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ZnO NANOPARTICLES: ROLE IN ENHANCING ANTIBACTERIAL ACTIVITY OF VARIOUS ANTIBIOTICS AGAINST *ESCHERICHIA COLI*

Manyasree D., Kiranmayi P.*

Department of Biochemistry, Acharya Nagarjuna University, Nagarjuna Nagar – 522510, India.

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ABSTRACT

Zinc oxide (ZnO) nanoparticles were synthesized by using wet chemical method. The synthesized sample was characterized by X-ray diffraction technique (XRD). The average crystallite size of the sample was calculated from the full width at half maximum of XRD peaks by using Debye-Scherrer's formula and was found to be 27 nm. *E.coli* was used as test microorganism. Disk diffusion method was used to determine the antibacterial activity of various classes of antibiotics in the absence and presence of zinc oxide nanoparticles. We also compared antibacterial activity of ZnO nanoparticles and bulk ZnO. These results indicated that the ZnO nano particles potentiate bactericidal efficacy of fluoroquinolone, cephalosporin, carbapenem, polymyxine and amino glycoside antibiotics.

Corresponding author

Kiranmayi.P

Department of Biochemistry,
Acharya Nagarjuna University,
Nagarjuna Nagar – 522510, India.
kiranmayikodali@rediffmail.com

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INTRODUCTION

Development of antibiotic resistant strains has become a serious global problem now a day. One of the promising approaches for overcoming bacterial resistance towards the antibiotics is the use of metallic nanoparticles [1]. The use of nanoparticles along with antibiotics may reduce the toxicity of both agents towards human cells and requirement of high dosages are decreased as well as enhancing their antimicrobial activities [2]. The particles which are having their size range 1-100 nm are known as nanoparticles. Compared to the macrosized particles nanoparticles exhibit larger surface areas and their size related properties are considerably different from bulk materials [3]. Nanoscale reduction in size can change their structural, morphological, optical, chemical, electrical and mechanical properties. These modified properties make easy the physical transfer of nanoparticles into living cells and allow them to interact with various biomolecules in the cell [4].

ZnO is a functional and inorganic material with a wide variety of applications. ZnO is used to treat a variety of skin conditions, in products such as baby powder, barrier creams to treat diaper rashes and in calamine cream, antidandruff shampoos and antiseptic ointments [5]. ZnO nanoparticles are reported by several studies as non-toxic to human cells [6], this aspect imposed their usage as antibacterial agents, harmful to microorganisms, and hold good biocompatibility to human cells [7]. Thus, developing novel antibacterial agents against pathogenic bacteria that contaminate food and water like *E.coli*. In this study the antibacterial activity of various classes of antibiotics was evaluated against *E.oli* either in presence and absence of ZnO nanoparticles, using disc diffusion technique.

MATERIALS AND METHODS

The zinc oxide nanoparticles were prepared by a wet chemical method using zinc nitrate and sodium hydroxide as precursors and soluble starch as a stabilizing agent [5]. The crystal structure of the sample was analyzed by XRD-6100 diffractometer (Shimadzu), and the patterns were recorded with Copper K α radiation ($\lambda=1.54060 \text{ \AA}$).

Disk diffusion method

The test organism, *E.coli* (MCC 2412) was procured from MCC, Pune, India. Once the medium was solidified, a suspension of each sample of the bacteria was diluted prior to 10^{-1} , 10^{-2} and 10^{-3} (1 ml of 10^8 cells/ml) and was spread on a solid agar medium in petri plates. To determine the effect of antibiotic the antibiotic discs were purchased from National Scientifics, Guntur and to study the combined effects, each standard paper disk was further impregnated with ZnO nanoparticles with concentration of 10 mg/ml. The plates were incubated at 37°C for 24 h, the zone of inhibition was measured [8].

RESULTS AND DISCUSSION

The crystallite size (t) of the prepared nanopowder can be calculated by using Scherrer's formula

$$t = 0.9\lambda / \beta \cos\theta$$

Where D is the crystallite size, λ is the wavelength (1.5406 \AA for Cu K α) of the X-ray radiation, β is the full width at half maximum of the peaks at the diffracting angle θ [9]. The crystallite size of the prepared nanopowder is found to be around 27nm.

The antibacterial activity of antibiotics along with ZnO nanoparticles and individual antibacterial activity of ZnO nanoparticles and antibiotics was investigated against gram negative strain (*E. coli*) using the disk diffusion technique. The diameter of inhibition zones around each disk is represented in Figure 1. The highest increase in the inhibition zones (antibiotic with ZnO nanoparticles) was observed for imipenem, tobramycin, ertapenem and ciprofloxacin (11 mm). The moderate increase in inhibition zones was observed against.

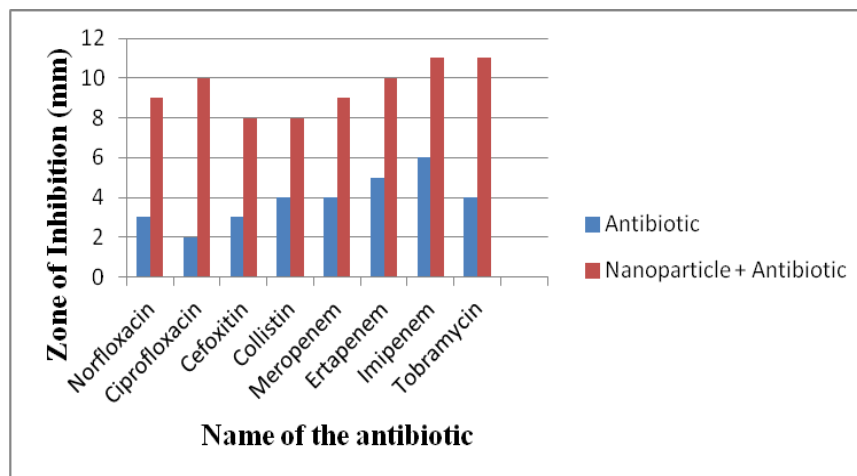


Figure 1: Antibacterial activity of ZnO nanoparticles and their combination with various antibiotics.

Norfloxacin and meropenem (9 mm) followed by cefoxitin and colistin (8 mm) in combination with ZnO nanoparticles. The selected antibiotics represent major classes of antibiotics (Carbapenem, Cephalosporins, Aminoglycoside, Polypeptide and Quinolones/Fluoroquinolones). The bioactivity of smaller particle is due to the higher surface area to volume ratio [9]. According to the results, it can be concluded that ZnO nanoparticles are effective antibacterial agents on Gram negative bacteria. The same results were confirmed in the study of Zhongbing et al. [10] in which Gram negative membrane disorganization was observed by transmission electron microscopy of ultrathin sections of bacteria. In addition, a comparative study was carried out to know the antibacterial activity of bulk ZnO and ZnO nanoparticles by disk diffusion method (Figure 2). *E.coli* was sensitive to ZnO nanoparticles with 9 mm inhibitory zone and bulk ZnO did not show any antibacterial activity. The antibacterial efficacy increased with decreasing particle size from bulk ZnO [11,12]. This could be due to ZnO nanoparticles were found to be more abrasive than bulk ZnO [13], and thus contribute to the greater mechanical damage of the cell membrane and the enhanced bactericidal effect of ZnO nanoparticles [14].



Figure 2: Antibacterial activity of bulk ZnO and ZnO Nanoparticles.

CONCLUSION

Compared to various broad spectrum antibiotics, ZnO nanoparticles have shown the best antibacterial behavior. The *E.coli* was resistant against ciprofloxacin and cefoxitin (2mm and 3 mm) but the highest increase in the inhibition zones was observed for antibiotic with ZnO nanoparticles. By comparing the results the ZnO nanoparticles potentiate the bactericidal efficacy of various classes of antibiotics, including Carbapenem (Imepenum, Ertapenem and Meropenem), Cephalosporins (Cefoxitin), Aminoglycoside (Tobramycin), Polypeptide (Colistin) and Quinolones/Fluoroquinolones (Ciprofloxacin and Norfloxacin). Finally, it can be concluded that, due to the increased resistance of microorganisms to usual drugs, it is important to find out new way out to avoid the development of multi resistant strains.

Conflicts of interest. None

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