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Research Article

Modulatory Effects of Chitosan Nanoparticles Against Alloxan-Induced Diabetes in Rats

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Abstract | Background: Diabetes is a metabolic condition characterized by chronic hyperglycemia and oxidative stress, which can cause organ damage. Bioactive chemicals have medical features such as antidiabetic and antioxidant capabilities, helping to avoid chronic disease by regulating physiological processes and improving metabolism and immunity. Aim: The current study aims to determine whether biocompatible chitosan nanoparticles are a natural adjuvant antidiabetic therapy with potential benefits, as well as to provide a systemic, quantifiable estimate of their effects on glycemic control, organ protection, and oxidative stress in a diabetic rat model. Methods: In rats, hyperglycemia was induced by a single intraperitoneal injection of alloxan monohydrate (120 mg/kg). Diabetic rats were given chitosan nanoparticles (300 mg/kg BW) orally for six weeks. The nanoparticles were studied with transmission electron microscopy (TEM), zeta potential, and FTIR spectroscopy. Biochemical, oxidative stress, histological, and immunohistochemical tests were performed. Results: Chitosan nanoparticles had a positive zeta potential (+47.7 mV) and distinct FTIR absorption bands. Treatment increased insulin levels by 65.15 percent and decreased blood glucose by 46 percent. The levels of glycosylated hemoglobin and amylase have been corrected. Liver enzymes (AST, ALP, and bilirubin) returned to near-normal values. The lipid profile improved with reductions in total cholesterol (↓40%), LDL-C (↓46.5%), and triglycerides (↓33.7%), while HDL-C increased (↑40%). CNPs reduced hepatic malondialdehyde by 19.4% and increased antioxidant enzyme activity (SOD by 49.3% and GST by 56.4%). Vitamin C, E, and glutathione levels were all partially restored. A histological examination revealed that chitosan nanoparticles preserved the normal structure of the liver and pancreas, remarkably reduced diabetic-induced damage, restored hepatocyte and islet morphology, and alleviated inflammation, necrosis, and β-cell atrophy compared to untreated diabetic rats. As regards immunostaining, chitosan nanoparticles retained strong insulin-positive immunoreactivity of pancreatic β-cells, enhancing β-cell expression in diabetic rats and reducing necrosis relative to poor staining in untreated diabetic animals. Conclusion: Chitosan nanoparticles have anti-diabetic, antioxidant, and tissue-protective properties, indicating their promise as a natural treatment for diabetes. Additional clinical trials are recommended to validate their safety and efficacy in humans.

Keywords | Alloxan, β-cells, Chitosan nanoparticles, Diabetes mellitus, Histopathology, Immunohistochemistry, Insulin, Nanotechnology, TEM

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INTRODUCTION

Diabetes mellitus is a long-term metabolic disorder caused by compromised metabolic processes. It affects people of all ages, genders, and populations. It is one of the world's leading causes of morbidity and mortality in adults. (Hossain *et al*., 2024). Diabetes is on the rise, especially in low-income countries, where many patients may not receive adequate treatment, management, or medical follow-up (Pradeepa, 2021). Diabetes is a disorder in which blood sugar levels remain continually elevated, either due to insulin insufficiency or insulin resistance. This disease impairs insulin receptors and metabolic processes in muscles, fat, and the liver, reducing functional capacity and quality of life (Shilbayeh, 2022). Diabetes mellitus has long-term effects such as retinopathy, renal failure, cardiovascular disease, and neuropathy, as well as an increased susceptibility to infections due to reduced immune function and an increased risk of mortality and amputation. According to recent studies, more than one-third of diabetes-related deaths occur among people below the age of sixty, emphasizing the chronic disease's considerable health burden across all age groups (Kropp *et al*., 2023).

Several antidiabetic medicines, such as sulfonylureas, thiazolidinediones, and alpha-glucosidase inhibitors, have been linked to a variety of side effects, the most notable of which include gastrointestinal problems and hepatotoxicity, which may limit the therapeutic efficacy of such pharmaceuticals (Weinberg *et al*., 2023). Furthermore, current evidence suggests that insulin medication may be linked to an increased risk of dementia, as indicated by Secnik *et al*. (2020), while the application of basal insulin or sulfonylureas is connected to a higher incidence of cardiovascular complications (Mannucci *et al*., 2015). Therefore, natural bioactive compounds have brought about renewed interest in antidiabetic medication development due to their potential efficacy, which prompted researchers to investigate natural options that may provide effective glucose regulation with fewer long-term consequences (Salehi *et al*., 2019).

Natural substances are gaining prominence in the treatment of diabetes due to their established therapeutic efficacy as well as a better safety profile. Their low side effects make them an appropriate choice for minimizing long-term consequences, supporting their usage as complementary or alternative medicines in diabetes treatment plans (Tran *et al*., 2020). Their medicinal advantages are further enhanced by their low cost and powerful antioxidant capabilities. However, the use of these natural agents in diabetes patients should be done under medical supervision and in conjunction with a healthy diet and frequent physical activity to maximize glucose control and enhance overall health outcomes. This integrative approach improves both safety and efficacy (Ansari *et al*., 2023).

Chitosan is one of the most important bioactive compounds. It is a biodegradable natural biopolymer resulting from the deacetylation of chitin, which is often derived from shrimp and crab exoskeletons, mollusk shells, and some fungi (Jiménez, 2020). Chitosan has amazing features including biodegradability, nontoxicity, and weak potential to provoke immune responses, making it a very biocompatible substance. These properties make it suitable for different types of biomedical applications, involving drug administration and tissue engineering applications, and wound healing (Muthu *et al*., 2021). Its level of deacetylation influences its solubility, biological activity, and overall efficacy in interacting with biological systems. Chitosan's strong biocompatibility has led to substantial research into its potential use in pharmaceuticals, food preservation, wound healing, and tissue engineering (Desai *et al*., 2023). Chitosan has been the subject of numerous studies examining its metabolic benefits, particularly in the areas of weight management and improving fat metabolism. Research has demonstrated its effectiveness in reducing weight gain by reducing the absorption of dietary fats, lowering triglyceride and cholesterol levels in the blood, and preventing excess fat accumulation in the liver (Shagdarova *et al*., 2023).

Nanochitosan, an advanced nanotechnology-based version of chitosan, is attracting widespread interest due to its distinct physical and chemical features. It is distinguished by the surface area which is significantly large in comparison to the volume, increased bioactivity, and exceptional environmental sensitivity. These nanoparticles have a high surface reactivity, so they are appropriate for a variety of applications, including medicine, biotechnology, and environmental science (Akdaşçi *et al*., 2025). Furthermore, nanochitosan has strong antioxidant capabilities and is essential for boosting the immune system, increasing resistance to bacterial infections, and accelerating tissue regeneration (Xia *et al*., 2022). In medicine, nanochitosan is very useful for regulated drug distribution. Because of its mucoadhesive qualities and capacity to encapsulate medicines, it improves drug stability, increases bioavailability, and enables targeted and prolonged drug release. This makes it an ideal option for generating new therapeutic treatments, particularly for chronic disorders such as diabetes, cancer, and infections (Jafernik *et al*., 2023). Moreover, nanochitosan has been extensively used in environmental applications, particularly water treatment, as it facilitates ˡeavy metal removal, including lead, cadmium, and chromium., as well as organic pollutants such as pesticides and oil residues (Benettayeb *et al*., 2023). Also, nanochitosan plays a significant role in modulating lipid metabolism, stimulating fat dissolution, and enhancing the composition of gut microbiota, which is vital for sustaining metabolic balance (Liu *et al*., 2021).

Alloxan was utilized in this investigation to develop diabetes in rats. This chemical substance preferentially dam-

ages pancreatic beta cells, causing insulin insufficiency and long-term hyperglycemia. Alloxan causes oxidative stress and cellular damage because it produces reactive oxygen species. Because of its beta-cell toxicity, it is frequently used in research to develop diabetic animal models for researching prospective therapies (Fajarwati *et al*., 2023).

Given the scientific evidence of the distinct biological properties of chitosan nanoparticles, this study aims to evaluate their antidiabetic potential, with a particular focus on their therapeutic effects in a model of alloxan-induced diabetes. The research also explores the effects of chitosan nanoparticles on glucose homeostasis, insulin sensitivity, and diabetes-related complications, as an adjuvant natural therapeutic agent for diabetes management.

MATERIALS AND METHODS

Chemicals

Alloxan monohydrate was bought from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Chitosan powder was obtained from Oxford Lab Chem. Company in India. The chemicals employed for this analysis were all obtained in analytical grade purity from commercial sources.

Chitosan Nanoparticle Development (ChNP)

Chitosan nanoparticles have been generated via ionic gelation, a process that takes advantage of the electrostatic contact between chitosan and a crosslinking agent (Hoang *et al*., 2022) with some modifications. Chitosan powder with molecular weight 161.16 and degree of deacetylation is 93%, solution was, stirred for 30 minutes with a magnetic stirrer. Twenty mL of an aqueous tripolyphosphate (TPP) solution (0.25% w/v) were introduced into 100 mL of chitosan solution while being magnetically stirred. Centrifugation at 5000 rpm for 30 minutes was used to extract nanoparticles at 4°C. Milky colored emulsion like appearance of nanochitosan was formed upon the ionic cross linking between TPP and chitosan solution. Nanochitosan was centrifuged at 10,000 rpm for 10 minutes to be isolated. The pellets formed were washed using distilled water and ethanol, air-dried, and stored before further use or analysis.

Physicochemical Analysis of Chitosan Nanoparticles

The following measurements were used to determine the properties of chitosan nanoparticles.

Effect of pH on nanoparticles: The effects of pH on chitosan nanoparticle solutions were investigated because it is one of the most important elements affecting zeta potential and nanoparticle size.

Determining the size and structural features of chitosan nanoparticles through transmission electron microscopy (TEM): The particle size and morphological examination of chitosan nanoparticles was determined by TEM (Ahmed and Aljaeid, 2016). A 100 µL sample of well-distributed nanoparticles was dropped onto a 200-mesh carbon grid covered with amorphous carbon, and the sample was then dried at room temperature. The dried samples are analyzed to determine the individual size. The JEOL JEM 2100 High Resolution Transmission Electron Microscope (HRTEM) was utilized to examine the individual particle size of nano synthesized chitosan. The HRTEM has a lattice resolution of 0.14 nm and a point-to-point resolution of 0.19 nm.

Zeta potential: The chitosan nanoparticles' charge was determined using a zeta potential instrument. The measurement was conducted within disposable polystyrene cuvettes at 25 °C, employing a detection angle of 90°. To determine the zeta potential, nanoparticle samples were diluted with de-ionized distilled water. The zeta potential of chitosan nanoparticles g was applied by Malvern Instruments.

Infrared absorption spectroscopy (FTIR): Fourier transform infrared (FTIR) analysis was applied to ascertain specific chemical groups. The FTIR spectra of chitosan nanoparticles were recorded using Spectrum Tow (PerkinElmer Spectrum IR Version 10.6.0).

Experimental Animals

This study utilized 40 male albino rats (Rattus norvegicus) aged 12 weeks, with an average body weight of 140 ± 5 grams. The rats were from the Animal Housing Facility at the National Research Centre in Dokki, Giza, Egypt. In a controlled environment and clean room, the animals were kept in cages made of polypropylene under pathogen-free environment. The rats were maintained at room temperature (27 ± 5 °C) with natural light cycles, fed a standard commercial pelleted diet, and had access to flowing water throughout. Prior to any experimental technique, there was a one-week acclimatization period. All experimental techniques were properly following the approved policy for ethical handling and treatment of experimental animals. The study protocols were ethically approved by the Research Ethics Committee of the Faculty of Science, Damanhour University, Egypt, under the number: DMU-SCI – CSRE- 241104).

Induction of Hyperglycemia

Following a 16-hour overnight fast with free access to drinking water, rats' blood glucose concentrations were evaluated. Type 1 diabetes mellitus hyperglycemia was generated by a single intraperitoneal administration of newly

prepared alloxan monohydrate solution in physiological saline at a dose of 120 mg/kg body weight. Type 1 diabetes was induced because alloxan selectively destroys pancreatic β-cells, leading to insulin deficiency and persistent hyperglycemia. To avoid drug-induced hypoglycemia, treated animals were orally administered 5% glucose solution for the first 24 hours after alloxan injection. The development of diabetes was verified six days after alloxan administration by measuring blood glucose levels. Animals with blood glucose higher than 250 mg/dL were considered diabetics and were exposed to the remaining experimental treatments. Throughout the course of the experiment, the control group's blood glucose levels remained normal (Fajarwati *et al*., 2023).

Experimental Design

In this experiment, rats were separated into four groups, with ten animals in each., and treated for six weeks using the following protocol:

Group 1 (G1): Comprised as the reference control group, remaining untreated.

Group 2 (G2): Non-diabetic rats were given an oral dose of chitosan nanoparticles (300 mg/kg body weight) every day.

Group 3 (G3): Diabetes was induced in rats at this group with alloxan as previously described in *(2.5)* with no other treatment.

Group 4 (G4): Alloxan-treated diabetic rats received the same dose of chitosan nanoparticles as G2.

Then, the animals were overnight fasted. After 24 hours after the final dose, they were subjected to inhalant anesthesia with isoflurane and sacrificed in the morning. Blood samples were drawn immediately from the tail vein using a sterile needle, ensuring minimal trauma, and collected into sterile tubes for further analysis. Next, the blood samples underwent centrifugation at 860×g for 20 minutes to obtain the serum. Liver and pancreas tissues were also taken for biochemical and histological investigations. All experiments were conducted in the laboratories of the Faculty of Science, Damanhour University, Egypt.

Biochemical Analyses

Liver tissues were quickly obtained after dissection, weighed, and placed in ice-cold 0.9% sodium chloride solution (normal saline). The tissue was then immersed in physiological saline for the removal of blood cells, applied to filter paper, after which they were minced, and homogenized in 50 mM potassium phosphate buffer (pH 7.4) using a mechanical Teflon apparatus equipped with a Potter-Elvehjem homogenizer. The tissues were centrifuged at 3000 × g for 10 min at 4°C. The clear supernatant that resulted was frozen and kept at -80°C until it was needed to measure different biochemical parameters.

Assessing of Glucose Concentration

Fasting blood glucose levels were quantified using commercially available Accurax blood glucose kits ensuring accuracy and reliability in glucose quantification.

Determination of Insulin Levels

The serum fasting insulin level was determined using ELISA. (Enzyme Linked Immuno-Sorbent Assay) method (Calbiotech ELISA kits).

Measurements of Glycosylated Hemoglobin (Hb)

Glycosylated hemoglobin (HbA1c) was measured using a commercial Elabscience kit, following the manufacturer's instructions for accurate assessment.

Measurment of Serum a-Amylase

Serum α-amylase was quantified using a commercially available test kit (Span diagnostic).

Investigation of Liver Function Parameters

Spectrophotometric analysis using commercial reagents (Sigma Diagnostics, India) was performed to measure serum AST, ALT, ALP, LDH, and total bilirubin. The activity of gamma-glutamyl-transferase (GGT) enzyme was measured using a kinetic assay with γ-glutamyl-p-nitroanilide as a substrate, as described by Li *et al*. (2011). Total cholesterol (TC) in serum, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) concentrations were measured using a commercially available enzymatic colorimetric reagent kit (Genzyme Diagnostics) and then on an automated clinical biochemical analyzer (Bayer ope-RA).

Analysis of Lipid Peroxidation and Antioxidant Enzyme Activities

The study involved a detailed biochemical assessment of malondialdehyde (MDA), a significant indicator of oxidative stress-induced lipid damage, as well as the functional analysis of crucial enzymatic components of the antioxidant system, namely superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) in liver using a Shimadzu UV2530 spectrophotometer (US) and commercially available assay kits, following standardized protocols.

Malondialdehyde (MDA) levels in liver homogenates were determined using the TBARS test, a spectrophotometric approach based on the interaction of MDA with thiobar-

bituric acid (Cheng *et al*., 2018). Superoxide dismutase (SOD), catalase (CAT), Glutathione peroxidase (GPx) and glutathione S-transferase (GST) levels were determined spectrophotometrically according to a previously established protocol (Gusti *et al*., 2021).

Evaluation of Nonenzymatic Antioxidants

The concentration of Vitamin C was determined using the technique of (Omaye *et al*., 1979). In brief, 0.5 mL of homogenized liver was subjected to 1.5 mL of 6% trichloroacetic acid (TCA) before being centrifuged at 10,000g for 20 minutes. The resulting supernatant was then treated with 0.5 mL of 2% dinitrophenylhydrazine (DNPH) and 4% thiourea in 9 N sulfuric acid for 3 hours at room temperature. Following incubation, 2.5 mL of 85% sulfuric acid was added, and the generated chromophore was measured spectrophotometrically at 530 nm after 30 minutes. Vitamin E concentrations were determined using Saran's technique (Saran *et al*., 2003). Vitamin E was extracted from hepatic tissue using 1.6 mL ethanol and 2.0 mL petroleum ether, followed by centrifugation. The top organic supernatant was carefully collected and evaporated in ambient air. The residue was then dissolved in 0.2 mL of 0.2% 2,2'-dipyridyl and 0.2 mL of 0.5% ferric chloride, which was left in the dark for 5 minutes. The addition of 4 mL of butanol produced a red color layer whose absorbance was measured at 520 nm. To assess reduced glutathione (GSH), one milliliter of supernatant was mixed with 0.5 mL of Ellman's reagent (19.8 mg of 5,5'-dithiobis 2-nitrobenzoic acid) (DTNB) diluted in 100 mL of 0.1% sodium citrate) and 3.0 mL of phosphate buffer (0.2 M, pH 8.0). The absorbance of the resulting yellow product was measured spectrophotometrically at 412 nm. To avoid spontaneous oxidation of GSH before analysis, the samples underwent reduction using potassium borohydride before beginning the investigation.

Assay of Protein

For protein quantification in the tissue samples, proteins were initially precipitated using a 10% trichloroacetic acid (TCA) solution and subsequently solubilized in 0.1 N sodium hydroxide to facilitate measurement. The protein content was then determined spectrophotometrically at a wavelength of 560 nm using the Lowry assay, as described by (Lowry *et al*., 1951), with bovine serum albumin (BSA) serving for calibration.

Histopathological Examination and Semi-Quantitative Scoring

Histopathological examination of pancreatic and liver tissues was conducted following (Gurina and Simms, 2025) standard procedures. The tissues were cleaned thoroughly in ice-cold physiological saline solution (0.9% sodium chloride) immediately after dissection to prevent any left-behind blood and surface debris that could interfere with proper fixation. After that, small representative tissue samples were carefully cut out and immediately fixed in 10% neutral buffered formalin to ensure optimal fixation and preservation of cellular architecture and to prevent autolysis and tissue destruction. The tissues were then air dried naturally and filtered to discard the excess fixative before processing the tissue. Fixed tissues were then processed for routine dehydration in increasing grades of alcohol, cleared in xylene, and embedded in paraffin wax to create solid tissue blocks. Paraffin-embedded tissues were then sliced into thin sections of approximately 4 micrometers (µm) thickness with a rotary microtome. The tissue sections obtained were fixed onto glass slides and stained with hematoxylin and eosin (H and E) to differentiate cellular and structural components. Hematoxylin stained the nuclei blue, while eosin stained the cytoplasm and extracellular matrix pink. Histological examination was then performed using a Leica binocular light microscopy with DMC4500 Digital Microscope Camera.

Immunohistochemical (IHC) Studies

Immunohistochemical staining for insulin in pancreatic tissue was performed using the avidin-biotin-peroxidase complex (ABC) technique, as previously described (Chang, 2000). 4-µm-thick paraffin-embedded pancreatic tissue sections were deparaffinized in xylene and rehydrated in graded ethanol. Antigen retrieval was performed by heating the sections in a microwave oven with citrate buffer at pH 6.0. To inactivate endogenous peroxidase activity, slides were immersed in 0.3% hydrogen peroxide (H_2O_2) in methanol for 30 minutes at room temperature. Antibody binding to non-specific sites was reduced via incubation in 5% normal goat serum for 1 hour. The tissues were then treated for 1 hour at room temperature in a humidified chamber with a mouse monoclonal anti-insulin primary antibody (Nova Castra Laboratories Ltd., UK). The slides were then washed and treated with a biotinylated secondary antibody (mouse anti-IgM) for 30 minutes before being incubated in Extra-avidin peroxidase conjugate (Sigma-Aldrich, USA) for 45 minutes at 37°C. The color was developed using 3,3'-diaminobenzidine (DAB) as a chromogenic substrate. Sections were then counterstained with Mayer's hematoxylin. Light microscopy was used to evaluate immunostaining and identify insulin-positive beta cells as brown cytoplasmic staining.

Statistical Analysis

Statistical analysis was done using the SPSS software (version 26). Data were expressed as mean ± standard deviation (SD). Normality of data distribution was confirmed using the Kolmogorov–Smirnov test, which revealed a normal distribution. Experimental groups' differences were compared using one-way analysis of variance (ANOVA), and

subsequently Duncan's post hoc test for multiple comparisons. A p-value < 0.05 was used to declare statistical significance (Cooksey, 2020).

RESULTS

Characterization of Chitosan Nanoparticles

For this research, the chitosan solution's pH was brought to a level of 4.8. This is because the average size of chitosan nanoparticles is affected by a variety of parameters, including the pH of the solution. Figure 1A and 1B, shows a TEM image of the prepared chitosan nanoparticles. The nanoparticles are nearly uniform in size and shape and approximately spherical with a smooth surface, with nanoparticle diameters ranging between 23 to 90 nm, indicating that all particles were under 90 nm. The chitosan nanoparticles had a positive zeta potential, measuring +47.7 mV. (Figure 2). Furthermore, characterization was carried out using FTIR absorption spectroscopy. Figure 3 shows FTIR spectral details of the chitosan nanoparticles. A broad band was measured at nearly 3434.73 cm^{-1}, this can be explained by the stretching vibrations of the –NH2 and –OH groups. The peak obtained at 2083.50 cm^{-1} corresponds to the aliphatic –CH asymmetric stretching.

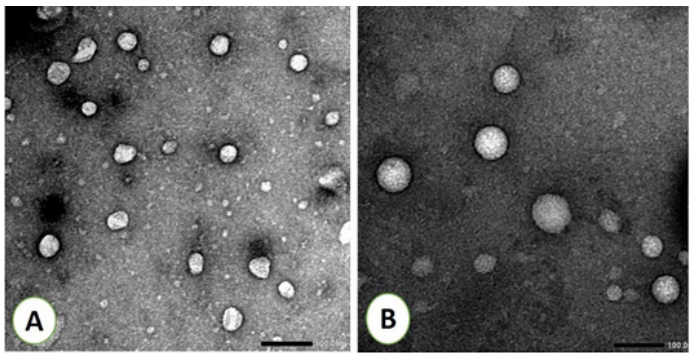

Figure 1: Images of high-resolution transmission electron microscopy (HR-TEM). Chitosan nanoparticles were analyzed for morphology and homogeneity, exhibiting consistent size and shape distributions. The particle diameters range from 23 to 90 nm, as measured at a 100 nm scale, confirming their nanoscale size.

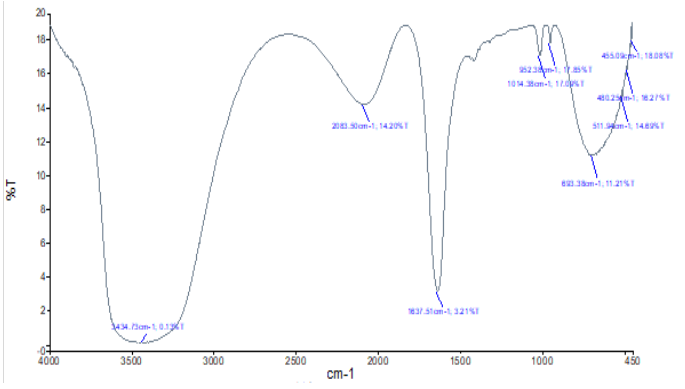

Figure 2: Zeta potential profile for chitosan nanoparticles.

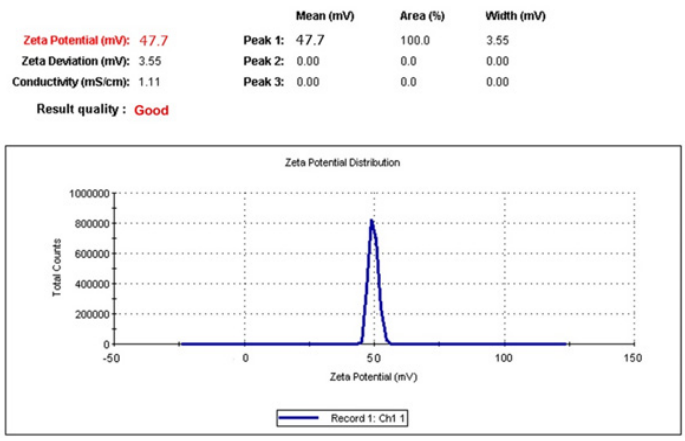

Figure 3: FTIR spectrum of chitosan nanoparticles.

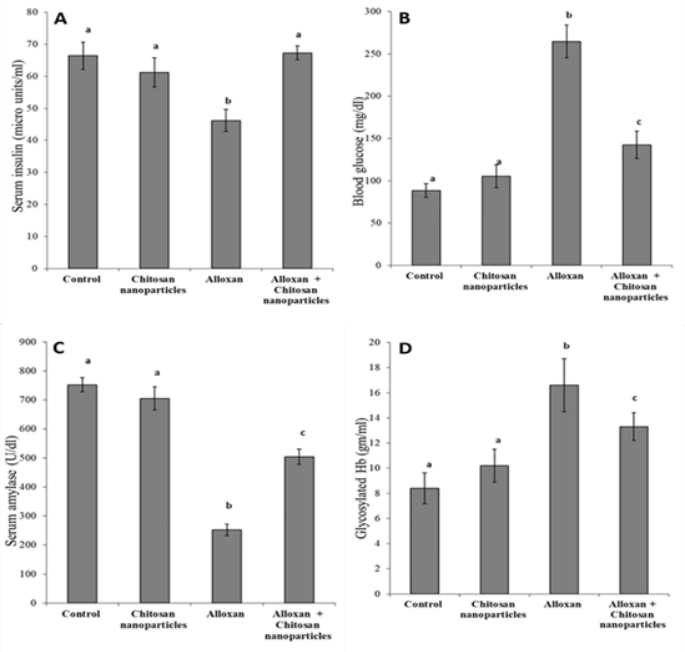

Figure 4: The effects of Chitosan nanoparticles on levels of **A:** serum insulin; **B:** plasma blood glucose; **C:** serum amylase; **D:** glycosylation of Hb. Data are shown as mean ± standard deviation (n = 7 per group), with different letters indicating significant differences between groups ($p < 0.05$) as determined by Duncan's Multiple Range Test (DMRT).

Effects of Chitosan Nanoparticles on Serum Insulin, Plasma Blood Glucose, Serum Amylase and Hb Glycosylation

Alloxan-treated rats showed decreased insulin levels (-30.4%) as compared to control rats. Co-treatment with chitosan nanoparticles for alloxan-treated rats considerably improved insulin levels (65.15%) as compared to alloxan treated group (Figure 4A). Chitosan nanoparticles also reduced serum glucose levels. Figure 4B shows that the diabetic group had significant (p<0.05) high levels of glucose in the blood serum of 200% in comparison with the normal control group. Using chitosan nanoparticles in diabetic rats resulted in a marked reduction of glucose lev-

els by 46% as compared to the alloxan group. In addition, results in Figure 4C show that the alloxan-treated group had a significant ($p<0.05$) reduction in serum amylase concentration (-66.42%) as compared to the control. Chitosan nanoparticles reversed this alloxan reduction of serum amylase ($p<0.05$). The rats treated with alloxan also exhibited a significant ($p<0.05$) increase in hemoglobin glycosylation, exceeding 97%. Alternatively, a significant decline ($p<0.05$) in glycosylated hemoglobin (-19.88%) was observed in the chitosan nanoparticles/alloxan co-treated group (Figure 4D).

Chitosan Nanoparticles Reversed the Elevation of Liver Functional Markers

Results of liver functions are shown in Table 1. Rats that received alloxan developed significant ($p<0.05$) hepatic damage, as illustrated by the increase of hepato-specific marker serum levels like AST, ALT, ALP, LDH, and GGT by 37.2%, 51.1%, 31.6%, 47.4%, and 149%, respectively, as compared to control. Conversely, administration of chitosan nanoparticles significantly ($p<0.05$) attenuated the elevated levels of AST (-36%), ALP (-27.1%), and total bilirubin (45%) compared with the alloxan-exposed rats, while ALT, LDH, and GGT showed a non-significant modulation.

Table 1: The effects of chitosan nanoparticles on the activity of liver markers in the serum of alloxan-treated diabetic rats.

Groups	Control	Chitosan nanoparticles	Alloxan	Alloxan and Chitosan nanoparticles
AST (U/l)	44.47 ± 2.51[a]	42.29 ± 2.07[a]	61.04 ± 5.17[b]	39.11 ± 2.55[a]
ALT (U/l)	29.50 ± 2.47[a]	30.04 ± 2.18[a]	44.58 ± 5.66[b]	33.54± 2.80[c]
ALP (U/l)	97.04 ± 7.03[a]	91.28 ± 8.51[a]	127.71 ± 9.72[b]	93.03 ± 6.16[a]
LDH (U/l)	127.22 ±10.75[a]	122.28 ±11.48[a]	187.55 ±14.04[b]	142.78 ±12.25[c]
GGT (U/l)	0.63 ±0.06[a]	0.71 ±0.07[a]	1.57 ±0.09[b]	0.84 ±0.07[c]
Bilirubin (mg/dl)	0.71 ±0.07[a]	0.76 ±0.06[a]	1.03 ±0.09[b]	0.83 ±0.06[a]

Data are shown as mean ± standard deviation (n = 7 per group), with different letters indicating significant differences between groups ($p < 0.05$).

Chitosan Nanoparticles Reversed the Elevation of Diabetic Lipid Profiles

Table 2 shows that alloxan administration led to prominent hypercholesterolemia by 84%. Serum LDL-C and Tg levels showed a significant increase ($p<0.05$) by 79.49% and 96.25%, respectively, in comparison with the control. Whereas the level of serum HDL-C sharply dropped by 28.12% for the diabetic group compared to control. Administration of chitosan nanoparticles with alloxan eliminated these increases in total cholesterol (Tc) (-40.0%), LDL-C (-46.5%), and Tg (-33.7%) concentrations induced by alloxan. Chitosan nanoparticles also significantly increased HDL-C concentration by more than 40% in comparison with the diabetic group.

Table 2: The effects of chitosan nanoparticles on the activity of serum total cholesterol (Tc), low density lipoprotein-cholesterol (LDL-c), high density lipoprotein-cholesterol (HDL-c) and triglyceride (Tg) on alloxan-treated diabetic rats.

Groups	Control	Chitosan nanoparticles	Alloxan	Alloxan and Chitosan nanoparticles
Tc (mg/dL)	80.22 ± 4.23[a]	84.62 ± 5.72[a]	147.65 ± 9.13[b]	88.67 ± 5.63[a]
LDL-c (mg/dL)	18.70 ± 2.63[a]	20.82 ± 3.11[a]	36.70 ± 4.24[b]	19.61 ± 3.12[a]
HDL-c (mg/dL)	146.82 ± 6.48[a]	141.70 ± 7.23[a]	105.53 ± 6.33[b]	148.72 ± 6.24[a]
Tg (mg/dL)	96.16 ± 7.58[a]	92.44 ± 8.44[a]	172.60 ± 10.31[b]	114.36 ± 8.28[c]

Data are shown as mean ± standard deviation (n = 7 per group), with different letters indicating significant differences between groups ($p < 0.05$).

Table 3: The effects of chitosan nanoparticles on hepatic lipid peroxidation (MDA) and the enzymatic antioxidants in alloxan-induced hyperglycemic rats.

Groups	Control	Chitosan nanoparticles	Alloxan	Alloxan and Chitosan nanoparticles
MDA (nmol/g)	16.47 ± 0.73[a]	18.53 ± 0.85[a]	27.38 ± 1.22[b]	22.07 ± 1.03[c]
SOD (units/mg protein)	12.25 ± 0.46[a]	11.23 ± 0.33[a]	6.51 ± 0.15[b]	9.72 ± 0.35[c]
CAT (µmole/min/mg protein)	63.51 ± 11.46[a]	68.47 ± 13.36[a]	42.32 ± 6.14[b]	66.21 ± 10.13[a]
GPx (µg/min/mg protein)	17.28 ± 4.26[a]	15.11 ± 3.47[a]	10.53 ± 2.05[b]	16.74 ± 3.83[a]
GST (µmole/min/mg protein)	13.85 ± 1.73[a]	14.30 ±2.79[a]	7.83 ± 1.82[b]	11.04 ± 2.06[c]

Data are shown as mean ± standard deviation (n = 7 per group), with different letters indicating significant differences between groups ($p < 0.05$).

Chitosan Nanoparticles Modulate MDA Level and Enzymatic Antioxidants Status

Table 3 shows parameter values related to oxidative stress in rat livers. The levels of MDA, a biomarker for lipid peroxidation, were significantly ($p<0.05$) elevated by 66.24%

in the liver tissues of rats with alloxan-induced hyperglycemia when compared to the control. The application of chitosan nanoparticles during alloxan administration was associated with a significant decrease (-19.4%, p <0.05) in MDA levels in contrast to the alloxan-treated group. Besides, alloxan administration caused a significant (p<0.05) reduction in antioxidant enzymes (SOD, CAT, Gpx, GST) by 46.8%, 33.3%, 39.0%, and 43.4%, respectively, when contrasted with the control group. The utilization of chitosan nanoparticles with alloxan significantly (p<0.05) improved both SOD and GST activities by 49.3% and 41% in co-treated rats, while CAT and GPx activities were not significantly different from control.

Chitosan Nanoparticles Modulates Diabetic Nonenzymatic Antioxidants

Hepatic vitamin E and C levels were significantly (p<0.05) reduced by 36.84% and 40.86%, respectively, in alloxan-treated rats compared to control. The treatment of Chitosan nanoparticles resulted in an increase of 19.44% and 29%. Alloxan furthermore caused a significant (p<0.05) reduction in GSH amount by 34.72% relative to the control group. This reduction was prevented by co-treatment with chitosan nanoparticles (Table 4).

Table 4: Effects of chitosan nanoparticles on the levels of non-enzymatic antioxidants in liver of alloxan-induced hyperglycemic rats.

Groups	Control	Chitosan nanoparticles	Alloxan	Alloxan and Chitosan nanoparticles
Vitamin C (µmol/mg protein)	0.93 ± 0.07[a]	0.85 ± 0.06[a]	0.55 ± 0.03[b]	0.71 ± 0.06[c]
Vitamin E (µmol/mg protein)	0.57 ± 0.05[a]	0.52 ± 0.05[a]	0.36 ± 0.01[b]	0.43 ± 0.04[c]
GSH (mg/g protein)	5.27 ± 0.26[a]	4.84 ± 0.66[a]	3.44 ± 0.38[b]	4.02 ± 0.58[c]

Data are shown as mean ± standard deviation (n = 7 per group), with different letters indicating significant differences between groups (p < 0.05).

Histological Examination of Liver

Examinations of the transverse liver sections by light microscopy for the control (G1) (Figure 5A) and chitosan nanoparticles treated (G2) (Figure 5B) rats show normal architecture, lobules containing well-formed hepatocytes with a well-preserved cytoplasm and prominent nucleus, and nucleolus with distinct portal triads. Hepatic cells were spatially structured like a cord and separated by sinusoids, with a clear central vein. For liver sections of diabetic animals (G3), on the other hand, extensive damage was seen, as evidenced by dramatic hepatocellular degeneration and inflammatory cell infiltration, vascular congestion, sinusoidal expansion, and cytoplasmic vacuolization, (Figure 6C, 6D and 6E), and large necrotic areas with neutrophil and hemorrhaging. The severe hepatic lesions of diabetic animals were remarkably reduced by treatment with chitosan nanoparticles (G4) (Figure 5F), revealing a histology very similar to the control (G1).

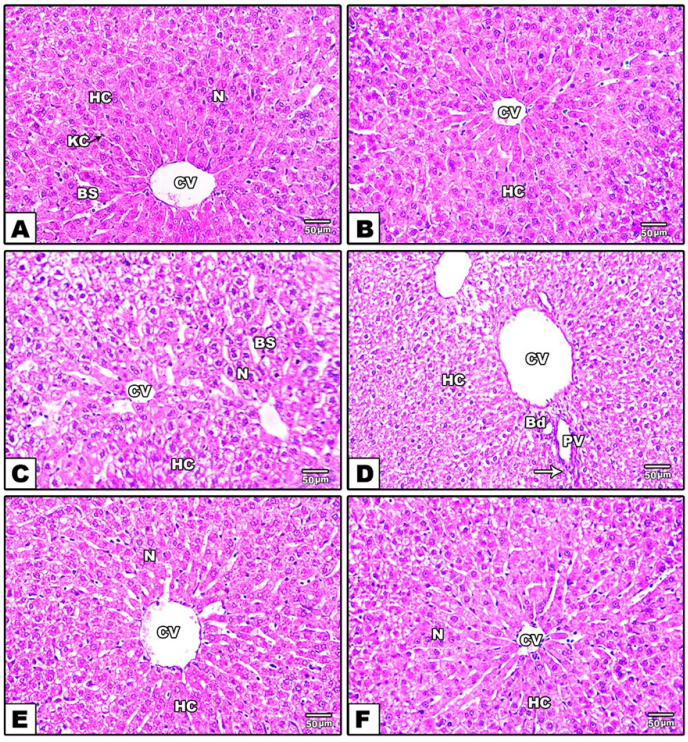

Figure 5: Photomicrographs of H and E-stained liver tissue (x200), sections for **A:** control rats; **B:** chitosan nanoparticles treated group; **C**, **D** and **E:** alloxan-treated group; **F:** alloxan + chitosan nanoparticles treated group. Labels are included for central vein (CV), hepatocytes (HC) with round nuclei (N), blood sinusoid (BS), Kupffer cell (KC), bile ductuli (BD), portal vein (PV), and leukocytic infiltration (arrow). Scale bare: 50µm.

Histological Examination of Pancreas

In the hematoxylin and eosin -stained histological sections of the pancreas in the control group (G1) and chitosan nanoparticles group (non-diabetic rat group G2), the acini and islet of Langerhans showed normal architecture. The pancreas is divided into lobules and is encased in a thin connective tissue capsule. The pancreatic acini looked rounded or oval. Pyramidal epithelial cells were organized radially around the narrow acinar lumen, forming its structural lining. Acinar cells had amphophilic cytoplasm and basal rounded nuclei. Scattered inside the pancreatic lobules are the Langerhans islets, showing up as light pink rounded or oval areas with islet cells arranged in trabecular and acini pattern with plentiful eosinophilic cytoplasm and tiny nuclei in the middle, divided by thin vessels and connective tissue. The islets size, location, and shape varied, with numerous β-cells of normally round shape and distinctly round nuclei (Figure 6A and 6B).

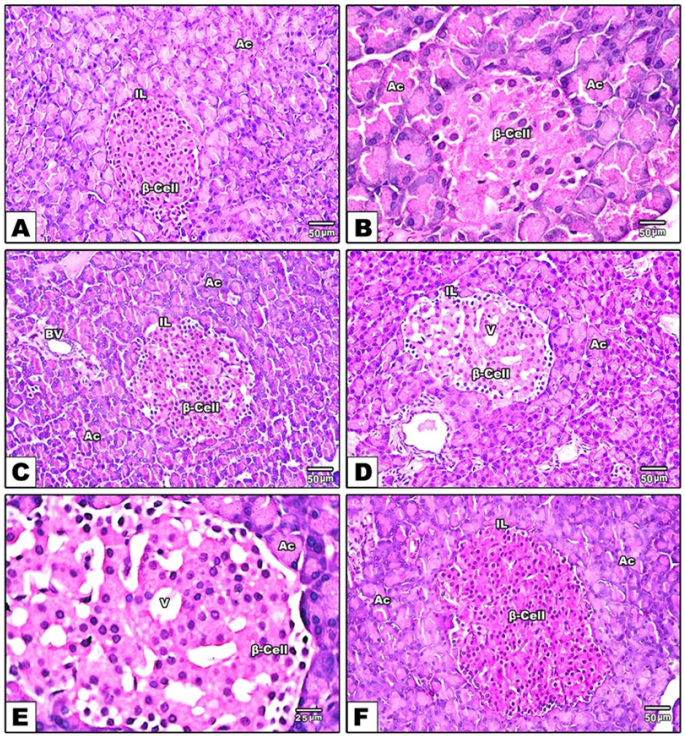

Figure 6: Photomicrographs of H and E-stained sections of pancreatic tissues (x200 and x400); **A:** Control rats (G1) show normal architecture of pancreatic tissue. Notice the pyramidal pancreatic acinar cells with a peripheral darkly stained nucleus, with the islet of Langerhans (IL) appearing as a pale oval area among dark acini of different sizes and shapes (Ac); **B:** Non-diabetic rats treated with chitosan nanoparticles (G2) show no pathological changes with well-organized pancreatic acini (Ac) and an islet of Langerhans (IL); **C**, **D** and **E:** Alloxanized diabetic rats (G3) show complete loss of normal architecture of pancreatic acini (Ac) and islet of Langerhans (IL) with dilated and congested blood vessels (CBV). Notice atrophy and degenerated β-cell with multiple cytoplasmic vacuoles of variable sizes (V) in the cytoplasm of the islet of Langerhans; **F:** Alloxanized diabetic rats supplemented with chitosan nanoparticles (G4) show normal acini (AC) and islet of Langerhans (IL) similar to control. Scale bare: 50µm (A, B, C, D, and F) and 25µm (E).

The pancreatic tissues of diabetic rats (G3) (Figure 6C, 6D and 6E) exhibited extensive damage to the normal pancreatic structure. Blood vessels were dilatated and congested with leucocytic infiltration, the acini exhibited a process of degeneration, and their cellular components were swollen. A clear shrinkage of islets' size, irregular shape, and many cytoplasmic vacuoles were observed. However, the alloxanized diabetic rats given chitosan nanoparticles (G4) with no significant changes of pancreatic architecture in comparison to the normal pancreatic tissues of G1 (Figure 6F). Langerhans islets had restored size, the cells occurred in more regular numbers with restored shapes and an entire connective tissue sheet.

Immunohistochemistry of Insulin Secreting Pancreatic β-Cell

The pancreas of rats from the control group (G1) in addition to rats without diabetes that were treated with chitosan nanoparticles (G2) expressed strong positive immune reactivity (brown color) to the insulin secreting β-cell (Figure 7A and 7B). Reaction sites and nuclei were stained brown and blue, respectively. The alloxanized diabetic rats (G3) showed weak β-cell expression with distinctly necrotic regions in the islets of Langerhans (Figure 7C). In contrast, alloxanized rats with diabetes that were given chitosan nanoparticles (G4) had a clear improvement in β-cell expression (Figure 7D).

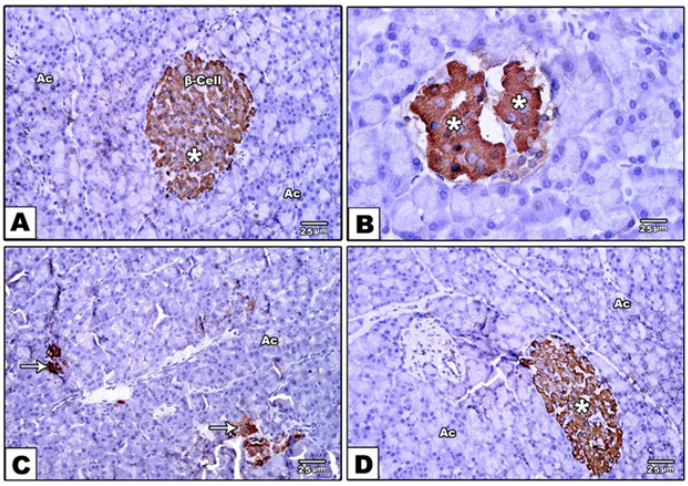

Figure 7: Photomicrographs of anti-insulin immune-reactivity-stained sections of rat pancreatic tissues (x200); **A:** Control rats (G1) show clearly positive immune-expression of insulin secreting β-cell (star); **B:** Non-diabetic rats treated with chitosan nanoparticles (G2) show an obvious increase in immune-reaction of insulin secreting β-cell (star) similar to the control expression; **C:** Alloxanized diabetic rats (G3) showing weak positive immune-reaction of β-cell (arrows) and a clearly necrotic islet area; **D:** Alloxanized diabetic rats treated with chitosan nanoparticles (G4) showing clear improvement and increased expression of insulin secreting β-cell (star). Scale bare: 25µm.

DISCUSSION

This study demonstrated that chitosan nanoparticles have potential antidiabetic properties in alloxan-induced diabetic rats. In terms of initial chitosan nanoparticle preparation, our use of ionic coagulation is consistent with previous studies (El-Naggar *et al.*, 2022), ensuring biodegradability, stability, and compatibility. The optimized pH (4.8) facilitated the formation of intact nanoparticles, prevented fine particle aggregation, and resulted in smaller and more organized particles through effective tripolyphosphate (TPP) crosslinking. The produced chitosan nanoparticles

had spherical shape and a consistent size distribution (23-90 nm), as validated by TEM examination. Because of the amino groups, they possess a positive zeta potential, implying robust colloidal stability, which is critical for biological applications (Yin *et al*., 2018). Fourier Transform Infrared Spectroscopy (FTIR) examination further validated the successful nanoparticle preparation.

Our results showed that diabetes induced by alloxan increased fasting blood glucose levels due to pancreatic β-cell destruction, leading to insulin deficiency (289-298 mg/dL) which was consistent with previous research articles (Farid *et al*., 2024). Nevertheless, giving chitosan nanoparticles led to a notable decrease in blood sugar and a significant rise in insulin release., suggesting β-cell regeneration and improved pancreatic function (Gwarzo *et al*., 2014). Chitosan nanoparticles (CNPs) optimize blood glucose levels and increase insulin levels via a variety of ways. They improve pancreatic beta cell activity by reducing oxidative stress and inflammation, both of which induce beta cell damage in diabetes patients. Their improved performance can also be linked to their mechanism of modulating insulin receptor signaling cascades and glucose uptake by peripheral organs. Furthermore, their antioxidant action protects pancreatic tissue from alloxan-induced oxidative damage, hence maintaining insulin secretion. Finally, these operations are linked to better blood glucose management and higher insulin levels. Moreover, the present investigation revealed that alloxan-induced diabetic rats showed elevated malondialdehyde (MDA) levels, reflecting enhanced lipid peroxidation, together with a decrease in the activity of important antioxidant enzymes (SOD, CAT, GPx, and GST) (Mosa *et al*., 2020). Treatment with chitosan nanoparticles significantly reduced MDA levels while restoring antioxidant enzyme activity, indicating their role in neutralizing free radicals and reducing oxidative stress-induced cellular damage (Canbolat *et al*., 2024).

In this research, applying chitosan nanoparticles significantly reduced blood sugar levels, which may be attributed to its ability to inhibit carbohydrate-digesting enzymes, such as alpha-amylase, sucrase, and maltase, thus delaying glucose absorption. These results are consistent with previous research indicating that alpha-amylase inhibition plays a crucial role in controlling hyperglycemia (Tzeng *et al*., 2022). Furthermore, our results demonstrate that chitosan nanoparticles enhance insulin secretion, promote beta cell regeneration, and increase glucose uptake in skeletal muscle, which is consistent with previous studies suggesting chitosan's role in improving insulin sensitivity and pancreatic function. Additionally, we observed a significant reduction in glycated hemoglobin (HbA1c) levels, supporting previous evidence of chitosan's potential to combat diabetes through its effects on glucose metabolism and the preservation of pancreatic β cells (Guo *et al*., 2020).

Hepatic damage is among the most prevalent and clinically severe consequences linked with diabetes. This damage is characterized by elevated levels of ALT, AST, ALP, and LDH, indicating liver injury and endothelial instability (Mohamed *et al*., 2016). In this investigation, diabetic rats had significantly increased levels of liver enzymes, confirming impaired liver function. However, therapeutic treatment with chitosan nanoparticles restored enzyme levels to normal, indicating the protective effects of these particles on the liver. This protective effect may be attributed to their ability to prevent oxidative damage caused by diabetes and to support liver cell regeneration which was indicated in previous studies and confirmed by the current study AlKandari *et al*. (2024).

Diabetic rats also showed dyslipidemia, marked by elevated total cholesterol, LDL cholesterol, and triglycerides, while leading to decreased levels of HDL cholesterol. Lipid abnormalities are a key contributor for cardiovascular disease in diabetics (Oksal *et al*., 2020). Interestingly, Chitosan's ability to modulate lipid levels may be related to its potential to bind fats and inhibit their absorption in the intestine (Liu *et al*., 2024).

Data collected in this research highlights the potential of chitosan nanoparticles as therapeutic agents, this is indicated by its ability to effectively restore hepatic architecture in diabetic rats, as evidenced by the preservation of hepatocyte integrity and reduction of inflammatory damage, mitigating liver injury associated with diabetes, and promoting cellular health and function, this coincides with other researches (Hashim *et al*., 2022).

Given the importance of studying the pancreas as a key organ in type 1 diabetes, histopathological analysis of diabetic rat pancreas revealed reduced pancreatic islet size, beta cell degeneration, and necrosis, confirming the severe pancreatic damage caused using alloxan to induce diabetes (Shah, 2014). However, rats treated with chitosan nanoparticles showed significant improvements, such as repaired pancreatic islet shape, increased beta cell density, and improved insulin secretion. These findings indicate that chitosan nanoparticles promote pancreas regeneration, most likely due to their antioxidative and anti-inflammatory potential.

Our findings indicated a considerable drop in GSH, vitamin C, and vitamin E levels in rats with experimentally induced diabetes by alloxan administration, which is consistent with previous research on oxidative stress in diabetes. However, chitosan nanoparticle treatment considerably restored antioxidant levels, demonstrating its potential for improving antioxidant defense and lowering lipid peroxidation, which was confirmed by previous research (Ibuki *et al*., 2020).

The current findings confirm that alloxan-induced diabetes cause severe liver and pancreatic damage, including hepatic disorganization, inflammatory infiltration, and β-cell degeneration, as previously reported (Lucchesi *et al*., 2015). The elevation in SOD and CAT activities proved that oxidative stress caused tissue damage. However, chitosan nanoparticle therapy greatly improved liver architecture by reducing cellular necrosis and inflammation. Additionally, pancreatic tissue analysis showed better islet integrity, β-cell regeneration, and insulin expression in treated rats. These observations are in agreement with earlier reports revealing chitosan's protective benefits, indicating that it can reduce diabetes-induced organ damage while also encouraging pancreatic cell regeneration and functional restoration (Tzeng *et al*., 2022).

Our data, which includes immunohistochemical investigation of chitosan nanoparticles reveals their potential to mitigate beta cell damage induced by alloxan in diabetic models. By enhancing cellular integrity and reducing apoptosis, these nanoparticles promote beta cell survival and function. This study highlights the therapeutic promise of chitosan nanoparticles in diabetes management through targeted cellular protection (Wardani *et al*., 2022).

The antidiabetic benefits of chitosan nanoparticles demonstrated in this study can be attributed to a variety of mechanisms. Chitosan nanoparticles dramatically reduced oxidative stress by scavenging free radicals and restored antioxidant enzyme activity. This protected β-cells of the pancreas from damage produced by alloxan and improved insulin production (Ivanova, 2020). Additionally, inhibiting α-amylase delays carbohydrate digestion, resulting in slower glucose absorption and better glycemic control (Giuntini *et al*., 2022). Furthermore, chitosan nanoparticles efficiently controlled lipid metabolism, lowering hyperlipidemia and the cardiovascular risks associated with diabetes (Souto *et al*., 2019). The observed improvements in blood glucose and insulin levels add to the expanding evidence of chitosan's powerful antioxidant and anti-diabetic effects, making it a promising therapeutic agent for diabetes control (Ghavimishamekh *et al*., 2019).

CONCLUSION AND RECOMMENDATIONS

In conclusion, this research significantly supports chitosan nanoparticles' therapeutic potential for diabetes and associated consequences. Their multifaceted impact, which includes improved glycemic management through improved insulin function and reduced carbohydrate digesting, hepatoprotection, favorable lipid profile modulation, and pancreatic islet regeneration, demonstrates their efficacy. Furthermore, their antioxidant and anti-inflammatory capabilities aid in cellular defense. These findings establish chitosan nanoparticles as a potential, biocompatible alternative to diabetes management, necessitating more clinical research to evaluate their safety and efficacy in humans.

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NOVELTY STATEMENTS

This study reveals chitosan nanoparticles significantly improve glycemic control, antioxidant defense, and organ protection in diabetic rats, suggesting their potential as natural antidiabetic therapeutics.

AUTHOR'S CONTRIBUTIONS

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Writing - Original Draft: Ali M. Eldib.
Writing - Review and Editing: Mamdooh H Ghoneum.
Supervision: Ali M. Eldib and Mohamed S. El-Gerbed.
Consent for publication: All authors have evaluated the work and approved its publication in this journal. Each author acknowledges approving the final version and be accountable for all elements of the study, including ensuring that questions of accuracy and integrity are properly addressed.

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Data Availability

Data will be available when requested by the corresponding author.

Clinical Trial Number

Not applicable.

List of Abbreviations

Abbreviation: Full Term
ABC: Avidin-Biotin-Peroxidase Complex
ALP: Alkaline Phosphatase
ALT: Alanine Aminotransferase

AST: Aspartate Aminotransferase
BSA: Bovine Serum Albumin
CAT: Catalase
CNPs: Chitosan Nanoparticles
DAB: 3,3'-Diaminobenzidine
DNPH: Dinitrophenylhydrazine
DTNB: 5,5'-Dithiobis-(2-Nitrobenzoic Acid)
ELISA: Enzyme-Linked Immunosorbent Assay
FTIR: Fourier Transform Infrared Spectroscopy
GGT: Gamma-Glutamyl Transferase
GPx: Glutathione Peroxidase
GST: Glutathione-S-Transferase
HbA1c: Glycosylated Hemoglobin
HDL-C: High-Density Lipoprotein Cholesterol
HRTEM: High-Resolution Transmission Electron Microscope
H_2O_2:Hydrogen Peroxide
LDH: Lactate Dehydrogenase
LDL-C: Low-Density Lipoprotein Cholesterol
MDA: Malondialdehyde
SOD: Superoxide Dismutase
TC: Total Cholesterol
TCA: Trichloroacetic Acid
TEM: Transmission Electron Microscopy
TG: Triglycerides
TPP: Tripolyphosphate

Highlights

Chitosan nanoparticles (CNPs) significantly improved insulin release (↑65.15%) and decreased hyperglycemia (↓46%) in alloxan-induced diabetic rats.
The treatment restored glycosylated hemoglobin, amylase, and primary liver function indicators (AST, ALP, and bilirubin).
The lipid profile improved significantly, with a 40% decrease in total cholesterol, a 46.5% decrease in LDL-C, a 33.7% decrease in triglycerides, and a 40% increase in HDL-C.
CNPs reduced oxidative stress and increased antioxidant enzyme activity (SOD ↑ 49.3%, GST ↑ 56.4%).
Non-enzymatic antioxidants were partially restored, including vitamins C, E, and glutathione.
Histological and immunohistochemical studies also verified pancreatic and hepatic tissue regeneration, indicating that CNPs are safe to use as an adjuvant natural treatment for diabetes.

Conflict of Interest

The authors declare no known financial or personal conflicts of interest that could be perceived as influencing this work.

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