Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Cytotoxic Study of Zinc Oxide Nanoparticles on Cervical Cancer Cell Line

Harshyini Maheswaran[1], Sinouvassane Djearamane[1,4*], Anto Cordelia Tanislaus Antony Dhanapal[2], Wong Ling Shing[3]

[1]Department of Biomedical Sciences, Faculty of Science, UniversitiTunku Abdul Rahman (UTAR), Kampar Campus, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia
[2]Department of Chemical Science, Faculty of Science, UniversitiTunku Abdul Rahman (UTAR), Kampar Campus, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia
[3]Faculty of Health and Life Sciences, INTI International University, Persiaran Perdana BBN, Putra Nilai, 71800 Nilai, Negeri Sembilan, Malaysia
[4]Biomedical Research Unit and Lab Animal Research Centre, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 602 105, India

Received – June 05, 2023; Revision – August 01, 2023; Accepted – October 31, 2023
Available Online – November 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(5).815.821

KEYWORDS

Zinc oxide nanoparticles

Chemical synthesis

HeLa cell

FESEM

EDX

MTT assay

ABSTRACT

The advancement of nanomedicine drugs as an outcome of nanotechnology offers tremendous potential to enhance cancer-fighting tactics. Scientists have begun studying the role of NPs in immunotherapy, an area that is particularly beneficial in treating malignancies. Conventional treatment of cancer uses medications known as chemotherapy that frequently cause adverse effects on healthy tissues. Zinc is a vital micronutrient for the well-being of humans; therefore, nanomaterials such as zinc oxide nanoparticles (ZnO NPs) are progressively appealing as cutting-edge medical agents with implementations like anticancer properties. A bottom-up approach was utilized to chemically produce the ZnO NPs, which were characterized using Field Emission Scanning Electron Microscope (FESEM) and Energy Dispersive X-ray analysis (EDX). MTT assays have been carried out to study the cell viability percentage against multiple ZnO NPs concentrations and durations. The white ZnO NPs displayed a diverse morphology within the nanoscale range, featuring rod and spherical shapes. This synthesis was confirmed through EDX, which revealed distinct peaks corresponding to zinc and oxygen, affirming the formation of pure ZnO NPs. MTT assay data showed that ZnO NPs had a dose and time-dependent cytotoxicity against HeLa cells. This observation suggests that the ZnO NPs possess the potential to combat cancer and may hold promise for applications in biomedical research, particularly in the development of anticancer drugs.

* Corresponding author
E-mail: drsino31@gmail.com (Sinouvassane Djearamane)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Cancer is a condition of unrestricted, random cell division and invasiveness, which is caused by environmental variables, including radiation and pollutants exposure, unhealthy lifestyles like a poorly balanced diet, and stress, as well as inherited genetics (Anand et al. 2008). Cancer is most commonly triggered by mutations or changes in the expression patterns of protooncogenes, tumour suppressor genes, and DNA repair genes. The focus of present cancer treatment is to kill the cancerous cells with chemotherapeutic medicines, typically administered through chemotherapy or radiation therapy. Nevertheless, these options frequently lead to several adverse consequences, owing to the effect on healthy neighbouring tissues (Sztandera et al. 2019; Amjad et al. 2022).

Nanotechnology enables a novel approach to biomedical operations at the molecular level. Nanoparticles (NPs) with sizes ranging from 1 to 100 nm show distinct properties that vary significantly from fine particles or bulk materials (Moore and Chow 2021). NPs used in biomedical studies are extremely diversified in size, structure, morphology, chemical composition, hydrophobicity, electrostatic charge, surface chemistry, and other characteristics (Sahu et al. 2021). Nanomaterials are widely used in cosmetics, sunscreens, sporting goods, electronics, tyres, and other everyday items (Sim and Wong 2021). Furthermore, nanotechnologies have transformed medical advances, especially in imaging, diagnostic methods, and drug delivery. NPs can diagnose cancer because of the leaky characteristics of malignancy blood vessels that allow NPs to penetrate and accumulate in cancer cells due to their minuscule size (Gavas et al. 2021). Nanoxel, Eligard, and Onivyde are a few examples of FDA-approved nanomedicines (Sim and Wong 2021).

Zinc oxide nanoparticles (ZnO NPs) are among the top essential metal oxides applied in materials science on account of their noteworthy physical, chemical, and biological characteristics such as biocompatibility, environmental friendliness, relatively inexpensive, and non-toxicity (Maheswaran et al. 2021). Reports have shown that ZnO NPs also have antibacterial (Sirelkhatim et al. 2015), antifungal (Ahmadpour Kermani et al. 2021), antiviral (Kumar et al. 2018), wound healing (Metwally et al. 2022), and many other notable biopotencies. Furthermore, ZnO NPs notably impact cancer cells (Dobrucka et al. 2017; Rajeshkumar et al. 2018; Zhao et al. 2018; Motazedi et al. 2020). Zinc ion (Zn^{2+}), a soluble form of ZnO, is a trace element the human body requires and participates in enzymatic reactions throughout protein and nucleic acid production (Singh et al. 2021; Mandal et al. 2022). The Food and Drug Administration (FDA) lists zinc oxide as one of the harmless metal oxides that can be applied in the food industry (Shaba et al. 2021). Thus, the application of ZnO NPs seems to be non-toxic to normal tissues while being effective enough to act against malignant apoptosis sensitization (Wang et al. 2009; Hassan et al. 2017; Singh et al. 2021).

The chromogenic MTT (dimethylthiazol-diphenyltetrazolium bromide) assay developed by Mosmann (1983) is a redox chemical reaction extensively used as a cell metabolic activity assay. It quantifies metabolic activity because it is primarily based on tetrazolium ring cleavage in active mitochondria, and therefore, the action takes place merely in alive cells (Pintor et al. 2020). Consequently, it is frequently utilized to evaluate cell viability or drug cytotoxicity. Yellow tetrazolium MTT salt undergoes a redox reaction to become formazan by a plethora of intracellular oxidoreductase enzymes, prominently nicotinamide adenine dinucleotide (NADH) reductase (Luis et al. 2019).

In this study, we strived to investigate the cytotoxicity of chemically synthesized ZnO NPs at various concentrations (3.13, 6.25, 12.5, 25, 50 and 100 µg/mL) and durations (24 hrs, 48 hrs and 72 hrs) on cervical (HeLa) cell lines using MTT Assay.

2 Materials and Methods

2.1 Synthesis of ZnO NPs

To chemically synthesize ZnO NPs, 1 mol of sodium hydroxide (NaOH) was added dropwise into 0.5 mol of zinc nitrate ($Zn(NO_3)_2$). The solution was continuously stirred for two hours at 50 °C. After that, the white suspension was spun in a centrifuge at a speed of 3000 revolutions per minute (rpm) for 10 minutes. The sediment was rinsed using distilled water and centrifuged again. The resultant pellet was transferred into a crucible and calcinated at 400 °C for two hours.

2.2 Characterization of ZnO NPs

FESEM was employed to study the size in addition to the morphology of the ZnO NPs. EDX was implemented to characterize the chemical make-up of the ZnO NPs.

2.3 Cultivation of HeLa Cells

The human cervical cancer cell line (HeLa) was bought from the American Type Culture Collection (ATCC, USA). The cells were cultivated in a 25 mL flask with Dulbecco's Modified Eagle's Medium (DMEM), enriched with 10% fetal bovine serum (FBS), and incubated in a 37 °C incubator with 5% CO_2. The cells were passaged into four flasks every week using Trypsin-EDTA.

2.4 Cytotoxicity Assessment

The cytotoxic impact of ZnO NPs on cancer cells was assessed using the MTT assay. Once the cells were about 70% confluent, they were trypsinized and seeded in 96-well plates. After 24 hours, the cells were treated with varying ZnO NPs concentration for 24, 48 and 72 hours. A volume of 20 µL of 5 mg/mL MTT solution was put into each well, and the plate was kept in the dark for 4 hours. The

supernatant was then aspirated, and 100 µL of dimethyl sulfoxide (DMSO) was introduced to every well. At 550 nm, the absorbance was measured using a microplate reader. The following equation was used to quantify the percentage of cell viability using optical density (OD), and the graph of the percentage of cellular viability against ZnO NPs concentrations was plotted. Negative control and Cisplatin acted as the positive control were used in the experiment.

$$\text{Percentage of cellular viability (\%)} = \frac{\text{OD of treated cells}}{\text{OD of negative control}} \times 100 \quad \text{(Eq.1)}$$

3 Results

3.1 Synthesis of ZnO NPs

Chemical ZnO NPs were effectively produced through the precipitation process. Zinc nitrate and sodium hydroxide were used as the precursors. Figure 1 (a) shows the white suspension formed after continuous stirring for two hours. Figures 1 (b) and 1 (c) depict the white pellet after washing with distilled water and the ZnO NPs powder after heat treatment at 400 °C, respectively.

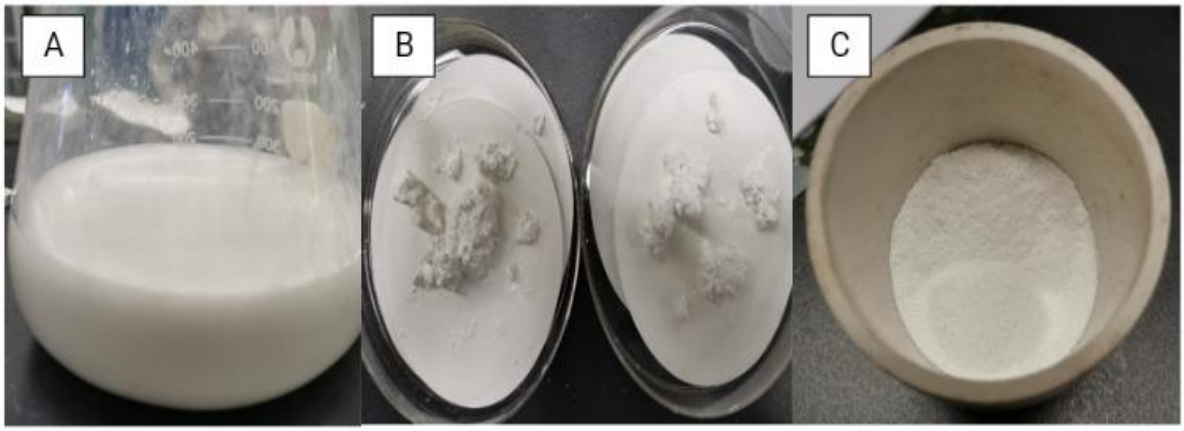

Figure 1 Process of chemical synthesis of ZnO NPs (a) white suspension of zinc hydroxide ($Zn(OH)_2$), (b) $Zn(OH)_2$ pellet after washing, (c) ZnO NPs powder after calcination.

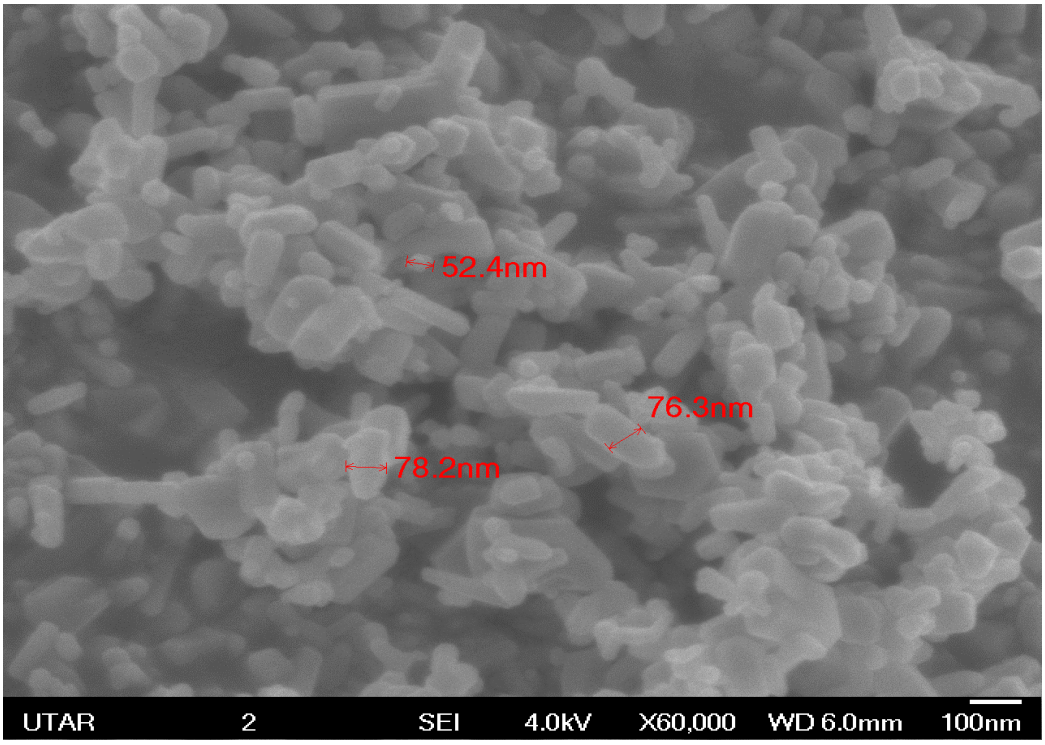

Figure 2 FESEM imaging of chemically synthesized ZnO NPs.

3.2 Characterization of ZnO NPs

3.2.1 Field Emission Scanning Electron Microscope (FESEM)

FESEM examination of the chemically synthesized ZnO NPs at a magnification of 60000x was carried out. Figure 2 revealed rod and spherical-shaped particles with smooth surfaces and some agglomeration. The size of the ZnO NPs was within the nano scale, ranging between 52.4 – 78.2 nm.

3.2.2 Energy dispersive X-ray (EDX)

EDX investigation of the synthesized ZnO NPs was performed to study the chemical components. Figure 3 illustrates peak absorbance corresponding to zinc and oxygen molecules in Spectrum 1, affirming the synthesis of clean ZnO. The inset image shows the percentage of zinc, oxygen, and carbon in the ZnO NPs sample.

3.3 Cytotoxicity Assessment

HeLa cells were exposed to ZnO NPs at concentrations of 3.125, 6.25, 12.5, 25, 50 and 100 µg/mL for 24, 48, and 72 hours and the cytotoxicity was evaluated through MTT assay. The cytotoxic consequence of ZnO NPs revealed that the growth inhibitory activity against HeLa cells was dose and time-dependent, as noted in Figure 4. HeLa cells were also treated with similar concentrations and durations of Cisplatin, the positive control. All the tested concentrations documented distinguishable elevated cytotoxicity effects (Figure 5).

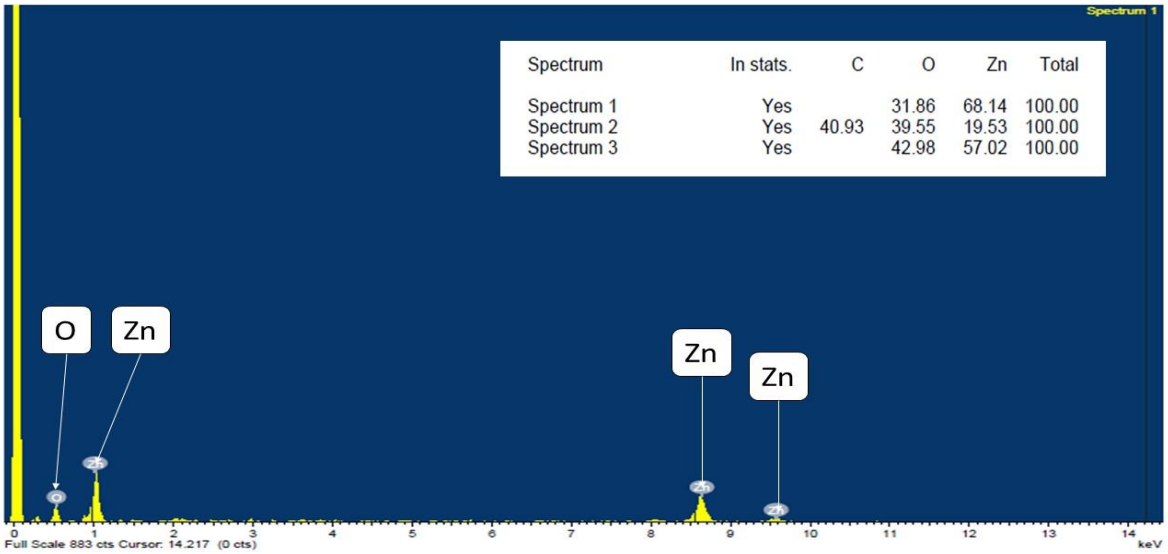

Figure 3 EDX of chemically synthesized ZnO NPs.

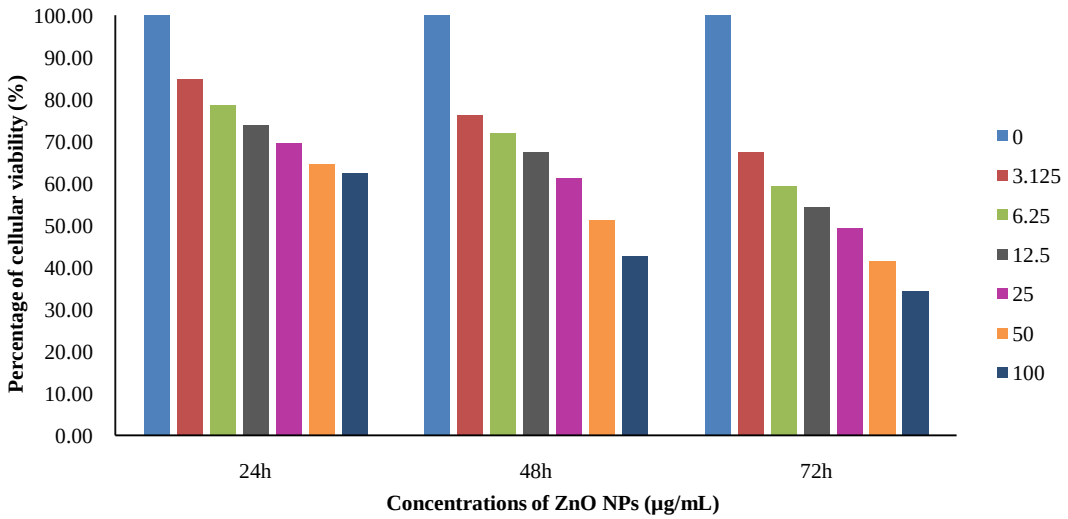

Figure 4 Percentages of cell viability on HeLa cancer cells upon treatment with ZnO NPs.

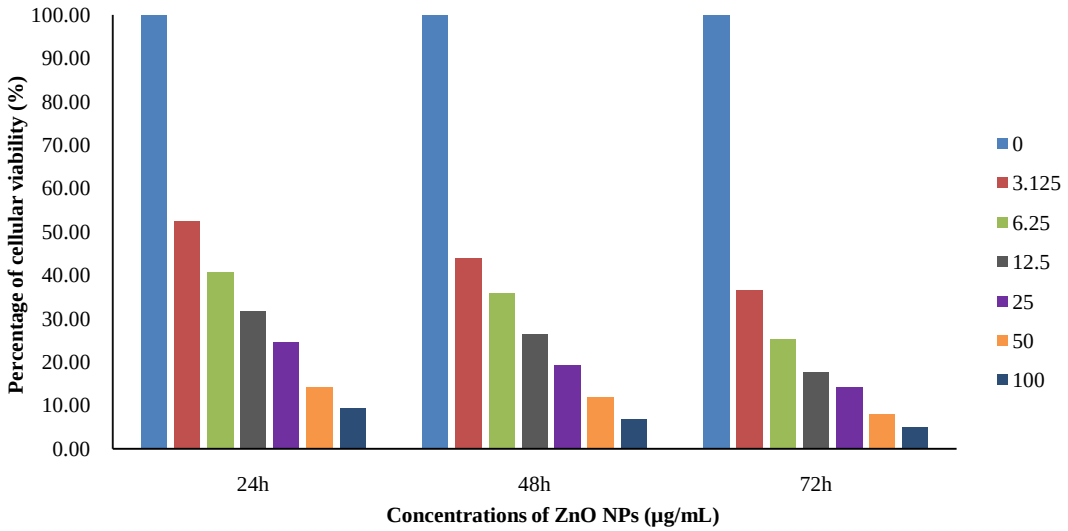

Figure 5 Percentages of cell viability on HeLa cancer cells upon treatment with Cisplatin.

4 Discussion

The chemical synthesis of ZnO NPs is depicted in Figure 1. Zinc nitrate was the precursor salt, and sodium hydroxide was the reducing agent. Once both the chemicals were mixed, a white suspension (Namasivayam et al. 2020) of $Zn(OH)_2$ formed, which was rinsed with distilled water to eliminate any residues from the synthesis (Norouzi Jobie et al. 2021). During calcination, the water evaporates; consequently, $Zn(OH)_2$ was transformed into ZnO (Biron et al. 2020).

The SEM micrograph of the chemically synthesized ZnO NPs showed a mixture of rod and spherical-shaped particles, as observed in Figure 2. The NPs have an estimated size range of 52.4 – 78.2 nm in diameter, proving that the ZnO NPs are in the nano range. It was also observed that there was some form of agglomeration between the ZnO NPs. This could be because the synthesis did not include capping agents to regulate the size of the ZnO NPs (Biron et al. 2020). The EDX spectrum of the synthesized ZnO NPs shown in Figure 3 visualized a singular peak of an oxygen molecule at 0.5 keV, and triple peaks of a zinc molecule were noted at 1.00, 8.65 and 9.60 keV. The elemental composition of ZnO NPs from the EDX spectrum inset indicated zinc and oxygen elements with a weight percentage of 68.14% and 31.86% of Spectrum 1, respectively. Similar EDX results were previously reported by Norouzi Jobie et al. (2021). Minimal carbon traces were noticed, which could be due to the contribution of carbon tape used during the EDX analysis (Taheraslani and Gardeniers 2019).

Viability assays such as MTT, a sensitive colourimetric assay, determine the cellular response to a toxicant. This study aimed to study the cytotoxic impact of ZnO NPs against HeLa cells at 24, 48 and 72 hrs, as evidenced in Figure 4. There was a notable decrease in cell viability at the highest concentration in 48 and 72 hrs, where the maximum cell death was 57.36% and 65.61%, respectively. IC_{50} of about 50 µg/mL and 25 µg/mL were recorded at 48 and 72 hrs. Although no IC_{50} was noted at 24 hrs, the cell viability decreased to 62.39% at 100 µg/mL. The data showed a predictable concentration and time-dependent cell viability loss of the HeLa cell line upon interaction with ZnO NPs. ZnO NPs showed considerable killing effects on the HeLa cell line. Figure 5 displays the percentage of cell viability on HeLa cancer cells upon treatment with the positive control, Cisplatin. The IC_{50} value of Cisplatin at 24 hrs was about 3.13 µg/mL; at 48 hrs and 72 hrs, it was lower than that. This indicated that Cisplatin is a good measure of positive control due to the cell mortality it brings about.

The cytotoxicity of the HeLa cell line against ZnO NPs increased with higher concentration and longer duration. The possible toxicity mechanism of ZnO NPs is that they generate ROS, which causes mitochondrial damage, lipid peroxidation and DNA damage. There is a possibility of membrane rupture and cytotoxicity when ZnO NPs enter the cell membrane (Ali et al. 2018). The action mechanism of ZnO NPs on cancerous cells could also most likely be based on the discharge of solubilized Zn^{2+} ions within the cells, which consequently destroyed the electron transport chain in the mitochondria by releasing a massive sum of ROS. This then causes mitochondrial dysfunction, protein activity imbalance, and the inevitable death of cancer cells by apoptosis signalling pathways (Khashan et al. 2020).

Conclusion

ZnO NPs were chemically synthesized using $Zn(NO_3)_2$ as the precursor. The synthesized ZnO NPs were analyzed using SEM

and EDX. SEM visualized that the ZnO NPs were a combination of rod and spherical-shaped particles with 52.4 – 78.2 nm in diameter. EDX analysis confirmed the yield of pure ZnO NPs. The cytotoxicity study of ZnO NPs on the HeLa cell line was studied using an MTT assay, which showed a typical cytotoxicity pattern. The study revealed a duration-dependent loss in the survival of the cells in tandem with increasing the dose of the ZnO NPs. Maximum cell death of 65.61% was observed at the highest concentration in 72 hrs on HeLa cells. This reveals that the synthesized ZnO NPs have cancer-killing potential and can be helpful in biomedical research for anticancer drug development.

Conflict of Interest

The authors have declared no conflicts of interest. All co-authors have read and reviewed the manuscript, and there are no financial conflicts to disclose.

References

Ahmadpour Kermani, S., Salari, S., & Ghasemi Nejad Almani, P. (2021). Comparison of antifungal and cytotoxicity activities of titanium dioxide and zinc oxide nanoparticles with amphotericin B against different Candida species: In vitro evaluation. *Journal of clinical laboratory analysis*, *35*(1), e23577. https://doi.org/10.1002/jcla.23577

Ali, A., Phull, A.R., & Zia, M. (2018). Elemental zinc to zinc nanoparticles: Is ZnO NPs crucial for life? Synthesis, toxicological, and environmental concerns. *Nanotechnology Reviews*, *7*(5), 413–441. https://doi.org/10.1515/ntrev-2018-0067

Amjad, M. T., Chidharla, A., & Kasi, A. (2022). Cancer Chemotherapy. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK564367/

Anand, P., Kunnumakara, A. B., Sundaram, C., Harikumar, K. B., Tharakan, S. T., Lai, O. S., Sung, B., & Aggarwal, B. B. (2008). Cancer is a Preventable Disease that Requires Major Lifestyle Changes. *Pharmaceutical Research*, *25*(9), 2097–2116. https://doi.org/10.1007/s11095-008-9661-9

Biron, D., Santos, V., & Bergmann, C. (2020). Synthesis and Characterization of Zinc Oxide Obtained by Combining Zinc Nitrate with Sodium Hydroxide in Polyol Medium. *Materials Research*, *23*, e20200080. https://doi.org/10.1590/1980-5373-mr-2020-0080

Dobrucka, R., Dlugaszewska, J., & Kaczmarek, M. (2017). Cytotoxic and antimicrobial effects of biosynthesized ZnO nanoparticles using of Chelidonium majus extract. *Biomedical Microdevices*, *20*(1), 5. https://doi.org/10.1007/s10544-017-0233-9

Gavas, S., Quazi, S., & Karpiński, T. M. (2021). Nanoparticles for Cancer Therapy: Current Progress and Challenges. *Nanoscale Research Letters*, *16*, 173. https://doi.org/10.1186/s11671-021-03628-6

Hassan, H. F. H., Mansour, A. M., Abo-Youssef, A. M. H., Elsadek, B. E. M., & Messiha, B. A. S. (2017). Zinc oxide nanoparticles as a novel anticancer approach; in vitro and in vivo evidence. *Clinical and Experimental Pharmacology & Physiology*, *44*(2), 235–243. https://doi.org/10.1111/1440-1681.12681

Khashan, K. S., Sulaiman, G. M., Hussain, S. A., Marzoog, T. R., & Jabir, M. S. (2020). Synthesis, Characterization and Evaluation of Antibacterial, Anti-parasitic and Anticancer Activities of Aluminum-Doped Zinc Oxide Nanoparticles. *Journal of Inorganic and Organometallic Polymers and Materials*, *30*(9), 3677–3693. https://doi.org/10.1007/s10904-020-01522-9

Kumar, R., Sahoo, G., Pandey, K., Nayak, M. K., Topno, R., Rabidas, V., & Das, P. (2018). Virostatic potential of zinc oxide (ZnO) nanoparticles on capsid protein of cytoplasmic side of chikungunya virus. *International Journal of Infectious Diseases*, *73*, 368. https://doi.org/10.1016/j.ijid.2018.04.4247

Luis, C., Castaño-Guerrero, Y., Soares, R., Sales, G., & Fernandes, R. (2019). Avoiding the Interference of Doxorubicin with MTT Measurements on the MCF-7 Breast Cancer Cell Line. *Methods and Protocols*, *2*(2), Article 2. https://doi.org/10.3390/mps2020029

Maheswaran, H., Wong, L. S., Dhanapal, A. C. T. A., Narendhirakannan, R. T., Janakiraman, A. K., & Djearamane, S. (2021). Toxicity of Zinc Oxide Nanoparticles on Human Skin Dermal Cells. *Journal of Experimental Biology and Agricultural Sciences*, *9*(Spl-1-GCSGD_2020), S95-S100. https://doi.org/10.18006/2021.9(Spl-1-GCSGD_2020).S95.S100.

Mandal, A. K., Katuwal, S., Tettey, F., Gupta, A., Bhattarai, S., et al. (2022). Current Research on Zinc Oxide Nanoparticles: Synthesis, Characterization, and Biomedical Applications. *Nanomaterials*, *12*(17), Article 17. https://doi.org/10.3390/nano12173066

Metwally, A. A., Abdel-Hady, A.N. A. A., Haridy, M. A. M., Ebnalwaled, K., Saied, A. A., & Soliman, A. S. (2022). Wound healing properties of green (using Lawsonia inermis leaf extract) and chemically synthesized ZnO nanoparticles in albino rats. *Environmental Science and Pollution Research*, *29*(16), 23975–23987. https://doi.org/10.1007/s11356-021-17670-5

Moore, J. A., & Chow, J. C. L. (2021). Recent progress and applications of gold nanotechnology in medical biophysics using artificial intelligence and mathematical modeling. *Nano Express*, *2*(2), 022001. https://doi.org/10.1088/2632-959X/abddd3

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, *65*(1–2), 55–63. https://doi.org/10.1016/0022-1759(83)90303-4

Motazedi, R., Rahaiee, S., & Zare, M. (2020). Efficient biogenesis of ZnO nanoparticles using extracellular extract of Saccharomyces cerevisiae: Evaluation of photocatalytic, cytotoxic and other biological activities. *Bioorganic Chemistry*, *101*, 103998. https://doi.org/10.1016/j.bioorg.2020.103998

Namasivayam, S. K. R., Shyamsundar, D., Prabanch, M. M., Bharani, R. A., & Avinash, G. P. (2020). Inhibitory potential of molecular mechanism of pathogenesis with special reference to biofilm inhibition by chemogenic zinc oxide nanoparticles. *Letters of Applied NanoBioScience*, *10*, 1862–1870.

Norouzi Jobie, F., Ranjbar, M., Hajizadeh Moghaddam, A., & Kiani, M. (2021). Green synthesis of zinc oxide nanoparticles using Amygdalus scoparia Spach stem bark extract and their applications as an alternative antimicrobial, anticancer, and anti-diabetic agent. *Advanced Powder Technology*, *32*(6), 2043–2052. https://doi.org/10.1016/j.apt.2021.04.014

Pintor, A. V. B., Queiroz, L. D., Barcelos, R., Primo, L. S. G., Maia, L. C., & Alves, G. G. (2020). MTT versus other cell viability assays to evaluate the biocompatibility of root canal filling materials: A systematic review. *International Endodontic Journal*, *53*(10), 1348–1373. https://doi.org/10.1111/iej.13353

Rajeshkumar, S., Kumar, S. V., Ramaiah, A., Agarwal, H., Lakshmi, T., & Roopan, S. M. (2018). Biosynthesis of zinc oxide nanoparticles using *Mangifera indica* leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. *Enzyme and Microbial Technology*, *117*, 91–95. https://doi.org/10.1016/j.enzmictec.2018.06.009

Sahu, T., Ratre, Y. K., Chauhan, S., Bhaskar, L. V. K. S., Nair, M. P., & Verma, H. K. (2021). Nanotechnology based drug delivery system: Current strategies and emerging therapeutic potential for medical science. *Journal of Drug Delivery Science and Technology*, *63*, 102487. https://doi.org/10.1016/j.jddst.2021.102487

Shaba, E. Y., Jacob, J. O., Tijani, J. O., & Suleiman, M. A. T. (2021). A critical review of synthesis parameters affecting the properties of zinc oxide nanoparticle and its application in wastewater treatment. *Applied Water Science*, *11*(2), 48. https://doi.org/10.1007/s13201-021-01370-z

Sim, S., & Wong, N. K. (2021). Nanotechnology and its use in imaging and drug delivery (Review). *Biomedical Reports*, *14*(5), 1–9. https://doi.org/10.3892/br.2021.1418

Singh, Th. A., Sharma, A., Tejwan, N., Ghosh, N., Das, J., & Sil, P. C. (2021). A state of the art review on the synthesis, antibacterial, antioxidant, antidiabetic and tissue regeneration activities of zinc oxide nanoparticles. *Advances in Colloid and Interface Science*, *295*, 102495. https://doi.org/10.1016/j.cis.2021.102495

Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N. H. M., Ann, L. C., et al. (2015). Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism. *Nano-Micro Letters*, *7*(3), 219–242. https://doi.org/10.1007/s40820-015-0040-x

Sztandera, K., Gorzkiewicz, M., & Klajnert-Maculewicz, B. (2019). Gold Nanoparticles in Cancer Treatment. *Molecular Pharmaceutics*, *16*(1), 1–23. https://doi.org/10.1021/acs.molpharmaceut.8b00810

Taheraslani, M., & Gardeniers, H. (2019). High-Resolution SEM and EDX Characterization of Deposits Formed by CH4+Ar DBD Plasma Processing in a Packed Bed Reactor. *Nanomaterials*, *9*(4), 589. https://doi.org/10.3390/nano9040589

Wang, H., Wingett, D., Engelhard, M. H., Feris, K., Reddy, K. M., et al. (2009). Fluorescent dye encapsulated ZnO particles with cell-specific toxicity for potential use in biomedical applications. *Journal of Materials Science: Materials in Medicine*, *20*(1), 11–22. https://doi.org/10.1007/s10856-008-3541-z

Zhao, C., Zhang, X., & Zheng, Y. (2018). Biosynthesis of polyphenols functionalized ZnO nanoparticles: Characterization and their effect on human pancreatic cancer cell line. *Journal of Photochemistry and Photobiology B: Biology*, *183*, 142–146. https://doi.org/10.1016/j.jphotobiol.2018.04.031