Antimicrobial Activity of Zinc Oxide Nanoparticles

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Abstract - **The purpose of this review is to summarize published data regarding synthesis and antimicrobial behaviour of zinc oxide nano particles. Zinc oxide is low cost, nontoxic, exhibit outstanding optical, physical, photocatalytic, and antimicrobial properties. Anti-microbial behaviour depends on shape and size of nano particles. Toxicity of zinc oxide nano particles toward both gram positive and gram-negative bacteria make them a potential antimicrobial agent. Due to this, zinc oxide nano particles are well accepted in the field of cosmetics, medicine, textile, food packaging etc. This paper reviews method of synthesis and application of synthesized material as anti-microbial agent. Zinc oxide nano particles are very much effective against Campylobacter jejuni, A. baumannii, S. aureus, S. Typhimurium, methicillin resistant S. aureus, Salmonella typhi and many more. They inhibit the growth of microorganisms by causing oxidative stress and by permeating into the cell membrane.**

Index Terms - **Anti microbial, Gram Positive & Gram negative, Inhibition, Nano particles, Synthesis, Zinc oxide**

I.INTRODUCTION

Principles of nanoscience and nanotechnology are used greatly in the field of microbiology and biotechnology to study antimicrobial behavior of metals like copper, silver, zinc, iron, or any other transition metals. Metal nanoparticles possess the unusual ability to get into any biological systems. Therefore, they are largely used in research and innovation. Inorganic nanoparticles offer a separate benefit over traditional antimicrobials synthesized from different chemical compounds. Many microorganisms have developed resistance to drugs over some generations. As a result, antimicrobial materials synthesized from chemical compounds have been competent for therapy but, they are insufficient to be used in the preparation of devices used in the field of medicine and in other antimicrobial services. Therefore, it is an urgent need for another technique to minimize the resistance towards the drugs in case of a variety of microbes, particularly in field of medicine and in health care sector.

Nanotechnology offers the use of materials with specific shape, size and desired properties. It also gives benefit of their orientation at the atomic or molecular scale. Advantage of nanoscience may be taken to kill or diminish the activity of many dangerous microbes. Availing the advantage of huge surface area to volume ratio and their exceptional physical and chemical properties, nanomaterials have come up as potential antimicrobial materials and nanomaterials like copper, zinc, titanium magnesium, silver and gold is being synthesized and used for this purpose.

II.LITERATURE REVIEW

Zinc oxide is known as promising inorganic material owing to their multifunctional properties and wide range of applications. Zinc oxide nanoparticles are low cost, nontoxic, exhibit outstanding optical, physical, and antimicrobial properties, photocatalytic activity. Food and Drug Administration listed ZnO nano particles as “generally recognized as safe (GRAS)” material. They are used in biosensor, window materials for displays drug-delivery, optical devices, cosmetics, gas sensor, food storage and solar cells. Shape and size of ZnO nano particles decide their properties, and accordingly method of synthesis ie, precursor temperature, pH, solvents, and other reagents be chosen. Zinc oxide nano particles between 20 nm and 45 nm enhance antimicrobial activity of the ciprofloxacin when added to it.

Zinc oxide nanoparticles generate reactive oxygen spices causing damage to cell wall as a result of ZnO-localized interaction, membrane permeability enhanced, internalization of nano particles cause proton motive force is lost with uptake of toxic dissolved zinc ions.[1] ZnO nanoparticles with more than 99.7% purity and average size of 30 nm with Brunauer-Emmett-Teller (BET)-specific surface area 35 m2 /gram were obtained from Inframat Advanced

Materials LLC (Manchester, CT). A stock suspension of concentration of 100 mg/ml was made by resuspending the nanoparticles in double-distilled water and the suspension was kept at 4°C.This suspension is checked for antimicrobial activity against Campylobacter jejuni. These bacteria showed sensitivity very much when treated with ZnO nanoparticles. The Minimum Inhibitory Concentration found to be 0.05 to 0.025 mg/ml of ZnO nanoparticles for C. jejuni. The antimicrobiall action of ZnO nanoparticles is most probablyly because of rupture of the cell membrane. ZnO nanoparticles caused oxidative stress in Campylobacter.[2]

ZnO nano particles were synthesized by using acetate precursor and sol-gel method. ZnO-NPs having size of 30 nm demonstrated good antibacterial activity against carbapenem resistant A. baumannii. The proposed mechanism of action of ZnO involves the production of reactive oxygen species, which elevates membrane lipid peroxidation that causes membrane leakage of reducing sugars, DNA, proteins, and reduces cell viability. [3] Zinc nano particles synthesized the by the solo chemical method were recognized as nanorods having length between 90.1 and 100 and wurtzite structure. This nano particles showed a strong antimicrobial effect against both gram negative S. aureus and S. Typhimurium The Minimum Inhibitory Concentration (MIC) equal to 0.05 mg mL/l for S. aureus and Minimum Bactericidal Concentration (MBC) equal to 0.5 mg mL/l for S. Typhimurium.[4]

ZnO nanoparticles were prepared in diethylene glycol (DEG)medium by forced hydrolysis of zinc acetate at 160°C. A surface layer of 8–13 nm sized ZnO nanoparticles were confirmed when characterized by x-ray photoelectron spectroscopy (XPS), XRD,TEM. Fluorescence properties were determined using photoluminescence spectroscopy and flow cytometry. Zinc oxide nano particles offer cell selective toxicity against potential disease-causing cells and find its application in the treatment of cancer and/or autoimmunity.[5] Zinc oxide nanoparticles with 99.9% purity were obtained from Nanostructured and Amorphous Materials Inc, Houston, TX. The synthesis procedure efficiently involves preparation of the nanoparticles by solid vapor deposition method. The nanoparticles obtained having size between 90 mm and 200 nm. They cause death of cancerous cells at the same time having no cytotoxic effect upon normal cells. Toxic behaviour of zinc oxide nanoparticle is because of generation of reactive oxygen species released by these nanoparticles.[6]

ZnO nano pyramids with hexagonal base (ZnO-NPYs) were prepared in laboratory without using surfactants or capping agents. Zinc oxide nanoparticles are smart as broad-spectrum antibiotics. Introduction to these nano particles resulted in over three-log reduction in colonies of methicillin resistant S. aureus with minimal increase in ROS or lipid peroxidation.[7]

ZnO nanoparticles are prepared by sol gel method with slight modification, using zinc acetate, ethylene glycol,2-propanol and glycerol having particle size 19.82nm. They inhibit the growth of methicillin-sensitive S. aureus (MSSA), methicillin-resistant S. aureus (MRSA), and methicillin-resistant S. epidermidis (MRSE) strains. These nano particles are efficient bactericidal agents and do not get affected by the drug-resistant mechanisms of MRSA and MRSE [8].Nano particles of zinc oxide showed remarkable effect against a variety of bacteria including pathogens such as Klebsiella pneumonia [9], Listeria monocytogenes, Salmonella enteritidis [10], Streptococcus mutans, Lactobacillus [11], and E. coli [10, 12] demonstrating little toxicity to human cells [13] Biological synthesis of zinc oxide nano particles plays major role in offering size and shape of nano particles, make them effective against pathogens. ZnO nano particles exhibit antimicrobial properties against of a wide spectrum of bacterial species at possibly lower doses. Mechanism of action of ZnO NPs is different from conventional antibiotics, therefore microbes do not develop multidrug-resistant against these nano particles[14] Zinc oxide nanoparticles with average size in the range of 20 nm were synthesized following the method of (Jaisai et al., 2012), with slight modifications using Zinc acetate dihydrates and Sodium hydroxide .ZnO nanoparticles exhibited remarkable ($p < 0.05$) decrease in viability of test bacteria with increase in time duration and absolute elimination (0 Log CFU/mL) was observed after 8 h for S. typhimurium and 12 h for S. aureus, demonstrating the bactericidal effect of nanoparticles. Due their safe status and cheap cost, zinc oxide nano particles may be used for food products as a preservative and packaging material.[15] ZnO nanoparticles were prepared from two different sources, zinc acetate by chemical co-precipitation method, and green synthesis using Sesbania grandiflora leaf extract method. Nano particles with spherical and flakes-shape and the estimated sizes 70–150 nm were obtained. Anti-bacterial activity was determined by agar well diffusion method. Zinc oxide nanoparticles (ZnO NPs) have come out as a good anticancer and antibacterial activity, which are involved with their extraordinary capability to trigger excess reactive oxygen species (ROS) generation, release zinc ions and encourage cell apoptosis. zinc oxide nanoparticles could elicit hemolysis and harshly impact the proliferation of lymphocytes at concentrations 25 μg/mL, 50 μg/mL, 75 μg/mL and 100 μg/mL.[16] Different concentrations of ZnO nanoparticles 0.2 mol/L, 0.5 mol/L, 0.7 mol/L and 1.0 mol/L were synthesized by a low-temperature sol-gel method. Slow cooling was done at 400 and 550 °C. The antimicrobial activity of various concentrations of nanoparticles against Salmonella typhi PTCC 1609 was determined by disk diffusion and agar dilution method at five concentrations of 10, 5, 2.5, 1.25 and 0.625 mg/mL. Results showed that the synthesized ZnO nanoparticles are extremely effective against Salmonella typhi. [17]

The ZnO nanoparticles synthesized by wet chemical method and directly applied on to the 100% cotton woven fabric using pad-dry-cure method. The antibacterial behaviour of the finished fabrics was assessed qualitatively by agar diffusion and parallel streak method, quantitatively by percentage reduction test. The results show that the finished fabric demonstrated extraordinary antibacterial activity against S. aureus in both qualitative and quantitative tests. The SEM analysis revealed the embedding of ZnO nanoparticles in treated fabrics. The wash durability study of the treated fabric was also carried out and found to withstand up to 25 wash cycles. [18]

The fractional dissolution of zinc oxide nanoparticles gives Zn2+ ions in aqueous suspension that are responsible for the antimicrobial action of Zinc oxide. In the broth Zn2+ ions are released that showed antimicrobial activity of ZnO. The complexation of Zn2+ ions by the components of the broth increased the solubility of the zinc in the liquid medium.[19] Zinc oxide quantum dots (ZnO QDs) are nanoparticles of purified powdered ZnO. Antimicrobial activity of these quantum dots was checked against, Escherichia coli O157:H7, Salmonella Enteritidis, and Listeria monocytogenes. The ZnO QDs were used as a powder, bound in a polystyrene film (ZnO-PS), or suspended in a polyvinylprolidone gel. (ZnO-PVP). ZnO-PVP coating had less inhibitory effect than the direct addition of ZnO-PVP. No antimicrobial behaviour of ZnO-PS film were observed.[20] Green synthesis of Zinc oxide nanoparticles (ZnO Nps) was carried out using the aqueous extract of green tea (Camellia sinensis) leaves. present amino acid, protein and lipids helped to stabilize the growth of the nanoparticles. Agar well -diffusion method was used to study the antibacterial and antifungal activities on selected pathogenic species. The synthesized ZnO Nps showed better and comparable antimicrobial activities with respect to the activities of synthetic drugs.[21] Nanorod-rich Zinc Oxide was homogenized by sonication and then added into sago starch solutions at different concentrations. The effects of zinc oxide nanorod incorporation on the flow properties of sago starch solution and sorption isotherm, water vapor permeability, antimicrobial, and UV transmission of sago starch films were investigated. ZnO-N sago starch films showed brilliant antimicrobial activity against Staphylococcus aureus. These behaviour suggest that ZnO nanorod has the potential as a filler in starch-based films for use as active packaging materials in the food and pharmaceutical and industries.[22] Zinc Oxide Nanoparticles (ZnO NPs) were synthesized by green method using the aqueous extract of Tabernaemontana divaricata green leaf. Characterization studies showed presence of pure hexagonal wurtzite crystalline structure, formation of spherical shape- sizes ranging fom 20 to 50 nm and these nano particles have been stabilized through the action of phenolic acids, steroids, flavonoids, phenyl propanoids, terpenoids and enzymes present in the extract of green leaf. The Higher antibacterial activity was determined against E. coli and S. aureus and smaller antibacterial behaviour against S. paratyphi in comparison with the standard pharmaceutical formulation. [23] A chemical method (precipitation method) was used to synthesize Zinc oxide nanoparticles using sodium hydroxide and zinc nitrate. These Zinc oxide nanoparticles have renowned inhibitory and bactericidal effects. The antimicrobial behaviour of zinc oxide nanoparticles was determined against human pathogens like Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Enterococcus faecalis, and Pseudomonas aeruginosa with well diffusion method. The antibacterial action improved with increasing concentration of zinc oxide nanoparticles. The results of the study showed that the zinc oxide nanoparticles may behave as potential antimicrobial agents.[24] Alkaline hydrolysis of zinc acetate in 2-propanol was carried out to prepare ZnO nano particles. The pecipitation method using Zn $(NO_3)_2$ and NaOH was also carried out to synthesize zinc oxide nano particles. Characterization of the synthesized material was done by transmission electron microscopy (TEM) x-ray diffraction (XRD), and photoluminescence (PL) spectroscopy. Antibacterial activity of ZnO nanoparticles enhanced as particle size decreased. It is determined that equally the harshness and the surface oxygen species of ZnO nanoparticles enhances the bactericidal effect of ZnO nanoparticles.[25] Antibacterial activity of photoactivated zinc oxide nanoparticles (ZnO NPs) against human pathogens Listeria monocytogenes ATCL3C 7644, Escherichia coli O157:H7, and plant pathogen Botrytis cinerea was checked. Evaluation of antifungal activity of ZnO NPs reveal that significant photoinactivation (58%) of B. cinerea was observed at NPs concentration 5×10^{-3} M and incubation time of 24 h. These particles, in presence of visible light display tough antifungal and antibacterial activity. The properties of zinc oxide nano particles definitely be used for developing powerful fungicides in agriculture or an antibacterial agent in field of medicine and pharmacy.[26] ZnO nano particles were synthesized by precipitation method. Characterization was done using scanning electron microscopy (SEM) and X-ray diffraction (XRD). Antimicrobial behaviour was studied against K. pnumoniae using the disk diffusion method. The Minimum Inhibitory Concentration of nano particles was found 40 µg/ml. The standard growth curve showed that ZnO Nps of 0.75 mM inhibited K. pneumoniae after 4 h. The quantity of nucleic acid and protein released from the cells increased with zinc oxide nano particle concentration used. Optical density of the ZnO Nps-treated cells decreased within 30 min of incubation.[27] Synthesis of zinc oxide nanoparticles was achieved by using zinc acetate, polyethylene glycol and ammonium carbonate by precipitation method. zinc oxide nanoparticles were calcinated at 450º C. Antimicrobial activity was determined against Bacillus subtilis and Escherichia coli using disc diffusion method. The zone of inhibition increases with the increase in Zinc oxide nanoparticle concentration and decrease in particle size.[28]

Nontoxic biomaterial, ZnO nanoparticles were prepared from the $ZnNo_3$ using green process from leaf extracts of the Costus pictus D. Don medicinal plant. The biosynthesized zinc oxide nanoparticles exhibit strong antimicrobial behavior against bacterial and fungal species when employing the agar diffusion method. Synthesized ZnO nanoparticles exhibit anticancer activity against Daltons lymphoma ascites (DLA) cells as well as antimicrobial activity against some bacterial and fungal strains.[29] Zinc oxide nano particles are synthesized by solgel method, calcined at different temperature and various properties like molecular structure, morphological optical, and antimicrobial activity were studied. Characterization of synthesized sample done X-ray Diffraction (XRD), High Resolution Scanning Electron Microscope (HRSEM)High Resolution Transmission Electron Microscope (HRTEM), Fourier Transform Infrared (FT-IR), and Ultraviolet–Visible Spectroscopy (UV–VIS). Rod shaped ZnO NPs found to be stable at 300 and 400 °C. and have the superior antibacterial effect on the gram positive and gram-negative pathogenic bacteria.[30] Films incorporated with Besil Extraction Oil, especially in combination with zinc oxide nano particles, showed strong antibacterial activity against food borne pathogenic and spoilage bacteria and thus could be used as an active food packaging material to ensure safety and to extend the shelf-life of packaged foods.[31] ZnO-NPs were synthesized the by the solochemical method. Antimicrobial efficacy against Escherichia coli Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans Aspergillus brasiliensis, was determined using disc diffusion susceptibility tests and a broth dilution method. Zinc oxide showed bactericidal and antifungal activity against all five tested micro-organisms. ZnO particles having smaller size, larger specific area and higher porosity exhibit higher antimicrobial activity. [32] The integration of ZnO into Fuji Ortho LC, a dental product, added antimicrobial properties to the original compound without significantly altering the shear bond strength. ZnO holds potential for preventing decalcification associated with orthodontic treatment. Antibacterial effects were 1.6 times greater with 23.1% ZnO than with 13% ZnO.[33] Anti-microbial activity of ZnO NP with sizes of 70 nm and concentrations of 0, 3, 6 and 12 mmol l^{-1} is checked against E. coli O157:H7. Inhibitory action increases as concentration of nano particles increases. A total inhibition of microbial growth was achieved at 12 mmol l−1. zinc oxide nanoparticles can deform and injure bacterial cell membrane, leading a seepage of intracellular contents and ultimately the death of bacterial cells.[34] Zinc oxide nano particles when tested for biofilm-producing MRSA isolates showed zone of inhibition of maximum 16 and 17 mm at 500 lg/ml against strong biofilm-producing MRSA isolates and a minimum zone of inhibition of 12 and 14 mm at 100 lg/ml against weak biofilm-producing MRSA isolates.[35] Antibacterial behaviour of ZnO increased with decreasing particle size and increasing powder concentration. Effect of particle size on the antibacterial activity of ZnO powders was investigated using powders with different particle sizes from 0.1 to 0.8 μm. The changes of antibacterial action for Staphylococcus aureus were similar to those for Escherichia coli. However, the influence of particle size for Staphylococcus aureus was less than that for Escherichia coli.[36]

Coprecipitation method (5-10% manganese doped in ZnO) was used to prepare the antimicrobial material. The effect of Mn doping on the photocatalytic, antibacterial action and the result of doping concentration on optical and structural properties of nanoparticles were determined. The toxicity of nanosized ZnO and Mn doped ZnO were investigated using both Gram positive and Gram-negative bacteria as test organisms. The results showed that Mn doped ZnO nanoparticles enhanced the antibacterial activity than ZnO nanoparticles.[37] ZnO nanoparticles have a broad spectrum of antibacterial activities. The antimicrobial efficiency of ZnO nanoparticles on Campylobacter jejuni was determined for inactivation and inhibition of growth of bacterial cell. The Campylobacter jejuni was very much responsive to action of ZnO nanoparticles. The MIC of ZnO nanoparticles for Campylobacter jejuni was estimated to be in the range of 0.05 - 0.025 mgml -1 Antibacterial action of nanoparticles is probably because of oxidative stress in C.jejuni eventually damaging the cell membrane.[38] Toxicological effects of synthesized zinc oxide nanoparticles having size 1.5 nm were compared to aqueous zinc chloride, in the free-living nematode Caenorhabditis elegans. Zinc oxide nanoparticles induced transgene expression in the mtl-2: GFP transgenic C. elegans in a way similar to that of zinc chloride. This may be indication of intracellular biotransformation of the nanoparticles have happened. Results showed that synthesized nano particles possess toxicity to the nematode C. elegans like that of aqueous ZnCl2.[39] Zinc oxide nano particles were synthesized through clean and green synthesis and their anti-microbial behaviour is determined. The synthesis was done a natural sweetener (Stevia) extract. Synthesized nano particles possess a rectangular shape with a size range of 10 to 90 nm. The minimum inhibitory concentration was found to be 2.0 μg mL -1.[40] Zinc oxide nanoparticles were prepared from a natural source Cuminum cyminum (cumin) and anti-microbial ability was checked. Parameters like concentration, temperature, pH and time have a a big role to play on the preparation of zinc nanoparticles. From characterization data the shape of nano particles found to be spherical or oval with an average size of 7 nm. antimicrobial activity was determined by disk diffusion method. The minimum inhibitory concentration and Minimum bactericidal concentration of synthesized nano particles were calculated against various strains of bacteria. The sensitivity of ZnO nanoparticles was more in gram-negative bacteria than gram positive bacteria.[41]

III. FINDINGS AND SUGGESTIONS

Zinc oxide nano particles due to small size and large surface area are potential antimicrobial agents. Research shows that the various chemical and physical parameters can modify the antimicrobial activity of ZnO nano particles, the significant parameters found in this review concerning antimicrobial behaviour was the size, and method of synthesis (preparing composite, ecofriendly synthesis, effect of doping, sol-gel method) of ZnO NPs. These nanoparticles show a broad spectrum of antibacterial actions. They are toxic to both gram positive and gram-negative bacteria.

IV. CONCLUSION

Due to extraordinary biocompatibility, low toxicity and low cost, zinc oxide nanoparticles are used in the field of cosmetics, pharmacy, textile, dentistry, food packaging and many more. These nano particles act as selective killers They inhibit the growth of microorganisms by causing oxidative stress and by permeating into the cell membrane.

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