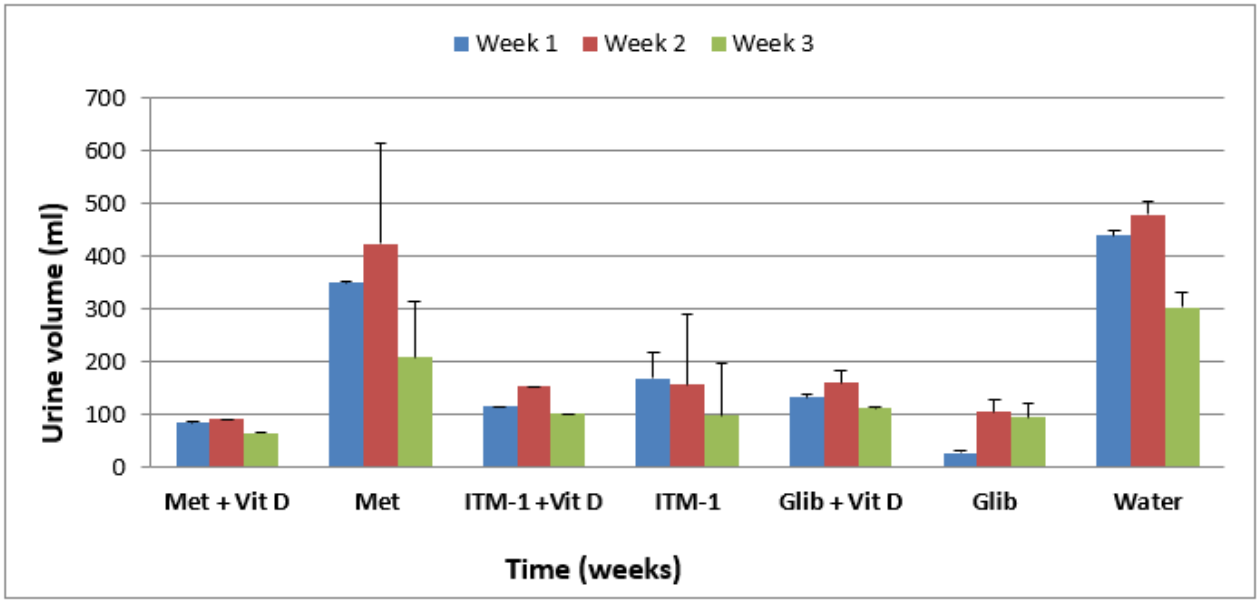

Figure 4. Effect of vitamin D on urine volume in diabetic rats

ITM-1: Capsule Improved Traditional Medicine from lyophilized aqueous extracts of *Mangifera indica*, *Persea americana* and *Ageratum conyzoides* leaves (25 mg/Kg); ITM-1+Vit D: ITM-1 (25 mg/Kg) + Vitamin D (500 UI/kg); Glib: Glibenclamide (5 mg/Kg); Glib + Vitamin D: Glibenclamide (5 mg/Kg) + Vitamin D (500 UI/kg); Met : Metformin (10 mg/kg); Met + Vit D : Metformin (10 mg/kg) + Vitamin D (500 UI/kg); Water: distilled water (10 mL/Kg).

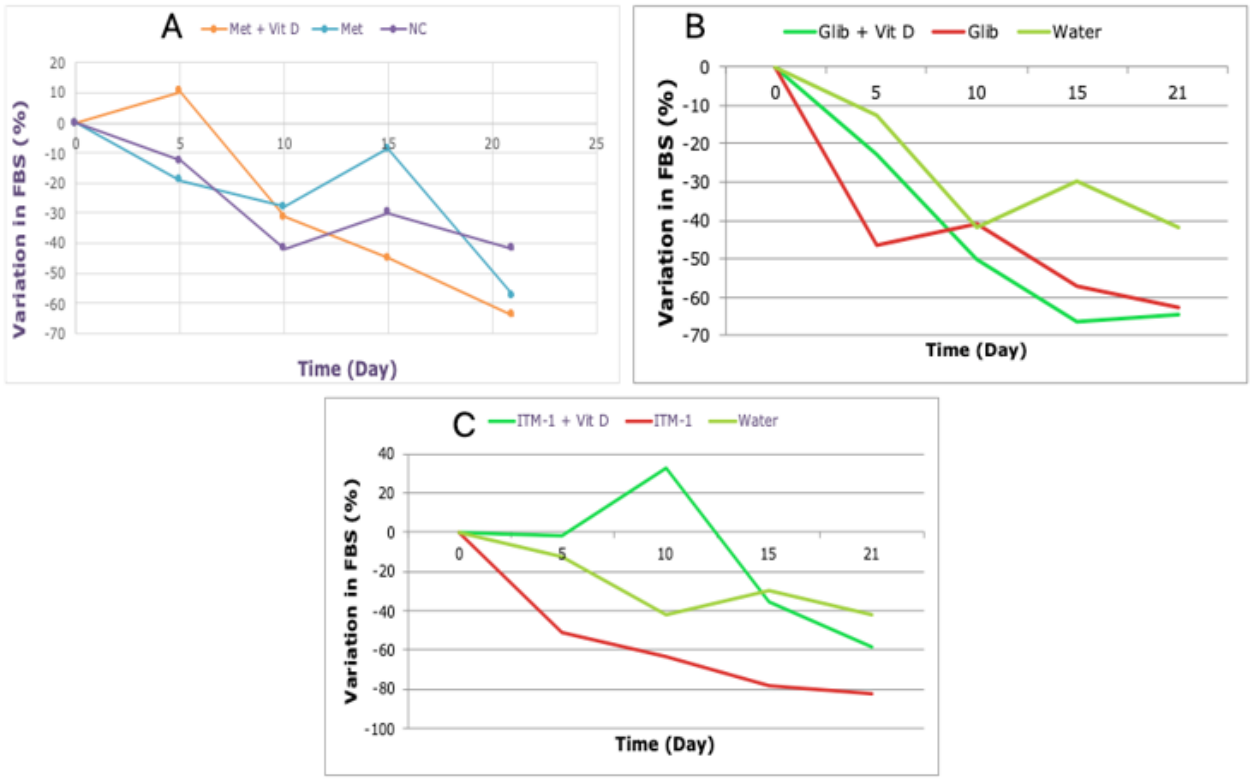

Figure 5. Effect of Vitamin D supplementation on glycemia variation rate of Metformin
ITM-1: Capsule Improved Traditional Medicine from lyophilized aqueous extracts of *Mangifera indica*, *Persea americana* and *Ageratum conyzoides* leaves (25 mg/Kg); ITM-1+Vit D: ITM-1 (25 mg/Kg) + Vitamin D (500UI/kg); Glib: Glibenclamide (5 mg/Kg); Glib + Vitamin D: Glibenclamide (5 mg/Kg) + Vitamin D (500 UI/kg); Met : Metformin (10 mg/kg); Met + Vit D : Metformin (10 mg/kg) + Vitamin D (500 UI/kg); Water: distilled water (10 mL/Kg).

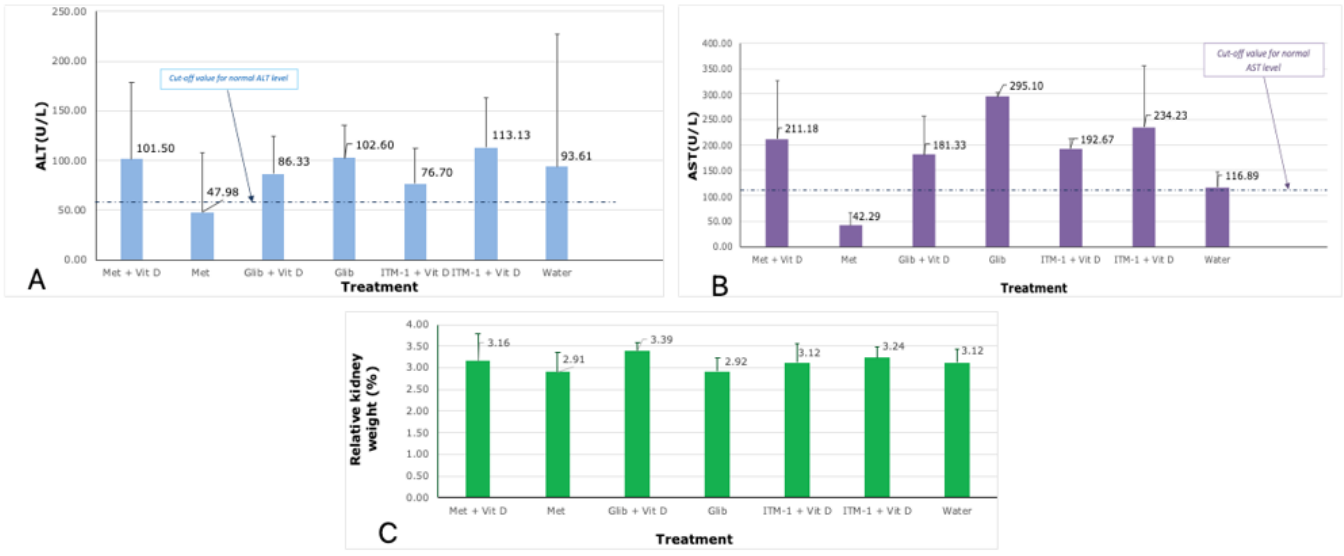

Figure 6. Effect of Vitamin D supplementation on liver function.
A: ALT level; B: AST level; C: relative liver weight; ITM-1: Capsule Improved Traditional Medicine from lyophilized aqueous extracts of *Mangifera indica*, *Persea americana* and *Ageratum conyzoides*leaves (25 mg/Kg); ITM-1+Vit D: ITM-1 (25 mg/Kg) + Vitamin D (500 UI/kg); Glib: Glibenclamide (5 mg/Kg); Glib + Vitamin D: Glibenclamide (5 mg/Kg) + Vitamin D (500 UI/kg); Met : Metformin (10 mg/kg); Met + Vit D : Metformin (10 mg/kg) + Vitamin D (500UI/kg); Water: distilled water (10 mL/Kg).

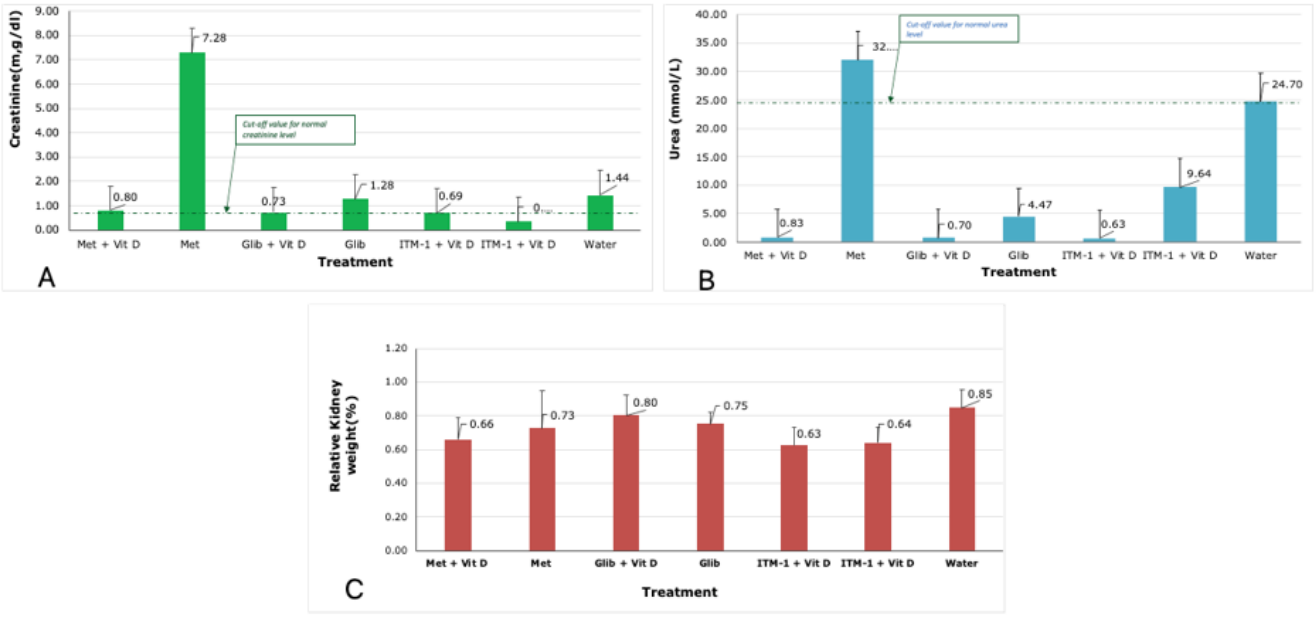

Figure 7. Effect of vitamin D supplementation on kidney function

A: Creatinine level; B: Urea level; C: relative kidney weight; ITM-1: Capsule Improved Traditional Medicine from lyophilized aqueous extracts of *Mangifera indica*, *Persea americana* and *Ageratum conyzoides*leaves (25 mg/Kg); ITM-1+Vit D: ITM-1 (25 mg/Kg) + Vitamin D (500 UI/kg); Glib: Glibenclamide (5 mg/Kg); Glib + Vitamin D: Glibenclamide (5 mg/Kg) + Vitamin D (500 UI/kg); Met : Metformin (10 mg/kg); Met + Vit D : Metformin (10 mg/kg) + Vitamin D (500 UI/kg); Water: distilled water (10 mL/Kg).

Table 1. Evolution of body weigh in the different experimental groups

Treatment	Day 0	Day 5	Day 10	Day 15	Day 21
Met + Vit D	153.6 ± 28.02	145 4 ± 18.8	140.2 ± 13.5	131.25 ± 7.2	140.3 ± 17.3
Met	195.3 ±60.5	195.1 ± 32.3	188.3 ± 53.3	173.5 ± 47.3	165.6 ± 41.7
ITM-1 +Vit D	173.5 ± 36.01	174.3 ± 19.5	173.6 ± 19.5	167.67 ± 26.6	182.2 ± 32.5
ITM-1	171.5 ± 12.3	172.5 ± 11.4	168.7 ± 23.03	168.5 ± 25.2	168.7 ± 34.6
Glib + Vit D	198.5 ± 36.01	185.8 ± 30.51	181.7 ± 27.2	181.3 ± 23.1	204.2 ± 22.1
Glib	163.5 ± 7.4	156.7 ± 12.8	162.7 ± 17.1	164.5 ± 21.4	166.2 ± 23.1
Water	140.2 ± 19.7	146.4 ± 19.06	145.1 ± 19.7	138.2 ±21.6	141.6 ± 23.5

Conclusion

Vitamin D supplementation enhances the efficacy of glibenclamide, metformin, and the ITM-1 potential drug, by preventing post-prandial glycemic peak, enhancing hypoglycemic effects, and improving clinical markers. Vitamin D supplementation consistently enhanced kidney function, suggesting its potential role as a treatment adjuvant for diabetes management. Further work including safety evaluation and exploration of the mechanisms through which vitamin D exerts its effect are envisaged for more insights.

Abbreviations

ITM-1: Capsule Improved Traditional Medicine from lyophilized aqueous extracts of *Mangifera indica*, *Persea americana* and *Ageratum conyzoides*leaves (25mg/Kg); T2DM: Type 2 Diabetes Mellitus; WHO: World Health Organization; IDF: International Diabetes Federation.

Authors' Contribution

DZ conceived the work, oversaw the laboratory work and drafted the manuscript; HNM and SKA carried out the bench work and took part in drafting the manuscript, NSA and CTM edited the manuscript. All the authors approved the last version of the work and its submission to the journal.

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Conflict of interest

The authors declare no conflict of interest.

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