# THE CYTOTOXICITY OF ZNO NPS ON BREAST CANCER CELL LINES MCF-7 DEPENDING ON THE DISPERSION SOLUTION

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However, the number of in vitro studies are being published regarding the cytotoxicity of nanomaterials is on the increase, the components of the media for toxicity assays have often varied according to the needs of the scientists. Our aim for this study was to: 1)evaluate the influence of serumin this case, fetal bovine serum FBS, Phosphate Buffer Saline PBS, water for injection in a cell culture medium on the toxicity of nano-sized (20–30 nm) ZnO on human breast cancer cell line (MCF-7) and 2) use very small dose that make 50% of cancer cell death after treatment in order to the normal cells can get rid of it and be safe on body normal cells. Dispersion solution is effective factor of cell viability in presence of ZnO NPs as a treatment. The nano sized ZnO exhibited their highest toxicity when exposed to Phosphate buffer saline, incontrast to exposure in media containing 10 % serum. This mainly comes from the absence of protein protection, and lower cell **growth** rate, but also that when we used sterilized water for injection as the dispersion solution it has an observed toxicity and a negative osmotic effect on cells that make cells increase till exploding.

**Keyword:** ZnO nanparticles, MCF-7, Cytotoxicity, Dispersion Solution.

## Introduction

The behaviour and structure of nanoparticles (NPs) are unique. By triggering cell death and suppressing cell development, some NPs, such as metal oxide NPs, can be used in biomedical applications and cancer treatment. Zinc oxide NPs (ZnO-NPs) are one of the most widely used metal oxide NPs in nanoscale applications. ZnO is a wide-band-gap semiconductor used in pharmaceutics, cosmetics, solar cell production, photocatalysis, gas sensors, and coatings, among other applications. Toxicity of ZnO NPs is low or absent. Increased amounts of ZnO NPs, on the

Other hand, cause cancer cells to become more cytotoxic. When analysing the toxicity of NPs, several obstacles and issues remain at the interface between NPs and biological systems. As shown here, the state of NPs in biological contexts is more intricate than previously imagined, necessitating a more comprehensive knowledge of what happens as NPs spread in local biological habitats and what cells interact with them. Not only should the biological interface of NPs be recognised and managed, but they should also be viewed as biological rather than inorganic entities. The understanding of nano-bio interactions could help in promoting applications of NPs in the biomedical fields and reducing/preventing possible adverse effects tothe biological systems caused by NPs.

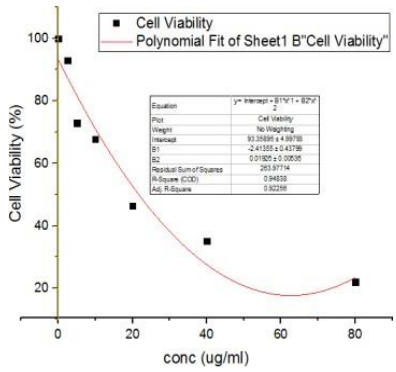
## Materials and Methods

**1-** Cell culture: MCF-7 cancer cells were obtained from Medical Technology Centre (Alexandria), cells were maintained in high glucose DMEM media supplement with 1%Pen strip (AB) and 10% heat-inactivated fetal bovine serum in humidified, 5% CO2 at 37°C.

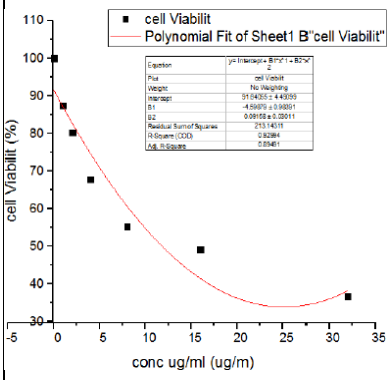
**2-** Cytotoxicity assay: Cell viability was assessed by Three-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT assay. 5*10^3 cells were seeded in 96-well plate and incubate in complete media for 24 hrs. Cells were treated media containing ZnO NPS that dispersed in different solutions (working solution) at various concentrations, after 24 hrs of drug exposure; wells were washed by PBS twice. Aliquots of 20 µl of MTT add to 180µl of PBS and placed into incubator for 3 hrs. The solution was removed then 200 µl was added and the plate was placed into dark with shaking for 15 minutes. The absorbancewas measured at 490 nm using Elisa reader.

**3-** Preparing of working Solution: ZnO NPs were purchased from local company (Nano Gate company) in Egypt that provided us that the prepared zinc oxide with average size30±5 nm and spherical like shape. ZnO NPs powders are suspended in(1- DMEM with 10% FBS , 2-Phosphate buffer saline, 3-Water for injection). The Woking solutions are exposed to UV under cytosafe cabinet UV lump for 1 hour. With the aid of water bath sonication the working solutions are well dispersed for 30 minutes.

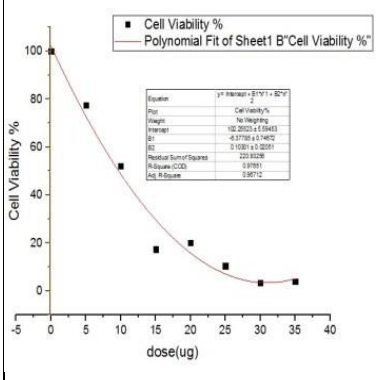
## Results and Discussion.




**(a) MCF-7 exposed to ZnO NPs dispersed in complete media with FBS**




**(b) MCF-7 exposed to ZnO NPs dispersed in PBS**




**(c) MCF-7 exposed to ZnO NPs dispersed in Sterile water for injectin**

**Fig1** MCF-7 breast cancer cell line exposed to different conditions

- In Fig.1 by using OriginPro 2018 version we can determined the IC50 (The half maximal inhibitory concentration) of MCF-7 breast cancer cell line in different dispersion solutions that illustrate in fig1 (a) the dose which achieve 50% of cancer cell death is approximately 19.5 µg/mldispersed in DMEM High glucose medium with 10% FBS, graph (b) IC50 is 12.5 µg/ml dispersed in phosphate buffer saline, and fig1 (c) IC50 is 10.5 µg/ml dispersed in water for injection.

**Cell morphology:** Cell morphology was assessed using an inverted optical microscope (OPTIKA XDS-3FL4) coupled to a digital compact camera system (Optika,BG,Italy).

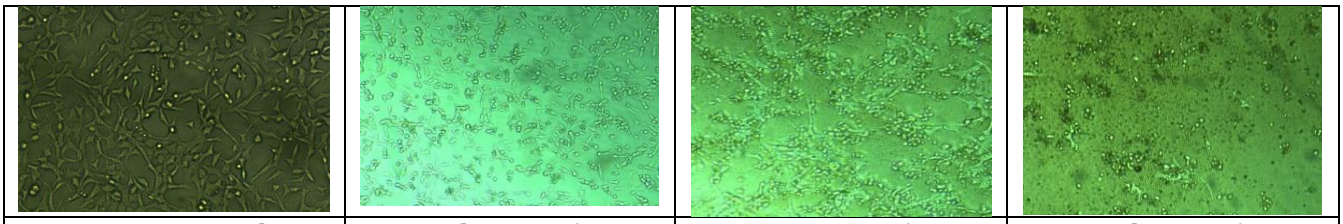
**Microscopic observation of MCF-7 cancer cells with ZnO-NPs:** Microscopy was used to observe the growth of cancer MCF-7 cells in the presence of different concentrations (2.5–80 µg/ml) of dispersed ZnO-NPs in complete media following a 24-hour incubation period.

- In Fig.2, the proliferation of MCF-7 cancer cells and their interactions with various doses of ZnO-NPs dispersed in complete medium are revealed.

On day 1, the cells were obviously mononucleotide; however, when the cells reached about 70-80% confluence and were treated with various doses of the produced NPs, the cell density was reduced due to their dose-dependent interaction with the NPs. At a NPs concentration of 2.5 µg/ml, no significant difference was seen, while increasing the NPs dosage from 5 to 20 µg/ml inhibited cancer cell development. At the highest concentration of nanoparticles (80 µg/ml), themost of cells were harmed.

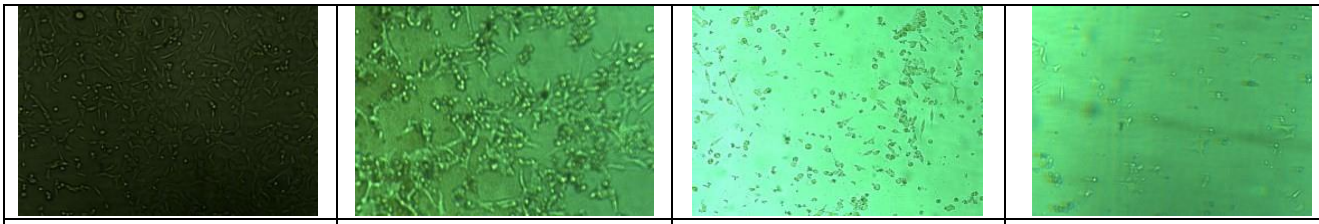
- In Fig.3, the results were observed in MCF-7 cells treated with dispersed NPs in Phosphate buffersaline with a pH of 7.4, which is close to the pH of the cells. The action of ZnO NPs is effective at 4 µg/ml and the cells are fully dead in the concentration of 32 µg/ml.

- Fig 4 Although the fact that dispersed NPs in water for injection have a dynamic effect on cells, microscopic observation showed dark batches rising with increasing doses, particularly the concentration of 35 µg/ml due to water's osmotic property.



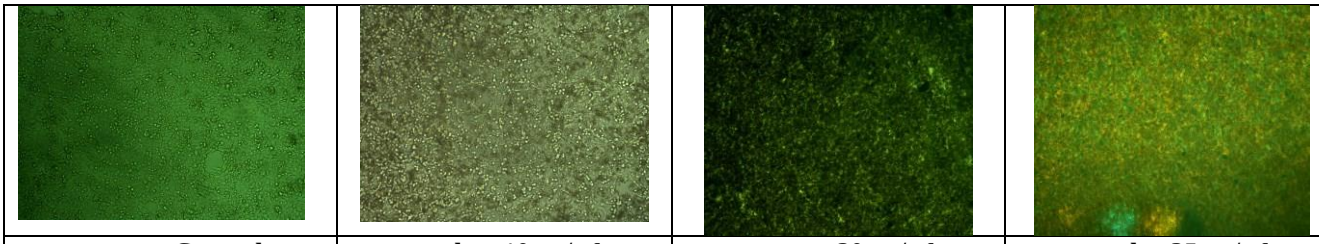
| a. Control | b. 20 µg/ml | c. 40 µg/ml | d. 80 µg/ml |
|---|---|---|---|

**Fig 2** Cellular morphology changes after 24 hrs treatment with different concentration of ZnO-NPs (20,40,80µg/ml) dispersed in complete medium containing 10% FBS.
(a) Untreated MCF-7 cells, (b) The MCF-7 cells treated with 20µg/ml of ZnONPs, (c) The MCF-7cells treated with 40 µg/ml of ZnO-NPs, (d) The MCF-7 cells treated with 80 µg/ml of ZnO-NPs



| a. Control | b. 8 µg/ml | c. 16 µg/ml | d. 32 µg/ml |
|---|---|---|---|

**Fig 3** Cellular morphology changes after 24 hrs treatment with different concentration of ZnO-NPs (8,16,32 µg/ml) dispersed in phosphate Buffer Saline.
(a) Untreated MCF-7 cells, (b) The MCF-7 cells treated with 8µg/ml of ZnONPs, (c) The MCF-7 cells treated with 16 µg/ml of ZnO-NPs, (d) The MCF-7 cells treated with 32 µg/ml of ZnO-NPs



| a. Control | b. 10 µg/ml | c. 20 µg/ml | d. 35 µg/ml |
|---|---|---|---|

**Fig 4** Cellular morphology changes after 24 hrs treatment with different concentration of ZnO-NPs (10,20,35/ml) dispersed in sterile water for injection .
(a) Untreated MCF-7 cells, (b) The MCF-7 cells treated with 10µg/ml of ZnONPs, (c) The MCF-7 cells treated with 20 µg/ml of ZnO-NPs,
(d) The MCF-7 cells treated with 35 µg/ml of ZnO-NPs

## Conclusion

1. Because of ZnO NPs are undissolved substance We investigate how to exposebreast cancer cell line MCF-7 to NPs, The sonication (the process of applying sound energy to agitate particles in a liquid) helps us for NPs dispersion.
2. In the various working solution the IC50 differ from one to other that we have proved in fig.1 But the microscopic observation give us an image about the natureof the reaction with cells, in complete medium the cells consumed ZnO NPs greater than that dispersed in PBS which when applying on human body the probability of normal cells will be damaged increasing.
3. We recommend using PBS as a dispersion solution because it has a safe effect oncells and is very good of ZnO NPs dispersion**,** not recommend exposing cells to water that may be has a role in cell death that not preferable, and the medium that containing FBS that reduce the cytotoxicity action of ZnO NPs.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