Antibacterial activity of zno nanoparticle on some gram-positive and gram-negative bacteria

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Abstract

The synthesis and bioactivity of zinc oxide nanoparticles has been extensively studied. The antibacterial activity of different antibiotics individually (ceftriaxone (C), chloramphenicol (CRO), penicillin (P) and amoxicillin (Ax)) and Zinc oxide nanoparticles (60µg/ml) in combination with the previously mentioned antibiotics has been demonstrated in the present study by using the disk diffusion assay method. The results showed a synergistic effect between Zinc oxide nanoparticles (ZnO NPs) and both Ax and P for most of the studied Gram-positive isolates (Staphylococcus aureus1, Staphylococcus aureus2, Staphylococcus epidermidis1, Staphylococcus epidermidis2, Enterococcus faecalis1, Enterococcus faecalis2) and between ZnO NPs and both CRO and C for most of Gram-negative isolates (Pseudomonas spp1, Pseudomonas spp2, Escherichia coli1, Escherichia coli2, Acinetobacter spp, Proteus spp) .So there was no effect or antagonism relationship was observed between ZnO NPs and both CRO and C for Gram-positive isolates and ZnO NPs and Ax, P for Gram-negative isolates.

Key words
Nanoparticles, Zinc oxide, Antibacterial activity, Disk diffusion.

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الفعالية الضد حيوية للجسيمات النانوية لأوكسيد الزنك على بعض البكتريا الموجبة لصبغة غرام والسالبة لصبغة غرام.

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الخلاصة

تم دراسة الفعالية الضد حيوية لبعض المضادات (سيفترايكزون C وكلورامفينكولCRO وبنسلين P و اموكسيسليينAx) على بعض انواع البكتريا السالبة والموجبة لصبغة غرام وتم اعادة الدراسة بعد خلط محلول الجسيمات النانوية لأوكسيد الزنك مع المضادات الاربعة بواسطة استخدام طريقة (الانتشار بواسطة الاقراص) .النتائج اوضحت التأثير الايجابي بين الجسيمات النانوية لاوكسيد الزنك وكل من الاموكسيسليين و البنسلين لأغلب العزلات الموجبة لصبغة غرام وبين الجسيمات النانوية وكل من سيفترايكزون و كلورامفينكول لأغلب العزلات السالبة لصبغة غرام في حين لا يظهر اي تأثير او تثبيط بين الجسيمات النانوية لاوكسيد الزنك وكل من سيفترايكزون و كلورامفينكول للعزلات الموجبة لصبغة غرام والجسيمات النانوية وكل من البنسلين و اموكسيسليين مع العزلات السالبة لصبغة غرام .

Introduction

Nanotechnology has attracted global attention because nanoparticles (NP) have properties unique from their bulk equivalents. NP of Ag, CuO and ZnO are being used industrially for several purposes including amendments to textiles, cosmetics, sprays, plastics and paints [1]. A common feature of these three NP is their antimicrobial activity[2,3]. The antimicrobial activity of NP largely has been studied with human pathogenic bacteria, mainly Escherichia coli and Staphylococcus aureus. These microbes exhibited

sensitivity to nano-CuO and nano-ZnO [4, 5]. NP of Ag, CuO and ZnO are reported to attack bacterial membranes [6]. The considerable antimicrobial activities of inorganic metal oxide nanoparticles such as ZnO, MgO, TiO_2, SiO_2 and their selective toxicity to biological systems suggest their potential application as therapeutics, diagnostics, surgical devices and nano medicine based antimicrobial agents [7,8]. The advantages of using these inorganic oxides nanoparticles as antimicrobial agents are their greater effectiveness on resistant strains of microbial pathogens, less toxicity and heat resistance [9,10]. Also in recent years ZnO has received considerable attention because of its unique optical, piezoelectric, and magnetic properties [11]. In addition ZnO nanoparticles has the potential to impact many aspects of food and agricultural system because of its antimicrobial efficacy especially with the growing need to find alternative methods for formulating new type of safe and cost-effective antibiotics in controlling the spread of resisted pathogens in food processing environment [12,13]. Nano-ZnO has been reported to have extremely good safety profile and no toxicity observed when taken at different nano sizes of the zinc particles [14].

Taken together nano-ZnO compound as a highly safe compound may be considered for combination therapy against bacterial pathogens to its potential synergistic effect with important antibiotics. In light of these, the present study was undertaken to investigate the effect of zinc oxide nanoparticle on the antibacterial activity of different antibiotics against different bacterial species (12 isolates) and the result showed a synergistic effect between ZnO NPs and both Ax and P for most of the gram-positive isolates and between ZnO NPs and both CRO and C for most of gram-negative isolates and no effect or antagonism relationship was observed between ZnO NPs and both CRO and C for gram-positive isolates and ZnO NPs and Ax, P for gram-negative isolates.

Materials and Methods

Synthesis of zinc nano-particles

Zinc Oxide NPs were prepared by pulsed laser ablation of Zinc target in double distilled water (DDW) which was used as carrier media for nanoparticles at room temperature. The Zinc target (purity of 99.99%, 0.8cm*0.8cm with thickness= 1mm) was immersed into 1 ml DDW in a glass dish (diameter=1cm, depth= 1cm) was applied as the container. The ablation was achieved using Nd: YAG laser (type HUAFEI) operating with number of pulses 40 pulse. Ablation is carried out with laser operating at 1064 nm with energy pulses 550 mJ. The laser beam was focused on the Zn target using convex lens of 11 cm focal length. The typical laser beam diameter on the target was varied in the range of 0.4–2.3 mm in diameter by changing the distance between the focusing lens and the Zn target and that shown in Fig. 1.

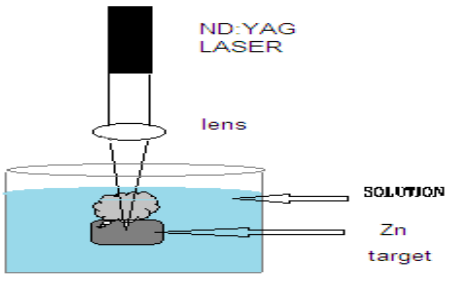

Fig. 1: The experimental setup of pulse laser ablation in liquid (PLAL) system.

Antibacterial activity study

Antibacterial activities of the synthesized ZnO nanoparticles were determined in comparison with ceftriaxone(CRO), chloramphenicol(C), penicillin(P), and amoxicillin(AX), using the disc diffusion assay method, is a means of measuring the effect of an antimicrobialagent against bacteria gro wn in culture. The bacteria in question are swabbed uniformly across a culture plate. A filter-paper disk, impregnated with the compound to be tested, is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. The concentration of the compound will be highest next to the disk, and will decrease as distance from the disk increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. Thus, the size of the zone of inhibition is a measure of the compound's effectiveness: the larger the clear area around the filter disk, the more effective the compound. Approximately (20 mL) of molten and cooled media (Nutrient agar) was poured in sterilized Petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. The bacterial test organisms were grown in nutrient broth for 24 h. A (100 mL) nutrient broth culture of each bacterial organism (1×10^8 cfu/mL) was used to prepare bacterial lawns (cfu=number of colony forming unite). Antibiotic disks of 5 mm in diameter were impregnated with ZnO nanoparticles solution (60µg/ml).The plates were incubated at 37ºC and were examined for evidence of zones of inhibition, which appeared as a clear area around the wells. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter.

Statistical Analysis

The mean and standard deviation (SD) reported for ZnO nanoparticle and antibiotics with each microbial isolates were based on three replicate.

Results and Discussion

Table1. shows the sensitivity of the twelve bacterial isolates against the four antibiotics (ceftriaxone, chloramphenicol, penicillin and amoxicillin) where used in this investigation. S.aureus 2, Pseudomonas 2 and Proteus spp. are being show the most sensitive pathogens to CRO with a diameter of inhibition zone ranges between (26-33mm). While all of the bacterial pathogens showed sensitivity to C except Proteus spp. and most of the Gram-positive isolates showed sensitivity to P and AX. On contrary, Gram-negative isolate showed resistance to those antibiotics except for Acinetobacter spp. This was sensitive to P and AX and Proteus spp. which revealed sensitivity to P .The antibacterial activity of ZnO nanoparticles was tested by the disk diffusion method. And Fig.(2) shows the absorption spectrum of ZnO NPs.

The synergism and the antagonism effect of the Zinc oxide nanoparticles determined as observed by the increase or decrease in diameter of inhibition zone (mm) around the different antibiotic disk (C, CRO, P, Ax) with Zinc Oxide nanoparticles have been recorded in respect of certain antibiotics (Table 2 and 3). The presence of an inhibition zone clearly indicated the antibacterial effect of ZnO nanoparticles. The size of inhibition zone was different according to the type of bacteria. Inhibition zone values were obtained for the mixture of the four former antibiotic with synthesized ZnONPs, tested against seven species (12 isolates*)* Staphylococcus aureus, Streptococcus epidermidis, Enterococcus faecali, E.coli, Pseudomonas spp., Acinetobacter spp., and Proteus spp.

Table (1): Bacterial isolates* and inhibition zone in mm

	Mean ± SD											
	S.aureus 1	S.aureus2	S.epidermidis1	S.epidermidis2	E.faecalis1	E.faecalis2	Pseudomonas1	Pseudomonas2	E.coli1	E.coli2	Acinetobacter	Proteus
CRO	0	26±1	0	0	0	11±1	0	15±0	0	0	8±2	33±2
C	22±1	30±2	33±1	10±1	21±1	38±2	23±1	15±1	22±1	18±1	12±1	0
P	14±2	16±1	20±2	6±1	16±2	33±2	0	0	0	0	9±2	12±1
Ax	0	12±1	16±1	0	8±2	25±1	0	12±1	0	0	10±1	0

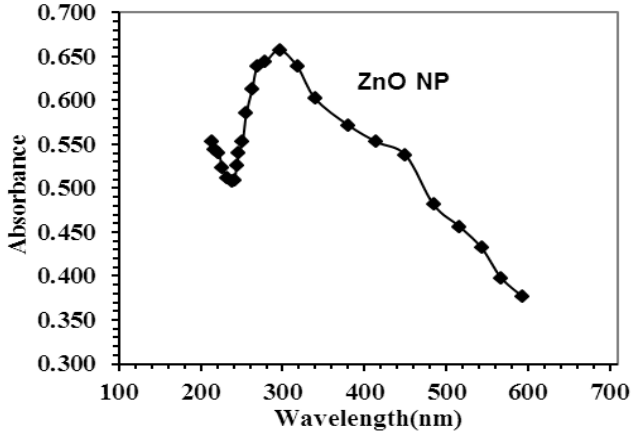

Fig. 2: Optical absorption spectrum measured from the NPs colloidal solution

The results in Tables 2 and 3, showed a synergistic effect between ZnONPs and both AX and P for most of the studied Gram-positive isolates and between ZnONPs and both CRO and C for most of Gram-negative isolates. The results are presented in image shown in the Fig.3. The statistical analysis showed that there is a significant positive effect was observed for the addition of ZnO NPs to Ceftriaxone (C) against Pseudomonas 2 isolate. The same effect was also shown for E. faecalis 1 isolate when we used amoxicillin (Ax) and a negative effect when we used chloramphenicol (CRO).The antibacterial activity of the ZnO particles were studied by Zhang et al. [15]. It seems that active oxygen species generated by ZnO particles could be a mechanism although there is no direct evidence from the results of this study. The presence of active oxygen species has been detected by Yamamoto et al. [16]. It has already been proved that both nano-sized and micron–sized ZnO suspensions are active in inhibiting the bacteria growth; the macro nano-sized ZnO suspension clearly has much higher activity than the micron-sized ZnO suspension. While no effect or antagonism relationship was observed between ZnO NPs and both CRO and C for Gram-positive isolates, and ZnO NPs AX, P for Gram-negative isolates. A low antibacterial activity of ZnO NPs compounds may due either to decrease in particle size or to low concentration of those compounds [17]. Furthermore reported that antibacterial effect was size and dose dependent and was more pronounced against Gram negative bacteria than Gram positive bacteria .It is also reported that bactericidal efficiency is affected by the type of microorganism[18]. Venubabu Thati et al., (2010)[19] observed that the

inhibition zone of ZnO in concentration of combination of ZnO NPs with antibiotic give higher inhibition zone than that observed in this investigation since he was used 100µg/ml, Zarrindokht Emami-Karvani and Pegah-Chehrazi[20] have reported the same results. Furthermore, the result showed that the differences in the susceptibility of bacteria to the ZnONPs could be related to differences in cell wall structure, cell physiology, metabolism or degree of contact.

Table (2): Bacterial isolates** and inhibition zone in mm

****mean antibiotic alone, **mean antibiotic combined with chemical compound ($ZnONp_s$).***

	Mean ± SD											
	S.aureus 1	S.aureus2	S.epidermidis1	S.epidermidis2	E.faecalis1	E.faecalis2	Pseudomonas1	Pseudomonas2	E.coli1	E.coli2	Acinetobacter	Proteus
CRO	0	23±1	0	0	0	11±2	0	36±2	0	0	8±1	34±1
C	17±1	26±1	32±1	10±1	9±1	38±2	23±1	17±1	22±2	21±1	15±2	0
P	14±2	17±1	23±1	7±2	23±1	34±2	0	0	0	0	0	11±1
Ax	10±1	12±1	18±2	0	16±2	25±1	0	12±1	0	0	10±1	0

Table (3): The overall effect of combination of ZnONPS and the four antibiotics on DIZ of the twelve bacterial isolates
(0= mean no effect, - = mean antagonism, + = mean synergism)

	Gram-positive isolate	**Gram-negative isolate**
CRO	0 -	0 +
C	0 -	0 +
P	+ 0	0 -
Ax	+ 0	0 0

Fig (3): The antibacterial effect of addition of ZnO NPs on different antibiotics(C, Ax, P, CRO).

Conclusions

We have demonstrated the antibacterial activity of addition of ZnO NPs to some antibiotics by the disk diffusion method. The result showed synergistic and antagonistic effect between ZnO NPs and some antibiotics for different bacterial isolate. The differences in susceptibility of bacteria due to the differences in cell wall structure, cell physiology, metabolism or degree of contact and the resistant of bacteria to some antibiotics, also the concentration of ZnO NPs have some effect.

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