



# Green Synthesis of Zinc Oxide (ZnO) Nanoparticles, Toxicological Assessment and Potential Biomedical Applications – A Review

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## Abstract

The development of solid, environmentally-friendly techniques for the enhanced production of plant-based nanoparticles is a critical advancement in the regulation of nanotechnology. Nanoparticles (NPs) have been extensively evaluated and utilized in various industrial and biomedical applications over the past two decades. In particular, the application of zinc oxide (ZnO NPs) has garnered significant attention due to their unique properties, such as UV-Vis absorption, photochemical activity, antifungal, catalytic, and antimicrobial properties. Due to the high toxicity of conventional chemicals and the harsh environmental conditions involved in traditional synthetic methods, green synthesis approaches have been adopted using plants, fungi, bacteria, and algae for the production of nanoparticles. This paper explores various green synthesis methods for the preparation of ZnO NPs and their application in several domains, with a focus on biomedical applications and their associated toxicity levels. The review draws on various secondary sources to present a comprehensive literature survey. The green synthesis approach is relatively safe and environmentally friendly compared to traditional

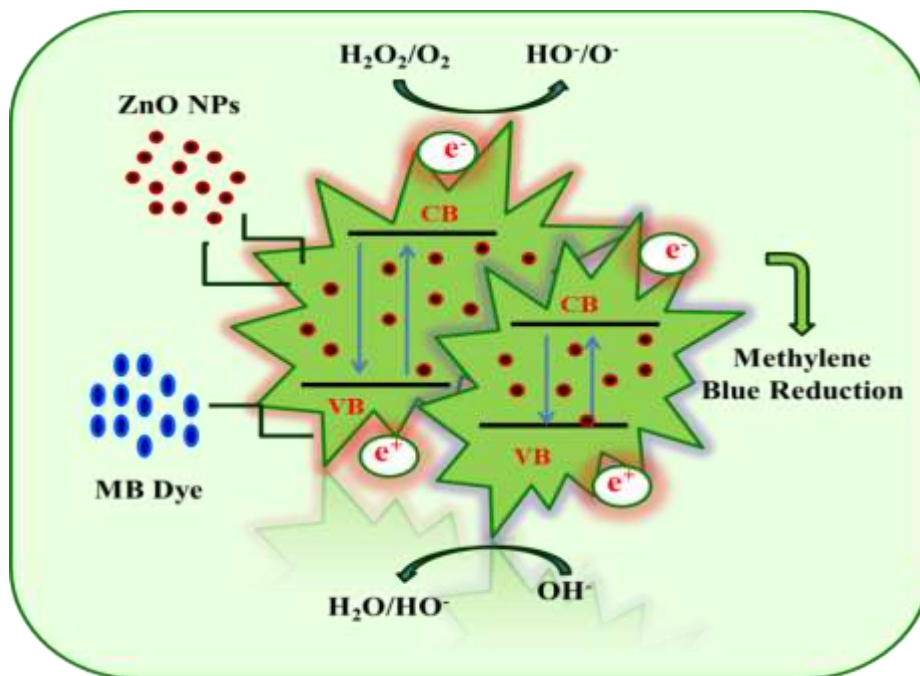


physical and chemical methods. Moreover, its biomedical applications have expanded progressively, with various processes such as bioimaging, drug delivery, biosensing, and gene delivery. Concerning toxicity, ZnO NPs show promise as effective agents against drug-resistant microorganisms and as potential alternatives to traditional antibiotics.

**Keywords:** Zinc oxide nanoparticles; Green synthesis; Apoptosis; Antimicrobial susceptibility analysis; Cell signaling.

## 1. Introduction

Science and innovation, particularly focused on the rapid development of ideas in recent decades, have led to significant advancements in nanotechnology. Nanomaterials, which possess distinct physicochemical properties, have the potential to drive the development of new systems, structures, devices, and nano-platforms, with promising applications across a wide range of disciplines [1][2]. Nanomaterials are materials at the nanoscale that enhance thermal transport, catalytic efficiency, nonlinear optical performance, and chemical stability due to their high surface area-to-volume ratio [3]. This characteristic has attracted numerous researchers to explore novel methods for their synthesis [4].



**Figure 1: Schematic diagram of efficient photo-catalytic degradation of synthesized zinc oxide nanoparticles by photon acting**

Although traditional methods (physical and chemical techniques) can produce large quantities of nanoparticles in a relatively short time, they often require toxic chemicals, such as stabilizing agents, to maintain stability, which can lead to environmental toxicity [5]. Zinc oxide nanoparticles, for example, have been reported to exhibit photocatalytic activity, particularly in the removal of carbon dioxide, as shown in Figure 1.

In light of this, green technology utilizing plants has emerged as an eco-friendly, non-toxic, and sustainable alternative. Hence, plant-extract mediated biosynthesis of nanoparticles (NPs) has proven to be economically viable and offers natural capping agents like proteins [6]. To mitigate chemical toxicity in the environment, the biological synthesis of various metal and metal oxide NPs via plant extraction has been utilized, providing an efficient method for controlling the chemical composition, size, and shape of NPs, including a precise mixture [7].



Biomedical applications, which offer significant advantages in terms of biodegradability, biocompatibility, and the use of sustainable nanomaterials, remain a prominent area of research. Among the various biomedical applications, the most studied NPs include paramagnetic NPs, quantum dots (QDs), nanoshells, and carbon nanotubes (CNTs) [8]. A broad range of nanostructures based on zinc oxide (ZnO) exhibits unique properties such as semiconducting, piezoelectric, and optical functionalities. Consequently, ZnO-based nanomaterials are considered to have extensive applications, including energy storage, nanosensors, cosmetic products [9], nano-optical devices [10], and nanoelectronic devices [11], among others. Biodegradability and lower toxicity are some of the most notable attributes of zinc oxide nanomaterials [12]. Zinc ( $Zn^{2+}$ ) is an essential trace element for adults and plays a vital role in various metabolic processes [13]. Under acidic or strongly basic conditions, zinc oxide nanoparticles may dissolve slowly. Solubilized ZnO NPs have been shown to release  $Zn^{2+}$  ions, which can apply pressure on cells and negatively affect various organisms [14].

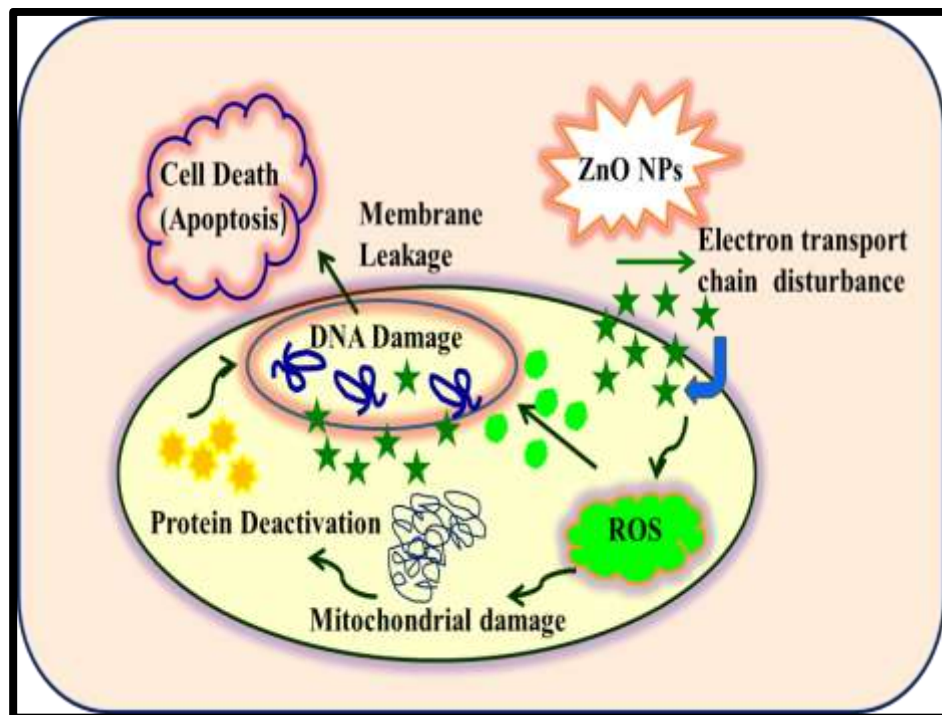
The essential actions of ZnO nanomaterials (NPs) have raised concerns regarding their biomedical applications. The toxic effects of ZnO NPs are primarily attributed to their solubility [15]. In extracellular environments, ZnO NPs dissolve, thereby increasing intracellular  $Zn^{2+}$  levels. The dissolution of ZnO NPs in suspension and their potential to elevate intracellular  $Zn^{2+}$  concentrations remain a topic of ongoing speculation. This review summarizes the current understanding of the use of ZnO nanomaterials in biomedical applications, their green synthesis methods, and their toxicological impacts [16].

Based on their characteristics, nanoparticles can be classified into two categories: natural NPs and inorganic NPs. Natural NPs include fullerenes, while inorganic NPs include magnetic NPs, noble metal NPs (e.g., Ag and Au), and semiconductor NPs (e.g., ZnO and  $TiO_2$ ) [17].



Nanoparticles can also be classified based on their origin, size, and chemical composition [18]. Nanomaterials are further categorized into natural and synthetic types based on their origin. Nanomaterials can also be classified by their structural dimensions, such as zero-dimensional (0D), one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D) materials. Zero-dimensional nanomaterials have nanoscale dimensions in all three directions; one-dimensional nanomaterials have a single nanoscale dimension, typically in the nanometer range; two-dimensional nanomaterials have two nanoscale dimensions, while three-dimensional nanomaterials possess nanoscale dimensions in all three directions. These bulk particles typically range from 1-100 nm in size [19].

According to their shape and structure, nanomaterials are classified into aggregated particles and nano-dispersions. Dendrimers, which are highly branched macromolecules, also exist on the nanoscale. Metal-mediated nanomaterials include nano-zinc [20], nano-magnesium, metal oxides such as  $\text{TiO}_2$ , and tightly packed semiconductors like quantum dots. Carbon-based nanoparticles are typically found in tube-like structures, such as empty cylinders or ellipsoids. Carbon nanostructures, such as spherical and ellipsoidal forms, are referred to as buckyballs, while round and hollow structures are called nanotubes. Nanotubes, resembling membrane leakage structures, can lead to apoptosis, as illustrated in Figure 2.



**Figure 2: Schematic summary of cell death apoptosis through membrane leakage after treating with synthesized zinc oxide nanoparticles**

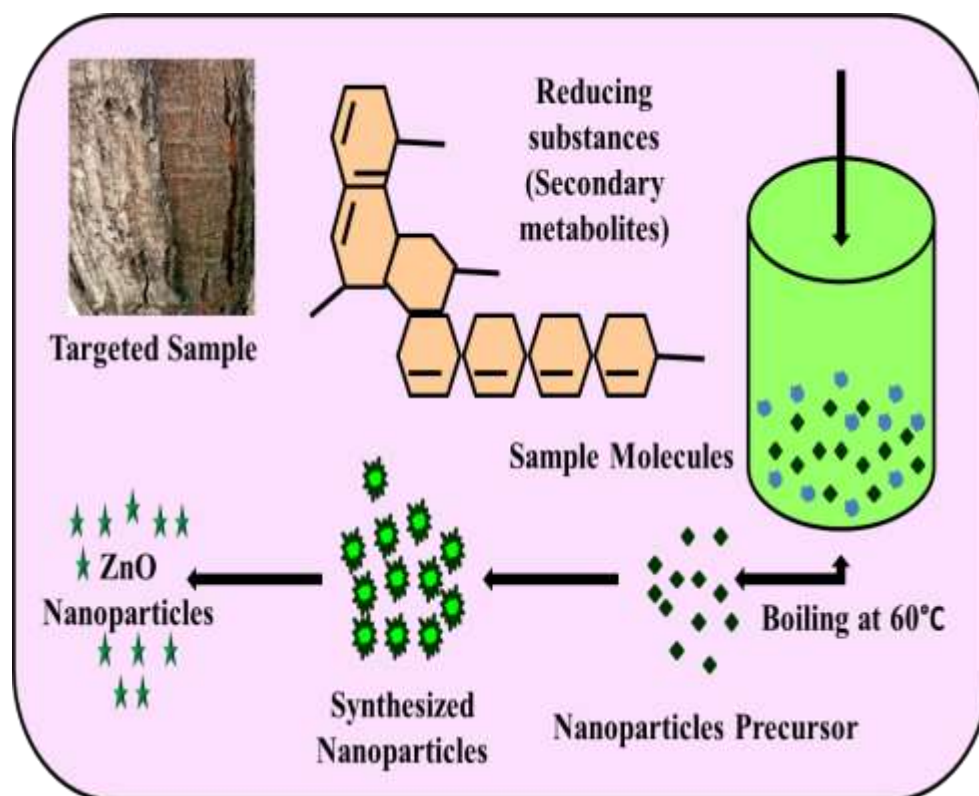
## 2. Nanoparticle Union Strategies

The two approaches proposed for the biosynthesis of nanoparticles (NPs) are 'bottom-up' and 'top-down' methods [21]. In terms of the granular perspective, the primary reaction involved is oxidation/reduction. The synthesis of nanoparticles is currently a significant area of research, aiming to develop eco-friendly methods and green materials for nanoparticle production [22]. Key steps involved in NP preparation, when viewed from the perspective of green chemistry, include the use of solvent suspensions for synthesis, environmentally benign reducing agents, and non-toxic capping agents to control nanoparticle formation. Most of the chemical and physical methods referenced typically rely on organic solvents [23]. This is primarily due to the hydrophobic nature of the capping agents used. Biosynthesis in living organisms aligns with the principles of green chemistry: eco-friendly processes, the use of reducing agents, and the



selection of capping agents for the reactions. The synthesis of inorganic metal oxide NPs using biological components has gained significant attention due to their unique properties, such as optical, electronic, and chemical characteristics [24].

### 3. Zinc Oxide Nanoparticles

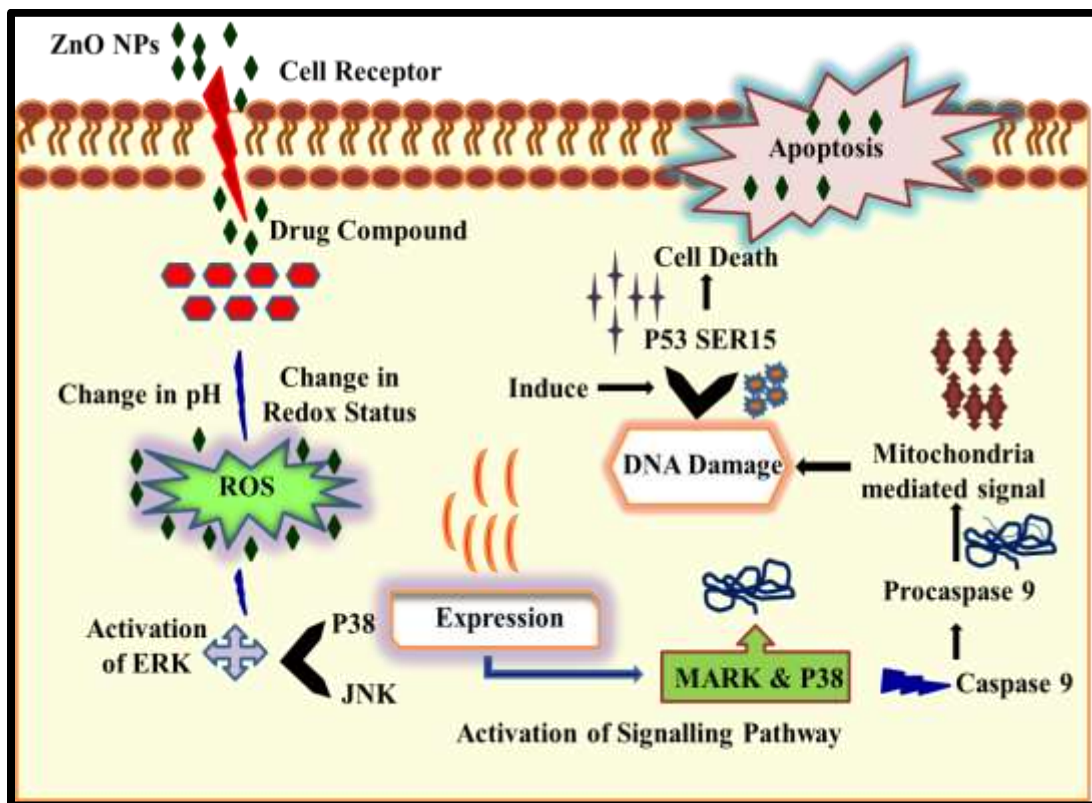


**Figure 3: Schematic diagram represent the synthesis of zinc oxide nanoparticles using raw plant bark sample**

Metal oxides play a crucial role in various areas of research, such as the development of microelectronic devices, sensors, piezoelectric devices, energy cells, photoresist coatings, and corrosion-resistant catalysts, as shown in Figure 3. Metal oxides are also used as protective agents against environmental toxins [25]. In the field of nanotechnology, oxide nanoparticles



(NPs) can exhibit remarkable chemical reactivity due to their small size and the increased surface area resulting from their high surface-to-volume ratio.



**Figure 4: Zinc oxide nanoparticles based activation of signaling pathway resulting in cell apoptosis (cell death)**

Zinc oxide (ZnO) is an n-type semiconducting metal oxide. Over the past few decades, researchers have been drawn to ZnO nanoparticles (NPs) due to their wide range of applications, particularly in biomedical systems, optics, and electronics [26]. Among various metal oxides, ZnO nanoparticles attract considerable attention due to their unique properties, such as a wide direct band gap of 3.3 eV at 37 °C, a high exciton binding energy of 60 meV, optical activity, high catalytic activity, anti-inflammatory properties, wound healing effects, and UV-filtering



capabilities. Several studies have reported the use of ZnO-based biosensors for detecting cholesterol, enzymatic activities, and other biosensing applications [27].

ZnO is a non-hygroscopic, non-toxic, inorganic, polar, and translucent material that is inexpensive, protective, and readily available. It has gained significant attention due to its involvement in various biological processes, sensors, transparent conductors, and surface acoustic wave devices. Zinc oxide nanoparticles possess unique properties such as semiconducting, piezoelectric, pyroelectric activities, and versatile applications in transparent devices, UV light emitters, chemical sensors, spintronics, personal care products, catalysts, coatings, and paints [28].

Due to these novel properties, ZnO nanoparticles have found applications in antireflection coatings, transparent electrodes for solar cells, UV light emitters, diode lasers, varistors, piezoelectric devices, spintronic devices, surface acoustic wave propagators, as antibacterial agents, and as photonic particles for gas sensing. Bio-molecules in plant extracts act as effective capping agents, playing a crucial role in nanoparticle synthesis and the activation of signaling pathways, as shown in Figure 4.

Capping agents stabilize nanoparticles through various mechanisms, including electrostatic stabilization, steric stabilization, hydration forces, and van der Waals interactions. Stabilization of nanoparticles is critical for their functionality and applications [29]. Zinc oxide nanoparticles have also been applied in the food industry, particularly in packaging. Their use in biodegradable polymers improves the quality of food and packaging through three main mechanisms: the release of antimicrobial particles, disruption of bacterial cell integrity, and the generation of reactive oxygen species (ROS) under light irradiation [30].

#### **4. Green Blend of zinc oxide nanoparticles**



**Table 1: Importance of ZnO NPs in the field of herbal medicine**

Plant	Precursor	Morphology	Active Properties	Ref
<i>L. aculeata</i>	Zinc nitrate ( $Zn(NO_3)_2$ )	Spherical	Antifungal activity against ( <i>A. niger</i> , <i>F. oxysporum</i> & <i>P. funiculosum</i> )	[31]
<i>Solanum torvum</i>	Zinc nitrate ( $Zn(NO_3)_2$ ) with Hydrogel Polymer	Spherical	Tissue Regeneration	[32]
<i>Nigella Sativa</i>	Zinc nitrate ( $Zn(NO_3)_2$ )	Homogeneous spherical	To remove food deficiencies	[33]
<i>M. fragrans fruits</i>	zinc acetate ( $Zn(NO_3)_2$ )	semispherical shape	Can be used to cosmetics, and to treat cancers	[34]
<i>A. elatus</i>	zinc nitrate ( $Zn(NO_3)_2$ )	Layers	Drug development	[35]
<i>P. hysterochrous</i>	zinc nitrate ( $Zn(NO_3)_2$ )	spherical	Antibacterial agent for biomedical application	[36]
<i>Pistia Stratiotes (PS)</i>	Zinc acetate Dihydrate ( $ZnC_4H_6O_4$ )	spherical	Anti-inflammatory and anti-fungal properties	[37]
<i>P. caerulea L. (Passifloraceae)</i>	Zinc acetate ( $ZnC_4H_6O_4$ )	Spherical	Anti-bacterial activity against urinary tract infection	[38]
<i>Raphanus sativus var. Longipinnatus</i>	Zinc acetate ( $ZnC_4H_6O_4$ )	spherical, hexagonal shaped	Solid waste management	[39]
<i>C. gigantea</i>	Zinc acetate dihydrate ( $ZnC_4H_6O_4$ )	spherical agrigates	Military camouflage application	[40]
<i>Ailanthus altissima</i>	Zinc sulfite ( $ZnSO_4$ )	spherical	Antibacterial, antioxidants and economic viability	[41]
<i>sheep faecal matter</i>	Zinc sulfite ( $ZnSO_4$ )	spherical structures	Environmental friendly NPs for biomedical application	[42]
<i>Phyllanthus niruri and stevia</i>	Zinc sulphide ( $ZnS$ )	Spherical	Antimicrobial properties	[43]
<i>Brassica oleracea var italica</i>	Zinc sulfite ( $ZnSO_4$ )	Non-homogeneous round structure	Nano-fertilizers	[44]



Recently, green-synthesized ZnO nanoparticles (NPs) have gained attention due to their antibacterial activity, biocompatibility, and antioxidant properties. The precursor sources containing relevant green components play a significant role in tailoring the structural properties of the nanoparticles [45]. In this context, it has been observed that ZnO NPs predominantly contribute to the field of medicine; most reports (Table 1) suggest that spherical morphology exhibits favorable properties for medical applications [46].

Elmer and White reported the pesticidal activities of zinc oxide nanoparticles by spraying incorporated ZnO on eggplants and tomatoes, observing a 30% reduction in disease severity compared to the control group [47]. Due to the growing popularity of green methods, several strategies have been implemented to produce ZnO NPs using various sources such as microorganisms, fungi, algae, and plants [48], among others. In vitro studies on plant extracts have shown that zinc salts (such as sulfate, zinc nitrate, chloride, and others) are reduced and synthesized into nanoparticles at elevated concentrations. Plant metabolites, such as tannins, saponins, terpenoids, starches, flavonoids, polypeptides, and phenolics [49], act as reducing and capping agents. Mild solvents like water, methanol, and ethanol are used to extract plant metabolites, which are then allowed to react with zinc salt solutions under various conditions to achieve optimal nanoparticle formation [50].

## 5. Plant-Mediated Biosynthesis of ZnO NPs

The biosynthesis of natural nanoparticles presents an alternative to physical and chemical methods for nanoparticle formation. Most researchers have focused on the green synthesis of metal oxide NPs, with plants providing a fast, low-cost, and eco-friendly option for human applications [51]. For example, the plant *Vitex negundo* can be used for the synthesis of zinc oxide nanoparticles, utilizing zinc nitrate hexahydrate as the precursor. The bio-synthesized ZnO



nanoparticles exhibited antimicrobial activities against *E. coli* and *S. aureus* bacteria. Dobrucka and Dugaszewski used *Trifolium pratense* (red clover) for the synthesis of ZnO nanoparticles, which were found to have a hexagonal shape with a size range of 50-65 nm. ZnO nanoparticles synthesized using *Lagenaria siceraria* (bottle gourd) extract exhibited antimicrobial, anti-arthritic, and anti-dandruff activities [52].

ZnO nanoparticles synthesized from green tea leaf extract have been evaluated for their capacitance properties for use in supercapacitor applications. Additionally, ZnO nanoparticles synthesized from *Leea asiatica* leaf extract showed antibacterial properties against urinary tract infection pathogens isolated from patient urine samples. These studies demonstrated the potential of low-cost, non-toxic plant-mediated synthesis of zinc oxide nanoparticles [53]. The efficiency of ZnO nanoparticles as photocatalysts for the degradation of organic dyes like methylene blue (MB) and methyl orange, as well as their antioxidant activity as assessed by DPPH assays, has been studied using extracts of *Phyllanthus niruri* and *Stevia*. Green synthesis of monophasic transparent ZnO nanoparticles, with a size range of approximately 15.8 nm, has been shown using these plant extracts as effective oxidizing/reducing agents. Stable and spherical ZnO nanoparticles have been synthesized using zinc nitrate and *Manilkara littoralis* leaf extract, with the properties characterized by FTIR, photoluminescence, UV-Vis spectroscopy, SEM, XRD, and TEM analysis [54].

## 6. Microbe-Mediated Biosynthesis of ZnO Nanoparticles

The microbial synthesis of nanoparticles involves a combination of cellular biochemistry, transport of ionic metals within cells, metal resistance mechanisms, metal-binding sites, ion



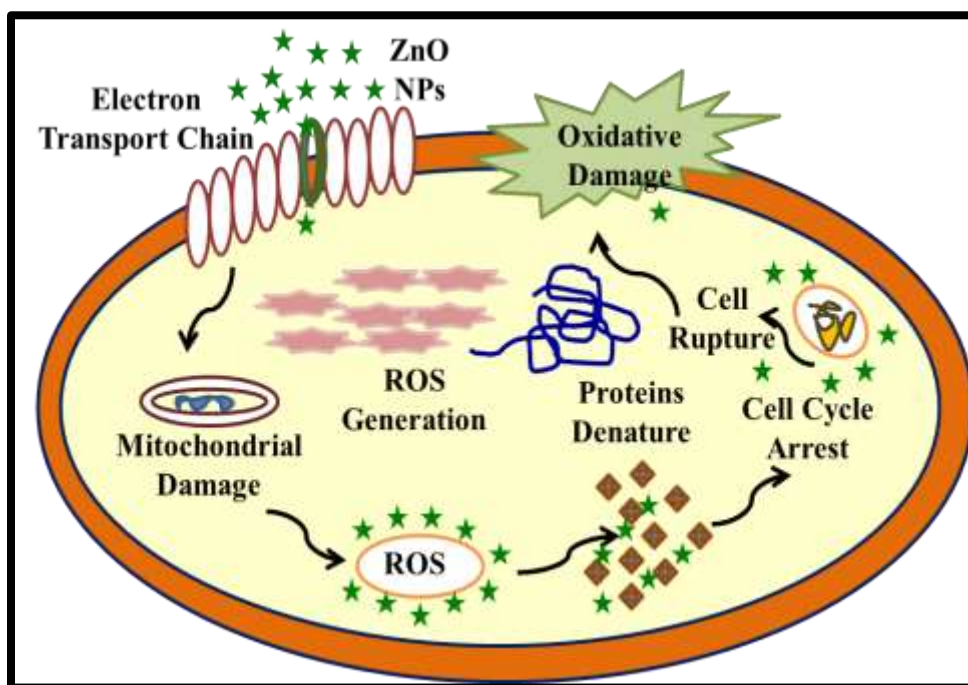
accumulation, and nucleation of metal oxides [55]. Zinc oxide nanoparticles have been synthesized from sheep fecal matter, producing moderately stable, round nanoparticles with a size range of 120-140 nm. *Serratia ureilytica*-mediated ZnO nanoparticles have found applications in the food industry. A novel method for biosynthesizing ZnO nanoparticles involves the use of the probiotic bacterium *Lactobacillus plantarum*. ZnO nanoparticles have also been synthesized using the actinobacterium *Rhodococcus pyridinivorans* NT2 from a zinc sulfate solution [56]. Bio-synthesized ZnO nanoparticles have been investigated for their potential as multi-functional materials, such as in vitro anticancer drug delivery (HT-29 colon carcinoma cell line) [57]. Green biosynthesis of ZnO NPs using *Candida albicans* as a reducing and capping agent has been described [58]. ZnO nanoparticles synthesized in this way have been shown to catalyze the rapid and efficient synthesis of steroidal pyrazoline compounds [59].

*Bacillus cereus* has been used as a bio-template for the formation of ZnO nanoparticles with raspberry-shaped and plate-like morphologies through simple thermal decomposition of zinc acetate, maintaining the pH of the reaction mixture. ZnO nanoparticles synthesized using *Aspergillus terreus* extract were produced extracellularly, resulting in spherical particles with a size range of 63.4 to 92.2 nm. The extracellular synthesis of ZnO NPs by fungi is particularly advantageous due to economic feasibility, ease of downstream processing, and scalability for large-scale production [60]. Fungal strains are often preferred over bacteria due to their enhanced metal bioaccumulation activity and resistance mechanisms [61]. ZnO NPs synthesized by *Aspergillus fumigatus* using *Candida albicans* also exhibited similar properties, with nanoparticles ranging from 15 to 25 nm, as confirmed by SEM, XRD, and TEM analyses [62].

## 7. Mechanisms of Natural Biosynthesis of ZnO NPs



Several studies have focused on the biogenic synthesis of silver nanoparticles, though fewer have explored the exact mechanisms underlying the formation of ZnO NPs. Understanding the fundamental mechanisms of green synthesis can aid in controlling the size and shape of nanoparticles.



**Figure 5: Pictorial represent shows electron transport chain through mitochondrial damage (oxidative damage)**

The biomolecules and phytochemicals present in microbial and plant extracts act as reducing and stabilizing agents during the synthesis of metallic nanoparticles [63], often facilitating cellular uptake and rupture, as shown in Figure 5. Studies have also reported that polyols and water-soluble heterocyclic compounds in *Cinnamomum camphora* leaf extracts are responsible for the bio-reduction of ZnO and chloroaurate ions, as well as the modification of zinc and gold nanoparticles [64]. Additionally, FTIR analysis has identified the presence of functional groups on the surface of zinc nanoparticles synthesized from *Rhizophora apiculata* mangrove leaf extract [65].



The characteristic peaks observed include the aldehydic C-H stretching ( $2,813\text{ cm}^{-1}$ ), the C=C stretching of the aromatic ring ( $1,570\text{ cm}^{-1}$ ), the O-H bending ( $1,240\text{ cm}^{-1}$ ), C-O stretching of phenols ( $1,236\text{ cm}^{-1}$ ), the C-H stretching in the benzene rings (between  $1,210$  and  $950\text{ cm}^{-1}$ ), and the C-H bond in the phenolic rings ( $910\text{-}740\text{ cm}^{-1}$ ). All these peaks correspond to the characteristic vibrational modes of polyphenols. The leaves of *Rhizophora apiculata* are rich in polyphenols, acting as effective reducing and stabilizing agents.

## 8. Applications of Biogenically Synthesized Nanoparticles

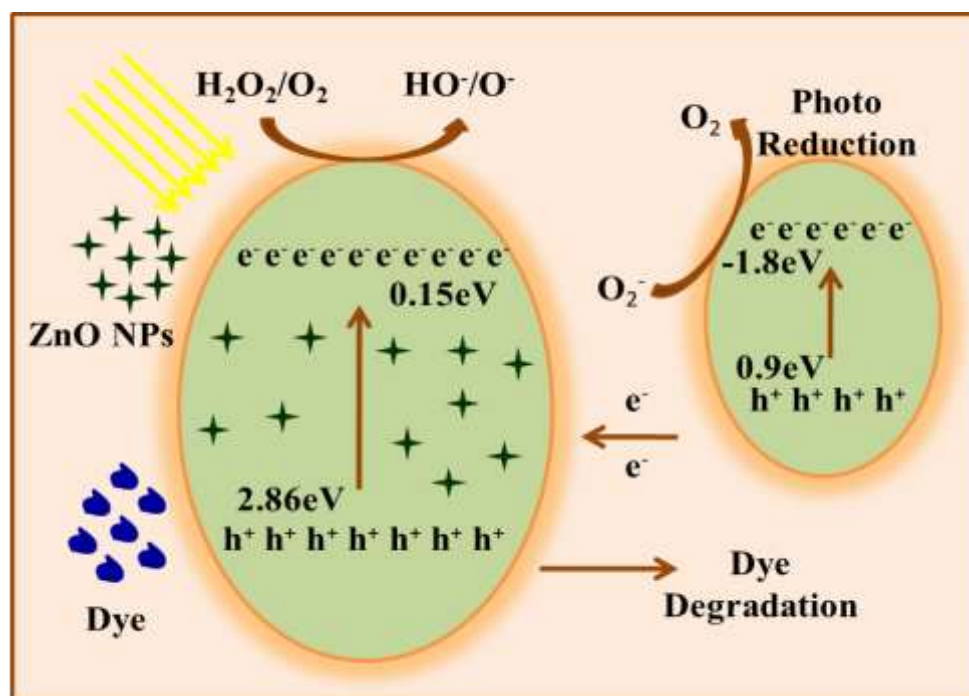
Biosynthesis offers an environmentally friendly method and has found applications in various scientific fields, including the detection of contaminants in food, water, soil, crop protection, pesticide applications, wastewater treatment, and more [66]. Some of the key applications of biosynthesized nanoparticles, including dye degradation, are discussed below and illustrated in Figure 6.

## 9. Biosensors

Currently, the use of silver nanoparticles (AgNPs) as biosensors has gained significant attention in various sectors, including environmental research, pharmaceuticals, food, and healthcare diagnostics [67]. Among these industries, the food sector has been particularly focused on exploring the potential of silver nanoparticle-mediated biosensors [68]. For example, a protocol for the calorimetric detection of caffeine in beverages using silver nanoparticle biosensors, which are incorporated into microspheres, has been developed [69]. Excessive caffeine consumption can cause various types of damage to the nervous system in humans. Therefore, these biosensors can be useful in accurately detecting caffeine levels in beverages [70]. Another study has developed a method for detecting mercury ions using biologically synthesized silver nanosensors. In the dairy industry, these biosensors play a significant role in



detecting microorganisms that affect the production and shelf life of dairy products, as well as chemical contaminants that impact the quality of dairy goods [71]. Beyond these applications, many biological assays can also be conducted using these biosensors [72]. Although the chemical stability of silver nanoparticles is lower compared to gold nanoparticles, silver nanoparticles offer excellent biosensor sensitivity, with the localized surface plasmon resonance (LSPR) being a key mechanism [73].



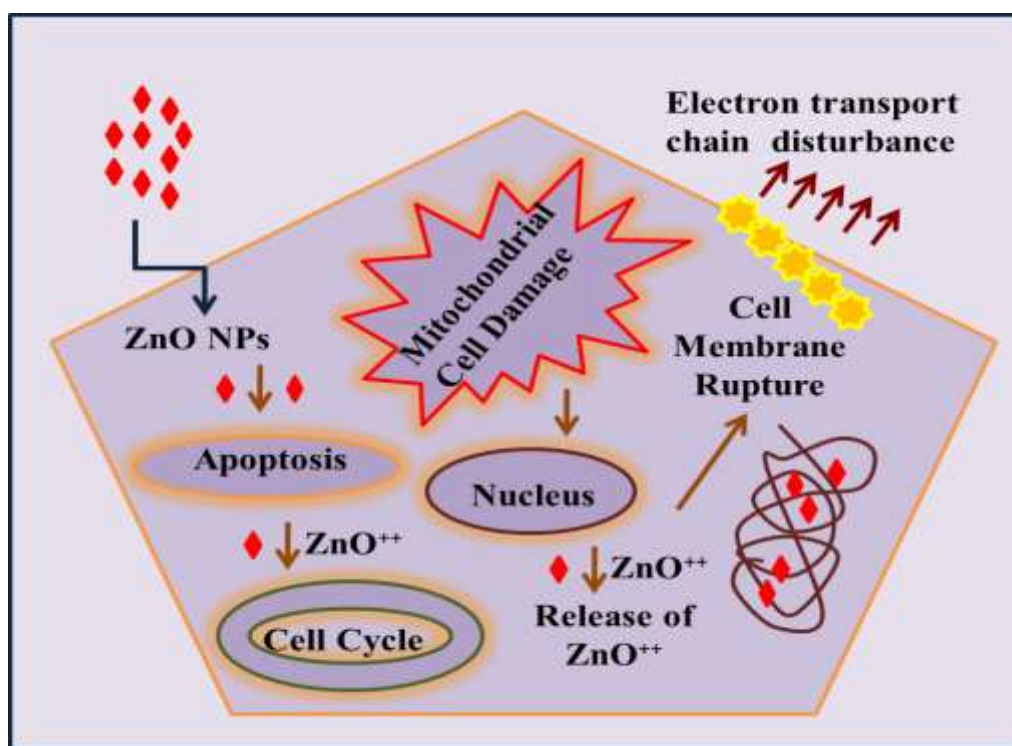
**Figure 6: Visible light irradiation of photo catalytic degradation through photo reduction**

## 10. Wastewater Treatment

Silver nanoparticles (AgNPs) are known for their excellent optical and catalytic properties. Biologically synthesized silver nanoparticles exhibit enhanced catalytic and electrical activity [74]. These catalytic properties are widely utilized in the degradation of wastewater, as illustrated in Figure 7. Biologically synthesized silver nanoparticles, using Konjac glucomannan extract [75], serve as effective reducing and stabilizing agents and have demonstrated catalytic



degradation of mono-azo and di-azo dyes in wastewater. These nanoparticles show significant catalytic activity in breaking down pollutants (such as dyes), which can be applied to treat wastewater streams, thereby managing the release of harmful by-products during chemical wastewater treatment. The stem extract of *Coscinium fenestratum* has been used for synthesizing zinc nanoparticles for wastewater degradation, while synthesized silver nanoparticles have been used for detecting the fungicide Thiram in wastewater through spectroscopic analysis [76]. Furthermore, silver nanoparticles have demonstrated excellent efficacy in degrading methyl blue (MB), methyl orange, and naphthol green B, which are acidic contaminants in wastewater [77].



**Figure 7: Pictorial diagram represents vicious cycle for nanoparticles based reaction involving in reactive oxygen species**

Extracellular synthesis of silver nanoparticles from fungal extracts has been studied, with the resulting materials exhibiting antimicrobial activity, useful for water purification processes.



In this study, polyurethane foam, which can be used for large-scale industrial applications, was coated with silver nanoparticles to combat microorganisms in water [79]. Silver nanoparticles possess a wide range of physical and chemical properties that make them valuable for various industrial applications, as well as for numerous scientific investigations [80].

## 11. Silver Nanoparticles for Crop Protection

Silver nanoparticles are known for their excellent photocatalytic and antimicrobial properties. Due to these activities, silver nanoparticles have been utilized in agricultural industries for crop protection [81]. For example, silver nanoparticles synthesized from *Capparis zeylanica* extract demonstrated antimicrobial activity against *Staphylococcus paratyphi*, *Enterococcus faecalis*, *Staphylococcus dysenteriae*, *Staphylococcus epidermidis*, *Candida albicans*, and *Aspergillus niger*. Another study revealed the sporicidal and bactericidal effects of silver nanoparticles synthesized from *Litchi chinensis* [82]. This research concluded that silver nanoparticles could be economically applied as sporicidal and bactericidal agents to protect crops and improve yields [83]. The synergistic antifungal effects of silver nanoparticles synthesized via a biological method using *Ligustrum lucidum* leaf extract have also been reported, as well as the use of zinc nanoparticles in agricultural applications [84]. Numerous studies have focused on the potential use of zinc nanoparticles in agriculture, exploring modern technologies and methods for crop enhancement [85].

## 12. Air Sanitization

Clean air systems have gained significant attention, especially in the aftermath of the COVID-19 pandemic, as they are essential for improving indoor air quality. Recent studies have explored the antimicrobial properties of ZnO nanoparticles, which have garnered the interest of various research groups and healthcare industries.



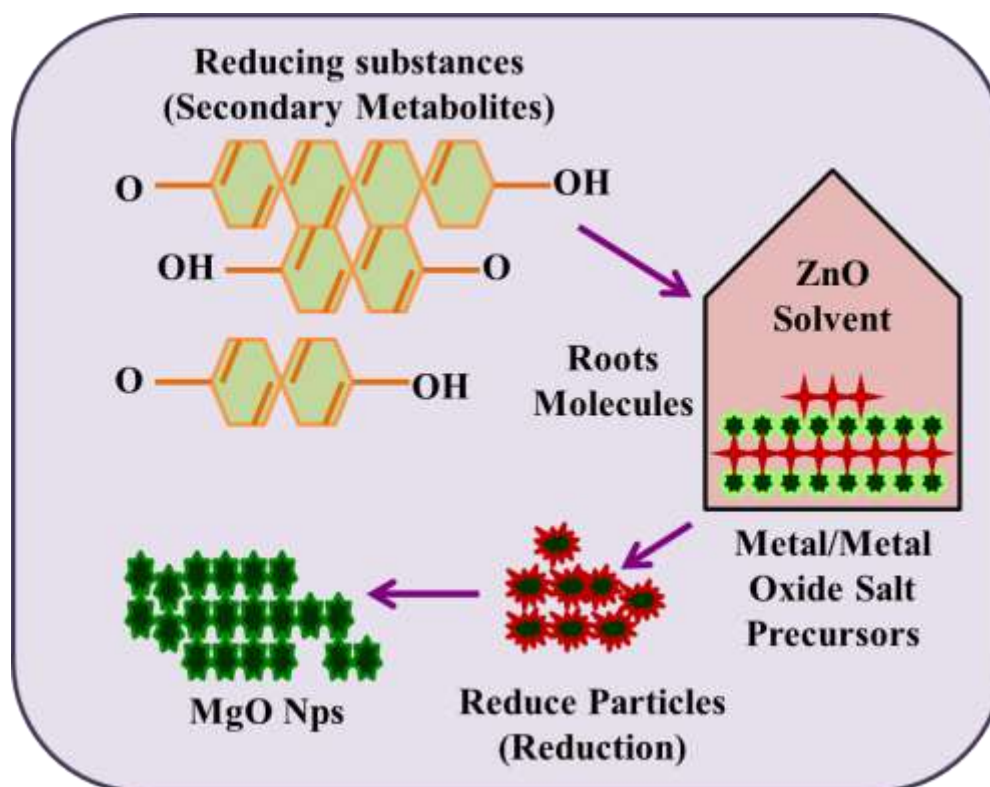
### 13. Characterization of Zinc Oxide Nanoparticles

The synthesized nanoparticles were characterized using several techniques, including Fourier Transform Infrared Spectroscopy (FTIR), Energy Dispersive X-ray Analysis (EDAX), Atomic Force Microscopy (AFM), X-ray Photoelectron Spectroscopy (XPS), Attenuated Total Reflection (ATR), UV-Visible Diffuse Reflectance Spectroscopy (UV-DRS), X-ray Diffraction (XRD), Transmission Electron Microscopy (TEM), Thermogravimetric-Differential Thermal Analysis (TG-DTA), Dynamic Light Scattering (DLS), Field Emission Scanning Electron Microscopy (FE-SEM), Photoluminescence (PL), Raman Spectroscopy, and Scanning Electron Microscopy (SEM). Extensive studies have been conducted on plants, particularly those belonging to the Lamiaceae family, such as *Vitex negundo*, *Plectranthus amboinicus*, and *Anisochilus carnosus*, which are known to promote nanoparticle synthesis with varying morphologies [86]. These morphologies include hexagonal, rod-shaped agglomerates, quasi-spherical, and circular forms, with a range of sizes. Results from characterization techniques such as XRD, TEM, and FE-SEM revealed that nanoparticle size was reduced with increasing concentrations of plant extract. Comparative analysis using techniques like XRD, EDAX, and SEM provided consistent results, though variations in particle size were noted depending on the method used.

The Debye-Scherrer equation was applied to determine the particle size of nanoparticles synthesized from *Vitex negundo* flowers and leaves, which exhibited an average size of 45.38 nm, as confirmed by X-ray diffraction (XRD) analysis. Zinc oxide nanoparticles synthesized from the leaves of *Azadirachta indica* (Meliaceae family) were also widely used in various studies [87]. The nanoparticle size distribution observed across these studies was consistent, with typical shapes, including nanobuds, hexagonal plates, and spherical forms, as confirmed by XRD



and Transmission Electron Microscopy (TEM). These studies revealed that nanoparticle formation occurred in the presence of functional groups such as carboxylic acids, amines, alkanes, carbonates, alcohols, and amides, which were further verified by Fourier Transform Infrared (FTIR) spectroscopy.



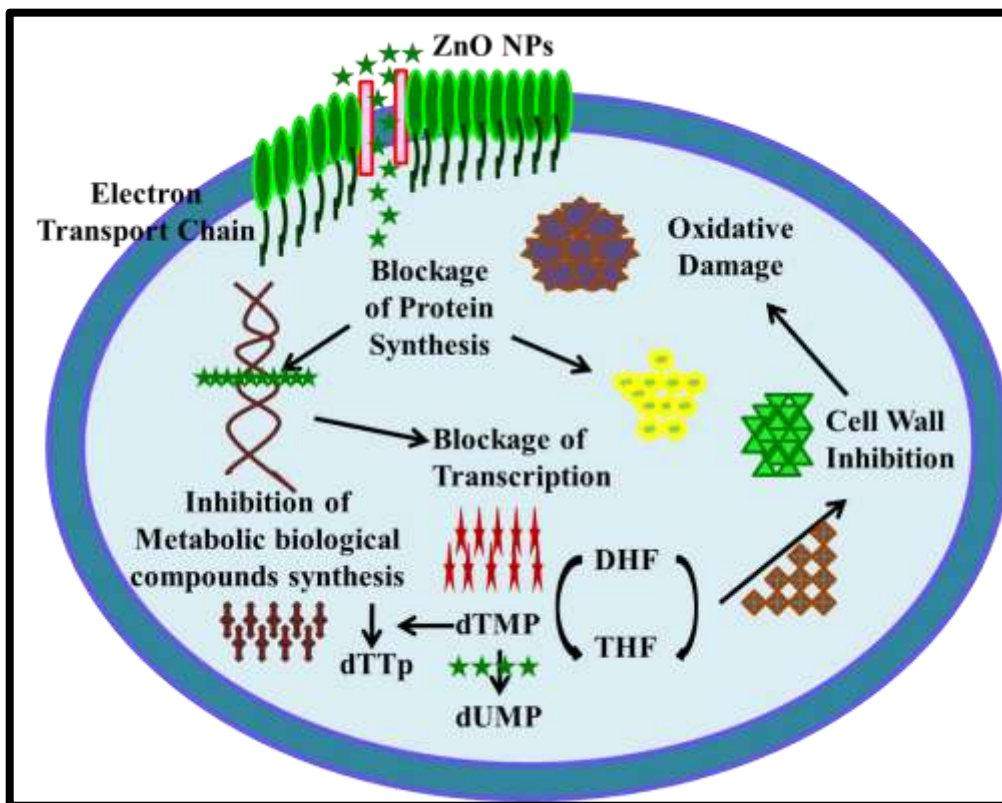
**Figure 8: Synthesis of nanoparticles using reducing substances with metal precursors**

Extracts from Aloe vera leaves, belonging to the Liliaceae family, showed the formation of aggregates in the nanoparticle synthesis process, as illustrated in Figure 8. Similar aggregated nanoparticle formations were also observed in nanoparticles synthesized from *Calotropis gigantea*, *Plectranthus amboinicus*, *Moringa oleifera*, *Agathosma betulina*, *Nephelium lappaceum*, and *Pongamia pinnata* [88]. To confirm the synthesis of nanoparticles, UV-Visible (UV-Vis) spectroscopy was employed. Crystalline nanoparticles were obtained by centrifuging the reaction mixture and drying the resulting pellet in a hot air oven.



## 14. Bio-medical uses of zinc oxide nanoparticles

### a. Drug Conveyance



**Figure 9: Systematic diagram represent the blockage of protein synthesis through oxidative damage**

The advantages of utilizing zinc oxide nanoparticles (ZnO NPs) for drug delivery arise from their two primary functions. First, due to their smaller size, nanoparticles can penetrate smaller blood vessels and be absorbed by cells, allowing for efficient drug accumulation at targeted sites. Second, the use of biodegradable particles in the preparation of NPs enables controlled and sustained drug release over a period of days.



The impact of zinc oxide nanoparticles on drug release has been studied using metronidazole benzoate as the model drug, observing its distribution through egg membranes [89]. The results revealed that zinc oxide nanoparticles, when loaded with the drug, interact with biological membranes, causing oxidative damage as shown in Figure 9.

## **b. Bio-Imaging of Zinc Oxide Nanoparticles**

In pre-clinical research, fluorescence imaging has been widely employed for therapeutic assessment. ZnO nanomaterials exhibit intrinsic excitonic blue and near-UV emission, which is associated with green fluorescence in O<sub>2</sub> vacancy cell imaging. Several studies have explored the application of ZnO nanomaterials in this context [90]. For instance, transferrin-conjugated green fluorescent zinc oxide nanoparticles have been used for cancer cell imaging with minimal cytotoxicity [91]. The optical properties of ZnO nanoparticles can be modified through doping with various elements. One study showed that ZnO NPs, doped with cations such as Ca, Cu, or Ni, exhibited improved stability in colloidal solutions and were applied to various cells for imaging studies.

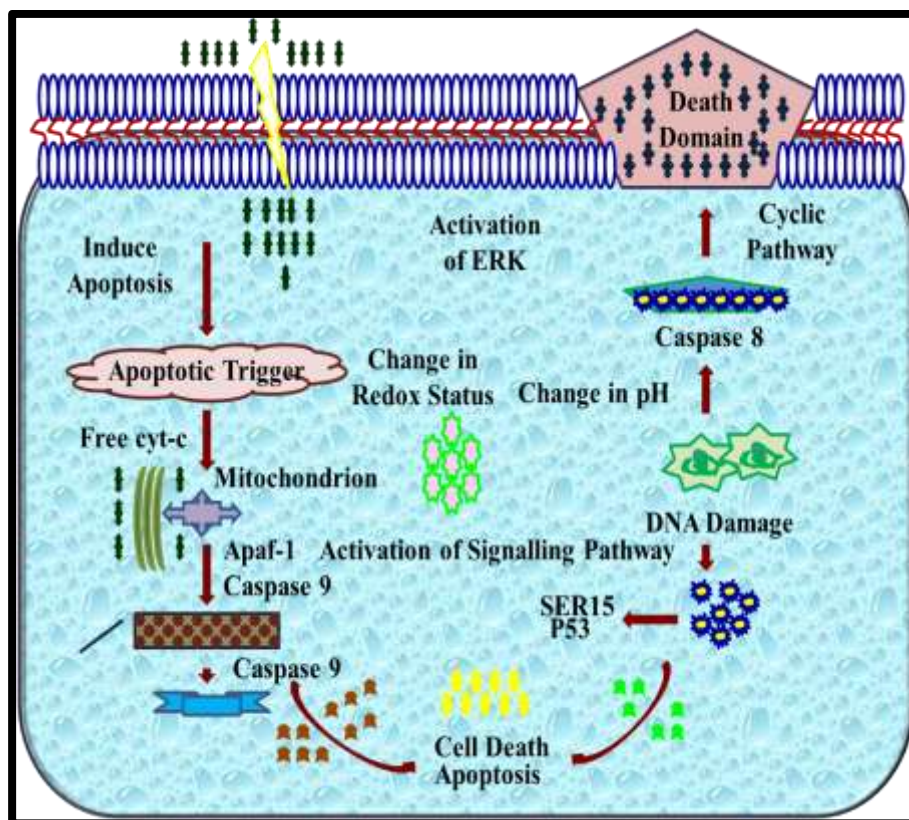
Microscale zinc oxide NPs are capable of entering the cell nucleus, demonstrating biocompatibility and optical activity. Heterostructural ZnO/Au nanocomposites, which involve gold nanoclusters (NCs) either on the exterior or at the tips of zinc oxide nanorods, have been synthesized and analyzed for their imaging potential [92]. In vitro imaging of cancer cells using anti-epidermal growth factor receptor (EGFR) antibody-functionalized zinc oxide nanoparticles has also been explored [93]. Quantum dots (QDs), with their unique optical properties, are another class of nanoparticles used for imaging. Zinc oxide QDs, which are incorporated into the cytoplasm, exhibit strong iridescence under UV light without significant cytotoxicity [94]. These QDs have been tested in mice following intravenous and intradermal injections.



Each imaging technique has its advantages and limitations. Different imaging modalities, combined with nanomaterials, can be optimized for synergistic benefits [95]. Nanomaterials, with their small size and large surface area, offer increased binding sites for functionalization and allow for the development of multifunctional imaging agents [96]. One specific study demonstrated the use of Gd-mediated zinc oxide QDs (with sizes under 8 nm) for both magnetic resonance imaging (MRI) and optical imaging [97]. Another study by Singh explored Fe<sub>3</sub>O<sub>4</sub>-zinc oxide core-shell magnetic QDs for targeted cancer imaging and therapy. For clinical applications, imaging techniques such as one-photon emission computed tomography (SPECT) and positron emission tomography (PET) are widely used due to their sensitivity and quantitative capabilities, with no tissue penetration limitations [98].

### **c. Drug Conveyance with zinc oxide Nanomaterials**

Zinc oxide nanomaterials are versatile nanoplatfroms used in both bio-imaging and drug delivery due to their adaptable surface chemistry, large surface area, and phototoxic effects, among other properties [99]. In vitro studies have shown that zinc oxide nanoparticles (ZnO NPs) can be highly toxic to cancer cells, microorganisms, and leukemic lymphocytes [100]. The intrinsic blue fluorescence of zinc oxide quantum dots (QDs) can be combined with folate-conjugated chitosan through electrostatic interactions. When loaded with doxorubicin (DOX), a widely used chemotherapy drug, the drug loading efficiency can reach up to ~80% [101]. The interaction between DOX and the ZnO QD surface, facilitated by hydrogen bonding, ensures the encapsulation of the drug. Additionally, the stability of the zinc oxide QDs in solution is enhanced by the hydrophilic chitosan coating, which improves the overall stability and dispersibility of the nanoparticles [102].



**Figure 10: Zinc oxide nanoparticles induced apoptosis through activation of signaling pathway**

At physiological pH (7.4), DOX is rapidly released from the nanoparticles, making them suitable for in vivo and in vitro studies. Dendritic cell (DC)-based cancer immunotherapy has been explored, with significant advances in delivering antigens efficiently to DCs using nanoparticles [103]. Due to the large surface area of nanomaterials, they offer significant potential for such applications. For example, Fe<sub>3</sub>O<sub>4</sub>-zinc oxide core-shell nanoparticles with an average diameter of 16 nm have been developed for antigen delivery into DCs and are capable of inducing imaging contrast for tracking purposes [104].

## 15. Gene Delivery with ZnO Nanomaterials



In recent years, gene therapy has garnered significant attention in cancer treatment. One of the major challenges in gene therapy is developing safe gene vectors that can protect DNA from degradation while improving the efficiency of cellular DNA uptake [105]. Zinc oxide nanomaterials have demonstrated positive results in gene delivery applications. For example, zinc oxide nanostructures, specifically three-dimensional tetrapods, have been studied as vectors for delivering pEGFPN1 DNA (encoding green fluorescent protein) into A375 human melanoma cells [106]. The electrostatic interactions between plasmid DNA (pDNA) and zinc oxide nanostructures facilitate DNA delivery into cells. The unique three-dimensional structure of these nanomaterials aids in cellular uptake, as illustrated in Figure 10.

Surface modification of nanomaterials plays a crucial role in efficient gene delivery. One study reported the coating of zinc oxide QDs with positively charged poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) polymers to enhance pDNA binding for gene delivery. The polymer-coated zinc oxide QDs exhibited fluorescence at 570 nm and were capable of delivering large amounts of pDNA, such as the luciferase reporter gene, with minimal cytotoxicity. The use of PDMAEMA-modified zinc oxide QDs showed significantly lower cytotoxicity compared to uncoated QDs, likely due to the stabilizing effects of the negatively charged polymethacrylate layer on the QDs [107][108].

## 16. Biosensors Based on Zinc Oxide Nanomaterials

Biosensors are widely utilized in various sectors, including the food industry, environmental monitoring, and healthcare, for chemical and biological analysis. Biosensors can be categorized into electrochemical, piezoelectric, photometric, and calorimetric types based on their detection principles. Nanomaterials, either alone or combined with biologically active substances, are increasingly gaining attention due to their ability to provide efficient platforms



for high-performance biosensors. This is attributed to their unique properties, such as enhanced surface area, which allows for the immobilization of biomolecules like antibodies, enzymes, and proteins [109]. Furthermore, nanomaterials facilitate direct electron transfer to electrodes and provide active binding sites for biomolecules.

Zinc oxide nanomaterials offer several advantages beyond their semiconducting properties, including bio-sensing capabilities, high adsorption capacity, and increased isoelectric point (IEP  $\sim 9.5$ ), which makes them suitable for the adsorption of specific proteins such as antibodies and enzymes with low IEPs through electrostatic interactions. Other key characteristics of zinc oxide nanomaterials include low toxicity, increased electron transfer ability, large surface area, and enhanced biocompatibility and stability [110][111]. Zinc oxide-based biosensors are widely used for detecting small molecule analytes, including phenol, cholesterol, urea, hydrogen peroxide ( $H_2O_2$ ), glucose, and many other substances. In addition, there are biosensors designed for detecting physical and chemical changes, such as pH variations.

## 17. Toxicity Studies of ZnO Nanoparticles

As the use of nanoparticles continues to grow, it is critical to assess their toxicity. A study on the toxicity of three metal oxide nanoparticles—CuO NPs,  $TiO_2$  NPs, and ZnO NPs—was conducted using zebrafish embryos [112]. Zinc oxide nanoparticles are considered relatively low in toxicity compared to their ionic form, which is associated with higher toxicity. Further studies have shown that the toxicity of zinc oxide nanoparticles is partially concentration-dependent. A study on the toxicity of green-synthesized zinc oxide nanoparticles from *Amaranthus caudatus* leaf extract revealed reduced antibacterial activity against *Staphylococcus epidermidis* and *Enterobacter aerogenes* compared to chemically synthesized zinc oxide nanoparticles [113].



A toxicity study conducted on zebrafish embryos with zinc oxide nanoparticles at a concentration of 10 mg/mL showed no significant effects on survival or deformities. In a 90-day toxicity study, 100 nm zinc oxide nanoparticles with varying surface charges (negative: AE100[-], and positive: AE100[+]) were administered to Sprague-Dawley rats to assess organ toxicity. Significant toxic effects were observed at concentrations greater than 125 mg/kg, while no adverse effects were seen at concentrations of approximately 31.25 mg/kg for both male and female rats [114].

## 18. Conclusion

In conclusion, green synthesis of zinc oxide nanoparticles is considered safer and more environmentally friendly compared to physical and chemical synthesis methods. Zinc oxide nanoparticles are highly significant and versatile materials due to their diverse properties, functionalities, and broad range of applications. Green agents used in nanoparticle synthesis act as reducing and stabilizing agents, contributing to precise control over size and shape. The application of zinc oxide nanoparticles in agriculture promotes crop growth and productivity, which is crucial as the global demand for food increases, and the need for sustainable agriculture becomes more pressing. Furthermore, the biomedical applications of ZnO nanoparticles continue to expand in various fields, including bio-imaging, drug delivery, biosensors, and gene therapy. In terms of toxicity, zinc oxide nanoparticles can serve as effective agents against drug-resistant microorganisms, presenting an alternative to traditional antibiotics. This review aims to streamline research in the development of innovative systemic and clinical applications of ZnO nanoparticles, which could provide solutions to various medical challenges.

In recent years, nanotechnology has gained significant attention due to the evolving demands of modern society. The need for sustainable processes and environmental



considerations has driven the development of nanomaterials. Nanoparticles, especially metallic nanoparticles, have opened new avenues in various fields. Among these, silver nanoparticles have attracted particular interest due to their excellent physical and chemical properties. Nanobiotechnology has paved the way for sustainable and eco-friendly technologies. This review highlights several synthesis methods for silver nanoparticles, with an emphasis on biological synthesis. The advantages of this approach, including the use of non-toxic compounds, cost-effectiveness, and enhanced stability of products, are discussed. Additionally, the emerging applications of these green-synthesized nanoparticles in environmental remediation, sensing, wastewater treatment, and crop protection are explored. Despite the widespread use of ZnO nanoparticles in various research and industrial sectors, their environmental impact is an important consideration due to potential effects on human health and ecosystems.

## **19. Novelty of the study**

This study presents a novel green synthesis approach for the production of Zinc Oxide (ZnO) nanoparticles using plant-based methods, highlighting their unique biomedical potential while addressing the toxicological concerns of conventional synthesis techniques. This statement emphasizes the innovation of using plant-based methods for nanoparticle synthesis, the focus on toxicity reduction, and the potential biomedical applications of the resulting ZnO nanoparticles.

## **Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### **Ethical Approval**

Not applicable.

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Authors declare that there is no funding source from any private or government agencies.

### **Author Contribution**

**Saheb Ali:** Conceptualization, Data validation, Review & Editing.

**Chitra Shivalingam:** Conceptualization, methodology, validation, formal analysis, and interpretation of data.

**George Franklin:** Drafting of the original manuscript.

### **Competing Interest**

The authors declare no conflict of interest.

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