Cytotoxicity of Green biosynthesized selenium nanoparticle on PC3 and WRL 68

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Abstract

Because of its importance in numerous physiological process , SeNPs research has gotten greater interest in recent years. In comparison to selenium, selenium nanoparticles have a high level of absorption in nutritional supplements . SeNP has been synthesized using a variety of chemical and biological techniques. Because of its anti-oxidant, anti-bacterial, anti-diabetic, and anti-cancer properties, selenium nanoparticles have biological and pharmacological applications . This article reviews biosynthesis of SeNPs using Na_2SeO_3 and *Bacillus clausii* under aerobic conditions .The structural and morphological properties of SeNPs were determined by UV_Vis spectroscopy and the absorption peak was observed at (260 nm) wavelength , in SEM size range between (37.58 – 75.16 nm) , the average diameter of SeNPs was 19.28 in AFM . Results showed that no significant cytotoxic effect of SeNPs against PC3 cells at concentrations (25 and 50 µg/mL) . Nevertheless, SeNPs at (100, 200 and 400 µg/mL) exhibited a dose_dependent_decrease in PC3 cell viability with maximum inhibition rate of 51.27±2.77% of PC3 cells at 400 µg/mL.

Keywords: *SeNPs , Bacillus clausii ,* UV- Vis spectroscopy , SEM , AFM , PC3 and WRL 68

DOI Number: 10.14704/nq.2022.20.12.NQ77098 **NeuroQuantology 2022; 20(12): 1173-1182**

1- Introduction

Nanotechnology is a new field of study that combines nanotechnology and biotechnology to give nano science . The diameter of the nanoparticles ranges between (1-100) nm , their chemical activity , large surface area , charge density and ability to interact with the bacterial-cell enabled them to enhance the antimicrobial activity by generating free toxic metal ions or ROS. (1, 2 , 3 , 4) .

Nanoparticles are manufactured by physical, chemical and biological methods. In biological methods (green synthesis) plants, fungi and bacteria are used to prepare nanomaterials. One of the benefits of this method is that it is environmentally friendly, economical, and less toxic when compared to other methods. As for the physical and chemical methods used in manufacturing, they produce high radiation and toxic reducing materials , which have the ability to affect human and other living organisms (5,6,7). In biological systems, se is a necessary micronutrient . SeNPs have many nanomedicine applications due to their anti-cancer anti-microbial , and anti-oxidant properties, and the cytotoxicity is lower than that of silver nanoparticles (8) . Polymers, dendrimers, liposomes, metal NPs (Eu , Ag , Fe , Au , Ti , Ce , Cu , Se and others), silicon, and carbon-based nanomaterials have all been employed as effective therapeutic agents and drug delivery carriers (9 , 10 ,11 , 12 , 13 , 14) . Because of their high stability and low toxicity , SeNPs are now widely accepted and recommended for use in a variety of scientific branches (15) . SeNPs have shown a major therapeutic platforms, especially in anti-cancer therapy , including combination with well-known chemotherapeutic agents

eISSN 1303-515

like 5-Fluorouracil , irinotecan and doxorubicin (16,17,18, 19) . As well as with oligonucleotides like small interfering RNA , exhibiting synergistic anticancer activity and overcoming multidrug resistance (16,20,21,22) .

2- Material and methods

2.1 SeNPs biosynthesis

Extracellular biosynthesis was used due to the advantages of this procedure over the intracellular method. The advantages of extracellular biosynthesis include easier, low artifacts workflow (Youssef et al., 2014). Sodium selenite (Na_2SeO_3) was used as precursor for biosynthesis of selenium nanoparticles . *Bacillus clausii* were grown in (BHIB) at 37 C^0 for 24h. After that , the suspension was centrifuged for 15 minutes at 10,000 rpm, the supernatant was taken and the precipitate was removed , these supernatant was supplemented with sodium selenite solution (3 mM) . The pH was adjusted to 8 with (2M) NaoH . In shaking incubator (150-200 rpm) , the resultant solutions were incubated in aerobic condition for 24-48 hours at 37 C°.

After incubation , the changing in color was observed and the mixture was centrifuged for 10 minute at (10000 rpm , 4 c°) , the supernatant was discarded and the precipitate was taken. In order to purify selenium nanoparticles, these sediment was washed with ddH_2O , these step were repeated three times. The final suspension was dried for 18-24 hours in oven at 40 C°. The powder was skilfully collected and stored in a vial for further examination (23).

2.2 SeNPs characterization

- **UV-Visible Spectra analysis**

The concentration of selenium nanoparticles in solution was determined using this method . Two ml of SeNPs were scaned at a medium scan rate two nano-meter/ second in the range of (200 – 800) nm and measured in a (1 cm) path-length-quartz-cuvette (24).

- **Scanning Electron Microscopy (SEM)**

SEM was used for characterization the morphological and size of selenium nanoparticles. The sample was smeared on adhesive carbon tape that was fixed to a brass stub for the SEM. The sample was then coated with gold using a sputtering unit for 10 seconds at 10 mA of current. The gold-coated sample was placed in SEM chamber, then, images of secondary electrons /back scattered electrons were captured . The microscope operated with different magnification ranging from x15000 to x35000 and voltage 20-30 KV (25) .

- **Atomic Force Microscope(AFM)**

AFM was used for SeNPs characterization in University of kashan , On a slide, a drop of SeNPs was applied and a thin smear was produced. The smear was air-dried before being scanned and examined with an Atomic Force Microscope (Broker, Germany) (26).

2.3 Assessment the SeNPs Cytotoxicity

To study the cytotoxic of SeNPs , Prostat cancer cell line (PC3) and normal hepatic cell line (WRL 68) was prepared ,the cell were cultured in monolayers according to (27). The cytotoxic effect of SeNPs in different concentration (25 ,50 ,100, 200 and 400 µg/ml) was performed by using MTT kit (Intron Biotech) . The grown cells were incubated using a 5% CO2 incubator for 24 h at 37°C . PC3 and WRL 68 cells were then removed by trypsinizatien . The cells were inculated in a 96-well-plate with approximately (106 cells / well) in their exponential growth phase and inoculateed

eISSN 1303-515

with different concentrations (25–400 μg/mL) of SeNPs in CO2 incubator for (24 , 48 ,72 h) after incubation to achieve the best results . The supernatant of both cells without stimuli was used as negative control . Each test was carried out in conjunction with a control containing complete medium devoid of cells as a blank; nanoparticles and MTT reagent devoid of cells were used as blanks . Following , (100 μL) of MTT was added to each well and incubated again for (4 h) . Finally, a pipet was used to add detergent reagent in a volume equal to the original culture medium (100 μL) , to entirely dissolve the resulting MTT formazan crystals . After well mixing , the optical density of resulting solution was immediately read in a microplate reader using a microplate spectrophotometer at the background absorbance of multi-well plates at (690 nm) and subtract at (570 nm) . All assays were performed in triplicate . The viability of cells was represented and compared to controls .

3- Results & discussion

3.1 SeNPs biosynthesis

Bacillus clausii used in SeNPs biosynthesis, showed up the ability of cell free supernatant in extracellular biosynthesis of SeNPs after the addition of Na2SeO3 as a substrate . Colour changing from yellow to red served as indicators for the biosynthesis of SeNPs by *Bacillus clausii* , (figure 1) .

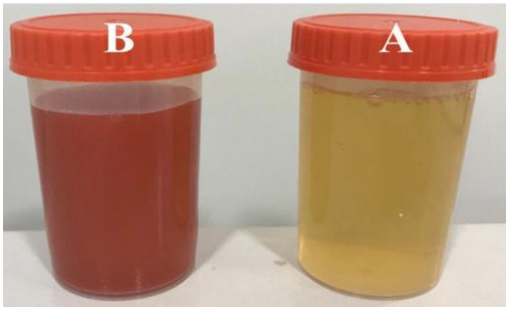


Figure (1) this images displayed biosynthesis of selenium nanoparticles product process. image (A) showed the supernatant of *bacillus clausii* without Na_2SeO_3 , image (B) showed the supernatant of *bacillus clausii* with Na_2SeO_3 after 48 hours.

In the biosynthesis of nanoparticle , The reaction solution's unique red hue was caused by the Se particles' surface plasmon vibrations being excited, providing a useful spectroscopic signature of their production . Earlier reports also showed the biosynthesis of selenium nanoparticles using several microbes . (28) biosynthesized SeNPs by using three species of non-pathogenic, *Lactobacillus* , where as synthesis of $SeNp_s$ using *Bacillus* sp. MSh-1 and further antimicrobial activity has been reported by (29), The results of our study also indicate that reduction of Na_2SeO_3 by the supernatant of *Bacillus clausii* took longer time than previous reports (28) .

3.2 characterization of selenium nanoparticles

- **UV-Visible Spectra analysis**

  UV-Vis spectroscopy research between 200 and 800nm was used to characterized the synthesized selenium nanoparticle. At 260 nanometers, the presence of SeNPs was detected (Figure 2) .

eISSN 1303-515

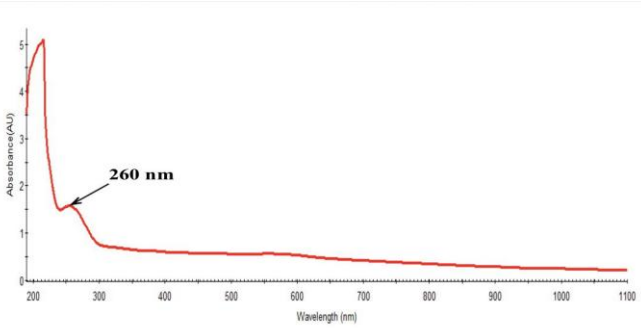


Figure (2): This curve of SeNPs in solution was determined by UV-vis spectroscopy

Many research in the literature support these findings and demonstrating that the reducing agent *Bacillus clausii* is capable of completely converting precursor molecules into nano-sized selenium particles when spherical particles are generated with size 50-100 nm , a consistent absorption maximum in the wavelength area of around 300 cm^{-1} is observed, depending on the experimental conditions (30,24) . Other research showed that the UV-visible spectroscopy for surface plasmon resonance was and (262 nm) and size range from (35 nm _ 75 nm) (24) .

- **Scanning Electron Microscopy (SEM)**

To confirm the size and morphology of the SeNPs , SEM were used . Rod shaped of SeNPs with a size range between 37.58 – 75.16 nm were reported . Figure (3) showed the SEM micrograph of purified SeNPs .

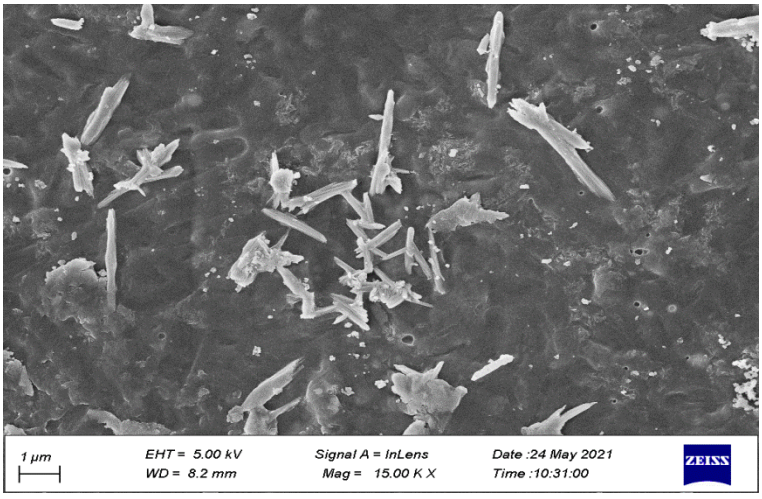


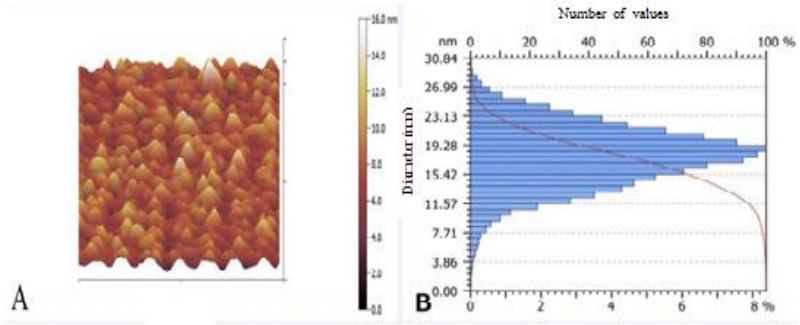
Figure (3) : SEM Micrograph of biogenic selenium nanoparticle synthesized by Bacillus clausii

eISSN 1303-515

The shapes and sizes of bio-genic metallic NPs can be controlled by exchanging the bio_reduction conditions , including : organism , types of culture , incubation time and nature of medium (31) . Rods-shape of SeNPs showed similarity with result that cited by (25,32,33) . Whereas spherical shape were reported by (34,31,35). Recently, numerous microscopic techniques are commercially available , whenever (TEM) and (SEM) are the most popular microscopes for the analysis of the NPs (36) .

- **Atomic force microscope(AFM)**

AFM imaging validated the shape and surface topography of the Selenium NPs. The AFM pictures revealed that the Selenium NPs had a spherical form with a diameter of 19,28 nanometer , indicating that nanoparticles were present in the medium after incubation (Figure 4).



(Figure 4) : A - AFM image of SeNPs biosynthesized by *Bacillus clausii* , B- Size (diameter) distribution histograms of corresponding structures

The morphology and distribution of selenium nanoparticles, which adhere to one other and create a layered structure, are also confirmed by AFM images . By detecting a sharp probe for bio-effective component of SeNPs and the attractive / repulsive forces between the sample surface , AFM images were obtained (37). The image of AFM showed the shape of the surface and the size of the particles for the samples that have been identified , and it also showed a three-dimensional (3D) and two-dimensional (2D) image of the sample (38) .

3.3 Cytotoxicity of SeNPs on PC3 and WRL68

The cytotoxic response of prostate cancer PC3 cell line and normal hepatic WRL68 cells treated with increasing concentrations of Se NPs (25, 50, 100, 200, 400 µg/mL) was investigated using MTT assay after 24 h exposure. Results in table (1) , showed that no significant cytotoxic effect of Se NPs against PC3 cells at concentrations (25 and 50 µg/mL) . Nevertheless, Se NPs at (100, 200 and 400 µg/mL) exhibited a dose dependent decrease in PC3 cell viability with maximum inhibition rate of 51.27±2.77% of PC3 cells at (400 µg/mL) .

Table (1): Multiple comparisons of mean±SD cell inhibition between PC3 and WRL68 treated with Se NPs (25, 50, 100, 200, 400 µg/mL) for 24 h.

Se NPs	PC3	WRL68	Sig.	*p* Value
	Mean Inhibition ± SD (%)	Mean Inhibition ± SD (%)		
400 µg/mL	51.27±2.77[a]	36.86±3.38[a]	**	<0.00001
200 µg/mL	36.3±2.12[b]	25.54±0.85[b]	**	<0.00001
100 µg/mL	22.38±2.41[c]	9.03±3.3[c]	**	<0.00001
50 µg/mL	8.08±1.36[d]	5.36±1.51[c]	NS	0.5243
25 µg/mL	4.05±0.53[d]	4.71±1.05[c]	NS	0.9981

****: p < 0.01, NS: non-significant. Different letters (a, b, c, d) consider significant (p < 0.05) in column.**

Regarding WRL68 cells, the sensitivity of the cells to Se NPs treatments was less than that of PC3. Se NPs concentrations at 25, 50 and 100 µg/mL showed no significant differences in pattern of cell inhibition. On the other hand, Se NPs at 200 and 400 µg/mL showed significant ($p < 0.05$) reduction in cell viability with maximum inhibition of 36.86±3.3% at 400 µg/mL . Multiple comparisons study , table (1) between PC3 and WRL68 with regard to Se NPs toxicity showed significant differences ($p = <0.0001$) in the pattern of cell inhibition at 100, 200 and 400 µg/mL, which obviously PC3 cells were more susceptible to Se NPs treatment than WRL68 cells. IC50 of Se NPs against PC3 and WRL86 was calculated with values of 164.4 and 175.4 µg/mL, respectively , figure (5) .

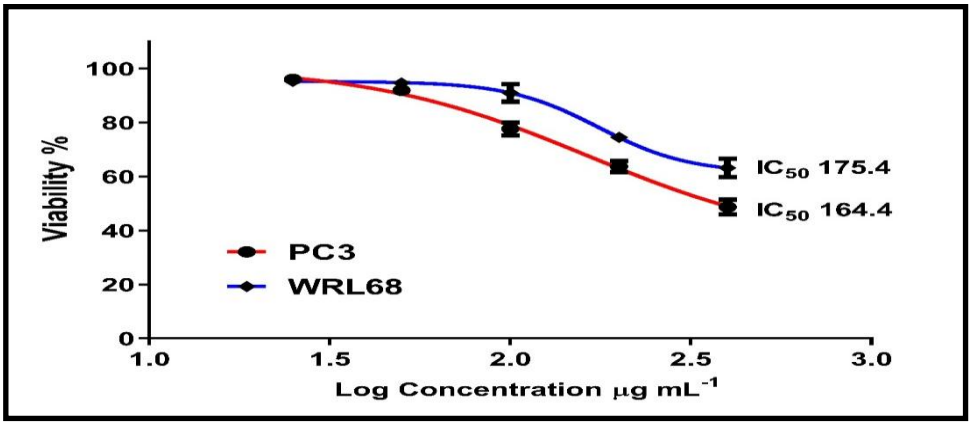


Figure (5): Dose-response curve (IC50) for PC3 and WRL68 cells treated with Se NPs after 24 h incubation at 37ºC.

One of the most common materials used in the production of solar cells and photography is selenium, which exhibits a well-known photoelectrical property. It is also an essential component of the human body, as it can protect tissues and cells from free radicals in vivo (39). A dose-dependent assessment of the effects of Se NPs on the viability of PC3 cells revealed that the cytotoxic effects of Se NPs were detrimental. The results showed that the presence of Se NPs negatively affected the

eISSN 1303-515

cell viability of PC3 cells. Depending on their physiochemical characteristics, Se NPs exhibits the tendency to induce the production of reactive oxygen species in exposed cells and thus responsible for the cytotoxic effect (40).

It was indicated that the method of NPs preparation, structure and size of Se nanomaterials have high impact on the antitumoral effect of Se NPs (41). It was reported that Se NPs in combination with other nano-metals exhibited antiproliferative effect against HepG2 cell line through induction of apoptosis (20). Moreover, Sonkusre, (2020) showed that a significant decrease in the viability of prostate LNCaP cell line was observed when treated with different concentrations of Se NPs with signature feature of necrosis due to the decrease in caspases and no LDH release from the cells (42).

SeNPs were observed to be effective in inhibiting a wide range of cancer cells while having little effect on normal cells . Similarly, studies have been suggested that Se NPs have potent cytotoxicity against tumor cells, but not against normal cells, like cervical carcinoma, hepatocarcinoma and colorectal cancer (43). However, studies confirmed that Se NPs are significantly potent against many cell lines and that their cytotoxicity on prostate cancer cell lines is more convincing than other types of tumor cell lines (44, 16).

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