İSTANBUL UNIVERSITY PRESS

RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ
Journal of Advanced Research in Health Sciences /
Sağlık Bilimlerinde İleri Araştırmalar Dergisi 2024
DOI: 10.26650/JARHS2024-1405790

CYTOTOXIC AND GENOTOXIC EFFECTS OF NICKEL OXIDE NANOPARTICLES ON HeLa CELLS

NİKEL OKSİT NANOPARTİKÜLLERİNİN HeLa HÜCRELERİNDE SİTOTOKSİK VE GENOTOKSİK ETKİSİ

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Citation/Atıf: Patan O, Zengin ÖS, Abudayyak M. Cytotoxic and genotoxic effects of nickel oxide nanoparticles on HeLa cells. Journal of Advanced Research in Health Sciences 2024;7(3):220-226. https://doi.org/10.26650/JARHS2024-1405790

ABSTRACT

Objective: Nickel oxide nanoparticles (NiO-NPs) are used invarious applications, and their increasing use has stimulated extensive studies both *in vivo* and *in vitro*. Nevertheless, their activity in cancer cells and thus the possibility of developing new anticancer drugs still remain controversial. Therefore, this study was conducted to specifically investigate the cytotoxicity and genotoxicity of NiO-NPs in human cervical carcinoma (HeLa) cells.

Material and Methods: The cytotoxicity of NiO-NPs was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), lactate dehydrogenase (LDH), and neutral red uptake (NRU) cytotoxicity assays. A comet assay was performed to determine the genotoxicity of NiO-NPs.

Results: The half maximal inhibitory concentration (IC_{50}) values were 419.6, 316.4, and 119.3 µg/mL in the MTT, NRU, and LDH assays, respectively. The comet assay revealed that NiO-NPs caused significant induction of DNA damage in the exposed HeLa cells. The tail intensity was 18.20% at 120 µg/mL.

Conclusion: NiO-NPs were cytotoxic and genotoxic to HeLa cells. Although NiO-NPs may be hazardous for a normal cell line, the effects observed on HeLa cells indicate that NiO-NPs can be proposed as a novel anticancer agent. However, the potential for NiO-NPs in cancer treatment will require additional and comprehensive studies on other cancer cell lines.

Keywords: HeLa cells, cytotoxicity, genotoxicity, NiO-NPs

ÖZ

Amaç: Nikel oksit nanopartikülleri (NiO-NP) endüstride geniş bir uygulama alanına sahiptir. Artan kullanım alanları ve miktarları ile birlikte kapsamlı araştırmalar ile araştırmacıların da ilgi odağı olmaktadır. Ancak, kanser hücrelerindeki etkinlikleri ve dolayısıyla yeni kanser ilaçları geliştirme olasılığı hala tartışmalıdır. Çalışmamızda NiO-NP'lerin insan servikal karsinom (HeLa) hücreleri üzerine sitotoksik ve genotoksik etki potansiyeli incelenmiştir.

Gereç ve Yöntem: NiO-NP'lerin sitotoksisitesi 3-(4,5-dimetiltiyazol-2-il)-2,5-difeniltetrazolyum bromür (MTT), laktat dehidrojenaz (LDH) ve nötral kırmızı alımı (NRU) testleri ile değerlendirilirken genotoksisitesi Comet testi ile araştırılmıştır.

Bulgular: Yarı maksimal inhibitör konsantrasyon (IC_{50}) değerleri MTT testi için 419,6 µg/mL, NRU testi için 316,4 µg/mL ve LDH testi için 119,3 µg/mL olarak bulunmuştur. Comet testi verilerine göre, NiO-NP'lerine maruz bırakılan hücrelerde DNA hasarı önemli ölçüde indüklenmiştir. Kuyruk yoğunluğunun 120 µg/mL maruziyet için %18,20 düzeylerinde olduğu tespit edilmiştir.

Sonuç: NiO-NP'ler HeLa hücrelerinde sitotoksik ve genotoksik etkili olduğu tespit edilmiştir. Normal bir hücre hattı için tehlikeli gibi görünse de kanser hücresi üzerine bu etkiler yeni bir antikanser ajan olarak NiO-NP'lerin umut verici olabileceğini göstermektedir. Ancak, NiO-NP'lerin kanser tedavisinde bir alternatif olma potansiyelini değerlendirmek üzere diğer kanser hücre hatlarında ve ileri çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: HeLa hücreleri, Sitotoksisite, Genotoksisite, Nikel oksit nanopartikülü

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Submitted/Başvuru: 16.12.2023 • **Revision Requested/Revizyon Talebi:** 07.01.2024 • **Last Revision Received/Son Revizyon:** 18.02.2024 • **Accepted/Kabul:** 27.02.2024 • **Published Online/Online Yayın:** 22.10.2024

INTRODUCTION

Nickel oxide nanoparticles (NiO-NPs) are desired in various fields because of their advantageous chemical and physical properties, including large surface area, high surface energy, magnetic properties, and low-melting point. NiO-NPs are widely used in battery construction, alloys, pigmentation, and biomedical materials. Nevertheless, the particle size, which is an advantage in industrial applications, increases the risk of toxicity to biological systems; nanoparticles, due to their extremely small size, can be more easily transported to cells and tissues and accumulate in target organs (1, 2). Because of their widespread use, it has been questioned whether they pose a threat to humans, and there is also some research addressing this issue (2). Conversely, the super small size and easy passage through cell membranes, and the fact that NiO-NPs have been confirmed to exert a cytotoxic effect on some cancer cells, make it possible to use these NPs to kill cancer cells as a type of chemotherapy and suggest their use as anticancer agents. Hence, several studies have investigated the cytotoxic effect of different NPs in general and NiO-NPs in particular in different cell lines (3, 4). Nickel-based NPs, which are widely used in industries, pose a high risk for airborne dermal and respiratory toxicity to workers. In this regard, studies primary evaluate their effects after inhalation exposure. *In vivo* studies have explored their effects on the cardiovascular system, lungs, and other organs, whereas *in vitro* studies have generally focused on cellular mechanisms. The results of different studies indicate that NiO-NPs generally caused cytotoxicity, increase in reactive oxygen species (ROS) levels, apoptosis, and genotoxicity (1, 2).

In the present study, it was hypothesized that NiO-NPs could exert an anticancer effect, because they could exert cytotoxic effects on human cervical cancer cells. It was also hypothesized that DNA damage could be a mechanism of this effect. A well-known human cancer cell line, HeLa, was used in this study. HeLa cells are derived from the epithelial cells of human cervical cancer and are the first immortal human cells to be grown in culture. These cells possess an extraordinary capacity for continuous proliferation, rendering them immortal in laboratory cultures. Moreover, HeLa cells exhibit a high growth rate, dividing rapidly and facilitating the generation of large cell populations for experimentation. Hence, they are widely used in different research areas (5). Various cytotoxicity assays were then used in this study to investigate the affected cellular organelle and, in addition, to test whether DNA damage was a cytotoxicity mechanism. The NiO-NP-exposed cells were also evaluated using the comet assay.

MATERIAL AND METHODS

Particle size characterization

NiO-NPs (Sigma-Aldrich, MO, USA) were suspended in double-distilled water or complete cell culture medium. Then, they were subjected to sonication for 15 min before examination under a transmission electron microscope (TEM) (JEM-2100 HR, JEOL, USA). The image of more than 100 particles in arbitrary TEM fields was captured and analyzed by measuring their diameters. Dynamic light scattering (DLS) was also performed to determine the size of NPs using a ZetaSizer Nano-ZS instrument (Malvern, UK). For DLS analysis, the NiO-NPs (1 mg) were dissolved in cell culture medium and sonicated at 40 W for 15 min at room temperature, then, a 10 µg/mL freshly prepared suspension was used in the measurement.

Cell culture procedure and exposure conditions

HeLa cells, a human cervical carcinoma cell line (CCL-2, ATCC, USA), were cultured in Eagle's minimum essential medium supplemented with penicillin–streptomycin (1%) and fetal bovine serum (FBS, 10%). Subculturing was conducted when the cells reached 50% confluence.

For exposure, the NP suspension was freshly prepared before each experiment. For this, the NiO-NPs were dispersed in the complete cell culture medium and sonicated for 15 min at room temperature, from which different concentrations were prepared. The exposure concentrations were 0–1000 µg/mL for the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), lactate dehydrogenase (LDH), and neutral red uptake (NRU) cytotoxicity assays and 15–120 µg/mL for the comet assay. The exposure time was set at 24 h. For the cytotoxicity assays, the percentage of cell viability was determined in comparison with the untreated cell group (negative control, NC).

Cytotoxicity

For the MTT assay, the MTT dye (5 mg/mL) was added to each well. After 3 h of incubation, the formazan crystals formed were dissolved using dimethyl sulfoxide (DMSO, 100 µL) per well. The optical density (OD) at 590 nm was measured using a microplate reader (Epoch, Germany) (6). For the NRU assay, the cells were treated with a neutral red solution (10 mg/mL NR dye in the cell culture medium) for 3 h. After incubation, the cells were washed with phosphate-buffered saline (PBS, 1×), and a dissolution reagent (100 µL/well) was added. OD was measured at 540 nm using the same microplate reader (7). For the LDH assay, the microplate was centrifuged at 250 *g* for 10 min. The supernatant was carefully transferred to new plates. LDH assay kit solutions were added and incubated according to the kit instructions, a stop solution was added, and the OD was measured at 490 nm using the same microplate reader (8).

Genotoxicity

Cells were cultured in 6-well plates (5×10^5 cells/well), incubated for 12 h, and then treated with NiO-NPs for 24 h. The cells were collected by trypsinization, centrifuged, washed, and suspended in PBS (1 mL). The cell suspension was mixed with prewarmed low-melting agarose and then applied to microscope slides previously coated with normal-melting agarose. After solidification at 4°C, the slides were incubated in a lysis solution at 4°C for at least 2 h. Then, the slides were transferred to fresh, cold electrophoresis buffer at 4°C for 20 min to dissolve the DNA strands. Electrophoresis was performed at 4°C for 20 min.

After electrophoresis, the slides were washed, neutralized, and then fixed in ethanol. DNA staining was performed using ethidium bromide, and the slides were examined under a fluorescence microscope (Olympus BX53, Tokyo, Japan) at 400X. The comet assay IV image analysis software (Perceptive Instruments, Suffolk, UK) was used to visualize and analyze the results. A minimum of 100 cells were scored for each sample, and the

amount of DNA damage in each cell was expressed as the tail intensity. The NC groups consisted of cells not treated with any chemical, whereas cells exposed to hydrogen peroxide (H_2O_2, 100 µM) were identified as the positive control (PC) group (9).

Statistical analysis

All assays were performed three times independently, each time in triplicate (n=9). One-way analysis of variance or Dunnett's test was used to examine the results, which were expressed as mean ± standard error. Statistical analyses were conducted using the SPSS software for Windows version 22.0, (IBM SPSS Corp., Armonk, NY, USA), and a *p* value cut-off of 0.05 indicated statistical significance.

RESULTS

According to the manufacturer, the nanoparticles are cubic, the mean particle size is ≤50 nm, and they are highly pure (99.8%). Based on TEM data, the particle size of NiO-NPs in water ranged from 4.2 to 38.1 nm, with a mean size of 15.0 nm. When the particles were dispersed in the cell culture medium, their average diameter increased to 21.4 nm, which could be due to agglomerated and aggregated NPs or adsorbed proteins from the medium. NiO-NPs have a size range of 7.70–194.1 nm and an average hydrodynamic size of 135.81 nm in the cell culture medium. The results of DLS revealed that 33% of NiO-NPs were smaller than 24.8 nm (Figure 1).

Cytotoxicity evaluation revealed that NiO-NPs caused cell death in a concentration-dependent manner. The half maximal inhibitory concentration (IC_{50}) values were 419.6, 316.4, and 119.3 µg/mL in the MTT, NRU, and LDH assays, respectively. Figure 2 depicts the relationship concentration and cell death (%).

The results of the comet assay revealed an accumulation of DNA damage according to the concentration of NiO-NPs, where the tail intensity was 3.78% (1.23-fold of NC) and 18.20% (5.92-fold of NC) at the lowest and highest concentrations, res-

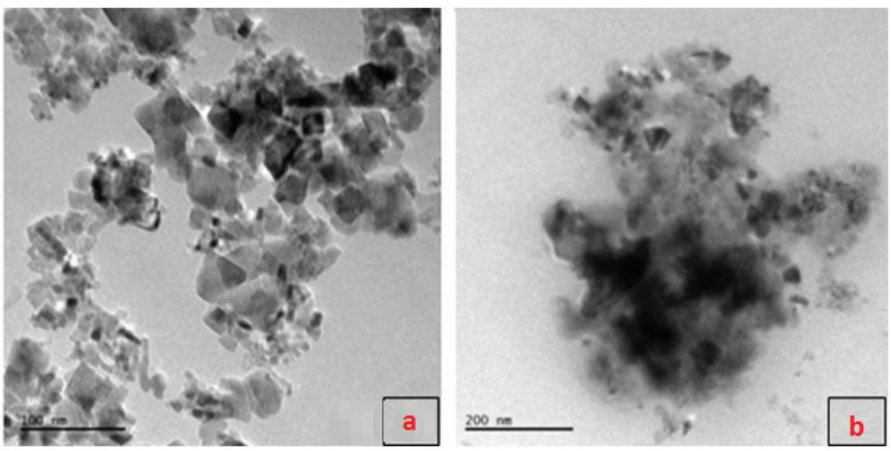

Figure 1: TEM images of NiO-NPs in water (a) and cell culture medium (b) (22)

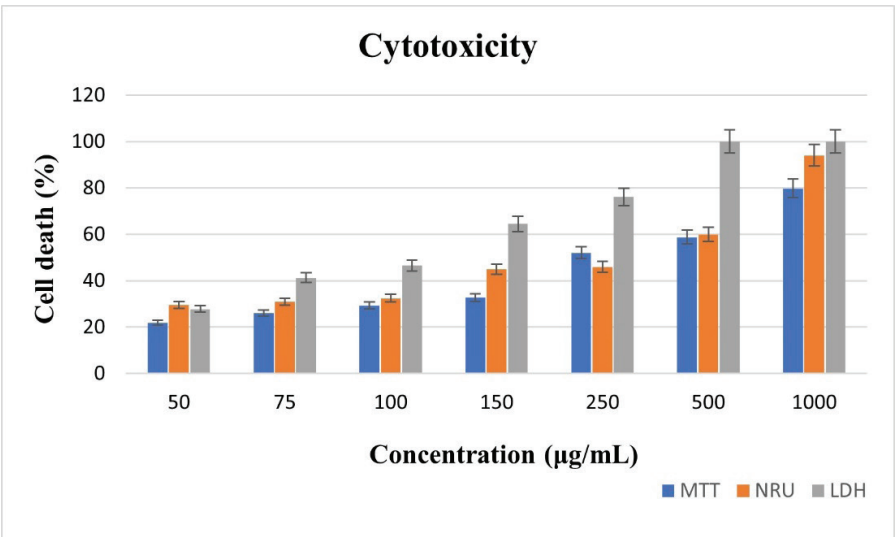

Figure 2: Cytotoxic potential of nickel oxide nanoparticles (NiO-NPs) evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), neutral red uptake (NRU), and lactate dehydrogenase (LDH) assays. Cells were exposed to NiO-NPs at different concentrations for 24 h.

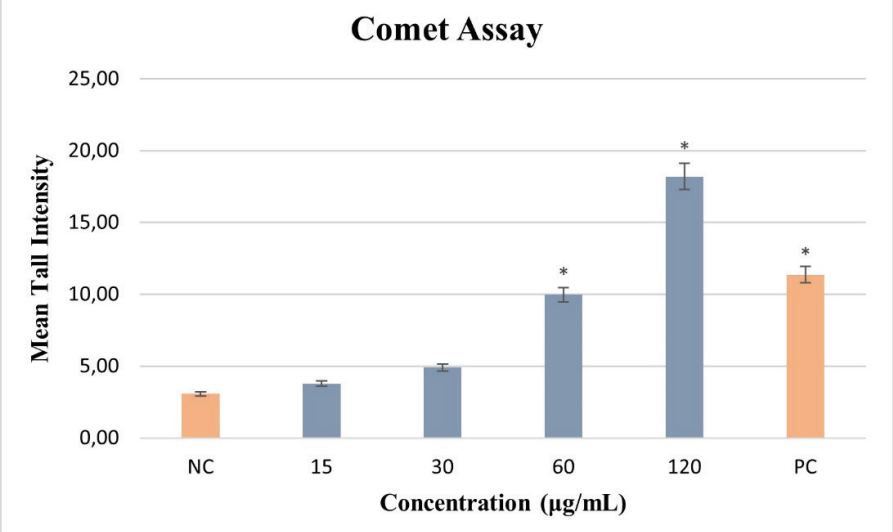

Figure 3: Genotoxic potential of nickel oxide nanoparticles (NiO-NPs) in HeLa cells evaluated using the comet assay. Cells were exposed to NiO-NPs at 15–120 µg/mL concentrations for 24 h. Unexposed cells and cells exposed to hydrogen peroxide (H_2O_2, 100 µM) served as negative control (NC) and positive control (PC), respectively

pectively. In the positive control, the tail intensity was 11.36% (3.7-fold of NC) (Figure 3). This increase in tail intensity was statistically significant ($p \leq 0.05$) for the 60 and 120 µg/mL groups but not for the 15 and 30 µg/mL groups.

DISCUSSION

NiO-NPs are one of the most widely utilized nanoparticles in various medical and commercial fields. In addition to their physicochemical properties, the possibility of their application as drugs has been evaluated by different research groups (1, 2). In particular, some studies have demonstrated that NiO-NPs cause cytotoxicity in several cancer cells, and other studies have explained their molecular and cellular effects such as ROS induction, lipid peroxidation, oxidative stress, apoptosis, necrosis, and DNA and chromosomal damage (Table 1). Based on these effects on cancer cells, we explored whether NiO-NPs might be a material that could be used in cancer treatment. For this purpose, we evaluated cytotoxicity and DNA damage induction in the well-known human cervical cancer cell line (HeLa) after 24 h of exposure.

The effects of NiO-NPs were evaluated in both normal and cancerous cells; previous studies have demonstrated low or no toxicity of NiO-NPs on normal cells. Khan et al. reported no cytotoxicity of NiO-NPs on normal splenic cells at concentrations up to 1000 µg/mL (10). Similarly, Abbaszadeh et al. reported low cytotoxicity for modified NiO-NPs in Hek293 normal cells, where the IC_{50} was 475 µg/mL (11). In human normal keratinocytes (HaCaT cells), the cytotoxic effects were also lower than those in the tested cancerous cells, with the IC_{50} being 312.5 µg/mL in the MTT test (12). No significant cytotoxicity was reported for NiO and other Ni-based salt NPs in human normal umbilical vein endothelial (HUVEC) cells at concentrations up to 1000 µg/mL (13). In contrast, NiO-NPs resulted in significant cell death in different cancerous cells, causing cytotoxicity in the leukaemia monocyte cell line THP-1 after 24 h of treatment, with the IC_{50} being 23.31 µg/mL (14). According to the studies of Horie et al., Ahamed et al., and Capasso et al., NiO-NPs are cytotoxic to A549 lung cancer cells (15-17). A549 cells were exposed to NiO-NPs at concentrations of 0–25 µg/mL for 24 and 48 h, which resulted in a gradual decrease in cell viability with increasing exposure concentrations and/or duration as evaluated using MTT and LDH assays. The oxidative stress caused by NiO-NPs was demonstrated by Horie et al. and Ahamed et al. Moreover, Horie et al. examined ROS production and lipid peroxidation in their study, and Ahamed et al. examined apoptosis as a cell death pathway in their study (16, 17).

Ahamed et al. and Ahmad et al. conducted studies demonstrating the cytotoxic and apoptotic effects of NiO-NPs on the HepG2 liver cancer cell line (18, 19). Ahamed et al. showed that exposure of HepG2 cells to NiO-NPs at concentrations of 0–200 µg/mL for 24 h resulted in cell viability of only 25% at 200 µg/mL concentration. Ahmad et al. exposed the cells to NiO-NPs (0–100 µg/mL) for the same duration and conducted MTT and NRU assays, which revealed a reduction in cell viability, decreasing to 50% at 100 µg/mL, in a concentration-dependent manner. Both studies concluded that NiO-NPs induced oxidative stress in a dose-dependent manner in addition to their observed cytotoxic effects (18, 19).

Siddiqui et al. conducted a study using Hep-2 cells containing HeLa marker chromosomes and observed that NiO-NPs at concentrations of 0–100 µg/mL induced dose-dependent cytotoxicity and apoptosis during 24-h exposure. Similar results were found in MCF-7 breast cancer cells in the same study. In the MTT assay, the viability of Hep-2 cells was 11% and that of MCF-7 cells was 9% at 100 µg/mL of NiO-NPs (4).

NiO-NP treatment for 24 h at different concentrations (0–640 µg/mL) induced cytotoxicity and apoptosis in HT-29 and SW620 colon cancer cell lines, with the IC_{50} values being 13.72 and

394.41 g/mL, respectively (3).

NiO-NPs have been demonstrated to cause cytotoxicity, apoptosis, DNA damage, and oxidative stress in SH-SY5Y neuroblastoma cell lines at concentrations of 50–500 µg/mL for 24 h. The IC_{50} values were 249.92 and 229.34 µg/mL in the MTT and NRU assays, respectively (20). In another study, treating Caco-2 colorectal adenocarcinoma cells with NiO-NPs (50–500 µg/mL) for 24 h resulted in apoptosis and cytotoxicity, with the IC_{50} values being 351.6 and 479.15 µg/mL in the MTT and NRU assays, respectively. At 30–150 µg/mL, NiO-NPs caused DNA and oxidative damage (21). In another study, Annexin V-FITC/PI apoptosis detection was used to determine whether NRK-52E kidney epithelial cells suffered necrosis or apoptosis. The results showed that the primary mechanism of cell death in NiO-NP-treated NRK-52E cells was apoptosis, wherein approximately 62.5% of the overall cell count at the maximum dose (300 g/mL) consisted of apoptotic cells and 14.4% of the overall cell count consisted of necrotic cells (22).

In the study by Ada et al., NiO-NPs were synthesized in an aqueous solution using a homogeneous precipitation method. Laboratory-synthesized NiO-NPs were evaluated for their cytotoxic, apoptotic, and necrotic effects on HeLa cells. For cytotoxicity, HeLa cells were exposed to NiO-NPs at 50–500 µl/mL concentrations for 2, 6, 12, and 16 h. Results showed that cytotoxicity increased with concentration and exposure time, and necrosis appeared to be the major cell death pathway at all concentrations for all exposure times (23).

Because the properties of NPs exert a significant influence on their biological effects, and the particle size, size distribution, chemical residues for synthesis process, and surface chemistry of NPs can impact the biological potential (1, 2), we believe that reference NiO-NPs should be evaluated in HeLa cells, so that the results could be compared with those of previous studies that used the same particles. The results of the present study indicated that NiO-NPs triggered cytotoxic effects in HeLa cells with IC_{50} values of 419.6, 316.4, and 119.3 µg/mL in the MTT, NRU, and LDH assays, respectively. Remarkably, although previous studies demonstrated a higher toxicity of antineoplastic agents that are already in use in HeLa cells compared with our study results related to NiO-NPs, some of those studies reported IC_{50} values not far from those of NiO-NPs or even lower than the IC_{50} of NiO-NPs. A previous study reported that 5-fluorouracil (5-FU) at 200 µM (26.016 µg/mL) decreased cell viability to 70% after 24 h of exposure (24), whereas another study reported that 7.8125 µg/mL of 5-FU did not induce any

Table 1: Previous studies that investigated the effects of NiO-NPs on cancer cells

Cell line	NiO-NP-induced toxicity	IC_{50} values by MTT	Reference
A549	ROS production, lipid peroxidation, oxidative stress	Not available	11
A549*	Cytotoxicity	38% cell viability at 10 µg/mL (24 h)	13
Caco-2*	Cytotoxicity, apoptosis, DNA damage	351.6 µg/mL (24 h)	17
HeLa	Cytotoxicity, apoptosis, necrosis	41% cell viability at 350 µg/mL (16 h)	19
HEPG2	Cytotoxicity, ROS production, apoptosis, oxidative stress	41% cell viability at 100 µg/mL (24 h)	14
HEp-2*	Cytotoxicity, apoptosis	43% cell viability at 25 µg/mL (24 h)	4
HT-29	Cytotoxicity, apoptosis	13.72 µg/mL (24 h)	3
MCF-7*	Cytotoxicity, apoptosis	42% cell viability at 10 µg/mL (24 h)	16
SH-SY5Y*	Cytotoxicity, apoptosis, DNA damage, oxidative stress	≥229.34 µg/mL (24 h)	16
SW620	Cytotoxicity	394.41 µg/mL (24 h)	3
THP-1	Cytotoxicity	23.31 µg/mL (24 h)	10

* The same nickel oxide nanoparticles (Sigma-Aldrich, Product No.: 637130) were used in this study. DNA: deoxyribonucleic acid, NiO-NPs: nickel oxide nanoparticles, IC_{50}: half maximal inhibitory concentration, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, ROS: reactive oxygen species.

cytotoxicity to the cells, with the IC_{50} being approximately 125 µg/mL (25). Cisplatin treatment to the same cells caused cytotoxicity, with the IC_{50} being 34.2 µM (26). However, in another study, the IC_{50} of cisplatin was 0.5–1 µM (27).

The comet assay revealed a significant induction of DNA damage at 60 and 120 µg/mL of NiO-NPs, suggesting that DNA damage could be a mechanism by which NiO-NPs induced cell death in HeLa cells. Compared with studies that used the same NiO-NPs and assays but different cell lines, HeLa cells may be more resistant than some cell lines (Table 1).

In conclusion, different studies have reported different results on the toxicity of NiO-NPs to cancer cells. These inconsistencies can be attributed to differences in the cell lines used; the type, shape, and size of NPs; and the synthesis method of NPs. Our results showed that NiO-NPs caused cell death at moderate concentrations (IC_{50} 119.3–419.6 µg/mL). The observed increase in DNA damage in exposed cells suggests that DNA damage contributes to the death of HeLa cells after exposure to NiO-NPs. These data further support the notion that Ni-based NPs, particularly NiO-NPs, have the potential for targeted cancer treatment by eliminating cancerous tissue. Nevertheless, further *in vitro* and *in vivo* research is required to gain a better understanding of the underlying mechanism of action of NiO-NPs. Furthermore, such studies are essential to determine the safety profile and feasibility of NPs for use in cancer therapy.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- O.P., Ö.S.Z.; Data Acquisition- O.P., Ö.S.Z.; Data Analysis/Interpretation- O.P., Ö.S.Z., M.A.; Drafting Manuscript- O.P., Ö.S.Z.; Critical Revision of Manuscript- O.P., Ö.S.Z., M.A.; Final Approval and Accountability- M.A.; Material and Technical Support- M.A.; Supervision- M.A.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

REFERENCES

1. More SL, Kovochich M, Lyons-Darden T, Taylor M, Schulte AM, Madl AK. Review and evaluation of the potential health effects of oxidic nickel nanoparticles. Nanomaterials 2021;11(3):642.
2. Magaye R, Zhao J. Recent progress in studies of metallic nickel and nickel-based nanoparticles' genotoxicity and carcinogenicity. Environ Toxicol Pharmacol 2012;34(3):644-50.
3. Khan S, Ansari AA, Malik A, Chaudhary AA, Syed JB, Khan AA. Preparation, characterizations, and in vitro cytotoxic activity of nickel oxide nanoparticles on HT-29 and SW620 colon cancer cell lines. J Trace Elem Med Biol 2019;52(1):12-7.
4. Siddiqui MA, Ahamed M, Ahmad J, Khan MM, Musarrat J, Al-Khedhairy AA, et al. Nickel oxide nanoparticles induce cytotoxicity, oxidative stress, and apoptosis in cultured human cells that are abrogated by the dietary antioxidant curcumin. Food Chem Toxicol 2012;50(3-4):641-7.
5. American Type Culture Collection (ATTC). HeLa CCL-2. https://www.atcc.org/products/ccl-2 2022
6. Van Meerloo J, Kaspers GJ, Cloos J. Cell sensitivity assays: the MTT assay. Methods Mol Biol 2011;731:237-45.
7. Repetto G, Del Peso A, Zurita, JL. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. Nature protocols 2008;3(7):1125-31.
8. Kumar P, Nagarajan A, Uchil PD. Analysis of cell viability by the lactate dehydrogenase assay. Cold Spring Harb Protoc 2018;6:465-8
9. Speit G, Hartmann A. The comet assay (Single-Cell Gel Test) a sensitive genotoxicity test for the detection of DNA damage and repair. Methods Mol Biol 1999;113:203-12.
10. Khan A, Shkir M, Ibrahim EH, Kilany M, AlFaify S, Sayed MA, Siddiquei MM. Effect of Bi contents on key physical properties of NiO NPs synthesized by flash combustion process and their cytotoxicity studies for biomedical applications. Ceram Int 2020;46(12):19691-700.
11. Abbaszadeh N, Jaahbin N, Pouraei A, Mehraban F, Hedayati M, Majlesi A, Salehzadeh A. Preparation of novel nickel oxide glutamic/thiosemicarbazide nanoparticles: Implications for cytotoxic and anti-cancer studies in MCF-7 breast cancer cells. J Clust Sci 2020;(10):1-9.
12. Rincon-Granados KL, Vázquez-Olmos AR, Rodríguez-Hernández AP, Prado-Prone G, Garibay-Febles V, et al. Bactericidal and Cytotoxic Study of Hybrid Films Based on NiO and NiFe2O4 Nanoparticles in Poly-3-Hydroxybutyrate. J Clust Sci 2023;35:167-78.
13. Zhang Y, Mahdavi B, Mohammadhosseini M, Rezaei-Seresht E, Paydarfard S, Qorbani M, et al. Green synthesis of NiO nanoparticles using Calendula officinalis extract: Chemical charactrization, antioxidant, cytotoxicity, and anti-esophageal carcinoma properties. Arab J Chem 2021;14(5):103-5.
14. Lanone S, Rogerieux F, Geys J, Dupont A, Maillot-Marechal E, Boczkowski J, Hoet P. Comparative toxicity of 24 manufactured nanoparticles in human alveolar epithelial and macrophage cell lines. Part Fibre Toxicol 2009;6(1):1-12.
15. Horie M, Fukui H, Nishio K, Endoh S, Kato H, Fujita K, Iwahashi H. Evaluation of acute oxidative stress induced by NiO nanoparticles *in vivo* and *in vitro*. J Occup Health 2011;53(2):64-74.
16. Ahamed M. Toxic response of nickel nanoparticles in human lung epithelial A549 cells. Toxicol In Vitro 2011;25(4):930-6.
17. Capasso L, Camatini M, Gualtieri M. Nickel oxide nanoparticles induce inflammation and genotoxic effect in lung epithelial cells. Toxicol Lett 2014;226(1):28-34.
18. Ahamed M, Ali D, Alhadlaq HA, Akhtar MJ. Nickel oxide nanoparticles exert cytotoxicity via oxidative stress and induce apoptotic response in human liver cells (HepG2). Chemosphere 2013;93(10):2514-22.
19. Ahmad J, Alhadlaq HA, Siddiqui MA, Saquib Q, Al-Khedhairy AA, Musarrat J, Ahamed M. Concentration-dependent induction of reactive oxygen species, cell cycle arrest and apoptosis in human liver cells after nickel nanoparticles exposure. Environ Toxicol 2015;30(2):137-48.
20. Abudayyak M, Guzel E, Özhan G. Nickel oxide nanoparticles

are highly toxic to SH-SY5Y neuronal cells. Neurochem Int 2017;108(1):7-14.

21. Abudayyak M, Güzel E, Özhan G. Cytotoxic, genotoxic, and apoptotic effects of nickel oxide nanoparticles in intestinal epithelial cells. Turk J Pharm Sci 2020;17(4):446.
22. Abudayyak M, Guzel E, Özhan G. Nickel oxide nanoparticles induce oxidative DNA damage and apoptosis in a kidney cell line (NRK-52E). Biol Trace Elem Res 2016;178:98-104.
23. Ada K, Turk M, Oguztuzun S, Kilic M, Demirel M, Tandogan N, Latif O. Cytotoxicity and apoptotic effects of nickel oxide nanoparticles in cultured HeLa cells. Folia Histochem Cytobiol 2010;48(4):524-9.
24. Mavrikou S, Tsekouras V, Karageorgou MA, Moschopoulou G, Kintzios S. Detection of superoxide alterations induced by 5-fluorouracil on HeLa cells with a cell-based biosensor. Biosensors 2019;9(4):126.
25. Arabnezhad S, Mani H, Gordi P, Ahmadi R. The Cytotoxic Effects of 5FU on Hek293 and HeLa Cells in vitro. Int J BioLife Sci 2022;1(3S)128-33.
26. Jurado R, Lopez-Flores A, Alvarez A, García-López P. Cisplatin cytotoxicity is increased by mifepristone in cervical carcinoma: an in vitro and in vivo study. Oncol rep 2009;22(5):1237-45.
27. Fathy M, Fawzy MA, Hintzsche H, Nikaido T, Dandekar T, Othman EM. Eugenol exerts apoptotic effect and modulates the sensitivity of HeLa cells to cisplatin and radiation. Molecules 2019;24(21):3979.