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# *IN-VITRO* EVALUATION OF ANTIBACTERIAL ACTIVITY AND CYTOTOXICITY EFFECT OF CHEMICALLY SYNTHESIZED ZnO NANOPARTICLE

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## ABSTRACT

Nanoparticles, generally considered as particle with a size of upto 100nm, exhibit completely new or improved properties as compared to the larger particles of the bulk material that they are composed of based on specific characteristics such as size, distribution and morphology. In this study ZnO nanoparticle was synthesized by a simple and easy chemical method from $ZnCl_2$. The synthesized nanoparticle was characterized by SEM and EDAX. Antibacterial activity of ZnO was observed against *E. coli, Enterobacter sp., Bacillus subtilis, Bacillus cereus, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi, Vibrio cholerae* by disc diffusion method. Preliminary Cytotoxicity of nanoparticle was studied by MTT assay against vero cell line. ZnO nanoparticle had shown antibacterial activity against all the tested bacteria and maximum activity was observed against *salmonella typhi*. ZnO nanoparticles has shown less cytotoxicity effect on vero cell than $ZnCl_2$.

**INTRODUCTION:** Nanotechnology deals with the nanomaterial and nanoparticle which are in the size of about 100nm. Nanoparticle has wide application in various fields like chemical industry electronics, biomedicine, cosmetics, etc. [1, 2], due to its novel properties. Novel property is due to the alteration in their physical, chemical, electrical and magnetic property [3]. When reduced to nanosize, unique size-dependent properties of nanoparticles are manifested [4]. The reason believed to cause this property change from their bulk is increase in relative surface area, a greater percentage of atoms at the material's surface, quantum effects which can affect chemical reactivity, and other physical and chemical property [4, 5]. Many nanoparticles have been synthesized and used in various field due to this altered properties. The metal nanoparticles are most widely used in the field of medicine.

ZnO is a conventional wide band-gap semiconductor [6, 7] that has been highly explored in multiple areas of science. ZnO nanomaterials have been used as semiconductor in microelectronic device and for accelerating degradation of water pollutants via photocalalytic activity.

ZnO are used in cosmetic industry, due to its UV irradiation absorbing ability and optical transparency [8, 9] and also used in photodetectors [10], surface acoustic wave devices [11], ultraviolet nanolaser [12], varistors [13], solar cells [14], gas sensors [15], biosensors [16], ceramics [17], field emission [18], and nanogenerator [19].

ZnO nanoparticles were synthesized by various methods like spray pyrolysis, thermal decomposition, molecular beam epitaxy, chemical vapor deposition, laser ablation etc. Chemical synthesis is one of the important techniques. It can be performed using a range of precursors and synthesis conditions like

temperature, time, concentration of reactants etc. Variation of these parameters leads to different size selection and geometries of resulting particles [20]. ZnO nanoparticle synthesized using capping agents like thioglycerol have been reported by Shriwas [20].

In this study, a simple and easy method to synthesis ZnO nanoparticle from $ZnCl_2$ without any capping agent was employed and the synthesized nanoparticle was focused for its application in field of biomedicine by evaluating its antibacterial and cytotoxic effect.

**MATERIALS AND METHODS**

**Materials:** Chemicals used for the study were bought from Himedia. Microorganisms (*E. coli, Enterobacter sp., Bacillus subtilis, Bacillus cereus, Klebsiella pneumonia, Staphylococcus aureus, Salmonella typhi,* and *Vibrio cholerae*) used for antibacterial activity study were purchased from MTCC, India. Vero cell line was bought from NCCS, Pune. Research work was carried out during the year 2011 at Sathyabama University, Chennai, India-119

**Methods:**

1. **Synthesis of ZnO nanoparticle:** To prepare ZnO nanoparticle, 0.5M aqueous solution of $ZnCl_2$ and 1M of NaOH were prepared in MilliQ water. The method for the preparation of ZnO nanoparticle is a slight modified method of Abdolmajid *et al* [21]. 1M NaOH solution was taken in a beaker and heated to 50$^{o}$C. After 5 mins of heating the NaOH solution, $ZnCl_2$ was added dropwise to the above heated solution under high magnetic stirring. After the complete addition of $ZnCl_2$, the beaker was sealed and kept at stirring condition for 10min at 50$^{o}$C. The white precipitate formation was observed at above said condition. The precipitation was collected and washed with milliQ water and with 100% ethanol then air dried at 50$^{o}$C.

2. **Characterization of ZnO nanoparticle:** The synthesized ZnO nanoparticle was characterized by SEM, EDAX analysis.

a. **SEM analysis of ZnO nanoparticles**: Scanning Electron Microscopic (SEM) analysis was done in Karunya University using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

b. **EDAX measurements**: In order to carry out EDAX analysis, the white precipitate ZnO nanoparticles were dried and drop coated on to carbon film and performed on Hitachi S-3400 NSEM instrument equipped with a Thermo EDAX attachments.

3. **Antibacterial analysis:** The antibacterial analysis was done for the ZnO against the following microorganisms: *E.coli, Enterobacter sp., Bacillus subtilis, Bacillus cereus, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi, Vibrio cholerae* by disc diffusion method.

4. **Cytotoxicity study:** Cell line maintenance and growth conditions. Vero cell line was purchased from NCCS (National Centre for Cell Sciences) Pune, India. The cell lines were maintained at 37$^{o}$C at 5% $CO_2$ in $CO_2$ incubator. Cultures were viewed using an inverted microscope to assess the degree of confluency and the absence of bacterial and fungal contaminants was confirmed.

a. **Cytotoxicity test**: MTT Cell Proliferation Assay was employed to estimate the cytotoxicity of synthesized nanoparticle. Cells were first transferred (200µL/well at 7.5×104mL) into 96 well plates and incubated for 24 hr. The original media was removed and 100 µL fresh media was added. Synthesized nanoparticle was added at concentration of 20-160 µg and then final volume was made to 200µl with the media and incubated for 4 hr.

After incubation media containing nanoparticle was removed. MTT reagent 20 µL was added to each well containing media and incubated for 3.5 hr at 37°C under an atmosphere of 5% $CO_2$ until a purple precipitate was visible. Media was removed carefully (Do not disturb cells and do not rinse with PBS). 150µl DMSO (MTT solvent) was added to dissolve the purple precipitate. Absorbance was read at 570 nm with a reference filter of 630 nm.

**RESULTS AND DISCUSSION:**

1. **Synthesis of ZnO nanoparticle**: The ZnO nanoparticle was synthesized by chemical method from $ZnCl_2$. The synthesized nanoparticle was characterized by SEM (**fig. 1**) and EDAX (**fig. 2**). The SEM analysis of the particle was found to be in nanometer size and the EDAX result confirmed that the particles were ZnO.

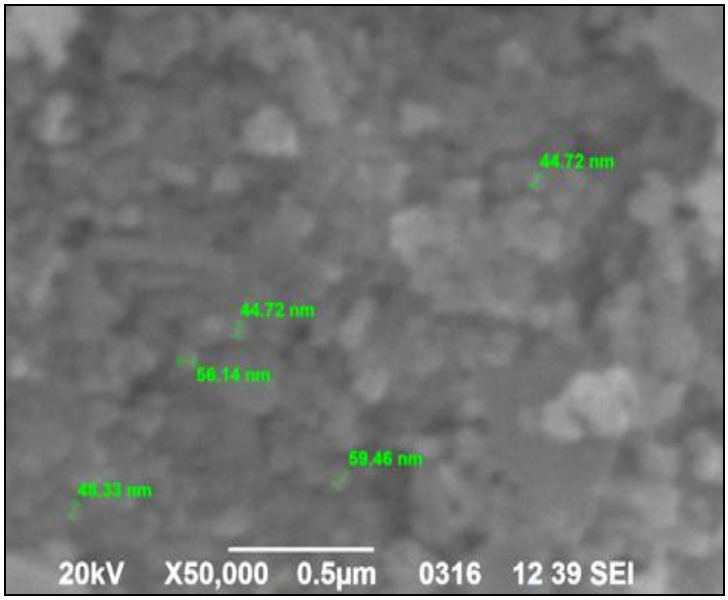


**FIG. 1: SEM ANALYSIS OF CHEMICALLY SYNTHESIZED ZnO NANOPARTICLE**

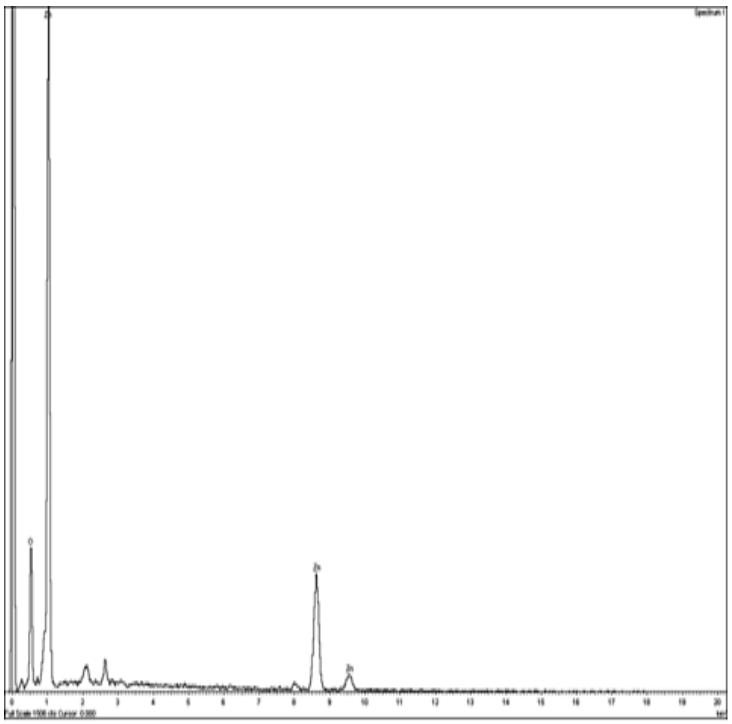

**FIG. 2: EDAX ANALYSIS OF THE CHEMICALLY SYNTHESIZED ZnO NANOPARTICLE**

2. **Antibacterial activity of ZnO nanoparticle:** The antibacterial activity of nanoparticle was assessed for the following microorganism *E.coli, Enterobacter sp., Bacillus subtilis, Bacillus cereus, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi, Vibrio cholereae* by disc diffusion method. Zone of inhibition was recorded and results were tabulated in **Table 1**. The nanoparticle and $ZnCl_2$ has shown antibacterial activity. The maximum antibacterial activity was found against *salmonella typhi* with the zone of inhibition of 25mm and second maximum of 24 mm against *Bacillus subtilis* for ZnO nanoparticle.

**TABLE 1: ANTIBACTERIAL ACTIVITY OF ZNO NANOPARTICLE**

| Microorganism | Zone of inhibition in mm | |
|---|---|---|
| | ZnO nanoparticle (10µg) | $ZnCl_2$ (10µg) |
| *E. coli* | 16 | 14 |
| *Enterobacter sp.* | 14 | 12 |
| *Bacillus subtilis* | 24 | 24 |
| *Klebsiella pneumoniae* | 15 | 14 |
| *Staphylococcus aureus* | 20 | 15 |
| *Salmonella typhi* | 25 | 16 |
| *Vibrio cholrea* | 22 | 20 |
| *Bacillus cereus* | 13 | 12 |

3. **Cytotoxicity (MTT) assay:** The Cytotoxicity of the ZnO nanoparticle and $ZnCl_2$ was studied In-vitro by MTT assay against Vero cell line at different concentration (20, 40, 60, 80, 100, 120, 140, 160µg). The percentage cytotoxicity was calculated and used for finding the $IC_{50}$. $IC_{50}$ (concentration required for 50% cell death) value for the nanoparticle and $ZnCl_2$ was found from the graph (**fig. 3**). The $IC_{50}$ value was found to be 159µg for ZnO nanoparticle.

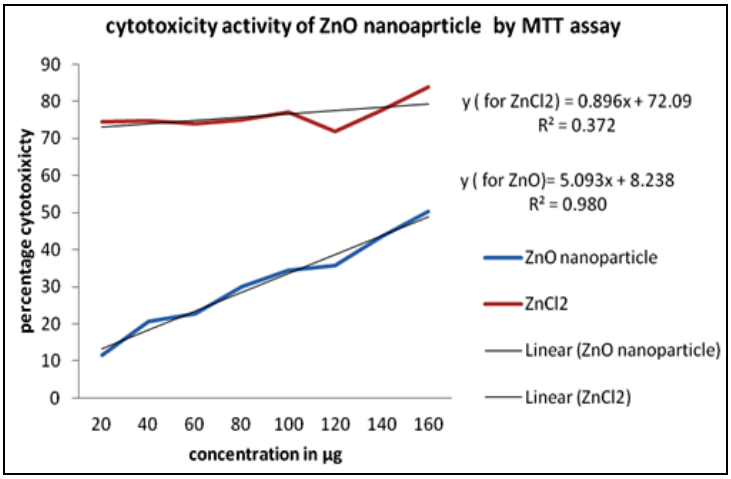


**FIG 3: CYTOTOXICITY ACTIVITY OF ZnO NANOPARTICLE BY MTT ASSAY**

**DISCUSSION:** In this study, a simple and rapid method was employed to synthesis ZnO nanoparticle. The NaOH was subjected to 50$^{o}$C and the $ZnCl_2$ solution was added drop by drop under stirrer condition at 50$^{o}$C. While adding the $ZnCl_2$ solution to the heated NaOH white precipitate formation was noted. After complete addition of ZnCl2 solution, set up was kept under same condition for another 15min. White precipitate formation indicates the formation of ZnO nanoparticle. This indicates the Zn ions oxidized to ZnO. The entire process took around 20min. The white precipitate was washed, dried and stored for further analysis.

The ZnO nanoparticle was characterized by SEM and EDAX (Fig. 1 and Fig. 2). The SEM results of the ZnO nanoparticle revealed the size of the particles were in nanosize and its ranges from 44nm-60nm. The elements of the nanoparticle were found to be Zn and O by EDAX analysis. From the EDAX, the weight % was found to be 28.19 and 71.81 for O and Zn respectively.

The antibacterial activity of ZnO nanoparticle was assessed against *E.coli, Enterobacter sp., Bacillus subtilis, Bacillus cereus, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi, Vibrio cholerae* for the ZnO nanoparticle and $ZnCl_2$ solution. When compared to $ZnCl_2$, ZnO nanoparticle has shown maximum activity against all the tested bacteria and the maximum activity was found to be against *salmonella typhi* (Table 1).

The cytotoxicity effect of synthesized ZnO nanoparticle and $ZnCl_2$ was studied on vero cell at different concentration (20-160µg) by MTT assay. After 4 hours of treatment, the ZnO nanoparticles were found to less toxic than $ZnCl_2$ to normal cell. Both the ZnO nanoparticle and ZnO has shown different pattern of dose-dependent cytotoxicity response (Fig. 3).

The ZnO has shown less toxicity of 11.68% at 20µg but $ZnCl_2$ at same concentration has shown toxicity of 74.53%. IC 50 value for the ZnO was found to be 159µg. The $ZnCl_2$ has shown more toxic at the tested minimum quantity than ZnO. So, ZnO can be used for in vivo studies like drug carrier and also in wound healing studies.

**CONCLUSION:** In this study, a simple and rapid method was employed for the synthesizing of ZnO nanoparticle from 0.5M ZnCl2. Nanoparticle were subjected to SEM and EDAX analysis to confirm the nanosize and element composition. The SEM and EDAX analysis revealed the particles are Zn and size of particles were in the range of 40-60nm. Synthesized nanoparticles were subjected to antibacterial assay by disc diffusion method against the following bacteria *E. coli, Enterobacter sp., Bacillus subtilis, Bacillus cereus, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi* and *Vibrio cholereae*.

Antibacterial activity was observed against the tested microorganism and maximum activity was observed against *salmonella typhi.* The cytotoxicity study by MTT assay revealed that the particle was very less toxic to normal cell than the $ZnCl_2$. This preliminary cytotoxicity study of ZnO nanoparticle might contribute to the comprehensive of this compound *in vivo* study like in drug delivery system.

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