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Intestinal Epithelial Cell Toxicity Induced By Nickel Oxide Nanoparticles
Nikel Oksit Nanopartikülleri Tarafından İndüklenen İntestinal Epitelyal Toksisite

Nickel oxide nanoparticles induced intestinal toxicity

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ABSTRACT

INTRODUCTION: The superior properties of nickel oxide nanoparticles (NiO-NPs) lead to wide uses in various fields; however, there has been little comprehensive knowledge about their toxicity, especially oral exposure. Because there was no study on the intestinal toxicity of NiO-NPs, the toxicity of The NiO-NPs (average size 15.0 nm) was investigated in Caco-2 (human intestinal epithelial) cells.

METHODS: Following identification of their particle size distribution and cellular uptake potential, the risk of NiO-NPs' exposure have been evaluated with the crucial toxicity features including the cellular morphologic changes, the cyto- and genotoxic potentials, oxidative damage and apoptotic induction.

RESULTS: According to our results, NiO-NPs caused 50% decrease in cell viability at 351.6 µg/mL, and induced DNA damage and oxidative damage at 30-150 µg/mL. It was observed that apoptosis could be the main cell death pathway at in intestinal cells exposed to NiO-NPs.

DISCUSSION AND CONCLUSION: The exposure to NiO-NPs could be hazardous to the gastrointestinal system, the results should rise the concerns about using NiO- NPs in food-contacts appliance and NiO-NPs containing wastes. Further in vivo and in vitro research should be conducted to explain the specific mechanism of these particles and reduce their risk to the human.

Keywords: Nickel oxide nanoparticles; Intestinal cells; Genotoxicity; Oxidative stress; Apoptosis

Özet

GİRİŞ ve AMAÇ: Nikel oksit nanopartiküller (NiO-NP) eşsiz özellikleri nedeniyle farklı alanlarda geniş kullanıma sahiptir. Ancak, NiO-NP'lerinin, özellikle oral maruziyet durumunda, toksisiteleri hakkında bilgi yetersizdir. Bu sebeple NiO-NP'lerin intestinal toksisitesini aydınlatmak üzere bu çalışma tasalanmıştır.

GEREÇ VE YÖNTEM: Caco-2 (insan intestinal epitelyal) hücrelerinde NiO-NP'lerinin (ortalama büyüklük 15,0 nm) partikül büyüklüğü, dağılımı ve hücresel alım potansiyeli belirlendikten sonra, NiO-NP'lerin maruz kalan hücrelerde sebep olduğu morfolojik değişiklikler, sito- ve genotoksik potansiyelleri, oksidatif hasar ve apoptoz indüksiyonu gibi kritik toksisite profilleri değerlendirildi.

BULGULAR: NiO-NP'ler 351,6 µg/mL'de hücre canlılığında %50 azalma, 30-150 µg/mL'de DNA ve oksidatif hasar indüksiyonu gözlendi. Apoptozun NiO-NP'lere maruz kalan intestinal hücrelerde ana hücre ölüm yolu olabileceği düşünülmektedir.

TARTIŞMA ve SONUÇ:NiO-NP'ler gastrointestinal sistem üzerine risk oluşturabilir. Araştırma sonuçlarına göre; gıdayla temas eden cihazların yapımında NiO-NP'lerin kullanımı ve NiO-NP'ler ile kontamine içme suyu ve gıda tüketimi durumunda potansiyel NiO-NP maruziyetinin kaygıyı oluşturacağı yönündedir. Ayrıca, NiO-NP'lerin spesifik toksik etki mekanizmalarını aydınlatmak ve insan sağlığı için muhtemel risklerini azaltmak için ayrıntılı in vivo ve in vitro araştırmaların oldukla gerekli olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Nikel oksit nanopartikülleri; İntestinal epitelyal hücreleri; Genotoksisite; Oksidatif stres; Apoptoz

INTRODUCTION

NiO-NPs have been led to be widely used as catalyst, pigment and sensor in different medical and industrial application because of their physicochemical features (Capasso et al., 2014; Martin et al., 2017; Morimoto et al., 2011). NiO and other Ni compound have been classified as carcinogenic to humans (Group 1) (IARC, 1990). Additionally, NiO-NPs are thought to be more toxic than that of fine particles because they have high solubility and release (Horie et al., 2011). Researches have mainly focused on their pulmonary toxicity, and reported that the particles induced oxidative stress and inflammatory responses in airway system (Capasso et al., 2014; Chang et al., 2017; 2017B; 2018; Horie et al., 2011; Li et al., 2018; Marzban et al., 2017; Morimoto et al., 2011; Senoh et al., 2017; Yu et al., 2017). The NiO-NPs were detected in the intestinal tissue after pulmonary exposure (Shinohara et al., 2017). Although it is known that NiO-NPs could be absorbed through the intestinal tract, harmful to the cells of mucosa, there are limited knowledge about the intestinal toxicity of NiO-NPs (Dumla et al., 2017; 2017B). On the other hand, the unabsorbed portion has been still considered a risk for the intestinal cells following oral exposure. In this study, the NiO-NPs' toxicity was assessed using various endpoints in Caco-2 human intestine cell line, a highly differentiated human cell line and extensively used to study the apical uptake, absorption of nutrients and chemicals as *in vitro* model for toxicological studies (Abudayyak et al., 2016; Kakinen et al., 2016; Martin et al., 1997). Several studies have also reported that the chemical permeability to Caco-2 cells could be correlated well with that of intestinal membrane *in vivo* (Yamashita et al., 2000).

MATERIALS AND METHODS

The NiO-NPs and the chemicals were purchased from Sigma (MO, USA). The nanoparticles also were characterised using TEM (Transmission Electron Microscopy) (JEM-2100 HR, JEOL, USA) and dynamic light scattering (DLS) (ZetaSizer Nano-ZS, Malvern Instruments, Malvern, UK) in distilled water and cell culture medium (Abudayyak et al., 2017a; 2017).

Caco-2 human intestinal epithelial cells (HTB-37) was purchased from American Type Culture Collection (ATCC) (MD, USA). The cell culture medium and all supplements were purchased from Multicell Wisent (Quebec, Canada). The cells were incubated according to the manufacturer' instruction. The cell densities were 10^5 - 10^6 cells/mL in exposure conditions, and the exposure time was 24 hrs.

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Thermo Elemental X series 2, USA) method and TEM (Jeol-1011, Tokyo, Japan) with an accelerating voltage of 80 kV and an attached digital camera (Olympus-Veleta TEM Camera, Tokyo, Japan) were used to evaluate the cellular uptake potentials of NiO-NPs, and both the cellular uptake and morphology changes at 50 and 100 µg/mL, respectively (Abudayyak et al., 2017a; 2017).

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) and neutral red uptake (NRU) assays were used to determined the cytotoxic activities of NiO-NPs in the range of 50-500 µg/mL (Repetto et al., 2008; Van Meerloo et al., 2011). The optical densities (ODs) were read using a microplate spectrophotometer system (Epoch, Germany) at 590 and 540 nm for MTT and NRU, respectively. The inhibition of enzyme activity observed in cells was calculated and compared to that of unexposed cells. The half maximal inhibitory concentration (IC_{50}) values were expressed as the sample concentration that caused an inhibition of 50% in enzyme activities in cells.

The genotoxic activities of NiO-NPs were determined in the range of 15-120 µg/mL by comet assay (Abudayyak et al. 2017; 2017a; Collins, 2004). Hydrogen peroxide (H_2O_2) (100 µM) was used as positive control. Fluorescent microscope (Olympus BX53, Olympus, Tokyo, Japan) at 400 magnification using an automated image analysis system was used to score the number of DNA breaks. DNA damage of individual cells was expressed as a percentage of DNA in the comet tail (tail intensity %).

GSH, MDA, 8-OHdG and PC ELISA kits for oxidative stress and dye reagent for protein assay were obtained from Yehua Biological Technology (Shanghai, China) and Bio-rad (Munich, Germany), respectively (Abudayyak et al. 2016; 2017). The cells were exposed to 50-150 µg/mL. The results were expressed for g of protein using a standard calibration curve

Annexin V-FITC/PI apoptosis-necrosis detection kit was obtained from Biolegend (CA, USA). The exposure concentrations was in the range of 400-700 µg/mL. The cells were counted under a phase-contrast fluorescent microscope (Olympus BX53, Olympus, Tokyo, Japan). The results were expressed as the percent of the total cell amount (Abudayyak et al. 2016; 2017).

The cytotoxicity assays were done in triplicate and repeated in different four days (n=12). Other tests were done in triplicate in different three day (n=9). Data was expressed as mean ± standard deviation (SD). The statistically analysis was conducted using one-way ANOVA Dunnett t-test by SPSS version 23 for Windows (SPSS Inc., Chicago, IL). *p* values of less than 0.05 were selected as the levels of significance.

RESULTS AND DISCUSSION

According to the TEM and DLS results obtained by our previous study (Abudayyak et al. 2017a; 2017), the average sizes of NiO-NPs were 15.0 nm (4.2-38.1 nm) in water and 21.4 nm (7.2-60.5 nm) in the cell culture medium. The increase could be due the adsorbiton of medium proteins in the surface of the particles (Abudayyak et al. 2017a; 2017).

The ICP-MS results showed that the NiO-NPs were up taken by the exposed cells at 50 and 100 µg/mL concentration (Table 1). The agglomeration of NPs at the high concentration could explain the decrease in the cellular uptake of NiO-NPs at the high concentration.

Table 1

The NiO-NPs were obtained within the cytoplasmic vacuoles at 50 and 150 µg/mL. The particle sizes were larger in the exposed cells at 150 µg/mL compared to those at 50 µg/mL. Also, the number of particle-containing vacuoles in the exposed cells decreased when the concentration of NiO-NPs was increased. Cytoplasmic organelles appeared normal and nuclear and plasma membranes were intact in the exposed cells, as in the negative control cells. Electron-dense bodies were visible in the cytoplasm of some cells in both treatment groups. The most notable change in the cells at 50 and 150 µg/mL included the presence of electron-lucent large vacuoles filled with NPs. In a few cells at 50 µg/mL, the cytoplasmic vacuoles were so large that they induced disruption of the cytoplasm. Some cells exhibited abnormal nuclei with chromatin condensation and indentations of the nuclear membrane in both treatment groups (Figure 1A; 1B; 1C).

In all the cells, lipid droplets were observed to increase with increasing NP concentrations, which could be an indicator or feature of the oxidative stress process (Khatchadourian and Maysinger, 2009; Lee et al., 2015). The results revealed by TEM pictures analysis, and ICP-MS analysis confirmed the uptake and accumulation of NiO-NPs into the cells, indicating that the observed toxicities were results of exposure to NiO-NPs.

Figure 1

The results of cytotoxicity evaluation showed a decrease in the cell viability within depending on concentration manner. The IC_{50} values were 479.15 and 351.6 µg/mL by NRU and MTT assay, respectively (Figure 2). According to the earlier studies, The NiO-NPs induced cellular death in human neuron, liver, lung, airway epithelial, and breast cells and rat kidney epithelial cells (Abudayyak et al. 2017a; 2017; Ahamed, 2011; Ahamed et al., 2013; Ahmad et al., 2013; Chang et al., 2018; Di Bucchianico et al., 2018; Horie et al., 2011; Lanone et al., 2009; Lee et al., 2015; Mohamed et al., 2018; Siddiqui et al., 2012). We could indicate that Caco-2 cells were more vulnerable than other previously studied cells to cytotoxic damage induced by NiO-NPs.

Figure 2

The genotoxic potential of NiO-NPs was evaluated using Comet assay (Figure 3). The NiO-NPs damaged to DNA (1.2-1.5 fold; $p \leq 0.05$). At the highest concentration of NiO-NPs, the tail intensities was 8.0%. In positive controls (100 µM H_2O_2), the tail intensities were 13.3% (1.85 fold). Previously, researchers showed that the NiO-NPs could induce DNA damages in different cells (Abudayyak et al. 2017a; 2017; Ahamed, 2011; Ahamed et al., 2013; Ahmad et al., 2013; Åkerlund et al., 2018; Chang et al., 2018; De Carli et al., 2018; Di Bucchianico et al., 2018; Horie et al., 2011; Lee et al., 2015; Siddiqui et al., 2012). Dumla et al. (2017) reported significant DNA damages in liver, lung and kidneys of rats exposed orally. Also, the NiO-NPs induced cell cycle alteration in consequence of genotoxicity via the nuclear translocation of phosho-ATM and phosphor-ATR in the human pulmonary epithelial cell lines (Dumla et al., 2017). The NiO-NPs induced DNA-damage signalling cascade at 20-100 µg/mL (1). Li et al. (2018) indicated DNA damages in pulmonary cells after intratracheal instillation similar to our results (li et al., 2018).

Figure 3

The oxidative damage potential of NiO-NPs was evaluated with the levels of GSH, MDA, 8-OHdG, and PC (Table 2). The NiO-NPs induced oxidative damage as a significant increase in the levels of PC (≤1.5 fold) and significant decrease in GSH levels (33.1-37.5%) were observed. However, the 8-OHdG and MDA levels did not change significantly. In general, the potential of oxidative stress by the NiO-NPs on Caco-2 cells was statistically significant ($p \leq 0.05$).

Table 2

Previous studies indicated the NiO-NPs caused damage in lung and liver and induce pulmonary inflamations via reactive oxygen species (ROS) (Chang et al., 2017; 2018; Li et al., 2018; Marzban et al., 2017; Senoh et al., 2017; Shinohara et al., 2017; Yu et al., 2017; Dumala et al., 2018; Ogami et al., 2009). *In vitro* researches confirmed with the *in vivo* studies that the NiO-NPs induced an increase in ROS and caused oxidative damage in the cells (Abudayyak et al. 2017a; 2017; Åkerlund et al., 2018; Di Bucchianico et al., 2018; Mohamed et al., 2018; Saquib et al., 2018). Previous data confirmed with our results; oxidative stress could be the mechansim underlying the cyto- and genotoxicity induced by the NiO-NPs .

The results of Annexin V-FITC/PI assay demonstrate that the NiO-NPs led to an induction of apoptosis (Figure 4). The percentage of apoptotic cells were observed to be 84.6-99.6% of the dead cells, while the percentage of necrotic cells were observed to be 0.4-15.4% of the dead cells (Figure 4). The results indicated that apoptosis could be the main cell death pathway in exposure to NiO-NPs. De Bucciani et al. (2018) and Saquib et al. (2018) observed increase in the apoptotic cells in dose dependent manner (De Bucciani et al., 2018; Saquib et al., 2018). Similarly, Chang et al. (2017b) indicated endoplasmic reticulum stress related apoptosis in rat exposed to NiO-NPs by intratracheal instillation (Chang et al., 2017b).

Figure 4

The NiO-NPs induced apoptosis by functional alterations in mitochondri and lysosome at 25-100 µg/mL (Ahmad et al., 2013) similar to our results. Previously, the changes of mRNA levels in the genes related to apoptosis pathway were reported (Ahamed et al., 2013; Saquib et al., 2018; Siddiqui et al., 2012). Duan et al. (2015) investigated the impacts of NiO-NPs on sirtuin 1, a NAD-dependent deacetylase involved in apoptosis in human bronchial epithelial cells (BEAS-2B). The NiO-NPs decreased the cell viability by an apoptotic process at 5-20 µg/cm^2, and indicated the repression of sirtuin 1, which might underlie the NiO-NP-induced apoptosis via tumour protein p53 and bcl-2-associated X protein (Duan et al., 2015). Similar to previous researchers, we observed the NiO-NPs increased the percentage of apoptotic cells in a dose-dependent manner. The degree of apoptosis/necrosis corresponded with severity of cytotoxicity (Figures 2 and 4).

In conclusion, the toxicity profile of NiO-NPs (15.0 nm) was evaluated in Caco-2 cell line. The NiO-NPs showed cytotoxic potential by disrupting the mitochondrial and lysosomal activities; inducing the cells apoptosis. Oxidative stress could be the mechansim of DNA damages induced by NiO-NPs. The results should raise concern about the safety of NiO-NPs contains products. Further studies should be conducted to clear up the probable toxicity in gastrointestinal tract and clarify the pathways of apoptosis and DNA damage.

Competing interests: The authors declare that there is no conflict of interest.

Authors' contributions: All authors contributed equally to this work. They read and approved the final manuscript.

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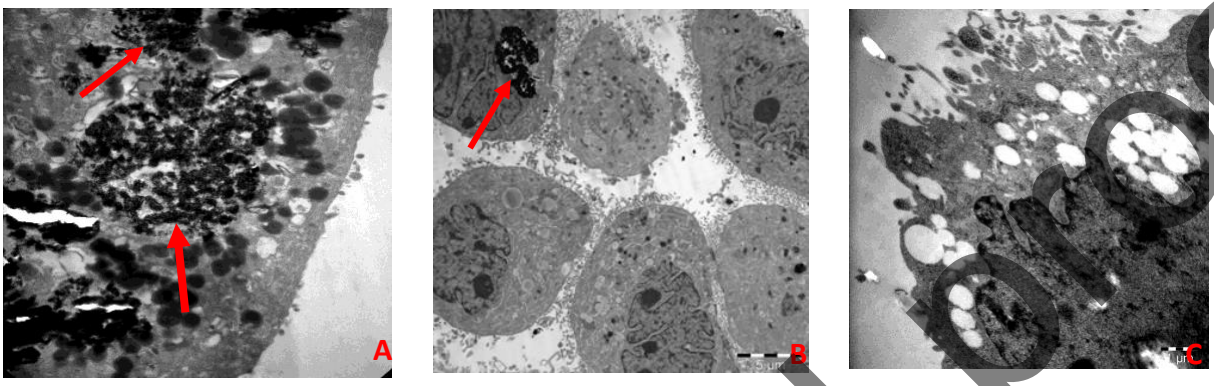
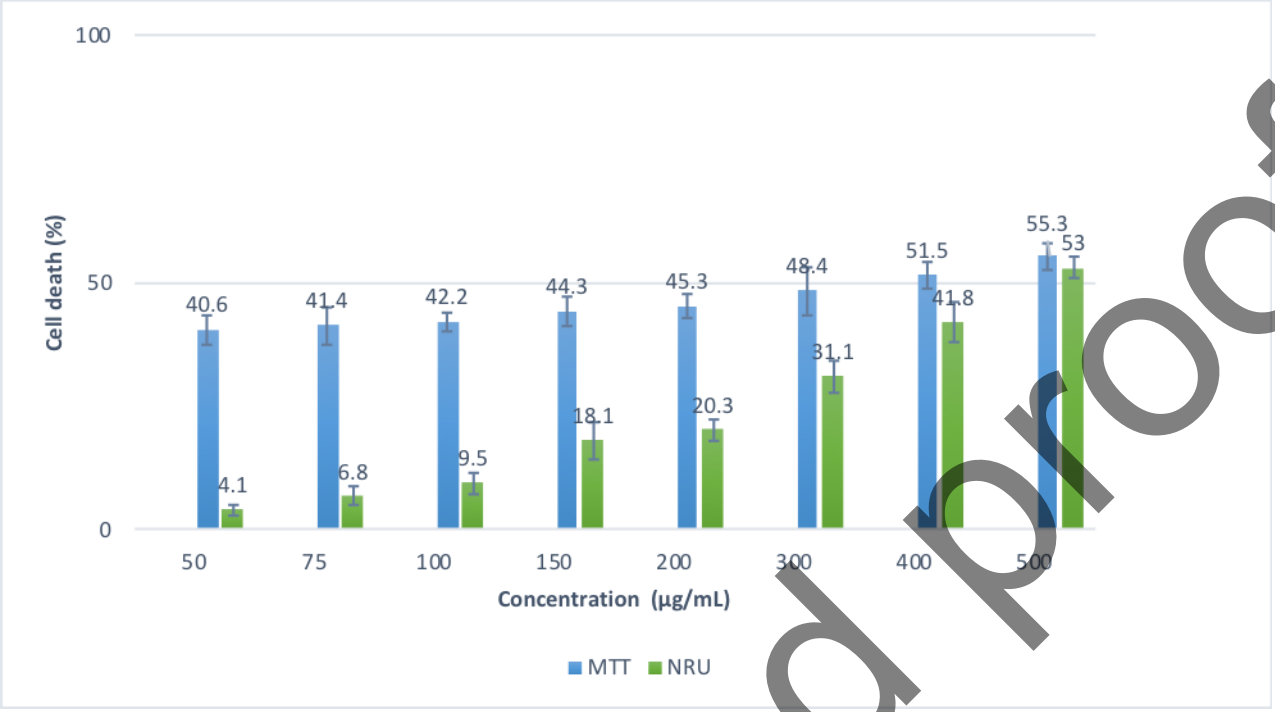
Figure 1: The TEM observations of NiO-NPs uptake by the Caco-2 cells.



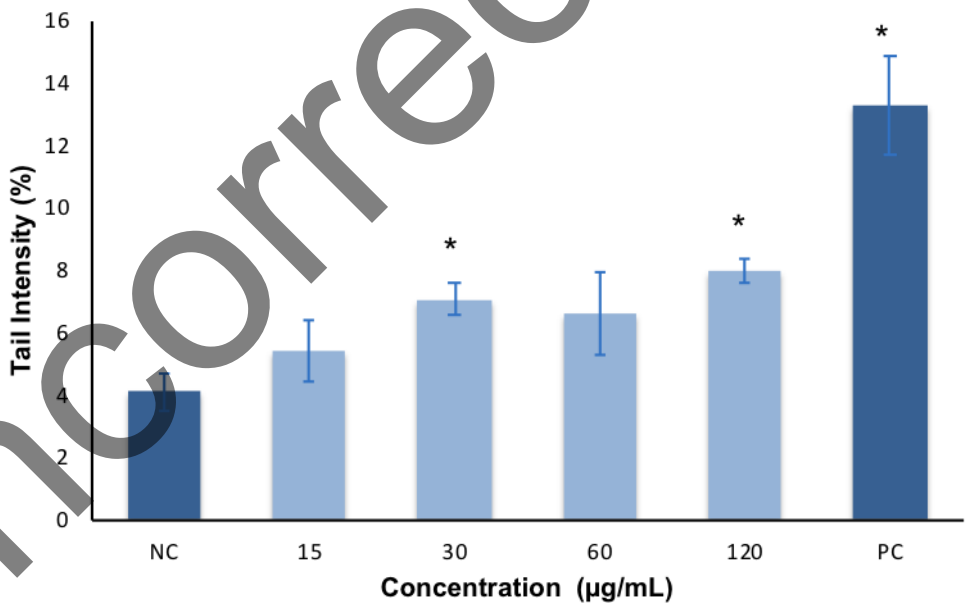
(A) Caco-2 cells exposed to NiO-NPs at 50 µg/mL, (B) Caco-2 cells exposed to NiO-NPs at 100 µg/mL, (C) Caco-2 unexposed cell (negative control).

Figure 2: The cytotoxic potential of NiO-NPs.



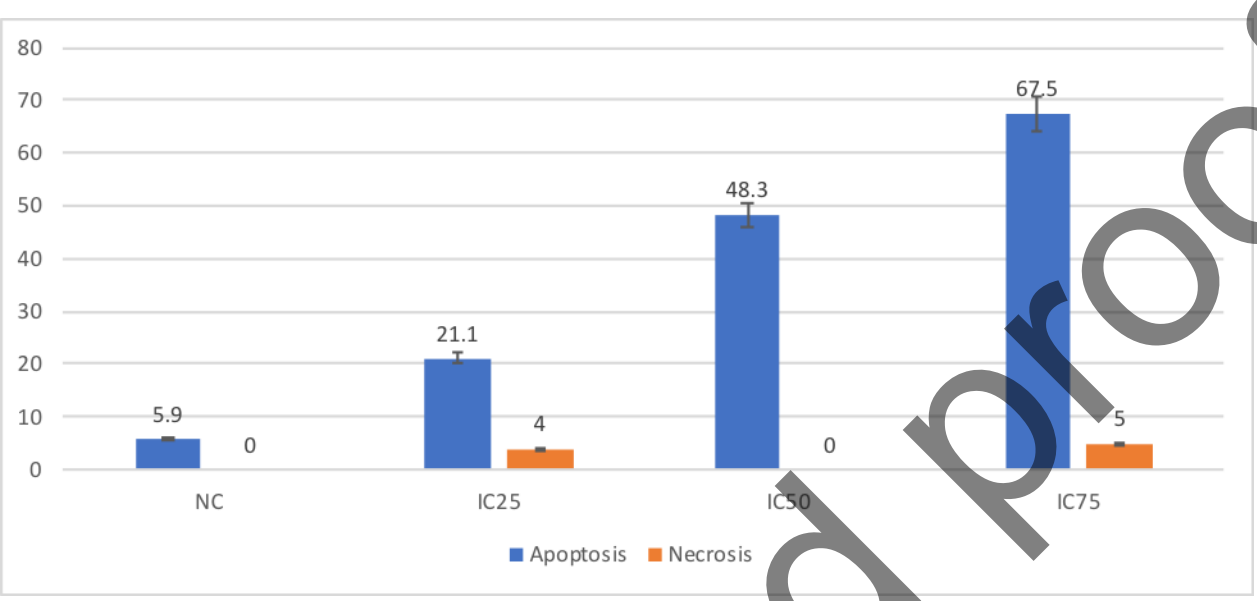
All experiments were done in triplicates and each assay as repeated four times. Data was expressed as mean ±SD. The IC_{50} values were 251.6 µg/mL and 479.2 µg/mL in MTT and NRU, respectively.

Figure 3: The genotoxic potential of NiO-NPs.



All experiments were done in triplicates and each assay as repeated three times. The results were presented as mean tail intensity (%) with ±SD.
*****$p \leq 0.05$ were selected as the levels of significance by one-way ANOVA Dunnett t-test.

Figure 4: The apoptotic/necrotic potential of NiO-NPs.



All experiments were done in triplicates and each assay as repeated twice.
The results were expressed as the percent of the total cell amount with ±SD.
$p \leq 0.05$ were selected as the levels of significance by one-way ANOVA Dunnett t-test, Apoptotic ratio was significant at all exposure samples.

Table 1: The NiO-NPs were taken up by the Caco-2 cells.

Exposure concentration (μg/mL/10^5 cells)	**Ni amount (μg/10^5 cells)**
Negative control	0.19±0.05
50	3.29±0.38
100	1.31±0.22

The assay was repeated four times.The results were presented as mean ±SD.

Table 2: The NiO-NPs induced oxidative damage to the Caco-2 cells.

Exposure concentration (µg/mL)	**8-OHdG (µg/g protein)**	**MDA (µmol/g protein)**	**GSH (µmol /g protein)**	**PC (µg/g protein)**
0	0.917±0.132	0.350±0.059	41.142±2.009	5.160±0.108
50	0.964±0.205	0.375±0.108	25.744±3.891*	5.435±0.814
75	0.990±0.242	0.481±0.090	27.500±6.082*	6.667±1.078*
100	1.111±0.310	0.408±0.158	38.092±3.782	7.115±0.877*
150	0.985±0.164	0.442±0.098	39.099±2.82	7.907±1.007*

All experiments were done in triplicates and each assay as repeated twice.
The results were presented as mean ±SD.
*****$p \leq 0.05$ were selected as the levels of significance compared to the negative controls by one-way ANOVA Dunnett t-test.