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ORIGINAL ARTICLE

Antimicrobial activity of Zinc oxide Nanoparticles on selected gram positive and Gram Negative Bacteria

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ABSTRACT
Nano biology is a field where interdisciplinary collaborations are essential and disciplines converge. Nanotechnology is promising fileld with various applications in the field of medicine and drug delivery. In the present study effect of zinc oxide were tested for antibacterial effect on four bacterial species. Two gram positive bacterial strains such as Staphylococcus aureus, Bacillus cereus and two gram negative bacterial strains such as Escherichia coli and Salmonella typhimurium were selected. The gram positive bacteria B.cereus showed that the maximum activity as compared to S.aureus . In gram negative bacteria S. typhimurium showed the maximum activity .The present study demonstrated that concentration of ZnO nanoparticles possess a great role in antibacterial effect. The high concentration (100 µg) showed the maximum antibacterial activity. The present study reveals that ZnO nanoparticles have great importance in treating pathogenic microbes since it has non – toxic to human beings. In future, ZnO nanoparticles has wide variety of the medicinal application.
***Key words**: Zinc oxide, antimicrobial activity, Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella typhimurium, gram positive, gram negative.*

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INTRODUCTION

Nanotechnology is the science that deals with matter at the scale of 1 billionth of a meter and is also the study of manipulating matter at the atomic and molecular scale. A nanoparticle is the most fundamental component in the fabrication of a nanostructure, and is far smaller than the world of everyday objects .The goal of nano biology is to assist in probing these systems at the appropriate length scale, heralding a new era in the biological, physical and chemical sciences [13]. Computational modeling will enhance the application of nanotechnology to key areas such as drug delivery and biomaterial design. These networks dictate how a cell responds to external stimuli, which in turn activate signalling cascades. One of the most promising application of nanotechnology is in the field of medicine. Indeed a whole new field of "nano medicine" is emerging. Nano medicine has been defined as the monitoring, repair, construction and control of human biological system at the molecular level using engineered nano devices and nanostructures [5]. Applications of nanotechnologies in medicine are especially promising, and area such as disease diagnosis , drug delivery targeted at specific sites in the body and molecular imaging are being intensively investigated and some products are undergoing clinical trials. Nanotechnology based delivery system could mitigate these problems by combining of tissue or organ specific targeting with therapeutic action. Multi functional nano – delivery systems with also combine targeting, diagnostic and therapeutic actions. Metallic nanoparticles have different physical and chemical properties from metals. Nanoparticles are currently made out of a very wide variety of materials, the most common of the new generation of nanoparticles being ceramics, which are best split into metal oxide ceramics, such as titanium, zinc, aluminium and iron oxides, to name a prominent few, and silicate nanoparticles, generally in the form of nano scale flakes of clay [18]. Nanoparticles with antimicrobial activity is a good effective method for drug delivery. There numerous nanoparticles are there they providing microbial resistance. the main nanocompounds with antimicrobial activity is Alumina Nanoparticles [17], Copper Oxide

nanoparticles [16], Gold Nanoparticles [4, 3],Magnesium Oxide nanoparticle [11], Silver nanoparticles [20],Titanium oxide nanoparticle [7].
Application of Zinc oxide nanoparticles in food systems may be effective at inhibiting certain food borne pathogens. ZnO nano particles possess strong antimicrobial activity against *Listeria monocytogens*, *Salmonella enteritidis* and *Escherichia coli* O157:H7 [9]. ZnO nanoparticles have a good potential to be coated on a plastic film to make antimicrobial packaging against bacteria such as *Escherichia coli* and Staphylococcus [12]. Zinc oxide nanoparticles are toxic on mesophilic and halophilic bacteria like *Enterobacter* sp., *Marinobactor* sp., and *Bacillus subtilis*. The nanotoxicity is more pronounced on gram negative bacteria. Zinc oxide nanoparticle reduces the growth of Enterobactor sp. Nanotoxicity towards gram positive cells is significantly less, due to the presence of thicker peptidoglycan layer. There are electrostatic interactions between nanoparticles and self surface as the primary step towards nanotoxicity, followed by cell morphological; changes, increase in membrane permeability and their accumulation in the cytoplasm[19]. Present study trying to test the antibacterial effects of metal nanoparticles, zinc oxide to certain gram-positive and gram-negative bacteria.

MATERIAL AND METHODS

Zinc oxide nanoparticles

The zinc oxide nanoparticles were prepared by following the procedure of Ghorbani *et.al*.,[8] and it is procured from the Research Laboratory of Physics,Dept.of Physics,Sree Narayana College for Women, Kollam, Kerala.

Bacterial strains.

Four bacterial species were selected for the present study. Two gram positive bacterial strains such as *Staphylococcus aureus*, *Bacillus cereus* and two gram negative bacterial strains such as *Escherichia coli* and *Salmonella typhimurium* were collected from the laboratory of Department of Biotechnology, CPCRI, Kollam, Kerala on January 2018.

Experimental design.

The method adopted for present bacteriological study is the disc diffusion method(Mouny *et.al*.,2015).Inoculated nutrient broth with a loop full of culture bacteria. Prepared nutrient agar (Himedia, batch 159609) filled the plate until solidification and 100micro litre culture was spread with a sterile rod placed three 6mm sterile disc having 10,20 and 30 µg of zinc oxide nanoparticles along with antibiotic (streptomycin) disc of Himedia (batch 176937) at the four corners of a culture plate. It is labelled as A,B,C respectively.In other culture plate, 50, 75, 100 µg of zinc oxide nanoparticle disc were used at three corners, were denoted as D,E,F. The cultural plate were labelled and allowed to incubate by measuring the diameter of zone of inhibition (mm) against the gram positive bacteria such as *S.aureus*, *B.cereus* and gram negative bacterial strains such as *E.coli* and *S.typhimurium* antibacterial activity determined.

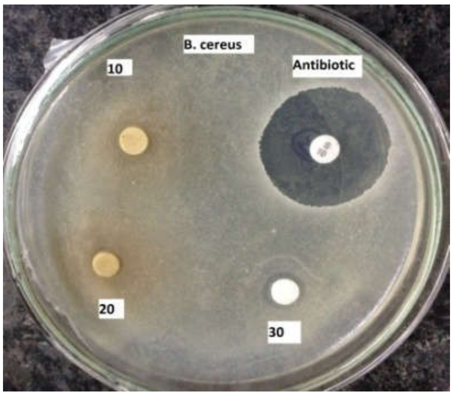
RESULT

The antibacterial activity of Zinc oxide nanoparticle sample was studied against both gram positive (*S.aureus*, B. Cereus) and gram negative (*E.coli* and *S.typhimurium*) bacteria. The result of antibacterial screening of Zinc oxide nanoparticles are shown in Table 1 and Figure 1.1 to 4.1 . The result shows the inhibitory action of samples. All the samples showed the antibacterial activity. Among two gram positive bacteria used sample F(100 µg) shown the maximum activity (diameter of zone of inhibition is 23mm) against B. Cereus . Moderate activity showed against the *Staphylococcus aureus* (diameter of zone of inhibition 21mm) (Figure.1.1&1.2,2.1& 2.2).Similarly among the two gram negative bacteria tested, the sample F(100 µg)showed the maximum activity (diameter of zone of inhibition 24mm) against *S. Typhimurium* and have a moderate activity against *E.coli* (diameter of zone of inhibition 20.8mm)(Figure.3.1&3.2,4.1&4.2).The result demonstrated that the bactericidal activity of zinc oxide nanoparticles depends upon the concentration. The size of the nanoparticle also had an important factor to determine its antibacterial effect. The lower concentration of Zinc oxide nanoparticles (10 µg, 20 µg, 30 µg) produced only mild antibacterial effect. The diameter of zone of inhibition produced by zinc oxide nanoparticles become 10mm, 13mm, 15.8mm in B. Cereus and 8mm, 11mm, 13mm in *S.aureus*, 12mm, 15mm, 16.2 mm *S.typhimurium* and 9.5mm, 11mm, 12.8mm in *E.coli* respectively.
The higher concentration of zinc oxide nanoparticles (50 µg, 75 µg, 100 µg) would produce the significant effect on the antibacterial action. *B.cereus* showed 17mm, 20mm and 23mm diameter zone of inhibition, *S.aureus* showd about 16.2mm, 18mm, 21mm, *S.typhimurium* showed about 19mm, 21.2mm, 24mm and *E.coli* showed about 17mm, 19mm, 20.8mm diameter zone oh inhibition respectively. Almost all bacteria show maximum growth inhibition at 100 µg concentration of zinc oxide. Thus the result indicate that the

maximum concentration of zinc oxide nanoparticles have a significant antimicrobial activity. On comparison the gram negative bacterium (*S.typhimurium*) shows the maximum antibacterial activity (diameter of zone of inhibition 24mm) against zinc oxide than that of gram negative bacteria. In addition, the 100-µg zinc oxide nanoparticles have very low bactericidal activity as compared with the antibiotic streptomycin. Because the diameter of zone of inhibition in the case of *B.cereus* , *S.aureus*, S. typhimurium, *E.coli* were 57.8mm, 55.6mm, 57mm, 59.6mm respectively. The study clearly indicates that low concentration of zinc oxide nanoparticle (10 µg, 20 µg, 30 µg) had mild bactericidal action. But concentration such as 50 µg, 75 µg ZnO nanoparticles showed moderate activity. High concentration (100 µg) showed maximum antibacterial activity.

Table.1. Antibacterial activity of ZnO on different bacterial strains.

Bacteria	**Zone of Inhibition (mm)**					
	A(10µg)	**B(20µg)**	**C(30µg)**	**D(50µg)**	**E(75µg)**	**F(100µg)**
B.cereus	10mm	13mm	15.8mm	17mm	20mm	23mm
S.aureus	8mm	11mm	13mm	16.2mm	18mm	21mm
S.typhimurium	12mm	15mm	16.2mm	19mm	22.2mm	24mm
E.coli	9.5mm	11mm	12.8mm	17mm	19mm	20.8mm



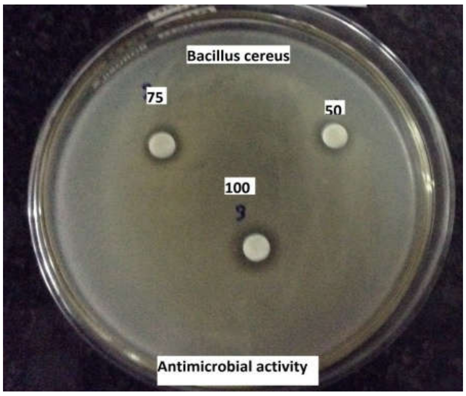
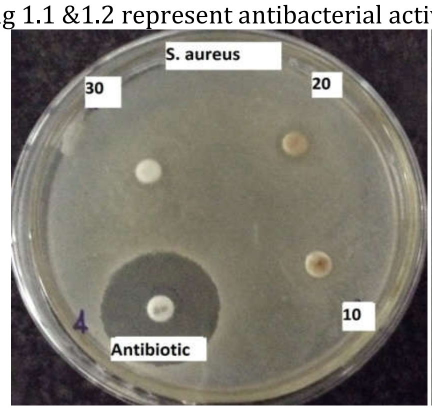


Fig 1.1 Fig 1.2

Fig 1.1 &1.2 represent antibacterial activity of ZnO nanoparticles against *B.cereus*,



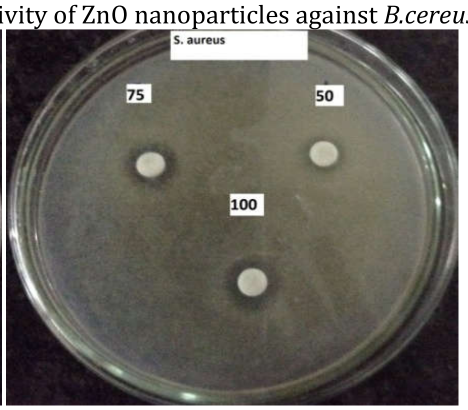
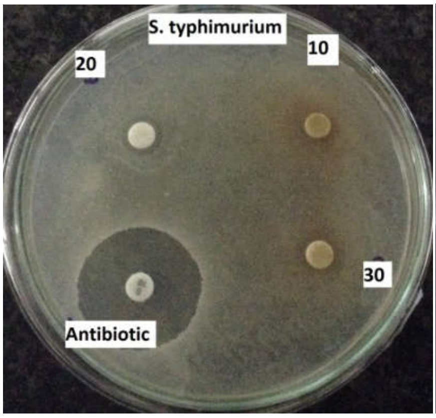


Fig 2.1 Fig 2.2

Fig 2.1 and 2.2 represent the antimicrobial activity of ZnO nanoparticle against *S.aureus*



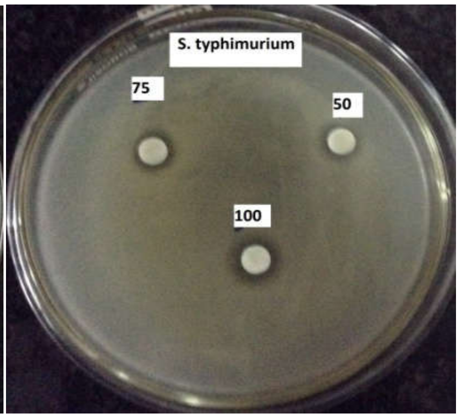
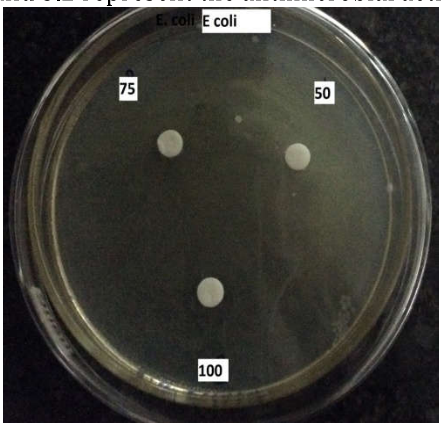


Fig 3.1 Fig 3.2

Fig 3.1 and 3.2 represent the antimicrobial activity of ZnO nanoparticle against *S.typhimurium*



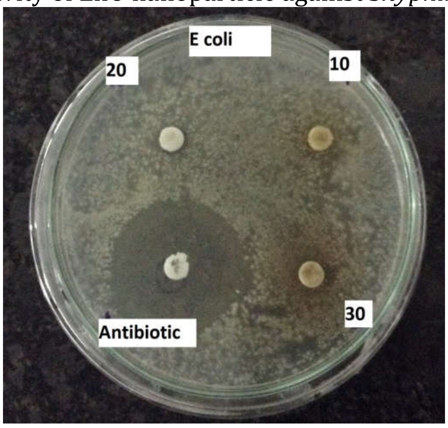


Fig 4.1 Fig 4.2

Fig 4.1 and 4.2 represent the antimicrobial activity of ZnO nanoparticle against *E.coli*

DISCUSSION

There are some reports on the considerable antibacterial activity of CaO, MgO and ZnO, which is attributed to the generation of reactive oxygen species on the surface of oxides, studied by a conductometric method. The nano structures of ZnO have increased the antibacterial effect. The particles exhibit both qualitatively and quantitatively better antibacterial properties than bulk ZnO with a particle size of 2 µm [21]. The presence of an inhibition zone clearly indicate that the mechanism of biocidal action of ZnO nanoparticle involves disrupting the membrane. The high rate of generation of surface oxygen species from ZnO leads to the death of bacteria [1]. Interestingly the size of the inhibition zone increased significantly with increasing the amount of ZnO nanoparticle. 10 µg, 20 µg, 30 µg ZnO nanoparticle could not produce a clear inhibition zone in almost all bacteria, 50 µg, 75 µg and 100 µg of ZnO nanoparticle could produce a significant inhibitory zone. It is believed that cell death is caused by the decomposition of the cell wall fallowed by subsequent decomposition of cell membrane. The damage to the cell membrane leads directly to the leakage of minerals, proteins and genetic materials causing cell death.The study of Durgaprasad *et al* [6] revealed that *E.coli* bacteria produce diameter of zone of inhibition about 24mm in diameter at 100mg/l concentration of ZnO nanoparticles. The generation of H_2O_2 from ZnO leads to the penetration of particles into the cell membrane of bacteria, to the formation of injuries and finally the death of bacterium has occurred [15]. The antimicrobial effect of ZnO nanoparticles against food born pathogen may leads to the proficient application in food packaging and preservation process [10]. Toxicity studies have shown that Zinc ions do not cause any damage to human cells [2]. The study with the disc diffusion method showed that the best antibacterial activity at 100 µg concentration of ZnO. The mechanism may be the ZnO nanoparticles could decompose the cell wall and damaged cell membrane, leads to the leakage of cytosolic components and kills the bacterial cells. It was concluded that the ZnO nanoparticles could potentially be an antibacterial substance to treat the diseases caused by bacteria.

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