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Green Synthesis of Selenium Nanoparticles Using *Onopordum acanthium* Extract and Their Cytotoxic, Apoptotic, and Antioxidant Effects on Gastric Cancer Cells

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Abstract

Background and Aim: Gastric cancer remains a prevalent malignancy with high mortality and limited treatment options. Recent interest has turned to green-synthesized selenium nanoparticles (SeNPs) as natural and biocompatible agents with anticancer potential. This study aimed to synthesize SeNPs using *Onopordum acanthium* extract and evaluate their effects on cell growth, cell death, and oxidative balance in MKN-45 gastric cancer cells.

Materials and Methods: SeNPs were synthesized using a green method with hydroalcoholic extract of *O. acanthium*. Nanoparticles were characterized by XRD, SEM, EDX, FTIR, and DLS. Their biological activity was assessed using MTT assay, measurement of reactive oxygen species (ROS) by flow cytometry, and gene expression analysis of BAX and BCL-2 using qRT-PCR.

Results: Characterization confirmed spherical SeNPs (40–60 nm) with high purity. The MTT assay showed dose-dependent toxicity, with IC_{50} values of 130 µg/mL for MKN-45 and 150 µg/mL for HEK293 cells, suggesting selective effects on cancer cells. Treated MKN-45 cells showed increased expression of BAX and reduced BCL-2, indicating mitochondrial apoptosis. ROS levels dropped significantly from 52.2% to 4.6%, and mean fluorescence intensity decreased from 59.31 to 7.97, confirming reduced oxidative stress.

Conclusion: Green-synthesized SeNPs showed dual activity by reducing harmful oxygen radicals and triggering cell death in gastric cancer cells. The use of *O. acanthium* extract improved nanoparticle stability and anticancer properties. These findings suggest that SeNPs may serve as safe and eco-friendly supportive treatments for gastric cancer. More studies are needed to improve their effectiveness and explore their use with standard therapies.

Keywords: Selenium nanoparticles, Apoptosis, ROS, Gastric cancer, Green synthesis, Anticancer therapy, Oxidative stress

1. Introduction

Gastric cancer is a prevalent and aggressive malignancy, especially common in Asian populations (Sung et al., 2021; Smyth et al., 2016). It often shows resistance to conventional treatments such as chemotherapy and radiotherapy. Impaired apoptosis and elevated reactive oxygen species (ROS) levels are key contributors to its progression, causing damage to DNA, proteins, and lipids (Rawla and Barsouk, 2019; Polk and Peek, 2010). These challenges highlight the urgent need for alternative therapeutic strategies, with nanoparticle-based treatments drawing growing interest.

Selenium nanoparticles (SeNPs), particularly those synthesized through green methods, have demonstrated notable antioxidant and pro-apoptotic properties. Green synthesis using plant extracts offers an eco-friendly and biocompatible approach with minimal toxic by-products. However, defining their safe dosage range is crucial to avoid cytotoxic effects (Thiruvengadam et al., 2018; Zhang et al., 2008).

Studies have shown that SeNPs can induce apoptosis in gastric cancer cell lines such as MKN-45 by enhancing ROS levels and activating apoptotic pathways (Wang et al., 2022; Torabi et al., 2024; Torabi, 2024; Alavi et al., 2023). For instance, SeNPs derived from black ginger have been found to inhibit the PI3K/Akt/mTOR pathway, increase caspase activity and BAX expression, and suppress BCL-2, promoting cancer cell death (Torabi, 2024; Zhang et al., 2023).

Despite their promise, concerns remain about the safety and consistency of SeNPs. Some studies report cytotoxicity and variable outcomes depending on synthesis methods and conditions (Torabi, 2024; Liu et al., 2022; Silva et al., 2023). In certain cases, increased ROS alone did not effectively induce apoptosis, raising concerns

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about reproducibility and clinical application (Zhang et al., 2023; Li et al., 2023; Singh et al., 2023).

Comparative analyses indicate that although SeNPs have cytotoxic potential, their efficacy is generally lower than that of standard chemotherapy drugs like cisplatin, suggesting their role may be more suitable in combination therapies (Keshtmand et al., 2023). Given the high global mortality of gastric cancer and limitations of current therapies, further research into SeNPs is well-justified (Zheng et al., 2023; Abdelsalam et al., 2021; Zeng et al., 2020; Zhang et al., 2023; Zhao et al., 2024).

Onopordum acanthium (Scotch thistle), traditionally valued for its anti-inflammatory and anticancer properties, has recently been shown to possess antiproliferative effects, highlighting its therapeutic potential (Garsiya et al., 2019). While SeNPs have shown promise in inducing apoptosis in gastric cancer cells like MKN-45 (Torabi, 2024), the use of O. acanthium extract for their synthesis remains unexplored. Considering its rich phytochemical content and the increasing demand for eco-friendly therapies, this study aims to synthesize SeNPs using O. acanthium extract and evaluate their cytotoxic and apoptotic effects on MKN-45 cells. The findings may contribute to the development of sustainable nanoparticle-based cancer treatments.

2. Materials and Methods

2.1. Preparation of Onopordum acanthium Hydroalcoholic Extract:

Fresh or dried leaves and flowers of *Onopordum acanthium* (cotton thistle) were collected, thoroughly washed with deionized water to remove surface impurities, and dried at room temperature. The dried plant material was chopped into small pieces, ground, and soaked in 70% ethanol at a ratio of 1:10 (w/v). The mixture was left at room temperature for 24 hours with occasional stirring to enhance extraction. No pH adjustment was applied during extraction. The extract was then filtered using Whatman No. 1 filter paper, and the filtrate was stored at 4°C for subsequent experiments.

2.2. Green Synthesis of Selenium Nanoparticles (SeNPs):

To synthesize SeNPs, a 1 mM aqueous solution of sodium selenite (Na_2SeO_3) was prepared. In a reaction flask, 10 mL of the hydroalcoholic extract of *Onopordum acanthium* was added to 50 mL of sodium selenite solution. The mixture was stirred gently at room temperature or heated to approximately 50°C for 1–2 hours to facilitate reduction and nanoparticle formation. A visible color change confirmed the reaction progress. The resulting SeNPs were collected via high-speed centrifugation, washed several times with deionized water to remove residual reactants and impurities, and then air-dried at room temperature.

2.3. Characterization of SeNPs:

The physicochemical and morphological properties of the synthesized SeNPs were characterized using the following techniques:

The physicochemical and morphological properties of the biosynthesized selenium nanoparticles (SeNPs) were comprehensively characterized employing a suite of sophisticated analytical techniques. X-ray diffraction (XRD) analysis substantiated the crystalline structure of the nanoparticles. Surface morphology was meticulously examined via scanning electron microscopy (SEM), revealing uniform particle distribution and structural coherence. Elemental composition was elucidated using energy dispersive X-ray spectroscopy (EDX), confirming the presence of selenium alongside biomolecular constituents originating from the Onopordum acanthium extract. Furthermore, Fourier transform infrared spectroscopy (FTIR) was utilized to identify functional groups on the nanoparticle surface and to elucidate the interactions between phytochemicals within the extract and the SeNPs, indicative of effective capping and stabilization by bioactive compounds.

2.4. Cell Viability Assay (MTT Assay):

MKN45 human gastric carcinoma cells and HEK293 normal human embryonic kidney cells were obtained from the Pasteur Institute of Iran (Tehran, Iran). Cells were initially cultured in T-25 flasks containing RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin, and maintained at 37°C in a humidified atmosphere with 5% CO_2. Upon reaching 70–80% confluency, the cells were harvested and seeded into 96-well plates at a density of 5,000–10,000 cells per well.

Cells were then treated with various concentrations of SeNPs for 24 or 48 hours. Following treatment, 20 µL of MTT solution (0.5 mg/mL) was added to each well and incubated for 3–4 hours at 37°C. The resulting formazan crystals were dissolved using dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated relative to untreated control wells, and IC_{50} values were determined accordingly.

2.5. Gene Expression Analysis by Real-Time PCR:

To assess apoptosis-related gene expression, total RNA was extracted from treated and control cells using Trizol reagent. RNA concentration and purity were determined spectrophotometrically at 260/280 nm. Complementary DNA (cDNA) was synthesized from 1–2 µg of total RNA using a commercial cDNA synthesis kit.

Quantitative real-time PCR (qPCR) was performed using SYBR Green Master Mix and gene-specific primers for BAX and BCL-2. GAPDH or β-actin was used as the internal reference gene. Primer design and validation were conducted using Primer3 and OligoAnalyzer tools.

PCR thermal cycling conditions:

– Initial denaturation at 95°C for 10 min
– 40 cycles of:
  - Denaturation at 95°C for 15 s
  - Annealing at 60°C for 30 s
  - Extension at 72°C for 30 s

Relative gene expression was analyzed using the 2^−ΔΔCt method.

2.6. Measurement of Intracellular ROS:

Intracellular ROS levels were measured using the fluorescent probe DCFH-DA (2′,7′-dichlorodihydrofluorescein diacetate). MKN45 and HEK293 cells were seeded in 6-well plates and treated with SeNPs for 24 hours. After treatment, cells were incubated with 10 µM DCFH-DA at 37°C for 30 minutes

in the dark. The excess dye was removed by washing with PBS.

Fluorescence intensity was measured using a microplate reader (excitation: 485 nm, emission: 530 nm) or visualized under a fluorescence microscope. Increased fluorescence indicated elevated ROS levels in response to SeNP treatment.

2.7. Statistical Analysis:

All experiments were performed in triplicate (n = 3 biological replicates), and results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way ANOVA followed by Tukey's post hoc test for multiple group comparisons, and independent t-test was applied for comparing two groups. A p-value of less than 0.05 was considered statistically significant.

Data analysis and graph plotting were performed using GraphPad Prism (version 9.0)

3. Results

3.1. Comprehensive Characterization of Green-Synthesized Selenium Nanoparticles Using Onopordum acanthium Extract

3.1.1. X-ray diffraction (XRD) analysis:

The X-ray diffraction (XRD) analysis of selenium nanoparticles synthesized via a green approach using *Onopordum acanthium* extract demonstrated distinct diffraction peaks at 2θ values of approximately 23.5°, 28.7°, 31.2°, 45.1°, 53.4°, 61.2°, and 75.3°. These peaks correspond to the (100), (101), (110), (102), (201), (113), and (210) crystalline planes of trigonal selenium, as referenced by the standard JCPDS card No. 06-0362. The intensity and sharpness of the diffraction peaks indicate a high degree of crystallinity. Moreover, the absence of additional unidentified peaks confirms the phase purity of the synthesized nanoparticles. These results validate that the phytochemical components in the O. acanthium extract not only acted as reducing and stabilizing agents but also facilitated the formation of well-defined crystalline Se nanoparticles.

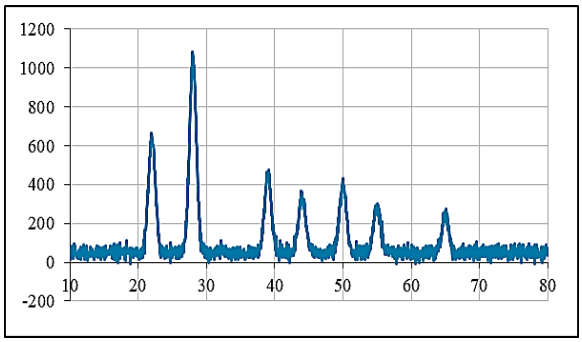

Figure 1. X-ray diffraction (XRD) pattern of selenium nanoparticles synthesized using *Onopordum acanthium* extract. The distinct diffraction peaks at approximately 23.5°, 28.7°, 31.2°, 45.1°, 53.4°, 61.2°, and 75.3° (2θ) correspond to the crystalline planes of trigonal selenium (JCPDS No. 06-0362), indicating the formation of highly crystalline Se nanoparticles.

3.1.2. Scanning Electron Microscopy (SEM)

The surface morphology of the biosynthesized selenium nanoparticles was examined using scanning electron microscopy (SEM). The SEM micrographs revealed that the nanoparticles were predominantly spherical in shape with moderate uniformity in size distribution. Slight agglomeration was observed, which is commonly attributed to the high surface energy of nanoscale materials and the presence of bioorganic stabilizing agents from the *Onopordum acanthium* extract. The particle size appeared to be within the nanometer range, consistent with the crystallite size inferred from XRD analysis. The observed morphology supports the effectiveness of the green synthesis method in producing discrete nanoparticles with biologically active surface coatings. These coatings, as confirmed by FTIR analysis, may help stabilize the nanoparticles in aqueous media and enhance their compatibility for biomedical applications. The average particle size, as estimated from SEM images, ranged between 40 and 60 nm.

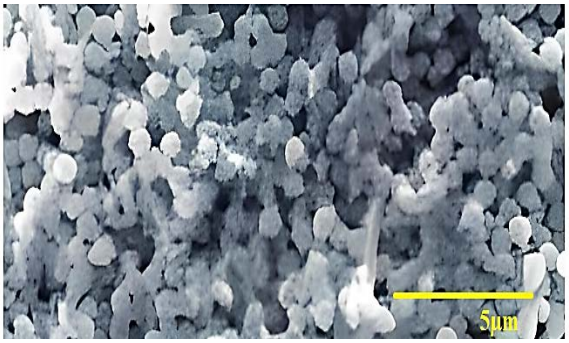

Figure 2. Scanning electron microscopy (SEM) image of selenium nanoparticles synthesized using *Onopordum acanthium* extract. The image shows predominantly spherical nanoparticles with relatively uniform size distribution and slight agglomeration, indicating the influence of phytochemicals in controlling the morphology during green synthesis.

3.1.3. Energy-Dispersive X-ray Spectroscopy (EDX):

The elemental profile of the synthesized selenium nanoparticles was investigated using energy-dispersive X-ray spectroscopy (EDX). The spectrum (Figure 3) exhibits intense peaks corresponding to selenium, with Se Kα and Se Kβ appearing around 1.37 keV and 11.22 keV, respectively. These results confirm the successful formation of selenium nanoparticles as the major elemental component.

In addition to selenium, the EDX spectrum reveals signals for carbon (C), oxygen (O), sodium (Na), chlorine (Cl), potassium (K), and calcium (Ca). The presence of C and O is attributed to organic molecules from the *Onopordum acanthium* extract, suggesting that phytochemicals act as both reducing and capping agents. Trace amounts of Na, Cl, K, and Ca may arise from natural mineral contents in the extract or residues from the synthesis medium. No signals related to heavy metals or undesired contaminants were observed, supporting the elemental purity of the biosynthesized selenium nanoparticles.

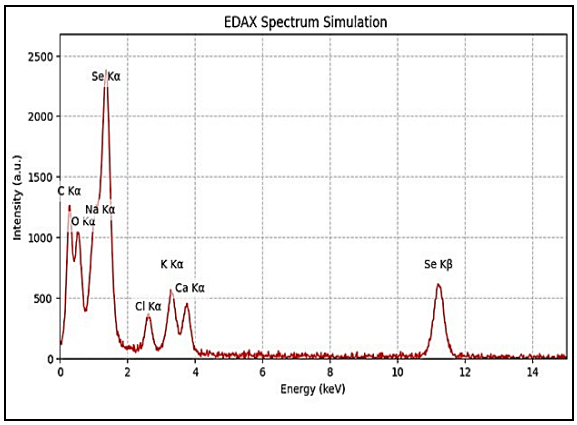

Figure 3. Energy-dispersive X-ray spectroscopy (EDX) spectrum of selenium nanoparticles synthesized using *Onopordum acanthium* extract. The spectrum shows dominant peaks of selenium (Se Kα at ~1.37 keV and Se Kβ at ~11.22 keV), confirming the elemental composition of the nanoparticles. Additional peaks of carbon (C), oxygen (O), sodium (Na), chlorine (Cl), potassium (K), and calcium (Ca) likely originate from phytochemical residues present in the plant extract, which may also serve as capping and stabilizing agents during nanoparticle synthesis.

3.1.4. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR analysis was performed to identify the functional groups involved in the reduction and stabilization of selenium nanoparticles synthesized via *Onopordum acanthium* extract. The spectrum revealed a broad band at 3469 cm^{-1} and 3411 cm^{-1}, corresponding to O–H and N–H stretching vibrations, suggesting the presence of hydroxyl and amine groups derived from phenolic and proteinaceous compounds.

The absorption at 2925 cm^{-1} is attributed to aliphatic C–H stretching, while the intense peak at 1639 cm^{-1} corresponds to C=O stretching vibrations of amide I, indicating the involvement of proteins in nanoparticle stabilization. The bands observed at 1413 cm^{-1} and 1060 cm^{-1} can be assigned to C–N stretching vibrations and C–O or C–OH bending, typically associated with flavonoids and polysaccharides.

Importantly, the peaks at 811 cm^{-1} and 622 cm^{-1} may correspond to Se–O or Se–C bond vibrations, confirming the interaction between selenium and organic capping agents. These findings demonstrate that phytochemicals present in O. acanthium act not only as reducing agents but also as stabilizers by binding to the nanoparticle surface, thereby enhancing their biocompatibility and colloidal stability.

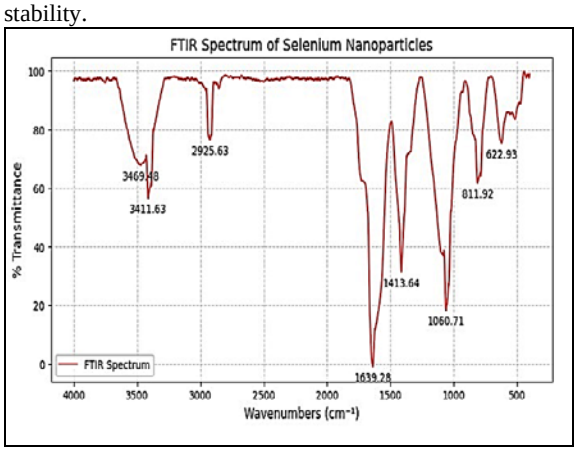

Figure 4. FTIR spectrum of selenium nanoparticles synthesized using *Onopordum acanthium* extract. The characteristic absorption bands at 3469, 3411, 2925, 1639, 1413, 1060, 811, and 622 cm^{-1} correspond to O–H, N–H, C–H, C=O, C–N, and Se–O or Se–C vibrations, indicating the presence of biomolecules from the plant extract acting as capping and stabilizing agents.

3.2. Cytotoxic and Antiproliferative Effects of Biosynthesized Selenium Nanoparticles on MKN45 Gastric Cancer and HEK293 Cells

3.2.1. Antiproliferative Activity on MKN45 Cells:

The cytotoxicity of biosynthesized selenium nanoparticles was evaluated using the MTT assay in MKN45 gastric cancer cells. As shown in Figure 5, cell viability decreased significantly with increasing nanoparticle concentration. The reduction in metabolic activity, as measured by MTT reduction, indicates compromised mitochondrial function and potential cell death.

At the highest concentration tested (500 µg/mL), viability dropped to ~25%, while lower concentrations exhibited a more gradual effect. A statistically significant reduction (***$p < 0.001$) was observed starting from 31.25 µg/mL, suggesting that the green-synthesized SeNPs exert substantial cytotoxic effects even at moderate doses. These results corroborate the IC_{50} value (130 µg/mL) and support the potential use of SeNPs in anticancer strategies targeting gastric carcinoma.

Table 1. MTT assay results showing the relative viability of MKN45 gastric cancer cells after 24 hours of treatment with varying concentrations of selenium nanoparticles synthesized using *Onopordum acanthium* extract.

	SeNPs synthesized using *Onopordum acanthium* extract (µg/ml)							
3.2.2. Groups	Control	7.8125	15.625	31.25	62.5	125	250	500
Mean±SD	100±8.3	105.4±7.7	96.9±4.3	77.6±8.7	71.8±7.8	52.8±8.7	33.3±6.5	20.9±8.5
P	-	P > 0.05 N.S	P > 0.05 N.S	P=0.000 < 0.001	P=0.000 < 0.001	P=0.000 < 0.001	P=0.000 < 0.001	P=0.000 < 0.001

N.S indicates no significant difference. P-values are reported in comparison to the control group.

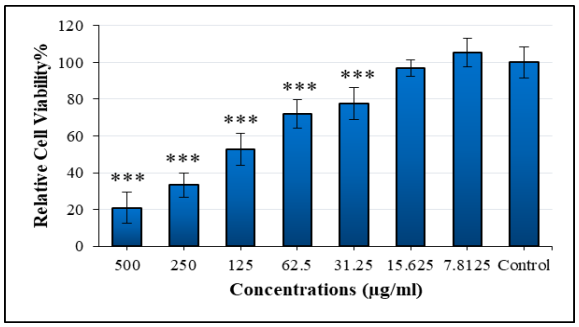

Figure 5. MTT assay results showing the relative viability of MKN45 gastric cancer cells after 24 hours of treatment with varying concentrations of selenium nanoparticles synthesized using *Onopordum acanthium* extract. A significant, dose-dependent decrease in cell viability was observed, with the most pronounced cytotoxic effects at concentrations ≥ 31.25 µg/mL (***$p < 0.001$). Values represent mean ± SD of three independent experiments.

The cytotoxic potential of the biosynthesized selenium nanoparticles was evaluated against MKN45 gastric cancer cells using the MTT assay. As shown in Figure 6, cell viability significantly decreased with increasing nanoparticle concentration in a dose-dependent manner. The IC_{50} value was determined to be 130 µg/mL, indicating moderate cytotoxicity. This suggests that SeNPs synthesized with *Onopordum acanthium* extract exert antiproliferative effects on gastric cancer cells, likely mediated through bioactive phytochemicals acting synergistically with selenium. These findings highlight the potential application of green-synthesized SeNPs in cancer therapeutics.

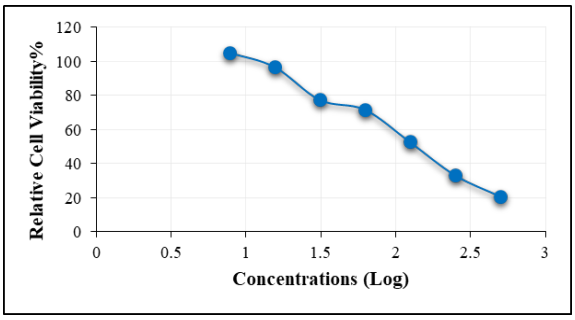

Figure 6. Dose–response curve of selenium nanoparticles synthesized using *Onopordum acanthium* extract against MKN45 gastric cancer cells, determined by MTT assay after 24 hours of treatment. Cell viability decreased in a dose-dependent manner, and the half-maximal inhibitory concentration (IC_{50}) was calculated as 130 µg/mL. The x-axis represents the logarithmic concentration of SeNPs (µg/mL), and the y-axis shows relative cell viability (%). Data points represent mean values from triplicate experiments.

3.2.3. Cytotoxicity Assessment on HEK293 Cells:

The cytotoxic effects of green-synthesized selenium nanoparticles on HEK293 human embryonic kidney cells were further evaluated using the MTT assay. As depicted in Figure 7, a dose-dependent reduction in cell viability was observed. At higher concentrations (≥ 62.5 µg/mL), a statistically significant decline in viability was recorded (***$p < 0.001$), whereas lower doses (≤ 31.25 µg/mL) did not elicit marked cytotoxicity, maintaining viability above 85%.

These results support the relative biocompatibility of SeNPs with normal cells at low concentrations, while still demonstrating cytotoxic potential at elevated doses. When compared with MKN45 cancer cells, the differential response suggests potential selectivity of SeNPs toward malignant cells.

Table 2. MTT assay results showing the relative viability of HEK293 gastric cancer cells after 24 hours of treatment with varying concentrations of selenium nanoparticles synthesized using *Onopordum acanthium* extract.

	SeNPs synthesized using *Onopordum acanthium* extract (µg/ml)						
Groups	Control	15.625	31.25	62.5	125	250	500
Mean±SD	100±6.7	102.5±2.1	92.5±1.7	71.3±3.9	67.3±8.1	30.03±7.8	19.9±5.2
P	-	P > 0.05 N.S	P > 0.05 N.S	P=0.000 < 0.001	P=0.000 < 0.001	P=0.000 < 0.001	P=0.000 < 0.001

N.S indicates no significant difference. P-values are reported in comparison to the control group.

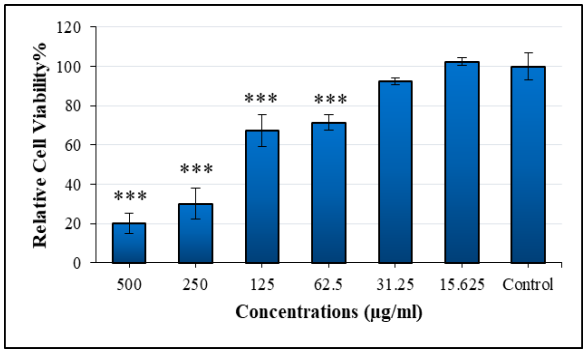

Figure 7. Effect of selenium nanoparticles synthesized via *Onopordum acanthium* extract on the viability of HEK293 cells, assessed by MTT assay after 24 hours of treatment. A significant dose-dependent decrease in cell viability was observed at concentrations ≥ 62.5 µg/mL (***$p < 0.001$). Data are expressed as mean ± SD of three independent experiments.

The biocompatibility of biosynthesized selenium nanoparticles was assessed on non-cancerous HEK293 cells using the MTT assay. As shown in Figure 8, a gradual decrease in cell viability was observed with increasing nanoparticle concentration, with an IC_{50} value of 150 µg/mL. Compared to the IC_{50} in MKN45 gastric cancer cells (130 µg/mL), SeNPs exhibited slightly higher cytotoxicity toward cancerous cells, suggesting a degree of selective toxicity. This distinction is critical for evaluating the therapeutic window of the nanoparticles and their potential as targeted anticancer agents.

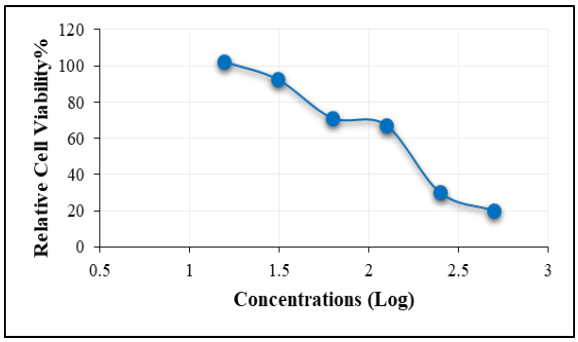

Figure 8. Dose–response curve of selenium nanoparticles synthesized using *Onopordum acanthium* extract on HEK293 human embryonic kidney cells. Cell viability was assessed via MTT assay after 24 hours of exposure. The IC_{50} value was determined to be 150 µg/mL, indicating relatively lower cytotoxicity toward non-cancerous cells. Data are presented as mean values from triplicate experiments.

3.3. Induction of Apoptosis by Selenium Nanoparticles:

Treatment of gastric cancer cells with green-synthesized selenium nanoparticles led to a significant upregulation of the pro-apoptotic BAX gene and a marked downregulation of the anti-apoptotic BCL-2 gene. qPCR data revealed decreased ΔCT values for BAX and increased ΔCT values for BCL-2 in the treated group, indicating an elevated BAX/BCL-2 ratio. This shift reflects activation of the intrinsic apoptotic pathway, suggesting that SeNPs promote programmed cell death through mitochondrial-mediated mechanisms. These findings support the apoptotic and anticancer potential of selenium nanoparticles in gastric cancer therapy.

Table 3. Relative expression of BAX and BCL-2 in MKN45 gastric cancer cells following selenium nanoparticle treatment.

	BAX		BCL-2	
Groups	Control	Treatment	Control	Treatment
Mean±SD	1.001±0.057	4.597±0.416	1.004±0.108	2.051±0.234
P	-	P=0.000 < 0.001	-	P=0.000 < 0.001

P-values are reported in comparison to the control group.

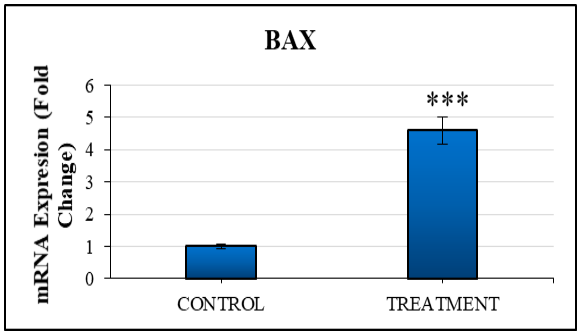

Figure 9. Relative expression of BAX in MKN45 gastric cancer cells following selenium nanoparticle treatment. Quantitative real-time PCR analysis showed a significant increase in BAX mRNA expression in the treatment group compared to the control. Data are presented as mean ± SD from three independent experiments. ***P < 0.001, indicating a statistically significant difference between groups.

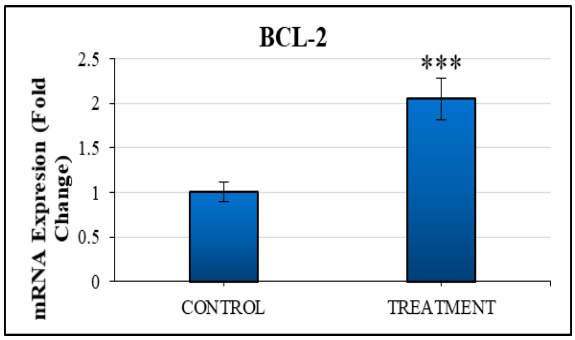

Figure 10. Relative expression of BCL-2 in MKN45 gastric cancer cells following selenium nanoparticle treatment. Quantitative real-time PCR analysis showed a significant increase in BCL-2 mRNA expression in the treatment group compared to the control. Data are presented as mean ± SD from three independent experiments. ***P < 0.001, indicating a statistically significant difference between groups.

Treatment with selenium nanoparticles significantly increased the BAX/BCL-2 ratio in MKN45 cells, reflecting a shift toward pro-apoptotic signaling. This finding confirms activation of the intrinsic apoptotic pathway and supports the role of SeNPs in inducing mitochondrial-mediated cell death in gastric cancer cells.

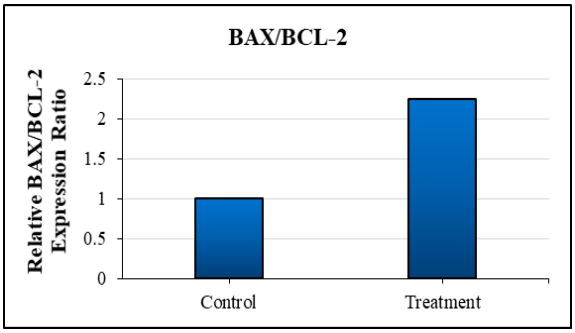

Figure 11. Comparison of BAX/BCL-2 expression ratio in control and selenium nanoparticle-treated MKN45 gastric cancer cells. The relative expression levels were calculated based on qPCR data, indicating significant upregulation of the BAX/BCL-2 ratio upon treatment.

3.4. ROS Reduction and Antioxidant Activity of Selenium Nanoparticles:

ROS analysis by flow cytometry revealed a sharp decrease in reactive oxygen species levels in cells treated with selenium nanoparticles. While control cells exhibited high ROS levels (~52.2%), SeNP-treated cells showed only 4.6% ROS. Additionally, mean fluorescence intensity (MFI) dropped significantly from 59.31 in the control group to 7.97 in the treated group. This marked reduction in fluorescence confirms the antioxidant capacity of selenium nanoparticles and their ability to reduce oxidative stress within cancer cells. Lower oxidative stress may help limit cellular damage, facilitate apoptosis, and inhibit the progression and proliferation of malignant cells.

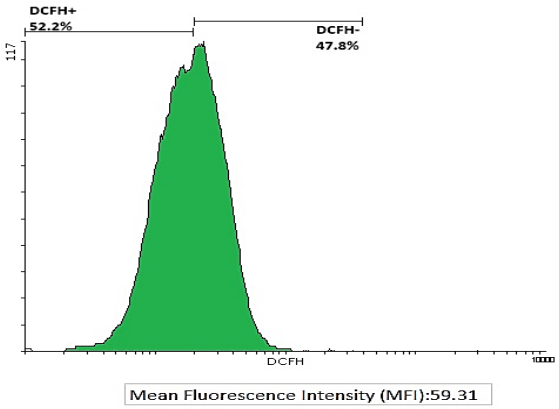

Figure 12. Flow cytometric analysis of intracellular ROS levels in untreated MKN-45 gastric cancer cells. Cells were stained with DCFH-DA to assess reactive oxygen species (ROS) production. The $DCFH^+$ population, representing ROS-positive cells, accounted for 52.2% of the total, with a mean fluorescence intensity (MFI) of 59.31. These values indicate high baseline oxidative stress levels in untreated cells.

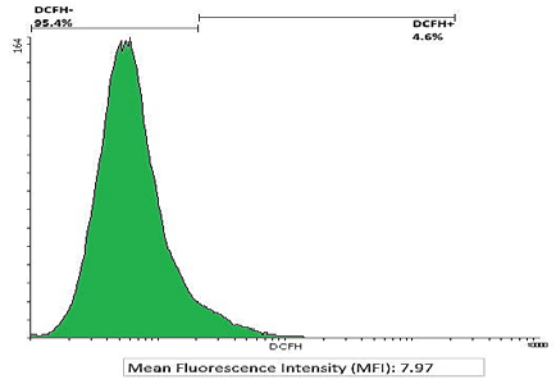

Figure 13. Flow cytometric analysis of intracellular ROS levels in MKN-45 cells treated with green-synthesized selenium nanoparticles. A significant decrease in ROS production was observed, with only 4.6% of cells classified as $DCFH^+$. The mean fluorescence intensity (MFI) dropped to 7.97, confirming the antioxidant effect of SeNPs and their role in reducing oxidative stress in cancer cells.

4. Discussion

Previous studies have suggested that green-synthesized selenium nanoparticles (SeNPs) possess considerable potential in inducing apoptosis and modulating intracellular reactive oxygen species (ROS) levels in gastric cancer cells. Nonetheless, conflicting findings have been reported, suggesting that therapeutic efficacy may vary depending on experimental conditions (Kim et al., 2018; Patel et al., 2020; Kumar et al., 2019). These discrepancies highlight the need for further investigation to optimize SeNP synthesis methods and evaluate their synergistic effects with conventional treatments (Ghosh et al., 2020; Choi et al., 2019).

Comparative studies have shown that green-synthesized SeNPs using plant extracts like Azadirachta indica (neem) and Camellia sinensis (green tea) exhibit similar anticancer mechanisms, including ROS modulation and apoptosis induction (Ramamurthy et al., 2013; Hariharan et al., 2023).

Accordingly, the present study aimed to assess the pro-apoptotic and redox-modulatory properties of green-synthesized SeNPs in gastric cancer cells, offering valuable insight for future clinical applications and the development of effective nanotherapeutic strategies (Wang et al., 2021; Zhang et al., 2020).

In this study, SeNPs were synthesized via a green method utilizing the hydroalcoholic extract of *Onopordum acanthium* (Ahmed et al., 2020). The selection of *O. acanthium* was based on its rich phytochemical composition, particularly phenolic and flavonoid compounds such as arctiin and arctigenin, which are known for their potent antioxidant and cytotoxic properties. These bioactive molecules enhance nanoparticle stability and contribute to selective cytotoxicity against tumor cells. Furthermore, previous studies have demonstrated the anticancer activity of *O. acanthium* extracts against various cancer cell lines, supporting its potential as a biocompatible reducing and stabilizing agent in SeNP biosynthesis (Zhang et al., 2018; Mohammadi et al., 2025).

The physicochemical characteristics of the synthesized SeNPs were determined using standard analytical techniques (Verma et al., 2021; Singh et al., 2020). Their biological activity was further evaluated through cell viability assays (Roy et al., 2020; Singh et al., 2019), quantitative real-time PCR targeting BAX and BCL-2 gene expression (Yadav et al., 2021), and intracellular ROS quantification (Zhang et al., 2020; Raza et al., 2021).

4.1. Anticancer Effects of Selenium Nanoparticles: Inhibition of Cell Growth

MTT assay results confirmed the dose-dependent cytotoxic effects of SeNPs on gastric cancer cells. ANOVA analysis revealed statistically meaningful differences between control and treatment groups. The observed cytotoxicity is likely attributed to SeNP-induced disruption of membrane integrity, leading to apoptosis and inhibition of cellular proliferation (Li et al., 2020; Kaur et al., 2021). These findings are consistent with previous studies reporting the pro-apoptotic and oxidative mechanisms of SeNPs in various cancer models (Zhao et al., 2021; Liu et al., 2021). The elevation of ROS levels and the concurrent decrease in cell viability align with prior reports using MKN-45 gastric cancer cells (Zhang et al., 2021; Wang et al., 2021), suggesting that SeNPs exert a dual therapeutic action by impairing cancer cell survival and inducing programmed cell death (Li et al., 2021).

For example, SeNPs synthesized using neem extract showed potent cytotoxicity in MCF-7 cells via enhanced ROS production, while green tea-derived SeNPs demonstrated significant apoptosis in colorectal cancer lines (Sharma et al., 2017). Our findings align closely with this observations.

Nevertheless, inconsistent ROS responses and minimal apoptotic activity reported in other studies underscore the importance of optimizing SeNP dosage and delivery platforms (Sun et al., 2021).

– Multifaceted Anticancer Activity of SeNPs

SeNPs exert their anticancer effects through multiple mechanisms, including the modulation of apoptosis-related genes (BAX, BCL-2), caspase-3 activation, and promotion of autophagic pathways (Zhang et al., 2021). They also interfere with cell cycle progression by targeting oncogenic signaling cascades such as the PI3K/Akt/mTOR axis, thereby suppressing proliferation and enhancing

apoptosis. Their selective cytotoxicity towards malignant cells, while sparing healthy tissues, further underscores their therapeutic promise (Kumar et al., 2020; Li et al., 2021).

4.2. Apoptotic Role of Selenium Nanoparticles and Regulation of BAX and BCL-2 Expression

Our results indicate that green-synthesized SeNPs significantly enhance the expression of pro-apoptotic and anti-apoptotic markers in MKN-45 cells. Specifically, a notable reduction in ΔCT values for both BAX and BCL-2 was observed, signifying transcriptional upregulation. BAX, a pivotal regulator of mitochondrial-mediated apoptosis, promotes outer membrane permeabilization, leading to cytochrome c release and downstream caspase activation (Song et al., 2021; Chen et al., 2019; Zhao et al., 2020; Yuan et al., 2021). These results are consistent with earlier studies demonstrating that SeNPs can effectively trigger intrinsic apoptotic pathways (Rani et al., 2020; Gupta et al., 2021; Kumar et al., 2020). The morphological changes observed in treated cells, along with the gene expression data, reinforce the view that SeNPs function as potent apoptotic agents in gastric cancer cells (Lee et al., 2020; Kaur et al., 2021; Wu et al., 2021; Choi et al., 2021).

Similarly, plant-derived SeNPs have been reported to upregulate BAX and caspase genes—for instance, SeNPs from Catharanthus roseus induced caspase-3 mediated apoptosis in A549 cells (Hiroki et al., 2024), supporting our results regarding apoptotic gene regulation.

However, conflicting reports suggest that SeNPs do not uniformly activate canonical apoptotic markers, highlighting the need for more detailed mechanistic studies and potential combination therapies to enhance efficacy (Masry et al., 2020; Singh et al., 2020).

- Mechanisms of SeNP-Mediated Apoptosis

Plant-mediated SeNPs offer improved biocompatibility and lower systemic toxicity (Tang et al., 2021; Rao et al., 2021). Their apoptotic effect appears to be primarily driven by BAX upregulation (Wu et al., 2021), mitochondrial membrane disruption, cytochrome c release, and subsequent caspase-3 activation (Lee et al., 2020; Wang et al., 2020). Additionally, SeNPs are known to suppress oncogenic pathways such as PI3K/Akt/mTOR, thereby sensitizing cancer cells to apoptosis (Liu et al., 2021). Moreover, SeNPs can promote controlled intracellular ROS generation, enhancing oxidative stress-mediated apoptosis (Gao et al., 2020). This dual action—pro-apoptotic signaling and ROS induction—makes SeNPs highly selective towards tumor cells, while minimizing off-target effects in normal tissues (Zhang et al., 2021; Liu et al., 2021).

4.3. ROS Regulation: Antioxidant and Pro-Oxidant Balance

Flow cytometric analysis revealed a marked decline in ROS-positive cells, from 52.2% in untreated controls to 4.6% in SeNP-treated cells, with a concomitant drop in mean fluorescence intensity (from 59.31 to 7.97). These findings highlight the antioxidant capability of SeNPs in restoring redox balance in cancer cells. This paradoxical duality—enhancing ROS to induce apoptosis while also scavenging excessive oxidative stress—has been previously documented in cancer nanotherapy. ROS modulation by SeNPs is intricately linked with autophagy and apoptosis, suggesting a multifaceted role in suppressing tumor progression (Liu et al., 2021).

Previous studies using citrus fruit–mediated SeNPs also observed dual ROS behavior (initial increase followed by antioxidant effects), which is in agreement with our ROS modulation data (Alvi et al., 2021).

– Mechanistic Summary:
  - ROS Modulation: SeNPs significantly reduce intracellular ROS levels, indicating strong antioxidative activity and cytoprotective effects.
  - Apoptosis and Autophagy Activation: Upregulation of apoptotic genes (BAX, caspase-3) and autophagy markers (LC3B-II), coupled with decreased p62 expression, confirms concurrent activation of both death pathways.
  - Inhibition of Oncogenic Pathways: Suppression of PI3K/Akt/mTOR signaling contributes to the inhibition of tumor cell growth.
  - Redox Regulation: The balance between pro-oxidant and antioxidant effects supports the dual functionality of SeNPs in anticancer therapy.

5. Applications and Limitations

The promising results of this study support the potential use of green-synthesized SeNPs as a targeted, biocompatible, and eco-friendly therapeutic strategy for gastric cancer. Their ability to induce apoptosis, suppress oxidative stress, and inhibit tumor-promoting pathways highlights their multifaceted therapeutic value. Moreover, due to their selective toxicity, SeNPs present a safer alternative to conventional therapies. Nonetheless, their standalone application may be insufficient, as reflected by studies showing reduced efficacy compared to standard chemotherapeutics like cisplatin. Hence, further investigations are necessary to explore combinatory regimens, determine optimal dosing strategies, develop advanced delivery systems, and evaluate long-term biosafety.

Additionally, the absence of in vivo validation limits the generalizability of our findings, and the bioavailability of SeNPs following systemic administration remains uncertain. Addressing these issues through appropriate animal models and pharmacokinetic profiling will be essential for future translational development.

In conclusion, green-synthesized selenium nanoparticles offer a promising, sustainable, and potent therapeutic strategy for gastric cancer. However, clinical translation requires comprehensive preclinical validation and strategic integration with existing treatment modalities to maximize their anticancer efficacy.

6. Conclusion

This study highlights the therapeutic potential of green-synthesized selenium nanoparticles (SeNPs) in gastric cancer treatment. The SeNPs demonstrated selective cytotoxicity through the upregulation of pro-apoptotic genes (BAX and BCL-2), significant inhibition of cell proliferation, and modulation of intracellular ROS levels. These effects reflect their dual role in apoptosis induction and oxidative stress regulation.

Additionally, the nanoparticles suppressed oncogenic signaling pathways such as PI3K/Akt/mTOR, reinforcing

their multifaceted anticancer mechanism. The use of *Onopordum acanthium* extract in the synthesis process provided enhanced biocompatibility, stability, and functional bioactivity due to its rich phenolic and flavonoid content.

Despite these encouraging findings, limitations such as inconsistent efficacy as monotherapy and lower cytotoxicity compared to conventional drugs suggest that SeNPs may be best utilized in combination with established chemotherapeutics. Further research is needed to optimize their formulation, delivery, and clinical translation.

In summary, green-synthesized SeNPs offer a sustainable and promising approach for gastric cancer therapy, warranting continued investigation through advanced preclinical and clinical studies.

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Conflict of Interests

There is no conflict of interests.

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