Green-Synthesized Silver Nanoparticles and the Effect on Human Neuroblastoma Cells

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***Abstract*— Neuroblastoma is a complex disease that shares some cellular traits with neurons involved in neurodegenerative diseases like Alzheimer's and Parkinson's diseased. The human neuroblastoma SH-SY5Y cell line is commonly used to model these conditions, providing a useful platform for exploring potential treatments. Silver nanoparticles (AgNPs) have gained attention as promising candidates due to their distinctive properties, although there are ongoing concerns regarding the toxicity and environmental effects linked to traditional synthesis methods. This study investigated the green synthesis of AgNPs using *Aloe vera* extract and assessed their effects on the viability of human neuroblastoma SH-SY5Y cells. Various techniques were employed to characterize the synthesized AgNPs, confirming their successful formation and stability. The MTT assay demonstrated a dose-dependent cytotoxic effect of AgNPs on SH-SY5Y cells, with lower concentrations showing acceptable biocompatibility. This research highlights the potential of green synthesized AgNPs as a viable therapeutic option for neuroblastoma, while also emphasizing the importance of careful concentration management. Moreover, this study aims to add to this expanding field by concentrating on the green synthesis of AgNPs using Aloe vera and assessing their therapeutic potential in relation to neurodegenerative diseases.**

Keywords— Green synthesis, Silver nanoparticles, Neuroblastoma, SH-SY5Y cell line, Biocompatibility, Cytotoxicity

INTRODUCTION

Over the past two decades, nanotechnology has been recognized as one of most powerful tools in scientific research and its application [1]. An increasing focus on the application of nanomaterials in medical fields has spurred research efforts across a wide range of nanoparticle classes, bimetallic or alloy, metal oxide, metal also magnetic nanoparticles [2]. This is the phenomenon of so-called quantum effects, which are features that occur not in bulk materials but at atomic and molecular scales; silver nanoparticles (AgNPs) has been amongst the most extensively studied cases with respect to such unique physicochemical properties seen primarily by them [3]. Partly on account of their high surface-to-volume ratio, these properties have led to the potential broad spectrum applications areas reflected in traditional health care use as antimicrobial agents, cosmetics and food spoilage prevention application textiles through more contemporary attention into electronics, magnetics catalysis, and optics [4]. Recently, nanotechnology has become a revolutionary impact on several scientific fields as well as biomedicine sector experiences maintain/growing applications of the next generation health technologies [5].

This convergence of fields has developed into the dynamic arena of nanobiotechnology, where nanoparticles are important components in diagnostics therapeutics including drug delivery systems [6]. The increasing trend in searching for nanomaterials designed specifically for biomedical applications has prompted advancements across nanoparticle classifications ranging from metallic, to bimetallic or alloy nanoparticles and metal oxides down through magnetic variants [2].

AgNPs are gaining notice in the realm of biochemistry because they exhibit property profiles at a cellular, atomic and molecular scale that differ from bulk materials [2]. This characteristic, in which they are frequently called into question for their high surface-to-volume ratio stimulates them to be utilized within an extensive range of investigations; starting from traditional applications antimicrobial agents in healthcare cosmetics, food storage [4], and to more recent investigations in electronics, magnetics, catalysis, and optics [2, 4].

Various methods have been applied for production of AgNPs such as physicochemical and biological ones [4]. While it's true that chemical reduction methods using sodium citrate or sodium borohydrate are widely used due to their ease of use and low cost, issues related essentially to environmental aspects and residual chemicals toxicity lead research towards more sustainable alternatives [4, 7].

Recently, green synthesis approaches using plant extracts as natural reducing agents are receiving special attention for AgNP production. There are several reports available on the use of neem [7], *Aloe vera*, Phyllanthus, turmeric, seaweeds, coffee and tea to act as reducing agents for synthesis of AgNP. The benefits of these environmentally friendly methods over the conventional physical and chemical approaches lead to low ecological damage, being cost-effective, the ability of the use of natural capping agents stabilizing nanoparticles [8 – 10].

Nevertheless, the introduction of silver nanoparticles into biological systems for healthcare-related applications requires attention in relation to biocompatibility and possible toxicity [11]. Williams introduced the new term biocompatibility in 2008 as: "biocompatibility as the ability of a biomaterial to perform its desired function without eliciting undesirable effects, while generating the most appropriate beneficial cellular or tissue response" [11]. This highlights a key definition of success, whereby the AgNPs are achieved and work therapeutically as required (e.g., targeted drug delivery or photoablation but with no detrimental effect. In contrast,

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nanoparticle toxicity means the tendency of these particles to impair normal physiology and cause structural perturbations in organs or tissues. Nanoparticles are able to be manipulated in terms of morphology, shape, particle size, surface charge, chemistry also composition in addition to providing colloidal stability, which could influence the bio-interactions with cells or tissues including cell transport across biological barrier such as cellular membrane thus allowing them migrate along nerve tracks and deposition at target sites is firmly established concept called dose response toxicity [11].

Although the antimicrobial performance of AgNPs has been investigated, less is known with respect to their overall biocompatibility [4], specifically in terms of neuronal well-being. While existing data report the antioxidant properties of these nanoparticles, there is a paucity regarding their effect on mammalian cells. However, despite the mounting relevance of green synthesized silver nanoparticles for several biomedical applications, their utility in combating neurodegeneration has not been fully explored. The SH-SY5Y cell line is widely used in neuroblastoma researches and other neurological disorders including Parkinson's disease (PD) and Alzheimer's disease (AD) provides the unique opportunity to investigate whether our nanoparticles may have therapeutic benefits. Although green synthesized nanoparticles have taken a boom in other areas but there application effect on SH-SY5Y cell line and its ability to modulate disease pathways, especially those associated with these multifactorial neurological disorders need further insight.

The present study was aimed to fill this gap by evaluating the cytocompatibilities and possible effects of *Aloe vera* green-synthesized AgNPs on SH-SY5Y human neuroblastoma cell line. Due to the fact that *Aloe vera* has an inherent antimicrobial properties and gelation property, they are considered a suitable source for biocompatible AgNPs production [12]. This study is designed to report the comprehensive safety and application of these green synthesized AgNPs on biomedical aspects.

METHODS

Synthesis of AgNPs by using aloe vera extract and characterization

Twenty-five grams of Aloe vera were weighed, crushed, and combined with 100 mL of distilled water dH2O. The mixture was heated at 90°C for 45 minutes, then incubated at room temperature overnight. After filtering with filter paper, a 25% Aloe vera extract solution was obtained. This solution was further diluted with distilled water to produce a 5% *Aloe vera* extract. 0.1 M silver nitrate solution was prepared by dissolving 1.7 grams of silver nitrate in 100 mL of distilled water (dH_2O) within a glass beaker. The resulting solution was then transferred to a dark bottle for storage at room temperature. 10 mL of 5% aloe vera extract were combined with 10 mL of 1M silver nitrate and heated at 100°C for 15 minutes. The solution's colour transitioned from transparent to yellow-brown, indicating the formation of silver nanoparticles (AgNPs). The solution was cooled to room temperature and was subjected to ultrasonic agitation for 10 minutes and then stored in a dark bottle for five days. On the sixth day, the solution was centrifuged at 500 rpm for 10 minutes. The supernatant was discarded, and the pellet was resuspended in autoclaved distilled water and stored at 4°C. Prior to use, the AgNPs were dried in an oven at 60°C, weighed, and then dissolved in distilled water to prepare the desired concentration.

The synthesized nanoparticles were characterized using the confirmative tools such as UV-Vis spectroscopy, FTIR to know about molecular structure and analyze interaction of functional groups with aloe vera components. Zetasize was used for charge and stability analysis, DLS study provided hydrodynamic diameter measurements while SEM and EDX was used to determine the size of the shape of the AgNPs.

Cytotoxicity Analysis

SH-SY5Y cells were grown on polystyrene cell culture plates in Dulbecco's Modified Eagle Medium/Hams Nutrient Mixture F12 (DMEM/F12; 1:1) (Sigma-Aldrich, Germany) supplemented with 10% fetal bovine serum (FBS) and 100 g/mL penicillin/streptomycin (Biological Industries, USA) (Sigma-Aldrich, Germany). The plates were incubated at 37°C with 5% CO2 for two days before the medium was replaced. The adherent cells were treated in 0.05 percent Trypsin-EDTA (Sigma-Aldrich, Germany) for 5 minutes at 37°C before being collected by centrifugation at 2.500xg for 5 minutes.

Following the cell counting and cell seeding SH-SY5Y cells were seeded in a 96-well plate with a density of 10,000 cells/well and incubated for 24 hours at 37°C with 5% CO_2. On the following day 50 µl of AgNPs at concentrations of 50, 25, 10, 5, 2.5,1.25 µg/µl were prepared and subsequently administered to the selected wells. Control wells were treated with 50 µl dH_2O in place of AgNPs, while negative control wells were treated with 50 µl of 10% DMSO. The plate was then incubated overnight at 37°C with 5% CO_2. As a starting point 10µl of MTT solution (5mg/ml) (Sigma Aldrich, USA) was added to all wells, and incubated for 2 hours at 37°C with 5% CO_2. Thereafter, all solutions were carefully aspirated, and 100 µL of DMSO was added to the well. The plate was then incubated for 15 minutes at 37°C on a shaker. Finally, the absorbance was read in a microplate reader (Thermo Scientific, Multiscan Go) at 570nm.

Frequency distribution for descriptive statistics, arithmetic mean and standard deviation for continuous variables were calculated by using Student's t-test. For all studies, significance value (p), which was *$p<0.05$, obtained using a paired two-tailed Student's t- test. Data are expressed as mean ± standard error.

RESULTS AND DISCUSSION

AgNPs were successfully synthesized multiple times using the green synthesis method from the *Aloe vera* plant, and silver nanoparticle solution has been observed to change it color

from transparent to brown (Figure 1a and 1b). The color shift confirmed the creation of AgNP.

The solution containing biosynthesized silver nanoparticles was analyzed by UV–visible spectrophotometry between 300 and 900 nm. The observed absorbance peak at 420 nm is consistent with the characteristic absorption range for green synthesized silver nanoparticles (Figure 1c).

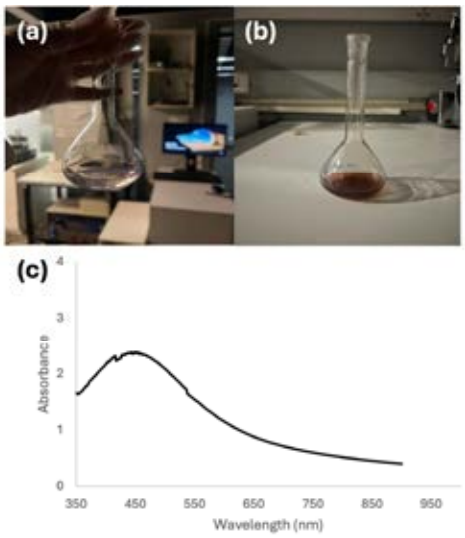


Figure 1. (a) Initial $AgNO_3$ and *Aloe vera* extract mixture (light yellow); (b) Final AgNP solution (brownish color); (c) UV-Vis absorbance spectra of AgNPs shows a peak at 420nm.

FTIR spectroscopy was employed to investigate the functional groups involved during the synthesis and stabilization of AgNPs using *Aloe vera* extract. The FT-IR spectra of green synthesized AgNPs revealed typical peaks at 3297.25 cm^{-1} (O-H stretching) and 1629.97 cm^{-1} (C=O stretching), as well as peaks in the area between 2275.65 and 2347.39 cm^{-1} (similar to C≡N or C≡C stretching) and at 1509 cm^{-1} (possible N-H bending). The 5% aloe vera extract solution exhibited peaks at 3297.25 cm^{-1} (O-H stretching), 2146.52 cm^{-1}, and 1633.71 cm^{-1} (C=O stretching). Overall, the FTIR study confirms the interaction between biomolecules in *Aloe vera* extract with synthesized AgNPs (Figure 2a).

The hydrodynamic diameter of the AgNPs, dispersed in *Aloe vera* was measured using DLS at 25 °C. The Z-Average mean diameter was determined to be 493.3 ± 3.4 nm, with a polydispersity index (PdI) of 0.240. The size distribution by intensity revealed a major peak at 571.4 ± 73.1 nm, while the size distribution by number indicated the presence of smaller particles (<136.5 nm) constituting approximately 25% of the total population and larger particles accounting for around 75% (Figure 2b). The zeta potential measurement for the AgNPs were measured at 25°C and it was determined to be -32.74 mV. The negative zeta potential value indicates that the AgNPs have a net negative surface charge.

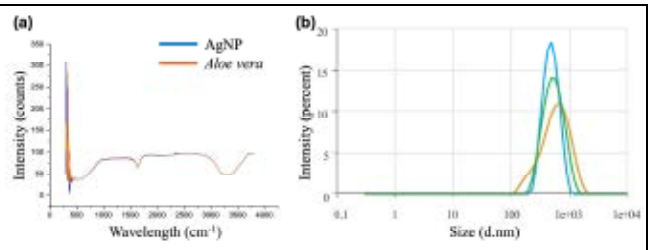


Figure 2. (a) FT-IR spectra of AgNP synthesized by using *Aloe vera* extract; (b) DLS analysis of AgNP.

SEM imaging was utilized to investigate the morphology, shape and size of the AgNPs (Figure 3a). EDX analysis revealed the presence of silver (Ag) in the produced nanoparticles at a weight percentage of 59.0%. Figure 3b demonstrates the EDX spectrum and the other components observed were carbon (C), nitrogen (N), oxygen (O), sodium (Na), which presumably derived from the *Aloe vera* extract and the synthesis process. Silicon (Si) peak is the result of the substrate to analyze AgNP with SEM.

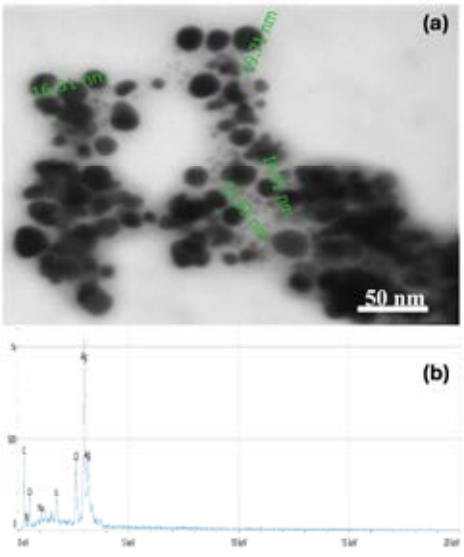


Figure 3. (a) SEM image of synthesized AgNPs; (b) EDX spectrum of AgNPs, confirming the presence of carbon (C), nitrogen (N), oxygen (O), sodium (Na), silicon (Si), and silver (Ag)

To analyze the cytocompatibility of green synthesized AgNPs, SH-SY5Y cells were assessed using the MTT assay and it was demonstrated that there is a concentration-dependent decrease in SH-SY5Y cell viability after 24 hours of exposure to AgNPs (Figure 4). Lower concentration (1.25, 2.50, 5 µg/µl) had minor effects, while 10 µg/µl and 25 µg/µl showed a considerable reduction in viability, with AgNP 50 µg/µl having the most marked cytotoxic impact. To calculate the proportion of viable cells, each sample and control group's vitality was compared to the positive control using the following formula:

Cell Viability (%) = (OD of sample / OD Control) × 100 %

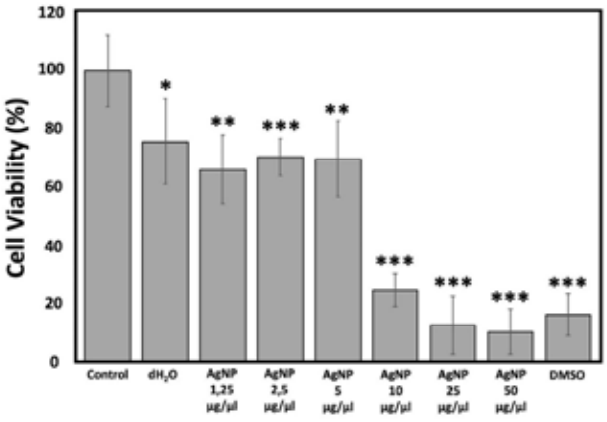


Figure 4. MTT analysis reveals AgNPs impact on SH-SY5Y cells viability (*p<0.05, **p<0.01, ***p<0.001).

CONCLUSION

In conclusion, this study showed the green synthesis of silver nanoparticles (AgNPs) using *Aloe vera* extract, providing a sustainable and environmentally friendly alternative to traditional approaches. The synthetic AgNPs were thoroughly studied, revealing their physicochemical features such as size distribution, shape, surface charge, and elemental composition. The presence of biomolecules from the *Aloe vera* extract on the nanoparticle surface, as demonstrated by FTIR and EDX tests, shows that they play a role in the AgNPs' stability and possible biocompatibility. The MTT experiment findings revealed the AgNPs' concentration-dependent cytotoxicity, with good biocompatibility reported at lower doses. This result emphasizes the significance of precise concentration considerations while investigating the possible biological uses of these nanoparticles.

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