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GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM OKRA (*ABELMOSCHUS ESCULENTUS*) EXTRACT AND THEIR ANTI-DIABETIC EFFICACY IN STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

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ABSTRACT

Diabetes mellitus is a global healthcare problem that deserves new therapies. Green synthesis of silver nanoparticles (AgNPs) from okra (Abelmoschus esculentus) pod aqueous extract is a new anti-diabetic therapy. This study synthesized and characterized okra-mediated AgNPs and evaluated their anti-diabetic activity in streptozotocin-induced diabetic Wistar rats compared to okra extract and metformin. AgNPs, synthesized by mixing okra extract with 5 mM AgNO3, were identified by UV-Vis spectroscopy (450 nm absorbance), FTIR, XRD, and TEM as spherical nanoparticles (mean 18.01 nm). Thirty-five Wistar male rats were divided into seven groups (n=5): non-diabetic control, diabetic control, and diabetic groups administered okra extract (1 mL/kg), AgNPs (0.2 mL/kg), AgNO3 (1 mL/kg), and metformin (500 mg/kg) via oral gavage for four weeks. AgNPs and okra extract also reduced fasting blood glucose (34.1% and 45.9%, respectively) and HbA1c (up to 65.1%) compared to diabetic controls ($p<0.05$). They also increased superoxide dismutase and glutathione while decreasing malondialdehyde, indicating amelioration of oxidative stress. AgNPs had anti-diabetic parameters comparable to metformin and hence a potential candidate that warrants further mechanistic and clinical studies for a green, potent anti-diabetic drug.

Keywords: Silver Nanoparticles, *Abelmoschus esculentus*, Diabetes Mellitus, Anti-Diabetic Activity, Streptozotocin

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by impaired insulin secretion or utilization, resulting in defective glucose homeostasis and pancreatic β-cell dysfunction (Antar et al., 2023). Diabetes mellitus imposes a tremendous burden on global public health, as the International Diabetes Federation (IDF) estimated that 537 million adults had diabetes in 2021, and this is projected to rise to 783 million by 2045 (Hossain et al., 2024). Diabetes mellitus is classified as type 1 diabetes mellitus (T1DM, 5–10% of the cases, resulting from autoimmune destruction of insulin-producing β-cells), type 2 diabetes mellitus (T2DM, ~90% of the cases, characterized by insulin resistance), and gestational diabetes (Choudhury & Devi Rajeswari, 2021). Current treatments, such as insulin therapy and oral hypoglycemic medications, are often hampered by side effects, variable efficacy, and lack of patient adherence, necessitating new therapeutic strategies (Suprapti et al., 2023).

Nanotechnology has transformed the area of diabetes mellitus therapy by allowing the development of nanoparticles (1–100 nm) with unique physicochemical properties, such as high surface area and enhanced bioavailability (Simos et al., 2021). Silver nanoparticles (AgNPs) have drawn much attention due to their broad spectrum of biomedical applications, including anti-diabetic, anticancer, and antibacterial activities (Jangid et al., 2024; Meher et al., 2024). Green synthesis of AgNPs using plant extracts is a non-toxic, cost-effective alternative to chemical synthesis, where phytochemicals act as reducing and stabilizing agents (Fahim et al., 2024). Okra (*Abelmoschus esculentus*), a medicinal plant containing bioactive molecules like flavonoids, phenolic acids, and polysaccharides, has been reported to possess antihypertensive, antimicrobial, and anti-diabetic properties (Elkhalifa et al., 2021; Kwok et al., 2025). These phytochemicals can be used to enhance the therapeutic potential of AgNPs by increasing stability and bioactivity (Liu et al., 2023).

Therefore, this study aimed to:

i. Synthesize and characterize AgNPs using aqueous extract of okra pods via a green synthesis method.
ii. Evaluate the anti-hyperglycemic effects of the synthesized AgNPs in STZ-induced diabetic rats.
iii. Compare the efficacy of the AgNPs with crude okra extract, metformin, and $AgNO_3$ control.
iv. Investigate the potential mechanism of action by assessing oxidative stress markers and metabolic parameters.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh okra (Abelmoschus esculentus) pods were procured from Waso Market, Ogbomoso, Nigeria (longitude 4°15'27.61"E, latitude 8°9'56.96"N) in March 2023. Pods were authenticated by a botanist at the Department of Plant Biology, Ladoke Akintola University of Technology (LAUTECH; Voucher No. LAU/2023/012). Pods were washed under tap water to remove debris, rinsed with distilled water, and air-dried at 25–28°C for 14 days. Dried pods were ground into a fine powder using an electric blender (Philips HR2056, 350 W) and stored in an airtight glass container at room temperature. For extract preparation, 5 g okra powder was dissolved in 200 mL distilled water for 24 hours at 25°C, filtered through Whatman No. 1 filter paper (pore size 11 µm), and the filtrate was stored at 4°C for up to 48 hours before use.

Biosynthesis of Silver Nanoparticles

Silver nanoparticles (AgNPs) were synthesized through dropwise addition of 4 mL of 5 mM silver nitrate (AgNO3,

Sigma-Aldrich, purity ≥99%) to 36 mL of okra aqueous extract under vigorous stirring (800 rpm) at 25°C for 3 hours. AgNPs formation was indicated through color change from pale brown to yellowish-brown (Jalab et al., 2021). The solution was centrifuged at 10,000 rpm for 15 minutes (Eppendorf 5810R centrifuge), and the pellet was washed three times using distilled water to remove unreacted components. The purified AgNPs were dried in a hot-air oven at 60°C for 12 hours and stored in a desiccator at room temperature for characterization. The yield and stability of AgNPs were analyzed using UV-Vis spectroscopy for 7 days to verify consistency. The percentage yield of AgNPs were not quantified during this experiment, as it is addressed in the limitation.

Characterization of Silver Nanoparticles (AgNPs)

The synthesized silver nanoparticles (AgNPs) were characterized to determine their optical, chemical, structural, and morphological properties using a series of sophisticated analytical techniques, as described below:

UV-Visible Spectroscopy

Optical properties of AgNPs were evaluated using UV-Visible spectroscopy to determine the presence of the characteristic surface plasmon resonance (SPR) band. Absorption spectra were recorded within the range of 300–800 nm on a PerkinElmer ANALYST 4000 double-beam spectrophotometer (PerkinElmer, Inc., Waltham, MA, USA) having a spectral resolution of 1 nm (Mahmudin et al., 2015). The measurement was performed in 1 cm path length quartz cuvettes, and deionized water was used as the blank.

Fourier-Transform Infrared Spectroscopy (FTIR)

To identify the functional groups responsible for the reduction and stabilization of AgNPs, Fourier-Transform Infrared (FTIR) spectroscopy was carried out. Spectra were measured using a PerkinElmer Spectrum Two FTIR spectrometer (PerkinElmer, Inc., Waltham, MA, USA) in the wavenumber range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} (Balčiūnaitienė et al., 2022). For sample preparation, AgNPs were blended with potassium bromide (KBr) to form pellets, enabling minimum interference and clear spectra.

X-Ray Diffraction (XRD)

The crystalline structure and phase purity of AgNPs were identified by X-ray diffraction (XRD). The analyses were performed on a Bruker D8 Advance diffractometer (Bruker Corporation, Billerica, MA, USA) using Cu Kα radiation (λ = 1.5406 Å) at 40 kV and 40 mA (Ali et al., 2023). Diffraction patterns were collected at a 2θ range of 10°–80° with a step size of 0.02° and a scanning speed of 0.5°/min. The patterns thus obtained were matched with the standard reference data to confirm the crystallographic nature of the nanoparticles.

Transmission Electron Microscopy (TEM)

The morphology, size, and size distribution of AgNPs were analyzed using transmission electron microscopy (TEM). Micrographs were recorded on a JEOL JEM-2100 transmission electron microscope (JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 200 kV (Kubheka et al., 2024). The samples were prepared by dropping diluted AgNP suspension onto carbon-coated copper grids (200 mesh) and air-drying at room temperature. Particle shape and size were established from high-resolution images.

Animal Studies

Experimental Animals

Thirty-five male Wistar rats (110–185 g, 2–3 months old) were obtained from the Animal House Facility at LAUTECH, Ogbomoso, Nigeria. Rats were acclimatized for 7 days in stainless steel cages under controlled conditions (12-h light/dark cycle, 20–22°C, 45–55% humidity) with ad libitum access to standard rodent chow (Ladokun Feeds, Nigeria) and filtered water. All procedures were in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals and approved by the LAUTECH Animal Ethics Committee (Approval No. LAUTECH/2023/03/015, dated 15th February 2023).

Induction of Diabetes

Diabetes was induced in 30 rats via a single intraperitoneal injection of streptozotocin (STZ, Sigma-Aldrich, ≥98% purity, 70 mg/kg body weight) dissolved in 0.1 M citrate buffer (pH 4.5). Five rats received citrate buffer alone as non-diabetic controls (Group A). After 7 days, fasting blood glucose (FBG) levels were measured via tail vein blood using an Accu-Check Active glucometer (Roche Diagnostics, Dubai, UAE). Rats with FBG >200 mg/dL were confirmed diabetic (Ghasemi & Jeddi, 2023).

Experimental Design

Male Wistar rats were allocated randomly to seven experimental groups (n=5 each group) for evaluating the effects of various treatments in diabetic models. Group A (non-diabetic control) received saline (1 mL/kg). Group B consisted of diabetic rats treated with silver nanoparticles (AgNPs) at 0.5 mg/kg, administered as 0.2 mL/kg from a 2.5 mg/mL stock suspension. Group C (diabetic control) received saline (1 mL/kg). Group D received okra extract at 100 mg/kg, administered as 1 mL/kg from a 100 mg/mL stock solution. Group E received silver nitrate ($AgNO_3$, 5 mM), equivalent to 0.85 mg/kg Ag, administered as 1 mL/kg of the prepared solution. Group F received AgNPs at 5 mg/kg, administered as 2.0 mL/kg from the 2.5 mg/mL stock. Group G received metformin at 500 mg/kg, administered as 1 mL/kg from a 500 mg/mL stock solution. All treatments were administered once daily by oral gavage for 4 weeks (500 mg/kg, Sigma-Aldrich, St. Louis, MO, USA) as a positive control. Treatments were administered daily by oral gavage using a stainless steel cannula for 4 weeks. Body weight and FBG levels were measured weekly at 8:00 AM following a 12-hour overnight fast to monitor the physiological response of the treatments (Adeoye et al., 2017).

Fasting Blood Glucose and Oral Glucose Tolerance Test (OGTT)

FBG was measured at weeks 0, 2, and 4 using the Accu-Check Active glucometer. For OGTT (week 4), rats were overnight fasted (12 hours) and administered 2 g/kg glucose orally (Stephen et al., 2025). Blood glucose was collected at 0, 30, 60, 90, and 120 minutes' post-glucose loading by tail vein blood.

Table 1: Oral Glucose Tolerance Test (OGTT) Results at 30-, 60-, 90-, And 120-Minutes Post-Glucose Load (2 G/Kg)

	Time(Min)			
Group	**30**	**60**	**90**	**120**
A	72.50±2.5	75.75±2.51	47.00±2.7	59.50±2.0
B	363.50±7.3	371.20±12.6	298.50±10.1	278.25±9.2
C	270.75±9.0	321.00±10.7	273.50±9.2	246.50±8.4
D	245.00±8.1	275.25±9.7	187.75±10.9	165.00±6.9
E	200.75±5.8	199.75±8.8	185.75±8.1	159.25±8.2
F	333.75±11.3	398.00±13.8	194.75±11.2	153.25±8.8
G	256.25±11.0	261.25±11.7	229.50±11.3	191.50±9.9

Biochemical and Oxidative Stress Analysis

Rats were anaesthetized at the end of week 4 using ketamine/xylazine (80/10 mg/kg, intraperitoneal) and euthanized via cervical dislocation. Blood was collected by cardiac puncture into EDTA tubes for plasma analysis. Plasma insulin, urea, and HbA1c were quantified using ELISA kits (Abcam, Cambridge, UK; Catalog Nos. ab277390, ab83362, ab285294). Liver glycogen was quantified using a colorimetric assay kit (Sigma-Aldrich, MAK016). Pancreatic tissue was homogenized in phosphate-buffered saline (pH 7.4) for the assay of superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA) levels using Cayman Chemical kits (Catalog Nos. 706002, 703002, 10009055).

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM, n=5 per group). Statistical analysis was performed using GraphPad Prism 9.0 (GraphPad Software, USA). One-way analysis of variance (ANOVA) followed by Tukey's post-test was used for group comparison. Significance was evaluated at $p<0.05$.

RESULTS AND DISCUSSION

Characterization of Silver Nanoparticles (AgNPs)

The UV-Vis spectrum of AgNPs revealed a surface plasmon resonance (SPR) peak typical at 450 nm, confirming the synthesis of unoxidized silver nanoparticles (Figure 1). The peak is consistent with the optical properties of colloidal silver nanoparticles synthesized by green methods, indicating successful reduction of AgNO3 by phytochemicals in okra extract (Wan Mat Khalir et al., 2020). The SPR peak at 450 nm reveals a uniform particle size distribution, in accordance with successful nanoparticle synthesis.

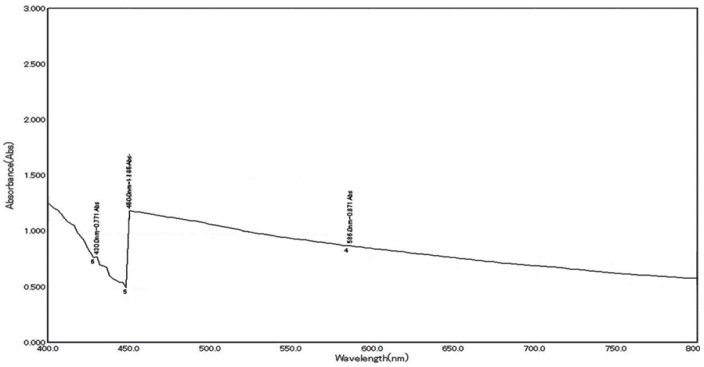


Figure 1: UV-Vis Spectrum of Agnps

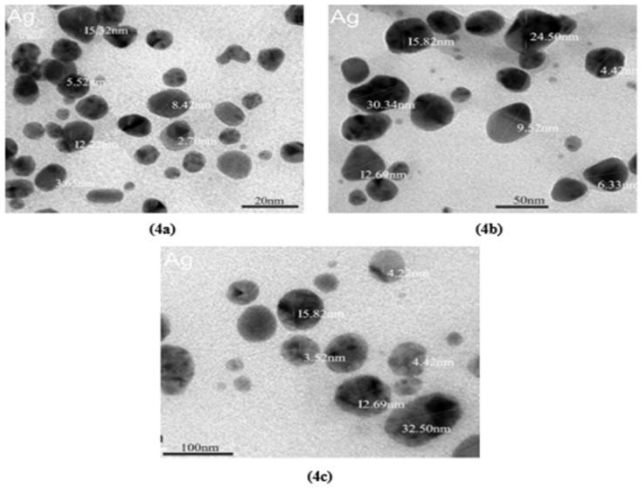
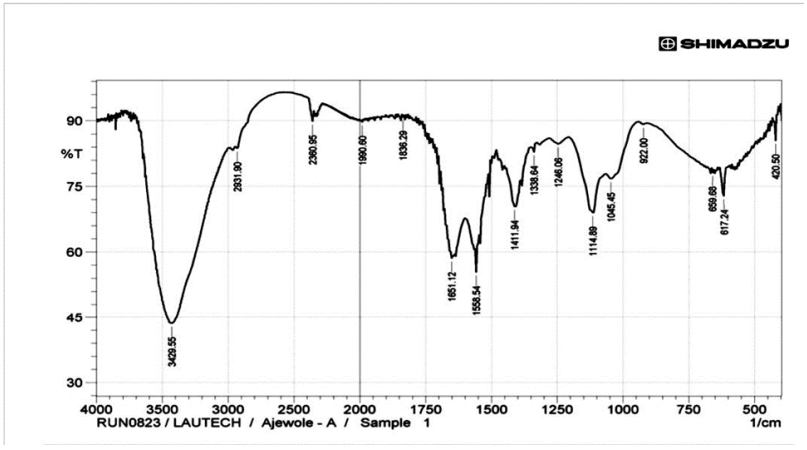


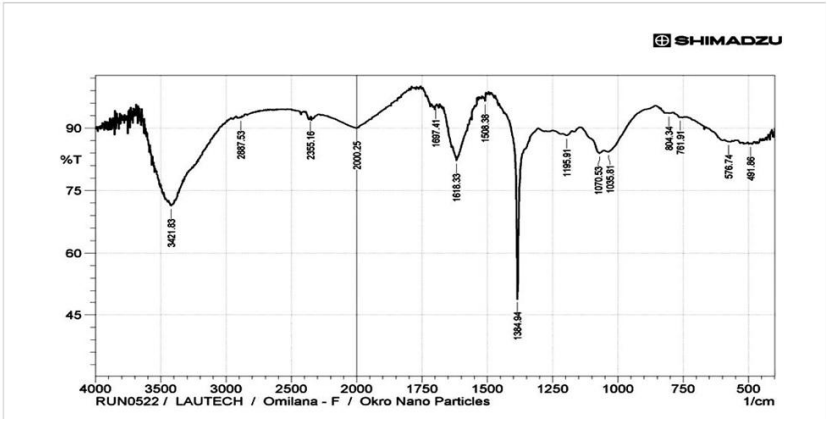
Figure 2: TEM Images of Agnps at (A) 20 Nm, (B) 50 Nm, And (C) 100 Nm Magnifications

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of okra extract showed prominent functional groups of bioactive compounds responsible for the synthesis and stabilization of silver nanoparticles (AgNPs). Peaks at 3429.55 cm^{-1} (O-H stretching), 2360.95 cm^{-1} (O=C=O stretching), 1651.12 cm^{-1} (C-H aromatic bending), 1558.54 cm^{-1} (N-O stretching, nitro compounds), and 1114.89 cm^{-1} (C-O stretching, secondary alcohols) were characteristic of flavonoids and phenolic acids, which are reducing and stabilizing agents (Zahra et al., 2024). These phytoconstituents present in the okra extract facilitate the reduction of silver ions and stabilize the synthesized AgNPs. Complementing this, the FTIR spectra of AgNPs showed absorption peaks at 3421.83 cm^{-1} (stretching of O-H, alcohols/phenols), 1697.41 cm^{-1} (bending of C-H aromatic), 1618.33 cm^{-1} (conjugated alkene C=C), and 1384.94 cm^{-1} (bending of O-H, alcohols), confirming the use of phytochemicals like phenolic acids and flavonoids as reducing and capping agents (Velgosova et al., 2024). The presence of them proves that okra extract bioactive compounds are important in enhancing the biocompatibility and therapeutic uses of AgNPs by efficient reduction of silver ions and nanoparticle stabilization (Figure 2).



(3a)



(3b)

Figure 3: FTIR Spectra of (a) Okra Extract and (b) AgNPs

X-Ray Diffraction (XRD)

XRD patterns of AgNPs exhibited Bragg reflections at 2θ values of 38.1°, 44.3°, 64.4°, and 77.4° corresponding to (111), (200), (220), and (311) planes of a face-centered cubic (FCC) crystalline structure, respectively (Figure 3). These peaks were in agreement with the typical Joint Committee on Powder Diffraction Standards (JCPDS) card No. 04-0783, which confirms the crystalline nature of AgNPs. The crystallite size, as calculated by the Debye-Scherrer equation, was 18 nm, in agreement with prior observations for plant-mediated AgNPs (Ganguli et al., 2023). The sharp diffraction peaks indicate a high degree of crystallinity, which can be accountable for the nanoparticles' stability and bioactivity.

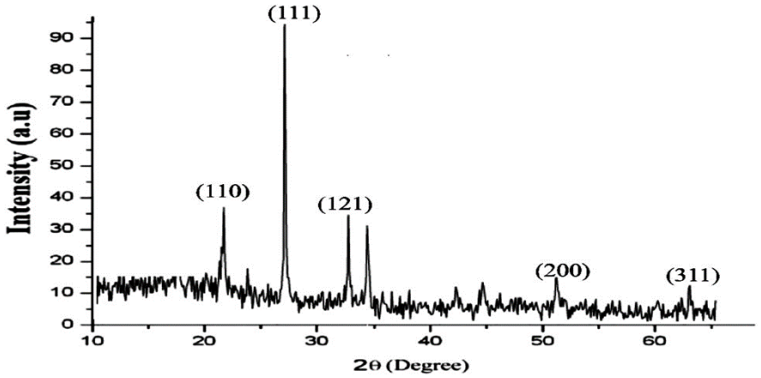


Figure 4: XRD Pattern of Agnps

Transmission Electron Microscopy (TEM)

TEM images revealed monodispersed spherical AgNPs of different sizes in the range of 10 nm to 50 nm and minimal aggregation (Figure 4a–c). At the magnifications of 20 nm, 50 nm, and 100 nm, the nanoparticles exhibited a polydisperse character consistent with green-synthesized AgNPs (Oliveira et al., 2025). The size of the particles was 18.01 nm on average, as calculated using ImageJ software. Spherical nature and small size enhance the surface area-to-volume ratio, which can contribute to increased cellular uptake and therapeutic action in biological systems.

Anti-Diabetic Activities

Oral Glucose Tolerance Test (OGTT)

The OGTT results revealed that significant improvements in glucose tolerance existed in treated groups (Table 1). The non-diabetic control rats (Group A) exhibited blood glucose concentration below 100 mg/dL throughout the 120 minutes. Diabetic untreated rats (Group C) indicated prolonged hyperglycemia (>250 mg/dL). Groups D (okra extract, 165.0 ± 6.9 mg/dL) and F (AgNPs, 153.3 ± 8.8 mg/dL) showed declines in blood glucose at 120 minutes significantly reduced compared to Group C (246.5 ± 8.4 mg/dL, $p < 0.05$), which translates to enhanced glucose tolerance. These were similar to Group G (metformin, 191.5 ± 9.9 mg/dL, $p < 0.05$ vs. Group C). The increased glucose tolerance in Groups D and F also shows that okra-derived AgNPs and extract enhance Insulin sensitivity, although GLUT4 was not measure, but it has a potential for increasing GLUT4 as a flavonoid-rich extracts, as reported in other researches (Abbigeri et al., 2025).

Fasting Blood Glucose (FBG)

FBG levels significantly decreased in treated diabetic groups after 4 weeks (Table 2). Group D (okra extract) reduced FBG from 325.3 ± 1.4 mg/dL to 176.0 ± 7.4 mg/dL, and Group F (AgNPs) from 375.3 ± 1.7 mg/dL to 247.0 ± 7.2 mg/dL ($p < 0.05$ with respect to Group C: 291.3 ± 3.3 mg/dL at week 4). Group E shows a significant decrease compared to Group C and F, which can be ascribed to the effect of nitrate oxide known to be cytotoxic and oxidative but harmful over long term to the pancreatic β-cell. Group G (metformin) was lowered to 285.3 ± 6.7 mg/dL ($p < 0.05$ with respect to Group C). These decreases (45.9% for okra extract, 34.1% for AgNPs) reflect strong anti-hyperglycemic activities, attributable to the synergistic interaction of okra's bioactive molecules and AgNPs' improved bioavailability (Shahzad et al., 2024). The therapeutic efficacy of AgNPs was as effective as that of metformin, an established anti-diabetic drug, demonstrating their value as therapeutics.

Table 2*:* Fasting Blood Glucose (FBG) Levels (Mg/Dl) At Weeks 0, 2, And 4

	Week		
Group	**0 (After initiation)**	**2 (Mid initiation)**	**4 (End initiation)**
A	100.25±5.5	87.75±3.92	82.00±3.44
B	369±1.88	313.50±3.0	264.0±9.0
C	333.75±3.4	313.50±3.0	291.25±3.3
D	325.25±1.4	273.75±5.4	176.0±7.40
E	236±4.34	181±2.08	140±4.50
F	375.25±1.74	267.75±6.0	247.00±7.2
G	306±6.27	292.75±7.3	285.3±6.7

Plasma Insulin and Urea

Plasma insulin concentrations in Group D (0.39 ± 0.034 µIU/mL) and Group F (0.41 ± 0.024 µIU/mL) showed minor changes compared with Group C (0.42 ± 0.034 µIU/mL, $p < 0.05$), reflecting lower insulin sensitivity (Figure 5a). Plasma urea, a marker of kidney function, was elevated in Group C (90.9 ± 2.7 mg/dL) but significantly reduced in Groups D (73.9 ± 2.4 mg/dL) and F (76.9 ± 1.8 mg/dL, $p < 0.05$), reflecting impaired renal damage (Figure 5b). These findings confirm that AgNPs and okra extract reduce diabetes-induced renal stress, which could be mediated by antioxidant effects, as elevated urea is a common complication of diabetic nephropathy (Bahreini et al., 2024).

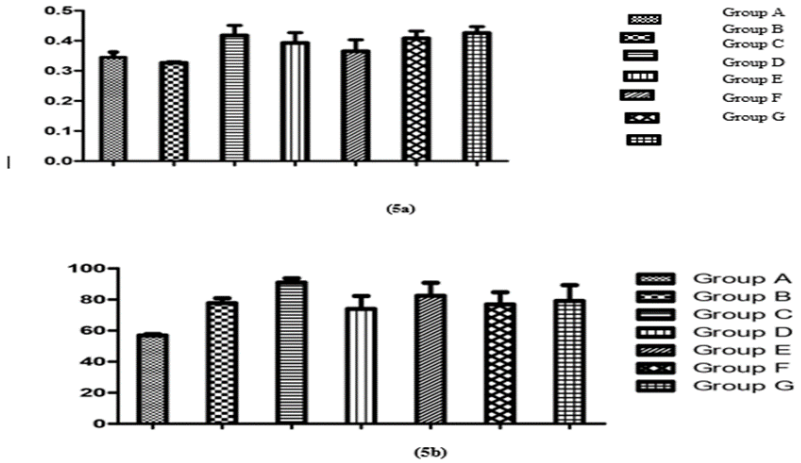


Figure 5: Plasma Levels of (5a) Insulin (µIU/mL) and (5b) urea (mg/dL) across Groups

Oxidative Stress Markers

Pancreatic antioxidant enzyme levels were drastically increased in the treated groups (Figure 6). Group D (1.42 ± 0.047 µmol/min/mg protein) and Group F (1.05 ± 0.026 µmol/min/mg protein) SOD activities were nearly equivalent to Group A (1.17 ± 0.025 µmol/min/mg protein, $p < 0.05$ vs. Group C: 0.80 ± 0.044 µmol/min/mg protein). Significant increases in glutathione (GSH) levels also showed this tendency, with the Group D value (0.71 ± 0.054 mM) and the Group F value (0.51 ± 0.076 mM) significantly exceeding the Group C value (0.40 ± 0.007 mM, $p < 0.05$). Malondialdehyde (MDA) levels, the lipid peroxidation marker, were significantly lower in Groups D (0.52 ± 0.02 µM) and F (0.55 ± 0.03 µM) than in Group C (0.70 ± 0.02 µM, $p < 0.05$). These improvements indicate that okra-extracted AgNPs and extract amerliorate oxidative stress, potentially due to flavonoids and phenolic acids eliminating reactive oxygen species (ROS), which provides pancreatic β-cells with a protective effect against damage (Wang & Wang, 2017).

HbA1c and Liver Glycogen

HbA1c levels, indicative of long-term glucose control, were similarly lower in Groups D (2.98 ± 0.37%) and F (1.45 ± 0.94%) compared to Group C (4.16 ± 0.39%, $p < 0.01$; Figure 7a). Liver glycogen levels were greater in Group D (0.28 ± 0.02 mmol/L) and Group F (0.24 ± 0.02 mmol/L) compared with Group C (0.21 ± 0.01 mmol/L, $p < 0.05$), demonstrating improved storage of glucose (Figure 7b). Downregulation of HbA1c also suggests good long-term glycemia control, possibly being mediated through enhanced glucokinase activity, as documented in plant compound research studies (Deen et al., 2022). Improved glucose metabolism is again evidenced by enhanced glycogen storage.

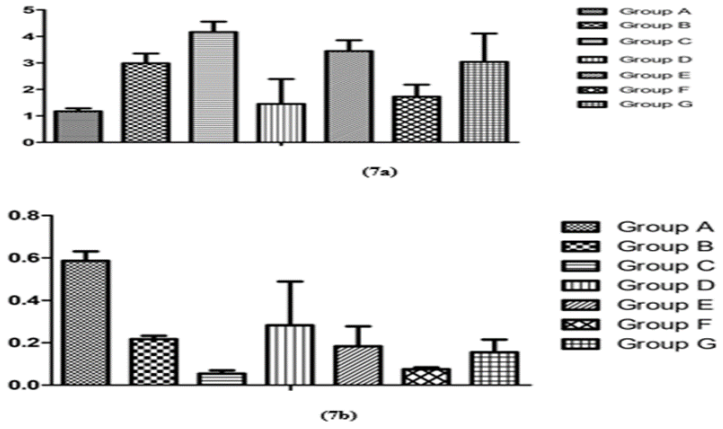


Figure 6: (6a) HbA1c (%) and (6b) Liver Glycogen (mmol/L) Levels

Green synthesis of AgNPs using okra extract provided nanoparticles with desired features (18 nm, spherical, crystalline), consistent with previous studies (Deen et al., 2022; Devanesan & AlSalhi, 2021). Anti-diabetic activity of AgNPs and okra extract could be attributed to phytochemicals such as flavonoids that enhance insulin signaling by means of pathways like PI3K/Akt and suppress oxidative stress (Situmorang et al., 2025; Sayem et al., 2018). Compared with Phagnalon niveum-derived AgNPs (Ul Haq et al., 2022), okra-derived AgNPs exhibited similar anti-hyperglycemic activities with smaller particle sizes, hence greater bioavailability. Its drawback is its short duration (4 weeks), small sample size (n=5), and lack of toxicity information. Chronic efficacy and safety must be determined using long-term studies, particularly in AgNP tissue accumulation. Further research must explore molecular mechanisms, e.g., glucose transporter (e.g., GLUT4) interactions, to explain the anti-diabetic effect of AgNPs.

CONCLUSION

This paper demonstrates the potential of okra-derived AgNPs as anti-diabetic agent evidence. Further research on the efficacy of green synthesis of silver nanoparticles (AgNPs) from aqueous okra (Abelmoschus esculentus) pod extract for new applications as an anti-diabetic medicine in streptozotocin-induced diabetic Wistar rats should be investigated. Characterization confirmed spherical AgNPs of 18 nm average diameter and crystalline structure, stabilized by bioactive flavonoids and phenolic acids present in okra. Okra extract and AgNPs significantly reduced blood glucose levels up to 45.9%, enhanced insulin sensitivity, and prevented oxidative stress, and were on par with metformin. The finding positions okra-derived AgNPs as a green, cost-effective therapeutic agent for diabetes management. Future research should focus on long-term toxicity, biodistribution, and molecular mechanisms, such as GLUT4 modulation, to enable clinical translation. Our work emphasizes the potential of nanotechnology from plants for addressing the world's diabetes epidemic, and sustainable therapeutic advancement.

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