Synthesis of zinc oxide nanoparticles and evaluated its activity against bacterial isolates

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Received: March 28, 2020; **accepted:** May 4, 2020.

Zinc oxide nanoparticles (ZnO NPs) have acquired large attention against microbial activity because of their distinctive properties associated with their size and shape. In current research, ZnO NPs produced by the Sol-gel method at room temperature were applied to evaluate its antibacterial activity against pathogenic bacterial strains of *Staphylococcus aureus* and *Escherichia coli* obtained from Al-Sadr Hospital, Maysan, Iraq. The prepared nanoparticles were investigated by dynamic light scattering (DLS), X-ray diffraction (XRD), and scanning electron microscopy (SEM). Antibacterial activity was tested by using the agar diffusion method, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) after 24 h of exposure to prepared ZnO NPs. The size distribution, characterization, and morphology of nanoparticles showed around 100 nm as observed by DLS, and the formed nanoparticles were spherically confirmed by SEM. Additionally, results of the disc diffusion test showed significant inhibition of growth of both bacterial strains by ZnO NPs. In conclusion, ZnO NPs created by the sol-gel method had an excellent antibacterial effect, that could be used to produce ZnO NPs for biomedical applications.

Keywords: zinc oxide nanoparticles; antibacterial activities; nanotechnology; antibiotic minimum inhibitory concentration.

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Introduction

The development of inorganic metal oxide nanoparticles has much interest because of its importance in multiple biomedical and environmental applications [1, 2]. These nanoparticles having a nanoscale size of 1–100 nm with functional properties have possessed unique physicochemical characteristics because of their high surface area and quantum effects [3]. Zinc oxide nanoparticles (ZnO-NPs) are among inorganic metal oxide and are considered a safe material being significantly used for antibacterial activity due to their physical and chemical properties [4]. Many researchers have reported that ZnO NPs, as an antibacterial factor for pathogenic microbes [5, 6], are currently progressing prominent antimicrobial agents and can be produced by several synthetic methods such as chemical co-precipitation [7], Gas condensation [8], Sono-chemical [9], and Sol-gel [10]. The synthesis method largely influences the characterizations of prepared nanoparticles.

In comparison to some above-mentioned methods, the sol-gel process is a relatively easy and simple method used to fabricate the fine and large quantity of pure composites under low-temperature conditions and at low cost. Therefore, it has been widely used to produce

zinc oxide nanostructures [11]. Antibiotic-resistant bacteria have become more usual in the hospital environment such as methicillin-resistant *Staphylococcus aureus* (MRSA) and third-generation cephalosporin-resistant *Escherichia coli*, and remain a challenge for global health [12]. Multidrug-resistant bacteria (MRB) appear to be the causes of morbidity associated with a long stay in hospitals, global mortality, and high medical costs. Conventional antibiotics used for the treatment of MRB infection are frequently limited. However, nanoparticles such as ZnO NPs have emerged as one of the alternatives to antibiotics due to their characteristics such as nano-scale size and high specific surface area, which make them capable to penetrate the bacterial cell membrane and interact with bacterial molecular components as their distinctive antibacterial mechanism [13].

Due to the increased resistance of bacteria to conventional antibiotics, the re-emergence of bacterial infections has become a big threat to global health. Therefore, the development of novel materials such as ZnO NPs is of great clinical importance. It has been proven that these nanoparticles are one of the most promising alternatives to antibiotics. It interacts with major cellular components and biomolecules such as genetic material, ribosomes, enzymes, and lysosomes, and has effects on cellular permeability, oxidative stress, gene expression, enzyme, and protein activation. NPs targets multiple biomolecules simultaneously, so it becomes extremely difficult for bacteria to develop resistance to them [14]. ZnO NPs are bio-safe material that exhibits attractive properties such as photocatalyst and photo-oxidation on biological species. It has been reported as non-toxic to human cells and it is safe to use in food additive [15]. These nanoparticles possess a high specific surface area as the reduced particle size leading to increased particle surface reactivity, which make them attractive agents for antibacterial applications [16].

The aims of this study are to evaluate the antibacterial effects of the prepared zinc oxide nanoparticles by applying it to against both pathogenic bacterial isolates *S. aureus* and *E. coli* obtained from Al-Sadar Hospital, Maysan, Iraq, and to estimate the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Materials and Methods

Zinc oxide nanoparticles synthesis

Synthesis of ZnO NPs was performed by using the typical sol-gel method under room temperature [13]. Briefly, 2.5 g of zinc acetate dihydrate with high purity of ≥99% and 8 g of NaOH (Sigma–Aldrich-Gmbh, Albuch, Germany) were dissolved in 20 mL of deionized water under stirring for 10 minutes, respectively. The solution of NaOH was added dropwise into the zinc acetate solution with vigorously stirring at room temperature for 15 min before 100 ml of ethanol was added dropwise to the mixture. After chemical reaction, the mixed solution was dried at 100°C, and the white powders were then collected.

ZnO NPs characterization

The compound was analysed by using X-ray diffraction (XRD). The size distribution analysis was done by using dynamic light scattering (DLS) method by dissolving ZnO NPS powder in Milli-Q water and then applying to Zetasizer Nano Range for analysis (Malvern Panalytical, Malvern, UK). The morphological features were examined by using scanning electron microscope (SEM).

Bacterial samples preparation

Bacterial samples were kindly provided by the laboratories of Al-Sadr Hospital in Amarah town, Maysan, Iraq. Two strain samples (*S. aureus* and *E. Coli*) of gram-positive and gram-negative bacteria were cultured in nutrient agar (5 g/L gelatin peptone, 1 g/L beef extract, 2 g/L yeast extract, 5 g/L sodium chloride, and 15 g/L Agar) at 37°C for 24 h.

Preparation of ZnO NPs solution

An appropriate quantity of ZnO NPs (10 mg) was weighed and dissolved in 10 mL of dimethyl

sulfoxide (DMSO) in order to obtain a stock solution at the concentration of 1 mg/mL. The following concentrations were prepared for agar diffusion test (20, 30, and 40 µg/mL) and identification of MIC and MBC (50, 25, 12.5, 6.25, 3.75, and 1.8 µg/mL) from stock solution.

Anti-bacterial activity of ZnO NPs for agar diffusion test

The antimicrobial effect of the prepared ZnO NPs was tested on both *S. aureus* and *E. Coli* by using agar diffusion test according to Kirby–Bauer protocol (18). The ZnO NPs were seeded with old culture (24 h) of the bacterial strains by using a sterilized cotton swab immersed into the broth of these cultures. After solidified of 20 mL nutrient agar in Petri dishes, wells were punctured by using sterilized cork borer (8 mm diameter). 100 µL of ZnO NPs suspension with various concentrations of 20, 30, and 40 µg/mL was applied to these wells, respectively. The bacterial inoculums were determined by transferring 10^8 colony-forming units (CFU)/mL, referenced by the turbidity of a sample of the 0.5 McFarland standards, to the wells. All Petri dishes were incubated in the refrigerator (4-6°C) for 3 h to let adequate diffusion before transferred to the incubator at 37°C for 24 h incubation. Evaluation of ZnO NPS activity against bacteria was performed based on the calculation of bacterial zone of inhibition (mm) comparing to that in the control group (DMSO only) [17, 18].

Estimation of MIC and MBC for ZnO NPs

MIC and MBC values of prepared ZnO NPs were determined by serial dilution method [19]. Briefly, 1 mL of medium was added to 1 mL of test solution in a test tube before 0.1 mL of each bacterial strain prepared in 0.9% NaCl was added. Serial dilution (two-fold) at concentrations of 50, 25, 12.5, 6.25, 3.75, and 1.8 µg/mL was performed by using nutrient medium. Sterilized cotton was used as stopper for each test tube. The test tubes were placed in 37°C incubator for 24 h. The control tests were carried out with DMSO only at each dilution group.

The MIC was determined by checking the lowest concentration of ZnO NPs, which inhibited the bacterial growth after the incubation period (no turbidity appeared in the test tube), while the MBC was identified as sub-cultured 30 µL of mixture from the test tube that showed no visible growth. If there was no indication for bacteria growth after additional sub-culture, the concentration of ZnO NPs in that particular test tube was defined as MBC [20].

Results and Discussion

ZnO NPs characterization

The results of XRD analysis have indicated a typical hexagonal pattern of nanoparticles prepared by sol-gel method. Figure 1 demonstrated ZnO NPs at the diffracted angles (30° to 70°) with a distinctive crystalline peak (2θ = 34) that matched to other reported literatures [21, 22]. The results of the size distribution (Figure 2) were fully consistent with the results of the SEM (Figure 3). The SEM image was taken at ×10,000 magnifications and revealed that ZnO NPs were spherical in shape and the homogenous in distribution, which also agreed with other reported findings [22].

Bacterial inhibition zone determination

The results of ZnO NPs antibacterial effects on *S. aureus* and *E. Coli* by the disc diffusion tests were shown in tables 1. An obvious zone of growth inhibition for both *S. aureus* and *E. Coli* by ZnO NPs at the concentration of 40 µg/mL was observed as 29 mm and 27 mm, respectively (Figure 4). Narayanan, *et al* [23] reported that the antibacterial effects of synthesized ZnO NPs against *S. aureus* and *E. coli* were 19 mm and 14 mm in inhibition zone at the concentration of 40 µg/mL of ZnO NPs, respectively. Azam *et al*. [24] also indicated that the inhibition zones of ZnO NPs against *E. coli* and *S. aureus* at the concentration of 50 µg/mL were 18 ± 0.5 mm and 16 ± 0.2 mm, respectively. These previous reported results do not agree with ours, which may be the reason of utilizing various methods to synthesis ZnO nanoparticles and ending up with

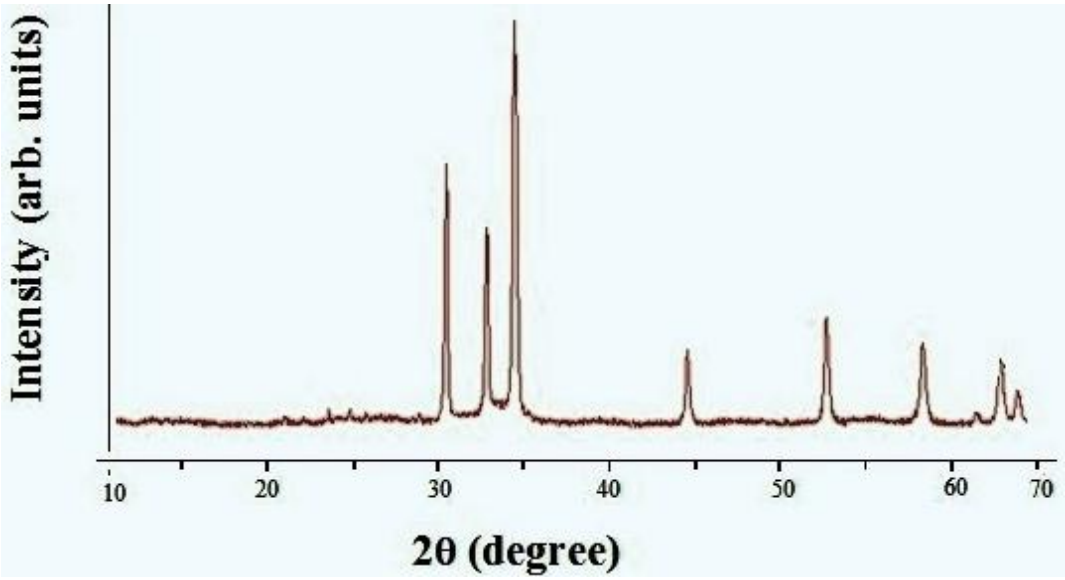

Figure 1. X-ray diffraction of prepared ZnO NPS by sol-gel process.

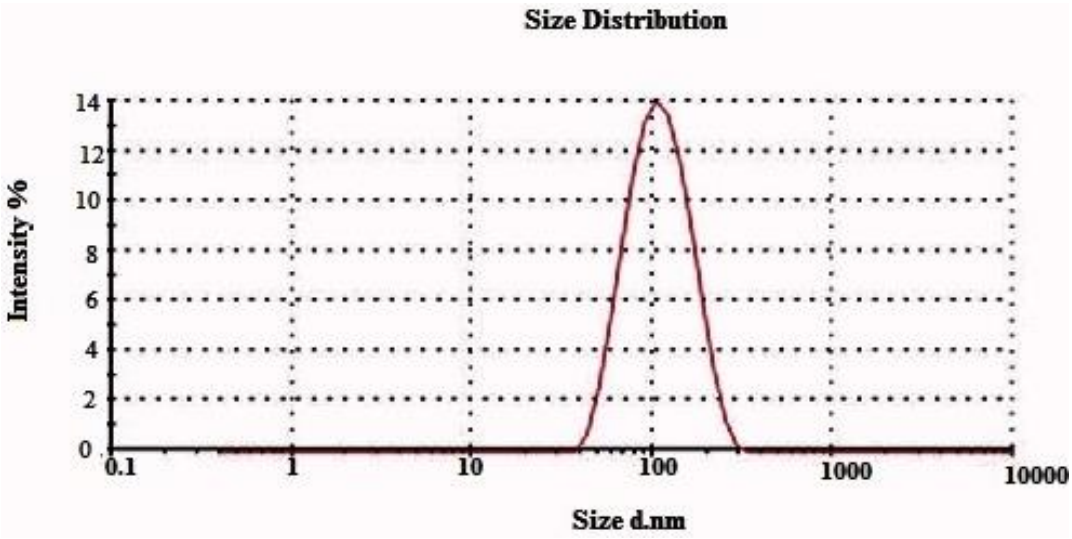

Figure 2. Results of the size distribution of prepared ZnO NPS by dynamic light scattering DLS.

different sizes of these nanoparticles. It has been indicated that physical and chemical characterizations such as shape, size, surface area, and other parameters of nanoparticles may play important roles in their biological responses [16, 25]. The differences in the results of inhibition zone may be also due to the constitutional difference between these strains such as genetic makeup and enzyme possession, acquisition of antibiotic resistance mechanisms, especially in pathological isolates obtained from hospitals [26].

MIC and MBC determination

In current study, the antibacterial activity of prepared ZnO NPs against pathogenic *S. aureus* and *E. Coli* isolates were examined through MIC and MBC by using different concentrations of

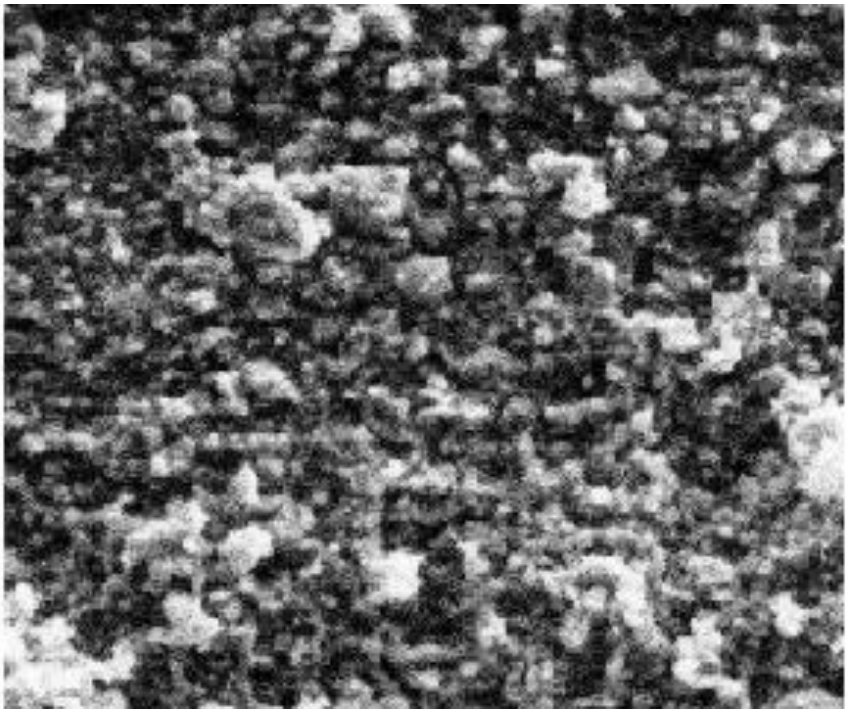

Figure 3. SEM images of prepared ZnO NPS (Scale bar: 50 µm).

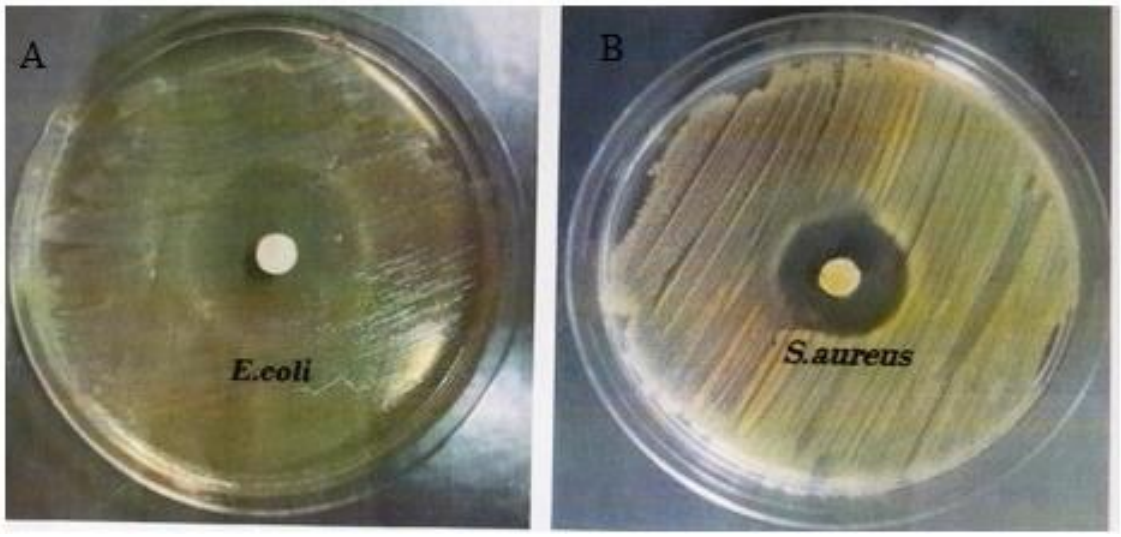

Figure 4. Visible clear of inhibition zone produced by prepared ZnO NPs against bacterial strains. **A.** *E. Coli* and **B.** *S. aureus*.

ZnO NPs. The results showed that both bacterial strains were absolutely inhibited on the concentration of 6.25 µg/mL (MIC) while no remarkable antibacterial effect was observed at the concentrations below that. (Table 2). In addition, minimum bactericidal concentration (MBC) was shown the same as MIC with the concentration of 6.25 µg/mL for both bacterial isolates. These results agree with previous literature report [27]. The potential ZnO NPs antimicrobial mechanism may involve the induction of reactive oxygen species which

Table 1. Determination of inhibition zone diameters (mm) of ZnO NPs against *S. aureus* and *E. coli* samples.

S. aureus		*E. Coli*	
Conc. of ZnO NPs (µg/mL)	**Inhibition zone (mm)**	**Conc. of ZnO NPs (µg/mL)**	**Inhibition zone (mm)**
40	27	40	29
30	21	30	23
20	16	20	18
control	0	control	0

Table 2. Test results of MIC and MBC for ZnO NPs.

ZnO NPs effect	***E. Coli***	**ZnO NPs effect**	***S. aureus***
MIC	6.25 µg/mL	MIC	6.25 µg/mL
MBC	6.25 µg/mL	MBC	6.25 µg/mL

increase membrane lipid peroxidation and cause the leakage of membrane by reducing proteins and sugars, as well as reducing cell viability. Therefore, ZnO NPs may be a treatment choice for hospital associated bacterial infections.

Our ZnO NPs MIC and MBC test results of each bacterial isolate were different to that of Gunalan *et al* [17] whose MIC and MBC results on *E. coli* was 0.8 and 8 µg/mL, respectively. Sonia *et al*. [28] reported that MIC and MBC were 16 µg/mL and 16 µg/mL. The differences among those results may be due to the use of a preserved bacterial strain that was not isolated clinically from the hospital or the different methods for ZnO NPs preparation, which may affect the size and other properties of ZnO NPs [13, 29]. The particle size is important for obtaining high bacterial growth inhibition activity [30].

Conclusion

ZnO NPs, produced by a simple and low-cost method (sol-gel method), indicated strong antibacterial effect on both Gram negative and Gram positive bacterial strains *S. aureus* and *E. coli*. ZnO-NPs antibacterial activity was confirmed by utilizing agar diffusion tests. The results of this study may facilitate the understanding of the mechanism of ZnO NPs against the bacterial cell viability and demonstrated that the resistance mechanism of pathogenic bacterial strains could be inhibited by these novel nanoparticles. Additional molecular and plasmid profile analysis will be required to identify the resistant gene(s).

Acknowledgement

This work was supported by the Department of Medical Basic Sciences, University of Misan, Iraq and the College of Veterinary Medicine, University of Al-Qadisiyah, Diwaniyah, Iraq. The authors wish to thank for editorial assistance.

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