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# Improved Anticancer Activity of Doxorubicin Gold Nanohybrid on Breast Cell Line

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## Abstract

The use of nanotechnology in medicine and definitely drug delivery allows the development of novel platforms for the efficient transport of drug molecules in diseased tissues. The current study aimed to use gold nanoparticles (GNPs) for Doxorubicin (Dox) drug carrier forming Dox/GNPs. Moreover, the cytotoxic effect was assessed on breast cancer cell line (MCF7) for different cultivation times. Dox/GNPs was examined by UV–visible spectroscopy, fluorescence spectroscopy and TEM. GNPs and Dox/GNPs have spherical and small size10±2 nm and 13±2 nm. The Anticancer Activity of the Dox/GNPs nanohybrid on the MCF7 cell line was increased compared to free Dox which indicate that AuNPs can be used as anticancer drug carrier for Doxorubicin in breast cancer treatment.

**Keywords***:* Nanoparticles, Doxorubicin, multidrug resistance, nanocomposite, drug carrier, breast carcinoma.

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## 1-Introduction

Despite the fantastic work done in cancer treatment, it is challenging to attain a cure rate without harming healthy cells. Anticancer medications have a number of issues, including their toxicity, which can cause both healthy and cancer cells to die, and the emergence of multidrug resistance (MDR)[i]. A significant global health issue nowadays is cancer. Every year, the world invests tens of billions of dollars and a significant amount of human capital in the creation of new anti-tumor medications[ii, iii]. The administration of anti-cancer medications has become safer and more effective because to developments in biocompatible nanoscale drug carriers, which have also improved pharmacokinetics and decreased side effects[iv]. The improved permeability and retention effect of nanoparticles in cancer treatment determines how effective they are[v,vi] .

Doxorubicin (Dox) is one of many chemotherapy medications that is frequently used as an anti-cancer medication. The three primary issues with Dox are first significant toxicity and a huge volume of distribution, second a short lifetime in the body, and third limited solubility which results in a narrow therapeutic index despite the fact that it kills cancer cells by preventing the synthesis of nucleic acid in the cell[vii]. Numerous researchers have suggested conjugating doxorubicin to nanoparticulate delivery devices to lessen the toxicity level in order to combat the non-specificity and high toxicity of the drug [viii]. Due to their features, including shape, size, and surface dependence, GNPs have been exploited as distinctive drug delivery vehicles in therapeutic applications. In addition to being biocompatible, it is also non-cytotoxic and has a functionalize surface[ix].

Here, we describe the synthesis of AuNPs using trisodium citrate acting as a reducing and capping agent, followed by the creation of a Dox/GNPs nanohybrid by Dox surface modification and direct interaction with AuNPs. UV-visible, TEM, and fluorescence spectroscopy have all been used to track the development of the novel composite. According to the data, the Dox/GNPs hybrid had a far more toxic effect on MCF7 than an equivalent dose of free Dox, which decreased side effects and improved therapeutic effectiveness.

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## 2-Materials and Methods

### 2.1. Preparation of GNPs

GNPs were synthesized according to the standard wet chemical method [x]. Trisodium citrate (0.0388 M, 2mL) was added to HAuCl4 boiling solution (0.001 M, 10mL). The formed nanoparticle size and shape were investigated using a TEM and UV-Visible spectrophotometer.

### 2.2 Preparation of Dox/GNPs nanocomposite

Dox/GNPs nanocomposite was made according to *Vaithilingam* method [xi]. 2ml of different concentrations of DOX (1, 0.1, 0.01, 0.001 mM) were mixed dropwise with 2ml of 1mM of AuNPs until ruby become violet.

### 2.3. Characterization of GNPs and Dox/GNPs nanocomposite

UV-visible absorbance spectra were measured using ***a*** double beam spectrophotometer ***(PG instrument, T80$^{+}$, UK.)***.400ul from (GNPs, Dox and Dox/GNPs nanocomposite) were diluted to 4ml with distilled water then the spectra were taken against distilled water.

The morphology was carried out using TEM – Nanotechnology& Advanced Material Central Lab. (NAMCL), Agriculture Research Center (ARC). Company name: FEI, Netherland. Model: Tecnai G20, Super twin, double tilt, and Applied voltage: 200 kV, Magnification Range: up to 1,000,000 X, and Gun type: LaB6 Gun.

Photoluminescence spectra of 400ul of (free DOX, and DOX-AuNPs) diluted to 4ml with distilled water were recorded, using UV-quartz cuvettes of 1x1 cm$^2$, with a *Perkin Elmer LS55* Spectrofluorometer. equipped with a xenon short-arc lamp as an exciting source. The spectra were measured at right angle excitation.

### Cytotoxicity of DOX and DOX-AuNPs on MCF7

This method was carried out according to that of Skehan et al. (1990)[xii]. Cells were seeded in 96-well microtiter plates at a concentration of 5x10$^3$ Cell/well in a fresh medium and left to attach to the plates for 24 hrs. Cells were incubated with different concentrations of free DOX (5x10$^{-3}$, 10$^{-2}$, 2x10$^{-2}$,4x10$^{-2}$, 8x10$^{-2}$mM) and Dox/GNPs with the same concentrations then completed to a total of 200 µl volume/well using fresh medium and

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incubation was continued for 24, 48 and 72 hrs. For each drug concentration, three wells were used. Following 24, 48, and 72 hrs treatment, the cells were fixed with 50 µl cold 50% trichloroacetic acid for 1 hr at 4 0C. Wells were washed 5 times with distilled water and stained for 30 min at room temperature with 50 µl 0.4 % SRB dissolved in 1 % acetic acid. The percentage of cell survival was calculated as follows: Survival fraction = O.D. (treated cells)/ O.D. (control cells). The $IC_{50}$ values (the concentrations of thymoquinone required to produce 50 % inhibition of cell growth).

## Results and discussion

### Characterization of Dox /GNPs nanohybrid

Figure 1, illustrates how the produced GNPs exhibit visible-range absorption caused by Surface Plasmon Resonance (SPR) at 520 nm[xiii],[xiv]. According to figure 1, pure Dox exhibits a single band in the visible spectrum starting at 400 nm for UV-visible absorption. Anthracyclines are what make this band distinctive. Dox has absorption peaks that vary from 200 to 300 nm[xv], therefore it absorbs in the UV spectrum as well. Dox band intensity dropped when colloidal GNPs were added. A new band at 630 nm also appears along with this reduction.

After the addition of medicines, the nearby GNPs began to aggregate as a result of interparticle contact, which led to the development of this new band. A color change from wine red to blue and a little rise in the GNPs size in figure 1 are indicators of the aggregation. The Dox/GNPs complex that results from the substitution of citrate molecules with Dox may be what causes GNPs to aggregate. The extra band at higher wavelengths may also be due to the stronger electrostatic attraction of active groups in Dox with GNPs than citrate group[xvi].

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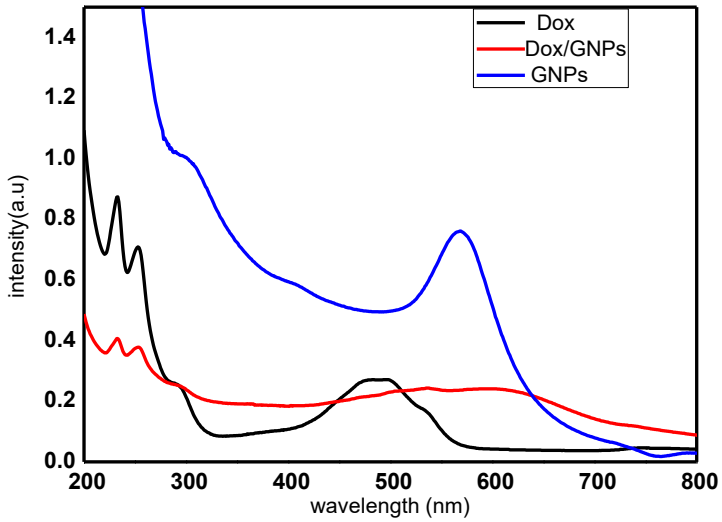


**Figure 1.** UV- Visible of GNPs, native Dox and Dox/GNPs nanohybrid

To better understand the morphology of the generated GNPs, TEM studies were used; the particles have a uniform size distribution and are roughly spherical in shape and (10±2nm) nm in size. According to TEM pictures of the Dox/GNPs nanohybrid in figure 2, the nanocomposite had a smooth surface and a consistently spherical form, with only small increases in particle size from 10±2 nm to13±2nm.

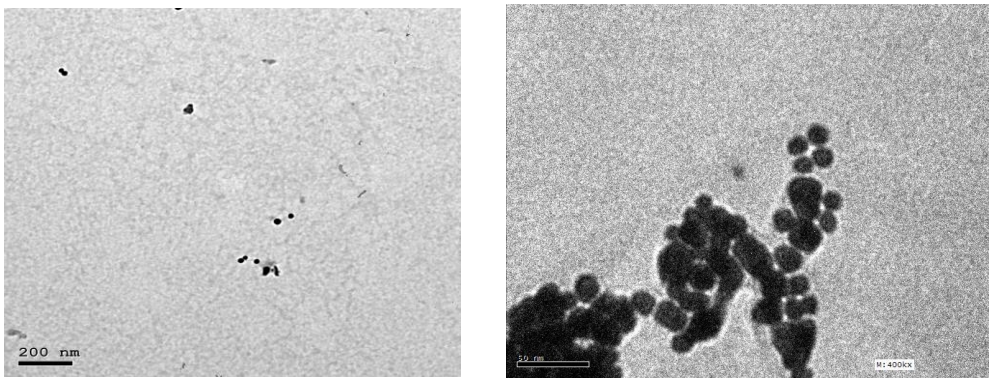


**Figure 2.** TEM of GNPs and Dox/GNPs .

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**Fluorescence measurement**

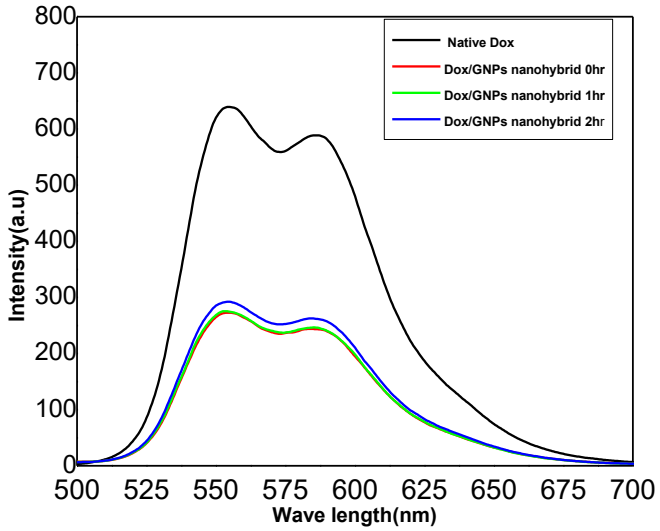


**Figure 3.** Fluorescence of n Dox and Dox@GNPs at changed time.

Since gold metal effectively quenches the emission of many fluorophores, fluorescence studies provide a great probe for verifying the binding of medicines with GNPs. In Fig. 3, the recorded spectrum is displayed. The peaks at 550 and 590 nm that are seen for pure Dox were maintained in the presence of GNPs, and the spectral profile of Dox-loaded GNPs did not significantly change. The -NH group's binding to the surfaces of GNPs causes an alteration in the electronic environment, which causes the fluorescence to become less intense. This quenching of fluorescence can be linked to the electronic interactions between Dox and GNPs. Nevertheless, the fluorescence signature's persistence supports the claim that Dox structure is retained following complexation with AuNPs.

**Effect of Dox and Dox/GNPs nanohybrid on MCF7**

Figure 4, shows the impact of various Dox and Dox/GNPs nanohybrid concentrations (0.005, 0.01, 0.02, 0.04, and 0.08 mM) on the percentage of breast cancer cell line (MCF7) survival after 24, 48, and 72 hours of drug treatment. It should be highlighted that the Dox/GNPs nanohybrid was used at each concentration so that the Dox concentration was comparable to that in free solution, allowing for a direct comparison. Cellular proliferation was observed to be concentration- and time-dependently lower at all study times compared to the corresponding control. The surviving fraction shows cytotoxicity when measured in relation to

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untreated control cells. Table1 compares the IC50 values for free Dox and Dox/GNPs nanocomposite at different concentrations at 24 hours, 48 hours, and 72 hours. When compared to free drug, where the reported IC50 values were 37.3 M and 1.05 M in 24 h and 48 h, respectively, Dox/GNPs nanohybrid showed a reduced IC50 value of 14.6 M and 0.857 M. While the IC50 values for free Dox and Dox /GNPs nanocomposite in 72h were both 0.667 M, which was lower than those in 24h and 48h.

Dox has been successfully used to treat a variety of malignancies. The capacity of doxorubicin to interfere with DNA activity and cause DNA damage is one of the mechanisms through which it exerts its proapoptotic effects. Dox has the ability to cross-link DNA strands, block topoisomerase II, and intercalate into DNA double helices. In addition, it is understood that doxorubicin generates reactive oxygen species (ROS) through one-electron reduction to the equivalent semiquinone free radicals, which subsequently quickly react with oxygen to produce superoxide radical anions. [xvii,xviii]. DNA damage and ROS buildup can both contribute to doxorubicin-mediated apoptosis. Due to targeted delivery, the Dox/GNPs nanocomposite may have increased cytotoxicity as a result of better drug accumulation at the site of action. The improvement caused by the internalisation of Dox/GNPs nanocomposite through an endocytosis process is one reason for the activity boost of Dox/GNPs nanocomposite. Contrary to the passive diffusion route of free DOX into cells, nanoparticles are often nonspecifically internalised into cells by endocytosis or phagocytosis [xix,xx].

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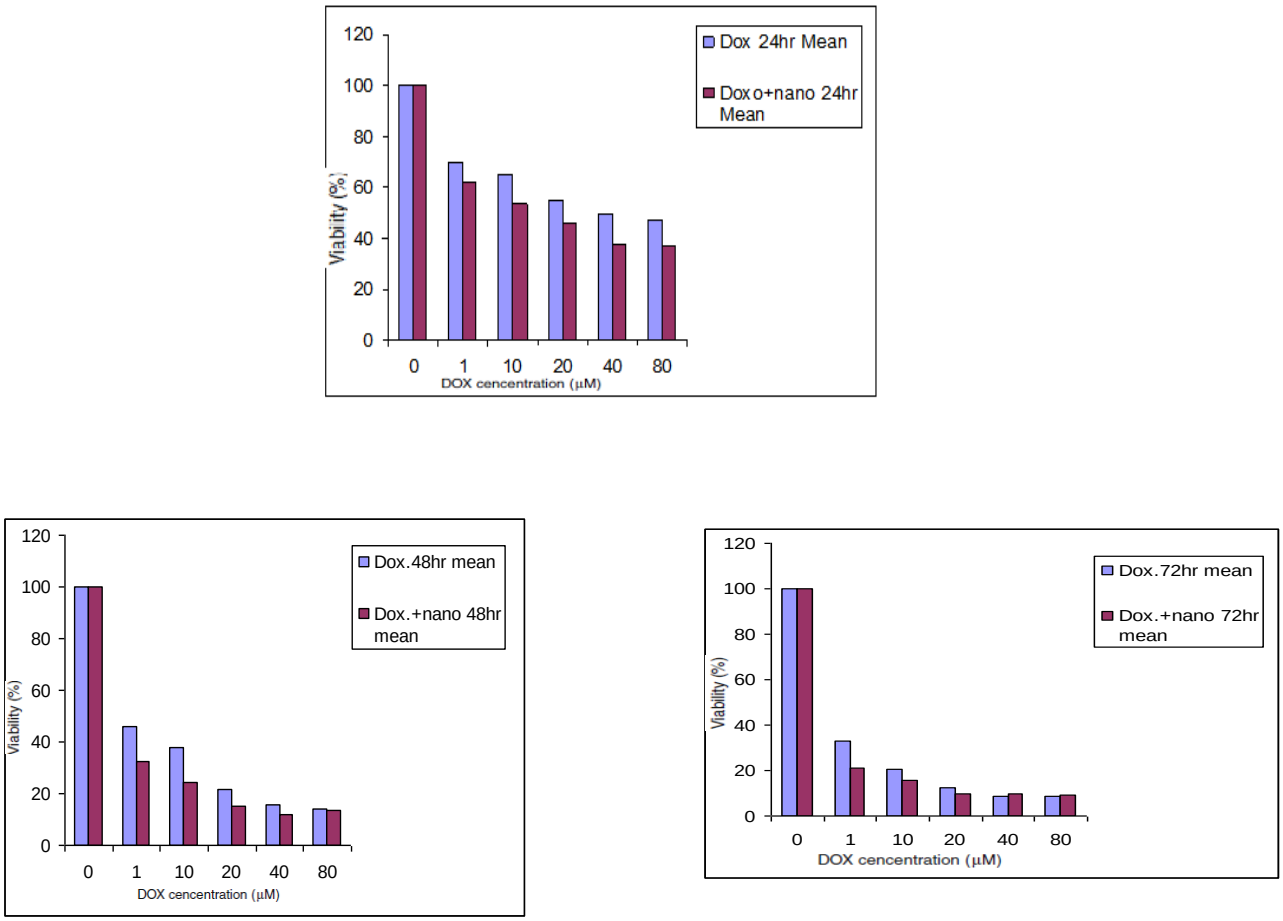


**Figure 4.** Cytotoxicity of Dox and Dox/GNPs nanocomposite on MCF7 cell line following 24, 48, and 72 hr.

## 4. Conclusion

The current work illustrated a technique for coating Dox on GNPs to create a nanocomposite for the treatment of breast cancer. The synthesized GNPs and Dox/GNPs nanocomposite had average particle sizes of 10 nm and 13 nm, with spherically shape . The anti-proliferation abilities of free Dox and Dox/GNPs nanohybrid against the MCF7 breast cancer cell line was then evaluated and contrasted. As a result of coating Dox on GNPs, the anti-proliferation activity against the MCF7 cell line was greatly enhanced, according to the data.

## ETHICAL STATEMENT:

**Ethics approval and consent to participate: Not Applicable**

**Consent for publication:** Not Applicable

**Competing interests**

The authors declare that they have no conflict of interest.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Authors' contributions:**

**“**All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by [Amna H.Faid]. The first draft of the manuscript was written by Marwa A Ramadan and Elham M.Mostafa and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.”

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