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Anticancer Potentials of Zinc Oxide Nanoparticles against Liver and Breast Cancer Cell Lines

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The aim of the present study was to evaluate the *in vitro* cytotoxic effects of zinc oxide nanoparticles (ZnONPs) with particle size of 40 and 50 nm against human hepatocellular carcinoma and human breast cancer cell lines. The effects of nanoparticles were analyzed after 24 hours of incubation using standard MTT assay. Incubation of ZnONPs with different cells produced a dose-dependent inhibition of cell growth. The IC_{50} values of ZnONPs for both prepared samples (40 and 50 nm) were comparable. It has been noticed that MCF-7 and MDA cells were more affected by the prepared 50 nm than the 40 nm ZnONPs. On the other hand, HepG2 cells showed inverse relation in terms of their response to the size of the prepared NPs.

Key words: Nano-biotechnology, Zinc oxide nano-particles, Cancer cells, In vitro

Introduction

Nanotechnology is considered to be one of the fastest growing technologies in the last two decades. It has been involved in various academic as well as industrial fields, including chemistry, biology, medicine and physics[1]. Nanotechnology is the field dealing particles having a general size in the nano-range[2]. Nanomaterials have been widely appreciated for possessing characteristics, in terms of size and shape, which are uniquely different from their main constituting atoms[3]. These novel characteristics offered nanoparticles great application range in almost all industrial sectors. Bionanotechnology is the field that concerns with the application of nanoparticles in biological fields. It has proved to be an efficient and attractive field in many biotechnological processes[4]. Zinc oxide nanoparticles (ZnONPs) are prepared form zinc metal oxides, and they have large specific area and high surface energies[5]. Therefore, they found many interesting applications in solar cells, gas sensors, photpcatalysts, pharmaceutical drugs[6,7]. In the recent years, nanoparticles ((ZnONPs) have been investigated widely for their potential application as bioactive materials against many pathogenic microorganisms and cancer cell lines[4,8,9].ZnONPs exhibited great selectivity towards cancer cells, and were able to avoid interfering with normal cell structure and metabolism[10]. Accordingly, they have been investigated in the treatments of many cancer diseases with the aim to overcome chemotherapeutic toxic side effects[11]. The aim of the current work was designed to evaluate the potential anticancer activities of chemically synthesized ZnONPs. The work was performed on two sizes of ZnONPs (40 and 50 nm). Cytotoxic activities were performed against different hepatocellular and breast cancer cell types. Also, the morphology of the affected cells was investigated microscopically..

Materials and methods

Preparation of stock and working solutions

The prepared ZnO nanoparticles (40 and 50 nm) were used to prepare a stock solution of 10 mg/mL in DMSO. Serial dilution with cultivation medium was used to prepare working nanoparticle solutions (0.0-0.1 mg/mL).

Cell cultivation and cytotoxic assay

HepG2, human hepatocellular carcinoma; MCF-7 and MDA-MB-231, human breast cancer cell lines were obtained from Sigma-Aldrich Chemical Company, USA, and were maintained in DMEM

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medium supplemented with 10% FBS and antibiotic solution. Cell cultivation and propagation was performed as per our developed protocol[12]. MTT standard cytotoxicity assay[13] was used to assess different cytotoxic effects of ZnONPs. The assay protocol previously used in our work was followed[14,15]. Treated cells were examined with an inverted contrast microscope for possible morphological changes (Nikon Eclipse T500, Japan) at 10x magnification.

Statistical analysis

Experiments were repeated thrice, and results are presented as mean ± standard deviation (S.D). ANOVA (One Way Analysis of Variance) was used statistically analyzethe obtained results, and significantly different results were denoted at $p \leq 0.05$.

Result and Discussion

The cytotoxic effects of the prepared ZnO nanoparticles on different cell lines (HepG2, MCF-7 and MDA) have been evaluated. Results illustrated in Figure 1 showed clearly that increasing the concentration of both prepared ZnO nanoparticles (40 and 50 nm) adversely decreased the viability of treated cell lines, with a highly significant effect ($p<0.001$). Moreover, the highest concentration applied (1 mg/mL) resulted in the highest decrease in cell viability in all cell lines, where the viability recorded 14.3±0.99, 25.33±0.2.37 and 50.93±3.57 for MDA, HepG2 and MCF-7 cells, respectively, in case of ZnO nanoparticles with 40 nm diameter. On the other hand, the 50 nm ZnO nanoparticles showed more or less improved cytotoxic effects, where the highest effect obtained upon using 1 mg/mL resulted in viabilities of 12.76±0.51, 27.02±0.55 and 46.95±1.95 for MDA, HepG2 and MCF-7 cells. Furthermore, the results obtained for MCF-7 cells treated with 40 nm of ZnO nanoparticles revealed that the effect on cell viability was not statistically

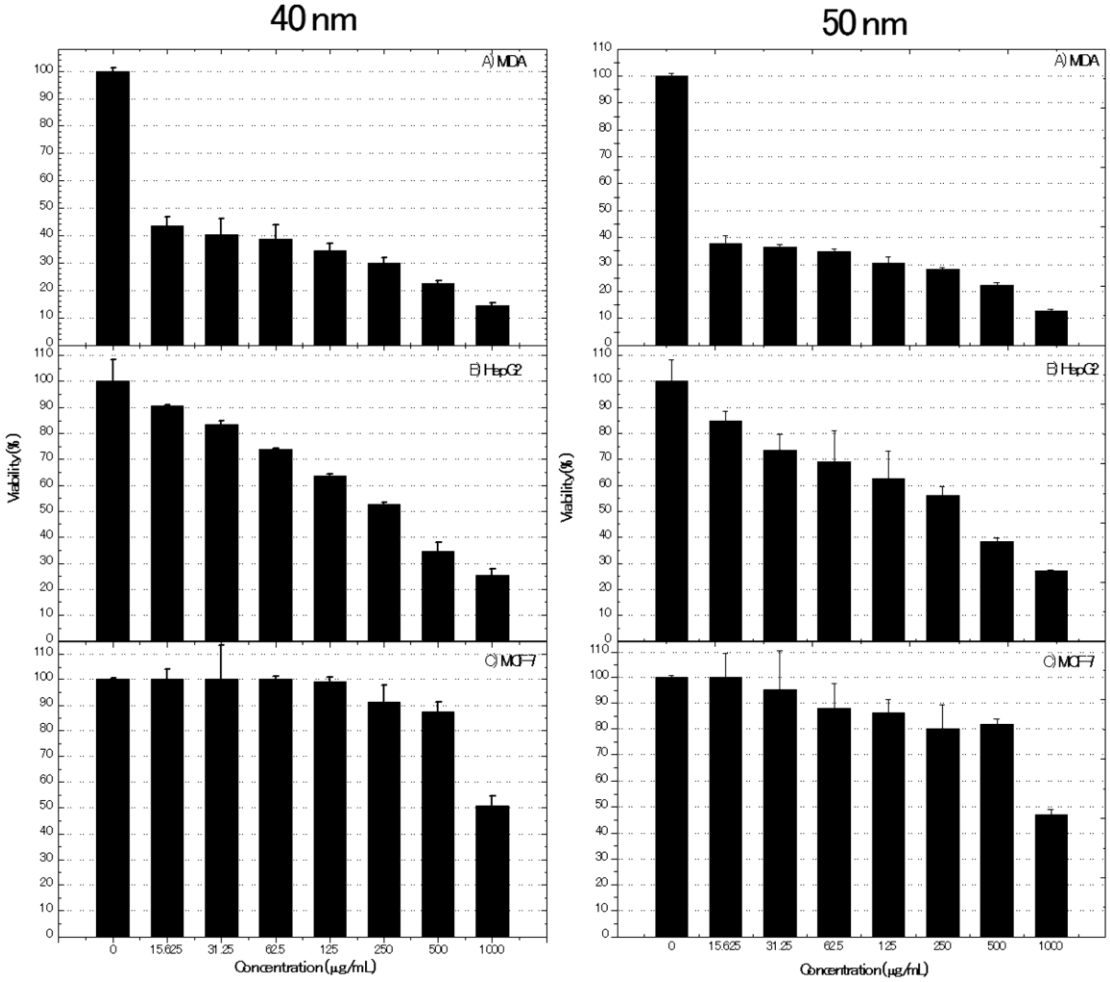

Fig.1 — Effect of different concentrations of both 40 and 50 nm of prepared ZnO nanoparticles on the viability of different cell lines. Data are expressed as means ± SD.

Table 1 — IC_{50} values obtained for different cell lines in response to ZnO nanoparticles

Size	IC_{50} (μg/mL)		
	MDA	MCF-7	HepG2
40 nm	198.97	522.51	236.61
50 nm	191.70	468.49	247.59

significantly changed upon treating the cells with concentrations ranging from 15.6 to 125 ug/mL. Additionally, MCF-7 cells were not also significantly affected when they were treated with 15.6 ug/mL of 50 nm of ZnO nanoparticles. The results tabulated in Table (1) represent the obtained IC_{50} values calculated for different cell lines as affected by different concentrations of the prepared nanoparticles. Results showed that different cell lines differ also in their obtained IC_{50} values, which also agrees with the aforementioned results for the dose-response data. Additionally, IC_{50} values obtained for both prepared samples (40 and 50 nm) were comparable, although it can be seen that MDA and MCF-7 cells were more affected by the prepared 50 nm of ZnONPs than the 40 nm NPs. On the other hand, HepG2 cells showed inverse relation in terms of their response to the size of the prepared NPs. Figures 2 and 3 showed morphological changes in MDA, HepG2 and MCF-7 cells in response to different concentrations of ZnO nanoparticles. Increasing nanoparticle concentration resulted in a drastic change in the morphological characteristics of the tested cell lines, which was concentration dependent. Cells shrank and lost their adherence capacity to the surface of the cultivation plate. Furthermore, maximal applied concentration resulted in cell rounding and complete floating compared to control plates. The above mentioned results showed clearly that different cell lines greatly differed in their reaction towards prepared nanoparticles depending on cell types and nanoparticle size. These results are in accordance with those previously reported[16,17]. Various cell types differ in their response to bioactive molecules due to their intrinsic dissimilarities in membrane structural composition and organization. Additionally, morphological examination results agree with that reported[18-20]. Authors observed similar severe morphological changes in MCF-7, HepG-2 and Vero cells upon treatment as a result for cell death through autophagy mechanisms. The disruption of the cell membrane activity by direct contact between nanoparticles and cell membrane is also reported to be responsible for antifungal activity of ZnO

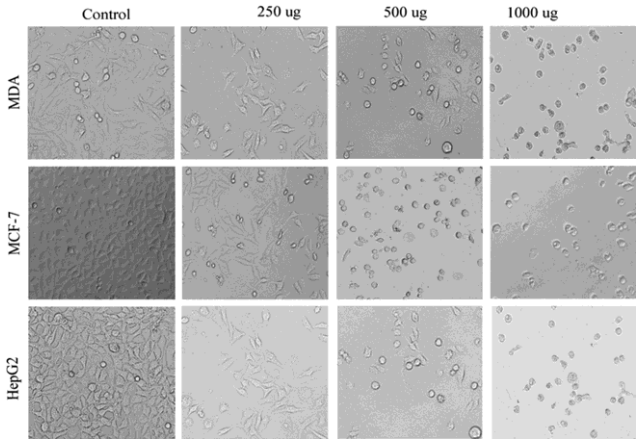

Fig. 2 — Changes in cell line morphology as affected by ZnO nanoparticles (40 nm) after 24 h. Images are captured using inverted contrast microscope (Nikon Eclipse T500) at 20x magnification.

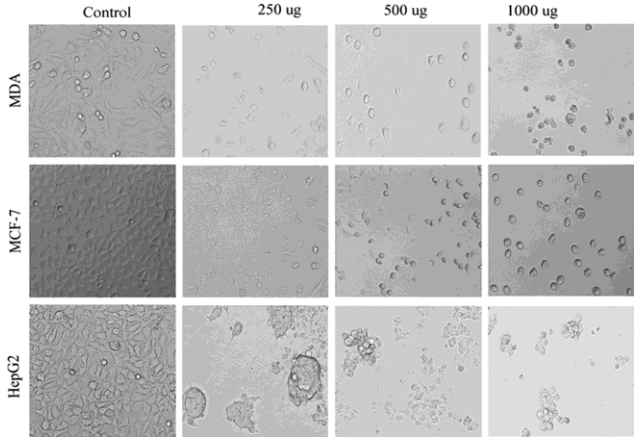

Fig. 3 — Changes in cell line morphology as affected by ZnO nanoparticles (50 nm) after 24 h. Images are captured using inverted contrast microscope (Nikon Eclipse T500) at 20x magnification.

nanoparticles. Binding nanoparticles to microbial cell membrane by direct or electrostatic forces affectsmembrane permeability and induces an oxidative stress status, thus preventing cell growth[21]. It was previously shown that the smaller the particle size, the greater will be the biological effect.In our current work, ZnO nanoparticles displayed remarkable anticancereffects. The inhibitory effect on cell viability suggests the generation of ROS to be the most likely mechanism. ZnO with its intrinsic defects (zinc vacancies) produced electron-hole pairs, which reacts with water producing H+ and OH− ions. These electrons converts dissolved oxygen into superoxide radical anions, which in turn react with H+ producing HO2• radicals. Those radicals are finally converted into hydrogen peroxide anions (HO2−), which then

react with hydrogen ions and produce hydrogen peroxide. Hydrogen peroxides have the ability to penetrate and disrupt cell membranesthrough their oxidative stress effect[22].

Conclusion

The current work aimed at investigating the anticancer activities of different sizes prepared ZnONPs (40 and 50 nm) against different cancer cell lines. Results revealed that both prepared nanoparticles affected cancer cells adversely depending on the applied concentration. Moreover, treated cells suffered from serious morphological changes due to nanoparticle exposure. ZnONPs possible affect cancer cells by enhancing ROS generation, and finally leading to cell death. Results suggest that ZnONPs can be effectively used to inhibit the growth and proliferation of different cancer cell types.

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