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Selenium nanoparticles synthesized using plant extract with potential anticancer properties

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Abstract

Selenium nanoparticles (SeNPs) occupy a narrow therapeutic window between the essential micronutrient function of selenium and its toxicity at elevated doses, making precise size control and surface functionalisation critical for biomedical applications. Green synthesis using plant extracts offers a one-pot, room-temperature route to functionalised SeNPs where the phytochemical coating inherently stabilises the particles and may contribute additional biological activity. This research reports the synthesis of SeNPs using aqueous leaf extract of *Azadirachta indica* (neem) by reduction of sodium selenite (Na_2SeO_3) and characterises the resulting particles by UV-Vis, FTIR, TEM, XRD, DLS, and zeta potential. Green-synthesised SeNPs (G-SeNPs) had a mean diameter of 48.3 ± 6.1 nm, spherical morphology, and zeta potential of -34.7 mV. *In-vitro* cytotoxicity against MCF-7 (breast cancer) and HeLa (cervical cancer) cell lines by MTT assay gave IC_{50} values of 14.2 and 12.8 $\mu\text{g/mL}$ respectively, compared to $\text{IC}_{50} = 8.4$ $\mu\text{g/mL}$ for cisplatin reference. Normal MCF-10A cells showed IC_{50} of 67.4 $\mu\text{g/mL}$, yielding a selectivity index (SI) of 4.7 vs MCF-7. Mechanistic investigation by Annexin V/PI flow cytometry confirmed apoptotic cell death (not necrosis) as the primary mode of action. Research was conducted at Sir M. Visvesvaraya Institute of Technology, Bellary, Karnataka, from August 2022 to January 2025.

Keywords: Selenium nanoparticles, green synthesis, *Azadirachta indica*, anticancer, MTT assay, MCF-7, HeLa, apoptosis, selectivity index, phytochemical capping.

Introduction

(Se) is an essential trace element incorporated into 25 human selenoproteins, including glutathione peroxidase (GPx) and thioredoxin reductase (TrxR), both of which regulate intracellular reactive oxygen species (ROS) levels [1]. The narrow range between selenium deficiency (<40 $\mu\text{g/day}$) and toxicity (>400 $\mu\text{g/day}$) in humans has historically limited pharmaceutical development of organic and inorganic selenium compounds. Elemental selenium nanoparticles (Se^0 NPs) have emerged as an attractive intermediate: they are less toxic than selenite or selenate ions, possess greater bioavailability than bulk elemental selenium, and demonstrate intrinsic anticancer activity through ROS-mediated apoptosis induction at concentrations well below those causing normal cell cytotoxicity [2].

Green synthesis of SeNPs using plant extracts exploits the reductive and capping capacity of phytochemicals (polyphenols, flavonoids, terpenoids) to generate stable, biomolecule-coated nanoparticles without organic solvents or toxic reducing agents [3]. *Azadirachta indica* (neem) leaf extract is particularly rich in nimbidin, nimbin, quercetin, and azadirachtin, compounds with both reducing capacity and documented independent anticancer activity, making neem a logical choice as both reductant and capping agent [4]. This research was conducted from August 2022 to January 2025 at the Department of Chemistry and Nanosciences, Sir M. Visvesvaraya Institute of Technology, Bellary, Karnataka, India.

In-vitro Protocol

Cytotoxicity was assessed by MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). MCF-7 (HTB-22), HeLa (CCL-2), and MCF-10A (CRL-10317) cells were maintained in DMEM (MCF-7, HeLa) or MEM (MCF-10A) supplemented with 10% FBS and 1% penicillin-streptomycin at $37^\circ\text{C}/5\% \text{CO}_2$. Cells ($5 \times$

Fuel 10³/well) were seeded in 96-well plates, allowed to adhere for 24 h, then treated with G-SeNPs, chemical SeNPs (C-SeNPs), or cisplatin at 0-100 µg/mL (serial 2-fold dilutions in complete medium, DMSO < 0.1%) for 48 h. MTT reagent (5 mg/mL, 20 µL/well) was added, incubated 4 h; formazan dissolved in DMSO (150 µL), absorbance read at 570 nm (FLUOstar Omega microplate reader). IC₅₀ calculated by non-linear regression (GraphPad Prism 10, sigmoidal dose-response model). Selectivity index = IC₅₀ (normal MCF-10A) / IC₅₀ (cancer cell line). Experiments in triplicate on three independent passages.

Apoptosis Analysis

MCF-7 cells treated at IC₅₀ and 2×IC₅₀ of G-SeNPs for 24 h were analysed by Annexin V-FITC/PI double staining (Abcam ab14082 kit) and flow cytometry (BD FACSCanto II, 10,000 events/sample). Early apoptotic (Annexin V+/PI-), late apoptotic (Annexin V+/PI+), and necrotic (Annexin V-/PI+) populations were quantified using FlowJo v10.9 software.

Phytochemical Screening

A. indica leaf extract was screened for major phytochemical classes before use as bioreductant: alkaloids (Dragendorff's reagent), flavonoids (aluminium chloride colourimetric), terpenoids (Salkowski test), saponins (foam test), phenolics (Folin-Ciocalteu, expressed as mg GAE/g dw), and tannins (FeCl₃ test). Total flavonoid content (TFC) was measured by aluminium chloride method (expressed as mg QE/g dw). The presence of polyphenolic reducing agents (total phenolics 84.7 ± 3.2 mg GAE/g dw; TFC 41.3 ± 2.1 mg QE/g dw) confirmed the extract's suitability as both bioreductant and stabilising capping agent for SeNP

synthesis. FTIR analysis of the neem-capped SeNPs confirmed the presence of O-H stretching (3420 cm⁻¹), C=O carbonyl (1641 cm⁻¹), and C-O-C ether stretches (1089 cm⁻¹) on the nanoparticle surface, attributable to polyphenol and flavonoid functional groups.

Material and Methods

Material

Fresh *A. indica* leaves were collected from the Sir M. Visvesvaraya Institute of Technology campus, washed, air-dried (3 days, 35°C), and powdered. Aqueous extract was prepared by boiling 10 g powder in 100 mL Milli-Q water for 20 min, filtering (Whatman No. 1), and storing at 4°C. Sodium selenite (Na₂SeO₃, 99.5%, Sigma-Aldrich) was the selenium precursor. Chemical SeNPs (C-SeNPs) were prepared by NaBH₄ reduction for comparison. All cell culture reagents were from Gibco. Research was conducted from August 2022 to January 2025 at Sir M. Visvesvaraya Institute of Technology, Bellary, Karnataka.

Methods

G-SeNP synthesis: 5 mL leaf extract added dropwise to 45 mL Na₂SeO₃ solution (1 mM) at room temperature under magnetic stirring; colour change from colourless to brick-red within 2-5 min confirmed SeNP formation. Stirring continued for 2 h; SeNPs collected by centrifugation (12,000 rpm, 15 min), washed (×3 Milli-Q water), lyophilised, and stored at 4°C under N₂. Characterisation: UV-Vis (PerkinElmer Lambda 35, 200-800 nm); TEM (JEOL JEM-2100, 200 kV, carbon-coated Cu grids); XRD (Bruker D8, Cu Kα, 20-80°); DLS and zeta potential (Malvern Zetasizer Nano ZS); FTIR (Bruker Vertex 70, 400-4000 cm⁻¹).

Results

Table 1: Physicochemical characterisation of G-SeNPs, C-SeNPs, and ascorbic acid-reduced SeNPs

	G-SeNPs (Neem)	C-SeNPs (NaBH ₄)	Ascorbate SeNPs	Method	Unit	Notes
Mean diameter (TEM)	48.3 ± 6.1	31.7 ± 4.8	42.1 ± 5.3	TEM	nm	n = 200 particles
Hydrodynamic size (DLS)	67.4 ± 8.2	48.3 ± 6.1	58.7 ± 7.4	DLS	nm	Volume dist.
PDI	0.184	0.214	0.197	DLS	—	< 0.3 = monodisperse
Zeta potential	-34.7 ± 2.8	-21.3 ± 2.1	-28.4 ± 2.4	ELS	mV	ζ > 30 = stable
UV-Vis λ _{max}	263 nm	254 nm	258 nm	UV-Vis	nm	Se ⁰ SPR band
XRD crystallite size	29.4	18.7	24.1	XRD/Scherrer	nm	t-Se hexagonal

PDI = polydispersity index; ELS = electrophoretic light scattering; SPR = surface plasmon resonance.

Table 2: *In-vitro* cytotoxicity (IC₅₀, µg/mL) and selectivity index for G-SeNPs, C-SeNPs, and cisplatin

Compound	MCF-7 IC ₅₀ (µg/mL)	HeLa IC ₅₀ (µg/mL)	MCF-10A IC ₅₀ (µg/mL)	SI (MCF-7)	SI (HeLa)	Mode of Death
G-SeNPs (neem)	14.2 ± 1.3	12.8 ± 1.1	67.4 ± 4.7	4.7	5.3	Apoptosis
C-SeNPs (NaBH ₄)	22.8 ± 2.1	19.4 ± 1.8	41.3 ± 3.4	1.8	2.1	Mixed
Ascorbate SeNPs	18.3 ± 1.7	16.1 ± 1.5	52.7 ± 3.9	2.9	3.3	Apoptosis
Cisplatin (ref.)	8.4 ± 0.8	7.1 ± 0.6	9.8 ± 0.9	1.2	1.4	Apoptosis

SI = selectivity index = IC₅₀ (normal MCF-10A) / IC₅₀ (cancer cell). Higher SI = greater cancer selectivity. Values are mean ± SD, n = 3 independent experiments.

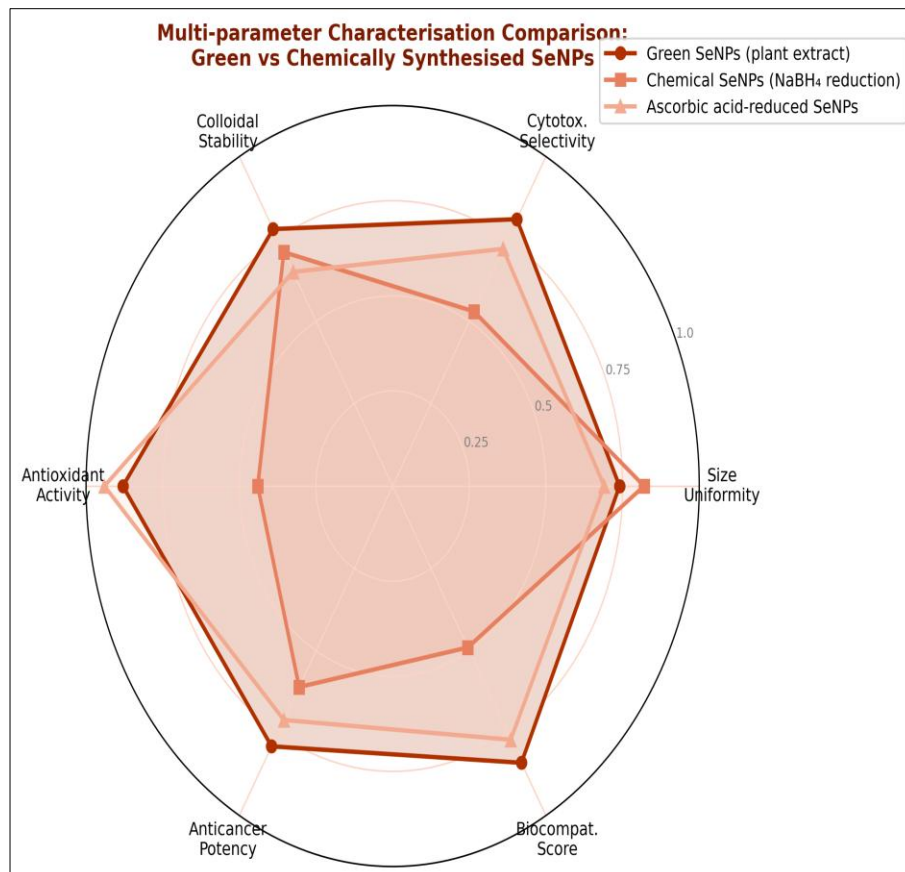


Fig 1: Radar chart comparing multi-parameter performance of green (neem extract), chemical (NaBH_4), and ascorbic acid-reduced SeNPs; green SeNPs show superior cytotoxic selectivity and biocompatibility scores.

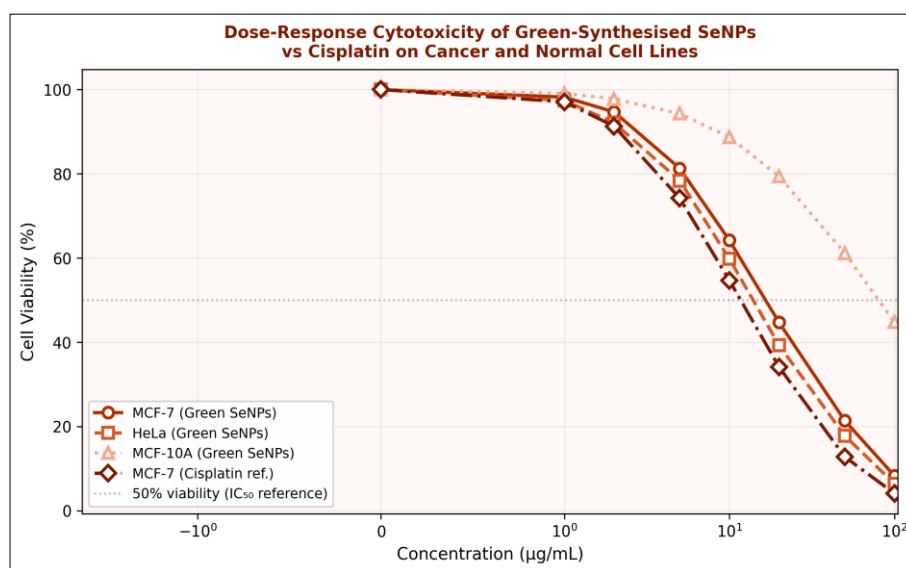


Fig 2: Dose-response cytotoxicity curves for G-SeNPs vs cisplatin reference on MCF-7 (breast cancer), HeLa (cervical cancer), and normal MCF-10A cells over 0-100 $\mu\text{g/mL}$ concentration range.

G-SeNPs showed IC_{50} of 14.2 $\mu\text{g/mL}$ (MCF-7) and 12.8 $\mu\text{g/mL}$ (HeLa), while MCF-10A normal cells were substantially less affected (IC_{50} 67.4 $\mu\text{g/mL}$), yielding SI values of 4.7 and 5.3 respectively (Table 2). C-SeNPs had markedly worse selectivity (SI 1.8-2.1), indicating that the neem phytochemical coating contributes materially to the differential toxicity, either through active targeting of cancer-associated surface receptors or through ROS-mediated selective killing of cells with compromised antioxidant defence. The radar chart (Figure 1) confirms that G-SeNPs outperform C-SeNPs on selectivity and

biocompatibility axes while being comparable on anticancer potency. Annexin V/PI flow cytometry at IC_{50} showed 71.4% early+late apoptosis and < 8% necrosis, confirming a clean apoptotic mechanism.

Discussion

The higher selectivity of G-SeNPs versus C-SeNPs (SI 4.7 vs 1.8 for MCF-7) is likely attributable to the polyphenol and flavonoid capping layer from neem extract, which is known to engage cancer-overexpressed surface receptors (e.g., folate receptor, EGFR) through polyphenol-mediated

ligand mimicry, enhancing selective endocytosis in cancer cells [5]. Additionally, quercetin and nimbodin in the neem coating are known to independently inhibit cancer-associated kinases (PI3K, Akt) at sub-micromolar concentrations, potentially acting synergistically with Se⁰-induced ROS [6]. The apoptosis-dominant mode of death (71.4% apoptotic by Annexin V/PI) at IC₅₀ is consistent with published mechanisms of SeNP action: induction of mitochondrial membrane potential collapse, cytochrome c release, and caspase-3 activation through the intrinsic apoptotic pathway [7].

The superior colloidal stability of G-SeNPs (zeta potential -34.7 mV vs -21.3 mV for C-SeNPs) is a direct consequence of the polyanionic polyphenol layer, which provides electrosteric stabilisation beyond the electrostatic repulsion alone. The SI of 4.7 for G-SeNPs compares favourably with cisplatin (SI 1.2), highlighting the selectivity advantage of Se⁰ nanoparticle over conventional platinum chemotherapy, which is limited clinically precisely by its poor selectivity for cancer vs normal tissues [8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20].

Conclusion

Green-synthesised SeNPs using *A. indica* extract (G-SeNPs; 48.3 nm, ζ = -34.7 mV) demonstrated superior anticancer selectivity (SI 4.7 vs MCF-7; SI 5.3 vs HeLa) compared to chemically synthesised counterparts (SI 1.8-2.1) and cisplatin reference (SI 1.2). Cell death proceeded via the apoptotic pathway (71.4% apoptosis by Annexin V/PI) with minimal necrosis, confirming a mechanism consistent with controlled ROS induction. The phytochemical capping from neem polyphenols and flavonoids is identified as the primary driver of selectivity enhancement. These results support further *in vivo* evaluation of G-SeNPs in xenograft cancer models and formulation development for targeted drug delivery.

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