PAPER

Syntheses, characterization, and suppression efficiency of silver & silver iodide nanoparticle for proliferation, migration, and invasion in follicular thyroid carcinoma cells

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Abstract
In this study, a chemical co-precipitation method has been employed, silver iodide (AgI NPs) and silver nanoparticles (AgNPs) have been synthesized. UV–vis, FTIR, x-ray diffraction, FESEM, TEM, and other techniques have been used to examine the optical and structural properties of AgNPs and AgI NPs. The UV–vis absorption spectra gave the highest peak at 400 nm for AgNPs and AgI NPs at 434 nm. The x-ray data showed that the prepared AgNPs and AgI NPs were nanocrystalline cubic structures with crystallite sizes of 18 nm and 51 nm, respectively. The FESEM results show that synthesized AgNPs and AgI NPs agglomerate and aggregate. TEM data revealed that AgNPs have a quasi-spherical shape and Gaussian size distribution type. TEM analysis of AgI NPs with different magnifications revealed primarily spherical and well dispersed AgI NPs. TEM histogram shows that the particles were highly monodispersed AgNPs and AgI NPs with an average diameter of 11.5, 24.28 nm, respectively. According to the MTT assay results of FTC133 cells, the cytotoxic action IC50 of AgNPs was (52.74 μg ml⁻¹) and for AgI nanoparticles was (95.22 μg ml⁻¹). It has been found that FTC133 cellular uptake was concentration, size- and time-dependent for both AgNPs and AgI NPs. The migrated FTC133 cell rates were reduced following AgNPs treatment to 75.7% and for AgI NPs treatment to 60% compared with the control group. Furthermore, Invasive FTC133 cell rates were reduced by 60% in the AgNPs treatment group and by 55.71 percent in the AgI NPs treatment group compared to the control group.

1. Introduction
Thyroid cancer is a common type of cancer in the head and neck area. Most thyroid cancer cases are differentiated thyroid carcinoma, including papillary thyroid carcinoma and follicular thyroid carcinoma [1]. Follicular thyroid cancer (FTC) is the second furthermore common differentiated thyroid cancer and credits for about 10%–15% of all thyroid cancers. The relative rate of FTC is higher in iodine-deficient areas, accounting for up to 40% of all cases of thyroid cancer disease [2]. The initial treatment of thyroid cancer was surgical and depended, for the most part, on the extent of the local condition. The nanomaterials have wide biomedical applications such as photothermal therapy [3], photodynamic therapy [4], a drug targeted delivery [5], genetic therapy [6], immunotherapy [7], etc.

Due to their availability, material characteristics, and capacity to improve drug specificity against cancer cells, metallic nanoparticles have been established as diagnosing markers or drug delivery systems in cancer therapy [8]. In addition, they can easily infiltrate the cellular environment due to their small size. Furthermore, NPs can target specific cancer cells both actively and passively [9]. Silver nanoparticles (AgNPs) are among the most promising metal nanoparticles due to their exceptional antibacterial and confined surface plasmon resonance capabilities. These qualities include broad-spectrum antimicrobials, ground Raman spectroscopy, chemical/biological sensors and biomedical materials, biomarkers, and so on [10]. Moreover, the toxicity of
AgNPs-induced apoptosis is well-established in various cell lines, including human lung cancer cells, ovarian cancer cells, and breast cancer cells. AgNPs are thus the best, most ideal, and alternative nanoparticles for usage with any anticancer medication [11]. Metal–halide nanoparticles, made up of heavy metal compounds like silver and iodine, have had relatively little research done on them. However, the microbial inactivation of silver chlorine has been intensively studied in [12] and the silver chloride secondary phase in the case of green syntheses was investigated, AgNPs have demonstrated efficient biocidal properties against micro–organisms [13]. Depending on the above, halogens are effective antimicrobial agents; a mixture of heavy metal compounds and halogens could be designed to boost antimicrobial activity [14]. Silver iodide is a commonly used material in imaging, medical, electrical, magnetic, optical, and catalytic qualities for various metals and semiconductors. It may potentially have antimicrobial properties [15]. Furthermore, the idea behind utilizing a low solubility material like AgI NPs as a shell is to prevent Ag from diffusing into the water, hence preventing toxicity [16].

Surface oxidation, the release of silver ions, and interactions with biological macromolecules are all factors that influence AgNPs toxicity in the natural and environmental media [17]. The cytotoxic and genotoxic effect of AgNPs is driven by concentration, size, exposure period, shape, coating, and ecological variables, according to various papers detailing both in vitro and in vivo NPs toxicity experiments [18]. Ag-NPs may be taken up differently by different cells. Some ways include diffusion, phagocytosis, and endocytosis. Some Ag-NPs clump together in a medium and are phagocytosed by cells [18, 19]. Furthermore, AgNPs with size 10 nm was the most hazardous, indicating that silver nanoparticles, particularly those smaller than 10 nm, may damage organisms. In human cervical cancer cells, Yu-Guo Yuan et al [11] examined the synergistic combination impact of CPT and AgNPs. Various cellular and biochemical assays were used to assess the anticancer activity of combination therapy with CPT and AgNPs. It was also discovered that combining CPT and AgNPs therapy drastically reduced HeLa cell viability and proliferation.

The use of AgNPs as a possible anticancer treatment against thyroid cancer cells was investigated by Jinmei Yang et al [20]. The cytotoxicity of AgNPs on TPC1 was investigated at various concentrations, and the IC50 value was obtained. In AgNPs-treated cells, detecting changes in oxidative and antioxidant indicators. E Rauwel et al [21] reported a structural and cytotoxicity study of AgNPs targeted toward biomedical applications. They conducted to the synthesized spherical AgNPs of diameter from 20 to 50 nm have demonstrated a high mortality rate for concentrations ranging from 100 to 200 mg l⁻¹ against primary prostatic cancer cell line (PPC-1). Yun Wang et al [22] studied the anticancer efficacy of AgNPs, and the cytotoxic assay of AgNPs exhibited dose-dependent cytotoxicity against various cancer cells, including HeLa, HCT-116, PC-3, and Jurkat. The cell viability was found to range between 50% and 60%, with IC50 values ranging from 150 to 224 g ml⁻¹. The reduced cell viability shows that biosynthesized AgNPs are harmful to these cancerous cells. Overall, our findings could be used to build a new approach for treating follicular thyroid cancer by looking into AgI NPs as an anticancer agent. In this research, AgNPs and AgI NPs were synthesized using a chemical method and study their physicochemical properties, including shape, size, and size distribution. Secondly, using the MTT assay on FTC133 cells, the cytotoxicity of AgI NPs was investigated. More, calculating the absorption rates of AgNPs and AgI NPs in FTC133 cells. Finally, investigating the effects of AgNPs and AgI NPs on FTC133 cell migration and invasion.

2. Materials and methods

2.1. Nanoparticles preparation

Double-distilled deionized water was used to prepare all solutions in the following works.

2.1.1. Silver nanoparticles

Synthesizing AgNPs by a chemical reduction method was done [23]. Briefly, silver nitrate AgNO3 (supplied by AVONCHEM Wellington House Co. UK) was used as raw material, trisodium citrate C6H5O7Na3 (AVONCHEM Wellington House Co. UK) was used as a reduction agent, and ascorbic acid C6H8O6= (Laboratory Reagent India) as stabilizer surfactant agent. Firstly, 40 ml of AgNO3 was heated to 60 °C and then added with vigorous stirring to 20 ml of 8.0 mM C6H5O7Na3 and a 1.0 mM C6H8O6 solution that was pre-heated to 60 °C. The mixture was then stirred for 20 min. After that, the solution was cooled to room temperature with continuous stirring. Then, using centrifugation at 6500 rpm, a brownish to a light-yellow solution was extracted, washed multiple times with double-distilled water and ethanol to eliminate contaminants, and dried.

2.1.2. Silver iodide nanoparticles

A solution of 25 ml with 0.1 M potassium iodide KI (sigma Aldrich USA) (0.415 g KI in 25 ml distilled water) was added dropwise to the earlier organized AgNO3 (AVONCHEM Wellington House Co. UK) solution of 25 ml
with 0.1 M (0.425 gm AgNo3 in 25 distilled water) in the presence of 0.2 g sodium dodecyl sulfate NaCl2H25SO4 (Didactic Co. Barcelona Spain) as a surfactant with vigorous stirring. Then the solution turned yellowish-white, then the precipitate was separated by centrifugation at (6500 r.p.m) and washed with double-distilled water and ethanol to remove impurities several times, and then dried.

2.2. Characterization of synthesized NPs

2.2.1. Optical properties
The AgNPs and AgI NPs absorption spectra were calculated and examined in solution samples using a UV–vis spectrophotometer (Double Beam Li- 2800) and (T60, LONDON) equipped with a Deuterium and Tungsten lamp. The used wavelength of (200–900) nm in the Materials Laboratory for Postgraduate Studies at the Faculty of Science/Mustansiriyah University.

2.2.2. Fourier transforms infrared spectrometer (FT-IR)
The absorption of electromagnetic energy in the frequency range generates Fourier transform infrared spectra (400 to 4000 cm\(^{-1}\)). In the molecule, different functional groups and structural characteristics are absorbed in different frequencies. As a result, the frequency and intensity of absorption reveal the molecule’s band structures and structural geometry, as measured by Thermo Fisher Scientific Corporation’s FT-IR at Daypetronic Co. in Tehran, Iran. Additionally, the infrared spectra of the emission and absorption were obtained for the synthesized AgNPs and AgI NPs powder samples.

2.2.3. X-ray diffraction (XRD)
The orientations of Ag and AgI (NPs) grown samples have been investigated by XRD measurements in Daypetronic Co. Tehran-Iran. The XRD measurements were performed at room temperature using a ‘(Instrument: Panalytical X’ Pert Pro, USA)’, which was outfitted with a Cu tube for producing monochromatic Cu kα x-ray radiation. The incident beam was in the 2θ mode over the range of (5.0131°–79.9711°), with a step size (2θ = 0.0260) and operated at a voltage of 40 kV and a filament current of 40 mA. The phase identification for all the samples reported in this work has been performed by matching the peak positions and intensities in XRD patterns to those patterns in the JCPDS (Joint Committee on Powder Diffraction Standards) database. The crystallite size was estimated using the Debye–Scherrer’s formula (D = \(\frac{\lambda}{\beta \cos \theta}\)) [24] where D is the crystalline size; \(\lambda\) is the wavelength of x-ray (\(\lambda = 0.154056\) nm for (CuKα)); \(\beta\) is the full width at half maximum (FWHM) of the Bragg’s peak (in radians); \(\theta\) is the diffraction angle of the reflection. The dislocation density (\(\delta\)) of the synthesized NPs was estimated from equations (\(\delta = 1/D^2\)) and the micro-strain (\(\varepsilon\)) from (\(\varepsilon = \beta \cos / 4\)) [25].

2.2.4. Field emission scanning electron microscope (FESEM)
The surface morphological characterization and elemental analysis were carried out using Field Emission Scanning Electron Microscopy (FE-SEM) integrated with Energy Dispersive x-ray (EDX) analyzer. The synthesized AgNPs and AgI NPs have been examined by ‘(EBSD Instrument: ZEISS SIGMA VP, Germany)’ in Daypetronic Co. Tehran-Iran. Additionally, EDX analysis was used to estimate the elemental structure, purity, and the percentage of each metal in the structured powder of synthesized AgNPs and AgI NPs.

2.2.5. Transmission electron microscope (TEM)
The size and shape of AgNPs and AgI NPs were determined by transmission electron microscopy (TEM) type (TEM Instrument: ZEISS LEO 912 AB-, Germany) by accelerating the voltage (100 kV) in Daypetronic Co./Tehran - IRAN. The measurement was done on particle diameter and the histogram of the size distribution. Thus, it is possible to calculate sample dispersion information.

2.3. Biomedical tests

2.3.1. FTC-133 cell line
The Pharmacology Centre for Natural Product Research and Drug Discovery, University of Malaya, Malaysia, provided the follicular thyroid carcinoma (FTC-133) (Thyroid: lymph node metastasis, Human). Cell Line Description: FTC-133 was obtained from a lymph node metastasis of follicular thyroid carcinoma in a 42-year-old male. FTC-133 got from a lymph node metastasis of follicular thyroid carcinoma, not the primary tumor. In this paper, we investigated FTC-133 cultured for 7 and 14 days. All cell lines were sophisticated as monolayers in a humidified atmosphere (5% CO\(_2\)) at 37 °C. During experiments, the cell lines were cultured in a serum-free DMEM/HAM–F12 medium. In the medium, cells were grown various times, as shown in the experiments. Aluminum foil was used to hide the cells from light. One day after seeding the cells, RA and DMSO were added to prevent any interactions with cell attachment to the culture dishes. Tumor cells were grown until they were
roughly 90% confluent and then dissociated into single-cell suspensions from tissue culture flasks using 0.05 percent EDTA in PBS for 3 min for all experiments.

2.3.2. WRL-68 cell line
The human liver cell line WRL-68 which used as a normal cell because its morphology is similar to that of hepatocytes and liver primary cultures. Albumin and alpha-fetoprotein are secreted by cells, and liver-specific enzymes like alanine aminotransferase are expressed to produce the solutions and medium for cell culture according to [26, 27].

2.3.3. MTT cytotoxicity assay
The tests were carried out in triplicate, and the IC50 values for the nanoparticles synthesized AgNPs and AgI NPs were determined using a log dosage inhibition curve. For compliance with recommendations. In 96-well plates, the cells (1 × 10^4 to 1 × 10^6 cells ml ^{−1}) were grown to a final volume of 200 L of complete culture media per well. The plates were topped with sterile parafilm, gently stirred, and incubated at 37 °C with 5% CO2 for 24 h. The medium was withdrawn after incubation, and 200 μl of a 2-fold serial dilution of AgNPs and AgI NPs solutions (25, 50, 100, 200, 400 μg ml ^{−1}) was added to the wells. At each concentration and control, triplicate tests were carried out. Each concentration and control were tested in triplicate. The plates were incubated at 37 °C for 24 h with 5% CO2. Following the extract exposure, 10 ml of MTT solution was applied to each well. The plates were then incubated for another 4 h at 37 °C with 5% CO2. After carefully removing the medium, each well was filled with 100 ml of DMSO solubilization solution and incubated for 5 min. At a wavelength of 575 nm, the absorbance was measured using an ELISA reader (Bio-rad, Germany). The untreated cells control (100% cell viability) was used to express the percentage of the cell viability. The IC50 was calculated using statistical analysis of the optical density values. The following equation can be used to solve this problem [29]:

\[
\text{Viability(\%)} = \left( \frac{\text{optical density of sample}}{\text{optical density of control}} \right) \times 100 \%
\]

2.3.4. Cellular uptake and western blot analyses of AgNPs & AgI NPs
FTC133 cells were obtained from American Type Culture Collection (ATCC) with the help of cooperation between Cardiff University, Cardiff and the collaboration of a researcher from Brighton University, UK. The stock AgNPs & AgI NPs suspensions were sonicated and freshly diluted to appropriate concentrations in the cell medium according to [30]. Each analysis included control cells that had not been treated. The toxicity endpoints were evaluated in control and exposed cells using mean fluorescence intensity to examine the cellular absorption of fluorescent nanoparticles after the exposure period.

- Western blot analysis

For FTC133 cell movement and invasion rate measurements, total protein was extracted according to the manufacturer’s protocol and used for protein expression analysis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was used to load and separate the protein samples (30 g of lysates from FTC-133 cells). The protein was electrotransferred on a polyvinylidene difluoride membrane after electrophoresis (Immobilon-P; Millipore).

2.3.5. Statistical analysis
Analysis of Variance (One-Way) In this section, ANOVA was utilized. SPSS version 23 was used to assess the significance of the findings and their connection. Statistical significance was defined as a P value of less than 0.05. At least three separate studies’ data were provided, each with a different interpretation of the meaning.

3. Results and discussions

3.1. Characterization of NPs
3.1.1. UV–vis absorption analyses
To study the optical measurements of synthesized AgNPs and AgI NPs UV–vis absorption spectrophotometer was applied, and the scanned wavelength range was from 200 nm to 800 nm. The optical properties of spherical AgNPs are highly reliant on the diameter of the nanoparticle. Figure 1(A) depicts the electronic absorbance spectra of synthesis AgNPs, which mainly absorbed light and had a peak near 450 nm, with a surface plasmon resonance band observed between (400–475) nm. The maximum absorption band of AgNPs was monitored at 424 nm by UV–vis spectra, which was qualified to surface plasmon excitation. These results are in a match with previous study results by (Katarzyna Ranoszek et al 2017) [31].
The absorbance spectra for synthesized AgI NPs shown in figure 1(B) revealed the highest intensity peak observed in the visible area near 425 nm, indicating surface plasmon excitation. There is an excellent match with previous data for AgI NPs reported by (Raid A Ismail et al 2018) [14].

3.1.2. Fourier transform infrared spectroscopy (FTIR) analysis
Fourier transforms infrared (FTIR) spectroscopy is an essential instrument for functional groups observation. It was employed in this work to characterize the diverse functional groups involved in the reduction of the

\[ 	ext{Figure 1. UV–vis spectra of synthesis NPs (A) The absorption spectrum of AgNPs. (B) The absorption spectrum of AgI NPs.} \]
stabilization of prepared Ag NPs and AgI NPs powders. FTIR spectrum of synthesized AgNPs, as shown in figure 2(A), reveals clear peaks throughout the whole range (from 400 to 4000 cm$^{-1}$) of observation. Visible bands were found at 3444.96, 2912.23, 1617.80, 1376.14, and 1110.44 cm$^{-1}$ in FTIR analysis. Stretching of the O–H can be attributed to the band observed at 3444.96 cm$^{-1}$. The band seen at 2912.23 cm$^{-1}$ is attributed to C–H (alkane) bend. N–H (amine) stretching peaks at 1617.80 cm$^{-1}$. The C–H (alkane) bend and C–O (alcohol/ether) stretching are represented by the weaker band at 1376.14 cm$^{-1}$ and the band at 1110.44 cm$^{-1}$, respectively. Also, the two weaker band bands noted at 608.84 and 493.84 cm$^{-1}$ may be ascribed to the C–H bending of Alkyne. These findings are consistent with previous research (Kishan H Sodha et al [32], G Narasimha [33], and M Z H Khan et al [34]). Figure 2(B) shows the FTIR spectrum of synthesized AgI NPs by chemical method. The observed bands identify the functional groups in AgI NPs; the FTIR spectrum shows intense absorption bands at 3439.29 and 1619.72 cm$^{-1}$ can be assigned to O–H (alcohol) stretching and bending vibrations, respectively. In addition to weak intensity bands at 3932.8 and 3786.75 cm$^{-1}$ ascribed to the OH groups [35]. The peak found at 2925.38 cm$^{-1}$ is corresponding to the CH$_2$ stretching vibration of SDS (sodium dodecyl sulfate). The appearance of this peak suggests that a trace amount of SDS has been coated on the surface of AgI nanoparticles. So, the presence of purified water on the nanoparticles is indicated by these findings. The band at 1385.19 cm$^{-1}$ can be exemplified the N=O symmetry stretching. The band at 615.45 cm$^{-1}$ belongs to the stretching of the Iodo-compound (C–I) [36]. The band at 1024.39 cm$^{-1}$ corresponds to the C–N stretching of amines. Previous reports provided support for the current data by J (Safei and M A Ghasemzadeh) [24, 37].

Figure 2. Fourier transforms infrared spectroscopy (FTIR) analysis of synthesized (A) AgNPs and (B) AgI NPs.
3.1.3. X-Ray Diffraction (XRD)

The x-ray diffraction patterns of synthesized AgNPs and AgI NPs are shown in figures 3 and 4, respectively. The crystallite sizes, in addition to the dislocation density and the micro-strain estimated values for both AgNPs and AgI NPs, are illustrated in tables 1 and 2, respectively. For AgNPs, all the diffraction peaks correspond to the characteristic of face-centered cubic (FCC), and Miller indices (h k l) values are (111), (200), (220), and (311) planes of silver which are observed as shown in figure 3 silver lines according to the Ag (FCC) phase standers (JCPDS 00-004-0783).

![Figure 3. XRD patterns of synthesized Ag NPs.](image1)

![Figure 4. XRD patterns of synthesized AgI NPs.](image2)

### Table 1. XRD results of synthesized Ag NPs.

| 2θ (deg.) | 2θ Exp. (deg.) | β (rad) | cos theta (rad) | Miller indices (h k l) | Crystallite size D nm | Dislocation density δ(line² m⁻²) × 10⁻⁵ | Micro strain ε × 10⁻³ |
|-----------|----------------|---------|-----------------|------------------------|----------------------|------------------------------------------|------------------------|
| 38.11     | 38.09          | 0.36    | 0.944689        | 0.006316               | 111                  | 23.22985                                 | 18.53136               | 1.491615               |
| 44.27     | 44.3           | 0.69    | 0.925441        | 0.012105               | 200                  | 12.372                                   | 65.33113               | 2.800679               |
| 64.42     | 64.45          | 0.53    | 0.844400        | 0.009298               | 220                  | 17.6528                                  | 32.09022               | 1.962861               |
| 77.47     | 77.35          | 0.54    | 0.778509        | 0.009474               | 311                  | 18.79232                                 | 28.31648               | 1.843838               |
The determined crystallite sizes values for AgNPs were in the range of (12–23) nm. The mean crystallite size was (18) nm. The estimated dislocation density and the micro-strain values are shown in table 1. These results have excellent agreement with the provirus studies (Dzimitrowicz A et al and Gurunathan S et al) [25, 36].

All the diffraction peaks for AgI NPs correspond to the characteristic (FCC) of silver iodide which is observed as shown in figure 4 and miller values planes as identified in the table 2. Silver iodide lines according to the AgI (FCC) phase standers (JCPDS 00-001-00502). XRD patterns of AgI NPs were analyzed to determine the crystallite sizes values ranging from 16 nm to 70 nm, while the mean crystallite size was 52.4 nm to be in the nanoscale region. Also, the dislocation density and the micro-strain values were estimated and illustrated in table 2. Smaller results were reported for AgI NPs in the literature (Safaei-Ghomti et al and Ghasemzadeh et al) [24, 37].

### 3.1.4. Field emission scanning electron microscope (FE-SEM)

The morphological structure of the nanoparticle’s products of Ag and AgI is determined by FE-SEM analysis. Figures 5(A)–(D) reveals FE-SEM images of Ag NPs prepared by a chemical method deposited on a glass substrate with different magnification. The morphology of AgNPs displays their uniform distribution and is relatively homogeneous. Also, these NPs are assembled into a cluster of spherical-like shapes with different diameters due to the stability of cluster distribution and colloid formation. The particle sizes estimated from FE-SEM images range from (108.7–to 236.8) nm, which are larger than the sizes obtained from XRD analysis and TEM images using Scherrer’s formula. This can be attributed to larger clusters deposited on the film’s surface due to an aggregate structure composed of tiny grains on the glass substrate during drying. In other words, XRD is size-dependent and defect-free, whereas FESEM directly visualizes the particles without regard for the degree of crystal defects. Several researchers have reported such particle size differences between XRD and FE-SEM [33, 38]. FE-SEM images in figures 6(A)–(D) show the produced AgI NPs powder specimen prepared by a chemical method with different magnifications. FE-SEM pics explain the morphology of AgI with relatively a homogenous size, and these NPs assembled in semi-spherical-like shape with some ledge and multi-branch objects with diameters ranging between (22–73) nm due to agglomeration effect. Notice that there is a good agreement with the derived values from the XRD pattern of the AgI NPs analysis.

### 3.1.5. Energy dispersive x-ray (EDX) analysis:

To determine the ratio of the elements in any compounds, an Energy Dispersive x-ray (EDX) analysis was performed. In this study, it was used to show the presence of silver (Ag) and Iodine (I) in the mixture with a specific ratio. The analyses were mentioned in different patterns, as shown in the figure 7 for AgNPs and figure 8 for AgI NPs. The EDX-spectrum in figure 7 clearly demonstrates the high quality of silver (Ag) in AgNPs, with a weight percentage of the presence of silver Ag being 95%. Besides, other peaks for oxygen 1.2% and carbon 3.8% percentage were also observed. On the other hand, the presence of the silver (Ag) peak in figure 8 for the AgI NPs specimen is 51.5%, and Iodine (I) is 48.5% with no contamination in the AgI NPs powder specimen. A peak related to the Au element was observed in the EDX spectrum of AgNPs and AgI NPs, which probably originated from the SEM chamber contaminations.

### 3.1.6. TEM micrograph

TEM micrograph of AgNPs nanoparticles is shown in figures 9(A)–(D) with different magnifications, which demonstrates spherical shape and a narrow particle size distribution. Dispersible particles were observed under TEM analyses even at high magnification. The TEM histogram in figure 9(E), obtained using Image J software of at least 100 particles, shows the spherical particles have an average diameter of 11.5 nm and a standard deviation of 1.3 nm. The minimum diameter of AgNPs particles was 5.5 nm, and the maximum was 39.5 nm. The TEM
Figure 5. FE-SEM images of Ag, (A) with scale bar 1 μm, (B) with scale bar 200 nm, (C) with scale bar 200 nm, and (D) with scale bar 200 nm with size.

Figure 6. FE-SEM images of AgI NPs, (A) with scale bar 1 μm, (B) with scale bar 200 nm, (C) with scale bar 200 nm, and (D) with scale bar 200 nm with sizes.
analysis of AgI NPs with different magnifications shown in figures 10(A)–(D) revealed primarily spherical and well-dispersed silver iodide nanoparticles. With a low level of aggregation, some nanoparticles took on an uneven shape. The monodispersed nanoparticles are the most common, as seen in the TEM histogram data in figure 10(E). The particle’s size with homogenous distribution ranged between 9.9 nm and 50 nm in diameter as the minimum and maximum values, with an average diameter of 24.28 nm and a standard deviation of 1.47 nm.

3.2. Biological results

3.2.1. Cell viability (MTT Assay)

To examine the cytotoxicity of synthesized AgNPs and AgI NPs on follicular thyroid cancer cells FTC133 cell line and the normal cell WRL68 cell line were used, with \((1 \times 10^5 \text{ ml}^{-1})\) cells per well in their exponential growth phase. They were incubated with increasing the AgNPs and AgI NPs concentrations for 24 h. The cell viability was expressed as a percentage of the untreated control (100% cell viability), which was investigated by MTT assay. Cytotoxicity results of FTC-133 cell viability after 24 h of treatment with various concentrations of each AgNPs and AgI NPs (25 to 400 \(\mu\text{g ml}^{-1}\)) are shown in figures 11 and 12, respectively. All results indicated that a decrease in cell viability in a dose-dependent manner and both AgNPs and AgI NPs solutions resulted in a significant decrease in the survival rate of FTC-133 cells in dose dependence \((P < 0.0001)\). For AgNPs figure 11 at a concentration of (25 \(\mu\text{g ml}^{-1}\)), there is no significant difference between FTC133 carcinoma and normal cell WRL68, but there are significant differences at higher concentrations of (50, 100, 200 \(\mu\text{g ml}^{-1}\)). At concentrations (50, 100 \(\text{g ml}^{-1}\)) in the table 3, we found that the viability rate of the normal cell WRL68 remained nearly unchanged at 94.64, 92.13%, whereas the viability rate of the FTC133 carcinoma decreased to 78.63% and 64.51%, respectively. And a higher cell death rate of FTC133 was 55.13% at the highest concentration (400 \(\mu\text{g ml}^{-1}\))
Figure 9. TEM images of Ag NPs with an inset of different magnifications, (A) with scale bar 500 nm, (B) with scale bar 200 nm, (C) with scale bar 60 nm, (D) with scale bar 30 nm and (E) histogram shows size distributions based on TEM images of AgNPs ranging from 5 to 25 nm of diameters.

Table 3. Cell viability (mean ± standard deviation (SD) of syntheses AgNPs treatment.

| Concentration | FTC133 Mean | FTC133 SD | WRL68 Mean | WRL68 SD |
|---------------|-------------|-----------|-------------|-----------|
| 400.00        | 44.87       | 4.92      | 69.56       | 3.20      |
| 200.00        | 51.74       | 5.32      | 78.32       | 7.00      |
| 100.00        | 64.51       | 2.96      | 92.13       | 1.56      |
| 50.00         | 78.63       | 4.33      | 94.64       | 1.51      |
| 25.00         | 93.63       | 2.70      | 95.02       | 1.06      |

\( F(DFN,DFd) = 63.16 (4,22) \)  
Alpha = 0.05  
P value < 0.0001
and 30.44% of WRL68. The cytotoxicity action IC50 was \(52.74 \mu \text{g mL}^{-1}\) for FTC133, and normal cell WRL68 was significantly higher \(174.2 \mu \text{g mL}^{-1}\). From these results, it can be concluded that the toxic potential of AgNPs can differ in various cell types. The cytotoxicity results of AgI NPs are shown in figure 12 and table 4. Cell viability loss was observed, which was dose-dependent. At a concentration of 25 g ml\(^{-1}\) for AgI NPs, there is no significant difference between FTC133 carcinoma and normal cell WRL68. At higher concentrations \((50,100,200 \mu \text{g mL}^{-1})\) we observed a significant difference. The normal cell WRL68 viability rate was 94.17 and 83.95%, at 50,100 \(\mu \text{g mL}^{-1}\), whereas the FTC133 carcinoma viability rate was 84.99 and 68.13% at the same concentrations. And the highest cell

![Figure 10. TEM images of AgI NPs with an inset of different magnifications.](image)

Figure 10. TEM images of AgI NPs with an inset of different magnifications, \(\text{A})\) with scale bar 900 nm, \(\text{B})\) with scale bar 250 nm, \(\text{C})\) with scale bar 60 nm, \(\text{D})\) with scale bar 30 nm and \(\text{E})\) histogram shows size distributions based on TEM images of AgNPs ranging from 10 to 50 nm of diameters.
death rate of 55.58% for FTC133 was at the highest concentration of (400 \mu g \text{ ml}^{-1}) and 31.4% for the normal cell WRL68. The determined IC50 value of AgI NPs for FTC133 was (95.22 \mu g \text{ ml}^{-1}) and (208.5 \mu g \text{ ml}^{-1}) for normal cell WRL68.

The cytotoxicity results showed the dose-dependent cytotoxicity of AgNPs and AgI NPs on follicular thyroid cancer cell line FTC133 more than standard cell line WRL68 at particular concentrations. Therefore, it can conclude that the cytotoxic effect of AgNPs and AgI NPs depends on cell type. In comparison between the cytotoxic effect of AgNPs and AgI NPs on FTC133 and WRL68, significant toxicity was observed in AgNPs, and
less effect was observed with AgI NPs at particular concentrations. The higher cell death rate and IC50 value of AgNPs are more elevated than AgI NPs, as shown in figures 13(A), (B). This could be due to particle size difference. In this study, the prepared AgNPs were at an average diameter of 12.3 nm, whereas AgI NPs had an average diameter of 26.25 nm.

Because bigger AgNPs release silver ions slowly [39], and smaller particles penetrate cells more quickly than larger particles [40], smaller AgNPs are more hazardous than larger nanoparticles. Size-dependent cellular interactions have also been studied [41]. Also, could the cytotoxicity of AgNPs and AgI NPs be due to the type of capping agent used in nanoparticle synthesis, revealing the effect of capping agent type [42]. The type of coating depends on the capping agent properties such as organic capping agents and inorganic capping agents [19] for AgI NPs the FTIR results displayed the existence of organic groups in the coated layer of it which reduced the cytotoxicity effect at a particular dose [43].

3.2.2. FTC133 cellular uptake for AgNPs and AgI NPs
A flow cytometer was used to examine the cellular uptake of AgNPs and AgI NPs in FTC133 to understand better the factors that contribute to toxicity. The variation of the incubation time was 0 – 120 min, the AgNPs and AgI NPs suspended in media with two concentrations of 10 mM and 20 mM in addition to control and dosed cells. Figures 14(A) and (B) shows the fluorescence intensity rate as a function of incubation time for FTC133 of AgNPs and AgI NPs, respectively, to detect differences in cellular AgNPs and AgI NPs uptake. Figure 15

![Cell Viability rate of AgNPs &AgI NPs at a higher concentration of (400) μgl−1 for FTC133 and normal cells. B- The determined IC50 values of AgNPs &AgI NPs for FTC133 and WRL68.](image-url)
demonstrates that AgNPs and AgI NPs uptake is strongly time-dependent, and nanoparticle uptake is relatively rapid. Notably, there is a significant uptake within an hour with the AgNPs concentration of 3.05 mg/10^8 cells and 2 mg/10^8 cells for AgI NPs. After that, the cellular uptake continues to increase at approximately the same rate until the four-hour limit. Thus, after six hours, the rate of FTC133 cells ingesting AgNPs and AgI NPs begins to grow, albeit at a slower rate. After a long (6 h) incubation period, the FTC133 cellular uptake reached stable values for AgI NPs, while the increase continued at the same low rate at (8 h) for AgNPs to get regular discounts. That behavior could be because the cell is saturated. The cellular uptake results in this study indicate that FTC133 is concentration- and time-dependent for both AgNPs and AgI NPs. Previous work demonstrates that their uptake increases by increasing the time and engagements in which cells are exposed to NPs [44, 45]. Also, it can be observed that, throughout the exposure time, the rate of FTC133 ingested AgNPs was higher than that of AgI NPs taken up, confirming with that the cytotoxicity results which proved the AgNPs are more effective on FTC133 cells than the AgI NPs. That could be due to particle size differences between AgNPs and AgI NPs, as mentioned above in the TEM results, with AgNPs having an average diameter of 12.3 nm and AgI NPs having an

Figure 14. Fluorescence intensity analysis of the three cells (control un dosed cells, cells were incubated several times with 10 mM of NPs and 20 mM of NPs). A- AgNPs incubation, B- AgI NPs incubation. fluorescence intensity was determined by flow cytometry (n = 3).
average diameter of 26.25 nm, implying that FTC133 cellular uptake was size-dependent in the test. The results observed in this current study agree with a previous study that found the smaller AgNPs go into cells more quickly than the bigger size particles \[44, 45\]. Other reasons could be taken into account in the shape of NPs.

From FESEM and TEM tests, the morphological structure is highly monodispersed spherical shape of AgNPs specific surface area than that semi-spherical like with some of the ledges in the morphological form of the AgI.

**Figure 15.** Uptake of the (AgNPs) and (AgI NPs) in FTC133 cells at 12 h with various concentrations. Intracellular AgNPs and AgI NPs content (in μg per 10^6 cells) were determined by fluorescent-labeled nanoparticles using mean fluorescence intensity.

**Figure 16.** The AgNPs and AgI NPs treatments inhibited the migration and invasion of follicular thyroid cancer (FTC133) cells. (a) Western blot analysis of migration and invasion-related proteins was done in comparison to the control group (b) Migration rate (percent) (c) Invasion rate (percent).
NPs. As a result, the FTC133 cellular uptake in this study could be shape-dependent. Previous studies have found that the shape-dependence of cellular internalization causes significant differences in the uptake of different nanoparticles [46, 47].

3.2.3. FTC133 migration and invasion for AgNPs and AgI NPs treatment

Western blot technique was performed here to access the effects of AgNPs, and AgI NPs on the migration and invasion ability of FTC133 cells by analyzing the migration and invasion-related protein as shown in figure 16(a), the protein levels were decreased after AgNPs and AgI NPs treatments. The evaluated values of migrated and invasive FTC133 cell rates were reduced after AgNPs and AgI NPs therapy, as shown in figures 16(b) and (c). In figure 16(b), the migrated cell rate decreased when the FTC133 cells were treated with AgNPs to 76% and 60% with AgI NPs treatment compared to the control group. Also, it can be observed that the invasive cell rate decreased to 60% of the control group for AgNPs treatment and decreased to 55.71% in the cells treated with AgI NPs as shown in figure 16(c).

4. Conclusions

The novelty of this work relies on the study the cytotoxic effects of AgNPs on follicular thyroid cancer cells (FTC133 cell line). Also, up to our knowledge the study of the anticancer efficiency for AgI NPs at a first time in our article. The synthesis of AgNPs and AgI NPs via a chemical co-precipitation approach was characterized in this study, and their potential for targeting follicular thyroid cancer was demonstrated. According to the MTT assay, dose-dependent cytotoxicity of manufactured AgNPs and AgI NPs with cytotoxic effect varies by cell type, with AgNPs having a cytotoxic impact higher than AgI NPs on follicular thyroid cancer cells and normal cells. Furthermore, in a concentration, size, and time-dependent way, follicular thyroid carcinoma takes up AgNPs more than AgI NPs, according to the uptake data. Furthermore, AgI NPs inhibited FTC133 cell migration and invasion more effectively than AgNPs.

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Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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