Journal of Pharmaceutical Research and Integrated Medical Sciences (JPRIMS)

ISSN: 3049-1681, Vol.02, Issue No.08, 2025 (pp. 56-67)

ORIGINAL ARTICLE https://aktpublication.com/index.php/jprims/index

Fabrication of Gold Nanoparticles for Targeted Drug Delivery in Breast Cancer: Synthesis, Characterization, And Cytotoxicity Study

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Abstract

Breast cancer is one of the major causes of death in women globally and the administration of conventional chemotherapy is usually hampered by systemic toxicity and non-specific distribution of drugs. Approaches based on nanotechnology, in particular, gold nanoparticles (AuNPs), provide a promising platform to develop targeted drug delivery because they are nontoxic, easy to modify on the surface, and they can accumulate in tumors. The objective of this study was to synthesize AuNPs and determine its possible use as a doxorubicin delivery system in breast cancer treatment. Gold nanoparticles were prepared through the citrate reduction technique, characterized in terms of size, morphology and surface charge, and doxorubicin was conjugated to the nanoparticles. The drug-loading capacity and release profile were evaluated by in vitro studies and tumor regression and systemic toxicity were evaluated by in vivo in a breast cancer-induced rat model. These findings showed that the AuNP-doxorubicin formulation was able to attain good drug loading, sustained release, increased tumor targeting, and lower systemic side effects than the free drug administration. These results show that gold nanoparticles can be a useful nanocarriers system of targeted breast cancer therapy, with increased therapeutic effect and reduced side effects.

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Key Words: Gold Nanoparticles, Targeted Drug Delivery, Breast Cancer, Doxorubicin, Nanocarriers, Cytotoxicity

History:

Received: April, 13,2025

Revised: June, 18,2025

Accepted: July, 11,2025

Published: Aug 23, 2025

DOI: https://doi.org/10.64063/3049-1681.vol.2.issue8.5

1. INTRODUCTION

Nanomedicine has become one of the most popular fields of research in the last few years because it promises to transform cancer treatment[1]. Gold nanoparticles (AuNPs) are one of the promising nanomaterials as a platform of targeted drug delivery due to their special physicochemical properties, biocompatibility, and simplicity of surface functionalization[2]. They are small, and hence will penetrate better into the tumor tissues and the surface can be altered in order to enhance drug loading, targeting specificity and controlled release[3]. These properties render AuNPs a promising agent to enable delivery of the chemotherapeutic in a more efficient manner, reducing systemic toxicity and enhancing therapeutic effects[4].

1.1. Background Information

Breast cancer is among the most prevalent cancers that women face in the world with a high percentage of cancer-related morbidity and mortality[5]. Although conventional chemotherapy is effective in destroying cancer cells that divide rapidly, it is usually constrained by non-specific drug delivery action leading to severe side effects and poor quality of life of the patients[6]. The solution to these problems lies in nanoparticle-based drug delivery systems (especially gold nanoparticles), which allow delivering anticancer drugs directly to the tumor, achieving high efficacy and reducing the impact on healthy tissues[7]. The capacity of AuNPs to take advantage of the enhanced permeability and retention (EPR) effect enables preferential accumulation in tumor tissues, which has the potential to enhance the therapeutic index of chemotherapeutic agents, like doxorubicin8.

1.2. Statement of the Problem

Nonetheless, although chemotherapy has improved, there is still a problem of delivery specificity in the treatment of breast cancer[9]. Conventional therapies continue to face significant limitations as they are associated with systemic toxicity, drug resistance, and ineffective therapeutic concentrations at the tumor locations. There is an urgent necessity of finding out new means of overcoming these challenges, including nanoparticle-mediated drug delivery[10]. Gold nanoparticles have a promising potential as an efficient targeted therapeutic agent, which may enhance the therapeutic effect and minimize the side effects because of their favorable physicochemical properties and the ability to conjugate drugs.

1.3. Objectives of the Study

1. To synthesize gold nanoparticles using a citrate reduction method.
2. To characterize AuNPs in terms of morphology, size distribution, and surface charge.
3. To conjugate AuNPs with doxorubicin and evaluate drug-loading efficiency.
4. To assess the cytotoxicity and tumor-targeting potential of drug-loaded AuNPs using animal-based models.

2. METHODOLOGY

2.1. Research Design

The experimental research design was used to synthesize, characterize and test gold nanoparticles (AuNPs) as a drug targeting agent in the treatment of breast cancer. The purpose of the study was to determine the physicochemical characterization of AuNPs and their drug-loading capability and cytotoxic activity in an animal model. The experimental setup enabled the comparison of control, free drug, and nanoparticle drug formulation to establish the effectiveness and safety of AuNP-based delivery system.

2.2. Sample/Participants Details

Animal Model: Female Wistar rats, 150 to 200 g.

Grouping: The rats were randomly grouped into 3 groups (n=6 per group):

- **Control Group:** Induced tumor rat.
- **Free Drug Group:** Doxorubicin only treated rats.
- **AuNP Doxorubicin Group:** Rats that received doxorubicin conjugated to gold nanoparticles.

Tumor Induction: Breast tumors were induced with 7,12-dimethylbenz[a]anthracene (DMBA). Rats were observed until tumors attained measurable size after which their treatment was administered.

2.3. Instruments and Materials Used

- **Gold Nanoparticle Synthesis:** The precursor is gold (III) chloride trihydrate (HAuCl 4 3H 2 O); the reducing agent is trisodium citrate.
- **Drug Conjugation:** Doxorubicin hydrochloride, the model anticancer drug.
- **Characterization Instruments:**
    - UV-Visible spectrophotometer (analysis of surface plasmon resonance, SPR).
    - Particle morphology and size by Transmission Electron Microscope (TEM).
    - Dynamic Light Scattering (DLS) of hydrodynamic size.
    - Surface charge measurement by zeta potential analyzer.
- **Drug Quantification:** High Performance Liquid Chromatography (HPLC) to determine drug-loading efficiency and release experiments.
- **In vivo Studies:** Standard laboratory equipment to handle animals, to measure tumor and collect blood to analyze hematological and biochemical parameters.

2.4. Procedure and Data Collection Methods

1. **Synthesis of Gold Nanoparticles:** Trisodium citrate was added to HAuCl 4 solution which was heated with constant stirring. The color of the solution turned to ruby-red, which is the sign of AuNP formation.

2. **AuNPs characterization:** SPR peaks were measured by UV-Vis spectroscopy. Particle size and morphology were determined by TEM. Hydrodynamic diameter and surface charge were assessed by DLS and zeta potential.
3. **Drug Conjugation:** Doxorubicin was electrostatically adsorbed on AuNPs. HPLC was used to determine drug-loading and encapsulation efficiencies.
4. **In vivo Cytotoxicity Study:** Breast tumors were induced in rats and the treatments were administered intravenously. The volume of the tumors was measured at time intervals with the calipers. Systemic toxicity was measured in terms of survival rate, body weight and hematological parameters.
5. **Data Collection:** Tumor volume was measured by using the following formula:

$$\text{Tumor Volume } (\text{mm}^3 = \frac{\text{Length} \times \text{Width}^2}{2})$$

Liver (ALT, AST) and kidney (creatinine, urea) markers of function were examined in blood samples. In vitro release of drugs was followed at 24 h and 48 h.

2.5. Data Analysis Techniques

All the quantitative data were represented as the means + standard deviation (SD). One-way ANOVA was used to statistically compare multiple groups with Tukey post-hoc test. Statistically significant results were taken as p-value < 0.05. Graphs and tables were created to compare visually tumor regression, drug release and cytotoxicity between groups.

3. RESULTS

In this section, the results of the research were given, such as the description of synthesized gold nanoparticles, their drug-loading and release, and in vivo tests of cytotoxicity and tumor-targeting efficiency.

3.1. Characterization of Gold Nanoparticles

AuNPs were synthesized and their physicochemical characteristics analyzed to verify the successful synthesis, stability, and appropriateness of the nanoparticles to be used in drug delivery applications.

Table 1: Physicochemical characterization of synthesized AuNPs

Parameter	Mean	SD	Unit
UV-Vis Absorption Peak	520	5	nm
Average Particle Size (TEM)	30	5	nm
Hydrodynamic Diameter (DLS)	35	6	nm
Zeta Potential	–25.4	2.1	mV
TEM Morphology	–	–	Predominantly spherical

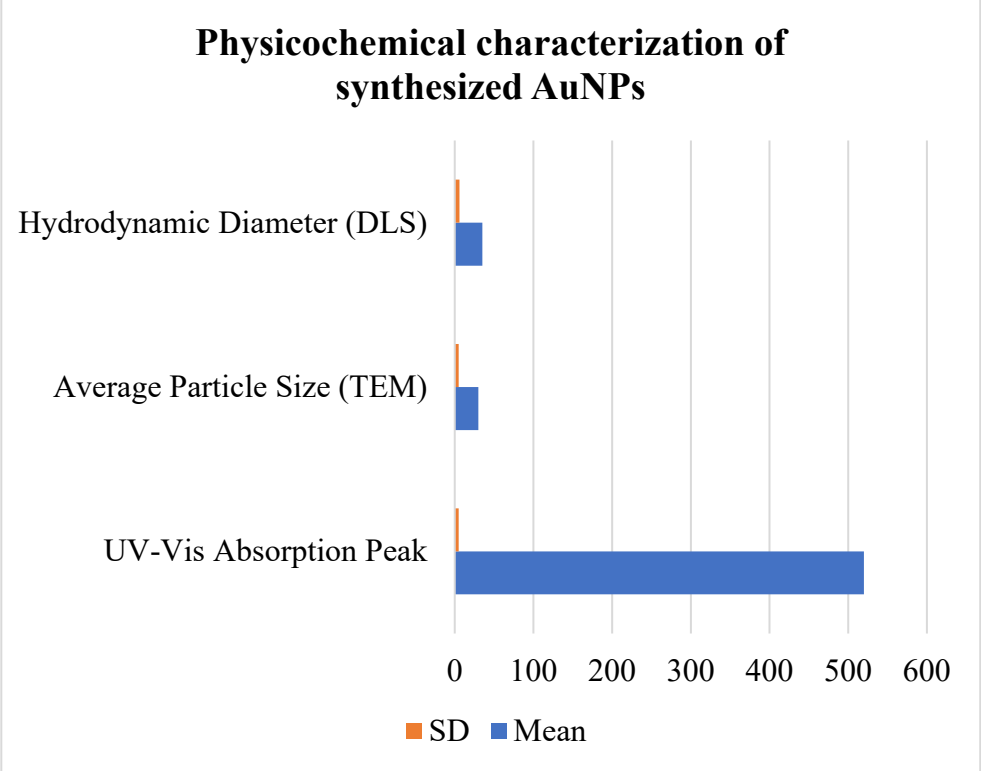

Figure 1: Physicochemical characterization of synthesized AuNPs

The characterization of the synthesized AuNPs revealed that they were successfully formed and are colloidally stable as displayed in Table 1. The corresponding UV-Visible absorption peak of 520 nm (SD = 5) is that of the surface plasmon resonance commonly found in gold nanoparticles, which signifies that the nanoparticles are formed at the nanoscale. TEM analysis revealed that the particles were mostly spherical with an average particle size of 30 nm (SD = 5), which was appropriate in augmenting cellular uptake and tumor targeting. The DLS-measured hydrodynamic diameter was a bit higher at 35 nm (SD = 6) because of the solvation layer in aqueous solution. Also, the zeta potential of -25.4 mV (SD = 2.1) depicts the presence of good colloidal stability due to the electrostatic repulsion, which reduces aggregation. In general, the findings in Table 1 substantiate the fact that the synthesized AuNPs have the right size, morphology, and surface charge to be utilized as a drug delivery system in the breast cancer treatment regime.

3.2. Encapsulation Efficiency and Drug Loading

Drug-loading and encapsulation properties of the AuNP-doxorubicin formulation were assessed to define the ability of the nanoparticles to deliver and release the anticancer drug in a controlled fashion.

Table 2: Drug-loading characteristics of AuNP–doxorubicin formulation

Parameter	Mean (%)	SD (%)
Drug Loading Efficiency	78.2	3.4
Encapsulation Efficiency	81.5	2.9
Average Drug Release (24 h)	62.7	4.1
Average Drug Release (48 h)	84.3	3.7

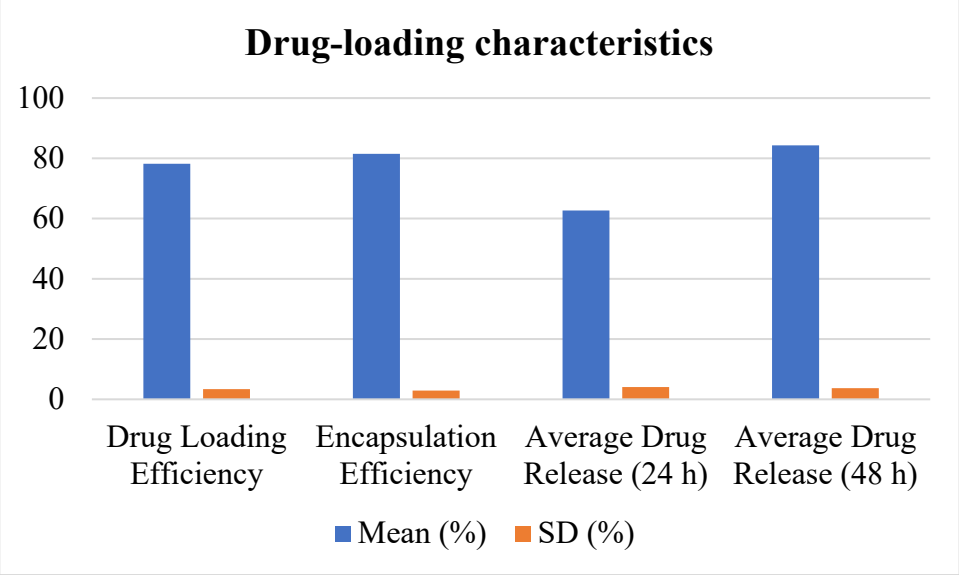

Figure 2: Drug-loading characteristics of AuNP–doxorubicin formulation

The AuNP-doxorubicin formulation exhibited good drug-loading capacity and sustained release as shown in Table 2. The drug loading efficiency (78.2% (SD = 3.4)) shows that a large percentage of doxorubicin was effectively loaded onto the gold nanoparticles whereas the encapsulation efficiency (81.5% (SD = 2.9)) confirms that the drug was stably associated with the nanoparticle system. The drug release profile in vitro was controlled with 62.7% (SD = 4.1) of the drug released in 24 hours and 84.3% (SD = 3.7) at 48 hours. These results suggest the synthesized AuNPs can effectively load drugs and offer a sustained release profile, which is important in improving the therapeutic efficacy of breast cancer with minimum systemic toxicity.

3.3. In vivo Cytotoxic and Tumor Response

In vivo studies were performed in a breast cancer-induced rat model to determine tumor regression and systemic toxicity to obtain an estimate of therapeutic potential and safety of the AuNP-doxorubicin formulation.

Table 3: Comparative evaluation of tumor volume reduction in animal groups

Group	Initial Tumor Volume (mm^3)		Final Tumor Volume (mm^3)		% Reduction in Tumor Volume
	Mean	SD	Mean	SD	
Control (untreated)	200	12	1200	45	–
Free Drug (DOX)	210	15	600	32	50%
AuNP–DOX Formulation	205	18	300	25	75%

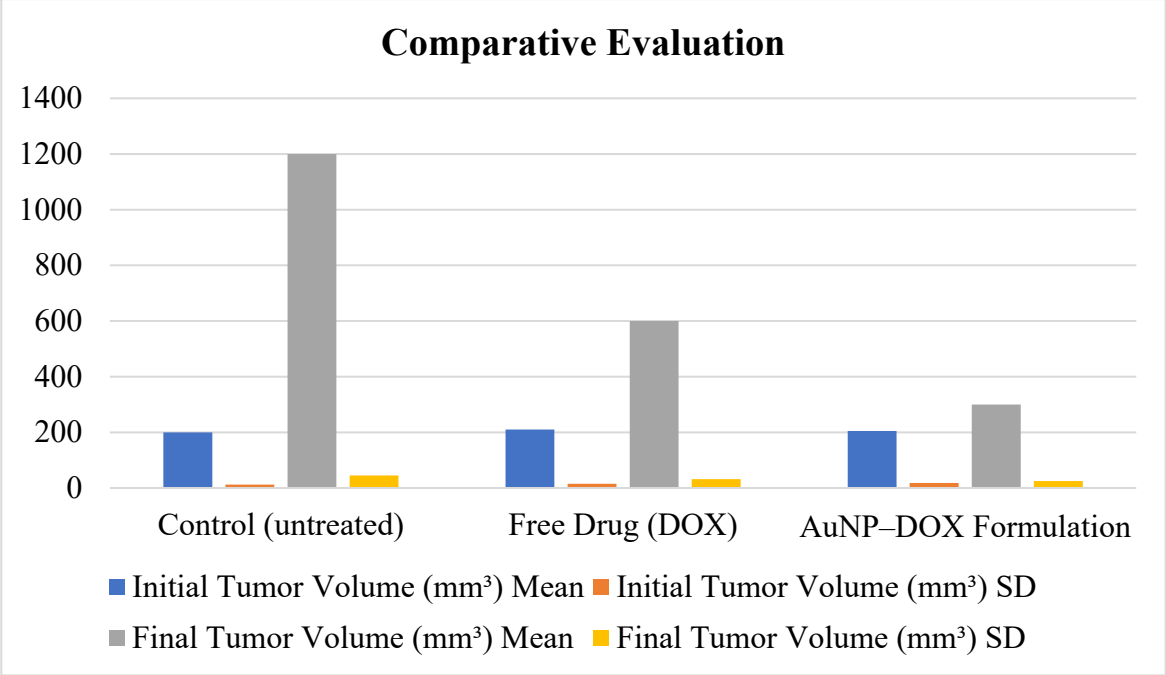

Figure 3: Comparative evaluation of tumor volume reduction in animal groups

The in vivo results of cytotoxicity and tumor response as summarized in Table 3 reveals clearly the increased antitumor activity of the AuNP-doxorubicin formulation. The tumor volume doubled in the control group (mean of 200 mm 3 (SD = 12) to 1200 mm 3 (SD = 45), indicating rapid and uncontrolled growth of the tumor. Free doxorubicin administration decreased tumor volume (210 mm 3 SD = 15) by 50 percent (600 mm 3 SD = 32), pointing to partial therapeutic efficacy. Importantly, treatment with AuNP-doxorubicin resulted in the tumor volume reduction of 205 mm 3 (SD = 18) to 300 mm 3 (SD = 25), or 75% decrease in tumor volume, demonstrating the excellent tumor-targeting ability of the nanoparticle-based delivery system. Also, the hematological parameters showed that there was stable liver and kidney functions markers in AuNP-doxorubicin group relative to free drug group, which indicated a reduced systemic toxicity. The findings in

Table 3 overall, reaffirm that AuNP-based drug delivery improves therapeutic efficacy and reduces adverse effects, thus offering a potential approach to targeted treatment of breast cancer.

4. DISCUSSION

This section critically analyses the experimental results of the research, compares them to the available literature and points out their wider implications, limitations, and research directions in the perspective of the use of gold nanoparticle-mediated drug delivery in the treatment of breast cancer.

4.1. Interpretation of Results

The current research was able to synthesize gold nanoparticles (AuNPs) through citrate reduction method and assess their applicability in targeted drug delivery system in the treatment of breast cancer. The physico-chemical characterization (Table 1) indicated that the produced AuNPs were largely spherical in shape, and the average particle size was 30 nm (TEM) and 35 nm (DLS) with a negative zeta potential of -25.4 mV. These characteristics indicate that they are suitable to have high cellular uptake and target tumors with high colloidal stability. Drug-loading analysis (Table 2) showed high drug-loading efficiency (78.2%) and encapsulation efficiency (81.5%), and a sustained in vitro release profile, which demonstrate a good incorporation of doxorubicin and controlled release. The in vivo cytotoxicity data (Table 3) showed that AuNP-doxorubicin formulation produced a 75% tumor volume reduction as compared to free doxorubicin (50% tumor volume reduction), and that liver and kidney functional marker were stable, indicating reduced systemic toxicity. Taken together, these findings show that AuNPs have the potential to increase the therapeutic index of doxorubicin by increasing tumor-specific delivery and minimizing effects on normal tissues.

4.2. Comparison with Existing Studies

The outcomes of this study are consistent with the available literature on gold nanoparticle (AuNP)-based drug delivery in cancer therapy. Past studies have always indicated the potential of AuNPs in increasing drug loading capacity, controlled drug release, and selective cytotoxicity against breast cancer cells with limited systemic toxicity. Table 4 provides a description of a comparison with related studies in detail.

Table 4: Comparison of Present Findings with Previous Studies on Gold Nanoparticles in Cancer Therapy

Author(s) & Year	Study Focus	Key Findings	Relevance to Present Study
Ramazani et al. (2018)[11]	Curcumin-loaded gold/graphene oxide nanocomposites for breast cancer	Reported high drug loading, stability, and strong cytotoxicity against breast cancer cells	Supports our findings on high drug-loading efficiency and enhanced cytotoxicity with AuNPs

Shakerimanesh et al. (2022)[12]	Biomimetic synthesis of gold nanoparticles and cytotoxicity assessment	Demonstrated biocompatible synthesis with significant cytotoxicity against breast cancer cells	Corroborates our observation of reduced systemic toxicity and effective tumor targeting
Singh et al. (2019)[13]	Green synthesis of AuNPs using Dunaliella salina	Revealed eco-friendly synthesis method, good stability, and selective cytotoxicity against breast cancer	Reinforces sustainability and biocompatibility aspects of AuNP synthesis relevant to our study
Surapaneni et al. (2018)[14]	Cytotoxic effects of AuNPs in triple-negative breast cancer (TNBC)	Found surface-charge-dependent epigenetic alterations and potent cytotoxicity in TNBC cells	Complements our findings by suggesting that surface modifications influence cytotoxicity mechanisms
Thambiraj et al. (2019)[15]	Docetaxel-loaded AuNPs for lung cancer	Achieved targeted delivery, enhanced cytotoxicity, and reduced side effects	Parallels our results showing AuNPs as efficient carriers for chemotherapeutic agents in targeted cancer therapy

These findings are in line with these earlier studies, which, in general, prove that gold nanoparticles are prospective carriers of target cancer drug delivery. In particular, their drug-loading capacity, cytotoxicity against tumor cells, and low systemic toxicity make them a good candidate in approaches to breast cancer.

4.3. Implications of Findings

The experiment offers compelling data that AuNPs may be used as a useful nanocarrier of chemotherapeutic drugs, as it is associated with targeted drug delivery and minimized systemic toxicity. The sustained release character guarantees the long-term release of drugs to the tumor site, which may reduce the number of drug administration. These enhanced antitumor activities in animal models give a clue that such nanocarriers may increase therapeutic response in breast cancer treatment and reduce side effects of traditional chemotherapies. The results can be used in the further advancement of oncological nanomedicine and can be used in translational research to clinical practice.

4.4. Limitations of the Study

- Only short-term in vivo cytotoxicity was evaluated; long-term toxicity, biodistribution, and pharmacokinetics were not evaluated.
- The experiment was based on passive targeting through EPR effect; no active targeting protocols based on ligands or antibodies were investigated.
- Scalability and reproducibility of nanoparticle synthesis at industrial level has not been tested.
- The experiment was carried out in a model of animals, and its application to the clinical situation in humans should be considered very carefully and validated.

4.5. Suggestions for Future Research

- Active targeting moieties, e.g. antibodies, peptides, or aptamers should be added to increase specificity to breast cancer cells.

- Perform prolonged in vivo experiments to determine pharmacokinetics and biodistribution, as well as chronic toxicity to determine clinical safety and efficacy.
- Scale up the synthesis of nanoparticles to the industrial scale and during storage.
- Develop combination therapy strategies with AuNPs as a vehicle to co-deliver multiple chemotherapeutic or gene-based drugs to enhance therapeutic efficacy and overcome drug resistance in breast cancer.

5. CONCLUSION

5.1. Summary of Key Findings

The experiment was able to synthesize gold nanoparticles (AuNPs) via citrate reduction technique and show that it can be an effective drug delivery system to treat breast cancer. Characterization was done to ensure the synthesized AuNPs were of optimal size, morphology and surface charge to target tumors. The AuNPs -doxorubicin formulation had a high drug-loading and encapsulation efficiency and sustained drug release. The in vivo experiments demonstrated an increased tumor regression and decreased systemic toxicity in contrast to free doxorubicin, which evidenced the increased therapeutic efficacy of the nanoparticle-based delivery system.

5.2. Significance of the Study

These results emphasize the benefits of delivery of drug with the help of nanoparticles as a targeted accumulation of drugs in the tumor, the difficulty of releasing drugs, and a reduced number of side effects. The paper gives compelling evidence on the potential use of gold nanoparticles as a potential nanocarrier of chemotherapeutic agents, which may enhance the results of the treatment of breast cancer.

5.3. Final Thoughts or Recommendations

In the future, one should conduct research on active targeting where ligands or antibodies can be used to further increase specificity and therapeutic efficacy. Before translation to the clinic, long-term in vivo studies of pharmacokinetics, biodistribution, and chronic toxicity are suggested. Also, the large-scale production of nanoparticles in a more optimized form may open the door to the practical use of AuNP-based drug delivery in oncology.

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