RESEARCH ARTICLE

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Modification of Gold Nanoparticles Provides Antioxidant and Anti-Tumor Properties

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Abstract

Background: Metal nanoparticles such as gold (Au NPs) are less poisonous than those used in drug transporters through several features, making them predominantly suitable for biological or pharmacological requirements. **Materials and Methods:** manufactured Au-NPs-PEG, then followed by encapsulation with SH-PEG-COOH to create a conjugate, which was validated via X-Ray diffraction analysis (XRD), in addition to Field-emission scanning electron microscopy (FE-SEM). The effectiveness of the Au NPs and Au NPs-PEG to MCF-7 (a cell line from human breast cancer) was estimated using antioxidant activity and clonogenicity assays. **Results:** The results showed that the produced nanoparticles were generally sphere-shaped with a smooth surface, with sizes of about 20.57 ± 2.51 nm and 29.14 ± 3.31 nm for gold nanoparticles and gold nanoparticles -PEG conjugate, respectively. Investigations were conducted into the antioxidant capacity of Au NPs and Au NPs-PEG conjugate against DPPH, and the Au NPs-PEG conjugate showed the maximum radical scavenging (84.661.79%) in concentration-dependent increments. A clonogenic survival assay investigated Au NPs and Au NPs-PEG conjugates for cytotoxicity against the MCF-7 cell line. Au NPs conjugate lowered the number of MCF-7 clones more effectively than the control. **Conclusions:** The generated Au NPs-PEG had a strong cytotoxic effect on the MCF-7 cells, accelerating cell death and enhancing cellular absorption of the chemical.

Keywords: Gold nanoparticles- PEG- Antioxidant- Anti-tumor

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Introduction

Cancer is a significant medical problem that affects millions around the world. Over 11 million individuals are diagnosed with diverse categories of cancer each year. By 2022, this number is predicted to climb to 17 million. The common type of cancer leading to malignancy and its primary reason for the decrease in females is breast cancer [1, 2]. May cancer treatments, for example, radiation, immunotherapy, and hormone therapy, are unsatisfactorily active due to the non-specific transport to the target site of the drug. The likelihood of reappearance, restricted value of the drug, with unfavorable consequences [3, 4]. As a result, A new, highly valuable chemotherapeutic drug is desperately needed, as well as strategies for delivering these mediators to transformed cells in a targeted manner. The field of nanotechnology remains in its infancy, but it has the potential to solve many issues in a wide range of fields [5]. Standard cancer therapy methods have changed in recent years, benefiting from nanotechnology-based diagnosis and treatment systems. Current advancements have nano-systems coupled to imaging probes and targeting aspects such as antibodies, aptamers, and enzymes. Nanoparticles are routinely used in modern medicine and have even established more innovative applications [6]. An attractive benefit of its nanoscale manufacturing capability and functionalization is due to the presence of SH (thiol) and NH3 (amine) groups, permitting the conjugation of different functional groups, specific antibodies, or pharmaceutical products [7, 8]. Au NPs are also biocompatible and easily conjugatable with other bioactive molecules like polyethylene glycol (PEG). They also have unique optical properties, which lead to a faster binding to active groups like NH3 and SH, resulting in a broader range of possibilities for altering the cell surface [9, 10].

Despite these advantages, the biocompatibility and cytotoxicity of nanoparticles, which vary depending on properties such as size, form, surface, and chemical structure, are critical factors that should be considered

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before clinical applications [11, 12]. When the nanoparticles are taken by the cells, proteins of the adjacent environment form a corona around the nanoparticles' surface, resulting in a nanoparticle-protein combination. The corona of this protein structure is remarkably diverse and flexible, and it is very significant in the nanoparticle's biodistribution in the tissues [13, 14].

Au NPs in the corona combination have an opsonin surface, by which immune cells identify this substance, which is a reticuloendothelial system component (RES). Such substances, in essence, regulate the path of internalization of nanoparticles, and, as a result, influence the destiny of the nanoparticles in the tissue [15, 16]. Albumin, fibrinogen, immunoglobulin G, immunoglobulin M, transferrin, and other proteins are extracted from the plasma's corona complex [17]. Changes in particle size and a charge may be caused by the corona complex that affects the uptake process of RES immune cells and their body's general distribution [18, 19]. Many proteins help immune cells distinguish nanoparticles; they have been shown to increase phagocytosis and then eliminate nanoparticles from specific tissues. However, dies-opsonins, for instance, albumins, have been shown to enhance nanoparticles to circulate through the blood for an extended time [20]. To prevent immune recognition of nanoparticles, scientists have developed a process known as "PEGylation," in which nanoparticles "cover" their surfaces with a PEG layer. It shields them from antibody detection, thereby extending their blood supply. The modification of nanoparticles with PEG can be applied to a surface by either adsorbing or covalently attaching the PEG chains to nanoparticles [21, 22]. According to what was previously stated, the goal of this study was to design and test the ability of the Au NPs-PEG conjugate as a candidate for anti-tumor therapy in vitro [23].

Materials and Methods

The Iraqi Center for Cancer and Medical Genetic Research, AL-Mustansiriyah University, Baghdad, Iraq, provided the human breast (MCF-7) cancer cell line. Trypsin/EDTA, serum of fetal bovine, DMSO (di-methyl sulfoxide), DPPH 32,2- (Diphenylpicrylhydrazyl), SH-PEG-COOH (Poly ethylene (2-mercaptoethyl) ether acetic acid) MTT Kit (Intron Biotech, Korea), antimicrobials as bacitracin and trimethoprim were provided from Sigma-Chemical Company (USA).

Generation of Metallic Nanoparticles

The Au NPs were produced using the technique described by Wu and his colleagues [19] with minor changes. Au NPs of 21 nm were created through boiling 100 milliliters of tri-sodium citrate with 2 mL of gold salts. Within 10 minutes, the solution altered to a wine-colored colour, suggesting the production of Au NPs. The mixture was subsequently cooled to room temperature with periodic stirring. The Au NPs–PEG preparation was mixed 1 mL SH-PEG-COOH with 100 mL of Au NPs. The mixture was stirred at 300 RPM. The citrate was exchanged thoroughly with the thiol at room temperature overnight. The mixture was cooled to 4°C and centrifugation at 13000 rpm for 30 minutes. The supernatant was discarded, The pellet was re-dispersed in phosphate-buffered saline (PBS). The Au NPs-PEG were completely purified for confirmation, and the centrifugation step was repeated.

Au NPs-PEG Characterization

FE-SEM is a scanning electron microscope that uses field emission. Examination was shown via MIRA 3 TE-SCAN to recognize the structural features of the produced Au NPs-PEG. A diffractometer for X-rays was used to clarify the crystals of the produced Au NPs-PEG. A Cu incident beam was used to determine the particles' diffraction patterns.

Free radical scavenging capacity

According to [24]. The DPPH test was used to assess the ability to scavenge free radicals' ability of Au NPs and Au NPs–PEG Briefly, in a cuvette, A combination of DPPH (60 mM) and each compound's IC50 concentration (0.5 mL) was produced and allowed to stay at 25 ˚C for 35 min. At 517 nm, absorbance was measured using a UV/VIS spectrophotometer (Lambda 19, PerkinElmer, Waltham, MA).

Maintenance and culture of cell lines

MCF-7 cells were cultivated in tissue-culture flasks (T 25 cm2, Falcon, USA) at 5 percent CO2 and 37 °C in RPMI-1640 supplied with L-glutamine (2 mM), HEPES (20 mM), and FBS (10%) for an in vitro cytotoxicity experiment.

Cytotoxicity Assays

The MTT assay was conducted utilizing 96-well plates to assess the cytotoxic effects of AuNPs. [25]. Cell lines were seeded at a density of 1×10^4 cells per well following 24 hours. A confluent monolayer was formed. MCF-7 cells underwent treatment with AuNPs. After 72 hours of treatment, cell viability was evaluated by removing the medium, adding 28 µL of a 2 mg/mL MTT solution, and incubating the cells for 2.5 hours at 37°C. Following the removal of the MTT solution, the crystals in the wells were solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) and incubated at 37°C for 15 minutes with shaking [26, 27].

The absorbance was quantified at 492 nm utilizing a microplate reader, and the experiment was conducted in triplicate. The cell growth inhibition rate (percentage of cytotoxicity) was calculated using the following equation [28].

Rate of Inhibition = A- B/A*100

A represents the control's optical density, while B denotes the samples' optical density [29].

A and B represent the optical densities of the control and test, respectively, for observing cell morphology under an inverted microscope. Cells were inoculated into 24-well microtiter plates at a density of 1×10^5 cells mL−1 and incubated for 24 hours at 37 °C. Subsequently, cells were subjected to AuNPs for 24 hours. After exposure, the plates were stained with crystal violet and incubated

at 37°C for 10 to 15 minutes. The stain was meticulously rinsed with tap water until the dye was entirely eliminated. The cells were observed using an inverted microscope at 100× magnification, and images were captured with a digital camera linked to the microscope [30].

Data was analyzed using an unpaired t-test using GraphPad Prism 6. Values were provided as the mean ± SD of triplicate measurements [31].

Ethical consideration

The research followed the ethical guidelines specified in the Helsinki Declaration. To get this permission, the local ethics committee amended and approved a research protocol by Document Number 61 on August 4, 2024.

Results

Creating Au NPs and Au NPs-PEG

Many techniques, including chemical, physical, and biological ones, have been used to create gold nanoparticles (Au NPs) of regulated size and form, and they have significantly improved human health. Citrate reduction of gold salts resulted in the creation of Au NPs. As tetra-chloroauric acid trihydrate solution was added dropwise to boiling sodium citrate dihydrate, the color progressively converted from pale yellow to brilliant red, suggesting the creation of spherical Au NPs with a size of less than 50 nm, as shown in Figure 1. The second phase was to functionalize Au NPs via Au-S bonds with SH-PEG-COOH to produce the final conjugate Au NPs-PEG.

X-ray diffraction characterization (XRD)

XRD was used to investigate the crystal-like phase of Au NPs and Au NPs-PEG. The physical investigation of Au NPs and Au NPs-PEG using XRD is shown in Figure 2A and B. Firm diffraction peaks at (38.2o), (44.4o), (64.7o), and (77.7o) connect the Bragg's reflections of the 111, 200, 220, and 311 planes to the face-centered cubic of typical Au (Figure 2C).

Scanning electron microscopy using field emission (FE-SEM)

Figure 3 shows the Au NPs developed uniform surfaces by perfectly separating, then a sphere appearance through a size distribution of about 22.57 1.51 nm for Au NPs and 28.14 2.31 nm for Au NPs-PEG. Most particles were distributed in a single, uniform pattern without accumulations. This might be because the Au NPs were in a citrate-reduced mixture.

Capacity for Free Radical Scavenging

Free radical scavenging action of DPPH of Au NPs

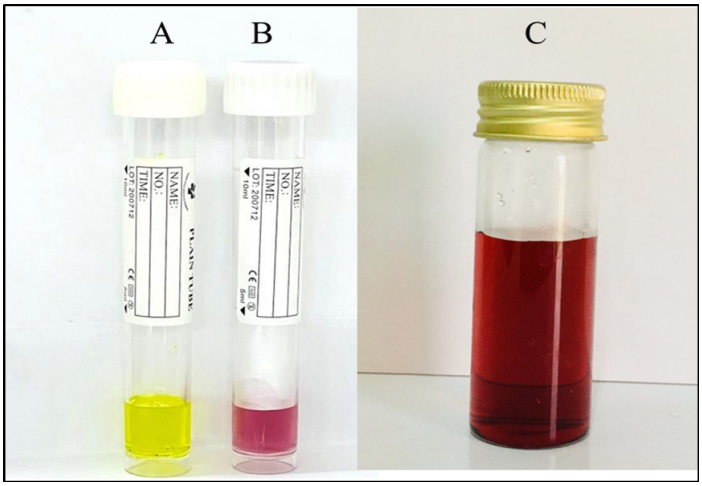

Figure 1. Shows the Gold Salts (A), Au NPs (B), and Au NPs-PEG (C).

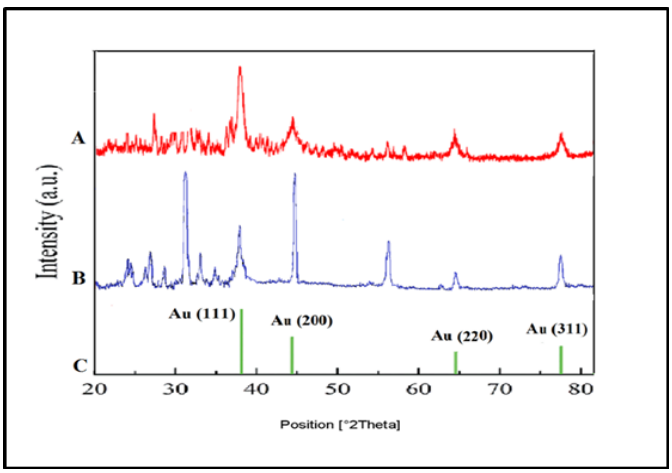

Figure 2. The XRD Shapes of Au NPs (A), Au NPs-PEG (B), and Au (JCPDS file: 04-0784) Standard Diffraction Peaks of Gold (C).

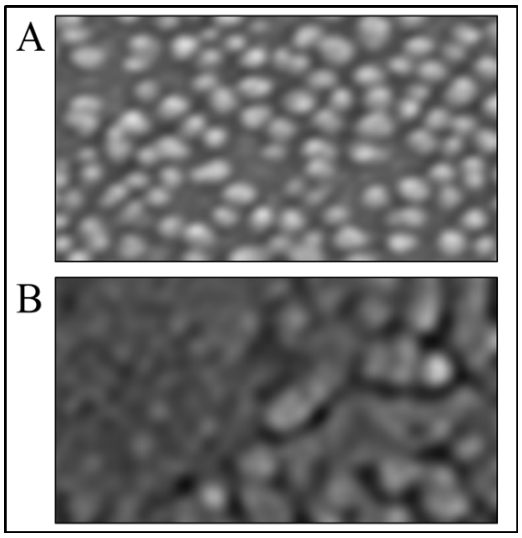

Figure 3. Scanning Electron Microscopy with Field-Emission. Au NPs (A) and Au NPs-PEG (B).

and conjugate was determined using a stable DPPH free radical scavenging assay at 100 g mL-1 for each treatment (Figure 4). The mean absorption at 517 nm was calculated and compared to the results obtained with vitamin C. The results revealed that Au NPs had an antioxidant activity of 55.24, with significant differences (p 0.0001) when compared to vitamin C. Surprisingly, Au NPs-PEG had the highest radical scavenging activity of 78.334.73 percent, with no significant difference in scavenging activity when compared to vitamin The results show that Au NPs-PEG was more efficient than Au NPs in terms of hydrogen atom donation and the elimination of DPPH's unstable electrons.

The cytotoxic effect of (Au NPs)

Figures 5 and 6 show the viability of MCF-7 cells after treatment with varying doses of Au NPs-PEG. The compound treatment for 24 hours resulted in considerable cellular growth suppression ($P > 0.5$), which was concentration-dependent. The highest cytotoxicity was seen at a concentration of 50 µg ML-1. The conjugate's cytotoxic impact on MCF-7 cells was investigated. The antiproliferative effect of (Au NPs-PEG) was investigated by examining their capacity to suppress cell growth. The results showed that the AuNPs have a high cytotoxic impact against breast cancer cell types.

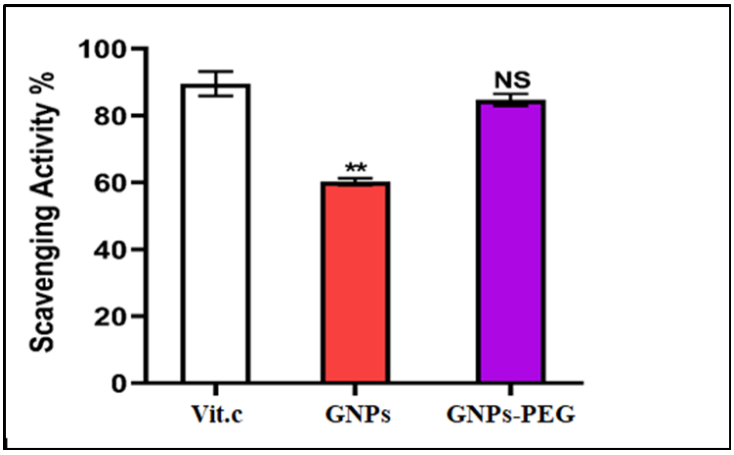

Figure 4. DPPH (Free Radical Scavenging) Activity of Au NPs and Au NPs-PEG. Vitamin C was used as a positive control. The data shows that the percent of antioxidant potential is at 517 nm (mean SD). **, p 0.01; NS, nonsignificant; SD, standard deviation. (n = 3).

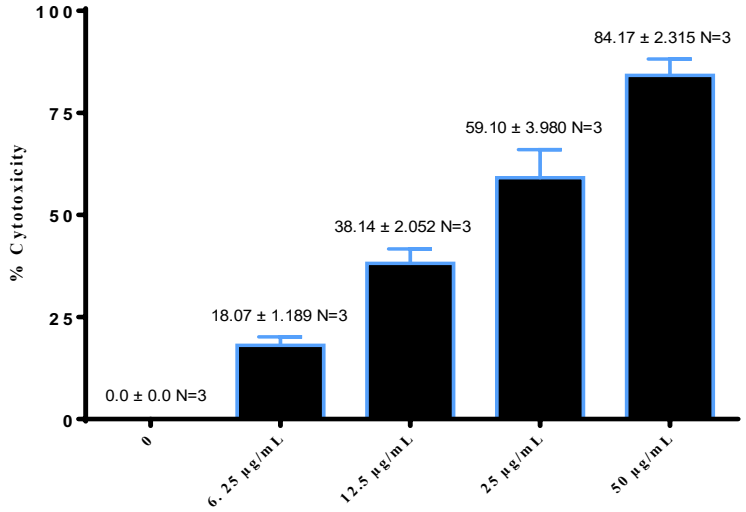

Figure 5. Cytotoxicity Effect of (AuNPs-PEG) in MCF-7 Cells

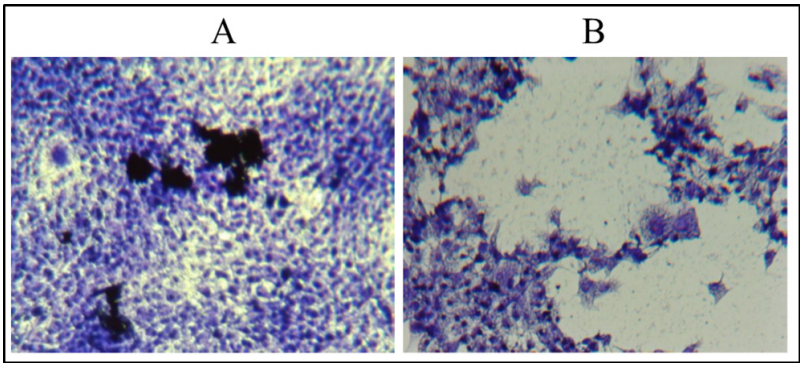

Figure 6. Control Untreated MCF-7 Cells. A. Morphological changes in MCF-7 cells after being treated with AuNPs-PEG. B. Magnification power 10x.

Discussion

Citrate reduction is an excellent approach for creating Au NPs since the citrate ions on the surfaces of Au NPs get negatively charged, and Au NPs spread effectively in polar solvents like water [32]. A colour change has most probably been caused by the appearance of maximum absorption of localized plasmon resonances (LSPR), which is characteristic of containing nanostructures such as gold. Additionally, Au NPs are surprisingly small nanoparticles in which electrons cannot flow as easily as in bulk metal. Due to this limitation in mobility, Au nanoparticles react to visible light differentially [33].

Compared to traditional procedures, our synthetic process streamlines the steps, reduces reaction time, and cost. As a result, many such Au NPs-PEG may be easily generated using a similar ecologically favorable method. Au NPs are small spheres ranging from 19 to 55 nm, commonly utilized in cancer treatment because they can swiftly enter cancer cells, functionalize, and interact with various chemicals. As a result, Au nanoparticles are frequently recognized as the most efficient drug-carrier choice for treating a variety of malignancies [34]. Au NPs coating with a single PEG layer or many PEG layers in combination with other compounds would aid in GNP internalization within target cells. As a result of their capacity to connect to cell membranes, these Au NPs might be effective medication carriers [35].

To improve antitumor efficacy, this study aims to look into the therapeutic potential of PEGylated Au NPs to assess their influence on human breast cancer. According to XRD, it indicated. Furthermore, Au NPs and Au NPs-PEG were primarily crystalline in the whole set of Au NPs-PEG diffraction peaks of Au NPs, indicating the structural stability of Au NPs under the influence of PEG. The newly identified diffraction peaks for Au NPs-PEG at 32.3 ° and 56.1 indicated the good crystallization of PEG on a GNP's surface. A few other intense peaks were discovered in the neighborhood of the Au NPs-PEG unique peaks. These peaks might have been generated by intermediary chemicals formed during production.

The appearance of these unselected external peaks did not influence the gold-specific Bragg reflection peaks, suggesting that their presence may help stabilize Au NPs. Citrate ions can provide a negative charge on the surface of Au NPs, allowing them to disperse [36]. efficiently. Au NPs-PEG spherical nanoscale particles had a typical size of 29.14 ± 3.31 nm, as shown in Figure 3-B, which could be attributed to the PEG existence, which caused size shifting. However, compared to Au NPs, Au NPs-PEG were not as monodisperse. Because of the presence of PEG, the nanoparticles formed hydrophobic interactions. Similarly, smooth and spherically formed Au NPs FE-SEM profiles revealed little homogeneity, and clusters of spherical structure appeared [24, 37]. Indeed, the antioxidant activity of citrate-capped Au NPs is achieved by donating hydrogen atoms, which removes DPPH's unstable electron. The PEG may be responsible for the significant antioxidant activity of Au NPs-PEG. The latter was found to have strong antioxidant properties against radicals such as peroxide, nitric oxide, hydroxyl, superoxide, and hydrogen, and the potential to be used as a therapeutic agent for leukemia [25].

Furthermore, the increased antioxidant activity of Au NPs-PEG could be due to functional groupings, such as amine and hydroxyl groups, which aid in better radical scavenging ability [38]. Meanwhile, Au NPs-PEG demonstrated greater antioxidant activity than citrate-capped Au NPs and may be useful in treating a broad variety of harmful diseases. The functionalized PEG on Au NPs demonstrated long-term stability, which could be helpful in long-term chemotherapy for cancer cells [39, 40]. The fast loss of MCF-7 clonogenic potential revealed that 24 hours of continuous exposure to the Au NPs-PEG resulted in MCF-7 cell death. The numerous pathways of necrosis and apoptosis are almost certainly implicated in cell viability suppression following Au NPs-PEG conjugate treatment. As a result, further research was necessary to determine the precise mechanism of cell death induction.

In conclusions, Au NPs can be directly functionalized with PGE, resulting in a conjugate with excellent biocompatibility and stability. The produced Au NPs-PEG revealed a very cytotoxic outcome. The breast cancer cell line (MCF-7) induces cell death and increases cellular absorption of the compound. These findings validated the ability to generate cytocompatible Au NPs-PGE that induce apoptosis and develop new anticancer medicines. Further research is needed to fully appreciate the influence of Au NPs-PEG in inhibiting cancer progression and killing

malignant cells accurately. Finally, we are optimistic that our work will include groundbreaking information for applying diverse materials and multifunctionality in cancer therapy via programmed cell death (apoptosis).

Author Contribution Statement

Ali G. Al-Dulimi1,Samah Ali Al Lateef : Study design. Ahmed Flayyih Hasan: Writing the manuscript and corresponding with the journal. Mustafa Hadi , Zina F.H. Al-Obaidi: Conducting data and statistical work.

Acknowledgements

Conflicts of interest

There aren't any interests.

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