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Research Article

# ROLE OF ZnO NANOPARTICLES IN ENHANCING THE ANTIBACTERIAL ACTIVITY OF ANTIBIOTICS

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**ABSTRACT**

The synthesis of ZnO nanoparticles, antimicrobial activity of antibiotics and in combination with ZnO nanoparticles against gram positive and gram negative bacteria has been demonstrated. *E.coli* and *Bacillus subtilis* were used as test microorganisms. Disk diffusion method was used to determine the antibacterial activity of various classes of antibiotics in the absence and presence of zinc oxide nanoparticles. The highest increase was observed for erythromycin and tetracycline compared to penicillin and ampicillin. These results signify that the ZnO nano particles potentiate bactericidal efficacy of macrolides, tetracyclins and beta lactum antibiotics.

**Keywords:** ZnO nanoparticles, antimicrobial activity, wet chemical method.

## INTRODUCTION

Nanoparticles are a special group of materials with unique features and extensive applications in diverse fields [1]. In the present scenario nano scale materials have emerged up as novel antimicrobial agents owing to their high surface area to volume ratio and its unique chemical and physical properties [2, 3]. The availability of a wide range of nanostructures makes ZnO an ideal material for piezoelectric nanogenerators [4] as well as in biotechnology [5]. Furthermore, ZnO appears to strongly resist microorganisms [6]. The antimicrobial activity of zinc oxide nanoparticles have been studied against the food related bacteria *Bacillus subtilis, Escherichia coli* and *Pseudomonas fluorescens* [7]. ZnO powder is an active ingredient for dermatological applications in creams, lotions and ointments on account of its antibacterial properties [8]. The anticancer effects of ZnO nanostructures on human brain tumor U87 and cervical cancer Hela were obtained and indicate promising activity that varies with the changes in the structure and the size [9]. In light of these, the present study was undertaken to investigate the effect of zinc oxide nanoparticles on the antibacterial activity of different antibiotics.

## MATERIALS AND METHODS

### Preparation of ZnO nano particles

### Wet chemical method

The zinc oxide (ZnO) nanoparticles were prepared by wet chemical method [10] using zinc nitrate and sodium hydroxide as precursors and soluble starch as stabilizing agent. Different concentrations of soluble starch (0.1%, 0.5% and 1.0%) were dissolved in 500 ml of distilled water by using microwave oven. Zinc nitrate, 14.874 g (0.1 mol), was added in the above solution. Then the solution was kept under constant stirring using magnetic stirrer to completely dissolve the zinc nitrate for one hour. After complete dissolution of zinc nitrate, 0.2 mol of sodium hydroxide solution was added under constant stirring, drop by drop touching the walls of the vessel. The reaction was allowed to proceed for 2 h after complete addition of sodium hydroxide. After the completion of reaction, the solution was allowed to settle for overnight and the supernatant solution was then discarded carefully. The remaining solution was centrifuged at 10,000 X g for 10 min and the supernatant was discarded. Thus obtained nanoparticles were washed three times using distilled water. Washing was carried out to remove the byproducts and the excessive starch that were bound with the nanoparticles. After washing, the nanoparticles were dried at 80°C for overnight. During drying, complete conversion of zinc hydroxide in to zinc oxide takes place.

### Determination of antimicrobial activity

Bacterial cultures, *E.coli* (ATCC 35218) and *Bacillus subtilis* (ATCC 6633) were procured from ATCC , USA. The agar disc diffusion method was employed to determine the antimicrobial activities of the ZnO nanoparticles. Disc-assay was found to be a simple, cheap and reproducible practical method [11]. A suspension of each sample tested micro organism diluted prior to $10^{-1}$, $10^{-2}$ and $10^{-3}$ (1 ml of 108 cells/ml) was spread on a solid agar medium in petri dishes (Nutrient agar). Filter paper discs (4 mm in diameter) were soaked in 5 µl of the sample and placed on the inoculated plates and allowed to dry for 15 min, then incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in millimeters [12].

## RESULTS AND DISCUSSION

The antibacterial activity of antibiotics along with ZnO nanoparticles was investigated against various pathogenic bacteria of gram positive (*Bacillus subtilis)* and gram negative strains (*E. coli)* using disc diffusion technique fig-1-4. The diameter of inhibition zones around each disc is represented in table-1 and 2. *Bacillus subtilis* was resistant against penicillin and ampicillin but the highest increase in the inhibition zones (antibiotic with ZnO nanopraticles) were observed for erythromycin and tetracyclin (3.2 mm, 4 mm). The moderate increase in inhibition zones were against penicillin (2 mm) followed by Ampicillin (2 mm) in combination with ZnO nanoparticles. In the present study 5 µl of the nanoparticles was taken as final product for antimicrobial assay. *E.coli* exhibits resistance against penicillin but in combination of nanoparticles the zone of inhibition was 2.5 mm. By comparing the results the ZnO nanoparticles potentiate bactericidal efficacy of macrolides, tetracyclins and beta lactum antibiotics.

**Table 1: Zone of inhibition for *Bacillus subtilis***

| S.NO. | ANTIBIOTIC | ZONE OF INHIBITION BY ANTIBIOTIC WITH ZnO | ANTIBIOTIC WITH ZnO NANOPARTICLES (mm) |
|---|---|---|---|
| 1 | Erythromycin | 1 - 2.8 | 1 – 3.2 |
| 2 | Penicillin | - | 1 – 2 |
| 3 | Tetracyclin | 1 - 3.5 | 1 - 4 |
| 4 | Ampicillin | - | 1 - 2 |

**Table 2: Zone of inhibition for *E.coli***

| S.NO. | ANTIBIOTIC | ZONE OF INHIBITION BY ANTIBIOTIC WITH ZnO | ANTIBIOTIC WITH ZnO NANOPARTICLES (mm) |
|---|---|---|---|
| 1 | Erythromycin | 1 - 3 | 1 – 3.5 |
| 2 | Penicillin | 1 - 1.8 | 1 – 2.5 |
| 3 | Tetracyclin | 1 - 3.5 | 1 - 4 |
| 4 | Ampicillin | - | 1 - 2 |

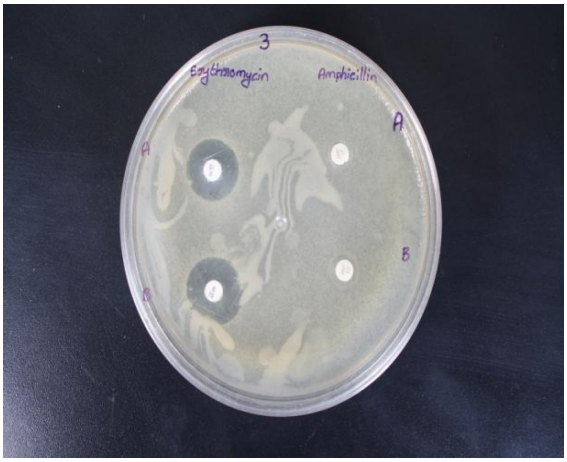


**Fig 1: Erythromycin and Ampicillin**

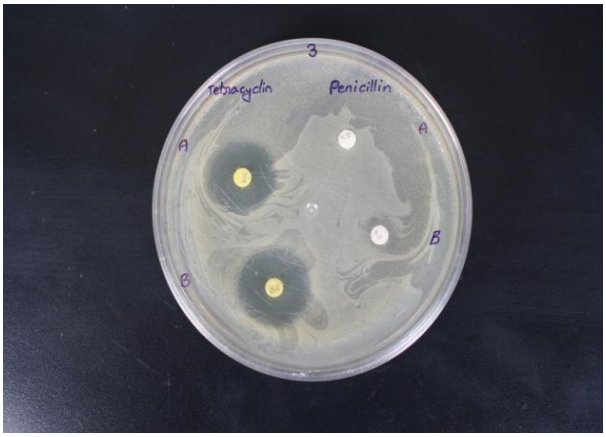


**Fig 2: Tetracyclin and Penicillin**

**A: Zone of inhibition by Antibiotic**
**B: Zone of inhibition by Antibiotic with ZnO Nanoparticles**


**Fig 3: Erythromycin and Ampicillin**

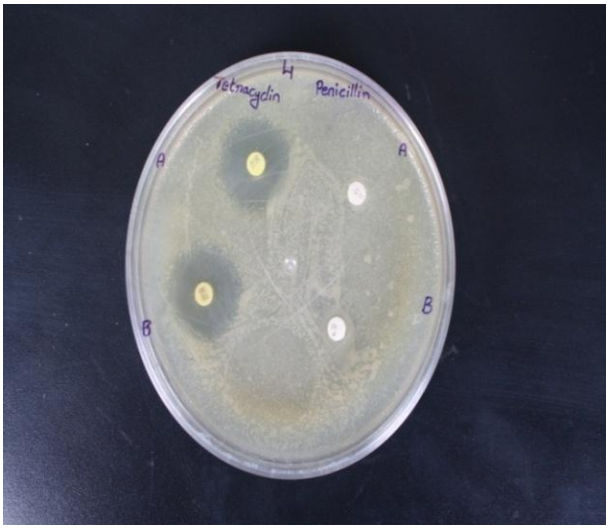


**Fig 4: Tetracyclin and Penicillin**

**A: Zone of inhibition by antibiotic**
**B: Zone of inhibition by antibiotic with ZnO Nanoparticles**

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