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Research Article

Antidiabetic Activity of Green-Synthesized Zinc Oxide Nanoparticles Mediated by *Balanites aegyptiaca* in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus remains a major global health challenge associated with complications such as neuropathy, nephropathy, and cardiovascular disorders. This study evaluated the antidiabetic potential of zinc oxide nanoparticles (ZnO-NPs) synthesised using the fruit extract of *Balanites aegyptiaca* through a green synthesis approach. The plant extract served as both a reducing and stabilising agent, and the formation of crystalline ZnO-NPs was confirmed by Fourier Transform Infrared (FTIR) and X-ray Diffraction (XRD) analyses. Diabetes was induced intraperitoneally in Wistar albino rats using streptozotocin (STZ, 55 mg/kg). STZ induction caused a significant increase in fasting blood glucose, total cholesterol, triglycerides, and LDL levels, along with a reduction in HDL, anaemia, and elevated liver enzymes (ALT, AST, ALP), indicating hepatic dysfunction and haematological disturbances. Oral administration of ZnO-NPs (2.5, 5, and 10 mg/kg) for 14 days significantly reversed these alterations in a dose-dependent manner. Treated rats exhibited reduced fasting blood glucose, normalised lipid profiles, restored haematological parameters, and improved liver enzyme levels. Histopathological examination of pancreatic tissues revealed marked β-cell regeneration and restored islet architecture, comparable to glibenclamide-treated rats. These findings suggest that *B. aegyptiaca*-mediated ZnO-NPs possess potent antidiabetic, hepatoprotective, and haematoprotective effects. The synergistic action between zinc and plant-derived phytochemicals enhances therapeutic efficacy, providing an eco-friendly and cost-effective nanotherapeutic for diabetes management.

Keywords: Antidiabetic activity; *Balanites aegyptiaca*; Diabetes mellitus; Nanomedicine; Streptozotocin; Zinc oxide nanoparticles

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (ADA, 2023). The condition remains one of the most challenging global health issues, with increasing prevalence and associated morbidity and mortality, particularly in developing countries (Cho *et al.,* 2018). Persistent hyperglycemia is linked to multiple complications, including cardiovascular disease, neuropathy, nephropathy, and retinopathy, which significantly reduce patients' quality of life (Abou *et al.,* 2023). Despite the availability of several pharmacological agents such as metformin, sulfonylureas, and insulin, these therapies often produce adverse effects and may become less effective over time (Maritim *et al.,* 2003). Consequently, there is a growing interest in the development of safer, plant-based alternatives with improved efficacy and minimal toxicity.

Nanotechnology has emerged as a promising tool in biomedical science, providing novel strategies for disease diagnosis and therapy (Akhtar *et al.,* 2012). Among the different metal oxide nanoparticles, zinc oxide nanoparticles (ZnO-NPs) have gained significant attention due to their unique physical, chemical, and biological properties. Zinc plays a vital role in insulin synthesis, secretion, and action, while ZnO-NPs possess antioxidant, anti-inflammatory, and glucose-regulating potentials (Gotteparthi *et al.,* 2023). Conventional chemical synthesis methods for nanoparticles, however, often involve toxic reagents and generate hazardous byproducts. Green synthesis, which utilizes plant extracts as natural reducing and capping agents, offers a more eco-friendly, cost-effective, and biocompatible alternative (Nkemzi *et al.,* 2024).

Balanites aegyptiaca (L.) Delile, commonly known as desert date, is a medicinal plant traditionally used across Africa and Asia to treat diabetes, jaundice, and inflammation. The plant is rich in phytochemicals, including saponins, flavonoids, alkaloids, terpenoids, and phenolics, which contribute to its pharmacological activities (Chothani and Vaghasiya, 2011). Studies have shown that *B. aegyptiaca* extracts exhibit antihyperglycemic and antioxidant effects, improve lipid profiles, and restore pancreatic β-cell architecture in diabetic animal models (Bello *et al.,* 2019). The phytochemicals present in the extract not only confer antidiabetic effects but also facilitate the biosynthesis and stabilization of metal nanoparticles (Bello *et al.,* 2019).

Combining the medicinal properties of *B. aegyptiaca* with the physicochemical and therapeutic advantages of ZnO-NPs may enhance their biological efficacy through synergistic mechanisms. Such an approach could improve glucose metabolism, reduce oxidative stress, and support pancreatic regeneration. Therefore, this study aimed to synthesize zinc oxide nanoparticles using *Balanites aegyptiaca* fruit extract via a green route and to evaluate their antidiabetic, hypolipidemic, hepatoprotective, and hematological effects in streptozotocin-induced diabetic Wistar albino rats.

MATERIALS AND METHODS

Study Design

This study investigated the antidiabetic effects of zinc oxide nanoparticles (ZnO-NPs) synthesized using *Balanites aegyptiaca* fruit extract on streptozotocin (STZ)-induced diabetic Wistar albino rats. The experiment was conducted over 14 days, with rats divided into six groups (n = 6) to evaluate biochemical, haematological, and histopathological parameters.

Collection and Preparation of Plant Material

Fresh fruits of *Balanites aegyptiaca* were collected from Birnin Kebbi, Kebbi State, Nigeria, and authenticated at the Department of Plant Science, Federal University Birnin Kebbi. The fruits were thoroughly washed, air-dried at room temperature, and ground into fine powder using a mechanical grinder. Fifty grams (50 g) of the powdered sample was extracted with 500 mL of distilled water by boiling for 30 minutes. The extract was filtered using Whatman No.1 filter paper and stored at 4°C for further use.

Green Synthesis of Zinc Oxide Nanoparticles

For the synthesis, 50 mL of *Balanites aegyptiaca* extract was mixed with 100 mL of 0.01 M zinc oxide solution and an equimolar amount of NaOH dissolved in deionized water. The mixture was stirred continuously at 70°C until a homogeneous solution was obtained and then allowed to stand at room temperature for 1 hour to form a white precipitate. The precipitate was centrifuged at 5000 rpm for 15 minutes, washed three times with deionized/distilled water to remove soluble salts and plant residues, and twice with ethanol to eliminate organic impurities. The purified product was dried at 80°C and calcined at 400°C for 2 hours to obtain fine ZnO nanopowder (Dilaveez *et al.,* 2017).

Characterization of ZnO-NPs

The synthesized zinc oxide nanoparticles (ZnO-NPs) were subjected to various analytical techniques to confirm their formation, structural properties, and functional characteristics.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis was conducted to identify the functional groups present in the *Balanites aegyptiaca* extract that were involved in the reduction and stabilization of ZnO-NPs. The FTIR spectrum typically showed characteristic absorption bands corresponding to O–H stretching vibrations (around 3400 cm^{-1}), indicating the presence of

hydroxyl groups from alcohols and phenolic compounds. Peaks observed near 2920 cm^{-1} and 2850 cm^{-1} were attributed to C–H stretching vibrations of alkanes, while the bands around 1620–1650 cm^{-1} represented C=O stretching of amides or carbonyl groups. The absorption peaks near 1380 cm^{-1} and 1040 cm^{-1} corresponded to C–N stretching of amines and C–O stretching of alcohols, respectively.

A strong and broad band observed in the region of 400–600 cm^{-1} confirmed the presence of Zn–O stretching vibrations, which is a clear indication of the formation of ZnO nanoparticles. The shift or reduction in intensity of some functional group peaks after nanoparticle synthesis suggested that biomolecules in the plant extract acted as capping and stabilizing agents, preventing particle aggregation and enhancing nanoparticle stability.

X-ray Diffraction (XRD) Analysis

The crystalline structure and average particle size of the synthesized ZnO-NPs were determined using X-ray diffraction (XRD). The XRD pattern displayed distinct and sharp diffraction peaks at 2θ values around 31.7°, 34.4°, 36.2°, 47.5°, 56.6°, 62.8°, and 67.9°, corresponding to the (100), (002), (101), (102), (110), (103), and (112) planes of hexagonal wurtzite ZnO (JCPDS Card No. 36-1451). The sharpness and intensity of these peaks indicated a high degree of crystallinity of the synthesized nanoparticles.

Experimental Animals

Thirty-six healthy Wistar albino rats (150 to 200 g) of both sexes were obtained from the Animal House, Federal University Birnin Kebbi. The rats were acclimatized for two weeks under standard laboratory conditions (12-hour light/dark cycle, 25 ± 2°C, 50–60% humidity) and fed with standard pellets and water ad libitum. The experimental procedures were approved by the Institutional Animal Ethics Committee in accordance with international guidelines for the care and use of laboratory animals.

Induction of Diabetes

Diabetes mellitus was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) at 55 mg/kg body weight, dissolved in 0.1 M citrate buffer (pH 4.5). After 72 hours, fasting blood glucose (FBG) levels were measured using a glucometer. Rats with FBG ≥ 200 mg/dL were considered diabetic and selected for the study.

Experimental Grouping and Treatment

A total of twenty-four (24) adult Wistar albino rats (150–200 g) were used for the study. The animals were acclimatized for two weeks under standard laboratory conditions and provided standard feed and water *ad libitum*. Diabetes was induced by a single intraperitoneal injection of streptozotocin (50 mg/kg) prepared in 0.1 M citrate buffer (pH 4.5). Seventy-two hours post-induction, rats with fasting blood glucose levels above 200 mg/dL were considered diabetic and included for treatment. Due to post-induction casualties, a few animals were lost and replaced to maintain a minimum of three (3) rats per group. The animals were randomly divided into six groups (n = 3).

Biochemical Analyses

At the end of the treatment period, all animals were fasted overnight and sacrificed under light anesthesia. Blood samples were collected via cardiac puncture into plain and EDTA-coated tubes for serum and hematological analyses, respectively. Pancreatic tissues were also harvested for histopathological examination.

Lipid Profile

Serum was analyzed for total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) using enzymatic colorimetric methods with commercially available diagnostic kits (Randox Laboratories, UK). LDL levels were calculated using the Friedewald equation.

Liver Function Tests

Liver function markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, albumin, and total protein, were determined using standard spectrophotometric techniques with diagnostic reagent kits according to the manufacturer's instructions.

Haematological Parameters

Whole blood samples collected in EDTA tubes were analyzed for hematological indices such as red blood cells (RBC), white blood cells (WBC), hemoglobin (HGB), hematocrit (HCT), and mean corpuscular volume (MCV) using an automated hematology analyzer.

Histopathological Examination

Pancreatic tissues were fixed in 10% buffered formalin, dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax. Thin sections

(5 µm) were stained with hematoxylin and eosin (H&E) and examined under a light microscope for histoarchitectural changes in pancreatic islets.

Haematological Parameters

Whole blood samples were analyzed for hemoglobin concentration (Hb), packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), and differential leukocyte counts using an automated hematology analyzer.

Histopathological Examination

Pancreatic tissues were excised, fixed in 10% buffered formalin, and processed for histopathological analysis. Sections (5 µm) were stained with hematoxylin and eosin (H&E) and examined under a light microscope to assess histo-architectural changes and β-cell regeneration.

Data Analysis

All data were expressed as mean ± standard error of the mean (SEM). Statistical differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Statistical significance was set at $p < 0.05$.

RESULTS

Characterization of ZnO-NPs Using FTIR and XRD analysis

Fourier Transform Infrared (FTIR) spectroscopy was carried out to identify the functional groups present in the synthesized ZnO nanoparticles and to confirm the role of phytochemicals from *Balanites aegyptiaca* in their stabilization. The spectra revealed characteristic absorption bands corresponding to O–H, C=O, C–H, and N–H groups, indicating the presence of bioactive compounds acting as reducing and capping agents. A strong absorption band observed in the range of 450–550 cm^{-1} confirmed the stretching vibration of Zn–O bonds, validating the successful synthesis of ZnO nanoparticles (Figire 1).

XRD Analysis

X-ray Diffraction (XRD) analysis was performed to determine the crystalline structure and phase purity of the synthesized ZnO nanoparticles. The diffraction pattern showed distinct and sharp peaks corresponding to the hexagonal wurtzite structure of ZnO, consistent with standard JCPDS data. The crystallite size was calculated using the Debye–Scherrer equation, confirming the nanoscale dimension of the particles. The absence of additional peaks indicated high purity without significant impurities or secondary phases (Figire 2).

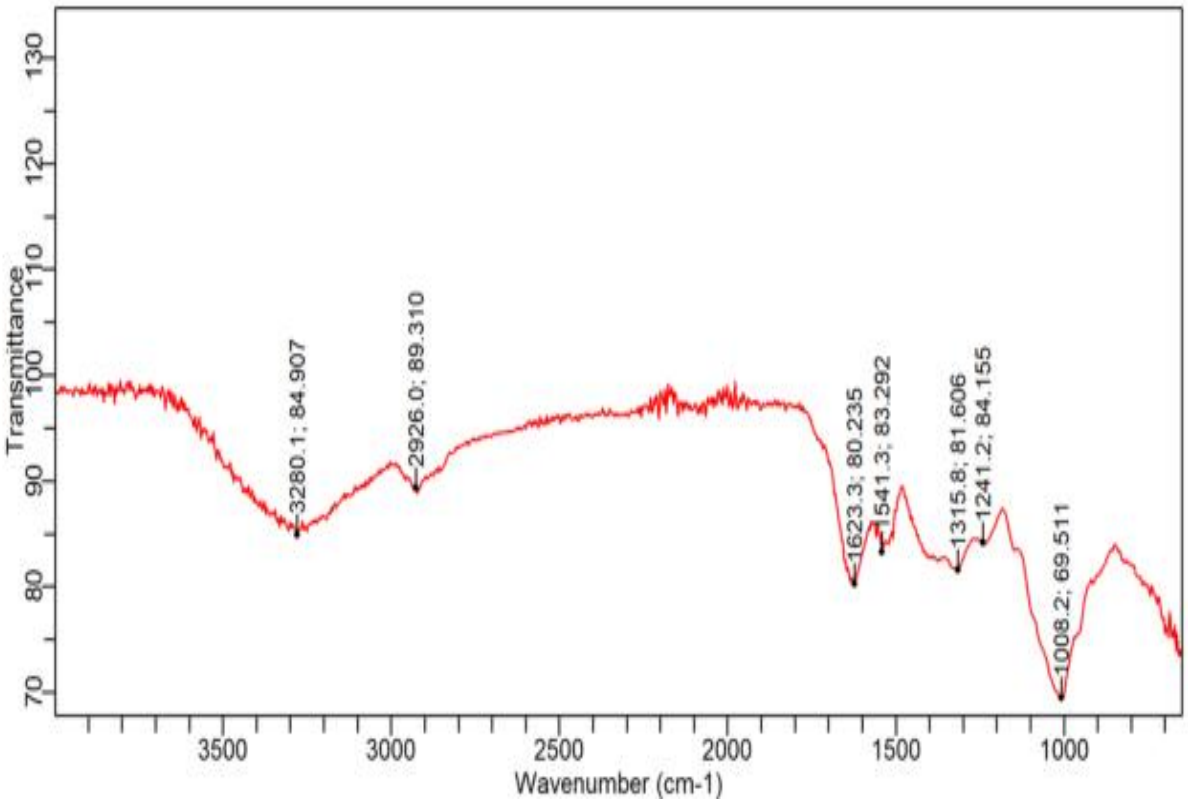

Figure 1. FTIR analysis of zinc oxide nanoparticles

The graphical representation of zinc oxide nanoparticles with absorption peaks of 3280.1 cm^{-1} (84.907), 2926.0 cm^{-1} (89.310), 1623.1 cm^{-1} (80.235), 1541.3 cm^{-1} (83.292), 1415.8 cm^{-1} (84.155), 1315.8 cm^{-1} (81.606), and 1008.2 cm^{-1} (69.511) associated with bending vibration of N–H, C–H, C=O, N–H, O–H, C–N, C–O of zinc oxide nanoparticles respectively.

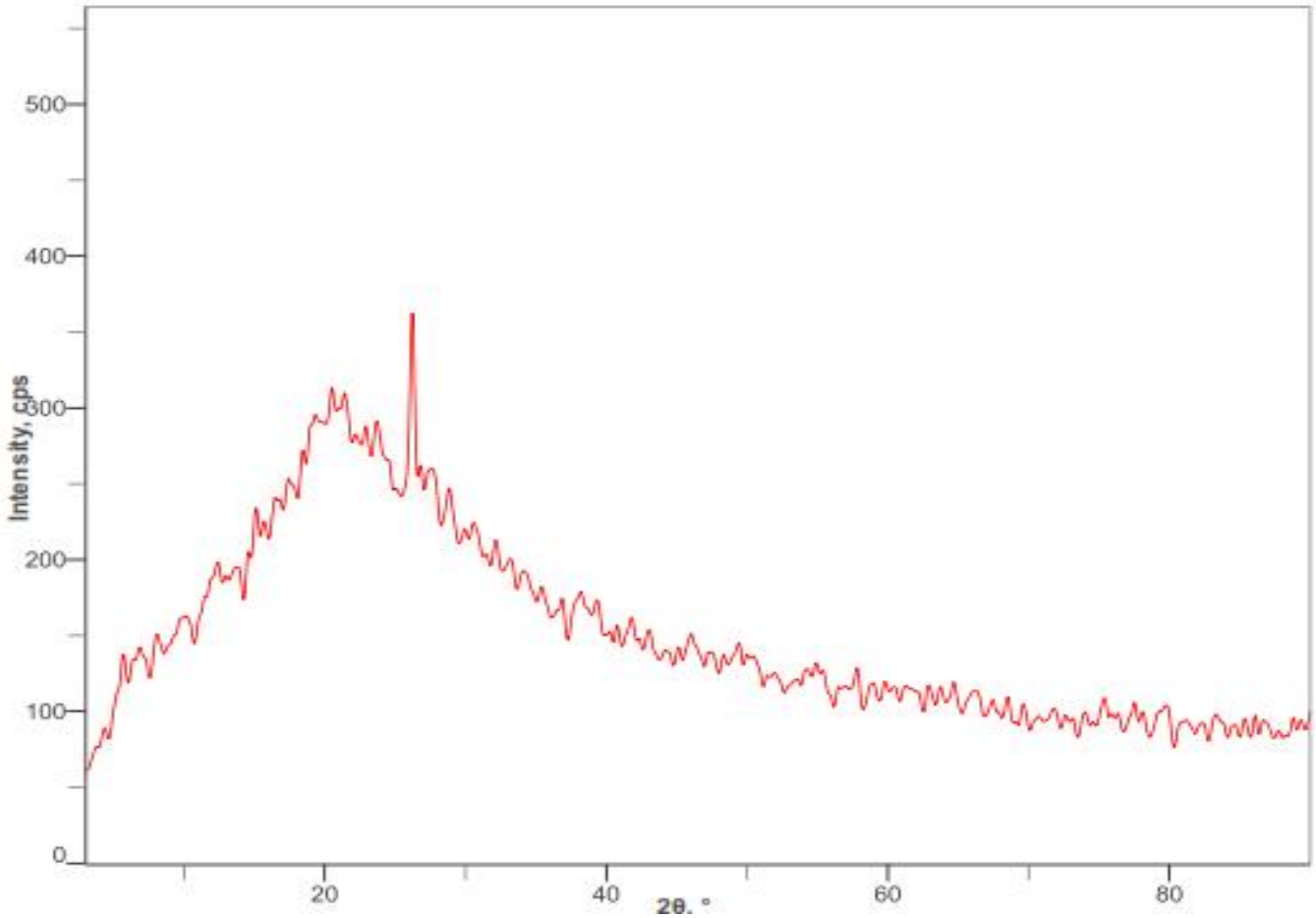

Figure 2. XRD analysis

5.176°, 3.905°, 3.400°, 2.651°**.** These peaks suggest the presence of a crystalline structure in the zinc oxide nanoparticles, where 17.13°, 22.77°, 26.21°, and 33.81° angles correspond to specific planes within the crystalline regions.

Effect of ZnO-NPs on Body Weight

Diabetic rats exhibited significant ($p < 0.05$) weight loss compared to normal controls. Treatment with ZnO-NPs significantly reversed the weight loss in a dose-dependent manner, similar to the glibenclamide group, suggesting improved metabolic status (Table 1).

Effect of ZnO-NPs on Fasting Blood Glucose Levels

Table 3.2 shows fasting blood glucose (FBG) levels of control and treated groups. Induction of diabetes with streptozotocin significantly ($p < 0.05$) increased FBG levels compared to the normal control. Administration of ZnO-NPs for 14 days caused a dose-dependent reduction in glucose levels. The 10 mg/kg ZnO-NP-treated group exhibited the most significant reduction, comparable to glibenclamide (Table 2).

3.5 Effect of ZnO-NPs on Lipid Profile

Table 3.3 presents serum lipid profile results. Streptozotocin-induced diabetes caused significant increases in total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL), with a reduction in high-density lipoprotein (HDL). Administration of ZnO-NPs significantly improved all lipid parameters, particularly at 10 mg/kg, restoring values close to normal control levels (Table 3).

Table 1. Effect of ZnO-NPs on Body Weight in STZ-Induced Diabetic Rats

Groups	Body weight (kg)		
	Week 0	Week 1	Week 2
Group 1 (Normal Control)	125.00 ± 2.00 [a]	169.73 ± 0.67[a]	186.29 ± 0.37[a]
Group 2 (Diabetic Control)	181.17 ± 1.04 [b]	170.50 ± 1.41[a]	165.30 ± 0.32[b]
Group 3 (Glibenclamide)	120.33 ± 0.58[c]	143.26 ± 3.00[b]	159.36 ± 0.43[c]
Group 4 (ZnO NPs 2.5mg/kg)	105.33 ± 1.53[d]	114.84 ± 0.66 [c]	118.97 ± 1.00 [d]
Group 5 (ZnO NPs 5 mg/kg)	108.33 ± 1.53[d]	127.14 ± 1.98[d]	133.96 ± 1.67[e]
Group 6 (ZnO NPs 10 mg/kg)	108.02 ± 0.97[d]	135.79 ± 1.19 [e]	147.33 ± 1.81 [f]

Values are expressed as mean ± standard deviation. Values with different superscripts down the column are statistically different

Table 2. Effect of ZnO-NPs on Fasting Blood Glucose Levels in STZ-Induced Diabetic Rats

Groups	Blood Glucose Level (mmol/L)		
	Week 0	Week 1	Week 2
Group 1 (Normal Control)	5.97 ± 0.32 [a]	5.50 ± 0.26[c]	5.30 ± 0.20[c]
Group 2 (Diabetic Control)	10.73 ± 0.80[ab]	14.57 ± 0.71[a]	16.60 ± 0.79[a]
Group 3 (Glibenclamide)	10.47 ± 0.75[ab]	6.10 ± 0.20[c]	4.83 ± 0.15[c]
Group 4 (ZnO NPs 2.5mg/kg)	11.27 ± 0.30[ab]	6.00 ± 0.98[c]	5.20 ± 0.70[c]
Group 5 (ZnO NPs 5 mg/kg)	14.27 ± 4.30 [b]	10.87 ± 1.63[b]	9.60 ± 1.42[b]
Group 6 (ZnO NPs 10 mg/kg)	11.03 ± 1.89[ab]	9.53 ± 1.32[b]	7.80 ± 0.61[b]

Values are expressed as mean ± standard deviation. Values with different superscripts down the column are statistically different

Table 3. Effect of ZnO-NPs on Lipid Profile in STZ-Induced Diabetic Rats

Groups	Lipid Profile Parameters (mg/dl)			
	Cholesterol	Triglyceride	HDL	LDL
Group 1 (Normal Control)	81.37 ± 0.23[b]	90.99 ± 0.28[b]	79.07 ± 0.49[d]	46.98 ± 0.72[b]
Group 2 (Diabetic Control)	183.51 ± 0.43[c]	171.11 ± 0.32[c]	24.64 ± 0.05[c]	166.35 ± 0.58[d]
Group 3 (Glibenclamide)	105.57 ± 0.14[e]	100.41 ± 0.49[d]	55.92 ± 0.43[b]	71.14 ± 0.52[f]
Group 4 (ZnO NPs 2.5mg/kg)	149.41 ± 0.89[f]	141.17 ± 1.02[f]	46.36 ± 1.32[a]	84.06 ± 1.82[e]
Group 5 (ZnO NPs 5 mg/kg)	118.81 ± 1.14[a]	127.28 ± 1.05[a]	59.11 ± 2.29[e]	50.63 ± 2.20[a]
Group 6 (ZnO NPs 10 mg/kg)	92.53 ± 1.15[d]	103.89 ± 0.37[e]	73.27 ± 2.08[c]	37.91 ± 0.48[c]

Values are expressed as mean ± standard deviation. Values with different superscripts down the column are statistically different.
HDL = High-density lipoprotein;
LDL = Low-density lipoprotein

Liver Function Parameters

As shown in Table 4, diabetic rats had significantly elevated serum ALT, AST, and ALP levels, indicating hepatic dysfunction. Treatment with ZnO-NPs significantly reduced these enzyme levels toward normal, suggesting hepatoprotective effects.

Haematological Parameters

The haematological assessment revealed significant alterations in diabetic and treated groups. Diabetic control rats exhibited a marked decrease in red blood cell (RBC) count, hemoglobin (HGB), and hematocrit (HCT) values, indicating anemia commonly associated with hyperglycemia. Conversely, white blood cell (WBC) counts were significantly elevated relative to the normal control group, reflecting possible inflammatory or immune responses to diabetic stress. Treatment with ZnO-NPs and glibenclamide significantly restored hematological indices toward normal levels. Rats treated with ZnO-NPs, particularly at 5 mg/kg and 10 mg/kg, showed significantly higher RBC, HGB, and HCT values ($p < 0.05$) compared to the diabetic control, indicating improvement in erythropoietic activity and oxygen-carrying capacity. WBC levels

were significantly lower ($p < 0.05$) in treated groups than in the diabetic control, suggesting attenuation of systemic inflammation. Mean corpuscular volume (MCV) showed no significant difference among groups, implying stable red cell morphology (Table 5).

Histopathological Findings

Histological examination of pancreatic tissues (Figure 3) revealed normal pancreatic architecture in the control group, while the diabetic control showed severe necrosis and degeneration of pancreatic β-cells. Treatment with ZnO-NPs, particularly at 10 mg/kg, resulted in marked regeneration of β-cells and restoration of islet morphology, comparable to the glibenclamide-treated group.

Table 4. Effect of ZnO-NPs on Liver Function Markers

Groups	Liver Function Parameters				
	AST (u/l)	ALT (u/l)	Total Bilirubin (mg/dL)	Albumin (g/L)	Total Protein (g/dL)
Group 1 (Normal Control)	18.18 ± 0.03^{f}	17.54 ± 0.42^{e}	0.67 ± 0.01^{e}	41.07 ± 0.41^{a}	9.81 ± 0.35^{a}
Group 2 (Diabetic Control)	76.63 ± 0.34^{a}	88.66 ± 0.34^{a}	2.34 ± 0.04^{a}	16.10 ± 0.23^{e}	4.31 ± 0.38^{d}
Group 3 (Glibenclamide)	32.01 ± 0.58^{d}	30.38 ± 0.38^{d}	0.99 ± 0.01^{d}	33.34 ± 0.09^{c}	8.96 ± 0.41^{b}
Group 4 (ZnO NPs 2.5mg/kg)	53.74 ± 1.46^{b}	50.25 ± 2.69^{b}	1.86 ± 0.03^{b}	31.46 ± 0.43^{d}	6.21 ± 0.13^{c}
Group 5 (ZnO NPs 5 mg/kg)	37.83 ± 1.01^{c}	38.22 ± 1.78^{c}	1.18 ± 0.09^{c}	39.68 ± 0.39^{b}	6.97 ± 0.16^{c}
Group 6 (ZnO NPs 10 mg/kg)	20.76 ± 0.89^{e}	20.37 ± 0.58^{e}	0.87 ± 0.03^{d}	41.15 ± 0.56^{a}	9.43 ± 0.14^{a}

Values are expressed as mean ± standard deviation. Values with different superscripts down the column are statistically different.

AST = Aspartate aminotransferase;

AlT = Alanine aminotransferase;

Table 5. Effect of ZnO-NPs on Hematological Parameters

Groups	Full Blood Count				
	WBC (x10^{9}/L)	RBC (x10^{12}/L)	HGB (g/dL)	HCT (%)	MCV (fL)
Group 1 (Normal Control)	5.27 ± 0.06^{b}	4.82 ± 0.08^{bc}	10.67 ± 0.15^{d}	34.80 ± 0.20^{d}	72.13 ± 1.18^{a}
Group 2 (Diabetic Control)	6.50 ± 0.10^{a}	3.83 ± 0.10^{c}	8.37 ± 0.31^{e}	26.73 ± 0.97^{e}	70.07 ± 0.85^{a}
Group 3 (Glibenclamide)	4.94 ± 0.06^{bc}	5.92 ± 0.08^{ab}	11.50 ± 0.30^{c}	37.00 ± 0.50^{c}	62.53 ± 0.15^{c}
Group 4 (ZnO NPs 2.5mg/kg)	5.33 ± 0.25^{b}	6.59 ± 0.09^{a}	12.47 ± 0.17^{b}	41.40 ± 0.50^{b}	65.37 ± 0.15^{b}
Group 5 (ZnO NPs 5 mg/kg)	3.93 ± 0.15^{d}	5.9 ± 1.06^{ab}	11.55 ± 0.50^{c}	45.03 ± 0.99^{a}	65.20 ± 1.44^{b}
Group 6 (ZnO NPs 10 mg/kg)	4.63 ± 0.31^{c}	7.11 ± 0.17^{a}	13.57 ± 0.23^{a}	43.79 ± 0.30^{a}	62.43 ± 0.83^{c}

Values are expressed as mean ± standard deviation. Values with different superscripts down the column are statistically different.

WBC = White Blood Cells,

RBC = Red Blood Cells,

HGB = Haemoglobin

HCT = Haematocrit,

MCV = Mean Corpuscular Volume.

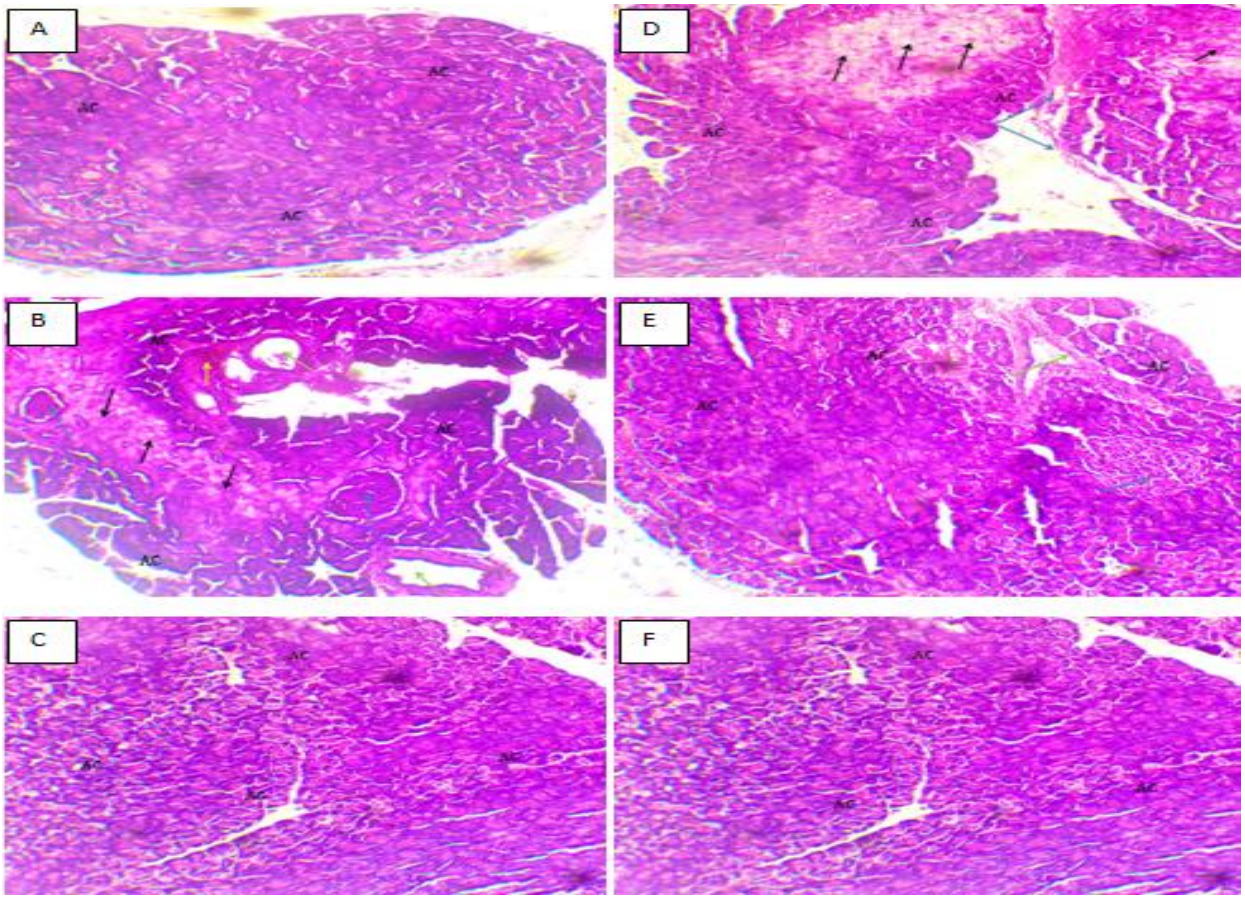

Figure 3. Histopathology of pancreas tissue (H&E stains at X100)

(**a**) **Normal Control** showed normal architecture closely packed serous acini (ac) and islet of Langerhans – (blue arrow) containing α-cells and β-cells (**b**) **Untreated Control** showed architecture packed serous acini (ac) and islet of Langerhans (blue arrows) - containing α-cells and β-cells, Blood vessel (green arrow). Serous acinar degeneration, atrophy, and vacuolation (black arrow), Inflammatory cell infiltration (yellow arrow). (**c**) **Glinbenclamide** showed normal architecture, packed serous acini (**d**) **2.5mg/kg,** showing normal architecture, closely packed serous acini (ac). Serous acinar atrophy (black arrow), dilation of interlobular connective tissue septa(blue arrow) (ac). (**e**) **5mg/kg** showed a normal architecture, closely packed serous acini (ac) and islet of Langerhans (blue arrow) - containing α-cells and β-cells, blood vessel (green arrow). (**f**) **10mg/kg** showed normal architecture, packed serous acini (ac)

DISCUSSION

The present study evaluated the anti-hyperglycemic effect of zinc oxide nanoparticles (ZnO-NPs) synthesized using *Balanites aegyptiaca* fruit pulp extract on streptozotocin (STZ) nicotinamide-induced diabetic Wistar rats. The findings demonstrate that the green-synthesized nanoparticles possess significant therapeutic potential, as evidenced by improvements in blood glucose regulation, lipid metabolism, liver function, hematological balance, and pancreatic histoarchitecture.

The FTIR spectrum revealed absorption bands corresponding to O–H, C–H, C=O, N–H, and C–O stretching vibrations, indicating the presence of phytochemicals (saponins, flavonoids, tannins, and proteins) from *B. aegyptiaca* on the nanoparticle surface. The ZnO stretching vibration observed around 500 – 600 cm^{-1} confirmed the formation of ZnO nanoparticles. This suggests that plant bioactive molecules acted as both reducing and stabilizing agents, consistent with earlier reports on plant-mediated synthesis of ZnO NPs (Gomathi *et al.,* 2020).

XRD analysis showed distinct diffraction peaks corresponding to crystalline ZnO with a hexagonal wurtzite structure, indicating high crystallinity and purity. Similar patterns were reported by Yousef and Ismail, 2024, confirming that green synthesis produces highly pure, nanosized ZnO with strong biological activity. The crystalline nature of the nanoparticles is significant because smaller, well-structured ZnO NPs are associated with higher surface area and enhanced bioavailability, improving their biological interactions and antidiabetic potential (Kumar *et al.,* 2023).

In the diabetic control rats, significant body-weight loss was observed a hallmark of uncontrolled diabetes resulting from increased protein and fat catabolism in the absence of effective insulin activity. Treatment with ZnO-NPs led to a progressive increase in body weight, with the highest dose restoring weight to near-normal levels. Similar trends have been reported in ZnO-treated diabetic rats, indicating improved metabolic efficiency and restoration of anabolic processes (Bai *et al.,* 2022).

A dose-dependent reduction in fasting blood glucose levels was observed in ZnO-NP-treated rats, with the highest dose showing comparable efficacy to glibenclamide. This finding is in agreement with Alkaladi *et al.* (2014), who reported significant hypoglycemic effects of ZnO NPs in STZ-induced diabetic models. The antidiabetic activity of ZnO-NPs can be attributed to zinc's role as a cofactor in insulin biosynthesis and storage, its ability to protect and regenerate pancreatic β-cells, and its enhancement of glucose uptake in peripheral tissues (Alkaladi *et al.* (2014). Thus, ZnO-NPs synthesized from *B. aegyptiaca* demonstrate potent anti-hyperglycemic effects and may serve as a cost-effective alternative to conventional therapy.

Diabetic control rats exhibited dyslipidemia, characterized by elevated total cholesterol, triglycerides, and LDL levels with reduced HDL concentrations. ZnO-NP treatment significantly normalized these parameters, reflecting improved lipid metabolism. This aligns with previous reports where both *B. aegyptiaca* extracts and ZnO NPs improved lipid profiles by enhancing antioxidant activity and modulating lipid peroxidation (Yousaf, 2024). This improvement suggests that ZnO-NPs may offer cardioprotective benefits alongside glycemic regulation.

Elevated ALT, AST, and bilirubin levels observed in untreated diabetic rats confirmed hepatic injury due to hyperglycemia and oxidative stress. Treatment with ZnO-NPs significantly reduced these enzyme levels, indicating hepatoprotective effects. Similar outcomes were reported by Abd El-Baset *et al.* (2023), who demonstrated that ZnO-NPs ameliorated diabetic hepatotoxicity and improved hepatic architecture. The stabilization of liver enzymes implies that ZnO-NPs enhance hepatic glucose metabolism while minimizing oxidative damage, underscoring their biochemical safety and therapeutic potential.

Diabetes-induced haematological alterations, including decreased RBC, hemoglobin, and haematocrit values and elevated WBC counts, were evident in untreated diabetic rats consistent with anemia and systemic inflammation (Ashfaq *et al.,* 2023). ZnO-NP treatment restored these indices toward normal levels, indicating improved hematopoietic function and reduced inflammatory response. Comparable hematological recovery has been documented in ZnO-treated diabetic animal models (Yousaf, 2024; Bai *et al.,* 2022), supporting the hematoprotective role of the nanoparticles.

Histopathological examination of pancreatic tissues revealed necrosis and vacuolation of β-cells in untreated diabetic rats, consistent with the cytotoxic effects of STZ. However, ZnO-NP-treated groups, especially those receiving 5 mg/kg and 10 mg/kg, showed restoration of islet cell integrity and reduced inflammatory infiltration. The high-dose ZnO-NP group displayed nearly normal pancreatic morphology comparable to glibenclamide treatment. Similar histological recovery has been observed in ZnO-NP-treated diabetic models, confirming β-cell regeneration and islet repair (Yousaf, 2024).

Overall, the biochemical and histopathological findings demonstrate that ZnO nanoparticles synthesized from *B. aegyptiaca* fruit pulp exert potent antidiabetic effects through reduction of hyperglycaemia, restoration of lipid and liver function profiles, stabilization of haematological indices and regeneration of pancreatic β-cells.

The dual contribution of Zn, an essential trace element in insulin metabolism, and the bioactive phytochemicals of *B. aegyptiaca* likely explains the enhanced efficacy observed. Moreover, the green-synthesis approach ensures an eco-friendly, cost-

effective, and locally sustainable method for nanoparticle production with potential biomedical applications.

CONCLUSION

This study demonstrated that zinc oxide nanoparticles (ZnO-NPs) synthesized using *Balanites aegyptiaca* fruit pulp extract exhibit significant antidiabetic potential in streptozotocin–nicotinamide-induced diabetic rats. The green synthesis approach yielded stable, crystalline ZnO-NPs capped with bioactive phytochemicals, enhancing their biological activity. Treatment with ZnO-NPs effectively reduced blood glucose levels, improved lipid and liver function profiles, normalized hematological parameters, and restored pancreatic architecture. These findings suggest that *B. aegyptiaca*-mediated ZnO-NPs hold promise as an eco-friendly and cost-effective nanotherapeutic agent for diabetes management.

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