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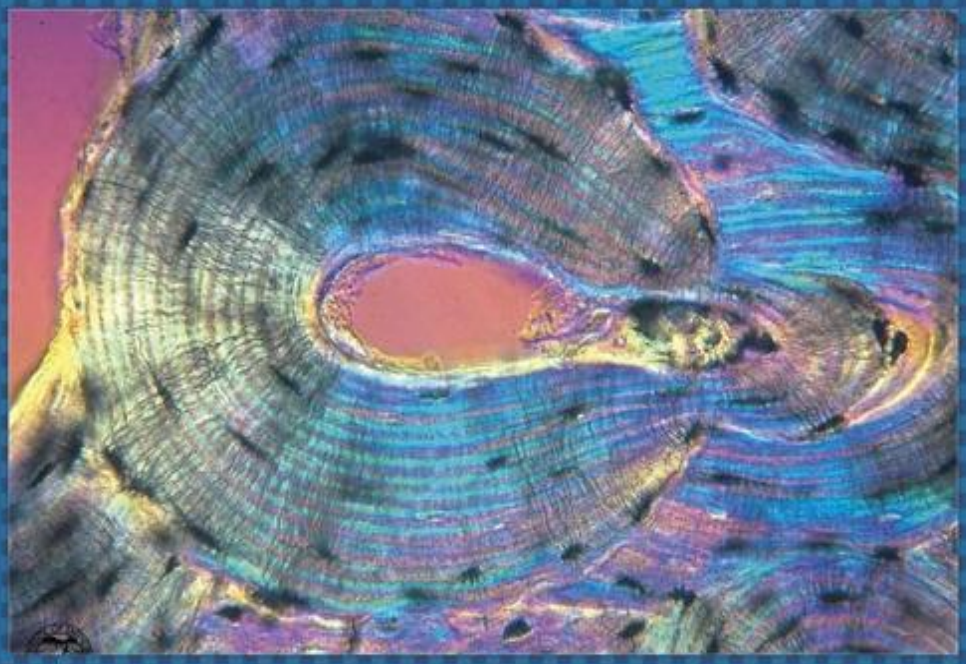

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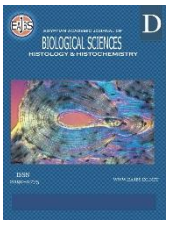

Synergistic Effects of Green-Synthesized Silver Nanoparticles and *Moringa oleifera* on Lipid and Renal Function in Diabetic Rats

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ABSTRACT

Diabetes mellitus (DM) is a chronic disease characterized by hyperglycemia, dyslipidemia, nephropathy, and oxidative stress, highlighting the need for innovative therapeutic strategies. This study investigated the potential of green-synthesized silver nanoparticles (Ag NPs), prepared using *Moringa oleifera* (*M. oleifera*) leaf extract, to mitigate diabetes-related complications in a streptozotocin (STZ)-induced diabetic rat model. Silver nanoparticles were synthesized via a green method and characterized using transmission electron microscopy (TEM). Fifty adult albino rats were divided into five groups: Group I (control), Group II (diabetic), Group III (diabetic treated with Ag NPs), Group IV (diabetic treated with *M. oleifera*), and Group V (diabetic receiving combination therapy). Biochemical parameters related to renal function, lipid profile, and antioxidant biomarkers were measured, along with a histological examination of kidney tissue. Combination treatment exhibited superior therapeutic efficacy, significantly reducing fasting blood glucose, HbA1c, total cholesterol, triglycerides (TG), and low-density lipoprotein cholesterol (LDL-c) while increasing high-density lipoprotein cholesterol (HDL-c). Additionally, antioxidant biomarkers improved, with elevated levels of glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT), alongside a reduction in malondialdehyde (MDA). These effects were accompanied by significant histological improvements in kidney architecture, indicating nephroprotective properties. Transmission electron microscopy confirmed the formation of uniform, spherical Ag NPs, while combination treatment demonstrated synergistic effects, suggesting that *M. oleifera* enhances nanoparticle efficacy through its antioxidant properties. This study underscores the therapeutic potential of green-synthesized Ag NPs and *M. oleifera* leaf extract as a promising approach for managing DM and its complications.

INTRODUCTION

Diabetes mellitus is a complex chronic disease characterized by persistent hyperglycemia due to impaired insulin secretion, insulin action, or both (Alberti & Aschner, 2009; Basevi, 2011; Goyal & Jialal, 2019; Schleicher *et al*., 2022; Yameny, 2024). The condition is often accompanied by secondary complications such as dyslipidemia, nephropathy, and oxidative stress, highlighting its significant impact on morbidity and mortality (Jin *et al.*, 2023; Kachhawa *et al*., 2017).

Dyslipidemia, particularly elevated levels of triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C), along with decreased levels of high-density lipoprotein cholesterol (HDL-C), is a major contributor to cardiovascular risk in diabetic individuals (Gonna & Ray, 2019; Kaze *et al*., 2021; Matsuzaka & Shimano, 2020; Wu & Parhofer, 2014; Zambon, 2020). Similarly, diabetic nephropathy, characterized by a progressive loss of renal function, is a leading cause of end-stage renal disease worldwide (Pelle *et al*., 2022; Samsu, 2021; Sun *et al*., 2013). These complications highlight the urgent need for innovative therapies addressing both lipid imbalances and renal dysfunction in diabetes management. Recent advancements in nanotechnology have introduced new strategies for therapeutic interventions in chronic diseases such as diabetes mellitus (DM). Among these, green-synthesized nanoparticles have gained significant attention due to their biocompatibility and enhanced biological efficacy (Harsoliya, 2012; Kumar *et al*., 2015; Singh *et al*., 2024). Silver nanoparticles are promising due to their well-documented antimicrobial, antioxidant, and anti-inflammatory properties (Burdușel *et al*., 2018; Eker *et al*., 2024; Gherasim *et al*., 2020). However, conventional synthesis methods for Ag NPs often involve toxic chemicals, which may restrict their biomedical applications (Devi *et al*., 2023; Hebbalalu *et al*., 2013; Khan *et al*., 2018; Prasad *et al*., 2021). An alternative approach involves the use of plant-based extracts for nanoparticle synthesis, utilizing phytochemicals as natural reducing and stabilizing agents while also enhancing the nanoparticles' bioactivity (Singh *et al*., 2023).

Moringa oleifera, commonly referred to as the drumstick tree or miracle tree, is widely recognized for its rich phytochemical composition, including flavonoids, phenolics, saponins, and alkaloids, which exhibit diverse pharmacological properties (Bhattacharya *et al*., 2018; Singh *et al*., 2022). These properties make *M. oleifera* a suitable candidate for synergistic applications in green nanoparticle synthesis (Jadhav *et al*., 2022; Perumalsamy *et al*., 2024). When combined with Ag NPs, *M. oleifera* enhances nanoparticle stability, restores antioxidant activity, and improves therapeutic outcomes, particularly in managing oxidative stress and inflammation—key drivers of diabetes-related complications (Kalakotla *et al*., 2022; Virk *et al*., 2023). Given the potential of *M. oleifera* and Ag NPs in addressing diabetes- related complications, the present study investigates their individual and synergistic effects on lipid metabolism and renal function in STZ-induced diabetic rat model.

MATERIALS AND METHODS

Characterization of Ag NPs:

The relative morphology and size distribution of silver nanoparticles were analyzed by transmission electron microscope (TEM) (JEOL Ltd, Tokyo, Japan). An aqueous suspension of nanoparticles was applied to a carbon-coated copper grid, dried, air dried, and subsequently examined using TEM.

Induction of Diabetes:

Diabetes mellitus was induced in rats that had fasted for 24 hours through a single intraperitoneal (IP) injection of STZ (50 mg/kg) dissolved in cold 0.1% M citrate buffer (pH 4.5). Following the injection, the rats were provided with a 15 g/L sucrose solution in their drinking water for 24 hours to minimize the risk of hypoglycemia-induced mortality. Diabetes was confirmed by measuring fasting blood glucose (FBG) levels three days after STZ administration. Rats with FBG levels exceeding 300 mg/dL were classified as diabetic and included in the experiment. After six weeks, serum urea, uric acid, and creatinine levels were assessed to evaluate kidney dysfunction associated with diabetes.

Experimental Design:

Fifty rats were obtained from the Animal Section of the King Fahad Medical Research Center at King Abdulaziz University and were classified into five groups (n = 10) as follows:

- **Group I:** Non-Diabetic Control received 0.5 mL citrate buffer intraperitoneally (IP).
- **Group II:** Diabetic Control received STZ (50 mg/kg, IP).
- **Group III**: Diabetic rats received STZ (50 mg/kg, IP) and were treated with Ag NPs (0.2 mg/kg, orally).
- **Group IV**: Diabetic rats received STZ (50 mg/kg, IP) and were treated with *M. oleifera* (0.2 mg/kg, orally).
- **Group V**: Diabetic rats received STZ (50 mg/kg, IP) and were treated with a combination of Ag NPs (0.2 mg/kg, orally) and *M. oleifera* (0.2 mg/kg, orally).

Glucose Metabolism:

Following blood collection, blood glucose levels were measured using the method described by Middleton and Griffiths (1957). Plasma insulin levels were quantitatively measured using the ELISA method with an insulin ELISA kit. Glycosylated hemoglobin (HbA1c) levels were determined using the Bannon (1982) method with a commercial diagnostic kit.

Kidney Function Biomarker Assessment:

Blood urea nitrogen (BUN) levels were determined using the Patton and Crouch (1977) method. Uric acid (UA) levels were estimated using the Burtis and Ashwood (1994) method. Creatinine (CR) levels were measured using the Moore and Sharer (2017) method.

Lipid Profile Biomarkers Assessment:

Triglyceride levels were determined using the Fossati and Prencipe (1982) method. Total cholesterol concentrations were measured using the Richmond (1973) method. High-density lipoprotein cholesterol levels were determined using the method of Warnick et al. (1983). Low-density lipoprotein cholesterol levels were calculated using the Friedewald *et al*. (1972) equation.

Antioxidant Biomarker Assessment:

The antioxidant parameters were examined as follows: blood samples were collected and analyzed using the Misra and Fridovich (1972) method for superoxide dismutase (SOD) and catalase (CAT), the Draper and Hadley (1990) method for malondialdehyde (MDA), and the Moron *et al.* (1979) protocol for glutathione (GSH).

Histopathological Examination:

Kidney tissues were fixed in 10% formalin, dehydrated through a graded series of alcohol concentrations, cleared with xylene, and embedded in paraffin wax. For histological examination, 5-µm sections were cut and stained with Harris' hematoxylin and eosin (H&E).

Statistical Analysis:

Data are expressed as the mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons, with $P < 0.05$ considered statistically significant. All analyses were conducted using GraphPad Prism (version 9.0; GraphPad, USA).

RESULTS

Transmission Electron Microscopy for Ag NPs:

Figure 1, confirms the successful synthesis of Ag NPs, as demonstrated by TEM analysis. The low-magnification TEM image (A) reveals uniformly dispersed nanoparticles with a spherical morphology, while the high-resolution TEM image (B) shows well-defined lattice fringes, indicating their crystalline nature. Additionally, the line profile analysis (C) highlights distinct interplanar spacings, consistent with the characteristic crystallographic planes of silver nanoparticles. These findings validate the structural integrity and

crystalline properties of the synthesized Ag NPs.

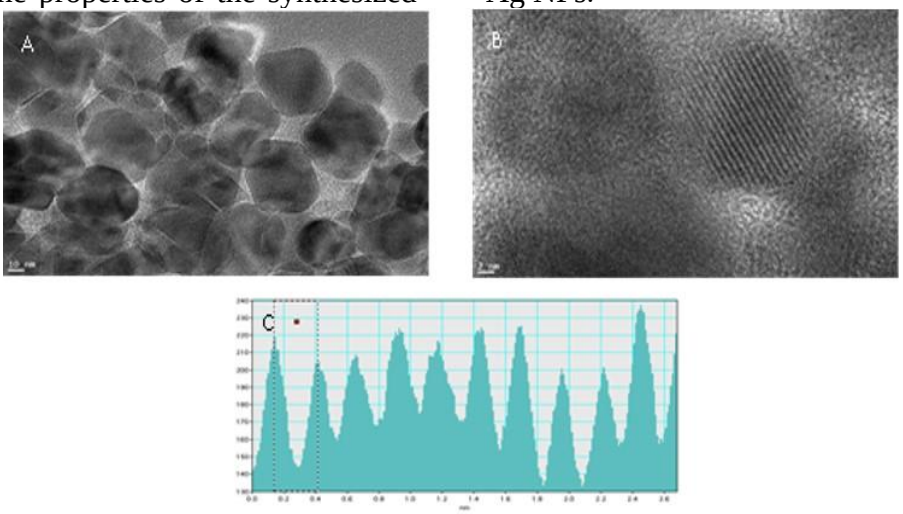

Fig. 1. Transmission Electron Microscopy characterization of Ag NPs. (A) TEM image showing the morphology and uniform distribution of Ag NPs. (B) High-resolution TEM image revealing lattice fringes, confirming the crystalline structure of Ag NPs. (C) Line profile analysis illustrating the interplanar spacing characteristics of Ag NPs.

***Moringa oleifera* Improves Glucose Metabolism and Mitigates Diabetes-Induced Dysregulation:**

Figure 2, illustrates the effects of Ag NPs, *M. oleifera* leaf extract, and their combination on serum glucose, insulin, and HbA1c levels in diabetic and control groups. The STZ-induced diabetic group exhibited a significant increase in glucose and HbA1c levels, along with a sharp decrease in insulin levels compared to the control group ($P < 0.001$). Treatment with *M. oleifera* leaf extract significantly reduced glucose and HbA1c levels while restoring insulin levels compared to the untreated diabetic group ($P < 0.001$). The combination of *M. oleifera* and Ag NPs further enhanced these effects, demonstrating a superior reduction in glucose and HbA1c levels, along with an increase in insulin levels, compared to individual treatments. Although treatment with Ag NPs alone resulted in significant improvements, the effects were less pronounced than those observed with the combination treatment. These findings suggest that *M. oleifera* and Ag NPs act synergistically to mitigate hyperglycemia and improve glucose metabolism in the diabetic group.

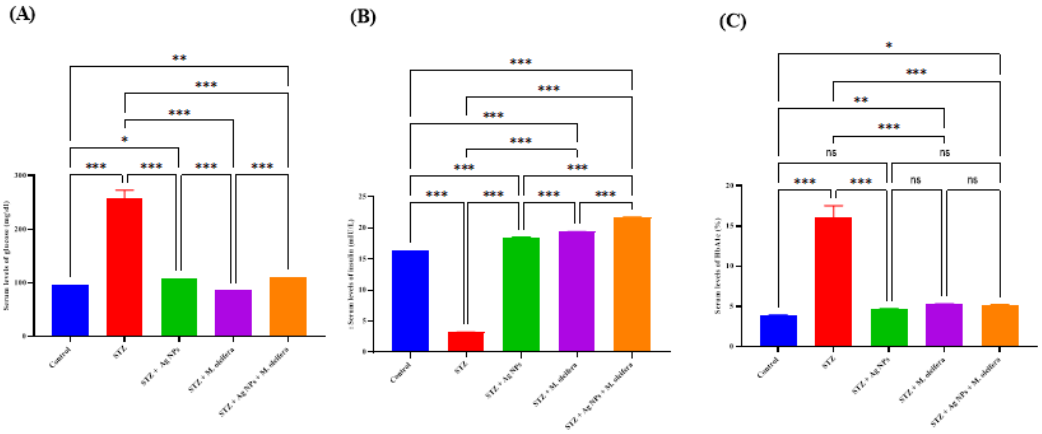

Fig. 2. Effects of *M. oleifera* and Ag NPs on serum glucose, insulin, and HbA1c levels in STZ-induced diabetic rats. (A) Serum glucose levels, (B) insulin levels, and (C) HbA1c levels. Results are expressed as the mean ± standard deviation (SD), with statistical significance set at $P < 0.05$.

M. oleifera Ameliorates Dyslipidemia Caused by DM

Figure 3, illustrates lipid profile analysis which reveals significant alterations across experimental groups. (A) Total cholesterol levels were significantly elevated in the STZ group compared to the control (P<0.001). Treatment with Ag NPs, *M. oleifera leaf* extract, and their combination significantly reduced cholesterol levels (P<0.001) compared to the STZ group. (B) Triglyceride levels were also significantly increased in the STZ group (P< 0.001), while treatment groups showed notable reductions, with no statistically significant differences between them. (C) High-density lipoprotein-cholesterol (HDL-c) was significantly reduced in the STZ group compared to the control (P<0.001), whereas it was significantly restored with all treatments. (D) Low-density lipoprotein-cholesterol (LDL-c) levels showed significant increases in the STZ group, and substantial decreases with all treatments (P<0.001), except for STZ + Ag NPs + *M. oleifera*, where reductions were not significant compared to the control group.

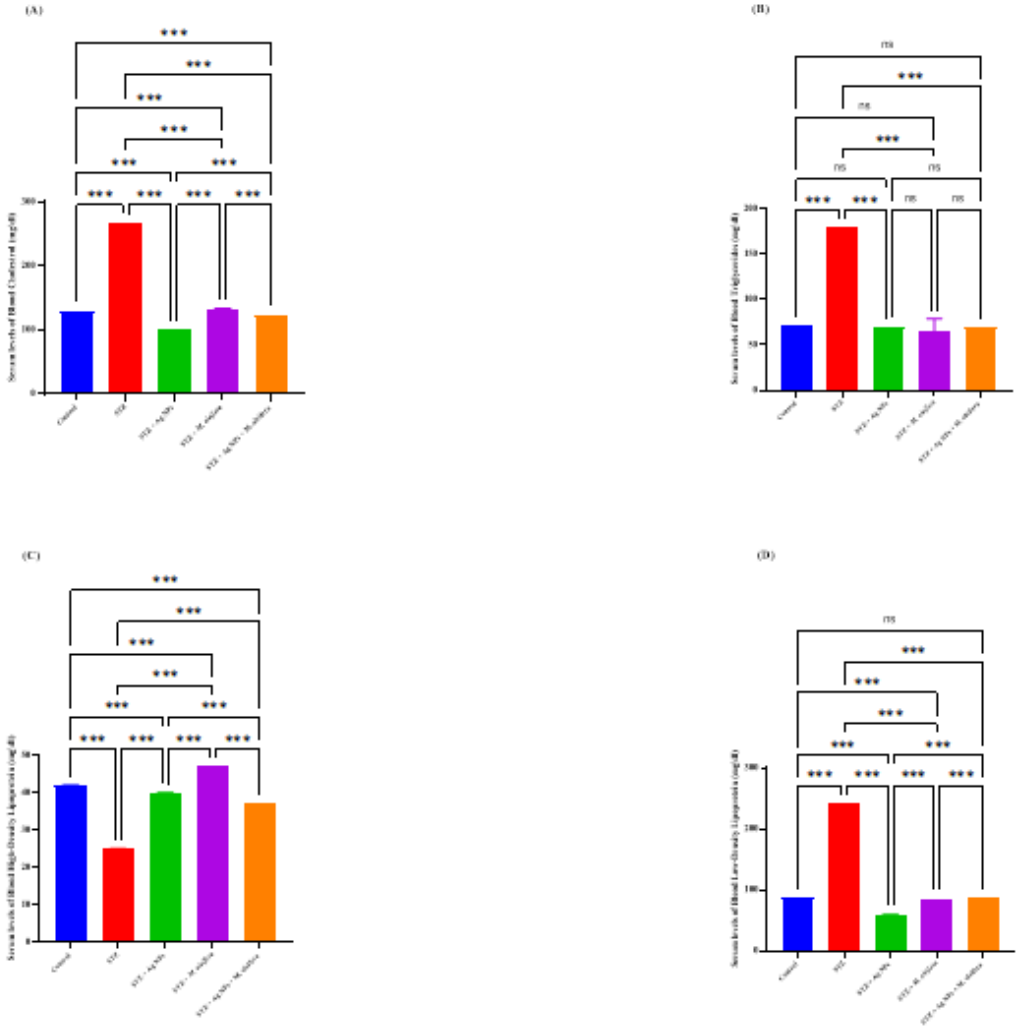

Fig. 3. Effects of *M. oleifera* and Ag NPs on serum cholesterol, triglyceride, and HbA1c levels in STZ-induced diabetic rats. (A) Serum cholesterol levels, (B) triglyceride (TG) levels, and (C) glycated hemoglobin (HbA1c) levels. Results are expressed as the mean ± standard deviation (SD), with statistical significance set at P < 0.05.

Ag NPs and *M. oleifera* mitigate the Diabetic Impairments in Antioxidant Biomarkers:

Figure 4, demonstrates the effects of Ag NPs and *M. oleifera* on STZ-induced oxidative stress and antioxidant biomarkers. The STZ-treated group exhibited a significant depletion in serum reduced glutathione (GSH) levels and decreased activities of superoxide dismutase (SOD) and catalase (CAT), along with a marked increase in malondialdehyde (MDA), a lipid peroxidation marker. These changes indicate elevated oxidative stress and impaired antioxidant defense. Treatment with Ag NPs and *M. oleifera*, particularly in combination, reversed these effects by significantly increasing GSH levels, restoring SOD and CAT activities, and reducing MDA levels. The combination of Ag NPs and *M. oleifera* was more effective than individual treatments, demonstrating a synergistic effect in mitigating oxidative damage and enhancing antioxidant capacity.

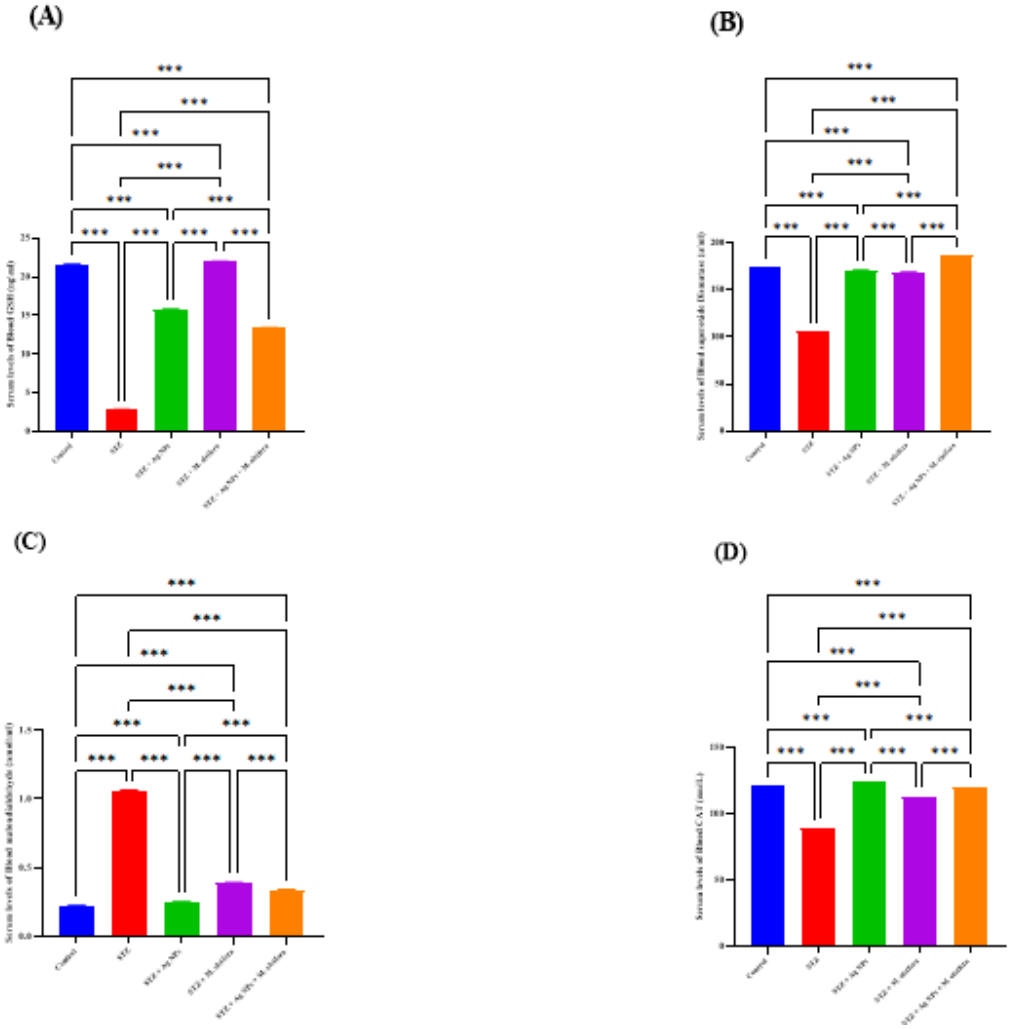

Fig. 4: Effect of Ag NPs, *M. oleifera*, and their combination on antioxidant biomarker levels in STZ-induced liver damage. The graphs illustrate the serum levels of antioxidant biomarkers, including (A) glutathione (GSH), (B) superoxide dismutase (SOD), (C) malondialdehyde (MDA), and (D) catalase (CAT). Results are expressed as the mean ± standard deviation (SD), with statistical significance at $P < 0.05$.

Figure 5, demonstrates that DM treatment caused significant kidney damage, including glomerular congestion, tubular degeneration, and inflammatory infiltration (Fig. 5B). Administration of Ag NPs and *M. oleifera* to the STZ-treated group mitigated these effects, showing partial

restoration of glomerular and tubular structures and reduced inflammation (Fig. 5C). The combination of Ag NPs and *M. oleifera* exhibited nearly normal kidney histology compared to the control group, indicating its nephroprotective potential (Fig. 5D).

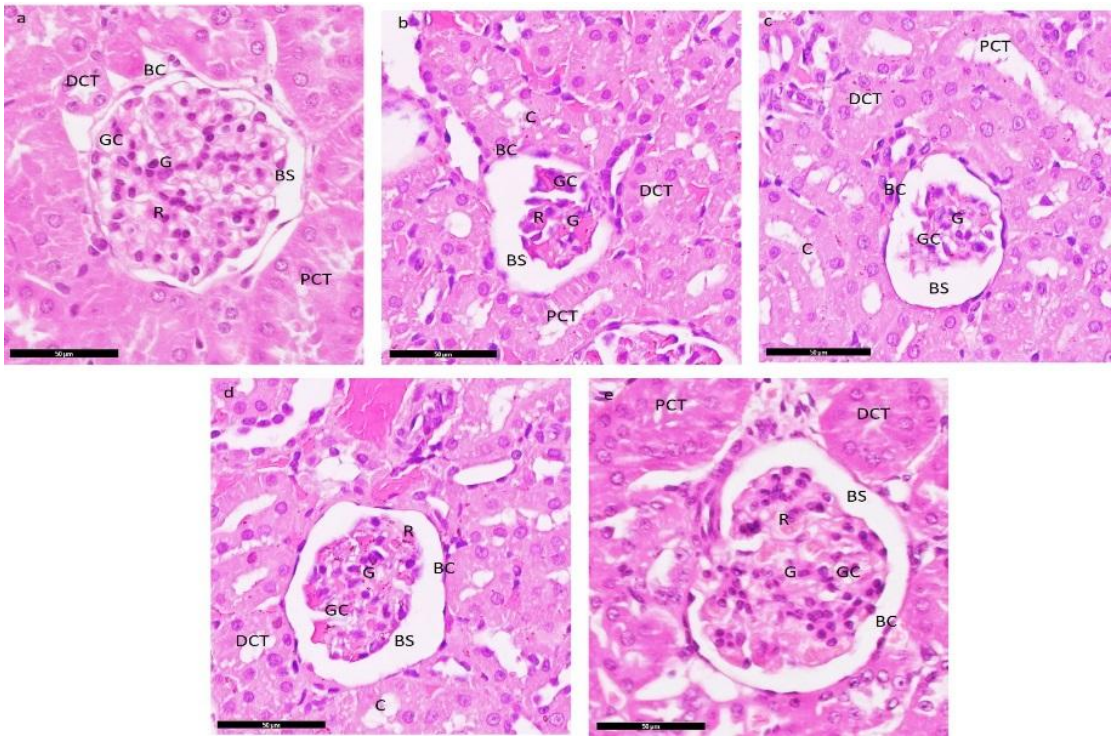

Fig. 5. Photomicrograph of kidney tissue showing H&E (scale bar 50 µm, 400 magnification). a: control group. b: Diabetic control (received STZ) group. c: Diabetic + Ag NPs. d: Diabetic + *M. oleifera* leaf extract. e: Diabetic + Ag NPs with *M. oleifera* leaf extract. BC: Bowman`s capsule, BS: Bowman`s space, PCT: proximal convoluted tubule. DCT: distal convoluted tubule, G: glomerulus, GC: glomerular capsule, R: red blood cells.

DISCUSSION

This study highlights the therapeutic potential of green-synthesized Ag NPs combined with *Moringa oleifera* leaf extract in addressing diabetes- related complications,particularly dyslipidemia, nephropathy, and oxidative stress. The combination treatment of Ag NPs and *M. oleifera* leaf extract significantly improved glucose metabolism by reducing blood glucose and HbA1c levels while enhancing insulin levels, demonstrating superior efficacy compared to individual treatments. These findings align with previous research suggesting that the anti-diabetic properties of *M. oleifera* are likely attributed to its rich phytochemical composition, including flavonoids and phenolics, which enhance insulin sensitivity and glucose uptake (Santos *et al*., 2022; Wang *et al*., 2021; Amir Hamza *et al*., 2023).

In addition to glucose regulation, lipid profile improvement was evident across all treatment groups, with a significant reduction in total cholesterol, triglycerides, and LDL-C levels, alongside an increase in HDL-C levels. The enhanced lipid metabolism observed in the combination treatment group aligns with existing evidence indicating that *M. oleifera* has a hypolipidemic effect (Chatterjee *et al*., 2013; Jain *et al*., 2010; Ogbuehi *et al*., 2014; Helmy *et al*., 2017) and that Ag NPs can regulate lipid biomarkers through oxidative stress modulation (Lawal *et al*., 2024). These effects are particularly important given the strong

association between dyslipidemia and cardiovascular risks in diabetes.

Oxidative stress, a pivotal factor in diabetic complications, was significantly alleviated by the treatments. STZ-induced diabetic rats exhibited depleted glutathione (GSH) levels and reduced superoxide dismutase (SOD) and catalase (CAT) activities, along with elevated malondialdehyde (MDA) levels, indicating heightened oxidative damage. Treatment with Ag NPs and *M. oleifera* leaf extract restored antioxidant biomarkers, with the combination treatment demonstrating the most pronounced effects. This outcome reflects the role of *M. oleifera* leaf extract as a powerful antioxidant (Aju *et al*., 2019; Jangir & Jain, 2016; Yassa & Tohamy, 2014) and Ag NPs as a potent free radical scavenger (Kumar *et al*., 2022; Luhata *et al*., 2022; Netala *et al*., 2016; Thanh *et al*., 2022).

The histological analysis of kidney tissues confirms these biochemical findings, as the combination therapy group showed marked improvement in renal architecture compared to diabetic control. These observations highlight the nephroprotective effects of *M. oleifera* leaf extract and Ag NPs, possibly mediated through their anti-inflammatory and antioxidant properties (Ahmad *et al*., 2022; Omodanisi *et al*., 2017; Oguntibeju *et al*., 2020). However, further research is necessary to clarify the specific mechanisms involved in these protective effects.

CONCLUSION

This study provides compelling evidence supporting the therapeutic potential of green-synthesized silver nanoparticles (Ag NPs) combined with Moringa oleifera leaf extract as a novel strategy for managing diabetes and its complications. Future studies should explore the underlying molecular mechanisms of these effects, focusing on signaling pathways, gene expression, and cellular processes. Additionally, clinical studies are essential to evaluate this treatment's long-term safety and efficacy in diabetic patients, particularly concerning its applicability to human health.

Declarations:

Ethical 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 EU Directive 2010/63/EU regarding animal research.

Competing interests: The authors declare that there is no conflict of interest.

Author's Contributions: Mohammed F. Al Khuzaee and Muhamed A. El Nobey contributed equally to this work and share the first authorship. Muhamed A. El Nobey wrote the manuscript, and Salim M El Hamidy revised it.

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Availability of Data and Materials: All data sets are available in the manuscript and supplementary file.

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