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The protective effect of *Moringa olifera* against complications of type2 diabetes mellitus in male albino rats.

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Background: Diabetes mellitus, particularly type 2 (T2DM), is one of the most common diseases. T2DM has been associated with stress and a change in lifestyle. Regulation of postprandial blood glucose levels and the use of some natural plant extracts with inhibitory actions against carbohydrate digestion enzymes like alpha-amylase and fewer adverse effects than synthetic medicines are potential methods for preventing dietary carbohydrate absorption. This study shows the anti-diabetic effects of Moringa olifera extract on insulin and blood glucose in diabetic rats given streptozotocin (STZ) treatment.

Methods: The experiment was performed on 40 Wistar male rats; the experimental study included 4 groups: (I) normal rats and (II)normal rats orally received an aqueous extract of Moringa (500 mg/kg/day) for consecutive 4 weeks (III)diabetic group; male albino rats injected with a single intraperitoneal dose of Streptozotocin 55 mg/kg b.w.t. (IV) Streptozotocin-treated rats received an aqueous extract of Moringa 500 mg/kg/day orally for consecutive 4weeks.

Results: The results of the following study showed that the injection of STZ resulted in a decline in insulin and an increase in glucose.

It was also revealed that ***Moringa olifera*** extract has more anti-diabetic effect against streptozotocin treatment due to its higher phenolic content and its effect on decreasing the activity of α-amylase. This

suggests that MAE leaf decreased postprandial glucose levels by slowing amylase-mediated glucose uptake.

Keywords: Diabetes mellitus, *Moringa olifera*, Streptozotocin, insulin, glucose, pancreas.

Introduction

Diabetes mellitus (DM) is a chronic metabolic and noncommunicable disease that can affect people all over the world. It is caused by an unhealthy lifestyle, a lack of exercise, being overweight, eating excessive amounts of sugar, genetic factors, and a variety of other variables. [1]

Type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), and gestational diabetes (GDM) are the three most prevalent types of diabetes. T2DM accounts for over 90% of all diabetes cases.

According to the International Diabetes Federation, 387 million individuals worldwide were diagnosed with diabetes in 2014, with that figure anticipated to climb to 592 million by 2035. The primary treatment for diabetes is to minimize hyperglycemia and intestinal glucose absorption by inhibiting carbohydrate metabolizing enzymes (e.g., α-amylase) [2].

Since its complications continue to plague the human race, several animal models are constantly being developed to study the pathogenic mechanism and therapeutic interventions. Streptozotocin (STZ) is a chemical that has been widely used to induce diabetes in mouse and rodent experimental models. It was first reported to have a diabetogenic effect in 1963, and since then, other researchers have utilized it in various combinations to induce diabetes [3]. Because of STZ's structural resemblance to glucose (a glucose analogue), the GLUT 2 transporter in the plasma membrane makes it easier for STZ to enter beta cells.

Diabetes management with minimal side effects is a big issue for specialists. Many attempts have been made to manage hyperglycemia in diabetes with synthetic drugs, but the drugs used in clinical practice are either prohibitively expensive or have undesirable side effects or contraindications. Plant-derived medicines have garnered a lot of interest since they have fewer adverse effects and are less expensive than manufactured drugs. As a result, there is

a need to develop natural therapies that can be utilized as alternatives to pharmaceutical drugs for type 2 diabetes [4]. Many herbal treatments are used to treat diabetes, and the most extensively used modern anti-diabetic drug (metformin) is derived from the active ingredients of the Western herbal cure's guanidine and galegine [5].

Moringa oleifera (MO), also referred to as the "tree of life" or "miracle tree," is a tree in the Moringaceae family, genus Moringa, that was originally native to the Himalayas but is now cultivated in many tropical and subtropical climates worldwide. MO's leaves, fruits, flowers, and roots have all been utilized as food and in traditional medicine to treat inflammation, liver illness, heart disease, cancer, ulcers, and diabetes. For example, the most widely utilized portion, the leaves, contains a variety of nutrients like beta-carotene, vitamins B, C, and E, minerals (calcium, iron, potassium, magnesium, and so on), necessary and non-essential amino acids, and carbohydrates [6].

The present study aimed to investigate the effects of *Moringa olifera* leaf aqueous extract on complication of diabetes mellitus.

MATERIALS AND METHODS

A-Materials

I-The experimental animals

Forty adults male Wistar rats weighing 120-150 g were obtained from King Saud University's College of Pharmacy. All rats were housed in cages of 10 rats per cage under standard laboratory conditions (19-25°C, 12 hours); light/dark cycles and a relative humidity of 50–60%. respectively for one week for adaptation to laboratory conditions. Fresh tap water and standard rodent pellets were always available. The pellets were bought from the General Organization for Grains (SAGO). Fodder No. 1005 is special for experimental animals.

II. Chemicals:

Streptozotocin

streptozotocin (purchased from Sigma Chemical Co., St. Louis, USA) in powder form and was dissolved in citrate buffer immediately before use.) is a small molecule antibiotic that contains N-nitroso groups. Nitric oxide (NO) donors and vasorelaxants are both functions of streptozotocin.

Induction of diabetes mellitus:

Rats were given a single dosage of Streptozotocin (55mg/kg bw) intraperitoneally after fasting for 16 hours. dissolved in ice cold 0.1 M sodium citrate buffer (20ml sodium citrate with 30ml citric acid, pH=4.0), followed by oral administration of 2-3 ml sucrose solution 10% (w/v) for one day. The blood glucose level was tested in a drop of blood from the lateral tail vein using sterile surgical scissors and the Gluco test strip Precichek AC-302, AutoCode glucometer, and its test strips after an overnight fast. Diabetic animals have blood glucose levels more than 240 mg/dl [7].

III.Preparation of *Moringa olifera* extract:

Moringa oleifera was bought from a local market, Al-Qassim region, Saudi Arabia. A skilled botanist from King Abdulaziz City for Science and Technology ridentified the plant scientifically. The aqueous extract was prepared according to [7]'s procedure. Ninety grams of powdered dry herb leaves were soaked for three hours in 900 ml of boiling distilled water, then filtered through sterile Whatman filter paper number 42 (Whatman International Ltd, Maidstone, England) and lyophilized using a freeze drier (Christ alpha1-4 Id plus, Germany) under a pressure of 0.1-0.5 mBar and a temperature of -35 °C to 41 °C; the dry extract was stored in a dark bottle at-80°C until usage.

Determination of total extract yield:

The filtrate was transferred to a quick fit round bottom flask with a known weight (W1), then freeze-dried and weighed again (W2). Finally, the yield was calculated from the following formula:

$$\text{Yield (g/ g crude herb)} = \frac{W_2 - W_1}{W_3}$$

Where, W1 is the weight of clear and dry quick fit flask in grams, W2 is the weight of the flask with the extract after lyophilization in grams, and W3 is the weight in grams of the dried, unprocessed plant [7].

IV.Experimental Design:

Forty adult male albino rats were arranged into four groups:

Group I: control group feed normal diet.

Group III: Male albino rats will be orally received aqueous extract of ***Moringa*** (500 mg/kg/day) for 4 consecutive weeks [8].

Group III: the diabetic group; male albino rats injected with a single intraperitoneal dose of Streptozotocin 55mg/kg b.wt. [7].

Group IV: Male albino rats treated with a single intraperitoneal dose of Streptozotocin 55mg/kg b.wt. [7]and after estimation of diabetic state; rats received an oral dose of aqueous extract of ***Moringa*** 500 mg/kg/day for 4 successive weeks.

V. Sampling:

At the end of the experiment, overnight fasting rats were sedated with diethyl ether before being dissected, and blood samples were taken into clean non-heparinized centrifuge tubes. Blood sera were separated, and stored at -20 °C for biochemical investigation. Pancreas specimens from all groups were kept in 10% formaline for subsequent histopathological analysis.

B: Methods

Analytical procedure:

Concentration Glucose level

The concentration of blood glucose was estimated by collecting a blood drop from the tail`s vein by using AutoCode-Pricicheck glucometer.

Estimation of biochemical parameters:

Serum values of Insulin were estimated by using "ELISA kits" that were purchased from Ejadah Medical Company, for scientific medical material chemical and laboratory and hospital equipment, Riyadh according to [9]. By using an ELISA device, DYNEX DSX® (Microplate Reader, USA).

Statistical analysis

All data were statistically analyzed using analysis of variance (ANOVA) and the Statistical Analysis System's General Linear Model Procedure (SAS 1982). By using the Waller-Duncan k-ratio, the significance of the differences between the various treatment groups was assessed (Waller and Duncan, 1969). A probability of $p \leq 0.01$ served as the foundation for all declarations of significance.

RESULTS AND DISCUSSION

Biochemical results:

Table (1): Mean values of blood glucose and Insulin levels of control, diabetic, and diabetic-treated animals' groups as compared to normal control animals' group.

Groups \ Parameters		Glucose (mg/dl)	Insulin (pg/ml)
Control	M ±SE	106 ±2.3^{C}	6.3 ±0.08^{A}
MAE	M ±SE	105.8 ±2.3^{C}	6.2 ±0.06^{A}
	% Change A	**-0.2%**	**-1.6%**
STZ	M ±SE	542 ±16.3^{A}	3.06±0.08^{C}
	% Change A	**411%**	**-51.4%**
STZ, then MAE	M ±SE	438 ±17.6^{B}	4.98 ±0.04^{B}
	% Change B	**-19%**	**62.7%**

Data are presented as mean ± standard error of mean. Data were subjected to one-way ANOVA followed by Duncan post hoc test at p≤0.01. Within the same column, means with different superscript letters are significantly different. MAE (Moringa aqueous extract).

Blood glucose level (mg/dl) of adult male Wistar albino rats (*Rattus norvegicus):*

The obtained data in table 1 & figure 1 showed that oral administration of healthy adult male rats or Moringa aqueous extract (500 mg/kg/day) separately for 30 consecutive days did not deteriorate ($p< 0.01$) the blood glucose level when these groups were compared to the corresponding values of the control animals' group (107 vs 106,104.8 vs 106 respectively). In contrast, a single i.p. injection of rats with STZ (55mg/Kg bwt) resulted in a more significant ($p\leq0.01$) increase (411 %) in the blood glucose level (542 Vs 106) when was compared to the control animals' group.
Interestingly, oral treatment with MAE resulted in a less decrease (-19%) in blood glucose level (438 vs 542) when was compared to the STZ-treated animals' group.

It may be interpreted because STZ resulted in a more significant increase in the blood glucose level these results are matched with those of [10,11, 12,13,14,7]. They attributed this elevation in blood glucose level to pancreatic β-cell malfunction, decreased β-cell bulk, and insulin shortage due to that STZ had at least partially damaged the pancreas, so it was expected that it would be unable to produce enough endogenous insulin to deal with the high levels of glucose [13].

While the oral gavage with Moringa olifera into male rats resulted in a significant decrease in glucose level compared to the STZ-treated animals' group, and these results are in line with those results [17-19]. They suggested that the decreases in blood glucose level due to high phenolic & flavonoids contents in MAE [10,22,24-27].

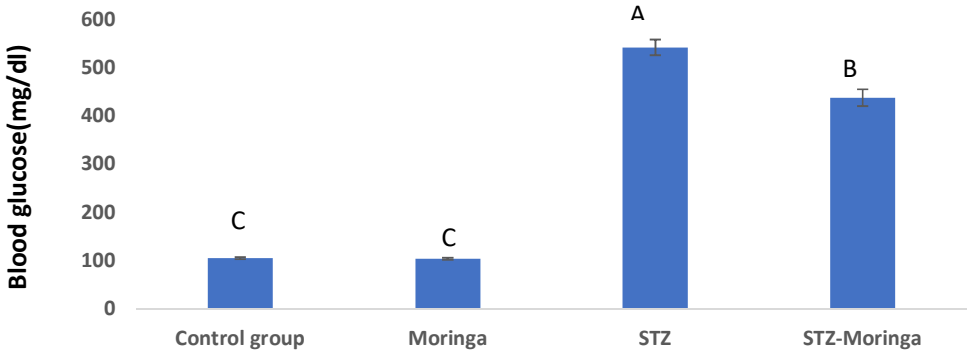

Fig.1:Means of Blood glucose level (mg/dl) of adult male albino rat *Rattus norvegicus.*

Serum Insulin level (pg/ml) of adult male Wistar albino rats (*Rattus norvegicus*):

The obtained data in table 1 & figure 2 showed that oral administration of untreated adult male rats with either Moringa aqueous extract (500 mg/kg/day) separately for 30 consecutive days did not deteriorate the serum insulin level when these groups were compared to the corresponding values of the control animals' group (6.2 vs 6.3 ,6.2 vs 6.3 respectively).

STZ-treatment in a single i.p. injection (55mg/Kg bwt) resulted in a highly-significant decline (-51.4%) in the levels of serum insulin (3.06 vs 6.3) when was compared to the corresponding values of the control animals' group. While the oral administration of diabetic animals with MAE induced a more significant increase (62.7%) in the levels of serum insulin when was compared (4.98 vs 3.06) with STZ-treated animals.

It may be interpreted because STZ resulted in a more significant increase in the blood glucose level These results are in line with [18,19,20,21]. This decrease may be due to that STZ causes pancreatic beta cell necrosis that inhibits insulin secretion and STZ inhibits insulin production and release by interfering with glucose metabolism and oxygen consumption [7,14]. STZ can also affect insulin receptor substrate 1 (IRS-1), protein kinase B (AKT), and glucose transporter isoform 4 (GLUT-4) mRNA expression [20].

While the oral administration of MAE to the diabetic animals induced a more significant increase in serum insulin levels when compared to STZ-treated animals, our findings are consistent with those of other studies [17,21]. They attributed these results due to the presence of potential Phenols, flavonoids such as quercetin, chlorogenic acid, coumaric acid, thamnolic acid and others found in MAE that protect and regenerate pancreatic beta-cells and increase insulin release (anti-diabetic effect) via the activation of the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway and the defense of pancreatic beta-cells against oxidative damage and stimulating the production of insulin. In contrary, the result of research [23] showed no changes to the levels of insulin.

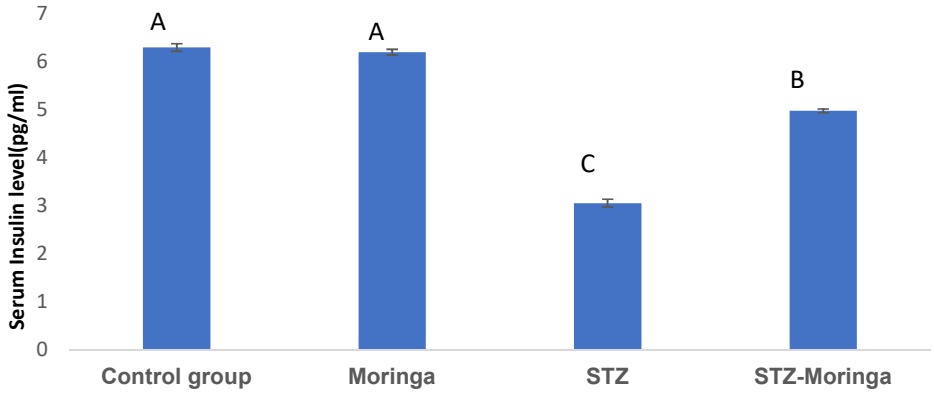

Fig.2:Means of serum Insulin level(pg/ml) of adult male albino rats *Rattus norvegicus*.

Histopathological results:

Showing normal structure & architecture of islets of Langerhans(arrow) which shows the endocrine portion of the pancreas formed of many small clusters of cells called islet of Langerhans, the islet is seen as pale staining groups of cells embedded in a darker staining exocrine tissue consisted of the acini, the acinus cell has a big basal spherical nucleus, the islet cells are well differentiated into edge small dark nuclei and mostly pale nuclei with large cytoplasm (Fig. 3).

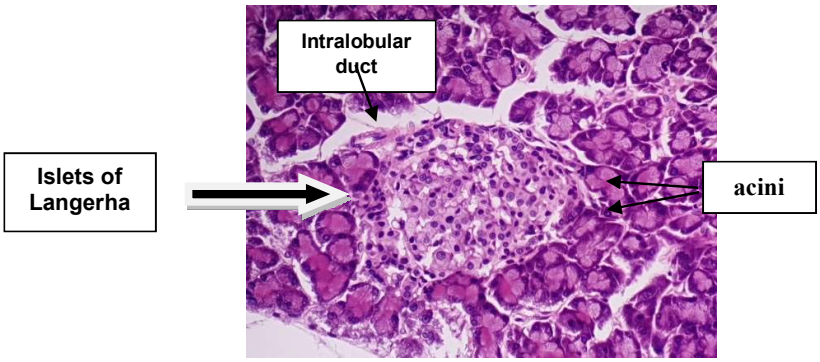

Fig.3: Section of pancreas of control group (H&E.)

The oral injection of healthy male rats with MAE 500 mg/kg/day orally in fig.4.for 30 days did not reveal any significant change in the normal pancreatic architecture or exocrine & endocrine functions as was declared in fig.5. STZ treatment in a single i.p. injection (55mg/Kg bwt) revealed mild focal degenerative and necrotic changes, such as shrunken or reduced islets of Langerhans surrounded by hyprechromatic epithelial cells of acini. The reduction of islet of Langerhans size (double arrows), surrounded by hyperchromatic epithelial cells of acini (arrow)

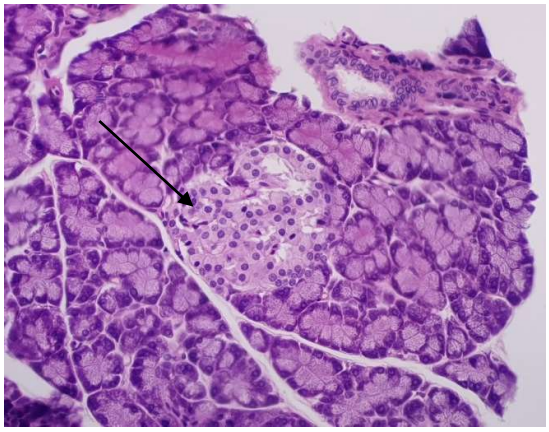

Fig.4: Section of pancreas of MAE-treated group 100x(H&E.).

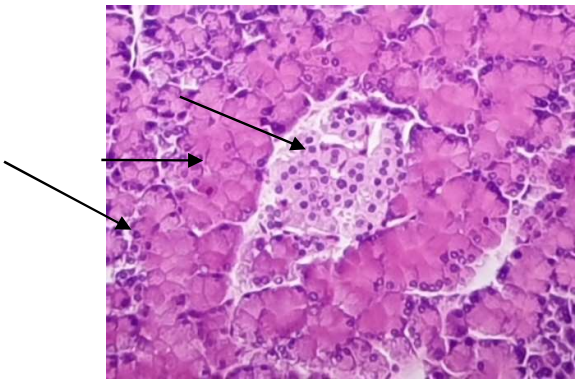

Fig.5a: Section of pancreas of diabetic group 100x(H&E.)

Fig.5b: Section of pancreas of diabetic group 400x(H&E.)

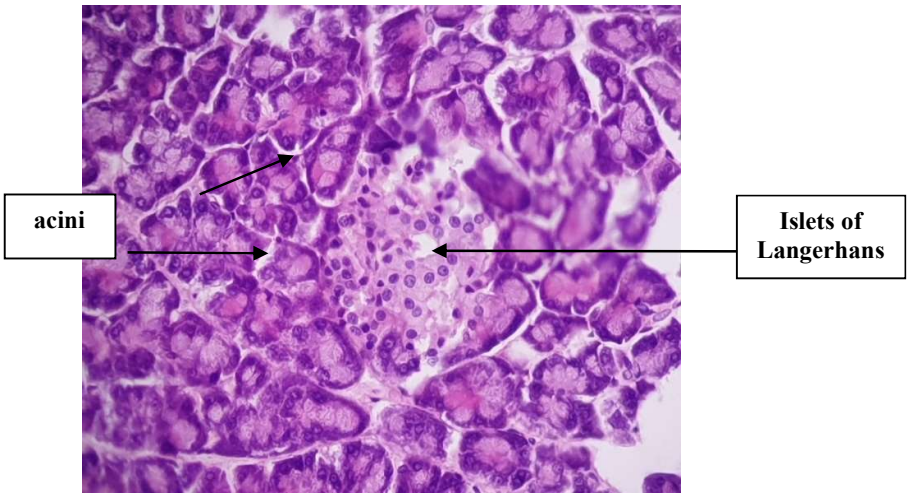

Fig.6: Section of pancreas of diabetic group treated with MAE 100x(H&E.)

Moringa aqueous extract treatment (500 mg/kg/day) orally for 30 consecutive days into diabetic rats showed mild regeneration of normal architecture of islets of Langerhans that was clear by the increase in number & size of islets of Langerhans, dilated interlobular duct and well differentiates and recovering of exocrine pancreas. The acini cells appeared with rounded basal nuclei and mild defatted intercalated duct fig.6.

CONCLUSION

Moringa olifera has ability to ameliorate the signs of degeneration the islets of Langerhans' and cellular damage caused by STZ-induced diabetes, as evidenced by decreasing blood glucose level, elevating insulin levels and restoring the normal pancreatic architecture.

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