

RESEARCH

Open Access



Induction of oxidative DNA damage, cell cycle arrest and p53 mediated apoptosis by calcium titanate nanoparticles in MCF-7 breast cancer cells

Hanan R. H. Mohamed^{1*}, Maria M. H. Ibrahim² and Ayman Diab²

Abstract

Background: The distinctive properties and high activity of calcium titanate nanoparticles (CaTiO₃-NPs) increase their use in many products. However, the cytotoxic and genotoxic effects of CaTiO₃-NPs in human cancer cell lines have not been well studied. Therefore, this study was conducted to explore CaTiO₃-NPs induced cytotoxicity, genomic instability and apoptosis in human breast cancer (MCF-7) cells.

Methods: Sulforhodamine B (SRB) and the alkaline comet assays were done to study cell viability and DNA damage induction, respectively. Apoptosis induction and cell cycle distribution were analyzed using flow cytometry. The level of intracellular reactive oxygen species (ROS) was studied, and the expression levels of p53, Bax and Bcl2 genes were also measured.

Results: The results of the Sulforhodamine B (SRB) cytotoxicity assay showed that viability of MCF-7 cells was not affected by CaTiO₃-NPs treatment for 24 h, however, exposure to CaTiO₃-NPs for 72 h caused concentrations dependent death of MCF-7 cells. Treatment with CaTiO₃-NPs for 72 h caused marked increases in intracellular ROS level and induced DNA damage. Treatment of MCF-7 cells with CaTiO₃-NPs also caused MCF-7 cell cycle arrest at the G0 and S phases and triggered apoptosis of MCF-7 cells by causing simultaneous increases in the expression levels of apoptotic p53 and Bax genes and a decrease in the expression level of anti-apoptotic Bcl2 gene.

Conclusion: Collectively, it was concluded that CaTiO₃-NPs cause time- and concentration-dependent cytotoxic effects in human MCF-7 cells through induction of ROS generation, genomic instability and apoptosis. Thus it is recommended that further in vitro and in vivo studies are therefore recommended to understand the cytotoxic and biological effects of CaTiO₃-NPs.

Keywords: Calcium titanate nanoparticles, Cytotoxicity, Genotoxicity, Apoptosis, Cell cycle arrest

Introduction

Titanium compounds such as titanium alloys, titanates and titanium dioxides are widely used in various biological applications. For example, the superior physical and

biological properties of titanium-oxygen alloys make them successfully used in dental implant and orthopedic hip replacement. For enhancing the bonding bioactivity of titanium alloys alkaline or heat treatments are applied to form a calcium-TiO₂ layer on the alloys surfaces, the calcium in this layer is fixed on the surface of the alloy and is thus not labile [1].

*Correspondence: hananeeyra@cu.edu.eg

¹ Zoology Department Faculty of Science, Cairo University, Giza, Egypt
Full list of author information is available at the end of the article



© The Author(s) 2022. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Calcium titanate (CaTiO_3), an inorganic compound, is a diamagnetic solid known also as perovskite. Recent discovering of the ability of CaTiO_3 to bind and release calcium ions increases the possibility of using CaTiO_3 to deliver calcium ions in various medical applications [2, 3]. However, CaTiO_3 induced-toxicity has been poorly studied. Monosodium calcium titanate has been found to cytotoxic to monocytes and fibroblasts [4, 5]. Moreover, monosodium titanate alone or with metal ions has been shown to inhibit cellular metabolism in different cell types [6–8].

Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. A bulk material should have constant physical properties regardless of its size, but at the nanoscale this is often not the case [9]. Size-dependent physical and chemical properties are observed, e.g. surface area/mass ratio increased, thereby greatly enhancing chemical/catalytic reactivity (amongst other properties) compared to normal-sized particles of the same substance [10].

Nowadays there is increasing ongoing interest on using nanoparticles instead of bulk materials because nano-sized materials have variable size-dependent physical and chemical properties rather than the constant size independent physical properties of bulk materials. For example increased surface area/mass ratio in nanoparticles, resulting in significantly improved chemical and catalytic activities compared to the same bulk material [10].

Ongoing with these unique promising characteristics of nanoparticles, nano-sized titanium compounds are now used extensively in various consumer products, paints, pharmaceuticals, and also in wastewater treatment [11]. The cytotoxicity of several calcium titanate compounds towards different human cell lines has been also detected [4–8]. However, the cytotoxic effects of CaTiO_3 -NPs in human cancer cells have not been well studied.

Therefore, this study was undertaken to estimate the effect of CaTiO_3 nanoparticles on the viability and genomic stability of human breast cancer cell line. Cells viability was detected using Sulforhodamine B assay and the alkaline comet assay was conducted to quantify DNA damage. Apoptosis was also estimated and cell cycle analyzed using flow cytometry. The production of intracellular reactive oxygen species was detected using 2,7-dichlorofluorescein diacetate dye, and the expression levels of the apoptotic (p53 and Bax) and anti-apoptotic (Bcl2) genes were quantified using real-time polymerase chain reaction.

Materials and methods

Chemicals

Calcium titanate nanoparticles (CaTiO_3 -NPs) were purchased from Sigma-Aldrich Chemical Company (Saint Louis, USA) with white appearance and product number (633801). Nano-powders of CaTiO_3 -NPs with 99% trace metals basis were suspended in deionized distilled water and ultra-sonicated to prepare the concentrations required for the experiments of this study.

Cell lines

Human breast cancer (MCF-7) cells were purchased from Nawah Scientific Company (Mokatam Cairo Egypt) and were preserved in Dulbecco's Modified Eagle Medium (DMEM) media provided with streptomycin (100 mg/ml), penicillin (100 units/ml) and heat-inactivated fetal bovine serum in humidified, 5% (v/v) carbon dioxide atmosphere at 37 °C.

Characterization of CaTiO_3 -NPs

X-ray diffraction (XRD) analysis was conducted to study the properties of CaTiO_3 -NPs using a charge coupled device diffractometer (XPRT-PRO, PANalytical, Netherlands). The size distribution and Zeta potential of the CaTiO_3 -NPs were also evaluated using a Malvern Instrument Zeta sizer Nano Series (Malvern Instruments, Westborough, MA) equipped with a He-Ne laser ($\lambda = 633$ nm, max 5mW). Finally, the suspended CaTiO_3 -NPs were imaged using transmission electron microscopy (TEM) to determine the morphology and mean particle size of CaTiO_3 -NPs.

Cells viability

The effect of CaTiO_3 -NPs on the MCF-7 cells viability was assessed using a Sulforhodamine B (SRB) assay [12]. A 100 μl of MCF-7 cell suspensions were cultured separately in 96-well plates and incubated for 24 h in complete media. After incubation, the cells were treated with five different concentrations of CaTiO_3 -NPs (0.01, 0.1, 1, 10 and 100 $\mu\text{g}/\text{ml}$) and incubated for 24 h or (0.1, 1, 10, 100 and 1000 $\mu\text{g}/\text{ml}$) and incubated for 72 h. After exposure to CaTiO_3 -NPs for 24 or 72 h, the cultured MCF-7 cells were fixed and washed with distilled water, then SRB solution (0.4% w/v) was added and the cells were incubated for 10 min at room temperature in a dark place. All plates were washed with acetic acid (1%) and left overnight to dry. The protein-bound SRB stain was then dissolved and the absorbance measured using a

BMG LABTECH®-FLUO star Omega microplate reader (Ortenberg, Germany) at 540 nm.

Treatment schedule

MCF-7 cells were cultured under the proper conditions and divided into control and treated cells. Control cells were treated with an equal volume of the vehicle (DMSO; final concentration, $\leq 0.1\%$), while treated cells were treated with the determined IC₅₀ of CaTiO₃-NPs. All MCF-7 cells were left for 72 h after treatment and harvested by trypsinization and centrifugation. Cells were then washed twice with ice-cold PBS and used for different molecular assays. Triplicate was done for each treatment in all conducted molecular assays.

Genomic stability

Alkaline Comet assay was done to study the effect of CaTiO₃-NPs on genomic stability in MCF-7 cells [13, 14]. Briefly, a mixture of cell suspensions and low melting agarose was dispersed on a clean slide covered with a layer of normal melting agarose and left to dry. Slides were then incubated in a cold lysis buffer, electrophoresed and finally immersed in neutral Tris buffer. Slides were immersed in cold absolute ethanol for permanent preparation. Immediately before examination slides are stained with ethidium bromide, photographed using epi-fluorescent microscope at magnification 200× and fifty comet nuclei were analyzed using Comet Score™ software for each sample.

Production of intracellular ROS.

The influence of CaTiO₃-NPs on the production level of intracellular reactive oxygen species (ROS) in MCF-7 cells was also studied based on the formation of a fluorescent dichlorofluorescein complex resulting from the reaction of a 2,7-dichlorofluorescein diacetate dye with intracellular ROS [15]. After cultured MCF-7 cells were washed with phosphate-buffered saline (PBS), 2,7-dichlorofluorescein diacetate dye was added and left for 30 min in the dark. The mixture of cells and dye was then dispersed on a clean slide and the emitted fluorescent light was examined using epi-fluorescent at 200× magnification.

The mRNA expression levels of p53, Bax and Bcl2 genes

The mRNA expression levels of p53, Bax and Bcl2 genes were measured using real time Polymerase chain reaction (RT-PCR) in all MCF-7 cells after 72 h of treatment. Total RNAs was extracted using the GeneJET RNA Purification Kit (Thermo scientific, USA) (Thermo scientific, USA) and then transcribed reversely into complementary DNA (cDNA) using the Revert Aid First Strand cDNA Synthesis Kit (Thermo scientific, USA). To measure mRNA

Table 1 Sequences of the used primers in RT-PCR

Gene	Strand	Primer's sequences
GAPDH	Forward	5'-GAAGGTGAAGGTCGGAGTCA-3'
	Reverse	5'-GAAGATGGTGTGGGATTTTC-3'
BAX	Forward	5'-CCGCCGTGGACACAGAC-3'
	Reverse	5'-CAGAAAACATGTCAGCTGCCA-3'
BCL-2	Forward	5'-TCCGATCAGGAAGGCTAGAGT-3'
	Reverse	5'-TCGGTCTCTAAAAGCAGGC-3'
P53	Forward	5'-CAGCCAAGTCTGTGACTTGCACGTAC-3'
	Reverse	5'-CTATGTCGAAAAGTGTCTGTATC-3'

expression levels, the p53, Bax and Bcl2 genes were amplified using the 7500 Fast system (Applied Biosystem 7500, Clinilab, Egypt). The primers previously designed by [16, 17] listed in Table 1 were used for RTPCR amplification and the comparative Ct (DDCt) method was undertaken to quantify the mRNA expression levels of amplified genes. Data of RTPCR were standardized using housekeeping GAPDH gene expression and the results were expressed as mean \pm S.D.

Cell cycle analysis

Analysis of MCF-7 cell cycle was conducted using flow cytometry. After 72 h of treatment MCF-7 cells were collected, washed with PBS and re-suspended in PBS with RNAase A and propidium iodide (PI). Control and treated MCF-7 cells were then incubated in dark for 20 min at 37 °C and the DNA contents were analyzed using FL2 (λ ex/em 535/617 nm) signal detector (ACEA Novocyte™ flow cytometer, ACEA Biosciences Inc., San Diego, CA, USA). For each sample, 12,000 events were acquired and cell cycle distribution is calculated using ACEA NovoExpress™ software (ACEA Biosciences Inc., San Diego, CA, USA).

Induction of apoptosis

The number of apoptotic and necrotic cells were also determined using Annexin V- Fluorescein isothiocyanate (FITC) apoptosis detection kit (Abcam Inc., Cambridge Science Park Cambridge, UK) coupled with two fluorescent channels flow cytometry. After 72 h of treatment MCF-7 cells were harvested and washed with ice-cold PBS (pH 7.4). Collected cells are then incubated with Annexin V-FITC/propidium iodide (PI) solution in dark for 30 min at room temperature, injected via ACEA Novocyte™ flowcytometer (ACEA Biosciences Inc., San Diego, CA, USA) and analyzed using FL1 and FL2 signal detector for FITC and PI fluorescent signals, respectively (λ ex/em 488/530 nm for FITC and λ ex/em 535/617 nm for PI). For each sample, 12,000 events were acquired and

positive FITC and/or PI cells were quantified by quadrant analysis and calculated using ACEA NovoExpress™ software (ACEA Biosciences Inc., San Diego, CA, USA).

Statistical analysis

All results of the present study are expressed as mean ± Standard Deviation (S.D) and were analyzed using the Statistical Package for the Social Sciences (SPSS) (version 20) at the significance level $p < 0.05$. The *student t-test* was used to compare between the untreated and treated cancer MCF-7 cells.

Results

Characterization of CaTiO₃-NPs

The results of XRD analysis confirmed the purity of the obtained CaTiO₃-NPs and the absence of any other oxides or impurities as manifested by the appearance of CaTiO₃ distinctive peaks at theta angles of 32.7°, 47.1°, 58.9°, and 69° (Fig. 1). Using a Zeta sizer analyzer it was found that CaTiO₃-NPs had a mean Zeta potential value of -3.38 mv and an average particle of 3.62 nm with a polydispersity index (PDI) value of 0.895 as seen in Fig. 2. Screening of CaTiO₃-NPs using TEM manifested

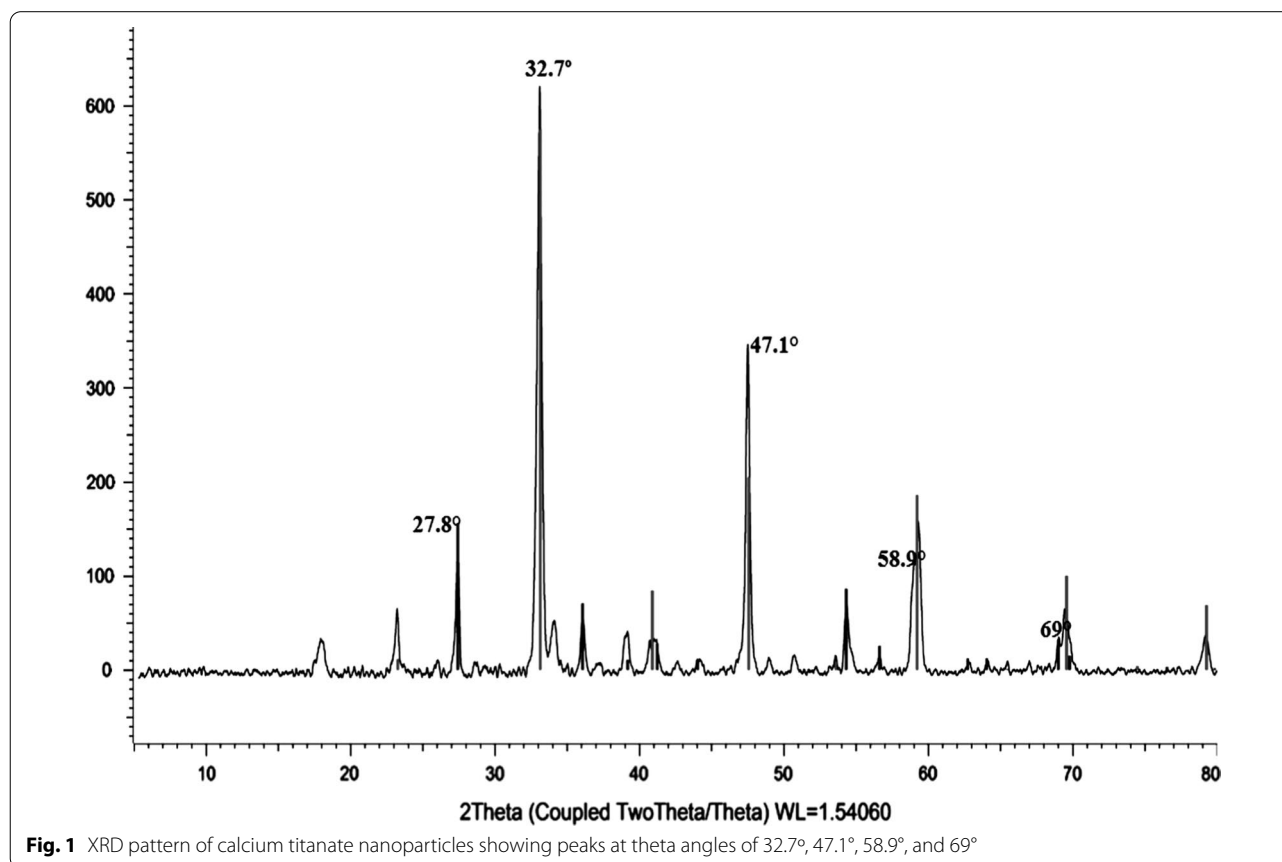
that CaTiO₃-NPs are well dispersed and have a spherical shape (Fig. 3).

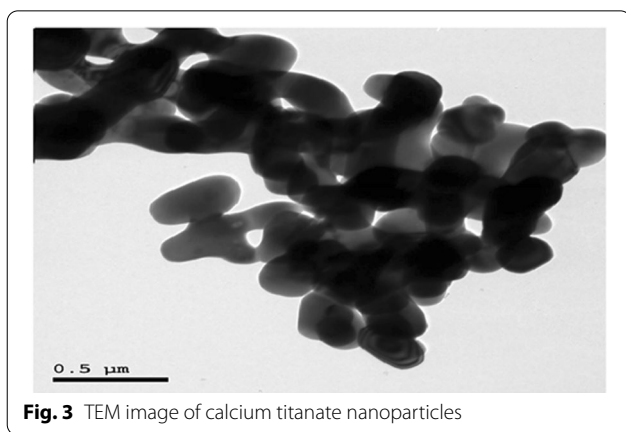
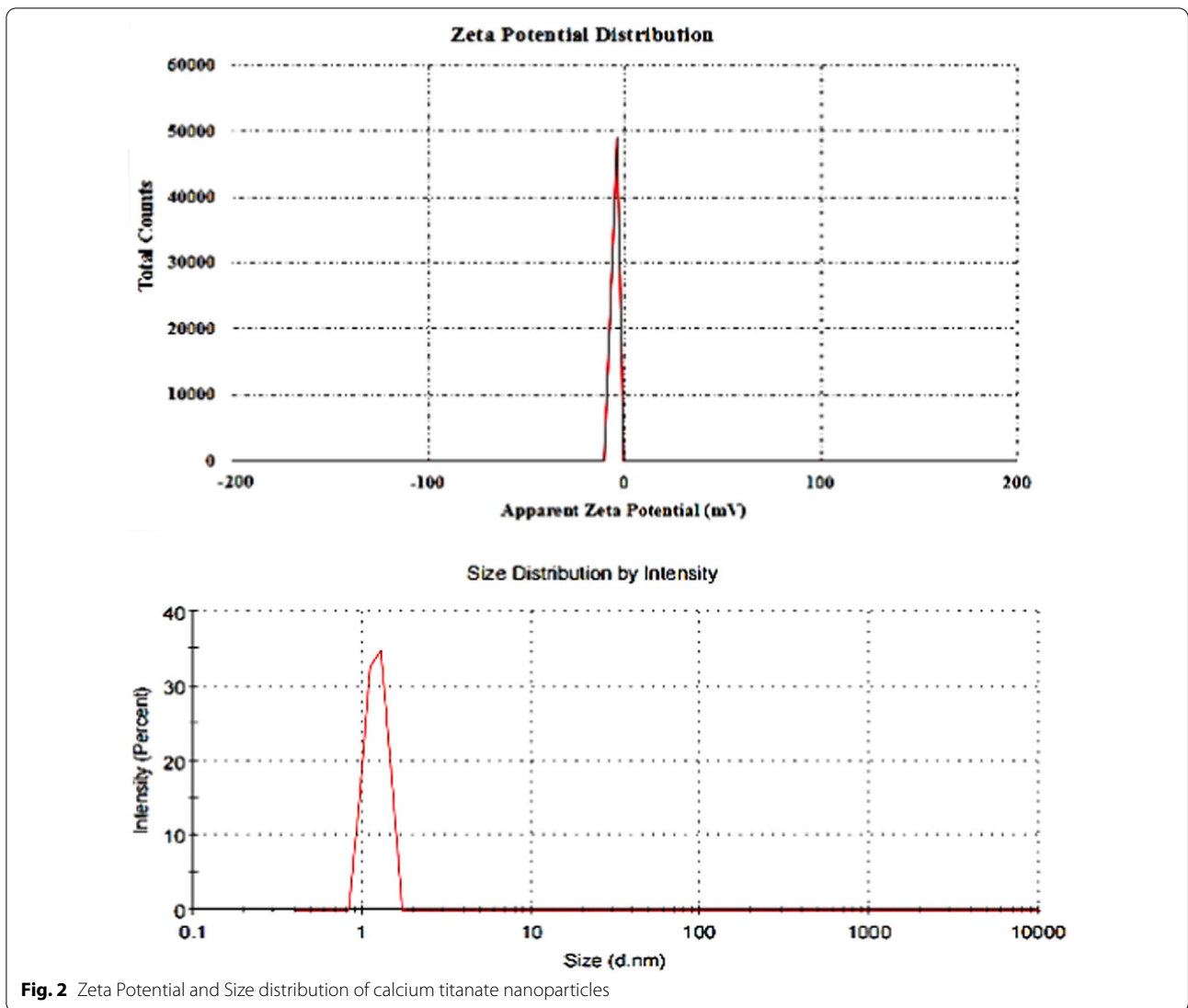
Cells viability

The results of the SRB assay showed that treatment of MCF-7 cells with CaTiO₃-NPs concentrations 0.01, 0.1, 1, 10 and 100 µg/ml for 24 h did not affect cell viability and slight cell death was observed in cells treated with CaTiO₃-NPs concentration greater than 10 µg/ml, and so the half maximal inhibitory concentration (IC₅₀) of CaTiO₃-NPs was greater than 100 µg/ml in cancer MCF-7 cells (Fig. 4). However, exposure of MCF-7 cells to CaTiO₃-NPs five concentration 0.1, 1, 10, 100 and 1000 µg/ml for 72 h markedly decreased cells viability in a concentration dependent manner and the IC₅₀ values for CaTiO₃-NPs was 71.82 µg/ml in MCF-7 cells (Fig. 4).

Genomic instability

As displayed in Table 2 treatment of MCF-7 cancer cells with IC₅₀ (71.82 µg/ml) of CaTiO₃-NPs for 72 significantly increased ($p < 0.05$) tail length and tail moment but the % DNA in the tail was not significantly changed compared to their values in MCF-7 untreated control cells. Representative examples for the examined and scored





Comet nuclei with intact and damaged DNA were shown in Fig. 5.

Intracellular ROS production

As shown in Fig. 6, the level of intracellular ROS production was markedly elevated in MCF-7 cancer cells treated with CaTiO₃-NPs at a 71.82 μg/ml (IC₅₀) concentration level compared to that of the untreated control MCF-7 cells. This high ROS generations was manifested by high increases observed in the intensities of fluorescent light emitted from MCF-7 cells stained with a 2,7-dichloro-fluorescein diacetate dye compared to light emitted from untreated MCF-cells (Fig. 6).

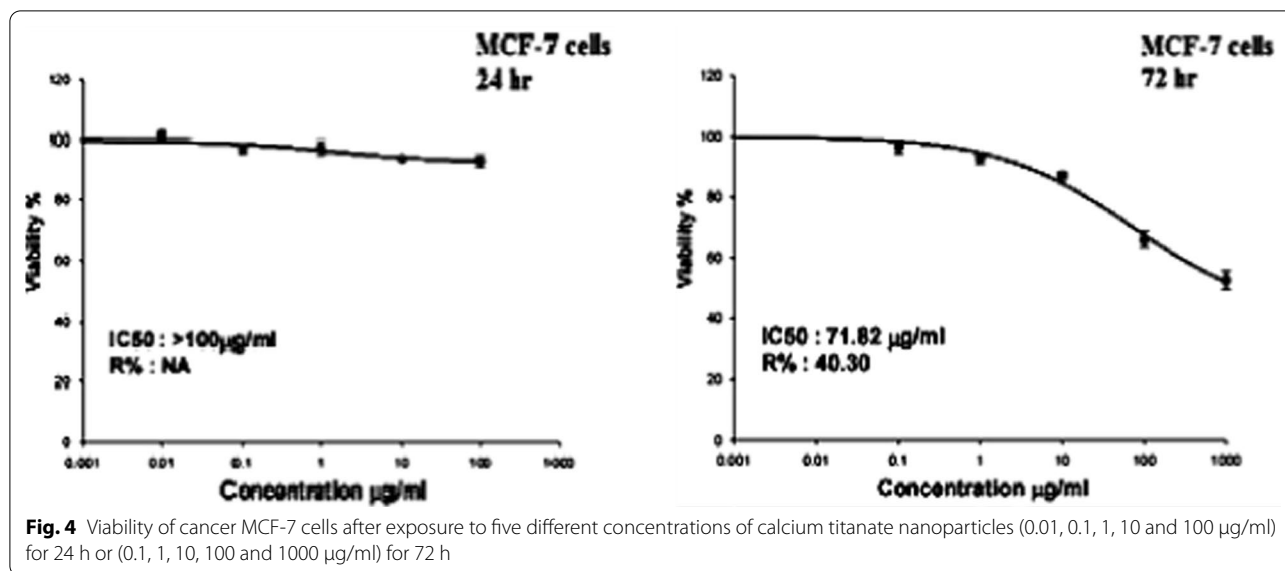


Table 2 Tail length, %DNA in tail and tail moment in the control and treated MCF-7 cells with IC50 of Calcium Titanate nanoparticles

Cell line	Treatment	Tail length	%DNA in tail	Tail moment
MCF-7	Control	5.18 ± 1.07	31.45 ± 5.4	1.93 ± 0.57
	treated	11.91 ± 0.65 ^a	34.91 ± 3.09	4.89 ± 0.79 ^a

Results are expressed as mean ± SD

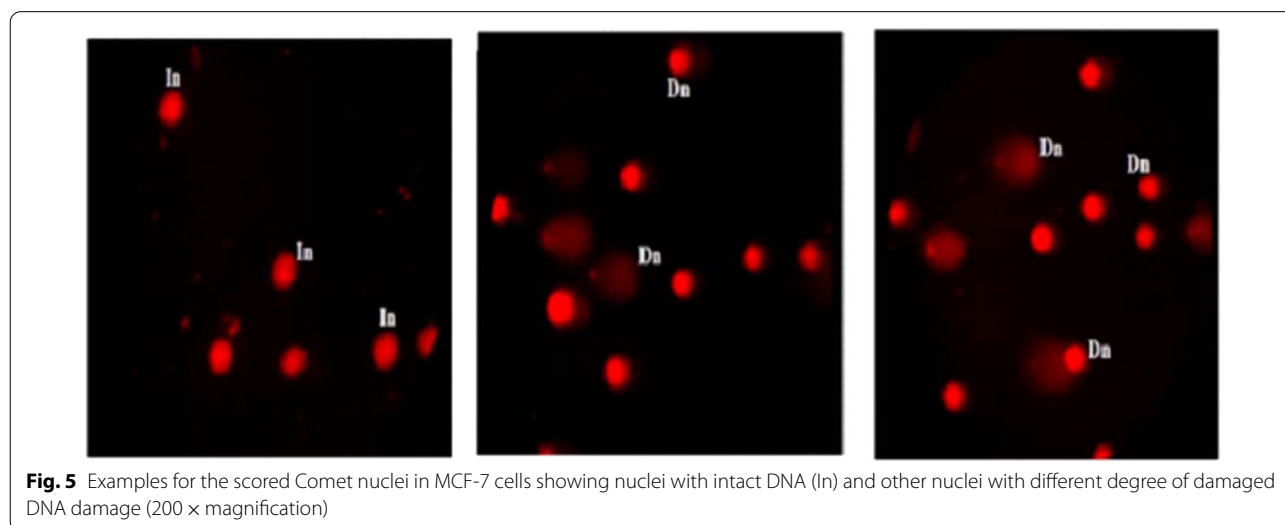
^a Indicates statistical significant difference from the compared control at p < 0.05 using student t-test

The mRNA expressions levels of the P53, Bax and Bcl2 genes

Figure 7 showed that treatment with CaTiO₃-NPs IC₅₀ (71.82 µg/ml) for 72 h caused significant increases (p < 0.05) in the mRNA expression levels of p53 and Bax (apoptotic) genes and a significant decrease in Bcl2 (anti-apoptotic) gene expression compared to the control untreated MCF-7 expression levels.

Cell cycle distribution.

As obvious in Fig. 8 treatment of MCF-7 cells with CaTiO₃-NPs at a concentration of 71.82 µg/ml (IC₅₀) for 72 h caused cell cycle arrest at the G₀ and S phases of cell cycle as indicated by the significant increases



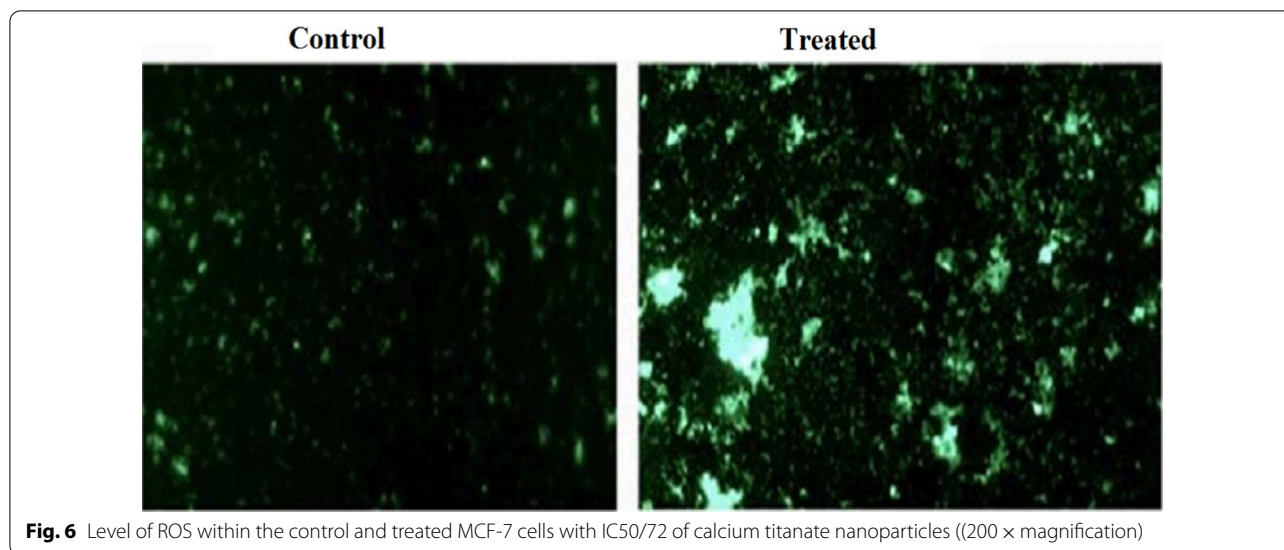


Fig. 6 Level of ROS within the control and treated MCF-7 cells with IC50/72 of calcium titanate nanoparticles ((200 x magnification)

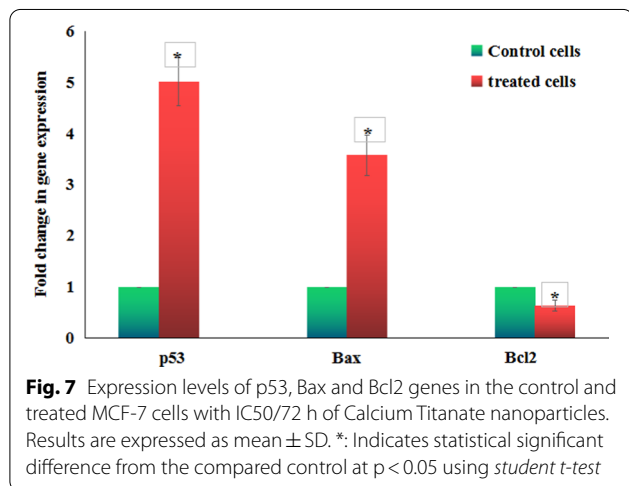


Fig. 7 Expression levels of p53, Bax and Bcl2 genes in the control and treated MCF-7 cells with IC50/72 h of Calcium Titanate nanoparticles. Results are expressed as mean ± SD. *: Indicates statistical significant difference from the compared control at $p < 0.05$ using *student t-test*

($p < 0.05$) noticed in the number of MCF-7 cells in the SubG1 (G0) and S phases of cell cycle compared to the MCF-7 control populations in these cell cycle phases. On the contrary, the number of MCF-7 cells in the G1 and G2 phases of the cell cycle was markedly decreased after treatment with CaTiO₃-NPs IC50 for 72 h compared to the control MCF-7 populations (Fig. 8).

Apoptosis induction

As noticed in Fig. 9 exposure of MCF-7 cells to a concentration level 71.82 µg/ml (IC50) of CaTiO₃-NPs for 72 h resulted in statistical significant elevations in the number of MCF-7 cells in the early and late apoptotic phases, while the number of necrotic MCF-7 cells was unchanged compared to the MCF-7 control cells.

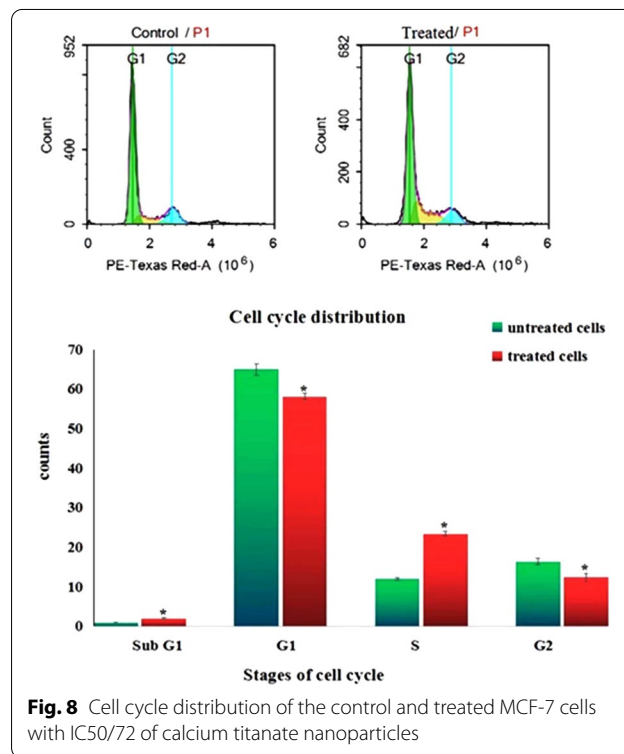
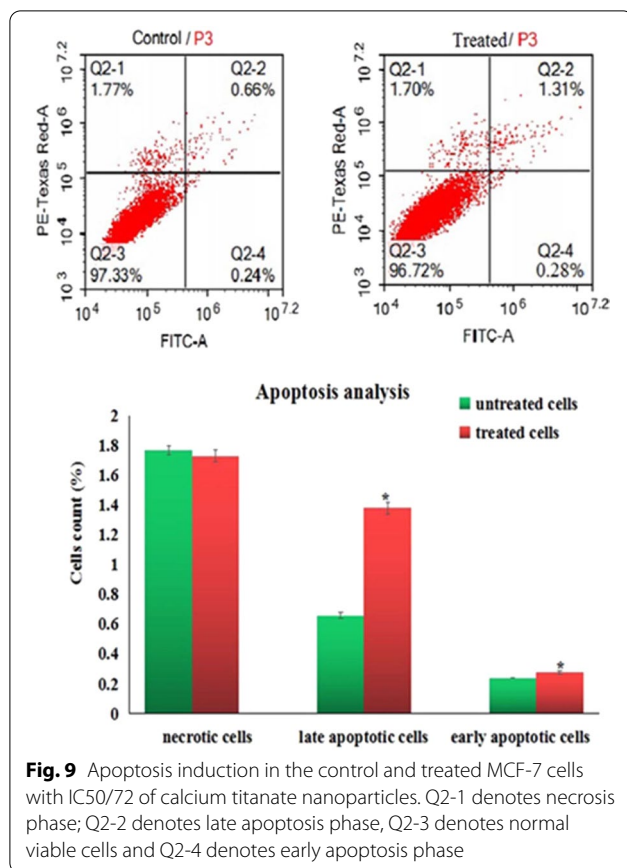


Fig. 8 Cell cycle distribution of the control and treated MCF-7 cells with IC50/72 of calcium titanate nanoparticles

Discussion

Recent discoveries of the antioxidant and microbial activities of CaTiO₃-NPs containing compounds together with a high capacity of CaTiO₃ to deliver calcium ions raise interest in the use of CaTiO₃-NPs in a wide range of medical applications [3, 18]. However, the cytotoxic and genotoxic effects of CaTiO₃-NPs in human MCF-7 cancer



cells almost have not been studied. Consequently, this study was performed to estimate CaTiO₃-NPs induced cytotoxicity, genomic instability and apoptosis in human cancer MCF-7 cells.

Although, the size of many nanoparticles measured by DLS is actually found to be larger than the size measured by TEM, there is no fixed rule for nanoparticles because the measured size of nanoparticles depends on the concentration of nanoparticles, ions and other variables. Consequently, the lower measured size of CaTiO₃-NPs in this study by Dynamic Laser Scattering (DLS) compared to that measured by TEM in our recent published manuscript [19] may result from using highly diluted samples in DLS and also the low Zeta potential value of -3.38 mv makes CaTiO₃-NPs highly aggregated in consistency with recent study [20] that showed that size of CaTiO₃-NPs by DLS was ranged from 15–30 nm, while sized measured by TEM was larger and ranged from 10–100 nm.

The results of the SRB cytotoxicity assay demonstrated that CaTiO₃-NPs is cytotoxic towards cancerous as manifested by the observed concentration-dependent reductions the MCF-7 cells viability after 72 h of CaTiO₃-NPs

treatment in consistent with the reported cytotoxic effects of normal-sized CaTiO₃ in previous studies [4, 5].

The inhibition of MCF-7 cells proliferation and cytotoxic effects of CaTiO₃-NPs demonstrated in this study can be attributed to the alterations in cell cycles distributions noticed after MCF-7 treatment with CaTiO₃-NPs for 72 h. Our finding of the marked increases in the number of MCF-7 cells in the SubG1 (G0) and S phases of the cell cycle determined using flow cytometry revealed that CaTiO₃-NPs caused MCF-7 cell cycle arrest at the G0/S phases and thus inhibited cells proliferation.

Reactive oxygen species (ROS) are highly reactive molecules and therefore increased ROS generations disrupt the equilibrium between oxidants and antioxidants within cells and harm cellular macromolecules: lipids, proteins, carbohydrates, and DNA [21]. Therefore, the CaTiO₃-NPs induced toxicity described in this study may result from the marked elevations in the intracellular ROS level observed after 72 h of MCF-7 treatment with IC50 of CaTiO₃-NPs.

Excessive ROS also attack DNA results in DNA breaks that severely damage DNA [22]. Results of Comet assay manifested DNA damage induction by CaTiO₃-NPs through the high increases in tail length, %DNA in tail and tail moment observed after 72 h of MCF-7 exposure to IC50 of CaTiO₃-NPs. Excessive intracellular ROS and high DNA breakages act as signals for various intracellular processes and stimulate apoptosis through upregulation of apoptotic genes and downregulation of anti-apoptotic genes expressions [23]. Consistency, apoptosis of MCF-7 cells demonstrated in this study after exposure to CaTiO₃-NPs for 72 h by the high elevations in MCF-7 counts detected in the early and late apoptotic phases could be attributed to the above described extra-production of ROS and DNA damage induction by CaTiO₃-NPs.

This was further confirmed by the demonstrated significant elevations in the expression levels of apoptotic p53 and Bax genes and a significant decrease in the anti-apoptotic Bcl2 gene expression after exposure of MCF-7 cells to CaTiO₃-NPs since p53 stimulates apoptosis and regulates the expression levels of apoptotic Bax gene and the anti-apoptotic Bcl2 gene [24, 25].

The cytotoxic and genotoxic effects seen after treatment of MCF-7 cells with IC50 of CaTiO₃-NPs for 72 h may also be explained by genomic instability and deregulation of different DNA repair pathways in highly proliferating MCF-7 cancer cells that makes MCF-7 cancer cells highly susceptible to DNA damage [26].

Similarly, treatment of normal cells with CaTiO₃-NPs caused the time and concentration-dependent death of normal human skin fibroblast (HSF) cells. However, the genomic integrity of normal HSF cells was still

normal and not disrupted after treatment with IC50/72 of CaTiO₃-NPs for 72 h [27].

Conclusion

Collectively from the above results, CaTiO₃-NPs induced concentration dependent cytotoxicity in cancerous MCF-7 cells. However, exposure to CaTiO₃-NPs IC50 inhibited MCF-7 cells proliferation through extra-generation of ROS that attack and damage genomic DNA damage stimulating apoptosis of MCF-7 cells by altering the expression levels of the p53, Bax and Bcl2 genes. More studies are thus recommended to further understand the toxic effects of CaTiO₃-NPs.

Acknowledgements

Many thanks and appreciation to the Department of Zoology, Faculty of Science, Cairo University, for providing chemicals and equipment required for conducting experiments.

Author contributions

HRHM: designed the study and conducted the molecular experiments, wrote manuscript and performed statistical analysis. MMH: performed experiments and wrote manuscript. All authors read and approved the final manuscript.

Funding

Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB). The present work was partially funded by Faculty of Science Cairo University and Faculty of Biotechnology, October University for Modern Sciences and Arts (MSA) Egypt.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Declarations

Ethical approval and Consent to participation.

Not applicable.

Consent for publication

Not applicable.

Competing interests

Author declared that there is no conflict of interest.

Author details

¹Zoology Department Faculty of Science, Cairo University, Giza, Egypt. ²Faculty of Biotechnology, October University for Modern Sciences and Arts, Giza, Egypt.

Received: 18 July 2022 Accepted: 4 November 2022

Published online: 15 November 2022

References

- Kizuki T, Takadama H, Matsushita T, Nakamura T, Kokubo T. Preparation of bioactive Ti metal surface enriched with calcium ions by chemical treatment. *Acta Biomater.* 2010;6(7):2836–42.
- Bakland LK, Andreassen JO. Will mineral trioxide aggregate replace calcium hydroxide in treating pulpal and periodontal healing complications subsequent to dental trauma? A review. *Dental Traumatol.* 2012;28(1):25–32.
- Pjetursson BE, Zwahlen M, Lang NP. Quality of reporting of clinical studies to assess and compare performance of implant-supported restorations. *J Clin Periodontol.* 2012;39(12):139–59.
- Hobbs DT, Messer RLW, Lewis JB, Click DR, Lockwood PE, Wataha JC. Adsorption of biometals to monosodium titanate in biological environments. *J Biomed Mater Res B Appl Biomater.* 2006;78(2):296–301.
- Davis RR, Lockwood PE, Hobbs DT, et al. In vitro biological effects of sodium titanate materials. *J Biomed Mater Res Part B Appl Biomater.* 2007;83(2):505–11.
- Davis RR, Hobbs DT, Khashaba R, et al. Titanate particles as agents to deliver gold compounds to fibroblasts and monocytes. *J Biomed Mater Res Part A.* 2010;93(3):864–9.
- Drury JL, Chen Y-W, Wong JJ, et al. Titanates deliver metal compounds to suppress cell metabolism. *J Exp Clin Med.* 2014;6(1):21–7.
- Drury JL, Jang Y, Taylor-Pashow KML, Elvington M, Hobbs DT, Wataha JC. *In vitro* biological response of micro- and nano-sized monosodium titanates and titanate-metal compounds. *J Biomed Mater Res B Appl Biomater.* 2015;103(2):254–60.
- Murray CB, Kagan CR, Bawendi MG. Synthesis and characterization of monodisperse nanocrystals and close-packed nanocrystal assemblies. *Annu Rev Mater Sci.* 2000;30:545–610.
- Reeves JF, Davies SJ, Dodd NJF, Jha AN. Hydroxyl radicals (•OH) are associated with titanium dioxide (TiO₂) nanoparticles-induced cytotoxicity and oxidative DNA damage in fish cells. *Mutation Res.* 2008;640(113):122.
- Jeon YM, Park SK, Rhee SK, Lee MY. Proteomic profiling of the differentially expressed proteins by TiO₂ nanoparticles in mouse kidney. *Mol Cell Toxicol.* 2010;6:419–25.
- Allam RM, Al-Abd AM, Khedr A, Sharaf OA, et al. Fingolimod interrupts the cross talk between estrogen metabolism and sphingolipid metabolism within prostate cancer cells. *Toxicol Lett.* 2018;291:77–85.
- Tice RR, Agurell E, Anerson D, Burlinson B, Hartmann A, Kobayashi H, et al. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environ Mol Mutagen.* 2000;35:206–21.
- Langie SA, Azqueta A, Collins AR. The comet assay: past, present, and future. *Front Genet.* 2015;6:266.
- Siddiqui MA, Kashyap MP, Kumar V, Al-Khedhairi AA, Musarrat J, et al. Protective potential of trans-resveratrol against 4-hydroxynonenal induced damage in PC12 cells. *Toxicol In Vitro.* 2010;24:1592–8.
- Suzuki K, Kazui T, Yoshida M, Uno T, Kobayashi T, Kimura T, et al. Drug-induced apoptosis and p53, BCL-2 and BAX expression in breast cancer tissues in vivo and in fibroblast cells in vitro. *Jpn J Clin Oncol.* 1999;29(7):323–31.
- Lai CY, Tsai AC, Chen MC, Chang LH, Sun HL, Chang YL, Chen CC, Teng CM, Pan SL. Aciclatin induces p53-dependent apoptosis via mdm2 depletion in human cancer cells in vitro and in vivo. *PLoS ONE.* 2013;7(8): e42192.
- Venkatesan K, Mathew AM, Sreya PV, Raveendran S, Archana Rajendran B, SubramanianPattanayak DK. Silver—calcium titanate—titanium decorated Ti6Al4V powders: an antimicrobial and biocompatible filler in composite scaffold for bone tissue engineering application. *Adv Powder Technol.* 2021;32(12):4576–86.
- Mohamed HRH, Ibrahim MMH, Soliman ESM, et al. Estimation of calcium titanate or erbium oxide nanoparticles induced cytotoxicity and genotoxicity in normal HSF cells. *Biol Trace Elem Res.* 2022. <https://doi.org/10.1007/s12011-022-03354-9>.
- Cesconeto FR, Borlaf M, Nieto MI, Novaes de Oliveira AP, Moreno R. Synthesis of CaTiO₃ and CaTiO₃/TiO₂ nanoparticulate compounds through Ca²⁺/TiO₂ colloidal sols: structural and photocatalytic characterization. *Ceram Int.* 2018;44(1):301–9.
- Mohamed HRH. Estimation of genomic instability and mitochondrial DNA damage induction by acute oral administration of calcium hydroxide normal- and nano- particles in mice. *Toxicol Lett.* 2019;304:1–12.
- Mohamed HRH. Induction of genotoxicity and differential alterations of p53 and inflammatory cytokines expression by acute oral exposure to bulk- or nano-calcium hydroxide particles in mice “Genotoxicity of normal- and nano-calcium hydroxide.” *Toxicol Mech Methods.* 2021;31(3):169–81.
- Chen M, Yin J, Liang Y, Yuan S, Wang F, Song M, Wang H. Oxidative stress and immunotoxicity induced by graphene oxide in zebrafish. *Aquat Toxicol.* 2016;174:54–60.

24. Di Micco R, Fumagalli M, Cicalese A, Piccinin S, Gasparini P, Luise C, Schurra C, Garre M, Nuciforo PG, Bensimon A, et al. Oncogene-induced senescence is a DNA damage response triggered by DNA hyper replication. *Nature*. 2006;444(7119):638–42.
25. Nehls O, Okech T, Hsieh CJ, Enzinger T, Sarbia M, Borchard F, Gruenagel HH, Gaco V, et al. Studies on p53, BAX and Bcl-2 protein expression and microsatellite instability in stage III (UICC) colon cancer treated by adjuvant chemotherapy: major prognostic impact of proapoptotic BAX. *Br J Cancer*. 2007;96(9):1409–18.
26. Alhmoud JF, Woolley JF, Al Moustafa AE, Malki MI. DNA damage/repair management in cancers. *Cancers*. 2020;12(4):1050.
27. Mohamed HRH, Ibrahim MMH, Soliman ESM, Safwat G, Diab A. Estimations of calcium titanate or erbium oxide nanoparticles induced cytotoxicity and genotoxicity in normal HSF Cells. *Biol Trace Elem Res*. 2022. <https://doi.org/10.1007/s12011-022-03354-9>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

