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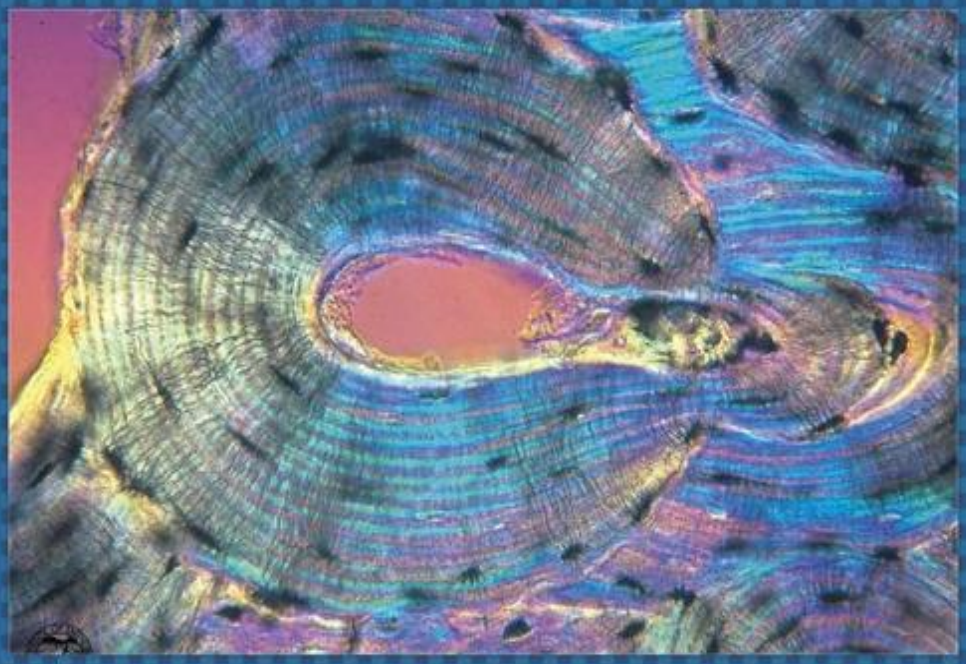

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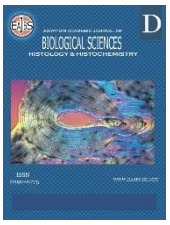

Efficacy of Fenugreek in Mitigating Renal Impairment, Dyslipidemia, and Oxidative Stress in a Diabetic Rat Model

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ABSTRACT

Type 2 diabetes mellites (T2DM) often leads to renal dysfunction, necessitating novel therapeutic interventions. This study aimed to evaluate the influence of zinc oxide nanoparticles (ZnO NPs) and *Trigonella foenum-graecum* (TFG) extract on glucose level, kidney function, lipid profile, and antioxidants in diabetic-induced rats, with a focus on its potential therapeutic role in mitigating diabetes-related complications accompanied with histological examination of kidney tissues in rat models of T2DM. Forty adult Wister albino rats were divided into five groups as follows: Group I (non-diabetic control group); Group II (diabetic control group); Group III (diabetic rats treated with ZnO NPs); Group IV (diabetic rats treated with TFG); Group V (diabetic rats treated with the combination of TFG and ZnO NPs). Biochemical parameters such as Glucose (GLU), Uric acid (UA), Blood urea nitrogen (BUN), Creatinine (CR), Triglyceride (TG), Cholesterol (CHOL), High-density lipoprotein-cholesterol (HDL-c), Low-density lipoprotein-cholesterol (LDL-c), Glutathione (GSH), Superoxide dismutase (SOD), Malondialdehyde (MDA) and Catalase (CAT) along with histological investigation for renal tissue. Treatment with TFG, ZnO NPs, and their combination exhibited a significant decrease in serum glucose (GLU) level, revised lipid profile to normalcy, reduced oxidative stress, and enhanced the histological pattern for kidney tissue, highlighting their therapeutic potential. These findings suggest that ZnO NPs and TFG extract significantly improve glucose metabolism, lipid profile, renal function, and oxidative stress in T2DM.

INTRODUCTION

Diabetes mellitus (DM) is a primary risk factor for kidney dysfunction, with diabetic nephropathy as a leading cause of end-stage renal disease (Selby & Taal, 2020). It is characterized by initial glomerular hyperfiltration and albuminuria, followed by a decline in renal function (Sagoo & Gnudi, 2020). DM can lead to kidney damage, while kidney dysfunction can contribute to insulin resistance and diabetes development (Kumar *et al*., 2023). Key risk factors for kidney disease include chronic hyperglycemia and hypertension (Pelle *et al*., 2022). Oxidative stress, driven by hyperglycemia and plays a crucial role in the pathogenesis and progression of diabetic kidney disease (DKD), a leading cause of end-stage renal disease (Su *et al*., 2023). Chronic hyperglycemia and mitochondrial dysfunction contribute to elevated reactive oxygen species (ROS) production, leading to cellular damage and dysfunction (Caturano *et al*., 2023).

Trigonella foenum-graecum (TFG) and zinc oxide nanoparticles (ZnO NPs) are gaining attention for their potential therapeutic effects on oxidative stress-related disorders affecting the kidney and pancreas (Elsherif *et al*., 2023). TFG, rich in bioactive compounds such as flavonoids and saponins, exhibits antioxidant and anti-inflammatory properties, protecting tissues from oxidative damage and supporting cellular function (Ahmad *et al*., 2022). ZnO NPs, known for their antioxidant potential, can scavenge reactive oxygen species (SOD) and enhance antioxidant defenses, through their effects depending on dosage and exposure (Włodarczyk *et al*., 2023).

In the kidney, oxidative stress is a key driver of fibrotic changes and functional impairment. TFG has been shown to mitigate oxidative stress by enhancing enzymatic antioxidants, while ZnO NPs may provide additional protection through ROS neutralization (Awadalla *et al*., 2022). Similarly, in the pancreas, oxidative stress contributes to beta-cell dysfunction and impaired insulin secretion, hallmarks of diabetes (Dludla *et al*., 2023). TFG, a hypoglycemic agent, has antioxidative properties combined with the ROS-reducing effects of ZnO NPs, offering promising effects for preserving pancreatic function and improving glucose metabolism. This study explores the combined impact of TFG and ZnO NPs on the functionality of the kidney, pancreas, and oxidative stress, highlighting their therapeutic potential.

MATERIALS AND METHODS

Characterization of ZnO NPs:

The relative morphology and dimensions of ZnO NPs were analyzed by transmission electron microscope (TEM) (JEOL Ltd, Tokyo, Japan). An aqueous suspension of nanoparticles was applied to a copper grid coated with carbon, dried, and subsequently examined.

Induction of Diabetes:

Diabetes mellitus was triggered in fasted rats for 24 h through a one-dose intraperitoneal (IP) injection of STZ (50 mg/ kg) dissolved in cold 0.1 % M citrate buffer (pH: 4.5). After the injection, rats were provided with sucrose (15g/L) in their drinking water for 24 hours to mitigate the risk of hypoglycemia-induced mortality. Diabetes was confirmed by assessing fasting blood glucose (FBG) levels three days post-STZ administration. Rats exhibiting an increase in fasting blood glucose (FBS) level of more than 250 mg/dL which were classified as diabetic and incorporated into the experiment. Six weeks later, Diabetes was assessed by assessing urea, uric acid, and creatinine serum levels.

Experimental Design:

The rats were obtained from the Animals section of the King Fahad Medical Research Center at King Abdulaziz University and classified into five groups, n=10, as follows:

- **Group I**: Non-diabetic control group, received (0.5 mL citrate buffer, IP).
- **Group II:** Diabetic group received STZ (50 mg/ kg, IP).
- **Group III:** Diabetic group received STZ (50 mg/ kg, IP) and was treated with ZnO NPs (10 mg/kg, orally).
- **Group IV:** Diabetic rats received STZ (50 mg/ kg, IP) and treated with TFG (500 mg/kg, orally).
- **Group V:** Diabetic rats received STZ (50 mg/ kg, IP) and were treated with a combination of ZnO NPs (10 mg/kg, orally) and TFG (500 mg/kg, orally).

Glucose Metabolism:

Following the blood collection, glucose levels for the studied groups were assessed using the glucose oxidase assay method using an Accu-Chek Performa glucometer (Roche, Switzerland). Plasma insulin level was measured quantitatively by the ELISA method. For this purpose, an insulin ELISA kit was used, and Glycosylated hemoglobin (HbA1C) was measured by Bannon (1982) method using a commercial diagnostic kit.

Kidney Function:

Following 5 weeks of treatment, after fasting for 8 hours, rats were administrated with diethyl ether to anesthetize rats. Samples were collected from the ocular venous plexus using serum separator tubes. Following centrifugation (2500 rpm for 15 minutes), the blood serum was obtained, and UA levels were determined by Burtis and Ashwood (1994) method, CR value was measured using the Moore and Sharer (2017) method, and BUN was assayed using Patton and Crouch (1977) method.

Lipid Profile Parameters Determination:

Triglyceride (TG) was determined using the Fossati and Prencipe (1982) method. CHOL concentration was determined using the Richmond (1973) method. HDL-c was determined using the Warnick *et al*. method (1983). LDL-c was measured by Friedewald *et al*., (1972) equation.

Antioxidant Parameters:

Following the collection of blood samples, the antioxidant parameters selected for this experiment were analyzed using the following procedures: GSH, based on the protocol described by Moron *et al*. (1979); MDA was determined according to Draper and Hadley (1990). SOD and CAT were measured based on the method of Misra and Fridovich (1972).

Histopathological Analysis of Kidney and Pancreatic Tissue:

Kidneys and pancreas were carefully dissected and analyzed for gross morphological changes, and the tissue was fixed in 10% buffered formaldehyde. Then, the tissue was dehydrated with an ascending grade of ethanol solution; the tissue was rinsed with xylene, embedded in molten paraffin, and set into paraffin blocks. After fine sectioning by a microtome, the slides were stained with hematoxylin and eosin (H & E) stain, which provided results that were investigated using a photomicroscope.

RESULTS

Scanning Electron Microscopy for ZnO NPs:

The examination of ZnO NPs was performed by scanning electron microscopy (SEM) (Quanta-FEG, FEI, Netherlands). The presence of several more significant ZnO NPs in the SEM images was related to aggregation. The ZnO NPs measurement ranged about 22 ± 4 nm.

Energy-dispersive X-ray Spectroscopy Screening ZnO NPs:

The samples of ZnO NPs were analyzed using EDS. The sample's composition is analyzed using a field emission scanning electron microscope with an EDX detector. The measurements were conducted using an acceleration voltage of 20 kilovolts. The EDS spectra revealed the distinct weight % of constituents present in the sample. Figure (1), displays the EDS spectra of ZnO-NP samples. The labeling displays the names and corresponding percentages of the components 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.

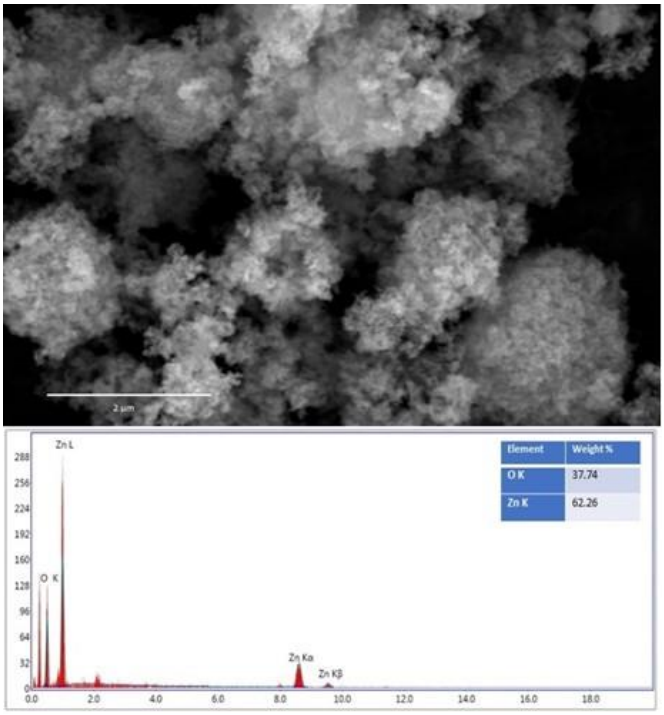

Fig. 1 shows the scanning electron microscopy and energy dispersive spectroscopy patterns of ZnO NPs.

Transmission Electron Microscopy for ZnO NPs:

High-resolution transmission electron microscopy (2021 FGG, JEOL, Japan) was used to determine the sizes and morphologies of ZnO nanoparticles, indicating the presence of spherical ZnO nanoparticles (ZnO NPs) (Fig. 2). The TEM micrographs showed that the ZnO nanoparticles consist primarily of spherical particles, with an average crystallite size ranging from around ~ 14 to 26 nm.

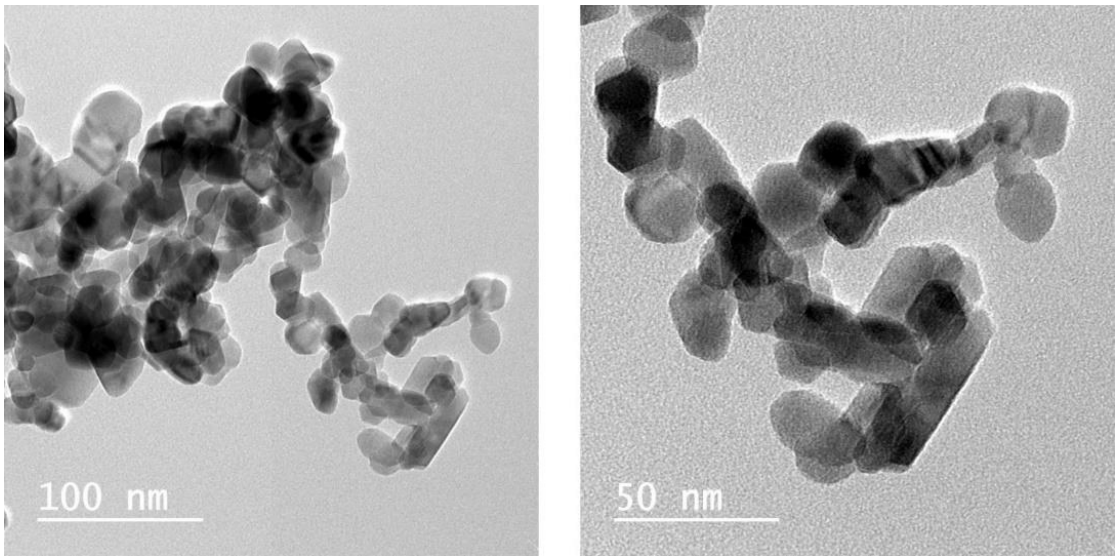

Fig. 2. A & B Transmission Electron Microscope micrograph of zinc oxide nanoparticles at different magnifications.

Histopathological Examination of Kidney:

Figure 3, illustrates the histological examination of hematoxylin and eosin (H&E)-stained kidney sections from the studied groups. Figure 3-a depicts the non-diabetic control group, showing a normal kidney structure in the renal cortex, including the glomerulus (G), proximal convoluted tubules (PCT),

and distal convoluted tubules (DCT). Figure 3-b represents the diabetic group, characterized by glomerular rapture and atrophy, dilatation of PCT and DCT, interstitial hemorrhagic exudate, hyaline cast formation in tubular lumen, vacuolated degeneration in the PCT, necrosis of tubular epithelial cells. Figure 3-c demonstrates mild improvement in the glomerular structure and partial recovery from STZ-induced damage, although the renal cortex still shows significant dilatation of PCT and DCT. Figure 3-d shows moderate improvement in glomerular and tubular morphology, with reduced but still evident dilatation of the PCT and DCT. Figure 3-e illustrates near-normal histological features, including an intact Bowman's capsule (BC) lined with simple squamous cells, a normal Bowman's space (BS), and proximal and distal tubules that are nearly restored to their typical appearance.

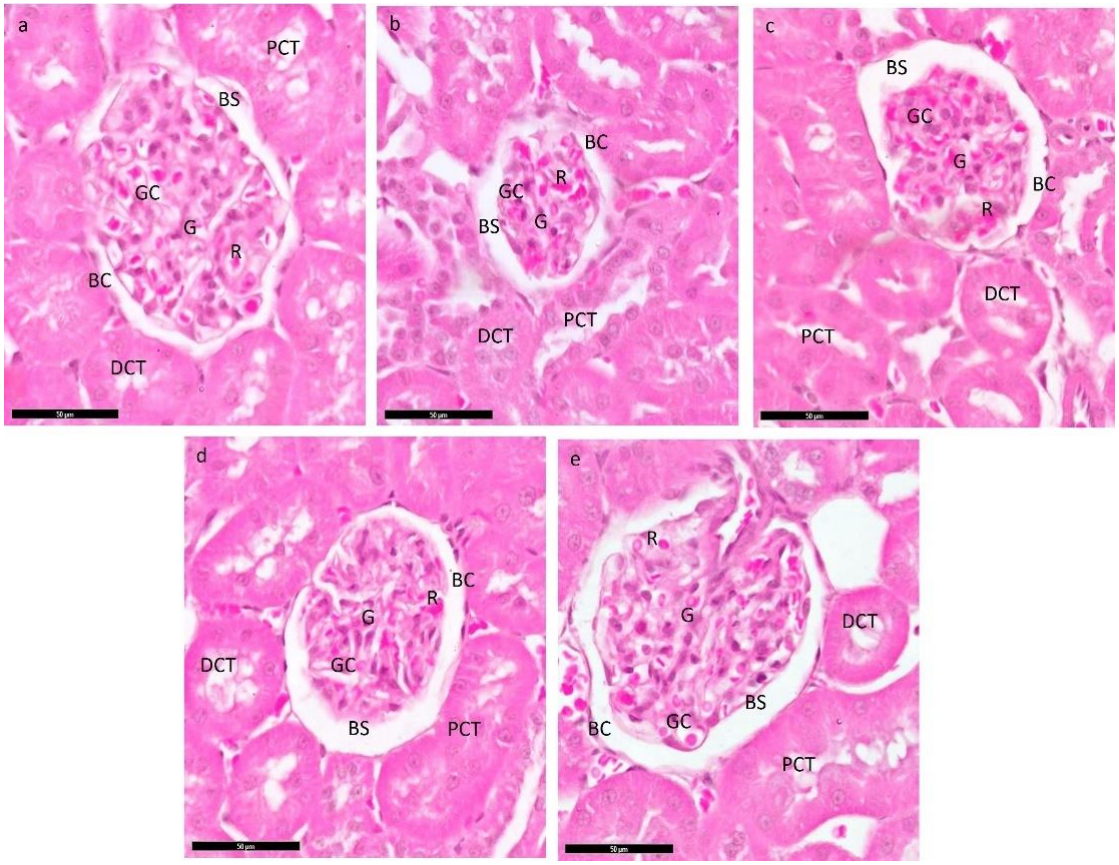

Fig. 3. Light photographs of histological sections from rat kidneys at magnifications X400 show different studied groups stained by H &E. a: control group. b: Diabetic control (received STZ) group. c: Diabetic + ZnO NPs. d: Diabetic + Fenugreek seeds extract. e: Diabetic + ZnO NPs with Fenugreek seeds extract. BC: Bowman`s capsule, BS: Bowman`s space, PCT: proximal convoluted tubule. DCT: distal convoluted tubule, G: glomerulus, GC: glomerular capsule, R: red blood cells. (H&E, X 400; scale bar: 50 µm).

Glucose Metabolism:

Table 1 and Figure 4, illustrate the effect of ZnO NPs and TFG on serum glucose, insulin, and HbA1c level in STZ-induced diabetic rats. Diabetic rats showed elevated serum glucose and HbA1c levels while markedly reducing insulin levels compared to control group ($p < 0.001$). Treatment with ZnO NPs, TFG, or their combination significantly reduced glucose and HbA1c levels and restored insulin levels compared to STZ group ($p < 0.001$). Notably, the combination of ZnO NPs and TFG exhibited the most pronounced effects, restoring these parameters approached to

the control levels. These findings highlight the synergistic potential of ZnO NPs and TFG in mitigating diabetic control groups and restoring the insulin function under diabetic conditions.

Table 1. Glucose Metabolism parameters levels 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	^{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	114.2±0.023	19.06±0.002	5.661±0.001
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
STZ + TFG + ZnO	104±0.032	18.86±0.001	4.982±0.001
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

Results are expressed as mean ± standard deviation (SD). ^{1}P: indicates a significant difference from control group; ^{2}P: indicates a significant difference from STZ group; ^{3}P: indicates a significant difference from STZ + TFG; ^{4}P: indicates a significant difference from STZ + TFG + ZnO NPs at P < 0.05 using One-way ANOVA with Tukey's post hoc test. *Trigonella foenum-graecum*, STZ: Streptozotocin, ZnO NPs: Zinc oxide nanoparticles.

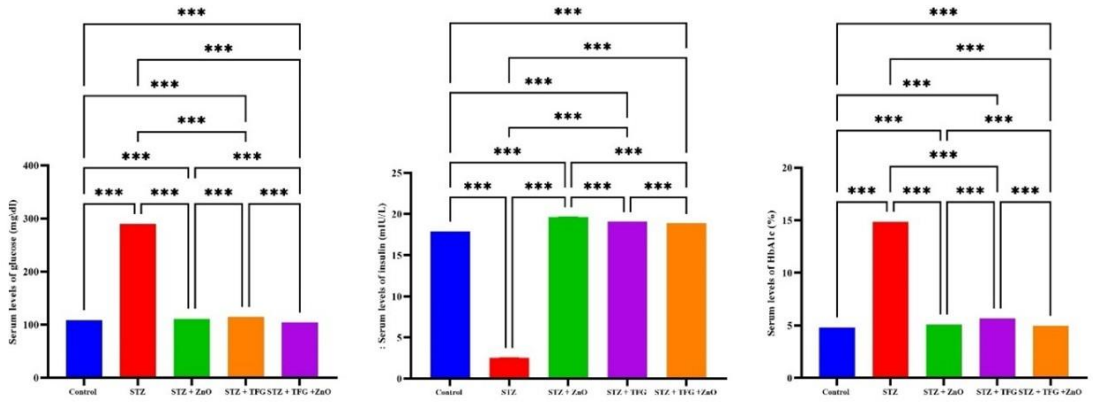

Fig. 4. Serum levels of glucose metabolism parameters in the studied groups. (A) Glucose (GLU), (B) insulin, and (C) Hemoglobin A1c (HbA1c). Results are expressed as mean ± standard deviation (SD). Statistical significance was determined at P < 0.05 using the One-way ANOVA with Tukey's post hoc test. TFG: *Trigonella foenum-graecum*, STZ: Streptozotocin, ZnO NPs: Zinc oxide nanoparticles.

Kidney Function Test:

The effects of ZnO NPs and TFG on renal function are illustrated in Figure 4. During the experimental period, serum levels of BUN, UA, and CR were significantly elevated (P < 0.05) in the STZ-treated group (G2) compared to the control group (G1), indicating impaired kidney function. However, by the end of this study, oral administration of TFG in (G3), ZnO NPs + TFG (G4), and ZnO NPs + TFG in (G5) resulted in a significant reduction in serum BUN, UA, and CR levels, reflecting an improvement in kidney function compared to STZ group (Table 2).

Table 2. Kidney function markers in different studied groups.

Groups	BUN (mg\dl)	UA (mg\dl)	CR (mg\dl)
Control	16.16±0.002	4.463±0.002	0.7284±0.0002
STZ	48.74±0.003	8.363±0.002	1.722±0.001
Significance	$^{1}P < 0.001$	$^{1}P < 0.001$	$^{1}P < 0.001$
STZ + ZnO NPs	14.88±0.004	4.522±0.002	0.6763±0.0002
Significance	$^{1}P < 0.001$; $^{2}P < 0.001$	$^{1}P < 0.001$; $^{2}P < 0.001$	$^{1}P < 0.001$; $^{2}P < 0.001$
STZ + TFG	17.82±0.002	4.623±0.023	0.6661±0.0001
Significance	$^{1}P < 0.001$; $^{2}P < 0.001$; $^{3}P < 0.001$	$^{1}P < 0.001$; $^{2}P < 0.001$; $^{3}P < 0.001$	$^{1}P < 0.001$; $^{2}P < 0.001$; $^{3}P < 0.001$
STZ + TFG + ZnO NPs	18.62±0.0023	5.481±0.001	0.5923±0.001
Significance	$^{1}P < 0.001$; $^{2}P < 0.001$; $^{3}P < 0.001$; $^{4}P < 0.001$	$^{1}P < 0.001$; $^{2}P < 0.001$; $^{3}P < 0.001$; $^{4}P < 0.001$	$^{1}P < 0.001$; $^{2}P < 0.001$; $^{3}P < 0.001$; $^{4}P < 0.001$

Results are expressed as mean ± standard deviation (SD). ^{1}P: indicates a significant difference from control group; ^{2}P: indicates a significant difference from STZ group; ^{3}P: indicates a significant difference from STZ + TFG; ^{4}P: indicates a significant difference from STZ + TFG + ZnO NPs at $P < 0.05$ using One-way ANOVA with Tukey's post hoc test. *Trigonella foenum-graecum*, STZ: Streptozotocin, ZnO NPs: Zinc oxide nanoparticles.

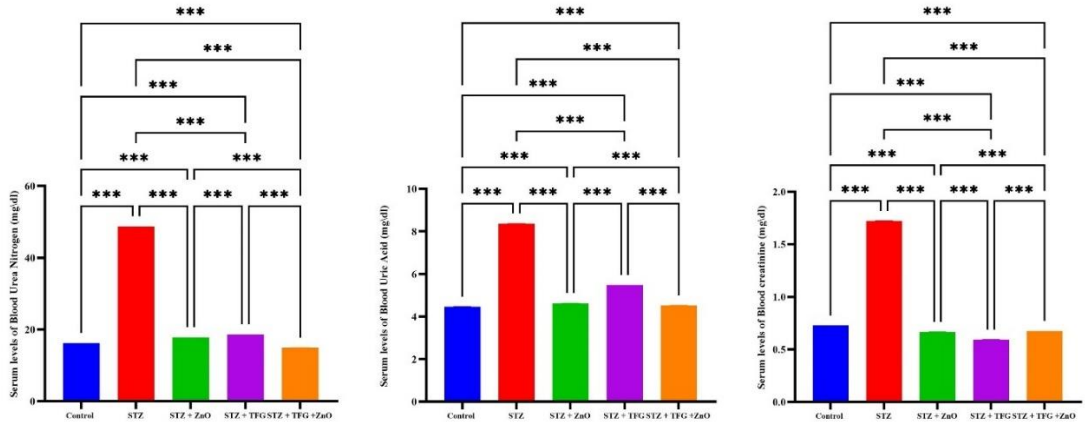

Fig. 5. Serum levels of kidney function markers in the studied groups. (A) Blood urea nitrogen (BUN), (B) Uric acid (UA), and (C) creatinine (CR). Results are expressed as mean ± standard deviation (SD). Statistical significance was determined at $P < 0.05$ using the One-way ANOVA with Tukey's post hoc test. TFG: *Trigonella foenum-graecum*, STZ: Streptozotocin, ZnO NPs: Zinc oxide nanoparticles.

Lipid Profile Test:

The effects of ZnO NPs and TFG on lipid profile parameters are illustrated in Figure 5. Serum levels of TG, CHOL, and LDL-c showed a significant increase in the STZ, STZ + ZnO NPs, and STZ + TFG groups compared to the control group ($p < 0.001$). However, these levels significantly decreased in the STZ + ZnO NPs + TFG group compared to the control group ($p < 0.001$). TG, total CHOL, and LDL-c levels were significantly reduced in the STZ + ZnO NPs, STZ + TFG, and STZ + ZnO NPs + TFG groups compared to the STZ group ($p < 0.001$). Furthermore, the STZ + ZnO NPs + TFG combination group showed the most pronounced reduction in the levels of lipid profile compared to the STZ + TFG and STZ + ZnO NPs groups ($p < 0.001$). Furthermore, the STZ + TFG group exhibited significantly lower lipid levels compared to the STZ + ZnO NPs

group (p <0.001). Serum level of HDL-c was significantly decreased in the STZ, STZ + ZnO- NPs, and STZ + TFG groups compared to the control group (p < 0.001). HDL-C levels showed a significant increase in the STZ + ZnO NPs, STZ + TFG, and STZ + ZnO NPs + TFG groups compared to the STZ group (p <0.001). Additionally, the HDL-C levels were significantly higher in the STZ + ZnO NPs + TFG group compared to the STZ + ZnO NPs and STZ + TFG groups (p <0.001), and in the STZ + TFG group compared to the STZ + ZnO NPs group (p <0.001) (Table 3).

Table 3. Lipid profile markers in different studied groups.

Groups	CHOL (mg/dl)	TRIG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)
Control	152.8±0.032	79.23±0.026	47.21±0.008	105.6±0.018
STZ	279.8±0.037	146.8±0.017	28.22±0.023	251.6±0.027
Significance	[1]*P*<0.001	[1]*P*<0.001	[1]*P*<0.001	[1]*P*<0.001
STZ +ZnO-NPs	121±0.027	76.82±0.017	42.63±0.023	78.43±0.026
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	121.8±0.022	84.02±0.022	44.02±0.017	77.82±0.017
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
STZ + TFG + ZnO-NPs	120.4±0.035	76.02±0.023	44.24±0.035	76.22±0.018
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

Results are expressed as mean ± standard deviation (SD). [1]P: indicates a significant difference from control group; [2]P: indicates a significant difference from STZ group; [3]P: indicates a significant difference from STZ + TFG; [4]P: indicates a significant difference from STZ + TFG + ZnO NPs at P < 0.05 using One-way ANOVA with Tukey's post hoc test. *Trigonella foenum-graecum*, STZ: Streptozotocin, ZnO NPs: Zinc oxide nanoparticles.

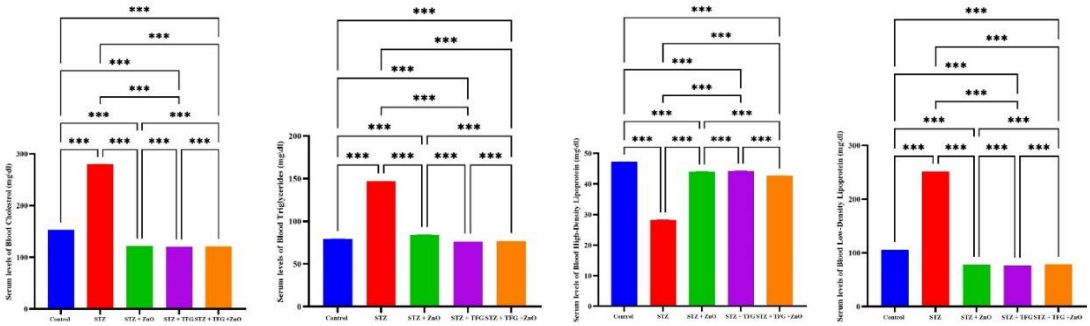

Fig. 5. Serum levels of Lipid profile markers in the studied groups. (A) Cholesterol (CHOL), (B) Triglyceride (TG), and (C) High-density lipoprotein-cholesterol (HDL-c). (D) Low-density lipoprotein-cholesterol (LDL-c). Results are expressed as mean ± standard deviation (SD). Statistical significance was determined at P < 0.05 using the One-way ANOVA with Tukey's post hoc test. TFG: *Trigonella foenum-graecum*, STZ: Streptozotocin, ZnO NPs: Zinc oxide nanoparticles.

Oxidative Stress Test:

The effect of STZ administration on oxidative stress markers is shown in Figure 6**.** Administration of STZ led to a significant decrease in levels of antioxidant markers (GSH, SOD and CAT) compared to negative control (P <0.0001). Administration of TFG to group treated with STZ led to a significant increase in antioxidants compared to the STZ group (P <0.0001)

but still lower than negative control (P <0.0001). Administration of ZnO to groups treated with STZ led to a significant increase in antioxidants compared to STZ and TFG + STZ groups (P <0.0001) but GSH, SOD, and CAT levels were still lower than control (P <0.0001). Meanwhile, levels of oxidative stress biomarkers, MDA were elevated versus the control group (P <0.0001). After the administration of TFG, the level of MDA was significantly decreased versus the STZ group (P <0.000) but still higher than the control (P <0.0001). Administration of ZnO NPs led to significantly decreased levels of MDA versus STZ and TFG + STZ groups (P <0.0001) but still level was still higher than control (P <0.0001) (Table 4).

Table 4. Antioxidant biomarkers 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-NPs	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	^{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	13.08±0.002	184±0.018	121.6±0.032	0.7342±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
STZ + TFG + ZnO-NPs	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; ^{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

Results are expressed as mean ± standard deviation (SD). ^{1}P: indicates a significant difference from control group; ^{2}P: indicates a significant difference from STZ group; ^{3}P: indicates a significant difference from STZ + TFG; ^{4}P: indicates a significant difference from STZ + TFG + ZnO NPs at P < 0.05 using One-way ANOVA with Tukey's post hoc test. *Trigonella foenum-graecum*, STZ: Streptozotocin, ZnO NPs: Zinc oxide nanoparticles.

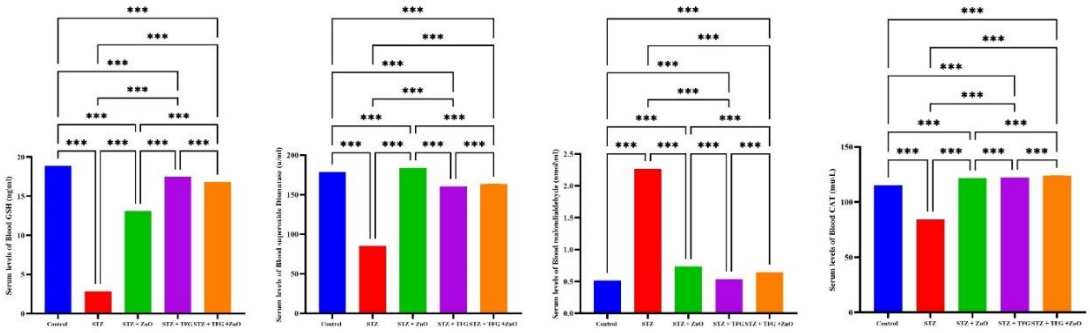

Fig. 6. Serum levels of antioxidant markers in the studied groups. (A) Glutathione, (B) Superoxide dismutase (SOD), (C) Malondialdehyde (MDA), and (D) Catalase (CAT). Results are expressed as mean ± standard deviation (SD). Statistical significance was determined at P < 0.05 using the One-way ANOVA with Tukey's post hoc test. TFG: *Trigonella foenum-graecum*, STZ: Streptozotocin, ZnO NPs: Zinc oxide nanoparticles.

DISCUSSION

The results indicate the potential of ZnO NPs and TFG in alleviating oxidative stress and enhancing kidney function in STZ-induced diabetic conditions. Compared to the STZ group, ZnO NPs and TFG reduced markers of kidney damage, lipid dysregulation, and oxidative stress, highlighting their therapeutic potential.

Histopathological analysis revealed several renal damages in the

STZ group, including glomerular atrophy, tubular dilatation, and interstitial hemorrhage. In contrast, groups treated with ZnO NPs, TFG, and their combination treatment restored renal function, with the combination restored renal architecture to near-normal levels, aligning with the study by Barakat *et al*., (2023), who reported the nephroprotective effects of TFG through its antioxidant properties, and Awadalla *et al*., (2022), who highlighted the ROS-scavenging ability of ZnO NPs.

Kidney function markers (BUN, CR, and uric acid) were elevated in the STZ group, indicating impaired renal function. Treatment with ZnO NPs, TFG, and their combination markedly reduced these markers, consistent with the findings of the earlier studies. For instance, Alsuliam *et al*., (2022) demonstrated the protective effects of TFG on kidney function in diabetic models, while Abd El-Baset *et al*., (2022) documented the renal benefits of ZnO NPs in oxidative stress models. The superior outcome in the combination group suggests a synergistic effect of TFG and ZnO NPs in alleviating renal dysfunction.

Oxidative stress, a major contributor to renal and pancreatic damage, was evident in the STZ group, as indicated by reduced levels of GSH, SOD, and CAT and elevated MDA. ZnO NPs and TFG significantly restored antioxidant markers and reduced MDA levels, with the combination group showing the most pronounced effects. These results are consistent with the antioxidant mechanisms described by Bafadam *et al*., (2021) and Al Hunduwan & El Hamidy, (2024) for TFG and Elmetwalli *et al*., (2022) for ZnO NPs where both agents modulate ROS levels and enhance enzymatic antioxidant defenses.

Lipid profile analysis further supports the protective roles of ZnO NPs and TFG. The STZ group exhibited hypercholesterolemia and elevated triglyceride, while treatment significantly normalized lipid levels, particularly in the combination group. This agrees with Sharma, M. & Choudhary, P. R. (2017) who reported the lipid-lowering effects of TFG, and Azab (2020), who reported hypolipidemic effects on ZnO NPs.

The combined treatment of ZnO NPs and TFG exhibited the most significant improvements across all parameters. This suggests that TFG's bioactive compounds, such as flavonoids and saponins, synergize with the ROS-scavenging properties of ZnO NPs to provide enhanced protection against oxidative damage. While the results are promising, further studies are needed to elucidate the exact mechanisms and assess long-term safety.

In conclusion, this study confirms the efficacy of ZnO NPs and TFG, individually and in combination, in alleviating oxidative stress and improving kidney and lipid parameters in diabetic conditions. These findings contribute to the growing evidence supporting the integration of nanotechnology and phytotherapy in managing diabetes and oxidative stress-related disorders.

Declarations:

Ethics Approval: The experiments were processed using the animal ethical rules of King Abdulaziz University's Animal Care and Use Committee (ACUC). Furthermore, all tests adhered to the Arrive standards and the EU Directive 2010/63/EU regarding animal research.

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

Author contribution: Khaled Al Hunduwan and Muhamed A El Nobey contributed equally to this work and shares the first authorship. Muhamed A El Nobey wrote the manuscript, and Salim M El Hamidy revised it**.**

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