Synthesis of Zinc Oxide Nanoparticles by Wet Chemical Method and Study of Enhancement of The Antimicrobial Activity of Various Antibiotics by Zinc Oxide Nanoparticles

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***Abstract*— The study was conducted with the aim of preparing Zinc Oxide nanoparticles within a short timeline by using standard laboratory technique. Zinc Oxide Nanoparticles were prepared by wet chemical method. Antibiotic Susceptibility and enhancement in the activity of those antibiotics when applied in combination with Zinc Oxide Nanoparticles for *Escherichia coli* and *Staphylococcus aureus* were performed. For *E. coli*, the enhancement was seen for the following Antibiotics –Ampicillin, Erythromycin and Tetracycline. For *S. aureus*, enhancement was seen for the following antibiotics- Ampicillin, Cloxacillin, Cefotaxime, Cotrimoxazole, chloramphenicol, tetracycline, erythromycin, penicillin, amoxicillin, ciprofloxacin.**

Key words- Nanoparticles, Antibiotic Susceptibility

I. INTRODUCTION

Zinc is an essential mineral of "exceptional biologic and public health importance. The antibacterial activity of ZnO has been found to be due to a reaction of the ZnO surface with water.The research was performed with the aim of preparing Zinc Oxide nanoparticles within a short timeline by using standard laboratory equipment and without the requirement of highly expensive and specialized instruments. The concentration has been kept low enough just to observe how it enhances the action of the antibiotics .

11. EXPERIMENTAL

A. MATERIALS

Substances used:1%starch soln, zinc nitrate.6H2O,0.8 g of NaOH, distilled water, nutrient agar

Instruments used : conical flasks, magnetic stirrer, oven for dehydration, centrifuges, micropipette, antibiotic discs, petridish.

B. METHODS

Zinc Oxide Nanoparticles were prepared by Wet Chemical Method. 500 ml 1% Starch solution 5gm in 500 ml D/W and boiling the mixture until transparent. 14.87 g Zinc Nitrate Hexahydrate was added to the above soln to obtain a 0.1M Zinc Nitrate soln. 100 ml 0.2M NaOH solution was prepared by dissolving 0.8gm NaOH crystals in D/Wout of which 25 ml of the 0.2M NaOH solution was added dropwise along the walls of the vessel to the 0.1M Zinc Nitrate soln continuously stirring with magnetic stirrer for 2 hrs and then kept undisturbed overnight to allow the precipitation of Zinc Hydroxide. Supernatant was discarded and the remaining solution was centrifuged at 11000 rpm for 10 min to form of a pellet. The pellet was washed and dehydrated by keeping it in an oven overnight at 80 deg C . Zinc Oxide formed by the dehydration was suspended in distilled water as per the concentration requirement to obtain a suspension of Zinc Oxide Nanoparticles. Antibiotic Discs containing Zinc Oxide Nanoparticles
were prepared (20 mg in 1 ml D/W) to make a conc of 20 µg / µl. 10 standard antibiotic discs were taken on a dry, sterile petri plate inside the laminar air flow hood. 5 µl nanoparticle suspension was pipetted with a micro-pipette and impregnated each disc with the suspension which was further sterlised. Thus each disc has 100 µg Zinc nanoparticles. Antibiotic susceptibility tests were performed against *E. coli* and *Staphylococcus aureus* following the protocol for the Kirby-Bauer disc diffusion method .

Kirby Bauer Disc Diffusion Method

Also called as agar diffusion method which is used to test the effectiveness of antibiotics on specific organisms.an agar plate is first spread with bacteria ,then paper disks of antibiotics are added.
If the antibiotic stops the bacteria from growing or kills the bacteria there will be an area around the disc where the bacteria have not grown enough to be visible ,this is called the ZONE OF INHIBITION.

Figure 1- Synthesized ZnO Nanopowder

C. Results

	E.*coli*		S.*aureus*	
	Diameter of zone of inhibition(mm)		Diameter of zone of inhibition(mm)	
Antibiotic	Without ZnNP	With Zn NP	Without Zn NP	With Zn NP
Penicillin	0	0	19	19
Amoxicillin	27	27	40	42
Ciprofloxacin	28	28	26	26
Erythromycin	10	15	35	35
Cotrimoxazole	30	30	28	33
Tetracycline	21	26	35	35
chloramphenicol	30	30	30	34
Ampicillin	22	26	30	34
Cefotazime	32	32	30	36

D. Discussion

We synthesized Zinc Oxide nanoparticles by using standard laboratory equipments and reagents that can be procured very easily. The procedure was not time consuming and wasn't costly. Hence, the procedure was economically feasible and a practical one for large scale applications. The antibiotic susceptibility test showed that the application Zinc Oxide nanoparticles increase the bactericidal effect of various antibiotics for both Gram-positive and Gram-negative bacteria up to varying extent.The reasons for not being able to obtain optimum results may be attributed to constraints of time and resources. The Zinc nanoparticle suspension used for the susceptibility test was prepared with simple laboratory equipment and by vortexing which was not the best way of forming a suspension since it does not ensure uniform disruption of nanopowder aggregates. Other methods to produce nanoparticles of zinc oxide is sol gel technique, precipitation method.

E. Conclusion

Zinc Oxide Nanoparticles were synthesized by the Wet Chemical Method by using Zinc Nitrate as starting material, with the help of simple laboratory equipment in a short time frame. The nanoparticles were applied to antibiotic discs at sub-lethal concentrations to find enhancement in their activity. *E.coli* and *S.aureus* were used as standard organisms for the susceptibility test. The preliminary findings suggested that ZnO nanoparticles can be used externally to control the spreading of bacterial infections. In the prevention and control of bacterial spreading and infections, the main target is the cell wall structure. The cell wall of most pathogenic bacteria is composed of surface proteins for adhesion and colonization, and components such as polysaccharides and teichoic acid that protect against host defences and environmental conditions. These components are charged macromolecules; therefore, specific interactions to disrupt their main function and location may be triggered by introducing specific groups on the surface of the nanoparticles. Hence application of antibiotics in tandem with nanoparticles increases their antibacterial activity

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