Synthesis, Characterization of Silver and Gold Nanoparticles from *Coffea arabica* Extract and Their Cytotoxic Potential Against Cancer Cells

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**Abstract**

Silver and gold nanoparticles have been getting great medical interest due to their potential physicochemical properties like higher surface to volume ratio and low molecular weight. In this study biogenic synthesis of silver and gold nanoparticles were successfully carried out using *Coffea arabica* extract. The aqueous extract of coffee was mixed with silver nitrate (1 mM) and chlorauric acid (10^{-4} M) ensuing formation of silver and gold nanoparticles. UV-visible spectroscopy shows absorption maxima at 428 nm for silver and 540 nm for gold nanoparticles. Further characterization techniques used were FTIR, EDX and HRTEM with SAED. The HRTEM analysis reveals spherical nature of silver nanoparticles and polymeric nature of gold nanoparticles with mean particle size 16 nm for silver and 12 nm for gold nanoparticles. The cytotoxic potentials of these nanoparticles were studied against Hela, HCT116, MCF-7, THP-1 and K562 cell lines using MTT assay for studying cellular response of these nanoparticles. The synthesized silver nanoparticles were cytotoxic to all cell lines under study with mean cytotoxicity of 90% with IC_{50} 18.36 µg/mL and IC_{50} 7.9 µg/mL. The gold nanoparticles have shown IC_{50} of 69.97 µg/mL for the cell lines under study.

1. Introduction

Nanotechnology arises from physical, chemical, biological and engineering science where novel techniques are being developed to probe and manipulate single atom and molecules. There is vast current anticipation in the study of nanoscale matter, having nanometer dimensions (1 nm = 10^{-9} m) with respect to their fundamental properties, organization to form superstructures and applications. The unique properties of nanoparticles that differentiate them from corresponding bulk materials arise from their large surface area to volume ratio, surface energy and spatial confinement [1]. Metal nanoparticles have received significant attention in recent years because of their unique properties and potential applications in catalysis [2], optoelectronics [3], biological sensor [4, 5] and pharmaceutical applications [6]. Inorganic nanomaterials have been widely used for cellular delivery due to their versatile features like wide availability, rich functionality, good biocompatibility, and capability of targeted drug delivery and controlled release of drugs [7].

Nanoparticles of silver and gold have received incredible research attention for their broad applications in recent times, which include their optical, catalytic, conducting and biological properties. Advances in nanobiotechnology have demonstrated many nanoparticles as novel drug and gene delivery vehicles, biosensors, cancer treatment and contrast agents for biological imaging. Metal nanoparticles have many potential applications including use in biomedical [8], optoelectronics [9] and catalysis system which relate to their size dependent properties. A gold nanoparticle enhances Raman scattering, which outcomes in detection and identification of single molecule [10]. The size of nanoparticle decides its role in biomedical application such as nanoparticles which are less than 2 nm in diameter can cross blood brain barrier [11-13] and those of less than or equal to 30 nm can be endocytosed by cells [14].

Gold nanoparticles are used in chemotherapy and diagnosis of cancer cells [15]. Glucose level in rats can also be detected using SERS sensor made up of gold nanoparticles. Gold nanoparticles are accomplished of delivering large biomolecules without limiting themselves as carrier of only small molecular drugs [16]. Knowledge of nanoparticles, their potential toxicity and impact on health is essential before these nanoparticles can be used in clinical use. Most of the nanoparticles synthesizing practices employ either expensive machines or corrosive or hazardous chemicals. Thus, there is necessity of greener way of synthesis. This paper deals with synthesis, characterization and cytotoxic effects of nanoparticles on selected cancer cell lines. In this context single step reduction process of silver and gold nanoparticles using *Coffea arabica* powder was carried out and cytotoxicity of the synthesized silver and gold nanoparticles has been studied.

Chemical synthesis methods lead to presence of some toxic chemicals absorbed on the surface that may have adverse effect in the medical applications. Green synthesis offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for synthesis. It also provides advancement over chemical and physical methods as it is cost effective, environment friendly [17, 18], easily scaled up for large scale synthesis and this method do not use high pressure, energy, and toxic chemicals.

2. Experimental Methods

2.1 Chemicals

Silver nitrate (AgNO\(_3\)), chlorauric acid (HAuCl\(_4\cdot\)H\(_2\)O) and MTT reagent [3-{4, 5-dimethylthiazolyl(2)-2, 5-diphenyltetrazolium bromide] were purchased from Sigma-Alrich®. All Cell lines were purchased from National Centre for Cell Sciences, Pune, India. All culture media were bought from Himedia, Pvt. Ltd., India. Coffee powder (Nescafe Classic., lot no 12310452, Nestle India Ltd., Mysore India) was used to make aqueous extract and Coffee beans collected from Kerala, were roasted and finely crushed into coffee powder.

2.2 Synthesis of Silver and Gold Nanoparticles

The optimized *coffe arabica* extract was added to 1 mM silver nitrate and 1 x 10^{-4} M chlorauric acid solution for the synthesis of silver and gold nanoparticles respectively. The silver nanoparticles (AgNPs) reaction mixture was stirred for 30 minutes whereas gold nanoparticle synthesis took not more than 5 minutes. The reduction of AgNO\(_3\) and HAuCl\(_4\) ions was monitored by periodic sampling of aliquots of aqueous component and measuring UV-visible spectra of the reaction mixture [19].
3. Results and Discussion

3.1 UV-Vis Spectral Analysis

UV-visible spectroscopy is one of the widely used techniques for structural characterization of nanoparticles. The silver nanoparticles and gold nanoparticles synthesized according to method described in previous section, colloidal solution turned from pale yellow to pale brown for silver and from pale yellow/pale grey to ruby red for gold nanoparticles [18-20]. The reduction of pure silver and gold ions was monitored by measuring UV-visible spectrum (SHIMADZU UV-visible spectrophotometer 1800) of the reaction mixture by diluting small aliquot of sample with double distilled water (Fig. 1). The surface plasmon resonance (SPR) band absorption depends on particle size, shape and dielectric constant surrounding the medium. The absorption spectrums show SPR band at 428 nm and 540 nm indicating presence of spherical or roughly spherical silver and gold nanoparticles respectively [21] which was then confirmed by TEM analysis.

![Fig 1](image)

**Fig. 1** UV-visible spectrum of (a) silver nanoparticles - AgNPs and (b) gold nanoparticles - AuNPs

3.2 EDX Analysis

The elemental analysis of AgNPs was performed using EDX with SEM. The EDX spectrum reveals strong signal in the silver region and confirms the formation of silver nanoparticles. Silver (91.70%) was major constituent element compared to phosphorus (4.22%), and sulphur (4.08%) as shown in Fig. 2. Metallic silver nanocrystals generally show typical absorption peak approximately 3 keV due to surface plasmon resonance. The EDX spectra showed peaks around 2.013 keV, 2.307 keV, 2.93 keV that corresponding to the binding energy of P, S and Ag L₂, L₃ (AgLb-Aglb2), indicates presence of AgNPs. The peaks between 2.98-3.50 keV corresponds to binding energies of AgLa, AgLb, and AgLb2 with 50-60 counts, peak near 1 keV corresponds to carbon, this peak is due to SEM holding grid. This result indicates that synthesized product is composed of high purity of silver nanoparticles [22] (Fig. 2).

![Fig 2](image)

**Fig. 2** ZAF Method Quantitative Analysis (Fitting Coefficient: 0.9704)

3.3 Fourier Transform Infra-Red Spectrum (FTIR)

The size distribution and characterization of silver and gold nanoparticles could be further verified by FTIR spectrum. From Fig. 3, the absorption characteristic bands are found as 1023.61 cm⁻¹, 1605.43 cm⁻¹, 2851.01 cm⁻¹ and 2919.07 cm⁻¹ for silver nanoparticles and 799.12 cm⁻¹, 1025.70 cm⁻¹, 1636.10 cm⁻¹ and 2919.01 cm⁻¹ for gold nanoparticles. Absorbance peak at 1380 cm⁻¹ can designate stretch vibrations of functional groups such as -C=O, -C-O-, -C=O, -C=O as well as 1636.10 cm⁻¹ can be associated with the stretch vibration of -C=C and 1025 cm⁻¹ can be displayed to C-N vibrations of amine [23].

3.4 Transmission Electron Microscopy and Selected Area Diffraction Pattern

The morphology of silver and gold nanoparticles was observed by high resolution transmission electron microscope. One drop of nanoparticle suspension was placed on copper grid, and was allowed to dry at room temperature before analysis. TEM image shows spherical, truncated triangles and decahedral morphologies ranging from 7.18 - 20.31 nm for silver nanoparticle (AgNPs) and 9.22 nm-12.92 nm for gold nanoparticles (AuNPs) with average size of 12.51 nm and 16.21 nm respectively. Most of the nanoparticles were nearly circular in shape with smooth edges. The phytochemical constituents in coffee extract (chlorogenic acid, caffeine and trigonelline) may act as reducing agents during synthesis of AuNPs and AgNPs. SAED patterns of AgNPs and AuNPs exhibit concentric rings, indicating nano particles highly crystalline nature [1] (Fig. 4).

![Fig 4](image)

**Fig. 4** Transmission electron microscopic analysis of silver nanoparticles (AgNPs) at different resolution (a) 50 nm (c) 20 nm and selected area diffraction (SAED) pattern of AgNPs at 51 nm (d) size distribution histogram of silver nanoparticles and (e) XRD pattern of silver nanoparticles

3.5 X-Ray Diffraction Crystallography (XRD)

The X-ray diffraction pattern of silver and gold nanoparticles synthesized have shown in Figs. 4(e) and 5(e). The XRD pattern of AgNPs and AuNPs were recorded using Philip PW1710 X-ray diffractometer. The scanning was done in Bragg’s angle region (2θ) from 10° to 80°. The full widths at half maximum values (FWHMs) of X-ray diffraction were used to calculate nanoparticle size using Debye-Scherrer equation D=Kλ/βcosθ. The foremost peaks were observed at 2θ = 37°, 9.44°, 65° for silver nanoparticles and for gold nanoparticles 2θ = 38.4°, 48°, 68° both corresponds to (111) (200) (220) in accord with the JCPDS-file no.04-0783. The calculated size of nano particles with Debye-Scherrer equation (Table 1) shows approximate match between the nanoparticles size measured from TEM image.

![Fig 5](image)

**Fig. 5** Transmission analysis of gold nanoparticles (AuNPs) at different resolution (a) 50 nm (c) 20 nm and (b) selected area diffraction pattern (SAED) of AuNPs at 51 nm (d)-size distribution histogram of gold nanoparticles and (e) XRD pattern of gold nanoparticles

Table 1 X-Ray diffraction analysis calculation based on Debye-Scherrer

<table>
<thead>
<tr>
<th>Nanoparticles</th>
<th>FWHM degree</th>
<th>20 (2θ)</th>
<th>0, Rad</th>
<th>d</th>
<th>Crystalite size, nm</th>
</tr>
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<tbody>
<tr>
<td>CEAuNPs</td>
<td>0.608</td>
<td>38.4</td>
<td>19.2</td>
<td>0.3351</td>
<td>0.0106</td>
</tr>
<tr>
<td>CEAuNPs</td>
<td>0.608</td>
<td>38.4</td>
<td>19.2</td>
<td>0.3351</td>
<td>0.0106</td>
</tr>
</tbody>
</table>

3.6 Cytotoxicity Study

3.6.1 Cell Culture Conditions

The cell lines used were Hela, HCT116, MCF-7, K562 and THP-1. These cell lines cultured in respective media and were supplied with 10% fetal bovine serum, penicillin/gentamicin (1%), glutamine (0.75%) and sodium pyruvate (1%). Cell lines used and their respective culture media are Hela, MCF-7 - Eagle’s minimum essential medium, THP1-RPMI1640 with 2-mercaptoethanol up to final concentration of 0.05 mM and HCT116- McCoy’s 5a modified medium. cells were maintained in 5% CO2 incubator at 37°C with 95% relative humidity.

3.6.2 MTT Assay

A standard cytotoxic assay (MTT) was employed as it is the most sensitive assay [24]. Metabolic activity-based assay measure mitochondrial activity of cultured mammalian cells relating to its energy metabolism and cell growth. Reduction of MTT to formazan within cells is considered to be a sensitive assay for cell proliferation. The absorbance was measured at 540 nm. The MTT assay determines the ability of viable cells mitochondria to reduce the yellow insoluble formazan therefore the absorbance of formazan formed directly associate to the number of cell, whose mitochondrial metabolism is not damaged even after exposure to AgNPs and AuNPs [24].

The effect of nanoparticles to inhibit cell proliferation was tested using MTT assay. The cell lines were maintained at 37°C in a CO2 incubator for 24 hours. Cells were then treated with nanoparticles with different concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.78125 µg/mL and allowed to incubate. Later, the cells were washed thoroughly with phosphate buffer saline to remove unattached cells. Furthermore, 10 µL MTT (5 mg/mL in PBS) was added to cells in growth medium, and the plates were incubated at 37°C for 4 hours for MTT assay. DMSO (100 µL) were used to dissolve formazan crystals.

The absorbances were recorded at 570 nm. Percent growth inhibition was calculated by following formula, Percent Growth inhibition = 100 - (At-Ab) x 100/(Ac-Ab), where, At=Absorbance of test compound; Ab=Absorbance of blank; and Ac=Absorbance of control. The experiment was performed in triplicate and the quantitative value was expressed as the mean ± standard deviation.

Table 2 Cytotoxic cell concentration (IC50/CC50) of AgNPs and AuNPs along with IC10

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>CC50 (µg/mL)</th>
<th>IC50 (µg/mL)</th>
<th>IC10 (µg/mL)</th>
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<tbody>
<tr>
<td>AgNPs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AuNPs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hela</td>
<td>8.52</td>
<td>76.46</td>
<td>100</td>
</tr>
<tr>
<td>HCT116</td>
<td>14.03</td>
<td>63.29</td>
<td>&gt;100</td>
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<tr>
<td>K562</td>
<td>7.9</td>
<td>&gt;100</td>
<td>83.65</td>
</tr>
<tr>
<td>THP-1</td>
<td>16.21</td>
<td>&gt;100</td>
<td>91.75</td>
</tr>
<tr>
<td>MCF-7</td>
<td>19.14</td>
<td>&gt;100</td>
<td>&gt;100</td>
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</table>

The nanoparticles were synthesized from Coffea arabica extract which have reduced the salt of the silver and chloroauric acid. Synthesis was confirmed primarily by the colour change followed UV-Visible spectroscopy which shows narrow peak at 428 nm for silver and 540 nm for gold nanoparticles. The TEM analysis of silver nanoparticle shows that synthesized particles are of 16 nm size while gold nanoparticles are of 12 nm in size. The FTIR spectra showed characteristic peaks at 1380 cm-1 which designate strong vibration of functional groups such as -C-O-, -C=O, -C=C=O as well as 1636.1 cm-1 is associated with the strong vibration of C-C and 1023 cm-1 presented to C-N vibrations of amine. The silver nanoparticles show 94.32% mean cytotoxicity against all cell lines under study whereas gold nanoparticles reveal 50.46% mean cytotoxicity against the same. Among the cell lines used in this study highest cytotoxicity of silver nanoparticles were found against HeLa cell line which was followed by HCT116, K562, THP-1 and MCF-7 cell lines shown in Table 2. As compared to this gold nanoparticles showed 50% mean cytotoxicity in all cell lines stated above.

Most of the scientific reports that examine the cellular influence of nanomaterials are ‘In vitro with limited work to understand the real situation ‘In vivo’ [44]. A standard cytotoxic assay was applied to study effect of AgNPs and AuNPs on different cancer cell lines. Methodology of cytotoxic test is implemented as given by National Cancer Institute [26-28]. The MTT assay determines the ability of viable cells mitochondria to reduce the yellow insoluble formazan therefore the absorbance of formazan formed directly associate to the number of cells whose mitochondrial metabolism is not damaged even after exposure to AgNPs and AuNPs [24]. Receptor mediated endocytosis has been proposed as the primary mechanism of cellular entry for gold nanoparticles [28-30]. The result of our studies suggests that AgNP possesses the strongest cytotoxic effects on all human cancer cell lines which entice its anticancer potential whereas AuNPs doesn’t show significant cytotoxicity up to 100 µg/mL to all cell lines except HeLa and HCT 116 cells (Table 2).

In the present investigation we have observed that the nanoparticles caused marked cell growth inhibition in human cancer cells K562, HeLa, HCT116, MCF-7 and THP-1 in dose dependent manner [31-33]. In fact, silver nanoparticles had extreme cytotoxic activity with lowest IC50 value of 18.36 µg/mL for HeLa cell line and highest IC50 found to be 36.19 µg/mL (Table 2). The results obtained from present study shows silver nanoparticles were cytotoxic to HeLa, HCT116, K562, THP-1 and MCF-7 cells (Fig. 6). The IC50 value of a good drug candidate should be sufficiently low to avoid any indefinite effects. The IC10, which is lower than 30 µg/mL, is considered to be a promising anticancer agent as defined by American National Cancer Institute [34]. Advance studies are required to explicate the precise molecular mechanism.

The possible route nanoparticle enters into the cell is by phagocytosis [35, 36] or by passive diffusion through membrane [37]. The entry of silver nanoparticle into cells and their toxic potential is size dependant [36]. Another mode of action is enhancement of reactive oxygen species inside the cells. Reactive oxygen species when formed produce subsequent cellular damage such as disrupting membrane integrity as well as damaging proteins and DNA [35-42].

4. Conclusion

Biogenic synthesis of silver and gold nanoparticles were successfully carried out using Coffea arabica extract. The synthesized nanoparticles were characterized using UV-visible spectroscopy, Electron diffraction spectrum (EDX), transmission electron micro scope (TEM) with SAED. The knowledge about its cytotoxicity is indispensable for further applications. The silver nanoparticles were found to have strong anticancer properties as it inhibits proliferation of cancerous cell lines.

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References


