

RESEARCH ARTICLE

In vitro toxicological assessment of MgO and Silica Nanoparticle in human colon carcinoma cells

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ABSTRACT

The usage of nanoparticles (NPs) has been expanded to many fields such as agriculture, food industry, medicine and biotechnological fields. Therefore, human exposure to NPs is increasing continuously. Accordingly, their potential biological effect and toxicity of NPs for humans and the environment is still a topic of concern. This study aimed to investigate the cytotoxicity effects of magnesium oxide (MgO) and silica (SiO₂) NPs on human colon adenocarcinoma (HT-29) after 24 hours of exposure. In this study, cytotoxicity of MgO and SiO₂ NPs was evaluated using MTT assay after 24 hours of MgO (50 nm) and SiO₂ (90-110 nm) NP sexposure at doses of 25-200 µg/ml. Moreover, to assess the rate of cell apoptosis, cells were stained with ethidium bromide/acridineorange stain. The staining was examined under a fluorescent microscope. The exposure of HT29 cells to SiO₂ and MgO NPs increased cytotoxicity in a dose-dependent response. Also, these results revealed a significant increase in apoptosis induction in groups treated with MgO and SiO₂ NPs. These results showed that SiO₂ and MgO NPs can cause cytotoxicity in HT29 cells and it is better to avoid using them in foodstuff and food packaging ingredients.

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INTRODUCTION

Nowadays, nanoparticles are widely used in different industries and businesses such as textiles, staining, medical imaging, and disease diagnosis. Therefore, global investment in nanotechnology research and development is increased significantly [1, 2]. This potential is due to unique physiochemical characteristics of materials such as large surface, different electronic properties, and reactivity level [3, 4]. Currently, NPs are used in different industrial processes and a vast spectrum of products and systems of daily life (e.g. food, drinks, and sunscreen creams). Moreover, nanotechnology has many promising applications in the food

industry, such as improvement of taste and texture and increased product shelf life [5, 6].

One of the useful nanoparticles in industry and medicine is MgO NPs. MgO NPs as an important alkali metal oxide is used in catalytic reactions and wastewater treatment. MgO NPs show high capacity in absorption and analysis of pollutants and fabrication of insulation and resistant materials against high temperature [7]. The results of different studies show that MgO NPs alone or with other antimicrobial groups are used as an effective antibacterial agent to increase the health of food products [8]. High stability, biocompatibility, and low toxicity are among the important properties of magnesium oxide [9].

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SiO₂ NPs is a non-metallic oxide and has different applications in chemical industries and is used as an additive in foodstuff, drugs, cosmetics, etc. This NP is used in biotechnology and medicine, too [10]. The usage of SiO₂ NPs as an anticaking agent in powder and flavoring products is taken into consideration. [11, 12]. Nowadays, SiO₂ NPs is one of the components used in foodstuff and is confirmed as an additive (E551). Therefore, finding information about the toxicity and safety of SiO₂ NPs as a food additive is urgently need [13]. So far, different studies are conducted on cytotoxicity of NPs and shows that size, structure, and surface properties are important factors for their toxicities [14, 15]. It has been indicated that the toxicity of NPs is dependent on their concentration [16]. Determination of suitable dose to evaluate cell toxicity is a key component to understand toxic effects of NPs under real physiological conditions [17]. Kyung et al. (2009) investigated SiO₂ NPs toxicity in rat keratinocyte cells (HEL-30) and showed that SiO₂ NPs toxicity is dependent on exposure and size of NPs. LDH leakage was only observed in cells exposed to NPs with the diameters of 30 and 40 nm and NPs with the diameters of 118 and 535 nm did not show any leakage. The results of MTT assay showed a certain degree of toxicity for 30-40 nm particles at high concentrations (100 µg) compared with 118 and 535 nm [4]. NPs due to their small size can pass through physiological barriers and enter the host circulation system and cause disorders in cell process when they reach the tissues and organs and this leads to disease [18]. Different studies have been conducted on the effect of NPs toxicity and toxicities of titanium oxide, zinc oxide, silver, and gold on the different cells, such

as skin, macrophage, heart, liver, and kidneys [19]. The distribution of NPs in tissues and organs due to their small size and creation of structural and histopathological changes and also tissue necrosis and dystrophy can be caused concerns [20]. NPs toxicity is mainly dependent on the action of NPs and biomolecules. Oxidative stress induction resulting reactive oxygen species (ROS) generation is one of the main toxicity mechanisms of NPs. High production of ROS leads to DNA damage, releases intracellular calcium and disorders in mitochondrial function followed by cell death [21, 22]. Recently, the effect of MgO NPs toxicity in human cardiovascular endothelial cells has been reported that is dependent on exposure duration, size, and NPs concentration [23, 24].

Modern nanotechnology is developing rapidly and the production of NPs as the main component of this science is increased. Therefore, human exposure to NMs increases consequently. Increased use of different nanoparticles that most of them are related to biological systems necessitates careful examination of the side effects. This research aims to investigate the cytotoxic effects of MgO and SiO₂ NPs on human colon adenocarcinoma cells (HT-29) after 24 hours of exposure.

METHODS

Nanoparticles synthesis

SiO₂ NPs and MgO NPs were synthesized according to the methods proposed by Ding et al. (2016) and Parvajanti et al. (2015), respectively [25, 26]. Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and X-ray diffraction (XRD) have been used in the characterization of NPs to acquire

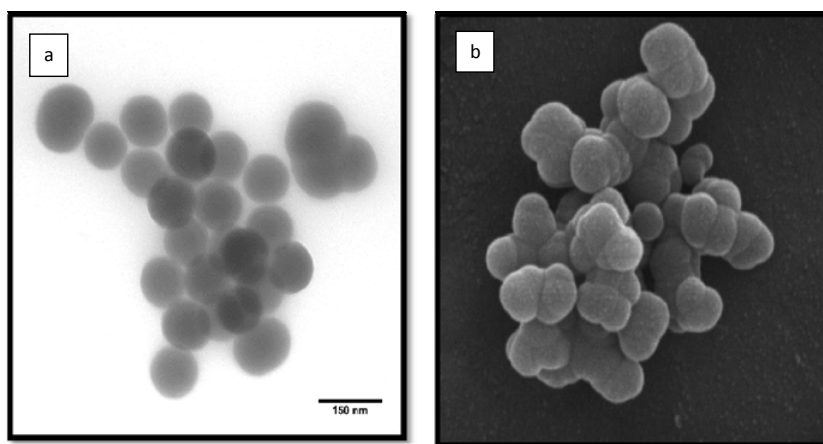


Fig. 1. The characterization of synthesized SiO₂ NPs. (a) Transmission electron microscopy (TEM); (b) Scanning Electron Microscopy (SEM)

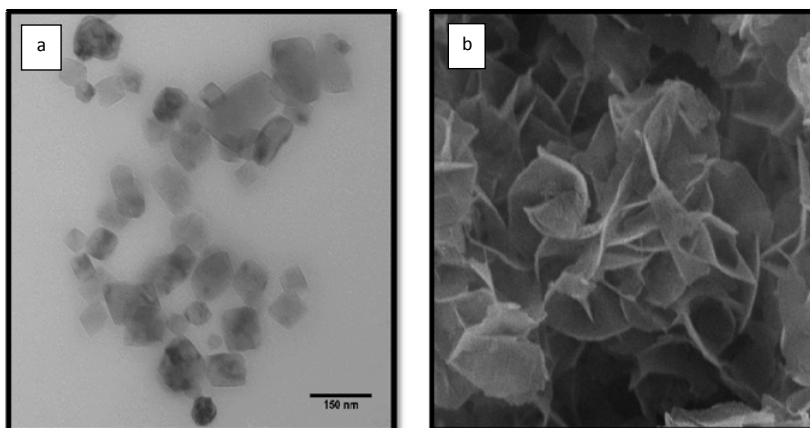


Fig. 2. The characterization of synthesized MgO NPs. (a) Transmission electron microscopy (TEM); (b) Scanning Electron Microscopy (SEM)

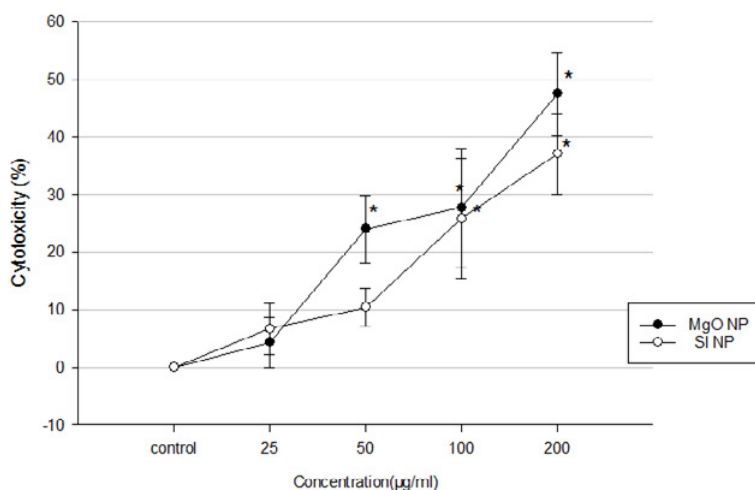


Fig. 3. Dose-dependent toxicity of SiO₂ and MgONPs in HepG2 cells which were treated with various concentrations of NPs for 24 h and the cytotoxicity was determined by the MTT assay. Values were the mean ± SD from three independent experiments. Significance is indicated by: *p < 0.05 versus the control cells.

the size, shape, and crystalline character (Fig. 1 and 2).

SiO₂ NPs and MgO NPs sample preparation

To prepare different concentrations of MgO (50 nm) and SiO₂ (90-110 nm) NPs suspension, MgO and SiO₂ NPs were suspended in deionized water and various concentrations of these NPs (25, 50, 100, and 200 µg/ml) were prepared. The suspension was homogenized with an ultrasonic bath (Sonica MH S3, Soltec, Italy) and sterilized using a syringe filter.

Cell cultures

HT-29 cells were obtained from Cancer Research Center, Shiraz University of Medical Sciences. This cell line category belongs to human colon adenocarcinoma.

The cells were cultured in RPMI1640 containing glutamate (Sell Max Company, Iran) supplemented with 10% fetal bovine serum (Gibco Company, USA), antibiotics penicillin-streptomycin 100 units/ml (Bio Idea Company, Iran), and amphotericin B 2.5 mg/ml (Sigma Company, USA). Cells were maintained in a humidified incubator at 37 °C in a 5% CO₂ atmosphere. The supernatant of cells was changed every 48 hours and at 80-90% confluence, cells were detached by using 0.25% trypsin and were sub-cultured into 25 cm² flasks.

Cytotoxicity Assays

The Cytotoxic effects of the MgO and SiO₂ NPs were evaluated by MTT assay, which is based on

the reduction of MTT to insoluble purple formazan crystals by mitochondrial dehydrogenases. A mitochondrial dehydrogenase activity is an important marker in cell viability [27]. Briefly, for this assay, the cells were seeded (1×10^4 cells per well) in a 96-well culture plate. After overnight incubation, the cells were exposed at various concentrations (25-200 $\mu\text{g/ml}$) of MgO and SiO₂ NPs for 24h. The MTT assay was performed according to the manufacturer's procedure (Pars Tous Company, Iran), following the treatment, 0.1 mg of MTT was added to each well, then incubation of the cell was performed at 37°C for 4h. After removing of culture media, 100 μl of DMSO was added to each well to dissolve the purple formazan crystals. Absorbance was read using a microplate reader at 570 nm (Tecan, Salzburg, Austria). All experiments were performed in triplicate. Cell viability was determined as a percent of the control culture value.

Fluorescence technique (Ethidium bromide/Acridine orange staining)

Fluorescence staining and cell counting under microscope is one of the simple methods to identify living and dead cells. In order to detect apoptotic or necrotic nuclei ethidium bromide and acridine orange was used. Acridine orange is a vital color that can be absorbed by living cells and penetrate into the cells' DNA. In this condition, living cells' chromatin under microscope appears uniformly green. Ethidium chromatin stains dead cells that have lost their

membrane uniformity. Necrotic cells stained orange with no condensed chromatin. The late apoptotic cells appeared green with condensed and often fragmented nuclei. [28].

HT-29 Cells were grown in 6-well culture plate at a density of 5×10^5 cells/well and allowed to attach for 24 h. After 24 h of cell attachment, plates were washed with phosphate buffered saline (PBS) and then cells were incubated with various concentrations (25-200 $\mu\text{g/ml}$) of MgO and SiO₂ NPs for 24 h. HT29 cells in culture medium without NPs were used as the control. After incubation, the supernatant medium was removed and the cells were washed with a sterile PBS. Then 200 $\mu\text{l/ml}$ of filtered EDTA (1mM) was used for each well to harvest the cells from 6 well culture plate. A centrifugation (200g, 10min) was performed and the cells were collected and suspended in 1 ml of fresh RPMI 1640 medium. 10 μl of dye-cell suspension was placed on hemocytometer and cells are viewed under a fluorescence microscope and counted to quantify apoptosis.

Statistical analysis

Data analysis was performed with SPSS 16 and the results were reported as mean and standard deviation. In order to investigate the changes in different treatments, one-way ANOVA was used. In cases in which a significant difference existed between treatments, means were compared with Duncan test. The level of $P < 0.05$ were considered as significance.

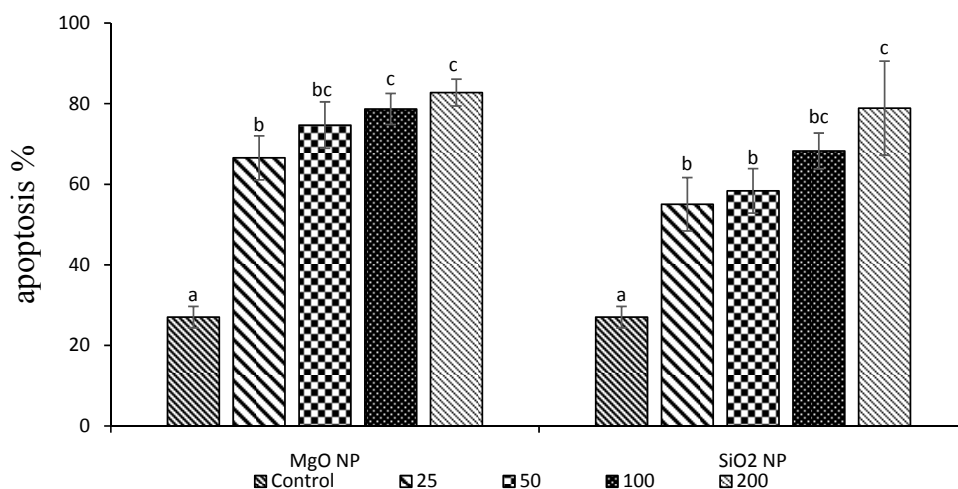


Fig. 4. The effect of different concentrations of MgO and SiO₂ NPs on HT-29 cells apoptosis after 24 hours of exposure (with fluorescence staining). Different letters show the significant difference between groups ($P < 0.05$).

RESULTS

In the current study, HT29 cells were exposed to MgO NPs (50nm) and SiO₂ NPs (90-110nm) at 25-200 µg/ml for 24 h. The amounts of cytotoxicity created by MgO and SiO₂ NPs were evaluated using the MTT assay and fluorescent (ethidium bromide/acridine orange) staining. As shown in Fig. 3, treating the HT29 cells with SiO₂ NP and MgO NP increased cytotoxicity in a dose-dependent manner. The results indicated that the percentage of cytotoxicity increased when the concentrations of both types of NPs increased.

The results of fluorescence staining confirmed the results related to MTT. A significant increase in the cytotoxicity was observed by increasing the NPs dosage. As shown in Fig. 4, high dosage of MgO and SiO₂ NPs showed a significant cytotoxicity. At 200 µg/ml dosage, the apoptosis percentages in the cells treated with SiO₂ and MgO NPs were 78/91 and 82/78, respectively. Also the results of fluorescence staining by using ethidium bromide/acridine orange stain showed that the living cells appeared in green and dead cells appeared in orange or brown (Fig. 5).

DISCUSSION AND CONCLUSIONS

In recent years, the studies that have focused on the negative effects of NPs on human health are increasing significantly but still is a concern about the potential biological effects and toxicity of these particles [29]. In the current study, the cytotoxicity of MgO and SiO₂ NPs was investigated using MTT assay and fluorescence technique. In the current

study, it was found that exposure to SiO₂ and MgO nanoparticles at a dosage level of 25-200 µg/ml caused dose dependent cytotoxicity as revealed by MTT assay and fluorescence technique. These findings are in accordance with previous studies. Schneider *et al.* (2017) investigated the cytotoxic effects of metal nanoparticles and metal oxide (copper, silver, gold, zinc, and titanium oxide) on human colon adenocarcinoma cells using MTT assay and trypan blue staining. Their findings showed a significant decrease in mitochondrial activity, that this value in cells treated by high silver concentration was 40% and in cells treated with gold was 60% [30]. These results were similar to our results. The results of previous studies proved that SiO₂ NPs can intervene with molecular cells and influence the cell growth and also, they showed cytotoxic effects [31]. In another study, Sergeant *et al.* (2012) investigated the cytotoxic and genotoxic effect of SiO₂ NPs with the diameters of 25 and 100 nm. Their results showed that the exposure of HT29 cells to SiO₂ NPs induced slightly cytotoxicity effect [32]. There is a concern about the distribution of nanoparticles in tissues and organs due to their small size and creating structural and hisopathologic transformation and also tissue necrosis and dystrophy [20]. Among metal oxide nanomaterials, MgO NPs due to low cost and biocompatibility are very important. Also, studies showed that MgONPs had lower toxicity compared with other NPs such as ZnO and TiO₂ [33]. The toxic effect mechanism of MgONPs in HT-29 cell after 48 hours exposure was investigated

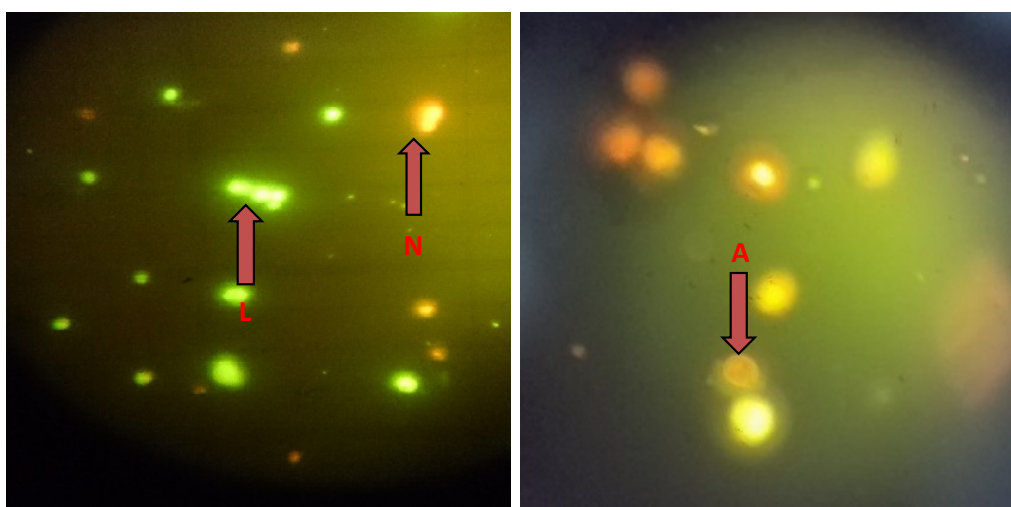


Fig. 5. Fluorescent microscope images of HT-29 cells after staining with acridine orange and ethidium bromide. In these images, L is living cells, N is necrotic cells, and A refers to cells with secondary apoptosis.

by Alqahtani *et al.* In their study cytotoxicity was evaluated by using MTT and neutral red uptake assays. The results showed that MgONPs reduced cell viability in concentration and time-dependent manner. MgO NPs caused oxidative stress by reducing glutathione (GSH) concentrations and enhancing reactive oxygen species (ROS) and lipid peroxidation levels [33]. Jebali *et al.* (2013) reported the toxic effect of SiO₂ NPs in the form of wire, rod, and sphere with different sizes (20, 50 and 100 nm) on rat pulmonary cells after 6 and 24 hours of exposure, using MTT and MTS cytotoxicity tests. Their results showed that NPs with smaller size (20 nm) have higher toxicity in compared with larger sizes. Also, silica nanoparticles in spherical form had higher cytotoxic effects. Moreover, 24 hours of incubation led to higher cell death [34]. Bohmert *et al.* (2012) in their study on NPs toxicity of silver coated with peptide, showed the strong cytotoxic effects of silver NPs on duplication and differentiation where these effects intensified with increased incubation time and dose and decreased particles size. Nanoparticles toxicity in the cell started with morphological transformation and decreased cell binding capacity and after 24-48 hours of exposure, cell death occurred [35].

In a recent study by Mai *et al.* (2012), MgO NPs showed considerable toxicity in AGS gastric cancer cells that was dose and time dependent. These findings are similar to the results of the present study. This study also showed structural and physiological transformations including chromatin compression and fragmentation [36].

In conclusion, our results showed that MgO and SiO₂ NPs can be toxic elements for HT-29 cells and this toxicity is dose-dependent. Therefore, it is better to avoid them in foodstuffs and packages that are directly connected to food.

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CONFLICT OF INTEREST

The authors did not report no conflict of interest.

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