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# Anticancer, Cytotoxicity and Antimicrobial of Zinc Oxide Nanoparticles

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## ABSTRACT

Zinc oxide nanoparticles (ZnO-NPs) are versatile inorganic metal oxide nanoparticles known widely, which use in a broad spectrum of the applications. In this study, chemical method was used to prepare ZnO-NPs.The shape and size of the prepared nanomaterial were visualizedbya transmission electron microscope (TEM). The average length and diameter of the prepared ZnO-NPs were approximately100-200 nm and 25-50 nm, respectively. In vitro, the prepared ZnO-NPs were used as an anticancer agent toward breast cancer cell lines (MCF-7). However, the results of MTT assay showedthat ZnO-NPs is a promising drug against MCF-7. The prepared ZnO-NPs was also tested as an antimicrobial agent against (gramnegative and gram positive) bacteria. *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S.aureus*) were selected as a test ofbacteria. The antibacterial profile of the prepared ZnO-NPs was also studied with different concentration of ZnO-NPs with different methods such as a disc method, well diffusion agar method, minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC).Moreover, an effect of time with different concentrations of Zn-ONPs at the growth of *S. aureus*and *E. coli*was studied. Five different concentrations of ZnO-NPs were tested to determine the MICthatincludes 15, 10, 5, 2.5, 1, and 0.5mg/ml. The MIC valueswere found to be 1 and 0.5 g/m for *E. coli* and *S. aureus*, respectively. The resistivity against gram-positive bacteria wasless as compared with gram negative bacteria. IncreasingZnO-NPs concentration was relatedwithhigh antibacterial activity of ZnO-NPs. The antibacterial effect of ZnONPs was depending on a time and the effect was gradually.

**Keywords:** ZnO-NPs, *E. coli*, *S. aureus*, Nanoparticles, TEM**.**

## INTRODUCTION

Nanoparticles canbe defined as a group of materials that have unparalleledcharacteristics and wide range of applications in variousareas [1-2]. On theother hand, there are a huge difference in term of properties between nanoparticles and their bulk size counterparts[3-4]. Several materials were classified as safe materials show a toxicity behavior when it turns to nano size particles [5-6].This phenomenon is basically due to the increasing in the which is mainly related to the increasing in surface area and chemically active at nano size scale level[7-8]. Increasing the surface area plays an important role as it increases the reactivity with the organic sites that presents in organism cell surface [9].

Furthermore, zinc oxide nanoparticles (ZnO-NPs) has become an interesting metal oxide material for researchers due to its remarkablechemical and physical characteristics such as nontoxic nature, high

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activity as a catalysis, good piezoelectric, stable mechanically and chemically, high absorption of radiation, etc. Synthesis of ZnO-NPs can be achieved using severalapproaches such as controlled precipitation, vapor transport process, micro emulsion synthesis, etc. [10].

The chemical method of ZnO-NPs preparation has several advantages as compared to other method such as the large surface area and better porosity, which play an important role to increase the possibility to interact with the bioorganic molecules that is found in the viable cell surface [11]. Other advantages of preparation ZnO-NPs by chemical method are simplicity, relatively inexpensive, and can obtain nanoparticles in high crystalline and low dimeter. In general, inorganic metal oxide nanoparticles and especially ZnO-NPs show a very toxicity selectivity toward bio-systems which make it a considerable option as antimicrobial agentto be used in various applications such as in surgical instruments, curative, diagnosis, and in nano-medication [12]. ZnO-NPs is considered as a great option as an antimicrobial agent due to their great effective to resist strains for pathogen of microbial as well as low toxicity and can provide a resistance toward heat. Moreover, ZnO-NPsofferthe essential mineral substances fororganism cells and can provide a strong activity when applied even in small amount.

Dueto its uniquecharacteristics, it has been used extensively in different areassuch as pharmaceutical and cosmetic industries, electro technology industries, rubber industry, textile industries, etc. It has also been investigated to be used in possible applications in medicine. It shows a high degree of selectivity toward cancer cells and itcanbeat the therapeutic indicators of some traditionally chemotherapeutic drugs [13].

## MATERIALS AND METHODS

Different chemicals were used to prepareZnO-NPs, which werezinc sulphate heptahydrate ($ZnSO_4.7H_2O$ M=287.54 g/mol, Merck), sodium hydroxide (NaOH, M=39.99 g/mol, Sigma Aldrich) and polyvinyl alcohol which was used as a surfactant (PVA, Sigma Aldrich). *Staphylococcus auerus*was derived from NCTC 12493 and *Escherichia coli*was obtained from drinking water sources in south of Iraq. The growth of these microbials were achieved in nutritioussolution for 24 h at 37°C under air prior to bethe aimed organisms. The density of these isolated strains was setto match the optimum density of 0.5 McFarland standards.

### ZnO-NPs Preparation

ZnO-NPswas prepared by adding 0.01% of PVA solution to 1M of $ZnSO_4.7H_2O$solution, then2M of NaOH was added drop wise to the mixture with continuing stirring for 24 h. After this period,it was noticed a big amount of white powder wasparticipated.Filtration and washing with distilled water for this residue was obtained and the powder was placed in a conventional oven to dry at 100 °C for 6 h. The dried powder then grinded by dome shape grinder and to obtain fine powder and finally it was calculatedunder $N_2$ gas at 450 °C for 3 h.

### Transmission Electron Microscope (TEM)

The structure of the prepared ZnO-NPs as well as the shape and particle size were examined by transmission electron microscopy (TEM). Hitachi H7500 instrument was used for TEM analysis and the voltage was accelerated to 70 kV. The prepared nanoparticles were dispersed in acetone and then a few drops of the mixture were on carbon-coated 400 mesh copper grids.The mesh was left for 10 minutes to dry the acetone at room temperature prior to insert it inside the TEM instrument.

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### Anticancer Activity by MTT Assay

The MTT assay was carriedout by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide in order to investigate the anticancer activity of the prepared ZnO-NPs. Trypsin of cells culture of the breast cancer fibroblast was carried outin addition to setting the number of the cells to be approximately 20,000 cells for 200 µL of the suspension. 200 µL of the diluted cell suspension was added to each well in the microtiter plate, followed by incubation at 37 °C and under 5% of $CO_2$gas for 24 h. After that 200 µL of the rested drugs were added to these wells with different test concentrations. Similarly, incubation of these plates was carried out at 37 °C and under 5% of $CO_2$gas for another 24 h. Then 10% of MTT was added as a reagent to each followed by incubation at 37 °C and under 5% of $CO_2$gas for 5 h. The absorbance of the samples was checked by using microplate reader a wavelength of 570 nm. The cell viability was calculated as follows:

$$Cell\ vialbility\ = \frac{Test}{Control} \times 100 \quad (1)$$

### Antibacterial Activity Assay

The prepared ZnO-NPs antibacterial activity against *E. coli* and *S. aureus*was carried out by suspending ZnO-NPs in sterile normal saline withcontinues stirring to form a suspension witha ratio of 1000 mg/ml. To measure the toxicity of ZnO-NPs, a test of bacteria was vaccinated in nutrient broth medium bya seriesof ZnO-NPsina range between 10 to 0.250 mg/ml. The bacterial samples were incubated at 37 °C for 24 h and quantified by Colony forming units (cfu).

### Determination of Minimum Inhibitory Concentration

Agar dilution test was used in order to measure MIC and MBC. The targeted bacteria were inoculated on nutritious agar and different concentrations of ZnO-NPs were applied.Quantitation of colony forming unit (cfu) was used to determine the growth levelsof the examined bacteria. The samples that the bacteria did not grow after being incubated was selected. These samples transferred to a new medium that did not have ZnO-NPs after adding 0.1 ml of distilled water.

### Time Dependent Test

The time dependent study was conducted by placing the same amounts of *E. coli* and *S. aureus* in nutritious broth with different concentrations of ZnO-NPs0.2 ml of both bacteria were distributed on nutrient agar medium individually with time respect.The bacterial samples were incubated at 37 °C for 24 h, then 0.1 ml of different cultures was distributedon nutrient agar individually with time respect. This followed by counting the cfu for each samples well as comparing the cfu of the control sample. All experiments were carried out in triplicate and the average value wasacquired.

## RESULTS AND DISCUSSION

Fig. 1 shows the TEMimage of ZnO-NPs. The ZnO-NPs image showedthat the particles are uniform distributed. The average ZnO-NPslength and diameter of the particles were nearly between 100 nm to 200 nm and 25 nm to 50 nm, respectively. the TEM image of the ZnO-NPs particles showed sizes of nano scale and in the range 25-50 nm in addition to their spherical shape.

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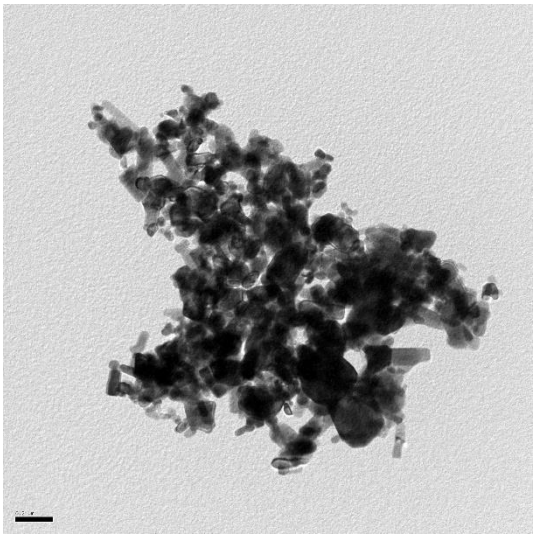

**Fig. 1.**TEM image of the prepared ZnO nanoparticles

Several advantages can be obtained in conducting in vitro experiments such as simple to perform,time consuming is less and can offeran acceptable range for *in vivo*study. The results of the *in vitro*ofthe cytotoxicity study were obtained after incubation of the sample for 24 h and using ZnO-NPs with different concentrations within the range from0.05 to 0.5 mg/ml.It was noticed various phases of cells death at different by applying numerousconcentrations of ZnO-NPs[14]. The cytotoxicity results revealed that by using 100 mg/ml of ZnO-NPs, MCF-7 cells were necrotizedwhich indicates that ZnO-NPs toxicity is nearly similar to the traditional camptothecindrug whichhas a toxicity value of 50 g [15]. In addition, the cytotoxic effect of ZnO-NPs on MCF-7 cell is illustrated in Fig. 2. The findingsshow a reverse relation between ZnO-NPs concentration and the cell viability. The required concentration of ZnO-NPs to preventthe growing of the cell by 50% was found to be 0.1mg/ml of ZnO-NPs.

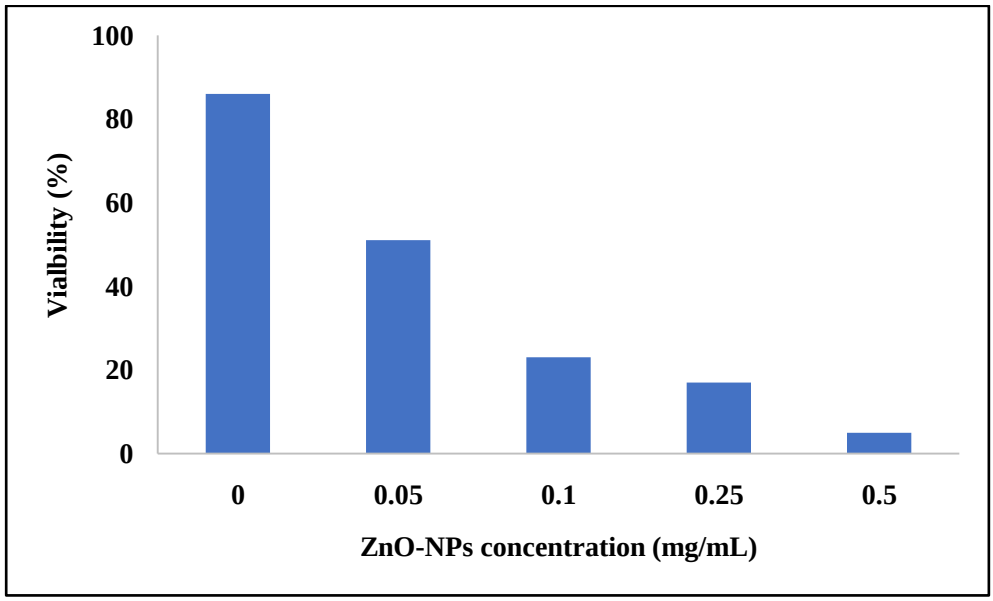


**Fig. 2.** Cell viability of MCF-7 cells calculated with MTT assay. Cells were incubated with the ZnO-NPs prepared for 24 h.

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Tables 1 and 2 show the zone of inhibition of*S. areus* and *E.coli*,respectively, of ZnO-NPs by the well and disc diffusion agar methods. The effect of prepared ZnO-NPs was indicated by the existence of an inhibition zone. It was observed that when the concentration of ZnO nanoparticles increased in both methods, the inhibition of growth was also increased, which was consistent withsome other reported studies Rizwan *et al*. [16].Moreover, the inhibition zone size was varied with the bacteria type, and ZnO-NPsconcentrations.

**Table 1.** Zone of inhibition for *S. areus*.

| ZnO concentration in each wells (mg/ml) | ZOI (mm) | ZnO concentration in each discs (mg/ml) | ZOI (mm) |
|---|---|---|---|
| **15** | 33 | 15 | 30 |
| **10** | 30 | 10 | 26 |
| **5** | 28 | 5 | 23 |
| **2.5** | 26 | 2.5 | 20 |
| **1** | 22 | 1 | 17 |
| **0.5** | 0 | 0.5 | 0 |
| **0** | 0 | 0 | 0 |

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

| ZnO concentration in each wells (mg/ml) | ZOI (mm) | ZnO concentration in each discs (mg/ml) | ZOI (mm) |
|---|---|---|---|
| **15** | 23 | 15 | 42 |
| **10** | 20 | 10 | 36 |
| **5** | 17 | 5 | 30 |
| **2.5** | 15 | 2.5 | 20 |
| **1** | 0 | 1 | 0 |
| **0.5** | 0 | 0.5 | 0 |
| **0** | 0 | 0 | 0 |

Fig. 3 show the cfu for*E. coli* and *S. aureus* after being incubated overnight with various concentrations of ZnO-NPs. The minimum concentration of ZnO-NPs required for growth inhibition of both bacteria were 2.5 mg/ml for *E. coli* and 1 mg/ml for *S.aureus*. Similar result was found in a previous published study which reported that 3.4 and 1 mg/mlfor *E. coli* and *S. aureus*, respectively, were the required MIC of ZnO-NPs [17-22].

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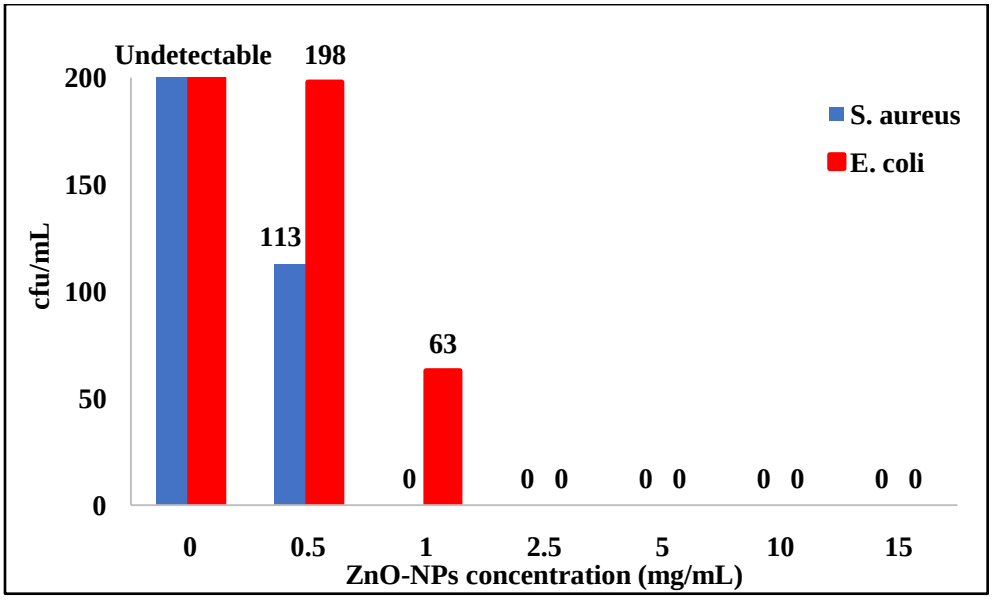


**Fig. 3.**Showed cfu for*E. coli* and *S. aureus* with various concentrations of ZnO-NPs

Tables 3 and 4 show a summary of MIC and MBC results for *E. coli* and *S. aureus*, respectively. It was found that higher concentration of ZnO-NPs is required for growth inhabitation for gram-negative bacteria comparedwith gram-positive bacteria. This could be due to different reasons such as the wall structure of the cell, contact degree or physiology of the cell [18].

**Table 3.** Detection of MIC and MBC for *E. coli.*

| Mode of effect | Concentration (mg/ml) |
|---|---|
| **Growth** | 0.5 |
| **Growth** | 1 |
| **Bacteriostatic** | 2.5 (MIC) |
| **Bacteriostatic** | 5 |
| **Bacteriostatic** | 10 |
| **Bactericidal** | 15 (MBC) |

**Table 4.** Detection of MIC and MBC for *S. aureus.*

| Mode of effect | Concentration (mg/ml) |
|---|---|
| **Growth** | 0.5 |
| **Bacteriostatic** | 1(MIC) |
| **Bacteriostatic** | 2.5 |
| **Bacteriostatic** | 5 |
| **Bacteriostatic** | 10 |
| **Bactericidal** | 15 (MBC) |

Fig.4 and 5 show the cfu of bothtest bacteria with time as a factor.The time-dependentstudy of ZnO-NPs antibacterial activity showed that for all the concentrations, the cfu of the tested bacteria wasgradually decreased via 72 hrs. Moreover,all the concentrations of ZnO-NPs,it was found that cfu was undetectable after 7hrsfrom the experiment.Based on the results, it mayhave suggested that ZnO-NPs play an efficacious antibacterial agent on both*E. coli*and *S. aureus* bacteria.

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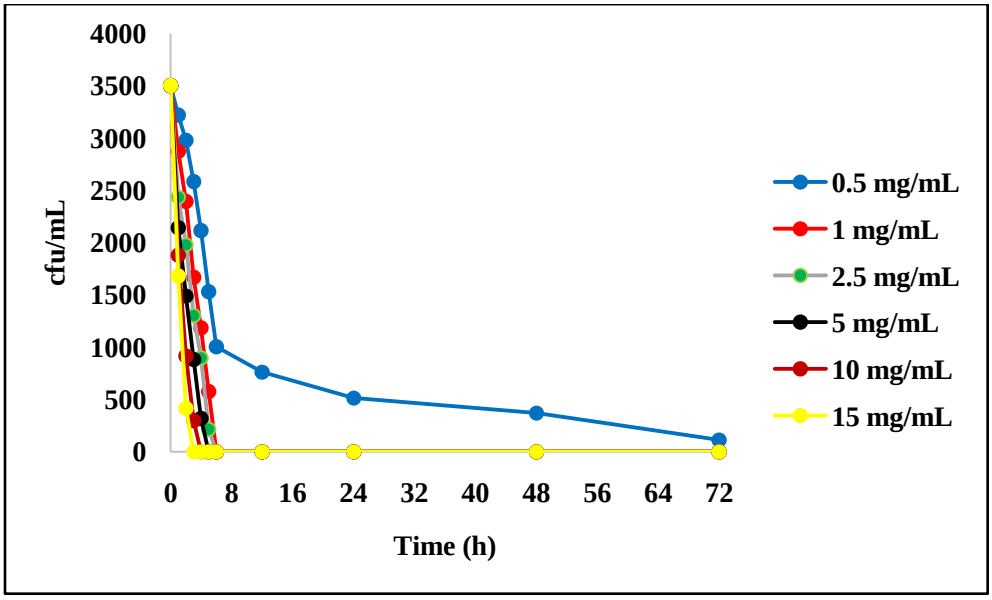


**Fig. 4.**cfu of *E. coli*with respect of time

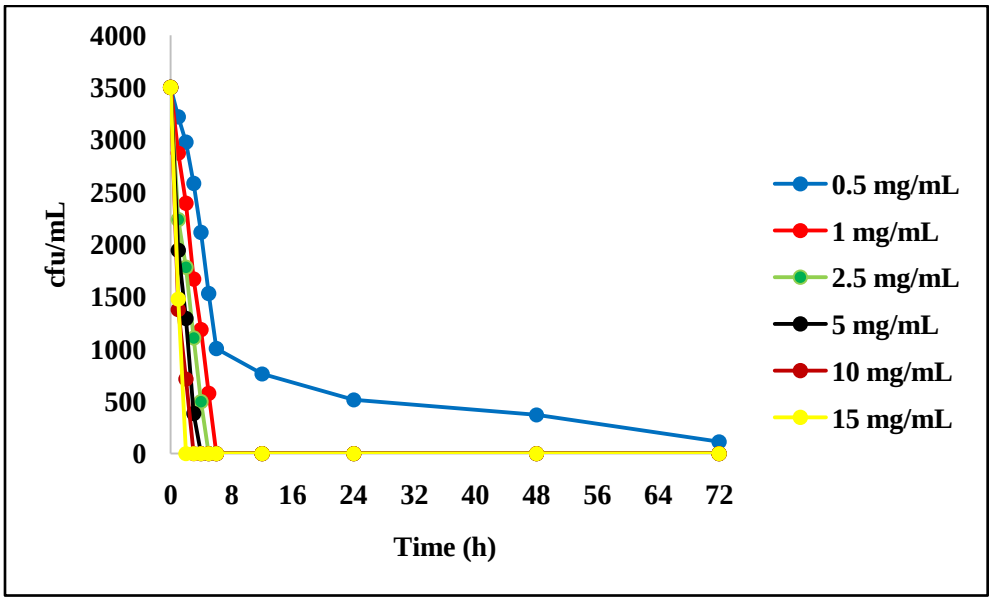


**Fig. 5.**cfu of *S. aureus*with respect of time

## COMCLUSION

Chemical method was used to successfully synthesized zinc oxide nanoparticles with using Poly Vinyl Alcohol (PVA) as a surfactant. The particles structure of the prepared ZnO-NPswas chocked bytransmission electron microscopy (TEM). TEM image revealed thatthe size ofZnO-NPswasata nano scale level.The synthesized ZnO-NPs was found to be an applicable potential alternative anticancer drug rather than the traditional other than the camptothecindrug. Different concentrations of ZnO-NPs were used in the cytotoxicity in vitro studyand the results indicated that varying the concentrations of ZnO-NPs resultedin several phases of the cell death. The prepared ZnO-NPs showed necrosis of the MCF-7 cells at 100 mg/ml. However, this study was concluded that ZnO-NPs havean effective role as an antibacterialagent on both *E. coli*and *S. aureus* bacteria.

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