RESEARCH PAPER

Improvement the Antibacterial Effect of Zinc Oxide Nanoparticles by Conjugation with Tetracycline

Zainab H. Kareem

Department of Biology, Collage of Science, University of Babylon, Iraq

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ABSTRACT

Nanoparticles when used along with antibiotics offer a novel strategy to combat bacterial infections. In this research, synergistic effect of Zinc Oxide (ZnO) nanoparticles with Tetracycline antibiotic in two types of bacteria including *Klebsiella pneumoniae* and *Staphylococcus aureus* were assayed. The result showed that ZnO nanoparticles and Tetracycline bound ZnO nanoparticles are both have antibacterial effect. Nevertheless, Tetracycline-conjugated ZnO NPs demonstrated strong antibacterial efficacy in comparison with ZnO NPs or Tetracyclene alone against both bacteria species tested. This increased potency indicates a possible additive effect of the nanoparticles and antibiotic. This research provides an important introduction to the ability of nanoparticles to potentiate the activity of classical antibiotics. It also highlights the possibility of synergistic effects of these combinations for surmounting resistance by bacteria. Altogether, this study opens up new ways of exploring nanoparticle-antibiotic conjugates as promising and innovative antibacterial products for clinical and industrial purposes.

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INTRODUCTION

Nanotechnology is at the top of the list of scientific and research interests in all countries of the world because of its impact on many fields such as agricultures, medicine and industrial... etc [1, 2]. Many researchers have studied the influence of NPs on bacteria for example: TiO2, silver, silver oxide, ZnO, calcium and copper oxides, silica and magnesium oxide as antibacterial agents [3-6].

Many researchers found that ZnO nanoparticles can inhibit bacteria even at low concentrations [7-9]. Combination of NPs with antibiotics also studied by many researchers, combination of NPs with antibiotics may or may not produce a synergistic effect [10]. The effect of Tetracycline mixed with ZnO NPs on bacteria need more scientific interest. Therefore, ZnO NPs and Tetracycline conjugated ZnO NPs against *Klebsiella pneumoniae* And *Staphylococcus aureus* have been tested.

MATERIALS AND METHODS

Zinc Oxide nanoparticles (50nm) were obtained from XFNano company, the characterization was made using X-Ray Diffraction and Transmission Electron Microscopy.

Two isolates *K. pneumoniae* and *S. aureus* bacteria

* *Corresponding Author Email: sci.zainab.mohammd@uobabylon.edu.iq*

were obtained from advanced microorganisms lab in biology department/ university of Babylon, Bacteria were characterized by conventional methods. Standard laboratory powder of Tetracycline was utilized in the present study.

To produce Tetracyclin-ZnONPs, 0.01g from ZnONPs and Tetracycline were suspended in 1ml deionized water separately). Then Conjugation was made by adding 1 mL of the antibiotic and ZnO NPs, then sonication was made for 30 seconds. The solution was left for 24h to help transfer of Tetracycline to the ZnO NPs. Unbounded Tetracycline were washed several times and removed by centrifuge at 10000 rpm for 15minutes. After that, the pellet was washed 3 times by centrifuge with suspending into sterile D.W., then t7he solution stored for overnight at dark to dry [11].

The antibacterial susceptibility test for ZnO nanoparticle and Tetracyclin conjugated ZnO nanoparticle against *K. pneumoniae* and *S. aureus* was made by agar diffusion method, determined of MIC and MBC was made from broth macro-dilution assay.

The concentration 2000 µg/ml for ZnO nanoparticle and Tetracyclin conjugated ZnO nanoparticle was prepared with distilled water, 20 ml of Muller Hinton agar was put in petri plates. After agar solidification, 0.2 ml of each isolate was spread, the petri plates were left for an 5 minutes then wells about 6mm were made with cork borer on each plate. Fifty microliter for ZnO nanoparticle and Tetracycline conjugated ZnO nanoparticle and Tetracycline was added to each well and diffuses for 10 minutes. wells that contain D.W. were considered as negative control, the plates then incubated at 37oC for 24hrs. Inhibition zones was measured as millimeter in diameter, the experiment was made in triplicate. The MICs for Tetracycline conjugated ZnO nanoparticle and ZnO nanoparticle against bacteria was determined using broth Macro-dilution method as reported by [12]. The turbidity of the bacteria was adjusted to 0.5 McFarland standard in Muller Hinton broth. The MIC was performed by two fold dilution series for ZnO nanoparticle and Tetracyclin conjugated ZnO nanoparticle. Initially, serial dilutions (2000,1000,800,400,200,100) µg/ml for ZnO nanoparticle and Tetracycline conjugated ZnO nanoparticle were performed in Muller Hinton broth. 100 µL of the bacteria was inoculated in each tube. Un-inoculated broth the negative control and tube containing broth medium and bacterial isolate is positive control. Experiments were assayed in triplicate, the tubes saved in incubator for 24h at 37 °C. Then MIC and MBC was tested. the MIC value. the lowest nanoparticles concentration that seems clear and did not show visible growth in the tubes was considered the MIC.

To determine MBC, the tubes that did showed any bacterial growth were sub-cultured in nutrient agar plates, the lowest concentration that did not show growth of bacterial colony on the nutrient agar plates was regarded as MBC [13].

RESULTS AND DISCUSSION

X-ray Diffraction Analysis

ZnO nanoparticles was tested by X-ray diffraction (XRD). The diffraction pattern (Fig. 1) exhibits distinct and sharp peaks, refearing a high degree of crystallinity. at 2θ values, the major diffraction peaks appeared of approximately 31.8°, 34.4°, 36.3°, 47.5°, 56.6°, 62.8°, 66.3°, 68.0°, and 69.1°, which correspond well to the (100), (002), (101), (102), (110), (103), (200), (112), and (201) planes, respectively, of the hexagonal wurtzite ZnO crystal structure (JCPDS Card No. 36-1451). No secondary phases or impurity peaks were observed, confirming the purity of the synthesized ZnO nanoparticles [14, 15]. The sharpness and intensity of the peaks further suggest well-defined crystalline domains. the average crystallite size was estimated to be in the nanometer range (typically between 20–40 nm) using Scherrer equation, indicating successful synthesis of ZnO in nanocrystalline form [16].

Transmission Electron Microscopy Analysis

The size and morphology of ZnO NPs were investigated using (TEM), as shown in Fig. 2. The TEM micrograph reveals agglomerated nanoparticles with quasi-spherical shapes. The individual particle size appears to range between 20–50 nm, which is consistent with the crystallite size calculated from XRD data. The particles show moderate agglomeration, which is typical for ZnO nanoparticles due to their high surface energy and tendency to form clusters. Despite agglomeration, the particle boundaries are distinguishable, and the nanoscale nature of the material is evident. Overall, the combined XRD and TEM analyses confirm the successful synthesis of pure, crystalline ZnO NPs with nanometer-scale dimensions and wurtzite phase structure.

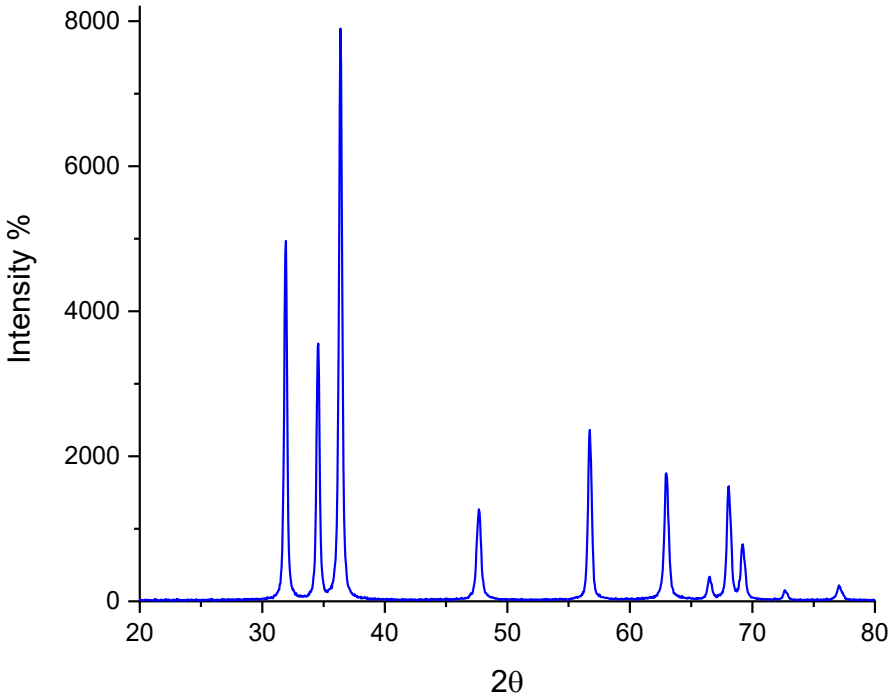

Fig. 1. XRD pattern of ZnO nanoparticles showing distinct peaks corresponding to the hexagonal wurtzite structure.

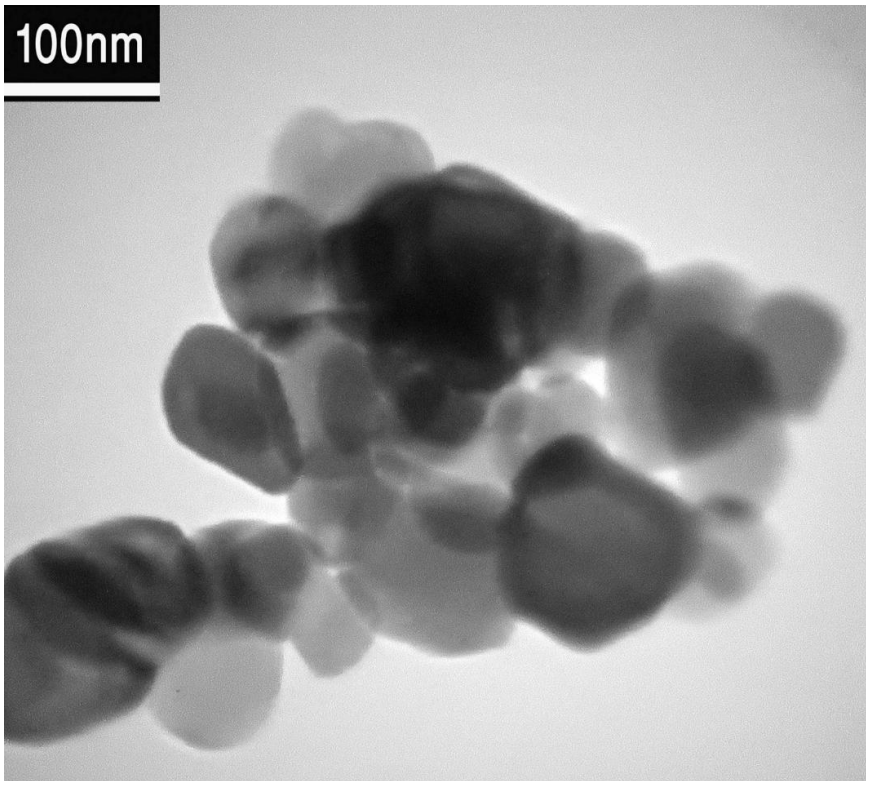

Fig. 2. TEM image of ZnO nanoparticles indicating quasi-spherical morphology and particle size in the range of 20–50 nm.

Antibacterial Effect

The antibacterial effect of synthesized ZnO nanoparticle and Tetracycline conjugated ZnO nanoparticle at concentration 2000µg/ml against *Klebsiella pneumoniae* and *Staphylococcus aureus* were investigated by agar well diffusion assay and the antibacterial sensitivity was measured by determinntion the zones of inhibition diameter in millimeter. The results showed that ZnO nanoparticle and Tetracycline conjugated ZnO nanoparticle have antibacterial effect against *Klebsiella pneumonia* and *Staphylococcus aureus*. The result showed that tetracycline conjugated ZnO Nanoparticles has more antibacterial effect

Fig. 3. Antibacterial activity of: 1- Tetracycline conjugated ZnO Nanoparticles at concentration 2000µg/ml, 2- ZnO Nanoparticles at concentration 2000µg/ml , 3- Tetracycline , 4- deionized water (control) against *Klebsiella pneumoniae* and *Staphylococcus aureus.*

Table 1. Antibacterial activity of ZnO nanoparticles and Tetracycline conjugated ZnO nanoparticles and Tetracycline against *Klebsiella pneumoniae* and *Staphylococcus aureus*, Data represent the mean ± SD.

Concentration 2000µg/ml	Zones of Inhibition in Millimeter		
	Tetracycline conjugated ZnO Nanoparticles	ZnO Nanoparticles	Tetracycline
Klebsiella pneumoniae	24.6±0.577	20.0±1.000	14.0±2.000
Staphylococcus aureus	26.6±0.6	23±0.7	13±1.000

Table 2. The MIC and MBC for synthesized ZnO nanoparticles

Bacteria	Tetracycline conjugated ZnO Nanoparticles		ZnO Nanoparticles	
	MIC	MBC	MIC	MBC
K.pneumoniae	100 µg/ml	200 µg/ml	1000 µg/ml	2000 µg/ml
S. aureus	100 µg/ml	200 µg/ml	1000 µg/ml	2000 µg/ml

than ZnO nanoparticle or tetracycline alone for both bacteria utilized in the study as shown in Fig. 3.

The MIC and MBC for synthesized ZnO nanoparticles and tetracycline conjugated ZnO Nanoparticles was determined from broth macro dilution method, two-fold dilution series of synthesized ZnO nanoparticles and tetracycline conjugated ZnO Nanoparticles (2000,1000,800,400,200,100) µg/ml were tested, the results are shown in Table 2.

The result showed that the nanoparticles have the same effect on both types of bacteria, this result disagree with [7], he found that gram negative bacteria is more resistant than gram positive bacteria. he explained that silver nanoparticle can inhibit bacteria by several mechanisms, and the main mechanism suggested is Reactive oxygen species generation.

ROS, including hydroxyl radicals, superoxide radicals, hydrogen peroxide and singlet oxygen, cause damage to DNA and proteins in bacteria [17, 18], In this case, ZnO nanoparticles could generated ROS that inhibit bacteria.

[19] found that ZnO nanoparticles inhibited bacteria even its multidrug-resistant as well, he found that ZnO nanoparticles damages the outer membrane of *E. coli*, by destroying the lipopolysaccharide, followed by damaging the inner membrane, then inter within the cell and create reactive oxygen species.

For all bacteria tested, the results showed that tetracyclene conjugated ZnO nanoparticles more activity than ZnO nanoparticles and Tetracycline alone used in the study, The effect of ZnONPs with Ampicillin and ciprofloxacin was reported against *Pseudomonas aeruginosa*, *E. coli* , *K. pneumoniae*, *B. subtilis, S. typhi* and *S. aureus* [20]. who showed that the activity of ciprofloxacin and Ampicillin against bacteria increased when conjugated with ZnONPs. the improvement in antibacterial activity may be because of the multiple mechanisms of influence of NPs. Conjugation of nanoparticles with antibiotic might increase penetration of the nanoparticles through the bacterial cell wall therefore enhance the effect against bacteria. In addition, the two antibacterial agents have different mechanisms of action, if bacteria give resistance to one of them, the other antibacterial factor would inhibit the bacteria [21]. As well as, high surface area and small size of NPs make them more drug loading which increase antibiotic concentration at the site of antibiotic-bacteria contact and this may cause increasing in the inhabitation of bacteria [22]. In addition, the conjugants have lower dose of both antimicrobial agents, which reduces the harmful effects [21].

CONCLUSION

Tetracycline conjugated ZnO nanoparticles and ZnO NPs possess antibacterial influence against *Klebsiella pneumoniae* (gram negative bacteria) *Staphylococcus aureus* (and gram positive bacteria). Conjugation of Tetracycline with ZnO NPs increases the antibacterial activity of ZnO NPs.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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