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Elucidating the antimicrobial and cytotoxic responses of silver nanoparticle synthesised using *Pongamia pinnata* leave extract

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Abstract

Biosynthesized nanoparticles, for their unique additional properties, have received global attention in nanomedicine. Medicinal plants have extensively been screened for eco-friendly and efficient synthesis of metal nanoparticles. Owing to the ever increasing applications of biosynthesized metal nanoparticles, the manuscript demonstrates the biogenic synthesis of silver nanoparticle (AgNP) using *Pongamia pinnata* leave extract. The characterization of biosynthesized AgNP indicated the highest yield of approximate 25 nm size face centred cubic silver nanocrystal with negative surface potential was obtained, when the plant extract taken in ten folds excess to metal salts. The biological corona, included bioactive molecules from the plant leave extract, is stabilising the AgNP core, as confirmed by elemental mapping of the nanoparticle. The biosynthesised AgNP found to have antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*, *via* predominantly enhanced intracellular ROS generation, at very low concentration. Interestingly, the nanoparticle showed higher cytotoxicity towards fibrosarcoma (HT1080) cells than differentiated monocytes (dTHP1), and also found to co-localise with nucleic acid. Additionally, the concentration inhibiting the microbial growth is found to be significantly higher than the cytotoxic concentration against HT1080 cell.

Keywords - Silver nanoparticle, biosynthesis, antimicrobial activity, internalization, cytotoxicity.

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Introduction

Medicinal plants have gained attention for its potential to reduce metal salts and capping the resulting elements growth at nanometre size, which it does either to counter metal ion toxicity and detoxify the system or use the metal ion as intrinsic factors and reduces ion while doing so.

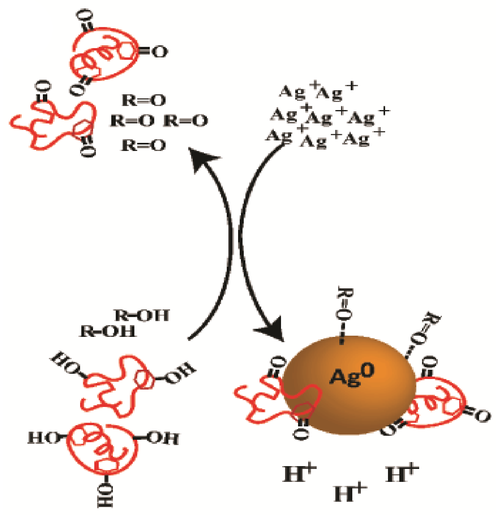

Figure 1. schematic diagram of biomolecules, like polyphenols, mediated Ag^+ reduction into Ag^0 and capping at nano-size crystal.

Objective

The objective of this study was to elucidate the antimicrobial and cytotoxic responses of biosynthesized silver nanoparticle (AgNP).

Results and discussion

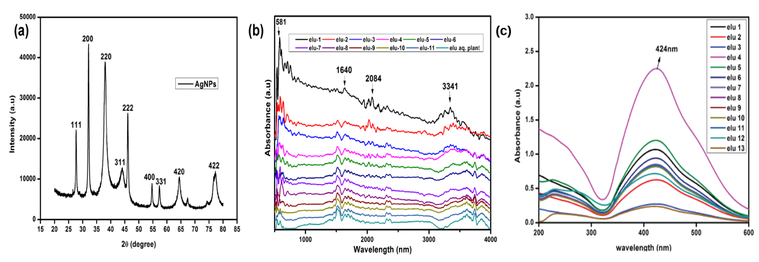

Figure 2. Characterization of biosynthesized AgNP. (a) XRD spectra (b) ATR-FTIR absorption spectra, and (c) Time dependent UV-Visible spectra M^+:PE ratio solutions 1:10 indicating the AgNP-specific SPR peak by arrow after SEC.

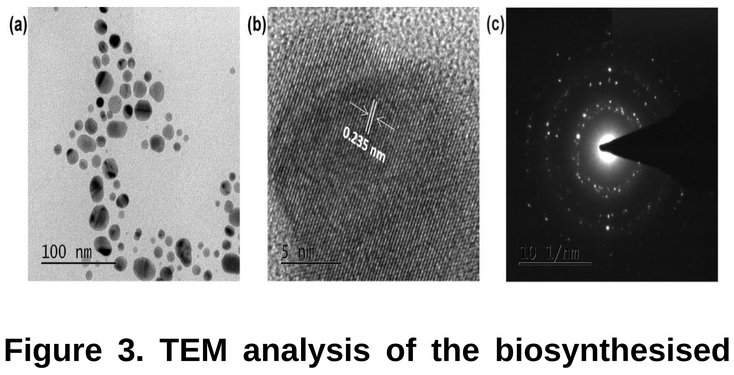

Figure 3. TEM analysis of the biosynthesised AgNP. (a) TEM micrograph of the monodisperse biosynthesized nanoparticle. (b) HR-TEM image, showing characteristic d-spacing in the crystalline biosynthesised AgNP. (c) SAED pattern, showing crystal plan of elemental Ag in the nanoparticle.

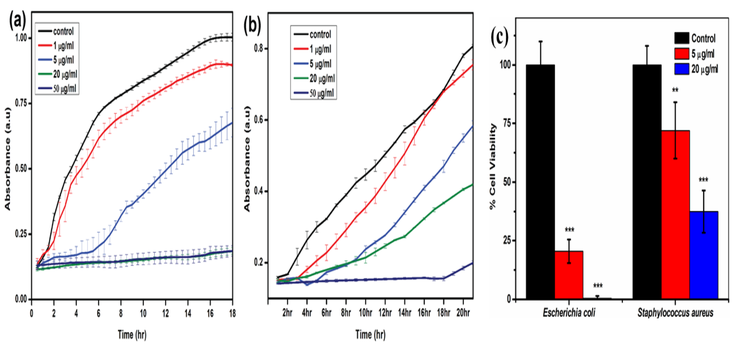

Figure 4. Effect of biosynthesised AgNP different concentrations on growth kinetics of *E. coli* (a) and *S. aureus* (b), and colony forming unit (CFU) of treated and untreated bacteria culture (c) expressed as relative cell viability compared to control. The error bar indicates S.E.M. of three independent experiments with respective significance, **:P<0.01; ***:P<0.001 compare to control.

Results cont....

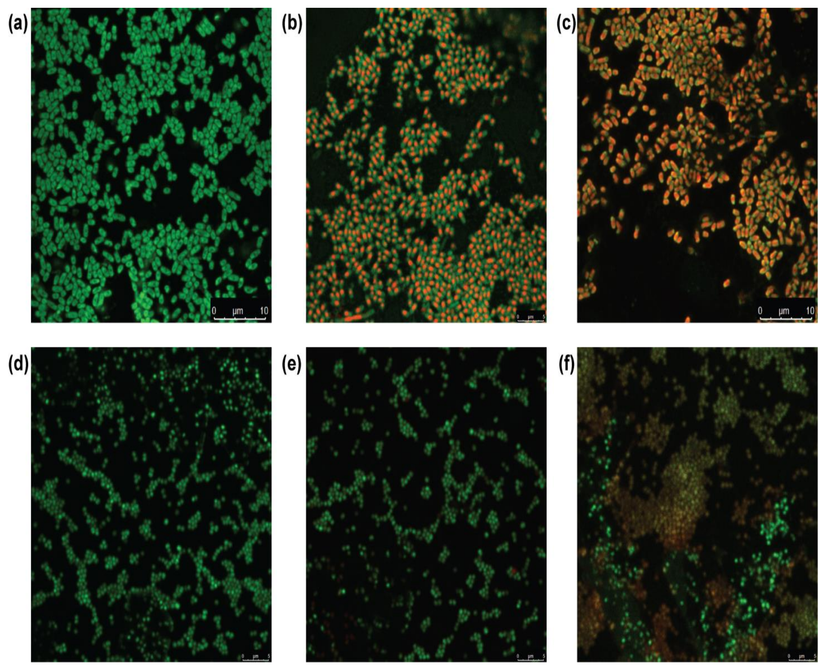

Figure 5. Confocal images of (a) intact *E. coli*, (b) 5 µg/mL AgNP treated *E. coli*, (c) 20 µg/mL AgNP treated *E. coli*, (d) intact *S. aureus*, (e) 5 µg/mL AgNP treated *S. aureus*, and (f) 20 µg/mL AgNP treated *S. aureus* cells stained with LIVE/DEAD BacLight bacterial viability stains.

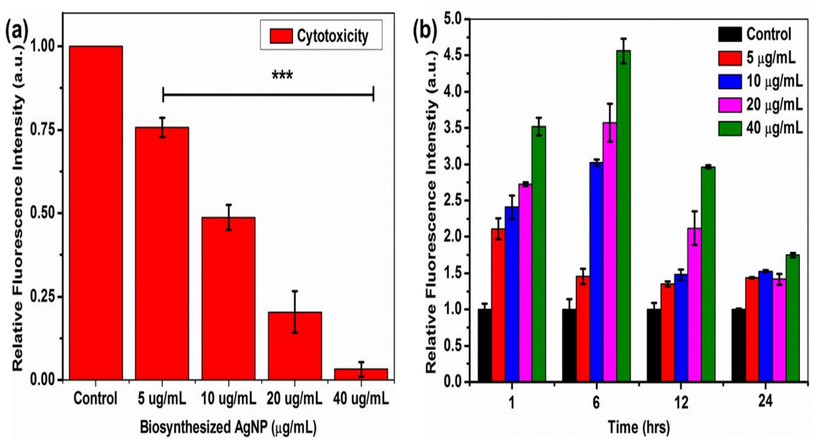

Figure 6. Alamar Blue reduction assay for cell viability (a) and time dependent DCFH-DA-mediated ROS detection HT1080 cells at 1, 6, 12 and 24 hrs of treatment with biosynthesised AgNP (b). The error bar indicates S.E.M. of three independent experiments with respective significance, **:P<0.01; ***:P<0.001 compare to control (untreated cells).

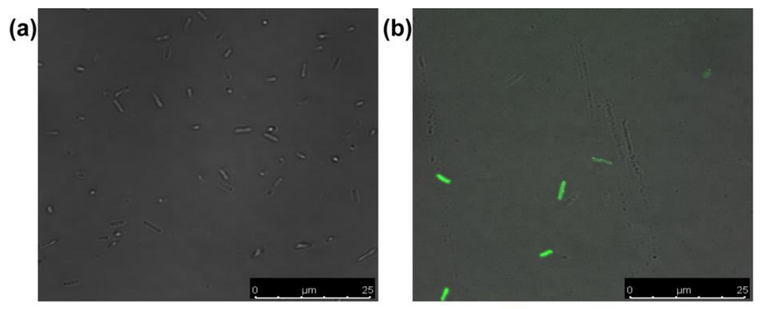

Figure 7. Confocal imaging of FITC-conjugate biosynthesized AgNPs and its sub-cellular localization. a) and c) Control images of *E. coli* and *S. aureus* (untreated), b) and d) *E. coli* and *S. aureus* treated with FITC-conjugate AgNPs (5 mg/mL), e) and f) Control images of HT1080 cells untreated and HT1080 treated with FITC-conjugate AgNPs (5 mg/mL) respectively (Scale bar 25 mm).

Discussion

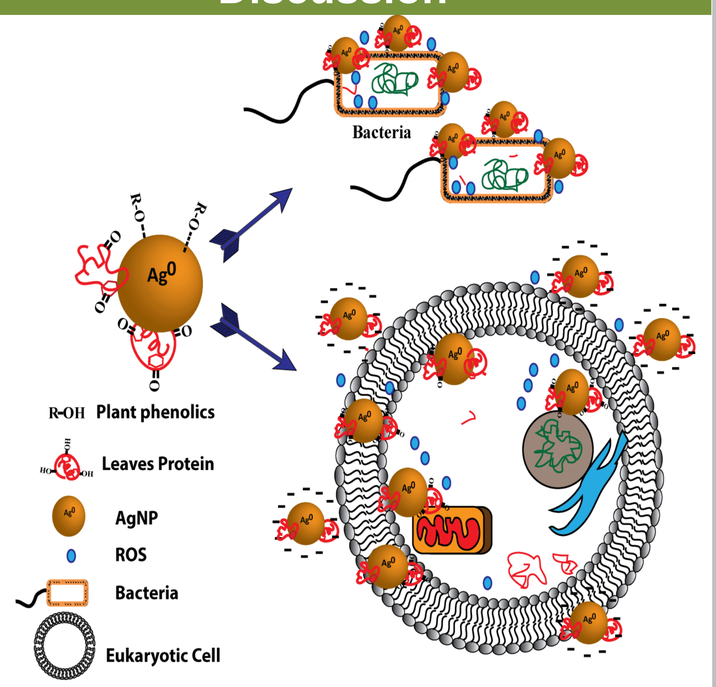

Figure 8. Schematic illustration of biofabricated AgNP interaction and toxicity with bacterial and cancerous cell.

Conclusion

The study demonstrated that the *Pongamia pinnata* leaf extract aids in efficient bio-reduction and stabilization of Ag (I) to Ag (0) during biosynthesis of AgNP. The antimicrobial propensity of biosynthesized AgNP which was studied *against E. coli* and *S. aureus* showed significant reduction in the growth of *E. coli* at very low concentration, whereas S. aureus showed significant reduction at relatively higher concentration. To this end, therapeutic potential of biosynthesized AgNP could help to opt for alternative antibiotics against pathogenic bacteria. Furthermore, AgNP gets internalize in cells, and exhibits effective toxicity against cancerous cells compared to normal cells, hence likely to be used in cancer therapy.

Reference

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