

Inhibition of Ovarian Cancer Cells Growth Using Gold Nanoparticles and Silica Coated Gold Nanoparticles: In-Vitro Study

Salah H. Mannoush¹, Abid A. Thaker², Majid S. Jabir³

¹Department of Biotechnology, College of Science, University of Anbar, Iraq.

²Department of Biology, College of science, University of Anbar, Iraq.

³Division of biotechnology, Department of applied science, University of Technology-Iraq.

Email: salah.bio2015@uoanbar.edu.iq

Abstract

This research aims to employ gold nanoparticles (GNPs) and silicon-coated GNPs (Si-GNPs) as in vitro cytotoxic drugs against ovarian carcinoma cells. UV-spectrophotometer was used to characterize gold nanoparticles. GNPs and Si-GNPs were applied to ovarian cancer cells at various doses, and their anti-proliferative activity was measured using the Methyl-Thiazolyl Tetrazolium (MTT) test. Acridine-Orange/ Ethidium-bromide AO/EB, a dual stain, was used to examine apoptotic cells; a Crystal violet stain was used to evaluate cancer cells colony formation. The findings indicated GNPs and Si-GNPs' capacity to cause apoptosis as a possible antitumor agent in and ovarian cancer cell line. According to the present results, GNPs and Si-GNPs might be employed for a variety of biological applications in the future, perhaps replacing chemotherapy in the treatment of several types of cancer disorders.

Keywords: GNPs; Si-GNPs; Cytotoxicity; Apoptosis; SKOV-3 cells.

INTRODUCTION

A group of diseases known as cancer is described by the unchecked growth and spread of abnormal cells, which, if the growth and spread are unchecked, can be fatal. Cancer is at an alarmingly high level in Iraq, and the blame has shifted to mutagenic and carcinogenic agents. According to the most recent Iraqi cancer registry, in 2014, 130 new instances of cancer in males were reported., while 4.902 cases were registered in women [1, 2]. Ovarian cancer, the most prevalent malignant tumor in the world, has an impact on women Asian and African women are less likely to be affected than white women [3]. It is avoidable and has a low prevalence in women under the age of 40 before peaking in the late 1970s. The fallopian tube is where malignant serous malignancies are most frequently discovered [4]. As a result of various variables connected to the environmental contamination brought on by decades of warfare, the prevalence of cancer has grown in Iraq.

The seventh most frequent malignancy among Iraqi women is ovarian cancer [5]. It has been demonstrated that it is largely resistant to traditional treatments [6].

As a result, it is critical to overcoming this resistance, which necessitates the development of novel treatment approaches [7, 8]. The term "nano-silica" refers to silica that has been structured as nano-scale particles [9]. By expanding the thickness of an area SiNPs and adding contemporary surface and quantum impacts, this structure changes the materials' physical characteristics [10].

Address for correspondence: Salah H. Mannoush
Department of Biotechnology, College of Science, University of Anbar,
Iraq.
Email: salah.bio2015@uoanbar.edu.iq

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SiNPs are commonly utilized as catalytic, medication transporters, coat enhancers, substance stabilizers, and other things in the medical and industrial industries. SiNPs have been shown in studies to enter the human body via drug injection, skin, and the respiratory and gastrointestinal systems, and to spread throughout the body via the flow of blood [11].

It has been reported that silica nanoparticles are more inflammatory than microparticles [12]. A novel method for producing efficient cancer therapies is nanotechnology. Nanoparticles' unique chemical and physical characteristics [13], created them for use in a variety of biological specialties [14, 15] and similar anti-cancer treatments [16, 17]. Numerous biological uses for gold nanoparticles (AuNPs) have been shown to be generally safe [18]. GNPs have been studied against a range of carcinoma cells, including breast and lung carcinoma cell lines, and have been effective against these cells [19, 20] by the activation of many processes, such as the production of oxidative stress [21]. Combination therapy has been proven to be more successful since it targets cancer cells in a variety of ways [22].

MATERIALS AND METHODS

GNPs' characteristics

GNPs and Si-GNPs were obtained from Sigma-USA. The samples were characterized using a UV- spectrophotometer with an absorbance range of 200-1000 nm [23,24].

Cell lines

AL Mustansiriyah University's Iraqi Center for Cancer and Medical Genetic Research in Baghdad, and its cell bank unit provided the SKOV3 ovarian cancer cell line and REF cells rat embryonic fibroblast cell line, respectively. The cells were kept alive in RPMI-1640 supplemented with 10% fetal bovine serum and 100 units/ml of antibiotics treatment (penicillin and 100 µg/mL streptomycin). Once a week, cells were re-suspended by trypsinization using a 0.25% trypsin-EDTA solution (US Biological, USA), and then incubated in 37°C in a CO2 incubator[25, 26].

MTT test for cytotoxicity

SKOV-3 and REF cells were planted in 96 well micro titer plates in full RPMI-1640 media at a density of 1× 10⁴ cells/ml. The cells were incubated overnight for attachment, after 24 hr. from incubation time and a monolayer cell culture was formed, cells were exposed GNPs and Si-GNPs. GNPs (3.12, 6.25, 12.5, and 25 µg/ml) were applied in triplicate at varying concentrations, then the cells were incubated at 37°C for 72 hours. Following two sterile PBS1x washes, the cells were stained using MTT dye (Sigma-Aldrich, USA) at a concentration of 2 mg/ml. The samples were incubated at 37°C, for 2 hr. Each well-received DMSO (Sigma-Aldrich, USA), and the micro plate reader assessed the absorbance at 492 nm[27]. Using the following equation,

the percentage of cytotoxicity was computed:

$$\text{Inhibition rate} = \frac{A-B}{A} \times 100$$

Where A and B, respectively, represent the optical densities of the control and the test [28]. 200 µL of cell suspensions were seeded in 96-well micro-titration plates at a density of 1x10⁴ cells mL⁻¹ and cultured for 24 hours at 37°C in order to examine the form of the cells under an inverted microscope. Following the removal of the medium, GNPs and Si-GNPs were introduced for 24 hours. After the exposure period, the plates were dyed with crystal violet and heated to 37 degrees for ten to fifteen minutes. The stain was then removed by gently washing with tap water. The cell was observed under an inverted microscope at 40x magnification power and photographed with a digital camera [29].

Test for Clonogenicity

The effect of GNPs, and Si-GNPs on the proliferation of clonogenic cells was performed according to previous work. Briefly, SKOV3 and REF cells were seeded on the 12 well plates with density (1x10⁴) cells/well, 24 hrs. when cells reached monolayer confluence, the cells were treated with 10µg/mL each of GNPs and Si-GNPs, after 24 hrs. the medium discarded and rinsed using PBS. The colonies were fixed with methanol (96%), stained with crystal violet (Sigma–Aldrich, USA), and incubated at 37°C with 5% CO₂ for 10-20 min, then washed to remove the excesses dye, dried, and then captured on camera with a digital device[30].

Staining with acridine orange and ethidium bromide (AO/EtBr)

AO/EtBr staining was carried out in accordance with our earlier investigations [31]. Briefly, Cells line was seeded with density(1×10⁴) on the cover slide that was located at the 12-well plate with GNPs and Si-GNPs were added in triplicate at different concentrations, and then incubated for 72 h (IC50 concentration), were used to treat the cells in 96- well plates. After incubation 24 hours, washing with PBS (twice), and addition of 100 µL of AO/EtBr (5 min), fluorescence microscopy was applied for cell visualization [32].

Statistic Evaluation

The results were statistically analyzed using Graph Pad Prism 6, and they were shown as the mean ±SEM of 3 replications for each experiment[33, 34].

RESULTS AND ANALYSIS

Characterization of Gold nanoparticles

A UV-visible spectrophotometer was used to confirm GNPs and Si-GNPs. Figure1 depicts the picture of gold nanoparticles taken from the UV spectrum. The peak of GNPs has been seen in UV spectrum assays at close to 530 nm.

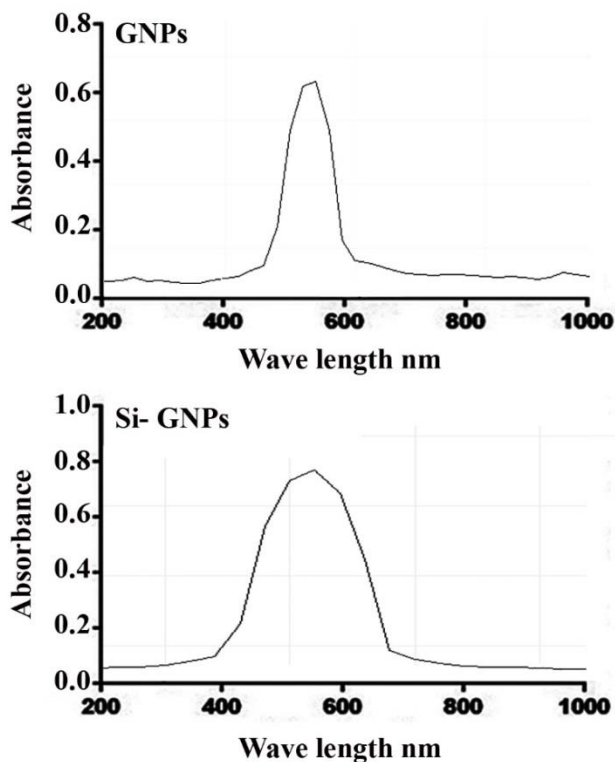


Figure 1: Uv-spectrum of GNPs, and Si-GNPs

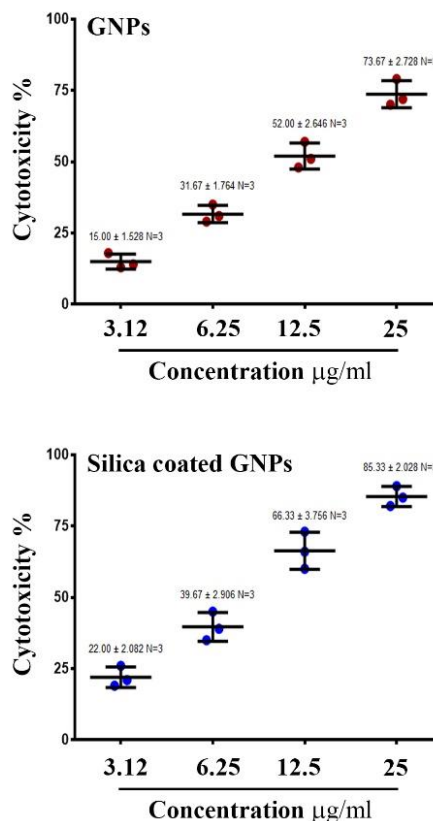


Figure 2: Cytotoxic effect of GNPs, and Si-GNPs against SKOV-3 cells.

Cytotoxic effect using MTT stain

Figures 2 and 3 show the results of the 72-hour examination of the cytotoxic activity of GNPs and Si-GNPs against the ovarian cancer cell lines SKOV3 and REF cells. The SKOV3 cell line's cell proliferation was significantly inhibited after 72 hours, according to the results. Depending on the concentration, During 72 hours, there was a noticeably greater restriction of cell growth. Si-GNPs significantly decreased cell viability after GNPs treatment of the cells. Incubation of ovarian cancer cells with GNPs showed significant and highest inhibition was 73.67±2.728 at a concentration of 25µg/ml, while cancer cells treated with Si-GNPs for 72 hrs showed 86.33±2.028 at a concentration of 25µg/ml. The findings showed that GNPs and Si-GNPs function as biocompatible materials for REF cells. Taken together, The findings showed that Si-GNPs are more effective than GNPs at killing and controlling the growth of ovarian cancer cells. The REF cell line was used as a normal cell and exposed to GNPs and Si-GNPs with four different concentrations after 72 hrs. The results showed that the toxicity of GNPs and Si-GNPs against REF cells was very low compared with results in cancer cell lines SKOV3 as in figures 4 and 5, respectively.

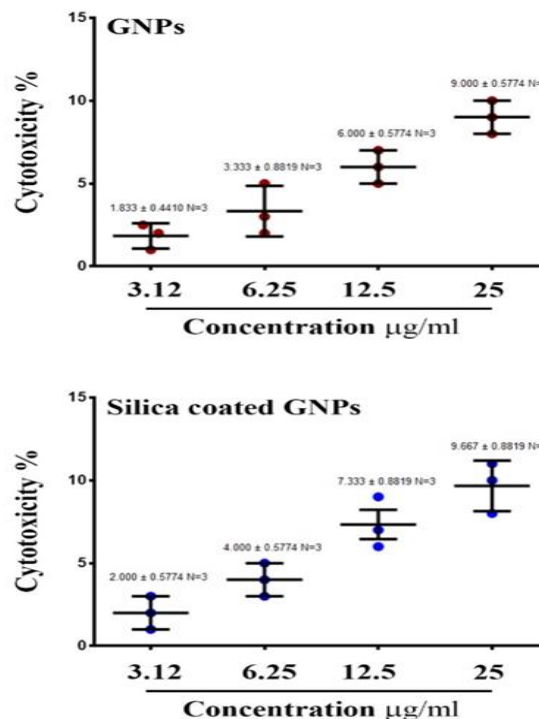


Figure 3: Cytotoxic effect of GNPs, and Si-GNPs against REF cells.

The cytotoxicity observed in SKOV3 and REF cells was confirmed by crystal violet staining, as shown in figures 4 and 5. The dye's affinity for the outside of the DNA double helix serves as the foundation for the crystal violet assay. The quantity of dye absorbed is dependent on the total DNA content of the cells and allows an estimation of the number of viable cells in the culture. As a result, SKOV3 cells exposed to Si-GNPs and GNPs appear to be clustered. Final results demonstrate that Si-GNPs and GNPs induced morphological changes such as cell shape and size changes, and inhibition in communication was accompanied by nuclear shrinkage.

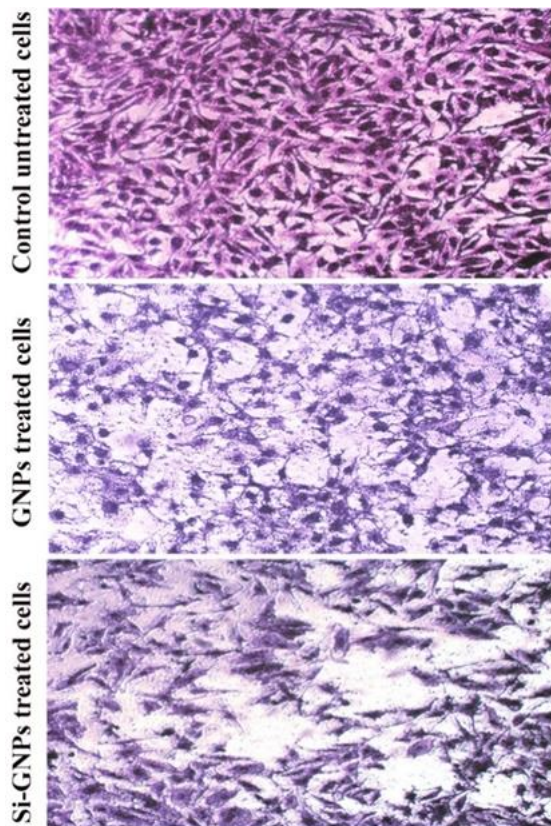


Figure 4: Morphological alterations in SKOV-3 cells after being handled with GNPs, and Si-GNPs. Cells were treated and then stained using Crystal violet for 15 minutes. Magnification power 400x.

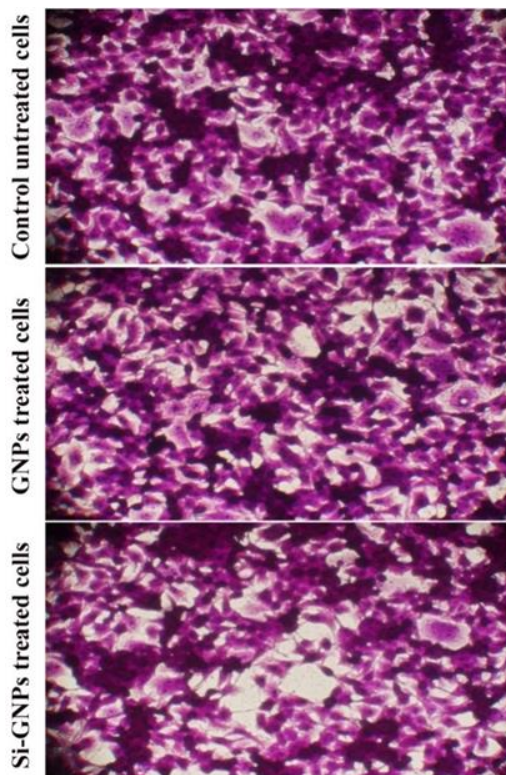


Figure 5: Morphological alterations in REF cells after being handled with GNPs, and Si-GNPs. Cells were treated and then stained using Crystal violet for 15 minutes. Magnification power 400x.

Effect of GNPs and Si-GNPs in Colony forming of SKOV-3 cells

This is an assay that evaluates a single cell's ability to grow into a colony and assesses the effectiveness of cytotoxic agents. The result of this assay was presented as in vitro. Based on a single cell's capacity to create a colony, cell survival was evaluated. This test can be used to determine the anti-proliferative effects of a compound. Si-GNPs and GNPs exhibited a significant impact on colonies' development of SKOV-3 cancer cells. The result was multiplied with silica activity to suppress the colony formation of SKOV-3, the effect shown in figure 6. Clonogenic assays were used to evaluate the therapeutic efficacy of GNPs and Si-GNPs, and We may assume that cancerous cells in the continuous therapy had been eliminated during the first 24 hours of treatment based on the decline in colony formation.

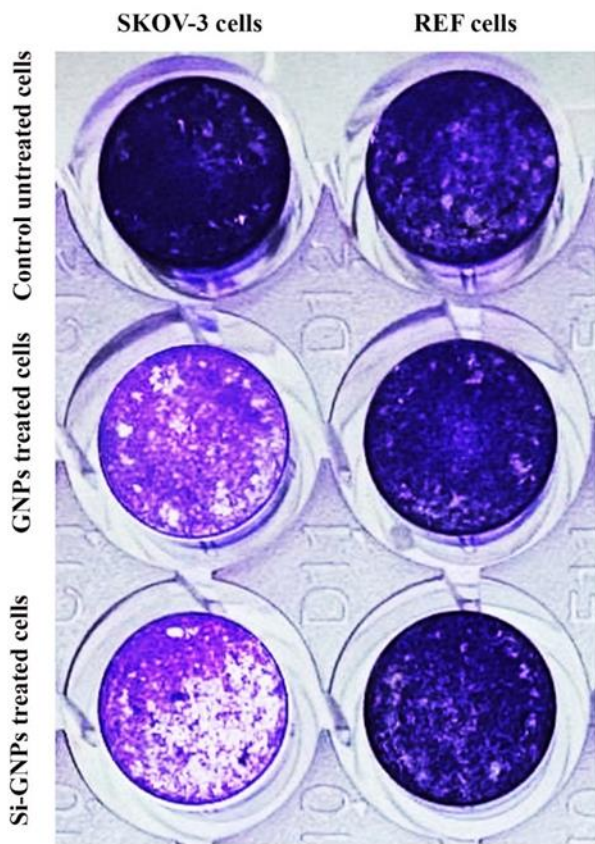


Figure 6: Effect of GNPs and Si-GNPs in colony forming of SKOV-3 and REF cells.

GNPs and Si-GNPs induce apoptosis in SKOV-3 cells

GNPs capability was measured using acridine orange-ethidium bromide dyes, and silicon GNPs, to induce apoptosis of SKOV-3 cells as in figures 7 and 8. In this assay, detect apoptosis has been developed that detects changes in cell morphology and surface markers associated with apoptosis. Acridine orange/ethidium bromide (AO/EtBr) fluorescence staining can be used to spot apoptosis-related alterations in the nucleus and cell membranes under a fluorescent microscope [35]. SKOV-3 and REF cells were treated with GNPs and Silicon GNPs. Double staining occurs after culturing, exposure, and incubation time. When bound with different cellular organelles, Fluorescence from an AO stain with unique characteristics comes in various hues. When stained with EtBr, apoptotic cells looked orange or red, while viable and non-apoptotic cells appeared green as they were stained with AO. The GNPs and Silicon coated GNPs have a clear effect on the cancer cell line and the bigger effect was for the GNPs, the Silicon GNPs having a greater effect than GNPs, as it clears in red and orange color. EtBr was a DNA intercalating agent that was designed to cause late apoptosis in the cell, including membrane disruptions and nuclear condensation. It was used to identify cells that were late apoptotic and had a highly disrupted membrane [36]. The intensity of the orange or red color depends on the strength of the materials'

impact on cancer cells, so the intensity of the color is stronger in the Silicon GNPs effect. GNPs and Silicon GNPs changed the morphology of the cell and altered the outer membrane, which had an effect on the structures of the cells and made them more permeable.

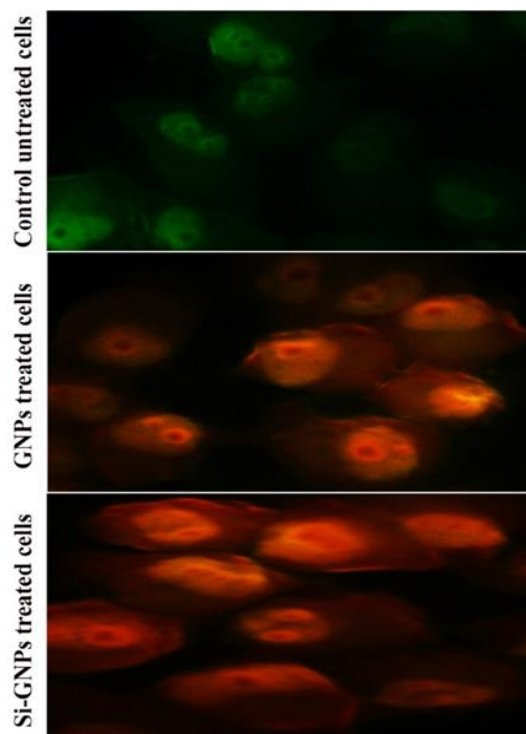


Figure 7: GNPs and Si-GNPs induce apoptosis in SKOV-3 cells. Scale bare 10 μm.

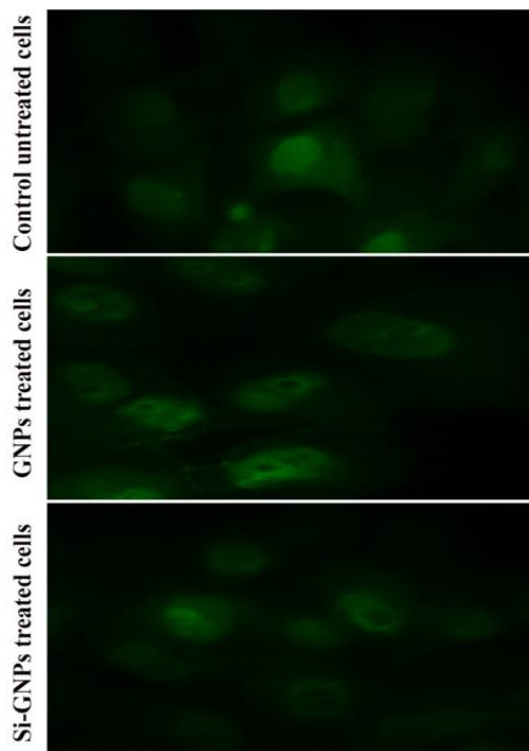


Figure 8: In REF cells, GNPs and Si-GNPs do not promote apoptosis. Scale bare 10 μm.

CONCLUSION

The current study looked at the cytotoxicity and antiproliferative efficacy of GNPs and Si-GNPs toward ovarian cancer cells. The cytotoxicity and apoptosis induction in ovarian cancer cell lines were significantly elevated by using GNPs and increased significantly after using Silicon/GNPs as a combination therapy. These compounds can be employed as therapeutic medications alone or in conjunction with other chemotherapeutics to treat distinct cancer cell types, according to the findings.

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