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IN VITRO CYTOTOXIC STUDY FOR COMPARISON BETWEEN EFFECT OF GOLD AND SILVER NANOPARTICLES AGAINST CANCER CELLS LINES

SUMAIAH I. HUSSEIN[1], NAWFAL N.R. AL-RAWI[2], SHAIMAA S. MUTLAK[1], MAYSSA K. AL-MALKY[3]*, HEBA F. HASSAN[1] AND NAHI Y. YASEEN[4]

[1]Department of Basic Science, College of Dentistry, University of Baghdad, Baghdad-Iraq
[2]Department of Life Science, College of Science, University of Anbar, Anbar-Iraq
[3]Tropical-Biological Research Unit, College of Science, University of Baghdad, Baghdad-Iraq
[4]Department of Oncology, Iraqi Center for Genetics and Cancer Research, Al-Mustansiriy University, Baghdad-Iraq

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Abstract – Cancer is a large problem in the world, so the traditional treatment is not efficient in eradication the body from cancer cells. Recently, Nanotechnology in medicine involves applications of nanoparticles currently under development, especially the nobel elements such as gold and silver. In the present study, we investigated the effects of AgNPs and AuNPs on cytotoxicity of two cell lines Hepatocyte cancer cell line for mice (HC) and breast cancer cell line for human (MCF-7), Five concentration (0.625-10) μg were used from each particle and for each cell line for comparison between the effect of AgNPs and AuNPs. The study reveals that Au NPs and Ag NPs have effect on this cell line but the AuNPs have more effect than the Ag NPs, so the effect is related to concentration and cell type-dependent manner.

INTRODUCTION

Cancer is a leading cause of death group worldwide. According to WHO, deaths from cancer worldwide are projected to continue rising, with an estimated 11.5 million deaths in 2030 (Plummer *et al.*, 2012). Cancer therapies are currently limited to surgery, radiation, and chemotherapy. All three methods risk damage to normal tissues or incomplete eradication of the cancer; therefore nanotechnology offers the means to target chemotherapies directly and selectively to cancerous cells and neoplasms, guide in surgical resection of tumors, and enhance the therapeutic efficacy of radiation-based and other current treatment modalities. All of this can add up to a decreased risk to the patient and an increased probability of survival (Bertrand *et al.*, 2014). Inorganic nanoparticles (NPs) have attracted increasing attention and are used in many fields, such as biomedicine, various industries, and electronics due to their excellent physicochemical properties (Sau *et al.*, 2010; Dziendzikowska *et al.*, 2012). Currently, silver nanoparticles (AgNPs) are one of the most widely used NPs in commercial products (*e.g.* wound dressings, contraceptive devices, and packaging materials) because of their strong antimicrobial and anti-inflammatory properties (Dziendzikowska *et al.*, 2012; van der Zand *et al.*, 2012). Also the gold nanoparticles (AuNPs) offer a wide range of applications including cosmetics, chemical sensing, drug carriers, bioimaging, and gene therapy (Boisselier *et al.*, 2009; Ghosh *et al.*, 2008). Several research are used Au NPs and Ag NPs in many application for cancer treated in vitro and in vivo such as anti-metastasis (Li *et al.*, 2008), anti-angiogenesis (Kovács *et al.*, 2016; Hussein, 2016), anti-inflammatory (Gnanasundaram *et al.*, 2017), photothermal therapy (Neshastehriz *et al.*, 2017), apoptosis, autophagy and necrosis activity (Sun *et al.*, 2018; Zielinska *et al.*, 2018).

MATERIALS AND METHODS

Characterization of Silver and Gold Nanoparticles

Colloidal gold nanoparticles and silver

nanoparticles were prepared by Chinese Company, then the analysis was done as following:

UV–VIS Absorbance Spectroscopy Analysis

An absorbance spectrum of the silver and gold nanoparticle solution was measured by UV-visible spectrophotometer (Metertech SP-8001-Tiwan).

Concentration Measurement

Concentration (μg/mL) of AgNPs and AuNPs were determined using atomic absorption spectroscopy (model Nov AA350, Germany).

Atomic Force Microscope Measurement

Atomic force microscope (AFM) examination (SPM AA3000 Angstrom Advanced Inc, USA) was employed to take topography. Surface roughness analysis and particle size distribution were carried out with CSPM software. Sample preparation for AFM examination was carried out. Briefly, a glass slide 10×10 mm was cleaned with D.W then absolute ethanol and dried in ear forced oven. A drop of the sample solution of proper dilution was placed on the cleaned glass slide; the drop was dried in an argon stream chamber, and then examined in AFM.

Cytotoxicity Experiment

Two cancer cell lines, Human breast cancer cell line (MCF-7) and Hepatocyte cancer cell line (hepatoma) for mice (HC) was provided by Iraqi Center for Genetics and Cancer Research, Al-Mustansiriy University (Baghdad, Iraq) and used throughout this study. it was propagated and maintained on Rosswell Park Memorial Institute medium (RPMI-1640, US biological, USA), To this media, 10% fetal bovine serum (Cellgro, USA) and 1% penicillin/ streptomycin (Cellgro, USA) were added and incubated in a humidified 5% CO_2 incubator at 37ºC. The cells were subculture after they had achieved 80-90% confluence which can be observed under inverted microscope (Majeed, 2015). Cell viability was assessed by using trypan blue exclusion test and found to be greater than 99% (Phelan, 2018).

The cytotoxicity of AuNPs and AgNPs on cancer cell lines was examined according to the inhibition of proliferation rate (IR %). In a 96 well tissue culture plate, seed about 2 x 104 cells per well in 200 μl antibiotic-free normal growth medium supplemented with FBS. Incubate the cells at 37° C in a CO2 incubator until the cells are 60-80% confluent. This will usually take 18-24 hours. Then it wills exposure with different concentrations of AuNPs (0.625, 1.25, 2.5, 5 and 10) μg/mL, as well as the same thing for Ag NPs. After that the plates were incubated at 37Cº for 24hrs. After incubation, 20 μL of MTT [3-(4, 5- dimethylthiazol-2-yl-2-2.5-diphenyltetrazolium bromide)] was added and incubated for further 4 hrs at 37 °C. The untreated cells were also done as control. The absorbance of treated and untreated cells was measured at 492 nm. The inhibitory rate of cell proliferation was calculated according the equation: IR%= A–B/A×100, Where A represents the absorbance of untreated cells, while B represents the absorbance of treated cells.

Statistical Analysis

The Statistical Analysis System, SAS (2012) was used to identify effect different factors in study parameters. Least significant difference –LSD test was used to assess differences between means**.**

RESULTS AND DISCUSSION

Characterization of Gold and Silver Nanoparticles

Figure 1 shows the percentage of diameter ranges for AgNPs is about (60-90 nm) by AFM analysis, their particle size distribution, 2-Dimension image, and 3-dimension image.

Figure 2 shows the percentage of diameter ranges for AuNPs is about (60-85 nm) by AFM analysis, their particle size distribution, 2-Dimension image, and 3-dimension image.

Stock Concentration

The stock concentrations of each AuNPs and Ag NPs were about 20 μg/mL from source, which it confirmed through re-measuring the concentrations by using atomic absorption spectroscopy (AAS) (Figure 3).

Uv–Vis Absorbance Spectroscopy Analysis

From our previous results, we showed that AgNPs and AuNPs inhibited proliferation of cancer cells. The inhibitory rate (IR%) which influence by activity of AgNPs and AuNPs compared with control (non-treated groups) was studied and shown in Figure (4) which reveal the inhibition rate between different concentration of gold and silver nanoparticles, the figure show increase in inhibition rate with increasing of concentration expect in third concentration for both of theme, as well as the figure revealed that gold nanoparticles has more effect on hepatoma cell line than silver nanoparticlesso there

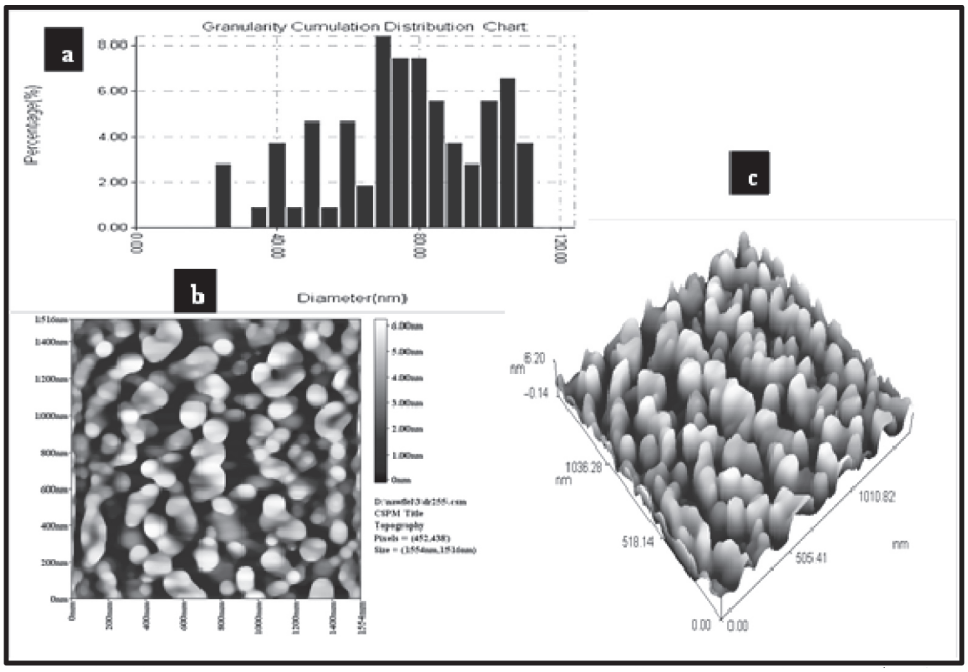

Fig. 1. Show the percentage of diameter ranges for AgNPs is about (60-90 nm) by AFM analysis; (a) particle size distribution; (b) 2-Dimension image; (c) 3-dimension image.

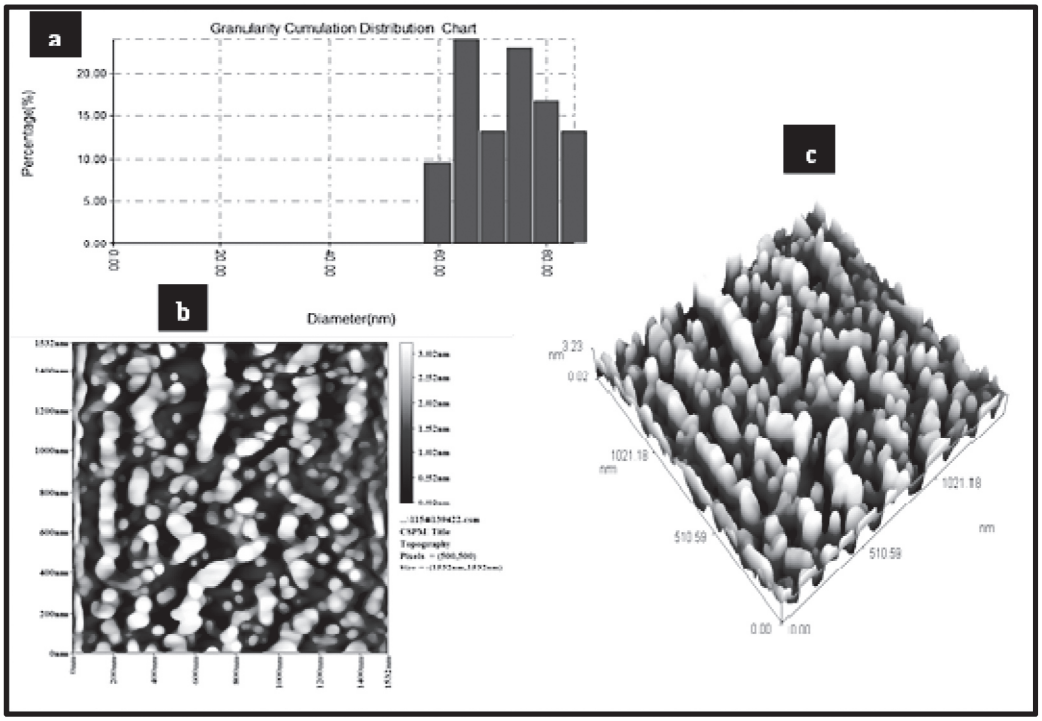

Fig. 2. Show the percentage of diameter ranges for AuNPs is about (60-85 nm) by AFM analysis; (a) particle size distribution; (b) 2-Dimension image; (c) 3-dimension mage.

is no significant difference in all concentration expect the last concentration ($p<0.05$), Figure 6(A and C).

In similar manner, Figure (5) shows the effect of AuNPs on breast cell line more than AgNPs with a significant difference between concentrations for both theme, although there is a simple down in second and fourth concentration for the Au NPs and Ag NPs respectively, there is increase in inhibition rate with increasing of concentration for both

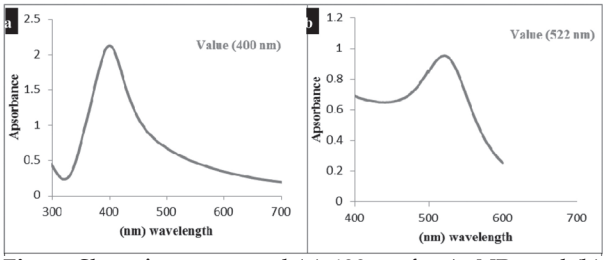

Fig. 3. Show ˆ max around (a) 400nm for AgNPs and (b) 522nm for AuNPs

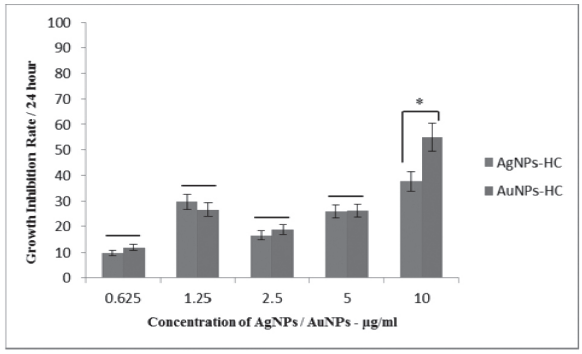

Fig. 4. Comparison between effect of AgNPs and Au NPs on HC cell line at 24 hrs

particles, as well as there is a highly significant difference between the effect of Au NPs and Ag NPs in all concentration expect the last concentration, in which, there is no significant difference (P<0.01), Figure 6(B and D).

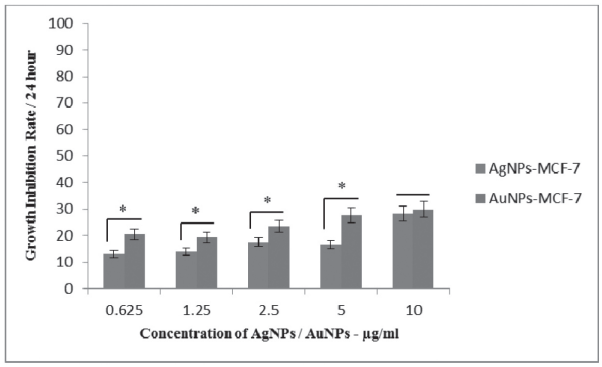

Fig. 5. Comparison between the effect of Ag NPs and AuNPs on MCF- 7 cell line at 24hrs

In dependent cell line manner, Figure (7) show the effect of Ag NPs on MCF-7 in all concentration expect the first and third concentration, in which, HC cell line more effect. While Au NPs has effect on MCF-7 cell line also in all concentration opposite HC cell line which effect by gold particles in second and especially fifth concentration, in which highly significant difference (Figure 8). Finally, the results of Figure 7 and 8 pointed to the MCF-7 more effect than HC cancer cell line.

Cytotoxicity is a complex process influence multiple parameters and pathways, after toxic insult, cells often undergo either apoptosis or necrosis accompanied by changes in nuclear morphology, cell permeability and mitochondrial function, resulting in loss of mitochondrial membrane potential and release of cytochrome c from mitochondria (Babu *et al.*, 2003). Al-Rawi *et al*, (2018), reported thatAgNPs were exhibited dose-dependent cell death in (AMJ13) human breast cancercell line, while the effect of AgNPs on lymphocytes was very low(Al-Rawi *et al.*, 2018). The growth inhibition rate depends on the type of the cell line, exposure period and concentration, but in the nanoparticles experiments, the growth inhibition rate is also depend on size, shape of nanoparticles and surface chemistry etc. (Qu *et al.*, 2012).

First, in this experiment; the size of Au NPs and Ag NPs within the range of effective (Jain *et al.*, 2006; Zhornik *et al.*, 2015). Hence, the results of this experiments are refer to the inhibition rate is related to dose, type of particles, concentration and type of cell line like in study of Patra and his team (2007)(Patra *et al.*, 2007), when they suggested that cell death was selective induce by Au NPs dependent on type of cell lines until in absence of any specific functionalization, as well as Ag NPs showed potential cytotoxicity against various cancer cells such as lung cancer A549 cells, breast cancer MCF-7 cells, colon cancer HT29 cells (He *et al.*, 2016), because The NPs can induce cancer cells death by the production of reactive oxygen species (ROS), DNA damage, and apoptosis (Li *et al.*, 2008; Kovács *et al.*, 2016). When the anti-proliferation activities of AgNPs and AuNPs on many cancerous cells have been studied *in vitro* experiment of (Yen *et al.*, 2009; Khan *et al.*, 2013), they remember that the inhibition of cell growth may be due to upregulated cytokine expression by AgNPs and AuNPs in cell lines (Khan *et al.*, 2013). Parnsamut and Brimson, (2015); found that AgNPs can inhibit TNF-α while AuNPs can inhibit IL-2 and IL-6 in leukemia cells in a concentration-dependent manner which leading to anti-cell proliferation (Parnsamut and Brimson, 2015). Also the results of this study refer to that effect of Au NPs more than the effect of Ag NPs in most part of concentration of nanoparticles, the reason is that both NPs can enter the cells but only AuNPs can up-regulate the expressions of proinflammatory genes interlukin-1 (IL-1), interlukin-6 (IL-6), and tumor necrosis factor (TNF-

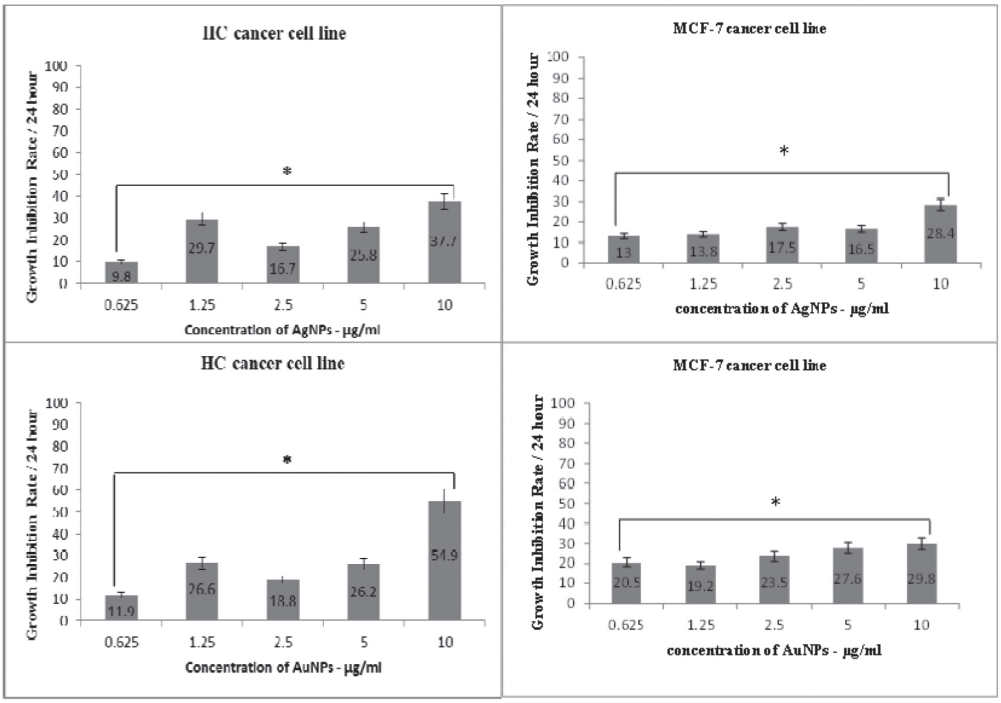

Fig. 6. (A) Effect of Ag NPs on HC cell line, (B) on MCF-7 cell line, (C) Effect of Au NPs on HC cell line, (D) on MCF-7 cell line

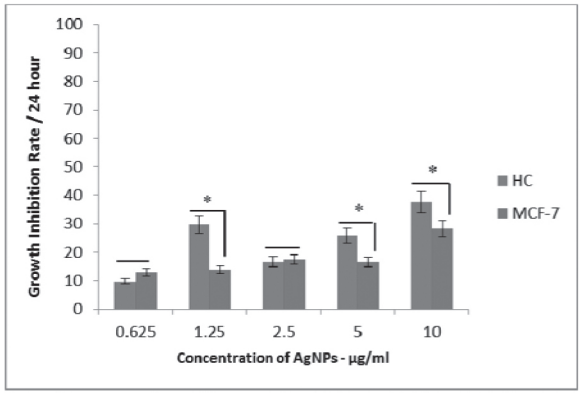

Fig. 7. Comparison between the effect of Ag NPs on HC and MCF- 7 cell line at 24hrs

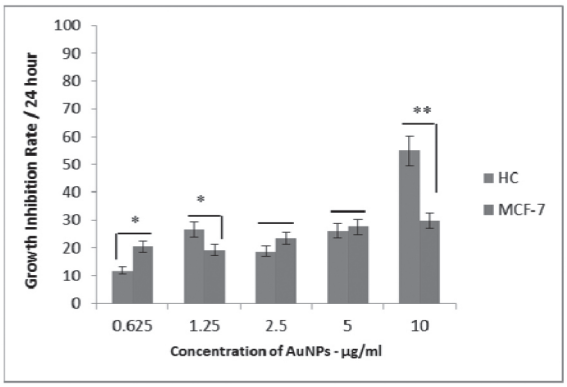

Fig. 8. Comparison between the effect of Au NPs on HC and MCF- 7 cell lineat 24 hrs

alpha), so the both of nanoparticles can trapped in vesicles in the cytoplasm, but only AuNPs are organized into a circular pattern and might adsorb serum protein and enter cells via the more complicated endocytotic pathway, which results in higher cytotoxicity and immunological response of AuNPs as compared to AgNPS (Yen *et al.*, 2009). Moreover, several researches pointed to that nanoparticles safety for normal cells, such as lymphocytes after exposed it to Au NPs and Ag NPs especially in size >200nm as in this study (Zhornik *et al.*, 2015; Hussein, 2016).

CONCLUSION

Our data provides useful information regarding possible treatments of anti-proliferation in both of them, MCF-7 and HC cell lines. In addition to anti-proliferation properties, nanoparticles may process other properties, which could damage cell functions or even induce cell death or apoptosis. Hence, further studies should be carried out to examine this. Our findings suggest that nanoparticles can be applied for alternative co-treatment, combined with conventional treatments for breast cancer and hepatoma.

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