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Green synthesis of selenium nanoparticles using *Azadirachta indica* leaves extract: evaluation of anthelmintic and biocompatibility potential

Maysar Abu HAWSAH[1], Rewaida ABDEL-GABER[1*], Saleh AL-QURAISHY[1], Hossam M. A. ALJAWDAH[1], Saleh N. MAODAA[1], Esam AL-SHAEBI[1]

Abstract

Various attempts have been made for the green synthesis of metal oxide nanoparticles, revealing the significance of plant extracts in reducing metal sources to nanoparticles and applications in various scientific domains. *Azadirachta indica* (family Meliaceae) is an evergreen tree that contains several phytochemicals within its leaves. This study aimed to perform the green synthesis of selenium nanoparticles (SeNPs) from *Azadirachta indica* leaves extract. In respective research attempts, NPs were evaluated for applications of anthelminthic and cytotoxic activities. Transmission electron microscopy analysis revealed the spherical morphology and size ranged from 62.1 to 77.6 nm of Bio-SeNPs. Phenolics and flavonoids were determined to be 0.83 ± 0.05 µg/g and 7.37 ± 0.32 µg/g, respectively, and antioxidant activity was 23.93 ± 2.05. For anthelminthic activity, the model earthworm, *Eisenia fetida*, was tested. Bio-SeNPs (5 mg/mL) have the lowest time for paralysis and death of earthworms (33.68 ± 1.13 and 50.10 ± 2.21 min, respectively). Moreover, histological examinations showed shrinkage of the worm body among treated groups in comparison to control group. In addition, IC_{50} of Bio-SeNPs was obtained at 127.212 µg/mL for the HCT116, 263.430 for MDA-MB-231, and 330.456 µg/mL for HUH7 cell lines. Our results suggest Bio-SeNPs possessed anthelmintic and biocompatibility activities.

Keywords: nanotechnology; plants; *Azadirachta indica*; anthelmintic; cytotoxicity.

Practical Application: Efficacy of biosynthsized selenium nanoparticles as antihelmintic and anticoccidial effects.

1 Introduction

Nanotechnology is one of the most rapidly developing fields of science in the previous decade (Bayda et al., 2020). This topic's research interest is growing, particularly in the field of nanoparticles (NPs) (particle sizes ranging from 1 to 100 nm) (Khan et al., 2022). These NPs' uses have expanded to the treatment of different complex diseases as well as other domains such as biomedical and food industries (Mohammad et al., 2022). Concurrent study in metallic NPs has also evolved its expanding usefulness in numerous industrial domains such as targeted drug delivery in the pharmaceutical field (Shnoudeh et al., 2022).

Due to the increasing demand among scientists for the synthesis of metal NPs and their applications, eco-friendly methods of production of metal oxide nanoparticles utilizing certain plant extracts have been discovered (Singh et al., 2018). The utilization of phytomolecules-based NP synthesis is of tremendous interest because it is a reasonably simple and quick procedure that does not require a reaction environment (Tavangar et al., 2013). Because of their numerous industrial applications, most investigations have focused on the synthesis of copper oxide, Zinc oxide, iron-based, titanium dioxide, cerium dioxide, and selenium nanoparticles (Patil et al., 2022).

Selenium nanoparticles 'SeNPs' have been introduced in the biomedical field due to their unique properties such as antimicrobial (Menon et al., 2020), antiprotozoal (Shirsat et al., 2015), anticancer (Wadhwani et al., 2016), free radicals scavenging (El-Sayed et al., 2021), and DNA protection against oxidative damage (Battin et al., 2011). There have been multiple reports of SeNPs being produced utilizing plant extracts (Alipour et al., 2021; Ikram et al., 2021; Souza et al., 2022).

Green synthesis using *Azadirachta indica* (AI) leaves extract, which is a biological approach employed in SeNPs synthesis, was chosen in this study because it is more ecologically friendly and less harmful than physical and chemical procedures. AI leaves have traditionally been employed in the therapy of numerous illnesses due to the diverse phytochemicals found in them (Durrani et al., 2008; Sharma et al., 2014; Alzohairy, 2016; Nagano & Batalini, 2021; Abu Hawsah et al., 2023).

The present *in vitro* study aimed to evaluate the anthelminthic efficacy and biocompatibility study to establish the applicability of the formulated NPs.

2 Materials and methods

2.1 Plant collection and extract preparation

Azadirachta indica leaves were collected from the botanical gardens (Riyadh, Saudi Arabia). The methanolic extract of the plant leaves was produced in accordance with the protocol of Manikandan et al. (2008) and then stored at -20 ºC until use.

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[1] Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia
*Corresponding author: rabdelgaber.c@ksu.edu.sa

2.2 Nanoparticles preparation and characterization

Selenium nanoparticles (SeNPs) were prepared by reducing sodium selenite (Nanocs Inc., Boston, MA, USA) following the protocol of Abdel-Gaber et al. (2023). The aqueous solution of the biosynthesized SeNPs was prepared using a methanolic plant extract in a 9:1 ratio and then allowed to cool at 27 °C. Using a transmission electron microscope (TEM) (JEM-1011, JEOL) at an accelerating voltage of 80 kV, the morphology of the Bio-SeNPs was examined.

2.3 Determination of phenolics and flavonoid contents and antioxidant activity

The phenolic components were evaluated using the Folin-Ciocalteu procedure, and data was calculated using a gallic acid standard curve, according to Al-Zharani et al. (2019). Furthermore, the flavonoid content was calculated as a quercetin calibration curve, according to Ghosh et al. (2013). The antioxidant activity was measured by the DPPH method of Akillioglu & Karakaya (2010) by mixing 80 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with 20 mL of Bio-SeNPs (1 mg/mL), incubated in dark for 30 min, and then measured the absorbance.

2.4 Anthelmintic activity

Earthworms, *Eisenia fetida*, (n=25) were collected from agricultural lands in Riyadh (Saudi Arabia) and identified by a specialist in the College of Food and Agriculture Sciences (King Saud University). Worms were divided into five groups with five per group of nearly the same body size, as follows: ***Group 1*** was served as control and supplied with distilled H_2O. ***Group 2*** was treated with 10 mg/mL of Mebendazole (reference drug). ***Group 3*** was treated with 5 mg/mL of Bio-SeNPs. ***Group 4*** was treated with 2.5 mg/mL of Bio-SeNPs. ***Group 5*** was treated with 1.25 mg/mL of Bio-SeNPs.

Paralysis and death time were expressed in minutes, according to Dkhil (2013). After that, different parts from the worm in all groups were collected and fixed in formalin (10%) for 24 hr, then dehydrated by ascending ethanol series and then embedded in paraffin wax. Sections of 5 µm were stained with hematoxylin and eosin (H&E). Sections were examined and photographed using an Olympus B×61 microscope (Tokyo, Japan).

2.5 Cytotoxicity assay

Using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoilum bromide (MTT) assay, the cytotoxicity of Bio-SeNPs was applied to the human colorectal carcinoma cell line (HCT116), human breast cancer cell line (MDA-MB-231), and human hepatic cell line (HUH7) that purchased from ATCC (USA). Cells were cultured at 37 °C and 5% CO_2 for 24 hr in Dulbecco's modified Eagle's medium (DMEM) containing fetal bovine serum (10%). Cells in dist. H_2O served as control as well as those in different concentrations of Bio-SeNPs were treated. After the addition of a yellow tetrazolium salt, the reduction was done to purple formazan crystals which dissolved by dimethyl sulfoxide (DMSO) then the absorbance of the colored solution was quantified. Untreated cells were used as a control group. Cell viability (%) was calculated according to Shirley & Lillehoj (2012).

2.6 Statistical analysis

Data analysis was performed using SigmaPlot® version 11.0 (Systat Software, Inc., Chicago, IL, USA) and presented as mean ± SD with p-value ≤ 0.05.

3 Results

Transmission electron microscopic analysis revealed that the Bio-SeNPs are spherical in nature of the particles, and these particles' size ranged from 62.1 to 77.6 nm (mean 67.97 nm) (Figure 1). The total concentration of phenolics and flavonoids in Bio-SeNPs was found to be 0.83 ± 0.05 µg/g and 7.37 ± 0.32 µg/g, respectively (Figure 2). Our results indicated that the antioxidant activity was evaluated using DPPH radical scavenging activity to be 23.93 ± 2.05.

Anthelmintic activity of Bio-SeNPs was tested in different *E. fetida* against the standard drug (Figure 3). A total of 25 worms (five in each group) were taken in respective solutions. Then, the paralysis and death time of the worms were recorded, and the obtained results were shown in Table (1). This experiment was carried out for up to 210 minutes and was compared with mebendazole (standard). The paralysis and death time for the most efficient dose of Bio-SeNPs (5 mg/mL) was 33.68 ± 1.13 and 50.10 ± 2.21 min, respectively. However, mebendazole took less time, with paralysis and death times of 6.622 ± 0.424 and 9.398 ± 0.770 min, respectively (Table 1). After treatment with Bio-SeNPs, a significant reduction in the body length of worms

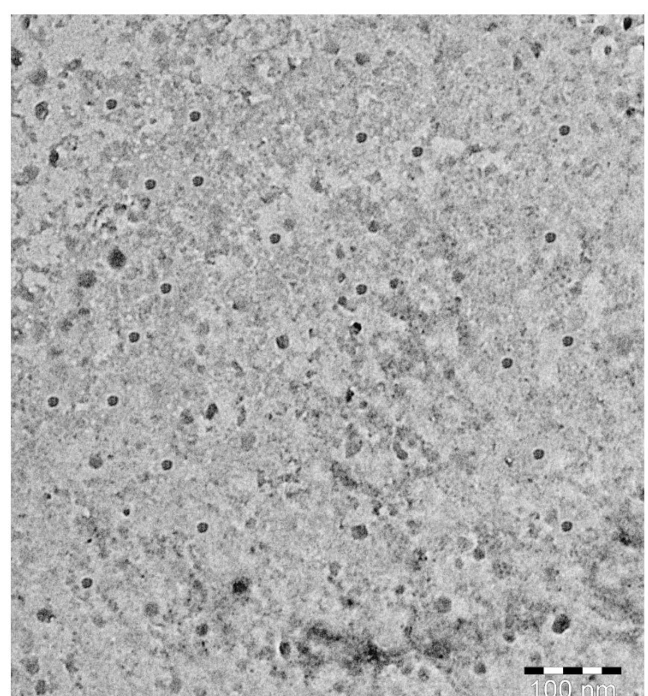

Figure 1. TEM of the Bio-SeNPs with magnification = 40000× and HV = 80 kV.

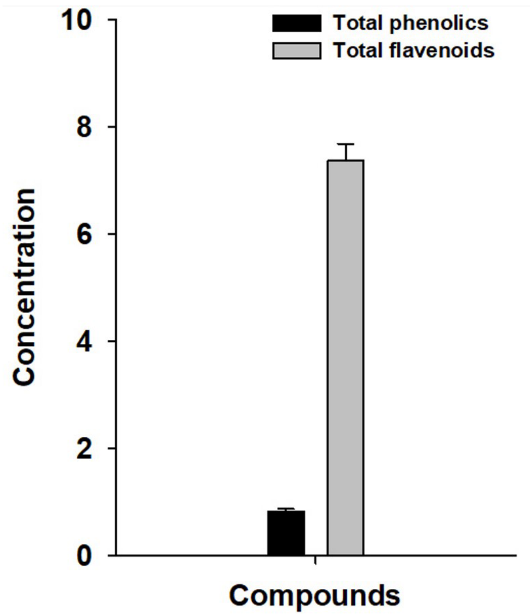

Figure 2. Concentration of phenolics (mg) and flavonoids (mg) in Bio-SeNPs.

than those in water and as well as the observable destruction of the cuticle layer in the mebendazole group (Figure 4).

Using an MTT assay, the cytotoxic effect of Bio-SeNPs was tested on three different cell lines HCT116, MDA-MB-231, and HUH7 (Figure 5). The IC_{50} of Bio-SeNPs was obtained at 127.212 µg/mL for the HCT116 cell line as well as 263.430 and 330.456 µg/mL for MDA-MB-231 and HUH7 cell lines, respectively (Figure 5).

4 Discussion

The chemical synthesis of NPs may result in hazardous toxic manifestations because of the presence of toxic organic residues within a product (Sharma et al., 2014). The biogenic synthesis of NPs utilizing natural products is currently widely used due to the lack of toxic residues and other hazardous matters (Mukherjee et al., 2001). There is some indication in the literature that the NPs generated using green synthesis have a low hazardous effect (Gour & Jain, 2019). Keeping this in view, the green synthesis technique was used to synthesize SeNPs in this study which revealed that the AI leaves extract provided satisfactory results. In this study, the characterization of NPs was done using TEM and revealed the spherical morphology

Figure 3. *In vitro* anthelmintic effect of *Allium sativum* juice against *Eisenia fetida*.

Table 1. *In vitro* anthelmintic activity of Bio-SeNPs.

Test samples	Concentration (mg/mL)	Time is taken for paralysis (min.)	Time is taken for death (min.)
Control (H_2O)	--	--	--
Bio-SeNPs	5 mg/mL	33.68 ± 1.13 ^{abde}	50.10 ± 2.21 ^{abde}
	2.5 mg/mL	75.25 ± 2.01 ^{abce}	90.32 ± 3.11 ^{abce}
	1.25 mg/mL	180.32 ± 6.32 ^{acd}	210.55 ± 7.18 ^{acd}
Mebendazole	10 mg/mL	6.622 ± 0.424 ^{acd}	9.398 ± 0.770 ^{acd}

Values are mean ± SD. All superscripts indicate significance at $p \leq 0.05$, [a]compared to untreated (H_2O), [b]compared to mebendazole, [c]compared to the lowest concentration of Bio-SeNPs, [d]compared to the moderate concentration of Bio-SeNPs, [e]compared to the highest concentration of Bio-SeNPs.

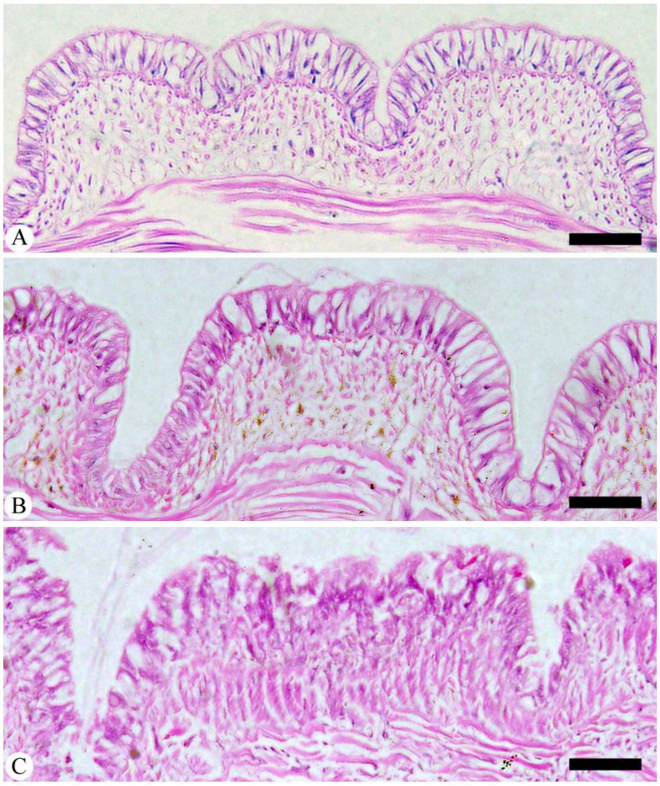

Figure 4. Cuticle thickness of *E. fetida* with various treatments. (A) worms in dist. H_2O (control); (B) worms in Bio-SeNPs (5 mg/mL); (C) worms in mebendazole. Scale bar = 25 µm.

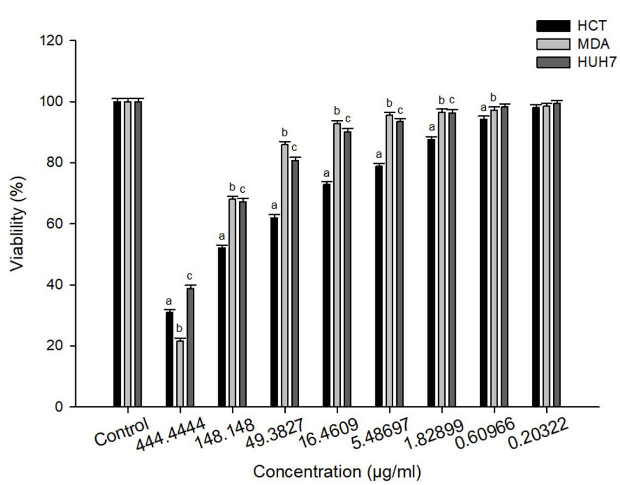

Figure 5. Cytocompatibility evaluation of Bio-SeNPs. [a]significance changes with respect to control group of HCT; [b]significance changes with respect to control group of MDA; [c]significance changes with respect to control group of HUH7.

with small particles' size, this in line with Luesakul et al. (2016) reported that smaller nanoparticles had a greater cytotoxic effect. Moreover, the particle size of NPs affects their properties and applications as well as their toxicity (Shah et al., 2019). This fact occurs due to the increased contact surface and the ease of interiorization of smaller nanoparticles.

Flavonoid contents in this study were evaluated in higher concentration than phenolics, which acting as a capping agent block the accumulation of the NPs and modify their biological activities, this result in line with Harale et al. (2022). Moreover, the Bio-SeNPs exhibited considerable antioxidant activity, this might due to the occurrence of phenolic compounds and flavonoids content in the nanoparticle's suspension besides, their small size. This agreed with Huang et al. (2007), Vyas & Rana (2017), Kumar et al. (2018) and Oka et al. (2020) everted the presence of these active compounds to the AI leaves extract (Kumar et al., 2018; Oka et al., 2020).

The mechanism of the inhibitory effects of Se ions on microorganisms is partially known. For that, the paralysis and death time of the worms that treated with Bio-SeNPs were recorded for up to 210 minutes. The most efficient dose of Bio-SeNPs (5 mg/mL) was 33.68 ± 1.13 and 50.10 ± 2.21 min, respectively. It might be a result of the net positive charge on the selenium ion being the reason for antimicrobial activity as it can attract the negatively charged cell membrane of microorganisms through the electrostatic interaction (Huang et al., 2020). On the other hand, *A. indica* leaves consist of alkaloids, amino acids, saponins, flavonoids, phenols, polysaccharides, and galactomannan (Ramamurthy et al., 2013). These phytochemicals can attach with free proteins in the gastrointestinal tract or glycoprotein on the parasite cuticle and cause death. Moreover, the change in the body worm length after treatment with Bio-SeNPs might be the AI leaves extract brings about the permeability changes in the cuticle of the worms, this is agreed with Hu et al. (2018) reported that rutile TiO_2 NPs negatively affect both the body size and worm population.

The effect of SeNPs on cancer cell lines was related to numerous factors, such as size, type of coating, and stability in solution (Souza et al., 2022). The cytotoxic effect of Bio-SeNPs was tested using an MTT assay on different cell lines. The viability of cells, in the present study, has a direct dose-dependent manner, which agreed with Rajkumar et al. (2020). Also, Ramamurthy et al. (2013) stated that the presence of chemical compounds in the plant extract increases the applicability and the potential of the plant-based SeNPs as a possible future solution to combat the devasting effects of different cancer cells. The IC_{50} of Bio-SeNPs on the examined cell lines were recorded with different concentrations, this result in line with Abd-Rabou et al. (2019) reported that MCF7 cell line is sensitive, while MDA-MB-231 cell line is resistant to Se and nano-Se.

5 Conclusion

Green-synthesized SeNPs exhibited *in vitro* anthelmintic and biocompatibility activities. This type of NPs could be considered as a novel chemo preventive agent with reduced toxicity risk. This study should contribute to the development of future research projects focusing on other biological activities of the bio-synthesized SeNPs from AI leaves extract.

Conflict of interest

The author(s) declare that they have no conflict of interest regarding the content of this article.

Availability of data and material

All the datasets generated or analyzed during this study are included in this published article.

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