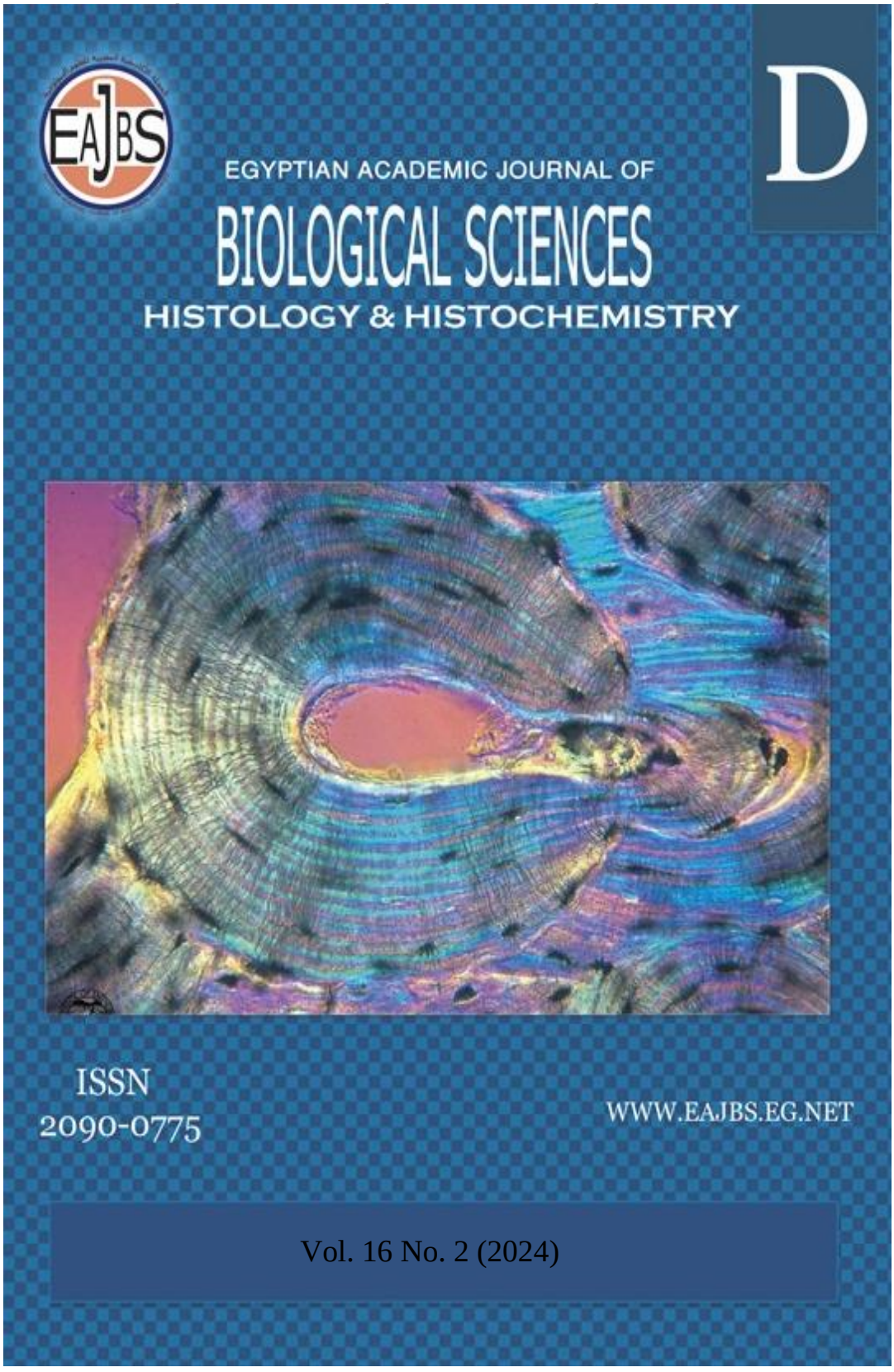

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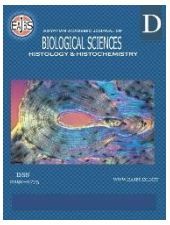

Effect of Green Zinc Oxide Nanocomposite with Fenugreek Seeds Extract on Streptozotocin-Induced Diabetic Rat

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Zinc Oxide, Fenugreek, Diabetes, Streptozotocin, Nanotechnology, Oxidative stress.

ABSTRACT

Background: With the increasing prevalence of type 2 diabetes mellitus (T2DM), exploring alternative therapeutic agents with antioxidant and glucose-regulating properties is crucial. **Objective**: This study aimed to evaluate the impact of zinc oxide nanoparticles (ZnO-NPs) and *Trigonella foenum-graecum* (TFG) on glucose metabolism, liver function, and oxidative stress alongside with histopathological examination for hepatic tissue in a rat model of T2DM. **Methods:** Seventy-five adult Wister albino rats were randomly categorized into five groups: non-diabetic control, diabetic control, diabetic rats treated with ZnO-NPs, diabetic rats treated with TFG, and treated with a combination of ZnO-NPs and TFG. T2DM was induced using Streptozotocin (STZ), one single dose (50 mg/kg) by intraperitoneal injection (IP). Treatment for diabetic rats was conducted using ZnO-NPs (200 mg/kg), TFG (500 mg/kg), and a combination of ZnO-NPs (200 mg/kg) and TFG (500 mg/kg) together. **Results:** The results showed significantly reduced blood glucose and HbA1c levels and improved insulin levels in treated groups. Liver function markers (ALT, AST, total bilirubin) were elevated in the diabetic group but improved significantly with treatment. Antioxidant markers, including glutathione, superoxide dismutase, and catalase, were enhanced, while malondialdehyde levels decreased in treated rats. **Conclusions:** These findings suggest that ZnO-NPs and TFG seed extract may have therapeutic potential in improving glucose metabolism, liver function, and oxidative stress in T2DM. Further studies are warranted to explore clinical applicability.

INTRODUCTION

Diabetes mellitus (DM) is a debilitating and persistent illness that occurs when insulin production is inadequate or ineffective in the body, causing a prolonged disruption in metabolism. The prevalence of diabetes among people globally exceeded 10% in 2021. The number of persons between the ages of 20 and 79 diagnosed with diabetes has more than tripled. It has risen from 151 million individuals, which accounted for 4.6% of the worldwide population then, to 537.5 million individuals, representing 10.5% of the current world population (Kumar *et al*., 2024). Type 2 diabetes mellitus (T2DM) is the most common form of diabetes, affecting millions worldwide.

Therefore, it is advisable to provide risk-reducing statin medication to almost all patients aged 40 or above with T2DM, irrespective of their cholesterol levels (Habte *et al*., 2020). However, exploring alternative agents with antioxidant, hepatoprotective, and glucose-regulating properties remains critical for comprehensive disease management.

Fenugreek (*Trigonella foenum-graecum*), a member of the Leguminosae family, has long been a traditional therapeutic herb. Vast pharmacological and clinical evidence supports that fenugreek possesses therapeutic properties (Yao *et al*., 2020). The plant's seeds and leaves are frequently applied and have exhibited various pharmacological properties, such as anti-diabetic, hypocholesterolaemia, anti-nociceptive, anti-carcinogenic, antioxidant, hepatoprotective, cardioprotective, neuroprotective, immunomodulatory, nephroprotective, anti-cholesterolemic, analgesic, emollient, laxative, anti-spasmodic, anti-atherogenic, appetite suppression, pain relief, worm expulsion, obesity prevention, and anti-inflammatory effects (Almatroodi *et al*., 2021; Shahrajabian *et al*., 2021; Tewari *et al*., 2024).

Nanotechnology, a rapidly advancing research topic, draws from diverse disciplines such as materials science, biological science, and related fields. The term 'nano' denotes smallness, and nanoparticles, with their unique attributes, find applications in various domains such as agriculture, medicine, textiles, and the environment (Saqib *et al*., 2022). ZnO nanocomposites, non-hazardous and biodegradable biopolymers, are extensively used in the biomedical and medicinal domains, improved biosensors, and enhanced drug delivery vehicles (Falfushynska *et al*., 2019; Kamal *et al*., 2022). Shwetha *et al*. (2020) highlight the potential of biologically synthesized zinc oxide nanoparticles (ZnO NPs) in treating diabetes and cancer. Owing to their significant antioxidant activity, these nanoparticles show promise in treating cancer, diabetes, microbial infection, and inflammation. They can also serve as drug carriers, imaging agents, and biosensors (Deka *et al*., 2022). Oxidative stress (OS) plays a role in the initiation and progression of calcification in vascular tissue (Greenberg *et al*., 2022). It is crucial in progressing both macro and microvascular complications in diabetes mellitus (Burgos-Morón *et al*., 2019). Evidence suggests that OS plays a vital role in the pathophysiology of T2DM, and the development of complications associated with diabetes (Dworzański *et al*., 2020).

This study presents a novel approach for synthesizing green ZnO nanostructures with fenugreek extract as a reducing agent and stabilizer. By combining the antioxidant properties of ZnO-NPs and fenugreek, the study also aims to explore the potential anti-diabetic effects of the synthesized nanostructures in vivo, specifically in hyperglycemia induced by streptozotocin. This investigation addresses the gap in understanding the synergistic effects of these agents on T2DM and its complications.

MATERIALS AND METHODS

Fenugreek Extraction:

Fenugreek seeds were extracted using ascending-grade ethanol in a Soxhlet apparatus with a condenser to prevent solvent loss. The ethanol volume was 3L for Fenugreek. The assembly was heated on a temperature controller heater to maintain at a controlled temperature. After 8 hours, the apparatus was stopped. The solvents and extracts were collected and evaporated using a rotary evaporator under vacuum at a temperature of 45°C. The extract obtained after evaporation weighed 77.13 gm for (*Trigonella foenum-graecum*).

ZnO NPs Preparation with A Suspension of Fenugreek Seed Extract:

Fenugreek seed extract (150 mg/ml) and zinc oxide nanoparticles (10 mg/ml were combined and dissolved in distilled water. The suspension was then sonicated using an ultrasonic cleaner sonicator (Branson Ultrasonic Corporation, Danbury, Connecticut, USA) for 40 minutes at 230 V while at room temperature. A vortex agitator was used to stir the suspension before treatment administration.

Experimental Design:

There are seventy-five adult male Wister albino rats. This research used rats weighing between 180 and 200 grams. The animals are housed in temperature-regulated enclosures constructed from stainless steel, with a temperature of 25 ± 2 ◦C. Additionally, they are given unrestricted access to pelleted food and purified drinking water. After a one-week acclimatization period, the rats were randomly allocated into five groups, each consisting of 15 rats:

Group I: non-diabetic control group.

Group II: Diabetic group (received 50mg/kg STZ Intraperitoneal injection).

Group III: Diabetic and ZnO NPs group (received10 mg/kg ZnO NPs).

Group IV: Diabetic + TFG group (received 150 mg/kg TFG seed extract).

Group V: Diabetic, TFG, and ZnO NPs group (received 150 mg/kg TFG + 10 mg/kg ZnO NPs).

As part of the unique methodology, following a duration of seven weeks, the test animals had a fasting period overnight, after which they were administered diethyl ether to induce an anesthetic state. Orbital venous plexus was used for blood sample collection into non-heparinized tubes. The tubes were centrifuged at 2500 rpm for 15 minutes to separate the blood sera. The sera were collected, divided into smaller portions, and kept at -80 °C until required (Kumar *et al*., 2017).

Histopathological Examination:

After the blood collection, the liver and pancreas were promptly extracted and stored in a 10% buffered formaldehyde solution. Once fixation, the samples were dehydrated, encased in wax, and subsequently sectioned into 5-micron slices. To conduct histological analysis, the slices were stained using the haematoxylin and eosin methods (Feldman & Wolfe, 2014). finally, a light microscope (Olympus BX 51, Olympus America, Melville, NY) at magnifications of 10x and 40x was used for observation under various magnifications (Al Suleimani *et al*., 2024).

Blood Biochemical Parameters:

The sera collected were used to assess biomarkers related to glucose metabolism, including glucose, insulin, and glycated hemoglobin A1C (HbA1C). Additionally, liver function parameters, including aminotransferase, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Albumin (ALB), Total Protein (TP), and Total bilirubin (TB) were also evaluated. Furthermore, the levels of oxidative stress and antioxidant biomarkers, including malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH), were analyzed using commercially available assay kits obtained from MyBioSource Co., following the instructions provided by the manufacturer (Yan *et al*., 2022).

Statistical Analysis:

The data are expressed as mean ± standard deviation. The data obtained from the experimental groups were subjected to statistical analysis using one-way ANOVA with Tukey's post hoc multiple comparisons tests. The study used GraphPad Prism software version 5 (San Diego, CA, USA). A significance level of $p < 0.05$ was employed to establish statistically significant distinctions between the groups (Ali *et al*., 2021).

RESULTS

1. Nanoparticle Characterization:

- **Scanning Electron Microscopy (SEM):**

The SEM analysis of ZnO NPs revealed the presence of aggregated nanoparticles with an average size of 22 ± 4 nm (Fig. 1).

- **Energy-dispersive X-ray Spectroscopy (EDS):**

The sample's composition is analyzed using a field emission scanning electron microscope with an EDX detector. Measurements were performed at an acceleration voltage of 20 kV. Figure (1), displays the energy-dispersive X-ray spectra of ZnO NPs samples. Labels indicate the elements and their corresponding weight percentages in the ZnO NPs sample. The sample primarily consists of Zinc (62.26) and Oxygen (37.74), with no detectable contaminants within the detection range of EDX. Therefore, ZnO NPs are of high purity.

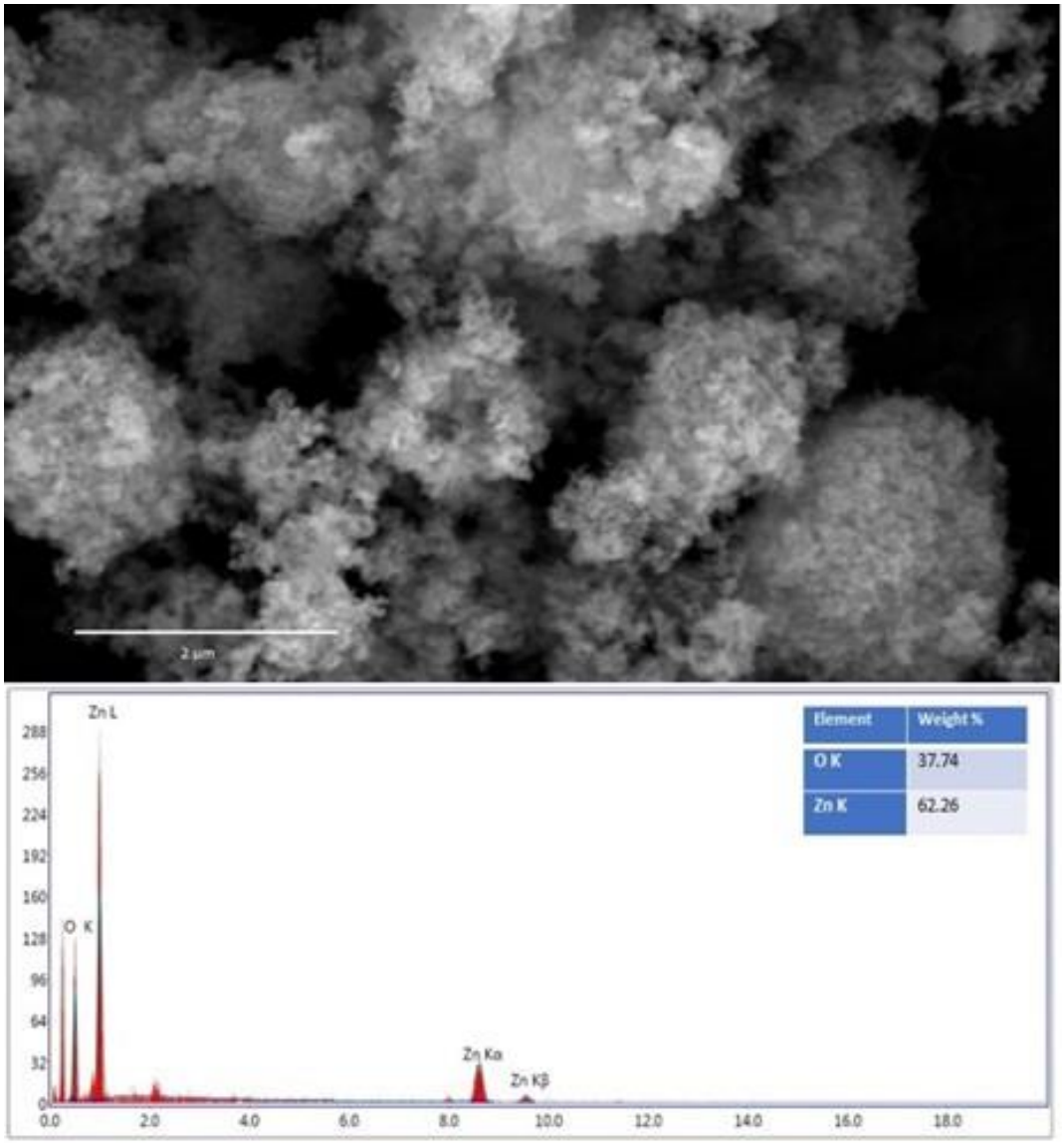

Fig. 1: Scanning electron microscope and Energy dispersive spectroscopy of ZnO NPs.

- **Transmission Electron Microscopy (TEM):**

High-resolution transmission electron microscopy (2021 FGG, JEOL, Japan) was used to determine the sizes and morphologies of ZnO NPs, revealing the presence of spherical ZnO NPs **(**Fig. 2**).** The TEM micrographs showed that the ZnO NPs consist primarily of spherical particles, with a typical crystallite size between approximately 14 to 26 nm.

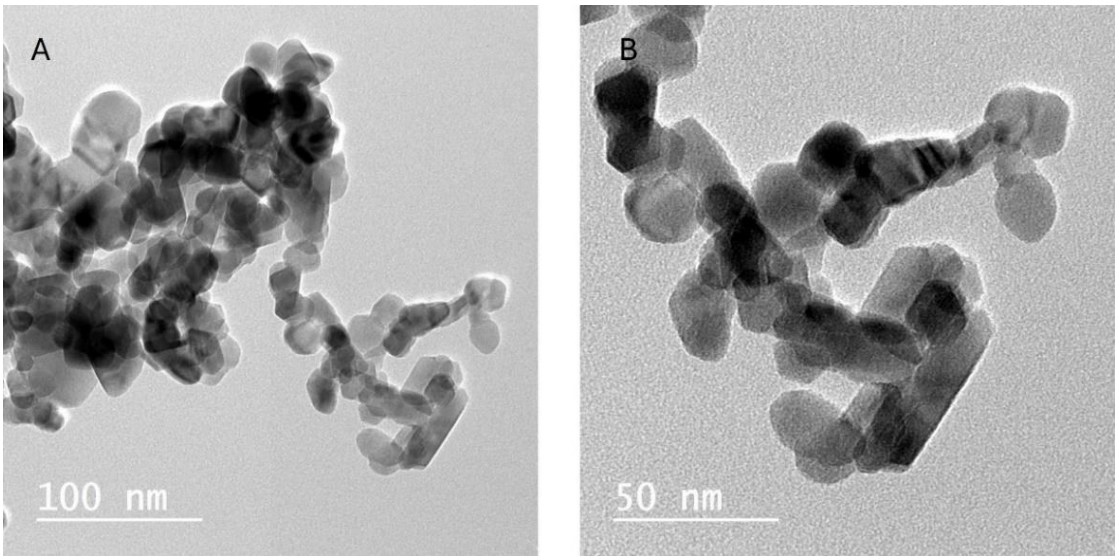

Fig. 2: A & B Transmission Electron Microscope micrograph of zinc oxide nanoparticles at different magnifications.

2. Histopathological Findings:

- **Pancreas:**

Figure (3 A)**:** Images from the control group show typical characteristic formations of the islets of Langerhans, which appear circular and substantial. The endocrine β-cells were in the centre and displayed larger, more translucent nuclei. (B): Microscopy images captured from STZ-induced diabetic rats showed a reduction in size (atrophy) and significant degeneration of the islets of Langerhans. Moreover, there is a substantial decrease in the α and β-cells, with numerous cells demonstrating pyknotic nuclei. (C): Diabetic rats receiving oral ZnO NPs showed partial recovery of cellular integrity and degenerative alterations in the cellular components of pancreatic islets, characterized by a small number of cells exhibiting pyknotic nuclei. (D): Oral administration of fenugreek extract in animals resulted in slight protective benefits and degenerative alterations in the cellular components of the pancreatic islets, with a significant number of cells showing pyknotic nuclei. (E): Images taken from ZnO NPs with a fenugreek seed extract group showed that the islets of Langerhans appeared nearly normal in size, with few degenerative and approximately average quantities of α and β-cells, with a few cells demonstrating pyknotic nuclei.

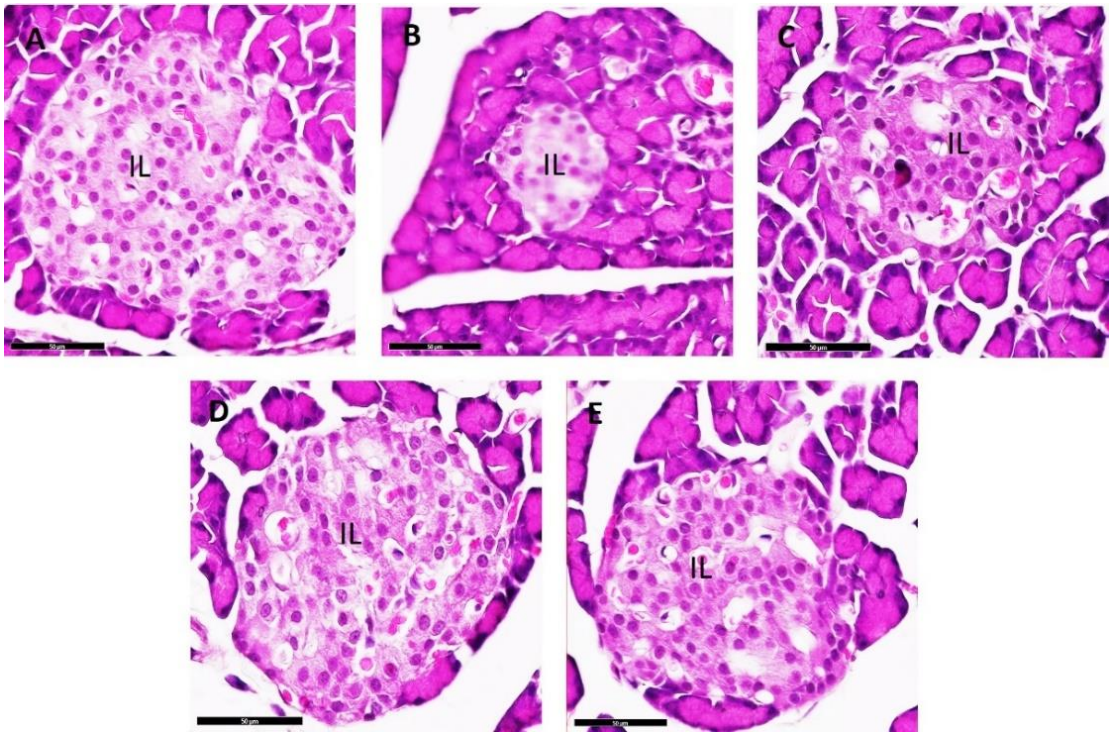

Fig. 3: Photomicrograph of pancreatic tissues staining H&E (scale bar 50 µm, 400 magnification). (A) Control group, (B) Diabetic group (received STZ), (C) Diabetic + ZnO NPs, (D) Diabetic + Fenugreek, (E) Diabetic + ZnO NPs + Fenugreek. IL: Islet Langerhans.

- **Liver:**

Figure (4): The liver histology of rats subjected to STZ and/or ZnO NPs, Fenugreek, and combining ZnO NPs with Fenugreek has been examined. (A): The liver section of the control rats exhibits the typical structure of the liver, including the central vein (CV), hepatocytes (HC), blood sinusoids (S), and Kupffer cells (KC). On the other hand, the liver section of the STZ-treated rats (B): displays moderate dilations in the blood sinusoids (S) and haemorhage, with red blood cells (RBC) observed in the central vein (CV), activated Kupffer cells (KC), vacuolization nuclei, pyknotic nuclei, some binucleated hepatic cells, and small focal necrotic area, mild degeneration of hepatocytes, (C): Liver sections of ZnO NPs showing enhancement in the liver tissue with moderate dilations in blood sinusoids (S) and mild bleeding as red blood cells (RBC) in central vein (CV), less activated Kupffer cells (KC), vacuolization nuclei, marked decrease in pyknotic nuclei, (D): The liver sections of rats treated with Fenugreek exhibit little alterations in liver structure. (E): The combination of ZnO NPs + Fenugreek in a liver slice shows a development in the structure with minor abnormal alterations in the central vein (CV) and a significant level of recovery (H&E, scale bar = 50 µm).

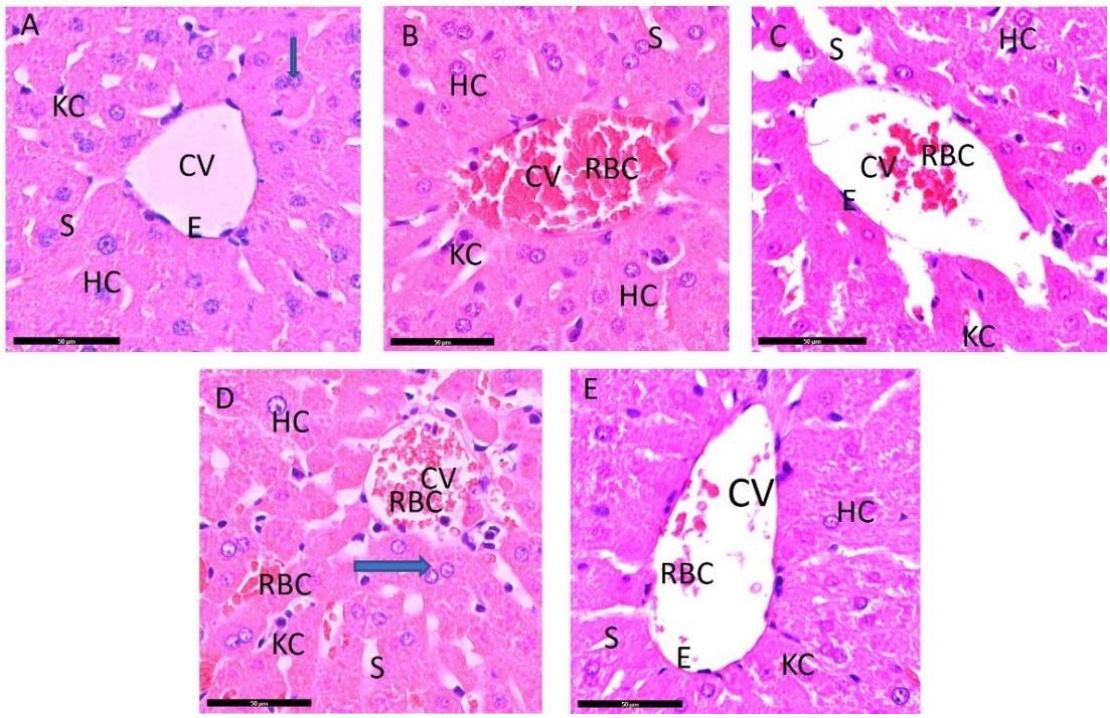

Fig. 4: Histological image of mice liver of various groups: (A) Control group. (B) STZ group. (C) STZ with ZnO NPs. (D) STZ + Fenugreek. (E) STZ plus ZnO NPs with fenugreek. Cv: Central vein; RBC: Red blood cell; HC: Hepatic cell; KC: Kupffer cell, S: Sinusoid, blue arrow: Binucleated hepatocyte, E: endothelial cell. (H&E, scale bar = 50 µm).

3. Biochemical Tests:

Glucose Metabolism:

Figure (5) and **Table** (**1**), shows the measured serum insulin, blood glucose, and HbA1c levels across various studied cohorts. The blood glucose and HbA1c levels were significantly increased in STZ but were significantly decreased in STZ + ZnO NPs, STZ + TFG, and STZ + ZnO NPs + TFG groups versus control ($p < 0.001$). Serum insulin was significantly decreased in STZ but increased dramatically in STZ + ZnO NPs, STZ + TFG, and STZ + ZnO NPs + TFG group versus control ($p < 0.001$ for all).

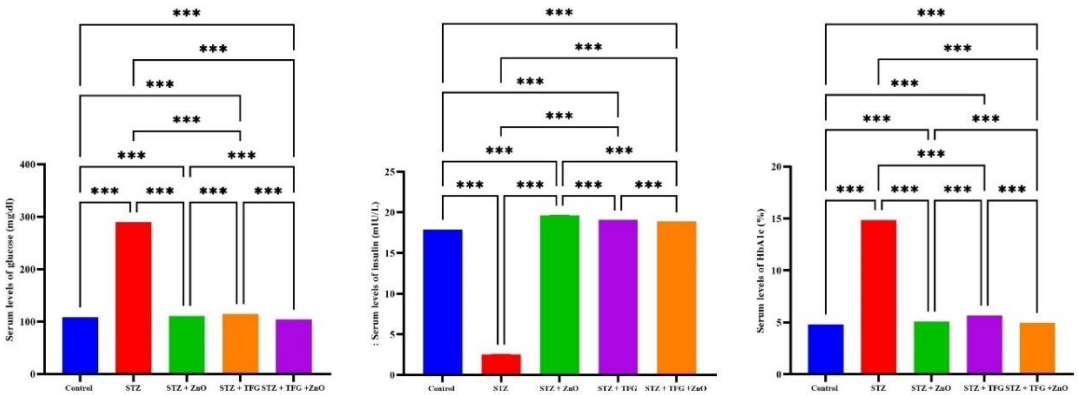

Fig. 5: Serum levels of Glucose Metabolism in different studied groups. Values are presented as mean ± standard deviation. [1]P: Importance difference from the control group; [2]P: Importance versus STZ group; [3]P: Importance versus STZ + ZnO; [4]P: Importance versus STZ + TFG at $P < 0.05$ using One-way ANOVA (Tukey) test. TFG: Trigonella foenum-graecum, STZ: Streptozotocin, ZnO: Zinc oxide nanoparticles.

Table 1: Glucose Metabolism in different studied groups.

Groups	Glucose (mg/dL)	Insulin (mIU/L)	HbA1c (%)
Control	108±0.017	17.88±0.001	4.821±0.0008
STZ	289.4±0.032	2.542±0.001	14.84±0.002
Significance	^1P<0.001	^1P<0.001	^1P<0.001
STZ + ZnO	110.8±0.018	19.63±0.026	5.083±0.002
Significance	^1P < 0.001; ^2P < 0.001; ^3P < 0.001; ^4P < 0.001	^1P < 0.001; ^2P < 0.001; ^3P < 0.001; ^4P < 0.001	^1P < 0.001; ^2P < 0.001; ^3P < 0.001; ^4P < 0.001
STZ + TFG	114.2±0.023	19.06±0.002	5.661±0.001
Significance	^1P < 0.001; ^2P<0.001	^1P < 0.001; ^2P<0.001	^1P < 0.001; ^2P<0.001
STZ + TFG + ZnO	104±0.032	18.86±0.001	4.982±0.001
Significance	^1P<0.001; ^2P<0.001; ^3P<0.001	^1P<0.001; ^2P<0.001; ^3P<0.001	^1P<0.001; ^2P<0.001; ^3P<0.001

- **Liver Function:**

The toxic effect of STZ in liver function tests is shown in Table (2) and Figure (6). In STZ-treated groups, serum levels of AST, ALT, and TBIL were significantly increased compared with the negative control group (P <0.001 for all). Meanwhile, serum levels of albumin and total proteins were significantly decreased in STZ versus negative control (P <0.001 for both). Treatment of the animals by TFG + STZ led to a substantial lowering in AST, ALT, and TBIL serum levels and a significant enlargement in albumin and total proteins versus the STZ group (P <0.001 for all). Still, it showed substantial changes versus the negative control group (P <0.001 for all). Treatment of the animals by ZnO NPs + TFG led to a significant decrease in serum levels of AST, ALT, and TBIL and a significant increase in albumin and total proteins versus STZ and ZnO NPs + STZ groups (P <0.001 for all). Still, it showed substantial changes versus the negative control group (P <0.001 for all)

Table 2: Liver function tests in different studied groups.

Groups	ALT (U/L)	AST (U/L)	TBIL(g\dl)	ALB (mg\dl)	TP (mg\dl)
Control	24.16±0.001	23.23±0.022	0.5422±0.0001	4.917±0.018	6.530±0.023
STZ	54.83±0.023	64.82±0.018	1.648±0.0002	1.862±0.001	3.521±0.001
Significance	^1P<0.001	^1P<0.001	^1P<0.001	^1P<0.001	^1P<0.001
STZ + ZnO	12.66±0.002	15.84±0.002	0.4863±0.0002	4.314±0.0003	5.561±0.001
Significance	^1P<0.001; ^2P<0.001; ^3P<0.001; ^4P<0.001	^1P<0.001; ^2P<0.001; ^3P<0.001; ^4P<0.001	^1P<0.001; ^2P<0.001; ^3P<0.001; ^4P<0.001	^1P<0.001; ^2P<0.001; ^3P<0.001; ^4P<0.001	^1P<0.001; ^2P<0.001; ^3P<0.001; ^4P<0.001
STZ + TFG	15.74±0.001	21.02±0.002	0.9341±0.0001	4.520±0.017	6.822±0.001
Significance	^1P<0.001; ^2P<0.001	^1P<0.001; ^2P<0.001	^1P<0.001; ^2P<0.001	^1P<0.001; ^2P<0.001	^1P<0.001; ^2P<0.001
STZ + TFG + ZnO	12.62±0.002	14.52±0.017	0.3617±0.0001	4.874±0.0003	6.563±0.002
Significance	^1P<0.001; ^2P<0.001; ^3P<0.001	^1P<0.001; ^2P<0.001; ^3P<0.001	^1P<0.001; ^2P<0.001; ^3P<0.001	^1P<0.001; ^2P<0.001; ^3P<0.001	^1P<0.001; ^2P<0.001; ^3P<0.001

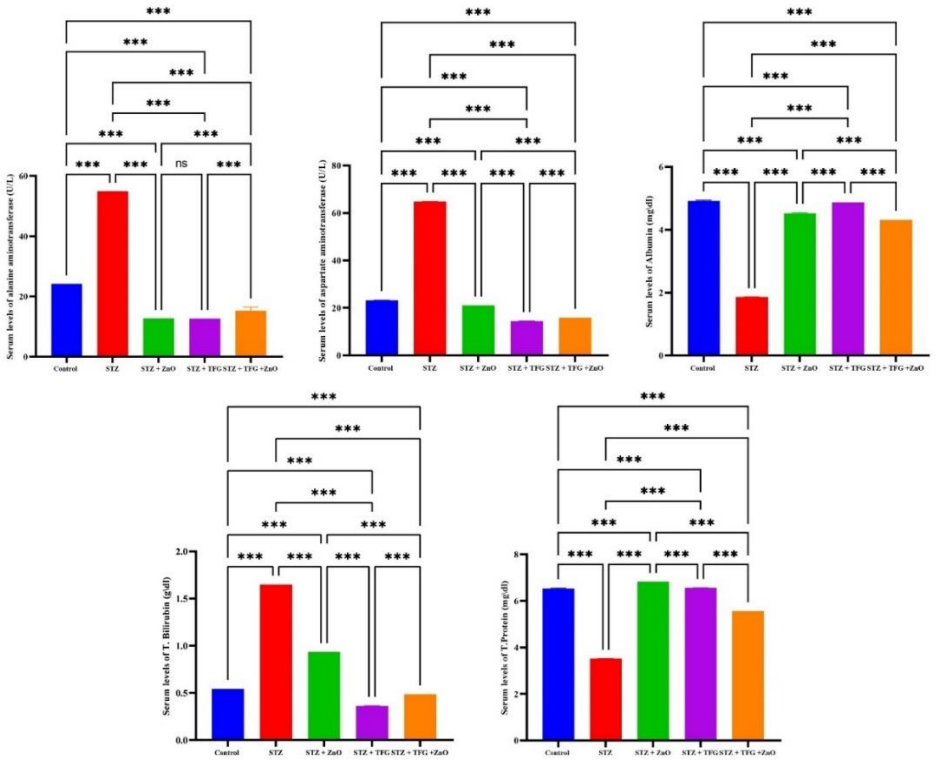

Fig. 6: Serum levels of Liver Function in different studied groups. Values are presented as mean ± standard deviation. [1]P: Importance difference from the control group; [2]P: Importance versus STZ group; [3]P: Importance versus STZ + ZnO; [4]P: Importance versus STZ + TFG at P< 0.05 using One-way ANOVA (Tukey) test. TFG: Trigonella foenum-graecum, STZ: Streptozotocin, ZnO: Zinc oxide nanoparticles.

4. *Oxidative Stress Markers:*

The effect of STZ administration on oxidative stress markers is shown in Table (3) and Figure (7). Administration of STZ led to a significant decrease in levels of antioxidant markers (GSH, SOD, and CAT) compared to the control group (P <0.001). Meanwhile, oxidative stress biomarkers, MDA, were elevated compared to the control group (P <0.001). Administration of TFG and ZnO led to a significant increase in antioxidants (GSH, SOD, and CAT) compared to the STZ group (P <0.001). After the TFG and ZnO administration, the MDA level was significantly decreased versus the STZ group (P <0.001).

Table 3: Serum levels of oxidative stress markers in different studied groups.

Groups	**GSH** (ng/mL)	**SOD** (u/ml)	**CAT** (Mu/L)	**MDA** (nmol/mL)
Control	18.86±0.003	178.6±0.028	115±0.027	0.5141±0.002
STZ	2.842±0.001	85.02±0.017	84.22±0.023	2.266±0.0001
Significance	**[1]*P*<0.001**	**[1]*P*<0.001**	**[1]*P*<0.001**	**[1]*P*<0.001**
STZ + ZnO	16.78±0.003	163.4±0.027	123.8±0.042	0.6442±0.0001
Significance	**[1]*P*<0.001; [2]*P*<0.001; [3]*P*<0.001; [4]*P*<0.001**	**[1]*P*<0.001; [2]*P*<0.001; [3]*P*<0.001; [4]*P*<0.001**	**[1]*P*<0.001; [2]*P*<0.001; [3]*P*<0.001; [4]*P*<0.001**	**[1]*P*<0.001; [2]*P*<0.001; [3]*P*<0.001; [4]*P*<0.001**
STZ + TFG	13.08±0.002	184±0.018	121.6±0.032	0.7342±0.0001
Significance	**[1]*P*<0.001; [2]*P*<0.001**	**[1]*P*<0.001; [2]*P*<0.001**	**[1]*P*<0.001; [2]*P*<0.001**	**[1]*P*<0.001; [2]*P*<0.001**
STZ + TFG + ZnO	17.46±0.002	160.4±0.013	122.0±0.036	0.5361±0.0001
Significance	**[1]*P*<0.001**; **[2]*P*<0.001; [3]*P*<0.001**	**[1]*P*<0.001**; **[2]*P*<0.001; [3]*P*<0.001**	**[1]*P*<0.001**; **[2]*P*<0.001; [3]*P*<0.001**	**[1]*P*<0.001**; **[2]*P*<0.001; [3]*P*<0.001**

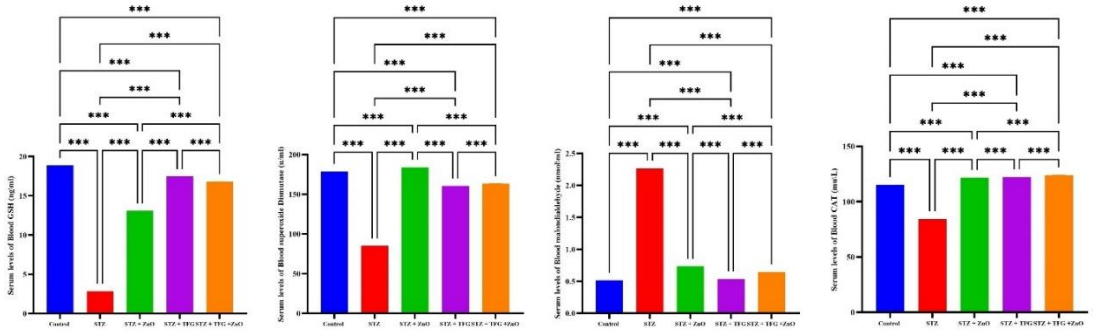

Fig. 7: Serum levels of oxidative stress in various examined groups. Values are presented as mean ± standard deviation. [1]P: Importance difference from the control group; [2]P: Importance versus STZ group; [3]P: Importance versus STZ + ZnO; [4]P: Importance versus STZ + TFG at P< 0.05 using One-way ANOVA (Tukey) test. TFG: Trigonella foenum-graecum, STZ: Streptozotocin, ZnO: Zinc oxide nanoparticles.

DISCUSSION

Pancreatic β cells release insulin, which promotes glucose absorption into cells for energy production and several other functions (Siddiqui *et al*., 2020). Diabetes mellitus (DM) is caused by administering only one intraperitoneal dosage of Streptozotocin (STZ), recognized for its high toxicity to pancreatic beta cells responsible for generating insulin (Ahmed *et al*., 2022). DM ranks among the top 10 leading causes of mortality worldwide, and its prevalence is increasing at a fast pace in developed nations (Khan *et al*., 2020). The primary pharmacological treatment for type 2 diabetes mellitus (T2DM) contains insulin secretagogues, incretin mimetics, insulin sensitizers, biguanides, alpha-glucosidase inhibitors, amylin antagonists, and sodium-glucose co-transporter-2 (SGLT2) inhibitors (Kanwugu *et al*., 2022).

Nanotechnology has not only enhanced the technological treatment of diabetes but also introduced novel approaches to enhance quality of life. It has enabled the development of a highly efficient and controlled method for administering insulin and novel sensing techniques for monitoring blood glucose levels (Chauhan *et al*., 2020). These techniques have significantly contributed to the diagnosis and treatment of diabetes in recent years (Siwach *et al*., 2019). Additionally, it exhibits commendable efficacy in identifying and treating diabetes-related problems (Siddiqui *et al*., 2020; Tan *et al*., 2020). Nanoparticles (NPs) possess unique benefits such as biocompatibility, bioavailability, targeting efficiency, and low toxicity, making them well-suited for diabetes treatment (Luo *et al*., 2021). Zinc oxide nanoparticles (ZnO NPs) can serve as a highly efficient nanocarrier for traditional pharmaceuticals because of their inexpensive nature and advantageous properties of biodegradability and biocompatibility (Mandal *et al*., 2022). Furthermore, zinc enhances insulin signaling by promoting the phosphorylation of insulin receptors, augmenting Phosphoinositide 3-kinases (PI3Ks) activity, and inhibiting glycogen synthase kinase-3 (GSK-3). Moreover, this element was proposed to enhance the outcomes of diabetes-related conditions, such as nephropathy. ZnO NPs, a new zinc delivery agent, have significant applications in several treatments for illnesses, including DM (San Tang, 2019a). ZnO NPs with tiny size, extensive surface area, and high binding capacity can be utilized as more effective carriers for drug delivery, less toxic alternatives to antifouling agents, and improved antioxidant and anti-diabetic agents (Falfushynska *et al*., 2019). ZnO NPs can reverse the alterations in pancreatic tissue caused by diabetes.

The study's results are consistent with Wahba *et al*.'s research in 2016, which investigated the therapeutic benefits of ZnO NPs in lowering

histological and functional alterations in the pancreas of rats with STZ-induced diabetes. According to Amiri *et al*. (2018), our findings demonstrated that ZnO NPs therapy is associated with the regeneration of pancreatic cells, resulting in elevated insulin output. Our histological findings provided additional evidence to support this idea, as ZnO NPs promoted the regeneration of the islets of Langerhans and effectively restored the typical morphology of β cells. Our findings align with those of Othman *et al*. (2020), who found that treatment with ZnO NPs led to significant enhancements in insulin levels, glucose tolerance, and the functioning of pancreatic cells. The study determined that ZnO NPs show great potential as an effective treatment for diabetes. Zinc oxide (ZnO) is a valuable nanocarrier for enhancing medication delivery and release mechanisms (Lakshmipriya & Gopinath, 2021).

The findings are consistent with those reported by Nazarizadeh and Asri-Rezaie (2016), who observed that ZnO NPs helped prevent a decrease in serum insulin levels in the animals. These results align with those of Wahba *et al*. (2016), who found that ZnO NPs effectively reversed the damage to the pancreas caused by diabetes. This was evidenced by the improvement in the pancreas' structure and the normalization of blood glucose and serum insulin levels, as shown in biochemical analysis. Our results align with Bai & Jarubula (2023), who noted a significant decrease in blood glucose levels and increased glycogen levels in diabetic rats due to ZnO NPs. ZnO NPs are commonly preferred for their anti-diabetic properties compared to other metal nanoparticles. In summary, ZnO NPs not only prevent and reverse the effects of diabetes but also stimulate the expression of Glucose transporter 4 (GLUT-4) and INS genes. This is achieved through various mechanisms, such as improved cellular uptake of synthesized ZnO NPs, enhancement of hepatic glycogenesis to promote glycolysis, and increased insulin concentrations. Furthermore, it amplifies the cumulative impact on the manifestation and operation of elevated glucokinase and the levels of IRA and GLUT-2 expression (Bayrami *et al*., 2018).

The data obtained from the investigation agrees with Salman and Qadeer (2021), who demonstrated that *Trigonella foenum-graecum* (TFG) effectively lowered fasting blood glucose levels to almost normal. As anti-diabetic medicines, botanical compounds are available with ample supplies, significant therapeutic efficacy, and low side effects. Phytocompounds typically operate through four hypoglycemic pathways, which involve reducing carbohydrate breakdown and glucose absorption, enhancing glucose uptake and metabolism, improving insulin action and sensitivity, and exerting antioxidant and anti-inflammatory effects. Nevertheless, the traditional method of orally administering anti-diabetic botanical compounds has many inherent shortcomings. Oral nano drug delivery systems for botanical compounds to treat T2DM possess the benefits of oral administration while also addressing the limitations of traditional oral drug delivery (Kambale *et al*., 2022). Natural products exhibit biocompatibility, are more cost-effective, and are anticipated to elicit fewer adverse effects than existing anti-diabetic medications. (Zolkepli *et al*., 2022). The anti-diabetic effects of TFG seeds may be attributed to steroidal compounds, dietary fiber, alkaloids, and saponins (Shahrajabian *et al*., 2021).

The current study's findings, compatible with those of Jiang *et al*. (2017, 2018) and Zin *et al*. (2019), demonstrated that diabetic rats exhibited impairment of the pancreatic islet cells in STZ. They pronounced pathological alterations in the exocrine and endocrine components, aligning with prior observations. Current metabolomics investigations have revealed a noteworthy effect of TFG flavonoids on the pancreas, kidney, and liver in

connection with STZ. According to Abeysekera *et al*. (2018), the researchers found that the TFG seed extract had both anti-glycation and glycation reversal effects in a BSA-glucose paradigm. The researchers have determined that the ability of TFG seed to reverse glycation is a discovery in terms of its anti-diabetic capabilities. This study suggests that TFG may potentially treat the complications linked to advanced glycation end products in individuals diagnosed with diabetes. The finding agrees with previous reports that demonstrated that TFG had a positive impact on regulating hemoglobin A1C (HbA1c) levels, reducing blood sugar levels in individuals with type 2 diabetes mellitus, and enhancing insulin secretion in the pancreas of rats and humans (Kandhare *et al*., 2018; Hassani *et al*., 2019). Our results are similar to those of this study, which suggested that TFG can enhance the body's sugar utilization, regulate insulin secretion, and reduce glucose uptake from the gut (Gaddam *et al*., 2015).

Also, the data obtained in the present study, like those examined by Kaur (2016), demonstrated the hypoglycemic efficacy of TFG. Although there are variations in the amount and length of the treatments, it has been observed that TFG seed can reduce plasma glucose and HbA1c levels in individuals with diabetes mellitus type 2. Our data, in line with Naicker *et al*. (2016) and Aldakinah *et al*. (2017), suggest that trigonelline enhances insulin's ability to work effectively. By regulating the renewal of pancreatic β cells and promoting the activity of enzymes that break down glucose, trigonelline reduces blood glucose levels. It helps manage type 2 diabetes (non-insulin-dependent). This collaborative research underscores the importance of shared knowledge in our scientific community. These results, like Al-Chalabi *et al*. (2019), observed an essential decrease in blood glucose levels in diabetic groups treated with *Trigonella foenum-graecum* extract.

While this study underscores the therapeutic potential of ZnO NPs and TFG, it is limited by its reliance on an animal model. Further research is needed to evaluate the long-term safety, optimal dosing, and efficacy of these treatments in clinical settings. Investigating their molecular mechanisms, particularly the role of TFG's trigonelline and flavonoids in regulating glucose transport, could provide deeper insights into their anti-diabetic effects.

Conclusion

This study demonstrated the therapeutic potential of ZnO-NPs and *Trigonella foenum-graecum* (TFG), both individually and in combination, significantly reduced blood glucose and HbA1c levels, enhanced insulin secretion, and improved oxidative stress markers. The combined treatment showed superior efficacy, with notable pancreatic β-cells regeneration and hepatic architecture restoration, indicating a synergistic effect between ZnO-NPs and TFG.

Declarations:

Ethics Approval: All international guidelines relevant to animal care and usage were strictly followed. All animal operations received clearance from the Institutional Animal Care and Use Committee (IACUC) of King Abdulaziz University, Saudi Arabia (protocol approval number: 130-2023).

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

Author contribution: Salim M. El Hamidy contributed to the paper by researching and editing the article.

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