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Anticancer Activities of Zinc Oxide Nanoparticles (ZnONPs) Synthesized from *Mentha longifolia* L. Leaf Extract

Polat İPEK[1], Mehmet Fırat BARAN[2], Abdulkerim HATİPOĞLU[3*], Ayşe BARAN[4]

Highlights:

- Green synthesis
- Metal nanoparticles
- Cancer cells inhibition

Keywords:

- Cytotoxic potential
- *Mentha longifolia* L.
- Zinc oxide nanoparticles
- MTT
- HCT-116
- OVCAR-3

ABSTRACT:

Recently, there has been a remarkable increase in cancer and cancer-related deaths. In this study, the impacts of zinc oxide nanoparticles (ZnONPs) produced from the aqueous leaf extract of *Mentha longifolia* L. (ML) on ovary adenocarcinoma (OVCAR-3), colorectal carcinoma (HCT-116), and healthy retinal pigment epithelial cell (RPE-1) lines were investigated. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test was performed to discover its antiproliferative properties. As a result of the application of ML-ZnONPs on RPE-1, OVCAR-3, and HCT-116 cell lines at doses (µg/mL) of 250, 500, and 1000 for 24 hours, the viability rates (%) in the cell lines were 18.73-30.56, 21.98-28.76, and 27.27-40.93, respectively. In the 48-hour application, the viability rates (%) of the same cells were between 29.51-46.83, 32.49-40.81, and 46.82-44.37, respectively. The MTT test revealed that ML-ZnONPs strongly suppressed the growth of RPE-1, OVCAR-3, and HCT-116 cell lines. The test showed that the effect of dose increase and time on the viability of both cancer cell lines was negative.

[1] Polat İPEK (**Orcid ID:** 0000-0003-1756-9757), Dicle University, Faculty of Veterinary Medicine, Department of Physiology, Diyarbakır, Türkiye
[2] Mehmet Fırat BARAN (Orcid ID: 0000-0001-8133-6670), Batman University, Vocational School of Technical Sciences, Department of Food Processing, Batman, Türkiye
[3] Abdulkerim HATİPOĞLU (**Orcid ID:** 0000-0002-1487-1953), Mardin Artuklu University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Mardin, Türkiye
[4] Ayşe BARAN (**Orcid ID**: 0000-0002-2317-0489), Malatya Turgut Özal University, Faculty of Health Sciences, Malatya, Türkiye

***Sorumlu Yazar/*Corresponding Author**: Abdulkerim HATİPOĞLU, e-mail: abdulkerimhatipoglu@artuklu.edu.tr

INTRODUCTION

Cancer, one of the most important reasons for mortality in the last quarter century, continues to threaten humanity (WHO, 2022). Chemotherapy, radiation, and hormone therapy are examples of cancer treatments that negatively affect the immunity of patients are applied (Johnson et al., 2018). As a result, research into the lethal effects of nanoparticles on cancer cells, which may be used as an alternative to current treatment approaches, has been increasing (Alyamani et al., 2021; Sabouri et al., 2021; İpek et al., 2023).

Chemical, physical, and biological processes are used to generate nanoparticles. The physical approach necessitates high temperatures and pressure, as well as a high cost and big space for machine installation. Toxic chemicals that are harmful to both nature and individuals are used in the chemical approach (Agarwal et al., 2017). As a result, low-cost, ecologically friendly, and green chemistry processes that do not use harmful chemicals have been used in the production of nanoparticles (Aktepe et al., 2022).

Nanoparticles such as gold (Hatipoğlu, 2021), silver (Baran et al., 2022), nickel (Uddin et al., 2021), platinum (Eltaweil et al., 2022), palladium (Arsiya et al., 2017), selenium (Baran et al., 2023), and zinc (Vijayakumar et al., 2018) have been synthesized from biological resources so far. The biological resources are mostly roots (Behravan et al., 2019), stems (Mahiuddin et al., 2020), flowers (Jamdagni et al., 2018), fruits (Baran et al., 2021) and leaves (Hatipoğlu et al., 2023), as well as microorganisms such as fungi (Vala, 2014), bacteria (Suba et al., 2021), and algae (Hassan et al., 2021).

Among the numerous nanoparticles, zinc oxide nanoparticles (ZnONPs) have a special place owing to their easy and inexpensive production as well as their environmental friendliness (Agarwal et al., 2017). ZnONPs are versatile semiconductors with excellent thermal and chemical stability, exhibiting significant optical transparency, and luminescent properties in the UV-vis regions (Fakhari et al., 2019). Furthermore, ZnONPs are of great interest to researchers as agents that increase antimicrobial and antioxidant activity, targeted drug delivery, biosensors, drought tolerance and nutrient supply (Bandeira et al., 2020; Pillai et al., 2020).

Mentha longifolia L. (*M. longifolia*, ML) is a Lamiaceae family member with square-sectioned, finely hairy leaves up to 1.5 m long, leaves up to 90 mm long and 22 mm broad, tiny (5 mm long) flowers, rhizomatous, perennial, herbaceous, medicinal and an aromatic herb (Golparvar et al., 2017; Rezaeinia et al., 2019; Gharib et al., 2020; Patonay et al., 2021). This plant is also called puneh, punk, and wild mint (Saedi et al., 2014; Mükemre et al., 2016). *M. longifolia* shows a wide genetic diversity with 22 subspecies. Since its distribution covers western and central Asia, moderate and subtropical regions of Europe, northern and southern Africa, it is considered to be the leading familiar untamed mint classification in the world (Patonay et al., 2021). Its leaves or fresh shoots are usually utilized as a mint scent and as a condiment in salads and prepared meals (Golparvar et al., 2017). It is used as a food additive and medicinal agent in the treatment of *M. longifolia*, hypertension, cough, cold, asthma, sinusitis, and digestive disorders (Anwar et al., 2017). Furthermore, *M. longifolia* plant extracts and/or essential oils have multipurpose uses in the food, pharmaceutical and hygiene industries thanks to their antimicrobial, antioxidant, anticancer, antispasmodic, anti-inflammatory, and biopesticide activities (Mokaberinejad et al., 2012; Rezaeinia et al., 2019; Gharib et al., 2020; Ali et al., 2021).

The goal of this research was to assess the cytotoxic activities of ML-ZnONPs derived from *M. longifolia* aqueous leaf extract in an ecologically friendly way.

MATERIALS AND METHODS

Materials

M. longifolia used in the research was obtained from public bazaars in Diyarbakır (Turkiye). $ZnSO_4.7H_2O$ salt was obtained from Sigma-Aldrich (USA).

To determine the cytotoxic effects of ML-ZnONPs on the cells, OVCAR-3 and HCT-116 cell lines obtained from the American Type Culture Collection (ATCC) and healthy RPE-1 cell lines were used. MTT used in cytotoxic studies was obtained from Merck (Germany). RPMI-1640 (Sigma-Aldrich, USA) was used as the medium for culturing cell lines.

Methods

Preparation of plant leaf extract

M. longifolia leaves were cautiously cleaned with distilled water and allowed to dry at room conditions. Using a laboratory grinder, the dried leaves were crushed into a fine powder. 20 grams of powder was boiled in 50 mL of deionized water and filtered through No. 1 Whatmann filter paper to make the extract. The filtrate was then kept in a refrigerator at +4 ^oC to be employed in the production of ZnONPs.

Biosynthesis of plant-compatible ZnONPs

A 50 mM aqueous zinc solution was produced from the solid form of $ZnSO_4$ for the biosynthesis of ZnONPs.$7H_2O$. 25 mL of extract was combined with 50 mM 10 mL of $ZnSO_4.7H_2O$ solution and left to react at 65 °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). The solid portion was then pulverized in a mortar. The synthesized nanoparticle was preserved for use in cytotoxic activity studies.

Cytotoxic activities of ML-ZnONPs via the MTT assay

All cell lines were grown in T75 culture flasks with RPMI-1640. Antibiotics (streptomycin and penicillin) were added to the culture media at 10% FBS and 100 U/mL, respectively. Cells were incubated at 37 °C in 5% CO_2 until they were 80-90% confluent. After incubation, the cells were extracted from the flasks using trypsins and counted using a hemocytometric technique. Each cell line counted was grown in 96-well microplates. ML-ZnONPs were introduced to the culture medium where the cells were situated at varied concentrations (1000, 500, and 250 g/mL), and interaction was monitored for 24 and 48 hours. Microplate wells were employed for control stages. The MTT test was done in the dark at the appropriate wavelength to assess changes in cell viability after the contact period. Using the test results, the concentration (IC_{50}) values at which the cells demonstrated 50% viability for ML-ZnONPs were determined using the GraphPad Prism 8 software (Kandemir and İpek, 2022).

The cytotoxic research was carried out at Dicle University's Faculty of Veterinary Medicine's Cell Culture Laboratory.

Statistical analysis

The study's data were assessed utilizing the SPSS software (IBM, 21.0). The statistical significance threshold was evaluated at $P<0.05$.

RESULTS AND DISCUSSION

MTT assay revealed that biogenic ZnONPs significantly inhibited the growth of RPE-1, OVCAR-3, and HCT-116 cell lines (Figures 1 and 2).

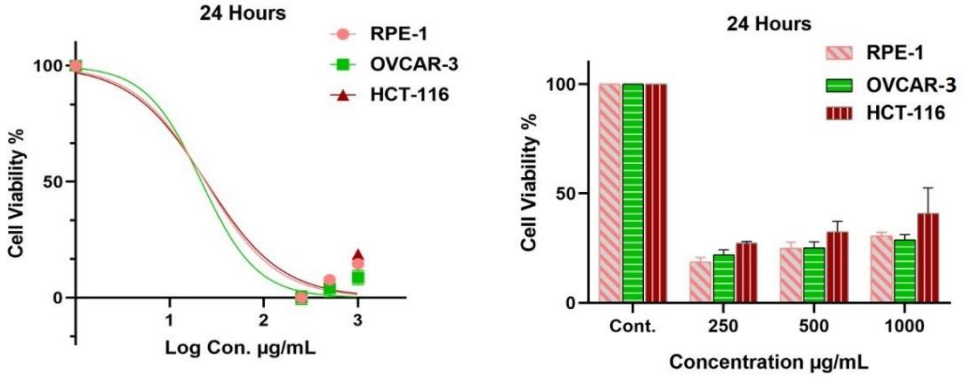

Figure 1. The half minimum inhibitory concentration (IC_{50}) results of ML-ZnONPs on OVCAR-3 (ovary adenocarcinoma), HCT-116 (colorectal carcinoma), and healthy RPE-1 (retinal pigment epithelial cell) cell lines at 24 hours

The application dose of ML-ZnONPs in the study was 250-1000 µg/mL (Tables 1 and 2). Cell inhibition was more significant at 250 µg compared to other doses. The dose increase was not statistically effective on cytotoxicity (P>0.05).

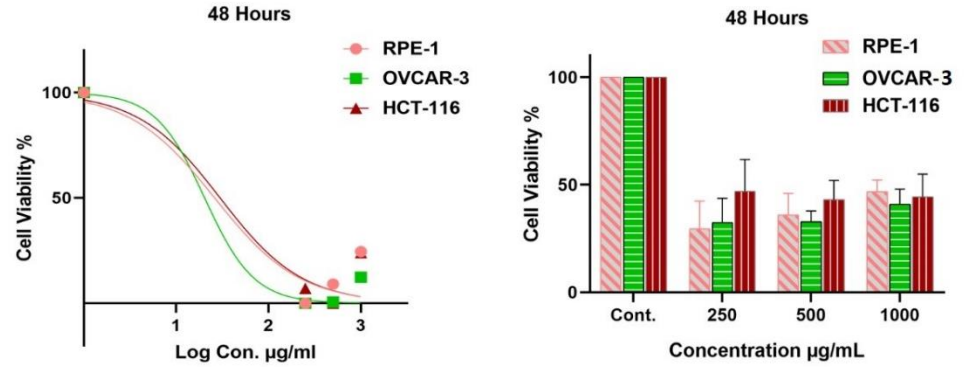

Figure 2. The half minimum inhibitory concentration (IC_{50}) results of ML-ZnONPs on OVCAR-3 (ovary adenocarcinoma), HCT-116 (colorectal carcinoma), and healthy RPE-1 (retinal pigment epithelial cell) cell lines at 48 hours

On the other hand, cell death observed in 48-hour administration was not significant compared to 24-hour administration. In 48-hour application, viability increased with increasing doses in RPE-1 and OVCAR-3 cell lines. In contrast, there was first a decrease and then an increase in the viability of HCT-116 cell lines. In summary, ML-ZnONPs exhibited both dosage and time-independent cytotoxicity.

In addition, it can be said that the phytochemicals (especially monoterpenoids) in *M. longifolia* increase this cytotoxic effect (Singh et al., 2020). These phytochemicals exert an antiproliferative impact by inducing apoptosis (Elansary et al., 2020).

Table 1. Cytotoxic effects data of ML-ZnONPs on OVCAR-3 (ovary adenocarcinoma), HCT-116 (colorectal carcinoma), and RPE-1 (retinal pigment epithelial cell) cell lines at 24 hours

Cytotoxic effects of ML-ZnONPs on the cell lines (n=3. $\bar{X} \pm S\bar{x}$. 24 hours)			
Cell lines	**250 µg/mL**	**500 µg/mL**	**1000 µg/mL**
RPE-1	18.73±1.99	24.97±2.76	30.56±1.63
OVCAR-3	21.98±2.29	25.1±2.78	28.76±2.42
HCT-116	27.27±0.84	32.51±4.73	40.93±11.66

Some researchers documented that ZnONPs had potent antiproliferative effects on HCT-116 cancer cells, both dose and time-dependent, at low doses (5-200 µg/mL) (Ahlam et al., 2020; Li et al., 2021; Mihailović et al., 2023). However, different researchers reported that the dose increase of ZnONPs above 0.25 µg/mL did not have a significant cytotoxic effect on OVCAR-3 cancer cells (Padmanabhan

et al., 2019). According to the comparisons, the IC_{50} levels of the current study are higher than the results of other researchers.

Table 2. Cytotoxic effects data of ML-ZnONPs on OVCAR-3 (ovary adenocarcinoma), HCT-116 (colorectal carcinoma), and RPE-1 (retinal pigment epithelial cell) cell lines at 48 hours

Cytotoxic effects of ML-ZnONPs on the cell lines (n=3. $\bar{X} \pm S\bar{x}$. 48 hours)			
Cell lines	**250 µg/mL**	**500 µg/mL**	**1000 µg/mL**
RPE-1	29.51±12.87	35.92±10.06	46.83±05.40
OVCAR-3	32.49±11.17	32.90±04.93	40.81±07.10
HCT-116	46.82±14.69	42.84±09.25	44.37±10.80

Moreover, it was reported that ZnONPs had high cytotoxic activity against human chronic myelogenous leukemia cells (K562), human placental choriocarcinoma cells (JEG-3) (Mihailović et al., 2023), human liver adenocarcinoma (HepG2) (Ismail et al., 2014) cell lines. It is thought that the particle dimension, shape, and stability of the ZnONPs produced in the studies determine the cytotoxic effect levels on the cell types.

CONCLUSION

In this document, the cytotoxic effects of zinc oxide nanoparticles (ZnONPs) derived from the leaves of *Mentha longifolia* L. (ML) on the deadly cancer cells OVCAR-3 (ovarian adenocarcinoma) and HCT-116 (colorectal carcinoma) were assessed. The tests showed that the effect of dose increase and time on the viability of both cancer cell lines was negative. In future studies, minimizing the nanomaterial application dose and testing it in the cells in question may help to monitor the cytotoxic activity better. In addition, the cytotoxic potential of ML-ZnONPs should be supported by in-vivo studies to be used as therapeutic agents in possible cancer treatments.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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