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# Enhancing the Efficiency of Doxorubicin -Conjugated with Gold Nanocomposites for Active Targeting and Laser Treatment for Human Esophagus Cancer Cells

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**Abstract**

Nanotechnology is being used in cancer treatment to deliver drugs to tumors, improve imaging, and detect cancer cells. Nanoparticles are being used to deliver drugs more effectively to tumors, while also reducing the side effects of the drugs. The current context shows the conceivable biomedical utility of gold nanoparticles (AuNPs) covered with doxorubicin (DOX) for double chemotherapy and photo-thermal therapy (PTT) of throat cancers (esophagus) in vitro. DOX as FDA-authorized anti-cancer drug was added to AuNPs to enhance the chemotherapy impact. The blended DOX - AuNPs showed exorbitant cytotoxicity at the cancer cells and strong light absorption for temperature expansion in vitro. In the wake of treating DOX– Au nanocomposites with inside the esophagus cell line, PTT-helped DOX-Au nanocomposites involved the fantastical cytotoxicity of the cell strains for 15 min after the laser treatment. Exceptionally cytotoxicity impact in esophagus cell line turned out to be more attractive due to specific light absorption with the aid of using the directed DOX-Au nanocomposites. Thusly, the proposed DOX-Au nanocomposites might be an ability nano-theranostic material for treating throat cancers. The gene expression levels withinside the esophagus cell line from every group with or without laser exposure have been compared individually with control group. Bax has been overexpressed in esophagus cell line organization post 15 min laser irradiation. Conversely, BCL-2 gene has been down regulated with the aid of using RT-PCR analysis post 15 min laser irradiation, while in comparison to control cell line.

*Keywords:* Laser, PTT, gold /DOX Nano composites, Bax and BCL-2 genes, RT-PCR.

## 1. Introduction

Cancer may be treated with the aid of using diverse strains of treatment such as surgery, chemo, radiotherapy, and immune therapy. Recently, nanotechnology has shown promise in cancer treatment by using lower quantities of chemotherapeutic drugs to target malignant cells while leaving healthy cells alone [1]. The esophagus is a component of the digestive system that transports water and nutritious materials from the throat to the stomach [2]. Diseases of the throat can include strep throat, tonsillitis, laryngitis, and epiglottitis. It can also include more serious conditions such as throat cancer, throat cysts, and infectious mononucleosis. Some of the symptoms of throat diseases can include a sore throat, hoarseness, and difficulty swallowing, coughing, as well as swollen lymph nodes. Among the disorders listed above, esophageal cancer has the highest fatality rate [3, 4]. Apoptosis is a genetically managed cell suicide pathway that performs a critical role in deleting excess, undesirable or damaged cells all through the development of tissue homeostasis. Apoptosis dysfunction contributes to a wide range of disease disorders in humans, including carcinogenesis [5]. Bcl-2 is a proto-oncogene that is primarily expressed in glandular cells towards the conclusion of the proliferative phase before disappearing when the secretory phase begins [6, 7]. Bax is a pro-apoptotic protein that promotes cell death by homo-dimerization and hetero-dimerization with bcl-2 and other members of the bcl-2 protein family. Nanomaterials are distinguished by their small size, which is less than a hundred nanometers. Nanoparticles exist in nature and may be created from carbon or minerals such as silver and gold. The ability to create and engineer chemicals on such a

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tiny scale allows them to gain medicinal, electric, and optical features [8]. Gold nanoparticle biomolecules (Au-NPs) are widely employed in medicine, pharmacy, and cosmetic items as biomarkers and bio-delivery vehicles [9].

Depending on the type of cancer cells and the type of photo-thermal treatment (PTT) being used, the rule for treating cancer cells with photo-thermal treatment can vary significantly. Generally speaking, the goal of photo-thermal treatment is to convert light energy into heat, to destroy cancer cells without causing damage to healthy cells nearby. In order to achieve this, the photo-thermal treatment must be precise and controlled in order to ensure that the cancer cells are killed to the fullest extent possible, while leaving healthy cells unscathed [10, 11]. PTT has the advantage of killing malignant cells without causing resistance regardless of the genetic background and hence may be carried out on all cancer patients. To get productive PTT and decline vague warming of healthy cells, photosensitizers need to have extreme assimilation withinside the NIR area [12]. Nanomaterial-based PTT has essentially been explored as a painless or insignificantly obtrusive and powerful healing strategy to treat different kinds of cancers in vitro and in vivo [13].

Doxorubicin is one of the maxima often used drugs in cancer chemotherapy. There are many advantages to using doxorubicin in cancer treatment. First, doxorubicin is a very powerful anticancer agent. It can kill cancer cells quickly and effectively. Second, doxorubicin is a relatively safe drug. It is not likely to cause any serious side effects, and it is generally well tolerated by patients. Third, doxorubicin can be used in combination with other anticancer drugs to improve its effectiveness. Fourth, doxorubicin can be used to treat a wide variety of cancers. [14-16].

The aim of this study was to enhance the efficiency of doxorubicin- conjugated with gold nanocomposites for active targeting and laser treatment of esophageal cancer.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Chemicals

All chemicals used in this study were analytical grade reagents and used without further purification. Sigma Chemical supplied the doxorubicin. Sigma-Aldrich provided chlorauric acid ($HAuCl_4$), 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), 100% methanol, trisodium citrate dihydrate ($Na_3C_6H_5O_7.2H_2O$), and phosphate-buffered saline (PBS, pH 7.2). (Darmstadt, Germany). Gibco® (Thermo Fisher Scientific, UK) provided RPMI-1640 cell culture media supplemented with L-glutamine and foetal bovine serum (FBS), while Biowest® provided penicillin-streptomycin and trypsin/EDTA.

#### 2.1.2. Cell line

Tow cell lines were used, first is BHK normal fibroblast cell line and others is one human carcinoma cell line this is esophageal carcinoma (OE33) cell line (ATCC) were obtained from the Amirican Type Culture Collection (ATCC). USA, and maintained in the VACSERA-Cell Culture Unit, Cairo, Egypt, were used throughout this study. Cells were cultured in RPMI-1640 L-glutamine medium enhanced with 10% FBS and penicillin-streptomycin antibiotic in a humidified 5% $CO_2$ incubator at 37ºC. Sub-culturing was done twice a week on a regular basis with trypsin/EDTA.2.2. 2.2. Methods

#### 2.2.1. Preparation of gold nanoparticles (Au-NPs)

The citrate reduction technique was used to create $5X10^{-3}$ mol/L gold nanoparticles according to Turkevich method [17]. The Turkevich method is an approach used to prepare gold nanoparticles in solution. It involves the reduction of a gold salt solution by sodium citrate to form aggregates of gold nanoparticles. The gold nanoparticles are formed via a diffusion limited aggregation process. The size and shape of the nanoparticles can be controlled by varying the amount of sodium citrate used in the reaction. In summary, a stock solution of gold nanoparticles was made by dissolving 0.1699 g of $HAuCl_4$ in 100 mL of double-distilled water, yielding a light yellowish solution. After that, 5 mL of this solution was mixed with 90 mL of double-distilled water. The solution was heated to boiling point, then 5 mL of 0.5% sodium citrate solution was added with strong magnetic swirling to the boiling solution. After another fifteen minutes of heating, the solution gradually developed a wine-red tint. The solution was produced up to 100 milliliters with double-distilled water, agitated for another 15 minutes, and kept at 4 degrees Celsius.

#### 2.2.2. Preparation of gold-doxorubicin Nanocomposites

Stock solutions of AuNPs and stock solution of Doxorubicin were prepared. 10 ml of doxorubicin was added to 20 ml of gold solution ($5 \times 10^{-3}$ mol/L). The mixture was stirred for 24 hours to complete granulation/surface adsorption of doxorubicin by gold nanoparticles, thus forming the corresponding Au/DOX nanocomposite. The preparation was carried out in a dark. The mixture was then diluted for next applications on cancer cells.

### 2.3. Characterization

A drop of the prearranged samples was dried on a carbon-covered copper lattice for transmission electron microscopy. Micrographs taken with a Joel-

100S transmission electron microscope with a resolution of 0.3 nm were used to calculate the sizes of the particles. An ultraviolet-visible spectrophotometer (Lambda 40, Perkin-Elmer, Redmond, WA) was used to attempt spectroscopic characterization of the colloidal AuNPs and Au/DOX nanocomposites with a matched quartz cell of 1 cm path length. The lyophilized samples of DOX, AuNPs, and AuNPs -DOX were kept in KBr plates and were recorded on a NICOLET 6700 FTIR Thermo spectrometer, England to concentrate on the functional groups and the binding of AuNPs, doxorubicin, and DOX-AuNPs.

**2.3.1. Anticancer activity method**

The two cell lines, the esophagus cell line (OE33) and the human normal fibroblast cell line (HBK) were gotten from VACSERA-Cell Culture Unit, Cairo, Egypt. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics were added (100 units/mL penicillin and 100 µg/mL streptomycin) at 37°C in a 5% $CO_2$ incubator. The cytotoxic effect of DOX; AuNPs and AU/DOX nanoparticles derivatives against OE33 cells and BHK were estimated by MTT (3-[4, 5-Dimethylthiazol]-2, 5-Diphenyltetrazolium bromide) assay [18]. The statistics were presented as the mean percentage of viable cells in comparison to the solvent-treated control cultures. The line equation of the dose-dependent curve was used to calculate the half-maximum growth inhibitory concentration (IC50 values) for each sample [19].

**2.3.1. Laser irradiation source**

Using a low-power diode laser (532 nm DPSS laser-LSR-PS-1, Lasever Inc., China), continuous wave (CW) laser illumination of nanoparticles was carried out. 1 $cm^2$ was the size of the laser spot. For each example, samples were illuminated with 80 mW and energy of approximately 75 $J/cm^2$ [20].

**2.3.2. Molecular studies**

RNA extraction from the OE33 cell line treated with nanoparticles compound was done using a commercially available kit RNeasy according to manufacture protocol. The levels of mRNA expression in the treated and control OE33 cells were analyzed using real-time PCR. As a housekeeping gene, the expression of the -actin gene was utilized. According to the manufacturer's instructions, RT-PCR reaction was carried out in triplicate with the Power master mix 2x (ABI reagent from Bio systems Applied, USA). The following were the conditions for amplification: 5 minutes at 95°C, followed by a two-step cycle of 95°C for 15 seconds and 64°C for 40 seconds for 40 cycles). The 2-ΔΔCT method was used to calculate differences in gene expression levels between treated and untreated OE33 cells [21, 22].

## 3. Results

### 3.1. Gold nanoparticles characterization

Figure 1 shows the ultraviolet-visible absorption spectra of gold nanoparticles and AuNPs-DOX, which display a peak with maximum absorption centered at of around 530 nm, corresponding to gold nanoparticles. The peak of the AuNPs-DOX nanocomposites is wider than that of the Au nanoparticles and is located at around 540 nm.

Fourier transform infrared (FTIR) spectroscopy was used to monitor different types of interactions and also the functional groups of the compounds formed. So, the functional groups of AuNPs; DOX; and AuNPs /DOX surfaces were investigated by FTIR analysis. Moreover, FTIR absorption spectra can provide the information about the chemical change of the functional groups involved in the mixture formation. FTIR spectra of AuNPs; DOX; and AuNPs /DOX were shown in (Figure 2).

In FTIR spectra of AuNPs synthesized by Turkevich method yielded a broad band from 500 to 700 $cm^{-1}$ and strong bands at 940, 1210, and1636 $cm^{-1}$(Figure 2). These bands correspond to the amide I, II, and III bands of polypeptides/proteins. In the same Figure, FTIR evaluation of Doxorubicin drug which confirmed wonderful peaks at wave numbers 667$cm^{-1}$, 1015 $cm^{-1}$, 1089$cm^{-1}$,1422$cm^{-1}$, 1635 $cm^{-1}$ and, 2041 $cm^{-1}$. The peaks corresponding to alkyne C-H, aromatic C-H, fragrant C-H, carboxylic acids, alkenyl C=C stretch, $NH_3$ structure. FTIR analysis of Doxorubicin loaded Gold nanoparticles showed broad band at 580 $cm^{-1}$corresponding to trans C-H stretch and a peak at 1220 $cm^{-1}$ corresponding to aromatic C-H stretching. The starching band of alkenyl C=C at 1636 $cm^{-1}$ still exist in the spectra of the mixture. In the spectra also peaks at 2040 $cm^{-1}$ and 2350 $cm^{-1}$ corresponding to $NH_3$ structure. The absence of many DOX pands is another indication of the complex formed from DOX and gold nanoparticles

The gold Nano spheres were around 4 ±1.5 nm in size, according to transmission electron microscopy. Transmission electron microscopy of the generated Au/DOX Nano composites, on the other hand, reveals roughly spherical forms with varied diameters ranging from 4 to 11 nm with a spherical shape (Figures 3).

### *3.2. Cytotoxicity assay (MTT)*

The applications of DOX; AuNPs and Au/DOX nanocomposites with laser or without laser treated revealed cytotoxicity on OE33 cell line as shown in (Figure 4). The results indicated that in OE33 cells, derivative AuNPs showed no cytotoxicity at low concentrations but had a moderate toxicity at high concentrations against OE33 as shown in (Figure 4). The derivative AuNPs was the best cytotoxic compound with IC50 about 2.55 ±0.31 µM compared to doxorubicin with 8.0 ± 0.03 µM IC50 against OE33 cells. The derivative Au/DOX nanocomposites

without laser treatment showed a moderate cytotoxicity, while derivative Au/DOX nanocomposites with laser treatment showed mild effect on normal cell viability against OE33 cells. The derivative Au/DOX nanocomposites with laser treatment showed mild cytotoxicity on normal cell, whereas it exhibited a moderate cytotoxicity against OE33 cancer cells. Results revealed that derivatives Au/DOX nanocomposites with laser treatment had the most potent cytotoxic effect with IC50 1.291 ±0.9 µM, compared to Au/DOX NPs without laser treatment with IC50 1.352 ±1.91 µM against OE33 cells.

### *3.3. q RT- PCR results*

Relative expression of mRNA of bax was significantly up regulated in OE33 laser treated or untreated cell lines when compared to control cell line. On the contrary, alterations in bcl2 genes level expression in OE33 laser treated or untreated cell lines were minimal in comparison to control cell lines (Figure 5).

**Table 1**
Summary of primer sequences for bcl-2, bax and β-actin [21, 22].

| Primers | | Sequence of Nucleotides |
|---|---|---|
| Bax | Forward | 5'- ATG GAC GGG TCC GGG GAG CA -3' |
| | Reverse | 5'- CCC AGT TGA AGT TGC CGT CA-3' |
| Bcl-2 | Forward | 5'- GTG AAC TGG GGG AGG ATT GT -3' |
| | Reverse | 5'- GGA GAA ATC AAA CAG AGG CC -3' |
| β-actin | Forward | 5′- CTG TCT GGC GGC ACC ACC AT -3′ |
| | Reverse | 5′- GCA ACT AAG TCA TAG TCC GC -3′ |

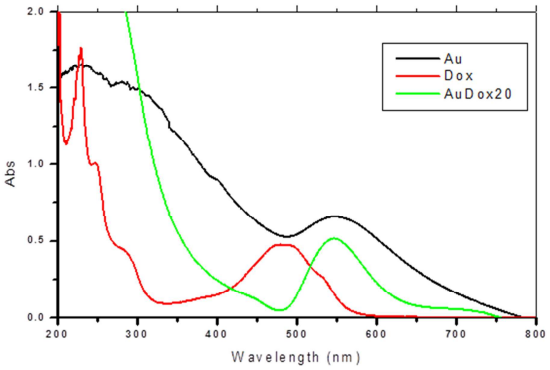


**Figure 1:** UV-Vis absorption spectra of DOX; Au and Au/DOX.

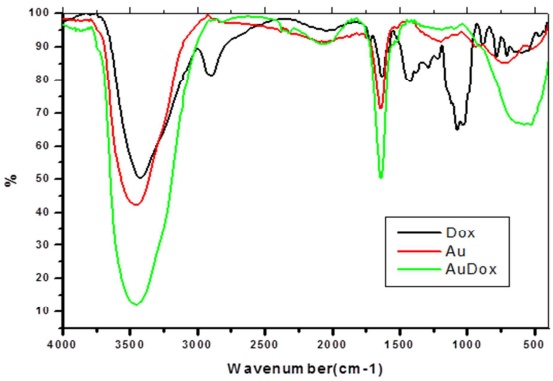


**Figure 2:** FTIR spectrum of DOX, Au, and Au/DOX.

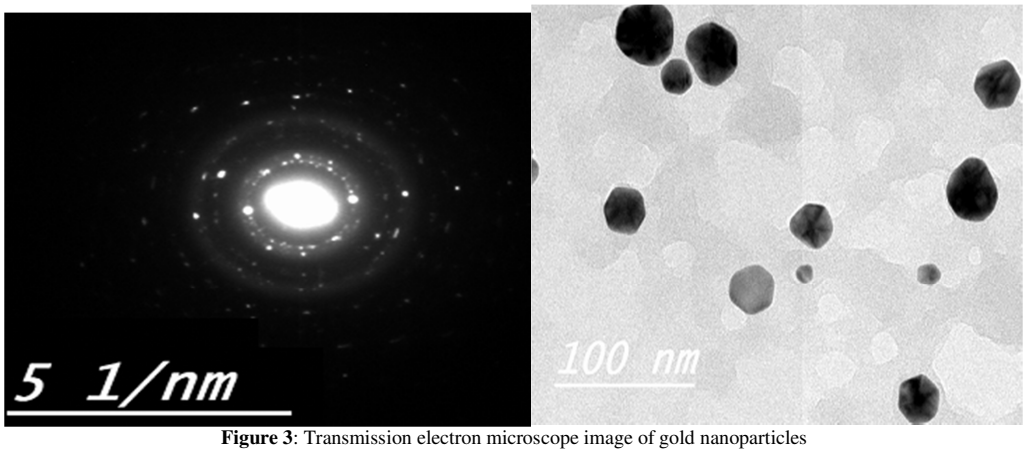


**Figure 3**: Transmission electron microscope image of gold nanoparticles

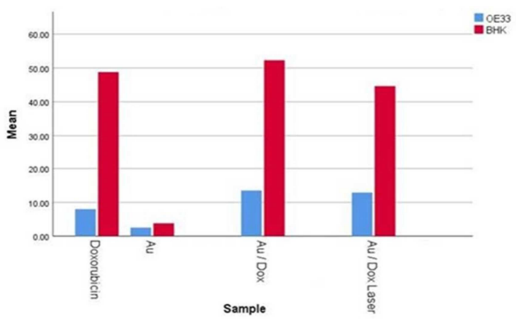


**Figure 4:** Cytotoxicity of Dox ; gold nanoparticles ; gold/DOX nanocomposites without laser and with laser against human cell lines

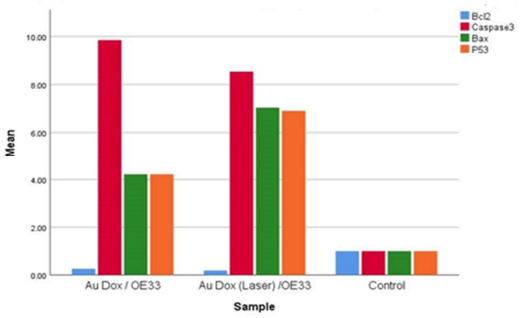


**Figure 5:** Gene Expression in Au/DOX without and with laser treated groups

## 4. Discussion

Nanotechnology could be used to develop targeted cancer therapies. For example, nanoshells containing antibodies against cancerous cells can be heated to kill cancerous cells while leaving healthy cells unharmed. This can be a powerful tool in cancer treatment, as it reduces the systemic side effects associated with traditional cancer treatments. In the present study, toxic responses of gold nanoparticles to human esophagus (OE33) cells were investigated. The obtained results demonstrated that exposure of gold nanoparticles and gold/doxorubicin nanocomposites to OE33 cells cause cytotoxicity. MTT assays revealed that the gold nanoparticles and gold/doxorubicin nanocomposites exert significant cytotoxicity to OE33 cells in laser treated. We analyzed the mRNA expression levels of three genes; bax, bcl-2 and B-actin as a control in response to gold nanoparticles and gold/doxorubicin nanocomposites exposure in OE33 cells.

In the present experiment, the treated cells with several concentrations of the present Doxorubicin, AuNPs, and Au/DOX Nanocomposites were examined by MTT test for 24 h regarding the cytotoxicity properties on normal fibroblast cell line (BHK) and human Caucasian esophageal carcinoma (OE33). The result of anti-esophageal cancer properties of gold/DOX nanocomposites with laser treatment were better than that of DOX, AuNPs and Au/DOX Nano composites without laser treatment, In agreement with Zhou et. al. [23]. Recently, many researchers have announced the utilization of gold nanoparticles for the fruitful delivery of drugs like doxorubicin for defeating drug resistance in malignant cells as well as peptide-functionalized gold nanoparticles for targeting cancer cells [24, 25]. Gold nanoparticles can possibly assume a critical rule to accomplish such goals. It is guessed that nanoparticle-mediated designated targeted delivery of drugs could essentially diminish the malignant growth drugs doses with better particularity, upgraded adequacy, and lower toxicities levels.

Quantitative real-time PCR results showed that the best result of gold/DOX Nano composites with laser treated up-regulated mRNA level of pro-apoptotic bax. Expression of anti-apoptotic bcl-2 was down-regulated in cells exposed to gold/DOX Nanocomposites with laser treatment, compared with gold/DOX Nano composites without laser treatment and control cell line, the activation of the pro-apoptotic family, for example, bax actuates permeabilization of the external mitochondrial layer, which lets dissolvable proteins out of the intermembrane space into the cytosol, where they advance caspase initiation Many comparable perceptions as noted by Lanvin et al., [26], Youle and Strasser [27].

## 5. Conclusions

In conclusion, this study reports a basic technique for the preparation of doxorubicin-stacked gold nanoparticles. Stacking of DOX was confirmed by UV and FTIR. Bio-distribution studies revealed that the gold-DOX could be effectively retained throughout the tumor in the presence reasonable laser. Organization of gold-DOX, in presence of laser, exhibited the best helpful anticancer activity and least fundamental harmfulness in contrast with that of free DOX and gold/DOX without laser treatment.

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