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Original article

Synthesis, characterization, biocompatible and anticancer activity of green and chemically synthesized silver nanoparticles – A comparative study

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ABSTRACT

Silver nanoparticles (AgNPs) are superior cluster of nanomaterials that are recently recognized for their different applications in various pharmaceutical and clinical settings. The objective of this work deals with novel method for biosynthesis of AgNPs using *Azadirachta indica* (neem) leaf extract as reducing agent. These bio and chemical synthesized nanoparticles were characterized with the help of UV–vis Spectroscopy, Nanotarc, Dynamic light scattering (DLS), Zeta Potential (ZP), Transmission Electron Microscopy and Fourier transform infrared spectroscopy (FTIR). The obtained results from Nanotrac and TEM revealed that the synthesized AgNPs possess spherical shape with a mean diameter at 94 nm for green and 104 nm for chemical method, the zeta potential values was −12.02 mV for green AgNPs and −10.4 mV for chemical AgNPs. In addition, FT-IR measurement analysis was conceded out to identify the Ag^+ ions reduced from the specific functional groups on the AgNPs, which increased the stability of the particles. Further, we compared the toxicities of green and chemical AgNPs against human skin dermal fibroblast (HDFa) and brine shrimp followed by anticancer activity in NCI-H460 cells. We observed green AgNPs cause dose-dependent decrease in cell viability and increase in reactive oxygen species (ROS) generation. Further, we proved to exhibit excellent cytotoxic effect and induction of cellular apoptosis in NCI-H460 cells. Furthermore, green AgNPs had no significant changes in cell viability, ROS production and apoptotic changes in HDFa cells. In contrary, we observed that the chemical AgNPs possess significant toxicities in HDFa cells. Hence, the green AgNPs were able to induce selective toxicity in cancer cells than the chemical AgNPs. Furthermore, green AgNPs exhibit less toxic effects against human red blood cells and brine shrimp (Artemia salina) nauplii than the chemical AgNPs. It was concluded, that apart from being superior over chemical AgNPs, the green AgNPs are effective and safer to the milieu as they show less toxic effect to normal cells and can be extensively applied in biomedical sciences particularly in cancer field.

1. Introduction

Natural greeneries are the monarch and socioeconomic source of medicinal plants globally owing rich biodiversity of natural products which can be sourced for developing drugs. Recently, many such plants have been gaining much more importance due to their unique constituents and their versatile applicability in various developing fields [1]. Nanobiotechnology is presently one of the most fascinating areas of research in modern material science with structural features where natural plants and different plant markers are found in the synthesis of NPs. In general scenario, particles with a size below 100 nm are denoted to as NPs [2]. NPs of gracious metals like platinum, gold and silver are well documented to have large applications in electronics, magnetic, optical receptors and catalysts in chemical reaction [3]. One such imperative element of the noble metal is silver. It has been used since time to manage medical and pharmaceutical products and it is involved directly encountered by human systems [4].

Recently, silver and silver nanoparticles (AgNPs) are widely being applied to consumer products and medical uses [5,6]. They are quickly gaining popularity for their multitude of uses. There are many methods available for the synthesis of AgNPs such as chemical [7], electrochemical [8], UV radiation [9], ultrasonic fields, photochemical methods [10], aerosol technologies and biological techniques [11]. As chemical synthesis is a well-known

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approach to the creation of AgNPs; however, it requires the use of toxic chemicals as reducing and/or capping substances [12]. Most of the reported methods comprise more than one additional step, high energy consumptions, low material conversions, difficulty in purification, and unsafe chemicals [54,55]. The chemical synthesis of NPs may lead to the presence of some toxic chemical species adsorbed on that may have adverse effects in its application [13]. In addition, the increased applications, human exposure to AgNPs has been increased. It has been reported that AgNPs are translocated into blood circulation and accumulated in some organs to cause hepatotoxicity or renal toxicity when administered through oral, inhalation or subcutaneously [14,15]. Due to the obvious disadvantages of the chemical reduction method, there is a need to design an inexpensive, biocompatible and environmentally useful route for synthesis of AgNPs in order to meet its growing demand in diverse sectors. Biological processes with the use of economic, efficient and eco-friendly accelerator are gaining much importance due to the benefits associated with their use. The major advantage of green synthesis of nanomaterials is their important role in protecting the environment and also the synthesized particles are stable [16]. Studies have shown that plant leaf extract of onion [17], Banana peel [18], *Cinnamomum camphora* [19], *Emblica officinalis* [20], *Aloe vera* [21], *Alfalfa* roots [22] have already been used as a reducing agents for the synthesis of metal nanoparticles. Previous studies also reports successful synthesis of AgNPs through a green route where the reducing and capping agent selected was the latex obtained from *Jatropha curcas*, *Crataegus douglasii* fruit extract as well as various other plant extracts as reducing agent [23–25]. Here, we have developed a rapid, eco-friendly and expedient method for the synthesis of AgNPs from silver nitrate using leaf extracts of Indian medicinal plant, namely, *Azadirachta indica* (neem). In this study, the plant mediated synthesized AgNPs were characterized and evaluated for their toxicity studies against human skin dermal fibroblast (HDFa) and brine shrimp toxicity along with anticancer activity in NCI-H460 lung cancer cells. Comparative studies were also performed between the toxicities of the biosynthesized and chemical synthesized AgNPs.

2. Materials and methods

2.1. Chemicals

Silver nitrate ≥99%, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), 2–7′ dichlorodihydrofluorescein diacetate (DCFH-DA), ethidium bromide (Et.Br), acridine orange (AO), RPMI-1640 medium, trypsin/EDTA solution, fetal bovine serum, glutamine-penicillin-streptomycin solution were purchased from Sigma Chemicals Co., St. Louis, USA. LSGS kit (fetal bovine serum 2% v/v, hydrocortisone 1 μg/mL, human epidermal growth factor 10 μg/mL, heparin 10 μg/mL and antibiotics) (Catalogue No: S-003-10) and medium 106 (Catalogue No: M-106-500), were procured from Cascade Biologics, Invitrogen cell culture, USA. All other analytical solvents, deionized water and sodium citrate were procured from Fisher Scientific, India.

2.2. Preparation of plant extract

Fresh leaves of *Azadirachta indica* (neem) were collected and authenticated from the Regional Medical Research Center (ICMR) with specimen No: RMRC-1255, Belagavi, Karnataka and India. For the purpose of chosen *Azadirachta indica* (Neem) is a good Indian medicinal plant, had well proved anti-oxidant, anti cancer, anti microbial, antifungal and etc [52]. Because of that plant leaves contains the active constituents includes alkaloids, flavanoids, carbohydrates and steroids etc [53]. The leaves were washed thoroughly with distilled water and air dried. The dried leaves were crushed to get the coarse powder. 150 g of dried powder was boiled in 1L Erlenmeyer flask with 1L of demineralized water for 20 min and were filtered with filter paper, centrifuged at 10,000 rpm for 20 min to the removal of particulate matter and to get clear solutions which were then refrigerated (4 °C) in 250 mL Erlenmeyer flasks for further experiments.

2.3. Preparation of silver nanoparticles (AgNPs)

The AgNPs were prepared by green synthesis with slight modifications [26]. 50 mL of 2 mM silver nitrate ($AgNO_3$) solution was prepared and added drop wise in to the 1000 mL flask containing 500 mL of *Azadirachta indica* leaves extract on the magnetic stirrer at 40 °C with 200 rpm for 30 min. Then the whole flask was wrapped with aluminum foil and kept in dark place for 24 h. Colour change was observed after 24 h which turns yellowish brown to thick brown colour, indicating that AgNPs were formed from silver ions. The AgNPs were separated by centrifugation at 4 °C with 14,000 rpm (round per minute) using (Kubota 6500, Tokyo) high speed refrigerated centrifuge, and finally spectra was checked by UV–vis spectroscopy. By chemical method the AgNPs were prepared using sodium citrate as reducing agent and stabilizer. 500 mL solution of 2 mM $AgNO_3$ was prepared in demineralized water and heated until it started to boil. 50 mL of 1% sodium citrate solution was added dropwise to the $AgNO_3$ solution as soon as boiling started. The boiling was continued till the colour of the solution slowly changes from colourless to grayish green colour indicating that reduction of the silver ions to AgNPs has occurred. Then a spectrum was checked by UV–vis spectroscopy.

2.4. Characterization of silver nanoparticles

The optical properties of AgNPs were determined by UV–vis spectrophotometer (Sican 2301, Germany). After formation of AgNPs, the spectras were taken between 350–500 nm at in different time intervals up to 48 h. Nanotrac was used to measure the average size and size distribution of the AgNPs, Dynamic Light Scattering (DLS) Nano Zetasizer (Malvern Instruments Ltd, UK) was used to measure the zeta potential of the AgNPs. The morphology of the AgNPs was characterized by the Transmission Electron Microscopy (TEM, Philips model CM 200, Tokyo, Japan), the analysis samples were prepared and dropped on to the carbon-coated copper grid, after drying the copper coated grid subjected TEM analysis. Fourier Transform Infrared Spectroscopy (FTIR) analysis was studied by using FTIR Spectrometer (Shimadzu-0815, Japan). The dried powders were carried out in the range 4000–400 cm^{-1} using KBr pellet method.

2.5. In vitro *cell viability and cytotoxicity assays*

2.5.1. Cell culture

The study carried out on NCI-H460 non-small cell lung cancer (NSCLC) cell line and human skin dermal fibroblast cells (HDFa). Lung cancer cells were obtained from National Centre for Cell Science, Pune, India and cultured as monolayer in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), penicillin and streptomycin. Human skin dermal fibroblast cells obtained from Invitrogen, Bioservices, USA and cells were cultured as monolayer in medium 106, supplemented with LSSG kit in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. Cells were grown in 75 cm^2 tissue culture flasks and used for experiments when in exponential growth phase.

2.5.2. Evaluation of cytotoxicity assay

The growth inhibitory activity of cells was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)