OKÜ Fen Bilimleri Enstitüsü Dergisi
6(2): 1408-1420, 2023

OKU Journal of The Institute of Science and Technology, 6(2): 1408-1420, 2023

Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi

Osmaniye Korkut Ata University Journal of The Institute of Science and Technology

Effects of *Gundelia dersim* Exosome-Like Nanoparticles on Cell Viability in Human Colon and Lung Cancer Cell Lines

Fazilet ERMAN[1*], Gül KAYA[2], Semra TÜRKOĞLU[3]

[1,2,3] Health Sciences Faculty, Department of Nutrition and Dietetic, Firat University, 23119, Elazig, Türkiye

[1]https://orcid.org/0000-0001-5181-4053
[2]https://orcid.org/0000-0001-5181-4053
[3]https://orcid.org/0000-0001-7682-0513
[*]Corresponding author: ferman@firat.edu.tr

Research Article

Article History:
Received: 12.08.2022
Accepted: 02.02.2023
Published online: 05.07.2023

Key words:
Gundelia dersim
Exosome
Cancer
Cell viability

ABSTRACT

Exosomes are nano sized (30-100 nm) bioactive vesicles that constitute the smallest subgroup of extracellular vesicles (EVs). They have properties such as transporting genetic materials, maintaining intercellular communication, regulating immunity, and transmitting signals. Although plant-derived nanoparticles, which exhibit a wide range of therapeutic potential, have not been studied as extensively as in mammalian cells, there is an increasing number of research supporting their cross-kingdom effects. This study aims to investigate the cytotoxic effects of exosome-like nanoparticles isolated from the *Gundelia dersim* plant (GdENs), which is endemic to Turkey, on cell viability in human lung cancer (A549) and human colon cancer (HCT 116) cell lines. The isolated GdENs were imaged through nanoparticle tracking analysis (NTA). Protein analysis was performed with BCA (Bicinchoninic acid). The nanoparticles were applied on A549 and HCT116 cell lines. By conducting a Water-soluble tetrazolium salt-1 (WST-1) assay, the cytotoxic effect on these cell lines was studied. According to our findings, the increasing concentrations of isolated GdENs on, demonstrated cytotoxic effects by reducing cell viability in HCT 116 cell lines at 75, 100 and 250 µg/mL within 24, 48 and 72 hours. In A549 cell lines, it was determined that nanoparticles exhibited cytotoxic effects at 24 hours in all concentration units, but did not exhibit any cytotoxic effect by increasing cell viability at 72 hours. In conclusion, GdENs are thought to have therapeutic potential as an anticarcinogen if supported by more comprehensive studies.

Gundelia dersim Eksozom-Benzeri Nanopartiküllerin İnsan Kolon ve Akciğer Kanser Hücre Hatlarında Hücre Canlılığı Üzerine Etkileri

Araştırma Makalesi

Makale Tarihçesi:
Geliş tarihi: 12.08.2022
Kabul tarihi:02.02.2023
Online Yayınlanma: 05.07.2023

Anahtar Kelimeler:
Gundelia dersim
Eksozom
Kanser
Hücre canlılığı

ÖZ

Eksozomlar; kalıtsal metaryal taşıma, hücreler arası iletişimi sağlama, bağışıklığı düzenleme ve sinyal iletme gibi özelliklere sahip olan ekstrasellüler vezikülllerin (EV) en küçük alt grubunu oluşturan, nano boyutlardaki (30-100 nm) biyoaktif keseciklerdir. Geniş bir terapötik potansiyel sergileyen bitki türevli nanopartiküller, memeli hücrelerindeki kadar çok çalışılmamış olsa da bunların cross-kingdom etkilerini destekleyen araştırmaların sayısı giderek artmaktadır. Bu çalışmanın amacı; Türkiye için endemik olan *Gundelia dersim* bitkisinden izole edilen eksozom benzeri nanopartiküllerin (GdEN) insan akciğer kanseri (A549) ve insan kolon kanseri (HCT 116) hücre hatlarındaki hücre canlılığı üzerine sitotoksik etkilerini araştırmaktır. İzole edilen GdEN'ler, nanopartikül izleme analiziyle (NTA) görüntülenmiştir. BCA

(Bikinkoninik asit) ile protein analizi yapılmıştır. Nanopartiküller A549 ve HCT116 hücre hatlarına uygulanmıştır. Suda çözünür tetrazolyum tuzu–1 (WST-1) analizi yapılıp bu hücre hatlarında GdEN'lerin sitotoksik etkisine bakılmıştır. Bulgularımıza göre, GdEN'lerin artan konsantrasyonları HCT 116 hücre hatlarında 24, 48 ve 72 saatte 75, 100 ve 250 µg/mL'de hücre canlılığını azaltarak sitotoksik bir etki göstermiştir. A549 hücre hatlarında ise nanopartiküllerin, tüm konsantrasyon birimlerinde 24 saatte sitotoksik etki gösterdiği, 72 saatte ise hücre canlılığını arttırarak sitotoksik etki göstermediği tespit edilmiştir. Sonuç olarak, GdEN'lerin daha ileri çalışmalarla desteklenmesi durumunda anti kanserojen etki gösterebileceği düşünülmektedir.

To Cite: Erman F., Kaya G., Türkoğlu S. Effects of *Gundelia dersim* Exosome-Like Nanoparticles on Cell Viability in Human Colon and Lung Cancer Cell Lines. Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi 2023; 6(2): 1408-1420.

1. Introduction

Gundelia dersim is a thick perennial plant with a milky latex, often called "Kereng" or "Kenger". The leaves are single-stemmed with strong-toothed thorns. The capitulum has few flowers, and it is morphologically homogeneous and discoid, densely concentrated in the head similar to *Eryngium* L. It is an endemic plant known for its milky gum-like secretion in the Tunceli region of Turkey (Figure 1). This secretion is used in ice cream production, for stomach disorders such as gastritis, ear disorders, to strengthen the jaw, and for chewing problems (Açar et al., 2021). Various species of the genus *Gundelia* have been used in traditional medicine systems to treat human diseases (gastric pain, vitiligo, chest pain, bronchitis, heart stroke, liver diseases, diarrhea, diabetes), and some species are found to harbour anti-cancer properties in their phytochemistry (Abu-Lafi et al., 2019; Dalar et al., 2019, Ertas et al., 2021).

Figure 1. *Gundelia dersim* plant (Açar et al., 2021)

The cell's ability to perform many tasks simultaneously in multicellular organisms is possible through intercellular communication. Extracellular vesicles (EVs) are nanovesicles heterogeneously released by eukaryotic cells and known to have important biological functions such as signal transduction, intercellular communication, immune regulation and genetic material transport. Exosomes constitute the smallest sub-group of EVs. They are bioactive vesicles of 30-100 nm size that can be obtained

from all body fluids (Kahraman et al., 2014; Ersöz et al., 2016; Yıldırım, 2018; İnanır and Ekici 2020, Nemati et al., 2022).

Exosomes are more common in mammalian cells. However, with recent studies, exosome-like nanoparticles have been obtained from many fruits and plants. Some examples of these sources include grapes, grapefruit, ginger, carrots, lemon, and blueberry. An increasing number of both in vivo and in vitro studies have been conducted to elucidate the roles of plant-derived exosome-like nanoparticles (PENs) in diseases and health (Taylor and Gercel-Taylor, 2008; Yıldırım, 2018; İnanır and Ekici, 2020; Kameli et al., 2021). In a study examining the effect of grape EVs on intestinal stem cells, it was reported that oral administration of these EVs might provide protection from dextran sulfate sodium (DSS)-induced colitis in mice (Ju et al., 2013). In a similar study where ginger-derived EVs were administered to mice with DSS-induced colitis, it was presented that ginger-derived nanoparticles were mainly taken up by intestinal epithelial cells and macrophages and were nontoxic. At the same time, using different mouse models of colitis, these nanoparticles were reported to reduce acute colitis, enhance intestinal repair and prevent chronic colitis and colitis-related cancer (Zhang et al., 2016).

Moreover, it was reported that EVs isolated from *Dendropanax morbifera* and *Pinus densiflora* plants could exhibit cytotoxic effects on tumor cells (Kim et al., 2020). In another study, citrus-lemon-derived nanovesicles were reported to inhibit cancer cell proliferation in various tumor cell lines (Raimondo et al., 2015). The enormous therapeutic applications of PENs, such as their anti-carcinogenic and anti-inflammatory effects, their role in cell-to-cell communication and regulation of intestinal homeostasis, have become an area of interest in the field of biomedicine (Rome, 2019). A study of exosome-like nanoparticles derived from edible tea flowers has shown that these nanovesicles cause mitochondrial damage, cell cycle cessation, cell apoptosis, microbiota modulation, and they inhibit the growth of breast tumours and lung metastasis (Chen et al., 2021). Research on exosomes from wheat derivatives (*Triticum aestivum* L.) has demonstrated the wound healing properties of exosomes using both in vitro and in vivo approaches (Koçak, 2021). Although it has been suggested that PENs mediate cross-kingdom communication, the detailed mechanisms about their roles in pathogenic processes have not yet been fully elucidated. (Kim et al., 2022).

The aim of this study was to evaluate the putative therapeutic potential of exosome-like nanoparticles isolated from the *G. dersim* plant in human lung cancer (A549) and colon cancer (HCT 116) cells using an in vitro approach. *G. dersim* is an endemic plant for Turkey and a study examining the effects of plant exosomes on cancer has not been done before. Therefore, every study to be done on this subject will be the first and will be illuminating about the characteristics of the plant. It will contribute significantly to the development of new drugs as an alternative treatment option for cancer, which is one of the most serious and increasingly widespread problems of today.

2. Materials and Methods

2.1. Exosome Isolation

The G*undelia dersim* plant was collected from Tunceli – Mazgirt (Gürbüzler road, Tunceli 14,2 km, 1390 m, 39°6'44" N / 39°42'30" E, 01,06.2021). The plant was ground in a mixer, and the mixture was filtered through a filter paper. The extract was centrifuged for 10 minutes at 1000 x g, 10 minutes at 20000 x g, and 30 minutes at 20 000 x g respectively, at room temperature. After each stage, the pellet was removed, and the supernatant was collected. Following the last centrifugation, the supernatant was filtered with a 0.22 μm filter. The PEG-DEX (Polyethylene Glycol- Dextran) solution that is used in the Aqueous two-phase systems (ATPS) isolation method (Kırbaş et al., 2019) was added at a 1:1 ratio (22.5 mL of PEG-DEX for 22.5 mL of extract). A wash solution with a 1:1 ratio (ATPS isolation solution with distilled water) was prepared for the washing process. The mixture was centrifuged at 1000 x g for 10 minutes at 4 °C. The wash solutions with buffered samples were centrifuged at 1000 x g for 10 minutes. 80% of the top phase was discarded. Wash solution at the same amount of the discarded part (36 mL) was added, and the solution was centrifuged at 1000 x g for 10 minutes at 4 °C. Repeating the washing process, the part above the phase line was discarded. The *G. dersim* exosomes obtained were stored at -20˚C.

2.2. Nanoparticle Tracking Analysis (NTA)

The determination of exosome amount and dimensional distribution is calculated by a blue laser beam at 488 nm via Nanoparticle Tracking Analysis (NTA) (Özkan et al., 2021). GdENs were diluted 1:1000 with sterile nuclease-free water before measurements. The samples were placed in the chamber of the test device (Nanosight NS300 (Malvern, UK)) and a video was recorded at 25 frames/s. The video was evaluated by the NTA software version 3.3.301 Nanosight. For each exosome, the two-dimensional Stokes-Einstein equation was used at least 5 times to analyze the particle size of exosomes and Brownian motions and particle motion velocities were utilized. Additionally, Zetasizer Nano ZS (Malvern Instruments) was used to evaluate the particle size of isolated exosomes.

2.3. Protein Analysis with BCA

The protein concentration was determined by using the BCA protein analysis (Thermo Fisher) kit. Bovine Serum Albumin (BSA) was used as standard. Serial dilutions of the standard were prepared. Exosomes were diluted at a 1:5 ratio (5 μL of exosomes, 20 μL of H_2O). Water was used as a blank and 10 μL of diluted exosome, water and standards were added in wells. Next, B and A solutions were mixed at a 1:50 ratio, and 200 μL of the mixture was supplied to each well. The plate was incubated for 30 minutes ($C0_2$ incubator. Absorbances were read at 570 nm in a spectrophotometer (Thermo Scientific, USA).

2.4. Cell Lines and Passaging

The human colon cancer (HCT116) and human lung cancer (A549) cell lines were bought from ATCC (American Type Culture Collection). Dulbecco's Modified Eagle's Medium (DMEM: High-glucose, glutamine) was used as the culture medium of the cell lines, and this medium was enriched with 10% (V/V) fetal bovine serum (FBS) and 1% (V/V) PSA (10.000 units/mL of penicillin, 10.000 µg/mL of streptomycin, 25 µg/mL of amphotericin B). The cells were incubated in the humidified 37°C incubator with 5% of $C0_2$ (Özkan et al., 2021). As the first step of the transition of the adhering cells, the surface of the bottles was washed with Ca^{+2} and Mg^{+2}-free 1 x DPBS (Dulbecco's phosphate-buffered saline solution). The cell-surface interaction was separated with 0.025 % (W/V) trypsin for 3-5 minutes at 37°C. The detached cells were collected on the live cell culture and centrifuged at 1300 rpm for 5 minutes. The pellet was dissolved in the live cell culture. DMEM with exosome-depleted FBS (%10) was used for the exosome treatment. To determine the number of cells per unit volume, 10 µL of cells with media were added to the hemocytometer (Hausser Scientific, Horsham, Pennsylvania, USA) and the cells in the 4 outer squares were counted under the microscope. To determine the average number in each square, the value resulting was divided by 4. The number of cells counted in the 4x4 area is the amount of cells in 0.0001 mL, so the result was multiplied by 10^4 to find the number of cells per milliliter. The value resulting was also multiplied by 10 (dilution factor) to obtain the final number of cells per milliliter.

2.5. Treatment with Exosomes and WST-1 Assay Analysis

100 µL of PBS was placed to each of the edge wells of a 96 well plate. 100 µL (5000 cell) of cell suspension with media (DMEM) were cultured into each well, and the plate was kept in the CO_2 incubator. Twenty four later, the preparation of the cell culture, the media in the wells were removed, and fresh media (DMEM) was added to control groups (150 µL to each well). 150 µL of exosomes with media ranged between 25-250 µg/mL, was added into the control groups, and they were left for incubation ($C0_2$ incubator) (Kocak et al., 2016). Exosome concentration was determined by NTA.

Following the exosome treatment for 24, 48, 72 h; the excess part was discarded leaving 90 µL in the wells. 10 µL of WST-1 stain was added to each well, and the plate was incubated for 30 minutes ($C0_2$ incubator). Measurements were made in the spectrophotometer device (at 540 nm wavelength). Four independent tests were performed to create the growth percentage against control cells while cell viability was calculated as a percentage based on the measured absorbance values.

2.6. Statistical Analysis

Statistical analysis of the data was performed using two-way ANOVA and IBM SPSS Statistics software (v 22.0, IBM, Armonk, NY, USA). $P < 0.05$ was considered statistically significant. Datas were evaluated at 95% confidence interval.

3. Results

3.1. Nanoparticle Tracking Analysis (NTA)

Isolated GdENs were characterized based on their size and morphology. According to Figure 2, the diameters of the particles are between 30 nm to 230 nm. This finding is in line with the literature (Raposo and Stoorvogel, 2013). Since the contents of nanoparticles may vary, they are distinguished by their distinct size and morphology. According to the results, D10 (particle diameter corresponding to 10% cumulative small size distribution) was 79,4, D50 (the median diameter of particle size distribution) was 113,8, and D90 (the particle diameter corresponding to 90% cumulative small particle size distribution) was 163.8 nm. Nanoparticle concentrations were obtained as $2{,}53\times10^{12}$ particles/mL. The mean mode size was 109.1 +/- 3.2 nm. The resulting graphs are demonstrated in Figure 2 and Figure 3.

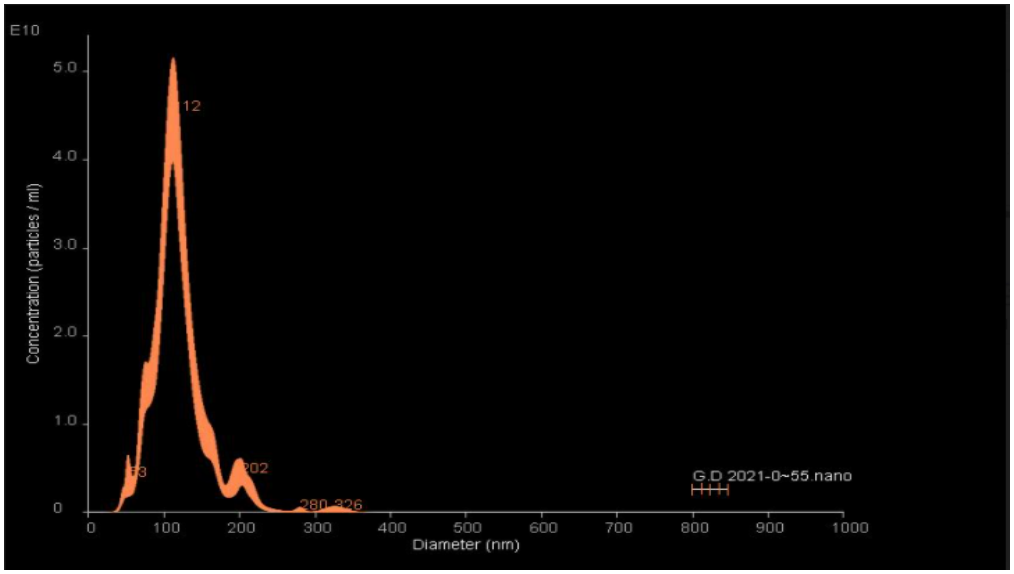

Figure 2. Concentration/Diameter Graph of Nanoparticles

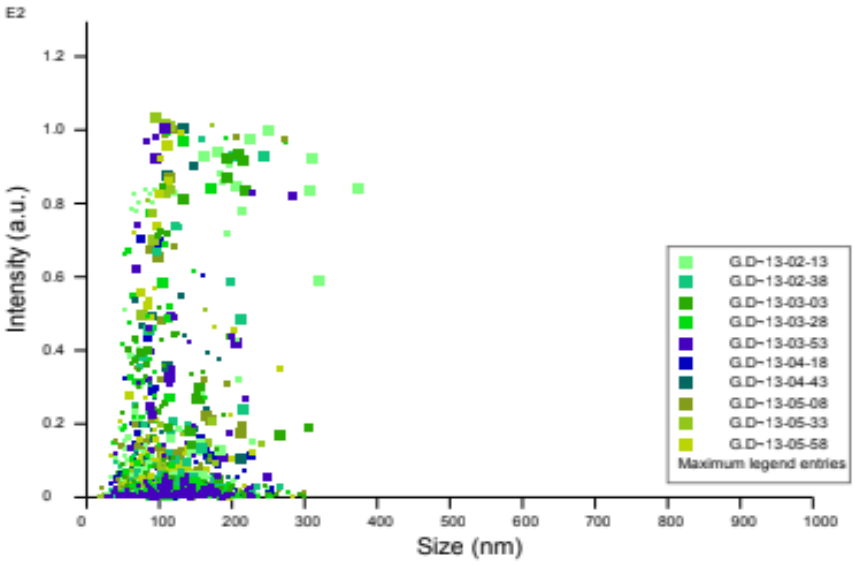

Figure 3. Intensity/Size Graph of Nanoparticles

3.2. Protein Analysis

The protein content of nanoparticles was calculated as 0.3875 µg/mL using the standard curve obtained from serial dilutions of Bovine Serum Albumin, which is used as a standard. The BCA test is a widely used colorimetric method to estimate the protein concentration in samples (Morton and Evans, 1992). The proteins detected in the tests are important indicators of the existence of GdENs.

3.3. Cell Viability (WST-1) Analysis

To determine the toxic dose of the exosomes like nanoparticles obtained from *Gundelia dersim* on cancer cells, a cell viability test was performed by employing a WST-1 assay test. The operation principle of WST-1 depends on the division of tetrazolium salt by a cellular mitochondrial dehydrogenase to form a formazan (Yin et al., 2013). The increase in cell viability increases the number of mitochondrial dehydrogenases, thus a saturated colour becomes generated. With this method, cell viability is measured through colorimetric ways (Koçak, 2021). The cell viability of the treated cells was calculated according to the viability of control cells.

In response to increasing doses of GdENs and treatment time periods, the cell viability of the HCT116 cell line was evaluated relative to control cells (n=4). A decrease of 20-30% at 100 and 250 µg/mL concentrations occurred in the 24-hour incubation period in the HCT116 cell line compared to control cells. For the 48-hour incubation at 75, 100, and 250 µg/mL concentrations, the decrease was 30-40%, while it was 10-30% in the 72-hour incubation at 75, 100 and 250 µg/mL concentrations. At the HCT116 cell line, no cytotoxic effect was observed at 24, 48, and 72 hours and 25 and 50 µg/mL, however, a significant cytotoxic effect ($p<0.05$) was observed by reducing the cell viability by a mean ratio of 25% at 75, 100 and 250 µg/mL (Figure 4).

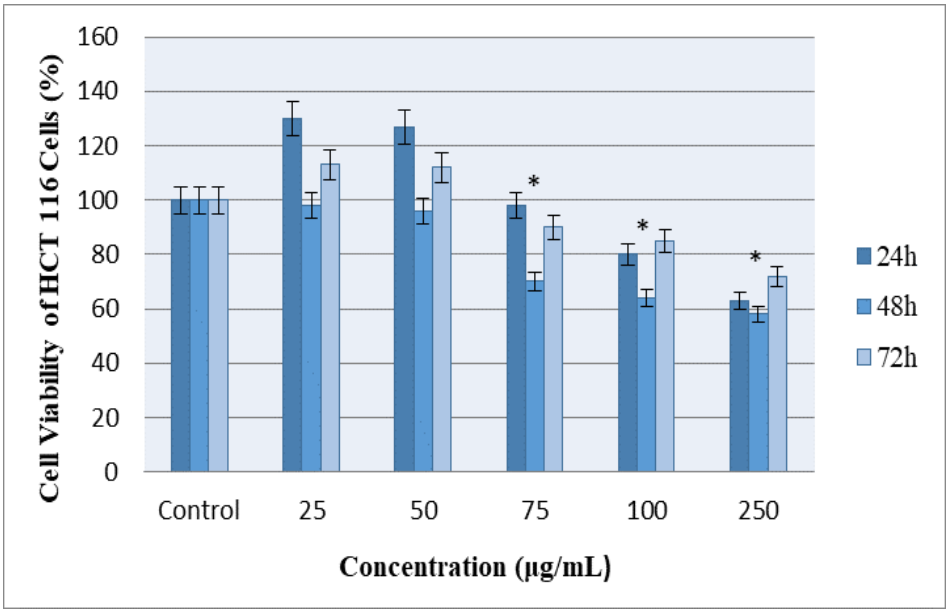

Figure 4. HCT116 Cell Viability (%)/Concentration Graph.

The cell viability results of the A549 cell line against the increasing doses and treatment periods with GEs are presented in Figure 5. Although in all 24, 48, and 72-hour incubation periods there were increases in cell viability proportional to the increased concentrations in the A549 cell line, after the 24-hour incubation a 30-35% decrease occurred in all concentrations compared to control cells, and following the 48-hour period, there was a 20% decrease in the 25 µg/mL concentration, and 10% decrease in the 50 µg/mL concentration. After the 72-hour waiting duration of the same cell line, an increase of 10% was observed in the 25 µg/mL concentration, and a 60% of the increase in the 100 and 250 µg/mL concentrations. Cell viability of the A549 cell line was evaluated relative to control cells in response to increasing doses of GdENs and treatment times (n=4). While it was observed that a significant cytotoxic effect ($p<0.05$) was observed in the A549 cell line, reducing cell viability by 21% at 24, 48, and 72 hours and only at 25 µg/mL, nanoparticles added to the cells at high concentrations did not demonstrate cytotoxic effects. On the contrary, they increased viability (Figure 5).

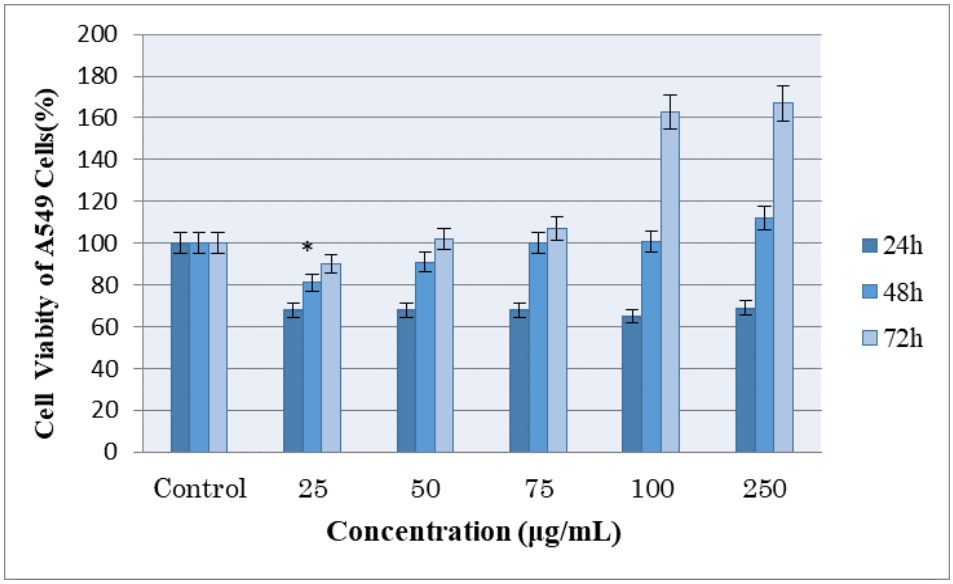

Figure 5. A549 Cell Viability (%)/Concentration Graph.

4. Discussion

Recently, PENs have become the center of attention due to their excellent biocompatibility, anti-inflammatory activities and antitumor capacities (Chen et al., 2022). Numerous studies reported that PENs exhibited anti-cancer effects. In the study with exosomes obtained from garlic, exosomes were given to metastatic breast cancer, small cell lung cancer, and colon cancer cells, and the effects of plant exosomes on cancer cells were examined. Garlic exosomes have not had significant toxicity on normal cells but have been shown to have an effect in reducing cancer cell viability (Yıldırım, 2018). In another study conducted on cancer cell proliferation of garlic-derived small extracellular vesicles (SEVs), it was reported that increasing doses of garlic SEVs did not demonstrate a significant

cytotoxic effect on the viability of normal human dermal fibroblast (HDF) cells. On the contrary, it significantly increased the proliferation of HDF cells when exposed to low doses of garlic SEVs (Özkan et al., 2021). In a study investigating the biological activities of exosome-like nanovesicles isolated from cabbage (Cabex) and red cabbage (Rabex) in human cells, cabex increased the proliferation of human keratinocyte (HaCaT) and murine macrophage (RAW 264.7) cells 72 hours after the treatment, and it was concluded that cabex and rabex generally increased the cell proliferation without demonstrating a cytotoxic effect (You et al., 2021).

In the current study, the effects of exosome-like nanoparticles isolated from *Gundelia dersim*, an endemic plant in Turkiye, on cell viability in HCT 116 and A549 cell lines were investigated. Polyethylene glycol was used in the isolation of GdENs by ATPS method to enrich the concentration and isolation efficiency. Dextran has surface properties that allow selective separation by specifically attacking exosomes and pushing/excluding the remaining extracellular vesicles. The two-phase separation allows exosomes to remain in the lower DEX phase, while contaminants and proteins remain in the upper PEG phase (Kim et al., 2015). This method provides a more inexpensive and easier way to isolate extracellular vesicles such as exosomes (Konoshenko et al., 2018).

In a study investigating the cytotoxic and antigenotoxic effects of different extracts of *G. dersim* plant, it was reported that the plant did not have any cytotoxic effect in the range of 0.25-50 mg/L concentrations (Aydin, 2022). In the literature review on this matter, no study on the cell viability of GdENs in cancer cells was found. However, certain studies were conducted with *Gundelia tournefortii* extracts. Jamshidzadeh et al. reported that these extracts could provide protection against CCI4-induced liver damage at doses of 200 and 300 mg/kg, but were less effective at doses higher than 300 mg/kg (Jamshidzadeh et al., 2005).

Various concentrations (25, 50, 100 and 200 μg/mL) of gold nanoparticles obtained from *Gundelia tournefortii* leaf extract were reported to have a significant cytotoxic effect on ovarian sarcoma (Skov-3), human colorectal adenocarcinoma (Caco-2) and glioblastoma (U118) cells and suppressed the viability of cancer cell lines by 14-59% at 25 μg/mL after 48 hours (Keskin et al., 2022). In another study, *Gundelia tournefortii* extracts exhibited anticancer effects on HCT 116 cell lines by MTT assay (Abu-Lafi et al., 2019). Additionally, the same plant extracts provided a significant cytotoxic effect on the breast cancer cell line (MCF-7) at 24 and 48 hours, and a decrease in cell viability was observed depending on the dose and time (Özaltun and Daştan, 2019). Lemon juice (*Citrus limon* L.) derived vesicles (5 or 20 μg/ml) were reported to inhibit tumor cell viability in A549, SW480 (human colorectal adenocarcinoma cell line) and LAMA84 cell lines for 24, 48 or 72 hours in a dose and time dependent manner compared to untreated cells by MTT viability assay. At 48 hours, 20 μg/ml nanovesicles demonstrated a 50% viability reduction in three cell lines (Raimondo et al., 2015).

In our study, GdENs exhibited a cytotoxic effect by decreasing cell viability by 25% ($p<0.05$) at 75, 100 and 250 μg/mL at all time points in the HCT 116 cell line (Figure 4). In the A549 cell line, GdENs decreased cell viability by 21% ($p<0.05$) at 25 μg/mL at all time points and by 35% ($p<0.05$)

at 24 hours at all concentrations. At 100 and 250 µg/mL, the cell viability increased (Figure 5). The findings obtained from both other plant-derived nanovesicles and *Gundelia tournefortii* studies are in agreement with our findings. However, we think that GdENs may not exhibit a cytotoxic effect at low concentrations in HCT 116 cells but may show cytotoxic effects at higher concentrations. Furthermore, we would like to point out that we did not have the opportunity to evaluate the apoptotic effects of nanovesicles on cancer cells with electron microscope images to support our results, which is a limitation of our study.

5. Conclusion

From our study, it was concluded that nanoparticles isolated for the first time from *G. dersim* can be evaluated as exosome-like nanoparticles in terms of size, morphology and protein content. In addition, this study showed that GdENs can exhibit a cross-kingdom communication and have an anticarcinogenic potential, given their cytotoxic effects on cancer cells at certain concentrations and at certain time periods. If our results are supported by more comprehensive studies (electron microscope images, apoptotic effects etc.) GdENs as a natural agent may become the focus of interest of drug researchers in the near future as promising new-generation therapeutics.

Acknowledgements

We thank Professor Doctor Fikrettin Sahin from Yeditepe University for all his support and contributions during the execution of our experimental studies. This work was supported by Scientific Research Projects Coordination Unit of Firat University (FUBAP Project number: SYO:20.03) and includes the results of the master's thesis.

Conflict of Interest

The authors declare that they do not any conflicts of interest.

Author's Contributions

The contribution of the authors is equal.

References

Abu-Lafi S., Rayan B., Kadan S., Abu-Lafi M., Rayan A. Anticancer activity and phytochemical composition of wild *Gundelia tournefortii*. Oncology Letters 2019; 17(1): 713-717.

Açar M., Akbulut GB., Taşar N. Anatomical, palynological and biochemical studies on *Gundelia dersim* Vitek, Yüce & Ergin (Asteraceae) an endemic of Turkey. Journal of the Institute of Science and Technology 2021; 11(3): 1781-1791.

Aydın S. Endemik *Gundelia dersim* bitkisinin farklı ekstraktlarının sitotoksik ve antigenotoksik etkilerinin araştırılması. Balıkesir Üniversitesi Fen Bilimleri Enstitüsü, Master's Thesis, page no:64, Balıkesir, Turkey, 2022.

Chen Q., Li Q., Liang Y., Zu M., Chen N., Canup BS., Luo L., Wang C., Zeng L., Xiao B. Natural exosome-like nanovesicles from edible tea flowers suppress metastatic breast cancer via ros generation and microbiota modulation. Acta Pharmaceutica Sinica B 2021; 12(2): 907-923.

Chen N., Sun J., Zhu Z., Cribbs AP., Xiao B. Edible plant-derived nanotherapeutics and nanocarriers: recent progress and future directions. Expert Opinion on Drug Delivery 2022; 19(4): 409-419.

Dalar A., Zengin G., Mukemre M., Bengu AS., İşler S. Gundelia rosea seed: Evaluation of biopharmaceutical potential and bioactive composition. South African Journal of Botany 2019; 125: 505-510.

Ersöz E., Can OB., Uzunoğlu S. Eksozomların kanserdeki rolü. Celal Bayar Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi 2016; 3(1): 144-152.

Ertas A., Firat M., Yener I., Akdeniz M., Yigitkan S., Bakir D., Cakir C., Yilmaz MA., Ozturk M., Kolak U. Phytochemical fingerprints and bioactivities of ripe disseminules (fruit-seeds) of seventeen *Gundelia* (kenger-kereng dikeni) species from anatolia with chemometric approach. Chemistry & Biodiversity 2021; 18(8): e2100207.

İnanır C., Ekici L. Eksozomlar: Kompozisyonları, biyolojik fonksiyonları ve biyo aktif bileşiklerin taşınmasındaki potansiyelleri. Akademik Gıda Dergisi 2020; 18(4): 421-432.

Jamshidzadeh A., Fereidooni F., Salehi Z., Niknahad H. Hepatoprotective activity of Gundelia tourenfortii. Journal of Ethnopharmacology 2005; 101(1-3): 233-237.

Ju S., Mu J., Dokland T., Zhuang X., Wang Q., Jiang H., Xiang X., Deng ZB., Wang B., Zhang L., Roth M., Welti R., Mobley J., Jun Y., Miller D., Zhang HG. Grape exosome-like nanoparticles induce intestinal stem cells and protect mice from dss-induced colitis. Molecular Therapy 2013; 21(7): 1345-1357.

Kameli N., Dragojlovic-Kerkache A., Savelkoul P., Stassen FR. Plant-derived extracellular vesicles: current findings, challenges, and future applications. Membranes 2021; 11(6): 411.

Kahraman T., Güçlüler G., Gürsel İ. Eksozomlar: Tanı ve isletmede doğal nanokesecik adayları. Türk İmmünoloji Dergisi 2014; 2(2): 34-40.

Keskin C., Baran A., Baran MF., Hatipoğlu A., Adican MT., Atalar MN., Husynova I., Khalilov R., Ahmadian E., Yavuz Ö., Kandemir Sİ., Eftekhari A. Green synthesis, characterization of gold nanomaterials using gundelia tournefortii leaf extract, and determination of their nanomedicinal (antibacterial, antifungal, and cytotoxic) potential. Journal of Nanomaterials, 2022; 2022, 7211066.

Kırbaş OK., Bozkur BT., Asutay A., Mat B., Ozdemir B., Öztürkoğlu D., Ölmez H., İşlek Z., Şahin F., Taşlı PN. Optimized isolation of extracellular vesicles from various organic sources using aqueous two-phase system. Scientific Reports 2019; 9(1): 1-11.

Kim J., Shin H., Kim J., Kim J., Park J. Isolation of high-purity extracellular vesicles by extracting proteins using aqueous two-phase system. Plos One 2015; 10(6): 0129760.

Kim K., Yoo HJ., Jung JH., Lee R., Hyun JK., Park JH., Na D., Yeon JH. Cytotoxic effects of plant sap-derived extracellular vesicles on various tumor cell types. Journal of Functional Biomaterials 2020; 11(2): 22.

Kim J., Li S., Zhang S., Wang J. Plant-derived exosome-like nanoparticles and their therapeutic activities. Asian Journal of Pharmaceutical Sciences 2022; 17(1): 53-69.

Kocak P., Canikya S., Batukan M., Attar R., Şahin F., Telci D. Comparison of enzymatic and nonenzymatic isolation methods for endometrial stem cells. Turkish Journal of Biology 2016; 40(5): 1081-1089.

Koçak P. The effect of wheat (*Trıtıcum Aestıvum L.*) Derıved exosomes on wound healıng. Yeditepe University Graduate School of Natural and Applied Sciences Department of Biotechnology, Doctora Thesis, page no: 129, İstanbul, Turkey, 2021.

Konoshenko MY., Lekchnov EA., Vlassov AV., Laktionov PP. Isolation of extracellular vesicles: general methodologies and latest trends. BioMed Research International 2018; 8545347.

Morton RE., Evans TA. Modification of the bicinchoninic acid protein assay to eliminate lipid interference in determining lipoprotein protein content. Analytical Biochemistry 1992; 204(2): 332-334.

Nemati M., Singh B., Mir RA., Nemati M., Babaei A., Ahmadi M., Rasmi Y., Golezani AG., Rezaie J. Plant-derived extracellular vesicles: a novel nanomedicine approach with advantages and challenges. Cell Communication and Signaling 2022; 20(1): 1-16.

Özaltun B., Daştan T. Evaluation of antimicrobial activities and in vitro cytotoxic activities of *Gundelia tournefortii* L. plant extracts. SDÜ Tıp Fakültesi Dergisi 2019; 26(4): 436-442.

Özkan İ., Koçak P., Yıldırım M., Ünsal N., Yılmaz H., Telci D., Şahin F. Garlic (Allium Sativum)-derived sevs inhibit cancer cell proliferation and induce caspase mediated Apoptosis. Scientific Reports 2021; 11(1): 1-11.

Raimondo S., Naselli F., Fontana S., Monteleone F., Dico AL., Saieva L., Zito G., Flugy A., Manno M., Bella MAD., Leo GD., Alessandro R. citrus limon-derived nanovesicles inhibit cancer cell proliferation and suppress cml xenograft growth by inducing trail-mediated cell death. Oncotarget 2015; 6(23): 19514-19527.

Rome S. Biological properties of plant-derived extracellular vesicles. Food & Function 2019; 10(2): 529-538.

Raposo G., Stoorvogel W. Extracellular vesicles: Exosomes, microvesicles and friends. Journal of Cell Biology 2013; 200(4): 373-383.

Taylor DD., Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecologic Oncology 2008; 110(1): 13-21.

Yin LM., Wei Y., Wang Y., Xu YD., Yang YQ. Long term and standard incubations of wst-1 reagent reflect the same inhibitory trend of cell viability in rat airway smooth muscle cells. International Journal of Medical Sciences 2013; 10(1): 68.

You JY., Kang SJ., Rhee WJ. Isolation of cabbage exosome-like nanovesicles and investigation of their biological activities in human cells. Bioactive Materials 2021; 6(12): 4321-4332.

Zhang M., Viennois E., Prasad M., Zhang Y., Wang L., Zhang Z., Han MK., Xiao B., Xu C., Srinivasan S., Merlin D. Edible ginger-derived nanoparticles: A novel therapeutic approach for the prevention and treatment of inflammatory bowel disease and colitis-associated cancer. Biomaterials 2016; 101: 321-340.