

# Cytotoxic potential of selenium nanoparticles (SeNPs) derived from leaf extract of *Mentha longifolia* L.

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## Abstract

The search for alternative methods in cancer treatments has been going on for many years. In the current study conducted for this purpose, selenium nanoparticles (ML-SeNPs) were produced from the aqueous leaf extract of *Mentha longifolia* L. easily and inexpensively, without harming the environment. The anticancer potential of ML-SeNPs on glioblastoma cell (U373), osteosarcoma cell (U2OS), and healthy retinal pigment epithelial cell (RPE-1) lines was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-difeniltetrazolium bromid) test. For the test, ML-SeNPs were applied at 100, 300, and 600 µg/mL levels and interaction was provided for 24 and 48 hours. The survival rates (%) in RPE-1, U373, and U2OS cell lines in the 24-hour application were 107.49-98.89, 97.66-86.82, and 87.81-83.37, respectively. The viability rates (%) of the cells in 48 hours of application were 72.27-87.39, 68.17-73.48, and 81.00-84.67, respectively. In general, it was discovered that the cytotoxic effect of ML-SeNPs on RPE-1, U373, and U2OS cell lines was greater at low doses and increased over time. In-vivo studies that support the antiproliferative action of ML-SeNPs may boost the prospect of using them as therapeutic agents in potential cancer treatment procedures in the following years.

**Keywords:** Anticancer, *Mentha longifolia* L., SeNPs, U373, U2OS

## INTRODUCTION

The increase in cancer cases worldwide in recent years is remarkable Who, (2022). According to the CBTRUS (The Central Brain Tumor Registry of the United States) Statistical Report, glioblastoma, the most common malignant central nervous system tumor, is the most aggressive form of brain cancer, with a 5-year overall survival rate of patients is 7% (Glioblastoma, 2022; Senhaji et al. 2022). Osteosarcoma is a very common form of malignant bone cancer, which is detected more in youth and young adults than in the previous cancer type, and the survival rate in patients is around 60-70% (Ni et al. 2022).

Conventional cancer treatment includes endocrinological therapy, surgical excision of cancerous tissues, and cell killing with chemotherapy or radiotherapy. Nevertheless, the patient's healthy cells, tissues, and organs are typically harmed by these treatments. (Ikram et al. 2021). In the recent several decades, efforts to produce medications with great therapeutic potency have intensified in order to decrease the aforementioned harm in patients. However, despite encouraging preclinical results for many novel medications, these results could not reflect the predicted effects in patients (Ntafoulis et al. 2023) During this time, with the advancement of nanotechnology, alternative nanomaterials to conventional pharmaceuticals emerged (Hatipoğlu et al. 2023; Baran et al. 2023; İpek et al. 2023). Some of these nanomaterials are NP forms of elements such as silver,

gold, zinc, selenium, palladium and platinum synthesized from biological sources (Yang et al. 2022; Hatipoğlu et al. 2023; Ashraf et al. 2023; Hashem et al., 2022, Aktepe et al. 2021; Gholami-Shabani et al 2023).

Researchers are becoming more interested in SeNPs, which are nanomaterials with a wide range of applications, including electronics, biosensors, food packaging, medicine, optics, and catalysis (Hussain et al., 2023). One of the elements required by the human body is selenium. However, while low quantities of selenium enable adequate human body function, excessive doses (such as 3200 g and higher per day) are harmful (Srivastava et al. 2015). Selenium is essential for thyroid hormone metabolism and immunological function. Furthermore, selenium and SeNPs shield cells from free radical harm by enhancing the activity of antioxidant enzymes (glutathione peroxidase and thioredoxin reductase). Cancer, cardiovascular disease, and inflammatory illnesses have all been related to selenium insufficiency (Shoeibi et al. 2017; Pyrzynska et al. 2012).

SeNPs can be generated by physical, chemical and biological (green synthesis) approaches Aktepe et al. (2022). Physical and chemical procedures necessitate the use of costly and ecologically hazardous severe poisonous substances (Hatipoglu, 2021; Baran et al. 2021). As a result, as in other NPs, low-cost, environmentally friendly and green chemistry procedures that do not contain toxic chemicals have begun to be adopted in the production of SeNPs (Saranya et al., 2023; Baran et al., 2023). Furthermore, in the biosynthesis process of SeNPs, biomolecules such as polysaccharides, phenolic compounds, saponins, flavonoids, enzymes, tannins, amino acids, proteins, and sugars in plant extracts are evaluated as possible reducing and stabilizing factors Pyrzynska, (2021).

*Mentha longifolia* L., which belongs to the Lamiaceae family, has a square section, finely hairy and up to 1.5 m long, leaves up to 90 mm long and 22 mm wide, tiny flowers, rhizomatous, perennial, herbaceous, rapidly growing, medicinal and aromatic plant (Mohammad Hosein et al., 2017; Gharib et al. 2020; İpek et al., 2023 Patonay et al., 2021). Strong-smelling *M. longifolia* has spike-shaped inflorescences with numerous flowers (Bahadori et al., 2018). Pune, punk, and wild mint are other names for this plant (Saeidi et al. 2014; Atalar et al. 2021). *M. longifolia* has 22 subspecies, indicating a high level of genetic variation. Because it is found in western and central Asia, temperate and subtropical Europe, and northern and southern Africa, it is regarded the world's most common wild mint taxon (Patonay et al., 2021). Its leaves or fresh shoots are usually used as a mint aroma and as a garnish in salads and prepared foods Mohammad Hosein et al. (2017). *M. longifolia* is used for the amelioration of cough, hypertension, cold, sinusitis, asthma, and digestive issues as a food additive and pharmaceutical ingredient Anwar et al., (2017). Because of their sedative, antimicrobial, antioxidant, antipruritic, anticancer, antispasmodic, antihistaminic, diuretic, anti-inflammatory, hepatoprotective, and biopesticide properties, *M. longifolia* plant extracts and/or essential oils have numerous applications in the pharmaceutical, food, and hygiene industries (Mokaberinejad et al., 2012; Ali et al. 2021). The primary synthetic method for producing selenium nanoparticles is chemical reduction, which makes use of a stabilizer and reducing agent. Stabilizer use, however, may impede the regular use of produced nanoparticles in biological applications, and because of its chemical makeup, stabilizer may also be hazardous. This work assesses the potential biological utility of selenium nanoparticles as an anticancer treatment and reports on a straightforward green production of the metal via phytochemical mediation. The purpose of this work was to reveal the anticancer activity of ML-SeNPs generated from the aqueous leaf extract of *M. longifolia* L. in an environmentally friendly way.

## MATERIALS AND METHODS

### Materials

*M. longifolia* L. used in the research was acquired from public bazaars in Diyarbakır (Türkiye). Sigma-Aldrich (USA) provided sodium selenite ( $\text{Na}_2\text{SeO}_3$ , 99% purity). The American Type Culture Collection (ATCC) cell lines U373 (glioblastoma), U2OS (osteosarcoma), and healthy RPE-1 (retinal pigment epithelial cell) were utilized to assess the cytotoxic effects of ML-SeNPs. MTT used in cytotoxic experiments was purchased from Merck (Germany). The cell lines were cultured in RPMI-1640 media (Sigma-Aldrich, USA).

### Methods

#### Biosynthesis of plant compatible SeNPs

To synthesize SeNPs, a 1 M  $\text{Na}_2\text{SeO}_3$  solution was produced. 75 mL of the extract was combined with 25 mL of  $\text{Na}_2\text{SeO}_3$  solution and allowed to react at 30 °C for 4 hours. Following the observation of the color change, the resultant solution was centrifuged for 30 minutes (6000 rpm). The collected solid phase at the bottom was rinsed many times with distilled water. The prepared NPs were dried in an oven (80 °C / 48 hours). In a mortar, the solid portion was crushed. The NPs was saved for use in cytotoxic activity investigations.

#### Cytotoxic Activities of ML-SeNPs Via the MTT Assay

Selected cell lines were cultured in T75 flasks in RPMI-1640 media containing 10% FBS, 2 mM L-glutamine, and 100

units/mL penicillin/streptomycin and incubated at 37 °C in a 5% CO<sub>2</sub> atmosphere. When the cells had attained 80-90% confluence, they were removed from the flasks and their cell counts were quantified using the hemocytometric technique. Cells were injected in triplicate into 96-well plates in 90 L media for each well of U373, U2OS (5×10<sup>3</sup>), and RPE-1 (10×10<sup>3</sup>) cell lines (two on microplates to execute two distinct periods of 24 and 48 hours). The cells were allowed to cling to the microplate base for 24 hours.

The next day, different concentrations of ML-SeNPs (600, 300, and 100 g/mL) were added to the seeded plates. The cells in the control group were treated with ultrapure water. The MTT assay was utilized to detect changes in cell viability 24 and 48 hours after treatment. Each well containing cells received 10 L of the prepared MTT (5 mg/mL) solution and was incubated for 3 hours at 37 °C in a humid atmosphere containing 5% CO<sub>2</sub>. The medium was withdrawn after 3 hours, and 100 L of DMSO was injected to each well. After 20 minutes in the shaker, the optical density (OD) values in the wells were measured using a UV-vis spectrophotometer (Multiskan GO, Thermofisher, USA) Irtegun and İpek (2023).

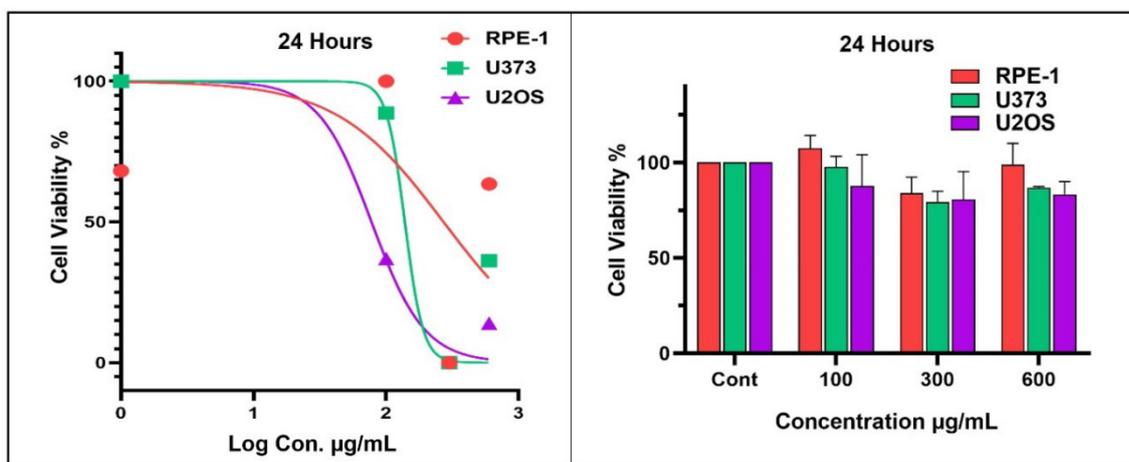
The absorbance values obtained by reading the control wells were averaged and regarded as 100% viable cell value. The absorbance readings from the ML-SeNP-treated wells were proportioned to the control absorbance value and accepted as % viability. MTT testing were carried out three times on various days. The inhibitor concentration value for ML-SeNPs was computed using the obtained data and the GraphPad Prism 8 tool. Cytotoxic experiments were conducted at Dicle University, Veterinary Faculty, Cell Culture Laboratory (Diyarbakır, Türkiye).

**Statistical analysis**

The SPSS package program (IBM, 21.0) was used to analyze the study’s data. P<0.05 was used as the statistical significance level.

**RESULTS AND DISCUSSION**

The 24-hour MTT assay revealed that a 100 g/mL dosage of ML-SeNPs increased the survival of healthy RPE-1 cells while inhibiting malignant U373 and U2OS cells some (Figure 1 and Table 1).



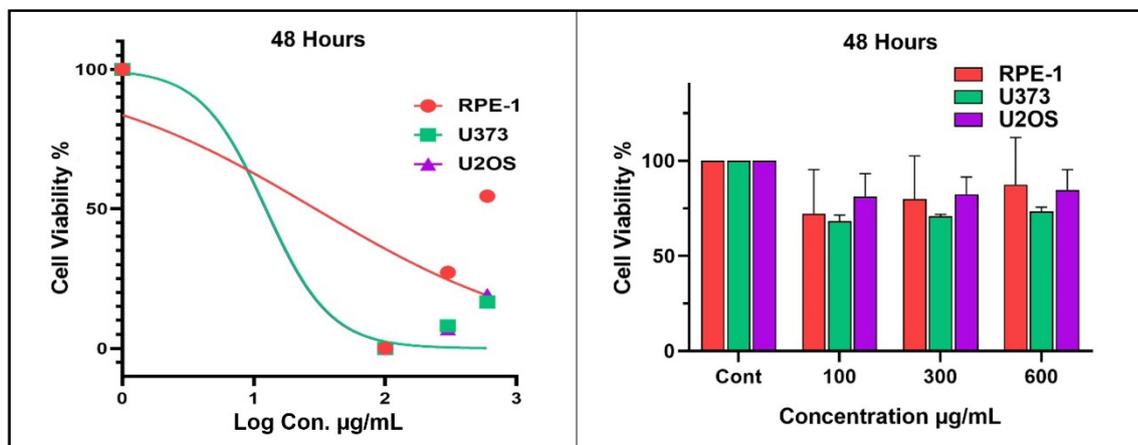
**Figure 1.** The half maximum inhibitory concentration (IC<sub>50</sub>) results of ML-SeNPs on U373 (glioblastoma), U2OS (osteosarcoma) and healthy RPE-1 (retinal pigment epithelial cell) lines at 24 hours.

Increasing the dose of biogenic SeNPs to 300 µg/mL decreased the viability of all cell lines, whereas increasing this amount to 600 µg/mL increased the viability of all cell lines.

**Table 1.** Cytotoxic effects data of ML-SeNPs on U373 (glioblastoma), U2OS (osteosarcoma) and RPE-1 (retinal pigment epithelial cell) lines at 24 hours.

Cytotoxic effects ML-SeNPs on the cell lines (n=3, $\bar{X} \pm Sx$ , 24 Hours)			
	100 µg/mL	300 µg/mL	600 µg/mL
RPE-1	107.49±06.62	83.97±08.36	98.89±11.16
U373	97.66±05.60	79.35±05.64	86.82±00.56
U2OS	87.81±16.24	80.66±14.55	83.37±06.59

The 48-hour MTT test revealed that a 100  $\mu\text{g/mL}$  dosage of ML-SeNPs produced a significant reduction in the viability of all cells (Figure 2 and Table 2). However, a progressive rise in cell viability was seen in line with the increase in NPs application dosage.



**Figure 2.** The half maximum inhibitory concentration ( $\text{IC}_{50}$ ) results of ML-SeNPs on U373 (glioblastoma), U2OS (osteosarcoma) and healthy RPE-1 (retinal pigment epithelial cell) lines at 48 hours.

In this situation, the inhibitory impact of ML-SeNPs owing to dosage increase is restricted or negative in cells, whereas time is a factor that promotes inhibition in cells. SeNPs were shown to have high cytotoxic effects on T98-G (glioblastoma), Skov-3 (human ovarian adenocarcinoma), and 4T1 (mouse breast cancer cells) cells in three prior studies (Wadhvani et al., 2017; Gharbav et al., 2022).

**Table 2.** Cytotoxic effects data of ML-SeNPs on U373 (glioblastoma), U2OS (osteosarcoma) and RPE-1 (retinal pigment epithelial cell) lines at 48 hours.

Cytotoxic effects of ML-SeNPs on the cell lines (n=3, $\bar{X} \pm S\bar{x}$ , 48 Hours)			
	100 $\mu\text{g/mL}$	300 $\mu\text{g/mL}$	600 $\mu\text{g/mL}$
RPE-1	72.27 $\pm$ 22.98	79.82 $\pm$ 22.67	87.39 $\pm$ 24.81
U373	68.17 $\pm$ 03.29	70.74 $\pm$ 01.10	73.48 $\pm$ 02.09
U2OS	81.00 $\pm$ 12.12	82.33 $\pm$ 09.07	84.67 $\pm$ 10.60

Moreover, other studies found that SeNPs given at low dosages (5-100  $\mu\text{g/mL}$ ) had a substantial inhibitory impact on HepG2 (hepatocellular carcinoma), MDA-MB-231 (breast cancer cells), and MCF-7 (breast cancer cells) (Cui et al., 2018; Cittrarasu et al., 2021).

One of the primary goals of nanomedicine is to solve the issues that are often associated with the use of conventional forms of drugs, particularly increased safety. Many studies highlight the unique medical applications of selenium nanoparticles (SeNP), which have various therapeutic benefits, including antioxidant, anti-inflammatory, anti-diabetic, and anti-tumor effects (Varlamova et al., 2021). Selenium nanoparticles (SeNPs) are considered superior to other metal nanoparticles, such as silver, gold, and platinum NPs, due to their better biocompatibility and in vivo degradability (Rajasekar & Kuppusamy, 2021). It is known that the pharmacological effect and toxicity of Se-based compounds depend on various factors, including concentration, redox, and type (Sonkusre & Cameotra, 2017; Varlamova et al., 2021). Several mechanisms have been suggested for selenium's anticancer activity, including cell cycle arrest, antioxidation, apoptosis, and interruption of the cell signaling pathway (Sonkusre & Cameotra, 2017). Nanoparticles had a different concentration-dependent effect on cancer cells of the studied human lines in our research. The study showed that SeNPs affected the survival of both cancer and normal cells in dose-dependent and time-dependent behaviors. The reduced cell viability was observed with increased time duration and decreased concentrations of SeNPs.

As a result, it is expected that by lowering the application dose, the nanomaterial employed in this study would have a stronger antiproliferative impact on cells.

## CONCLUSION

The SeNPs used in this work were produced using a straightforward, economical, eco-friendly, and ecologically compatible phytofabrication process using leaf extract from *M. longifolia*. The produced SeNPs were nanoscale, naturally crystalline, negatively charged, spherical, and exceptionally stable.

The investigation was carried out to discover the effects of the produced nanomaterial on cancer cells and to manufacture the plant-based NP form of selenium, which is a vital mineral for human health. When the study's findings were analyzed, it was discovered that modest dosages of ML-SeNPs had a greater antiproliferative impact on cancer cells, and that waiting time amplified this effect to some extent. In order to more clearly indicate the anticancer potential of ML-SeNPs, future investigations should minimize the nanomaterial application dosage. Better yet, if in-vivo research supports the lethal ability of ML-SeNPs, this nanomaterial may be a stronger option for involvement in prospective cancer therapy procedures.

## COMPLIANCE WITH ETCICAL STANDARTS

### Peer-review

Externally peer-reviewed.

### Conflict of interest

The authors in this study have no conflicts of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

### Ethics committee approval

This study does not require an ethics committee.

### Funding

There is no funder for this study.

### Data availability

Not applicable.

### Consent to participate

Not applicable.

### Consent for publication

Not applicable.

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