VOL. 10, NO. 12, JULY 2015 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences

www.arpnjournals.com

ANTI CANCER ACTIVITY OF ZnO NANOPARTICLES on MCF7 (BREAST CANCER CELL) AND A549 (LUNG CANCER CELL)

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ABSTRACT

ZnO nanoparticles have been proved to be promising in cancer treatment, including the tumor cells destruction with minimal damage to the healthy cells. In the present study highly pure ZnO nano particles with a narrow size distribution of 16-19 nm were prepared by the simple DMC (Dry Mechano-Chemical) method in the lab. The anticancer activity on MCF7 (Breast cancer cell) and A549 (Lung Cancer cell) were determined by the MTT (Methylthiazolydiphenyl-tetrazolium bromide) assay. A549 and MCF-7 cells were exposed to ZnO-NPs and it exhibited 50% reduction at a very low concentration 31.2 µg/ml. Thus, the reduction in cell viability with NPs induces cytotoxicity in cancerous cells. There is a size dependent effectiveness of ZnO nanoparticles in the removal of cancer cells and also a positive correlation with reduced toxicity.

Keywords: ZnO nanoparticles, anticancer activity, toxicity.

1. INTRODUCTION

One of the challenges in achieving a standard antitumor therapy is the technique to synthesize ZnO Nano size materials. The latest world cancer statistics (Lyon/Geneva, 12 December, 2013) done by the International Agency for research on cancer, WHO, show that since the 2008 estimates, breast cancer incidence has increased by more than 20%, while mortality has increased by 14%. Breast cancer is also the most common cause of cancer death among women (522 000 deaths in 2012) and the most frequently diagnosed cancer among women in 140 of 184 countries worldwide. It now represents one in four of all cancers in women. Statistics also reveals that the most common causes of cancer death were cancers of the lung (1.6 million, 19.4% of the total).An urgent need in cancer control today is to develop effective and affordable approaches to the early detection, diagnosis, and treatment of these deadly diseases [1]. Nano-medicine recently emerged as a better option for the treatment of some common cancers. As a result, many nanoparticles have been used to treat cancer cell lines. Of the various materials, zinc oxide exhibits biocompatibility [2]. Therefore, the aim of the present study was to investigate the action of zinc oxide nanoparticles (ZnO-NPs) against MCF7 (Human breast cancer cell) and A549 (Human lung cancer cell).

The ZnO nanoparticles (~16±3 nm) were prepared via a simple cost effective two step DMC method and were well-characterized through standard techniques. The study showed that treatment with NPs is notably effective against the MCF7 and A549 cells in a dose-dependent manner. The MTT (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide, a tetrazole) assays revealed the concentration-dependent cytotoxic effects of NPs in range of 2.5-1000µg/ml. MCF-7 and A549 were exposed to ZnO-NPs and exhibited a significant reduction in their cell viability(50%) in response to a very low concentration (31µg/ml) of the ZnO-NPs.

2. MATERIALS AND METHODS

The commercial ZnO powder [MERCK] was snow white in appearance, >99% in purity and XRD characterized to be in nanopowder form with an average size of 50-57 nm (Figure-2(B)).

a) Preparation and characterization of zinc oxide nanoparticles

i. Dry Mechano chemical method

In this present study, synthesis of nanoparticles is done by the Top-down approach. Solid-state mechano-chemical processing is not only a physical size reduction process with conventional milling but also a chemical reaction that is mechanically activated at the nano-scale. In the dry grinding method the solid substance is ground as results of shock, compression, or by friction, using such popular methods as jet mill, hammer mill, shearing mill, roller mill, shock shearing mill, ball mill, or tumbling mill. In general, mechano-chemical processing has been recognized as a powerful technique for the synthesis of a wide range of semiconducting nano-materials, magnetic materials, carbon nanotubes, etc., which could otherwise be difficult to prepare using conventional methods [3, 4].

ii. Synthesis of ZnO Nanoparticles

The ZnO nanoparticles were prepared by simple, cost effective dry mechano-chemical method. Zinc acetate dihydrate (AR) and Oxalic acid (GR) were mixed by grinding in mortar and pestle for 1 hour at room temperature. The molar ratio of the reactant mixture is taken as 1:1.5. The chemical reaction activated by the grinding yields zinc oxalate as an intermediate product. The intermediate product was then annealed at 450^0C in a muffle furnace in order to remove CO, $CO_2$and any moisture from the compound. An hour thermal decomposition of zinc oxalate at 450^0C results in zinc

oxide nanoparticles. All the chemicals (Merck) were used as bought, without further purification.

iii. Characterizations of ZnO Nanoparticles

The synthesized ZnO nanoparticles were characterized by XRD and SEM.Figure-1(A) shows the XRD of the synthesized ZnO nanoparticles. All peak positions and relative peak intensities of the ZnO product agree well with those of the standard XRD pattern (JCPDS data (file 36-1451)and no characteristic peaks of impurities, such as Zinc Oxalate (Zn (COO) $_2$) were observed.The purity of synthesized ZnO nanoparticles was indicated. The phase structure of ZnO nanoparticles belongs to a wurtzite structure (hexagonal phase). The ZnO nanoparticles have an average grain size of about 16-17 nm as calculated by the Debye-Scherrer equation.

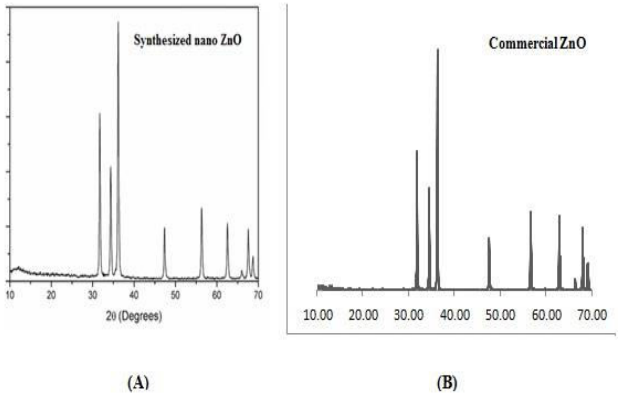

Figure-1. XRD images of (A) synthesized ZnO Nanoparticles (B) commercial ZnO powder.

Figure-2 (A) and (B), show the SEM image and EDX results of the ZnO nanoparticles. The shape and morphology of ZnO nanoparticles were studied from SEM. SEM analysis showed agglomerated nano-sphere morphology of ZnO nanoparticles of size 16-19 nm, well in agreement with the XRD analysis. The atomic weight percentage of the constituent elements was nearly 1:1 in the formed ZnOas given by the EDX result.

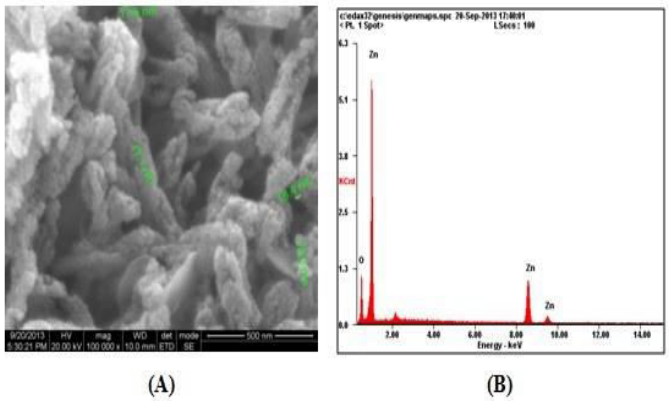

Figure-2. (A) SEM image and (B) EDX reports of synthesized ZnO nanoparticles.

Table-1. EDX analysis for synthesized ZnO nanoparticles.

Element	Wt.%	At%
OK	19.60	49.90
ZnK	80.40	50.10

b) Reagents

Minimal Essential Media, Fetal Bovine Serum (FBS), Trypsin, Methylthiazolyl Diphenyl- Tetrazolium Bromide (MTT) and Dimethyl (DMSO) were purchased from Hi media and Sigma Aldrich Mumbai and SISCO research laboratory chemicals Mumbai.

c) Cell culture

Two types of cancer cell (MCF7, A549) and VERO cell (normal cell-Monkey kidney) were used to determine the cell anticancer activity and cytotoxicity against ZnO nanoparticles and commercial ZnO. MCF7 (Human breast cancer cell), A549 (Human lung cancer cell) and VERO were obtained from National Centre for Cell Science, Pune, India (NCSS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100µg/ml) in a humidified atmosphere of 50µg/ml CO_2 at 37^0c.

Table-2. Details of cell lines.

Cell line	Origin	Species	Characteristics	Supplier
MCF7	Breast	Human	Anticancer Activity	NCCS, Pune
A547	Lung	Human	Anticancer Activity	NCCS, Pune
Vero	Kidney	Monkey	Toxicity	NCCS, Pune

d) In vitro anticancer activity of ZnO Nanoparticles

MCF7 cells were seeded at 1000000 cells/ well in 24-well plates, and A549, Vero cells were seeded at 100000 cells/ well in 96-well plates and both were incubated for 72h. Then 0.1% of DMSO (Dimethyl sulfoxide) was added tosynthesized and commercial ZnO nanoparticles at the various concentrations like 1000µg/ml, 500µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml, 31.2µg/ml, 15.6µg/ml,7.8µg/ml for 48h incubation. After the medium are removed it was washed with the phosphate saline solution. Then the sample was placed in a new medium containing MTTsolution (5mg/ml), incubate for 4h [5]. After the incubation 1ml of DMSO was added. The viable cells were determined by the absorbance at 540nm by micro-plate reader.

e) In Vitro Cytotoxicity On ZnO Nanoparticles

Vero cells were plated into a 96-well plate at density of 1×10^5 cells/ well for 72h incubation. 0.1% of DMSO was added for various concentrations of ZnO nanoparticles. Then the medium was removed and washed with the phosphate saline solution. The cells were incubated with MTT (5mg/ml) for 4h. The medium was removed and 1ml of DMSO was added into each well plates. The viable cells were determined by the absorbance at 540nm by micro-plate reader.

3. RESULT AND DISCUSSIONS

a) Anticancer activity of ZnO Nanoparticles

Result showed that the exposure of MCF7 and A549 cells to ZnO nanoparticles at the various concentrations for 72h significantly reduced the cell viability in a concentration dependent manner. However the cell viability at the higher concentration of 1000 µg/ml was not significant. As the concentration increases the cell viability% significantly decreases. For ZnO nanoparticles from7.8µg/ml to 31.2 µg/ml concentration the cell viability% significantly decreased from 78.7 to 54.2 (A549) and 82.8 to 50.8(MCF7) and for commercial ZnO the cell viability decreased from 91.4 to 75.5(A549).

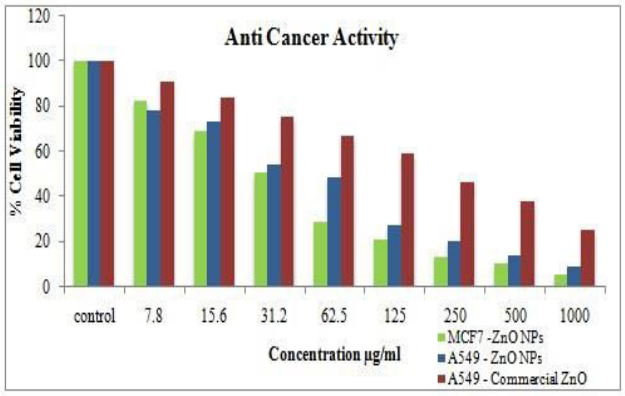

Figure-3. Cancer cell viability percentage on MCF7 cell line and A549 cell line at various concentrations of synthesized ZnO nanoparticles and Commercial ZnO.

b) In vitro cytotoxicity of ZnO Nanoparticles

Analysis showed that the exposure of VERO cells to ZnO nanoparticles at the various concentrations for 72h reduced the cell viability in a concentration dependent manner. However the cell viability at the higher concentration of 1000 µg/ml was not significant. As the concentration increases the cell viability decreases significantly in the case of commercial ZnO than with ZnO NPs. For ZnO nanoparticles at concentrationfrom62.5µg /ml the cell viability decreasedto68.6% whereas for commercial ZnO the cell viability significantly decreased to 55.6%.

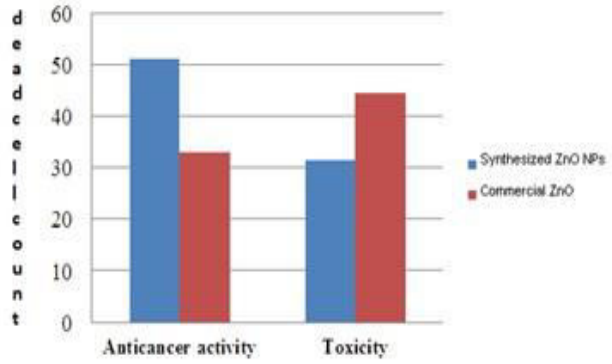

Figure-4. Cell viability percentage on normal Monkey kidney cells at various concentrations of synthesized ZnO anoparticles and Commercial ZnO.

c) Comparison between activity of synthesized and commercial ZnO Nanoparticles

Synthesized ZnO nanoparticles kills 51% of cancer cells while the commercial ZnO kills 33% of cancer cells (A549). Also, only 31.4% of normal healthy cells were removed by the smaller nano ZnO in comparison to 44.4%of cells by the larger size dcommercial ZnO at 62.5µg/ml concentration.

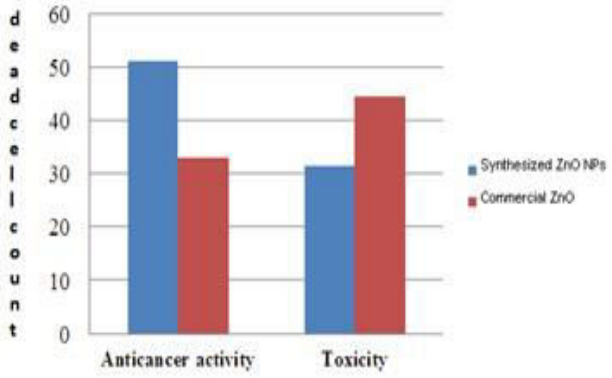

Figure-5. Anticancer activity(A549) and Toxicity- dead cell count: comparison of ZnO NPs and Commercial ZnO at 62.5 µg/ml concentration.

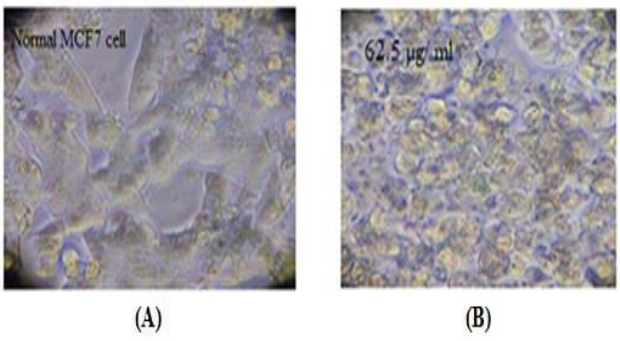

Figure-6. (A)MCF7- Human Breast Cancer Cell (B) MCF7after exposed to 62.5µg/ml synthesized ZnO nanoparticles.

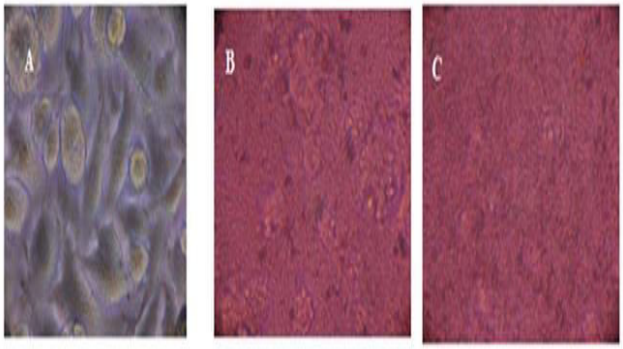

Figure-7. (A) A549-Human Lung cancer cell (B) A549 after exposed to 62.5µg/ml synthesized ZnO nanoparticles(C) A549after exposed to 62.5µg/ml Commercial ZnO.

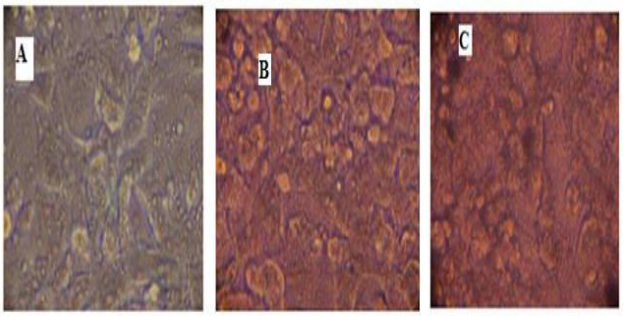

Figure-8. (A) VERO - Normal kidney cell (monkey) (B) VERO after exposed to 62.5µg/ml synthesized ZnO nanoparticles (C) VERO after exposed to 62.5µg/ml Commercial ZnO.

CONCLUSIONS

ZnO nanoparticles with an average size between 16 to 19 nm and sphere shapes were successfully synthesized using dry grinding method. The present study explores the potential anticancer activity of DMC synthesized ZnO nanoparticles on A549, MCF-7 cancer cell lines. The synthesis method reported here is easily scalable for large scale production, economically feasible, biocompatible and cost effective. The results suggest that with the aid of oxide based nanoparticles conditional chemotherapeutic agents may have even broader range of applications in the treatment of cancer cells. The dosage particles' size dependent activity against cancer cells and the variation in toxicity need to be further investigated to establish optimum standards.

ACKNOWLEDGEMENTS

The authors would like to thank the UGC, Government of India for the financial support in the form of minor project to one of the authors, D. Selvakumari and DST, FIST for equipment facilities provided.

The authors also like to acknowledge Mr. Nagarajan, Nuclear Physics Department, University of Madras, SAIF, IITM, India and Royal Bio Clinic, Velachery, India for the XRD, SEM analysis and Cell viability studies of the samples, respectively.

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