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Investigation the effects of green synthesized ZnO nanoparticles on the viability of L929 fibroblasts using MTT assay

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Abstract

Zinc oxide nanoparticles (ZnONPs) were prepared by eco-friendly method using the alcoholic extract of Ginger(*Zingiber officinale*) , this study conducted to evaluate the cytotoxic effect of Zinc oxide nanoparticles on viability of L929 fibroblast cell line for 72 hours and incubation with (1, 2, 4, 8, and 16 mg/ml) of GnZnO NPs , Several methods were used to characterize the biosynthesized GnZnONPs. including "UV-Visible Spectroscopy," Field Emission Scanning Electron Microscope ("FESEM"), " Infrared Spectroscopy (FTIR)" and X-Ray Diffraction (XRD) spectrum. the MTT assay was used to determine the cytotoxicity effect of Zinc oxide nanoparticles (ZnONPs) on the, cell line viability The results showed that there was no toxic effect of the synthesis GnZnONPs with an increase in concentration from (1-8) mg/ml after 72 hours of incubation at 37°C. The findings of this study, there was a time and dose-dependent reduction in cell viability in treated cell lines after exposure to GnZnO-NPs for 3 days, with no adverse effect on fibroblast cells till 8 mg/ml.

Keywords: ZnONPs , Zingiber officinale , MTT assay , L929 fibroblast

Introduction

Zinc is an important micronutrient that can be found in fortified foods and dietary supplement the ZnO nanoparticles exhibit antibacterial activity against a variety of bacteria, including *Salmonella enteritida, Staphylococcus aureus, E. coli, Listeria monocytogens*, and others, by creating Zn^{2+} ions and ROS, which cause cell organelle damage and cell death (1) (2). Nanoparticles can be produced in a variety of ways, including chemical, physical, and biological methods. Green synthesis utilizes plants, bacteria, fungi, yeast, enzymes, and other organisms. Researchers are increasingly turning to the green synthesis method because of the less toxic chemicals used, the eco-friendly nature of the method, and the one-step synthesis of nanoparticles, according to the researchers (3) Plant extracts were used to reduce, cap, and stabilize the metals that were made in the body. salts (ZnONPs). It has been found that ZnO nanoparticles have strong antibacterial properties, are the most effective because they are more toxic to microorganisms than larger ZnO nano particles (4). In a novel biosynthetic technique, zinc oxide nanoparticles (ZnO-NPs) are synthesized from a natural source in the presence of a catalyst. Compared to physical and chemical procedures, metal ions may be reduced in a manner that is easily scalable and

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harmless (5). Many different types of tests are used to determine the impact of potentially dangerous material (in this case, nanoparticles) on cells that have been grown in vitro. This test, which measures the influence of nanoparticles on cell viability, is known as the MTT. This colorimetric test may be used to determine cell viability, proliferation, and cytotoxicity in various cell types for assessing the capacity of nanoparticles to penetrate cells while simultaneously investigating cell distribution and the differences stages of the cell cycle (6) Zinc oxide is considered by the US Food and Drug Administration (FDA) to be a substance that is "generally regarded as safe" (GRAS) (7). ZnO has therefore been utilized in cosmetic and pharmaceutical products, including toothpaste, sunscreen, and textile coatings. Particularly in the biomedical field, environment, and industry—including electronics, textiles, tires, cosmetics, food processing and preservation, and so on—zinc oxide (ZnO) finds use in a variety of attractive applications. (8) The purpose of this experiment was to determine the cytotoxic activity of green synthesis of ZnO NPs by ginger extract at concentrations of 1, 2, 4, 8, and 16 mg/ml) on the viability of the L929 fibroblast cell line after incubation for 72 hours.

Material and method

Green-synthesis of ZnO nanoparticles (ZnONPs)

Green methods were used to synthesize ZnO nanoparticles; it is recommended to use 70% ethanolic ginger root (Zingiber officinale) extracts as mentioned in (9). Deionized water was used to perform 50 mL of 0.01 M zinc acetate dehydrate (Sigma Aldrich, Germany). While the magnetic stirrer was continually stirring, 500 ml of the extract solution was gradually added. After two hours of adding 1.0 M sodium hydroxide (Fluka, Germany) to the mixture to maintain the pH at 12, the liquid was centrifuged for ten min at 10,000 rpm. The pellets were cleaned in deionized water and then let dry for the full night at 200°C in a hot air oven. After carefully collecting the resulting white powder, it was then taken to be examined. The synthesized NPs were subjected to X-ray diffraction (XRD) techniques as well as the Fourier-transform infrared (FTIR) Shemadzu (Japan) spectrum in the 250–900 nm range and the UV spectrum (Schimadzu 1601 spectrophotometer).

Cytotoxicity and Cell Viability Assays:

Dimethyl sulfoxide (DMSO) was used to prepare ZnO nanoparticle solutions, which were then sonicated for a few minutes. Fibroblast cell lines and yellow tetrazolium dye were used . Fibroblast cell lines and yellow tetrazolium dye were used. In 96-well plates, cells were seeded in each well with 2 $X10^4$ cells in a 100-L volume/Dulbecco's Modified Eagle Medium, which was added to each well and incubated for 4 hours . The mixture was then incubated at 37 °C for 24, 48, and 72 hours at the desired concentration (1, 2, 4, 8, and 16) mg/mL in a 5% CO2 atmosphere. The control group was untreated. tetrazolium dye solution prepared as described in (11); the resulting colored solution was measured at absorbance at 570 nm with a microplate reader after the formation of purple formazan crystals (12). The darker the solution, the more metabolically active and viable cells there are in it. The growth of cultivated cells grown in the absence of a product was compared to the growth of cultivated cells grown in the presence of a NPs product as a negative control group, which represented 100% growth. The toxic effects of GnZnONPs on these cell lines were investigated and percentage of viable cells in each well was assessed by comparing them to the control cells, which were set to 100%.

percentage of cell viability formula

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Viable Cell % $\frac{\text{Viable Cells}}{Control} x\ 100\%$

Ethical approval:

The local Committee for Animal Care and Use at the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq, reviewed and approved all procedures. involved in the current study.

Statistical analysis

SAS was used to perform statistical analysis on the data (Statistical Analysis System - version 9.1). To assess significant differences between means, t -test and The ($P< 0.05$) was used to define statistical significance.

Results

2.1 Characterization of GnZnONPs

a-Fourier-transform infrared spectroscopy (FTIR) The N-H (amino) or amide groups, the Zn-O bond, and the C=O stretch, which indicate ketones, correspond to the peaks at (3430 cm-1), (415 cm-1), 1516 cm-1, 1384 cm-1, and 872 cm-1. Respectively. In this test was using to identified displays the biomolecular functional groups in ginger extract responsible for the reduction and stability of ZnO nanoparticles Figure (1) . The functional groups of biomolecules in the ginger extract that are in responsibility of ZnO nanoparticle reduction and stability , (13) (14.)

b-The X-ray diffraction pattern (XRD) :

the ZnO-NPs shows definite line broadening of peaks, indicating nanoscale particles. 31.79 °, 34.44 °, 36.27 °, 47.53 °, 56.57 °, 62.82 °, 66.32 °, 67.90 °, 69.03 °, 72.51 °, 76.88 °, 81.30 °, and 89.50 ° Bragg reflections with two values of 31.79 °, 34.44 °, 36.27 °, 47. (100), (0 02), (1 0 1), 102, (110), (103), (20 0), (112), (201), (004), (202), (104), and (203) are beyond the diffractions of ZnO nanoparticles to hexagonal planes (Figure 2).

c-UV-visible absorption spectrum

According to the spectra, the existence wavelength of 370 nm indicated that ZnO NPs exist (Figure. 3).

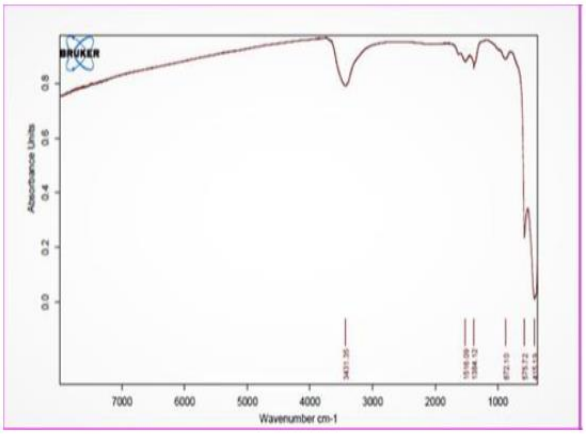

Figure (1): The FTIR spectrum of ZnO-NPs reveals the effective green creation of ZnO nanoparticles that were influenced by the metabolites in the plant extract

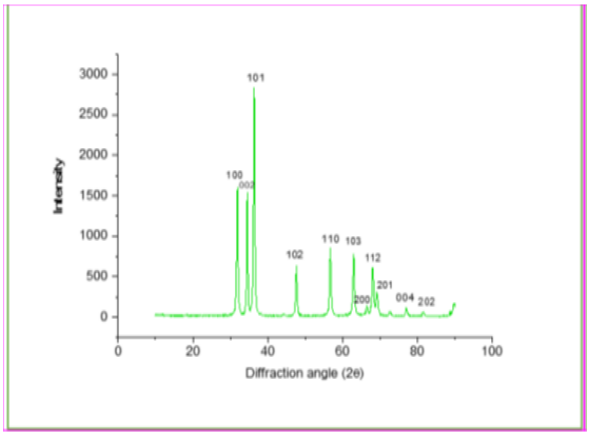

Figure (2) The X-ray diffraction pattern of ecofriendly synthesis ZnO nanoparticles.

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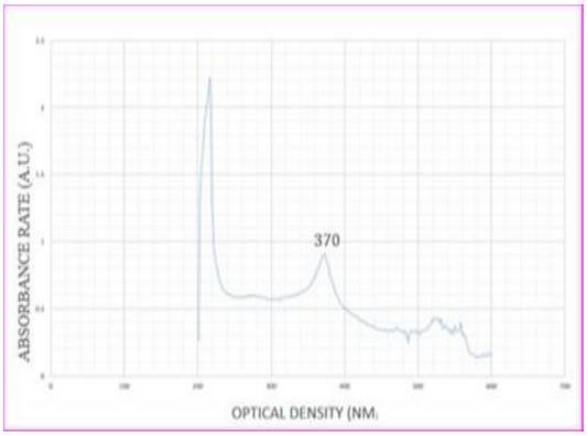

Figure (3) Green synthesis GnZnO NPs as measured by a UV-Vis spectrophotometer, showing a distinctive peak (at 370 nm).

d- Cytotoxicity and Cell Viability using MTT Assays

Table (1), shows the percentage of cell viability of L929 cells after incubation with (1, 2, 4, 8, and 16 mg/ml) for 24, 48, and 72 hours. of GnZnO NPs treatment. The L929 cell without treatment was used as control There was a significantly different ($P<0.05$) in cell viability between all concentrations during periods of treatments, the cell viability decreased significantly as ZnONPs concentrations increased from 1 mg/ml to 16 mg/ml,

Table (1). Cell viability After 24,48 and 72 hours of incubation, with Zno nanoparticles.

Zno nanoparticles (conc.)	Time of incubation		
	24 hr	**48 hr**	**72 hr**
Control (T1)	**A99.00±0.57a**	**B97.33±0.33a**	**B95.00±0.57a**
(T2) 1mg/ml	**A98.33±0.66a**	**B95.66±2.02a**	**B92.13±1.27a**
(T3) 2mg/ml	**A93.20±1.90b**	**B87.46±1.01b**	**C83.66±0.81b**
(T4) 4 mg/ml	**A87.40±0.96c**	**B82.53±0.98c**	**B79.36±0.49b**
(T5) 8mg/ml	**A78.36±0.43d**	**B76.40±1.00d**	**C73.43±1.79c**
(T6) 16/ml	**A75.00±1.73d**	**A71.77±0.57d**	**B69.06±2.31d**
LSD	**3.60**		

The experiments were carried out 3 times. The data was presented as the mean (standard error of the mean) and analyzed using one-way ANOVA. Means with a capital letter in the same row differ significantly ($P<0.05$). Means with capital letters in the same column differ substantially ($P<0.05$).). Control (T1)= Untreated (T2) 1mg/ml (T3) 2mg/ml (T4) 4 mg/ml (T5) 8mg/ml (T6) 16/ml cells At 24, 48, and 72 hours after treatment**.**

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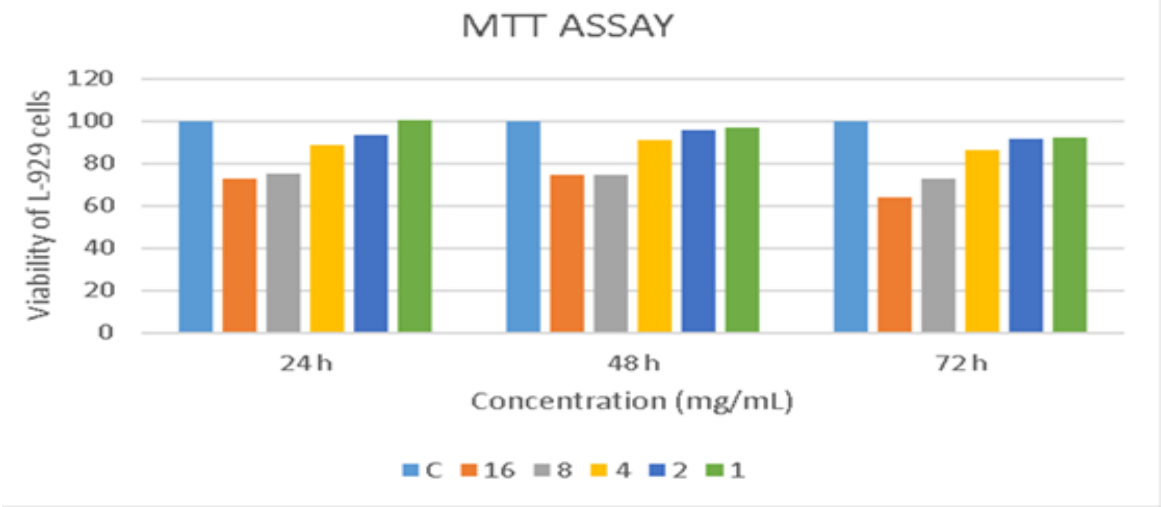

Figure (4). Cell viability After 72 hours of incubation, with Zno nanoparticles.

Discussion

3.1 characterization of ZnO nanoparticles

X-ray diffraction pattern (XRD) result showed capping agent stabilizing the nanoparticle may have caused these sharp Bragg peaks. All of the peaks matched the (JCPDF Card No. 01-079-2205) (15). The average crystallite size of the synthesized ZnO nanoparticles was estimated (24.36 nm) using Scherrer's equation from sharp diffraction peaks (100), (002), and (101). According to the spectra, the existence of a strong absorption band with a maximum wavelength of 370 nm indicated that ZnO NPs exist (Figure. 3). This sharp peak indicates that the particles are nanosized, which is consistent with previous observations of ZnO NPs (16).

3-2 Cytotoxicity and Cell Viability using MTT Assays

The MTT test was used to evaluate the cytotoxicity, proliferation, and viability of cells by metabolic activity while assessing the cytotoxicity effects of the green ZnO-NPs in vitro. GnZnO-NPs concentrations and time-dependently significantly (P 0.05) reduced the proliferation of the treated L929 cell lines, while the untreated (positive) control cell was set to 100% viability. (17), . In MTT assay Yellow tetrazolium dye is converted to formazan crystals by NAD(P)H-dependent oxidoreductase enzymes in life cells. The rate of formazan crystal generation directly correlates with cell viability. According to the findings of this research, the average cell viability was above the nontoxic limit for concentrations ranging from 1 mg/ml to 8 mg/ml. However, a concentration of 16 mg/ml may be poisonous, and the nanoparticle was considered unsafe when cell viability was less than 70%. (18). At 8 mg/ml considered harmless concentration, the average cell survival was (73.43%), although the exact toxicity mechanism is still unknown. According to certain research, ZnO properties like size, surface characteristics like surface charge and surface defect, and dissolution could all have an impact on how cytotoxic ZnO is to cells (19). Reactive oxygen species (ROS) produced by the ZnO surface may have been the source of the toxicity of ZnONPs. The dissolved Zn2+ ions in the mixture may also

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have contributed to the toxicity effect and cell membrane damage. Increased ROS levels cause serious damage to cells' DNA, which stops the cell cycle and ultimately leads to cell death. (20). The major mechanism behind ZnONP cytotoxicity is the intracellular release of soluble zinc ions, which is followed by ROS induction. This action is caused by a binary response, which includes the cell's pro-inflammatory response to ZnONPs as well as the nanoparticle's unique surface feature, which allows ZnONPs to behave as a redox system (21). The adverse effects of ZnO on normal cells are heavily influenced by their size, shape, and concentration, as well as the cultivation duration and cell type in general. Many cell lines are toxic to ZnO at different concentrations, sizes, and shapes. (22) L929 fibroblast cells, which were round and dull (23) due to greater dissolution Zn2+ ions and produced ROS, were killed by a high concentration of ZnO NPs. At all concentrations tested, ZnO NPs were cytotoxic to L929 normal fibroblast cells ($p < 0.05$). at 24 h and48 h .µg/ml after 72 hours. ZnO-NPs caused a slight decrease in the percentage of viable cells. Our result similar to (24)) All of these results indicated that variables in production, dosage, and duration of exposure all affected ZnO nanopowder's cytotoxicity., cell type, and proliferation, and the results were comparable to those reported in previous studies. (25) and (26), regardless of the type of synthesis.

Conclusions: According to the study's findings, treated cell lines exposed to GnZnO-NPs for three days exhibited a time- and dose-dependent decrease in cell viability; fibroblast cells were not negatively affected until 8 mg/ml.

Conflict of Interesst: The authors declare that there is no conflict of interest

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