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Natural polysaccharide functionalized gold nanoparticles as biocompatible drug delivery carrier

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ABSTRACT

Biocompatibility is one of the major concerns with inorganic nanoparticles for their applications as drug delivery system. Natural compounds such as sugars, hydrocolloids and plant extracts have shown potential for the green synthesis of biocompatible gold nanoparticles. In this study, we report the synthesis of gum karaya (GK) stabilized gold nanoparticles (GKNP) and the application of prepared nanoparticles in the delivery of anticancer drugs. GKNP were characterized using different analytical techniques. GKNP exhibited high biocompatibility during cell survival study against CHO normal ovary cells and A549 human non-small cell lung cancer cells and during hemolytic toxicity studies. Gemcitabine hydrochloride (GEM), an anticancer drug, was loaded on the surface of nanoparticles with 19.2% drug loading efficiency. GEM loaded nanoparticles (GEM-GNP) showed better inhibition of growth of cancer cells in anti-proliferation and clonogenic assays than native GEM. This effect was correlated with higher reactive oxygen species generation by GEM-GNP in A549 cells than native GEM. In summary, GK has significant potential in the synthesis of biocompatible gold nanoparticles that could be used as prospective drug delivery carrier for anticancer drugs.

1. Introduction

In recent years, inorganic nanoparticles have attracted much attention in the area of bio imaging, drug delivery and other therapeutic and diagnostic applications. A definite size and unique surface composition make them suitable carriers for the development of targeted drug delivery system. Commonly used inorganic nanoparticles include metal (gold, silver, and platinum) nanoparticles, quantum dots, carbon nanotubes, iron oxide magnetic nanoparticles and ceramic nanoparticles [1,2].

Among these inorganic nanoparticles, gold nanoparticles are most commonly investigated due to their unique optical-electronic properties, ease of synthesis, opportunity for surface modification, non-cytotoxicity and biocompatibility [3,4]. Gold nanoparticles can be synthesized using physical, chemical and biological approaches [1]. The chemical synthesis of gold nanoparticles relies on the reduction of electropositive gold (Au^{3+}) to neutral gold atoms. Several chemical agents are used as reducing agents in preparation of gold nanoparticles. Phosphorus was the first reducing agent, used by Michael Faradey to prepare colloidal gold, and after that sodium borohydride [5], citrate [6,7], polymers like poly (N-vinyl-2-pyrrolidone) [8,9], block copolymers [10], and G5 PAMAM dendrimers [11], were used to prepare and stabilize the gold nanoparticles. However, the toxicity and involvement of organic solvent are the major limitations of these reducing agents.

To overcome the above problems, natural compounds like gums [12–14], proteins [15], glucan [16], hemicellulose-arabinoxylan [17], hyluronan [18], and chitosan [19,20] have been encouraged in gold nanoparticle preparation. Gums are high molecular weight, hydrophilic polymers and are composed of monosaccharides attached with glycosidic bonds. Natural gums are obtained from plants and generally present in the intracellular parts of the plant or as extracellular exudates. Gum karaya (GK) is the dried exudates obtained from Sterculia urens Roxd and other related species of Sterculia (family Sterculiaceae) or Cochlospermum gossypium AP De Candolle or other species of C kunth (family

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Bixaceae). GK is partially acetylated polysaccharide with a molecular weight of about 16×10^9 Da, anionic in nature and on hydrolysis it produces rhamnose, galactose, galactouronic acid and glucuronic acid [21,22]. Because of its non-toxic nature, low-cost and easy availability, it has been found to have wide applications in food and pharmaceutical industries as binder, viscofier, food additive and emulsifier and as drug release modifier in the design of oral controlled release formulations [23–25].

Recently, inorganic nanoparticles such as copper-oxide nanoparticles and silver nanoparticles have been synthesized using GK [26,27]. Both the studies clearly demonstrated the cationic metal reducing capabilities of GK. The primary objective of this study was to explore the potential of GK as reducing agent and stabilizer in the preparation of gold nanoparticles. Secondly, to find out the possibility of prepared nanoparticles as prospective drug delivery carrier for an anticancer drug gemcitabine hydrochloride (GEM).

2. Materials and methods

Hydrochloroauric acid ($HAuCl_4$), dimethyl sulfoxide (DMSO), Dulbecco's modified eagle medium (DMEM), RPMI-1640, trypsin-EDTA, antibiotic antimycotic solution, MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] were purchased from Sigma Aldrich (St. Louis, MO, USA). Gum karaya was obtained from Girigen co-operative society, (Hyderabad, India). Gemcitabine hydrochloride was kindly provided by Ther-Dose Pharma Pvt. Ltd. (Hyderabad, India). Human lung cancer cells (A549) and Chinese hamster ovary (CHO) purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). Foetal bovine serum (FBS) was purchased from Gibco, USA. High performance liquid chromatography (HPLC) grade solvents were purchased from Merck specialties (Mumbai, India).

2.1. Preparation of Gum karaya solution

Gum karaya (GK) was powdered using End Perger Mill and passed through 250 μm sieve. The obtained gum powder was dissolved in MilliQ water and kept for stirring overnight. The gum solution was centrifuged at 15,000 rpm for 30 min. The supernatant obtained was collected, lyophilized (Modulyod freeze-dryer-230, USA) and was used for the experiment.

2.2. Preparation of gold nanoparticles

One hundred microliter of $HAuCl_4$ solution (10 mM) was added to the 5 mL of GK solution (15 mg/mL) and stirred magnetically for 1 h at 90 °C. A colour change was observed from colourless to wine red at the absorbance of 536 nm which indicated the formation of gold nanoparticles. The preparation of gum karaya stabilized gold nanoparticles (GKNP) was optimized for various parameters such as temperature, reaction time, gum concentration, gold concentration and gold to gum solution ratio.

2.3. Characterization of nanoparticles

GKNP were characterized by UV/Vis absorption spectroscopy, dynamic light scattering, transmission electron microscopy (TEM), powder X-ray diffraction (PXRD) analysis, differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) analysis. The UV/Vis absorption spectra of nanoparticle dispersion were monitored in the range of 200–700 nm using UV/Vis spectrophotometer (Lambda 25, Perkin Elmer, USA). Nanoparticles were dispersed in deionised water and analyzed for hydrodynamic diameter, size distribution and zeta potential using a particle size analyzer (Nano ZS, Mavern instruments, UK). Samples were run in triplicates and average value was considered as particle diameter or zeta potential of nanoparticles. For morphology studies, a drop of sample was placed on carbon coated copper grid, after 5 min drained the excess with the help of filter paper and washed with distilled water. Finally, it was stained with 2% uranyl acetate, air-dried and observed under transmission electron microscope (Hitachi, H-7500). For FTIR studies, samples were palletized with potassium bromide and scanned from wavenumber 4000–450 cm^{-1} using FTIR spectrophotometer (Perkin Elmer, Spectrum One, USA). Percent transmittance was recorded against wave number with a resolution of 4 cm^{-1}. Powder X-ray diffraction measurements were carried out using an X-ray diffractometer (D8 Advance, Bruker, Germany) at 40 kV and 30 mA with CuKα radiation. The samples were scanned between 2θ angles, 5–90°. Both gum karaya and GKNP were studied for thermal properties. About 5 mg of sample was placed in aluminium pan and heated from 30–250 °C at a scanning rate of 10 °C/min under nitrogen environment using DSC-Q100 (TA Instruments, USA).

2.4. Hemolytic toxicity studies

To study the hemolytic toxicity, GKNP were dispersed in normal physiological saline (0.9 w/v% NaCl). Varying concentrations (25–250 μg/mL) of these dispersions were added into red blood cells suspension (2 w/v%), mixed well and incubated at 37 °C. After 1 h, the samples were centrifuged at 1000 rpm for 10 min and absorbance of supernatant was measured at 540 nm spectrophotometrically. The absorbance values of supernatant of RBC suspension treated with Triton X-100 (1 v/v%) and normal saline were taken as standard and control; respectively. The % hemolysis was calculated as: $\%\ \text{Hemolysis} = (A_{\text{sample}} - A_{\text{control}})/(A_{\text{standard}} - A_{\text{control}}) \times 100$.

2.5. Biocompatibility studies of GKNP

Biocompatibility of GKNP was determined in Chinese Hamster Ovary (CHO) cells and A549 human lung cancer cells by MTT assay. CHO and A549 cells were maintained in DMEM and RPMI-1640 medium, respectively. Both media were supplemented with 10% foetal bovine serum (FBS), 1% streptomycin and penicillin and 5% L-glutamine, in a humidified atmosphere of 5% CO_2 incubator at 37 °C. Cells were seeded in 96-well plate at a density of 5×10^3 cells/well for 24 h. The medium was then replaced with 100 μL of fresh medium containing different concentrations of GKNP. After 48 h of incubation, medium was discarded and 0.5 mg/mL MTT solution in serum free media added. Cells were incubated for another 4 h. The medium was then replaced with dimethyl sulfoxide 150 μL and the absorbance was measured with a microplate reader at a wavelength of 570 nm. The cytotoxicity of formulations was expressed as the percentage of cell viability compared to the untreated control cells.

2.6. Drug loading

GKNP were dispersed in MilliQ water and 5 mg of GEM was added in the GNP dispersion and stirred for overnight at room temperature. GEM loaded gum karaya stabilized gold nanoparticles (GEM-GNP) were collected by centrifuging the nanoparticles dispersion at 15,000 rpm for 45 min. Drug content in supernatant was determined using a high performance liquid chromatography system with photodiode array detector (Waters, USA). Mobile phase (acetonitrile and water, 40:60 v/v) was pumped through a C_{18} Waters Reliant column (5 μm particle size, dimensions 4.6 mm × 250 mm) at a flow rate of 0.8 mL/min. After filtering through 0.2 μm nylon filter the mobile phase was degassed by bath sonication for 30 min. The injection volume was 25 μL and