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RESEARCH ARTICLE

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Cytotoxic Effects of Titanium Dioxide Nanoparticles on MCF-7 Cancer Cell Line

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ABSTRACT

Cancer is a widespread disease of various types worldwide that affects many people. Today, titanium dioxide nanoparticles have substantial therapeutic applications. We investigated how harmful titanium dioxide is to breast cancer cells. MCF-7 cancer cells and HFF cell lines were cultured. The survival of cells exposed to different amounts of titanium dioxide nanoparticles was tested. The examined concentrations were 25, 50, 100, and 200 µg/ml. The survival rate was measured after 48 and 72 hours and IC50 was determined. We found that the highest toxicity occured while MCF-7 and HFF cells were exposed to 200 µg/ml of titanium dioxide. Apoptosis in MCF-7 and HFF cells emerged as shown with Annexin V-PI staining and flow cytometry. Under a microscope, it was found that titanium dioxide nanoparticles could be harmful in specific amounts. At a dose of 200 µg/ml, after 48 and 72 hours of treatment, MCF-7 and HFF cells were affected. The mitochondrial membrane broke when breast cells were exposed to titanium dioxide nanoparticles. The matrix leaked into the cytoplasm, and the rough endoplasmic reticulum swelled. These observations occurred after 72 hours of treatment with a concentration of 200 µg/ml. Considering the acquired effects, titanium dioxide nanoparticles may be advocated as potential medicinal candidates for pharmaceutical purposes even though further research is required.

Keywords

TiO_2 Nanoparticles, Flow Cytometry, Micronucleus Assay, MTT Assay, TEM

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Abbreviations

NP: Nanoparticle
TiO_2: Titanium dioxide
FBS: Fetal bovine serum
TEM: Transmission electron microscope
DMSO: Dimethyl Sulfoxide

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Introduction

Nanotechnology is a broad field of applied physical sciences, chemical engineering, and biological engineering. NPs are tiny particles with dimensions less than 100 nm. Nanotechnology is used to supply cosmetics and medications. NPs have ancient records and were utilized by artisans in the 9th century to polish the surface of pottery. TiO_2, known as titanium IV, was first commercially extracted from ore in 1923. Titania exists in three forms: rutile, anatase, and brookite. Due to its luster, TiO_2 is used as a light-reflecting coating in papers and tablets as a white pigment, and also as an ultraviolet inhibitor in sunscreen.

Oxidative stress caused by environmental pollution and harmful gases, such as greenhouse gases, plays an essential role in aging. Moreover, the accumulation of reactive oxygen species damages the nucleic acid of the cells and can lead to aging. NPs build up rapidly in the environment, and the unique behavior of NPs, especially their high surface-to-volume ratio, is the reason for their high reactivity and ability to pass through cell membranes. NPs cause cell damage and inflammation due to the production of free radicals in cells [1].

The autosomal cell cycle has four phases, the first of which is called the S phase, where DNA synthesis and replication occur. The second phase is the M phase, where cell separation happens. In addition to the S and M phases, the cell cycle has three other phases: G1, G2, and G0. Cyclin kinases are responsible for controlling these phases. There are two types of tumors: benign and malignant. Malignant tumors are cancerous and grow fast. They can spread to other tissues and invade the body [2]. Cancerous cells can enter the bloodstream or lymphatic system and spread to distinct elements of the frame. All forms of most cancers are a consequence of troubles inside the cells. Normal cells usually divide to make new cells at the same time as they are needed, preserving stability amongst cell increase and cellular loss of life. While the managing system that regulates cellular boom is disrupted, cells divide uncontrollably and form tumors. Mutations that increase cell branches and prevent the loss of cell existence can cause cancers . In the modern day, scientists have discovered mutated genes in human cancer cells. These genes are divided into three groups: proto-oncogenes, tumor suppressors, and DNA restore genes. Mutations in these genes can set off proto-oncogenes or inhibit tumor suppressor genes, leading to out-of-control cell growth and immortality. Mutations in DNA repair genes can also cause the accumulation of more significant mutations. Cancers are now the second leading cause of death worldwide. Breast cancer is the most cancer for women. It causes more deaths than lung and stomach cancers. A survey reported 40,430 deaths from breast cancers (40,000 women and 430 men) in 2014 [3]. Cancer is the leading cause of death in Iran, after coronary heart disease and accidents [4]. Reducing death rates from breast cancer is difficult in developing countries, including Iran [5]. Treating breast cancer is difficult because chemotherapy has limited effectiveness and side effects. Therefore, today the focus is on controlled and targeted drug delivery systems. NPs can target and deliver anticancer agents to maximize their effectiveness and minimize the side effects [6]. There is an urgent need to find new substances for treatment. NPs are an alternative for treating diseases due to their biological effects and small and unique size. TiO_2 is used in many industries, such as paper, plastic, cosmetics, and paint. It is also used as a disinfectant and biological sensor to kill tumor cells [7]. The anticancer influences of TiO_2 have been investigated on several cancers, and the experiments showed that the viability of most cancers depends on the particle doses and counseled that TiO_2 NPs can be used for cancer treatment. TiO_2 NPs affect MCF-7 and HFF cell traces in distinctive concentrations. This cell line has been considerably used as a human breast cancer cell line for the development of recent antitumor treatments. The purpose of this was to assess the cytotoxic activity of TiO_2 NPs.

Result

Cell Growth Inhibition by TiO_2 NPs

TEM Technique

TEM image represents the morphology and organelles of MCF-7 and HFF cells via the NPs. TEM analysis shows the structural adjustments and damages occurring following treatment with TiO_2. Intracellular uptake of TiO_2 NPs was established by TEM. The MCF-7 and HFF cells were incubated for 72 h with 0 and 200 µg/ml of TiO_2 NPs.

Figure 1 (A and B) demonstrates the fibroblast cells without treatment, where the organelles are seen as healthy and intact. After 72 h, the results showed that the mitochondrial organelle was elongated and the rough endoplasmic reticulum was inflamed. Figure 2 (A and B) shows breast cancer cells without treatment. Compared to normal cells, these cells have many mitochondrial organelles due to multiple and misplaced mitotic divisions. Figure 2 (C and D) indicates the breast cancer cells treated with a concentration of 200 µg/ml for a period of 72 h. Electron microscope images show the presence of TiO_2 NPs inside the cell, as well as mitochondrial membrane rupture

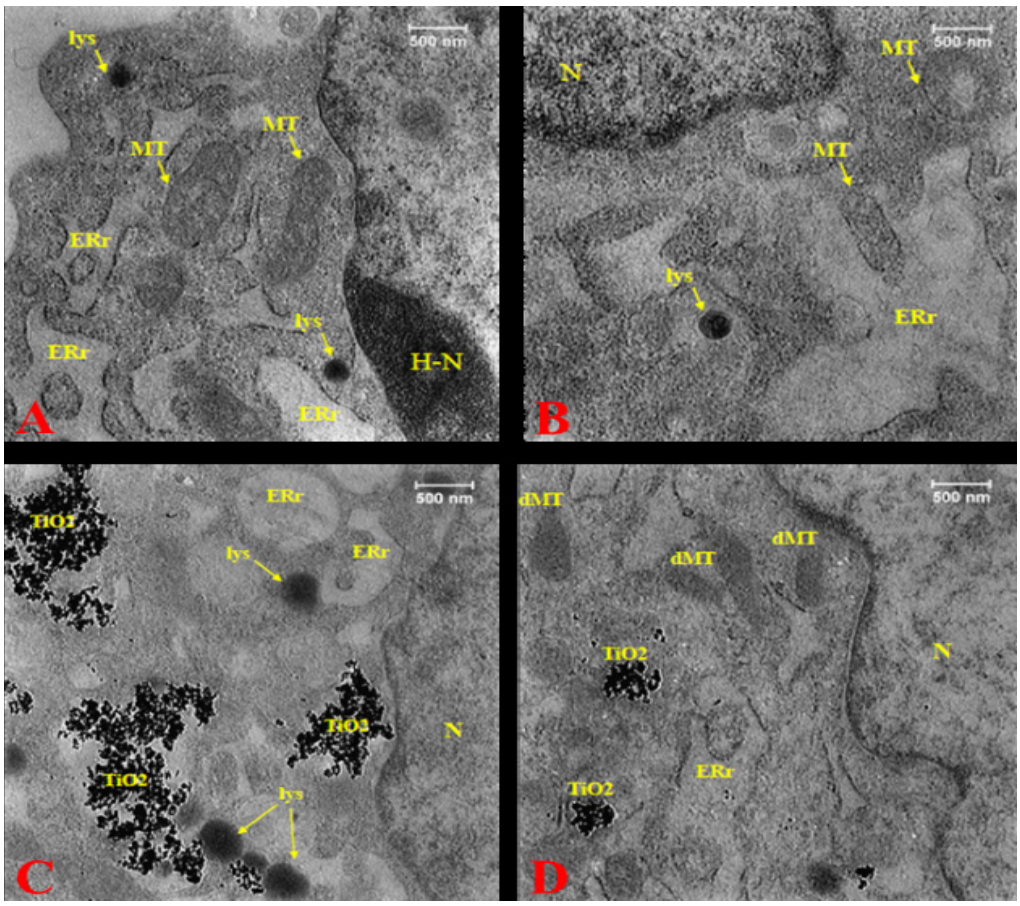

Figure 1.
Effect of 200 µg/ml concentrations of TiO_2 NPs on HFF cell line. A, B: Control group-HFF cell, ×4000; C, D: Dose 200 µg/ml-HFF cell, ×4000. Lys, lysosome; MT, mitochondria; ERr, rough endoplasmic reticulum; H-N, heterochromatin nucleus; N, nucleus; TiO_2, TiO_2 nanoparticle; dMT, damage mitochondria; N, nucleus.

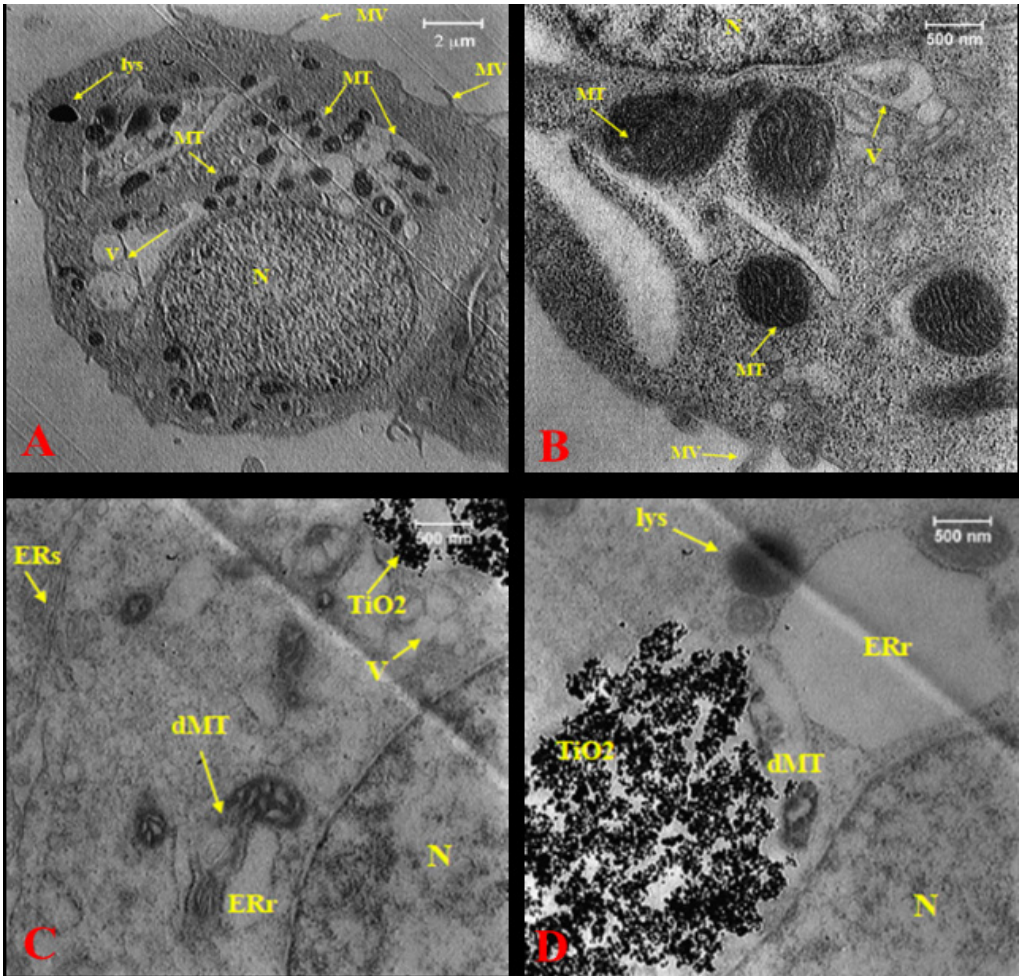

Figure 2.
Effect of 200 µg/ml concentrations of TiO2 NPs on MCF-7 cell line.
A, B: Control group-MCF-7 ×4000; C, D: Dose 200 µg/ml-MCF-7. Lys, lysosome; MT, mitochondria; MV microvilli; V, vacuole cytoplasm; N, nucleus; ERs, smooth endoplasmic reticulum; dMT, damage mitochondria; ERr, rough endoplasmic reticulum; TiO_2, TiO_2 nanoparticle.

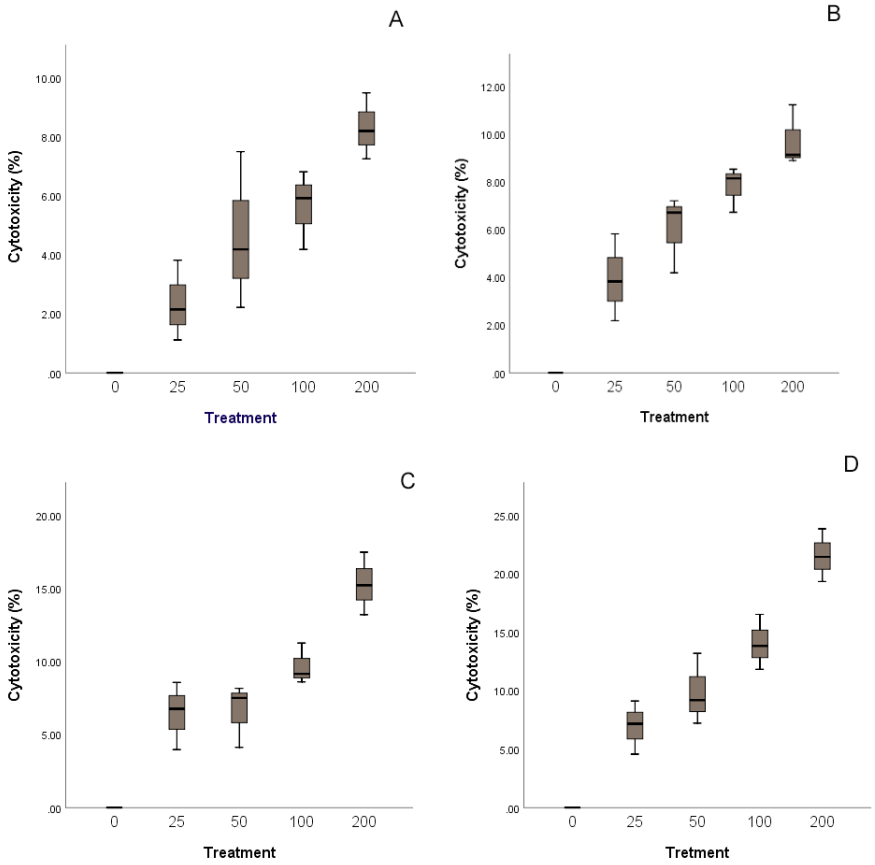

Figure 3.
Effect of different concentrations of TiO2 NPs (µg/ml) on HFF and MCF-7 cells line. A: 48h, HFF cell line; B: 72h, HFF cell line; C: 48h, MCF-7 cell line; D: 72h, MCF-7 cell line.

and leakage. Moreover, they present the swelling of the rough endoplasmic reticulum.

MTT Assay

In our study, we used TiO_2 NPs to treat MCF-7 and HFF cell lines. The used concentrations were in the range of 25-200 µg/ml. We used the MTT assay to measure cell growth inhibition. As the concentration of TiO_2 NPs increased, we found that cell growth was increasingly inhibited. This was observed after 48 and 72 h of exposure to the NPs. Cell viability decreased in a dose-dependent manner. Figure 3 shows that the amount of inhibition of cells depends on the concentration. At the concentration of 200 µg/ml, cell death percentages were significantly lower after 48 and 72 h of treatment ($p = 0.01$, $p < 0.05$). We calculated the IC50 values for TiO_2 NPs. The values represent the concentrations that cause 50% toxicity or death in the MCF-7 cancer cells and normal HFF cells. The IC50 was 420 and 1000 µg/ml for MCF-7 and HFF, respectively.

Micronucleus Assay

The micronucleus technique is a very convenient and fast method for examining the structural abnormalities of chromosomes. This technique is extensively used to study the cell morphology. These groups included the absence of or presence of NPs. The results of light microscopy showed that TiO_2 NPs can cause concentration-dependent toxicity at a dose of 200 µg/ml in the treatment periods of 48 and 72 h in the MCF-7 and HFF cell lines. As a result, at the concentration of 200 µg/ml, the percentage of cell death in the treatment periods of 48 and 72 h ($p = 0.01$) is significantly lower than other groups ($p < 0.05$) (Figure 4).

Flow Cytometry Assay

To assess the rate of cell death due to TiO_2 NPs, the MCF-7 and HFF cells were handled with IC50 attention. The cells were then stained with FITC Annexin V and PI and were analyzed by flow cytometry. The flow cytometry results are presented in Figure 5. While exposed to 420 µg/ml of TiO_2 NPs, the MCF-7 and HFF cells experienced a substantial decrease in viable cells. Moreover, the proportion of apoptotic cells extended. In the meantime, the percentage of necrotic cells was tiny and negligible.

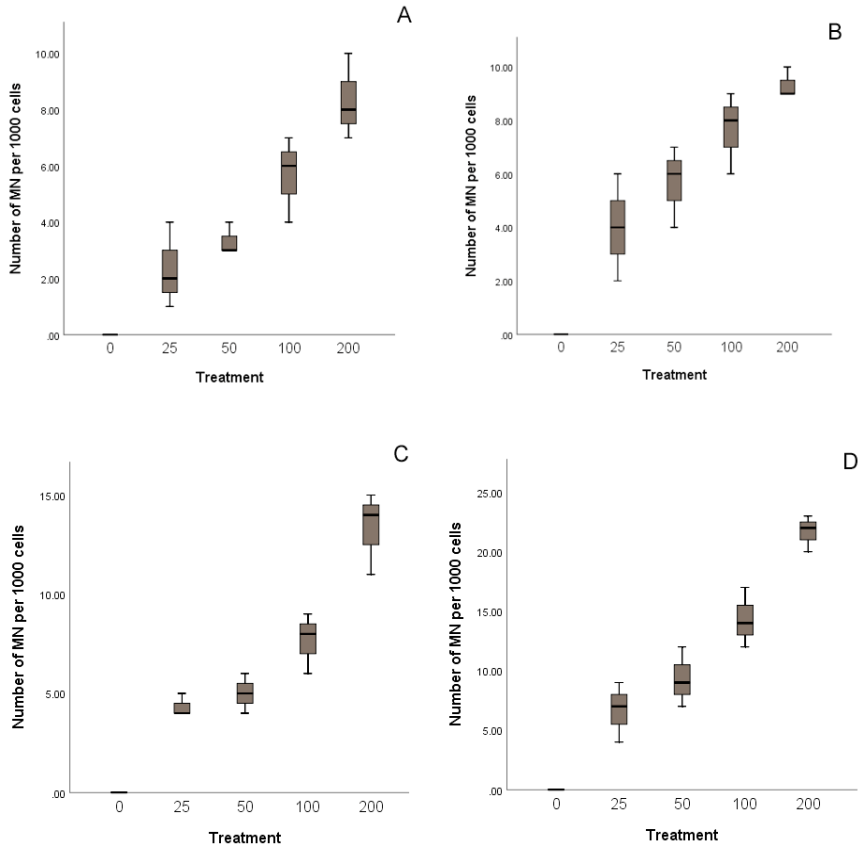

Figure 4.
Effects of different concentrations of TiO2 NPs (µg/ml) on HFF and MCF-7 cells line. A: 48h, HFF cell line; B: 72h, HFF cell line; C: 48h, MCF-7 cell line; D: 72h, MCF-7 cell line.

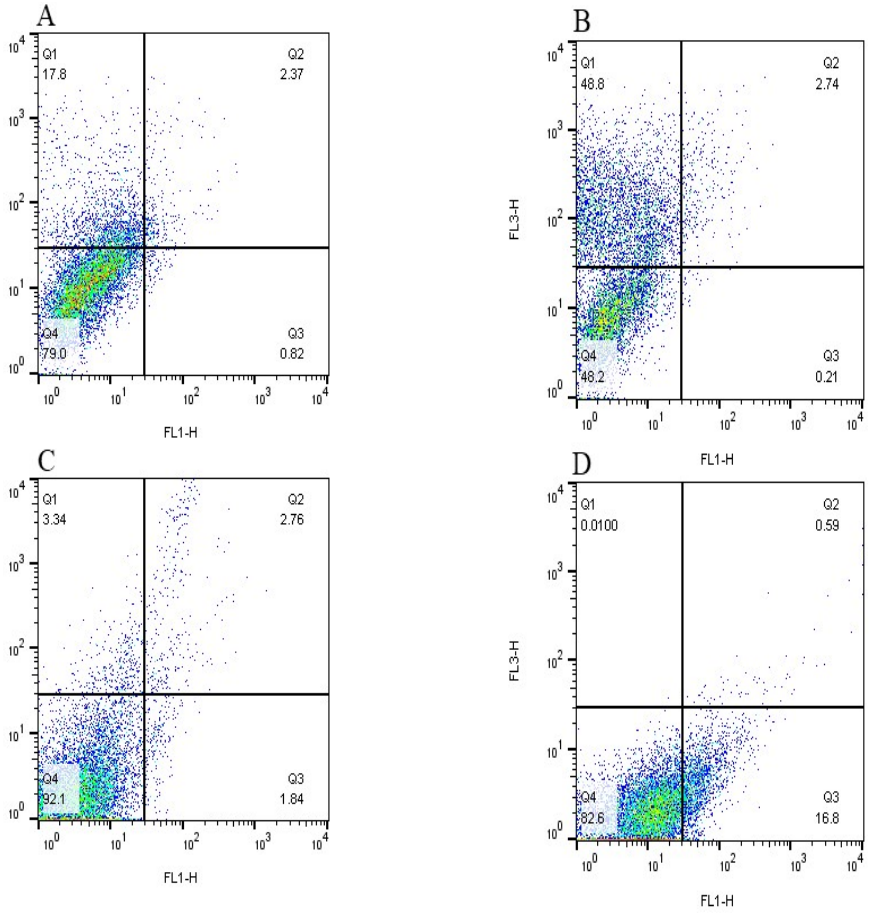

Figure 5.
Effect of TiO_2 NPs 200 (µg/ml) on HFF and MCF-7 cells line. A: control, HFF cell line; B: 72h, HFF cell line; C: control, MCF-7 cell line; D: 72h, MCF-7 cell line.

Discussion

NPs are a necessity in physics and dentistry because they can contest bacteria, fungi, and viruses (8-10). However, researchers have limited studies on the antineoplastic effects of TiO_2 NPs. The TiO_2 NPs did not harm glioma C6, RG2, mouse, or human glioma U373 cells. Copper-TiO_2 NP complex turned out to be much less poisonous than copper alone, indicating some protection from the harm of TiO_2 NPs. However, the complex became somewhat more venomous than cisplatin. The copper-TiO_2 complex can be a part of mitochondria and ATP composition. It may also lower the shaping of nitrogenic bases. Moreover, it may reach the cell nucleus and hook up with DNA base pairs. This occurs through interplay or groove binding. In the long run, it can trigger apoptotic cellular death [11].

Reports indicated that TiO_2 NPs, with added Au and Pt, effectively destroyed the K562 tumor cells [12]. The way TiO_2 NPs are taken up is not well understood. Some studies have suggested that TiO_2 NPs are taken up and stored in different cell parts, such as vacuoles, endosomes, and lysosomes. They may also be found in the cytoplasm because the lysosomal membrane breaks. Recently, it was found that TiO_2 NPs can enter human cells through a specific receptor. Human bronchial epithelial BEAS 2B cells were treated with nm uncoated anatase TiO_2-NPs smaller than 25 and SiO_2-lined rutile TiO_2-NPs of 10-40 nm.

Regardless of the truth that the uncoated TiO_2-NPs increased the micronucleus, the SiO_2-protected NPs no longer [13]. Guichard et al. [14] determined that none of the TiO_2-NPs or TiO_2 bulk behavior momentous starting of micronuclei shape after 24 h of the exposure of these particles to SHE cells. Determind that none of the TiO_2 NPs or TiO_2 bulk resulted in significant formation of micronulei shape after 24 hours exposure to SHE cells.The genotoxic potential of 20 n.m TiO_2 was assessed in SHE cells.The cells were treated with 0.1 mg/cm2 of the particles for 12,24,48,66 and 72 hours.The micronuleuse frequenies were increased by the treatment in a time dependet manner.[15] . The genotoxic capability of 20 nm TiO_2-NPs became charged in the SHE cells. The cells were treated with 1 mg/ml TiO_2-NPs for 12, 24, 48, 66, and 72 h. The micronucleus frequencies were extended by treatment in an age-setting method [15]. The HepG2 cells were treated with a low dose of 30 nm TiO_2-NPs and a vital increase inside the micronucleus commonness was placed in the treated cells [16].

Human epidermal cells (A431) were treated with 50 nm anatase TiO_2-NPs at a dose of 80 mg/ml. The treatment caused tremendous chromosome change at a dose of 80 mg/ml [17]. Human lung cancer cells, A549, were treated with 10 and 50 mg/ml of TiO_2-NPs for 24 h, and a micronucleus assay was performed to decide the genotoxicity of the debris. There was a high-quality response within the micronucleus induction for each of the treatment concentrations [18, 19]. The toxicity of NPs for cancer cells was compared to normal cells. Investigations showed that the rate of the inhibition of cells depended on the concentration. However, with raising the concentration, the rate of toxicity increased, and on the other hand, the rate of cell survival diminished. Further investigations are required to explain the TiO_2 NPs and to clarify the toxicity of TiO_2 NPs on cells for finding modern methodologies for treating cancer and other diseases.

Conclusion

According to the observed effects, TiO_2 NPs may be endorsed as potential medicinal candidates for pharmaceutical purposes. However, further investigations are required on this subject. Clinical studies on the animal and human models are necessary to confirm the effect of NPs on the normal cell line.

Materials and Methods

We obtained chemicals and reagents from different companies. RPMI1640 and FBS were purchased from Biosera in France. TiO2 NPs (nanopowder) were obtained from Sigma-Aldrich (Germany). We purchased MTT, plastic dishes, 6-well and 96-well plates, doxorubicin, and DMSO from BETACELL in Belgium .

TiO_2 Np Topography and NPs Characterization

We purchased TiO_2 NPs from Sigma-Aldrich company (United Kingdom). The TiO2 NPs used in this study were titanium (IV) oxide, and anatase, with a purity of 99.7%. We weighed the TiO2 NPs and placed them in natural water. In order to reduce the length of NP aggregates, NPs were sonicated three times for 30 min. We analyzed the TiO_2 NPs using a particle length analyz-

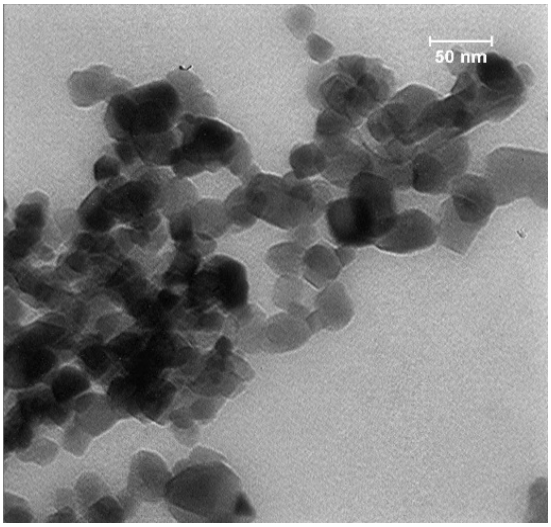

Figure 1. Detection of aggregation of TiO_2 nanoparticles (NPs) by transmission electron microscopy (TEM).

er (D mean number 35.51 n.m) (NanoQ Report). The topographical surface was then studied with a TEM (LEO 912 AB) (Figure 1).

Cell Culture

The MCF-7 and HFF cells (Ferdowsi University) were cultured at 37˚C in RPMI 1640 and Dulbecco's Modified Eagle Medium supplemented with 10% heat-inactivated FBS, 500 µg/ml penicillin-streptomycin, and 200 µg/ml amphotericin B in a humidified atmosphere with 5% CO_2. Cells were then harvested by treatment with 0.25% trypsin-EDTA.

Cytotoxic Activity

MTT test is used to check the share of living cells. A cell flask with 60% confluence was trypsinized, and after cell counting, it was transferred to a 6-well plate, so that about 150×103 cells were placed in 200 µl of way-of-life medium for every concentration of TiO_2 NPs, and were incubated at 37°C for 48 and 72 h. Cellular survival was assessed by adding 100 µl MTT (5 mg/ml in PBS containing 10% FBS) to each well, and the cells were incubated every other 4 hours. To dissolve the resultant formazan, 100 µl dimethyl sulfoxide was delivered, and absorbance was measured by a spectrophotometer at a wavelength of 540 nm. Moreover, cell death was calculated by the following formula. SPSS software and the Kruskal Wallis test were used for the statistical analysis of the data with a significance level of $p < 0.05$.

Survival Percentage = OD test/OD count ×100

Analysis of Apoptosis by Flow Cytometry

To measure cell death, we used a flow cytometry test called annexin V-FITC apoptosis detection kit. The test was performed following the instructions provided by the manufacturer. We treated the MCF-7 and HFF cells (5×105) with a solution of TiO2 NPs at different concentrations of 25, 50, 100, and 200 µg/ml. After 48 and 72 h, we gently removed the cells from the dishes, washed them once with a medium containing serum, and put them in 500 µl buffer. Next, we added 5 µl of annexin V-FITC and 5 µl of propidium iodide. The cells were incubated at room temperature for 15 min in the dark. Finally, cells were analyzed using a BD Falcon flow cytometer (USA).

Intracellular Uptake of TiO_2 NPs

We exposed cells to different amounts of TiO_2 NPs for 48 and 72 h. Afterwards, we washed the cells three times with PBS and stuck them for 1 h with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH: 7.4) at 4˚C. We collected the cells with a rubber scraper, dried them, and embedded them in Araldite M. Samples were stained with uranyl acetate and lead citrate. Finally, thin sections were observed under a LEO 912 AB TEM at a magnification of ×4,000.

Slide Preparation for Micronucleus Assay

We created two slides for each sample. The quantity of fixative varied depending on the pellet. We produced a total of four slides for each sample . On each slide, we introduced two 20 µl drops of cell suspension. Next, we allowed the slides to dry in the airand stained them with 10% Giemsa stain in phosphate buffer for 10 min. Afterwards, we allowed the slides to dry in the air for a single day. Subsequently, the slides were covered with a cover slip.

Statistical Analysis

The distribution of MTT and micronucleus values among the control and four test groups receiving different doses of TiO_2 was compared using the non-parametric Kruskal Wallis test. A pairwise comparison was performed using the Mann-Whitney U test with Bonferroni adjustment. MTT and micronucleus values were compared between MCF7 and HFF cell lines and also between two assessing times (48 and 72 h) using the Mann-Whitney U test. The data were analyzed using IBM SPSS Statistics version 26 software (IBM SPSS Statistics, Chicago, USA).

Authors' Contributions

Investigation, writing the original draft, and statistical analysis: RJ. Conceptualization, supervision, software, draft review, and editing: RJ, AR, HM, MA. All authors were involved in writing the article and accepted responsibility for its content.

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Competing Interests

The authors declare that there is no conflict of interest.

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