Review Article

Cytotoxic Activity of Zinc Oxide Nanoparticles against Cancer Cells: A Systematic Review

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ABSTRACT

Chemotherapy and radiation therapy have long been known as standard treatments for cancer. Despite the fact that these treatments appeared to be extremely effective against cancer cells, these non-selective therapies often lead to the failure of many biological processes, resulting in serious side effects. Recent studies have shown the capacity of nanoparticle-based treatment to target specific oncotic cells, thus overcoming these adverse side effects. The principal focus on the cytotoxic mechanism of these nanoparticles to induce apoptosis towards cancer cells makes them a promising anti-cancer agent. Zinc oxide nanoparticles (ZnO NPs) are one of the widely explored nanoparticles in cancer treatment methodology. This review outlined ZnO NP's unique physiochemical properties including its mechanisms with an essential focus given in size- and shape-induced cytotoxicity. The initial selection process identified a total of 3,204 literature works published from 2016 to the present year in various online databases such as ScienceDirect, PubMed, NCBI, and ResearchGate. The accumulated literature expound on the unique properties of the ZnO NPs, mechanism of cytotoxicity, and cytotoxic evaluation of the ZnO NPs towards cancer cells. Throughout the extensive analysis, the data gathered from this review had reported evidence of ZnO NP's anti-proliferative activity via different mechanisms of apoptosis-induced cytotoxicity. Smaller-sized nanoparticles appeared to be more potent than their large counterparts due to easier cell migration. The results of this review also concluded no direct correlation between the shape and cytotoxic potency, however, further studies are recommended to have a definite set of biological and exposure conditions when investigating shape-induced cytotoxicity.

Keywords: ZnO nanoparticles, Cytotoxicity, Cancer cells, Apoptosis, Reactive Oxygen Species (ROS).

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INTRODUCTION

Since the introduction of the nanotechnology field in 1959, there has been a steadily growing attraction to nano-based materials accompanying the creation of revolutionary research that provides an array of novel applications and improved technologies in biology and medicine. Nanoparticles (NPs) which are defined as 'tiny solid colloidal particles' with a dimension of less than 100 nm, are the most widely used nanomaterials largely as they offer ease of production from a variety of sources. Among its properties, the reason behind the intense interest in nanotechnology is because of its capacity to allow controlled synthesis of wide class materials at a nanoscale level.[1] The ultra-small size of nanoparticles, comparable to the size of the proteins and biomolecules that naturally occur in the cell, and smaller than many human cells, allows internalization and interaction of NPs within the cell surface, thus, enabling them to potentially influence cellular responses, dynamically and selectively.[2] This is why nanoparticles, especially metal oxide NPs, are used in many biomedical applications, including biosensing, targeted drug and gene delivery systems, bioimaging, and as well as in the provision of therapeutic interventions for cancer.[1,3]

Cancer, accompanying second place as the leading cause of mortality worldwide, is a disease wherein cells

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of the body multiply abnormally and potentially spread throughout other parts of the body.[4] There are various key differences between a normal and cancerous cell. One of which is their ability in cell signaling, also referred to as signal transduction, that controls basic cellular processes through various complex responses.[5] In normal cells, this signal transduction pathway generally ensures the coordination and constant intercellular communication between cells, thus, allowing cells to divide, differentiate, and even die. Unlike normal cells, the signal transduction pathways that regulate cell growth are usually disrupted and overridden in cancer cells. The overexpression and activation of oncogenes and the inhibition of the tumor suppressor genes that help cancer cells grow and metastasize results in the autonomous replication and resistance to apoptosis of cells. Other hallmark characteristics of cancer cells include abnormal metabolism, DNA damage, increased oxidative stress, and the ability to evade the body's immune cells.[5-6] Understanding the mechanisms of these characteristics has long been the focus of cancer research and is the key to finding a more effective cancer treatment.

In the past decades, chemotherapy and radiation therapy have long been commonly known as the standard treatments for cancer, which is characterized by the unchecked growth of malignant cells. However, even though these treatments appear to be extremely effective at killing cancer cells, many serious side effects have been associated with them, including myelosuppression, hair loss, neurotoxicity, and gastrointestinal reactions.[7] This is due to the indiscriminate mechanism of these treatments, which often leads to the failure of many biological processes. Although the surfacing of selective therapy has made its path, unavoidable adverse effects and the developing issue of drug resistance have still been a problem. Recent studies have shown that nanomaterial-based nanomedicine can overcome these chemotherapeutic side effects due to their high biocompatibility and a high degree of cancer cell selectivity.[8]

Zinc oxide (ZnO) nanoparticles, a remarkable wide optical bandgap (3.63 eV) semiconductor, are one of the most widely explored NPs in terms of cancer treatment methodology. This is due to the excellent cytotoxic ability of the ZnO NPs to induce selective apoptosis in cancerous cells with the liberation of dissolved metal ions leading to the high levels of Intracellular Reactive Oxygen Species (ROS) and oxidative stress.[9] Excess cellular ROS production causes damage in the cell and therefore, activates cell death such as apoptosis. The characteristic features of ZnO NPs that make them potent for cancer cells include: (1) Biocompatibility; (2) High selectivity; (3) Easy synthesis; and (4) Enhanced cytotoxicity. Additionally, with zinc as one of the most plentiful trace metals in human nutrition after iron, it plays a significant role in carrying cellular processes by responding to the repair and replication of DNA, oxidative stress, and the progression and apoptosis of the cell cycle.[10] With this, an increased alteration in zinc concentration can cause deleterious effects on cancer cells as an excess amount of zinc can cause disruption by exceeding the capacity of the zinc homeostasis system, ultimately activating apoptosis, hence the cell death.

A thorough understanding of the human body today, unfortunately, is not in part with the therapeutic cancer options provided to patients. Modern cancer therapies often fail to completely exhibit anti-cancer responses to drug resistance or their oversight in distinguishing between cancerous and normal cells. In response to this issue, this systematic review focuses on assessing the ZnO NPs' practical use as cancer treatments, which will greatly benefit medical institutions. If proven safe and effective, this may be utilized independently or in conjunction with existing cancer therapies such as radiation therapy, immunotherapy, chemotherapy, and other cancer treatments. Furthermore, given that nanoparticles can exist in various types of morphology due to their variation in synthesis, purification, and functionalization, an essential focus was given to their influence on the cytotoxic activity in terms of size- and shape-induced cytotoxicity.

MATERIALS AND METHODS

Literature Search

In conducting this review, the authors made use of research articles and journals to retrieve information regarding the cytotoxic effects of ZnO nanoparticles against cancer cells. Different search engines such as ScienceDirect, PubMed, National Center for Biotechnology Information (NCBI), and ResearchGate were used to collect data. Research articles and journals that were retrieved from these online databases were accessed in March 2022 and were consulted in April 2022 to verify the accumulated information.

Eligibility Criteria

The systematic review of Cytotoxic Effects of Zinc Oxide Nanoparticles Against Cancer Cells includes articles that met the following criteria: (1) cytotoxic effects of ZnO NPs against cells that are cancerous; (2) published studies from credible search engine sites such as ResearchGate, National Center for Biotechnology

(NCBI), PubMed, and Science Direct; (3) studies that were published in full-text English; and (4) studies that were published in 2016 and present year from credible search engine sites as aforementioned. Published studies that were excluded in this systematic review, however, have one of the following criteria: (1) articles that were published before 2016; (2) studies that are published from non-credible/predatory search engine sites; (3) studies that were produced in a foreign language other than English; and (4) studies that are not focused on the cytotoxic effectivity of the said nanoparticle.

Selection Strategy

Related literature were retrieved by inputting the combination of the following terms on credible search engines, including "Zinc Oxide," "Nanoparticles," "Cytotoxicity," "Cancer," and "Cancer cells." The dates of publication were also put into consideration in filtering the used sources, considering the requirement of only accessing articles and journals in the years 2016 to 2022. Additionally, the language chosen in the study is only English as it is the most convenient medium for a swift understanding of the published studies. Abstracts, potentially of foremost importance and value to the study conducted, were also reviewed and assessed thoroughly by the criteria given.

Data Extraction

The authors assessed the eligibility and anomalies of the chosen papers identified for inclusion. Further, both exclusion and inclusion criteria were established and taken into consideration before each eligible research article was chosen. Data from the eligible articles were further extracted with the use of appropriate study characteristics, which includes: (1) the title of the study or research article, (2) the year of publication, (3) the size and/or shape of the ZnO NPs, (4) specific cancer cell lines, (5) studies that utilized MTT assay as their cell viability assay, and (6) IC_{50} values of the cytotoxicity evaluation at 24 hr exposure. Detailed data extractions of each eligible article are shown in Table 1.

RESULTS

During the initial screening, a total of 3,204 research articles were retrieved from the chosen search engines, namely NCBI, ScienceDirect, PubMed, and ResearchGate. Duplicated studies were identified manually, removing a total of 86 duplicates and leaving a total of 3,118 literature sought for retrieval. Before the formal screening process, a total of 101 studies that are not available in full-text version were removed, with a total of 3,017 studies left to be screened. Based on the predetermined inclusion and exclusion criteria, the authors screened the title, abstract, and the year of publication of each literature, excluding 2,896 studies and leaving 121 articles to be assessed for eligibility. Each potential eligible research article was assessed using appropriate study characteristics, which were explicitly based on the size and/or shape of the ZnO NPs, cancer cell lines, and IC_{50} values of the cytotoxicity evaluation. After a full-text assessment for eligibility, a total of 15 studies were included in this review. A schematic diagram of the study selection process is shown in Figure 1.

Cytotoxicity of ZnO Nanoparticles against Various Cancer Cell Lines

The data of cytotoxicity of the eligible literature, which explicitly includes the shape, average particle size of ZnO NPs, specific cancer cell lines, and findings of each related literature, are presented in Table 1. With the precise evaluation using the MTT assay and half-maximal inhibitory concentration (IC_{50}) values, all studies demonstrated a reduction of cancer cell viability in a dosage-dependent manner following a 24 hr exposure. Additionally, the increased apoptotic activity through the formation of intracellular Reactive Oxygen Species (ROS), reduced levels of Mitochondria Membrane Potential (MMP), and cancer cell proliferation also contributed to the ZnO NPs-induced cytotoxicity.

Breast cancer cell lines (MDA-MB-231 and MCF-7); Prostate cancer cell line (DU-145); Human leukemia cell line (MOLT-4); Lung cancer cell line (A549); Cervical carcinoma cell line (HeLa); Osteosarcoma cell line (MG-63); Hepatocellular carcinoma cell lines (HUH-7 and HepG-2): Colorectal cancer cell lines (HCT-116 and Caco-2); Human multiple myeloma cell line (RPMI8226).

Cancer cell lines

At present, there have been various cancer cell lines that were used to evaluate the *in vitro* cytotoxicity of ZnO NPs. In this review, a total of 12 different cell lines were documented, including cell lines of prostate cancer, lung cancer, colorectal cancer, breast cancer, human leukemia, human multiple myeloma, cervical carcinoma, osteosarcoma, and hepatocellular carcinoma. As shown in Figure 2, the majority of the studies in this review make use of breast cancer (MCF-7) cells (15%) and colorectal cancer (HCT-116) cells (15%) against ZnO NPs to demonstrate its cytotoxicity. However, the cancer cell lines such as DU-145, MG-63, MDA-MB-231, RPMI-8226, HepG-2, and HUH-7 were the least used

Table 1: Cytotoxicity of ZnO NPs on Various Cancer Cell Lines Master Table.

Sl. No.	Title	Year	Shape and/or Size	Cancer Cell Lines	Findings
1	Bio-green synthesis ZnO-NPs in *Brassica napus* pollen extract: biosynthesis, antioxidant, cytotoxicity and pro-apoptotic properties[11]	2019	Shape: hexagonal Average particle size: 26 nm	MDA-MB-231	Decreased cell viability; increase the percentage of apoptotic cells; reduced cancer cell proliferation; IC_{50} value of 1 µg/ml
2	*Crotalaria verrucosa* Leaf Extract Mediated Synthesis of Zinc Oxide Nanoparticles: Assessment of Antimicrobial and Anticancer Activity[12]	2020	Shape: hexagonal; Average particle size: 27 nm	HeLa and DU-145	Increase ROS production; selective targeting within cancer cell; dose-dependent inhibition; decreased cell viability IC_{50} values of HeLa cells = 7.07 µg/mL and DU145 = 6.30 µg/mL
3	Green Synthesis of Zinc Oxide Nanoparticles (ZnO-NPs) Using *Arthrospira platensis* (Class: *Cyanophyceae*) and Evaluation of their Biomedical Activities[13]	2021	Shape: spherical Average particle size: 30-50 nm	Caco-2	Reduce cell viability in a time- and dose-dependent manner; IC_{50} value of 9.95 µg/mL
4	Facile green synthesis of ZnO-RGO nanocomposites with enhanced anticancer efficacy[14]	2022	Shape: Spherical Average particle size: 17 nm	MCF7 and HCT116	Increased production of intracellular ROS; IC_{50} values of 11 µg/ml = MCF7 and 12 µg/ml = HCT116
5	Zinc oxide nanoparticle synthesized from *Euphorbia fischeriana* root inhibits the cancer cell growth through modulation of apoptotic signaling pathways in lung cancer cells[15]	2020	Shape: spherical Average particle size: 30 nm	A549	Decreased cell viability; IC_{50} value of 14.5 µg/mL
6	Anticarcinogenic effect of zinc oxide nanoparticles synthesized from *Rhizoma paridis* saponins on Molt-4 leukemia cells[16]	2020	Shape: spherical	MOLT-4	Increased ROS production; decreased MMP; decreased cell viability IC_{50} value of 15 µg/mL
7	Employment of *Cassia angustifolia* leaf extract for zinc nanoparticles fabrication and their antibacterial and cytotoxicity[17]	2021	Shape: Hexagonal Average particle size: 26 nm	MCF-7	Decreased cell viability in a dose-dependent manner; IC_{50} value of 17.9 ± 1.4 µg / mL
8	*Bougainvillea* flower extract mediated zinc oxide's nanomaterials for antimicrobial and anticancer activity[18]	2019	Average particle size: 40 nm	MCF-7	Concentration-based cytotoxicity; decreased cell viability IC_{50} value of 19.2 µg/mL
9	Zinc oxide nanoparticles from *Cassia auriculata* flowers showed the potent antimicrobial and *in vitro* anticancer activity against the osteosarcoma MG-63 cells[19]	2021	Shape: hexagonal Average particle size: 41.25 nm	MG-63	Decreased cell viability in a dose-dependent manner; IC_{50} value of 20 µg/mL
10	Anticancer and apoptotic activity of biologically synthesized zinc oxide nanoparticles against human colon cancer HCT-116 cell line-*in vitro* study[20]	2019	Shape: Spherical Size: 12-24 nm	HCT-116	Increased apoptotic activity; decreased cell viability; increased cytotoxicity in HCT-116 cells than normal cells; IC_{50} value of 20 µg/mL
11	Zinc oxide nanoparticles synthesized from *Aspergillus terreus* induce oxidative stress-mediated apoptosis through modulating apoptotic proteins in human cervical cancer HeLa cells[21]	2021	Shape: spherical Average particle size: 43.72 ± 2.45 nm	HeLa	Concentration-dependent cytotoxicity; increased apoptotic activity by reduced levels of SOD, CAT, GPx, MMP, and increased ROS; IC_{50} value of 20.43 ± 0.98 µg/mL
12	Green synthesis of zinc oxide nanoparticles using *Elaeagnus angustifolia* L. leaf extracts and their multiple *in vitro* biological applications[22]	2021	Shape: Spherical Average particle size: 26 nm	HUH-7 and HepG2	Concentration-dependent cytotoxicity; IC_{50} values of HuH-7 = 29.8 µg/mL and HepG2 = 21.7 µg/mL
13	Zinc oxide nanoparticles induce human multiple myeloma cell death via reactive oxygen species and Cyt-C/Apaf-1/Caspase-9/Caspase-3 signaling pathway *in vitro*[23]	2020	Shape: spherical Average particle size: 30 nm	RPMI8226	Decreased cell viability in a dose- and time-dependent manner; elevated intracellular ROS levels; IC_{50} value of 33.83 µg/mL
14	Cytotoxic Potential of Biogenic Zinc Oxide Nanoparticles Synthesized From *Swertia Chiraiya* Leaf Extract on Colorectal Cancer Cells[24]	2021	Shape: spherical Average particle size: Ethanol: 32.11 ± 7.659 nm Methanol: 33.27 ± 5.851 nm	HCT-116 and Caco-2	Concentration-dependent cytotoxicity; decreased cell viability; IC_{50} values of Ethanol: HCT-116 = 34.356 ± 2.71 and Caco-2 = 52.15 ± 8.23 Methanol: HCT-116 = 32.856 ± 2.99 µg/mL; Caco-2 = 63.1 ± 12.09 µg/mL
15	Anticancer therapeutic efficacy of biogenic Am-ZnO nanoparticles on 2D and 3D tumor models[25]	2021	Shape: Spherical Average particle size: 80 nm	A549 and MOLT-4	Increased apoptotic activity by the depolarization of MMP; decreased cell viability IC_{50} values of A549 = 96.67 ± 10.75 and MOLT4 = 60.13 ± 7.13

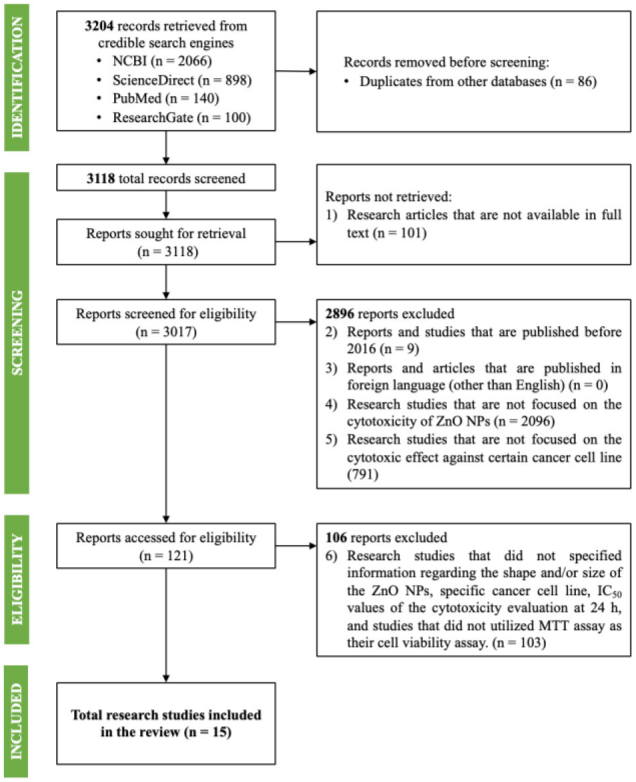

Figure 1: Schematic Diagram of Study Selection Process.

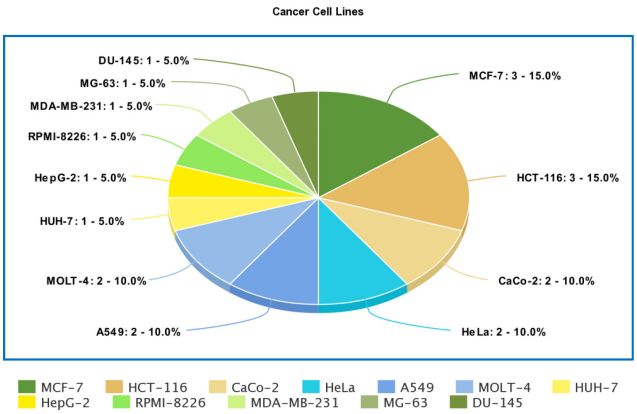

Figure 2: Cancer Cell Lines.

cell lines accounting for only 5% of all studies included in this review.

Size and cytotoxicity

The cytotoxicity results of ZnO NPs showed that the ZnO NPs induce an anti-metabolic activity and reduced cell viability against different-sized NPs. Among the ZnO NPs tested, smaller-sized NPs had shown higher cytotoxicity against cancer cells in terms of proliferation and viability inhibition. Generally, in cell-based cytotoxicity tests, the viability of cells is evaluated using the half-maximal inhibitory concentration (IC_{50}) value. The IC_{50} value is defined by the dosage of inhibitor against cell survival where the response is reduced by half (50%). Table 2 lists the IC_{50} values of ZnO NPs on various cancer cell lines against different-sized NPs. About the table, while the particle size of ZnO NPs ranged from 12 to 80 nm, with an average size of 34.29 nm, the IC_{50} values ranged from 1 μg/mL to 96.67 μg/mL.

Breast cancer cell lines (MDA-MB-231 and MCF-7); Prostate cancer cell line (DU-145); Human leukemia cell line (MOLT-4); Lung cancer cell line (A549); Cervical carcinoma cell line (HeLa); Osteosarcoma cell line (MG-63); Hepatocellular carcinoma cell lines (HUH-7 and HepG-2): Colorectal cancer cell lines (HCT-116 and Caco-2); Human multiple myeloma cell line (RPMI8226).

To demonstrate the cytotoxicity of ZnO NPs, the IC_{50} values of ZnO NPs were plotted against their respective particle size (Figure 3). Based on the Figure, it can be seen that there is a direct relationship between the particle size and IC_{50} values of ZnO NPs, which indicates that with smaller-sized nanoparticles, a lower concentration is required to reduce the cell viability by 50%, and therefore, demonstrates a higher cytotoxicity potency. This cytotoxic behavior is observed in all 12 types of cancer cell lines, although a minor discrepancy was observed in the study of Patnaik *et al.* (2021), wherein smaller-sized (32.11 ± 7.659 nm) ethanolic ZnO NPs induced a higher IC_{50} value (34.356 ± 2.71) when compared to the larger-sized methanolic ZnO NPs (33.27 ± 5.851 nm) that requires a lower IC_{50} value (32.856 ± 2.99 μg/mL) against HCT-116 cells.

Furthermore, based on Figure 3, the lowest IC_{50} value (1 μg/mL) was observed in ZnO NPs with a particle size of 26 nm against the cell line of breast adenocarcinoma (MDA-MB-231). However, with 80 nm in size against cancer cell line A549, ZnO nanoparticles yielded the highest IC_{50} value: 96.67 ± 10.75 μg/mL. This further justifies the inverse relationship between the cytotoxicity and particle size of the ZnO NPs, as well as their corresponding IC_{50} value. According to the review of Bisht *et al.* (2016), the higher potency of smaller-sized nanoparticles is due to their increased capacity to enter tumor cells and manipulate the cellular function of the cell, hence leading to cell death. Additionally, smaller-sized nanoparticles have a larger specific surface area (SSA). This creates more available space for cellular components to interact within the cell.[26] Such interaction of the ZnO NPs within the cell allows ROS concentration to increase, which leads to its cytotoxicity through the increased oxidative stress in the cell.

Table 2: Size-Dependent Cytotoxicity of ZnO NPs.

Sl. No.	Title	Size	Cancer Cell Lines	IC_{50} results
1	Anticancer and apoptotic activity of biologically synthesized zinc oxide nanoparticles against human colon cancer HCT-116 cell line-*in vitro* study[20]	12-24 nm	HCT-116 cells	20 µg/mL
2	Facile green synthesis of ZnO-RGO nanocomposites with enhanced anticancer efficacy[14]	17 nm	MCF-7 and HCT-116 cells	MCF7 = 11 µg/mL HCT116 = 12 µg/mL
3	Bio-green synthesis ZnO-NPs in *Brassica napus* pollen extract: biosynthesis, antioxidant, cytotoxicity and pro-apoptotic properties[11]	26 nm	MDA-MB-231 cells	1 µg/ml
4	Employment of *Cassia angustifolia* leaf extract for zinc nanoparticles fabrication and their antibacterial and cytotoxicity[17]	26 nm	MCF-7 cells	17.9 ± 1.4 µg / mL
5	Green synthesis of zinc oxide nanoparticles using *Elaeagnus angustifolia* L. leaf extracts and their multiple *in vitro* biological applications[22]	26 nm	HUH-7 and HepG-2 cells	HuH-7 = 29.8 µg/mL HepG2 = 21.7 µg/mL
6	*Crotalaria verrucosa* Leaf Extract Mediated Synthesis of Zinc Oxide Nanoparticles: Assessment of Antimicrobial and Anticancer Activity[12]	27 nm	HeLa and DU-145 cells	HeLa cells = 7.07 µg/mL DU145 = 6.30 µg/mL
7	Zinc oxide nanoparticle synthesized from *Euphorbia fischeriana* root inhibits the cancer cell growth through modulation of apoptotic signaling pathways in lung cancer cells[15]	30 nm	A549 cells	14.5 µg/mL
8	Zinc oxide nanoparticles induce human multiple myeloma cell death via reactive oxygen species and Cyt-C/Apaf-1/Caspase-9/Caspase-3 signaling pathway *in vitro*[23]	30 nm	RPMI-8226 cells	33. 83 µg/m
9	Green Synthesis of Zinc Oxide Nanoparticles (ZnO-NPs) Using *Arthrospira platensis* (Class: *Cyanophyceae*) and Evaluation of their Biomedical Activities[13]	30-50 nm	Caco-2 cells	9.95 µg/mL
10	Cytotoxic Potential of Biogenic Zinc Oxide Nanoparticles Synthesized From *Swertia chirayita* Leaf Extract on Colorectal Cancer Cells[24]	Ethanol: 32.11 ± 7.659 nm Methanol: 33.27 ± 5.851 nm	HCT-116 and Caco-2 cells	Ethanol: HCT-116 = 34.356 ± 2.71 Caco-2 = 52.15 ± 8.23 Methanol: HCT-116 = 32.856 ± 2.99 µg/mL; Caco-2 = 63.1 ± 12.09 µg/mL
11	*Bougainvillea* flower extract mediated zinc oxide's nanomaterials for antimicrobial and anticancer activity[18]	40 nm	MCF-7 cells	19.2 µg/mL
12	Zinc oxide nanoparticles from *Cassia auriculata* flowers showed the potent antimicrobial and *in vitro* anticancer activity against the osteosarcoma MG-63 cells[19]	41.25 nm	MG-63 cells	20 µg/mL
13	Zinc oxide nanoparticles synthesized from *Aspergillus terreus* induce oxidative stress-mediated apoptosis through modulating apoptotic proteins in human cervical cancer HeLa cells[21]	43.72 ± 2.45 nm	HeLa cells	20.43 ± 0.98 µg/mL
14	Anticancer therapeutic efficacy of biogenic Am-ZnO nanoparticles on 2D and 3D tumor models[25]	80 nm	A549 and MOLT4 cells	A549 = 96.67 ± 10.75 MOLT-4 = 60.13 ± 7.13

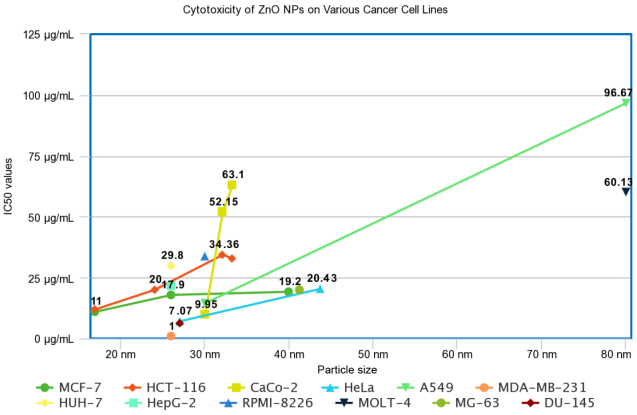

Figure 3: The correlation between the particle size and IC_{50} values of ZnO NPs against various cancer lines.

Shape and cytotoxicity

Aside from the particle size, the synthesis of ZnO NPs also allows them to be obtained in various types of morphology which have been theoretically considered to influence the cytotoxic activity of the ZnO NPs by affecting the capacity of the nanoparticle in cell migration. In the study of Fadoju *et al.* (2019),[27] it was observed that spherical nanoparticles migrate more easily in the cell than other irregularly-shaped nanoparticles due to their higher kinetics rate and diffusion coefficient. To date, the are diverse shapes of ZnO NPs used in cytotoxic studies ranging from spherical, hexagonal, rod-shaped, star-shaped, cylindrical, and cuboidal shapes, with spheroid ZnO NPs being the most common. Table 3 lists the shape of synthesized ZnO nanoparticles with their corresponding IC_{50} values against various cancer cell lines.

Breast cancer cell lines (MDA-MB-231 and MCF-7); Prostate cancer cell line (DU-145); Human leukemia cell line (MOLT-4); Lung cancer cell line (A549); Cervical carcinoma cell line (HeLa); Osteosarcoma cell line (MG-63); Hepatocellular carcinoma cell lines (HUH-7 and HepG-2): Colorectal cancer cell lines (HCT-116 and Caco-2); Human multiple myeloma cell line (RPMI8226). Figure 4 presents the forms of ZnO NPs used in various cytotoxic studies of this review. Out of the 15 eligible studies included in this review, only spherical and hexagonal shapes of ZnO NPs were documented, wherein the majority of these are in spheroid form (66.7%). Meanwhile, four out of 15 studies (26.7%) synthesized hexagonal-shaped nanoparticles, with the remaining study (6.7%) did not specify any shape characterization of the ZnO NPs.

The ZnO NPs demonstrated variable cytotoxic activity against cancer cells in terms of shape. Figure 5 represents the cytotoxicity of ZnO NPs against MCF-7 cells and HeLa cells of both hexagonal- and spherical-shaped ZnO NPs. Against MCF-7 cells, spherical ZnO NPs (11 µg/mL) have lower IC_{50} values than hexagonal ZnO NPs (17.9 µg/mL), therefore exhibiting higher cytotoxicity. Interestingly, against HeLa cells, the spherical ZnO NPs give an opposite cytotoxicity result. The IC_{50} values of spherical ZnO NPs (20.43 µg/mL) are higher than hexagonal ZnO NPs (7.07 µg/mL), exhibiting lower cytotoxicity. This opposite result might be because both hexagonal- and spherical-shaped ZnO NPs have different particle sizes, which could affect their cytotoxicity results.

DISCUSSION

The systematic review found evidence that ZnO nanoparticles have a potential for alternative cancer treatment because of their cytotoxic properties against cancer cells while still being selective and biocompatible. Primary concerns on biomedical uses of nanoparticles include their biocompatibility and selectivity as they raise potential use for drugs and therapy. Because of its small size, ZnO NP can pass and travel to the bloodstream unto the different parts of the body – that is why it is crucial that they must not induce cytotoxicity in healthy cells. Remarkedly, existing studies proved that ZnO NPs are highly selective, which is confirmed by a significantly higher dose and longer exposure requirement to reduce normal cell viability, such as in human red blood cells,[28] mesenchymal stem cells,[29] 3T3-L1 adipocytes,[30] as compared to cancer cells. While zinc is known to play critical roles in enzyme function and gene transcription, ZnO NP also exhibits biocompatibility, as shown by its efficient absorption and rapid distribution in body tissues.[31] In this review, all studies proved that ZnO NPs have size-dependent cytotoxicity that induces cell death via different mechanisms such as intracellular ROS, mitochondria membrane potential, and cell migration.

Intracellular ROS Production and Oxidative Stress

Once ZnO NPs have penetrated cancer cells, one of the three mechanisms is to produce cytotoxic effects in malignant cells, one of which is the generation of apoptosis-inducing species such as the intracellular Reactive Oxygen Species (ROS). The surface features of ZnO NPs make them a suitable candidate for a redox-reactive system to generate ROS. Lipid peroxidation is the most common cause of oxidative stress, which is caused by an overabundance of free radicals. The increased amounts of LPO and attenuation are widely recognized characteristics of oxidative stress-induced cell damage and include the presence of antioxidant indicators.[21]

Table 3: Shape-Dependent Cytotoxicity of ZnO NPs.

Sl. No.	Title	Shape	Cancer Cell Lines	IC_{50} results
1	Green Synthesis of Zinc Oxide Nanoparticles (ZnO-NPs) Using *Arthrospira platensis* (Class: *Cyanophyceae*) and Evaluation of their Biomedical Activities[13]	Spherical	Caco-2 cells	9.95 µg/mL
2	Facile green synthesis of ZnO-RGO nanocomposites with enhanced anticancer efficacy[14]	Spherical	MCF-7 and HCT-116 cells	MCF7 = 11 µg/mL HCT116 = 12 µg/mL
3	Zinc oxide nanoparticle synthesized from *Euphorbia fischeriana* root inhibits the cancer cell growth through modulation of apoptotic signaling pathways in lung cancer cells[15]	Spherical	A549 cells	14.5 µg/mL
4	Anticarcinogenic effect of zinc oxide nanoparticles synthesized from *Rhizoma paridis* saponins on Molt-4 leukemia cells[16]	Spherical	Molt-4 cells	15 µg/mL
5	Anticancer and apoptotic activity of biologically synthesized zinc oxide nanoparticles against human colon cancer HCT-116 cell line-*in vitro* study[20]	Spherical	HCT-116 cells	20 µg/mL
6	Zinc oxide nanoparticles synthesized from *Aspergillus terreus* induce oxidative stress-mediated apoptosis through modulating apoptotic proteins in human cervical cancer HeLa cells[21]	Spherical	HeLa cells	20.43 ± 0.98 µg/mL
7	Green synthesis of zinc oxide nanoparticles using *Elaeagnus angustifolia* L. leaf extracts and their multiple *in vitro* biological applications[22]	Spherical	HUH-7 and HepG-2 cells	HuH-7 = 29.8 µg/mL HepG2 = 21.7 µg/mL
8	Cytotoxic Potential of Biogenic Zinc Oxide Nanoparticles Synthesized From *Swertia chirayita* Leaf Extract on Colorectal Cancer Cells[24]	Spherical	HCT-116 and Caco-2 cells	Ethanol: HCT-116 = 34.356 ± 2.71 Caco-2 = 52.15 ± 8.23 Methanol: HCT-116 = 32.856 ± 2.99 µg/mL; Caco-2 = 63.1 ± 12.09 µg/mL
9	Zinc oxide nanoparticles induce human multiple myeloma cell death via reactive oxygen species and Cyt-C/Apaf-1/Caspase-9/Caspase-3 signaling pathway *in vitro*[23]	Spherical	RPMI-8226 cells	33. 83 µg/m
10	Anticancer therapeutic efficacy of biogenic Am-ZnO nanoparticles on 2D and 3D tumor models[25]	Spherical	A549 and MOLT-4 cells	A549 = 96.67 ± 10.75 MOLT-4 = 60.13 ± 7.13
11	Bio-green synthesis ZnO-NPs in *Brassica napus* pollen extract: biosynthesis, antioxidant, cytotoxicity and pro-apoptotic properties[11]	Hexagonal	MDA-MB-231 cells	1 µg/ml
12	*Crotalaria verrucosa* Leaf Extract Mediated Synthesis of Zinc Oxide Nanoparticles: Assessment of Antimicrobial and Anticancer Activity[12]	Hexagonal	HeLa and DU-145 cells	HeLa cells = 7.07 µg/mL DU145 = 6.30 µg/mL
13	Employment of *Cassia angustifolia* leaf extract for zinc nanoparticles fabrication and their antibacterial and cytotoxicity[17]	Hexagonal	MCF-7 cells	17.9 ± 1.4 µg/mL
14	Zinc oxide nanoparticles from *Cassia auriculata* flowers showed the potent antimicrobial and *in vitro* anticancer activity against the osteosarcoma MG-63 cells[19]	Hexagonal	MG-63 cells	20 µg/mL

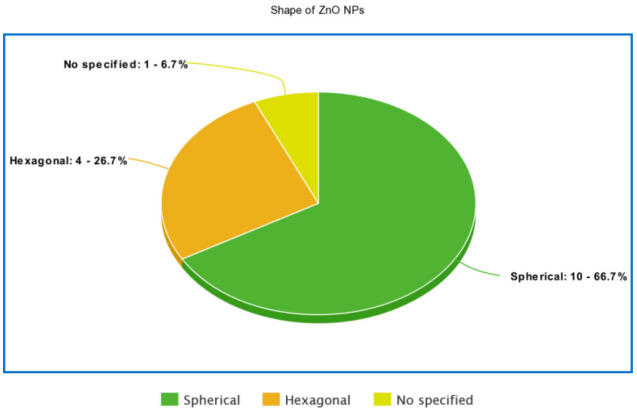

Figure 4: Shape morphology of the ZnO NPs.

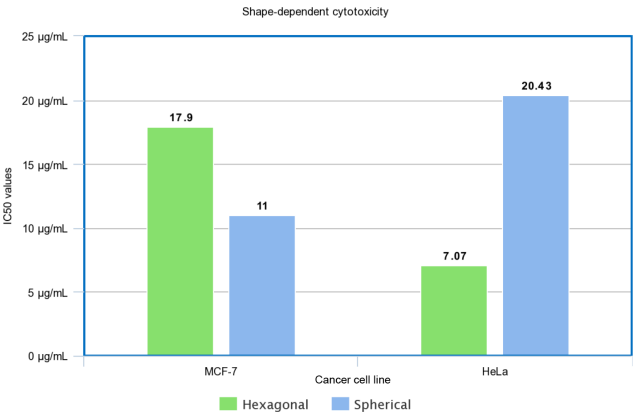

Figure 5: Cytotoxicity of hexagonal- and spherical-shaped ZnO NPs against MCF-7 and HeLa cells.

In the study of Li *et al.* (2020), when MM cells were exposed to ZnO NPs for 24 hr, the levels of ROS considerably rose. ROS are both a source and a target of ROS playing a crucial role in maintaining a cell's normal physiological function. Excessive ROS production, on the other hand, will disrupt the physiological equilibrium inside cells, leading to the loss of MMP, lysosomal activity, nuclear coagulation, and finally, cell apoptosis. Human MM cells treated with ZnO NPs can produce a lot of reactive oxygen species (ROS), which is a significant cause of cell death. The primary culprit of the oxidative stress-induced mitochondrial dysfunction is the activated Caspase-dependent signaling pathway which causes the apoptosis of multiple myeloma cells induced by the ZnO NPs. Due to the spherical shape of about 30nm in size of the nanoparticle, It has contributed to the decreased cell viability via intracellular ROS levels producing an IC_{50} value of 33.83 µg/mL against RPMI8226 cells.[23]

These findings suggest that a ZnO nanoparticle size of approximately 10-40 nm is a plausible reason for the higher cytotoxicity effect of the nanoparticle against cancer cells as they accumulate more efficiently and penetrate cell tumors than larger counterparts. Smaller sizes of less than 10 nm, however, might not be effective in inducing cytotoxicity as they can also be toxic in normal cells other than malignant cells.

Mitochondria Membrane Potential

The cytotoxicity impact indicates a variation in the MMP, a pre-apoptosis occurrence. In the entire living phase of cells, mitochondria utilize oxidable substrate to generate an electrochemical proton grade on the membrane to yield ATP and create vitality for cellular events. The assessment of the MMP of intact cells may require the essential data to measure their physical and systemic level.[23] Current interpretations in various cancer units have indicated that ZnO NPs augment the weakening of mitochondria; especially in mitochondrial layers, these compromise the capacity of a PI3K-like protein kinase to provoke mitochondria membrane potential and the delivery of apoptogenic proteins.[23] Aside from that, results showed that PINK1 expression increased following the treatment of ZnO NPs. This shows that the decreased levels of MMP in the damaged mitochondria further stabilize the PINK1 in the exterior parts the mitochondria.

The small dimension of ZnO NP, at the same time, facilitates the infiltration and preservation of nanoparticles in cancerous units, permitting them to take action. Metal zinc ions are released when ZnO nanoparticles interact and penetrate the plasma membrane in cancer cells and eventually enter their cytoplasm. This disrupts the membrane permeability, eventually causing damage to DNA structure, hence the cell death. Furthermore, given that biosynthesized ZnO NPs are semiconducting, cytotoxicity is also activated with the release of apoptosis-inducing species such as the intracellular ROS. ROS generation through the release of Zn^{2+} ions provides a more direct interaction between the NPs and cancer cell membrane eventually leading to increase oxidative stress and, ultimately causing apoptosis.[12]

Cell Migration

In the cytotoxicity of ZnO NPs, there is a difference in effectiveness due to different variations of sizes in NPs. The smaller the size of ZnO NPs (<10 nm) is more effective than the larger NPs. Smaller NPs can easily induce or penetrate through the body or blood vessels. Cells treated with concentrations of 10 to 15 µg/mL inhibit and increase the cell migration potential of the nanoparticle. Cell migration and invasion show great significance in metastatic diseases. There are

various approaches for cancer cells to disseminate and migrate across locations. These comprise amoeboid, mesenchymal, and collective cell migration (Wu *et al.,* 2021). With this, cell migration exhibits an integral part in the context of cancer cell invasion.

In line with this, studies conducted by Zhang *et al.* (2020) and Sana *et al.* (2020) showed similar findings. The study of Zhang *et al.* (2020) presented a significant inhibition of cell migration when cells were treated with the EF-ZnO NPs with a particle size of 30 nm. Contrary to that, the absence of treatment to the cells with EF-ZnO NPs showed increased proliferation. Additionally, Sana *et al.* (2020) concluded that the nanosized ZnO with an average particle size of 27 nm obstructs cancer cells' cell invasion and migration when proliferating. In fact, the study accumulated lower cell migration levels for the cancer cell lines when treated with ZnO NPs and higher levels of cell migration when they remained untreated. The two studies have utilized smaller-sized-ZnO NP and showed similar results of increased inhibition of cancer cell migration upon treating the cancer cell lines with ZnO NPs.

Shape and Size-induced Cytotoxicity

Against various cancer cell lines, the result of this review showed that the cytotoxic behavior of the zinc oxide nanoparticle is different in the type of cell lines they are exposed to. In the data presented, although the 26 nm-sized ZnO NP exposed against MDA-MB-231 cells is not the smallest nanoparticle to be documented, the lowest IC_{50} value (1 μg/mL) was obtained from this study. Therefore, although the cytotoxicity is highly dependent on the morphological characteristics of the nanoparticles, the malignancy of the cell line they are exposed to also plays a crucial role in the potency determination of the ZnO NPs.

Concerning cell viability and proliferation, it has been found that the particle size is directly proportional to IC_{50} values of ZnO NPs. This means that in smaller-sized nanoparticles, a lower dosage of the ZnO NPs is needed to induce cytotoxicity and reduce cell survival by half (50%). Therefore, the cell viability and proliferation are reduced in a higher cytotoxic environment as induced by smaller-sized nanoparticles. Additionally, the larger surface area of small-sized ZnO NPs that allows for such interaction within the cell, causes an increased apoptotic activity through ROS release, and reduced levels of MMP, leading to increased oxidative stress within the cell hence its cytotoxicity.

Likewise, although not proven yet, variations in shape have theoretically been believed to influence the cytotoxicity of nanoparticles by affecting the capacity of the nanoparticle in cell migration. There are diverse shapes of ZnO NPs used in various cytotoxic studies, with the spherical form being the most common. In the study of Fadoju *et al.* (2019),[27] it was observed that spherical nanoparticles migrate more quickly in the cell than other irregularly shaped nanoparticles due to their higher kinetics rate and diffusion coefficient. Both shape and size are directly related to the internalization process within the cell, which is primarily based on the principle of endocytosis. However, this review found no direct correlation between the shape and cytotoxicity of ZnO NPs as it is difficult to compare biological results of nanoparticles that are based on different exposure conditions.

CONCLUSION AND RECOMMENDATION

In conclusion, data from the gathered literature resulted in excellent cytotoxic activities of ZnO nanoparticles against various cancer lines via different mechanisms such as increased intracellular ROS production, cell migration, and reduced levels of mitochondria membrane potential. This review also concluded that apart from the time and dosage, the cytotoxicity of ZnO NPs is also highly dependent on its size, as smaller particle size results in a lower dose required to induce cell death. Furthermore, despite the morphological characteristics of the ZnO NP itself, the potency of ZnONPs varied according to the malignancy of different cancer lines they are against which played a crucial role in determining their potency. Before this review, it has been hypothesized that shape would influence the cytotoxicity of the ZnO NPs. However, this review found no direct correlation between the shape and cytotoxicity of ZnO NPs as it is difficult to compare biological results of nanoparticles that are based on different exposure conditions (e.g., time, dosage, cancer cell line, the particle size of NPs, its method of synthesis, and purification). Therefore, when investigating the effect of various shapes on the cytotoxicity of nanoparticles, it is recommended for further studies to have one set of synthesis protocols and biological evaluation parameters. Additionally, it is also important to note that there is still a vague understanding of nanoparticles that are less than 10 nm in size. Extensive research is, therefore, necessary as they may have toxic issues in healthy cells and the overall body.

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To God, all be the Glory!

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ZnO: Zinc Oxide; **NPs:** Nanoparticles; **NCBI:** National Center for Biotechnology Information; **CAT:** Catalase; **DNA:** Deoxyribonucleic Acid; **EF-ZnO NPs:** Euphorbia fischeriana-Zinc Oxide Nanoparticles; **GPx:** Glutathione Peroxidase; **IC_{50}:** Half-maximal Inhibitory Concentration; **LPO:** Lipid Peroxidation; **MM:** Multiple Myeloma cells; **MMP:** Mitochondria Membrane Potential; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; **ROS:** Reactive Oxygen Species; **SOD:** Superoxide Dismutase; and **SSA:** Specific Surface Area.

SUMMARY

The selective targeting capabilities of nanoparticles are becoming intrinsically significant in modern cancer treatments overshadowing the adverse effects of traditional cancer therapies such as chemotherapy and radiation therapy. ZnO nanoparticles, with their unique properties such as their high selectivity and biocompatibility, are the widely explored nanoparticles in cancer treatment methodology. This review article outlines the unique physiochemical properties, its shape and size, and the mechanisms of cytotoxicity of the ZnO NPs to assess its potential as a cytotoxic agent against cancer cells. Articles and journals that were collated from various databases were thoroughly screened and assessed by the predetermined eligibility criteria. Given that nanoparticles can exist in various types of morphology, due to the variation in synthesis, purification, and functionalization, chosen studies in this review centered on the morphological influence of the ZnO NPs in terms of size- and shape-induced cytotoxicity. The results generated had reported evidence of anti-proliferative activity of the ZnO NPs through a different mechanism of apoptosis-induced cytotoxicities such as increased intracellular ROS production, cell migration, and reduced levels of mitochondria membrane potential. Results from this review also suggested that smaller-sized nanoparticles are more potent than larger-sized nanoparticles as they accumulate and penetrate more efficiently than larger counterparts. Moreover, although no direct correlation was observed between shape and cytotoxicity, further studies are recommended to have one set of evaluation parameters when investigating shape-induced cytotoxicity.

Author's Contributions

All the authors participated equally from the first outline up to the last revision of the manuscript. Author K.F. led the group and set the cadence in theorizing the initial concept, which was similarly examined by Authors J.B., L.R., A.E., B.F., L.L., J.T., and J.V. While Author K.F. accomplished the original proof-of-concept, this was then expanded by Authors J.B. and L.L., who centered on cancer cells and several cancer therapies, Authors J.V. and J.T., who indicated the function of zinc in cancer and its discerning cytotoxicity, and Authors A.E. and L.R. who emphasized its significance by briefly discussing ZnO NPs as therapy for cancer and provided a brief explanation of the eligibility criteria. The information sources and search strategies followed were then devised by J.V. and J.T., with many valuable recommendations on the selection process from Authors K.F. and B.F. Author K.F., however, wrote the data extraction and led the justification of results, including the creation of charts, Figures, and tables. In the discussion part, everyone had a fair share of subsections. Author J.B. wrote the first part of the discussion, while Authors B.F., A.E., L.L., J.V., L.R., and J.T. wrote the mechanisms of cytotoxicity of the ZnO NPs, and data regarding the shape and size-induced cytotoxicity of ZnO NPs was discussed by Author K.F. Finally, Authors J.B. and K.F. provided the conclusion and recommendation of this paper. Author P.B. provided critical feedback, insights, and guidance in developing the manuscript. All authors have reviewed and authorized the final version of the paper.

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