In vitro antibacterial and anticancer studies of ZnO nanoparticles prepared by sugar fueled combustion synthesis

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Abstract

In this study we report the antibacterial and anticancer activity of ZnO nanoparticles (NPs) prepared by sugar fueled solution combustion synthesis. The structure and morphology of the sample were determined by XRD, UV-visible, FESEM and HRTEM. Surface area measurement was carried out by standard Brunauer-Emmett-Teller technique. Antibacterial activity of ZnO NPs was tested against *Clostridium perfringens* and *Salmonella enterica* by well diffusion method. The anticancer efficacy of ZnO nanoparticles was carried out on breast cancer cells MCF-7. The antibacterial results affirm that spherical ZnO NPs constitute as a successful bactericidal agent against both Gram-positive and Gram-negative bacteria. Anticancer result indicates that ZnO NPs exert dose dependent toxicity in MCF-7. Copyright © 2016 VBRI Press.

Keywords: Combustion synthesis, *clostridium perfringens, salmonella enterica,* MCF-7, MTT assay.

Introduction

ZnO is an important low cost semiconductor with Wurtzite structure. ZnO NPs have been extensively studied in the field of catalysis, paints, cosmetics, solar cells, gas sensors, food packaging materials, Piezoelectric nanogenarators, etc [**1-6**]. It is due to their ease of preparation in different morphologies, low cost, UV shielding properties, large surface to volume ratio, chemically alterable physical properties. The effect of zinc oxide nanoparticles on sex hormones and cholesterol in rat has also been studies [**7**]. Literature shows that ZnO NPs exhibit high toxicity against bacteria but minimum effect on human cells [**8, 9**]. The significance of ZnO structures against Herpes simplex virus type1 and type2 (HSV-1 and HSV-2) has also been reported [**10, 11**]. ZnO structures are also known for transparent-conductive activity, as UV sensors, as a polymers joining material and even in antifouling applications [**12-15**]. ZnO nano and microstructures find applications in microwave absorbing materials [**16**]. ZnO NPs have also received considerable attention in the field of cancer therapy in the recent days [**17, 18**].

The antimicrobial properties of nanoparticles depend significantly on their size, surface area, composition, surface charge and shape [**19**]. The antimicrobial activity of nanoparticles has been studied with different pathogenic and nonpathogenic bacteria such as *Staphylococcus aureus, Escherichia coli* and fungi such as *Botryis cinera, Penicillium expansum, Candida albicans* etc [**20-23**].

Clostridium perfringens is a bacterium that is usually responsible for food poisoning. This spore-forming bacterium may be found in many environmental sources. They may be also found in the intestines of human and animals. It has been assessed by the Centers for Diseases Control and the Food and Drug Administration of the United States of America that around 30 million individuals every year fall sick because of eating contaminated food [**24, 25**]. Studies have shown that *Salmonella enterica* is one amongst the more prevalent bacterial pathogens that causes food borne infections [**24**]. It is estimated that the medical and productivity losses caused by this bacterium are of high order. [**24**].

Solution combustion synthesis (SCS) is a simple, time saving and inexpensive method for preparation of oxide nano particles. It is an exothermic reaction between fuel and metal nitrate. SCS is a reliable technique for the preparation of ZnO NPs. Several organic compounds such as citric acid, urea, sugar, oxalyl dihydrazide, glycine have been used as fuels for this preparation process [**26-29**]. ZnO NPs have also been synthesized by SCS using various plant extracts as fuel [**30, 31**]. Though there are reports on the synthesis of ZnO NPs using sugar as

fuel, literature survey indicates that there is no detailed scrutiny on the antibacterial response of ZnO NPs prepared by sugar fueled combustion synthesis against *Clostridium perfringens* and Gram-negative *Salmonella enterica* and anticancer activity on human breast cancer cell line MCF-7. Hence, the present study reports the synthesis and antibacterial effect of ZnO NPs on Gram-positive *Clostridium perfringens* and Gram-negative *Salmonella enterica* by well diffusion method. The cytotoxicity of ZnO NPs has been investigated on human breast adenocarcinoma cell line MCF-7 by 3-(4,5-dimehtylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay.

Experimental

Materials / Chemicals

Zinc nitrate hexahydrate [$Zn(NO_3)_2.6H_2O$), AR 99% SD Fine], Nutrient agar [Himedia], Dulbecco's Modified Eagle's medium [Gibco], Dimethyl sulfoxide [C_2H_6SO, AR 99% Merck], MTT [C18H16BrN5S, 97.5%, Sigma aldrich] were used as such without further purification and sugar from the local market was purchased off the shelf.

Synthesis of ZnO NPs

The ratio of oxidizer to fuel needed for stoichimetric mixture was calculated as described by Patil et al [**26, 27**]. The synthesis of ZnO NPs was carried out by solution combustion synthesis (SCS) using sugar as fuel which is described in our previous work [**32**]. 5.0 g of $Zn(NO_3)_2.6H_2O$ and 1.2 g of sugar were dissolved completely in 20 mL of double distilled water under stirring. Here zinc nitrate and sugar act as oxidizer and fuel respectively. The crystallizing dish containing the mixture was introduced in to the preheated muffle furnace. The temperature of the furnace was maintained at 375 $\pm$ 10^0 C. Within a brief time the solution boiled to form a transparent gel followed by fast burning of the fuel. The reaction yielded a white and highly porous product.

Characterizations

ZnO NPs synthesized were characterized by various techniques. Powder X-ray diffraction pattern was recorded using Panalytical X'pert diffractometer with Cu Kα radiation (λ=1.5418 Å) as the source. The UV-vis measurement was carried out on JASCO (V-670) at room temperature**.** The morphology of the sample was characterized by Field Emission Scanning Electron Microscopy (FE-SEM) performed on FEI Quanta FEG 200 - High Resolution Scanning Electron Microscope. The shapes and particle size were investigated by High Resolution Transmission Electron Microscopy (HRTEM) carried out on JEOL 3010. Brunauer-Emmett-Teller (BET) surface area measurement was carried out on Micromeritics ASAP 2020.

Screening of ZnO NPs for antibacterial activity by well diffusion technique

The antibacterial activity of ZnO NPs was performed by well diffusion method in nutrient agar media [**12**]. About 20 mL of sterilized and molten nutrient agar media was poured in to the sterilized petri plates. The bacteria Gram-positive *Clostridium perfringens* and *Salmonella enterica* were cultured overnight at 37°C in nutrient agar and adjusted to a final density of 10^7 CFU/mL by 0.5 McFarland standards. 100 µL of the pathogenic bacteria cultures were transferred onto plate and made culture lawn. Homogeneous dispersions of NPs with different concentrations ranging from 500 µg/mL to 62.5 µg/mL were prepared by ultrasonication. Wells were prepared with sterilized stainless steel cork borer. The wells in each plate were loaded with 50 µL of different concentrations of ZnO NPs ranging from 500 to 62.5 µg/mL. The plates were incubated at 37 °C for 24 h. By measuring the diameter of the zone of inhibition (ZOI) formed around the wells, the antibacterial efficacy of ZnO NPs was determined.

Evaluation of anticancer activity in MCF-7 by MTT assay

Anticancer activity of ZnO NPs was carried out by MTT assay as reported in our earlier studies [30]. In brief, ZnO NPs with different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/mL) were dispersed in dimethyl sulfoxide (DMSO). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 11% fetal bovine serum at 37^0C with 5% CO_2. Cells were plated in 96-well plates (10^5 cells/well) containing 100 µL of medium. After 24 h, dispersions of ZnO NPs were loaded to each well and incubated for 24 h and same amount of DMSO was added to the control. Growth of the cells was quantified by the ability of living cells to reduce the yellow dye MTT to a blue formazan product. At the end of 24 h of incubation the medium in each well was replaced by fresh medium (100 µL) containing 0.5 mg/mL of MTT. Four hours later, the formazan product of MTT reduction was dissolved in DMSO and absorbance was measured using a microplate reader. Effect of ZnO NPs was quantified as the percentage of control absorbance of reduced dye at 570 nm.

Results and discussion

Crystal structure

The PXRD pattern of ZnO NPs is presented in **Fig.1**.

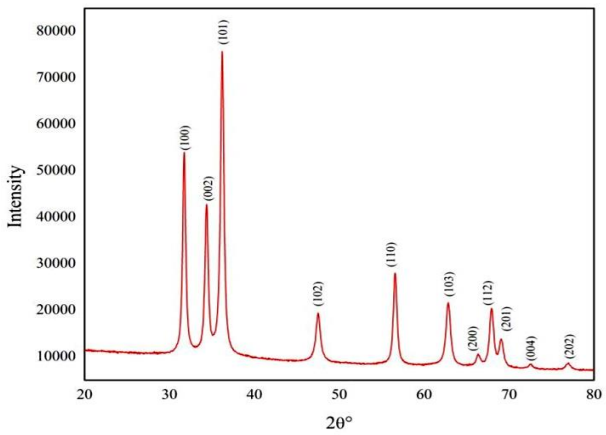

Fig. 1. PXRD pattern of ZnO NPs.

The result was examined with Crystallographica Search-Match (CSM). PXRD of the sample showed the crystalline nature of the sample having hexagonal structure with the standard Joint Committee on Powder Diffraction Standards (JCPDS) No. [36-1451] corresponding to zincite pattern. Hence it can be indexed as hexagonal Wurtzite type of ZnO. The crystallite size of the sample was calculated using Scherrer equation, D = k λ / β Cos θ, where D is the crystallite size, k is the Scherrer constant (0.9), λ is the X -ray wavelength, θ is the Bragg angle and β is the line broadening at half the maximum intensity (FWHM). The average crystallite size ZnO NPs calculated from this was found to be ~14 nm.

Evaluation of band gap energy

A plot of $(\alpha hv)^{1/2}$ vs Eg of ZnO NPs is shown in **Fig. 2**. The band gap energy value was determined by Wood-Tauc method [33, 34]. The band gap energy was found to be ~ 3.2 eV. This result is in concurrence with the literature [**35**].

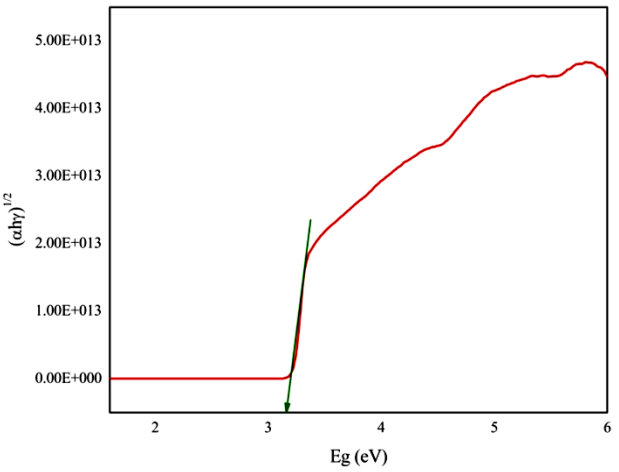

Fig. 2. Evaluation of band gap energy of ZnO NPs.

Morphological analyses

The FE-SEM micrograph of ZnO NPs is shown in **Fig. 3(a)**. The SEM micrograph of ZnO NPs shows that the morphology of particles is spherical.

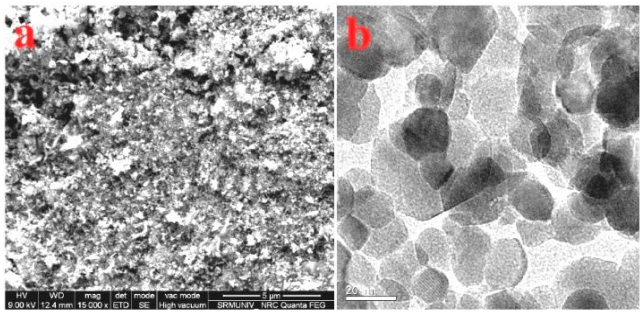

Fig. 3. (a) FE-SEM and (b) HRTEM images of ZnO NPs.

Micrograph also indicates that apart from spherical crystals the sample contains many voids and pores. The reason for which might be due to the release of large amount of gases during the process of combustion. The TEM study was carried out to comprehend the crystalline attributes of ZnO NPs. The particle size of powders can be determined from the TEM picture. The TEM method is better than X-ray line broadening in that it is direct and less likely to be affected by experimental errors and other properties of the particles such as internal strain or distribution in the size of the lattice parameter [**36**]. The HRTEM micrograph of ZnO NPs is shown in **Fig. 3(b)**. TEM studies show that ZnO particles have spherical shape with a mean particle size of 96 nm.

Surface area measurements

The surface area of Zno NPs was measured by the standard BET technique with N_2 adsorption-desorption isotherms on Micromeritics ASAP 2020. The BET surface area value of ZnO NPs was found to be 36.735m^2/g. These results showed high porosity of the sample compared to conventional ZnO NPs. All experimental parameters of BET surface area analysis are summarized in **Table 1**.

Table 1. BET surface area values.

Surface area (m^2/g)	Pore volume (cm^3/g)	Pore size (nm)	Micropores area (m^2/g)	BJH adsorption average pore diameter (nm)	BJH desorption average pore diameter (nm)
36.7350	0.421977	45.9481	36.4113	59.984	38.2813

Antibacterial studies

The antibacterial results on ZnO NPs against *Clostridium perfringens* and *Salmonella enterica* are presented in **Table 2** and the zone of inhibition is shown in **Fig. 4**.

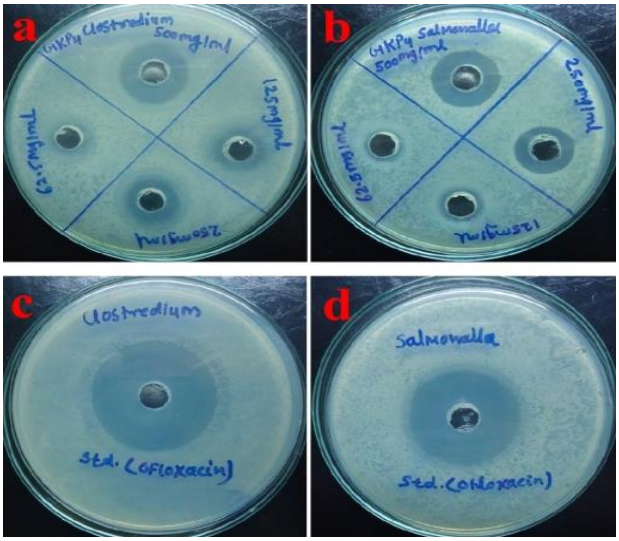

Fig. 4. Zone of inhibition produced by (a, b) ZnO NPs against *C. perfringens and S. enterica* respectively, (c, d) standard antibiotic against *C. perfringens and S. enterica* respectively.

Table 2. Results of antibacterial activity of ZnO NPs (Zone of inhibition in mm)

Test organism	Concentration of ZnO suspensions (µg/mL)				Positive control Ofloxacin (100 µg/mL)
Clostridium	25.50±1.291	21.75±0.957	17.75±0.957	12.50±1.291	40.75±1.258
Salmonella	23.25±1.500	19.75±0.957	17.00±0.816	12.50±1.291	38.00±0.816

It was noticed from the results that the ZOI is maximum for ZnO NPs at a concentration of 500µg/mL against both

the bacteria. The bacterial growth in presence of NPs concluded the bactericidal ability of NPs in a concentration dependent manner which is in well agreement with the literature [**37**]. The pictorial diagrammatic representation of action of of ZnO NPs against the bacteria has been shown in **Fig. 5**. The mechanism of action of ZnO NPs on microorganisms is still under discussion. The detailed mechanism of the bioactivity of ZnO is still under discussion. Many mechanisms have been proposed related to this: (a) One of the possible mechanisms is based on the abrasive surface texture of ZnO - binding of ZnO nanoparticles to the bacterial surface is due to electrostatic forces that directly kill bacteria [**38**], (b) mechanical destruction of the cell membrane caused by penetration of the nanoparticles [**39**], (c) release of Zn2+ ions from the nanoparticles [**39**] and (d) active oxygen generated from the powder [**41-44, 9**].

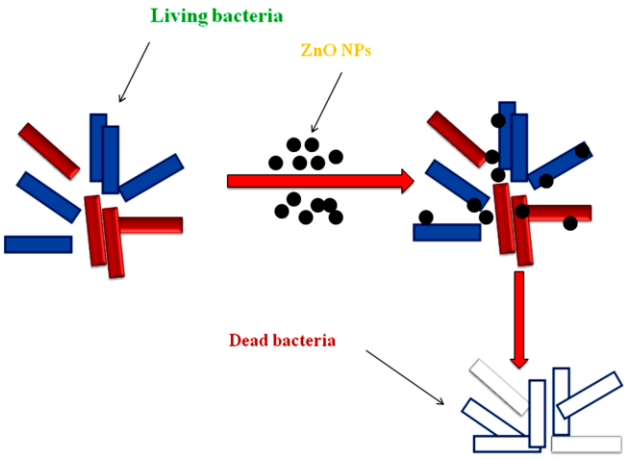

Fig. 5. Pictorial representation of action of ZnO NPs on bacteria.

As can be summarized from the antibacterial results, ZnO NPs exhibited higher zone of inhibitions for Gram-positive bacterial strain than the Gram-negative bacterial strain. This result might be indicative of higher Gram-negative strain tolerance against ZnO NPs over Gram-positive bacterial strains. Our these results are in agreement with the literature which reports that the ZnO NPs effect is more pronounced against Gram-positive bacterial strains than Gram-negative bacterial strains [**45, 46**].

Anticancer activity

Cytotoxic effect of ZnO NPs in cell line MCF-7 is presented **Fig. 6**. The pictorial diagrammatic representation of toxicity of ZnO NPs against the cancer cells has been shown in **Fig. 7**. These antiproliferative studies clearly demonstrate that treatments with ZnO NPs sensitize cancer cells. A dose dependent decrease was observed in the cell viability. The results indicate that ZnO NPs at their highest concentration of 100 µg/mL tested in our studies showed ~ 60 % cell viability. Literature shows that ZnO NPs induce cytotoxicity in a cell specific and proliferation dependent manner by rapidly dividing cancer cells being the most susceptible and quiescent cells being the least sensitive [**45, 47**]. Premanathan *et al.* have shown in their studies that the primary mechanism of ZnO NPs cytotoxicity might proceed by inducing the generation of reactive oxygen species, which are responsible for the induction of apoptosis [**45**]. However, the anticancer activity of ZnO NPs, specifically the mechanism of apoptosis in cancer cells due to ZnO NPs is still not clear.

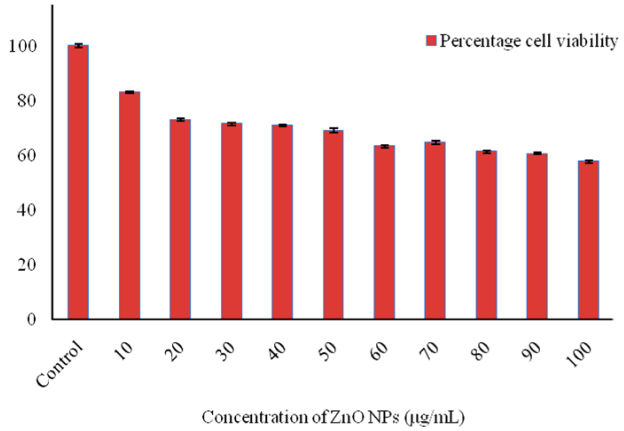

Fig. 6. Cytotoxic effect of ZnO NPs in MCF-7 cell lines. Cells were treated with various concentrations (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/mL) of ZnO NPs for 24 h. The percentage of cell death induced was determined using the MTT assay.

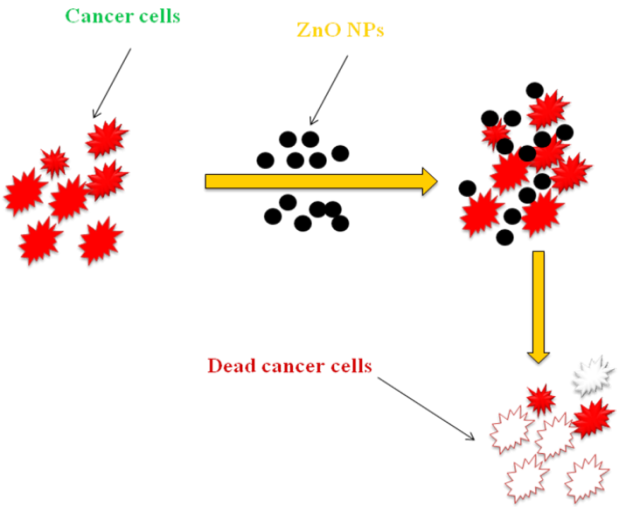

Fig. 7. Pictorial representation of cytotoxicity of ZnO NPs on MCF-7 cancer cells.

Conclusion

In this study, we focused on the antibacterial and anticancer activity of ZnO NPs prepared by simple and cost effective SCS using sugar as fuel. This work confirmed the antibacterial efficacy of ZnO NPs against both Gram-positive and Gram-negative bacteria namely *Clostridium perfringens* and *Salmonella enterica*. MTT assay results indicate that at a concentration of 100 µg/mL of ZnO NPs, the viability of MCF-7 cells dropped to ~ 60 %. However, the antibacterial and anticancer efficacy of ZnO NPs, their safety and detailed mechanism of action of ZnO NPs as an antibacterial and anticancer agent must be studied further in detail *in vitro* and *in vivo* in the near future to explore the plausible bio applications of ZnO NPs.

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