Gold Nanoparticles as anti-ovarian cancer therapy

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Abstract

Cancer of the ovary is one of the most common types of women cancer in the world. In Iraq, it is seventh cancer in Iraqi women. Nanomedicine emerged as a possible alternative for cancer therapy. Many nanoparticles have been tested on different cancer cell lines with promising results. Gold nanoparticles (AuNPs) showed interesting biocompatibility properties such as selective accumulation in tumor cells. The currentwork was aimed to study cytotoxicity and cell death induced by AuNPson ovarian cancer cells. Methods: Cytotoxicity of AuNPs was assessed by MTT viability assay. Results: AuNPs nanoparticles from 25 up to 100 µg/mL for 72 hours exerted concentration-dependent cytotoxicity. Conclusion: Gold nanoparticles induce cytotoxicity in ovarian cancer cells and Induce cell death and considered promising as cancer therapy.

Keywords: Gold nanoparticles (AuNPs), cytotoxicity. Cell death

Introduction

Ovarian cancer ranked seventh common cancer in females worldwide, and the eighth cause of cancer mortality, furthermore, it had five-year survival rates under 45% [1].Incidencediffers according to ethnicity,as white women are 30% higher than Asian and African women [2]. Ovarian cancer peaking in the late 70s, and it has a very low incidence inwomen of age below 40 years. Most of the high-grade serous cancers are recently showed to originate from the fallopian tube [3]. Cancer incidence has increased in Iraq, this elevation related to numerous factors connected to environmental pollution associated with conflicts for several years [4]. Ovarian cancer also ranked seventh in Iraqi women [5].Treatment resistance of ovarian cancerusuallyarises in 80–90% of cancer patients who are originally diagnosed with widespread ovarian malignancies[6]. Overcoming ovarian cancer resistance to conventional chemotherapy will need a variety of methods [7].

Nanoparticles considered a novel platform to produce safe and effective cancer treatment[8,9]. The unique chemical and physical properties of nanoparticles[10], led toa wide range ofapplications in the biomedical field[11] such as cancer[12,13]. Furthermore, the expected toxic effect of AuNPs may be multisided and is hard to predict, and othersrevealed no biological impact on health and safety [14,15]. AuNPs were reported to be to cytotoxic ondiverse cancer cells such breast cancer [16], and human lung carcinoma cells [17] which has been demonstrated that the AuNPs nanoparticles had inhibition to proliferation throughthe generation of oxidative stress, and changes in Wnt signaling pathway [18]. Moreover, AuNPs can be used for drug delivery such as for loading linalool,which

is monoterpene alcohol from aromatic plantsthat showed in-vitro anticancer activities against breast cancer[19]. Better treatment is necessary, which should show increased efficacy as well as a low toxicity, selective with high safety margin based on the patient and the clinical characteristics of the disease [20].In the current study, we testedAuNPs nanoparticles as promising anticancer therapy. The effect of AuNPs nanoparticles was examined on human ovarian cancer.

Materials and Methods

Maintenance of cell cultures

The human ovarian cancer cell line, SKOV3 were growing in a MEM medium (Usbiological, USA) supplied with 10% fetal bovine serum (FBS), 100 µg/mL streptomycin and 100 units/mL penicillin(Capricorn- Scientific, Germany). The cell lineis regularly evaluated for standard growth characteristics. SKOV3 cell line was cultured as a confluent adherent monolayer and incubated at 37°C in5% CO2 humidified atmosphere. Trypsin-EDTA was used for harvestingof the monolayer cells (Capricorn-Scientific, Germany) [21,22].

Gold nanoparticles

In this study was used gold nanoparticles(AuNPs), they are highly stable and suitable for biological applications and of 10nm size [19].

Cytotoxicity determination using MTT assay

The SKOV3 cells were seeded at 1× 10^4 cells/mL in 96 well microplates in MEM growth media and allowed for overnight incubation at 37ºC for adhesion and proliferation. AuNPs were exposed in triplicate at a different concentration from 3.2 to 100µg and incubated for 72 hrs. After that, the MTT viability stain was used at a concentration of 2µg/ml. After 3h incubation at 37ºC,Dimethyl Sulfoxide(DMSO) was added to all wells.The measurement of absorbance was done at 580 nm using biochrom microplate reader. Results of the assay were shown as a percentage of proliferation relative to control cells [23,24]

Statistical analysis

The collected data were statically analyzed using multiple comparison ANOVA testsusing GraphPad Prism 6.07; values were presented as the mean ± S.D of the triplicates**.**

Findings

GNPs inhibits growth of SKOV3 ovarian cancer cells

The cytotoxic effects AuNPs on the growth inhibition of human ovarian cancer cell lines SKOV3 for 72h were examined, as shown in figure 1. The study results revealed significant cytotoxicityon SKOV3 ovarian cancer cell line after 72 hrs. The cellscytotoxicity was significantly higher when compared to control untreated cells. After 72h treatment with AuNPs, the effective concentrations were 25, 50and100 µg/mL and the Cytotoxicity effect of AuNPs showed significant cytotoxic effect on the ovarian cancer cells, each concentration showed more significant effect with higher concentration as shown in table 1. The experiment results showed that AuNPs are suggested to be themostvalued source of actualcytotoxic and proliferation inhibitory agent. The apoptoticinduction was studied through cytopathological changes in SKOV3 cell line using a phase contrast inverted microscope. In Figure 2, the untreated cells displayed that the cells preserved their unique morphology; most of the untreated cells were attached to the tissue culture plate. Meanwhile, SKOV3 cells treated with AuNPs exhibited detachment and pathological morphological changes. IC50, which is the inhibitory concentration that kills 50% of the cells, was 11.28µg (Figure-2).

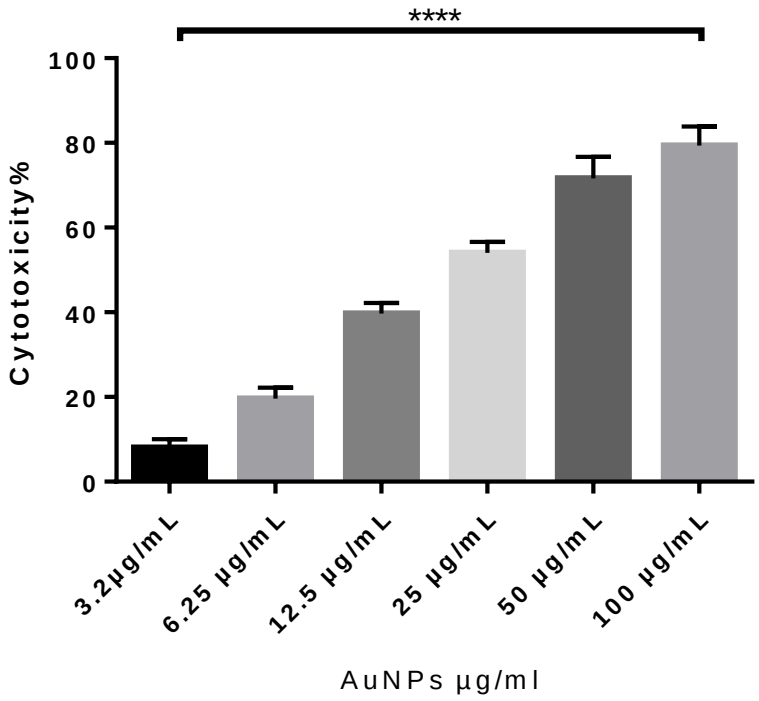

Figure-1, Cytotoxic effect of AuNPs nanoparticles in ovarian cancer SKOV3 cell lines. The cell lines were treated for 72 h with different concentrations of AuNPs nanoparticles (3.2 to 100 µg/ml). The cytotoxicity of the nanoparticles was determined using the MTT viability assay and is expressed in terms of the growth inhibition rate. All concertation showed significant inhibition in cancer cells.

Table-1, The collected data were statically analyzed using multiple comparison ANOVA tests

Tukey's multiple comparisons tests	Mean Diff.	95% CI of diff.	Significant?	Summary
3.2µg/mL vs. 6.25 µg/mL	-11.67	-20.99 to -2.344	Yes	*
3.2µg/mL vs. 12.5 µg/mL	-31.67	-40.99 to -22.34	Yes	****
3.2µg/mL vs. 25 µg/mL	-46.00	-55.32 to -36.68	Yes	****
3.2µg/mL vs. 50 µg/mL	-63.67	-72.99 to -54.34	Yes	****
3.2µg/mL vs. 100 µg/mL	-71.33	-80.66 to -62.01	Yes	****
6.25 µg/mL vs. 12.5 µg/mL	-20.00	-29.32 to -10.68	Yes	***
6.25 µg/mL vs. 25 µg/mL	-34.33	-43.66 to -25.01	Yes	****
6.25 µg/mL vs. 50 µg/mL	-52.00	-61.32 to -42.68	Yes	****
6.25 µg/mL vs. 100 µg/mL	-59.67	-68.99 to -50.34	Yes	****
12.5 µg/mL vs. 25 µg/mL	-14.33	-23.66 to -5.010	Yes	**
12.5 µg/mL vs. 50 µg/mL	-32.00	-41.32 to -22.68	Yes	****
12.5 µg/mL vs. 100 µg/mL	-39.67	-48.99 to -30.34	Yes	****
25 µg/mL vs. 50 µg/mL	-17.67	-26.99 to -8.344	Yes	***
25 µg/mL vs. 100 µg/mL	-25.33	-34.66 to -16.01	Yes	****
50 µg/mL vs. 100 µg/mL	-7.667	-16.99 to 1.656	No	ns

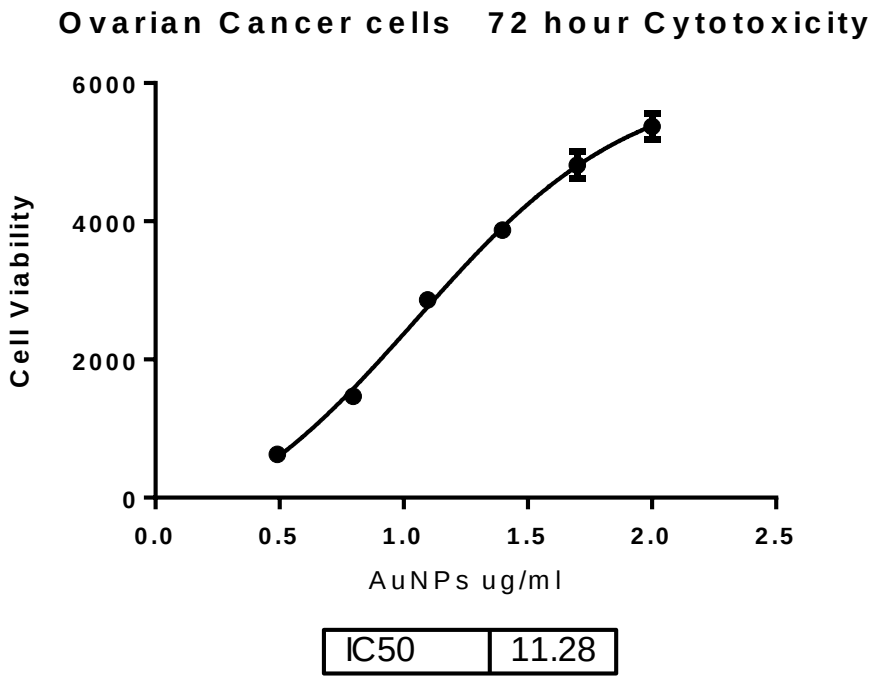

Figure-2, IC50 concentration was11.28µg/ml on ovarian cancer SKOV3 cell line, and increased doses inducemore cytotoxic effectin cancer cells.

Discussion

Nanoparticles have shown to be a promising tool for cancer treatment[25]. The use of AuNPs as an anti-cancer agentagainstovarian and breast cancer was reported[26]. In the currentexperiment, the antiproliferative activity of AuNPs nanoparticles to human ovarian cancer cells wasexamined. The study results showed that AuNPs exposure to cancer cells cause significant cytotoxicity in specific moderate concentrations. AuNPs exposure to cancer cells caused viability reduction and under the inverted microscope, detection of cell death typicalcytopathologicalcharacteristicssuch as rounding and loss of adherence. Moreover, reactive oxygen species (ROS) generation is enhanced by AuNPs administration, and apoptosis induction inAuNPs-treated ovarian cancer cells[16,27]. Furthermore,[28,29]group has proved that 20 nm AuNPs repressedovarian cancer cell proliferation, angiogenesis, and metastases in anexperimental mouse model.

The IC50 concentrations noticed in our current study which is 11.28 µg/ml on ovarian cancer cells, while it requires higher concentration IC50 on normal humandermal fibroblast (NHDF) cellswhich ranged from 17.9 to 19.3µg/ml noticed by another study[30]. This difference proves that cancer cells are more susceptible to AuNPs therapy than normal cells, which prove safety. Our study found that AuNPs was selective in killing cancer cells by causing cell death at less dose than in normal dermal fibroblast cells. As we found, it needs 11.28 µg/ml to induce cytotoxicity, and another investigator [30] found it needs 17.9 to 19.3 µg/ml to induce cytotoxicity in normal dermal fibroblast cells.The current study investigated the response to AuNPs nanoparticles exposure in ovarian cancer cells because apoptosis can be recognized morphologically.

Conclusion,

we have shown that AuNPs nanoparticles produce significant selective cytotoxicity against cancer cells through death pathway activation.

Conflict of interest

The authors declare that there is no conflict of interest.

Source of Funding

The work is self-funding and no specific funding was received.

Ethical Clearance

The work approved by the Al-Diwaniya maternity and child teaching hospital scientific committee.

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