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 current work was aimed to study cytotoxicity and cell death induced by AuNPs on 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. Incidence differs 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 in women 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 cancer usually arises 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 to a wide range of applications 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 others revealed no biological impact on health and safety 14,15. AuNPs were reported to be to cytotoxic on diverse cancer cells such breast cancer 16, and human lung carcinoma cells 17 which has been demonstrated that the AuNPs nanoparticles had inhibition to proliferation through the 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 monoterpenes alcohol from aromatic plants that showed in-vitro anticancer activities against breast cancer\textsuperscript{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 \textsuperscript{20}. In the current study, we tested AuNPs 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 in 5% CO2 humidified atmosphere. Trypsin-EDTA was used for harvesting of the monolayer cells (Capricorn-Scientific, Germany) \textsuperscript{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 \textsuperscript{19}.

**Cytotoxicity determination using MTT assay**

The SKOV3 cells were seeded at 1×10\textsuperscript{4} cells/mL in 96 well microplates in MEM growth media and allowed for overnight incubation at 37°C 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 37°C,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 \textsuperscript{23,24}.

**Statistical analysis**

The collected data were statically analyzed using multiple comparison ANOVA tests using 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 cytotoxicity on SKOV3 ovarian cancer cell line after 72 hrs. The cells cytotoxicity was significantly higher when compared to control untreated cells. After 72h treatment with AuNPs, the effective concentrations were 25, 50 and 100 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 the most valued source of actual cytotoxic and proliferation inhibitory agent. The apoptotic induction 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 concentration showed significant inhibition in cancer cells.

Table-1, The collected data were statically analyzed using multiple comparison ANOVA tests
**Figure-2**, IC50 concentration was 11.28 µg/ml on ovarian cancer SKOV3 cell line, and increased doses induced more cytotoxic effect in cancer cells.

**Discussion**
Nanoparticles have shown to be a promising tool for cancer treatment\(^{25}\). The use of AuNPs as an anti-cancer agent against ovarian and breast cancer was reported\(^{26}\). In the current experiment, the antiproliferative activity of AuNPs nanoparticles to human ovarian cancer cells was examined. 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 in AuNPs-treated ovarian cancer cells\(^{16,27}\). Furthermore,\(^{28,29}\) group has proved that 20 nm AuNPs repressed ovarian cancer cell proliferation, angiogenesis, and metastases in an experimental 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 human dermal fibroblast (NHDF) cells which ranged from 17.9 to 19.3 µg/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.

References


