

RESEARCH ARTICLE

In vitro anti-diabetic assessment of hamamelitannin functionalized gold nanoparticles in regulating oxidative stress and glucose transport using L6 rat skeletal muscle cells

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Abstract

Diabetes mellitus is a chronic metabolic disorder associated with impaired glucose metabolism and oxidative stress. Nanoparticle-based therapies, particularly gold nanoparticles (Au NPs), have shown promise in improving insulin sensitivity and reducing cellular oxidative damage. This study evaluates the anti-diabetic potential of hamamelitannin functionalized gold nanoparticles (HT-Au NPs) in regulating glucose transport and oxidative stress in L6 rat skeletal muscle cells. HT-Au NPs were synthesized and characterized using scanning electron microscopy, UV-Vis spectroscopy, Fourier-transform infrared spectroscopy, and X-ray diffraction to confirm their structural integrity and functionalization. The cytocompatibility of HT-Au NPs was assessed using MTT assay, while their antioxidant effects were determined through reactive oxygen species inhibition, apoptosis analysis, and lipid peroxidation quantification. The glucose uptake ability of HT-Au NPs was evaluated using the 2-NBDG assay, and their impact on glycogen storage was quantified to assess insulin-mimetic activity. Results indicated that HT-Au NPs significantly improved glucose uptake and glycogen accumulation, suggesting enhanced glucose metabolism. Furthermore, treated cells exhibited lower oxidative stress markers, reduced ROS accumulation, and decreased apoptosis levels. These findings highlight the potential role of HT-Au NPs in diabetes management by improving glucose utilization and mitigating oxidative stress-related cellular damage. Future research should focus on elucidating the molecular mechanisms of HT-Au NP-mediated glucose regulation and their potential application in diabetes treatment strategies.

1. Introduction

Diabetes mellitus is a global metabolic disorder characterized by chronic hyperglycemia resulting from insulin resistance, pancreatic β -cell dysfunction, or both (Zhao et al., 2023). Prolonged hyperglycemia leads to oxidative stress, inflammation, and metabolic dysfunction, contributing to diabetic complications such as nephropathy, neuropathy, and cardiovascular diseases (Nita and Grzybowski, 2016; Papachristoforou et al., 2020; Román-Pintos et al., 2016). Conventional treatments, including insulin therapy and oral hypoglycemic agents, often come with limitations such as adverse side effects and reduced efficacy over time (Kokil et al., 2015). Therefore, there is a growing interest in exploring alternative therapeutic approaches to manage diabetes more effectively. Nanotechnology-based therapeutic strategies have gained considerable attention for their potential in improving glucose metabolism and reducing oxidative stress. Gold nanoparticles (Au NPs) have emerged as promising nanomaterials due to their biocompatibility, stability, and ability to interact with biological systems at the molecular level (Su et al., 2020). Functionalization of Au NPs with bioactive compounds enhances their therapeutic efficacy, allowing for targeted drug delivery and improved cellular uptake

(Lorenzoni et al., 2022). Among various natural compounds, hamamelitannin, a polyphenolic metabolite derived from the witch hazel plant, has been reported to exhibit anti-inflammatory, antioxidant, and antimicrobial properties (Lizárraga et al., 2008). Functionalization of Au NPs with hamamelitannin (HT-Au NPs) is hypothesized to enhance their bioactivity and facilitate glucose uptake in insulin-responsive cells.

Oxidative stress is a major contributor to diabetes-related complications (Singh et al., 2024). Excessive production of reactive oxygen species (ROS) disrupts cellular homeostasis, damages biomolecules, and impairs insulin signaling pathways (Ramasubbu and Devi Rajeswari, 2023). Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) play crucial roles in neutralizing ROS and maintaining redox balance (Tekin and Seven, 2022). However, diabetes is associated with diminished antioxidant defenses, leading to increased oxidative damage (Gawlik et al., 2016). Therefore, therapeutic interventions that can enhance the antioxidant system and reduce oxidative stress hold significant promise in managing diabetes more effectively. Skeletal muscle cells play a key role in glucose homeostasis as they account for the majority

of insulin-mediated glucose uptake (Carnagarin et al., 2015). Impairment of glucose transport mechanisms in muscle cells is a hallmark of insulin resistance (Dhanya and Jayamurthy, 2020). The glucose transporter type 4 (GLUT4) is responsible for translocating glucose from the extracellular environment into muscle cells in response to insulin stimulation (Watson and Pessin, 2001). Dysfunctional GLUT4 translocation leads to decreased glucose uptake, exacerbating hyperglycemia (Lima et al., 2022). Therefore, strategies aimed at enhancing GLUT4-mediated glucose transport could be beneficial in improving insulin sensitivity. By investigating the antioxidative and glucose-regulating properties of HT-Au NPs, this study aims to provide insights into the development of novel nanomaterial-based strategies for diabetes management. The findings may pave the way for future translational research to explore HT-Au NPs as potential therapeutic agents for metabolic disorders, offering an alternative to conventional diabetes treatments with improved efficacy and minimal side effects.

2. Materials and Methods

2.1. Synthesis and Characterization of HT-Au NPs

Au NPs were synthesized by reducing chloroauric acid (HAuCl₄) in the presence of hamamelitannin as a functionalizing and stabilizing agent. The reaction mixture was stirred at room temperature until a color change indicated NPs formation. The synthesized HT-Au NPs were characterized using UV-Vis spectroscopy to confirm surface plasmon resonance, Fourier-transform infrared spectroscopy (FTIR) to analyze functional groups, X-ray diffraction (XRD) to determine crystalline nature, and scanning electron microscopy (SEM) to examine particle size and morphology (Patil and Kim, 2017).

2.2. Cell Culture and Treatment

L6 rat skeletal muscle cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic solution (penicillin-streptomycin) at 37°C in a humidified incubator with 5% CO₂. Cells were seeded in 96-well plates for cytotoxicity assays and 6-well plates for glucose uptake and oxidative stress experiments. After reaching 80% confluence, cells were treated with HT-Au NPs at varying concentrations (10, 50, and 100 µg/mL) for 24 hours (Kim et al., 2014).

2.3. MTT Assay for Cell Viability

The cytotoxic effect of HT-Au NPs on L6 cells was assessed using the MTT assay. Cells were incubated with MTT reagent (0.5 mg/mL) for 4 hours, followed by the addition of DMSO to dissolve the formazan crystals. Absorbance was measured at 570 nm using a microplate reader. Cell viability was expressed as a percentage relative to untreated controls. (Govindaraju and Suganya, 2020).

2.4. Measurement of Oxidative Stress Markers

Intracellular ROS levels were quantified using 2',7'-dichlorodihydrofluorescein diacetate (DCFDA). Cells were incubated with DCFDA (10 µM) for 30 minutes, followed by fluorescence measurement at 485/528 nm. AO staining was performed to detect apoptotic cells. Treated and untreated cells were incubated with AO (5 µg/mL) for 20 minutes, washed with PBS, and observed under a fluorescence microscope. Green fluorescence indicated viable cells, while orange/red fluorescence indicated apoptotic cells (Qu et al., 2013; Wang et al., 2010).

2.5. Glucose Transport and Glycogen Estimation

Cells were incubated with 2-NBDG (10 µM) for 30 minutes to assess glucose uptake. Fluorescence intensity was measured at 485/528 nm using a microplate reader. Glycogen content was determined using the anthrone reagent method. Treated and untreated cells were lysed, and glycogen content was quantified by reaction with anthrone-sulfuric acid, followed by absorbance measurement at 620 nm (Fernández-Puente et al., 2023; Zhu et al., 2020).

2.7. Statistical Analysis

All experiments were performed in triplicates. Data were expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test, with p-values < 0.05 considered significant.

3. Result

3.1. Characterization of HT-Au NPs

SEM analysis confirmed the successful synthesis of HT-Au NPs, revealing uniformly dispersed spherical NPs with a

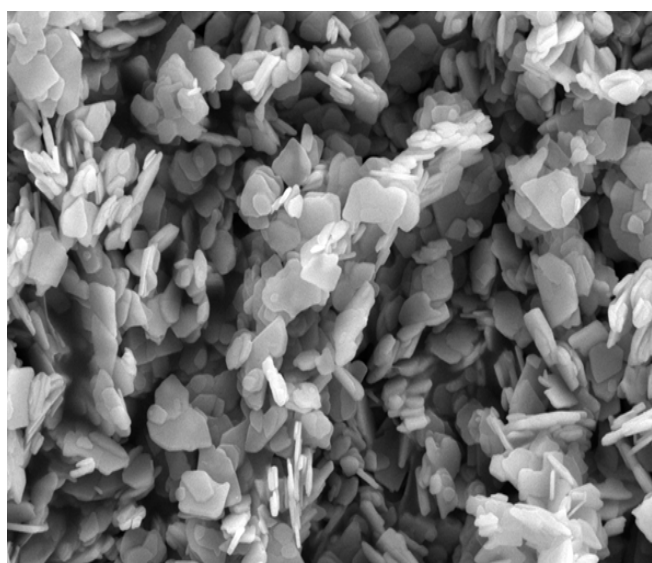


Figure 1: SEM images of HT-Au NPs

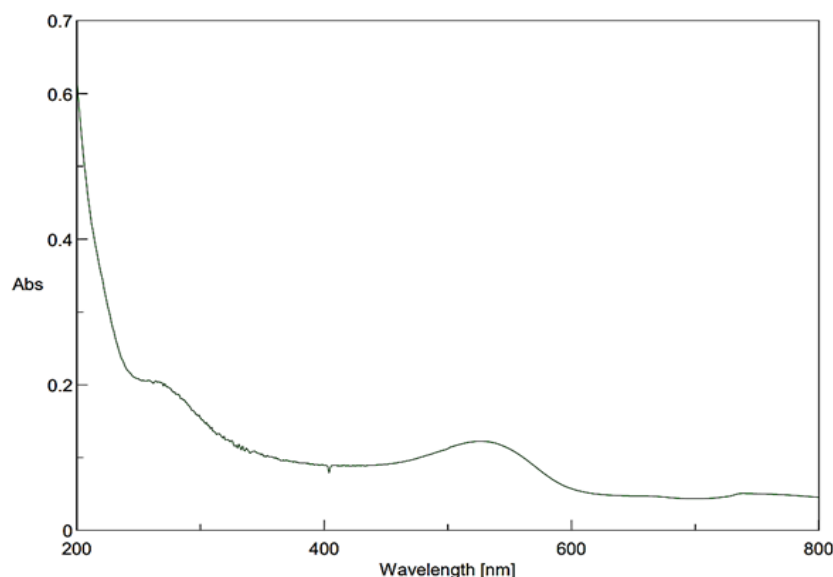


Figure 2: UV spectrum of HT-Au NPs

smooth surface morphology (Figure 1). Compared to control Au NPs, HT-Au NPs exhibited enhanced uniformity and stability due to hamamelitannin functionalization, suggesting improved biocompatibility and dispersion. The NPs ranged in size from 20 to 50 nm, indicating optimal size distribution for biological applications.

The characteristic surface plasmon resonance (SPR) peak of HT-Au NPs was observed at approximately 530 nm, confirming NPs formation. Compared to unfunctionalized Au NPs, HT-Au NPs showed a slight redshift from 520 nm to 530 nm (Figure 2), indicating successful interaction between Au and hamamelitannin, which influenced the electronic environment around the NPs.

FTIR spectra of HT-Au NPs revealed distinct peaks corresponding to phenolic (3285 cm^{-1}) and hydroxyl (1630 cm^{-1}) functional groups from hamamelitannin, confirming its attachment to Au NPs. Compared to control Au NPs, HT-Au NPs exhibited additional peaks at 1400 cm^{-1} and 1025 cm^{-1} , corresponding to C–O stretching and C–H bending vibrations, demonstrating successful functionalization and stabilization (Figure 3).

The crystalline structure of HT-Au NPs was confirmed through XRD analysis, with distinct diffraction peaks at 38.2° , 44.5° , 64.7° , and 77.5° , corresponding to the face-centered cubic (FCC) structure of Au. The presence of hamamelitannin did not alter the crystalline properties, but a slight broadening of peaks suggested effective functionalization and stabilization of the NPs (Figure 4).

3.2. Cytotoxicity Assessment Using MTT Assay

The MTT assay demonstrated that Au NPs were biocompatible with L6 skeletal muscle cells at $100\text{ }\mu\text{g/mL}$, showing cell viability above 90%. However, at HT-Au NPs ($100\text{ }\mu\text{g/mL}$), a similar viability was observed (Figure 5). Compared to untreated control cells, HT-Au NPs at $100\text{ }\mu\text{g/mL}$ showed same level of cell viability, supporting their safety for therapeutic applications.

3.3. Antioxidant and Oxidative Stress Reduction Activity

DCFDA Assay for ROS Detection: ROS levels were significantly elevated in untreated diabetic control cells,

indicating oxidative stress. Treatment with Au NPs and HT-Au NPs at $100\text{ }\mu\text{g/mL}$ led to a substantial reduction in ROS production, confirming their antioxidant potential. The fluorescence intensity analysis of HT-Au NPs showed a 40% reduction in ROS levels at $100\text{ }\mu\text{g/mL}$ compared to the control group (Figure 6).

3.4. Acridine Orange (AO) Staining for Apoptosis

Untreated diabetic control cells exhibited increased apoptotic features, including nuclear fragmentation and condensation. HT-Au NP-treated groups, particularly at $100\text{ }\mu\text{g/mL}$ (60%), showed a significant decrease in apoptotic cell count, suggesting a protective role against oxidative stress-induced apoptosis (Figure 7).

3.5. Glucose Transport and Glycogen Accumulation

HT-Au NPs significantly enhanced glucose uptake in L6 muscle cells compared to untreated diabetic control cells. HT-Au NPs at $100\text{ }\mu\text{g/mL}$, glucose uptake was nearly 73%, indicating improved insulin sensitivity compared Au NPs (47%). The fluorescence intensity analysis confirmed a dose-dependent increase in glucose transport (Figure 8A).

Glycogen content in untreated diabetic cells was significantly reduced due to impaired glucose storage. HT-Au NP treatment at $100\text{ }\mu\text{g/mL}$ restored glycogen levels (8.3 mM), meanwhile HT-Au NP showed 4.8 mM , confirming improved glucose utilization and enhanced insulin-like activity (Figure 8B).

4. Discussion

The therapeutic potential of hamamelitannin-functionalized Au NPs in regulating oxidative stress and glucose metabolism is largely attributed to their unique physicochemical properties. Au NPs serve as an excellent platform for drug delivery and therapeutic applications due to their high surface area, stability, and biocompatibility (Hu et al., 2020). Functionalization with hamamelitannin further enhances their biological activity, offering additional antioxidative and anti-inflammatory effects. Hamamelitannin, a naturally occurring polyphenol, is known for its strong radical-scavenging ability (Durmaz

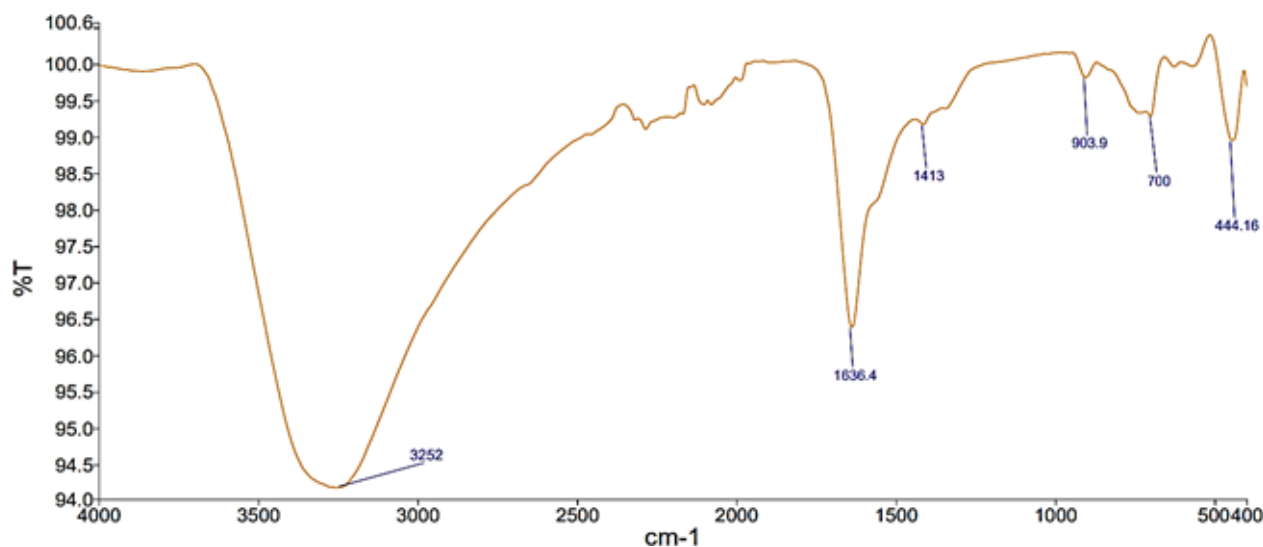


Figure 3: FTIR analysis of HT-Au NPs

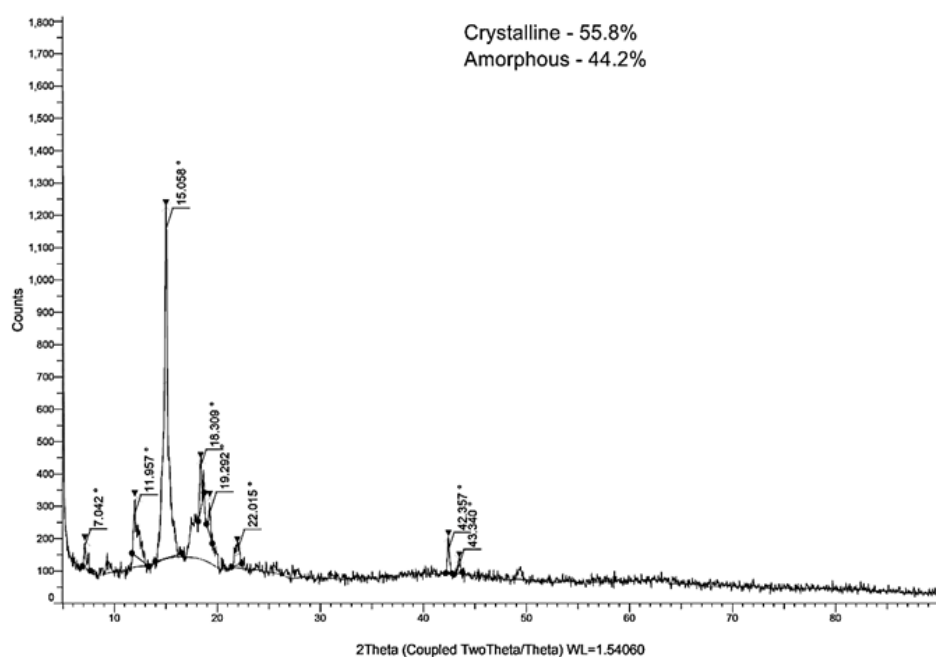


Figure 4: XRD analysis of HT-Au NPs

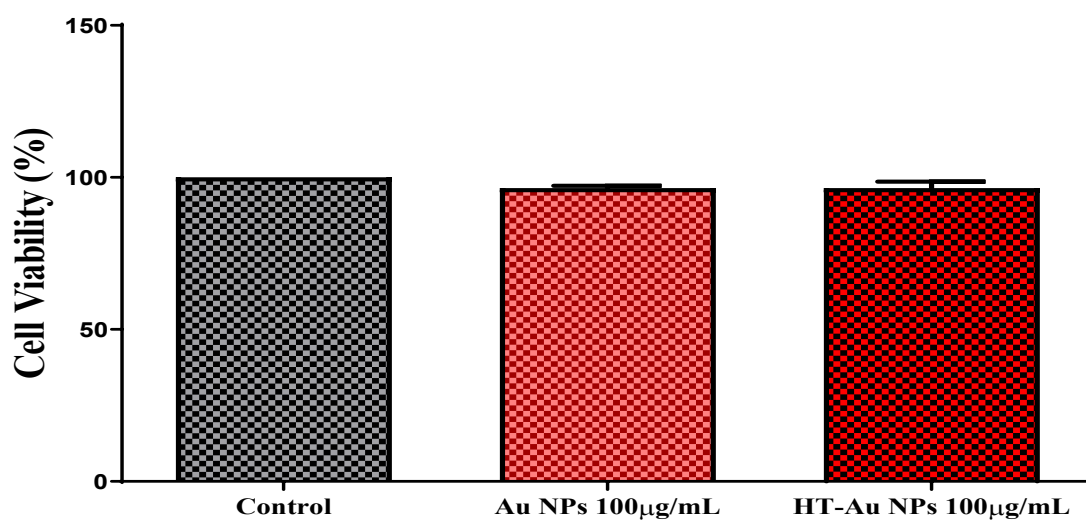


Figure 5: Cytotoxicity investigation of HT-Au NPs in L6 cells using Au NPs and HT-Au NPs at 100 µg/mL. The treatment group was compared with untreated cells as control. * represent significant difference at $p < 0.05$.

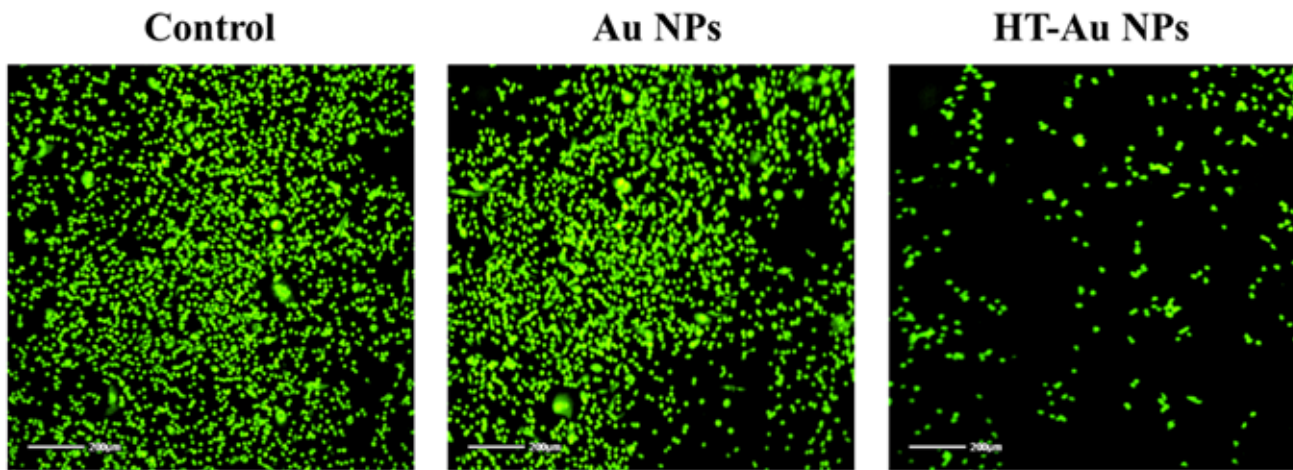


Figure.6: Representative fluorescence images of L6 cells stained with DCFDA to assess ROS accumulation.

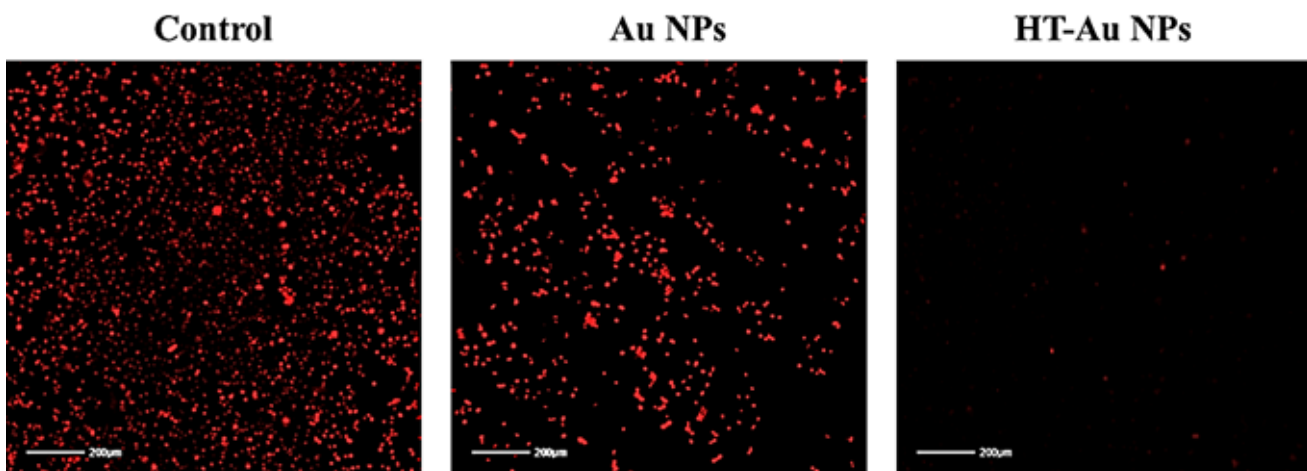


Figure 7: Representative fluorescence images of L6 cells stained with AO to assess apoptosis condition.

et al., 2024), which contributes to the reduction of oxidative stress in diabetes. Its functionalization onto Au NPs not only stabilizes the nanostructure but also amplifies its bioactivity by facilitating better cellular interaction and uptake.

One of the key properties of HT-Au NPs is their ability to counteract oxidative stress. In diabetes, excessive production of ROS leads to cellular damage, lipid peroxidation, and mitochondrial dysfunction (Newsholme et al., 2007). The polyphenolic structure of hamamelitannin allows it to donate electrons to neutralize ROS, thereby preventing oxidative damage (Janarathanam et al., 2024). Additionally, the nanoscale nature of HT-Au NPs enhances their cellular penetration, allowing them to reach intracellular compartments where ROS generation occurs. By mitigating oxidative stress, these NPs help preserve cellular integrity and function, which is crucial in maintaining normal metabolic activity in skeletal muscle cells.

The anti-apoptotic effects of HT-Au NPs are another critical aspect of their therapeutic potential. Diabetes-induced oxidative stress often triggers programmed cell death through mitochondrial dysfunction and activation of apoptotic pathways (Varga et al., 2015). The presence of hamamelitannin on Au NPs helps in stabilizing mitochondrial membranes and inhibiting the activation of pro-apoptotic proteins. This protective mechanism ensures that skeletal muscle cells maintain their viability, allowing them to efficiently participate in glucose metabolism. Furthermore, hamamelitannin is known to modulate inflammatory pathways by suppressing the activation of nuclear factor-kappa B (NF- κ B), a key regulator

of inflammation. This anti-inflammatory action further contributes to cell survival and improved metabolic activity.

HT-Au NPs also exhibit promising insulin-mimetic properties, which facilitate glucose uptake and storage. The ability of these NPs to enhance glucose transport is likely linked to their influence on insulin signaling pathways. Au NPs have been reported to activate the phosphoinositide 3-kinase (PI3K)-Akt signaling cascade, a crucial pathway for glucose transporter type 4 (GLUT4) translocation (Mahmoud et al., 2020). When GLUT4 translocation is enhanced, glucose uptake into skeletal muscle cells increases, improving overall glucose homeostasis (Zorzano et al., 2005). Additionally, polyphenols like hamamelitannin are known to activate AMP-activated protein kinase (AMPK), a key regulator of energy metabolism (Kumar et al., 2020). AMPK activation promotes glucose uptake, mitochondrial biogenesis, and fatty acid oxidation, all of which contribute to improved metabolic function (Han et al., 2015).

The role of HT-Au NPs in glycogen synthesis is another important aspect of their anti-diabetic potential. Glycogen storage is crucial for maintaining blood glucose levels, and its impairment is a hallmark of insulin resistance. The functionalized Au NPs enhance glycogen synthesis by modulating glycogen synthase activity, the key enzyme responsible for glycogen formation. Additionally, by reducing oxidative stress, HT-Au NPs prevent the

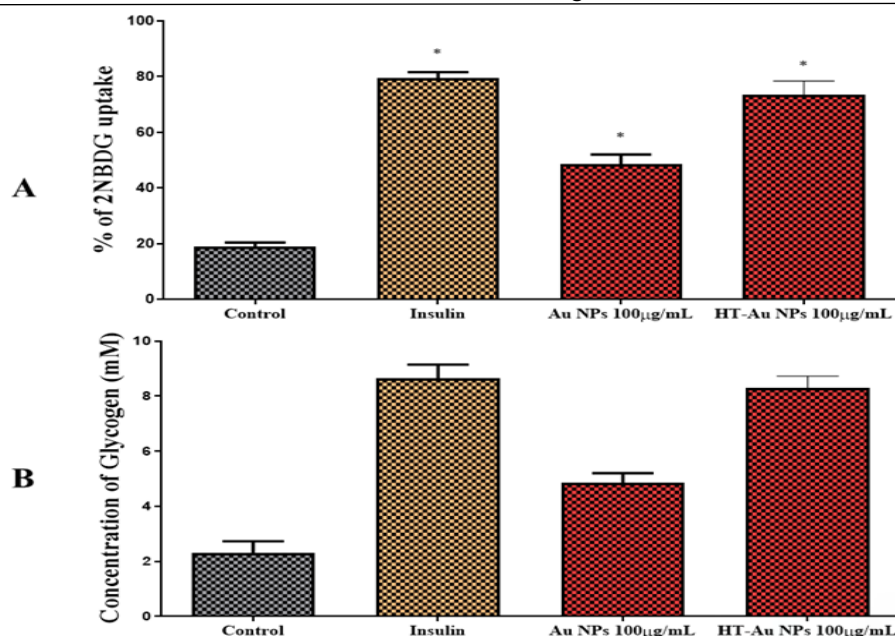


Figure 8: (A) The glucose uptake in L6 cells was performed through 2NBDG assay. (B) The concentration of glycogen content was estimated in L6 cells. The insulin treated and untreated L6 cells was used as positive control and control. * represent significant difference at $p < 0.05$.

inactivation of glycogen synthase, ensuring sustained glucose storage capacity in muscle cells. This dual action—enhancing glucose uptake and promoting glycogen storage—makes HT-Au NPs a highly effective therapeutic candidate for diabetes management.

Beyond their metabolic effects, HT-Au NPs exhibit excellent biocompatibility, which is essential for their clinical application. The stability of Au NPs prevents their aggregation, ensuring consistent bioavailability and controlled cellular interactions. Hamamelitannin further improves their biological compatibility by providing a natural, non-toxic surface modification that minimizes unwanted immune responses. This aspect is particularly important for the development of safe and effective nanomedicine-based treatments for metabolic disorders.

The synergistic interaction between Au NPs and hamamelitannin provides a multi-faceted approach to diabetes therapy. While Au NPs act as an efficient carrier and modulator of signaling pathways, hamamelitannin contributes to the antioxidant, anti-inflammatory, and metabolic regulatory effects. This combined action enhances therapeutic efficiency while minimizing potential cytotoxicity. Furthermore, the ability of HT-Au NPs to interact with key metabolic pathways suggests that they could be used in combination with existing anti-diabetic drugs to improve treatment outcomes. Future studies should focus on elucidating the precise molecular mechanisms through which HT-Au NPs exert their effects, particularly their interaction with insulin receptors and glucose metabolism-related enzymes.

The potential application of HT-Au NPs extends beyond diabetes management. Given their strong antioxidative and anti-inflammatory properties, these NPs could be explored for their role in preventing complications associated with diabetes, such as neuropathy, nephropathy, and cardiovascular diseases. Additionally, their ability to regulate oxidative stress and cellular metabolism suggests that they could be applied to other metabolic disorders, including

obesity and metabolic syndrome. Further research into in vivo models and clinical trials is necessary to validate these findings and establish HT-Au NPs as a viable therapeutic option for metabolic diseases.

Overall, the unique physicochemical properties of HT-Au NPs make them a promising candidate for diabetes treatment. Their ability to reduce oxidative stress, protect against apoptosis, enhance glucose uptake, and promote glycogen storage highlights their potential as a novel therapeutic agent. By leveraging the combined benefits of Au NPs and hamamelitannin, HT-Au NPs offer a powerful approach to restoring metabolic balance and improving insulin sensitivity. Continued research and development in this field will be critical in advancing nanomedicine-based interventions for diabetes and related metabolic disorders.

5. Conclusion

HT-Au NPs exhibited significant anti-diabetic properties by enhancing glucose uptake, increasing glycogen accumulation, and reducing oxidative stress in L6 skeletal muscle cells. Their strong antioxidant potential and cytocompatibility suggest that they could serve as a novel therapeutic strategy for diabetes management. Further studies are warranted to explore their mechanisms at the molecular level and their potential applications in clinical settings.

Declarations

Ethics approval statement

No ethical approval was required for the current study as it did not deal with any human or animal samples.

Consent to participate

Not applicable

Consent to publish

Not applicable

Data Availability Statement

The data are available from the corresponding author upon reasonable request

Competing Interests

The authors declare that they have no conflict of interest

Funding

Not Applicable

Author contribution

Conceptualization, Data curation, Investigation, Formal analysis: S.G. & S.N Writing—review and editing: S.G All authors have read and agreed to the published version of the manuscript

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Not Applicable

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