

Summary

In this study, selenium nanoparticles were synthesized biologically by *Bacillus licheniformis* JS2 strain, and a method was developed for the extraction and purification of intracellular nanoparticles. While following the earlier reported methods we observed association of NPs with the bacteria/bacterial debris and formation of NP agglomerates after the recovery. We improved the method and used lysozyme and a French press for complete bacterial cell lysis followed by Tris buffer washing and organic-aqueous extraction for the recovery of intracellular NPs. This method is a more successful method for the recovery of intracellular NPs than previously used techniques. Characterization of extracted nanoparticles with DLS, SEM, TEM, EDX, FTIR, SDS-PAGE, and silver staining indicated that the particles were uncharged, spherical, with diameters ranging between 40 and 180 nm, composed of selenium and capped with a few functional groups. Stability test under physiological buffered condition indicated that these neutral charged nanoparticles were more stable than that of the negatively charged particles. Considering the literature we hypothesized that coating of the functional groups was involved in providing steric stability to them. *In vitro* studies on prostate cancer cell lines (PC-3 and LNCaP) suggested that, compare to the commercially available selenium supplement, these NPs at a concentration of as low as 2 $\mu\text{g Se/ml}$ were very effective in inducing *tnf* and *irf1* mediated caspase independent necrosis/necroptosis without affecting the viability and integrity of hPBMC and RBCs. Endocytosis of SeNP and production of ROS were the additional results we observed in human prostate adenocarcinoma cells (PC-3). Moreover, these NPs were found to be free from bacterial endotoxins. *In vivo* toxicity studies (LD_{50} estimation, short term toxicity, and histopathology) on C3He/J mice suggested that the biogenic SeNPs are very less toxic compared to the commercially available selenium supplement (SeMet). However, further studies, including *in vivo*

bioavailability and anticancer property are required to claim the beneficial effects of these biogenic SeNPs.

Again, as selenium is reported to have anti-bacterial property, we have shown *Bacillus licheniformis* JS2 derived selenium nanoparticles inhibits *Staphylococcus aureus* adherence and micro-colony formation on polystyrene, glass and human urinary catheter surface. Confocal and electron microscopy results along with CFU counting strongly suggested that coating of these non toxic biogenic SeNPs, at a concentration of 0.5 mg Se/ml, prohibit bacterial load to more than 60 % on glass and catheter surface and obstruct their colonization, indicating that, SeNPs are also promising adherence inhibiting agent and can be applied on medical devices to control nosocomial infections. The activity of SeNPs can be increase by subjecting in combination with other antimicrobial agents.

To summarize, the study demonstrates the efficacious anticancer properties of *Bacillus licheniformis* derived SeNPs without any significant toxicity, suggesting a safest form of selenium for supplementation and prostate cancer chemoprevention. Again, *S. aureus* adherence inhibiting property of SeNPs can be utilized for the prevention of nosocomial infections by coating on medical devices.