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A Review on Green Synthesis, Cytotoxicity Mechanism and Antibacterial Activity of Zno-NPs

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Abstract

Recently, the development of eco-friendly methods for the synthesis of nanoparticles is an important key to nanotechnology. The use of green techniques using plants for the synthesis of nanoparticles is a replacement for chemical and physical techniques, because they are hazardous. Therefore, in this paper, we refer to green synthesis method using plant extracts and cytotoxicity and antibacterial mechanisms. Zinc oxide nanoparticles (ZnO-NPs) have been considered with regard to unique properties such as biocompatibility, selective cytotoxicity, anti-cancer and antibacterial activity. These nanoparticles are dissolved in the medium or in the cell and zinc ions are released, these zinc ions result in zinc-mediated protein activity disequilibrium and oxidative stress through reactive oxygen species, which may have a potential mechanism of action cytotoxicity of nanoparticles. Given the selective cytotoxic effects of ZnO-NPs due to the presence of more ROS in cancer cells, these nanoparticles can selectively target cancer cells and can be used as an anticancer agent. On the other hand, ZnO-NPs have significant antibacterial properties. Antimicrobial mechanism of ZnO-NPs may take place through the interaction of nanoparticles with bacterial cell surface and the production of reactive oxygen species (ROS) and release of zinc ions. ROS and free ions are important factors for several mechanisms, including increased membrane permeability, cell wall damage. These will weaken the mitochondria and cause oxidative stress and ultimately inhibit cell growth and cell death. Due to the selective toxicity effect against cancer cells, it can be used as a useful agent for the treatment of cancer and an alternative to antibiotics.

***Keywords*:** ZnO-NPs, anticancer, ROS, cytotoxicity, antibacterial activity

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Introduction

Nanotechnology has been considered as an applied technology in recent decades(1). Nanomedicine is the most important application of nanotechnology for medical problems and may be new methods for the treatment of certain diseases, such as cancer(2). Nanomaterials are particles with nanoscale dimensions and due to high rate of surface-to-volume, they have optical properties, catalytic reactions and chemical stability(3). These properties have attracted researchers to find new techniques for the synthesis of nanoparticles. Physical and chemical techniques use less time to produce nanoparticles, but to protect them, there is a need for protective materials that are poisonous and

cause toxicity in the environment. The green method by using plants is rising as a suitable, safe, non-toxic and eco-friendly method(4). Nanoparticles, such as iron oxide, zinc oxide, titanium dioxide have selective cytotoxicity to cancer cells and can be used in cancer treatment(5). Titanium dioxide nanoparticles, by binding to the cell membrane and formation of ROS on the surface of the membrane cell and the cytoplasm, can be used for cancer therapy under light irradiation(6). ZnO-NPs show selective cytotoxicity towards T98G cells and KB cells(7), whose mechanism of cytotoxicity is possible through zinc-mediated protein activity disequilibrium and ROS induction(8). Other nanoparticles, such as cobalt oxide, iron oxide, and copper oxide, have anticancer effects that have a cancerous effect by producing ROS or inducing apoptosis and necrosis(7). Among all nanoparticles, ZnO-NPs can be promising in treating cancer due to the highly selective nature of cancerous cells(5). Due to the small size of ZnO-NPs, they can easily interact with biological molecules. According to previous studies, ZnO can enter to bacterial surface and/ or with the bacterial core. ZnO is currently being considered as an antibacterial agent and exhibits significant antibacterial activity on a wide range of bacterial species (9). In this paper, we cover widespread views on green synthesis, anticancer and antibacterial activity of Zn-NPs, and focus on a wide range of cytotoxicity and antibacterial mechanisms, mainly ROS and the release of zinc ions which causes anticancer and anti-bacterial activity of ZnO-NPs.

Zinc Oxide Nanoparticles:

ZnO-NPs are one of the metal oxide nanoparticles, with several unique properties, such as good transparency, high electron mobility, and strong luminescence at room temperature (1), biocompatible and eco-friendly(10). ZnO is also a wide band gap semiconductor with band gap energy of ev3.3 in the near UV spectrum(11), which is important for scientific and industrial applications. These applications include transparent electrodes in a liquid crystal display and in energy saving or protective energy windows and other electronic programs (12) and as an additive in numerous materials as well as cement making in dentistry, foods(source of zinc nutrient), fire retardants, catalysts and photocatalyst, textile industry, health products such as sunscreen as a strong UV absorber, burn ointment, antibacterial treatments (9), gas sensors, tires (13), lubricants, as pigments in the manufacture of paints, ceramic(14), glass is used due to the ability to reduce the thermal expansion coefficient, reduce the melting point and increase chemical resistance(1).

ZnO-NPs synthesis Methods:

For synthesis of ZnO-NPs, various techniques including chemical, physical and biological methods are used. Chemical methods include precipitation, coprecipitation, colloidal methods, sol-gel processing, water-oil microemulsion, hydrothermal synthesis, cellulothermal, and sonochemical and polyol method (15). Chemical synthesis requires the use of a wide range of parameters and conditions, such as temperature, time, reactance concentration, etc. Changes in these conditions lead to morphological differences in the size and geometry of the nanoparticles. In some chemical techniques, chemical compounds / organic solvents such as H_2S and metal precursors are used as reducing agents that are poisonous and lead to the production of unusual side-effects, resulting in a significant risk to the environment (1). Physical methods of ZnO-NPs include ultrasonic, melt mixing, physical vapor deposition, laser ablation, electric arc deposition, and ion implantation(15). This method have disadvantages such as high energy consumption, low efficiency and the production of environmental pollutants.

Biological synthesis reduces the risk of pollution at the source level and uses non-polluting chemicals such as eco-friendly and safe solvents such as water and natural extracts to synthesize nanoparticles. Therefore, biological methods are suggested by using microorganisms and plants or plant extracts to synthesize nanoparticles as safe alternatives from other methods(3). The synthesis of nanoparticles by biological methods using microorganisms is somewhat difficult because it involves processes including careful

storage of cell culture, intracellular synthesis, and purification processes. Plants are eco-friendly and biodegradable in the process of synthesis (16). In fact, the green synthesis method is based on redox-based plants, in which the electron from the plant is transferred to metal ions and metal nanoparticles are created (17). Plants that contain more amounts of reducing agents produce more metal nanoparticles (18). Another useful feature of green synthesis is that it can be used from different parts of the plant such as stems, roots, fruits, seeds, cowls, latex, skin, leaves, flowers (19), even the biomass of dead and dried plants such as leaves, stems, dry straw, etc., to synthesize metal nanoparticles of different sizes and shapes (20). Some of the syntheses of ZnO-NPs using plant extracts including aerial parts of *Hibiscus rosa-sinensis* L.(21), *Ixora Coccinea* leaf extract (22), various parts of *Hybanthus enneaspermus* (23), aqueous extracts of *Hemidesmus indicus* (24), extracts of *Passiflora edulis Sims f. flavicarpa Deg* (25), seed extracts of *Celosia Argentea L.*(26), root extract of *Zingiber officinale* (27), leaf extract of *Hibiscus subdariffa* (28), Flower extract of *Nyctanthes arbor-tristis*(29), ethanol extract of *Mimosa Pudica* leaves(30), *Euphorbia Jatropa* latex(31), *Pyrus Pyrifolia* leaf extract(32) studied by other researchers. In general, the exact mechanism for the synthesis of nanoparticles remains unknown, but possible mechanisms suggest that plant extracts contain active alkaloids, phenolic acids, polyphenols, proteins, sugars and terpenes, play an important role in reducing ions metal and then make them stable (20). Zinc ions interact with active compounds such as proteins present in the extract, which results in the formation of a zinc compound. In addition, flexible connections of active molecules and proteins lead to the synthesis of stable spherical nanoparticles(15). The variation in the concentration and composition of these bioactive molecules in plants and their interaction with metal ions is the main factor for variation in the shape and size of nanoparticles(19). The synthesis of nanoparticles through plants is a relatively simple process and takes place at room temperature. The synthesis steps involve two nucleation and growth processes. At this stage, the plant extract is mixed with a solution of metal salt, the biochemical reduction of the metal salt occurs along with the change in the color of the reaction mixture, which indicates the formation of nanoparticles. Metal ions are converted from their mono or divalent oxidation states to zero-valant states, and the nucleation of reduced metal atoms takes place(33) (20). After nucleation, the growth process takes place and the small neighboring particles become smaller to form larger nanoparticles that are thermodynamically stable and reduce metal ions. Nanoparticles grow more and they come in many forms, such as cubes, spheres, triangles, hexagons, pentagons, rods and wires. In the final synthesis, plant extracts are able to stabilize the nanoparticles and be energetic and stable in terms of morphology. The concentration of metal salt, plant extract concentration, temperature, pH of the reaction solution and reaction time significantly affects the size, shape and quality of the synthesized nanoparticles(20). Recent studies have shown that ZnO-NPs synthesized from bioactive compounds of plants offer an opportunity to discover new drug agents for cancer treatment and antibacterial activity(17,28).

Properties of nanoparticles for biomedical applications:

Due to the high stability of ZnO nanoparticles, it has attracted much attention in biomedical applications, and the properties of the band-gap semiconductor in photocatalytic systems and the promotion of the production of ROS can be helpful (34), also to accelerate the destruction of water pollution and the destruction of cancer cells and bacteria are beneficial through photocatalytic activity and oxidative damage (35).

The effect of ZnO-NPs on cancer treatment:

The electrostatic characteristics of ZnO-NPs are another useful property for biomedical applications. ZnO-NPs are usually attached to the neutral hydroxyl groups and play an important role in their surface charge behavior. At low pH, protons are transferred from the environment to the particle surface, leading to be a positively charged from surface $ZnOH_2^+$ groups. While at high pH, chemical protons(H^+) move out from the

particle surface, causing them to be negatively charged surface with bonded oxygen atoms(35). ZnO-NPs have a positive charge under physiological conditions. Since cancer cells often have high concentrations of anionic phospholipids on their outer membrane, interaction with positive-charge ZnO-NPs are expected to be guided through an electrostatic bond, which increases cellular uptake and cytotoxicity(36). ZnO-NPs have the ability to decompose into ions that are absorbed by the body and are part of the feeding cycle, so they are recommended for biosensing in vivo(35). ZnO-NPs were also used as a convenient option for use in cancerous applications and as drug delivery(37). The characteristics of ZnO nanoparticles for cancer treatment include biocompatibility, selective effect and easy synthesis. ZnO-NPs have 28 to 35 times the selective cytotoxicity against cancer cells compared to normal cells. With more surface design, cytotoxicity can be increased(38, 5).

Mechanism of cytotoxicity of ZnO-NPs:

Several studies have shown that ZnO-NPs have a significant cytotoxicity compared to macro-scale ZnO against various types of cancer cells such as breast cancer, colon and lymphoma (35,17,39). The mechanism of cytotoxicity of ZnO-NPs includes intracellular release of zinc ions following ROS induction. This event causes zinc-mediated protein activity disequilibrium and oxidative stress, and ultimately killing of the cell (5). There is a strong correlation between the toxicity of ZnO-NPs and intracellular free zinc concentrations in human immune cells, which indicates a need to dissolve nanoparticles for the process of cytotoxicity, and when exposed to ZnO-NPs, the cytotoxicity at a concentration relatively high level was created(8). Extracellular solution Zn exhibits very little cytotoxicity. When the extracellular Zn solution is exposed to cell cultures, forms soluble amorphous zinc-carbonate phosphate precipitates. This precipitate should protect cells against cell cytotoxicity. On the other hand, by releasing soluble zinc ions into the cell, a cascade of pathways that are linked together is launched, which is responsible for the response of the cytotoxicity of the ZnO-NPs(5). The mechanism of cytotoxicity of ZnO-NPs is described in three steps:

ZnO-NPs and Zinc-mediated protein activity disequilibrium:

ZnO-NPs enter the cell by endocytosis. Some nanoparticles simply enter the cell, While some of them enter cells through pinocytosis and phagocytosis. By reducing pH, the speed of dissolution of ZnO-NPs increases rapidly, causing lysosome destabilization(40). To release zinc ions, acidic pH is required(41). This process leads to increased release of ions on the intracellular solution and zinc-mediated protein activity disequilibrium and increased ROS concentration. This would affect a wide range of vital cellular processes, such as DNA replication, repair of DNA damage, apoptosis, oxidative stress, electron transfer chains, cell homeostasis, etc.(5). Dissolution of ZnO-NPs in the extracellular medium and acid lysosomes causes cytotoxicity through the release of toxic ions Zn^{2+}. The release of Zn^{2+} in lysozomes and the intracellular environment can produce a series of harmful cellular effects, such as mitochondrial disorders, ROS production, lysosomal damage, and etc.(42).

ZnO-NPs and ROS production:

ZnO is a wide band-gap semiconductor with direct bang-gap of 3.37.eV and high-excitation binding energy of 60 MeV. The valence band and the conduction band are separated by a wide energy gap(i.e., 3.37 eV). UV light is required to detect electrons(e) from the valence band to the conduction band, causing holes in the valence band. Electricity conduction is performed by the movement of free electrons in the valence band. However, in the case of ZnO with nano-size, electrons jump to the conduction band in the absence of UV light(5). The electrons and holes(e^-, h^+) are the main carriers of the charge in the semiconductors and often rapidly combined and react with O_2 and OH in color solutions and can also be transferred to the surface of the nanoparticles. So, first, the electrons react with oxygen, and second, the holes react with hydroxyl ions or water to form superoxide and hydroxyl radicals(43), which

increases the number of electrons and holes at the surface of the nanoparticles. This particular feature of nanoparticles may be due to crystal defect in nanoparticles, due to their nano size. Holes act as a strong oxidant, water molecules turn into hydrogen and hydroxyl ions. Similarly, electrons act as a potent inhibitor, react with oxygen molecules, and produce superoxide radical anions($O_2^{\circ-}$)(35). This leads to the formation of hydroxyl radicals(OH°), $O_2^{\circ-}$ and hydroperoxyl radicals(OOH°)(44). These $O_2^{\circ-}$ more highly react with hydrogen ions(H^+), producing HO_2° radicals, these HO_2° molecules can produce hydrogen peroxide anions(HO_2^-) after the next interaction with electrons, then hydrogen peroxide anions can react with hydrogen ions to product hydrogen peroxide(H_2O_2). All of these radicals are reactive oxygen species, which act as strong oxidizing agents. The accumulation of these species in large quantities leads to an imbalance in the reduction of the oxidative homeostasis of the cell, resulting in oxidative stress, which is very harmful to the cell, and ultimately causes cell death. Therefore, holes and electrons in ZnO nanoparticles act as a redox reactive system and produce reactive oxygen species. Different molecules of ROS can create cascades in the cell or in adjacent cell membranes, which results in the destruction of cellular antioxidants, in which irreversible damage increases oxidative stress in the cell(45). ZnO nanoparticles show selective cytotoxicity toward cancerous cells. ROS production may be a reason for the selective cytotoxic response of ZnO nanoparticles to cancer cells. Due to the rapid metabolism of cancer cells compared to normal cells, ROS production is higher. When cancer cells are treated with ZnO-NPs, they, as a redox reactive system, increase the amount of chemicals and signaling molecules that produce more ROS, causing severe oxidative stress in the cell and eventually killing the cell. Resulting in high cytotoxic responses(46).

ZnO-NPs and apoptosis:

By increasing levels of ROS and oxidative stress, ZnO-NPs cause membrane damage through lipid peroxidation, protein denaturation(47) and DNA damage that causes cell death by apoptosis(48). DNA damage is mainly occurs by the failure of the DNA strands and the DNA-protein bonds. ROS can react with DNA components and lead to the fragmentation of DNA strands and mutations in DNA. OH radical, an oxygen species, defeats a string in DNA by forming 8-hydroxyl-2-dactylguanosine(8-OHdG) DNA(10). These DNA defects lead to the activation of the pathway of mitochondrial apoptosis and ultimately the death of the cell by apoptosis(49). Also, reactive oxygen species cause the opening of the mitochondrial membrane pores and release of some apoptotic proteins, including cytochrome C to cytosol, and activation of caspase. Studies show that the loss of mitochondrial membrane potential-mediated tumor cell apoptosis is mainly due to the decrease in mitochondrial membrane potential and the Bax / Bcl-2 ratios and the activation of caspase 9(50).

Antibacterial effect of ZnO-NPs:

Several studies have reported that ZnO-NPs for human cells are non-toxic(9) and due to its specific properties, such as the high ratio of surface-to-volume (50), physicochemical properties as well as reduced particle size, increase the particle surface reactivity. And thus uses it as an antibacterial agent, harmful to microorganisms and compatible with human cells(51). But its antibacterial activity is still unclear. The proposed mechanisms include: First, the interaction of ZnO-NPs with cellular levels, Zn^{2+} is linked to a negative-charge bacterial membrane(52) which is strongly absorbed due to forces(53) and leads to a change in permeability of the membrane, mitochondrial weakness(9) and oxidative stress in bacterial cells, leakage of reducing sugars and proteins and inactivation of the dehydrate respiratory chain. ZnO-NPs also create hole and gap in the bacterial membrane, the cell membrane is fragmented and leads to inhibition of cell growth and ultimately cell death (1). Second, the production of reactive oxygen species, including hydrogen peroxide, radical hydroxyl, and peroxide by these nanoparticles, can affect its antibacterial activity(9). ROS cause cell wall damage, increase

membrane permeability, enter the nanoparticles into cells due to lack of proton movement force and absorption of toxic Zn ions, resulting in mitochondrial weakness and oxidative stress, and ultimately cause cell death. The mechanism of toxicity in different media is different, and the ZnO solubility depends on the distribution of zinc ions in the medium, thus affecting its antibacterial activity(54). Zinc ions are transmitted from bacterial membranes through ion canals and consume more energy, thereby disrupting the living conditions of bacteria. Reducing the surface of ZnO-NPs can affect antibacterial activity(9). Previous studies have shown that free radicals or metal ions produced by metal nanoparticles penetrate the outer membrane of bacteria or peptidoglycans and cause bacterial cell fragmentation(55). In a study on the antibacterial activity of ZnO-NPs synthesized using *Trifolium pratense* flower extract, it was found that these nanoparticles have antibacterial effects against strains of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, causing cell and tissue damage in bacteria and was more effective than conventional antibiotics against bacterial strains(56). ZnO-NPs with a mean size of 30 nm by direct contact with two layers of lipid membrane can eliminate the integrity of the membrane(51). Also, the addition of radical release agents such as glutathione and mannitol results in an antibacterial effect of ZnO-NPs, which potentially produces ROS, especially hydroxyl radicals. However, free ions were not sufficient to produce antibacterial effects(57). The synthesized nanoparticles of *Cassia fistula* extract have significant antibacterial activity on *Klebsiella aerogenes*, *Escherichia coli* and *Staphylococcus aureus*(58). Other studies have reported similar results, and gram-negative bacteria are more resistant to gram-positive bacteria than ZnO-NPs, which in comparison with gram-positive bacteria, the growth of gram-negative bacteria is inhibited at higher concentrations of ZnO-NPs and the antibacterial activity of ZnO-NPs with particle size reduction and concentration increase, and the antibacterial effect of ZnO-NPs is time-dependent and gradually affects(59). Considering the importance of nanoparticles as an antibacterial agent against both gram-positive and gram-negative bacteria, we believe that in the future ZnO-NPs can be used as antibacterial agents such as ointment, lotion, mouthwash, nanoantibiotics and also in the food industry and to prevent bacterial bonding and spreading, these nanoparticles can be applied to various levels of medical devices.

Conclusion

The aim of this review was to investigate the green synthesis of ZnO-NPs and the cytotoxicity and antibacterial mechanisms of ZnO-NPs. Green synthesis of nanoparticles is much safer and more eco-friendly than chemical and physical methods. Due to the reducing and stabilizing agents in plants, nanoparticles can be synthesized with a certain shape and size. On the other hand, special focus is on the mechanisms of action that are considered as the most important issue in anti-bacterial and anti-cancer activity. Given the selective cytotoxic effect of cancer cells, it can act as a useful ingredient in the treatment of cancer as well as smart weapons against microorganisms as nano-antibiotics. ROS production is generated by the interaction of ZnO-NPs with the cell. The main mechanism of cytotoxicity is not related to the production of ROS; instead, the response is due to zinc-mediated protein activity disequilibrium, which leads to increased dissolution of free intracellular ions, the cause of most cell cytotoxicity. However, due to the unique properties of ZnO-NPs, some issues need to be addressed, including:(1) more research in vivo about the effects of anticancer, antibacterial and anti-inflammatory,(2) comparison biologically with other nanoparticles,(3) randomized treatment research, which, with better collaboration between scientists, can be understood as a deep understanding of the biology of cancer and the antibacterial effects of nanoparticles for clinical use. Considering the promising properties of ZnO-NPs, such as high ratio of surface-to-volume, long life and their intrinsic nature for selective toxicity toward cancer cells, they can clearly be considered as a key tool for cancer treatment and antibacterial agent.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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