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Phytofabrication of Silver nanoparticles: Novel Drug to overcome hepatocellular ailments

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ABSTRACT

This study aimed to treat hepatocellular ailments with biologically prepared silver nanoparticle (AgNPs). AgNPs were formulated using *Morus alba* leaf extract and their synthesis and characterization were determined by UV–visible spectroscopy, Transmission Electron Microscope (TEM), Scanning Electron microscope (SEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR) and Zeta analysis. *In vitro* studies on HepG2 cell lines for cytotoxic effect and *in vivo* studies in a rat model for hepatoprotective effect were carried out using biologically prepared AgNPs as curing agents. Dose response cytotoxicity on hepatic cancer (HepG2) cells was confirmed by 3-(4, 5-dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium (MTT) assay. The inhibitory concentrations (IC_{50}) were found to be 20 µg/mL and 80 µg/mL for AgNPs and *M. alba* leaf extract respectively against HepG2 cells at 24 h incubation. In addition, hepatotoxicity in Wistar rats (180 ± 10 g) was induced by intraperitoneal injection of *N*-nitrosodiethylamine (NDEA) and were treated with different doses of AgNPs (25, 50, 100 µg/kg). NDEA administration showed a significant rise in the biochemical parameters whereas the levels of enzymic antioxidants were decreased. Obtained results revealed that the elevated levels of Liver Function Test (LFTs) biomarkers were significantly reversed and the antioxidant levels were significantly recouped towards normal after the conjoint treatment of AgNPs in a dose-dependent manner. Thus green synthesized AgNPs showed a promising curing effect on hepatocellular ailments.

1. Introduction

Nanotechnology has played a crucial role and is the most proficient technology that can be functional in the fields of pharmaceutical, healthcare, biomedical and drug delivery [1,2]. Nanoscale particles are developed for use in drug delivery because they increase the drug dissolution rate, leading to enhanced drug absorption and bioavailability [3–5]. Silver nanoparticles possess electrical, optical as well as biological properties and are thus applied in biosensing, imaging, drug delivery, nanodevice fabrication and in medicine [6]. Various physicochemical properties such as low solubility or high lipophilicity were the foremost inevitable problems faced by the pharmaceutical industry in the development of a pharmacologically active substance with relevant activity and minimum toxicity [7]. Hence, various techniques are used for the improvement of the solubility of poorly soluble drugs. The metallic nanoparticles are used as valuable tools to enhance the effectiveness of the current therapies and to increase the compliances of the patient to the treatments. Thus finding a proper method to obtain a nanoparticle with a reduced number of inconveniences related to the physio-chemical properties of the nanoparticles, in terms of stability, biocompatibility, the proper size, and shape for biochemical uses, represented a real challenge for the researchers [8]. Transport of nanoparticles through the extracellular matrix (ECM) is complicated due to its mesh-like organization and the particles with a size larger than the network space are rejected by ECM, while smaller particles are able to pass through the matrix barrier [9,10].

Synthesis of AgNPs with the help of biological agents is more eco-friendly, cost-effective and highly focused research area compared to other chemical and physical methods due to reduced use of hazardous reagents and solvents, improved material and energy efficacy from the chemical process and enhanced design of nontoxic products [11,12]. The uses of plant extract for nanoparticles synthesis proved to be advantageous than microbiological processes, because pathogenic bacteria may contaminate the nanoparticles when used in biomedical fields

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[13].

Green synthesized silver nanoparticles are essential in medical applications because of appealing features such as the use of natural resources, rapidness, eco-friendliness, and benignancy. Such nanoparticles are devoid of contaminants and the process is easy to scale-up [14]. It is one of the best methods for the production of nanoparticles because it involves the plant extract as capping and reducing agent due to their reducing properties [15]. Various plant extracts *viz.*, *Ficus religiosa*, *Gymnema sylvestre, Embilica Officinalis, Moringa oleifera, Phyllanthus emblica, Melia azedarach, Annona squamosa, Andrographis paniculata, Cinnamon zeylanicum* were reported in literature with ability to develop nanoparticles as nano drug to treat medical implication [16–21] Aqueous leaf extract of *Azadirachta indica* has been found to be suitable reducing and caping agent for the synthesis of silver nanoparticles [22]. In this regard, leaf extract of *Morus alba* (mulberry) a species of family Moraceae, was used for fabrication of silver ion to nanoparticles. It is a deciduous tree that is widely distributed in Asia. All parts of this tree such as leaves, fruits, and roots have been used in traditional medicine [23]. *Morus alba* leaf contains triterpenes (lupeol) Sterols (β-Sitosterol), bioflavonoids (rutin, moracetin, quercetin-3-triglucoside and isoquercitrin), coumarins, volatile oil, alkaloids, amino acids and organic acids [24].

Hepatocarcinogenesis is one of the most common malignancies worldwide and the fourth most common causes of cancer mortality in Asia [25]. The conventional therapy for liver cancer including chemotherapy, radiation, surgical resection, and ablation gives little hope for the restoration of health because of poor diagnosis and serious side effects. Many challenges remain in treating cancer patients, including treatment-related adverse effects, poor outcomes, lack of a therapeutic target and balancing treatment toxicity with the quality of life in patients with metastatic cancer who have already received extensive therapy. Therefore, there is still an urgent need for new therapeutic options for cancer [26]. *N*-nitrosodiethylamine (NDEA), N-nitroso alkyl compound, is a potent hepatotoxin present in wide variety of foods such as cheese, soybean, smoked, salted and dried fish, cured meat and alcoholic beverages they are formed by the reaction of amines and amides with nitrosating agents derived from nitrite [27,28]. It is presumptive that NOCs formed endogenously in the stomach or intestines after consumption of nitrite-preserved foods like processed meat or fish and are absorbed into the bloodstream and reach the brain [29].

NDEA induced hepatocellular carcinoma (HCC) is considered as one of the most accepted and widely used experimental models to study hepatocarcinogenesis in rats [30]. NDEA metabolism in the liver by cytochrome isoform 2E1 (CYP 2E1) generates reactive oxygen species (ROS) causing oxidative stress [31] and oxidative damage leading to cytotoxicity, carcinogenicity, and mutagenicity [32].

A lot of work has been done on medicinal implications of AgNPs, but to the best of our knowledge, this is the first ever piece of work considering the two aspects. The first aspect is with regard to synthesis of AgNPs using *M. alba* leaf extract. The second important aspect was to evaluate the therapeutic efficiency of biosynthesized AgNPs against NDEA induced hepatocellular ailments in a rat model and reflect their cytotoxicity against *in vitro* HepG2 cells.

2. Materials and methods

2.1. Chemicals and reagents

N-Nitrosodiethylamine (PubChem CID: 5921), Silver nitrate (PubChem CID: 24470), Silymarin (PubChem CID: 5213), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), glutamine (PubChem CID: 5961), penicillin and streptomycin (PubChem CID: 86591708), were purchased from Sigma-Aldrich Chemical Company (USA). Trypan blue (PubChem CID: 9562061) and MTT [3-(4, 5- dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium] dye (PubChem CID: 64965) and 5-fluorouracil (5-FU) (PubChem CID: 3385) were obtained from Himedia laboratories (Mumbai, MH, India). Rest of the other chemicals, as well as solvents utilized, were of high purity in addition to analytical class sold by, E- Merck (Germany), Ranbaxy Pvt. Ltd. Company (India).

2.2. Preparation of aqueous leaf extract

Leaves of mulberry were collected from the maintained plants at CSR & TI, Central Silk Board, Pampore, Jammu and Kashmir, India, were identified by Dr. A.K. Jain, Director Institute of Ethnobiology, Jiwaji University Gwalior and were stored in same Institute under Voucher specimen No. 326. Leaves were rinsed thoroughly with tap water followed by distilled water to remove all the dust and unwanted visible particles. The leaves were shade dried at room temperature for 2–3 weeks and then powdered. About 10 g of leaf powder was added to 100 ml of sterile distilled water in a 250-ml Erlenmeyer flask and this mixture was boiled in a water bath at 60 °C for 1–2 h. After cooling to room temperature, the mixture was filtered by using muslin filter cloth. The filtrate was further filtered through 0.6 µm sized filters and then stored in an airtight container at +4 °C for further experiments.

2.3. Fabrication of AgNPs

An aqueous solution (1 mM) of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of AgNPs. The preparation of AgNPs was carried out by adding 5 ml of aqueous leaf extract to 95 ml of $AgNO_3$ solution in 250 ml Erlenmeyer flasks and kept in a rotary shaker (Remi elektrotechnik limited) at a different temperature such as 30 °C, 60 °C, 90 °C and 95 °C. The reaction mixture was monitored spectrophotometrically after incubation of 60 min. Reduction of silver nanoparticles was observed by a color change in the reaction mixture during temperature treatments. Complete reduction of $AgNO_3$ to Ag^+ ions and their restoration to metallic silver (nanoparticles) was confirmed by the change in color from light yellow to colloidal brown. The reactions were carried out in dark to avoid photoactivation of $AgNO_3$. After irradiation, the colloidal mixture was purified by centrifugation at 10,000 rpm for 10 min at 4 °C followed by redispersion of the pellet in Milli-Q water and dried in a vacuum distillation; powder form of fabricated AgNPs was weighed, sealed and stored in the dark at 4 °C [33].

2.4. Characterization of AgNPs

The alteration of color from yellowish to dark brown in the colloidal mixture was measured as an initial remark for the breakdown of silver nitrate. Suspension of samples (1 ml) were collected at regular increasing temperature to observe the completion of bioreduction of Ag^+ in aqueous solution, followed by dilution of the samples with 2 ml of deionized water and subsequently scanned in UV–vis spectra, flanked by 200–800 nm wavelengths by spectrophotometer (UV 3000$^+$ LABINDIA), having a resolution of 0.5 nm. The presence of functional biomolecules in *M. alba* mediated AgNPs were confirmed by FT-IR spectrometry (Perkin-Elmer, AD-6 Waltham, MA, USA). Transmission Electron Microscopy (FEI's Tecnai™ G2) was used to envisage the morphology and size of the AgNPs. Grids of TEM were organized by putting a 5 µl of the AgNP solutions on carbon-coated copper grids as well as dried beneath the lamp. The surface analysis of the synthesized AgNPs was studied using scanning electron microscope (SEM). The SEM images were recorded (Zeiss EVO 18, Germany) at 40,000 × magnifications operating with 20.00 kV. The crystal lattice of the synthesized NPs was determined by XRD measurements using an XRD-6000 X-ray diffractometer (Shimadzu, Kyoto, Japan). To study the zeta potential stability of the nanoparticles Malvern Zetasizer Nano series compact scattering spectrometer (Nano ZS 90, Malvern Instruments Ltd., Malvern U.K.) was used.