Original Article

Comparison the effects of Cds-Au nanoparticles on viability of normal and cancer cell lines and expression of P53 genes

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Abstract

Background and Objectives: Nanoparticles have been given a solution to many medical and industrial problems and other applications through their small size and ease of controlling their physical and chemical properties, as well as merging them with other materials to give them different properties used in multiple fields, including the field of medicine. This study aims to test the toxicity of Cds-Au NPS on cancerous and normal cell lines and apoptosis gene expression.

Results: IC50 value of Cds-Au NPS in Vero (normal cell model) 19.50 µg /ml while in MCF-7 (cancer cell model) 57 µg /ml. Effect of embedded nanoparticles on the phenotypical morphology of cell lines by AO and E & H The results show the degradation of the cytoplasm in cells and apoptotic cells vacuole degeneration, cell-free spaces, the remains of the dissolved cells, the floating cells, and enlarged and thickened nucleus. When the cell lines were treated with Cds-Au NPS, it showed an effect of elevation in p53 expression in treated cancer cells more than 14 times that of treated normal cells. While PTEN gene and P21 gene is higher in the cancer cells mor than normal cells.

Conclusions: CdS-Au NPs Show high toxicity effect on both cancer and normal cell lines also activate the P21, P53 and PTEN gene in MCF-7 cells.

Keywords: Antiapoptotic, AuNPs , Cds-AuNPs , P53, P21

INTRODUCTION

Cancer is a life-threatening disease and is considered the second most dangerous disease in the world. Previously, it was treated with familiar techniques such as chemotherapy, immunotherapy, gene therapy and other treatments. These remedies have a toxic effect on human life. Therefore, the current development in the world of research focuses on nanotechnology to address many problems in various fields, especially the field of nanomedicine.

Controversially, nanoparticles have several advantages, including their small size, equivalent to 100 nanometers, ease of control, shape, catalytic ability, the advantage of delivering drugs to target sites without harming the right places, and the ability to alter physical and chemical properties by combining them with other materials (1,2).

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Gold nanoparticles have several physical properties that made it unique, including its ability to absorb and scatter light depending on the refractive index and particle size, which gives different colors of gold. Gold nanoparticles with a spherical shape and a small size of about 30 nm have the ability to absorb LSPR at a wavelength of 530 nm, control the surrounding environment and also change their properties through the aggregation property (3).This study aims to test the toxicity of Cds-Au NPS on cancerous and normal cell lines and apoptosis gene expression.

Materials And Methods

Tessting the effects of nanoparticle on growth of cancer cell lines

The toxicity test of the combined nanomaterial using MTT was revealed according to (4) when monolayer of cells was obtained for (VERO & MCF-7) they were cultured at 96 wells for each well 100 µl and after 24 hours the cells were treated with a series of concentrations (1, 6,12.5,25,100 and 250) µg/ml for 48 hours later, the media and the nanomaterial are removed and MTT staining is performed, and the toxicity is calculated by the following equation:

Cytotoxicity = (A-B) / A X100

Where A: represent the means optical density of control wells.

B: represent the optical density of treated wells.

Characterization the morphology of dead cells

Eosin & Hematoxyline stain

After trypsnization and cell suspension in RPMI1640 medium containing 10% FBS Suspended cells were cultured at a ratio of 1×105 cell/well on clean and sterilized slide cover using petri dish capacity of 5 ml i.e. 1000 µl in Well, after 24 hours of culturing the cells on the slide cover and forming a monolayers presented cells to IC50 of Cds-Au NPS, and then the staining procedure were applied following the protocol of (5).

Staining with acridine Orange

Dissolve 5 mg of acardine dye powder in one ml of absolute ethanol, and 3 mg of dye powder is dissolved. Ethidium bromide in 1 ml of absolute ethanol. 1µl of the dye is taken and 1µl of the dye is diluted and dilute in 1 ml of PBS. The dye is prepared in dark conditions and at room temperature , this dye is for the purpose of detecting the viability of cells and the occurrence of programmed cell death of treated and untreated cells for the nanomaterial used in the current study, which are grown on the slide cover and examined by fluorescence microscopy . the red color indicates dead cells, and the green color indicates living cells. . Next , stain cover slid (6).

Converting the RNA of cell lines to cDNA

For converting the genome of cell lines from RNA to cDNA the kit of Accupower RocketScript ^{RT} Premix from (Bioneer) kit, CA: K-2101 was selected which work on reverse transcriptase enzymes principle.

Steps to convert RNA to cDNA according to Accupower RocketScript ^{RT} Premix from (Bioneer), as sumerızed below:

Maser mix preparation: The final volume of master mix reaction consist from (Oligo dT 20 1µl, Template RNA 5 µl, Free DEPEC-D.W 14 µl) respectively.

Reverse transcriptase procedure: for applying this steps the following temperature and time was programmed , first (Primer annealing (oligo dT 20) was subjected at 25 °C for 10 minutes, cDNA synthesis steps were at 45 °C for 60 minutes , while heat inactivation step were programmed at 45 °C for 5 minutes).

qPCR Primers

Sequences of PTEN, P21, P53 and β-actin primers that were used in qPCR techniques as mentioned in (7,8,9).

Real Time PCR reactions preparation

The components of Real Time PCR reaction were prepared according RealMOD™Green SF 2X qPCR mix from iNtRON kit (9).

Results

Cds-Au NPs

Absorption results were shown after cancer cell lines (MCF-7) and normal cell lines (VERO) were exposed to CdS-Au NPs with different concentrations (1, 6, 12.5 , 25 , 100 , 250 µg / ml) for 48 hours, the highest rate of viability was at a concentration of 250 µg / ml and the lowest rate of viability at a concentration of 12.5 µg/ml. The IC50 value for Vero is 19.50 µg/ml and MCF-7 57 µg/ml (Figure-1).

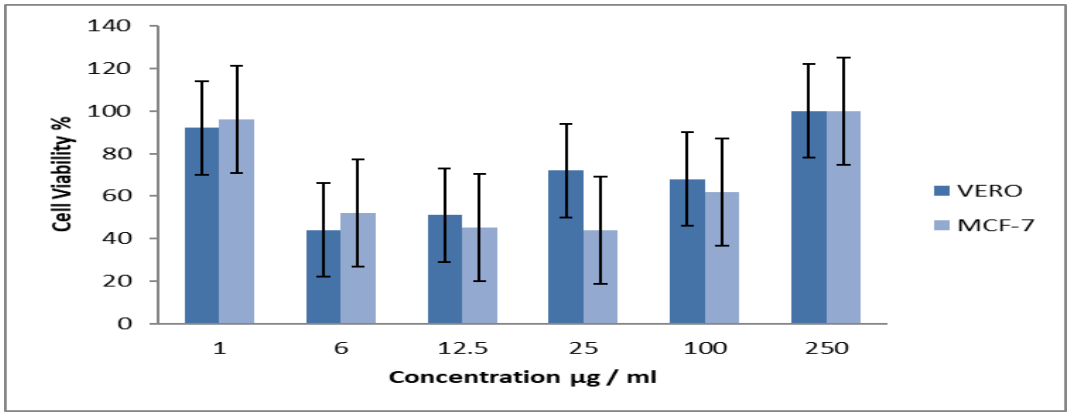

Figure 1 : Cytotoxic effects of concentration of CdS-Au NPS on viability of Vero and MCF-7 cell line after 48 h . Cells were treated with (1, 6, 12.5, 25, 100, 250 µg / ml) of CdS-Au NPS for 48 hours (n =3).

Effect of CdS-Au NPs on morphology of Vero and MCF-7cell lines

The VERO and MCF-7 were stained on the cover slide after treatment IC50 of CdS-Au NPs for 48h by E & H and examined under light and fluorescent microscopy phenotypic changes were diagnosed and examination results revealed that CdS-Au NPs lead to cellular pathological changes that include the distribution of cells in the cell culture and their shape as well as changes in the cell structure (nucleus and cytoplasm) (Figures 2,3 & 4).

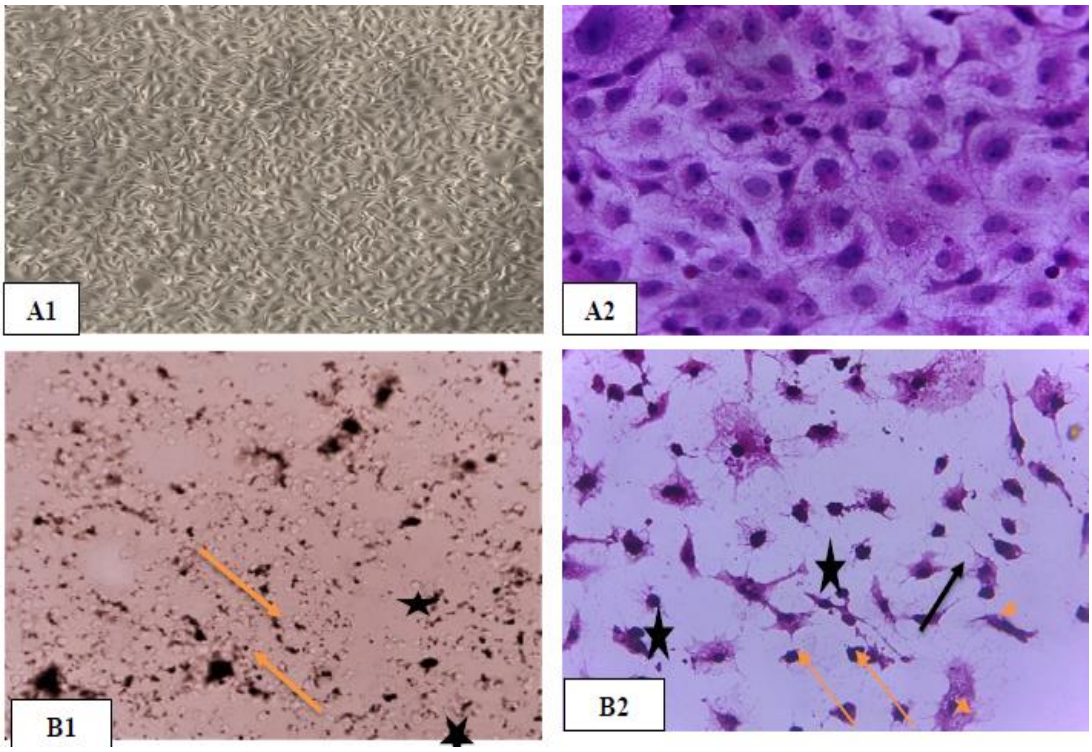

Figure 2: Vero cell lines after 48 hours under the light microscope (10x). A1;control confluent monolayer (unstained), A2; stained of control confluent monolayer,B1; The treated cells with IC50 of CdS-Au NPS 10X (unstained), B2; stain cell treated with IC50 of CdS-Au NPS (40X). The degradation of the cytoplasm in cells (yellow arrow), apoptotic cells (arrowhead), vacuole degeneration (black arrow), and cell-free spaces (stars).

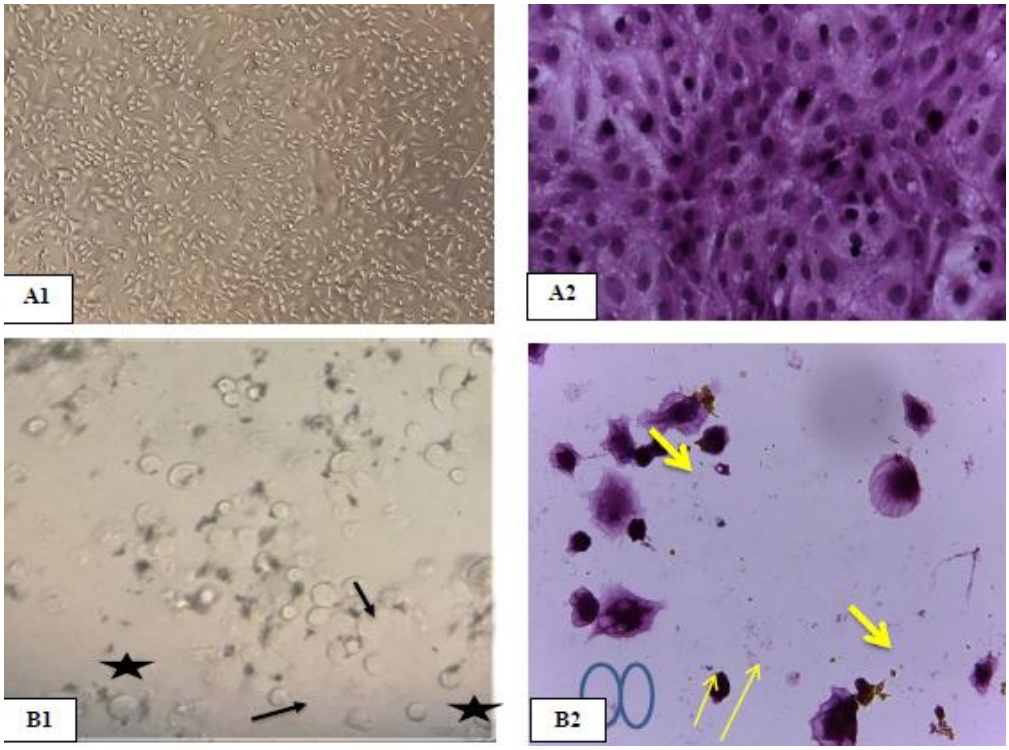

Figure 3: Vero cell line after 48 hours under light microscope. A1; of control confluent monolayer (unstain), A2; stain of control confluent monolayer,B1; unstain Cell treated with IC50 of CdS-Au NPS (10X),B2; stain Cells treated with IC50 of CdS-Au NPS (40X). The degradation of the cytoplasm in cells (yellow arrow), apoptotic cells (circle), cell-free spaces (stars), the remains of the dissolved cells (double arrow), the floating cells (black arrow), and enlarged and thickened nucleus (blue arrow).

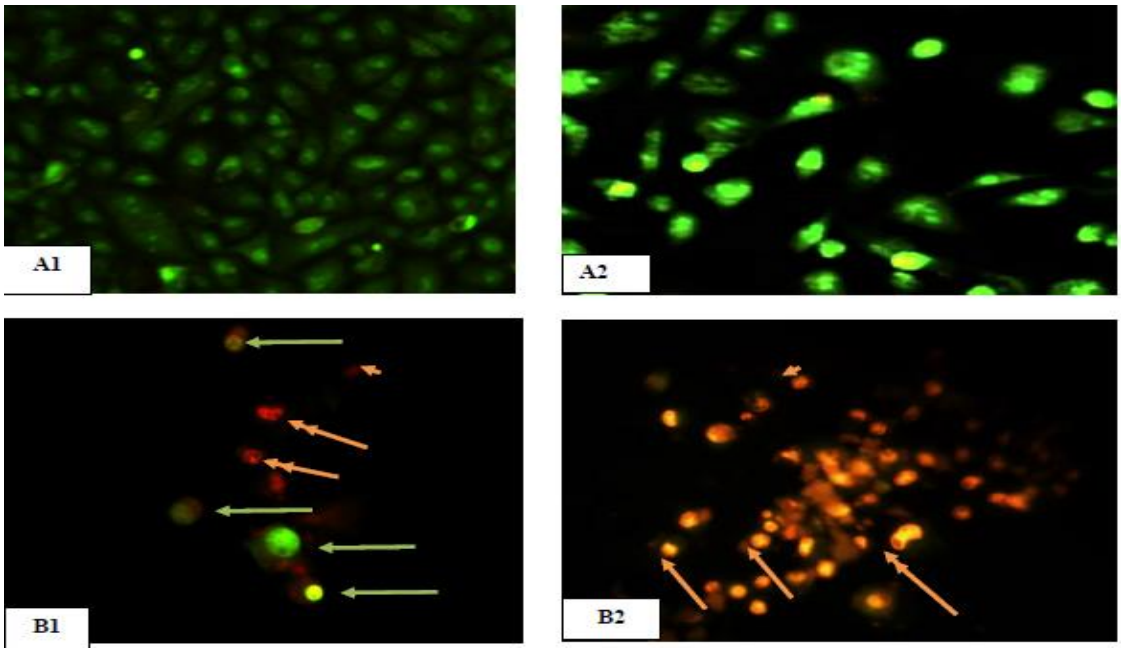

Figure 4: Fluorescent Microscope of of VERO & MCF-7 cells (A1) monolayer at control of vero cell line (40X) (A2) Monolayer at control of MCF-7 cell line (100X) (B1) Vero cell line were treated with the IC50 dose of CdS-Au NPs for 48 hours. the cell death (yellow arrow), the remains of dead cells (arrowhead), the fragmentation of nucleus (double arrow), and the early stage of apoptosis (green arrow). (B2) MCF-7 cell line with IC50 of CdS-Au NPs for 48 hours. The cell death (yellow arrow), the remains of dead cells (arrowhead), and the fragmentation of nucleus (double arrow).

Detectıon of relative expression of P53 genes in Vero and MCF-7 cell lines

Gene expression of p53 gene was detected in cell lines treated with IC50 of Cds-Au NPS. The results showed that the gene was 14 times higher in cancer cells (MCF-7), in contrast to normal cells (VERO) a slight increase in the gene was observed (Figure 5).

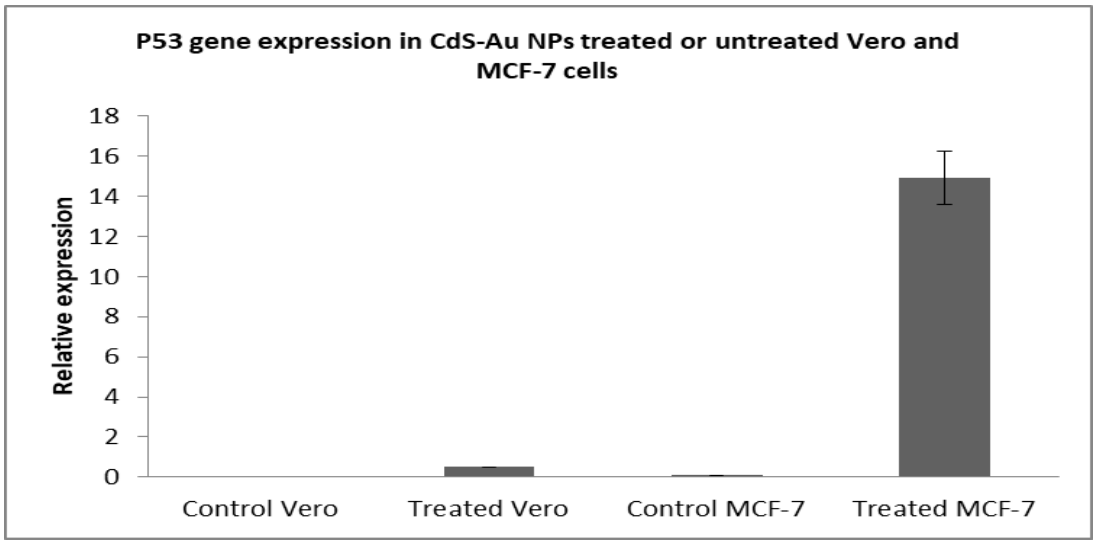

Figure 5: Detectıon of relative expression of P53 genes in Vero and MCF-7 cell lines under two treatments with and without CdS-Au NPs

The RNA genome were extracted and then subjected for reverse transcribed step, after that the cDNA genom were used as a template for assaying expression of P53 genes using SYBR green master mix reaction. For analyzing the data the ΔΔ CTs and normalized to (β-actin) house-keeping gene were used (p-value= 0.005,0.006) respectively.

Detection the expression of P21 genes in both Vero and MCF-7 cells lines

The effect of IC50 of Cds-Au NPS on gene expression assays of the p21 gene, one of the most important apoptosis genes, was tested. It was found that the effect of the prepared nanoparticles is high on the gene elevation of treated cancer cells (MCF-7) compared to the treated normal cells (VERO) (Figure 6).

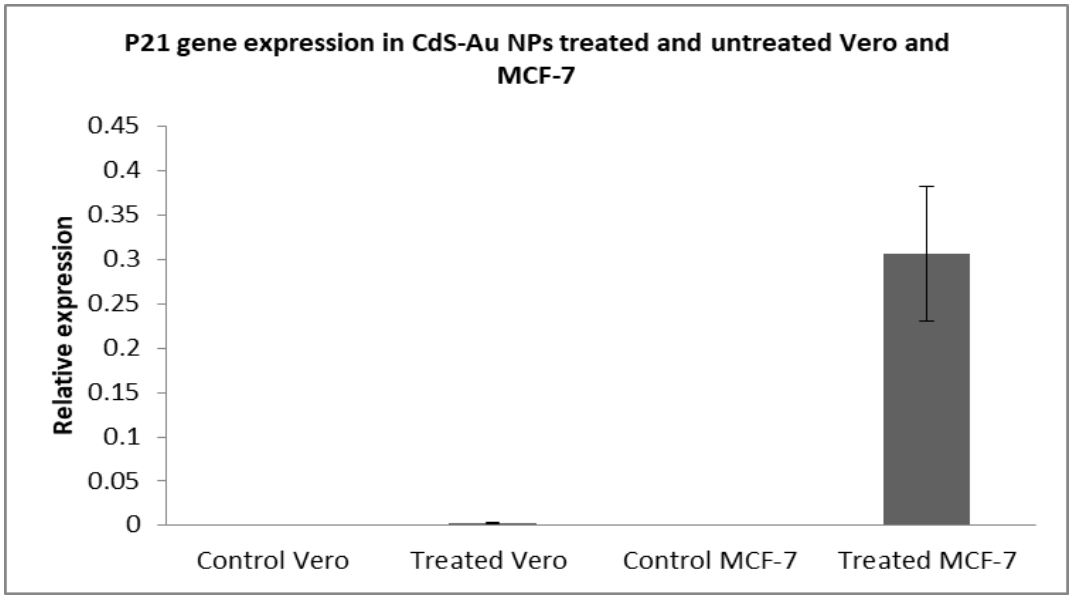

Figure 6: Detection the relative expression of P21 genes in both Vero and MCF-7 cells lines with and without treatement with CdS-Au NPs.

The RNA genome were extracted and then subjected for reverse transcribed step, after that the cDNA genom were used as a template for assaying expression of P53 genes using SYBR green master mix reaction. For analyzing the data the ΔΔ CTs and normalized to (β-actin) house-keeping gene were used (p-value= 0.005,0.007s) respectively

Gene expression of PTEN in Vero and MCF-7 cell lines

The gene works by manufacturing a protein that acts as a tumor suppressor and also maintains cell growth and division by removing the phosphorous group. Treatment with CdS-Au NPs increases its expression only in MCF-7 compared to the control sample and Vero cells (Figure 7).

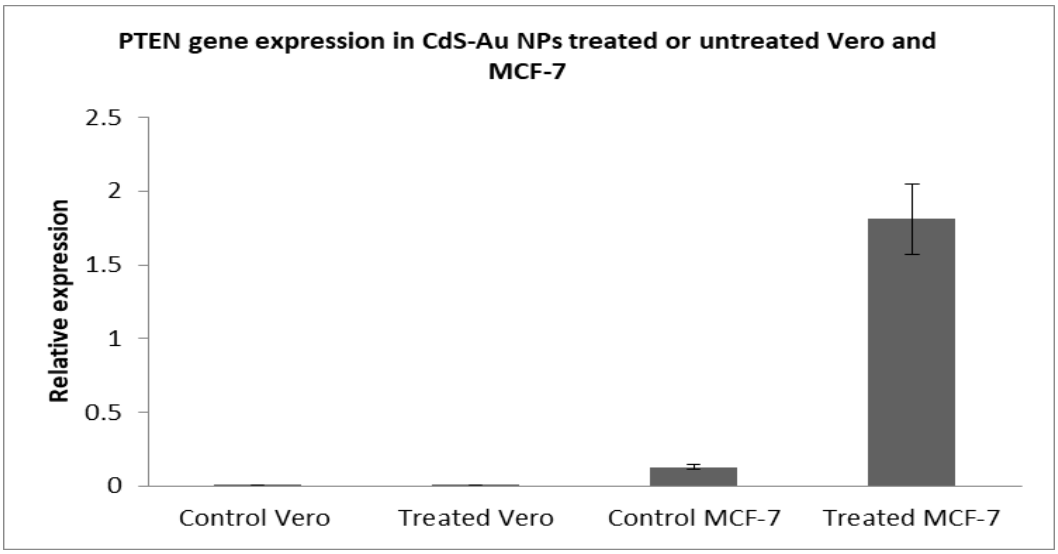

Figure 7: Relative gene expression of PTEN in Vero and MCF-7 cell lines under two type of treatment (with and without CdS-Au NPs).

The RNA genome were extracted and then subjected for reverse transcribed step, after that the cDNA were used as a template for assaying expression of P53 genes using SYBR green master mix reaction. For analyzing the data the ΔΔ CTs and normalized to (β-actin) house-keeping gene were used (p-value= 0.004, 0.005) respectively

Discussion

Effects of Cds-Au NPs on cell lines

Treating normal and cancer cells with different concentrations at IC50s of CdS-Au for Vero and MCF-7 for 48h . Our data come consistent with Hossain and Mukherjee (10) results as exposing HeLa cells to CdS NPs to upregulate the reactive oxygen response and kill the cells . CdS NPs stimulate the programmed cell death by producing ROS, mitochondrial damage, oxidative stress, and chromatin condensation (11). Many biological systems are affected with the treatment with CdsNPS since it is highly toxic by releasing Cd2+ ions inside the cells (12).

In this study, the prepared CdS-Au NPs is made up of Cds shell and Au core. Cadmium toxicity is attributed to cadmium ions that enter the cell through the calcium channels in the plasma membrane and adhere to the cytoplasmic and nuclear materials. The incoming causes DNA damage, chromosomes abnormalities, and blocks the synthesis of nucleic acids and proteins (13) . Thus, its effect eventually leads to induce oxidative stress and the triggers the programmed cell death (14). Agree with (15), treating HepG2 cells with CdS NPS reduces the cell viability and stimulates peroxide due to increased ROS, DNA damage, and programmed death. Low levels of glutathione and Cd affinity to proteins lead to the interaction with sulfhydryl groups cause structural deformation and changes in catalytic activity of enzymes (16). Cd genetic toxicity and disturbing effect belonging to the catalytic actions of enzymes are attributed to stimulate several responses inside the cells such as, increased oxidative stress, inhibited antioxidant response, stimulation of lipid peroxide, abolished Calcium balance,, interaction of Cd with sulfidyl-peptides, and upregulated production of reactive oxygen species (17) .

Morphological Analysis of Nanoparticles effect on cell lines (Apoptosis detection)

Effect of embedded nanoparticles (Cds-Au NPS) on the phenotypical morphology of (MCF-7 and VERO) cell lines by AO and E & H for 4 8h. The effect is detected as apoptosis or necrosis compared with the untreated controls. We found when treating VERO and MCF-7 cells with CdS-Au NPs and analyzing the results with acridine orange staining show presence of necrotic cells, cell atrophy, cellular extensions, and formation of cytoplasmic cavity (18) mention that treatment of IP15 and HK-2 cells with CdS NPs, results in morphological changes such as spherical shape, weakened

cell adhesion and irregular nucleus shape, especially with CdS NPs. In HeLa cells, exposure to CdS NPs changes their morphology by intense and segmented nuclei (19).

Effect of Nanoparticles on P53 expression

By treating VERO and MCF-7 with IC50 concentration of CdS-Au NPS for 48 hours, the results reveal that the expression of P53 in MCF-7 treatment is 16 times higher compared to the control while relative increases of P53 in VEO cells. Another study proves that nanoparticles and reactive oxidizing species cause DNA damage and activation of P53 and proteins associated with DNA repair (20).

Au NPs shows a cytotoxic effect in a dose dependent manner accompanied with high elevation of P53 mRNA (21). In general, cadmium augments the gene expression of P53 and other early genes such as fos and c jun and other defensive proteins (22). Combination of Au and other nanoparticles can be used to treat lung cancer with a significant increase in P21 and P53 (23).

Effect of Nanoparticles on P21 expression

The effect of CdS-Au NPs on gene expression of P21 in Vero and MCF-7 cells where there is an increase P21 expression in treated cancer cells is compared to control and there is no expression in normal cells. Combination of Au and other nanoparticles can be used to treat lung cancer with a significant increase in P21 and P53 (24).

Effect of Nanoparticles on PTEN expression

The resulting data show a higher elevation of this gene in treated cancer cells compared to normal cells. PTEN is an antitumor protein that when the gene is switched on, it suppresses the growth and development of cancer cells (25,26). The enzyme manufactured by PTEN sends signals that stop cell division and fight cells to programmed death (27) ,By activating it. In prostate cancer apoptosis was enhanced by PTEN by treatment with embedded nanoparticles (PL) (28).

Conclusion

In cytotoxicity testing, CdS-Au NPs Show high toxicity effect on both cancer and normal cell lines. Moreover, CdS-Au NPs activate the P21, P53 and PTEN gene in MCF-7 cells.

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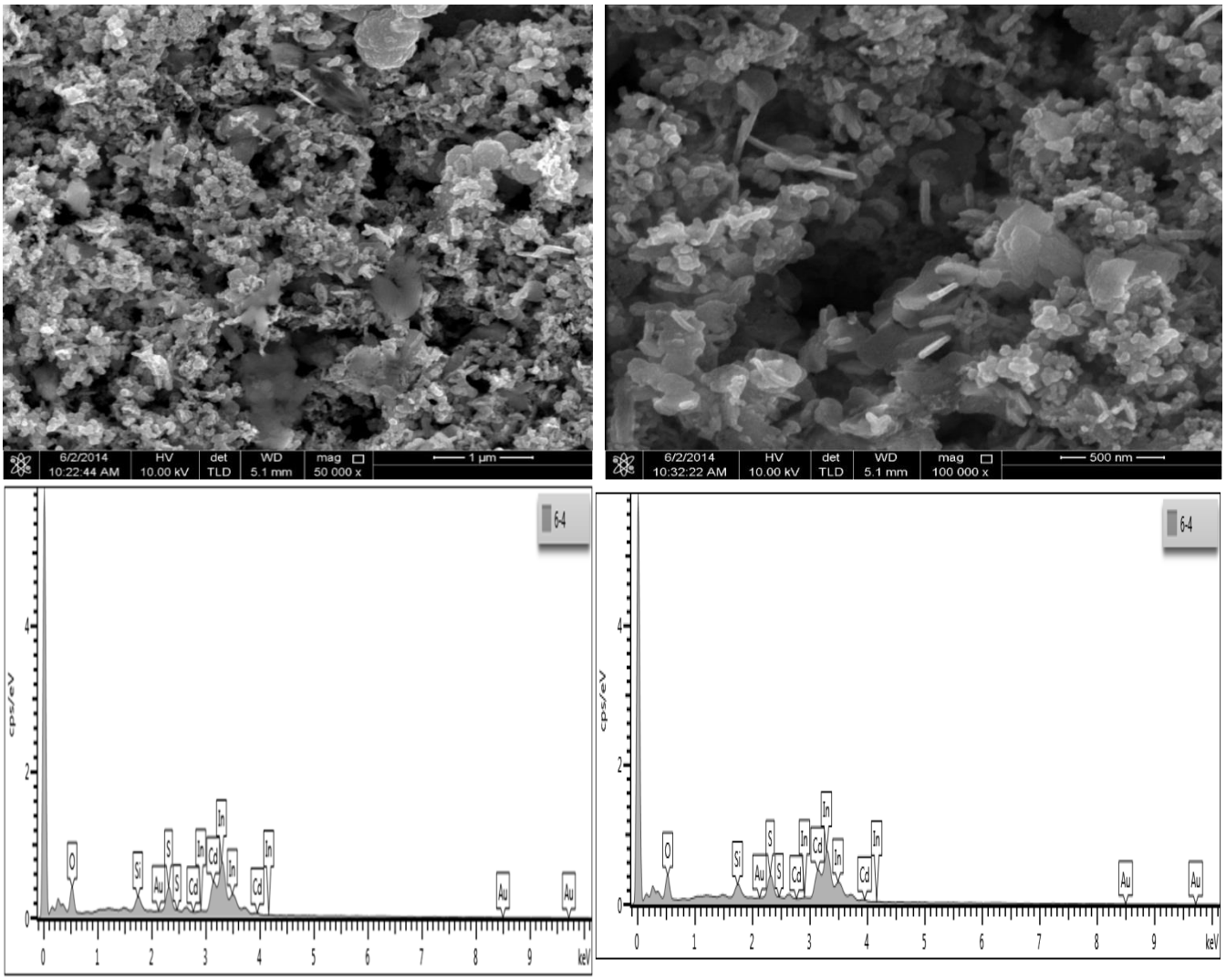

Figure: FESEM image for Cds-AuNPs prepared by A) Electrochemical method, B) particles size distribution