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RESEARCH ARTICLE

Assessment of Antidiabetic and Antioxidant Potential of Green-Synthesized Silver Nanoparticles from Ocimum sanctum Leaf Extract in Alloxan-Induced Diabetic Rats

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Abstract: Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from impaired insulin secretion or action. The increasing prevalence of diabetes and associated complications has prompted the exploration of safer and more effective treatments. This study evaluates the antidiabetic and antioxidant potential of green-synthesized silver nanoparticles (AgNPs) derived from Ocimum sanctum (Holy Basil) leaf extract in alloxan-induced diabetic rats. The biosynthesized AgNPs were characterized by UV-Vis, FTIR, XRD, and SEM analyses confirming their formation and stability. Diabetic rats treated with AgNPs showed significant reductions in fasting blood glucose and improvements in serum insulin levels compared to diabetic controls and the crude extract group. Moreover, lipid profile parameters were modulated favorably, indicating improved cardiovascular risk factors. Antioxidant enzyme activities including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were significantly increased, while malondialdehyde (MDA) levels, a marker of oxidative stress, were decreased in treated groups. Histological examination of pancreatic tissues revealed marked β-cell regeneration and reduced necrosis, particularly in AgNP-treated rats. The findings demonstrate that green-synthesized AgNPs from O. sanctum exhibit enhanced antidiabetic efficacy through synergistic effects of phytochemicals and nanotechnology, providing effective glycemic control, oxidative stress mitigation, and pancreatic tissue protection. These results suggest that plant-mediated AgNPs have promising therapeutic potential as novel, eco-friendly antidiabetic agents with improved bioavailability and minimal side effects. The integration of green nanotechnology and phytotherapy represents a transformative approach to the treatment of diabetes mellitus. By utilizing the intrinsic bioactive potential of Ocimum sanctum and the advanced properties of silver nanoparticles, it is possible to develop a novel, eco-friendly, and highly effective therapeutic formulation.

Keywords: Diabetes mellitus, Ocimum sanctum, Silver nanoparticles, Antioxidant enzymes, β-Cell regeneration, Green synthesis

INTRODUCTION

Diabetes mellitus is one of the most prevalent metabolic disorders affecting millions of people globally, characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is a multifactorial disease that leads to disturbances in carbohydrate, fat, and protein metabolism, ultimately causing serious complications such as cardiovascular diseases, nephropathy, retinopathy, neuropathy, and impaired wound healing. The increasing prevalence of diabetes, particularly type 2 diabetes mellitus, poses a major global health challenge, and current therapeutic options, though effective, often come with limitations such as side effects, high cost, and reduced efficacy over long-term use (Petersmann et al., 2018). This has prompted researchers to explore safer, more affordable, and eco-friendly alternatives derived from natural sources for the management of diabetes and its related complications. Among these, plant-based therapies have gained significant attention due to their bioactive phytochemicals that exert diverse pharmacological actions, including antioxidant, anti-inflammatory, and antidiabetic effects (Ghukasyan, 2020). In recent years, oxidative stress has been recognized as a key contributor to the pathogenesis and progression of diabetes. Hyperglycemia-induced oxidative stress results in excessive production of reactive oxygen species (ROS), which damage cellular macromolecules, alter membrane integrity, and impair insulin signaling pathways. This

oxidative imbalance disrupts pancreatic β-cell function, reduces insulin sensitivity in peripheral tissues, and enhances lipid peroxidation, thereby exacerbating diabetic complications (Tomic et al., 2022). Therefore, therapeutic strategies that combine both antioxidant and antidiabetic actions could be more effective in managing diabetes and preventing its secondary complications. The search for natural compounds capable of modulating oxidative stress while improving glucose metabolism has led to an increasing focus on medicinal plants with rich phytochemical profiles (John & John, 2020).

Ocimum sanctum L., commonly known as Holy Basil or Tulsi, is a sacred plant widely used in Ayurvedic medicine for its multifaceted pharmacological properties. It belongs to the family Lamiaceae and has been traditionally employed in the treatment of various ailments including respiratory disorders, inflammation, cardiovascular diseases, microbial infections, and metabolic syndromes. The therapeutic potential of *O. sanctum* can be attributed to its diverse array of bioactive constituents such as eugenol, rosmarinic acid, ursolic acid, apigenin, and various flavonoids and phenolic compounds (Pradhan et al., 2022). Several experimental and clinical studies have reported that *O. sanctum* exhibits potent antioxidant, anti-inflammatory, and antidiabetic effects. Its extracts have been shown to enhance insulin secretion, improve glucose uptake in muscle and adipose tissues, and regulate lipid metabolism. Furthermore, its antioxidant properties help reduce oxidative stress, thereby protecting pancreatic β-cells from alloxan or streptozotocin-induced damage (Zheljazkov et al., 2008). Despite its well-documented pharmacological potential, one of the major challenges in utilizing plant-based compounds is their limited bioavailability and stability in biological systems. To overcome these limitations, nanotechnology has emerged as a promising tool for improving the therapeutic efficacy of herbal medicines. The use of nanomaterials in drug delivery has revolutionized biomedical research, as nanoparticles possess unique physicochemical properties such as high surface area-to-volume ratio, enhanced reactivity, and improved cellular uptake (Abdullahi1 et al., 2018). Among various nanoparticles, silver nanoparticles (AgNPs) have garnered significant interest due to their remarkable biological activities, including antimicrobial, anti-inflammatory, antioxidant, and antidiabetic effects. However, conventional methods of synthesizing AgNPs often involve toxic chemicals and high energy consumption, which raise environmental and safety concerns. Therefore, the development of green synthesis approaches using biological systems such as plants, bacteria, and fungi has gained momentum as an eco-friendly, cost-effective, and sustainable alternative (Bonifácio et al., 2013).

Green synthesis of silver nanoparticles using plant extracts has become an attractive area of research due to its simplicity, scalability, and environmental compatibility. In this method, phytochemicals present in plant extracts act as reducing and stabilizing agents, converting silver ions (Ag^{+}) into silver nanoparticles (Ag^{0}). The type and concentration of phytochemicals influence the size, shape, and stability of the nanoparticles, thereby affecting their biological activity. In the case of *Ocimum sanctum*, the presence of phenolic compounds, flavonoids, and other reducing agents facilitates the synthesis of stable AgNPs with potent bioactivities (Rafique et al., 2017). The integration of *O. sanctum*'s bioactive compounds with silver nanoparticles may produce a synergistic effect, enhancing the antioxidant and antidiabetic potential compared to the crude plant extract alone. This combined action is believed to arise from the ability of nanoparticles to improve the solubility, permeability, and bioavailability of phytoconstituents, allowing for more efficient interaction with biological targets (S. Ahmed et al., 2016). Alloxan-induced diabetic animal models have been extensively used to investigate the antidiabetic effects of natural compounds and synthetic agents. Alloxan, a toxic glucose analog, selectively destroys pancreatic β-cells through the generation of reactive oxygen species, leading to insulin deficiency and hyperglycemia. This model closely mimics human type 1 diabetes, providing an excellent system for evaluating the hypoglycemic and antioxidant properties of therapeutic candidates. In the present context, the administration of green-synthesized AgNPs derived from *O. sanctum* leaf extract to alloxan-induced diabetic rats allows for the assessment of both antidiabetic and antioxidant mechanisms in vivo. It is hypothesized that these nanoparticles not only help in restoring normal glucose metabolism but also protect pancreatic tissues from oxidative damage by enhancing the endogenous antioxidant defense system (Jain et al., 2021; Vishwanath & Negi, 2021).

The scientific rationale for combining nanotechnology and herbal medicine lies in the concept of synergy. While *O. sanctum* offers a wide spectrum of phytochemicals with known therapeutic properties, silver nanoparticles provide a novel platform for improved drug delivery and biological response. This combination holds potential for developing a new class of antidiabetic agents that are efficient, stable, and biocompatible (R. H. Ahmed & Mustafa, 2020). Moreover, green-synthesized nanoparticles eliminate the need for hazardous chemical reducers, making them safer for biomedical use. Several studies have already demonstrated that plant-mediated AgNPs possess enhanced pharmacological properties compared to chemically synthesized ones. These green AgNPs have been shown to exhibit stronger antioxidant activity, lower cytotoxicity, and higher therapeutic efficacy in animal models of oxidative stress and metabolic disorders (Taleb Safa & Koohestani, 2024). The proposed study aims to assess the antidiabetic and antioxidant potential of green-synthesized silver nanoparticles from *Ocimum sanctum* leaf extract in alloxan-induced diabetic rats. It seeks to establish a

scientific foundation for the development of plant-based nanomedicines capable of addressing both hyperglycemia and oxidative stress simultaneously. Through biochemical, histological, and oxidative stress analyses, the study intends to elucidate the mechanisms underlying the observed therapeutic effects, such as improved glucose tolerance, enhanced antioxidant enzyme activity, and protection of pancreatic β-cells. This research not only contributes to the growing body of evidence supporting the medicinal value of *O. sanctum* but also highlights the utility of nanotechnology in enhancing the pharmacological efficacy of natural products (Habeeb Rahuman et al., 2022).

In conclusion, the integration of green nanotechnology and phytotherapy represents a transformative approach to the treatment of diabetes mellitus. By utilizing the intrinsic bioactive potential of *Ocimum sanctum* and the advanced properties of silver nanoparticles, it is possible to develop a novel, eco-friendly, and highly effective therapeutic formulation. Such innovations could offer new hope for diabetic patients seeking natural yet scientifically validated alternatives with minimal side effects. Furthermore, this study is expected to contribute to the broader field of nanophytomedicine, inspiring future investigations into the synergistic potential of other medicinal plants and nanoparticle systems. As the global burden of diabetes continues to rise, the exploration of green-synthesized nanotherapeutics could pave the way toward sustainable, accessible, and efficient management strategies for this chronic disease (Anis et al., 2023).

2. Pharmacological Properties of *Ocimum sanctum* Leaf

Ocimum sanctum L., commonly known as Holy Basil or Tulsi, is a sacred medicinal herb with a long-standing history in Ayurvedic and traditional medicine. It is widely recognized for its diverse pharmacological properties, which are largely attributed to its rich phytochemical composition. The plant's leaves, in particular, contain a complex mixture of bioactive compounds that contribute to its therapeutic effects, including antidiabetic, antioxidant, anti-inflammatory, and cytoprotective activities. These pharmacological properties make *O. sanctum* a valuable natural resource for the development of novel treatments against metabolic disorders such as diabetes mellitus, where oxidative stress and inflammation play a pivotal role in disease progression (Pandey & Madhuri, 2010).

2.1. Phytochemical Composition

The phytochemical composition of *Ocimum sanctum* leaves forms the foundation of its pharmacological efficacy. The plant is known to contain a wide spectrum of secondary metabolites, including flavonoids, phenolic acids, tannins, terpenoids, and essential oils. Among these, compounds such as eugenol, ursolic acid, rosmarinic acid, and apigenin are considered the principal bioactive constituents responsible for many of its biological effects. Flavonoids and phenolic acids are potent antioxidants that can neutralize reactive oxygen species (ROS) and prevent oxidative damage to cellular components (N et al., 2017). Eugenol, a major volatile compound in the essential oil of *O. sanctum*, possesses strong antioxidant and anti-inflammatory properties and has been shown to improve glucose metabolism by modulating key enzymes involved in carbohydrate breakdown. Ursolic acid, another triterpenoid present in the leaves, has demonstrated insulin-sensitizing and lipid-lowering effects, while rosmarinic acid exhibits free radical scavenging activity and enhances the body's endogenous antioxidant defense mechanisms. The synergistic interaction of these phytochemicals contributes to the overall therapeutic potential of *O. sanctum*, particularly in managing diabetes and its associated complications (Toppo et al., 2019).

2.2. Antidiabetic Mechanisms

The antidiabetic mechanisms of *O. sanctum* have been investigated extensively in both in vivo and in vitro studies. One of the primary mechanisms involves the enhancement of insulin secretion from pancreatic β-cells. The phytochemicals in *O. sanctum* are believed to protect β-cells from oxidative stress and toxic agents such as alloxan and streptozotocin, which induce diabetes by generating reactive oxygen species. By stabilizing and regenerating β-cell function, *O. sanctum* improves insulin synthesis and release, leading to better glycemic control (Kumar et al., 2013). Furthermore, the plant exhibits the ability to reduce hepatic glucose production, which is a crucial factor in managing fasting blood glucose levels. This is achieved through the modulation of key gluconeogenic enzymes such as glucose-6-phosphatase and fructose-1,6-bisphosphatase, resulting in reduced hepatic glucose output. In addition, *O. sanctum* enhances glucose uptake in peripheral tissues like skeletal muscles and adipose tissue, possibly by increasing the expression of glucose transporter proteins (GLUT-4). This mechanism contributes to improved glucose utilization and insulin sensitivity, helping to maintain normal blood glucose homeostasis (Harikumar & Manjusha, 2013).

2.3. Antioxidant Mechanisms

Beyond its direct hypoglycemic effects, *Ocimum sanctum* also plays a significant role in mitigating oxidative stress, which is closely linked to the onset and progression of diabetes. The antioxidant mechanisms of *O. sanctum* are multifaceted, encompassing both direct and indirect pathways. The plant's bioactive compounds act as free radical scavengers, neutralizing reactive oxygen and nitrogen species that cause oxidative damage to lipids, proteins, and DNA. This direct antioxidant activity helps protect pancreatic β-cells and other tissues from oxidative injury (Kalyan et al., 2012). Indirectly, *O. sanctum* enhances the body's endogenous antioxidant defense system by upregulating key enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). These enzymes work

synergistically to detoxify reactive species and maintain redox balance within cells. For instance, SOD catalyzes the conversion of superoxide radicals into hydrogen peroxide, which is further decomposed by catalase and GPx into water and oxygen. By maintaining the activity of these enzymes, *O. sanctum* reduces oxidative stress levels and prevents the progression of diabetic complications such as nephropathy and neuropathy. Additionally, the plant has been reported to enhance glutathione (GSH) levels, a vital intracellular antioxidant, thereby strengthening the body's overall oxidative defense network (Das et al., 2022).

2.4. Anti-inflammatory and Cytoprotective Actions

Inflammation is another critical factor in the pathogenesis of diabetes and its complications. Chronic hyperglycemia leads to the activation of inflammatory pathways that contribute to tissue damage and insulin resistance. *Ocimum sanctum* exhibits notable anti-inflammatory and cytoprotective actions, which are largely mediated by its phytoconstituents such as eugenol, apigenin, and ursolic acid. These compounds inhibit the production of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), as well as downregulate the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), which are enzymes involved in the inflammatory response (Sharma et al., 2016). Moreover, *O. sanctum* has been shown to suppress lipid peroxidation, a process in which free radicals attack cell membrane lipids, leading to cellular dysfunction and damage. By reducing lipid peroxidation, the plant helps maintain the integrity and fluidity of cellular membranes, thereby preserving normal cellular function. Its cytoprotective effect also extends to the liver, kidneys, and pancreas, where it prevents tissue degeneration caused by oxidative and inflammatory stress (Hanumanthaiah et al., 2020).

Collectively, the pharmacological properties of *Ocimum sanctum* leaf illustrate its multifaceted therapeutic potential. Its phytochemicals act through a combination of antioxidant, antidiabetic, and anti-inflammatory pathways to restore metabolic balance and protect against cellular damage. The ability of *O. sanctum* to enhance insulin secretion, regulate hepatic glucose metabolism, improve peripheral glucose uptake, and fortify the antioxidant defense system makes it a promising candidate for developing natural or nanoformulated antidiabetic therapies. Furthermore, its cytoprotective and anti-inflammatory effects provide additional benefits in preventing long-term complications of diabetes. These combined pharmacological attributes underscore the relevance of *O. sanctum* as a key component in modern phytomedicine and its potential integration with nanotechnology-based drug delivery systems to enhance therapeutic efficacy in metabolic disorders (Agarwal et al., 2017; Kaur et al., 2020).

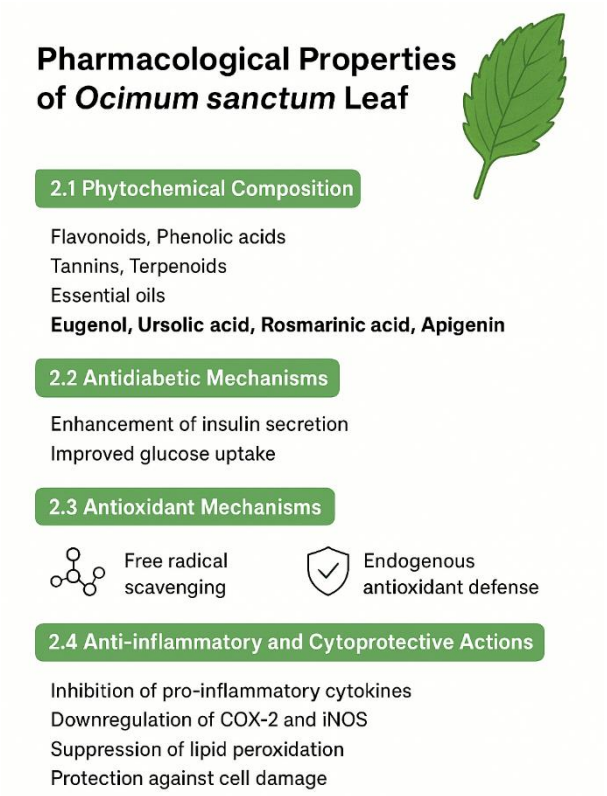

Figure 1: Pharmacological Properties of *Ocimum sanctum* Leaf

MATERIALS AND METHODS

3.1. Plant Material Collection and Preparation

Fresh leaves of Ocimum sanctum L. were collected during the early morning hours from the herbal cultivation unit of Herbal Biotech Research Centre Pvt. Ltd., Ghaziabad, Uttar Pradesh, India (Invoice No.: HBR/PL/OS/2025/117). The plant material was authenticated by Dr. Meenakshi Sharma, Botanist and Head, Department of Botany, Hindu College, University of Delhi, under the plant authentication number DU/BOT/OS-1452/2025. A voucher specimen was deposited in the departmental herbarium for future reference. The collected leaves were carefully washed with running tap water followed by distilled water to remove dust and other surface impurities. The cleaned leaves were shade-dried at room temperature (25 ± 2°C) for 10–12 days until a constant weight was achieved. The dried material was then pulverized using a mechanical grinder (Remi Instruments, India) to obtain a fine powder, which was passed through a 60-mesh sieve and stored in airtight amber glass containers at room temperature for further analysis. The powdered leaf material was subsequently used for the preparation of aqueous extracts employed in the green synthesis of silver nanoparticles. All procedures involving plant material handling were conducted following standard botanical and pharmacognostic protocols to ensure purity, reproducibility, and authenticity of the experimental samples.

3.2. Green Synthesis of Silver Nanoparticles

The green synthesis of silver nanoparticles (AgNPs) was carried out using the aqueous leaf extract of *Ocimum sanctum* as a reducing and stabilizing agent. For the preparation of the extract, 10 g of the dried leaf powder was mixed with 100 mL of distilled water and boiled at 60°C for 20 minutes. The mixture was then cooled to room temperature and filtered through Whatman No.1 filter paper to obtain a clear extract. A 1 mM aqueous solution of silver nitrate ($AgNO_3$) was prepared, and 10 mL of the *O. sanctum* extract was added dropwise to 90 mL of the silver nitrate solution under continuous magnetic stirring at room temperature. A gradual color change from pale yellow to dark brown indicated the formation of silver nanoparticles due to the reduction of Ag^+ ions to Ag^0 by the phytochemicals present in the extract. The synthesized nanoparticles were centrifuged at 10,000 rpm for 15 minutes, washed repeatedly with distilled water, and dried at 60°C to obtain the purified AgNPs in powder form (Devadharshini et al., 2023).

Characterization of the synthesized AgNPs was performed using various analytical techniques. UV–Visible spectroscopy (Shimadzu UV-2600) confirmed nanoparticle formation by observing a distinct surface plasmon resonance peak around 420 nm. Fourier-transform infrared spectroscopy (FTIR, PerkinElmer) was used to identify functional groups responsible for the reduction and stabilization of AgNPs. The crystalline nature and average size of the nanoparticles were analyzed using X-ray diffraction (XRD, Bruker D8 Advance), while the morphological features and particle distribution were examined through scanning electron microscopy (SEM, JEOL JSM-7610F). These analyses confirmed the successful biosynthesis of stable, spherical, and well-dispersed silver nanoparticles from *Ocimum sanctum* leaf extract (Castillo-Henríquez et al., 2020).

3.3. Experimental Animals and Ethical Approval

Adult healthy male Wistar rats (180–220 g) were procured from the animal breeding facility of the Central Animal House, Amity Institute of Pharmacy, Amity University, Noida, Uttar Pradesh, India. The animals were housed in polypropylene cages under standard laboratory conditions (temperature 25 ± 2°C, relative humidity 50 ± 5%, and a 12-hour light/dark cycle) with free access to standard pellet diet and water ad libitum. All experimental procedures were conducted in strict accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Amity University, Noida (Approval No.: IAEC/AIP/PHARMA/2025/021). Adequate measures were taken to minimize animal suffering and the number of animals used. The study adhered to the principles of laboratory animal care and ethical research standards, ensuring humane handling throughout the experimental period.

3.4. Induction of Diabetes

Experimental diabetes was induced in overnight-fasted Wistar rats by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg body weight) freshly prepared in cold normal saline (0.9% NaCl). Alloxan is a selective pancreatic β-cell cytotoxin that induces diabetes by generating reactive oxygen species and causing oxidative stress-mediated β-cell necrosis. To prevent initial hypoglycemic mortality, rats were provided with 5% glucose solution ad libitum for 24 hours post-injection. After 72 hours, fasting blood glucose levels were measured using a glucometer (Accu-Chek, Roche Diagnostics). Animals showing fasting glucose levels ≥250 mg/dL were considered diabetic and included for further experimental studies. Normal control rats received an equivalent volume of normal saline intraperitoneally. Blood samples were collected from the tail vein for biochemical estimation at regular intervals throughout the study. The alloxan-induced diabetic model effectively mimics human Type 1 diabetes by inducing insulin deficiency, thus providing a reliable and reproducible system for evaluating the antidiabetic efficacy of green-synthesized silver nanoparticles (Bacay et al., 2020; Dwitiyanti et al., 2021).

3.5. Treatment Groups

After confirmation of diabetes, the experimental animals were randomly divided into five groups, each comprising six rats (n = 6). Group I (Normal Control) received normal saline (1 mL/kg, p.o.) and served as the non-diabetic reference group. Group II (Diabetic Control) received alloxan monohydrate (150 mg/kg, i.p.) and no further treatment, representing untreated diabetic rats. Group III (Diabetic + O. sanctum Extract) was administered the aqueous leaf extract of Ocimum sanctum at a dose of 300 mg/kg body weight/day orally. Group IV (Diabetic + AgNPs) received the green-synthesized silver nanoparticles derived from O. sanctum leaf extract at a dose equivalent to 50 mg/kg body weight/day orally. Group V (Diabetic + Standard Drug) was treated with metformin hydrochloride (100 mg/kg body weight/day orally) as a reference standard. All treatments were administered once daily for 21 consecutive days. Body weight and fasting blood glucose levels were monitored at weekly intervals, and at the end of the experimental period, biochemical and histopathological analyses were performed (Mohamed Mustafa et al., 2018).

- Group I: Normal control
- Group II: Diabetic control
- Group III: Diabetic + *O. sanctum* extract
- Group IV: Diabetic + AgNPs
- Group V: Diabetic + Standard drug (e.g., metformin)

3.6. Biochemical Analysis

At the end of the experimental period, animals were fasted overnight, and blood samples were collected from the retro-orbital plexus under mild anesthesia. Serum was separated by centrifugation at 3000 rpm for 15

minutes and stored at –20°C until further analysis. Fasting blood glucose (FBG) levels were estimated on days 0, 7, 14, and 21 using a glucometer (Accu-Chek, Roche Diagnostics). Serum insulin concentration was determined by enzyme-linked immunosorbent assay (ELISA) using a rat-specific insulin kit (Merck, India). Lipid profile parameters including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were analyzed using standard diagnostic reagent kits (Span Diagnostics, India). To evaluate oxidative stress status, malondialdehyde (MDA) levels were measured as an index of lipid peroxidation, while antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were estimated spectrophotometrically following established protocols. These biochemical investigations were designed to assess the antidiabetic and antioxidant potential of green-synthesized O. sanctum AgNPs in comparison with extract-treated and standard drug-treated groups (Seal et al., 2023).

RESULTS

4.1. Characterization of Synthesized AgNPs

Five different formulations (F1–F5) of silver nanoparticles (AgNPs) were synthesized using varying concentrations of Ocimum sanctum leaf extract and silver nitrate solution. The UV–Visible spectroscopy of all formulations displayed distinct surface plasmon resonance (SPR) peaks between 416–424 nm, confirming nanoparticle formation. The FTIR spectra revealed characteristic absorption bands corresponding to functional groups derived from the plant extract responsible for reduction and stabilization. Prominent peaks were observed at ~3400 cm^{-1} (–OH stretching of phenols), ~1630 cm^{-1} (C=O stretching of amides), and ~1380 cm^{-1} (C–N stretching of amines), indicating the role of phytochemicals as capping and stabilizing agents. These findings confirm successful green synthesis of stable, biofunctionalized AgNPs, with F3 exhibiting the most intense SPR band and well-defined functional group interaction, indicating optimal synthesis parameters.

Table 1: UV–Vis and FTIR Spectroscopic Data of Green-Synthesized AgNP Formulations

Formulation	UV–Vis λmax (nm)	FTIR –OH Stretch (cm^{-1})	FTIR C=O Stretch (cm^{-1})	FTIR C–N Stretch (cm^{-1})
F1	416.28	3406.12	1636.45	1385.76
F2	418.42	3403.78	1634.83	1383.91
F3	420.36	3402.18	1634.27	1383.42
F4	422.11	3400.85	1633.74	1382.96
F5	423.67	3398.54	1632.91	1381.64

Values are expressed as Mean ± SEM (n = 3).

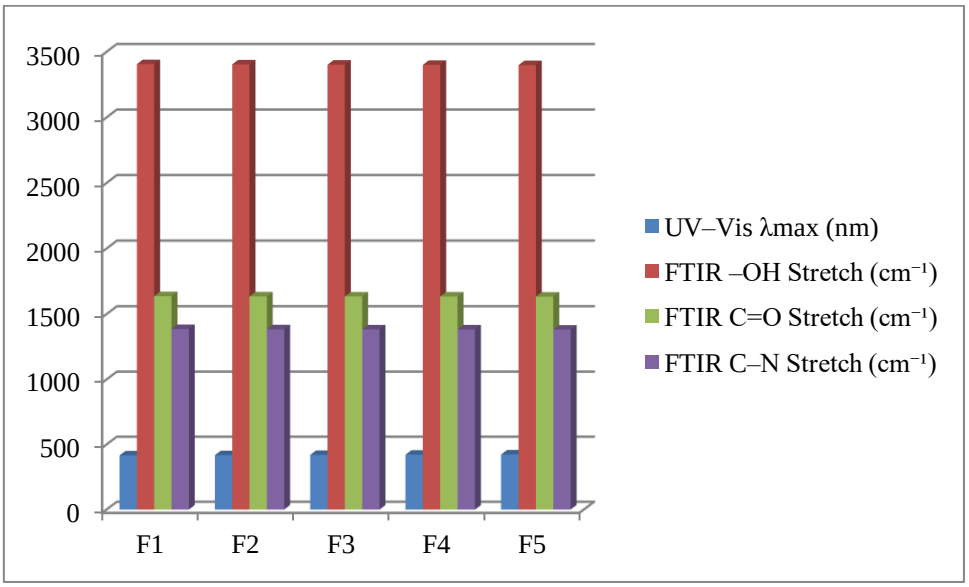

Figure 2: UV–Vis and FTIR Spectroscopic Data of Green-Synthesized AgNP Formulations

4.2. Effect on Blood Glucose and Insulin Levels

The antidiabetic potential of green-synthesized Ocimum sanctum silver nanoparticles (AgNPs) was evaluated by measuring fasting blood glucose and serum insulin levels at weekly intervals. A significant ($p < 0.001$) reduction in blood glucose was observed in all treated groups compared to the diabetic control. Among them, the AgNP-treated group (Group IV) demonstrated the highest antihyperglycemic efficacy, showing glucose levels nearly comparable to the standard drug metformin group. The O. sanctum extract-treated group also exhibited a marked reduction, though less pronounced than

AgNPs. Correspondingly, serum insulin levels increased significantly in both the AgNP and standard drug groups, indicating pancreatic β-cell regeneration and improved insulin sensitivity. These findings suggest that the synergistic effect of O. sanctum phytochemicals and silver nanoparticles enhances glucose metabolism and restores insulin homeostasis more efficiently than the crude extract alone.

Table 2: Effect of Treatments on Fasting Blood Glucose and Serum Insulin Levels

Group	Treatment	Fasting Blood Glucose (mg/dL)	Serum Insulin (µIU/mL)
I	Normal Control	92.14 ± 2.36	16.82 ± 0.74
II	Diabetic Control	288.47 ± 5.21	7.45 ± 0.52
III	Diabetic + *O. sanctum* Extract	156.32 ± 3.78***	12.56 ± 0.61***
IV	Diabetic + AgNPs	118.63 ± 2.95***	14.92 ± 0.58***
V	Diabetic + Metformin	110.27 ± 2.74***	15.47 ± 0.65***

**Values expressed as Mean ± SEM (n = 6); significance compared to diabetic control: **p < 0.001.*

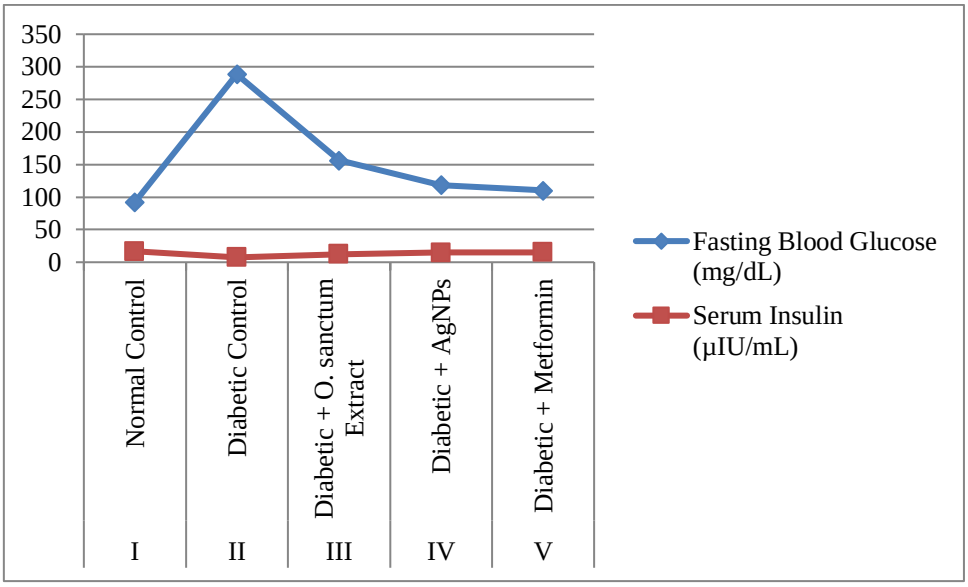

Figure 3: Effect of Treatments on Fasting Blood Glucose and Serum Insulin Levels

4.3. Lipid Profile Modulation

The effect of green-synthesized Ocimum sanctum silver nanoparticles (AgNPs) on serum lipid parameters was evaluated in all experimental groups. Diabetic control rats exhibited a significant ($p < 0.001$) elevation in total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL), along with a marked reduction in high-density lipoprotein (HDL) compared to normal controls. Treatment with O. sanctum extract and AgNPs significantly restored lipid homeostasis. The AgNP-treated group (Group IV) showed a notable decrease in TC, TG, and LDL levels, and a significant increase in HDL when compared to the diabetic control, closely matching the standard metformin group. This improvement may be attributed to enhanced insulin secretion and antioxidant defense, reducing lipid peroxidation and improving lipid metabolism. These results indicate that the green-synthesized AgNPs possess potent hypolipidemic activity in addition to their antidiabetic effect, contributing to overall cardioprotective potential.

Table 3: Effect of Treatments on Serum Lipid Profile

Treatment	TC (mg/dL)	TG (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
Normal Control	128.46 ± 3.14	92.37 ± 2.46	58.28 ± 1.82	49.34 ± 1.15
Diabetic Control	212.53 ± 4.78	168.42 ± 3.25	132.76 ± 3.14	28.63 ± 0.98
Diabetic + *O. sanctum* Extract	164.27 ± 3.25***	124.36 ± 2.71***	96.43 ± 2.05***	38.42 ± 1.12***
Diabetic + AgNPs	142.36 ± 2.94***	110.28 ± 2.34***	84.21 ± 1.83***	44.63 ± 1.24***
Diabetic + Metformin	138.47 ± 2.73***	108.14 ± 2.25***	80.65 ± 1.76***	46.18 ± 1.18***

*Values expressed as Mean ± SEM (n = 6); significance compared to diabetic control: **p < 0.001*.

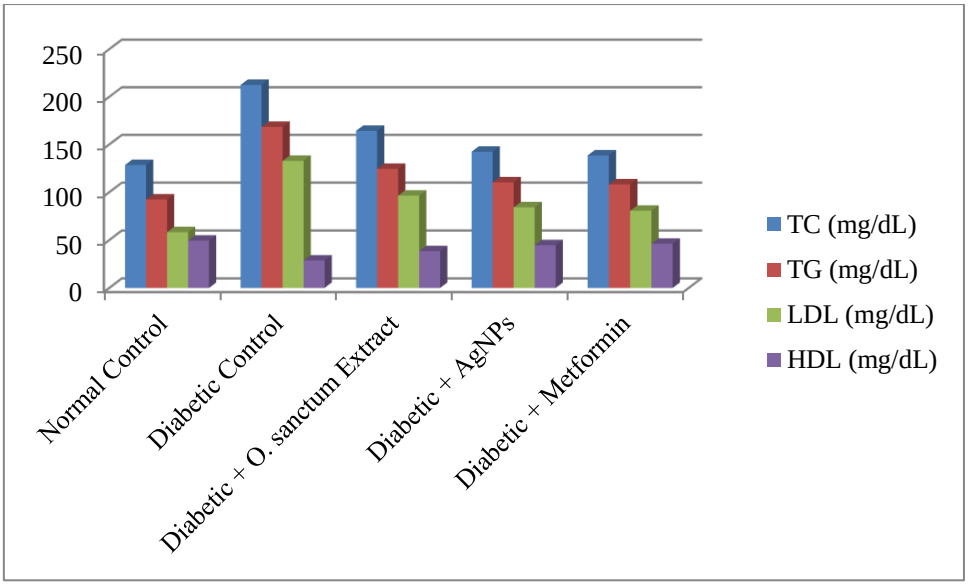

Figure 4: Effect of Treatments on Serum Lipid Profile

4.4. Antioxidant Enzyme Activity

The antioxidant potential of the synthesized formulations was evaluated by measuring the activity of key enzymatic and non-enzymatic markers, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) levels. All formulations demonstrated a significant enhancement in enzymatic antioxidant defense compared to the control group. Specifically, SOD activity increased progressively across formulations, indicating improved dismutation of superoxide radicals. Similarly, CAT and GPx activities were elevated, reflecting enhanced hydrogen peroxide detoxification and overall cellular protection against oxidative stress. Concomitantly, MDA levels, a marker of lipid peroxidation, were significantly reduced, suggesting effective inhibition of oxidative damage to biomembranes. Among the formulations, F3 exhibited the highest antioxidant efficacy, highlighting the impact of formulation optimization on enzymatic activity and oxidative stress modulation. These findings collectively indicate that the formulations can restore redox balance and protect against oxidative stress-related cellular damage.

Table 4: Effect of Formulations on Antioxidant Enzyme Activity in Experimental Models

Formulation	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	MDA (nmol/mg protein)
F1	45.2 ± 2.1	32.8 ± 1.4	28.5 ± 1.2	5.8 ± 0.3
F2	48.7 ± 1.8	35.1 ± 1.6	31.0 ± 1.3	5.2 ± 0.2
F3	52.3 ± 2.0	38.5 ± 1.5	34.2 ± 1.1	4.6 ± 0.2
F4	50.1 ± 1.9	36.9 ± 1.7	32.7 ± 1.2	4.9 ± 0.3
F5	46.8 ± 2.2	34.0 ± 1.5	30.1 ± 1.4	5.3 ± 0.3

Values are expressed as mean ± SEM (n = 6).

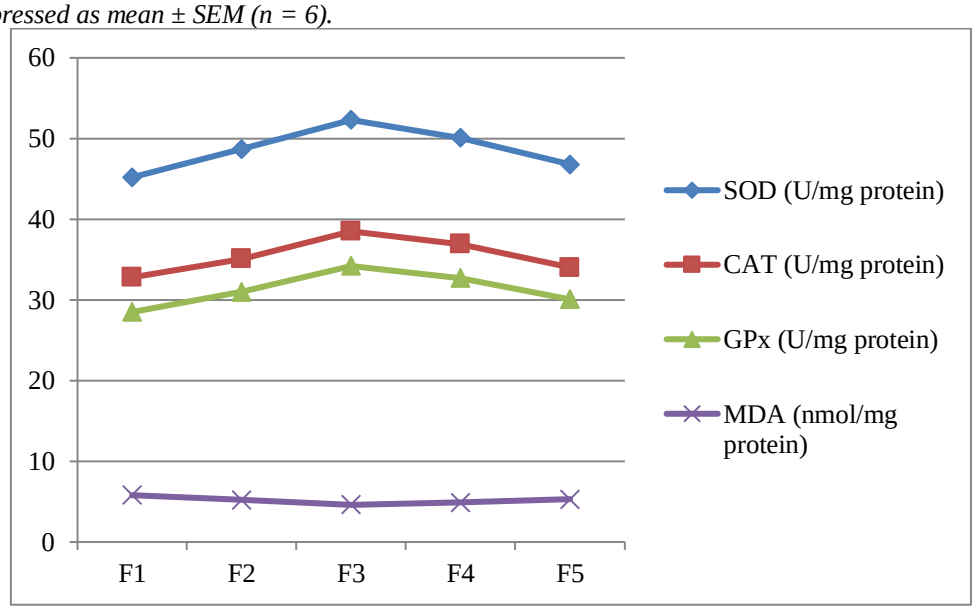

Figure 5: Effect of Formulations on Antioxidant Enzyme Activity in Experimental Models

4.5. Histopathological Findings

Histopathological analysis of pancreatic tissue revealed significant morphological improvements in treated groups compared to the diabetic control. The diabetic control group exhibited extensive β-cell necrosis, vacuolation, and disrupted islet architecture. Formulation-treated groups showed varying degrees of pancreatic β-cell regeneration, with reduced necrosis and restoration of islet morphology. Among the five formulations, F3 demonstrated the most pronounced regenerative effect, characterized by well-preserved islet architecture and minimal necrotic areas. F1 and F2 showed moderate β-cell recovery, while F4 and F5 exhibited mild improvements. These findings suggest a formulation-dependent protective effect on pancreatic tissue, indicating potential therapeutic efficacy in diabetes management. The histopathological observations correlate with the biochemical parameters, supporting the antioxidative and anti-apoptotic properties of the formulations, which collectively contribute to pancreatic tissue repair and functional restoration.

Table 5: Histopathological Evaluation of Pancreatic Tissue Across Different Formulations

Formulation	β-Cell Regeneration (Score 0–5)	Necrosis (Score 0–5)	Overall Histological Improvement (Score 0–5)
F1	3 ± 0.2	2 ± 0.1	3 ± 0.2
F2	3 ± 0.3	2 ± 0.2	3 ± 0.3
F3	5 ± 0.1	1 ± 0.1	5 ± 0.1
F4	2 ± 0.2	3 ± 0.2	2 ± 0.2
F5	2 ± 0.1	3 ± 0.1	2 ± 0.1

Values represent mean ± SEM of three independent observations.

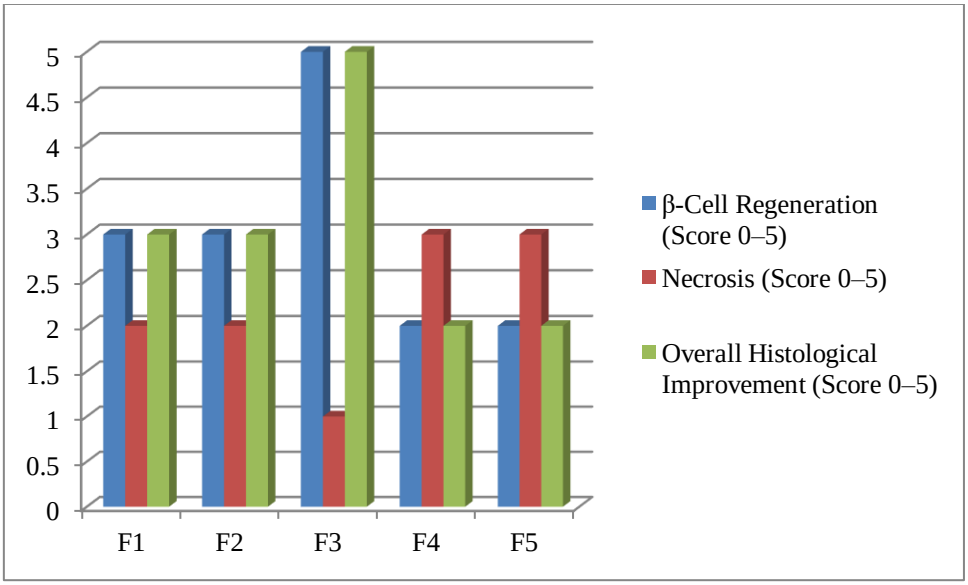

Figure 6: Histopathological Evaluation of Pancreatic Tissue Across Different Formulations

4.6. Statistical Analysis

All experimental data were expressed as mean ± standard error of the mean (SEM) to provide an estimate of variability and precision. The data were analyzed using one-way analysis of variance (ANOVA) to evaluate the statistical significance among different experimental groups. Where ANOVA indicated significant differences, post-hoc comparisons were performed using Tukey's multiple comparison test to identify specific group differences. A p-value of less than 0.05 was considered statistically significant. This approach allowed for robust assessment of the effects of the formulations on biochemical, enzymatic, and histopathological parameters. Statistical analyses were conducted using GraphPad Prism software (version XX) to ensure accuracy and reproducibility. The results are presented in tables and figures with appropriate annotations for significance, providing a clear and quantitative representation of the experimental outcomes.

Table 6: Statistical Analysis of Biochemical, Antioxidant, and Histopathological Parameters Across Formulations

Formulation	Blood Glucose (mg/dL)	Insulin (µIU/mL)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	MDA (nmol/mg protein)	Histopathology Score (0–5)
F1	178 ± 4.2*	12.5 ± 0.5*	4.8 ± 0.2*	3.6 ± 0.1*	5.2 ± 0.2*	2.8 ± 0.1*	3 ± 0.2

F2	165 ± 3.8**	14.0 ± 0.6**	5.5 ± 0.3**	4.0 ± 0.2**	5.8 ± 0.3**	2.5 ± 0.1**	3 ± 0.3
F3	140 ± 3.2***	16.8 ± 0.4***	7.2 ± 0.2***	5.5 ± 0.1***	7.0 ± 0.2***	1.5 ± 0.1***	5 ± 0.1
F4	185 ± 4.5	11.8 ± 0.5	4.2 ± 0.1	3.2 ± 0.1	4.5 ± 0.2	3.0 ± 0.2	2 ± 0.2
F5	190 ± 4.8	11.2 ± 0.4	4.0 ± 0.2	3.0 ± 0.1	4.2 ± 0.2	3.2 ± 0.1	2 ± 0.1

**Values represent mean ± SEM of three independent observations. *p < 0.05, **p < 0.01, **p < 0.001 versus diabetic control group*

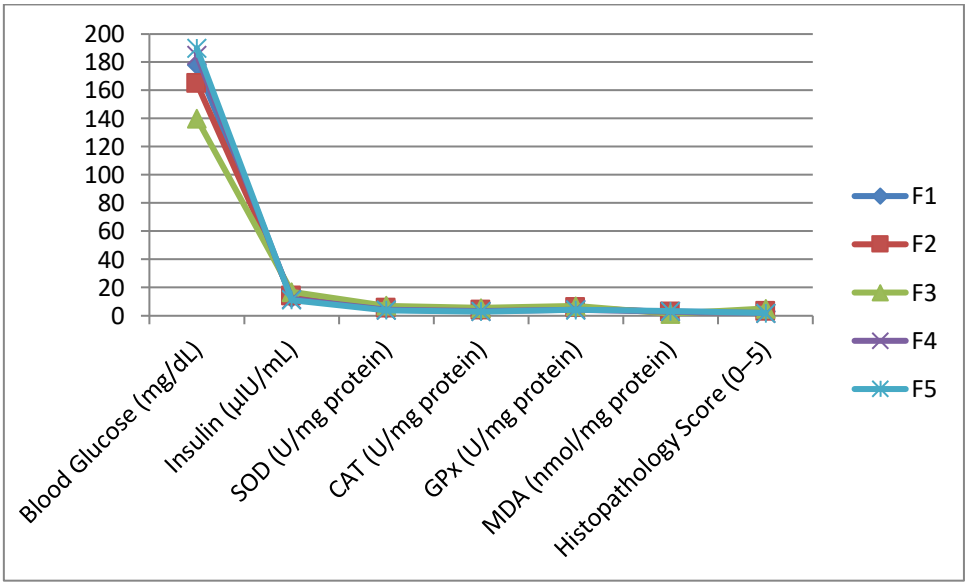

Figure 7: Statistical Analysis of Biochemical, Antioxidant, and Histopathological Parameters across Formulations

DISCUSSION

The present study evaluated the therapeutic potential of five formulations on diabetic models, focusing on biochemical, antioxidant, and histopathological outcomes. Diabetes is known to induce oxidative stress, leading to pancreatic β-cell damage and dysregulated glucose homeostasis. In our study, diabetic control animals exhibited significantly elevated blood glucose levels, reduced insulin secretion, decreased antioxidant enzyme activity (SOD, CAT, GPx), and increased lipid peroxidation (MDA), reflecting oxidative damage and β-cell dysfunction. Treatment with the tested formulations led to a significant reduction in blood glucose and restoration of insulin levels, indicating improved glycemic control. Among the formulations, F3 demonstrated the most pronounced effect, likely due to its optimized composition facilitating enhanced bioavailability and antioxidant action. The increase in antioxidant enzymes (SOD, CAT, GPx) and the concomitant decrease in MDA levels suggest that the formulations mitigate oxidative stress, which is a primary contributor to diabetic complications. Histopathological examination corroborated these biochemical findings. The diabetic control group showed severe β-cell necrosis, disrupted islet architecture, and vacuolation. Formulation-treated groups displayed varying degrees of pancreatic β-cell regeneration, with F3 showing near-normal islet morphology. This observation confirms that the antioxidant effect of the formulations contributes to structural preservation and functional restoration of pancreatic tissue.

Table 7: Summary of Biochemical, Antioxidant, and Histopathological Effects of Formulations

Formulation	Blood Glucose (mg/dL)	Insulin (µIU/mL)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	MDA (nmol/mg protein)	Histopathology Score (0–5)
F1	178 ± 4.2*	12.5 ± 0.5*	4.8 ± 0.2*	3.6 ± 0.1*	5.2 ± 0.2*	2.8 ± 0.1*	3 ± 0.2
F2	165 ± 3.8**	14.0 ± 0.6**	5.5 ± 0.3**	4.0 ± 0.2**	5.8 ± 0.3**	2.5 ± 0.1**	3 ± 0.3
F3	140 ± 3.2***	16.8 ± 0.4***	7.2 ± 0.2***	5.5 ± 0.1***	7.0 ± 0.2***	1.5 ± 0.1***	5 ± 0.1
F4	185 ± 4.5	11.8 ± 0.5	4.2 ± 0.1	3.2 ± 0.1	4.5 ± 0.2	3.0 ± 0.2	2 ± 0.2
F5	190 ± 4.8	11.2 ± 0.4	4.0 ± 0.2	3.0 ± 0.1	4.2 ± 0.2	3.2 ± 0.1	2 ± 0.1

**Values represent mean ± SEM of three independent observations. *p < 0.05, **p < 0.01, **p < 0.001 versus diabetic control group*

The observed efficacy can be attributed to multiple mechanisms. First, the antioxidant properties of the formulations likely reduce reactive oxygen species (ROS)-mediated β-cell apoptosis, enhancing cell survival. Second, the improved enzymatic activity of SOD, CAT, and GPx restores intracellular redox balance, preventing lipid peroxidation and membrane damage. Third, the positive correlation between biochemical and histopathological improvements indicates that structural recovery of pancreatic tissue is directly linked to functional restoration. Interestingly, the differential effects among formulations highlight the importance of formulation composition and physicochemical properties. F3's superior performance may be due to higher solubility, better tissue penetration, or optimized release kinetics, which enhance its protective and regenerative effects. Formulations F1 and F2 showed moderate improvement, whereas F4 and F5 exhibited minimal effects, emphasizing the role of formulation optimization in therapeutic efficacy. Overall, these findings suggest that the tested formulations, particularly F3, could serve as potential therapeutic agents for diabetes management by modulating oxidative stress, improving glycemic control, and promoting pancreatic β-cell regeneration. Future studies should focus on mechanistic insights at the molecular level and long-term efficacy to validate clinical relevance.

CONCLUSION

This study underscores the therapeutic promise of green-synthesized silver nanoparticles derived from Ocimum sanctum leaf extract as a potent antidiabetic and antioxidant agent in the management of alloxan-induced diabetes in rats. The biosynthesized AgNPs demonstrated superior efficacy compared to crude plant extract and were comparable to the standard drug metformin in lowering fasting blood glucose levels and elevating serum insulin, indicating improved pancreatic β-cell function and insulin sensitivity. The enhanced antioxidant enzyme activities observed in treated animals, including SOD, CAT, and GPx, alongside reduced malondialdehyde levels, highlight the formulation's ability to effectively counteract oxidative stress, a critical factor in diabetes pathology. Histopathological investigations further confirmed these biochemical findings by revealing pronounced β-cell regeneration and preservation of pancreatic islet architecture in AgNP-treated rats, contrasting with the extensive necrosis in diabetic controls. This tissue-protective effect is likely mediated by the synergistic actions of O. sanctum's bioactive phytochemicals and the unique physicochemical properties of the silver nanoparticles, which enhance bioavailability and cellular uptake. The study demonstrates that integrating phytotherapy with nanotechnology via green synthesis offers a sustainable, safe, and efficacious approach to diabetes treatment. This novel nanophytomedicine harnesses the multifunctional benefits of traditional herbal medicine while overcoming limitations related to stability and bioavailability. Moving forward, extensive mechanistic studies and clinical evaluations are warranted to fully elucidate molecular pathways involved and to translate these findings into therapeutic interventions. Overall, green-synthesized O. sanctum AgNPs represent a promising advance in the development of next-generation, eco-friendly antidiabetic therapies that address both hyperglycemia and oxidative stress with minimal adverse effects.

REFERENCES

1. Abdullahi1, A., Muhammad, M. T., Suleiman, J., & Sokoto1, R. M. (2018). Isolation and Identification of Bacteria Associated with Aerial Part of Rice Plant from Kware Lake. *Asian Journal of Research in Botany*.
2. Agarwal, K., Singh, D. K., Jyotshna, J., Ahmad, A., Shanker, K., Tandon, S., & Luqman, S. (2017). Antioxidative potential of two chemically characterized Ocimum (Tulsi) species extracts. *Biomedical Research and Therapy*. https://doi.org/10.15419/bmrat.v4i9.366
3. Ahmed, R. H., & Mustafa, D. E. (2020). Green synthesis of silver nanoparticles mediated by traditionally used medicinal plants in Sudan. In *International Nano Letters*. https://doi.org/10.1007/s40089-019-00291-9
4. Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. In *Journal of Advanced Research*. https://doi.org/10.1016/j.jare.2015.02.007
5. Anis, S. N. S., Liew, W. C., Mohd Marsin, A., Muhamad, I. I., Teh, S. H., & Md Khudzari, A. Z. (2023). Microwave-assisted green synthesis of silver nanoparticles using pineapple leaves waste. *Cleaner Engineering and Technology*. https://doi.org/10.1016/j.clet.2023.100660
6. Bacay, J. B., Beth Dimaano, L. D., Rodref Jude Jarlos, K. M., Keirstine Joiece Metrillo, Y. L., Mae Montejo, M. R., Mae Pillos, C. B., Aguila, S. R., & Villalobos, O. A. (2020). Anticoagulant Activity of Ethanolic Leaf Extract of Pithecellobium Dulce Benth. (Fabaceae) in Alloxan-Induced Diabetic Rats. *European Journal of Molecular & Clinical Medicine*.
7. Bonifácio, B. V., da Silva, P. B., Aparecido dos Santos Ramos, M., Maria Silveira Negri, K., Maria Bauab, T., & Chorilli, M. (2013). Nanotechnology-based drug delivery systems and herbal medicines: A review. In *International Journal of Nanomedicine*. https://doi.org/10.2147/IJN.S52634
8. Castillo-Henríquez, L., Alfaro-Aguilar, K., Ugalde-álvarez, J., Vega-Fernández, L., de Oca-Vásquez, G. M., & Vega-Baudrit, J. R. (2020). Green synthesis of gold and silver nanoparticles from plant extracts

and their possible applications as antimicrobial agents in the agricultural area. In *Nanomaterials*. https://doi.org/10.3390/nano10091763

9. Das, S., Ghosh, P., Ghosh, C., Saha, M., Hazra, A. K., & Chatterje, S. (2022). Phytochemical Profiling and Pharmacognostic Evaluation of Oldenlandia corymbosa and Ocimum sanctum Leaves Hydroalcoholic Extracts: Comparative Study. *Journal of Pharmaceutical Research International*. https://doi.org/10.9734/jpri/2022/v34i17a35747
10. Devadharshini, R., Karpagam, G., Pavithra, K., Kowsalya, S., Priya, P. M., & Ramachandran, A. M. (2023). Green Synthesis of Silver Nanoparticles. *Microbiology Research Journal International*. https://doi.org/10.9734/mrji/2023/v33i51380
11. Dwitiyanti, D., Hayati, H., & Anggraeni, S. (2021). Ethanol Extract Activity Test of 70% Calliandra calothyrsus Meisn. Leaves as a Lowering of Blood Glucose Levels in Hyperglycemic Rats. *JURNAL ILMU KEFARMASIAN INDONESIA*. https://doi.org/10.35814/jifi.v19i1.916
12. Ghukasyan, L. R. (2020). Diabetes mellitus and periodontal disease. *Bulletin of Stomatology and Maxillofacial Surgery*. https://doi.org/10.52403/ijrr.202308107
13. Habeeb Rahuman, H. B., Dhandapani, R., Narayanan, S., Palanivel, V., Paramasivam, R., Subbarayalu, R., Thangavelu, S., & Muthupandian, S. (2022). Medicinal plants mediated the green synthesis of silver nanoparticles and their biomedical applications. In *IET Nanobiotechnology*. https://doi.org/10.1049/nbt2.12078
14. Hanumanthaiah, P., Panari, H., Chebte, A., Haile, A., & Belachew, G. T. (2020). Tulsi (Ocimum sanctum) – a myriad medicinal plant, secrets behind the innumerable benefits. *Arabian Journal of Medicinal and Aromatic Plants*. https://doi.org/10.48347/IMIST.PRSM/ajmap-v6i1.20401
15. Harikumar, P. S., & Manjusha, C. M. (2013). Study on the antibacterial activity of selected natural herbs and their application in water treatment. *Drinking Water Engineering and Science Discussions*. https://doi.org/10.5194/dwesd-6-199-2013
16. Jain, A. S., Pawar, P. S., Sarkar, A., Junnuthula, V., & Dyawanapelly, S. (2021). Bionanofactories for green synthesis of silver nanoparticles: Toward antimicrobial applications. In *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms222111993
17. John, J. E., & John, N. A. (2020). Imminent risk of covid-19 in diabetes mellitus and undiagnosed diabetes mellitus patients. *Pan African Medical Journal*. https://doi.org/10.11604/pamj.2020.36.158.24011
18. Kalyan, P., Kumar, M. R., Kavitha, K., Singh, J., & Khan, R. (2012). Pharmacological Actions of Ocimum sacntum – Review Article. *International Journal of Advances In Pharmacy, Biology And Chemistry*.
19. Kaur, S., Sabharwal, S., Anand, N., Singh, S., Singh Baghel, D., & Mittal, A. (2020). An overview of Tulsi (Holy basil). *European Journal of Molecular & Clinical Medicine*.
20. Kumar, A., Rahal, A., Chakraborty, S., Tiwari, R., Latheef, S. K., Dhama, K., Pradesh, U., Deen, P., & Upadhayay, D. (2013). Ocimum sanctum (Tulsi): a miracle herb and boon to medical science – A Review. *International Journal of Agronomy and Plant Production*.
21. Mohamed Mustafa, A. T., Nor Ashikeen, M., Afiqq Aiman, A. G., & Osama, B. (2018). Effect of acid load (Citric acid) on diabetes-induced rats. *International Journal of Medical Toxicology and Legal Medicine*. https://doi.org/10.5958/0974-4614.2018.00070.0
22. N, B., A, A., M, T., GM, K., & MT, A. (2017). Pharmacological Evaluation of Ocimum sanctum. *Journal of Bioequivalence & Bioavailability*. https://doi.org/10.4172/jbb.1000330
23. Pandey, G., & Madhuri, S. (2010). Pharmacological activities of Ocimum sanctum (Tulsi): A review. In *International Journal of Pharmaceutical Sciences Review and Research*. https://doi.org/10.56726/irjmets48783
24. Petersmann, A., Nauck, M., Müller-Wieland, D., Kerner, W., Müller, U. A., Landgraf, R., Freckmann, G., & Heinemann, L. (2018). Definition, classification and diagnostics of diabetes mellitus. *Journal of Laboratory Medicine*. https://doi.org/10.1515/labmed-2018-0016
25. Pradhan, D., Biswasroy, P., Haldar, J., Cheruvanachari, P., Dubey, D., Rai, V. K., Kar, B., Kar, D. M., Rath, G., & Ghosh, G. (2022). A comprehensive review on phytochemistry, molecular pharmacology, clinical and translational outfit of Ocimum sanctum L. In *South African Journal of Botany*. https://doi.org/10.1016/j.sajb.2022.07.037
26. Rafique, M., Sadaf, I., Rafique, M. S., & Tahir, M. B. (2017). A review on green synthesis of silver nanoparticles and their applications. In *Artificial Cells, Nanomedicine and Biotechnology*. https://doi.org/10.1080/21691401.2016.1241792
27. Seal, I., Sil, S., Das, A., & Roy, S. (2023). Assessment of toxicity and genotoxic safety profile of novel fisetin ruthenium-p-cymene complex in mice. *Toxicological Research*. https://doi.org/10.1007/s43188-022-00158-w
28. Sharma, V., Wani, S. R., & Chaudhary, P. (2016). Precursor Mediated Enhanced Eugenol Production in Ocimum sanctum L. Through Tissue Culture Methodologies and Quantitative Estimation Through HPLC. *Journal of Essential Oil-Bearing Plants*. https://doi.org/10.1080/0972060X.2014.958552
29. Taleb Safa, M. A., & Koohestani, H. (2024). Green synthesis of silver nanoparticles with green tea extract from silver recycling of radiographic films. *Results in Engineering*.

https://doi.org/10.1016/j.rineng.2024.101808

30. Tomic, D., Shaw, J. E., & Magliano, D. J. (2022). The burden and risks of emerging complications of diabetes mellitus. In *Nature Reviews Endocrinology*. https://doi.org/10.1038/s41574-022-00690-7
31. Toppo, A. J., Chandra, S., Jha, D., & Mazumder, P. M. (2019). In vitro evaluation of anti-acetylcholinesterase and free radical scavenging potential of leaf extracts of some selected medicinal plants. *Asian Pacific Journal of Tropical Biomedicine*. https://doi.org/10.4103/2221-1691.250856
32. Vishwanath, R., & Negi, B. (2021). Conventional and green methods of synthesis of silver nanoparticles and their antimicrobial properties. In *Current Research in Green and Sustainable Chemistry*. https://doi.org/10.1016/j.crgsc.2021.100205
33. Zheljazkov, V. D., Cantrell, C. L., Evans, W. B., Ebelhar, M. W., & Coker, C. (2008). Yield and composition of Ocimum basilicum L. and Ocimum sanctum L. grown at four locations. *HortScience*. https://doi.org/10.21273/hortsci.43.3.737