Research article

Comparative evaluation of the antibacterial and cytotoxic activity of green synthesized and commercially available ZnO nanoparticlesSushmit Mukherjee¹, Aparna Shil¹, Kunal Pal^{2,3}, Shinjini Pal⁴, Mausumi Sikdar (ne'e Bhakta)¹¹Microbiology, Nutrition and Dietetics Laboratory, Physiology Unit, Department of Life Sciences, Presidency University, Kolkata, 700073, India²Department of Life Science and Biotechnology, Jadavpur University, Kolkata, 700032, India³Division of Molecular Medicine and Centre for Translational Research, Bose Institute, Kolkata, 700056, India⁴Department of Physics, Jadavpur University, Kolkata, 700032, India

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ABSTRACT

Introduction and Aim: Emergence of different applications of metallic nanoparticles in various field leads to innovation of new synthetic strategies. Besides being non-toxic to mammalian cells, zinc oxide nanoparticles (ZnONPs) has gained paramount attention due to its excellent antibacterial potential. This study illustrates a comparative analysis of antibacterial and cytotoxic activity of both phytochemically synthesized and chemically synthesized commercially available ZnONPs.

Materials and Methods: As a source of reducing agent, leaf extract of *Coriander sativum* was employed in case of green synthesis of ZnONPs. Several techniques, such as X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Dynamic light Scattering (DLS) and Field emission Scanning Electron Microscopy (FESEM) were performed to characterize both green synthesized and commercial ZnONPs. Antibacterial potential of both the ZnONPs were investigated on Gram-positive and Gram-negative bacterial strains to draw a correlative outcome. Hepatocellular cell line was used to determine the cytotoxic activity of both ZnONPs.

Results: Both the nanoparticles showed antibacterial and cytotoxic activity with measurable degree of difference.

Conclusion: From these studies it can be concluded, the green synthesized nanoparticles showed greater antibacterial as well as cytotoxic activity in comparison to the commercial ZnONPs.

Keywords: Green synthesis; ZnONPs; *Coriander sativum*; antibacterial; cytotoxic activity.

INTRODUCTION

Extensively tiny size and unique properties of nanomaterial intensified their acceptance in the field of biomedical research. Emergence of nanoscience creates a possibility to encounter unique properties of various substances and also enhanced the scope of capping and doping technology (1). Among the several metal oxides, ZnONPs are worth of appreciation due to its unique electrical, optical, and chemical properties. Large excitation binding energy, large band gap (3.3eV), high electron mobility and high transparency equip it appropriate for serving the field of diagnostics, drug delivery, bio-molecular detection, DNA labelling, optoelectronics, biosensors, solar cells, cement ceramic sensors (2) etc., In addition, purification of water by removal of impurities such as, sulphur, arsenic along with waste water treatment also prospered by application of ZnONPs (3). Excellent antibacterial activity of ZnONPs have also been reported against high temperature and pressure resistant spores apart from its anticancer potential. Electrostatic adsorption of the particles to the bacterial surface and formation of Hydrogen peroxide are considered to be the reason of efficient antibacterial activity of ZnONPs (4). Based on this

potent antibacterial efficacy, ZnONPs gained enough advantage in the arena of food and wood preservation, wound dressing, nano-medicines, cosmetics and disinfectant agents. These innumerable numbers of applications of ZnONPs build up the demand for different synthetic strategies (5). Till date, multiple chemical and physical synthesis procedures are reported, of which all comprised of hazardous chemicals, resulting in usage of toxic substances. In addition, performing these synthesis protocols are tedious and expensive. Acceptability of microorganism-based synthesis procedure could have been universal, if the criterion of maintaining highly aseptic conditions were avoidable. Besides, necessity of conducting several purification steps has become a major hindrance for application at industrial scale. Green synthesis emerged as an inexpensive and eco-friendly alternative for synthesis of well structured, biocompatible and potent ZnONPs (6). Several plants such as *Azadirachta indica*, *Cassia auriculata*, *Ocimum sanctum*, *Aloe vera*, *Coriandrum sativum* etc., have been exploited for synthesis of ZnONPs from extract of their different parts (7). For this study, leaf extract of *Coriandrum sativum* was employed as the plant source and reducing agent to achieve green synthesized ZnONPs. *Coriandrum*

sativum also known as 'Dhania' belongs to Apiaceae family (8). This study illustrates a comparative analysis of therapeutic potential between the chemically synthesized, commercially available and green synthesized ZnONPs. Both Gram-positive and Gram-negative bacterial strains were taken into account as test organisms for this comparison. Comparison has also been made with respect to the cytotoxic activity of both the nanoparticles and a comparative resolution was made.

MATERIALS AND METHODS

Materials and cell types used in this study

Commercial ZnONPs, Zinc acetate dehydrate [$\text{Zn}(\text{CH}_3\text{CO}_2)_2 \cdot 2\text{H}_2\text{O}$] and Sodium hydroxide (NaOH) was purchased from Sigma Aldrich Chemicals. Mueller Hinton Broth and Nutrient agar media for bacterial culture were purchased from Himedia (Mumbai, India) and the MTT reagents were procured from Merck Ltd, and SRL Pvt. Ltd, Mumbai, India. All the reagents used in this study were of analytical grade and used without further purification. During the entire study, deionized (Millipore) water of 18 M Ω -cm resistivity has been used. Fresh coriander leaves (*Coriandrum sativum*) were purchased from the local market.

Bacterial strains: The microbial test organisms used for this study includes, *Enterococcus faecalis* (*E. faecalis*) 441, *Staphylococcus aureus* (*S. aureus*) 740 as the Gram-positive bacterial strains and *Pseudomonas aeruginosa* (*P. aeruginosa*) 1688, *Escherichia coli* (*E. coli*) 443 as the Gram-negative bacterial strains. All used bacterial strains were procured from the Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India. Subcultures were made once per month and stock cultures (in glycerol) were stored at -20°C for each bacterial strain to maintain the cultures properly, without any contamination. All strains were maintained throughout the study by performing subcultures once per month.

Mammalian cell line: Hepatocellular carcinoma cells (Hep G2 cell line) were used to determine cytotoxic activity of both green synthesized and commercially available chemically synthesized ZnONPs.

Coriander leaf extract preparation

Fresh coriander leaves were first segregated from their stalks to work with only leaves. Then the leaves were thoroughly washed several times with Millipore water to remove all the dust and unwanted particles and kept at room temperature for air drying until properly dried. In order to prepare the extract, 25gm of air-dried leaves were boiled at 80°C in 100ml deionized water for 20 minutes. The extract obtained was cooled at room temperature, filtered using filter paper (Whatman™) and the filtrate was placed in a

sterile container (9). Every study was conducted using freshly prepared leaf extract.

Zinc salt solution preparation

0.2 M Zinc acetate solution was achieved by dissolving 2.2 gm of zinc acetate powder into 50 ml Millipore water and was subjected to magnetic stirring until dissolved entirely (10).

Zinc oxide nanoparticle formation

50 ml of prepared Zinc acetate solution (0.2 M) was kept at stirring condition, followed by addition of 50 ml filtered coriander leaf extract at room temperature. 10 ml of 1 M NaOH was added drop wise into this solution. A characteristic yellowish white precipitate was instantly visible right after the addition of NaOH solution (11).

Characterizations of both synthesized and Commercial ZnONPs

Powder X-ray diffraction method was implied to investigate the crystal structure of synthesized and commercial ZnONPs. This study was done by X-ray powder Diffractometer (12) model D8, Bruker AXS, Wisconsin (USA), containing Cu-K α as a target, employing 1.5418 Å wavelengths within 20°- 80°2 θ range, and a scan speed of 1 s/step was maintained while operating at 35kV.

To assume the functional groups, present in the used spice extract, Fourier transform infrared spectroscopy (FTIR) analyses was carried out. A range from 400 cm⁻¹ to 4000 cm⁻¹ was studied (13) by FTIR-8400S, Shimadzu.

The stability and particle size distribution of synthesized ZnONPs and commercial one was evaluated by the help of DLS (Dynamic light Scattering) study (14) conducted by Zetasizer (NANO ZS90, Malvern Instruments Ltd., UK).

Finally, to verify the morphology of both green synthesized and commercial ZnONPs, field emission Scanning Electron Microscopy (FE-SEM; 15) was performed through INSPECTF50 (FEI, Netherland).

Antibacterial potential of ZnONPs

Determination of Minimum Inhibitory Concentration (MIC)

ZnONPs were thoroughly ultrasonicated in a water bath prior to each experiment. On the day before the experiment, liquid cultures of each bacterial strains (*S. aureus*, *E. faecalis*, *P. aeruginosa*, *E. coli*) were prepared in sterile Mueller Hinton Broth. Graded concentrations of synthesized ZnONPs solution ranging from 0 µg/ml to 100 µg/ml were added to several tubes each containing 1ml sterilized Mueller Hinton Broth. Each tube was then inoculated by 10µl bacterial culture containing 2.5×10^5 CFU ml⁻¹ bacteria and subjected to overnight incubation at 37°C. This procedure was carried out with the

commercial ZnONPs also, for each of the four bacterial strains. Optical density (at 530nm) of the content of each tube was measured using spectrophotometer to determine minimum inhibitory concentrations (16).

Determination of Minimum Bactericidal Concentration (MBC)

10 µl from each tube used for MIC study, containing ZnONPs treated bacterial cultures were further spread on nutrient agar plates. These plates were then subjected to overnight incubation at 37°C. The result was observed on the next day, and the minimum concentration at which no bacterial colony was observed, was considered as the MBC value. In order to determine the MBC value of the commercial ZnONPs, the same procedure was followed for each of the study strains (17).

Tolerance level

The evaluated MIC and MBC values were used to express the tolerance level of each bacterial strain against both the green synthesized and commercial ZnONPs with the help of the following formula (18). $MBC/MIC = \text{Tolerance level}$

Agar well diffusion method

Several tubes containing 1ml freshly prepared Mueller Hinton Broth were inoculated with each bacterial strain and kept for an overnight incubation at 37°C. On the next day, each of the grown bacterial cultures was diluted using sterile Mueller Hinton Broth to adjust the turbidity, as per the standard of 0.5 McFarland (10^8 CFU/ml). From these diluted cultures, 100 µl were spread on a nutrient agar plate followed by punching of 4 wells in each plate measuring 0.563 cm² diameter. Two of the wells contained the experimentally obtained MBC values ZnONPs (one well was filled with green synthesized and the other with commercial), ampicillin was poured in a well as positive control and sterile distilled water in another well. These plates were kept for overnight incubation at 37°C. The diameters of the obtained zones of inhibition were measured and compared (19).

Kinetics of bacterial growth inhibition

A series of five glass containers with sterilized Mueller Hinton Broth were prepared. Fresh bacterial culture of a strain was added to all containers, leaving one, which was used to set the blank during the measurement of optical density, at 530 nm. The three containers were also provided with specific antibacterial agents like commercial ZnONPs (Sigma), green-synthesized ZnONPs (coriander leaf) and an antibiotic (Ampicillin), at pre-determined MIC values. One among the four containers, were deprived of any antibacterial agent and was used as control for the experiment. These containers were then incubated at 37°C. These set of containers were

prepared for all four strains of bacteria (*S. aureus*, *E. faecalis*, *P. aeruginosa* and *E. coli*). Optical density of the contents of each container was measured at an interval of one hour, starting from the zero hour (just before incubation). The obtained values were used to plot the growth curve of each strain of bacteria used for this study (20).

Bacterial cell viability assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was used in a standard protocol to determine the cell viability of bacteria. Bacterial cells were incubated overnight with different concentrations of both the synthesized and commercial ZnONPs at 37°C. Next day, bacterial cells were collected after performing centrifugation at 1400 rpm for 10 minutes at 4°C followed by washing thrice with sterile phosphate buffered saline (PBS, pH 7.4). These cells were further incubated at 37°C for 3 hours, after replacement of the previous media, by fresh 0.5 mg.ml⁻¹ of MTT reagent containing media. After this incubation, cells were again incubated at room temperature for 15 minutes after adding isopropanol-HCl solution. Spectrophotometer was used to measure the absorbance of the formed formazan-MTT product in a Shimadzu UV-Vis 2600 spectrophotometer at 570 nm (21).

Determination of bacterial intracellular ROS generation

2, 7-dichlorofluorescein di-acetate (DCF-DA) mediated protocol (22) was implemented to measure ROS generation within bacterial cells. 2,7-dichlorofluorescein (DCF), which is a highly fluorescence compound, is generated as a result of reaction between ROS generated within the bacterial cells and passively entered DCF-DA into the cells. The bacterial cells were incubated overnight with both synthesized and commercial ZnONPs and subjected to wash with phosphate buffer solution on the next day, followed by addition of required amount of DCF-DA. After incubating the bacterial cells for half an hour at 37°C, results were obtained spectroscopically (23).

Bacterial Scanning Electron Microscopy (SEM) study

The extent of the physical interaction of both the green synthesized and commercial ZnONPs with the treated bacterial cells, followed by damage of bacterial cell morphology, was analysed by SEM study. Samples were prepared following the previously reported method by Ansari et al (24). Centrifugation (4 min, 4000 RPM) of 6 hrs incubated 1ml treated bacterial cells, along with control was performed to acquire the pellet. Filtered sterile PBS containing 2% glutaraldehyde was utilized to wash the pellets thrice and then subjected to dehydration using serially diluted ethanol. Drop casting of the cells were then performed on cover slips and kept under laminar air flow for drying. Samples were

further gold coated after placing on carbon tape for bacterial SEM analysis (FEI, INSPECT F50, Netherlands).

Effect of ZnONPs on eukaryotic cells

Evaluation of mammalian cell ROS generation

Generally, the green fluorescence color developing compound dichlorofluorescein (DCF) is generated as a reaction product of reactive oxygen and DCFDA. 10 mM methanolic stock solution of DCFDA was prepared and to get 100µM working concentration further dilution with PBS was done. Treatment of Hep G2 cells were done by both the green synthesized and commercial ZnONPs using LD₅₀ concentrations following overnight incubation at 37°C. Next day, the incubated cells were washed thoroughly by ice-cold 1× PBS. Incubation for 30 minutes in dark at 37°C was done after adding 100µM DCFDA (3). Both spectroscopy and fluorescence microscopy were used to measure the fluorescence intensity at an excitation wavelength of 485nm and emission at 520 nm.

Cytotoxicity assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to find out the viable Hep G2 cell count after treatment with graded concentrations of both green synthesized and chemical ZnONPs (3). In a 96 well plate, each well containing approximately 10⁴ cells were treated, using different concentrations of both green synthesized and commercial ZnONPs ranging from 20 µg/ml – 100 µg/ml along with a control (untreated) and kept for 24hrs incubation at 37°C with 5% CO₂. After this, incubation for 4hrs was done at 37°C with 5% CO₂, after addition of 10 µl MTT solution of 1 mg/ml strength, followed by thorough washing with the help of PBS. Finally, the crystals of formazan produced were dissolved in solubilization buffer and absorbance was measured at 570nm. Interpretation of data was done in comparison to control.

RESULTS

Physical characterization of ZnONPs

XRD analysis

Result of X-ray diffraction study (Figure 1) of both green synthesized and commercial ZnONPs revealed nice crystalline structure having evident diffraction peaks and also portrayed the influence of the

experimental conditions maintained throughout the synthesis procedure of green ZnONPs. At 2θ values 31.81, 34.59, 36.36, 47.62, 56.71, 63.03, 66.57, 68.09, 69.22 and 32.02, 34.68, 36.44, 47.65, 56.80, 63.14, 66.67, 68.15, 69.47 distinct characteristic peaks were observed for green and commercial ZnONPs respectively, corresponding to (hkl) values of (100), (002), (101), (102), (110), (103), (200), (112), (201). The absence of any additional peak confirms the formation of pure sample. Equation of Debye–Scherer was applied to calculate average size of both the green and commercial ZnONPs utilizing the highest peak. Table 1 represents the observed average diameter and Polydispersive index (P.D.I) of both the ZnONPs. The size of both the ZnONPs evaluated from this study confirms its nano-scale structure.

$$D = 0.9\lambda / \beta \cos\theta$$

Where, λ = wavelength (1.5418 Å), θ = Bragg's diffraction angle, β = full width at half maximum.

Fig. 1: XRD of commercial and green synthesized ZnONPs.

FE-SEM analysis

The structural evaluation of both the green and commercial ZnONPs were conducted through FE-SEM study (Figure 2). Resultant micrograph images display spherical shape along with identical ordination for both green synthesized and chemically synthesized commercial ZnONPs.

Commercial ZnONPs

Green ZnONPs

Fig. 2: FESEM images of commercial (a) and green synthesized (b) ZnONPs.

Spectroscopic analysis

Surface adsorption of functional groups was examined through FTIR for both the green synthesized and commercial ZnONPs (Figure 3). Absorption band of both the ZnONPs were observed within a range of 4000-500 cm^{-1} . Peak at around 450 cm^{-1} confirms the existence of M-O and ZnO vibrational band as this peak demonstrates, ZnO bond stretching frequency. Peak at 774 cm^{-1} accounts for the intra-molecular hydrogen bond's -OH stretching along with C=O and C-C stretching of alkanes. Peak at around 1104 cm^{-1} can be the contribution of N-C stretching vibration of aromatic and aliphatic amides, aliphatic amines, phenolic and alcohol groups along with secondary amines stretching vibration. Existence of atmospheric CO_2 and amide linkage within amino acid residues are expressed by band at 3376 cm^{-1} ¹⁴⁵.

Fig. 3: FTIR absorption spectrum of green synthesized and commercial ZnONPs.

Determination of hydrodynamic size and surface charge of ZnONPs

As shown in the table 1, the zeta potential of green synthesized ZnONPs is greater than that of the

commercial one. This result suggests that the biogenic nanoparticles are more stable in aqueous medium, which in turn is conducive for its biological applications.

Table 1: Dynamic Light Scattering, Zeta potential and Polydispersive index of green synthesized and commercial ZnONPs (The data are the average of three experiments $\pm$ SD).

Sample name	DLS Size (d.nm)	Polydispersive index	Zeta potential (mV)
ZnONPs from coriander leaves	87.6 $\pm$ 2.7	0.143	13.34 $\pm$ 2.7
Commercial ZnONPs	85.6 $\pm$ 2.2	0.152	11.45 $\pm$ 1.8

Antibacterial study

Comparative analysis of antibacterial efficacy of green and commercial ZnONPs

Antibacterial effectiveness of both the green synthesized and commercial ZnONPs was investigated against *P. aeruginosa* and *E. coli* (Gram-negative) in addition to *E. faecalis* and *S. aureus* (Gram-positive) bacterial strains. Obtained MIC values for each specific strain enlisted in Table 2 and the effect of ZnONPs on bacterial growth is depicted in Figure 4. Each bacterial strain was cultured in sterile Mueller Hinton agar plate containing the obtained MIC value. MBC was considered to be the dose of ZnONPs at which there is complete killing of bacteria without any further growth. Table 2 shows the MBC values obtained for all the bacterial strains used. It can be easily concluded that, the MBC for green ZnONPs is lower than that of the commercial one for each bacterial strains used.

Fig. 4: Concentration dependent bacterial growth inhibition exhibited by green synthesized ZnONPs (a) and commercial ZnONPs (b). The data are the average of three experiments $\pm$ SD.

Table 2: MIC and MBC values of *E. faecalis*, *S.aureus*, *P.aeruginosa*, *E.coli* (The data are the average of three experiments $\pm$ SD)

Bacterial strain	Antibacterial effectiveness of Green synthesized Zinc Oxide Nanoparticles from coriander leaves	
	MIC ($\mu\text{g/ml}$)	MBC ($\mu\text{g/ml}$)
<i>Enterococcus faecalis</i>	28.0 $\pm$ 2.1	104.3 $\pm$ 2.4
<i>Staphylococcus aureus</i>	26.8 $\pm$ 1.3	102.8 $\pm$ 1.8
<i>Pseudomonas aeruginosa</i>	42.3 $\pm$ 2.2	156.7 $\pm$ 1.4
<i>Escherichia coli</i>	38.0 $\pm$ 1.0	114.2 $\pm$ 1.6
Bacterial strain	Antibacterial effectiveness of Commercial Zinc Oxide Nano-particles	
	MIC ($\mu\text{g/ml}$)	MBC ($\mu\text{g/ml}$)
<i>Enterococcus faecalis</i>	34.6 $\pm$ 2.3	110.4 $\pm$ 1.9
<i>Staphylococcus aureus</i>	30.3 $\pm$ 2.8	108.8 $\pm$ 2.1
<i>Pseudomonas aeruginosa</i>	49.8 $\pm$ 1.8	161.3 $\pm$ 2.2
<i>Escherichia coli</i>	46.3 $\pm$ 1.5	121.6 $\pm$ 1.1

Comparison between the effective bactericidal activity of green and commercial ZnONPs

Tolerance level is a tool for the discrimination between bactericidal and bacteriostatic agents. The MBC and MIC value of an antimicrobial agent, for any bacterial strain, helps in the identification of this level. Reversible bacterial growth inhibition occurs by bacteriostatic agents, whereas, non-reversible, total destruction of bacterial growth occurs by bactericidal ones. Agents are said to be bacteriostatic when the MBC/MIC ratio is more than or equal to 16 and bactericidal when the ratio is less than or equal to 4. In this study, the MBC/MIC ratios for *E. faecalis*, *S. aureus*, *P. aeruginosa* and *E. coli* were 4.0, 3.8, 3.5, 3.3 and 2.9, 2.1, 2.5, 2.2 after treating the strains with commercial and green ZnONPs separately. Here, green synthesized ZnONPs showed excellent bactericidal potential in comparison to the commercial one.

Confirmation of bactericidal activity

Treatment of each bacterial strain with their respective MBC values of both green synthesized and commercial ZnONPs gave rise to clear zones of

inhibition after performing Agar well diffusion study (Figure 5). After comparing the obtained zones of inhibition with that of the positive control (antibiotic) (Table 3), it was found that, bactericidal effect exhibited by ampicillin, commercial and green synthesized ZnONPs was almost same for their respective MBC values against the test organisms.

Fig. 5: Zone of inhibitions obtained from Agar well diffusion assay against a) *Staphylococcus aureus*, (b) *Enterococcus faecalis*, (c) *Escherichia coli* (d) *Pseudomonas aeruginosa*.

Table 3: Diameters of the Zones of inhibition for *E. faecalis*, *S. aureus*, *P. aeruginosa*, *E. coli* using MBC values (The data are a mean of three experiments)

Strains	Diameter of Zone of inhibition (mm)			
	Antibiotic (Ampicillin)	CZnONPs	GZnONPs	Negative Control (H ₂ O)
<i>Staphylococcus aureus</i>	28	27	28	0
<i>Enterococcus faecalis</i>	26	25	27	0
<i>Escherichia coli</i>	27	25	29	0
<i>Pseudomonas aeruginosa</i>	25	28	25	0

Bacterial growth inhibition dynamics

Results of bacterial growth curves, for each strain of bacteria used here (*E. faecalis*, *S. aureus*, *P. aeruginosa* and *E. coli*) is presented in Figure 6. Growth curves, indicate the relation between growth inhibition and time. The interpretation of the plotted graph reveals the higher inhibition potential of green

ZnONPs compared to the commercial one. Besides, when each type of bacteria was cultured in presence of their respective MIC doses, the Gram-positive strains of bacteria showed greater inhibition than the Gram-negative strains, in presence of ZnONPs. This observation was similar in both types of ZnONPs.

Fig. 6: Bacterial growth curve in presence of (a) green and (b) commercial ZnONPs. The data are the average of three experiments $\pm$ SD.

ZnONPs significantly reduce the viability of bacterial cells

Figure 7 illustrates the results of the MTT assay performed to determine bacterial cell viability, in response to both green and commercial ZnONPs. It

was clearly observed that, the viability of bacterial cells notably diminished at their corresponding MIC doses, for both green synthesized and commercial ZnONPs.

Fig. 7: Bacterial cell viability response to commercial (a) and green (b) ZnONPs. The data are the average of three experiments $\pm$ SD.

Probable mechanism of action, justifying the bactericidal activity of ZnONPs

Figure 8 represents the results of the estimation of ROS generation inside the treated bacterial cells in presence of nanoparticles, which was measured by administering an indicator of intracellular ROS formation, DCF-DA. Both the green and commercial

ZnONPs proved to be responsible for augmentation of ROS generation inside the treated bacterial cells, leading to cell death. Although in comparison to commercial ZnONPs, green synthesized ZnONPs are evident to aggravate much more intracellular ROS formation.

Fig. 8: Effect of commercial (a) and green synthesized (b) ZnONPs in bacterial ROS generation. The data are the average of three experiments $\pm$ SD.

Effect of nanoparticles on bacterial morphology

Membrane destruction of bacterial cells occurred on treatment by both green and commercial ZnONPs, as revealed through SEM images (Figure 9). Possible penetration of ZnONPs was the underlying cause for membrane leakage, resulting in expulsion of cytoplasmic material. This was followed by clumping

of bacterial cells post treatment by ZnONPs. A slightly higher antibacterial effect was executed by green ZnONPs in comparison to the commercial one, as observed in the SEM images. As a whole, treating bacterial cells by both green and commercial ZnONPs leads to loss of its morphological integrity, followed by death of the microorganism.

Fig. 9: SEM images of both control (untreated) and ZnONPs (C=commercial;G=green synthesized) treated (a) *S. aureus* and (b) *P. aeruginosa*.

Cytotoxic study

Anticancer efficacy of ZnONPs

Hep-G2 cell line was taken to evaluate the *in-vitro* cytotoxicity of both the green synthesized and

commercial ZnONPs. Treatment of the cells were done by administration of graded concentrations (0-120 µg/ml) of both ZnONPs (green synthesized and commercial), followed by 24 hours incubation at 37°

C and then subjected to MTT assay. Observed result (Figure 10) clearly defines a reciprocal relationship between concentration of ZnONPs (both green synthesized and commercial) and eukaryotic cell viability i.e., cell survivability decreases with enhanced ZnONPs concentration. The interpreted

LD₅₀ values for green synthesized ZnONPs being $42 \pm 1.2 \mu\text{g/ml}$, is comparatively less than that of the commercial one, i.e., $47 \pm 1.8 \mu\text{g/ml}$, and can therefore be considered as a potent anticancer therapeutic over the commercial ZnONPs.

Fig. 10: Cancer cell viability in response to ZnONPs. The data are the average of three experiments $\pm$ SD.

ZnONPs induced ROS generation in eukaryotic cell

2', 7' dichlorofluorescence in di-acetate (DCF-DA) was recruited as a probe to measure generated intracellular ROS inside the HepG2 (hepatocellular carcinoma cells), in response to both green synthesized and commercial ZnONPs treatment. 80 μM and 100 μM concentrations of both the ZnONPs was utilized to treat the Hep-G2 cells for 12 hours at

37°C and then analysed by two-way analyses- Spectrofluorometer and fluorescence microscopy. Observed green fluorescence in images of fluorescence microscope indicates the generated ROS inside the cells were greater in case of green ZnONPs treated cells than commercial ZnONPs treated cells (Figure11).

Fig. 11: Evaluation of ROS generation by (a) Spectrofluorometric method [The data are the average of three experiments $\pm$ SD.] and (b) Fluorescence microscopy.

DISCUSSION

This detailed comparative study, to evaluate the therapeutic efficacies of green and commercial ZnONPs were performed for the very first time, as per our knowledge. Green ZnONPs, were synthesized using *Coriander sativum* (Dhania). The diffraction peaks, as observed by X-ray diffraction studies confirm the characteristic crystalline structure and size of both the commercial and green synthesized nanoparticles. Inexistence of any additional peak confirms the maintenance of uniform conditions and the formation of pure sample by the green synthesis procedure. Interpretation of the results of XRD studies, revealed that the hexagonal Wurtzite ZnONPs structure approved by Joint Committee on Powder Diffraction Studies Standards (JCPDS card number 008, 82-1042 and 5-0664) resembles the observed plane values obtained in case of synthesized ZnONPs (12). The morphology of green synthesized ZnONPs as revealed by FE-SEM in this study, are in accordance with that of other reported green nanoparticles (15). This satisfactory result of FE-SEM study further confirms that, there was no morphological variation between green synthesized and commercial ZnONPs. Results of Dynamic Light Scattering (DLS) analysis further confirmed the average size and Zeta potential of both commercial and green synthesized ZnONPs. The observed average diameter and Polydispersive index (P.D.I), as indicated by the results of DLS studies of both commercial and green synthesized ZnONPs are in accordance with literature (14). The greater zeta potential of green synthesized ZnONPs compared to that of the commercial one, suggests that the green synthesized nanoparticles are more stable in aqueous medium, which in turn is beneficial for its biological applications. Essential stabilization and capping of green synthesized ZnONPs were possibly due to the presence of numerous phytochemicals in the leaf extract, as revealed by the results of FTIR analysis, which are in accordance with literature (11,14). This study, therefore, indicates that eco-friendly ZnONPs can be synthesized by a much easier procedure in comparison to the conventional, hazardous and costly chemical synthesis methods.

The antibacterial activity of both commercial and green synthesized nanoparticles against all studied bacterial strains was demonstrated by the results of MIC and MBC values, agar well diffusion tests and bacterial growth curves. Interpretation of the results of Tolerance levels (MIC/MBC ratios less than 4) reveals that the green synthesized nanoparticles exert greater bactericidal effect than the commercial ones. The bactericidal activity of the nanoparticles was further confirmed by the results of MTT assay, estimation of ROS generation by DCF-DA and SEM studies in bacterial strains. The results of MTT assay and estimation of ROS activity in Hep-G2 cell line in

presence of both commercial and green synthesized ZnONPs reveal their cytotoxic activity in eukaryotic cells.

Results of this study indicate that the average size and crystalline structure of both ZnONPs (green synthesized and commercial) are almost similar. It is therefore evident that, the differences in the antibacterial and cytotoxic efficacies of both types of ZnONPs observed in this study, were not due to a consequence of their difference in size or crystalline shape. Analysis of results obtained from treating bacterial cells with DCF-DA, indicates that the penetration of ZnONPs inside the treated bacterial cells, followed by cessation of cell growth may account for the bactericidal activity of ZnONPs, which is in accordance to previous study by Pal et al (21). Literature survey suggests that greater antibacterial and cytotoxic effect is generally observed in case of green ZnONPs when compared to nanoparticles synthesized by other routes (12). Both the green synthesized and commercial ZnONPs potentially uplift intracellular ROS formation, which culminates in fatal cell damage (5). Generation of intracellular ROS and cell death shares a linear relationship, as increased generation of intracellular ROS leads to enhancement in cell death, as an obvious fate of damaged cell membrane, respiratory chain disruption and destruction of genetic material (23). Hence, results of our study on phyto-fabricated ZnONPs shows no deviation from existing research reports, in context of its antibacterial potential and intracellular ROS production.

CONCLUSION

From the results of this study, it can be concluded that, the green synthesized ZnONPs can be synthesized using *Coriander sativum* leaf extracts. Though these green synthesized ZnONPs are similar to commercial ZnONPs, with respect to their size, shape and crystalline nature, they exert a comparatively greater antibacterial and cytotoxic activity. The green synthesis procedure for preparing nanoparticles should therefore be an obvious choice of nanoparticle synthesis, regarding the ease of implementation, cost effectiveness and the stabilization of nanoparticles in aqueous solution, which normally becomes an issue of concern in its biological applications. This study draws our attention towards the beneficial effect of using green synthesized ZnONPs as an alternative to conventional antibiotic therapeutics. In addition, this study also highlights, on the basis of comparative assessment, that green synthesized ZnONPs, may be considered as a suitable alternative to commercial ZnONPs against pathogenic bacterial strains. This also holds correct with regard to its cytotoxic effects against hepatocellular cancer cells. According to the conclusions drawn from this comparative study, green synthesized ZnONPs, in recent future may be

effectively utilized as a therapeutic tool to combat bacterial infections, if and only convincing positive outcomes are available from *in-vivo* studies.

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CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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