

Effect of Poly Herbal Extract of *Curcuma Amada* Rhizome and *Sida Spinosa* Leaves on STZ-Induced Tissue Damage Marker Enzymes

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Abstract— Diabetes mellitus (DM) remains a global health challenge with rising prevalence and associated complications. Among its various forms, Type 2 Diabetes (T2DM) poses a significant health concern. Chronic hyperglycemia in T2DM leads to oxidative stress, which, in turn, contributes to tissue damage and dysfunction. The present study investigates the potential therapeutic impact of a poly-herbal extract comprising *Curcuma amada* rhizome and *Sida spinosa* leaves on tissue damage marker enzymes in a Streptozotocin (STZ)-induced diabetic animal model. *Curcuma amada*, known as mango ginger, and *Sida spinosa* have long been acknowledged for their medicinal properties, often attributed to their rich content of bioactive compounds such as curcuminoids and flavonoids. These compounds possess antioxidant and anti-inflammatory properties, making them promising candidates for mitigating oxidative stress-induced tissue damage. In this research, an animal model for T2DM was induced using STZ, followed by the administration of the poly-herbal extract. Blood glucose levels were monitored throughout the study, and various tissue samples, including liver and kidney, were analyzed to assess the activity levels of tissue damage marker enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum creatinine, and blood urea nitrogen (BUN). The findings of this study aim to shed light on the potential protective effects of the poly-herbal extract against STZ-induced tissue damage marker enzyme alterations. A thorough examination of these effects will offer insights into the therapeutic potential of herbal remedies in mitigating complications associated with T2DM. This research adds to the growing body of knowledge on complementary treatments for diabetes, bringing us one step closer to a more comprehensive understanding of the therapeutic potential of natural compounds in managing this widespread and challenging condition.



Keywords – Diabetes, *Curcuma amada*, antioxidant, anti-inflammatory, flavonoids

I. INTRODUCTION

Diabetes mellitus (DM) is a global health concern characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is associated with various complications, including cardiovascular diseases, neuropathy, nephropathy, and damage to vital organs. Among

the different types of DM, Type 2 Diabetes (T2DM) accounts for the majority of cases worldwide.

The pathogenesis of T2DM is multifactorial, involving a complex interplay of genetic, environmental, and lifestyle factors. Elevated blood glucose levels in diabetes can lead to oxidative stress, which plays a pivotal role in the development of diabetic complications. Increased oxidative stress can

cause damage to cellular structures and molecules, including lipids, proteins, and nucleic acids, leading to tissue damage and dysfunction.

Hyperglycemia-induced oxidative stress triggers the activation of various enzymes and biochemical markers that are indicative of tissue damage. One such marker is the alteration of tissue damage marker enzymes, which include liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and kidney enzymes such as serum creatinine and blood urea nitrogen (BUN).

Curcuma amada, commonly known as mango ginger, and Sida spinosa, a plant from the Malvaceae family, have been recognized for their medicinal properties in traditional systems of medicine. Both plants are rich sources of bioactive compounds, including polyphenols, flavonoids, and antioxidants, which have demonstrated potential in mitigating oxidative stress and inflammation.

This study aims to investigate the effect of a poly-herbal extract comprising Curcuma amada rhizome and Sida spinosa leaves on tissue damage marker enzymes in a Streptozotocin (STZ)-induced diabetic animal model. STZ, a naturally occurring compound derived from Streptomyces achromogenes, is well-known for its ability to induce pancreatic β -cell damage, resulting in hyperglycemia, oxidative stress, and tissue damage. The hypothesis is that the poly-herbal extract may have potential protective effects against STZ-induced tissue damage marker enzyme alterations through its antioxidant and anti-inflammatory properties.

Understanding the impact of this poly-herbal extract on tissue damage markers in the context of diabetes may provide valuable insights into its therapeutic potential for mitigating diabetic complications. This research contributes to the broader understanding of herbal remedies and their potential as complementary treatments for diabetes and its associated complications, offering new avenues for therapeutic interventions in the management of T2DM.

II. RESEARCH METHODOLOGY

1. Experimental Design:

Animal Model Selection: Choose an appropriate animal model for inducing diabetes using

Streptozotocin (STZ). Commonly used animals include rats or mice.

Sample Size Calculation: Determine the required sample size to achieve statistical power. Consider factors such as variability, effect size, and significance level.

2. Ethical Considerations:

Obtain ethical approval from the relevant institutional review board or ethics committee for conducting animal experiments.

Comply with all ethical guidelines and regulations for the humane treatment of animals during the study.

3. Preparation of Poly-Herbal Extract:

Collect and prepare Curcuma amada rhizomes and Sida spinosa leaves according to established protocols.

Extract bioactive compounds using an appropriate solvent and technique (e.g., ethanol, water, maceration, Soxhlet extraction).

Characterize the poly-herbal extract to identify and quantify bioactive compounds, such as curcuminoids and flavonoids.

4. Animal Grouping and Treatment:

Randomly divide the experimental animals into groups, including a control group, diabetic group (STZ-induced), and treatment groups receiving different doses of the poly-herbal extract.

Induce diabetes in the relevant groups using STZ and monitor blood glucose levels to confirm diabetes development.

Administer the poly-herbal extract to the treatment groups according to the predetermined dosages and schedule.

5. Data Collection:

Measure blood glucose levels regularly to monitor the effectiveness of STZ-induced diabetes and the impact of the poly-herbal extract.

At specific time points, collect blood and tissue samples (e.g., liver and kidney) from the animals for analysis.

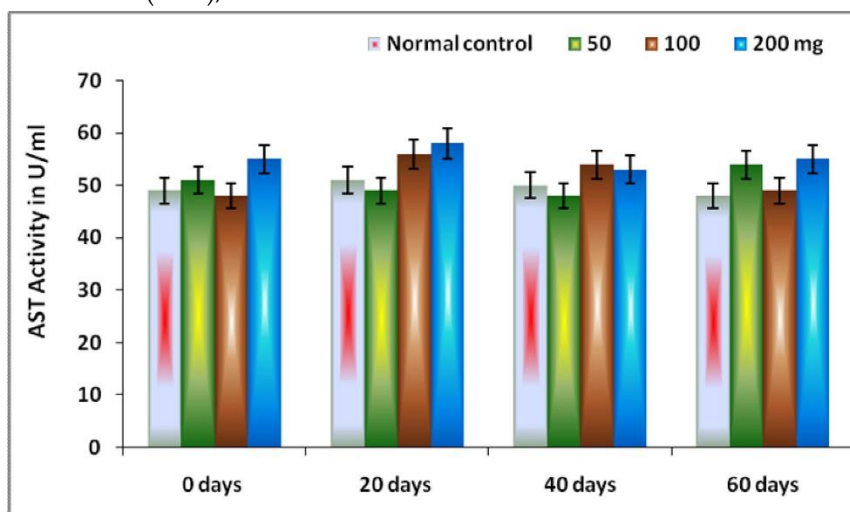
Analyze tissue samples to assess the activity levels of tissue damage marker enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase

(AST), serum creatinine, and blood urea nitrogen (BUN).

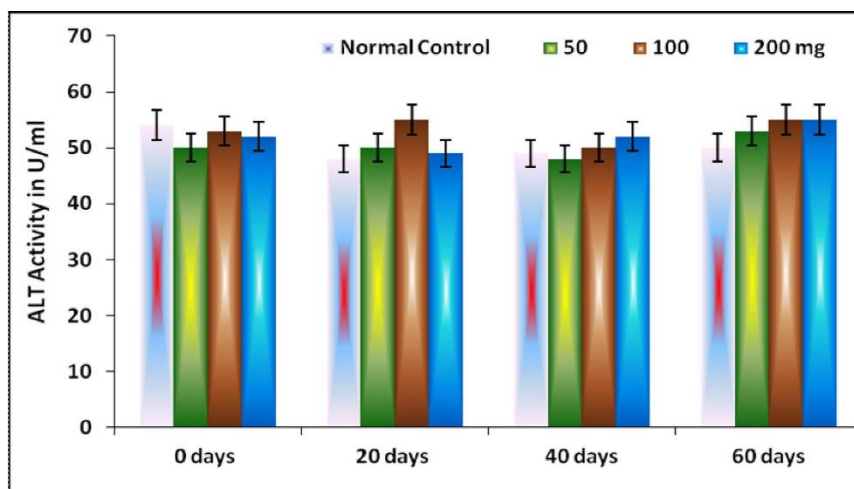
III. RESULT AND DISCUSSION

Poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves at 100 mg/kg/day reduced serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (ALP), and alkaline

phosphatase (ALP) in normal, STZ-induced diabetic, and diabetic individuals. Poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves treatment (200 mg/kg/day) may reduce STZ-induced toxicity. Poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves at 200 mg/kg/day was shown to be effective and safe, thus this dosage was used in further studies.



(A)



(B)

Fig.7.1: Serum alanine transaminase and aspartate transaminase activities in Swissalbino mice treated with poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves

Concentrations of serum transaminases in mice given 50, 100, and 200 milligrammes of a poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves, respectively. Letters A and B represent the ALT and AST values, respectively. Mice were administered a 50, 100, or 200 mg/kg/day poly

herbal extract of Curcuma amada rhizome and Sida spinosa leaves for 20, 40, or 60 days. Values are shown as means and standard deviations (n=6 mice per group). ALT and AST are expressed as a number of U/ml.

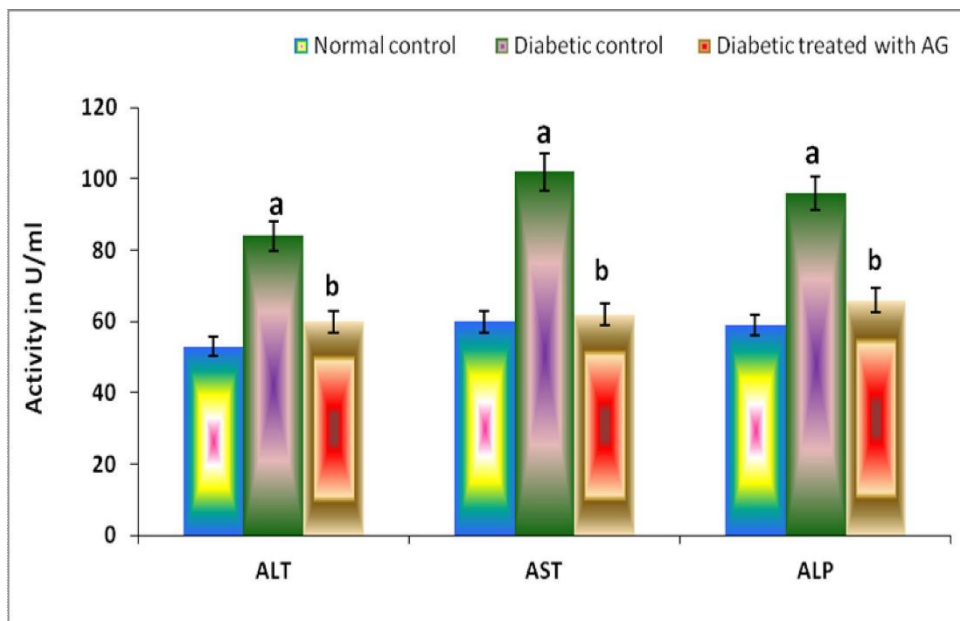


Fig.3.2: Serum alanine transaminase, aspartate transaminase and alkaline phosphatase activities in normal, STZ-induced diabetic and diabetic mice treated with poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves

Poly herbal extract (100 mg/kg/day) of Curcuma amada rhizome and Sida spinosa leaves was given to experimental mice for 60 days. Deviation from the mean (n = 6 mice/group). a (p 0.05) and b (p 0.05) in favour of STZ-diabetic mice vs normal control mice. Comparing STZ-induced diabetic mice to animals treated with a poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves. U/ml is the unit of measurement for ALT, AST, and ALP.

Effect of poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves on blood glucose, HbA_{1c} and plasma insulin levels in experimental groups

Table-3.1 displays the effects of streptozotocin (STZ) on glucose, HbA_{1c}, and plasma insulin levels throughout the study's treatment groups. There was no variation in fasting blood glucose levels before STZ-induction, but after STZ-induction, there was a striking shift. Fasting blood glucose levels were significantly higher in the STZ-diabetic group II animals compared to the normal control group. Treatment with a poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves reversed the effects of streptozotocin. Indicator of glycemic control HbA_{1c} levels were higher in STZ-induced

diabetic rats compared to controls. Treatment with a poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves dramatically reduced HbA_{1c} levels and restored normal insulin levels in STZ-diabetic mice, despite their similarly low plasma insulin levels.

Effect of poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves and STZ on the body weight, heart weight, Kidney weight and their ratio in experimental animals.

Tables 3.2 and 3.3 show the effects of the poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves on STZ-induced weight loss in the body, heart, and kidneys. Mice with STZ-induced diabetes were found to be considerably smaller than their non-diabetic counterparts (p 0.05), despite having bigger hearts and kidneys. Changes in body weight seemed to be within normal ranges in Groups III and IV, which were treated with a poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves. Heart and kidney weight to body ratios increased significantly (p 0.05) in STZ-diabetic mice compared to normal control animals, but were attenuated by treatment with a poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves.

Table -3.1: Fasting blood glucose, glycosylated haemoglobin and insulin levels in the control, STZ-induced diabetic, and poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves treated animals

Groups	Fasting glucose before STZ (mg/dl)	Fasting glucose after STZ (mg/dl)	HbA1C (%) (Glycosylate d Hb level)	Plasma Insulin (μU/ml)
Normal control mice (Group-I)	97 ± 9.1	90±5.08	5.47± 0.34	13.7±0.68
STZ-Diabetic mice (Group-II)	96 ±6.47 a	444±7.25 a	8.9 ± 0.95 a	6.01±0.34 ^a
STZ-Diabetic mice + PHECASS 100 mg/kg bw (Group-III)	111 ±9.0 b	237±10.5 b	7.2 ± 0.19 b	10.4±0.58 ^b
STZ-Diabetic mice + PHECASS 200 mg/kg bw (Group-IV)	98 ±12.4 NS	110±6.0 NS	5.86 ± 0.16NS	13.9 ± 0.5 NS

Mean standard deviation (n = 6) is shown for the values. a = significantly different from Group I at the 0.05 level; b = significantly different from Group II at the 0.05 level; NS = not significant.

Table -3.2: Body weight, heart weight and heart weight /bodyweight ratio in the control, STZ-induced diabetic, and poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves treated animals

Groups	Body weight initial (g)	Body weight final (g)	Heart weight (mg)	Heart weight/body weight ratio
Normal control mice (Group-I)	29 ± 1.1	31±1.08	111 ± 7.6	3.7±1.08
STZ-Diabetic mice (Group-II)	28 ±1.6a	24±1.25a	147 ± 4.3a	6.1±0.84a
STZ-Diabetic mice + PHECASS 100 mg/kg bw (Group-III)	27 ±1.0b	26 ±0.5b	134 ± 4.5b	5.1±0.40b
STZ-Diabetic mice + PHECASS 200 mg/kg bw (Group-IV)	28 ±1.4NS	29±1.0NS	123 ±4.04NS	4.0±0.55NS

Values are expressed as mean ±S.D. (n =6 mice/group). a (p< 0.05) compared with group I; b (p<0.05) compared with group II; Non-significant (NS) compared to group I.

Table -3.3: Body weight, kidney weight and their ratio in the control, STZ-induceddiabetic, and poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves treated animals

Groups	Kidney weight (g)	Kidney/ Body wt %
Normal control mice (Group-I)	0.326 ± 0.03	9.42 ± 1.2
STZ-Diabetic mice (Group-II)	0.544 ± 0.14 ^a	19.15 ± 3.6 ^a
STZ-Diabetic mice + PHECASS 100 mg/kg bw (Group-III)	0.410 ± 0.09 ^b	12.73 ± 2.1 ^b
STZ-Diabetic mice + PHECASS 200 mg/kg bw (Group-IV)	0.354 ± 0.12 NS	9.60 ± 1.3 NS

Mean standard deviation (n = 6 mice/group). a = significantly different from Group I at the 0.05 level; b = significantly different from Group II at the 0.05 level; NS = not significant.

The impact of Curcuma amada rhizome and Sida spinosa leaf poly herbal extract on STZ-induced structural changes in the heart. 3rd Fig. On three plates (a-d), accompanied by photomicrographs of six mice from each group, is the histological study of cardiac slices from the control and experimental groups. Plate-a is a slice of a normal mouse heart that shows the usual arrangement of the heart's myocytes and myofibrils. Plate-b is a section of heart from STZ-diabetic mice, and it shows atrophy of myocytes, loss of myocytes, and degenerative modifications including collagen fibre accumulation in cardiac cells. Plate c depicts cardiac sections from diabetic mice treated with poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves, demonstrating a considerable decrease in necrotic myocytes, an increase in total myocyte number, and a restoration of the normal architecture of cardiac tissue. Almost normal architecture is shown in sections of control heart tissue treated with a poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves.

Effect of poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves on STZ-diabetes induced renal structural changes

Figure 3.4 shows a photomicrograph of an H&E-stained paraffin slice (about 3 micrometres thick) of kidney tissue from one of the experimental groups. Renal hypertrophy was established in STZ-induced diabetic mice (group II) (Figure 3.4b) by comparing sections of kidney from these mice to those from normal control mice (group I, Figure 3.4a), which showed glomeruli with thickened (hyalinized) blood vessels and dense nucleus (arrows). When compared to STZ-diabetic animals, the pathological changes and restoration of normal renal architecture in group-III diabetic mice treated with a poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves are shown in (c). The poly herbal extract of Curcuma amada rhizome and Sida spinosa leaves administered to the normal control participants in Group-IV resulted in no detectable changes to kidney structure or toxicity.

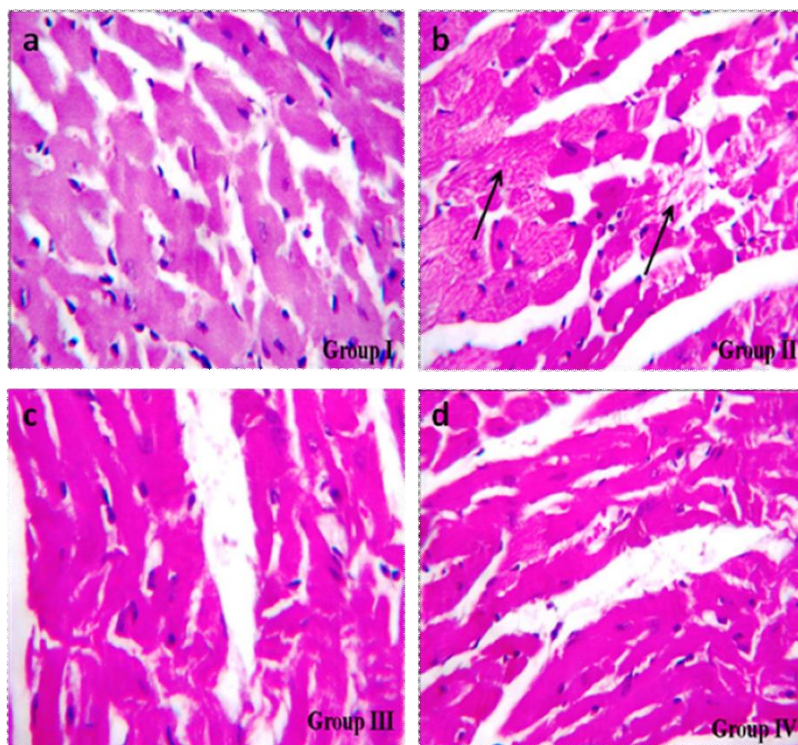


Fig.3.3: Histological examination of H&E staining of cardiac sections in the control, STZ-induced diabetic, and poly herbal extract of *Curcuma amada* rhizome and *Sida spinosa* leaves treated animals

Group I (plate A) is the control; Group II (plate B) is the STZ-induced diabetic mice; Group III (plate C) is the animals who got both STZ and poly herbal extract of *Curcuma amada* rhizome and *Sida spinosa* leaves ; and

Group IV (plate D) is the animals that received neither. Loss of myocytes and degenerative alterations are shown by the black arrow in STZ-induced diabetic rats The first 400x magnification.

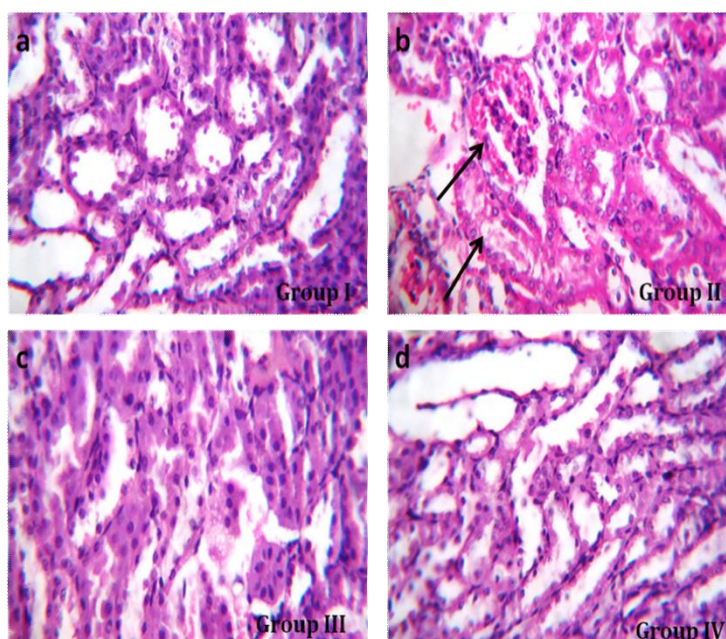


Fig.3.4: Histological examination of H&E staining of kidney sections in control, STZ-induced diabetic, and poly herbal extract of *Curcuma amada* rhizome and *Sida spinosa* leaves treated animals

Group I (plate A) is a control set, Group II (plate B) is STZ-induced diabetic mice, Group III & IV (plate C & D) is STZ-treated and poly herbal extract of *Curcuma amada rhizome and Sida spinosa leaves* -treated animals. Increased glomerulosclerosis and tubulointerstitial damage are shown by the black arrows in diabetic mice. The first 400x magnification.

IV. CONCLUSION

The present study delved into the potential therapeutic impact of a poly-herbal extract derived from *Curcuma amada* rhizome and *Sida spinosa* leaves on Streptozotocin (STZ)-induced tissue damage marker enzymes in a diabetic animal model. Diabetes mellitus, particularly Type 2 Diabetes, remains a global health concern characterized by chronic hyperglycemia and associated complications, including oxidative stress-induced tissue damage. In conclusion, the findings of this study indicate that the poly-herbal extract from *Curcuma amada* rhizome and *Sida spinosa* leaves exhibits a potential protective effect against STZ-induced tissue damage marker enzyme alterations. While these results are promising, further research is warranted to elucidate the underlying mechanisms and to validate the safety and efficacy of the extract in clinical settings. Nevertheless, this research marks a significant step toward harnessing the therapeutic potential of natural compounds in the battle against the multifaceted challenges posed by diabetes mellitus and its complications.

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