RESEARCH ARTICLE

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Antioxidant and Anticancer Activities of Bio-Synthesized Selenium Nanoparticles by *Escherichia coli*

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Abstract

Objective: Selenium, an essential micronutrient, plays a crucial role in various physiological processes, including immune function, fertility, and the prevention of chronic diseases like diabetes, cardiovascular disease, and certain cancers. While selenium nanoparticles (SeNPs) have garnered significant attention for their potential in cancer therapy, their biosynthesis using eco-friendly methods remains a key area of research. **Methods:** In this study, Escherichia coli isolates were utilized to biosynthesize SeNPs from sodium selenite (25, 50, and 100 mM) at pH 8 and 160 rpm for 48 hours. The biosynthesized SeNPs were characterized using Fourier Transform Infrared Spectroscopy (FTIR), UV-Visible spectroscopy, X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and Atomic Force Microscopy (AFM). The antioxidant and anti-proliferative activities of the synthesized SeNPs were evaluated against human colorectal carcinoma (SW480), human hepatocellular carcinoma (HepG2), and normal human embryonic kidney (HEK293) cell lines. **Results:** The biosynthesis process resulted in a reddish-brown coloration of the medium, indicating the formation of SeNPs. Characterization techniques confirmed the successful synthesis of SeNPs with a size range of 21.96-50.71 nm. The SeNPs exhibited significant antioxidant activity, with a maximum radical scavenging efficiency of 80.21% at a concentration of 100 µg/ml. Additionally, the SeNPs demonstrated potent anti-proliferative effects against both SW480 and HepG2 cell lines, with IC_{50} values of 3.9 and 4.5 µg/ml, respectively. Importantly, the SeNPs exhibited negligible cytotoxicity towards normal HEK293 cells. **Conclusion:** The eco-friendly biosynthesis of SeNPs using E. coli offers a promising approach for producing nanoparticles with potential applications in biomedical fields. The synthesized SeNPs demonstrated potent antioxidant and anti-cancer properties, suggesting their potential as a novel therapeutic agent.

Keywords: Selenium Nanoparticles- *Escherichia coli*- Biosynthesis- Antioxidant- Anticancer

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Introduction

Nanotechnology, a rapidly evolving field, has revolutionized various sectors, including medicine. By manipulating matter at the nanoscale, researchers have overcome significant challenges related to drug delivery and bioavailability [1]. Nanotechnology has introduced innovative materials and approaches to the pharmaceutical and medical industries, offering promising solutions to complex health problems [2]. Nanoparticles (NPs), with their exceptionally high surface-to-volume ratio, exhibit unique physicochemical properties that differ significantly from their bulk counterparts [3]. In the realm of bionanotechnology, green synthesis of NPs using biological systems has emerged as a sustainable and environmentally friendly approach. This method leverages the ability of living organisms, such as plants, fungi, and bacteria, to reduce metal ions into less toxic nanoparticle forms [4-6]. These biologically synthesized NPs offer several advantages, including simplicity, cost-effectiveness, safety, and high purity [7].

Selenium nanoparticles (SeNPs) have emerged as a promising class of compounds with potential applications in various medical fields, including antibacterial, anticancer, and antioxidant therapies. Selenium, an essential micronutrient, plays a crucial role in numerous biological processes, including immune function, antioxidant defense, and cancer prevention [8-10].

Biogenic SeNPs, synthesized using biological agents, offer several advantages over chemically synthesized nanoparticles. The biomolecular capping of these nanoparticles enhances their stability and prevents aggregation [11]. Furthermore, SeNPs exhibit unique redox properties, acting as both antioxidants and pro-oxidants depending on factors such as dosage, exposure time, and oxidation state [12, 13]. Notably, the biological activity of SeNPs is size-dependent, with smaller nanoparticles demonstrating superior free radical scavenging ability and reduced oxidative stress compared to larger particles [14].

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The anticancer mechanism of SeNPs involves multiple pathways, including the induction of oxidative stress, mitochondrial dysfunction, and apoptosis. SeNPs are internalized by cancer cells through receptor-mediated endocytosis. The acidic microenvironment of cancer cells promotes the pro-oxidant conversion of SeNPs, leading to the generation of reactive oxygen species (ROS) and subsequent cellular damage. This oxidative stress triggers endoplasmic reticulum (ER) stress and mitochondrial membrane disruption, ultimately leading to the release of mitochondrial proteins and cell death [15, 16].

SeNPs have demonstrated the ability to target tumor cells selectively, leading to increased selenium accumulation in cancerous tissues while minimizing exposure to healthy tissues. This targeted delivery can enhance the therapeutic efficacy of selenium. Additionally, SeNPs have been shown to inhibit angiogenesis, a process essential for tumor growth and metastasis. By disrupting various biological processes, including DNA damage, cell cycle arrest, and apoptosis, SeNPs can effectively combat cancer cells [17].

In this study, Escherichia coli bacteria were employed to biosynthesize selenium nanoparticles from sodium selenite. The synthesized nanoparticles were characterized using various techniques and evaluated for their antioxidant and anticancer properties against multiple cancer cell lines.

Materials and Methods

Bacterial Isolates

A total of 130 samples were collected from various sources (urine, blood, wounds, stool, and vaginal swabs) at different hospitals in Baghdad, Iraq, between September and December 2023. Sixty-five Escherichia coli isolates were identified using the VITEK 2 Compact System.

Biosynthesis and Purification of Selenium Nanoparticles

A modified version of the method described in [18] was employed to synthesize SeNPs. Briefly, a single colony of *E. coli* was inoculated into 5 mL of nutrient broth and incubated overnight at 160 rpm and 37°C. Subsequently, the bacterial culture was transferred to three separate flasks containing 200 mL of nutrient broth and 4 mL of filter-sterilized sodium selenite (Na2SeO3) (Central Drug House, New Delhi, India). The pH of the reaction mixture was adjusted to 8, and the flasks were incubated at 160 rpm and 37°C for 24-48 hours, during which a color change in the solution was observed.

The purification of SeNPs was carried out following the protocols described in [18, 19]. The reaction mixture was centrifuged at 10,000 rpm for 10 minutes to separate the SeNPs. The pellet was washed with 0.9% NaCl solution and resuspended in a pH 8.2 Tris/HCl buffer. The suspension was then subjected to 10 minutes of ultrasonic treatment at 100 W. After filtration through a 0.25 µm Millipore syringe filter, the filtrate was centrifuged at 10,000 rpm for 30 minutes at 4°C to isolate the SeNPs. The purified Se-NPs were resuspended in 5 mL of ultrapure water. To remove any remaining impurities, the nano-precipitate was washed with ethanol and water and dried in a hot air oven at 45-50°C.

Characterization of SeNPs

The UV-Vis spectrophotometer (Spectronic-20, England) was used to analyze the absorption spectra of the synthesized nanoparticles within a wavelength range of 200-1000 nm. Energy-dispersive X-ray spectroscopy (XRD) was employed to determine the elemental composition of the samples. X-ray diffraction (XRD) (Shimadzu, Japan) analysis was conducted to identify the crystalline phases and assess the crystallite size. The morphology of the synthesized SeNPs was examined using scanning electron microscopy (SEM) (Carl Zeiss Ultra 55, Japan). Fourier transform infrared spectroscopy (FTIR) (Shimadzu, Japan) was utilized to identify the functional groups present in the nanoparticles within the wavenumber range of 400-4000 cm^{-1}. The surface morphology and topography of the nanoparticles were characterized using atomic force microscopy (AFM) (Model AA300 Angstrom Advanced Inc., USA).

Antioxidant Activity

The antioxidant activity of the synthesized SeNPs was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, with slight modifications based on the method described in (14). DPPH (Alfa Aesar, Japan) was used to measure the free radical scavenging capacity of the SeNPs. Different concentrations of SeNPs (100, 50, and 25 µg/mL) were prepared in methanol. Added 200 µL of freshly prepared DPPH solution was added to the control well of a 96-well plate, while 200 µL of methanol was added to the blank well. Subsequently, 100 µL of the SeNPs solution was added to each well containing the DPPH solution, bringing the final volume to 200 µL. The plate was incubated in the dark for 30 minutes, and the absorbance was measured at 517 nm. The DPPH solution served as a control.

The percentage of DPPH radical scavenging activity was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Anticancer Activity

The cytotoxicity of SeNPs against human colorectal carcinoma (SW480), human hepatocellular carcinoma (HepG2), and normal human embryonic kidney (HEK293) cell lines was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, based on the method described in [20]. The assay involved three main steps: cell seeding, treatment, and MTT assay.

Briefly, 1×10^4 cells per well were seeded in a 96-well plate and incubated for 24 hours. The cells were then treated with various concentrations of SeNPs (25, 12.5, 6.25, 3.125, 1.56, 0.78, and 0.39 µg/mL) in triplicate. After 48 hours of incubation, 20 µL of MTT solution (0.05 mg/mL) was added to each well, and the plate was incubated

for an additional 4 hours. The formed formazan crystals were dissolved using 100 µL of dimethyl sulfoxide (DMSO, Merck, Germany), and the absorbance was measured at 570 nm. The Viability(%) was determined by applying the following equation:

$$\textbf{Viability (\%)} = \frac{\textbf{optical density of sample}}{\textbf{optical density of control}} \textbf{ x 100\%}$$

The IC_{50} values were determined by statistical analysis measurements using Graph Pad Prism software

Cellular morphological changes before and after treatment with Se-NPs were observed using a phase-contrast inverted microscope and a digital camera (Olympus, Japan).

Statistical Analysis

One-way ANOVA tests were performed using GraphPad Prism 8 to assess significant differences between groups. A p-value of less than 0.05 was considered statistically significant. Data are presented as mean ± standard deviation (SD). All experiments were performed in triplicate.

Results

Escherichia coli Isolates

Approximately 65 Escherichia coli isolates were identified using a light compound microscope. The isolates were characterized as rod-shaped, Gram-negative bacteria that exhibited a green metallic sheen on Eosin Methylene Blue agar and fermented lactose on MacConkey agar [21-23]. The identification of the isolates was further confirmed using the VITEK 2 Compact System 2 (ID-GNB) cards.

Biosynthesis and Production of SeNPs

The biosynthesis of SeNPs was indicated by the development of a red coloration in the reaction mixture (bacterial culture with Na_2SeO_3) at pH 8 and 160 rpm, in contrast to the control (nutrient broth only). A less intense red color was observed at a 25 mM concentration of Na_2SeO_3, while a more intense color was observed at 50 and 100 mM concentrations. Among (30) isolates, isolate number 10 exhibited the highest SeNP production, as determined by absorbance measurements (Figure 1).

Table 1. Antioxidant Activity of Se-NPs

Concentrations of SeNPs (µg/ml)	Antioxidant %
100 µg/ml	80.21
50 µg/ml	72.57
25 µg/ml	69.07

Characterization of SeNPs

UV-Vis Spectrum Analysis

The UV-Vis spectrum of the synthesized SeNPs, shown in Figure 2, exhibited a well-defined absorption peak at 266 nm, indicating the successful synthesis of SeNPs by *E. coli*.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of the SeNPs, shown in Figure 3, revealed the presence of various functional groups. The peaks at 3341 cm^{-1} and 3271 cm^{-1} corresponded to -OH groups in carbohydrates and amines. The peaks at 2966 cm^{-1}, 2928 cm^{-1}, and 2873 cm^{-1} were attributed to methylene groups and C-C bonds in unsaturated compounds. The peak at 1576 cm^{-1} represented the C-O group (amide I, II), while the peak at 1298 cm^{-1} corresponded to amide III bands. The peak at 1150 cm^{-1} was assigned to C-O-C bonds in carbohydrates, and the peak at 1045 cm^{-1} was attributed to phenol and alcoholic compounds. The peak at 879 cm^{-1} was assigned to aromatic C-H bending, and the peak at 569 cm^{-1} corresponded to the Se-O stretching vibration. These findings are consistent with previous studies [24, 25].

X-Ray Diffraction Analysis (XRD)

The XRD pattern of the SeNPs, shown in Figure 4, revealed sharp and narrow peaks, indicating the crystalline nature of the nanoparticles. The peaks at 2θ values of 23.772°, 46.00773°, 66.828°, and 75.744° corresponded to the (101), (110), (200), and (210) crystal planes, respectively, which matched the standard JCPDS card

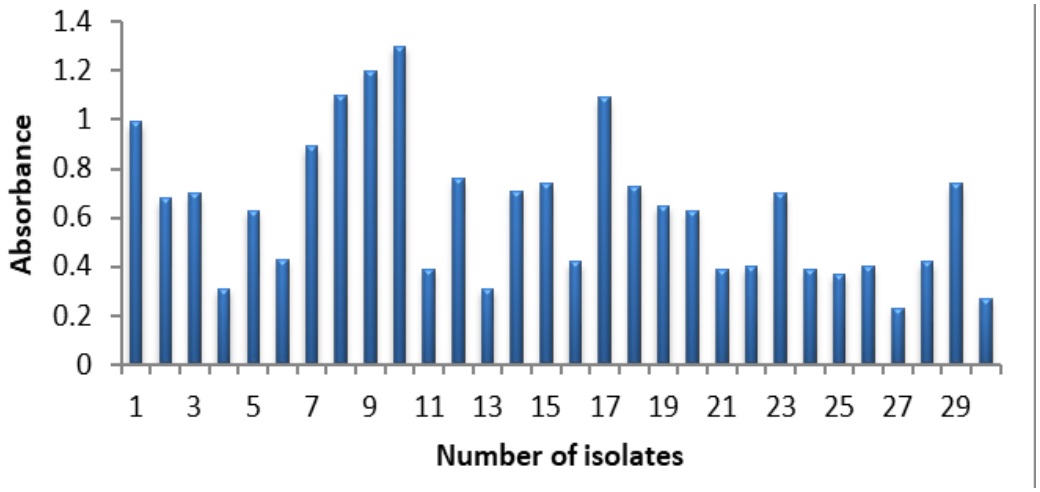

Figure 1. Values of the SeNPs' Absorbance Produced by *E. coli* Isolates

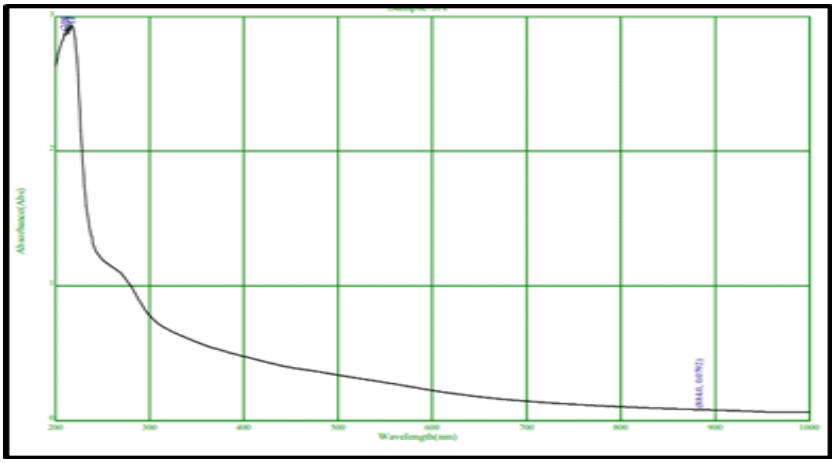

Figure 2. UV-Vis Spectroscopy of SeNPs

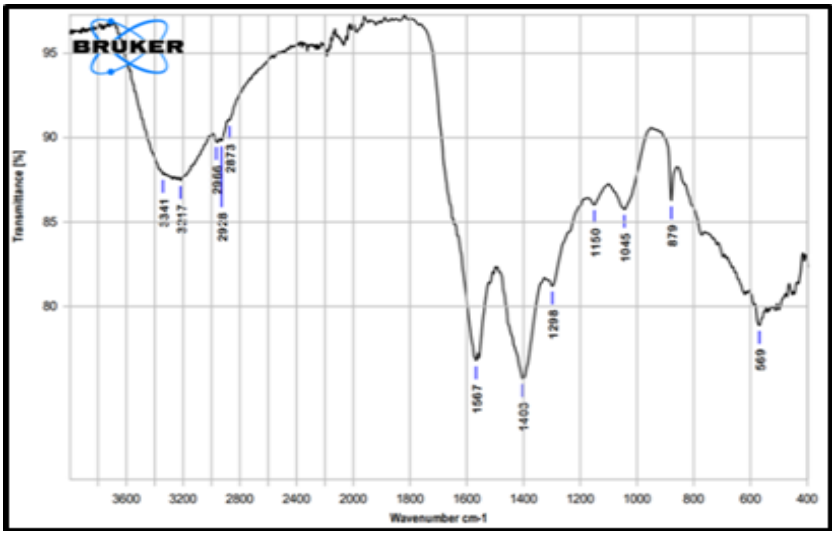

Figure 3. FTIR Spectra of SeNPs

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Scanning Electron Microscopy (SEM) Analysis

SEM analysis revealed that the bacteria successfully produced spherical SeNPs with a size range of 39.53 to 50.71 nm (Figure 5).

Atomic Force Microscopy (AFM)

AFM analysis confirmed the spherical morphology and surface topography of the SeNPs. Figure (6-A) shows the

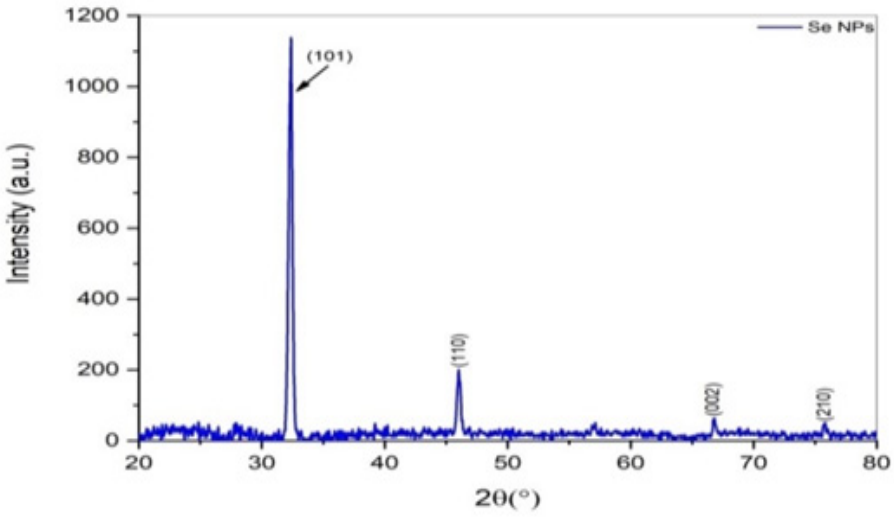


Figure 4. XRD Analysis of Selenium Nanoparticles

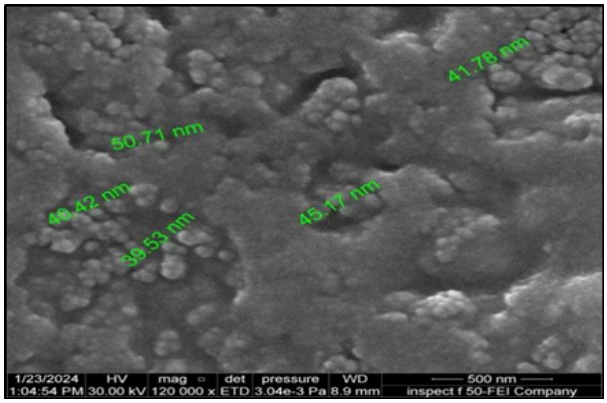
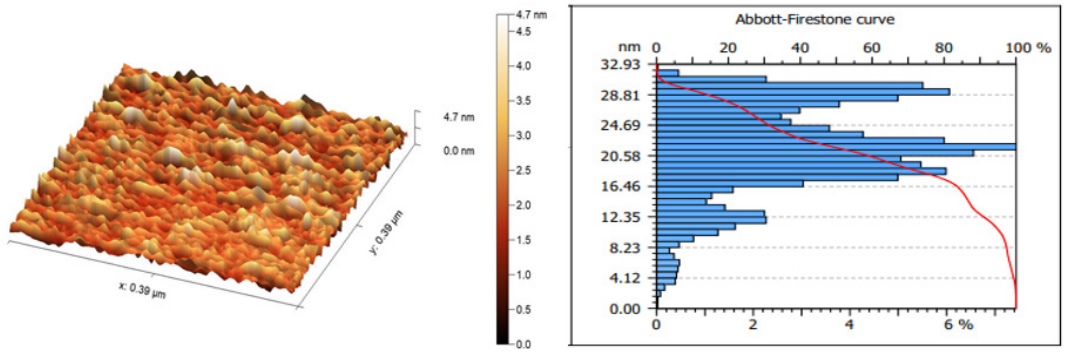


Figure 5. SEM Iimage of Se-NPs Nanoparticles Synthesized by *E. coli*



(A) **(B)**

Figure 6. (A) The 3D image of Se-NPs nanoparticles synthesized by *E. coli.* (B)Abbott-Firestone curve explain surface roughness of SeNPs.

spherical shape of the nanoparticles, while Figure (6-B) indicates an average diameter of 21.96 nm and an average roughness of 3.11 nm.

Antioxidant Activity

As shown in Table 1 and Figure 7, the synthesized SeNPs exhibited significant antioxidant activity, effectively scavenging free radicals at concentrations of 100, 50, and 25 µg/mL. The highest scavenging activity was observed at 100 µg/mL, with a value of 80.21%.

Anticancer Activity

The cytotoxic effects of SeNPs on human colorectal carcinoma (SW480), human hepatocellular carcinoma (HepG2), and normal human embryonic kidney (HEK293) cell lines were evaluated using the MTT assay. As shown in Table 2, Figures (8) and Supplementary Figure 1, SeNPs exhibited significant cytotoxicity against both

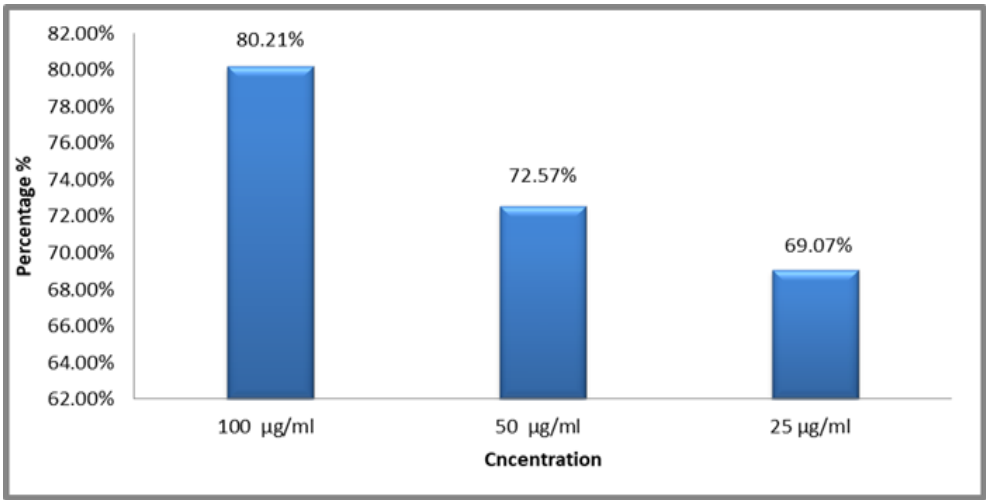


Figure 7. Percentage of Antioxidant Activity of SeNPs

Table 2. Effect of Different Concentrations of SeNPs on Viability (%) of SW480 and HPG2 Cancer Cell Lines

Concentrations (V/V)%	Average Viability (%) of SW480	Average Viability(%) of HPG2	Average Viability(%) of (HEK293)
25	12.65±6.68	13.81±3.46	97.25
12.5	11.36±2.99	20.40±5.74	98.19
6.25	36.82±4.46	40.44±4.40	99.21
3.125	60.64±5.54	64.91±7.96	100
1.56	77.42±4.19	80.85±5.19	100
0.78	89.83±6.80	92.13±8.65	100
0.39	102.11±7.16	98.69 ±9.1	100
Control	100±6.06	100 ±7.34	100

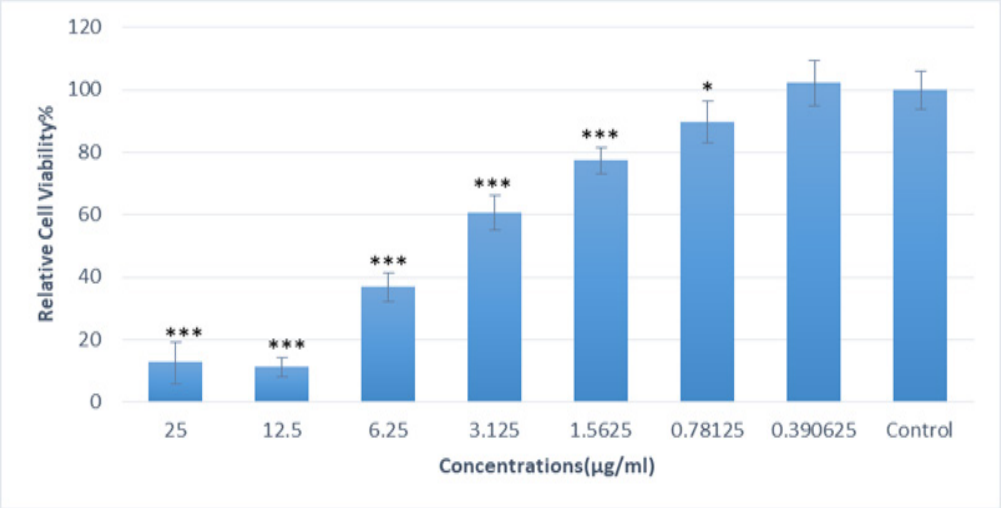


Figure 8. MTT Cell Proliferation assay of SeNP Treated Cells of SW480 Cell Line for 48 hr. Each value is the mean ± SD ($p < 0.05$). One-way ANOVA .Comparison with control, *** p-level < 0.001 , *p- level<0.05.

SW480 and HepG2 cells in a concentration-dependent manner. The IC_{50} values for SW480 and HepG2 cells were determined to be 3.9 and 4.5 µg/mL, respectively Supplementary Figures (3, 4).

In contrast, SeNPs showed negligible cytotoxicity towards normal HEK293 cells (Supplementary Figure 2. These findings suggest that SeNPs selectively target cancer cells, inducing cell death without affecting normal cells.

Morphological changes were observed in SW480 and HepG2 cells treated with SeNPs for 48 hours, as shown in Supplementary Figures 5 and 6, respectively. These changes, compared to the control cells, indicate the cytotoxic effects of SeNPs on cancer cells.

Discussion

In this study, *E. coli* was successfully employed to mediate the biosynthesis of SeNPs. The addition of sodium selenite to the *E. coli* culture resulted in a distinct red coloration, indicating the formation of SeNPs. The total selenium content in the SeNPs increased with higher concentrations of sodium selenite, which aligns with previous findings [26, 27]. The development of a red color within 24 hours suggested the efficient reduction of selenite to selenium nanoparticles by the bacteria. This observation is consistent with the ability of bacteria to transform inorganic substances, However, the method for producing selenium nanoparticles depends on the reaction conditions as reported in earlier studies [28].

UV-Vis spectroscopy analysis revealed a characteristic absorption peak at 266 nm, attributed to the surface plasmon resonance (SPR) of the synthesized SeNPs. This finding is in agreement with previous studies [11, 29]. FTIR spectroscopy confirmed the presence of functional groups involved in the bioreduction process, including amide groups that likely played a role in stabilizing and reducing the metal ions. These results are consistent with previous studies [26, 30]. The presence of proteins may have acted as capping agents to stabilize the SeNPs, as reported in [29].

XRD analysis confirmed the crystalline nature of the synthesized SeNPs, The formation of a narrow peak with a Bragg's angle indicates that the particles are crystalline [31]. The hexagonal structure of the SeNPs, as observed in the SEM analysis, is characteristic of metallic selenium nanocrystals. The size and shape of the biogenic NPs were further confirmed by SEM analysis [32], which aligns with previous findings [11]. AFM analysis provided information about the surface roughness and three-dimensional morphology of the SeNPs, and the size measurements were consistent with previous studies [33, 34].

In the present study, the antioxidant activity of SeNPs increased with increasing concentration. The highest scavenging activity was observed at 100 µg/mL, while a decrease in activity was noted at 50 µg/mL and 25 µg/mL. The pro- and antioxidant properties of SeNPs are influenced by factors such as dosage, duration of exposure, and oxidation state, as reported in [12]. Additionally, the size of SeNPs plays a crucial role in their antioxidant activity. Smaller SeNPs exhibit enhanced free radical scavenging ability compared to larger particles, leading to reduced oxidative stress, as reported in [12, 35, 36].

Selenium, an essential component of antioxidant enzymes like thioredoxin reductase and glutathione peroxidase, primarily exerts its biological effects through its antioxidant properties. These enzymes play a vital role in regulating the levels of reactive oxygen and nitrogen species (ROS and RNS) generated during metabolic processes, thereby preventing cellular damage and oxidative stress [8].

The results of the anticancer activity assessment demonstrated that SeNPs exhibited significant cytotoxicity against cancer cell lines (SW480 and HepG2) while having minimal impact on normal cells (HEK293). Nanoparticles within the size range of 10-100 nm possess the ability to penetrate tumor tissues and selectively target cancer cells, sparing healthy cells. This phenomenon, known as the enhanced permeation and retention (EPR) effect, is attributed to the larger pore sizes of blood capillaries in tumor tissues compared to those in healthy tissues [37].

One of the primary mechanisms underlying the anticancer activity of SeNPs is the induction of oxidative stress. SeNPs can elevate intracellular levels of reactive oxygen species (ROS) to lethal levels, leading to damage to DNA, proteins, and cell membranes, ultimately resulting in cell death. Excessive ROS production can disrupt the mitochondrial membrane potential (MMP), triggering the activation of the intrinsic apoptotic pathway. MMP is crucial for maintaining cellular function, and its loss can lead to the release of cytochrome c and other pro-apoptotic factors, initiating a cascade of events culminating in cell death [38, 39]. Also SeNPs can stimulate programmed cell death (apoptosis) by activating intracellular signaling pathways such as p53 and caspase pathways. SeNPs have demonstrated promising antitumor activity against HepG2 and SW480 cells. Additionally, the normal cells exhibit slight cytotoxic responses to SeNPs due to their more robust antioxidant defense systems, including enzymes like glutathione peroxidase and superoxide dismutase. These defenses mitigate ROS damage, maintaining cellular integrity [40, 41].

Morphological analysis of human colorectal carcinoma (SW480) and human hepatocellular carcinoma (HepG2) cells revealed significant changes after treatment with SeNPs for 48 hours. As depicted in Supplementary Figures 5 and 6, untreated control cells exhibited a normal, adherent morphology and reached approximately 95-100% confluence. In contrast, SeNP-treated cells displayed severe morphological alterations, including loss of the typical spindle shape, detachment from the surface, and inhibition of cell growth, leading to reduced cell density. These findings align with previous studies, such as [17], which reported similar dramatic morphological changes, including the complete loss of normal spindle shape.

In conclusion, the primary objective of this research was to develop a simple, reliable, cost-effective, and eco-friendly method for producing uniformly sized SeNPs. This goal was achieved through the biosynthesis of SeNPs using Escherichia coli culture supernatant and sodium selenite. The study further aimed to evaluate the antioxidant and anti-proliferative activities of the synthesized SeNPs.

The results demonstrated that SeNPs exhibited enhanced antioxidant activity with increasing concentration, effectively scavenging DPPH free radicals. Additionally, SeNPs displayed significant anti-proliferative effects against SW480 and HepG2 cancer cell lines, due to its multiple mechanisms including selective targeting of cancer cells, reduction of oxidative stress, and stimulation of immunity. Importantly, SeNPs were found to be non-cytotoxic to normal cells. Overall, the findings of this study suggest that selenium nanoparticles hold promise as a potential therapeutic agent for cancer treatment, exhibiting potent anti-tumor activity at low concentrations against HepG2 and SW480 cancer cell lines.

Author Contribution Statement

R. M.Y, N.H.Z authors contributed equally to all stages of the research, from study design to the final manuscript.

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Approval

This manuscript is part of a master's student's research approved by the College of Science, Mustansiriyah University.

Ethical Declaration

This study was ethically approved by Ethics Committee at Mustansiriyah University\ College of Science–Department of Biology reviewed and approved the study under code number Ref: BCSMU\0923\ 0005B authorization ID.

Conflict of Interest:

The authors declare no competing financial interests or personal relationships that could have influenced the research reported in this paper.

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