

# Bridging Green Nanotechnology and Biosafety: Cytotoxicity Assessment of Nickel oxide and cobalt oxide Nanoparticles

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**Abstract**— Green synthesis of metal oxide nanoparticles has emerged as an environmentally friendly approach for producing functional nanomaterials with improved biocompatibility and reduced chemical hazards. In the present study, nickel oxide (NiO) and cobalt oxide (Co<sub>3</sub>O<sub>4</sub>) nanoparticles were synthesized through a plant-mediated method and evaluated for their *in vitro* cytotoxic effects. The synthesized nanoparticles were characterized using standard analytical techniques to confirm their structural and morphological properties. Cytotoxicity was investigated using the MTT assay on human Dermal Fibroblast (HDF) to determine the dose-dependent cellular response to nanoparticle exposure. The results revealed that both NiO and Co<sub>3</sub>O<sub>4</sub> nanoparticles exhibited concentration-dependent cytotoxicity, with cell viability decreasing progressively as nanoparticle concentration increased. NiO nanoparticles demonstrated relatively higher biocompatibility, maintaining greater cell viability at lower concentrations, whereas Co<sub>3</sub>O<sub>4</sub> nanoparticles showed comparatively stronger cytotoxic effects at elevated concentrations. The observed cytotoxicity may be attributed to nanoparticle-induced oxidative stress and the generation of reactive oxygen species (ROS), which can affect cellular metabolism and membrane integrity. Despite the dose-dependent effects, both nanoparticles displayed acceptable levels of cell viability within moderate concentration ranges. These findings provide valuable insights into the biological safety of green synthesized metal oxide nanoparticles and support their potential application in environmental, biomedical fields and water treatment when used within safe exposure limits.

**Keywords**— Green synthesis, Nickel oxide nanoparticles, Cobalt oxide nanoparticles, Cytotoxicity, HDF cells, Nano biotoxicology.

## I. INTRODUCTION

Nanotechnology has emerged as a rapidly advancing interdisciplinary field due to the unique physicochemical properties of materials at the nanoscale. Nanoparticles typically possess high surface area-to-volume ratios, tunable surface chemistry, and enhanced catalytic and electronic properties compared with their bulk counterparts. These characteristics have enabled their widespread applications in environmental remediation, water treatment, energy storage, catalysis, sensors, and biomedical engineering [1,2]. However, the increasing production and application of engineered nanomaterials have raised significant concerns regarding their potential interactions with biological systems and their associated toxicological effects. Among the various nanomaterials, metal oxide nanoparticles have received considerable attention because of their chemical stability, catalytic efficiency, and multifunctional applications. In particular, nickel oxide (NiO) and cobalt oxide (Co<sub>3</sub>O<sub>4</sub>) nanoparticles have been extensively investigated due to their remarkable physicochemical and electrochemical properties. Nickel oxide nanoparticles exhibit semiconducting behaviour, high catalytic activity, and strong adsorption capabilities, making them suitable for applications in photocatalysis, sensors, batteries, and environmental pollutant removal [3,4]. Similarly, cobalt oxide nanoparticles, which typically possess a spinel crystal structure containing mixed oxidation states of cobalt ions, have demonstrated excellent catalytic, magnetic, and electrochemical properties.

As a result, Co<sub>3</sub>O<sub>4</sub> nanoparticles have been widely used in supercapacitors, lithium-ion batteries, gas sensors, and wastewater treatment technologies [5,6]. Despite their promising technological applications, the potential biological effects of these nanoparticles must be carefully evaluated before their large-scale implementation. Due to their extremely small size and high surface reactivity, nanoparticles can easily interact with biological membranes and intracellular components. These interactions may lead to alterations in cellular metabolism, oxidative stress, inflammatory responses, and damage to cellular macromolecules such as DNA, proteins, and lipids [7]. Numerous studies have demonstrated that the cytotoxicity of metal oxide nanoparticles is often associated with the generation of reactive oxygen species (ROS), which disrupt cellular redox homeostasis and trigger oxidative stress pathways [8]. The extent of nanoparticle-induced toxicity depends on several factors including particle size, morphology, surface charge, chemical composition, concentration, and exposure duration. Smaller nanoparticles generally exhibit higher biological reactivity due to their larger specific surface area, which enhances their interaction with cellular components [9]. Additionally, the dissolution of metal ions from nanoparticles may further contribute to cytotoxic effects by interfering with intracellular biochemical processes.

In recent years, green synthesis approaches using plant extracts have been increasingly explored as environmentally sustainable alternatives to conventional chemical and physical nanoparticle synthesis methods. Traditional synthesis

techniques often involve hazardous chemicals and high-energy processes that may generate toxic by-products. In contrast, plant-mediated synthesis utilizes naturally occurring phytochemicals such as flavonoids, phenolic compounds, alkaloids, and terpenoids as reducing and stabilizing agents, thereby offering an eco-friendly and cost-effective method for nanoparticle production [10]. Moreover, the presence of plant-derived biomolecules on the nanoparticle surface may enhance their stability and potentially improve their biocompatibility. Although several studies have reported the synthesis and functional applications of NiO and Co<sub>3</sub>O<sub>4</sub> nanoparticles, comparative investigations of their cytotoxic effects remain limited. Understanding the biological responses induced by these nanomaterials is crucial for evaluating their safety and guiding their responsible use in environmental and biomedical applications. In vitro cytotoxicity studies using mammalian cell culture models provide valuable insights into nanoparticle-cell interactions and help identify potential toxicological mechanisms. The human Dermal Fibroblast (HDF) cell line is widely used in toxicological and biomedical research due to its stable growth characteristics and high sensitivity to environmental stress factors. This cell line serves as a reliable model for evaluating nanoparticle-induced cellular responses, including alterations in metabolic activity, oxidative stress levels, and cell viability [11]. Therefore, investigating the cytotoxic effects of metal oxide nanoparticles using HDF cells can provide important information regarding their biological safety. In this context, the present study aims to evaluate the in vitro cytotoxic potential of green synthesized nickel oxide (NiO) and cobalt oxide (Co<sub>3</sub>O<sub>4</sub>) nanoparticles. The cytotoxicity of the synthesized nanoparticles was assessed using the MTT assay to determine the dose-dependent effects on cell viability. By comparing the cytotoxic profiles of NiO and Co<sub>3</sub>O<sub>4</sub> nanoparticles, this study provides valuable insights into their biological interactions and contributes to the development of safer nanomaterials for environmental and biomedical applications.

## II. EXPERIMENTAL

### *Preparation of ginger extract*

Fresh ginger rhizomes were purchased from a local market and thoroughly washed with distilled water to remove any dirt, dust, or contaminants. The rhizomes were then peeled to remove the outer skin, ensuring that only the inner fresh tissue was used for extraction. Then grinded the fresh ginger and filter the extract. The resulting clear filtrate was collected as the ginger extract and stored in a clean glass container at 4°C for further use in the synthesis of NiO nanoparticles.

### *Green Synthesis of Nickel Oxide (NiO) Nanoparticles*

The ginger extract was gradually added dropwise in a 1:1 ratio to the 0.01M nickel nitrate solution under continuous stirring at 70°C for 2 hours and NaOH used for the pH maintain. The addition rate and stirring speed were controlled to ensure the uniform mixing of the extract with the nickel salt solution. The reaction mixture gradually changed colour, indication the formation of nickel containing complexed. The resulting precipitates was centrifuged at 8000 rpm for 15 minutes,

washed repeatedly with deionized water and ethanol to remove impurities and dried overnight at 80°C in hot air. The dried powder was collected in the form of pure crystalline NiO nanoparticles.

### *Green Synthesis of Cobalt Oxide (Co<sub>3</sub>O<sub>4</sub>) Nanoparticles*

Cobalt oxide nanoparticles were synthesized using a similar plant-mediated method. A 0.1 M cobalt chloride solution was prepared in distilled water and mixed with the prepared ginger extract under continuous stirring at room temperature for 2 hours. The reaction mixture was allowed to proceed until a visible colour change to dark brown, confirming the initiation of nanoparticle formation. The reaction mixture was allowed to stand undisturbed for 24 h to complete the reduction process. The resulting precipitate was centrifuged at 8000 rpm for 15 min, and the collected pellet was washed repeatedly with distilled water and ethanol to remove unreacted species and organic residues. The purified nanoparticles were oven-dried at 80°C for 6 h and subsequently calcined at 400°C for 3 h in a muffle furnace to obtain phase-pure crystalline Co<sub>3</sub>O<sub>4</sub> NPs suitable for characterization and biological evaluation.

### *Characterization of NiO and Co<sub>3</sub>O<sub>4</sub> nanoparticles*

The synthesized nanoparticles were characterized using various analytical techniques to determine their structural and morphological properties. X-ray diffraction (XRD) analysis was performed to confirm the crystalline phase and structure of the nanoparticles. Fourier transform infrared spectroscopy (FTIR) was used to identify the functional groups associated with the plant-derived biomolecules involved in nanoparticle stabilization.

### *Cell Culture*

The Human Dermal Fibroblast (HDF) cell line was used to evaluate the cytotoxicity of the synthesized nanoparticles. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) and 1 % penicillin-streptomycin solution. The cells were maintained in a humidified incubator at 37°C with 5 % CO<sub>2</sub>. The culture medium was replaced regularly to maintain optimal cell growth, and the cells were subcultured when they reached approximately 80–90 % confluency.

### *Cytotoxicity Assessment by MTT Assay*

The cytotoxicity of NiO and Co<sub>3</sub>O<sub>4</sub> nanoparticles was evaluated using the MTT assay. HDF cells were seeded in 96-well culture plates at an appropriate density and allowed to attach overnight. After incubation, the cells were treated with different concentrations of NiO and Co<sub>3</sub>O<sub>4</sub> nanoparticle suspensions prepared in culture medium. The treated cells were incubated for 24 hours under standard culture conditions. After the exposure period, MTT solution was added to each well and incubated for several hours to allow the formation of purple formazan crystals by metabolically active cells. The culture medium was then carefully removed, and the formed formazan crystals were dissolved in dimethyl sulfoxide (DMSO). The absorbance was measured at 570 nm using a microplate reader. The percentage of cell viability was calculated using the following equation:

$$\text{Cell viability (\%)} = \left( \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \right) \times 100$$

### III. RESULTS AND DISCUSSION

#### Characterization of NiO nanoparticles

##### Fourier Transmission Infrared (FTIR) spectroscopy

A broad absorption band observed around 3400–3450  $\text{cm}^{-1}$  is attributed to the O–H stretching vibration of hydroxyl groups present in alcohols and phenolic compounds. These functional groups originate from ginger-derived phytochemicals and play a significant role in the reduction of nickel ions and stabilization of the formed nanoparticles. The absorption band appearing near 2920–2950  $\text{cm}^{-1}$  corresponds to C–H stretching vibrations of aliphatic hydrocarbons, which are commonly associated with organic biomolecules such as proteins and lipids present in the ginger extract. Another prominent band around 1630–1650  $\text{cm}^{-1}$  can be assigned to C=O stretching vibrations or C=C stretching of aromatic compounds, suggesting the presence of proteins or other biomolecules acting as capping agents for the nanoparticles. A peak observed around 1050–1100  $\text{cm}^{-1}$  corresponds to C–O stretching vibrations of alcohols, phenols, or polysaccharides present in the plant extract.

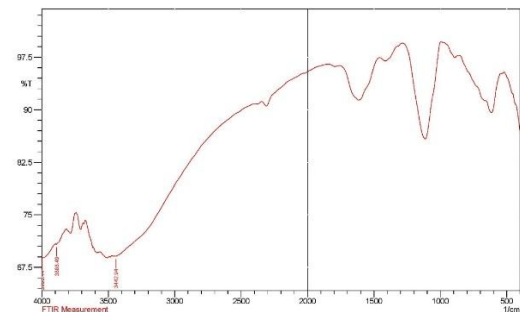


Fig. 1. FTIR spectrum of NiO nanoparticles using ginger extract

Most importantly, the characteristic absorption band observed in the lower wavenumber region around 450–600  $\text{cm}^{-1}$  corresponds to the Ni–O stretching vibration, confirming the successful formation of nickel oxide nanoparticles. This peak is considered a signature band for NiO nanoparticles and indicates the formation of metal–oxygen bonds in the crystalline structure. The presence of these functional groups suggests that phytochemicals such as phenolic compounds, flavonoids, proteins, and polysaccharides present in the ginger extract act as natural reducing and stabilizing agents during the green synthesis process. These biomolecules not only facilitate the reduction of nickel ions but also prevent nanoparticle aggregation by forming a protective capping layer around the particles.

##### X-ray diffraction (XRD)

The crystallographic structure of the synthesized nickel oxide (NiO) nanoparticles was analyzed using X-ray diffraction (XRD) and calculated by Debye–Scherrer equation. The measurement was carried out using Cu  $K\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ) over a  $2\theta$  range of  $5^\circ$ – $80^\circ$  in locked-coupled continuous scan mode. The XRD pattern, shown in Figure.1, revealed distinct

diffraction peaks at  $2\theta$  values of  $37.39^\circ$ ,  $43.97^\circ$ , and  $63.18^\circ$ , corresponding to the (111), (200), and (220) planes, respectively. These peaks are in good agreement with the standard JCPDS card No. 47-1049 for face-centered cubic (FCC) NiO, confirming the formation of a single-phase crystalline NiO structure.

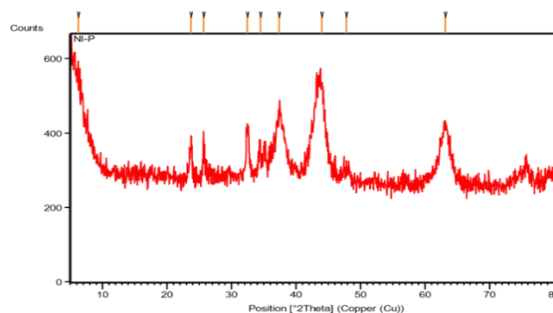


Fig. 2. XRD analysis NiO nanoparticles using ginger extract

The average crystallite size of the NiO nanoparticles was calculated using the Debye–Scherrer equation, no additional peaks corresponding to impurities or secondary phases were detected, indicating the high purity of the synthesized product. Using the most intense (200) peak at  $2\theta = 43.97^\circ$ , the average crystallite size was calculated to be approximately 6–8 nm. This nanoscale dimension suggests a high surface area, which is beneficial for applications. These results confirm the successful green synthesis of nanocrystalline NiO using ginger extract, with the extract likely acting as a natural chelating, reducing, and capping agent during synthesis.

##### Characterization of $\text{Co}_3\text{O}_4$ nanoparticles

##### Fourier Transform Infrared spectroscopy

In the FTIR spectrum, a broad absorption band observed around 3400–3450  $\text{cm}^{-1}$  corresponds to the O–H stretching vibration of hydroxyl groups, which may originate from phenolic compounds, alcohols, or absorbed water molecules present in the ginger extract. These hydroxyl groups are known to play an important role in the reduction of metal ions and stabilization of the synthesized nanoparticles. The peak appearing near 2920–2950  $\text{cm}^{-1}$  can be attributed to C–H stretching vibrations of aliphatic compounds, indicating the presence of organic biomolecules such as proteins and lipids associated with the nanoparticle surface. Another prominent band around 1630–1650  $\text{cm}^{-1}$  corresponds to C=O stretching vibrations or aldehyde or ketone groups in the extract. These biomolecules may act as capping agents, preventing aggregation of nanoparticles and enhancing their stability. Additionally, peaks in the region of 1050–1100  $\text{cm}^{-1}$  correspond to C–O stretching vibrations of alcohols, phenols, or polysaccharides present in the ginger extract.

Most importantly, the presence of strong peaks in the region of 504–664  $\text{cm}^{-1}$  corresponds to Co–O vibrations, confirming the formation of cobalt oxide nanoparticles with spinel structure. The presence of these functional groups suggests that phytochemicals such as phenols, flavonoids, proteins, and polysaccharides present in the ginger extract play a significant role in the reduction of metal ions and stabilization of the

synthesized nanoparticles. These biomolecules act as natural reducing and capping agents during the green synthesis process.

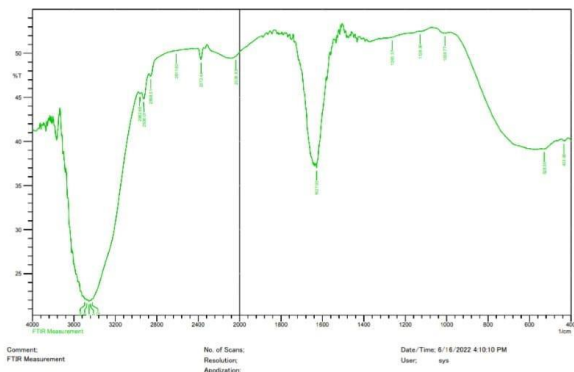


Fig. 3. FTIR spectrum of Co<sub>3</sub>O<sub>4</sub> nanoparticles using ginger extract

*X-ray diffraction (XRD)*

The XRD pattern of Co<sub>3</sub>O<sub>4</sub> nanoparticles showed prominent diffraction peaks at approximately  $2\theta = 31.90^\circ, 36.8^\circ, 44.44^\circ, 59.47^\circ,$  and  $65.29^\circ$ , which correspond to the crystal planes (220), (311), (222), (400) and (422), respectively. These peaks are characteristic of the spinel cubic structure of Co<sub>3</sub>O<sub>4</sub>, and they are in good agreement with the standard JCPDS data (JCPDS card No. 42-1467). The absence of additional impurity peaks indicates that the synthesized nanoparticles are phase-pure. These reflections are characteristic of the face-centered cubic (FCC) spinel structure of Co<sub>3</sub>O<sub>4</sub>. The average crystallite size, calculated using the Debye–Scherrer equation, ranged from 15 to 25 nm, with an estimated mean size of approximately 23.82 nm. These results confirm the formation of a pure, well-crystalline Co<sub>3</sub>O<sub>4</sub> phase.

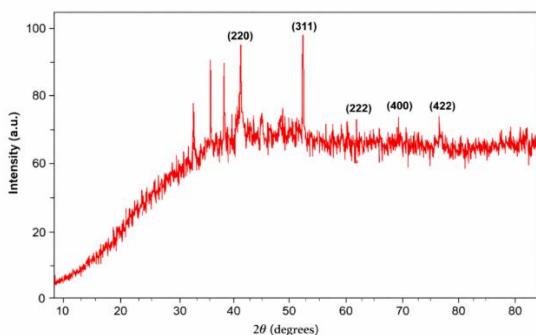


Fig. 4. XRD analysis Co<sub>3</sub>O<sub>4</sub> nanoparticles using ginger extract

*Cytotoxicity Assessment of NiO Nanoparticles*

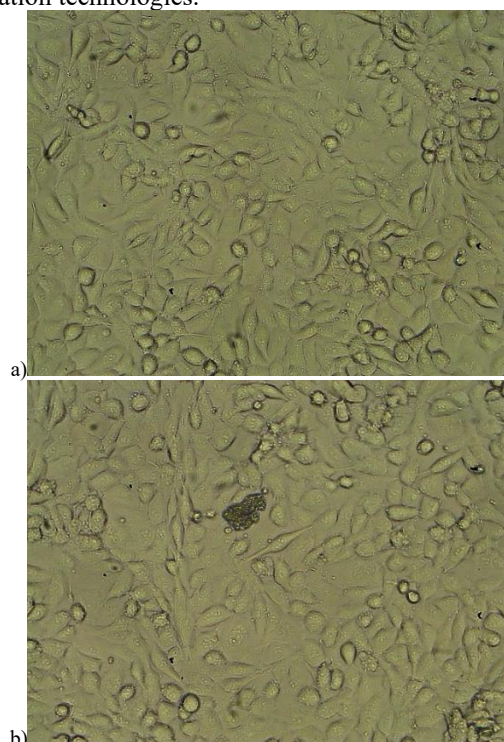
The cytotoxic effects of nickel oxide (NiO) nanoparticles on Human Dermal Fibroblast (HDF) cells were evaluated using the MTT assay after 24 h of exposure at different nanoparticle concentrations. The results revealed that NiO nanoparticles induced concentration-dependent cytotoxicity in HDF cells. At the lowest tested concentration 6.25 μg/mL, the nanoparticles showed relatively high cell viability 90.36%, suggesting minimal cytotoxic effects and good cytocompatibility at this dose. The observed reduction in cell viability at higher concentrations may be attributed to the physicochemical properties of NiO nanoparticles, particularly their high surface reactivity and ability to generate reactive oxygen species

(ROS). Excessive ROS production can lead to oxidative stress, resulting in damage to cellular components such as proteins, lipids, and DNA. This oxidative stress can impair mitochondrial function, thereby reducing the metabolic activity of HDF cells and ultimately leading to decreased cell viability in the MTT assay.

TABLE 1. Percentage cell viability of HDF cells treated with different concentrations of NiO nanoparticles after 24 h exposure, determined using the MTT assay, indicating the maximum safer concentration

Cell viability data - NiO nanoparticles vs HDF		
Culture condition	% cell viability	Max safer conc. (ug/ml)
Untreated	100.00	100ug
Toxin - 1ug	65.01	
NiO - 6.25ug	90.36	
NiO - 12.5ug	85.81	
NiO - 25ug	79.97	
NiO - 50ug	65.53	
NiO - 100ug	61.61	

Despite the observed cytotoxic effects at higher concentrations, the nanoparticles demonstrated acceptable cytocompatibility at lower concentrations, particularly at 6.25 μg/mL, which can be considered the maximum safer concentration based on the high cell viability observed. These findings suggest that NiO nanoparticles may be safely applied in environmental remediation processes such as water pollutant removal, provided that their concentration is carefully controlled to minimize potential biological risks. Overall, the results indicate that NiO nanoparticles exhibit dose-dependent cytotoxic behaviour, with lower concentrations showing minimal toxicity toward HDF cells. This highlights the importance of optimizing nanoparticle dosage to ensure safe and effective application in environmental and water purification technologies.



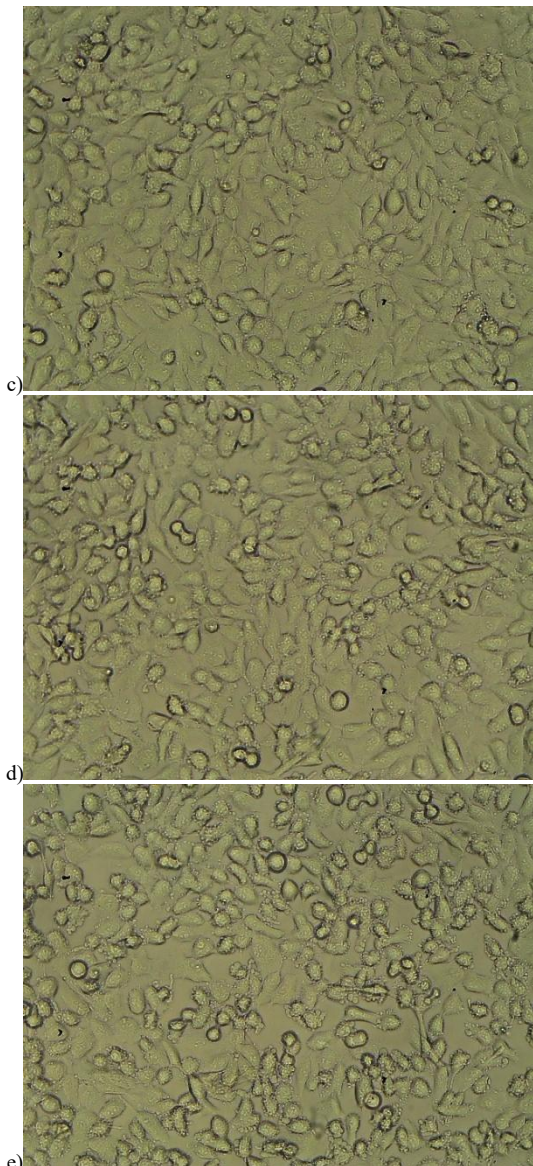


Fig. 5. Microscopic images showing the morphological appearance of Human Dermal Fibroblast (HDF) cells after 24 h exposure to different concentrations of cobalt oxide ( $\text{Co}_3\text{O}_4$ ) nanoparticles including control, a) 6.25, b) 12.5, c) 25, d) 0, and e) 100  $\mu\text{g}/\text{mL}$ , illustrating concentration-dependent changes in cell morphology and viability.

*Cytotoxicity Assessment of  $\text{Co}_3\text{O}_4$  Nanoparticles*

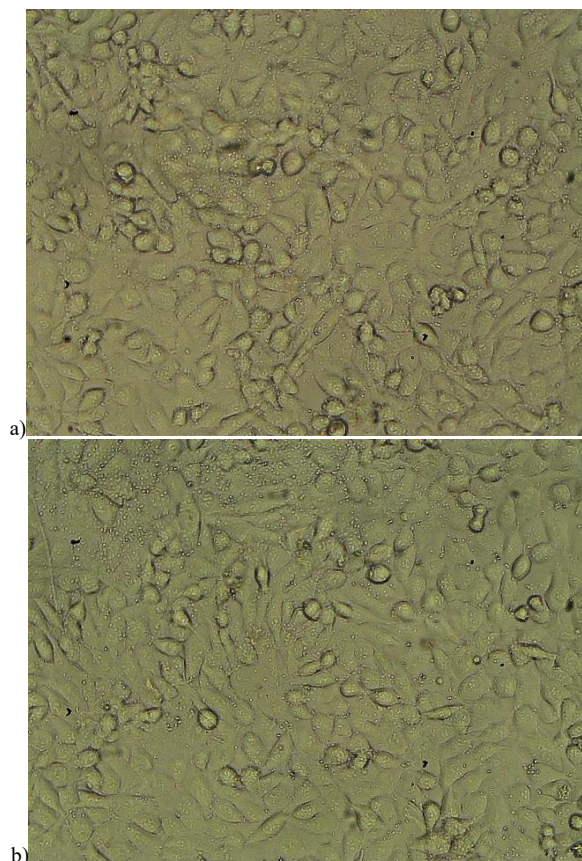
The cytotoxic potential of cobalt oxide nanoparticles  $\text{Co}_3\text{O}_4$  nanoparticles was evaluated using the MTT assay on Human Dermal Fibroblast (HDF) cells following 24 hours of exposure. The observed reduction in cell viability at higher nanoparticle concentrations may be attributed to nanoparticle-induced oxidative stress and the production of reactive oxygen species (ROS). Excessive ROS generation can disrupt mitochondrial function, damage cellular proteins and lipids, and ultimately impair cell metabolism. Additionally, cobalt oxide nanoparticles may interact with the cell membrane and intracellular components, leading to alterations in cellular homeostasis and reduced metabolic activity. Based on the obtained results, 6.25  $\mu\text{g}/\text{mL}$  was identified as the maximum safer concentration, as it maintained more than 90% cell

viability, indicating minimal cytotoxicity toward HDF cells. Concentrations above this level showed a progressive decline in cell viability, suggesting increased cellular stress and toxicity.

TABLE 2. Percentage cell viability of HDF cells treated with different concentrations of  $\text{Co}_3\text{O}_4$  nanoparticles after 24 h exposure, determined using the MTT assay, indicating the maximum safer concentration.

Cell viability data - $\text{Co}_3\text{O}_4$ nanoparticles vs HDF		
Culture condition	% cell viability	Max safer conc. ( $\mu\text{g}/\text{ml}$ )
Untreated	100.00	100ug
Toxin - 1ug	65.01	
$\text{Co}_3\text{O}_4$ - 6.25ug	92.18	
$\text{Co}_3\text{O}_4$ - 12.5ug	85.72	
$\text{Co}_3\text{O}_4$ - 25ug	79.81	
$\text{Co}_3\text{O}_4$ - 50ug	73.66	
$\text{Co}_3\text{O}_4$ - 100ug	63.28	

The cytotoxicity evaluation using HDF cells serves as a preliminary biosafety assessment to confirm that the synthesized nanoparticles do not cause severe damage to normal human cells. In the present study, the cytotoxicity assessment using HDF cells demonstrated that the synthesized cobalt oxide nanoparticles maintained high cell viability at lower concentrations, indicating minimal adverse effects on normal human cells. These findings indicate that the synthesized cobalt oxide nanoparticles exhibit acceptable biocompatibility at lower concentrations, supporting their potential application in water pollutant removal and purification technologies while maintaining minimal risk to human health when applied under controlled conditions.



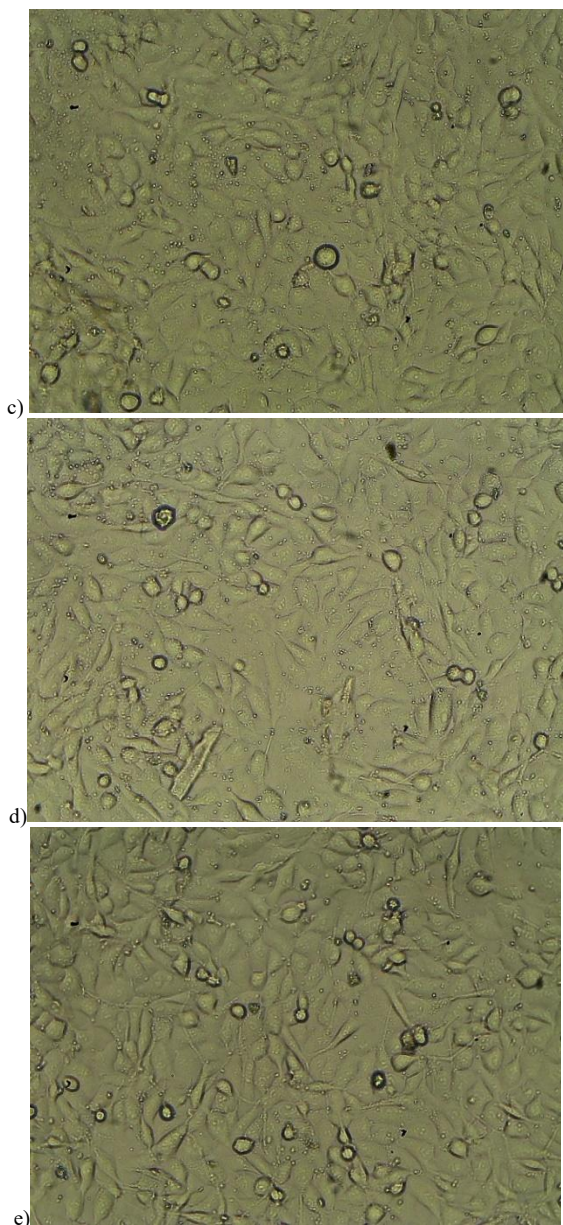


Fig. 6. Microscopic images showing the morphological appearance of Human Dermal Fibroblast (HDF) cells after 24 h exposure to different concentrations of cobalt oxide ( $\text{Co}_3\text{O}_4$ ) nanoparticles including control, a) 6.25, b) 12.5, c) 25, d) 0, and e) 100  $\mu\text{g}/\text{mL}$ , illustrating concentration-dependent changes in cell morphology and viability.

#### Comparative Cytotoxicity Analysis of NiO and $\text{Co}_3\text{O}_4$ Nanoparticles

A comparative evaluation of the cytotoxic effects of nickel oxide (NiO) and cobalt oxide ( $\text{Co}_3\text{O}_4$ ) nanoparticles on Human Dermal Fibroblast (HDF) cells revealed a concentration-dependent reduction in cell viability for both nanomaterials. In both cases, untreated control cells maintained 100% viability, while the toxin-treated positive control showed significantly reduced viability (65.01%), confirming the sensitivity of the MTT assay. At lower concentrations, both nanoparticles exhibited relatively high cytocompatibility toward HDF cells. For instance,  $\text{Co}_3\text{O}_4$  nanoparticles demonstrated 92.18% cell viability at 6.25  $\mu\text{g}/\text{mL}$ , whereas NiO nanoparticles showed 90.36% viability at the same concentration. This indicates that

both nanomaterials exert minimal cytotoxic effects at low exposure levels.

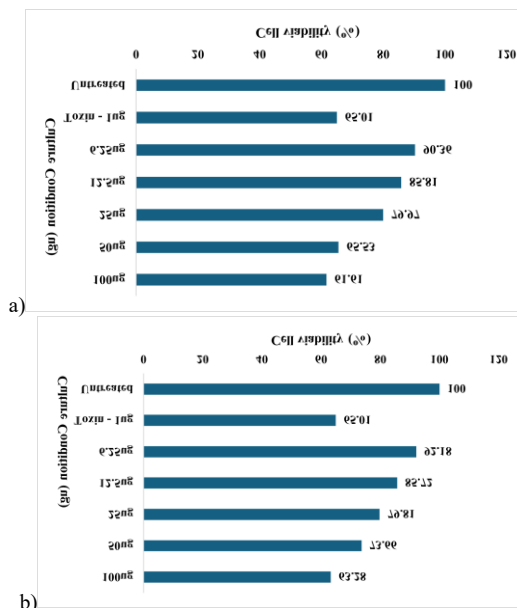


Fig. 7. Comparative dose-dependent cytotoxicity of a) NiO and b)  $\text{Co}_3\text{O}_4$  nanoparticles on Human Dermal Fibroblast (HDF) cells showing percentage cell viability after 24 h exposure determined by the MTT assay.

#### IV. CONCLUSION

The cytotoxic effects of nickel oxide (NiO) and cobalt oxide ( $\text{Co}_3\text{O}_4$ ) nanoparticles were evaluated using the MTT assay on Human Dermal Fibroblast (HDF) cells to assess their potential biological safety. The results demonstrated that both nanoparticles exhibited concentration-dependent cytotoxicity, with a gradual decrease in cell viability observed as the nanoparticle concentration increased. At lower concentrations, particularly 6.25  $\mu\text{g}/\text{mL}$ , both NiO and  $\text{Co}_3\text{O}_4$  nanoparticles showed high cell viability (>90%), indicating minimal cytotoxic effects and good cytocompatibility toward normal human skin cells. However, at higher concentrations (50–100  $\mu\text{g}/\text{mL}$ ), a noticeable reduction in cell viability was observed, suggesting increased nanoparticle–cell interactions and possible oxidative stress-mediated cellular damage. The inclusion of a toxin-treated positive control further confirmed the sensitivity and reliability of the MTT assay in detecting cytotoxic responses. The observed reduction in cell viability at elevated nanoparticle concentrations may be attributed to the surface reactivity of metal oxide nanoparticles and their ability to generate reactive oxygen species (ROS), which can induce oxidative stress and disrupt cellular metabolic activity. Overall, the findings indicate that NiO and  $\text{Co}_3\text{O}_4$  nanoparticles exhibit acceptable cytocompatibility at lower concentrations, highlighting their potential suitability for environmental applications such as water pollutant removal and purification processes.

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