



Cytotoxic activities of zinc oxide nanoparticles on non-invasive human breast and prostate cancer cell lines

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ABSTRACT

ZnO-NPs have displayed anticancer activity against a broad range of tumor cells. The present research work was planned to formulate highly pure and well-characterized ZnO-NPs, in addition, to investigate their cytotoxicity against two types of cancer cells. The cancer cells that had been investigated in the present study were breast (MCF7) and prostate (PC3) cancer cells. The aqueous sol-gel method is a powerful and cost-effective technique for the synthesis of high pure ZnO-NPs. While the anticancer activities of ZnO-NPs on both MCF7 and PC3 were determined by MTT assay. Characterizations of ZnO-NPs were fundamentally evaluated by their morphological and structural properties, in addition to their particle size distribution with the aid of SEM and EDX, respectively. ZnO NPs were successfully prepared using sol-gel method in a range of nano-scale. Scanning electron microscopy (SEM) has shown flower-like nanoparticles of zinc oxide, their nano-size has ranged from 4.497 nm. to 143.7 nm. Results of EDX characterization has exhibited a good purity of ZnO-NPs (zinc content of 50% and Oxygen content of 50 %). There was size-dependent effectiveness of ZnO-NPs as an anticancer agent on both MCF7 and PC3. In addition, there were positive correlation between anticancer activities and low toxicity with the size of ZnO-NPs. At 12.2 $\mu\text{g/ml}$, ZnO-NPs has exhibited around 51% reduction in the viability of cancerous cells. In conclusion, the results of this study have shown that there was an effective and notable dose-dependent manner in the treatment of MCF7 and PC3 with ZnO-NPs, in the cell line approaches.



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INTRODUCTION

The latest world cancer statistics have shown that the incidence of breast cancer has raised by more than 20%, with a 14% increase in the mortality rates. Breast cancer is the most common type of cancer that has frequently been diagnosed among females, all over the world (Bray *et al.*, 2020). While in males, prostate cancer is the most common causes of cancer death among males. So that, there is an urgent need for developing effective and affordable therapy for both. Nanoparticles have recently received great attention for their implications in the treatment of different diseases. One of the most common areas

of their application is in the field of antitumor therapy.

Zinc oxide (ZnO) nanoparticles (NPs) are the most recently used as anticancer. The pharmaceutical nanotechnology is the most emerging branch in pharmaceutical sciences. It is now well-established for drug delivery. This is especially so in recent years due to it has enormous potential applications in disease diagnosis and therapy. The nano-scale substances have had some unique features in their properties. They can provide an improved localization of drug and enhance the efficacy of drug (Bray *et al.*, 2020; Hasnidawani *et al.*, 2016). They are currently contributing substantially to the development of NPs against tumor cells. Although the mechanisms underlying the anticancer effect of ZnO-NPs remain unclear, they are going to be a promising substance in the treatment of tumor cells, because they induce selective cytotoxicity. The researchers claimed that the impact of ZnO-NPs against both types of cancer cell lines namely: MCF7 (Human breast cancer cell), and PC3 (Human prostate cancer cell) was size-dependent effectiveness. To the best of our knowledge, only few studies have hitherto been published on the use of ZnO-NPs on breast and prostate cancer cells (Hasnidawani *et al.*, 2016).

MATERIALS AND METHODS

Synthesizing of nanostructure of zinc oxide

Synthesizing ZnO-NPs using an aqueous sol-gel method in the present research work has included the use of several materials such as zinc acetate dihydrate ($\geq 99\%$ purity, Sigma Aldrich) as precursor, sodium hydroxide ($\geq 98\%$ purity, Sigma Aldrich), ethanol ($\geq 98\%$ purity, Sigma Aldrich) as a reagent. The distilled water was used as a solvent medium. This method is a well-established synthetic technique to deliver a high pure ZnO-NPs. The sol-gel technique fundamentally involves varying consecutive steps. Actually, there were series of chemical reactions that had been taken place, those are hydrolysis, condensation, and drying process. Initially, two grams of zinc acetate dihydrate were weighted and dissolved in 15 ml of distilled water. Afterwards, eight grams of sodium hydroxide (NaOH) were also weighted and dissolved in 10 ml of distilled water. Constant stirring was applied for about five minutes each. Then, the solution of NaOH was added to zinc acetate dihydrate with a constant stirring at room temperature for about five minutes. Afterward, 100 ml of ethanolic solution was added. The hydroxyl groups (-OH) of alcohol molecules bond with the zinc ions. The nanopowder was obtained after completing hydrolysis

of zinc acetate with the aid of NaOH in an ethanolic solution. Thereafter, the zinc acetate was heated forming acetate ions and zinc ions. Finally, a white precipitated powder (i.e., ZnO nano-powder) was yielded after drying the precipitate at 120 °C (Figure 1).

Characterizations of ZnO Nanoparticles

The morphology and structural properties of ZnO-NPs were investigated using scanning electron microscopy (SEM). While the particle size of ZnO-NPs were calculated from energy dispersive X-ray spectroscopy (EDX) data. The particle size of ZnO-NPs has profound effect on their release and effectiveness on the tumor cells. Characterizations of ZnO-NPs are fundamentally evaluated by their morphological and structural properties and particle size distribution with the aid of SEM and EDX, respectively. The characterizations of ZnO-NPs had been done after preparing the ZnO-NPs suspension containing the HU and control (Figure 1).

RESULTS

Scanning Electron Microscope Analysis (SEM)

The morphological shape of ZnO-NPs was determined and confirmed using the scanning electron microscope (SEM) (Quanta 250 FEG, Czech), which was operated at 20 kV. The shape and distribution of particles was firstly taken at low magnification, then at a higher magnification, focusing on typical particle. As showed in Figure 2 (A), Figure 2 (B) and Figure 2 (C) the SEM images of synthesized ZnO-NPs were taken at (X2000, X15000 and X60000) magnifications. The images showed ZnO-NPs that have a flower-like morphology with a different size ranging from 4.479 nm to 143.7 nm in length with a smooth surface. Comparable outcomes were additionally seen by other researchers. In fact, the smaller size of ZnO-NPs have larger surface area, which might fasten the releasing of drug. Comparable outcomes were additionally seen by other researchers (Haarindrapsad *et al.*, 2015; Perumal *et al.*, 2015).

Energy dispersive x-ray (EDX)

Figure 3 represents the EDX data for each of Zn and O₂ NPs. The zinc and oxygen content have had an average of about 80.34, and 19.68 wt.%, respectively. It is obvious that there was no characteristic peaks of impurities were observed, which indicates the purity of the synthesized ZnO-NPs were high. All peak positions and intensities of the ZnO-NPs product suggested that a high pure ZnO nanopowder was obtained.

As showed in Figure 2 (C) and Figure 2 (B), the SEM

Table 1: EDX analysis for synthesized ZnO -NPs

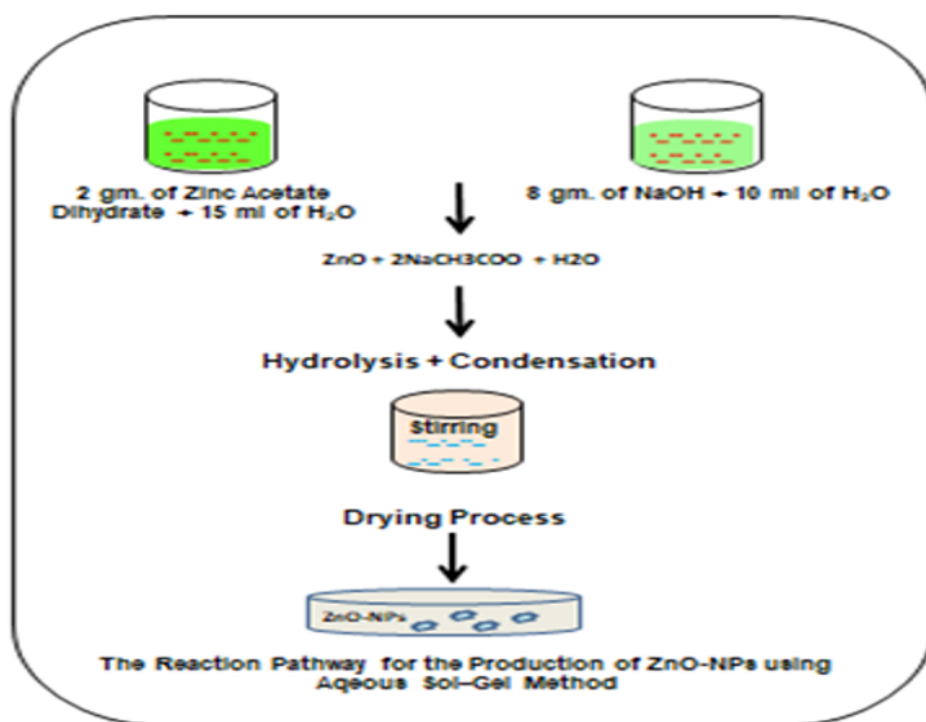
Element	Weight percentage (Wt. %)	Atomic percentage (At %)	Net Int.	Error %
ZnK	80.34	50	134.02	3.86
Oxygen	19.66	50	0	0

Table 2: Human MCF7 and PC3 cancer cells

Cells	Species	Activity of ZnO-Np
MCF7	Human breast cancer cells	Anticancer
PC3	Human prostate cancer cells	Anticancer

Table 3: Comparison between the Cytotoxic Effect of ZnO-NPs on MCF-7 Cells Viability (%) at different concentrations

ID	Conc. ug/ml	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50 ug
vero	dilution	0.289	0.282	0.299	0.290	0.005	100%	0%	
M	390.6	0.037	0.036	0.028	0.034	0.003	11.61	88.4	44.94
	195.3	0.051	0.049	0.057	0.052	0.002	18.05	82.0	
	97.65	0.094	0.083	0.097	0.091	0.004	31.49	68.5	
	48.82	0.142	0.139	0.142	0.141	0.001	48.62	51.4	
	24.41	0.194	0.183	0.194	0.190	0.004	65.63	34.4	
	12.2	0.283	0.294	0.287	0.288	0.003	99.31	0.69	

**Figure 1: Schematic diagram shows the Sol-gel technique**

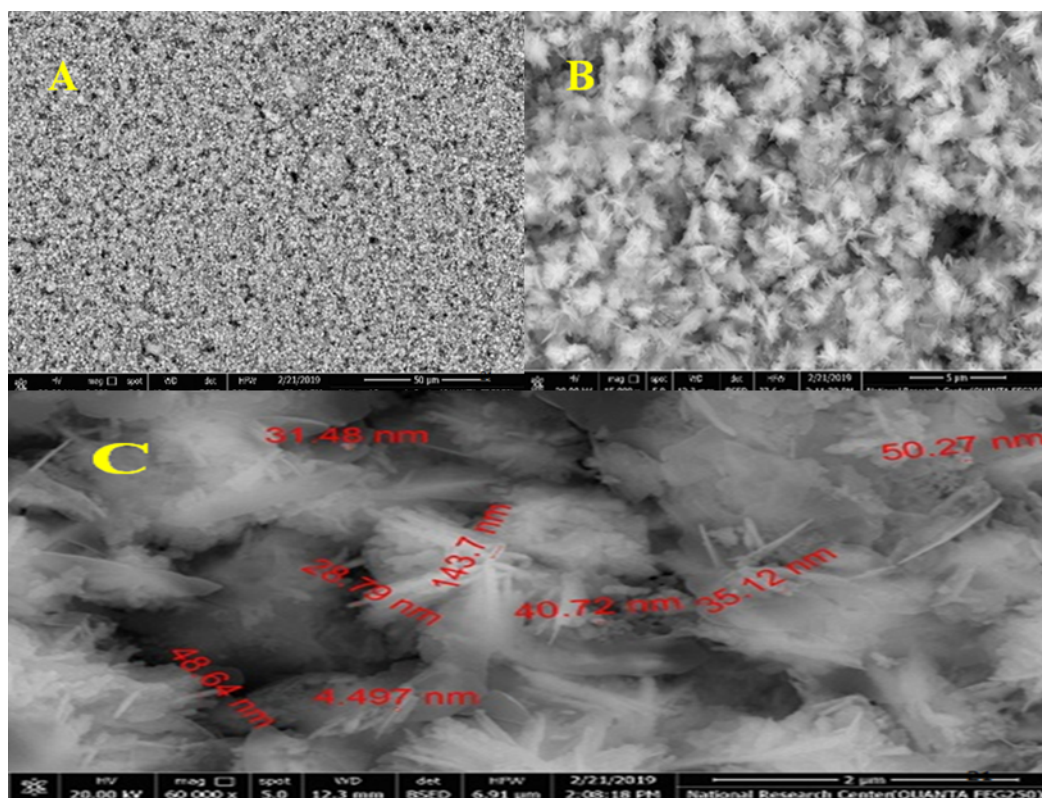


Figure 2: SEM images of synthesized ZnO-NPs at different magnifications: (A) X2000, (B) X15000, and (C) X60000, respectively.

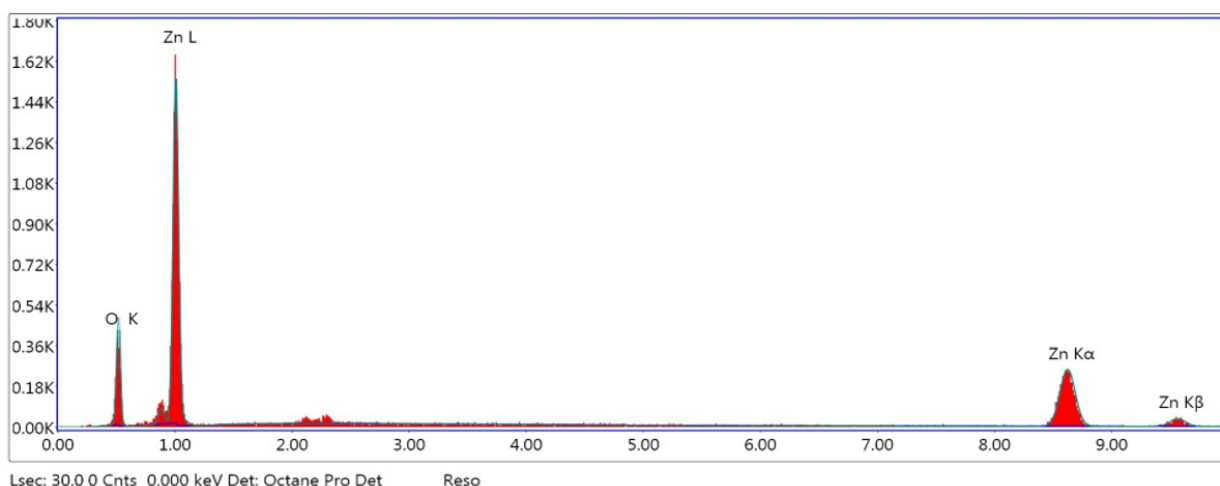


Figure 3: EDX analysis for Pure ZnO-NPs

analysis has showed particles have agglomerated in flower-like morphology their nano-size from 4.479 nm. to 143.7 nm. These results are commensurate with the EDX analysis as shown in Figure 2. The atomic percentage of the constituent elements was 1:1 in the formed ZnO as given by the EDX results (Table 1).

Cell Culture

MCF7 and PC3 were used to evaluate the cytotoxicity of ZnO-NPs and commercial ZnO (Table 2). The cells were cultured in media supplemented with

10% FBS, penicillin (100 U/ml) and streptomycin (100 μ g/ml) in a humidified atmosphere of 50 μ g/ml CO_2 at 37°C (Haarindradas et al., 2015).

One million cells had been seeded per well in 24-well plates to study the *In-vitro* cytotoxic ZnO-NPs as anticancer on human cells of MCF7 and PC3.

MTT Protocol

The viability of cells was determined using MTT (5 mg/mL) as previously described (Perumal et al., 2015; Van Meerloo et al., 2011).

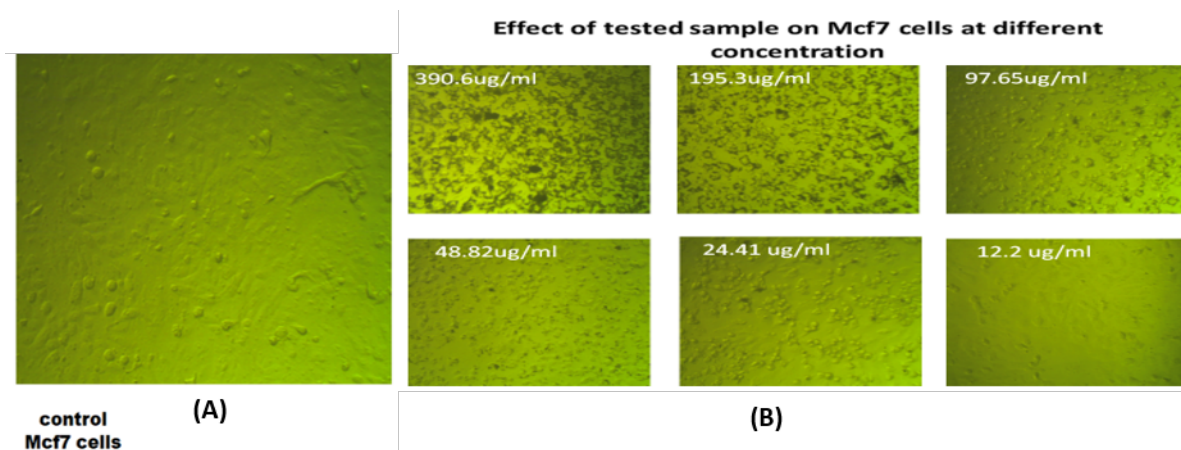


Figure 4: Inverted Microscope (40x) Showed Untreated MCF-7 Cell Line as a Control (A). MCF-7 treated with different six concentrations of ZnO-NPs after 24 h of Cell Exposure (B). The experimental groups than in the control.

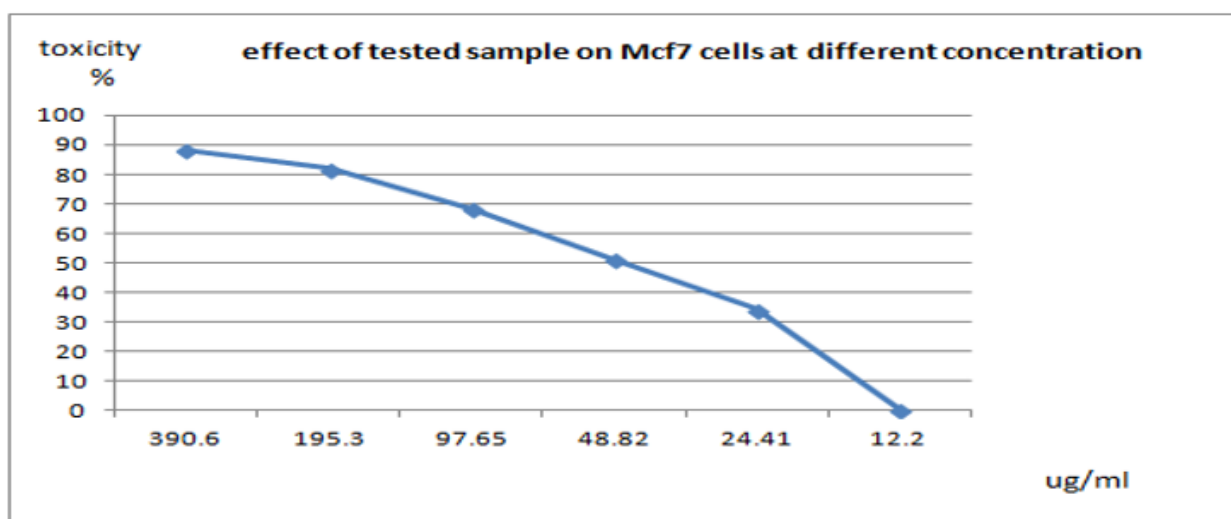


Figure 5: Effect of different concentrations from ZnO-NPs on MCF7 cells

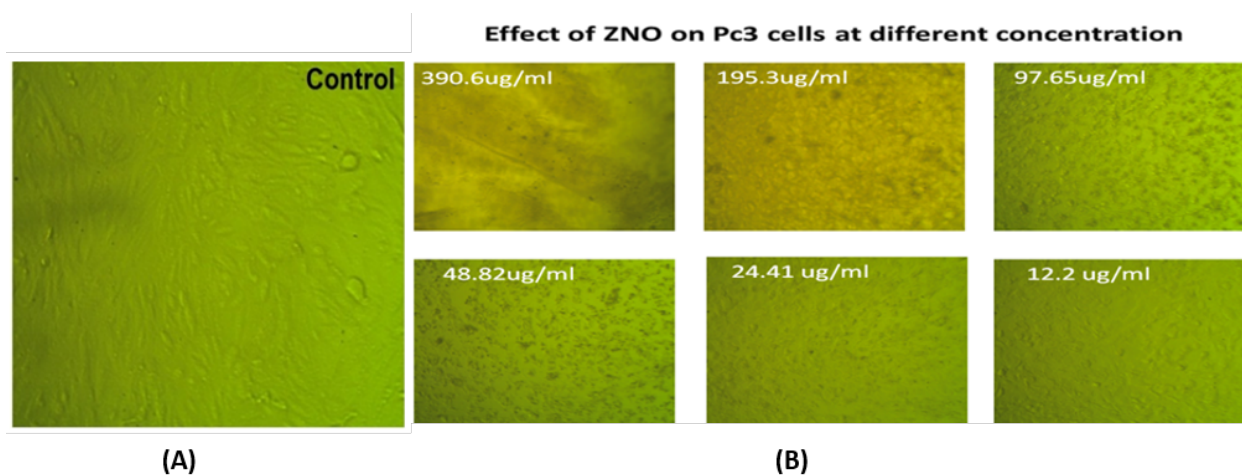


Figure 6: Inverted Microscope (40x) Showed Untreated PC3Cell Line as a Control (A). PC3 treated with different six concentrations of ZnO NP after 24 h of Cell Exposure (B). P<0.05 compared to control.

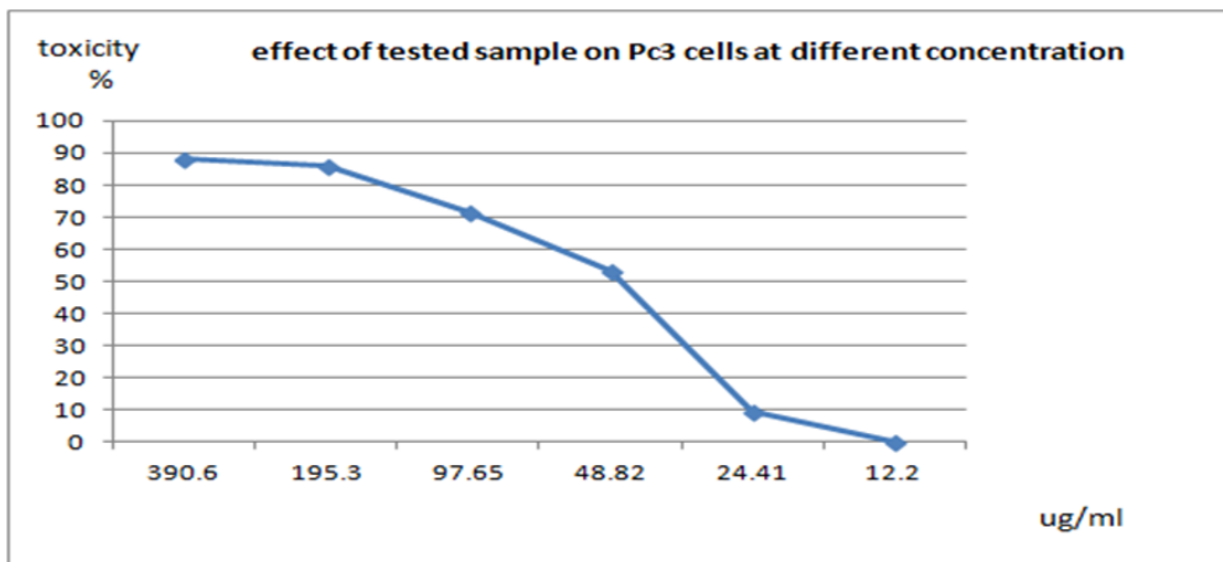


Figure 7: Effect of different concentrations from ZnO-NPs on PC3 cells.

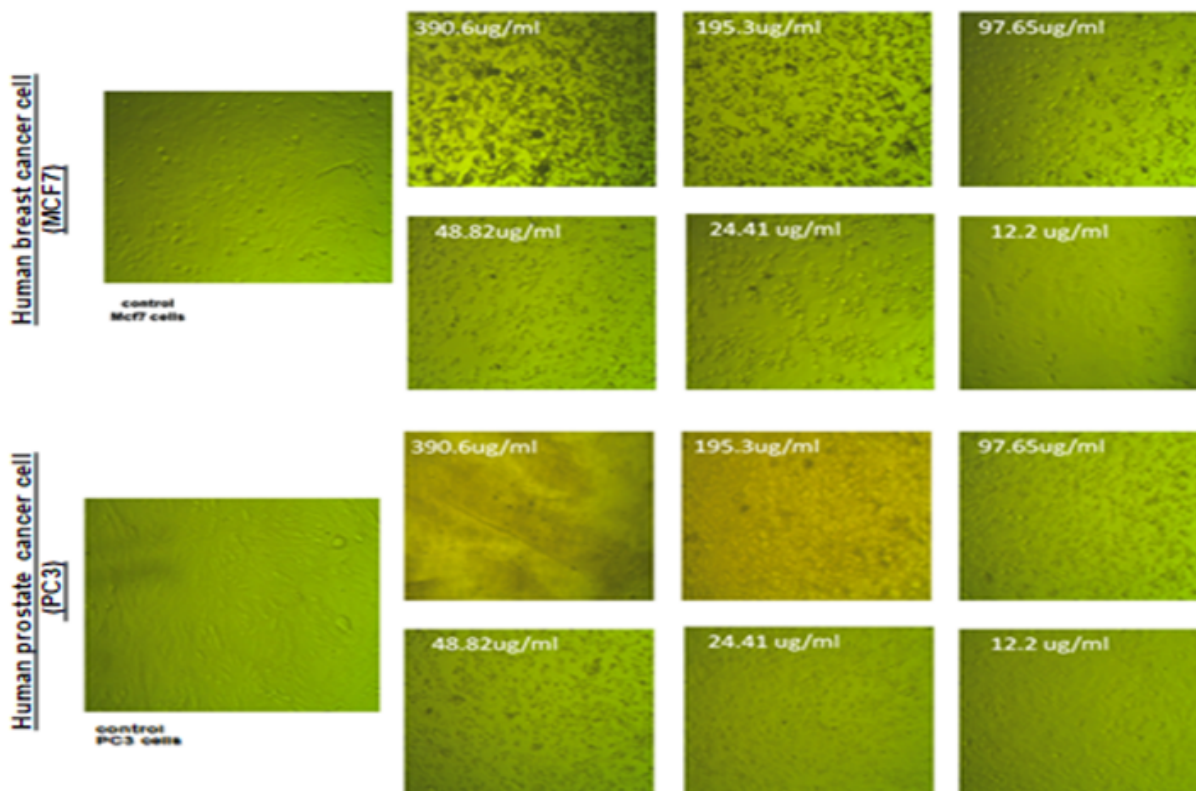


Figure 8: Effect of various concentration of ZnO-NPs on MCF-7 and PC3, P<0.05 compared to control.

Examination of interaction of ZnO-NPs with MCF-7 cells using inverted light microscopy; MCF-7 cells treated with different six concentrations of ZnO NP (12.2, 24.4, 48.8, 97.6, 195.3 and 390.6 $\mu\text{g/ml}$) for 24 h (Figure 4) indicate remarkable morphological changes characteristic of viability of these cells directly proportion with toxicity.

Examination of interaction of Zinc oxide Nanoparticles (ZnO-NPs) with MCF-7 cells using inverted light microscopy; MCF-7 cells treated with different six concentrations of ZnO-NPs (12.2, 24.4, 48.8, 97.6, 195.3 and 390.6 $\mu\text{g/ml}$) for 24h (Figure 3) indicate remarkable morphological changes characteristic of viability of these cells directly proportional with toxicity % ($P < .005$). The cytotoxic effect of various concentrations of ZnO-NPs was assessed in MCF-7 cell cultures using MTT assay (Table 3). Minimal inhibition concentration (MIC) of ZnO-NPs was 12.2 $\mu\text{g/ml}$. MTT results showed that ZnO-NPs inhibited MCF-7 and PC3 cell growth in a dose-dependent manner ($P < 0.05$) (Figures 5, 6, 7 and 8).

DISCUSSION

More recently, the Zinc Oxide has attracted extensive attention because it has multi-functional activities such as: antitumor, antibacterial, disinfectant, and UV shield agent. It is becoming increasingly evident that ZnO-NPs has cytotoxic activities. In this study the ZnO-NPs have shown a strong cytotoxic activity on both cancer cell types in vitro. Results of our study were consistent with findings of previous studies (Wang et al., 2018; Chao and Wei, 2015; Król et al., 2017).

Firstly, various concentrations of ZnO-NPs were used to evaluate the cytotoxicity of ZnO-NPs toward MCF7 and PC3 cells by evaluating the cells viability. Our results had revealed that the anticancer activity of ZnO-NPs non-invasive human breast and prostate cancer cell was in dose-dependent manner. The cytotoxic activity of ZnO-NPs is associated with ROS production. (Wang et al., 2018) and (Chao and Wei, 2015) reported that ZnO-NPs had displayed their anticancer properties due to their intracellular ability to stimulate the formation of ROS in high level within the tumor cells.

In a study done by Wang et al, the ZnO-NPs had increased the levels of ROS within the LTEPa-2 cells. In addition, it was claimed that the ZnO-NPs had reduced the levels of oxidative stress biomarkers. Moreover, many studies had suggested that the ZnO-NPs might cause swelling of mitochondria resulting in nonfunctional mitochondria of cancer cells (Xu et al., 2011).

Mitochondria are significant organelles required for regulating the signal transduction and apoptosis in mammalian cells. Therefore, cytotoxic activities of ZnO-NPs on non-invasive human breast and prostate cancer cell lines of the present study might be due to generating mitochondrial damage and high levels of ROS within that cell lines (Tanino et al., 2020).

CONCLUSIONS

This study showed that ZnO-NPs was successfully synthesized by sol-gel technique in nanometer size range from 4.479 nm to 143.7 nm. The development of ZnO-NPs through nanotechnology has yielded less cytotoxic and efficacious nanoparticles capable of extending the therapeutic options for the management of both breast and prostate cancer. In fact, the integration of the pharmacology and nanotechnology provides the opportunity for the development of new loaded-drugs in the nanometer size range that can be applied to different potential applications in pharmacology and pharmaceutical preparations.

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Conflict of interest

The authors declare that they have no conflict of interest for this study.

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