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Synthesis and characterization of Arabic gum capped gold nanoparticles for tumor-targeted drug delivery

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ABSTRACT

In this study, green synthesis of gold nanoparticles (GNPs) was achieved by sunlight-mediated method using the leaves extract of *Vitex negundo (V. negundo)* as a reducing agent and Arabic gum is used as a capping agent. Epirubicin-loaded Arabic gum-capped gold nanoparticles (E-GNPs) was synthesized and then functionalized with folic acid for tumor-targeted drug delivery. Folic acid-functionalized (Fa-E-GNPs) nanoparticles were characterized by different physicochemical characterization methods such as UV spectra, FESEM, particle size analysis, zeta potential, SAED and HRTEM. *In vitro* stability of the Fa-E-GNPs was also analyzed. The Fa-E-GNPs showed enhanced cytotoxic effects on lung adenocarcinoma (A549) cell line, suggesting that the folic acid functionalization led to cancer cell targeting and in turn increased the uptake of epirubicin by cancer cells.

1. Introduction

Lung cancer is considered the most frequent cause of major cancer incidence and mortality worldwide. Adenocarcinoma is the most common type of lung cancer, accounting for almost half of all lung cancers [1]. Potential applications of GNPs have been recently studied and administrated in phase I and II clinical trials for the treatment of cancer [2]. The major advantages of GNPs are the ease of preparation of monodispersed NPs [3] and low toxicity [4]. GNPs are one of the most commonly explored and used nanoparticles in drug delivery because of their controlled size, improved efficacy and targeted delivery [5–6]. Green chemistry-based eco-friendly methods are predominantly used for the synthesis of nanoparticles and it could be an alternative method for chemical synthesis. Numerous natural derived materials and biopolymers have been successfully used for well-organized and speedy green synthesis of silver, copper oxide, zinc oxide, selenium and platinum [7–11], GNPs [12–17]. Herein, we report on developing a novel Arabic gum-capped nanocarrier that was synthesized using natural and biocompatible *V. negundo* leaves extract as reducing agent. The usage of epirubicin is limited owing to serious non-specific toxicity to normal tissues, especially cardiac toxicity related to intramyocardial production of reactive oxygen species (ROS). Meanwhile, the rapid clearance rate by the reticuloendothelial system (RES) reduces the extravasation of epirubicin into tumor site and accordingly weakens drug efficacy [18]. To overcome this problem, we have synthesized Fa-E-GNPs for the targeted delivery of epirubicin. Coating of GNPs with anionic polysaccharide (Arabic gum) will make the GNPs more suitable for easy loading of cationic drug (epirubicin) through electrostatic interaction [19].

2. Experiment details

The optimized leaf extract of 7.5 ml was added to 10 ml of 1×10^{-7} M $HAuCl_4$ gold solution and then exposed to bright sunlight; the change of color in the reaction mixture will start taking place within 15 minutes from yellow to wine red color. The green reduction of $AuCl_4^-$ ions in solution was monitored by periodic sampling of aliquots (0.1 ml) of aqueous component and measuring UV-Visible spectra of the solution. Thirty milligrams of EDC was dissolved in 0.1 M NaOH and 30 mg of folic acid (FA) was added to it. This mixture was activated for 3 h. Twenty milligram of Arabic gum dissolved in 0.1 M NaOH was added to this mixture and allowed to stir for 24 h, followed by dialysis against water to remove unreacted EDC, FA from FA conjugates. Then 1 ml of conjugate was added to E-GNPs and allowed to stir for 12 h followed by centrifugation to form Fa-E-GNPs. The free drug present in the supernatant solution was determined by measuring its absorbance at 480 nm. *In vitro* drug release was performed using dialysis method. Fa-E-GNPs (equivalent to 10 mg of epirubicin) were

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dialyzed against 100 ml of sodium phosphate buffer, pH 7.4, at 37 °C in dark with continuous stirring at 100 rpm. For the determination of effect of pH on the stability, Fa-E-GNPs were incubated in different pH medium (pH 1.2 to pH 7.4). Nanoparticles incubated in saline were taken as control. Absorbance was measured after 10 days and compared with absorbance of control. Cytotoxicity study was performed using standard MTT assay. The *in vitro* cytotoxicity of free epirubicin, Fa-E-GNPs was investigated against cells A549 (human lung adenocarcinoma cell line).

3. Results and discussion

In the present study, GNPs were prepared by sunlight-mediated of *V. negundo* leaves extract-$HAuCl_4$ aqueous solution for 15 min. The initial yellow color solution became colorless, indicating the reduction of $AuCl_4$ to Au^{3+} atoms, later the solution turned to wine red color. The color changes occurred due to the excitation of surface plasmon vibrations of GNPs [20]. The yield of GNPs was optimized by changing the volume of extract from 25 μl to 250 μl and keeping the gold solution remain constant (1 ml water mixed with 750 μl of 1×10^{-7} gold solution). Our studies found that 150 μl of extract produced maximum yield of GNPs (Fig. 1A). The colloidal stability of GNPs was analyzed with different pH in room temperature for 10 days and our studies found the increased stability of Fa-E-GNPs in pH 7.4 (Fig. 1B), which could be due to the presence of Arabic gum [21]. Energy Dispersive X-ray (EDX) analysis of GNPs showed the presence of gold (Au) in the Fa-E-GNPs (Fig. 1C).

The mean particle size and zeta potential of Fa-E-GNPs were measured by DLS method. The average particle size was 98.65 ± 1.86 nm (Fig. 2A). The zeta potential value of GNPs is -30.4 mv (Fig. 2B) and this assures long-term stability. Moreover the PDI of 0.159 indicates the good homogeneity of synthesized GNPs. HRTEM results revealed (Fig. 2C) the surface morphology of GNPs is spherical in shape. Crystallinity of GNPs was confirmed by Selected Area Electron Diffraction pattern (SAED) (Fig. 2C inset). The FESEM image (Fig. 2D) showed that GNPs are nearly spherical in nature.

A549 cells were used to study the cell viability of epirubicin and Fa-E-GNPs by a standard MTT assay [22,23]. The results of MTT assay for free and Fa-E-GNPs on A549 cells are shown in (Fig. 3A). The IC_{50} value for Fa-E-GNPs was found to be around 4 μg/ml while that for free epirubicin ranged from 24 μg/ml on the lung cancer cells. These results revealed that the Fa-E-GNPs produced here showed better cytotoxic activity than what free epirubicin would. Our results confirmed that Fa-E-GNPs showed a lower IC_{50} value than free epirubicin in A549 cells. This could be due to the different uptake profile leading to better activity of folic acid functionalized nanoparticles (Fa-E-GNPs) as suggested by Pooja et al. (2014) [23]. FTIR spectrum (Fig. 3B) of *V. negundo* shows peaks at 3435, 3331, 2214, 1557, 1416, 1124 and 1051 cm^{-1} [24]. The peak at 3425,2942,1614,1426 and 1074 cm^{-1} becomes relatively narrow, which could be attributed to the stretching vibration of phenolic hydroxyls (OH bond), and confirms the interactions of phenolic hydroxyls to GNPs, resulting in the partial destruction of hydrogen bonds among *V. negundo* extract molecules. A shift in the peak from 1614 cm^{-1} to 1613 cm^{-1} also indicates that *V. negundo* extract interacts with GNPs through its adjacent phenolic hydroxyl group. Polyphenols present in *V. negundo* leaf extract react with Au^{3+} and reduce them to Au^0

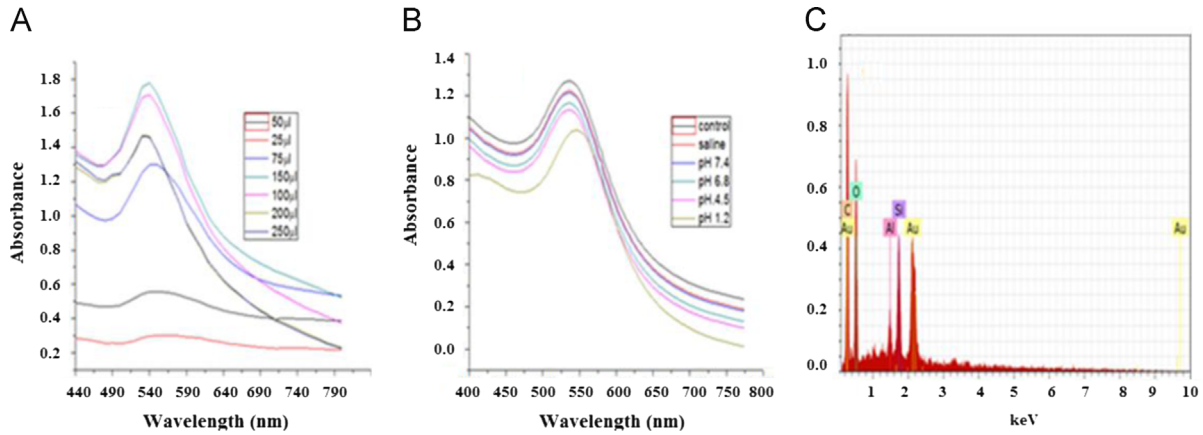

Fig. 1. (A) UV–vis spectrum of nanoparticle measured at the time of reaction of *V. negundo* leaf extract with aqueous solution of 10^{-7} mol/L HAuCl4. (B) Effect of pH on the stability of Fa-E-GNPs. (C) EDX profile of GNPs by *V. negundo* L. leaf extract.

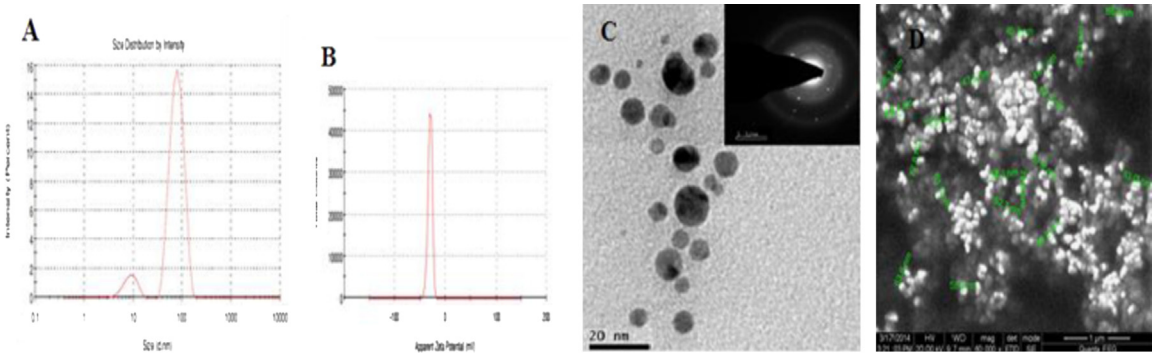

Fig. 2. Storage stability of Fa-E-GNPs: (A) particles diameter (B) zeta potential. (C) HRTEM (inset) - SAED pattern micrograph of GNPs by *V. negundo* leaf extract. (D) FESEM image of Fa-E-GNPs.