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RESEARCH ARTICLE

Anti-cancerous effect and biological evaluation of green synthesized Selenium nanoparticles on MCF-7 breast cancer and HUVEC cell lines

Negin Seifi [1, 2, a], ***Roghayeh Mansoori*** [1, 3, a], ***Parham Khoshbakht Marvi*** [1, 4], ***Sedigheh Niknam*** [1, 5], ***Hannaneh Zarrinnahad*** [1], ***Nooshin Amini*** [1, 2], ***Ghazaleh Chizari Fard*** [1, 2, 6], ***S. Ahmad Dehdast*** [1, 2*], ***Mohammad Shabani*** [1**]

a These authors contributed equally to this work as Co-First authors.

[1] *Department of Biochemistry, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*

[2] *"Pajoohesh BAMA" Knowledge Enterprise Co., 15847-15414 Tehran, Iran.*

[3] *Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.*

[4] *School of Biomedical Engineering, McMaster University, 1280 Main Street West, Hamilton, ON L8S 4L8, Canada*

[5] *Institute of Nanoscience and Nanotechnology, University of Kashan, Kashan, Iran.*

[6] *Clothing and Fabric Design Department, Art Faculty, Imam Javad University College, Yazd, Iran.*

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ABSTRACT

Green synthesis of nanoparticles is a safe and cost-effective process for creating nanoparticles using extracts from different parts of plants, such as flowers, leaves, stems, and roots. In this research, *Melissa Officinalis* L. (*MO*) aqueous extract was used for the synthesis of Selenium nanoparticles (Se NPs) for the first time. These extracts contain flavonoids, polyphenols, and alkaloids, which act as reducing agents in synthesizing nanoparticles. In this study, the extract serves both as a reducing agent and a capping agent during the fast and simple green synthesis of Selenium nanoparticles. The hydrodynamic size of the nanoparticles was investigated by using DLS. Further characterization of the shape and size of the nanoparticles was conducted through SEM and TEM studies. Moreover, the elemental composition of the NPs was identified through Energy Dispersive X-ray (EDX) elemental analysis. Microscopy analysis results showed that the Se NPs had a spherical shape. Furthermore, the particle size was determined to be 65 nm and 34 nm via DLS and SEM studies, respectively. Additionally, the biological evaluation of Se NPs demonstrated a non-toxic effect on the Human Umbilical Vein Endothelial Cells (HUVEC) normal cell line and anticancer activity on the MCF-7 breast cancer cell line.

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INTRODUCTION

In the past couple of decades, nanotechnology, as an exciting field of science, has made significant strides in various applied sciences and technologies, including material science, computation and electronics, energy science, environmental remediation, healthcare, and medicine [1]. Submicroscopic materials and suitable synthesis methods, often based on biological entities, are essential components of nanotechnology's versatile applications [2]. Due to their nanoscale size and remarkable surface-to-volume ratio, nanoparticles (NPs) possess unique characteristics that differ from those of bulk materials [3]. However, the morphology, size, and surface charge of NPs can be manipulated using various

* Corresponding Author Email: *arashdehdast@gmail.com*
shabani.mo@iums.ac.ir

synthesis approaches from different sources [4–6]. Numerous methods have been proposed, including physical, chemical, and biological procedures [7,8]. The latter, specifically biosynthesis of NPs using plant extracts, is widely adopted as a safe and sustainable alternative, replacing chemical processes and reducing or eliminating harmful substances detrimental to health and the environment [9]. By enabling reactions without hazardous solvents, reducing agents, and stabilizers, the green synthesis approach stands out for producing low-cost nanoparticles with eco-friendly properties [10]. NPs derived from specific plant extracts have demonstrated antifungal, antibacterial, and antimicrobial properties in practical applications [11,12]. There is a plethora of investigations on metallic NPs renowned for their particular optoelectronic properties and precise, tractable surface modification processes, which make them applicable to various biological purposes, such as bio-detection devices, pharmaceuticals, magnetic resonance imaging agents, and theranostic drug delivery systems [13–17]. Among noble metallic NPs, selenium NPs (Se NPs) have garnered significant attention from the scientific community, especially in medical applications. Se NPs are intrinsic trace elements in selenoproteins, acting as cofactors for enzymes like glutathione peroxidase and thioredoxin reductase, which are part of the antioxidant defense system in animal cells. They play a vital role in defending against oxidative stress and inflammation [18,19]. This biological activity arises from Se particles' ability to interact with biomolecules found in bacterial cells and plant extracts, including hydroxyl, carbonyl, carboxyl, cyanide, and amide functional groups [20–22]. As a result, Se NPs exhibit outstanding antibacterial, antioxidant, ROS scavenging (i.e., anticancer), and enzyme inhibition activities [23–27], with lower toxicity compared to other conventional organic and inorganic selenium compounds [28,29]. Notably, significant antimicrobial activity of these nanoparticles has been reported against fungi, pathogenic bacteria, and drug-resistant bacteria [30,31]. Although the tendency of Se NPs to aggregate due to poor water solubility is considered one of their significant drawbacks hindering their use in biological media, surface modification with various biomaterials can stabilize and enhance their potential features [32,33]. Apart from different polysaccharides [27,32–34], amphiphiles [25], ascorbic acid [35], etc., Se NPs can be obtained from various plant extracts, providing a less toxic, simpler, and more cost-effective approach [36,37]. Medicinal plants, in particular, can offer fewer side effects than synthetic drugs [38] and possess inherent antioxidant, antimicrobial, antifungal, immune-modulatory, and anticoccidial effects [39]. It has been shown that vitamins, amino acids, proteins, alkaloids, tannins, phenolics, saponins, terpenoids, and flavonoids, collectively prevalent in herbal extracts, are crucial in reducing, capping, and stabilizing metalloid nanoparticles [40,41]. One of the herbal extracts suitable for obtaining monodispersed NPs is the leaf of *Melissa Officinalis* L. (*MO*), commonly known as lemon balm, a perennial subshrub endemic to south-central Europe and Central Asia. It is primarily composed of flavonoids, polyphenolic compounds, monoterpenoid aldehydes, monoterpene glycosides, triterpenes, sesquiterpenes, tannins, and essential oils [42]. Several studies confirm that *MO* extract holds promising antitumor potential in some human cancer cell lines, enhances memory, exhibits antiviral, antispasmodic, antibacterial, and anti-anxiety capabilities, and has a significant impact on cell proliferation and neuroblast differentiation [42–49]. However, it has been validated that the biological effects of *MO* extracts, like other plants from the Lamiaceae family, primarily depend on the concentration of rosmarinic acid, the predominant constituent of *MO* [50]. In this contribution, we endeavor to introduce a safe and practical method for acquiring modified Se NPs using biomolecules from *MO* extracts. We assess the enhancement of the biological performance of synthesized NPs through surface observation and characterization using DLS, EDX, SEM, and TEM instrumental techniques. Furthermore, we investigate biological toxicity on the Human Umbilical Vein Endothelial Cells (HUVEC) cell line and assess the anticancer effect against the MCF-7 cell line to substantiate the improved properties of the produced biogenic Se NPs (Fig. 1).

MATERIALS AND METHODS

materials

Sodium selenite (Na_2SeO_3) was purchased from Sigma (USA). The MCF-7 breast cancer cell line and Human umbilical vein endothelial cells (HUVEC) cell line were purchased from the Iranian Biological Resource Center (Iran). All chemicals

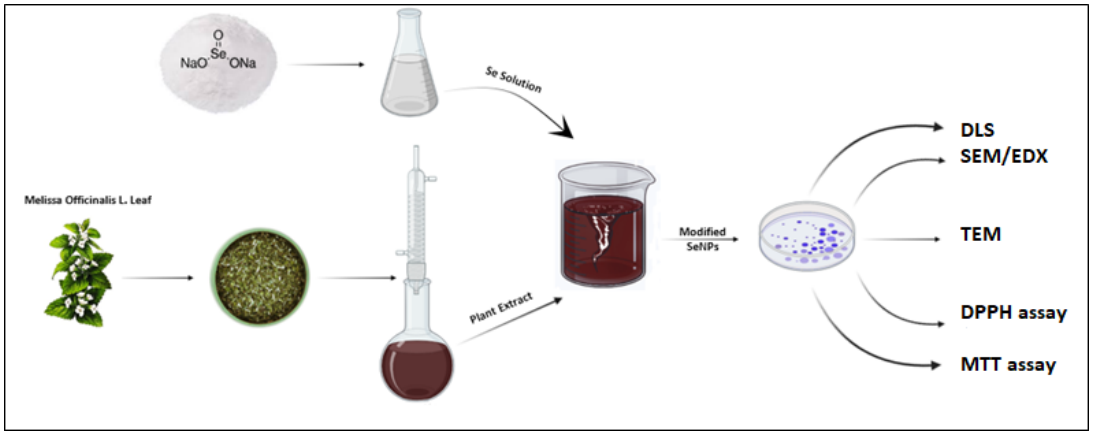

Fig. 1. Graphical abstract: Representing the green synthesis process, characterization and biological assessment of Se NPs.

used were of analytical grade, and all experiments were conducted using sterile double-distilled water.

Preparation of Plant Extract

Two grams of *Melissa Officinalis L.* (*MO*) leaves were ground into a powder and suspended in 20 ml of double-distilled water. The extraction process was carried out using a heater-stirrer, and suspension heated 70° C for 60 min. Subsequently, it was filtered through Whatman filter papers (No. 1) and stored in a sterilized glass container at 4° C until further use.

Synthesis of Se Nanoparticles

Selenium nanoparticles (Se NPs) were synthesized by gradually adding a Se solution to the *MO* plant extract to prepare a reaction mixture comprising 20 mL of the prepared plant extract and 5 mL of an aqueous solution containing 0.01 mmol of Na_2SeO_3. The reaction was conducted in a glass flask, and the solution was homogenized by stirring at 50°C for 30 minutes. The pH plays key role in the synthesis of nanoparticles, for this reason pH was adjusted between 8-9 using alkaline solution, which is suitable for synthesis. which is suitable for nanoparticle synthesis. The change in color of the mixture from light brown to dark brown provided a clear indication of the formation of Se nanoparticles through the green synthesis process using *MO* extract.

CHARACTERIZATION OF SE NANOPARTICLES

Hydrodynamic size of the nanoparticles in solution was determined using Dynamic Light Scattering (DLS) with a ZETA SIZER NANO zps instrument from Malvern Instruments, UK. Scanning Electron Microscopy-Energy Dispersive X-ray (SEM-EDX mapping) was employed to investigate the morphology and size of Se NPs, as well as assess their chemical composition and elemental distribution. The SEM analysis was conducted using a Sigma SEM microscope from Zeiss, Germany. Additionally, Transmission Electron Microscopy (TEM) was utilized to examine the nanostructure morphology, utilizing a Zeiss-EM10C microscope from Germany.

BIOLOGICAL EVALUATION AND ANTICANCER EFFECT OF SE NPS

DPPH pro-antioxidant assay:

To assess the pro/antioxidant activity of Se NPs, a 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging assay was performed [51]. Fresh DPPH solution was prepared in ethanol, and different concentrations of Se NPs were added to 1 ml of 0.1 mM of DPPH solution. The absorbance was measured at the wavelength λ = 517 nm at room temperature, after 20 min. Ascorbic acid was considered as positive control in the experiment.

Preparation of MCF-7 and HUVEC cell lines

The MCF-7 and HUVEC cell lines were cultured in a growth medium containing 1% penicillin/streptomycin and 10% fetal bovine serum (FBS). The MCF-7 cell line was incubated at 37°C with 5% CO2 in a CO2 incubator. When the cell growth reached approximately 70%, cells were detached from the culture flask using Trypsin-EDTA. The contents were then centrifuged at 1500 rpm for 5 minutes. Subsequently, a cell suspension was

prepared using the sedimented cells and 1 mL of cell culture. The viability of the cells was determined using trypan blue dye and observed under a light microscope. Only cell suspensions with a survival rate exceeding 90% were used for the study [52-54].

Investigation of cytotoxicity and anticancer activity

The cytotoxicity potential of Se NPs was assessed using a cell proliferation assay based on MTT. Both normal Human Umbilical Vein Endothelial Cells (HUVEC) and human breast carcinoma cells (MCF-7) were exposed to varying concentrations of nanoparticles for 24 and 48 hours. The yellow-colored MTT is reduced to the insoluble purple-colored formazan by the reductase enzymes found in the mitochondria of live cells. HUVEC and MCF-7 cells were seeded in a 96-well plate at a density of 5 × 103 cells/well and allowed to grow for 24 hours. The culture medium was then replaced with different concentrations of Se nanoparticles solution (five wells per group for each experiment), and cells were incubated for 24 and 48 hours. MTT solution was added to each well to achieve a final concentration of 5 mg/mL and incubated at 37°C for 4-6 hours. Formazan was subsequently solubilized using DMSO, and the concentration was determined by measuring optical density at 570 nm using a multi-well spectrophotometer [52,54]. The data obtained represent the mean of five independent experiments. To calculate viability or toxicity percentages based on OD values, the following formulas were used:

Cell viability (%) = OD of Sample/OD of Control × 100

Cytotoxicity (%) = 100 – Viability %

IC50 values (the concentration at which 50% maximum inhibition occurred) were calculated from curves plotting cell survival (%) versus nanoparticle concentration (µg/mL).

Statistical Analysis

The collected data were subjected to statistical analysis using Graph Pad Prism software. Values are presented as the mean ± SD of three replicates for each experiment. Values with $p<0.05$ were considered statistically significant.

RESULTS

In recent years, numerous plants and their extracts have been explored for the synthesis of various nanoparticles and their potential biomedical applications. According to a comprehensive literature review, this research represents the first documented instance of biosynthesizing Selenium nanoparticles using Melissa Officinalis L. (MO) extract. In this process, Se ions were reduced by the organic components present in the (MO) extract, resulting in the synthesis of Se nanoparticles. To assess the outcomes of this research, various analyses, including DLS, EDX-Mapping, SEM, and TEM, were employed. Additionally, biological and anticancer activity assessments were conducted using the DPPH assay and MTT assay.

DLS analysis

The Dynamic Light Scattering (DLS) method was employed to determine the hydrodynamic diameter of dispersed nanoparticles in a liquid medium. It also verified the distribution of nanoparticles within the solution. The size distribution histogram of Se nanoparticles synthesized through green synthesis, as analyzed using Dynamic Light Scattering (DLS), is presented in Figure 2. The average hydrodynamic diameter of the nanoparticles was approximately 64 nm (Figure 2).

SEM and TEM microscopy

SEM analysis was conducted to observe the topography, surface morphology, and diameter of the Se NPs. The results revealed the production of relatively monodisperse, uniform, spherical Se NPs with a consistent and similar size distribution ranging from 31 to 35 nm (Figure 3A). These SEM images further validate the formation of a high density of Se NPs achieved through the use of Melissa Officinalis L. (MO) leaves aqueous extract. It's worth noting that the difference between the particle size data obtained by SEM microscopy imaging and DLS studies arises because the DLS method reports the hydrodynamic diameter of nanoparticles in solution, whereas microscopic imaging provides the actual non-hydrodynamic diameter of the nanoparticles. Additionally, the morphology and size of the synthesized Se nanoparticles were examined using a Transmission Electron Microscope (TEM). The TEM results corroborate the SEM data, confirming that the Se NPs are indeed in the nanoscale range, exhibiting a spherical morphology with an approximate size of 30 nm (Figure 3B).

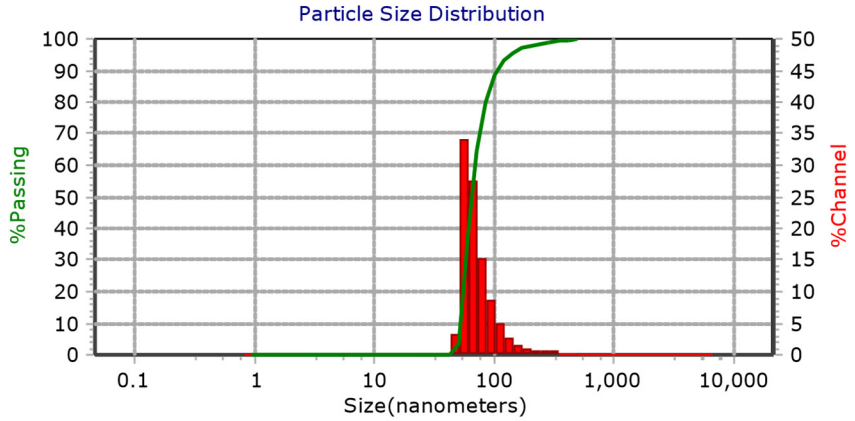

Fig. 2. Particle size distribution of the green synthesized Se NPs by DLS.

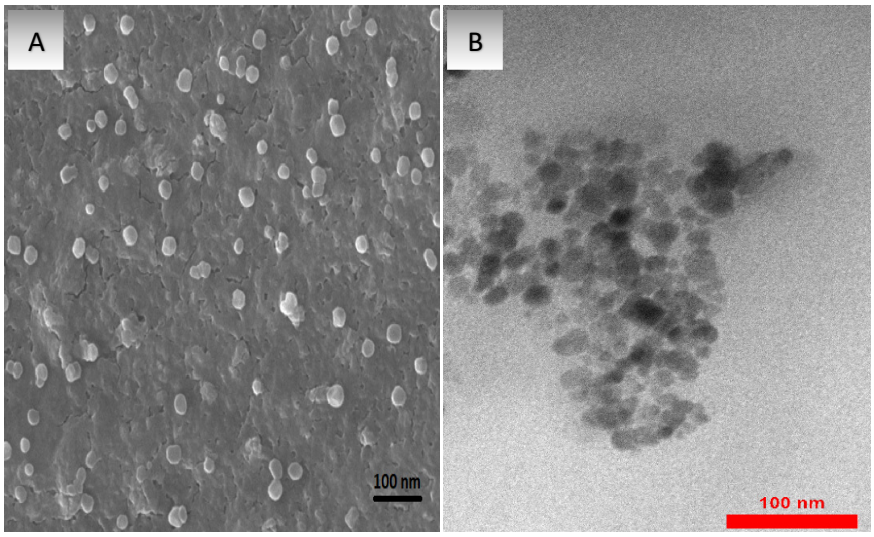

Fig. 3. Scanning electron microscopy (SEM)(A), and transmission electron microscopy (TEM)(B), images of green synthesized Selenium nanoparticles.

EDX and Elemental Mapping Analysis

EDX analysis was used to determine the elemental composition of the synthesized nanoparticles. The EDX analysis of Se NPs confirmed the presence of pure selenium (Se), along with carbon (C) and oxygen (O) elements within the synthesized nanostructure. The presence of carbon (C) and oxygen (O) as constituent elements in the Se NPs provides evidence for the green synthesis of these nanoparticles, indicating the presence of organic compounds within the nanostructure, either as a capping agent or contributing to the formation of Se-organic nanoparticles (Figure 4A) [55]. Furthermore, elemental mapping analysis of the Se nanoparticles demonstrated a well-distributed presence of metallic Se, carbon (C), and oxygen (O) within the scanned electron micrograph image of the biosynthesized nanoparticles solution (Figure 4B).

Pro-oxidant /Antioxidant activity

The pro/antioxidant activity of Se NPs biosynthesized using the MO aqueous extract was assessed through the DPPH assay. In this assay, antioxidants present in the sample reduce the stable nitrogen radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), resulting in a reduction of the absorption peak at the wavelength of 517 nm compared to the control sample. Oxygen donor substances within the sample generate the reduced form of DPPH, causing the solution to lose its violet color. The results of the DPPH assay demonstrated the

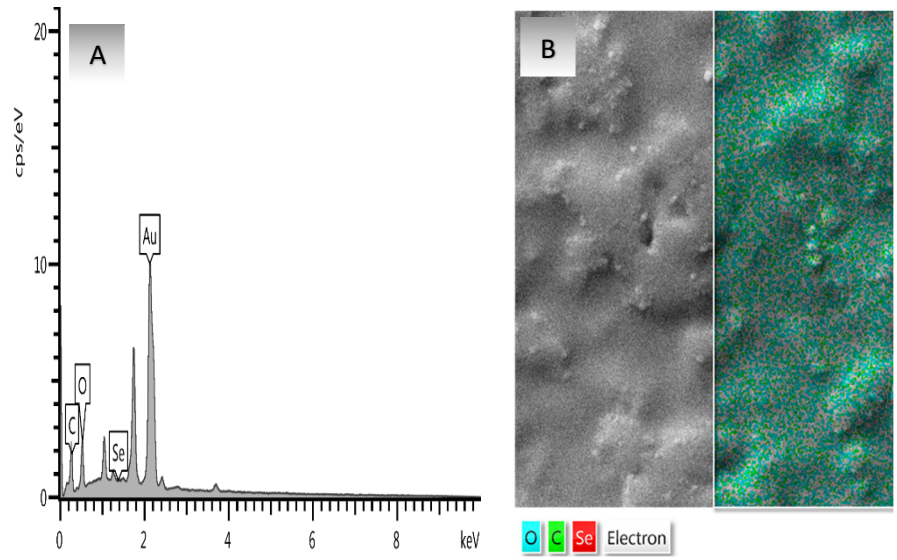

Fig. 4. EDX spectrum of green synthesized Selenium nanoparticles (A), and elemental mapping: distribution of Se, C, and O element in electron micrograph region of Se nanoparticles(B).

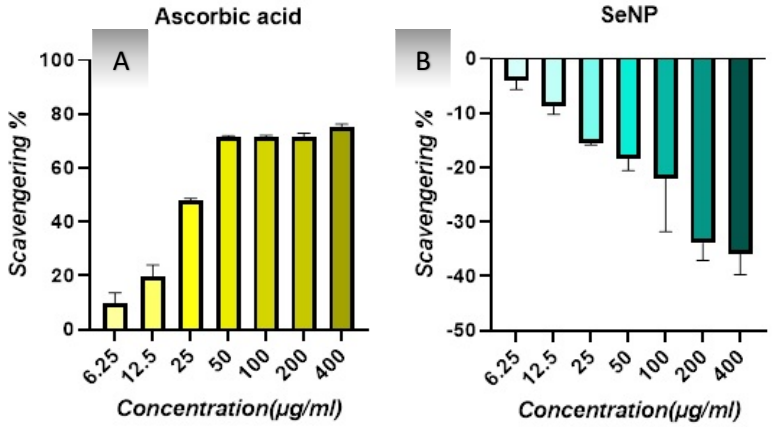

Fig. 5. DPPH scavenging activity of ascorbic acid (A), and biosynthesized selenium nanoparticles (B). The values are means ± SD, n = 3. The means with different superscripts are significantly different ($P < 0.05$).

high antioxidant activity of ascorbic acid, used as a positive control (Figure 5A). Additionally, the obtained results from the assay confirmed the pro-oxidant activity of the biosynthesized Se NPs (Figure 5B). The presence of Se nanoparticles led to a significant increase in pro-oxidant properties. Selenium nanoparticles have the unique ability to generate Reactive Oxygen Species (ROS), thus exhibiting pro-oxidant effects. The pro-oxidant and antioxidant properties of selenium nanoparticles depend on factors such as dose, duration, environmental conditions, and oxidation state. Furthermore, selenium ions and nano-selenium possess a high potential for ROS production, which can induce anti-carcinogenic mechanisms, making them valuable for their anticancer properties [56,57].

MTT assay

In the last decade, nanoparticles have garnered considerable attention for their potential in cancer treatment. Eco-friendly Selenium nanoparticles (Se NPs) synthesized from herbal components have demonstrated efficacy against various human

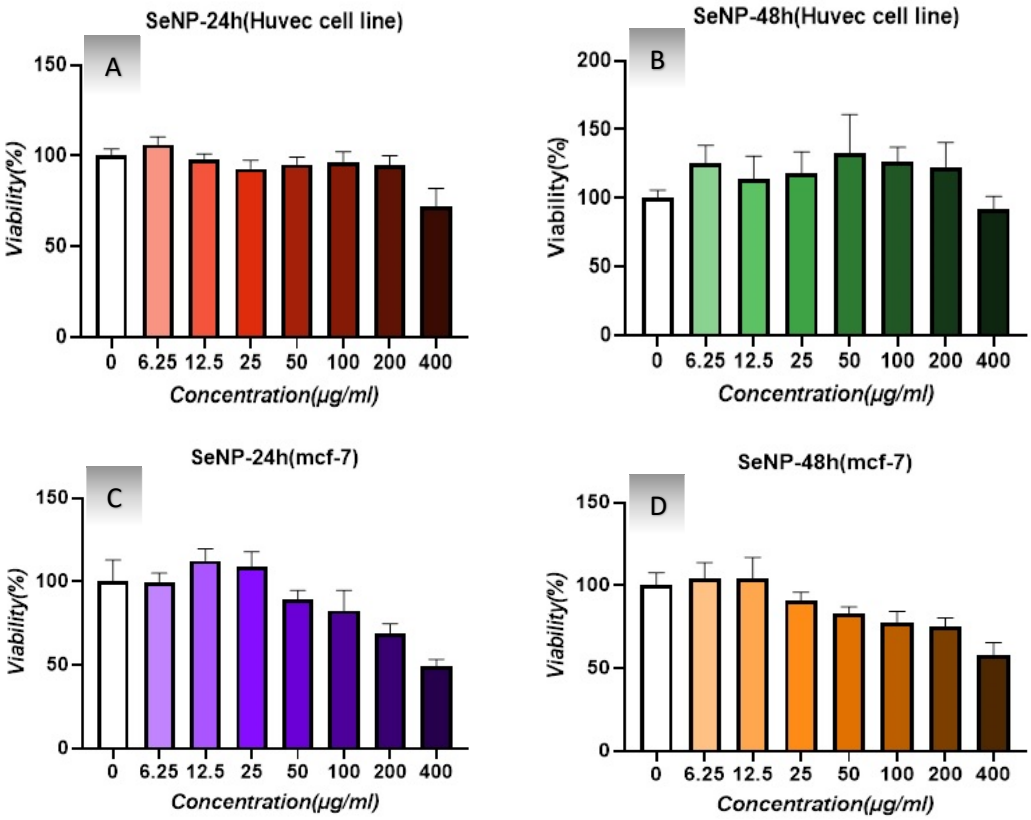

Fig. 6. Cell viability assay of Se nanoparticles at different concentrations against normal cell line (HUVEC) after 24 h(A) and 48 h incubation(B). Cell viability assay of Se nanoparticles against the breast cancer cell line (MCF-7) after 24 h(C) and 48 h(D) incubation. The values are means ± SD, n = 3. The means with different superscripts are significantly different ($P < 0.05$).

cancer cell lines, including MCF-7[58], human colorectal carcinoma cell line (HCT116), and human hepatic cell line (HUH7) [59], confirming their anti-cancerous effects. In this study, the in vitro biocompatibility of biosynthesized Se NPs was evaluated against the HUVEC cell line (normal cells), and their anticancer activity was assessed against MCF-7 cell lines using the MTT assay. HUVEC (Figure 6A and B) and MCF-7 (Figure 6C and D) cell lines were exposed to different concentrations of Se NPs (ranging from 0 to 400 µg/mL) for 24 hours (Figure 6A and C) and 48 hours (Figure 6B and D). The percentage of cell viability after incubation with Se NPs at various concentrations is depicted in Figure 6. The results indicated low toxicity of Se NPs on the normal HUVEC cell line (Figure 6A and B). In the MCF-7 breast cancer cell line, cell viability decreased to approximately 70% after treatment with 200 µg/mL of Se nanoparticles and further decreased to about 45% after treatment with 400 µg/mL of Se nanoparticles (Figure 6C and D). The IC50 values for Se nanoparticles were calculated to be 599 µg/mL for HUVEC and 372 µg/mL for MCF-7 cell lines, respectively. The lower IC50 value in the breast cancer cell line suggests the efficiency of Se NPs in inhibiting the growth of cancerous cells, while having a negligible effect on normal cells at the same concentration. The results of the anticancer activity of green-synthesized Se NPs against breast cancer cell lines confirmed the dose-dependent cytotoxicity of nanoparticles. In summary, the MTT assay results demonstrate lower toxicity of selenium nanoparticles against normal cells compared to cancer cells, indicating the biocompatibility and excellent anticancer properties of the synthesized Se nanoparticles. Thus, the prepared selenium nanoparticles show promise for anticancer applications against breast cancer cells.

Nanoparticles induce cytotoxic effects on human cells through various mechanisms, including the uptake of free nanoparticles and DNA damage, site-specific cytotoxicity, generation of reactive oxygen species (ROS), and free radicals, among others [60]. Moreover, since selenium is a pro-oxidant and pro-oxidants lead to ROS production, increased lipid oxidation, and dysfunction of mitochondria in cancer cells compared to normal cells, the higher toxicity of selenium nanoparticles in breast cancer cells can be attributed to their pro-oxidant capabilities [57,61].

CONCLUSION

Selenium nanoparticles (Se NPs) were successfully synthesized using a simple, fast, and eco-friendly method with Melissa Officinalis L. (MO) aqueous extract. The green-synthesized selenium nanoparticles (Se NPs) utilizing plant extract exhibited pro-oxidant activity, good biocompatibility with Human umbilical vein endothelial cells (HUVEC) cell lines, and demonstrated significant cytotoxic effects against breast cancer (MCF-7) cell lines. One of the advantages of this work compared to previous studies is the focus on rapidly preparing small-sized Se NPs using a novel plant extract. The proposed synthesis method utilizing MO aqueous extract offers a cost-effective approach for nanoparticle production compared to commercial methods. A dose-dependent MTT assay was employed to assess the toxicity and anticancer activity of the Se NPs on both HUVEC and MCF-7 cell lines. The cell viability data obtained confirm that the synthesized pro-oxidant Se NPs are biocompatible and possess promising anticancer activity potential. Consequently, these findings suggest the potential suitability of biosynthesized Se NPs for various medical applications, including as a food nutrient, anticancer agent, drug carrier, and more.

AUTHORS CONTRIBUTIONS AND CONFLICTS OF INTEREST

All authors contributed to the study design, experimental sections, analysis of data, writing the article, and drafting the manuscript. All authors read and approved the final manuscript. The authors of the present work declare no conflict of interest, financial or otherwise.

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