

Fabrication of Nickel Oxide Nanoparticles via *Morus alba* Leaf Extract and Investigation of their Biological Properties

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Nickel oxide nanoparticles (NiO NPs) were effectively produced through a green synthesis utilizing *Morus alba* leaf extract as stabilizing and capping agent. The obtained nanoparticles were characterized by various analytical techniques. The successful formation of NiO NPs showed a distinct absorbance peak at 338 nm, while other results suggested the role of plant-derived compounds in stabilizing the nanoparticles. Transmission electron microscopy displayed mainly spherical particles with an average diameter of 35.4 nm, whereas SEM-EDX confirmed their structural features and elemental compositions. The biological activities of the prepared NiO NPs were comprehensively investigated. The anticancer potential exhibited a concentration-dependent cytotoxicity against PC-3 prostate cancer cells and WI-38 normal cells, with greater selectivity toward cancer cells ($IC_{50} = 238 \mu\text{g/mL}$ for PC-3 and $402 \mu\text{g/mL}$ for WI-38). The lipase inhibition test indicated a moderate inhibitory effect of NiO NPs in comparison with Orlistat. In addition, the antidiabetic activity was assessed and the nanoparticles showed moderate inhibitory effects relative to acarbose. Moreover, the assays of antioxidant activity demonstrated a dose-dependent radical scavenging ability, although lower than that of ascorbic acid. Overall, these findings suggest that *Morus alba*-derived NiO NPs hold promise for biomedical applications; however, further optimization studies are still necessary to enhance their efficacy.

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INTRODUCTION

Nanotechnology has become one of the fastest growing and most dynamic areas in contemporary science, offering innovative solutions across biomedical, environmental, and industrial applications. At the nanoscale, the particles exhibit remarkable physicochemical properties, including high surface area and enhanced reactivity, and distinctive optical and

electronic characteristics (Alfattah *et al.* 2025; Soliman *et al.* 2026). These features significantly improve the functional performance of nanoparticles compared to their bulk counterparts, particularly in biological systems. As a result, nanoparticles have been widely explored for applications in drug delivery, cancer therapy, antimicrobial treatment, and enzyme inhibition (Khormi *et al.* 2025; Soliman *et al.* 2025). In parallel, increasing environmental concerns have driven the development of sustainable and green synthetic strategies to serve as an alternative to traditional chemical methods that typically rely on hazardous reagents and severe reaction conditions (Selim *et al.* 2025).

Among various metal oxide nanoparticles, nickel oxide (NiO) nanoparticles (NPs) have received growing attention owing to their excellent physicochemical stability, catalytic activity, and broad range of biological applications (Alotaibi *et al.* 2025). NiO NPs exhibit pronounced biological activities, including antimicrobial, antioxidant, and anticancer effects, mainly because of their ability to induce ROS production, causing oxidative stress and apoptosis in cells (Falemban *et al.* 2025). Notably, research indicates that NiO NPs may display selective toxicity toward cancer cells with minimal impact on healthy cells, making them attractive candidates for anticancer applications (Mahdi *et al.* 2025). Moreover, NiO NPs have been found to suppress the activity of key enzymes, suggesting their usefulness in controlling diabetes and obesity (Neethidevan *et al.* 2024).

Plant-based green synthesis of NPs is increasingly recognized as an effective, economical, and sustainable technique. Plant extracts are abundant in bioactive compounds such as flavonoids, phenolics, terpenoids, and alkaloids, which serve as natural stabilizing and capping agent during nanoparticle formation. This strategy minimizes the use of harmful chemicals and enhances the biological performance of NPs due to the presence of phytochemical residues on their surface (Soliman and Salem 2025a,b). The species *Morus alba* is particularly rich in such bioactive compounds and has widely been well known for its antioxidants and therapeutic properties. Previous research has indicated that *Morus alba* extracts can effectively facilitate nanoparticle synthesis while improving their stability and bioactivity (Rocha *et al.* 2026).

Accordingly, this study aimed to develop NiO NPs through a green synthetic approach utilizing *Morus alba* leaf extract as a natural stabilizing and capping agent. The prepared NPs were thoroughly characterized using a range of analytical techniques to investigate their structural and physicochemical features. In addition, their diverse biological activities were investigated, including cytotoxic effects against PC-3 prostate cancer and WI-38 normal cell lines, lipase inhibitory activity, antidiabetic potential inhibition, and antioxidant activity. This study is intended to provide insight into the probable application of plant-mediated NiO NPs as sustainable and multifunctional nanomaterials for biomedical use.

EXPERIMENTAL

Material

Nickel nitrate hexahydrate, NaOH, ethanol, methanol, and DMSO were used as received from standard suppliers. Cell culture media (DMEM and RPMI-1640), FBS, and antibiotics were obtained from Gibco (USA). Enzymes and reagents including α -amylase, α -glucosidase, lipase, acarbose, Orlistat, DPPH, and ABTS were purchased from Sigma-Aldrich (USA). *Morus alba* leaves were collected locally and used for nanoparticle

synthesis. All chemicals were of analytical grade, and distilled water was used throughout the study.

Preparation of *Morus alba* Leaf Extract

Fresh *Morus alba* leaves were washed thoroughly with distilled water and air-dried at room temperature. The dried leaves were ground into a fine powder using a laboratory grinder and passed through a 60-mesh sieve. Approximately 10 g of the powdered leaves were mixed with 100 mL of distilled water and heated at 60 °C under continuous stirring at 500 rpm for 30 min. The obtained extract was filtered through Whatman No. 1 filter paper and stored at 4 °C for further use.

Green Synthesis of NiO Nanoparticles

The NiO NPs were synthesized using a green method mediated by *Morus alba* leaf extract. Initially, a 0.1 M solution of analytical-grade nickel(II) nitrate hexahydrate [Ni(NO₃)₂·6H₂O] was prepared in distilled water. Approximately 50 mL of the plant extract was gradually introduced into 100 mL of the nickel precursor solution under continuous magnetic stirring at 600 rpm and 70 °C for 2 h. The pH of the reaction system was adjusted to nearly 9 by adding 1 M NaOH solution. The transition in colour from light green to dark green signified the formation of nanoparticles. The obtained precipitate was separated by centrifugation at 6000 rpm for 15 min, followed by repeated washing first with distilled water and then with 70% ethanol at room temperature to eliminate impurities. The product was then dried at 80 °C for 12 h. Subsequently, the dried material was calcined at 400 °C for 3 h in a muffle furnace to yield NiO NPs.

Characterization

The synthesized NiO NPs were characterized using various analytical techniques. Optical properties were examined by UV–Vis spectroscopy (200 to 800 nm) using a spectrophotometer (Model: UV-2600, Shimadzu, Kyoto, Japan) with a scanning rate of 200 nm/min, while FTIR analysis (4000 to 500 cm⁻¹) was performed using an FTIR spectrometer (Model: Nicolet iS10, Thermo Fisher Scientific, Waltham, MA, USA) at a resolution of 4 cm⁻¹ to identify functional groups involved in nanoparticle formation and stabilization. XRD analysis was carried out using an X-ray diffractometer operated with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) over a 2θ scanning range of 10 to 80° to investigate the crystalline nature of the synthesized NiO nanoparticles. Morphology and particle size were analyzed using TEM (Model: JEM-2100, JEOL, Tokyo, Japan) operated at an accelerating voltage of 200 kV, and size distribution was determined with ImageJ software (National Institutes of Health, Bethesda, MD, USA). Surface features were further studied by SEM (Model: JSM-6510LV, JEOL, Tokyo, Japan) at an operating voltage of 15 kV, while elemental composition was confirmed using EDX analysis (Model: X-MaxN, Oxford Instruments, Abingdon, UK). Additionally, elemental mapping was performed to assess the distribution of Ni and O within the nanoparticles.

Cell Culture and MTT Cytotoxicity Assay

Cell lines and culture conditions

The PC-3 and WI-38 cell lines were obtained from VACSERA, Egypt, and maintained under standard sterile cell culture conditions. Cells were routinely monitored microscopically to ensure normal morphology and contamination-free growth before biological assays.

The MTT assay

Cells were maintained in DMEM or RPMI-1640 medium supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin–streptomycin and incubated at 37 °C under 5% CO₂ in a humidified environment. Cytotoxicity was evaluated using the MTT assay. Cells were seeded in 96-well plates at a density of approximately 1×10^4 cells per well and incubated overnight for attachment. The medium was then replaced with fresh medium containing various concentrations of NiO NPs. After 24 or 48 h of exposure, 20 µL of MTT solution (5 mg/mL) was added, followed by incubation for 3 to 4 h to allow formazan formation. The supernatant was removed, and the crystals were dissolved in DMSO. Absorbance was recorded at 570 nm using a microplate reader, with a reference wavelength when required. Cell viability was calculated relative to untreated control cells using the following formula:

$$\text{Cell viability (\%)} = (A_{\text{treated cells}} / A_{\text{control cells}}) \times 100 \quad (1)$$

Pancreatic Lipase Inhibitory Assay

The inhibitory activity of NiO NPs against pancreatic lipase was evaluated and compared with Orlistat as a standard inhibitor. Different concentrations of NiO NPs and Orlistat were prepared in the appropriate solvent. Briefly, 100 µL of the test sample was mixed with 100 µL of pancreatic lipase solution prepared in Tris-HCl buffer (pH 8.0). The reaction mixture was pre-incubated at 37 °C for 15 min. Then, 100 µL of *p*-nitrophenyl butyrate (*p*-NPB) substrate solution was added to initiate the reaction, and the mixture was further incubated at 37 °C for 30 min. The release of *p*-nitrophenol was measured spectrophotometrically at 405 nm. A control was prepared under the same conditions without the test sample, while a blank contained all reagents except the enzyme. The percentage inhibition of pancreatic lipase activity was calculated using the following Eq. 2:

$$\text{Inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100 \quad (2)$$

Antidiabetic Assay

In vitro α-amylase inhibitory assay

The inhibitory activity of NiO NPs was assessed and compared with acarbose as a reference drug. Various concentrations of NiO NPs and acarbose were prepared in a suitable solvent. In brief, 500 µL of the sample was mixed with 500 µL of α-amylase solution in 0.02 M sodium phosphate buffer (pH 6.9) and incubated at 25 °C for 10 min. Subsequently, 500 µL of 1% starch solution was added, followed by an additional 10 min incubation. The reaction was stopped by adding 1 mL of DNS reagent and heating in a boiling water bath for 5 min. After cooling, the mixture was diluted with distilled water, and absorbance was recorded at 540 nm. A control was prepared without the test sample. The percentage inhibition was calculated using the following Eq. 3:

$$\text{Inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100 \quad (3)$$

In vitro α-glucosidase inhibitory assay

The inhibitory was determined using a standard *in vitro* method and compared with acarbose as a positive control. Different concentrations of NiO NPs and acarbose were prepared. In brief, 50 µL of the test sample was mixed with 100 µL of α-glucosidase enzyme solution in phosphate buffer (pH 6.8) and incubated at 37 °C for 10 min. Then, 50

μL of *p*-nitrophenyl- α -D-glucopyranoside (*p*NPG) substrate solution was added to initiate the reaction, and the mixture was incubated again at 37 °C for 20 min. The reaction was stopped by adding 1.0 mL of sodium carbonate solution. The absorbance of the released *p*-nitrophenol was measured at 405 nm using a spectrophotometer. A control was prepared under the same conditions without the test sample. The percentage inhibition of α -glucosidase was calculated according to the following Eq. 4:

$$\text{Inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100 \quad (4)$$

Antioxidant Activity

DPPH radical scavenging assay

The antioxidant activity of NiO NPs was assessed using the DPPH radical scavenging method, with ascorbic acid as a reference standard. A 0.1 mM DPPH solution in methanol was prepared, and 1.0 mL of this solution was mixed with 1.0 mL of the sample at different concentrations. The mixture was incubated in the dark at room temperature for 30 min. Afterward, the reduction in absorbance was recorded at 517 nm using a UV–Vis spectrophotometer. A control sample containing methanol instead of the test solution was used.

ABTS radical scavenging assay

The antioxidant activity of NiO NPs was also determined using the ABTS radical cation decolorization assay, with ascorbic acid as a standard. The ABTS radical cation (ABTS⁺) was generated by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate, followed by incubation in the dark at room temperature for 12 to 16 h. The resulting solution was diluted with ethanol or phosphate buffer to obtain an absorbance of $\sim 0.70 \pm 0.02$ at 734 nm. Then, 1.0 mL of ABTS⁺ solution was mixed with 1.0 mL of test sample at different concentrations. The mixture was incubated at room temperature for 6 to 10 min, and the absorbance was recorded at 734 nm.

Experimental controls

Untreated cells or reaction mixtures without nanoparticles were used as negative controls in all biological assays, while standard compounds including ascorbic acid, acarbose, and Orlistat were used as positive controls according to the assay type. The selected concentration ranges of NiO NPs were based on preliminary screening experiments to evaluate dose-dependent biological effects.

Statistical Analysis

All experiments were performed in triplicate ($n = 3$) and presented as mean $\pm$ SD. Statistical evaluation was carried out using one-way ANOVA followed by Tukey's test ($P < 0.05$) using Origin and GraphPad Prism software. The concentration range used was 1000 to 12.5 $\mu\text{g/mL}$, except for the anticancer study, which was conducted within a range of 1000 to 50 $\mu\text{g/mL}$.

RESULTS AND DISCUSSION

Characterization

The UV–Vis spectrum (Fig. 1a) showed a characteristic absorbance peak at around 338 nm, which is attributed to the surface plasmon resonance of NiO NPs. This peak

confirms the formation of NiO NPs and is consistent with previously reported values for biosynthesized NiO nanoparticles (Venkatalakshmi *et al.* 2023). Similar absorption peaks in the range of 330 to 370 nm have also been reported for green-fabricated NiO nanoparticles synthesized using different plant extracts, confirming the optical properties and nanoscale formation of NiO systems (Alotaibi *et al.* 2025).

The FTIR spectrum (Fig. 1b) revealed several prominent peaks corresponding to different functional groups. The broad band observed around 3380 cm^{-1} is assigned to O–H stretching vibrations, indicating the presence of phenolic compounds from the plant extract. Peaks at 1592 and 1372 cm^{-1} correspond to C=O and C–N stretching vibrations, respectively, suggesting the involvement of biomolecules in the reduction and stabilization process. The strong peak observed at around 618 and 457 cm^{-1} is characteristic of Ni–O stretching, confirming the formation of NiO NPs. Similar functional group assignments have been reported in green-synthesized NiO systems (Ezhilarasi *et al.* 2020). The observed biological activities of the synthesized NiO NPs may be partly attributed to bioactive phytochemicals present in *Morus* leaf extract, particularly phenolic and flavonoid compounds, which can act as reducing and capping agents during nanoparticle formation and may contribute synergistically to antioxidant, enzyme inhibitory, and cytotoxic effects (Chaikali *et al.* 2025).

XRD analysis was performed to investigate the crystalline nature of the synthesized NiO NPs (Fig. a3). The diffraction pattern exhibited characteristic peaks corresponding to crystalline nickel oxide phases, confirming the successful formation of NiO nanoparticles. The broad diffraction peaks observed in the XRD spectrum indicate the nanoscale nature and partial crystallinity of the synthesized particles, which is commonly reported for green-synthesized metal oxide nanoparticles. The broadened peaks may also be attributed to the small crystallite size and the presence of phytochemical capping agents derived from *Morus* leaf extract. Similar XRD patterns for biosynthesized NiO nanoparticles have been previously reported in the literature (Ezhilarasi *et al.* 2020).

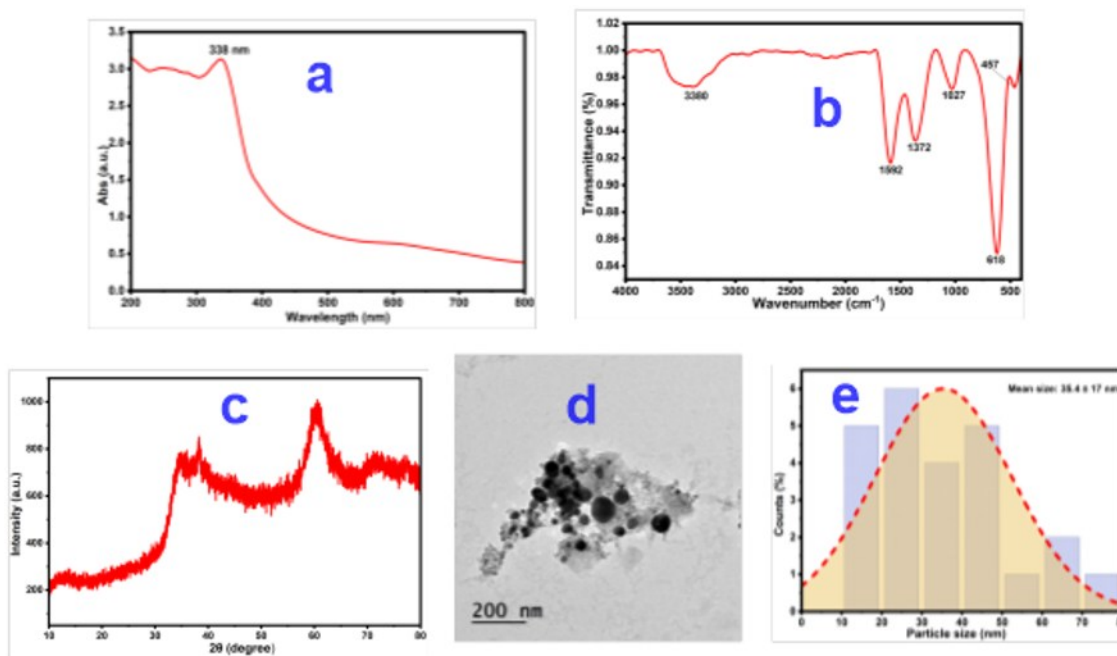


Fig. 1. (a) UV–Vis spectrum, (b) FTIR spectrum, (c) XRD pattern, (d) TEM image, and (e) particle size distribution histogram of NiO NPs synthesized using *Morus alba* leaf extract

The TEM analysis (Fig. 1d) revealed that the synthesized NiO NPs were predominantly spherical with slight agglomeration. The particles appeared well-dispersed with nanoscale dimensions, confirming the effectiveness of the plant extract played a role in stabilization. The histogram representing particle size distribution (Fig. 1e) showed that the nanoparticles ranged between approximately 20 and 60 nm, with an average size of 35.4 ± 17 nm. This size range is comparable to previously reported green-synthesized NiO NPs, which typically fall within the nanoscale range suitable for biomedical applications (Punitha and Saral 2024). Previous studies demonstrated that nanoparticles within this nanoscale range generally exhibit enhanced biological interactions due to their high surface-area-to-volume ratio and improved cellular uptake efficiency (Falemban *et al.* 2025).

The structural features and elemental distribution of the synthesized NiO NPs were analyzed using SEM, along with EDX and elemental mapping techniques (Fig. 2). The SEM image (Fig. 2a) revealed that the NiO NPs had an irregular, aggregated morphology with a rough surface texture. The formation of aggregates can be explained by the high surface energy of nanoparticles as well as bioactive phytoconstituents originating from *Morus* leaves extract acting as capping agents. Similar morphological features have been reported for green-synthesized NiO NPs (Madasamy *et al.* 2023). Comparable aggregation behavior has also been observed in other phytofabricated NiO nanoparticles, where partial agglomeration was attributed to intermolecular interactions among capped nanoparticles and residual phytochemical constituents on the nanoparticle surface (Neethidevan *et al.* 2024).

The EDX spectrum (Fig. 2b) verified the elemental composition of the prepared NPs. The presence of strong characteristic peaks corresponding to nickel (Ni) and oxygen (O) indicates the successful formation of NiO NPs. Minor signals for carbon (C) were also detected, which can be associated with trace organic compounds from the extract or the sample preparation process. The quantitative analysis showed that Ni and O were the dominant elements, confirming the purity of NPs. These findings are in agreement with previous studies on plant-mediated NiO NPs synthesis (Khodair *et al.* 2022). Similar EDX elemental profiles dominated by Ni and O peaks have been extensively reported for biosynthesized NiO nanoparticles, confirming the successful conversion of nickel precursors into nickel oxide nanostructures with minimal impurities (Said *et al.* 2025). Elemental mapping analysis (Fig. 2c) further demonstrated the consistent distribution of Ni and O throughout the material. The uniform dispersion of elements indicates the successful formation of NiO NPs without significant impurities or phase separation. The presence of carbon was evenly distributed as well, supporting its role as a stabilizing residue from the plant extract.

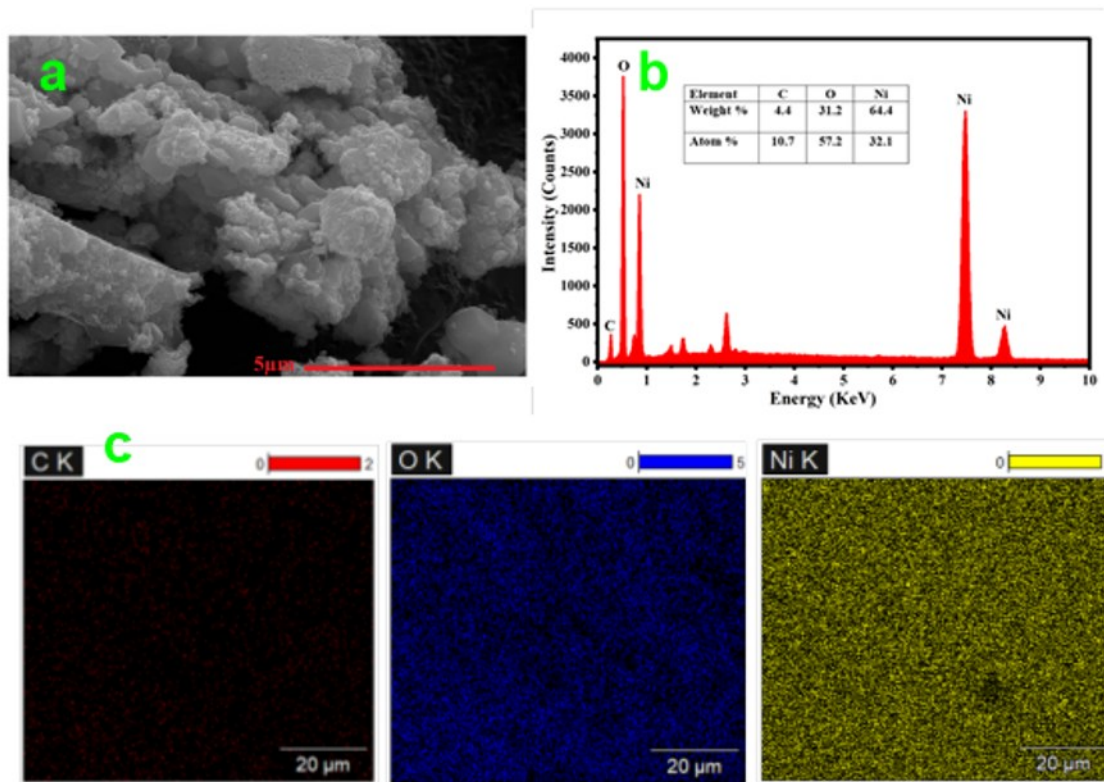


Fig. 2. (a) SEM image, (b) EDX spectrum, and (c) elemental mapping of NiO NPs synthesized from *Morus alba* leaf extract

Anticancer Activity

The anticancer behavior of the NPs was assessed alongside WI-38 (normal lung fibroblast) and PC-3 (prostate cancer) cell lines using the MTT assay (Fig. 3a). The results clearly demonstrated a concentration-dependent cytotoxic effect for both cell lines. Cell viability decreased from 95.5% to 17.6% in WI-38 cells and from 92.3% to 10.0% in PC-3 cells with increasing doses starting from 50 to 1000 $\mu\text{g/mL}$. Notably, NiO NPs exhibited higher cytotoxicity toward PC-3 cancer cells compared to WI-38 normal cells, indicating selective anticancer activity. This observation was confirmed by the IC_{50} values, where PC-3 cells showed a lower IC_{50} (238.6 $\mu\text{g/mL}$) compared to WI-38 cells (402 $\mu\text{g/mL}$). Such selectivity is a desirable feature for anticancer agents, as it minimizes damage to normal cells while effectively targeting cancer cells. The enhanced cytotoxic effect of NiO NPs can be attributed mainly to the formation of reactive oxygen species (ROS), which results in oxidative stress, mitochondrial damage, and programmed cell death in cancer cells. Previous reports have shown that NiO NPs trigger apoptosis through ROS-mediated pathways and the release of Ni^{2+} ions, leading to DNA damage and cell death. In addition, cancer cells are generally more susceptible to oxidative stress than normal cells, which explains the higher sensitivity of PC-3 cells observed in this study (Zhou *et al.* 2025). Comparing the present findings with previous reports, green-synthesized NiO NPs have shown strong anticancer activity against various cancer cell lines. For instance, NiO NPs synthesized using *Sesbania grandiflora* extract against HeLa and breast cancer cells, indicating higher potency than the present study (Gobinath *et al.* 2023). Similarly, another recent study reported activity against A549 lung cancer cells using biogenic NiO NPs (UR *et al.* 2021). The difference in cytotoxic efficiency between studies may be attributed to

several factors, including particle size, morphology, surface chemistry, and synthesis method. In the present study, the relatively larger particle size (~35 nm) and possible aggregation observed in TEM and SEM analyses may have reduced cellular uptake and consequently, cytotoxic efficiency. It is well known that smaller nanoparticles with higher surface area exhibit enhanced cellular internalization and stronger biological activity.

Inhibitory Effect on Lipase Enzyme

The effect of NiO NPs was evaluated and compared with the standard drug Orlistat (Fig. 3b). Both NiO NPs and Orlistat exhibited increased lipase inhibition with increasing concentration. The inhibition percentage of NiO NPs increased from 3.2% at 6.25 $\mu\text{g/mL}$ to 55.5% at 1000 $\mu\text{g/mL}$, indicating moderate inhibitory activity. However, Orlistat exhibited significantly higher lipase inhibition at all tested concentrations, reaching up to 95.5% at 1000 $\mu\text{g/mL}$. This difference was further confirmed by the IC_{50} values, where Orlistat showed a much lower IC_{50} (36.4 $\mu\text{g/mL}$) compared to NiO NPs (570 $\mu\text{g/mL}$), indicating its superior potency as a lipase inhibitor. Despite the lower activity compared to Orlistat, the observed inhibitory effect of NiO NPs suggests their potential as a mild anti-obesity agent. The mechanism of lipase inhibition by nanoparticles is often attributed to their interaction with the enzyme active site, adsorption onto the enzyme surface, or conformational changes that reduce catalytic activity (Shuai *et al.* 2017). Additionally, the presence of phytochemicals from *Morus alba* extract on the nanoparticle surface may contribute to enzyme inhibition through synergistic effects. Comparing these findings with previous studies, several metal oxide nanoparticles have shown similar moderate lipase inhibitory activity (Cheah *et al.* 2025). Moreover, recent studies have reported that biogenic nanoparticles generally show lower lipase inhibition than Orlistat but offer advantages such as reduced side effects and better biocompatibility (Asghar *et al.* 2022).

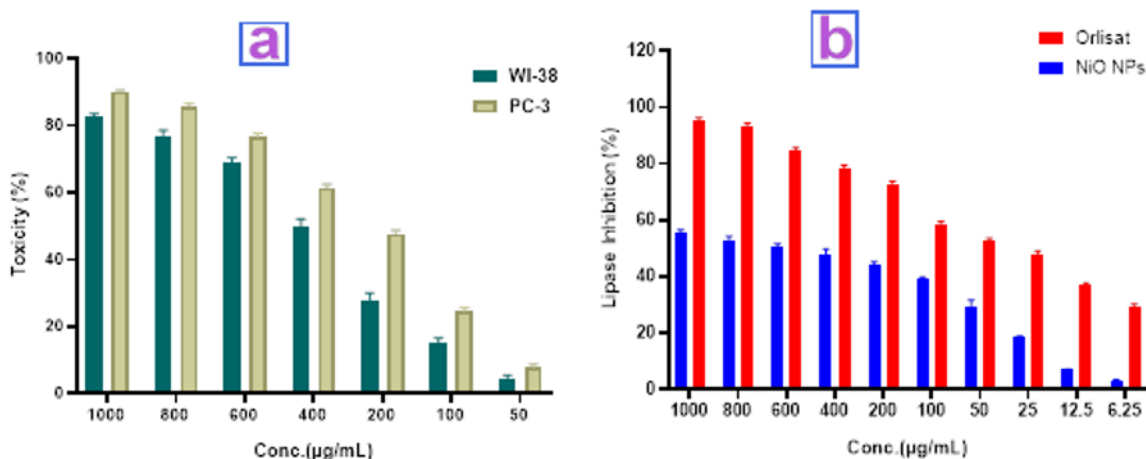


Fig. 3. (a) Effect of different concentrations of NiO NPs on cell viability (WI-38 and PC-3) and (b) lipase inhibitory activity compared to Orlistat

Antidiabetic Activity

The inhibitory activity of NiO NPs against α -amylase and α -glucosidase enzymes was evaluated (Fig. 4). The results revealed a clear enhanced enzyme inhibition corresponding to increasing concentration for both NiO NPs and acarbose. For α -amylase (Fig. 4a), the NiO NPs exhibited moderate inhibitory activity, increasing from 6.6% at 6.25

$\mu\text{g/mL}$ to 61.6% at 1000 $\mu\text{g/mL}$. In contrast, acarbose showed significantly higher inhibition, reaching 96.9% at the same concentration. This difference was reflected in the IC_{50} values, where acarbose exhibited a much lower IC_{50} (28.6 $\mu\text{g/mL}$) compared to NiO NPs (438.5 $\mu\text{g/mL}$), indicating its higher potency. Similarly, in the case of α -glucosidase (Fig. 4b), NiO NPs showed concentration-dependent inhibition ranging from 6.9% to 55.9%, whereas acarbose achieved inhibition values up to 93.9%. The IC_{50} values further confirmed this trend, with acarbose showing an IC_{50} of 23.8 $\mu\text{g/mL}$ compared to 726 $\mu\text{g/mL}$ for NiO NPs. Despite their lower activity compared to acarbose, NiO NPs demonstrated noticeable inhibitory effects against both enzymes, suggesting their potential role as mild antidiabetic agents. The mechanism of inhibition may involve interaction of nanoparticles with the enzyme active sites, leading to structural alterations and reduced catalytic efficiency. Additionally, phytochemicals from *Morus alba* extract adsorbed on the nanoparticle surface may contribute synergistically to enzyme inhibition (UR *et al.* 2021). Comparing these findings with previous studies, green-synthesized NiO NPs have shown similar moderate inhibitory activity against α -amylase and α -glucosidase, typically with low IC_{50} values (Said *et al.* 2025). Furthermore, recent studies have reported that biogenic nanoparticles generally exhibit lower inhibitory activity than synthetic drugs like acarbose but offer advantages such as improved biocompatibility and reduced side effects (Priyadharshini *et al.* 2025). The relatively higher IC_{50} values observed for NiO NPs in this study may be attributed to factors such as particle size (~ 35 nm), aggregation, and surface characteristics, which can influence enzyme–nanoparticle interactions. Smaller and well-dispersed NiO NPs with higher surface area are known to exhibit stronger enzyme inhibition (Talukdar *et al.* 2024).

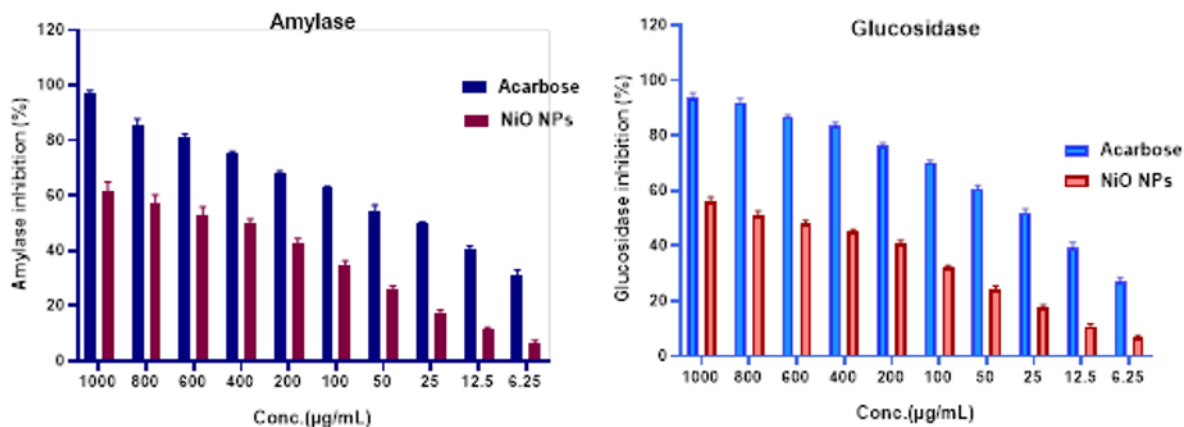


Fig. 4. Enzyme inhibition of NiO NPs: (a) amylase and (b) glucosidase compared with acarbose

Antioxidant Activity

The DPPH and ABTS assays were used to determine the antioxidant activity of NiO NPs (Fig. 5). The results demonstrated a concentration-dependent increase in radical scavenging activity for both NiO NPs and ascorbic acid. In the DPPH assay (Fig. 5a), the NiO NPs showed moderate antioxidant activity, increasing from 11.2% at 12.5 $\mu\text{g/mL}$ to 64.9% at 1000 $\mu\text{g/mL}$. However, ascorbic acid exhibited significantly higher scavenging activity, reaching 97.0% at the same concentration. This difference was further confirmed by the IC_{50} values, where ascorbic acid showed a much lower IC_{50} (46.3 $\mu\text{g/mL}$) compared to NiO NPs (452 $\mu\text{g/mL}$), indicating its superior antioxidant capacity. Similarly, in the ABTS assay (Fig. 5b), the NiO NPs demonstrated a concentration-dependent scavenging

activity ranging from 9.8% to 59.4%, while ascorbic acid achieved up to 97.2% inhibition. The IC_{50} values (589 $\mu\text{g/mL}$ for NiO NPs vs. 48.8 $\mu\text{g/mL}$ for ascorbic acid) further confirmed the relatively lower antioxidant efficiency of NiO NPs. Despite their lower activity compared to ascorbic acid, NiO NPs still exhibited noticeable antioxidant potential. This activity can be attributed to the presence of bioactive phytochemicals from *Morus* leaf extract adsorbed on the nanoparticle surface, which may act as electron or hydrogen donors to neutralize free radicals. Additionally, the nanoscale size and surface properties of NiO NPs can enhance their interaction with reactive species (Kaur *et al.* 2024). Comparing these findings with previous studies, green-synthesized metal oxide nanoparticles typically exhibit moderate antioxidant activity (Ashrafi-Saiedlou *et al.* 2025). Moreover, recent studies have highlighted that the antioxidant activity of nanoparticles is strongly influenced by particle size, surface area, and the type of plant extract used during synthesis (Chaikali *et al.* 2025). Similar concentration-dependent antioxidant behavior has been reported for plant-mediated NiO nanoparticles synthesized using olive leaf and *Moringa* extracts, where DPPH scavenging activities ranged from moderate to high depending on nanoparticle size and phytochemical composition (Mahdi Salih *et al.* 2025). Furthermore, Said *et al.* (2025) demonstrated that biosynthesized NiO nanoparticles exhibited antioxidant activity comparable to the present study, supporting the role of surface-bound phytochemicals in free radical neutralization. In another study, Priyadharshini *et al.* (2025) reported that aggregation state and surface reactivity significantly affect the antioxidant efficiency of biogenic metal oxide nanoparticles, which is in agreement with the moderate activity observed in the current work. The relatively lower antioxidant activity observed in this study compared to ascorbic acid may be due to nanoparticle aggregation and limited surface availability for radical interaction. Smaller, well-dispersed nanoparticles generally exhibit enhanced antioxidant performance due to increased surface reactivity. A schematic illustration of the proposed antioxidant mechanism of NiO NPs *via* electron/hydrogen transfer is presented in Fig. 5c.

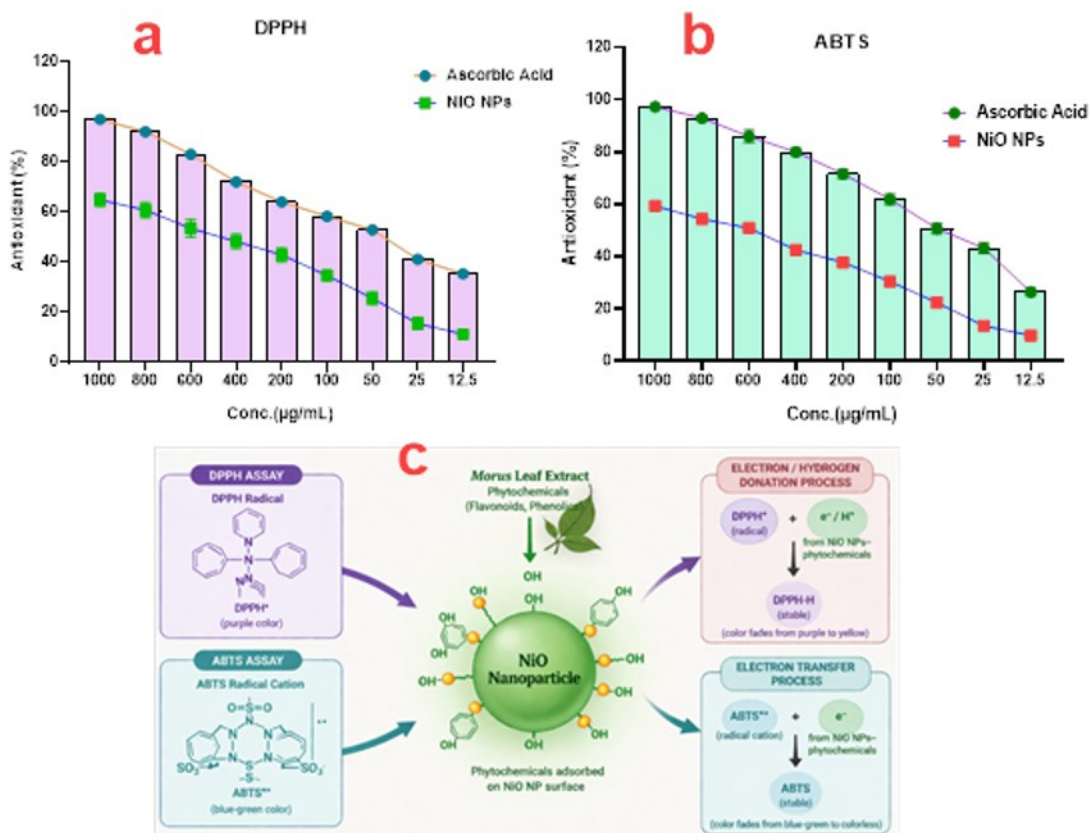


Fig. 5. Antioxidant activity of NiO NPs: (a) DPPH; (b) ABTS compared with ascorbic acid; and (c) proposed mechanism of antioxidant activity

CONCLUSIONS

1. NiO nanoparticles were effectively synthesized using *Morus alba* leaf extract through a simple and eco-friendly green method.
2. Various analytical results (UV-Vis, FTIR, TEM, SEM, and EDX) confirmed the development of NiO NPs with nanoscale size and suitable morphology.
3. The synthesized NiO NPs exhibited concentration-dependent cytotoxic activity, with higher selectivity toward PC-3 cancer cells compared to WI-38 normal cells.
4. NiO NPs demonstrated moderate lipase inhibitory activity compared to Orlistat, indicating potential anti-obesity properties.
5. The nanoparticles showed noticeable repressive effects alongside α -amylase and α -glucosidase enzymes, suggesting possible antidiabetic activity.
6. Moderate antioxidant activity was observed in both DPPH and ABTS assays.

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