Research Article

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Exploring the Role of Phytochemicals: Effect of [6]-Gingerol Combined with Colloidal Gold Nanoparticles on Thyroid Carcinoma Cells

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Abstract

Gold nanostructure can be manufactured in many forms, such as spherical by using simple chemical method. X-ray powder diffraction (XRD) pattern of gold nanoparticles (AuNPs) was derived from a 400-μL trisodium citrate solution. The diffraction peaks corresponding to the diffraction planes of (111), (200), and (220) were all indexed to the gold with a face-centered cubic structure. The lattice constant calculated from the XRD pattern is 4.078 Å, which matches the conventional cubic gold metal diffraction pattern well (Pattern card number (04-784)). The results of scanning electronic microscope show that the biological nanoparticles of gold have asymmetric shapes and different sizes grouped as a circular particle. It was observed that the fashioning of AuNPs increased with increase in the concentration of [6]-gingerol waste extracts. The viability of FTC-133 cells treated for 48 h with various concentrations of [6]-gingerol extract coated with AuNPs (12.5–400 g/mL) was used to determine the cytotoxicity. A dose-dependent reduction in FTC-133 cell viability caused by [6]-gingerol extract capped with Au NPs was substantial ($P < 0.05$), reaching 75% cell mortality at 400 g/mL; the IC_{50} was 90.5%.

Keywords: colloidal gold; 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity; [6]-Gingerol; 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

Introduction

As one of the most prevalent endocrine malignancies, thyroid cancer has steadily increased in incidence over the past 10 years [1]. About 80% of human thyroid malignancies are of the primary histological form known as follicular thyroid carcinoma (FTC-133) [2]. A number of well-known clinicopathologic factors, including age, tumor size, histologic subtype, extrathyroidal extension, and lymph node metastases, have been shown to influence the prognosis of FTC-133 [3]. Generally speaking, distant metastases or negative clinical outcomes are found in 10%–15% of FTC-133 patients [4]. Additionally, 5%–20% of all patients experience illness recurrence, and some even underwent total thyroidectomy [1]. Although most FTC-133 patients have a good prognosis, there are not many effective therapy options for cervical lymph

node metastases and early invasion [5]. Therefore, the need for a fresh and effective target therapy protocol is important. Due to their availability, material characteristics, and capacity to improve medication selectivity against cancer cells, metallic nanoparticles have been successfully used as diagnostic tools or drug delivery systems in cancer therapy [6, 7]. Gold nanoparticles (AuNPs), a diverse group of metallic nanoparticles, have drawn attention due to their unique characteristics, such as their nanosize, low toxicity, straightforward manufacturing, and ability to target a particular area [8, 9]. A promising therapeutic possibility for limiting tumor growth and metastasis may be monotherapy with AuNPs, according to recent research [10]. Through the suppression of the MAPK signaling pathway, AuNPs can reduce the growth of ovarian tumor cells and increase leukemia cell death by causing endoplasmic reticulum stress [11, 12]. *In vivo*, inflammation and apoptosis have been found to be induced by 13 nm AuNPs [13]. Additionally, human fibroblast migration and adhesion, as well as morphological alterations, are all influenced by AuNPs [14]. Additionally, AuNPs have been shown to harm cancer cells by a variety of mechanisms, including cell necrosis, the stimulation of proapoptotic protein (Bax) expression, prevention of tumor cell invasion and migration, and reduction of oxidative reactive species formation [15]. However, it has been noted that melanoma cells are cytotoxic to AuNPs of 1–2 nm in diameter [16].

In this work, AuNPs were prepared in different shapes for comparison and determination of the best non-toxic dose. After that, effect of [6]-gingerol combined with colloidal gold nanoparticles on thyroid carcinoma cells was investigated.

Experimental

Preparation of AuNPs

Synthesis of AuNPs was carried out by using chemical reduction technique as follows: 200 mL of 0.04% (mass fraction) chlorauric acid ($HAuCl_4 \cdot 3H_2O$) liquid was heated to boiling, while continuous stirring in a 500 mL volumetric beaker. Then a few hundred microliters of 2% (mass fraction) trisodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) liquid was speedily added to the auric liquid. The liquid changed color within several minutes from yellow to black and then to red or purple color, which may be due to the grain size of the AuNPs.

Preparation of ginger plant extract

Fresh ginger rhizomes were washed thoroughly to remove any trace amount of soil. Then the rhizomes were cut into thinly sliced pieces before being stored in a deep freezer at (–75 °C) overnight. The ginger specimens were freeze-dried in the following day for one week. The dried ginger was then ground to a fine powder using a blender and dissolved with ionic water and mixed for 1 h at room temperature and then filtered for a pure solution of ginger. Samples were taken for the analyses of [6]-gingerol content and bioactive properties.

Preparation of [6]-gingerol combined with colloidal AuNPs: 70 mg of ginger extract was extracted with deionized water, and 7 mL of a 3 mmol/L $HAuCl_4 \cdot 3H_2O$ solution was slowly added under magnetic stirring with hot plate at 70 °C for 60 min to uniformly coat the AuNPs; pH of mixture was adjusted to 8 using 1 mol/L NaOH solution at the beginning of the stirring step. A color change from pale yellow (plant aqueous extract) to purple indicated the successful formation of AuNPs. The solution was evaporated to collect the precipitate, which was washed thrice with sterilized distilled H_2O followed by oven drying at 100 °C for 2 h.

Cytotoxicity assay

The tests were run in triplicate, and a log dosage inhibition curve was used to determine the values IC_{50} of the compounds. For expressing human cell cultivation, in 100 μL of RPMI 1640, human follicular thyroid cancer with lymph node metastases (FTC-133) was grown (Roswell Park Memorial) in 10% fetal bovine serum (FBS)-containing institutional solution. For cell adhesion, FTC-133 cells were cultured for an entire night at 37 °C with 5% CO_2.

MTT cytotoxicity assay

According to the operator's guidelines [16], in 96-well plates, 200 mL of the cells (10^4–10^6 cells/mL) was grown. At 37 °C with 5% CO_2, the plates were gently stirred, covered with a sterile parafilm, and incubated for 24 h. The medium was taken out of the wells after the incubation period and 200 mL of a 2-fold serial dilution of the crude extract of [6]-gingerol combined with colloidal AuNPs (25, 50, 100, 200, and 400 mg/mL) was added. At each concentration

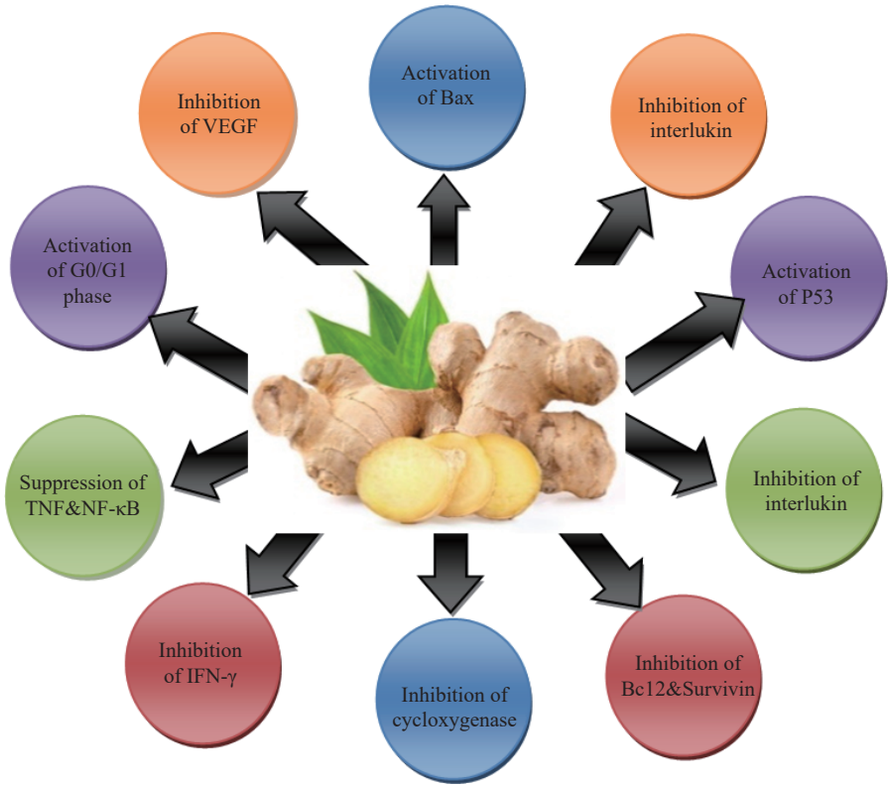

Fig. 1 Display of the research objectives.

and control, three replicates were run. 48 h at 37 °C and 5% CO_2 were spent incubating the plates. 10 mL of 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) solution were added to each well after each well had been exposed to the extract. The plates underwent a further 4 h of incubation at 37 °C and 5% CO_2. After carefully removing the medium, 100 mL of the dissolving solution was added to each well, and each was then given a 5-min incubation period. At a wavelength of 575 nm, absorbance was measured using an ELISA reader (Bio-rad, Germany). The optical density readings were statistically analyzed to determine the IC_{50}, as the following equation states:

$$\text{Viability} = \frac{\text{optical density of sample}}{\text{optical density of control}} \times 100\% \quad (1)$$

Multi-parameter cytotoxic assay

Five orthogonal FTC-133 cell health indicators were measured using a multiparametric cytotoxicity test [16] following *in vitro* exposure to nanoethanolic ginger extract. The variables were viable cell count, total nuclear intensity, permeability of the cell membrane, permeability of the mitochondrial membrane, and release of cytochrome C. Briefly, after being exposed to various doses of the [6]-gingerol combined with colloidal gold nanosheets extract for 24 h, treated FTC-133 cells were stained with a cell staining solution (MMP dye+osmotic dye) at 37 °C for 30 min. The main cytochrome C antibody and a second goat anti-mouse IgG probed with DyLight 649 for 60 min each were used to probe the cells after they had been fixed, permeabilized, and blocked. An ArrayScan HCS Analyzer was used to analyze the plates (Thermo Scientific, USA).

Results and Discussions

Figure 2 shows a typical XRD pattern of AuNPs derived from a 400-μL trisodium citrate solution. The diffraction peaks corresponding to the diffraction planes of (111) and (200) were all indexed to the gold metal with a face-centered cubic structure, as shown

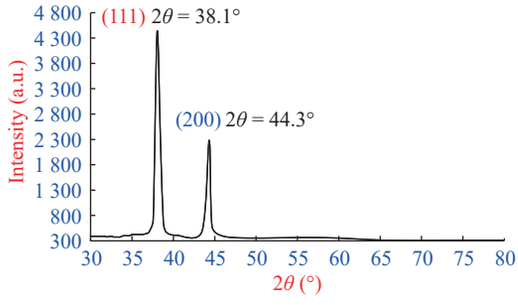

Fig. 2 XRD pattern of AuNPs synthesized using chemical reduction method.

in this figure. The lattice constant calculated from the XRD pattern is 4.078 Å, which matches the conventional cubic gold metal diffraction pattern well (Pattern card number (04-784)).

Figure 3 shows that gold nanoscale can be manufactured in many forms, such as spherical, cubic, rods, and stellar; each type depends on a special method of manufacture and the intended application. All types can be loaded with drugs to reach the target tissue and can be used in the process of detecting diseased cells. Generally, AuNPs have a tendency to easily aggregate, especially in the presence of a high concentration of salts and biological molecules such as nucleic acids and proteins. Although the aggregating of AuNPs is useful for specific cases to the identification of biomolecules, but AuNPs must be constantly differentiated into biological fluids in most applications. AuNPs was synthesized by a set of methods that mainly depended on gold chlorauric acid reduction in the presence of a stabilizer. The most widely used method is the synthesis method with citrates. The reduction gold salt with trisodium citrate leads to the formation of the nanoscale gold solution. The AuNPs size is mainly depended on the salt concentration, temperature, and rate of addition of the reactants, which results in a size of 10–25 nm. However, a size range of 1–100 nm or less can also be achieved by changing the salt concentration and temperature. Figure 3 shows the typical FESEM pictures of the AuNPs fabrication at various amounts of 2% (mass fraction) trisodium citrate solution. The grain size of the prepared AuNPs decreases from 40 to 10 nm when the amount of trisodium citrate increases from 100 to 600 μL.

Figure 4 shows the UV–visible spectra for AuNPs fabrication by using chemical reduction method. It was observed that the fashioning of AuNPs increased with increase in the concentration of [6]-gingerol waste extracts.

The high-performance liquid chromatography (HPLC) analysis revealed that [6]-gingerol is the major compound of the freeze-dried ginger and accounted to 22.63% ± 0.5%, as shown in Fig. 5.

Viability assay

The viability of FTC-133 cells treated for 48 h with various concentrations of [6]-gingerol extract coated with AuNPs (12.5–400 g/mL) was used to determine the cytotoxicity. A dose-dependent reduction in FTC-133 cell viability caused by [6]-gingerol extract capped with AuNPs was substantial ($P < 0.05$), reaching 75% cell mortality at 400 g/mL. The IC_{50} was 90.5% as shown in Fig. 6.

Multi-parameter cytotoxic activity

Multiparametric cytotoxic activity with [6]-gingerol extract capped with AuNPs was performed in HCS using FTC-133 cells. Five different measurements (cell count viability, nuclear intensity, cell membrane permeability, mitochondrial membrane potential and cytochrome C release) were detected in this assay (Figs. 7–11).

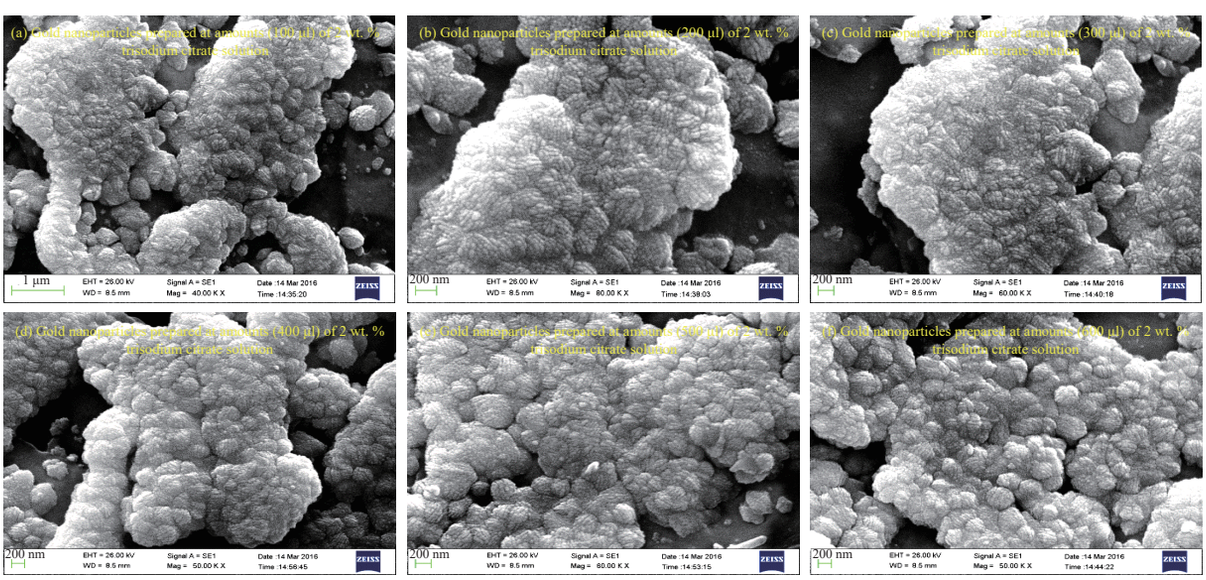

Fig. 3 FESEM pictures of AuNPs synthesized with different amounts (**(a)** 100, **(b)** 200, **(c)** 300, **(d)** 400, **(e)** 500, and **(f)** 600 μL) of 2% trisodium citrate solution.

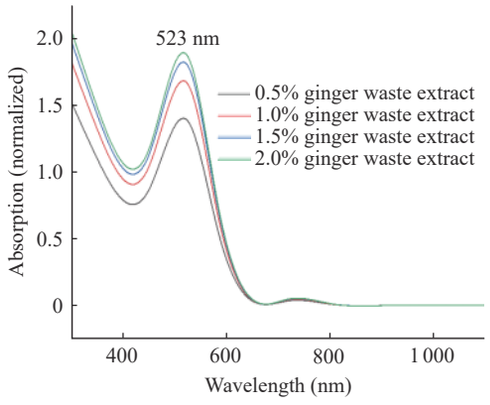

Fig. 4 UV–visible absorption spectra of AuNPs synthesized using chemical reduction method.

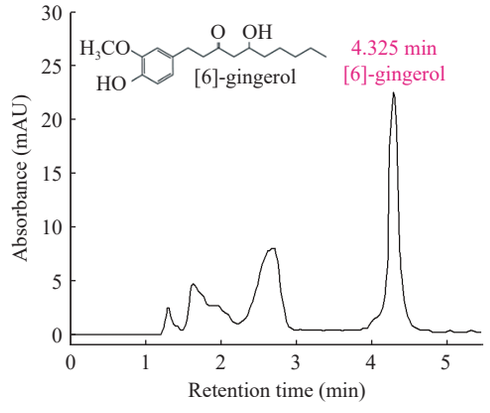

Fig. 5 The HPLC chromatogram of [6]-gingerol fresh ginger.

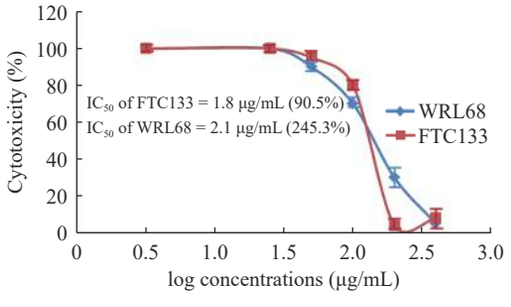

Fig. 6 Effect of [6]-gingerol extract capped with AuNPs treatment on cytotoxicity in FTC-133.

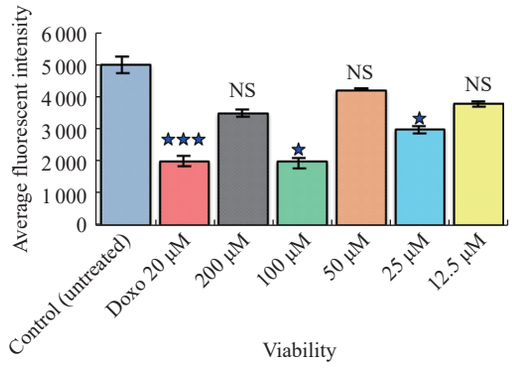

Fig. 7 Effect of [6]-gingerol extract capped with AuNPs treatment on cell viability in FTC-133.

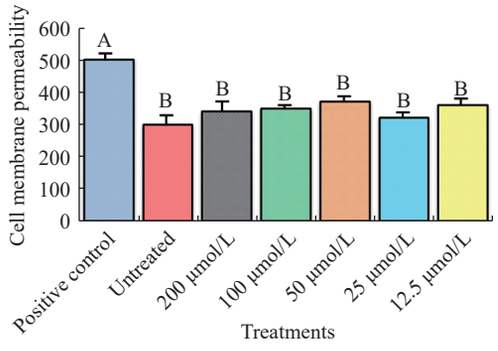

Fig. 8 Effect of [6]-gingerol extract capped with AuNPs treatment on cell membrane permeability in FTC-133.

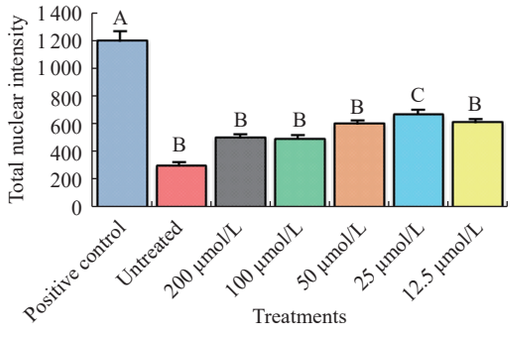

Fig. 9 Effect of [6]-gingerol extract capped with AuNPs treatment on total nuclear intensity in FTC-133.

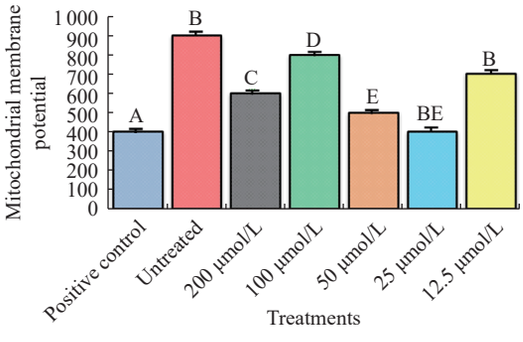

Fig. 10 Effect of [6]-gingerol extract capped with AuNPs treatment on cytochrome C in FTC-133.

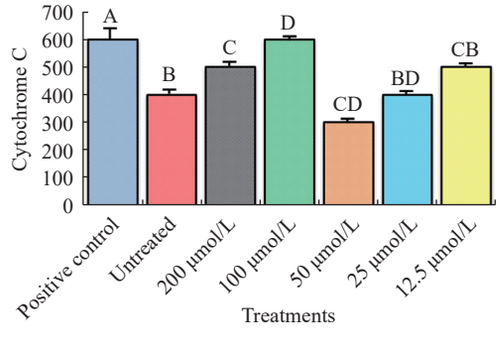

Fig. 11 Effect of [6]-gingerol extract capped with AuNPs treatment on mitochondrial membrane potential in FTC-133.

Conclusion

The viability of FTC-133 cells treated for 48 h with

various concentrations of [6]-gingerol extract coated with AuNPs (12.5–400 g/mL) was used to determine the cytotoxicity. A dose-dependent reduction in FTC-133 cell viability caused by [6]-gingerol extract capped with AuNPs was substantial ($P < 0.05$), reaching 75% cell mortality at 400 g/mL; the IC_{50} was 90.5%. Multiparametric cytotoxic activity with [6]-gingerol extract capped with AuNPs was performed in HCS using FTC-133 cells. Five different measurements (cell count viability, nuclear intensity, cell membrane permeability, mitochondrial membrane potential, and cytochrome C release) were detected in this assay.

CRediT Author Statement

Majid S. Khalaf: Methodology and software. **Marwa Abdul Muhsien Hassan:** Formal analysis, funding acquisition, writing, review, and editing. **Asmaa Hadi Mohammed:** Supervision and writing original draft.

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