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The Cytotoxic Properties and Anticancer Impacts of Zinc oxide Nanoparticles On Human Breast, MCF7 Cell Lines

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Abstract

Nanotechnology enable us to manipulate the substances at nanoscale level. Metallic nanomaterials such as Zn O nanoparticles synthesized with chemical methods, which has the disadvantage of using deleterious chemicals. Green chemistry allows the design and synthesis of nanoparticles, with reducing or elimination of hazardous materials. One of the important applications of zinc oxide nanoparticles is to use as an antibacterial and anticancer agent. In this study, we synthesized Zn O nanoparticle using Hyssopus officinalis plant extract and evaluate its their cytotoxicity on MCF7 breast cancer cell line.

Materials and Methods: The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell viability assay was done on MCF7 cell line. Cells were seeded on 96-well plates and incubated for 24 hours. After 24 hours, cells were treated with different concentrations of Zn O nanoparticles (8, 16, 32, 64 and 128 µg / ml) and incubated for next 24, 48 and 72 hours. Then, treatments removed and MTT was added to each well. DMSO was used in order to dissolve insoluble blue crystals and absorbance was read at 570 nm.

Results: Results showed that Zn O nanoparticles synthesized in Hyssopus officinalis plant extract are able to inhibit MCF7 cells. However, this inhibition was at low concentrations of Zn O nanoparticles and the values of IC50 (in 24, 48 and 72-hour treatment) respectively was calculated at 16, 10 and 5 µg/ml NPs.

Conclusion: Results of this study indicated the inhibitory effects of Zn O nanoparticles synthesized in Hyssopus officinalis plant extract on cancer cells. However, in vitro studies should perform in order to confirm these effects. Finally, these results demonstrate the anticancer potential of Zn O nanoparticles, which could be used as therapeutic agent in cancer therapy.

Keywords:

Green Chemistry,

Zn O Nanoparticles,

Hyssopus Officinalis,

MCF7 Cell Line,

Breast Cancer..

1. Introduction

In recent years, many attempts have been made to produce nanoparticles due to their specific optical, chemical, electrical and photoelectric properties, which confirmed the various uses of these materials in the fields of catalysts, optics, biomedical knowledge, mechanics, magnetism and Energy is[1] . Zinc is a rare element in the body and is also used as a food supplement in foods, so some researchers consider zinc oxide nanoparticles as low toxicity substances. Various studies have been conducted on the toxicity of zinc oxide nanoparticles, the main cause of which is the toxicity of zinc oxide nanoparticles, related to microorganisms and factors related to the release of metal cations [2]. Concerning the formation of zinc oxide nanoparticles that are synthesized both in chemical and green form, it has to be said that in the process of various plant extracts, which are very common today, it is completely safe and safe compared to chemical synthesis[3]. The different structures of nanoparticles make them possible to be used in many fields, including biosensors, Nano medicine, and bio nanotechnology [4]. Moreover, the applications of nanoparticles (NPs) in the health, food, chemistry and cosmetics industries are developing rapidly. It should be noted that the synthesis of nanoparticles is feasible by different methods, which are known as green, chemical or physical synthesis [5-7]. Expensive equipment and the difficult conditions of synthesis in the physical methods [8] as well as dangerous and toxic chemicals for patients and researchers in the chemical methods, have increased the applications of green synthesis approaches [9]. The

synthesis of nanoparticles through plants, bacteria, fungi and algae is called green synthesis. The advantage of this approach is the pure amounts of ZnO NPs production on a large scale [10].

It is known that ZnO has the individual potential for applying in the biomedical and cancer fields, due to its specific chemical properties [11]. However, numerous studies have shown that the acute and chronic proximity to ZnO ultimately leads to cytotoxicity and genotoxicity in plants and animals [12-14]. It has been shown that the ZnO-NPs toxicity is due to its involvement in the ROS generation and the oxidative stress induction [15-17].

Methods and materials

Green synthesis of Zinc oxide nanoparticles and approval tests

The fresh green leaves and stem of *Hyssops officinalis* were harvested from 10 g of the collected parts were washed thoroughly thrice with water followed by sterile distilled water (DW). After cutting and soaking the plant in 200 mL distilled water, they were shade-dried for

10-15 days and then powdered by a domestic blender. Heating the 0.3 g of produced powder was performed at 50°C for 2 h in 100 mL DW. 1 mm Zinc acetate [Zn $(O_2CCH_3)_2(H_2O)_2$] was added in 50 ml DW and mixed by magnetic stirrer at 25 ° C for 1 h. A mixture containing of 20 mL NaOH, 50 mL Zinc acetate, and 25 mL of plant extract was made subsequently and mixed for 3 h. The yellow color was indicated the ZnO NPs synthesis. Centrifuging at 8000 rpm at 60°C for 15 min was performed to collect the pellet. The pellet was dried at 80°C for 2 h and stored for future studies [18, 19].

The X-ray diffractometer (PAN analytical X-Pert PRO) was used to measure the pattern of density, purity, and size of the ZnO NPs. X-ray diffraction (XRD) analysis using CuKα radiation (1.54060 Å) X-ray crystallography was applied. FTIR spectroscopy was applied to define the FTIR spectra in a resolution of 4 cm^{-1} [20]. Finally, to analyze the shape and size of NPs, SEM and TEM were used respectively. The average size of produced nanoparticles was 20 nm.

MTT assy

To study the impact of ZnO NPs on cell viability, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was applied. 75×10^3 cells of Huh7 cell line were seeded into each plate of 17 culture plates. Moreover, HupG2 and Huvec cells were seeded in two groups of five plates by the same way. After an overnight incubation, the mediums were replaced with a fresh modified medium containing ZnO NPs. Incubation plane was designed into two classes of treatments, dose-dependent and time-dependent viability assays. For dose-dependent MTT assays, different ZnO NPs concentrations were added into the medium of all of 27 cultured cells and incubated for 24 hours. For time-dependent MTT assays, certain doses of ZnO NPs, were used to measure the viability of Huh7 cell line for 48 hour incubation. The doses, which were used are indicated in Figures 9 and 10.

Upon the incubations were completed, the ZnO NPs-modified medium was removed, and 100 µL of medium containing MTT 0.5 mg/mL was added into all culture plates. Then, 3 hours incubation at 37°C was performed. 100 µL of dimethylsulfoxide (Sigma) was replaced with MTT-medium and shaked for 10 minutes to stabilize the formed formazan. Versamax microplate reader (Molecular Devices, Sunnyvale, CA) was used at a wavelength of 570 nm to measure the absorbance (background effect was removed at 690 nm).

Statistical analysis

Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Significance was ascribed at $p < 0.05$.

Results

The viability of the treated hepatocyte cell lines by ZnO NPs using *Hyssopus officinalis* plant extract

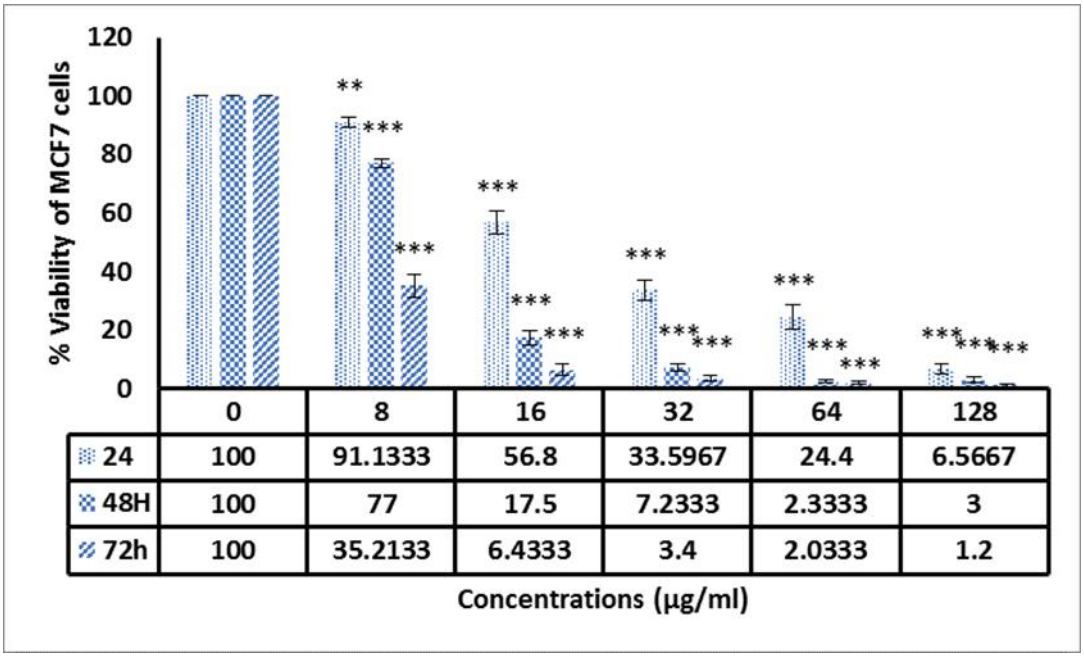
The results of MTT assay analysis for MCF7 cell lines were designed in Figures 1. The IC50 value for MCF7 cell lines (in 24, 48 and 72-hour treatment) respectively was calculated at 16, 10 and 5 µg/ml NPs.

According to the results obtained from breast cancer cells that were treated with ZnO nanoparticles in a green way from the *Hyssopus officinalis* plant extract, it was considered that with increasing concentration and increasing time, the effects of ZnO nanoparticles on the growth of cancer cells increased, so that these nanoparticles At a concentration of 128 µg / ml, it has the highest cytotoxic effect on MCF7 cells at different times.

Morphological Images of MCF7 Cells

According to the results, in the form of the effect of zinc oxide nanoparticle toxicity at concentrations of 12 and 24 µg / ml, compared to the control group. As it is seen, the size and number of cells treated with the nanoparticle is reduced and the cell deformation can be attributed to changes in their appearance, such as cytoplasmic decline, germination of cells, and changes in nucleus pigmentation. By increasing the concentration of the nanoparticle, the number of cells decreases and decomposes, which indicates the cytotoxic effects associated with the concentration of zinc oxide nanoparticles synthesized by the green method from the *Hyssopus officinalis* plant extract.

The viability of the treated hepatocyte cell lines by ZnO NPs



	0	8	16	32	64	128
24	100	91.1333	56.8	33.5967	24.4	6.5667
48H	100	77	17.5	7.2333	2.3333	3
72h	100	35.2133	6.4333	3.4	2.0333	1.2

Figure 1: MCF7 cells treated with zinc oxide nanoparticles produced from the *Hyssopus officinalis* plant extract (The significance levels was defined by (**P < 0.01) and (***P < 0.001)).

Morphological Images of MCF7 Cells

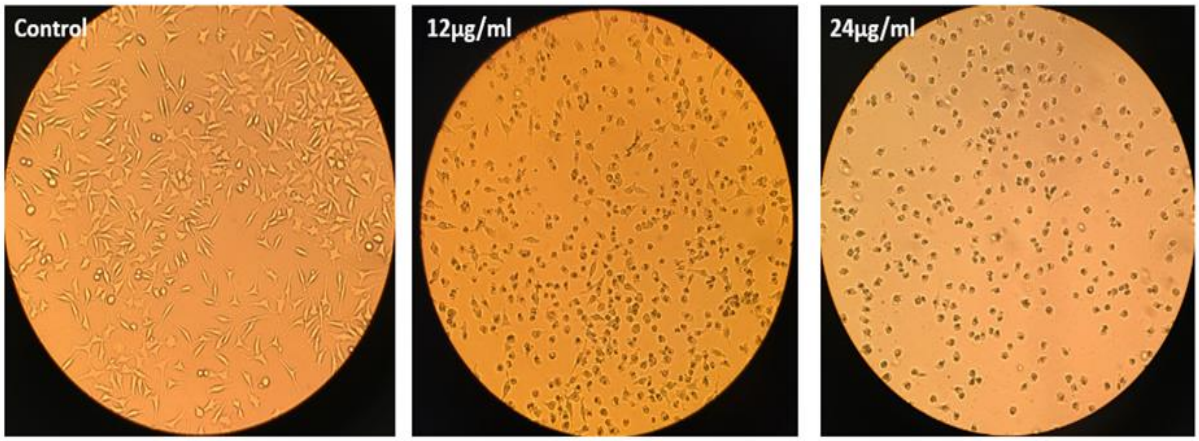


Figure 2: Morphology of cells treated with zinc oxide nanoparticles synthesized from *Hyssopus officinalis* plant extract compared to control.

Discussion

Cytotoxicity parameters such as MTT, NRU and LDH assays represent the damage in mitochondrial, lysosomal and cell membranes, respectively that eventually triggers cell death37. These assays serve as sensitive and integrated measures of cell integrity and of the inhibition of cell proliferation. cytotoxicity results were consistent with the observed higher MMP loss in cells treated with Al-doped ZnO nanoparticles than those of pure ZnO nanoparticles. Damage to

lysosomal membranes is known to release lysosome protease into intracellular spaces, which affects the neighboring cells and triggers cell death due to apoptosis. LDH leakage from cells is further evidence for the penetration of nanoparticles into cells and cell membrane damage16,38. Liver and breast cancer are the most traumatic diseases because they affect the major organs of the body. Nanomedicine recently emerged as a better option for the treatment of these deadly diseases. As a result, many nanoparticles have been used to treat cancer cell lines. Of the various nanoparticles, zinc oxide exhibits biocompatibility[21].

The toxicity effects of ZnO NPs are shown in several types of human cell lines including hepatocytes, kidney and alveolar adenocarcinoma, depending on the nanoparticle's size and dosage used [31-33]. It is known that the ZnO NPs toxicity is related to the Zn ionization to Zn2+ and production of free radicals from the NPs surface, which results in metabolic and ionic imbalance of the cell [34-36]. ZnO NPs lead to induce the oxidative stress by the generation of ROS, therefore, the lack of sufficient antioxidant system causes the cell death and genotoxicity [36]. Some studies argued that ZnO nanoparticles induce toxicity due to the dissolution of the particles into Zn^{2+} ions and metal ions doping can reduce the ZnO cytotoxicity by reducing ZnO dissolution[22]. However, some investigators demonstrated that ZnO nanoparticles liberate Zn^{2+} ions in aqueous state, but the levels of Zn^{2+}ions released were insufficient to promote cytotoxicity in human cells unless the particulate matter is in contact with the cells[23].

We found that zno NPs improves the inherent selective cytotoxicity of ZnO nanoparticles toward human breast cancer cells while posing no toxicity to normal cells. ZnO nanoparticles were found to induce apoptosis in MCF-7 cells. Overall, our data suggested a novel approach through which the inherent selective cytotoxicity of ZnO nanoparticles against human breast cancer cells can be improved. Further research on anticancer activity of ZnO nanoparticles from *Hyssopus officinalis* plant extract in different types of cancer cells is warranted.

Conclusion

Our results indicated a novel approach through which the inherent selective cytotoxicity of ZnO nanoparticles from *Hyssopus officinalis* plant extract against cancer cells can be further improved.

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Contributing Contributors

All authors had standards of writing on the basis of the International Committee's recommendations to medical journal publishers.

Conflict of interest

The authors state that there are no conflicts of interest in the current research.

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