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Polyvinyl polypyrrolidone attenuates genotoxicity of silver nanoparticles synthesized via green route, tested in *Lathyrus sativus* L. root bioassay

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ABSTRACT

The silver nanoparticles (AgNPs) were synthesized extracellularly from silver nitrate ($AgNO_3$) using kernel extract from ripe mango *Mengifera indica* L. under four different reaction conditions of the synthesis media such as the (*i*) absence of the reducing agent, trisodium citrate (AgNPI), (*ii*) presence of the reducing agent (AgNPII), (*iii*) presence of the cleansing agent, polyvinyl polypyrrolidone, PVPP (AgNPIII), and (iv) presence of the capping agent, polyvinyl pyrrolidone, PVP (AgNPIV). The synthesis of the AgNPs was monitored by UV–vis spectrophotometry. The AgNPs were characterised by the energy-dispersive X-ray spectroscopy, transmission electron microscopy, X-ray diffraction, and small-angle X-ray scattering. Functional groups on the AgNPs were established by the Fourier transform infrared spectroscopy. The AgNPs (AgNPI, AgNPII, AgNPIII and AgNPIV) were spherical in shape with the diameters and size distribution-widths of 14.0 ± 5.4, 19.2 ± 6.6, 18.8 ± 6.6 and 44.6 ± 13.2 nm, respectively. Genotoxicity of the AgNPs at concentrations ranging from 1 to 100 mg L^{-1} was determined by the *Lathyrus sativus* L. root bioassay and several endpoint assays including the generation of reactive oxygen species and cell death, lipid peroxidation, mitotic index, chromosome aberrations (CA), micronucleus formation (MN), and DNA damage as determined by the Comet assay. The dose-dependent induction of genotoxicity of the silver ion (Ag^+) and AgNPs was in the order Ag^+ > AgNPII > AgNPI > AgNPIV > AgNPIII that corresponded with their relative potencies of induction of DNA damage and oxidative stress. Furthermore, the findings underscored the CA and MN endpoint-based genotoxicity assay which demonstrated the genotoxicity of AgNPs at concentrations ($\leq$10 mg L^{-1}) lower than that ($\geq$10 mg L^{-1}) tested in the Comet assay. This study demonstrated the protective action of PVPP against the genotoxicity of AgNPIII which was independent of the size of the AgNPs in the *L. sativus* L. root bioassay system.

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1. Introduction

Nano-biotechnology is a rapidly evolving discipline of modern biology that focuses on the chemical, electronic, optical, and magnetic properties of the nanoparticles (NPs) with immense potential for application in nanomedicine, biosensing, and catalysis in addition to their use in plasmonics and dye-based solar cells [1]. Increased production, extensive usage, and disposal of the NPs pose environmental concerns owing to the fact that the NPs are non-degradable and their adverse effects on non-human biota are not thoroughly established [2,3]. A thorough understanding of the ecotoxicity and genotoxicity of the NPs is essential for their safety evaluation and risk assessment [4–6]. Silver nanoparticles (AgNPs) are among the most commonly encountered NPs in consumer and medical products including the deodorants, clothing materials, bandages, cleaning solutions, and sprays [7]. The AgNPs

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are known to exhibit antimicrobial and anticancer actions and thus offer pharmacological uses [8–10]. Although the genotoxicity of AgNPs has not been established unequivocally to date, it is reported that they are toxic only at high doses or after prolonged exposure in several mammalian *in vivo* and *in vitro* assays [11].

Certain bacteria, fungi and plants biosynthesize metallic NPs including the AgNPs as a strategy of adaptive mechanism(s) to cope with metal toxicity [12]. Those mechanisms may involve the alteration of the chemical nature of the toxic metal to a less toxic or non-toxic form and result in the formation of NPs of the metal. Thus, the formation of NPs is the metabolic outcome of resistance mechanism against a toxic metal, and this can be used as an alternative green route (green-synthesis) to synthesize the metallic NPs that are expected to be less toxic or non-toxic. Cost-effective synthesis of NPs is the primary step towards addressing the needs or applications of nano-biotechnology. Plant extracts have the potential to develop simple, cost-effective, and alternate processes for large-scale biosynthesis and production of the NPs [13,14]. Green-synthesis of the NPs offers many advantages such as the (*i*) minimizing or reducing the generation of large quantities of hazardous wastes from chemical synthesis of the NPs, (*ii*) easy accessibility/availability of plants that contain numerous secondary metabolites that mediate and participate in green-synthesis of the NPs, and (*iii*) the green NPs are expected to be ecofriendly and, biocompatible [15,16]. The basic characteristics of the NPs including stability, size, shape, composition, aggregation, and crystalline nature determine toxicity of the NPs, which can be tailored by reducing, stabilizing, and capping agents [17,18]. The commercially available AgNPs or for that matter the green AgNPs synthesized in the laboratory are reported to cause DNA damage and genotoxicity through generation of the reactive oxygen species (ROS) [19]. Trisodium citrate (TSC) is a commonly used surface reductant in synthesis of the AgNPs [20]. Jiang et al. [21] have predicted multiple roles of citrate ion and used the compound as a reducing agent, stabilizer, and complexing agent to synthesize the AgNPs. The capping agent, polyvinyl pyrrolidone (PVP) has also been used to disperse and stabilize the AgNPs and attenuate their genotoxicity [22,23]. Polyvinyl polypyrrolidone (PVPP), an insoluble, high molecular weight, and cross-linked polymer of PVP has been reported to remove the polyphenol and tannoid impurities from the plant extracts through adsorption [24,25]. Also, considering the adverse health effects of the residues of PVP, PVPP is preferred to PVP in the wine refining process [25]. Furthermore, reports are yet to be made on the effect of PVPP on green-synthesis of the AgNPs and also on the effect of TSC and PVPP on the AgNPs-induced genotoxicity and oxidative stress.

Among several plant extracts which we investigated in our preliminary screening to green-synthesize the AgNPs, we identified the kernel extract of the ripen mango (*Mangifera indica* L.) as the most effective and suitable plant material for the green synthesis of AgNPs. Here, we report production of the green AgNPs as influenced by TSC, PVPP, and PVP, their modulation of the genotoxicity, and their abilities to induce oxidative stress in our well-established grass pea (*Lathyrus sativus* L.) root bioassay system.

2. Materials and methods

2.1. Preparation of plant extract and greensynthesis of AgNPs

Leaf, bark and kernel from the unripe and ripe mango (*Mangifera indica* L., Family: Anacardiaceae) were used for preparation of the plant extracts. Extracts were prepared by boiling the chopped plant material (5 g) in sterile distilled water (100 mL) for 5 min in an Erlenmeyer flask followed by filtration of the extracts through Whatman Grade 1 filter paper. Biosynthesis of the AgNPs was initiated by adding 5 mL of the plant extract into 100 mL of 1 mM aqueous solution of $AgNO_3$ (Merck, Mumbai) at room temperature ($28 \pm 1\,^{\circ}C$) as described earlier [19]. In order to control the reduction of $AgNO_3$, the plant extract was added to $AgNO_3$ solution containing 1–5 mM trisodium citrate (Merck, Mumbai) without or with 0.1–0.5% polyvinyl polypyrrolidone (PVPP, Sigma-Aldrich, USA) and polyvinyl pyrrolidone (PVP, HiMedia, Mumbai). The pH of the synthesizing medium was recorded at every step of the synthesis. Upon addition of the plant extract, $AgNO_3$ solution changed from colourless to orange red and finally to dark brown indicating the ongoing process of greensynthesis of the AgNPs. Twenty four hours after the green synthesis as indicated by the complete colour change of the reaction medium to dark brown, the photoluminescence spectrum of the coloured solution, owing to surface plasmon resonance (SPR), was recorded between 300 to 600 nm at a resolution setting of 0.1 nm using a UV–vis spectrophotometer (UV-3000+, LabIndia Instruments, Mumbai). The air-dried powders of AgNPs biosynthesized from the plant extract alone (AgNPI), plant extract + TSC (AgNPII), and plant extract + TSC + PVPP (AgNPIII) and PVP (AgNPIV) were prepared by centrifugation at 12,000 rpm for 15 min followed by repeated dispersion and washing with double distilled water, ethanol, and finally by air-drying [19].

2.2. Physical characterisation of AgNPs

The sample was first analysed using Energy-Dispersive X-ray Spectroscopy (EDS, Bruker, USA) equipped with a XFlash® Detector with the energy resolution of 127 eV at 15 kV accelerating voltage, 20 nA of beam current, input count rate of 150000 cps, acquisition time of 5 min, and a mapping resolution of 600×450 pixels. This ascertained the composition to be silver. The dry powder samples were subjected to X-ray diffraction (XRD) for identification of the face-centered cubic (fcc) crystalline solid silver particles. The samples were smeared on a high index (911) Si plate which was spun continuously to reduce the effect of any preferred orientation of the particles. The measurements were carried out on a PANalytical Xpert Pro θ–2θ diffractometer using the Cu Kα radiation operating at 45 kV and 40 mA. The measurements were repeated on a Stoe diffractometer using the Cu Kα radiation operating at 40 kV and 30 mA. A coarse silver powder of uniform grain size of 25 μm diameter (Ag Bulk) was used for comparison of the silver peaks. Complexes of the AgNPs with different functional groups of the compounds were analyzed by the Fourier Transform Infrared Spectroscopy (FTIR) using an IRPrestige-21 FTIR Spectrophotometer (Shimadzu, Japan). For the FTIR, a small amount of dry powder sample of AgNPI, AgNPII, AgNPIII or AgNPIV was mixed with 50–100 mg of KBr, and then ground gently with a mortar and pestle and the KBr pellets of the samples were prepared. The transparent KBr pellets, containing the kernel extract without or with the AgNPs, were then subjected to the FTIR at a resolution of 4 cm^{-1} in the range of 4000–400 cm^{-1}.

The particle shape and size were determined by the transmission electron microscopy (TEM). For the TEM, samples of dry powder (~1 mg) of AgNP were mixed with ethanol in a 2 mL Eppendroff vial. The vials were then placed in an ultrasonic bath for 10 min to disperse the NPs into suspension. A drop of the suspension was applied onto a copper grid (300 mesh) coated with a carbon film. After air drying for 10–30 min, the copper grid was subjected to the TEM (JEOL, JEM-2100F, USA) operated at 200 kV. The size and size-distribution of the NPs were determined by the small angle X-ray scattering (SAXS). These measurements were carried out in transmission geometry using a setup with dual Mo (50 W, 50 kV and1 mA) and Cr (13 W, 30 kV and 0.46 mA) radiation with wavelengths of 0.709 Å and 2.24 Å, respectively. Incident x-ray beam was collimated with scatter-less single crystal blades with size of $0.8 \times 0.8\ mm^2$. Scattered photons from the sample

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