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Silver nanoparticles from leaf extract of *Mentha piperita*: Eco-friendly synthesis and effect on acetylcholinesterase activity

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ABSTRACT

Silver nanoparticles (AgNPs) have been used in various medicinal and commercial products because of their exceptional anti-microbial and anti-odor properties. On the other hand, increased commercialization of AgNPs containing products has led to its release into the environment. Thus, studies are needed to assess their impact on the environment as well as on human body. Several reports have shown that AgNPs could cause some serious neurotoxic effects. Most of these studies have been performed using chemically synthesized AgNPs. In contrast, green nanoparticles are usually considered safer than their chemically synthesized counterparts. Accordingly, in this research work, we have assessed the effect of AgNPs synthesized from aqueous-leaf-extract of *Mentha piperita* on one of the most important neurological enzymes i.e. acetylcholinesterase (AChE) to predict its neurotoxicity. *M. piperita* synthesized AgNPs were subjected to characterization by UV–visible-spectrometry, Scanning Electron-Microscopy as well as Transmission Electron-Microscopy. Here, the size of the AgNPs was found to be 35 nm with spherical shape. These AgNPs showed concentration-dependent inhibitory-effect on the AChE enzyme-activity displaying an IC_{50} of 150 nM. Further, kinetic analysis showed mixed type of inhibition, which means that AgNPs were capable of binding to both the free enzyme (AChE) and to the enzyme-substrate (AChE-acetylcholine) complex. These results suggest that even green synthesized AgNPs might cause neurotoxicity via inhibiting AChE activity. However, more studies are needed to elucidate the exact mechanism of neurotoxicity by AgNPs. Nevertheless, we could safely state that the present study provides relevant preliminary information regarding neurotoxicity of green synthesized AgNPs.

1. Introduction

The inimitable physical, chemical and biological features of nanoparticles allow their application in various interdisciplinary fields such as cosmetics, bioanalytics, optoelectronics, biomedicines and antibacterials [1–4]. However, among different nanoparticles, silver nanoparticles (AgNPs) are more widespread in industrial and consumer applications and have been quite rigorously studied [5]. There are broad ranges of medicinal as well as daily-use AgNPs containing products already available in the market like contraceptive devices,

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surgical instruments, room sprays, bone prostheses, paints, detergents, devices for water purification, etc. In addition, AgNPs are widely used in textile industries to reduce the undesirable effects of microbial growth, for instance, generation of the unpleasant odor, discoloration in the fabric and reduction of fabrics mechanical strength. On the other hand, this increased commercialization of AgNPs has led to its discharge into the environment and deeper understanding of the potential risk caused by AgNPs on the environment and biological system is urgently required [6]. It has been observed that transformation of AgNPs by surface-oxidation and Ag-ions-release increases its interactive contacts with various bio-macromolecules and influences its transport, fate and toxicity into the environment [7]. The size and concentration of AgNPs, route of AgNPs administration and the exposure time are also considered as significant influencing factors for the toxicity of AgNPs [8]. Due to the easy dissemination of AgNPs in the environment, the human body could be exposed to AgNPs through different routes. From deodorants and disinfecting sprays, AgNPs could enter in the human body through inhalation. Other routes of human exposure could be ingestion and dermal penetration. Further, these AgNPs could easily cross the blood-lung barrier or dermal barrier, enter in the circulatory system and eventually spread throughout the body. Moreover, it has been reported that nanoparticles once entered into the systemic circulation, might cross the Blood-Brain Barrier and cause neurotoxic effects. Importantly, in 2008, Tang et al. determined the ability of AgNPs to cross the Blood-Brain Barrier (BBB) after subcutaneous administration in rats. They found that AgNPs could accumulate in the brain and cause neuro-degeneration and necrosis [9]. Diminished cerebral blood-flow, oedema of the brain, neuronal-injuries, glial cell-activation, up-regulation of the 'heat-shock-proteins' and damage to myelinated-fibers are considered as some of the adverse neurotoxic effects of AgNPs [10]. As of now, neurotoxic effects of silver nanoparticles stay well-acknowledged [11].

Studies describing the toxic effect of chemically synthesized AgNPs on AChE have also been performed by researchers [12,13]. However, AgNPs synthesized by herbal extracts are generally considered safer than their chemically synthesized counterparts. In contrast, it has been reported by authors that even the silver nanoparticles which have been green-synthesized could alter the permeability of barrier-cells (e.g. cells of brain endothelium, intestine) thereby stimulating the 'oxidative-stress' alley ways in the nerve cells [14]. The aforementioned reports, prompted us to evaluate the effect of AgNPs on an important neurological enzyme i.e., acetylcholinesterase (AChE) so as to assess their possible neurotoxic effect. Accordingly, the present study is an attempt to investigate the effect of green synthesized AgNPs on the activity of AChE. Herein, we synthesized AgNPs from *Mentha piperita* leaf-extract which were duly characterized by UV–visible Spectrophotometry, SEM and TEM. Subsequently, the effect of these herbally synthesized AgNPs on AChE activity was assessed in vitro.

2. Material and methods

2.1. Preparation of plant leaf extract

We plucked farm-fresh, medium sized *Mentha piperita*-leaves that were available in the campus of IFTM-University which is located in the city of Moradabad, (UP), India. Three-times washing of these leaves was performed using distilled-water. The leaves were cut into smaller pieces that were subsequently air-dried using an air-oven at a temperature of 50 °C for 4 h. These pieces were finely ground to prepare their powder. 10 g of the aforementioned dried-powder was homogenized in 100 ml of distilled-H_2O. Leaf-extract was obtained boiling for 0.5 h on water-bath maintained at a temperature of 100 °C. The aqueous leaf extract so obtained was allowed to cool to room-temperature. Finally, the extract was filtered through Whatman no. 1 paper.

2.2. Biosynthesis of silver nanoparticles (AgNPs)

AgNPs were synthesized according to the protocol outlined by Ahmad et al. [15]. We prepared an aqueous-solution of $AgNO_3$ having a molarity of 1 mM which was subsequently utilized to synthesize the desired nanoparticles. We added 10 ml of filtered aqueous leaf-extract of *M. piperita* in 90 ml of the aforementioned $AgNO_3$-solution for reduction into Ag^0-ions. Next, this solution was incubated at room temperature for 15 min accompanied with a strong stirring by a magnetic-stirrer. We observed a fast alteration in the solution color which was indicative of initiation of AgNPs-formation. The ensuing mixture of AgNPs and extract thus obtained was maintained for 1 day accompanied with gentle stirring. In order to ensure the formation of AgNPs, we duly recovered samples at specified time-intervals. Accordingly, these samples were studied by UV–visible spectroscopic analyses. A reaction achieved without the addition of leaf extract was designated as a 'control-reaction'. Once the reaction was complete, we harvested the AgNPs employing the centrifugation technique (performed at 30,000*g* for half an hour). These AgNPs were again two—times washed in MilliQH_2O. Finally, the AgNPs were characterized by multipronged approaches e.g. UV–visible spectrometry [using Shimadzu dual-beam spectrophotometer (modelUV-160PC)]; Transmission Electron-Microscopy/(TEM) [using a Hitachi Model H-7500 (Hitachi, Japan)] which was operated at 100 kV accelerating-voltage and Scanning Electron-Microscopy/(SEM) [using JEOL JSM 6700F (JEOL, Japan)].

2.3. Determination of AgNPs-concentration

AgNPs-concentration was determined by the method reported by Liu et al. [16].

Firstly, we determined the average-number of atoms/nanoparticle employing the following relation:

$$N = \frac{\pi \rho D^3}{6M} N_A$$

Here, N represents the number of atoms/nanoparticle, $\pi = 3.14$, ρ represents the density of face centered cubic or fcc-silver ($= 10.5$ g/cm^3), D represents the average-diameter of AgNPs ($35\text{ nm} = 35 \times 10^{-7}$ cm), M represents the atomic-mass of silver (107.868 g), while N_A represents the Avogadro-number.

Hence, supposing 100% transformation of all of the Ag ions into AgNPs,

$$N = \frac{3.14 \times 10.5 \times (35.0 \times 10^{-7})^3 \times 6.023 \times 10^{23}}{6 \times 107.868}$$

i.e. N = 1315503.677.

Subsequently, we determined the molar-concentration of these nanoparticles employing the relation mentioned below:

$$C = \frac{N_T}{NVN_A}$$

Here, C represents the molar-concentration of the nanoparticle-solution, N_T represents the total number of silver-atoms added (given that $AgNO_3 = 1$ M), N represents the number of atoms/nanoparticle, V is the reaction solution-volume in litres, while N_A is the Avogadro-number i.e. 6.023×10^{23}.

$$C = \frac{1 \times 6.023 \times 10^{23}}{1315503.677 \times 1 \times 6.023 \times 10^{23}}$$

$$C = 7.60 \times 10^{-7}\text{ M/L} = 760\text{ nM}.$$

2.4. Calculating the percent-inhibition of acetylcholinesterase enzyme-activity

The percent-inhibition of acetylcholinesterase enzyme-activity was calculated employing the protocol of Ingkaninan et al. [17] with minor