# Chitosan-doped gold nanoparticles with potent and selective activity against diabetes mellitus

**Fatma Ashraf[1], Alaa Elmetwalli[2,3], Mohamed O. Abdel-Monem[1], Jihan Hassan[4]**
**and Mervat G. Hassan[1]**

[1]Botany and Microbiology Department, Faculty of Science, Benha University, Benha, Egypt

[2]Department of Clinical Trial Research Unit and Drug Discovery, Egyptian Liver Research Institute and Hospital (ELRIAH), Mansoura, Egypt

[3]Microbiology Division, Higher Technological Institute of Applied Health Sciences, Egyptian Liver Research Institute and Hospital (ELRIAH), Mansoura, Egypt

[4]Department of Applied Medical Chemistry, Medical Research Institute, Alexandria University, Alexandria, Egypt

**E-mail:** Fatmaasharf5@gmail.com

**Abstract:**

**Background:** Diabetes Mellitus (DM) poses a significant global health challenge, with rising prevalence and associated complications. Despite available treatments, the search for safer and more effective options continues. This study investigates the potential of chitosan-doped gold nanoparticles (AuNPs-chito) as a novel therapeutic approach for DM. Chitosan shows promise as a flexible biomolecule for the management of several pathophysiological problems linked to diabetes mellitus (DM) because of its dual roles as an antidiabetic agent and medication carrier**Methods:** Streptozotocin-induced diabetic rats were treated with AuNPs-chito, and various biochemical and histopathological parameters were assessed.

**Results:** Our data indicate that AuNPs-Chito administration led to significant improvements in blood glucose levels, lipid profiles, oxidative stress markers, and liver function compared to untreated diabetic rats. Histopathological examination revealed restoration of hepatic architecture in AuNPs-chito-treated rats. **Conclusion:** These findings suggest that AuNPs-chito may offer a promising strategy for managing DM by mitigating oxidative stress and improving metabolic parameters.

**Keywords:** gold nanoparticles; chitosan; diabetes mellitus; hepatotoxicity; oxidative stress

## 1.Introduction

The chronic and complex disease known as type 2 diabetes mellitus (T2DM) is linked to high rates of co-morbidity globally. It is typified by the degeneration of pancreatic β cells, which results in insulin resistance and metabolic disruptions in a number of tissues, including the liver, skeletal muscles, and adipose tissues. Through the secretion of enzymes, the pancreas is essential in controlling blood glucose levels [1]. The World Health Organization (WHO) estimates that 422 million people worldwide are anticipated to be afflicted with diabetes, which resulted in 1.6 million fatalities in 2016. It is now the seventh most common cause of illness and death globally, and by 2040, estimates suggest 640 million people will be affected by its prevalence [2].

The symptoms of type 2 diabetes (T2DM) include polyuria, polydipsia, increased appetite, weariness, non-healing wounds, impaired vision, numbness, weight loss, and exhaustion. The pathogenesis of T2DM is a complicated interaction between genetic predisposition and environmental variables [3]. Controlling one's diet, controlling weight, and engaging in physical activity are the main management strategies. Pharmacological interventions include metformin prescriptions, thiazolidinediones, α-glucosidase inhibitors, sulfonylureas, and repaglinide, among other medications.

Modern research investigates innovative ways, such as drug delivery systems based on nanoparticles, to improve therapeutic efficacy and minimize adverse effects. High specificity, extended circulation duration, and decreased toxicity are among the benefits of nanoparticles; gold nanoparticles (AuNPs) are particularly promising because of their adaptability and efficiency in a range of applications, including medicine [5].

With its diverse biomedical applications ranging from wound healing to antitumor and antimicrobial properties, chitosan, a carbohydrate biopolymer made of D-glucosamine and N-acetyl-D-glucosamine units linked by β-(1-4) bonds with varying degrees of deacetylation, has attracted a lot of attention [6]. Chitosan shows promise as a flexible biomolecule for the management of several pathophysiological problems linked to diabetes mellitus (DM) because of its dual roles as an antidiabetic agent and medication carrier [7]. Chitosan and gold nanoparticles together produce stable nanostructures with increased biological activity, as studies have shown [6]. In an animal model of diabetes mellitus caused by streptozotocin, the purpose of this study is to determine if AuNPs-Chito can improve its efficacy.

## 2.Materials and Methods
### Methods

This study involved animal testing with forty adult male Swiss albino rats, weighing between 110

and 130 g and aged between 4 and 4.6 months. We gave 5 mg/kg of streptozotocin to 28 of these rats to induce diabetes mellitus. After that, the AuNPs-chito were ready. Three groups of six rats were used: Group I was the control group, which received no therapy; Group II included diabetic rats who received no treatment; and Group III included diabetic rats who received treatment with AuNPs-chito (10 mg/kg) for 14 days [8]. An insulin syringe (1 mL capacity) was used to inject the test substances intraperitoneally (i.p) into the rats. The study followed the ethical criteria of the Alexandria University Ethical Committee and the rats were housed in the Medical Research Institute's Animal House of the Medical Technology Center. The rats were humanely grown and handled. After receiving streptozotocin for three days, rats with fasting glucose levels more than 180 mg/100 mL were classified as diabetic.

**Preparation of Biological Samples**

Following the sacrifice, isoflurane was utilized to prepare samples of liver tissue and blood. Centrifuging at $1000\times$ g was used to acquire serum samples, and collecting $3000\times$ g was used to collect supernatants from tissue samples [9]. Once further analysis was not possible, the samples were kept refrigerated at -80 ∘C.

**Glucose Test for the Evaluation of Diabetes**

A blood glucose monitoring device was used to test the rats' blood glucose levels at 7-8 a.m. after they went without meals from 5 p.m. to 7 a.m. [10]. Rats' tail veins were used to collect blood samples [11]. Diabetic rats were defined as those whose fasting glucose levels were more than 180 mg/100 mL. By verifying the animals' glucose readings twice, the diabetic state was established.

**Measurement of Glucose Level**

The commercial kit (Biomed, Egypt) was used to test the serum glucose level in accordance with the manufacturer's instructions [12], [13].

**Measurement of Redox Parameters**

To assess their function in controlling oxidative stress in vivo, two redox parameters—reactive oxygen species (ROS) [14], glutathione-S-transferase (GST), and malondialdehyde (MDA) [15]—were chosen. Using the sulfosalicylic acid and DTNB techniques, the absorbance of the reaction mixture was measured at 412 nm in order to quantify the amounts of GST. Based on how MDA reacted with 2-thiobarbituric acid (TBA) and trichloroacetic acid (TCA), MDA levels were calculated [16].

**Analysis of Liver Function Markers**

The serum samples were used to measure liver function indicators such as GGT and alkaline phosphatase (ALK). Serum indicators were measured using commercial kits in accordance with the manufacturer's recommendations [9], [17].

**Histopathological Analysis**

The dissected liver tissues were stored in a 10% formalin solution. After that, they were cut into pieces with thicknesses between 5 and 7 μM. Subsequently, the slices underwent H&E staining [18], [19], [20].

**3.Results**
**Effect of AuNPs-Chito on liver and blood glucose levels in different rat groups**

The results presented in **Table 1** demonstrate the effect of AuNPs-Chito on liver and blood glucose levels in different rat groups. The variables observed included FBS, PPBS, GGT, and ALK. The control group (Cnt) had the lowest levels of FBS, PPBS, GGT, and ALK, with mean values of 91.2±1.58, 122.5±0.34, 18.25±1.24, and 96.58±3.54, respectively. On the other hand, the STZ group had the highest levels of these variables, with mean values of 189.9±1.48, 244.5±2.78, 114.25±2.87, and 258.47±4.70, respectively. The AuNPs-chito group showed intermediate values for all variables, with mean values of 118.10±0.25, 155.78±1.86, 45.25±1.25, and 112.32±3.23 for FBS, PPBS, GGT, and ALK, respectively. These results suggest that AuNPs-chito may have a potential effect on liver and blood glucose levels in diabetic rats.

Table (1) Effect of AuNPs-chito on liver and blood glucose levels in different rat groups.

| Variable | Cnt | STZ | AuNPs-chito |
|---|---|---|---|
| **FBS** | 91.2±1.58 [a] | 189.9±1.48[b] | 118.10±0.25[c] |
| **PPBS** | 122.5±0.34[a] | 244.5±2.78[b] | 155.78±1.86[c] |
| **GGT** | 18.25±1.24[a] | 114.25±2.87[b] | 45.25±1.25[c] |
| **ALK** | 96.58±3.54[a] | 258.47±4.70[b] | 112.32±3.23[c] |

In each group results for 6 rats are expressed by means ± SE. Small (a-c) letters showing the marked change at P ≤ 0.05. The same letters show (non-significant) and the significance is expressed by dissimilar letters. **Cnt**: control group, **STZ**: streptozotocin .

**Table (2)** Effect of AuNPs-chito on oxidative and antioxidant markers in different rat groups.

| Variable | Cnt | STZ | AuNPs-chito |
|---|---|---|---|
| ROS | 0.89±0.25 [a] | 2.25±0.58[b] | 1.12±0.11[c] |
| MDA | 35.25±2.12[a] | 121.29±3.25[b] | 47.58±1.08[c] |
| GST | 14.25±1.37[a] | 5.27±2.18[b] | 11.25±0.87[c] |

In each group results for 6 rats are expressed by means ± SE. Small (a-c) letters showing the marked change at P ≤ 0.05. The same letters show (non-significant) and the significance is expressed by dissimilar letters. **Cnt**: control group, **STZ**: streptozotocin .
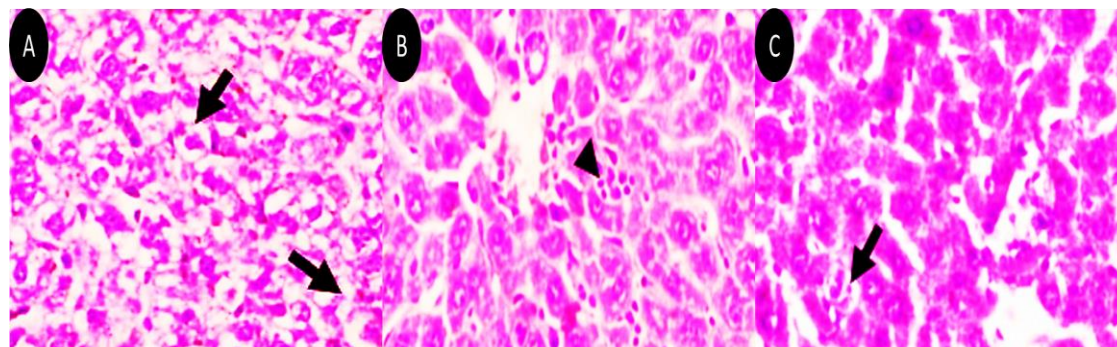


**Fig. (1) (A)** The photomicrograph of the control group's liver displays normal hepatic architecture, including CV. **(B)** In the liver photomicrograph of the STZ group, the CV is swollen, and there is a slight enlargement of the blood sinusoids. **(C)** The liver photomicrograph of the AuNPs-chito group reveals mild focal areas with pleomorphic darkly basophilic cells. The scale bar is 50 μm.

**Effect of AuNPs-chito on oxidative markers in different rat groups**

The results presented in (Table 2) describe the variables, Cnt, STZ, AuNPs-chito, ROS, MDA, and GST. The results indicate that the values of ROS, MDA, and GST were significantly different between the control group (Cnt) and the streptozotocin group (STZ). In particular, the values of ROS and MDA were significantly higher in the STZ group compared to the Cnt group, while the value of GST was significantly lower. The AuNPs-chito group showed intermediate values for these variables which signify the potentiality of AuNPs-chito as an anti-oxidative drug .

**Histopathology examination**

The liver of the control group may be seen in the photomicrograph with normal hepatic architecture, including the central vein (CV), the portal region (P), polyhedral-shaped hepatocytes (arrow), and blood sinusoids (arrowhead) (Figure 1a). A photomicrograph of the liver from the STZ group revealed a swollen CV, a little enlargement of the blood sinusoids (black arrow), a slight degradation of the hepatocytes (arrow), and a slight activation of the Kupffer cells (Figure 1b). The photomicrograph depicts the power horse group's liver, revealing tiny focal areas and diffuse pleomorphic darkly basophilic cells indicated by arrows in the perivascular region surrounding the congested CV. Additionally, moderate congestion of some blood sinusoids, denoted by arrowheads, is seen in (Figure 1c).

**4.Discussion**

Diabetes mellitus, commonly referred to as diabetes, is a metabolic syndrome characterized by elevated levels of blood glucose. Unfortunately, the prevalence of diabetes is increasing worldwide, with an estimated 600 million people projected to have the disease by 2045 [21]. While contemporary antidiabetic drugs are effective in reducing hyperglycemia and its consequences, natural remedies are preferred due to their safety, affordability, and lack of potential adverse effects [22].

Although several approaches to managing and controlling diabetes have been put forth, the condition is still challenging to manage. On the other hand, a recent research [23] proposed applying nanotechnology to enhance diabetes treatment results. According to the study, AuNP-chitosan dramatically enhanced a number of measures in diabetic rats. Numerous metrics improved in the AuNP-chitosan-treated rats. In diabetes circumstances, elevated glucose levels cause auto-oxidation, which deteriorates redox balance [24]. This procedure starts a chain reaction of hepatotoxicity that is mediated by inflammation and oxidative stress. Additionally, reactive oxygen species (ROS) infiltrate the hepatocytes of the liver, resulting in significant tissue damage and the release of many biochemical indicators into the bloodstream [11].

The study also found that in long-term diabetes settings, antioxidant enzymes of proteins and GST were depleted, and ROS may have an impact on target tissues' nuclear DNA, proteins, and carbs. AuNPs-chito are a prospective antidiabetic treatment because

they can increase the hepatic glucose transporter-2 (GLUT-2) gene, the liver glucokinase (GK) enzyme, and serum insulin levels. Because chitosan may both as a medication carrier and an antidiabetic agent, chitosan-based treatment techniques are looking like a potential strategy to control diabetes [25].

AuNP-chito works together to restore cellular structures and functions by reducing lipid peroxidation and suppressing oxidative stress [26]. Additionally, it has the ability to repair the histology and functioning of the pancreatic islets, potentially increasing insulin sensitivity and activity levels [19]. With these encouraging findings, the use of AuNP-chitosan may represent a major advancement in the management of diabetes, offering a more secure, cost-efficient, and potent substitute for conventional antidiabetic medications.

## 5.Conclusion

In conclusion, the study demonstrates the potential of chitosan-doped gold nanoparticles (AuNPs-chito) as a therapeutic intervention for Diabetes Mellitus. By targeting multiple facets of the disease, including blood glucose regulation, lipid metabolism, oxidative stress, and liver function, AuNPs-chito shows promise as an effective and safe alternative to conventional antidiabetic drugs. Further research is warranted to elucidate the underlying mechanisms and optimize the therapeutic application of AuNPs-chito in clinical settings. If validated, AuNPs-chito could contribute significantly to the management of DM, offering patients a more sustainable and affordable treatment option with fewer adverse effects.

**Conflict of interest:** no conflict of interest was declared

## References

[1] R. A. DeFronzo *et al.*, "Type 2 diabetes mellitus," *Nat. Rev. Dis. Prim.*, vol. 1, no. 1, pp. 1–22, 2015.

[2] A. Elmetwalli *et al.*, "Nanoparticle zinc oxide obviates oxidative stress of liver cells in induced-diabetes mellitus model," *Med. J. Viral Hepat.*, vol. 7, no. 1, pp. 8–12, 2022.

[3] A. Elmetwalli *et al.*, "Novel phloretin-based combinations targeting glucose metabolism in hepatocellular carcinoma through GLUT2/PEPCK axis of action: in silico molecular modelling and in vivo studies," *Med. Oncol.*, vol. 41, no. 1, p. 12, 2023.

[4] J. Blahova, M. Martiniakova, M. Babikova, V. Kovacova, V. Mondockova, and R. Omelka, "Pharmaceutical drugs and natural therapeutic products for the treatment of type 2 diabetes mellitus," *Pharmaceuticals*, vol. 14, no. 8, p. 806, 2021.

[5] K. B. Patil, N. B. Patil, S. V Patil, V. K. Patil, and P. C. Shirsath, "Metal based Nanomaterial's (Gold and Gold): Synthesis and Biomedical

application," *Asian J. Pharm. Technol.*, vol. 10, no. 2, pp. 97–106, 2020.

[6] A. Pellis, G. M. Guebitz, and G. S. Nyanhongo, "Chitosan: Sources, processing and modification techniques," *Gels*, vol. 8, no. 7, p. 393, 2022.

[7] [7] S. Sarkar, D. Das, P. Dutta, J. Kalita, S. B. Wann, and P. Manna, "Chitosan: A promising therapeutic agent and effective drug delivery system in managing diabetes mellitus," *Carbohydr. Polym.*, vol. 247, p. 116594, 2020.

[8] [8] C. Ong, J. Z. Z. Lim, C. T. Ng, J. J. Li, L. Y. Yung, and B. H. Bay, "Gold nanoparticles in cancer: therapeutic efficacy and toxicity," *Curr. Med. Chem.*, vol. 20, no. 6, pp. 772–781, 2013.

[9] A. Elmetwalli *et al.*, "Modulation of the oxidative damage, inflammation, and apoptosis-related genes by dicinnamoyl-L-tartaric acid in liver cancer," *Naunyn. Schmiedebergs. Arch. Pharmacol.*, pp. 1–13, 2023.

[10] A. Elmetwalli *et al.*, "Ammonia scavenger and glutamine synthetase inhibitors cocktail in targeting mTOR/β-catenin and MMP-14 for nitrogen homeostasis and liver cancer," *Med. Oncol.*, vol. 41, no. 1, p. 38, 2023.

[11] A. A. El-Shehawy *et al.*, "Thymoquinone, piperine, and sorafenib combinations attenuate liver and breast cancers progression: epigenetic and molecular docking approaches," *BMC Complement. Med. Ther.*, vol. 23, no. 1, pp. 1–21, 2023.

[12] T. El-Sewedy *et al.*, "Hepatocellular Carcinoma cells: activity of Amygdalin and Sorafenib in Targeting AMPK/mTOR and BCL-2 for anti-angiogenesis and apoptosis cell death," *BMC Complement. Med. Ther.*, vol. 23, no. 1, pp. 1–17, 2023.

[13] A. Elmetwalli *et al.*, "Diarylheptanoids/sorafenib as a potential anticancer combination against hepatocellular carcinoma: the p53/MMP9 axis of action," *Naunyn. Schmiedebergs. Arch. Pharmacol.*, pp. 1–17, 2023.

[14] A. Elmetwalli, A. M. Abdel Khalek, S. A. El-Naggar, M. A. El-Magd, and A. F. Salama, "Amygdalin Enhances the Antitumor Effect of Sorafenib," *Egypt. Acad. J. Biol. Sci. D. Histol. Histochem.*, vol. 13, no. 2, pp. 61–68, 2021.

[15] [15] A. Elmalla, A. Elmetwalli, M. E.-S. Rizk, and A. F. Salama, "The Effect of Vitamin B17 on Cardiomyopathy against Ehrlich Tumor Development in Female Mice," *Biochem. Lett.*, vol. 17, no. 1, pp. 69–76, 2021.

[16] H. M. Nasser, S. A. El-Naggar, M. E.-S. R. El-Sayed Rizk, A. Elmetwalli, and A. F. Salama, "Effect of Sorafenib on Liver Biochemistry Prior to Vitamin B17 Coadministration in Ehrlich Ascites Carcinoma Mice Model: Preliminary Phase Study," *Biochem. Lett.*, vol. 17, no. 1, pp. 40–49, 2021.

[17] R. Ali, H. G. El-Tantawi, M. E.-S. Rizk, S. A. El-Naggar, A. Elmetwalli, and A. F. Salama, "Is

Amygdalin Outcomes Weighing Detriments of Sorafenib Treatment In Female Mice With Kidney Injury Induced By Ehrlich Ascites Carcinoma Model? Preliminary study," *Biochem. Lett.*, vol. 17, no. 1, p. 0, 2021.

[18] N. F. Ismail, G. Hamdy, A. A. Hassan, A. Elmetwalli, M. Salah, and J. Hassan, "The Impact of Energy Drinks on Liver Health," *Med. J. Viral Hepat.*, vol. 7, no. 3, pp. 1–6, 2023.

[19] A. A. Attia *et al.*, "Amygdalin potentiates the anti-cancer effect of Sorafenib on Ehrlich ascites carcinoma and ameliorates the associated liver damage," *Sci. Rep.*, vol. 12, no. 1, pp. 1–9, 2022.

[20] S. M. Ebrahim, A. Elmetwalli, M. Abu El-Magd, S. A. El-Naggar, and A. F. Salama, "The Rationale Behind the Therapeutic Potential of Drugs Containing Hydroxycinnamic Acid on Hepatocellular Cancer Induced by 4-Dimethylaminoazobenzene: Technique-Related Implications," *Egypt. Acad. J. Biol. Sci. D. Histol. Histochem.*, vol. 14, no. 2, pp. 13–23, 2022.

[21] Y. Mukhtar, A. Galalain, and Ujej. Yunusa, "A modern overview on diabetes mellitus: a chronic endocrine disorder," *Eur. J. Biol.*, vol. 5, no. 2, pp. 1–14, 2020.

[22] M. F. Mahomoodally, D. Lobine, M. C. N. Picot-Allain, N. Sadeer, S. Jugreet, and G. Zengin, "Conventional and non-conventional targets of natural products in the management of diabetes mellitus and associated complications," *Curr. Med. Chem.*, vol. 28, no. 23, pp. 4638–4669, 2021.

[23] E. Papachristoforou, V. Lambadiari, E. Maratou, and K. Makrilakis, "Association of Glycemic Indices (Hyperglycemia, Glucose Variability, and Hypoglycemia) with Oxidative Stress and Diabetic Complications," *J. Diabetes Res.*, vol. 2020, 2020, doi: 10.1155/2020/7489795.

[24] V. Mishra *et al.*, "Pharmaceutical aspects of green synthesized gold nanoparticles: A boon to cancer treatment," *Anti-Cancer Agents Med. Chem. (Formerly Curr. Med. Chem. Agents)*, vol. 21, no. 12, pp. 1490–1509, 2021.

[25] H. Zhao, R. Zhang, X. Yan, and K. Fan, "Superoxide dismutase nanozymes: an emerging star for anti-oxidation," *J. Mater. Chem. B*, 2021.

[26] R. Bhanumathi, K. Vimala, K. Shanthi, R. Thangaraj, and S. Kannan, "Bioformulation of gold nanoparticles as berberine carrier cum anticancer agent against breast cancer," *New J. Chem.*, vol. 41, no. 23, pp. 14466–14477, 2017.