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**Original Research Article**

# Gold Nanoparticles Targeting Human Cervical Cancer Cells

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## Abstract

The most disturbing gynecologic malignancies are cervical cancer particularly in the developing world with the same high incidence in Iraqi women. Cervical carcinoma has a high rate of mortality. The high death rate is associated with presence of human papillomavirus (HPV) infection. Cervical carcinoma is hard to treat, and conventional therapies are very aggressive which lead for the need for new approaches of therapy. Nanoparticles is a promising treatment modality to produce non-toxic and efficient cancer therapy. Gold nanoparticles (AuNPs) accumulate in cancer cells selectively. The current work was aimed to study cytotoxicity and cell death induced by AuNPs on cervical carcinoma cells. Methods: Cytotoxicity of AuNPs was assessed by MTT viability assay and analysed using multiple comparison ANOVA tests. Results: AuNPs nanoparticles from 12.5 up to 50 µg/mL for 72 hours showed concentration-dependent killing activity. Conclusion: Gold nanoparticles have anti-cervical carcinoma cells activity by cell death induction.

**Keywords**: Gold nanoparticles (AuNPs), cytotoxicity. Cell death, cervical cancer, Hela cells.



## INTRODUCTION

The most disturbing gynecologic malignancies is cervical cancer particularly in the developing world [27]. The new cervical cancer cases are about 569,847 diagnosed annually worldwide (estimates for 2018) [12]. Human Papillomavirus (HPV) infection is associated with the cervical cancer [23]. In Iraq, cervical cancer ranked 9th most common cancer in Iraqi women aged 15 to 44 years [13]. The most frequent type of cervical cancers is squamous cell carcinoma (SCC) which is about 85% [11]. There are known risk factors associated with cervical cancer in addition to HPV infection, such as cigarette smoking and smoke carcinogen (benzo[a]pyrene, BaP) [7]. Human papilloma virus (HPV) subtypes that cause cancer is genotype 16 that cause SCC and genotype 18 that cause adenocarcinoma [25]. It is well reported the importance of HPV infection in different type of benign and malignant tumors. It is well established that there is association between HPVs with cervical cancer [29]. Moreover, environmental pollutions associated with conflicts may be another source for increase cancer related cases [5]. There were numerous attempts to overcome resistance to conventional therapies to accomplish a considerable therapeutic effect in malignant tumors by using gold nanoparticles [8, 18]. The resistant of cancer while there is treatment for long time, will cause progressive type of malignant tumors, requires the development of novel therapeutic modalities to overcome chemo-resistance and enhance prolong surviving effect [15]. Nanoparticles is a promising treatment modality to produce non-toxic and efficient cancer therapy [6, 26]. Nanoparticles carry unique chemical and physical features [31], these features utilized in biomedical applications [20] such as cancer [9]. Nanoparticles were combined with other cancer targeting agents to enhance therapeutic effects [6]. The search for more effective therapy is needed, therefore we designed the current study to use Gold (AuNPs) nanoparticles as anti-cervical cancer therapy which hold promise for clinical application.

## MATERIALS AND METHODS

### Maintenance of cell cultures

The human cervical cancer cell line, Hela were cultured in basic MEM media (Usbiological, USA) supplemented by 10% fetal bovine serum (FBS), 100 units/mL penicillin and 100 µg/mL streptomycin (Capricorn- Scientific, Germany). The cell line is authenticated regularly. Hela cell line was subcultured

using trypsin-EDTA when confluent monolayer was achieved and incubated at 37°C in 5% CO2 humidified incubator [2, 4].

**Gold nanoparticles**

The current work used highly stable gold nanoparticles (AuNPs) of 10nm size, they are designed for biomedical applications [18].

**Cytotoxicity determination using MTT assay**

The Hela cells were seeded at concentration of 10000 cells/well in 96 multi-well microplates. The cells were suspended in 10% FBS MEM medium and allowed to grow for 80% confluency. Gold NPs were added at several concentrations from 3.2 to 100µg in triplicate and incubated for 72 hrs. at the end point, the MTT stain was added 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 [1, 3].

## 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**.**

## RESULTS

**Gold NPs suppress Hela cervical cancer cells**

The killing effect of Gold nanoparticles on human cervical carcinoma cells Hela after 72h of exposure was shown in figure 1. The cytotoxicity of GNPs on Hela cervical carcinoma cells was significant in compare to control not treated cells. The GNPs cytotoxicity was significantly elevated with the increase in concentration as shown in table 1. the effective concentrations were 12.5, 25 and 50 µg/ml. The results of the cytotoxicity experiment suggest that gold nanoparticles can be valuable anti-cervical cancer therapy. Hela cells treated with AuNPs exhibited detachment and pathological morphological changes. IC50, which is the inhibitory concentration that kills 50% of the cells, was 8.713µg (Figure-2). The cytopathological changes revealed apoptotic induction in Hela cells when observed under phase contrast inverted microscope. The untreated cells displayed that the cells preserved their unique morphology; most of the untreated cells were attached to the tissue culture plate.

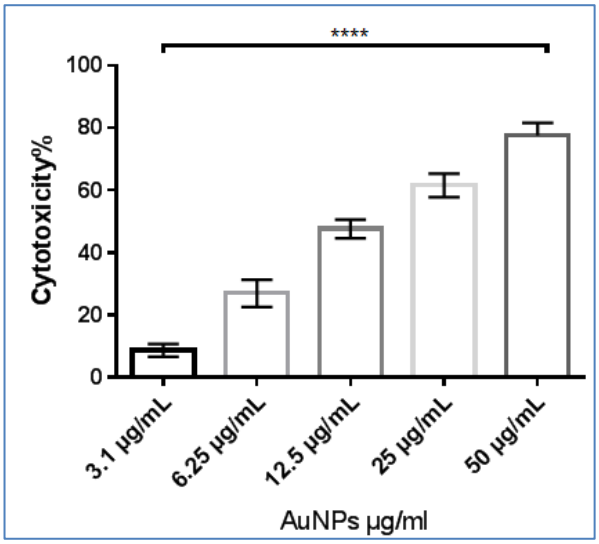


**Fig-1: killing effect of gold nanoparticles in cervical cancer Hela cells was measured via the MTT viability assay. The Hela cells were exposed to gold NPs for 72 h with different concentrations. The effective concentration of AuNPs nanoparticles were from 12.5 to 50 µg/ml**

**Table-1: The cytotoxicity assay effect were analyzed using multiple comparison ANOVA tests**

| Tukey's multiple comparisons test | Mean Diff. | 95% CI of diff. | Significant? | Summary |
|---|---|---|---|---|
| 3.1 µg/mL vs. 6.25 µg/mL | -18.33 | -27.90 to -8.770 | Yes | *** |
| 3.1 µg/mL vs. 12.5 µg/mL | -39.00 | -48.56 to -29.44 | Yes | **** |
| 3.1 µg/mL vs. 25 µg/mL | -53.00 | -62.56 to -43.44 | Yes | **** |
| 3.1 µg/mL vs. 50 µg/mL | -69.00 | -78.56 to -59.44 | Yes | **** |
| 6.25 µg/mL vs. 12.5 µg/mL | -20.67 | -30.23 to -11.10 | Yes | *** |
| 6.25 µg/mL vs. 25 µg/mL | -34.67 | -44.23 to -25.10 | Yes | **** |
| 6.25 µg/mL vs. 50 µg/mL | -50.67 | -60.23 to -41.10 | Yes | **** |
| 12.5 µg/mL vs. 25 µg/mL | -14.00 | -23.56 to -4.436 | Yes | ** |
| 12.5 µg/mL vs. 50 µg/mL | -30.00 | -39.56 to -20.44 | Yes | **** |
| 25 µg/mL vs. 50 µg/mL | -16.00 | -25.56 to -6.436 | Yes | ** |

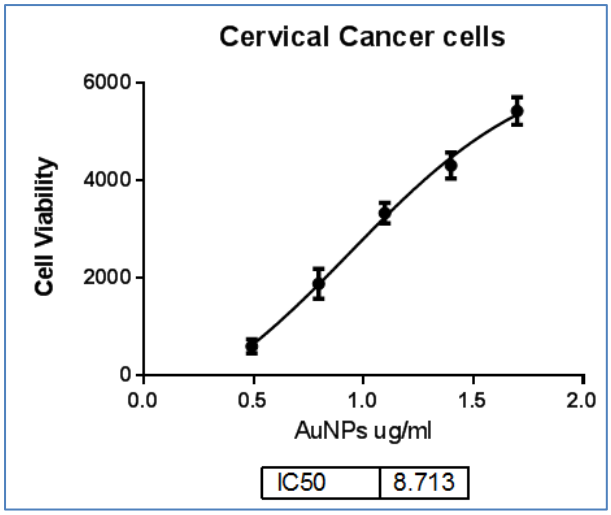


**Fig-2: IC50 value for Gold NPs in Hela cells was 8.7µg/ml, this dose reflects sensitivity of cervical cancer cells to nano gold**

## DISCUSSION

In the current experiment, the antiproliferative activity of AuNPs nanoparticles to human cervical cancer cells was investigated. Nanoparticles is considered an important drug delivery methods for cervical Cancer treatment [24]. Gold nanoparticles reported to have an anti-cervical cancer properties [19]. The study results showed that AuNPs exposure to cancer cells cause significant cytotoxicity in most doses tested. Gold nanoparticles caused viability reduction in exposed cancer cells. It is reported that biosynthesized gold nanoparticles exposed to Hela cervical cancer cells cause DNA damage, G2/M arrest, and apoptotic cell death via caspase activation [19]. Cell death cytological features of rounding and cell detachment as observed under the inverted microscope. Furthermore, generation of reactive oxygen species (ROS) is enhanced by gold nanoparticles treatment [8, 16]. Moreover, couple of studies [10, 22] showed that 20 nm gold nanoparticles decrease cancer cells growth mouse model. AuNPs may also used as delivery agents for phytochemicals such as Gallic acid or chemotherapeutic agents such as doxorubicin can be used as replacement for cervical cancer therapy to decrease radiotherapy and chemotherapy side effects [14, 28]. Retinoic acid loaded on gold nanoparticle showed promising anti-cervical cancer effect [30]. The IC50 dose estimated was 8.713 µg/ml in cervical carcinoma cells. Pharmacokinetic and bio-distribution investigation for AuNPs in tumor bearing animal model showed that gold nanoparticles have high stability when conjugated with therapeutic agents and radiotherapeutic enhancement [17]. Nanoparticles hold very promising activity against cervical cancer [32]. Furthermore, cervical cancer and HPV need to find alternative treatments. Nanoparticles suggested promising in cervical cancer treatment by the capacity to target and internalize cancer cells [21]. In conclusion, the present study proved that gold nanoparticles can be used for cervical cancer therapy.

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