Synthesis and characterization of Ammonium oxalate stabilized Gold Nanoparticles and their Anticancer activity on A549 Human Lung cancer cells

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ABSTRACT

Gold nanoparticles (AuNPs) have been used for various biomedical research applications such as cell labeling, imaging, biosensing, gene, drug delivery and cancer therapy. AuNPs are synthesized by physical, chemical and biological methods. The present study explain facile one-step synthesis of gold nanoparticles using ammonium oxalate under the bright sunlight irradiation, in which ammonium oxalate act as reducing as well stabilizing agent. The AuNPs synthesis was completed in 20min at pH 7.5. The characterizations of AuNPs were analyzed by UV-visible spectroscopy, FESEM, DLS and Zeta potential. The synthesized gold nanoparticles showed maximum surface plasmon resonance (SPR) spectra at 530nm and revealed spherical shape with 33nm in size under the electron microscopic observation. The anticancer efficacy of synthesized AuNPs on A549 lung cancer cells was demonstrated by cell viability analysis and apoptosis analysis. The obtained result revealed that the AuNPs had potential anticancer activity in the concentration manner and IC50 concentration of AuNPs was 100µg/mL. Therefore this potential candidate can be used for biomedical applications like anticancer therapy, drug delivery and photothermal therapy.

Keywords: Ammonium Oxalate, Chloroaucric acid, Sunlight irradiation, Gold nanoparticles, Cell viability.

1. INTRODUCTION

Nanoparticles are very tiny material of less than 100 nm in size and their applications have more attention over the past few years in many disciplines [1]. Generally used metal nanoparticles are gold, silver, platinum, iron and ceramic nanoparticles. Among these different metal nanoparticles, gold nanoparticles have great interest due to their unique physicochemical properties and can be synthesized through the physical, chemical and biological methods [2]. AuNPs are used in wide range of biomedical research applications including photothermal therapy, drug delivery, bioimaging, biosensing [3], anticancer therapy [4, 5], antibacterial [6, 7] applications.

Cancer is a group of disease, causes by various pathological and metabolic abnormalities in the cells
and develops through abnormal cell proliferation, angiogenesis and metastasis [8]. According to the world health organization (WHO) statement 2012, cancer patient has increased to 14 million and this number will be increased to 22 million in 2030 [9]. Among the various cancer types, lung cancer is most predominant and deadly cancer in both men and women and its death rate exceeds than the colon, breast and pancreatic cancer. More than 50% of lung cancer patient died in one year [10]. The physical and chemical treatment is limited for different stages of cancer and further they induce adverse effect on normal cells in over dosage and radiation exposure [8]. Nanotechnology is emerging field that involved in modern medicine and treatment of inflammatory disease, liver disease, HIV, arthritis and tumour [11]. They provide novel approaches to address the challenges in conventional medicine [12].

Recently, sunlight mediated photochemical synthesis of nanoparticles are more inspired than the existing methods, because of they are renewable, nontoxic and cost effective [13], minimum time consuming and synthesis size controlled NPs [14]. Previously, Ganeshkumar et al. 2012 reported sunlight mediated synthesis of AuNPs using nutrient agar [15], Devendiran et al. 2014 explained sunlight mediated biosynthesis of gold nanoparticles using Pisonia grandis leaf extract for biomedical applications [16] and Dane et al. 2016 described Pyoverdin mediated sunlight induced green synthesis of silver nanoparticles and their antibacterial activity [17]. Here we report, a simple one-step synthesis of AuNPs using ammonium oxalate as a reducing as well as stabilizing agent under the bright sunlight irradiation method. Sunlight irradiation is catalyst the reaction of NPs synthesis. The characterization of ammonium oxalate stabilized AuNPs (AOX-AuNPs) were analyzed in UV-visible spectroscopy, field emission scanning electron microscopy (FESEM), dynamic light scattering analysis (DLS), zeta potential. The anticancer efficacy of AOX-AuNPs were evaluated against A549 human lung cancer cell line through the cell viability analysis and apoptosis analysis using fluorescence staining.

2. MATERIALS AND METHODS

2.1. Materials

Chloroauric acid, Ammonium oxalate and Acridine orange/ Ethidium bromide (AO/EtBr) purchased from Sigma Aldrich, USA. Dulbecco’s Modified Eagle’s Medium (DMEM), Trypsin phosphate versene glucose (TPVG) were obtained from Himedia Laboratories Pvt Ltd, India. Fetal bovine serum (FBS), Antibiotic-antimycotic solutions were purchased from Life technologies corporation, USA. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Dimethyl sulfoxide (DMSO) were obtained from Sisco Research Laboratories Pvt Ltd, India. All the glassware were cleaned with aqua regia (HCl: HNO3 = 3:1) and were rinsed with double distilled water, and dried in a hot air oven prior to use.

2.2. Synthesis of AOX-AuNPs

Ammonium oxalate (100mM) 800µL was added to the 100mL conical flask containing 40mL of Chloroauric acid (1mM) and the pH was adjusted to 7.5. After stirring, the solution was placed under the bright sunlight for 20min. The pale yellow colour solution was turned into deep red colour of gold nanoparticles (AuNPs), it was confirmed by surface plasmon resonance (SPR) analysis in UV-visible spectrophotometer. The synthesized AuNPs were stored at 4°C for analysis.

2.3. Characterization of AOX-AuNPs

2.3.1. UV-visible spectrophotometer analysis

UV-visible absorption spectra of synthesized AuNPs were acquired using Shimadzu UV-1601 spectrometer (Japan) in the 200-800nm wavelength range and the wavelength data were used for the plotting the absorbance peak.

2.3.2. Field emission scanning electron microscopy (FESEM)

A drop (50µL) of gold nanoparticles were placed in the clean aluminium foil and allowed to dry it in room temperature for overnight. The shape and size of the AOX-AuNPs were determined through the FESEM (Hitachi, SU-6600) at 15.0KVand 500nm scale.

2.3.3. Dynamic light scattering (DLS) analysis

The hydrodynamic diameters of AuNPs were determined in the aqueous medium with Zetasizer Nano series (Malvern Instruments, United Kingdom). 1 mL of AOX-AuNPs was taken in a clean cuvette and placed in the sample holder. The instrument was operated at an accelerating voltage of 100 VA at 25°C, the count rate of 232 kcps with the duration of 60 s and the obtained data was plotted as column graph.
2.3.4. Zeta potential measurements

The surface net charge (zeta potential) of the AuNPs were determined in Zetasizer Nano series (Malvern Instruments, United Kingdom). The diluted AOX-AuNPs were filled with electrode cuvette and placed in the sample holder. The instrument was operated at an accelerating voltage of 100 VA at 25°C, the count rate of 232 kcps with the duration of 60s.

2.4. In vitro anticancer activity
2.4.1. Cell viability analysis

Anticancer activity of AOX-AuNPs on A549 human lung cancer cell line was analyzed using MTT assay [18]. Briefly, 1x10⁴ cells/well were seeded in 96 well culture plates and incubated at 37°C, 5% CO₂ for 24h. Then the cells were exposed to different concentration of AOX-AuNPs (25, 50, 75, 100, 125 and 150µg/mL) for 24h. After the incubation, 10µL of freshly prepared MTT was added to all the wells and the plate was incubated 3h in dark condition. Finally, 100µL of DMSO was added to dissolve the formazan which produced purple colour, which was measured by ELISA reader (BIO-TEK, Powerwave-XS) at 570nm. The percentage of cell viability was calculated as follows,

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\text{Cell viability} (\%) = \frac{\text{OD of test sample}}{\text{OD of control}} \times 100
\]

2.4.2. Apoptosis analysis

A549 cells (1x10⁵ cells/well) were seeded on a sterile coverslip containing six well plate and incubated for 24h in controlled atmosphere. Then the cells were treated with IC₅₀ concentration (100µg/mL) of AOX-AuNPs for 24h, after that the cells were stained with AO/EtBr and images were captured through the fluorescence microscope (Nikon Eclipse 80i).

2.5. Statistical analysis

All the data were evaluated using the statistical software SPSS/16. Hypothesis-testing methods included one-way analysis of variance (ANOVA) followed by least significant difference test. P-values less than 0.05 were considered statistically significant. All the results were expressed as mean ± standard deviation (n = 3).

3. RESULTS AND DISCUSSION

3.1. Synthesis of AOX-AuNPs

The present study described the synthesis of AuNPs using ammonium oxalate as reducing as well as stabilizing agent under the bright sunlight irradiation method. After the addition of ammonium oxalate into the chloroaauric acid solution, light yellow colour chloroaauric acid solution was changed to deep red colour in 20 min at pH 7.5 that indicates the synthesis of AuNPs (Figure 1). It was also confirmed in UV-visible spectrophotometer, exhibited fine single SPR absorption peak (λₘₐₓ) at 530nm with 1.42a.u intensity. Ammonium oxalate contains three major functional groups such as hydroxyl (~OH), amine (~NH) and carboxylate (~COO), which is responsible for synthesis of gold nanoparticles. In sunlight mediated synthesis of AuNPs, sunlight acts as a catalyst in the presence of reducing/stabilizing agent. Sunlight mediated preparation of NPs is facile, inexpensive, eco-friendly and no need of any sophisticated instruments.

3.2. Characterization of AOX-AuNPs

A specific bright colour is a distinguishing feature of noble metal nanoparticles. It is caused to the phenomenon of localized surface plasmon resonance (LSPR) that appears as absorption in the visible range of the spectrum [19]. Figure 2a, UV-visible spectrum reveals the optical characterization of AOX-AuNPs exhibited a well-defined single absorption peak at 530nm, which confirms the conversion of ionic gold to neutral gold nanoparticles and also suggest that the particles are narrow size distribution. The bioactivity of metal AuNPs depends on their size, shape and surface chemistry [20]. The FESEM analysis of AOX-AuNPs showed monodispersed particles with spherical shape and average size is ~ 33nm (Figure 2b)

The hydro dynamic diameter of the nanoparticles in the aqueous medium was ~ 58.77nm, which was measured by DLS techniques (figure 2c). The surface net charge (zeta potential) of the synthesized AuNPs was -27.5mV in the aqueous medium (figure 2d), this highly negative zeta potential repel each nanoparticles in the suspension. It helps to nanoparticles for long term stability and prevents the particles from aggregation [21].

3.3. Anticancer activity

MTT assay is fast and efficient method routinely used to estimate cytotoxicity of nanomaterials. Figure 3 shows the cytotoxicity of A549 cells were treated with different concentration of AOX-AuNPs (25, 50, 75, 100, 125 and 150µg/mL) for 24 h incubation. In MTT assay, 92.26%, 82.66%, 63.43%, 50.03%, 41.93% and 32.93%, of cell viability was found to be 25, 50, 75, 100, 125 and 150µg/mL concentrations of AOX-AuNPs. The synthesized AuNPs exhibited -
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Figure 1. Synthesis of ammonium oxalate stabilized gold nanoparticles in the sunlight irradiation.

Figure 2. Characterization of AOX-AuNPs a) SPR analysis in UV-visible spectroscopy b) Size and shape of AuNPs were determined by FESEM c) Hydrodynamic diameter of AuNPs were determined using DLS analysis d) Zeta potential measurement of AuNPs
**Figure. 3.** Cell viability analysis of AOX-AuNPs on A549 human lung cancer cell line

**Figure. 4.** Apoptosis analysis of AOX-AuNPs treated cells stained with AO/EtBr.
dosage dependent cytotoxicity and the cell viability was decreased while increasing the concentration of metal NPs. 50% of cell inhibition was attained at 100µg/mL (inhibitory concentration, IC₅₀), which showed significant cytotoxicity in A549 cells for 24 h of exposures. Previously, several report explained the anticancer activity of gold nanoparticles in various types of cancer cell lines. Dasguptha et al. 2015 reported anticancer activity of AuNPs against human leukemic cell lines U937 and K562 [22]. Yang et al. 2014 reported anti-liver cancer activity of dihydriodic acid capped AuNPs in HepG2 cell line [23].

3.4. Apoptosis analysis

Apoptosis is defined as programmed cell death and characterized by the cell membrane blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation. The effect of AOX-AuNPs in apoptosis was tested by AO/EtBr fluorescence staining. Acridine orange dye stains both live and dead cells and ethidium bromide stains the cells that have lost nuclear membrane integrity and binding with DNA. Figure 4, the control and AOX-AuNPs treated cells were stained after 24h incubation and the normal cells appeared in organized structure with intact nuclei stained with green fluorescence, where as early apoptotic cells were visible with bright green and light orange patches and late apoptotic cells were stained with orange to red patches [24]. Obtained result is also supported by MTT assay results of cell viability inhibition of AOX-AuNPs. Muthukumarasamyvel et al. 2017 reported hydrophobicity depending apoptotic effect of different cholic acid capped AuNPs in A549 lung cancer cell line [21].

4. CONCLUSION

In conclusion, the present study reports synthesis of gold nanoparticles using ammonium oxalate under the bright sunlight irradiation in aqueous medium at nearly biological pH (7.5). The synthesized AuNPs were monodispersed, uniform size and anionic nature, characterized by UV-Visible spectroscopy, FESEM, DLS and Zeta potential techniques. The anticancer efficacy of AOX-AuNPs has demonstrated by MTT assay and apoptosis analysis using fluorescence staining on A549 lung cancer cell line. AOX-AuNPs had anticancer activity in concentration manner and IC₅₀ concentration was (100 µg/mL). Therefore, this potential AOX-AuNPs could be used effectively for cancer therapeutics in biomedical applications.

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Conflicts of Interest

There are no conflicts of interest.

References


