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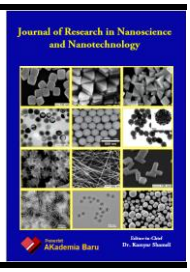

Short Review: Phytofabrication of Zinc Oxide Nanoparticles for Anticancer Applications

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ABSTRACT

Zinc oxide nanoparticles (ZnO NPs) are one of the most well-known materials in the field of nanotechnology. Adopting a more environmentally friendly synthesis methods of ZnO NPs have been the focus in the last few decades. Of all green synthesis methods of ZnO NPs, fabrication with the help of plant extracts has been the most popular due to its many benefits. The use of phytofabricated ZnO NPs in anticancer studies has been conducted increasingly over the last decade because of its high inhibition activity against various types of cancerous cells. This short review article will present the current update on the phytofabrication of ZnO NPs in recent years and discuss on their cytotoxicity mechanism against cancer cells.

Keywords:
Phytofabrication, zinc oxide,
nanoparticles, cancer.

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1. Introduction

Year by year, the number of people diagnosed with various types of cancer are increasing around the globe. It is estimated by the year 2030, the number of deaths from cancer will be around ~21 million, making it one of the major cause of morbidity and mortality in the world [1, 2]. Therefore, effective cancer screening and therapy have to be developed to solve this highly concerning issue. The main problems with current anticancer chemotherapy usually involve development of drug resistance and difficulty to differentiate between cancerous and healthy cells thus causing severe side effects to patients.

In recent years, researchers have been experimenting on the application of nanotechnology in cancer therapy using different types of nanoparticles. In this regard, several studies have shown that

zinc oxide (ZnO) nanoparticles have high potential to be used as anticancer agent due to its high selectivity of cancer cells and effective cytotoxic activities. ZnO also has good biocompatibility level as it is graded as a "GRAS" (generally recognized as safe) substance by the US Food and Drug Administration (FDA). Therefore, they are commonly used in food industries for packaging and improving shelf life of food [3]. Chemically, the surface of ZnO is rich in hydroxyl (–OH) groups, which allows ZnO to slowly dissolve in both acidic (tumor microenvironment) and strong basic conditions. By aiming the specific cites of cancer cells, drug delivery based on the use of ZnO NPs could reduce the dosage of drugs used for treatment and reduce other side effects. The biodegradable properties and low toxicity of ZnO NPs have increased its usage in cancer drug delivery compared to other nanoparticles. The solubility and toxicity of ZnO NPs can also be improved by loading it with current anticancer drugs such as baicalin, doxorubicin, curcumin, and paclitaxel [4]. Based on these promising properties, ZnO NPs have gained immense interest in biomedical applications especially in anticancer diagnostics and therapy [5].

Intermediate metals nanoparticles produced by physicochemical and or green synthesis methods include silver, gold, platinum, palladium, as well as metal oxides such as copper oxide, zinc oxide, etc., often have medical applications in inhibiting disease such as bacterial, microbe, fungi and viruses as well [6-11].

Among different approaches and techniques used to synthesize ZnO NPs, green synthesis using biological sources such as plants, microbes and other natural elements has been generally accepted by researchers due to its many advantages as compared to other methods. Conventional methods like chemical and physical approaches usually uses toxic chemicals that are detrimental to the environment and human health, requires high amount of energy and advanced laboratory equipment [7]. On the other hand, green synthesis methods are eco-friendly, uses non-hazardous reagents, simple and cost-effective [4]. This short review aims to report on the latest publications of ZnO NPs biosynthesized using plant sources as well as discussing their cytotoxic potential and general mechanism against cancerous cells.

2. Phytofabrication of zinc oxide nanoparticles

In the process of fabricating nanoparticles, several approaches can be adopted such as physical, chemical and green synthesis methods. Out of these three methods, green synthesis of nanoparticles has gained prominence in the last several decades due to its many advantages. Green synthesis is categorized under 'bottom to up' approach for creating nanoparticles, which means that the construction process starts from metal atoms assembling to form clusters, and eventually the desired nanoparticles. The main components needed to form nanoparticles are metal salt (precursor), reducing agent and stabilizing agent.

Green synthesis using plant sources or phytofabrication has been one of the most well studied technique in producing ZnO NPs. Phytofabrication makes use of biomolecules and secondary metabolites in plant extracts such as tannins, flavonones, saponins, polyphenols, alkaloids, and terpenoids [4]. These compounds act as a reducing agent and are mainly responsible for the effective reduction of zinc precursors to nanoparticles. Some compounds might also play the role of stabilizing agents as it can control the size of nanoparticles produced during the synthesis process. Plant extracts can be made from various parts of plants such as their leaf, flowers, fruits, fruit peels and many more. **Table 1** shows a list of plants that have been utilized to synthesize ZnO NPs and its application towards different types of cancerous cells. Compared to other green synthesis methods, phytofabrication is usually favored due to the high concentrations of chemical constituents they

contain, low cost, ecological, non-hazardous and bio-compatible. Other than that, it is generally safe as it does not involve the use of microorganisms and large-scale production is highly plausible [4].

Table 1. Zinc oxide nanoparticles synthesized from various plants and their cytotoxic potential.

No.	Type of plants	Parts	Zinc oxide NPs morphology	Target cancer cells	Ref.
1	*Bergenia ciliate*	Rhizome	~30 nm, flower shaped	Human cervical cancer (HeLa) and human colon cancer (HT-29)	[8]
2	*Spondias pinnata*	Leaf	30-48.5 nm, polygonal and hexagonal	Colon carcinoma (HCT 116) and chronic myelogenous leukemic (K562)	[9]
3	*Euphorbia fischeriana*	Root	30 nm, spherical	Human lung cancer (A549)	[2]
4	*Dendropanax morbifera*	Whole plant	65-239.6 nm, flower and spheroid shapes	Human lung cancer (A549)	[10]
5	Banana	Peel	81.22-526.52 nm, rod and sheet-like shapes	Skin cancer (A431), colorectal cancer (SW620) and liver cancer (HepG2)	[11]
6	*Geranium wallichianum*	Leaf	~18 nm, hexagonal	Liver cancer (HepG2)	[1]
7	*Clausena lansium* Lour. Skeels Wampi	Peel	28.42 nm, spherical	Neuroblastoma (SH-SY5Y)	[12]
8	*Acantholimun serotinum*	Aerial	20-80 nm, spherical	Human colon cancer (Caco-2), neuroblastoma (SH-SY5Y), breast cancer (MDA-MB-231) and embryonic kidney (HEK-293)	[13]
9	*Cycas pschannae*	Leaf	177-249 nm, irregular and rod-like	Lung cancer cell (A549)	[14]
10	*Deverra tortuosa*	Aerial	9.26-31.18 nm	Human colon cancer (Caco-2) and human lung cancer (A549)	[5]
11	*Ficus hispida*	Leaf	20-200 nm	Dalton's lymphoma ascites (DLA) cells	[15]
12	*Ocimum americanum*	Leaf	~45 nm, spherical	Human skin cancer (A431)	[16]
13	*Cratoxylum formosum*	Leaf	~500 nm, spherical or sheet-like structures	Non-melanoma skin cancer (A431) and liver cancer (HepG2)	[17]
14	*Arisaema triphyllum*	Whole plant	10-15 nm, spherical	Esophageal squamous cancer (EC109 and TE8)	[18]
15	*Delonix regia (Gul Mohar)*	Leaf	~20 nm, rose bud, Martian sedimentary lavers, Marigold flower, sedimentary	HeLa cancer cell line	[3]

			rock, brown diamond		
16	*Albizia lebbeck*	Stem bark	66.25 – 112.87 nm, spherical/irregular	Breast cancer (MDA-MB-231 and MCF-7)	[19]
17	*Ricinus communis*	Seed	10-30 nm, irregular	Breast cancer (MDA-MB-231)	[20]
18	*Leucaena leucocephala*	Leaf	50-200 nm,	Breast cancer (MCF-7) and human prostate cancer (PC-3)	[21]
19	*Punica granatum (pomegranate)*	Fruit peel	32.98 – 81.84 nm, hexagonal and spherical	Human colorectal cancer (HCT116)	[22]
20	*Chelidonium majus*	-	~10 nm, spherical	Human non-small cell lung cancer (A549)	[23]
21	*Sechium edule*	Leaf	36.2 nm, spherical	Breast cancer (MCF-7)	[24]
22	*Ziziphus nummularia*	Leaf	17.33 nm, spherical/irregular	HeLa cancer cell line	[25]

3. Cytotoxic potential of zinc oxide nanoparticles

Most published anticancer studies using phytofabricated ZnO NPs reported efficient *in vitro* cytotoxicity against various cancer cells in a dose dependent manner. A study by Banzeer et al. reported the highest inhibition potential of liver cancer cells (HepG2) of up to ~71% mortality at 1000 μg/mL and the potency of its phytofabricated ZnO NPs was decreasing in lower concentration [1]. Several studies also reported higher inhibition of cancer cells by ZnO NPs when compared to the respective plant extracts used to synthesize them. For example, *Z. Nummularia* leaf extract inhibited HeLa cancer cells to about 40% while the phytofabricated ZnO NPs successfully inhibited around 50% at 50 μg/mL concentrations [13, 25]. This is probably due to the synergistic effects of bioactive phytocompounds from plant extract present on the surface of ZnO NPs leading to enhanced bioavailability and cytotoxicity. While majority of papers reported increased cytotoxicity potential of ZnO NPs in higher concentration, a study by Kanika et al. found that low dosage of ZnO NPs has been very effective against liver cancer (HepG2) and breast cancer (MCF-7). The cell viability of HepG2 cells in particular was less than 10% at a low concentration of only 25 μg/mL [8].

In terms of selectivity, ZnO NPs have proven to be highly selective towards cancer cells in several papers. In a published report by Damita Jevapatarakul et al., all phytofabricated ZnO NPs were found to inhibit non-melanoma skin cancer cells without affecting normal cells. They also reported that ZnO nanosheets were observed to have more potent anticancer activities compared to spherical ZnO NPs [17]. A study by Jittiporn et al. also mentioned that their ZnO nanorods and nanosheets successfully inhibited viability of all tested cancer cells without affecting normal cell (Vero) [11]. Another method to significantly increase selectivity of ZnO NPs towards cancer cells include loading them with other conventional cancer drugs.

Figure 1 shows the illustration of generally discussed anticancer mechanisms of ZnO NPs. The ZnO NPs and ions usually enter into cancer cells by damaging their cell wall and membrane protein or through electron transport process. Positive charge of ZnO NPs surface plays an important role in enabling better binding of the NPs to the negatively charge cancer cells by endocytosis and cellular transportation [3]. Subsequently, as there are elevated amounts of intracellular zinc particles, protein action disequilibrium happens and cytotoxicity process will be initiated [20]. During this process, proteins and enzymes will undergo denaturation, and other organelles such as mitochondria

and DNA will be damaged. The ZnO NPs will stimulate reactive oxygen species (ROS) production which eventually result in oxidative damage of organelles and cell apoptosis. This ROS action is induced through pro-inflammatory reaction of the cell against ZnO-NPs as well as the characteristic surface property of the nanoparticles that makes ZnO NPs act as a redox system [5]. Other than that, ZnO NPs are known to stimulate cell surface receptors resulting in provoking of caspase-8 and commencement of caspace cascade in the death receptor pathway. This activation will lead to apoptosis and necrosis of cells [3]. Basic signs that indicate apoptosis in cells can be observed following 48 hours of incubation with the phytofabricated ZnO NPs. An example can be seen from a study by Huzaifa et al. where membrane blebs can be seen on MDA-MB 231 and MCF-7 cancer cells after incubation with ZnO NPs phytofabricated with *A. lebbeck* stem bark [19]. Similarly, Abdul Aziz et al. in his study found that the ZnO NPs-treated colon cancer cells showed a loss of anchorage from the culture flasks, a phenomenon known as "anoikis" after 48 hours. They also observed deformed and shrunken cells with distorted or fragmented nuclei, providing visual images of cellular damage. Interestingly, the ZnO-NP-treated colon cells further displayed the presence of single, conspicuously large, bubble-like cells clearly suggestive of the occurrence of necrosis. Therefore, the cytotoxicity of ZnO NPs are mediated by highly regulated and genetically controlled apoptosis-necrosis continuum ("necroptosis") [9].

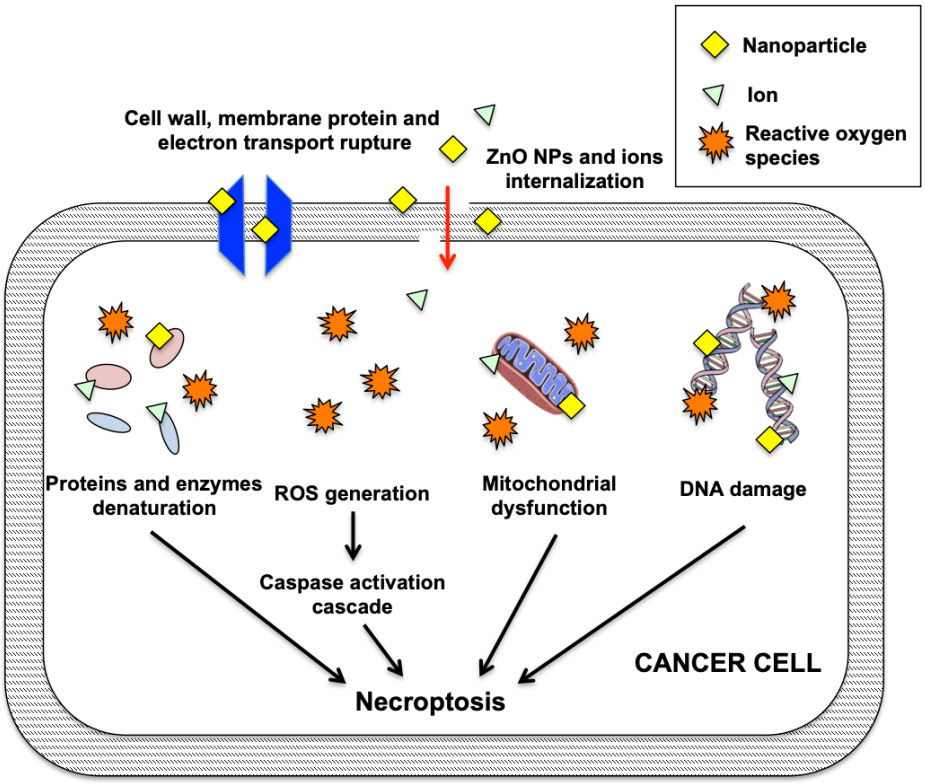

Figure 1. Anticancer mechanism of zinc oxide nanoparticles.

4. Future outlook and conclusions

Even though ZnO NPs shows an excellent potential to be used as an anticancer agent, there are also several loopholes that need to be studied and experimented. Firstly, there is a genuine absence of *in vivo* utilization of NPs in cancer treatment studies [20]. There should be a joint effort between clinicians, researcher and material researchers to further evaluate on the toxicity and biocompatibility of these phytofabricated ZnO NPs in different animal models and finally utilized in

clinical applications [1]. Without *in vivo* studies, all the *in vitro* research will be redundant and making no further progress. Next, one of the most prominent disadvantages of metal oxide nanoparticles such as ZnO NPs is its increased toxicity levels due to accumulation and agglomeration. As there are a lot of free energy in these nanoparticles, agglomeration process might occur making them less effective for drug delivery process. Therefore, future research should focus on minimizing this problem so that the cytotoxicity potential of ZnO NPs can be efficiently utilized [26]. Accumulation of these nanoparticles in the environment has also becoming a highly concerning problem as the field of nanotechnology has expanded. Regulatory agencies should play a crucial role in adopting new guidelines and assessing the safety measures of these nanoparticles to prevent unwanted ecological problems in the future [26].

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