Antibacterial Activity of Copper Nano-Particles against Pathogenic Bacteria

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Abstract

Copper nano-particle prepared by simple green method using copper sulphate, Chitosan & sodium borohydrate. The activity of copper nanoparticle encapsulated with Chitosan is studied by simple Cork Borer antibacterial assy. These partical shows significant antagonistic activity against most of pathogenic bacteria. The average zone of inhibition observed is in between 6mm to 30mm copper nano particle generally increase the surface activity such as ROS formation, photoreactivation, cellular uptake & catalysis. Nano particle also increase the ability of cell to adsorb proteins & salts from medium. As copper is a metal it releases metal ions in medium induces cytotoxicity. The efficiency of nano particle to inhibit the bacterial growth is depend on size & accumulation of nano particle in the bacterial cell.In present study it was found that pathogrnic bacteria such as Klebsiella pneumoniae, Staphylococcus aureus, Proteus vulgaris, Methanococcus spp, Salmonella typhi are highly sensitive to the copper nano-particle.

Keywords: Chitosan, antagonistic, ROS, cytotoxicity.

Introduction

Metals are used as antimicrobial agents because of their both bacteriostatic & bacteriocidal effect. Nowadays studies on nano material emerges different types of metallic & oxides of nano particle having potent antibacterial activity. The dynamic characters of nano particle are due to small size. Previous study showed that metallic nano particle having less effect on mammalian cells. Copper oxide nano particles are valuable, cheaper, stable antibacterial agent. There are several methods of preparing Copper nano particles such as chemical reduction, vacuum vapor & radiation microemulsion. Here we use the copper nano particle prepared in chitosan & Sodium Borohydrate. Chitosan is polymer obtained by deacetylation of chitin. It is cationic polysaccharide composed of β-D linked D-glucosamine & N-acetyl glucosamine units. It is nontoxic, degradable, & biologically active polymer with amino & hydroxyl group. Chitosan generally cheater metals so it stabilizes the copper nano particle & provide favorable conditions for nano particle synthesize.

Previously reported antibacterial activity of copper nano particle, it was found that it has significant potency to act as bacteriocidal agent than gold, silver, zinc nano particles. Combination of different Nano particles such as Silver & Copper may show more significant effect on bacterial growth. Gram-positive

bacteria have a thick cell wall containing multiple layers of peptidoglycan, while gram-negative bacteria have a relatively thin cell wall consisting of a single layer of peptidoglycan. Surfaces of copper nano particles interact directly with the bacterial cell wall & outer membrane, leads to rupture of cell wall & killing bacteria Mechanism of action of copper nano particle: 1) accumulation of nano particles in membrane alters permeability leads to release of lipopolysaccharides, integral proteins & intracellular fluid. 2) Oxidative damage to cell structure due to formation of Reactive Oxygen Species (ROS). 3) Bacterial cells generally take up whole metallic ions inside which deplete affect ATP production & DNA replication. Regardless of chemical & physical properties of copper nano particle it has extremely high surface area to volume ratio. Specifically copper nano particles having a potential to kill pathogenic bacteria.

Materials & methods

Copper Nano Particle-

The readymade solution of Cu-NP is used for antibacterial assy.

Media & Bacterial culture preparation

Antibacterial activity of Cu-NP was tested against both Gram positive & Gram negative bacteria. It is done by modified Cork well Diffusion Method. The bacterial culture is subculture in Nutrient broth at 37˚C on a rotary shaker at 150 rpm for bacterial growth. From this culture 100µ of culture were spread on Nutrient Agar plate by sterile glass spreader. The 10mm wells are punched in nutrient agar plate for testing antibacterial activity of copper nano particles. Using micropipette 100µi of sample was loaded in well on plate. After overnight incubation of plate in inverted position at 37˚C different zone of inhibition were observed & measured.

Results

The effect of different concentration of nano particle solutions is made by diluting it with distilled water. The Cu-NPs solution have been tested for their antimicrobial activities and an interesting antimicrobial profile has been observed against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebshiella Pneumonia Salmonella typhi, Proteus vulgaris,& Methanococcus*. The zones of inhibitions (mm) are mentioned in table-1

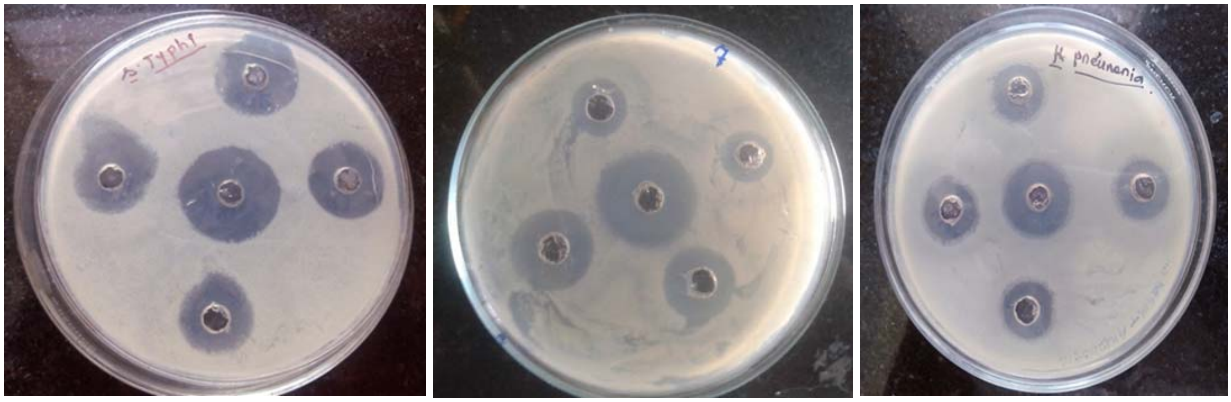
Effect of copper Nano particle on pathogenic organism

1) ***Salmonella typhi-*** it is obligate parasite, Gram negative enteric bacillus. Salmonella is motile facultative anaerobes its infection leads to typhoid or enteric fever. It also produce end toxin & has Vi antigen as well. Copper nano particle shows significant bacteriocidal effect against this strain of *Salmonella.*
2) ***Proteus vulgaris-*** it is Gram negative rod, enteric bacteria, facultative anaerobes. It is often infect hospitalized specific infection & community acquired infection

Table: 1 Zone of inhibition against each Bacteria.

		Zone of inhibition in mm				
Sr.no	Bacteria	1:1	1:10	1:100	1:200	1:500
1	*Escherichia coli*	21	18	15	13	11
2	*Bacillus subtilis*	20	14	10	09	08
3	*Bacillus thuringiensis*	30	20	16	13	11
4	*Klebsiella pneumoniae*	30	22	20	18	16
5	*Staphylococcus aureus*	21	16	15	08	06
6	*Pseudomonas aeruginosa*	24	15	14	13	12
7	*Proteus vulgaris*	26	20	18	15	14
8	*Methanococcus spp*	19	16	12	10	08
9	*Salmonella typhi*	25	21	14	10	09

3) ***Klebsiella pneumoniae-*** it is Gram negative, rod shaped, lactose fermenting bacteria. It is enterobacter belong to normal flora of human mouth & intestine. *Klebsiella pneumonia* infections are usually hospital acquired & patient with impaired host defense.



Salmonella typhi ***Proteus vulgaris*** ***Klebsiella pneumonia***

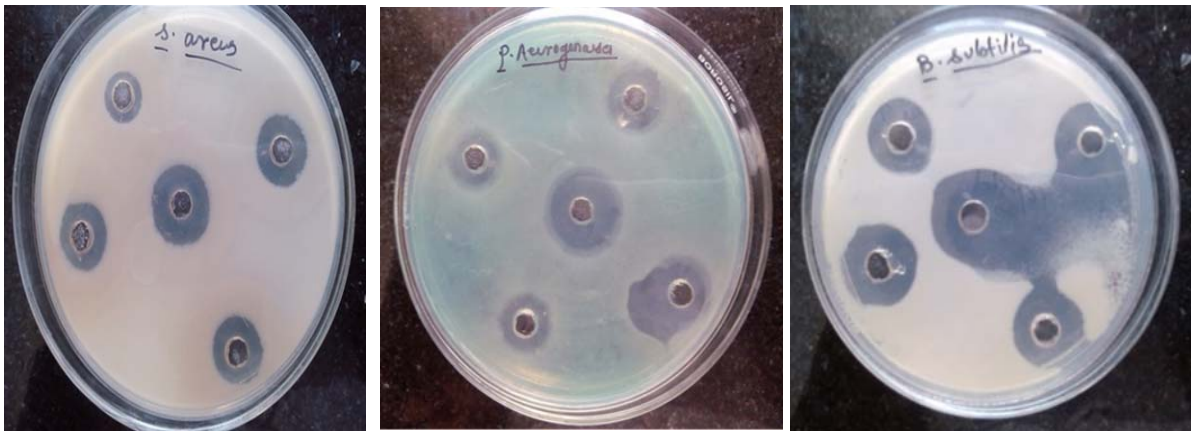
Fig. 1: Antibacterial activity of Copper Nano Particles.

4) ***Staphylococcus aureus-*** it is Gram positive spherical, cluster forming coccus, non motile, facultative anaerobic bacteria. *Staphylococcus aureus is a* human pathogen causes wide range of infections as well as food poisoning. Infection of *Staphylococcus aureus* also cause impetigo & toxic shock syndrome.

5) ***Pseudomonas aeruginosa****- it is* Gram Negative (γ- proteobacter) facultative arobic, produce soluble blue pigment being fluorescence. *Pseudomonas aeruginosa* free living, biofilm forming bacteria. It is common plant & animal (opportunistic) pathogen. If patient is immunosuppresed or has tissue

damage may invasive or toxigenic. It can infect ears, eyes, central nervous system & gastrointestinal infection. It is highly resistant to antibiotics.

6) ***Bacillus subtilis***- it is Gram positive, rod shaped, commonly found in soil. Usually non pathogenic. Spore forming, resistant to high temperature. Found on skin & human intestinal tract. *Bacillus subtilis* produce toxin called *Subtilisin*, it cause allergic reactions on repetitive exposure in high concentration.



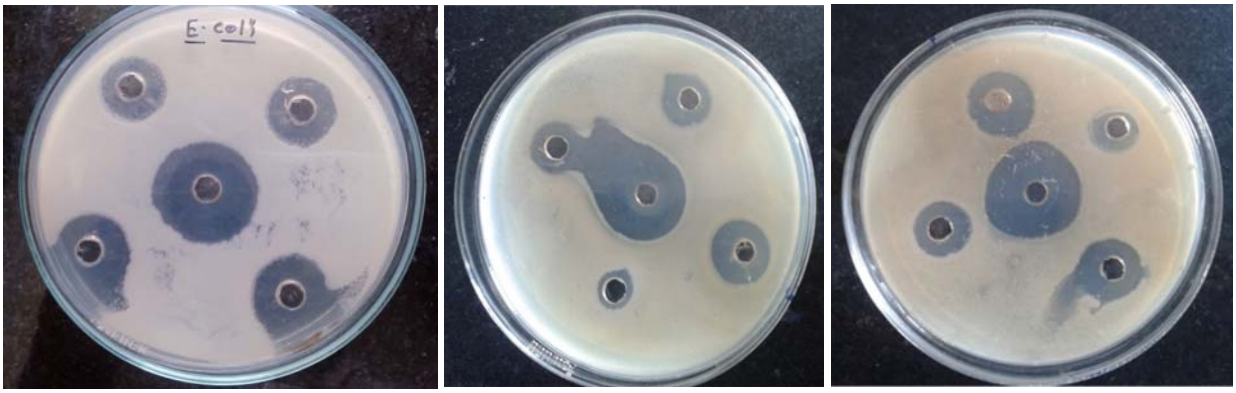
Staphylococcus aureus ***Pseudomonas aeruginosa*** ***Bacillus subtilis***

Fig. 2: Antibacterial activity of Copper Nano Particles.

7) ***Escherichia coli***- it is Gram negative, facultative anaerobic, rod shaped bacterium. Found in intestine of healthy person. Causes severe abdominal cramp, bloody diarrhea & vomiting, urinary tract infection.

8) ***Bacillus thuringiensis***- it is gram positive, soil dwelling bacterium. Infect insect, has host specificity & anthropogenic activity.

9) ***Methanococcus spp***- it is mesophilic, methane producing plant pathogenic bacteria.



Escherichia coli ***Bacillus thuringiensis*** ***Methanococcus spp***

Fig. 3: Antibacterial activity of Copper Nano Particles.

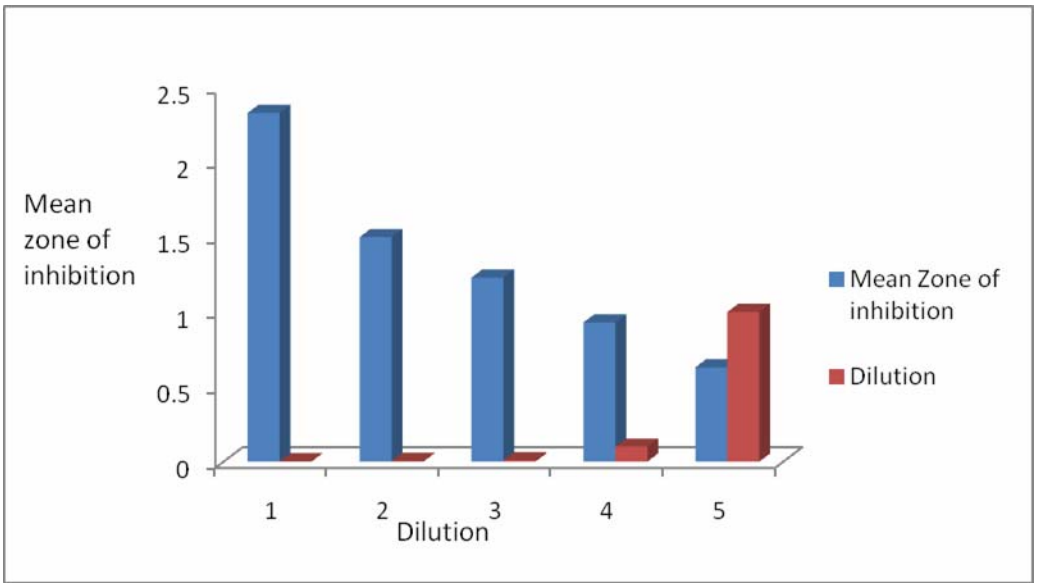


Fig. 4: Mean Zone of inhibition of Bacteria

Conclusion

Growth of different pathogenic bacteria was performed in presence in presence of copper nano particle. This study shows that Copper nano particle is significantly inhibit the bacterial growth against all nine pathogenic strain studied. *Klebsiella pneumonia* shows maximum susceptibility to copper nano particle at maximum dilution (1:500) shows 16 mm zone of inhibition. *Staphylococcus aureus* is more resistant than other bacteria, it shows 06 mm zone of inhibition at maximum dilution (1:500). The chemicals used in preparation of nano particle viz. Chitosan, Sodium borohydrate are non toxic which cannot inhibit the growth of bacteria. This shows that copper nano particle are significant bacteriocidal activity.

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