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Short-Communication

Co-formulation of ursolic acid and selenium nanoparticles modulates the mRNA expression of *MMP-2*, *MMP-9* and *ICAM-1* in triple-negative breast cancer cells MDA-MB-231

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Abstract

Objective: Metastasis drives cancer mortality. Triple negative breast cancer (TNBC) is highly invasive and often resistant to therapy. Ursolic acid combined with selenium nanoparticles may reduce proliferation and enhance treatment efficacy while minimizing toxicity in MDA-MB-231 cells.

Materials and methods: The cytotoxicity of ursolic acid and selenium nanoparticles on MDA-MB-231 cells was assessed via MTT assay. Effects on *ICAM-1*, *MMP-2*, and *MMP-9* expression were measured by real-time PCR, and cell migration was evaluated using the scratch assay.

Results: A combination of selenium nano particles (SeNPs) (150 µg/mL) and ursolic acid (20 µg/mL) showed greater cell inhibition compared to each substance alone. This co-formulation not only decreased the viability of cancer cells but also exhibited additive effects on reducing the migration and metastasis of MDA-MB-231 cells by effectively downregulating *MMP-9*, *MMP-2*, and *ICAM-1* expression.

Conclusion: These findings support further animal studies to validate the therapeutic potential for TNBC.

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Introduction

Breast cancer, accounting for 11.7% of all cancer cases in 2020 according to the World Health Organization(Organization ; Sung et al. 2021), remains one of the leading causes of cancer-related mortality among women (Dai et al. 2017). Breast cancer is further classified into subtypes based on receptor expression, including progesterone receptor (PR), human epidermal growth factor 2 receptor (HER2), and estrogen receptor (ER). Among these, triple-negative breast cancer (TNBC) is

particularly aggressive due to the lack of these receptors, affecting 15–20% of patients. TNBC tumors typically exhibit a high histologic grade and an increased rate of early relapses, often diagnosed at an advanced stage (Singh and Yadav 2021). In contrast to receptor-positive tumors, TNBC tumors are considered more aggressive and incapable of treatment. Patients with TNBC who developed distant metastases had a low overall survival rate, around eighteen months (Vagia et al. 2020). Breast tumor mortality is often caused by its ability to spread to other parts of the body, known as metastasis. This process consists of various stages including (i) tumor cells' transformation by degradation of extracellular matrix (ECM), (ii) intravasation that is denoted by tumor cells' migration to vessels, (iii) extravasation, and (iv) proliferation in the target organ (Van Zijl et al. 2011), during which cancerous cells spread from the primary tumor site to other parts of the body via the lymphatic system, bloodstream, or direct extension. The poor prognosis associated with TNBC stems from its aggressive nature and the limited success of conventional treatments, such as chemotherapy, which often result in significant toxicity and minimal improvement in survival rates. Therefore, discovering a new TNBC treatment strategy is critical to increase life expectancy (Chien et al. 2018; Wang et al. 2019).

Although not all natural products are beneficial, several compounds derived from plants, animals, and microorganisms, have shown significant potential in cancer prevention and treatment (Yao et al. 2020). They interfere with cancer initiation, development, and progression through mechanisms such as cellular proliferation, differentiation, apoptosis, angiogenesis, and metastasis (Muhammad et al. 2022). These compounds offer a multi-targeted approach, modulating various signaling pathways involved in cancer such as NF-κB (Nuclear Factor Kappa B), PI3K/Akt (Phosphatidylinositol 3-Kinase/Protein Kinase B), MAPK (Mitogen-Activated Protein Kinase), and JAK/STAT (Janus Kinase/Signal Transducer and Activator of Transcription) pathways, and can induce apoptosis, inhibit angiogenesis, and prevent metastasis(Hashem et al. 2022). Combining natural compounds with conventional therapies can enhance efficacy, reduce toxicity and overcome resistance compared to monotherapy (Deng et al. 2020; Naeem et al. 2022).

Ursolic acid, a pentacyclic triterpenoid, is a versatile compound found in various medicinal plants such as *Prunella vulgaris*, *Nepeta cataria and Lavandula stoechas*, fruits and vegetables with a wide range of pharmacological activities including anti-cancer, anti-inflammatory, antioxidant, and neuroprotective effects (Ryu et al. 2000). It demonstrates significant potential in inhibiting metastasis across various cancer types including colorectal cancer, non-small cell lung cancer, and breast cancer through various mechanisms including induction of apoptosis, cell cycle arrest, and inhibition of key signaling pathways (Yin et al. 2018; Zou et al. 2019). According to a study in 2010, Ursolic acid (UA) has shown anti-invasive effects on highly metastatic breast MDA-MB-231 cells by inhibiting the phosphorylation of Jun N-terminal kinase, Akt, and the mammalian target of rapamycin, mTOR. In addition, it appears to reduce nuclear NF-κB protein levels, followed by downregulation of *MMP-2* and u-PA expression (Yeh et al. 2010). A study shows that UA significantly enhances the sensitivity of MDA-MB-231 and MCF-7 cells to epirubicin, modulating autophagy-related pathways and improving cytotoxicity compared with single-agent treatment (Obidiro et al. 2023). UA also demonstrates potential to reverse chemoresistance to paclitaxel in resistant breast cancer cells through regulation of miR-149-5p/MyD88 signaling, indicating a supportive role in therapy (Wang et al. 2022). Furthermore, it has been shown that UA inhibits the proliferation of colorectal cancer cells by targeting multiple cells signaling pathways including NF-κB,

STAT3, and β-catenin, and downregulating proteins associated with cell survival, and metastasis (Prasad et al. 2012). Thus, the use of UA could enhance such therapeutic strategies by amplifying targeted effects on cancer cells while reducing side effects, positioning it as a valuable natural component in cancer treatment (Shanmugam et al. 2013; Wang et al. 2012).

Cell adhesion molecules (CAMs) are considered crucial to preserving tissue structure and function. CAMs participate as a binding agent in which they mediate cell-to-cell or cell-to-extracellular matrix (ECM) binding (Ren et al. 2011). Among them, intercellular adhesion molecule-1 (*ICAM-1*), a transmembrane glycoprotein of the immunoglobulin (Ig)-like superfamily, demonstrates overexpression in highly metastatic cancers (Schröder et al. 2011) such as TNBC, around 8 to 25-fold higher (Guo et al. 2014). It has been claimed that *ICAM-1* leads to the interaction between leukocytes and endothelium, which facilitates tumor cells' extravasation (Figenschau et al. 2018; Reina and Espel 2017).

Matrix metalloproteinases (MMPs), other biomarkers of invasive cancers, are from the zinc-dependent endopeptidases involved in tissue remodeling, and cancer development. MMPs elevate the cancer invasion rate by reducing cell adhesion through the degradation of the ECM which acts as a physical barrier to tumor metastasis (Tang et al. 2019). As reported in several studies, *MMP-2* and *MMP-9* are significantly increased in various metastatic cancers, leading to the eradication of basement membrane (BM) and ECM and, in turn, cancer metastasis (Katunina et al. 2011; Parsons et al. 1998; Vizoso et al. 2007).

Selenium (Se) is a trace element that plays a crucial role in living organisms. Among different forms of Se, red elemental selenium (Se^0), known as selenium nanoparticle (SeNP) has greatly attracted attention due to its therapeutic advantages, including anti-cancer, antioxidant, anti-inflammatory and antimicrobial effects(Sampath et al. 2024). Moreover, nanosized Se is more bioavailable, affordable, and less toxic than other selenium-containing compounds (Forootanfar et al. 2014). SeNPs can be synthesized through different methods including physical, chemical, and biological. Chemical synthesis is mostly a common and rapid method. In contrast, microbes and plants are used for the biological synthesis of SeNPs (Skalickova et al. 2017). Reports suggested that oral administrations of SeNP in mice affected by metastatic breast tumors enhanced their immune response and reduced metastasis to the liver (Faghfuri et al. 2015; Yazdi et al. 2012).

Given the complementary biological profiles of UA and SeNPs namely the anti-inflammatory and anti-metastatic activity of UA and the enhanced bioavailability and cellular uptake of SeNPs, their combination was selected to assess potential improvements in anti-TNBC efficacy.

Materials and Methods

Cell culture condition

Human breast cancer cell line MDA-MB-231 (IBRC C11325) was obtained from the Iranian Biological Resource Center (IBRC, Tehran, Iran). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Germany) supplemented with 10% fetal bovine serum (FBS) (Gibco, Germany), 100 Units of penicillin/ml (Gibco, Germany), and 100 µg of streptomycin/ml (Gibco, Germany), then incubated at 37°C with 5% CO_2.

Production of SeNPs

According to a previous study, biological SeNPs were produced using *Lactobacillus plantarum* (Zare et al. 2023). Briefly, 1 mL of a 254 mM stock solution of SeO2 along with 1 mL of *L. plantarum* culture with OD_{600}= 1 was added to 100 mL of fresh MRS (de Man, Rogosa and Sharpe) broth and incubated at 37°C for 72 hr. To

release the intracellular red elemental selenium, *L. plantarum* cell pellets were broken with liquid nitrogen and ultrasound treatment. SeNPs were purified using an n-Octyl alcohol/water extraction system (Shakibaie et al. 2010). The SeNPs solution was stored at 4°C until use.

Characterization of SeNPs

The morphology, particle size distribution, and surface charge of selenium nanoparticles (SeNPs) were characterized using scanning electron microscopy (SEM), dynamic light scattering (DLS), and zeta potential analysis, respectively. Scanning electron microscopy (SEM) SEM was used to examine particle shape and surface morphology. Dynamic light scattering (DLS) DLS determined the hydrodynamic diameter and polydispersity index, while zeta potential measurements assessed surface charge and colloidal stability.

Ursolic acid (UA) preparation

Purified UA powder was previously obtained from the Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Tehran University of Medical Sciences (Tehran, Iran). A stock solution was prepared by dissolving 10 mg of UA in 500 µL of dimethyl sulfoxide (DMSO) (Sigma, UK) and stored at 4 °C. For experimental use, the stock was diluted to the desired concentrations (DMSO <0.1%) in DMEM (Gibco, Germany) without FBS and refrigerated for subsequent procedures.

Selection of SeNPs and UA concentrations

The selected concentration ranges for SeNPs (50–250 µg/mL) and UA (5–25 µg/mL) were based on preliminary experiments assessing cytotoxicity and biological activity, as well as published studies reporting effective doses in similar cell models. For SeNPs, prior studies have reported significant cytotoxic effects and IC_{50} values within the tens to low hundreds of µg/mL range in cancer cells, justifying exploration up to 250 µg/mL to capture the full dose-response (Medina-Cruz et al. 2023). For UA, multiple *in vitro* studies report effective inhibitory and apoptotic effects at concentrations corresponding to approximately 5–25 µg/mL (≈10–50 µM), supporting our use of this range to assess dose response (Zhang et al. 2019)

MDA-MB-231 cell viability assay

Cell viability was evaluated by MTT assay (Sigma, United Kingdom). To this end, 2×10^4 MDA-MB-231 cells/well were seeded in 96-well cell culture plates and incubated at 37° C for 24 hr. Then, the medium was changed with the fresh one containing different concentrations of SeNPs (50, 100, 150, 200, and 250 µg/mL) and UA (5, 10, 15, 20, and 25 µg/mL). The treated cells were incubated separately for 24, 48, and 72 hr to evaluate the time dependency of the treatment. 20µL MTT reagent (5 mg/mL) was added to each well after the mentioned times. Then, the plates were further incubated for 2 hr in darkness. Finally, the complete cell culture medium was replaced with 200 µL of DMSO. After 15 min, the absorbance was measured at 570 nm to quantify the amount of formazan crystals. The cell viability percentage of treated cells was calculated using the following formula:

$$\%\text{Cell viability} = \frac{\text{MeanODtreated cells}-\text{MeanODblank}}{\text{MeanODcontrol cells}-\text{MeanODblank}} \times 100$$

RNA extraction and cDNA synthesis

Approximately 2.5×10^5 MDA-MB-231 cells/well were seeded in three separate six-well plates and incubated for 4h. Each plate was treated with one of three protocols including: 1) SeNPs group (150 µg/mL SeNPs); 2) UA group (20 µg/mL UA); and 3) combined group (150 µg/mL SeNPs + 20 µg/Ml UA and incubated for 24, 48, and 72 hr. Total RNA was extracted from both treated and control MDA-MB-231 cells using the RNX™ reagent kit (SinaClon, Iran), following the manufacturer's instructions. The RNA concentration and purity were determined by measuring

absorbance at 260 nm and 280 nm using a spectrophotometer. The integrity of RNA was confirmed by electrophoresis on a 1.2% agarose gel (Meryer, Shanghai) for 1 hr at 100 V. Equal amounts of high-quality RNA from each sample were used for complementary DNA (cDNA) synthesis (Sanaei et al. 2021). The complementary DNA (cDNA) was synthesized from 3 µL of total RNA by incubation in a thermocycler for 1 hr at 43°C with M-MLV reverse transcriptase enzyme (Fermentas, Lithuania) according to the manufacturer's instruction. Then, to visualize the PCR product, amplified cDNA with gene-specific primers (Table 1) was run on a 1.5% agarose gel with ethidium bromide staining (Meryer, Shanghai). The relative expression data were normalized using *β-actin* as a housekeeping gene.

Table1. Primers' sequences for qPCR

Target gene	Forward primer	Reverse primer
MMP-2	CAGGCTCTTCTCCTTTCSCAAC	AAGCCACGGCTTGGTTTTCCTC
MMP-9	GCACGACGTCTTCCAGTACC	CAGGATGTCATAGGTCACGTAGC
ICAM-1	CCTGATGGGCAGTCAACAGCTA	ACAGCTGGCTCCCGTTTCA
β-actin	GAGACCTTCAACACCCCAGCC	AGACGCAGGATGGCATGGG

Real-time polymerase chain reaction

Quantitative real-time PCR was performed with 25 µL of the PCR reaction mix, according to the manufacturer's instructions. Briefly, each PCR reaction contains 12 µL of SYBR Green master mix, 2 µL of primers (Table 1), 3 µL of cDNA template, and 8 µL of double-distilled water. The PCR procedure consisted of 95°C for 10 min followed by 40 cycles of 95°C for 15 sec, 60°C for 1 min, and 72°C for 5 min. All the tests were run in triplicate on an ABI 7500 Fast Real-time PCR system. Data were analyzed by a comparative threshold (C_t), using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001) and the mean fold inductions of the samples were compared with those of the untreated samples.

Scratch assay

Cells were plated into a 6-well cell culture plate and incubated to approximately 90% confluence. To minimize cell proliferation, 5% FBS was used in the culture media. Then, cells were carefully scratched using a yellow pipette tip, and cellular debris was washed away with Phosphate buffered saline (PBS) PBS (Di et al. 2016). After that, the cells were treated with 150 µg/mL SeNPs, 20 µg/mL UA, or a combination of the two. Each cell-containing well was monitored during the following time (0, 24, 48, and 72 hr) by taking the photos under an inverted microscope (Olympus Corp.), and the closure of the wound in each treated cell was compared with the control to observe the potential of cell migration.

Statistical analysis

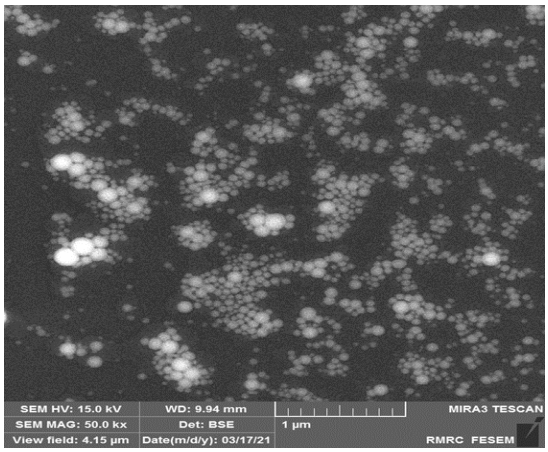
Statistical analysis was performed using GraphPad Prism software version 8.4.3 (GraphPad Software, San Diego, CA, USA). Data are reported as group means ± SEM and were analyzed via two-way ANOVA followed by Tukey's test. Differences were considered significant when the p-value was < 0.05.

Results

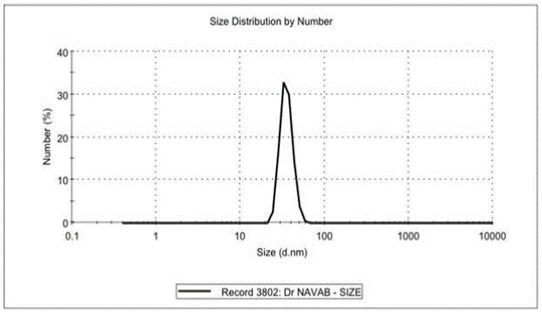
Characterization of SeNPs

The synthesized selenium nanoparticles (SeNPs) were characterized using SEM, DLS, and zeta potential analysis. SEM images revealed predominantly spherical nanoparticles with relatively smooth surfaces and a fairly uniform size distribution (Figure 1a). DLS analysis

showed an average hydrodynamic diameter of approximately 35–40 nm, with a narrow and unimodal size distribution (Figure 1b). Zeta potential analysis showed a negative surface charge centered at −40 mV, indicating strong electrostatic repulsion between particles and good colloidal stability of the SeNPs (Figure 1c).



a



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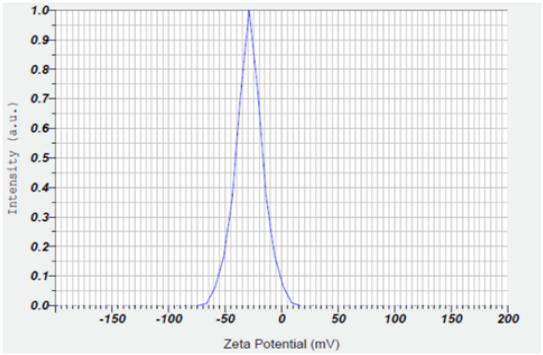

c

Figure 1. Characterization of the SeNPs. SEM images of the SeNPs revealing predominantly spherical particles with smooth surfaces and good dispersion(a). DLS analysis showing the particle size distribution of the SeNPs(b). Zeta potential measurement of the SeNPs, displaying a strongly negative value (c).

Cell viability by MTT assay

To evaluate the cytotoxic effect of different doses of SeNPs (50-250 µg/mL), UA (5-25 µg/mL), and the combination of them (SeNPs 50-200 µg/mL+ UA 20 µg/mL) on MDA-MB-231 cells, MTT assay was performed. A significant decrease in MDA-MB-231 cell viability was observed in cells treated with 150 µg/mL of SeNPs (Figure 2a), 20 µg/mL of UA (Figure 2b), and the combination dose of 150 µg/mL SeNPs + 20 µg/mL UA (Figure 2c). The percentage of viable cells after treatment with SeNPs, UA, and combined group treatments was about 10%, 30%, and 20%, respectively compared to the control group (Figure 2). Therefore, the most effective doses of SeNPs and UA for cell viability were determined by MTT analysis and considered for the following experiments on expression and metastasis development.

Effect of SeNPs and UA on *MMP-2, MMP-9,* and *ICAM-1* expression levels

Our study aimed to investigate the effect of SeNPs and UA on tumor metastasis and invasion. In this regard, the expression levels of genes involved in tumor invasion, including *MMP-2*, *MMP-9*, and *ICAM-1* were measured by real-time PCR. MDA-MB-231 cells were incubated in three separate cell culture plates with selective concentrations of 150 µg/mL SeNPs, 20 µg/mL UA, and a combination of 150 µg/mL SeNPs + 20 µg/mL UA for 24, 48, and 72 hr (Figure 3). As shown in Figure 3a, the *MMP-9* expression level was significantly decreased in all treated cells compared to the control group (***p<0.001). Likewise, *MMP-2* expression level indicates significant reduction after treatment with 20 µg/mL UA (**p<0.01) and the combination of 150 µg/mL SeNPs plus 20 µg/mL UA (***p<0.001) and 150 µg/mL SeNPs (*p<0.05) (Figure 3b).

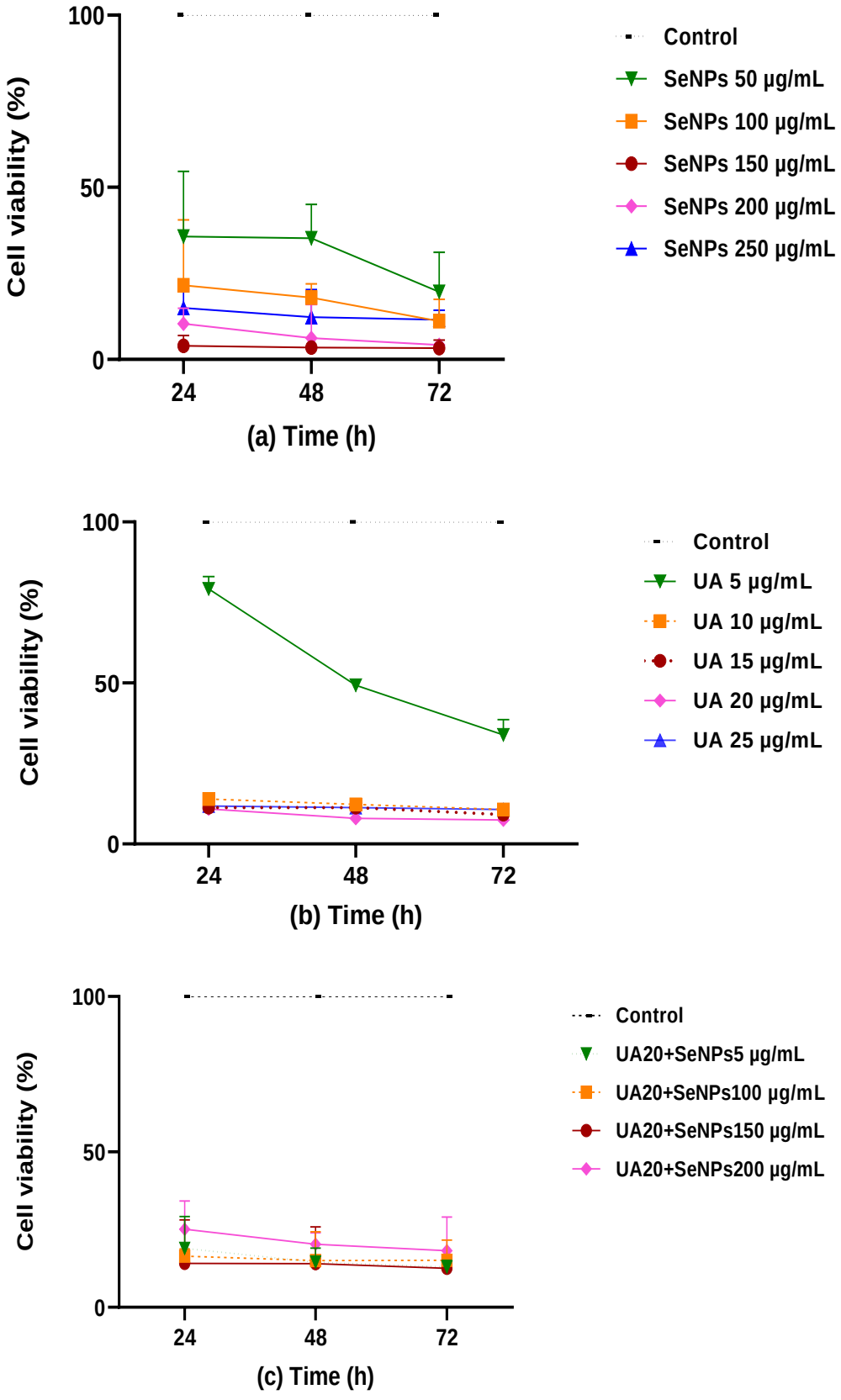

Figure 2. Effect of SeNPs (a), Ursolic acid (UA) (b), and SeNPs plus UA (c) on cell viability of MDA-MB-231 cells. Cells were treated with various concentrations for 24, 48, and 72 h. Cell viability was assessed by the MTT method. Each point represents mean ± SEM (n=3).

Additionally, *ICAM-1* expression showed a significant reduction after incubation with 150 µg/mL SeNPs plus 20 µg/mL UA (***p<0.001) and with 20 µg/mL UA alone (**p<0.001). However, 150 µg/mL SeNPs did not significantly affect *ICAM-1* expression levels (Figure 3c). Of note, the most reduction of *MMP-9*, *MMP-2*, and *ICAM-1* gene expression belonged to the combination-treated compared with 150 µg/mL SeNPs or 20 µg/mL UA alone (Figure 3).

Moreover, the inhibition of *MMP-2*, *MMP-9*, and *ICAM-1* gene expression using UA, SeNPs, and their combination showed a time-dependent manner, in which treated cells indicated significant results after 72 hr of incubation with all treatments (Figure 3).

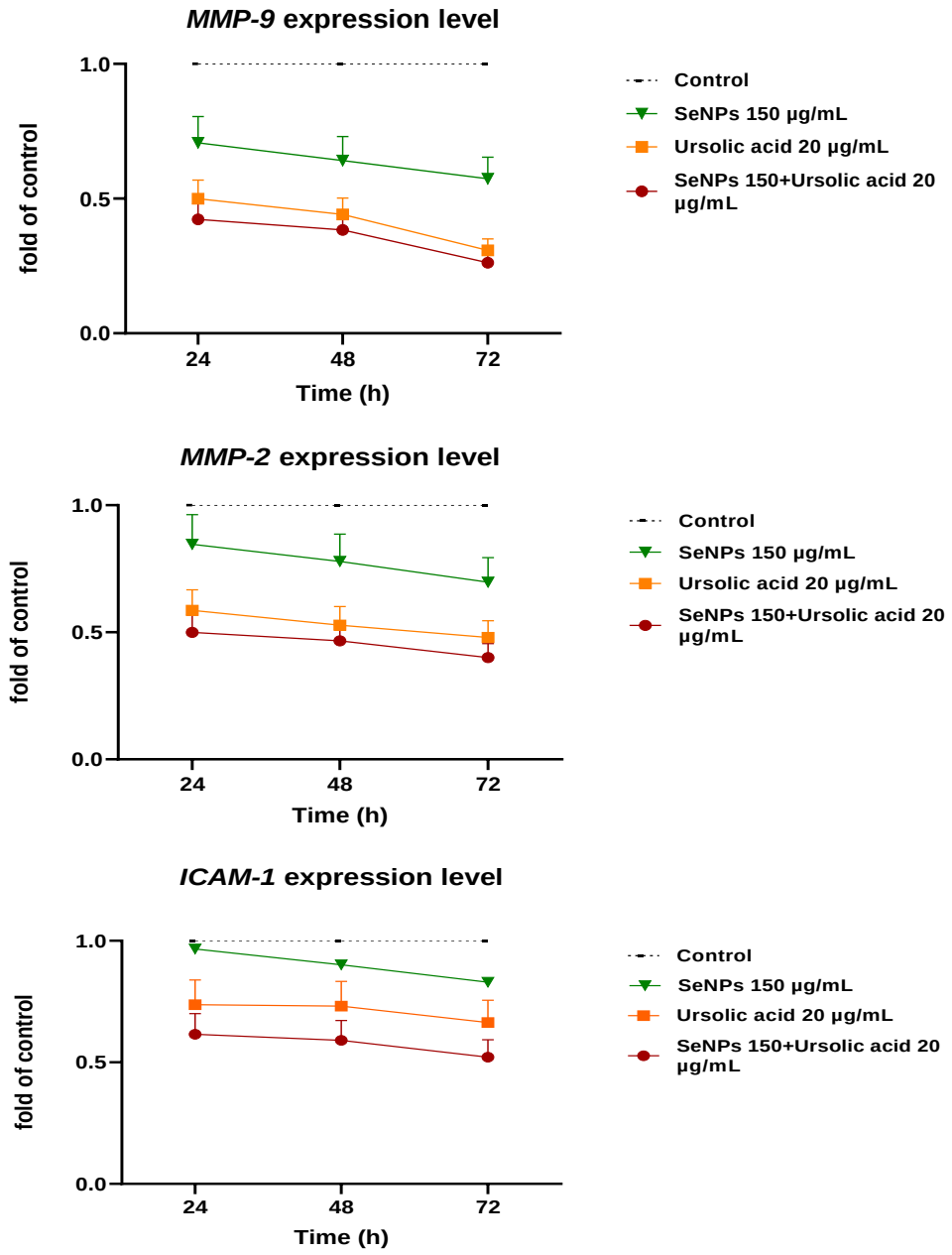

Figure 3. Effect of 150 µg/mL SeNPs and 20 µg/mL ursolic acid, and their combination on the expression level of *MMP-9* (a), *MMP-2* (b), and *ICAM-1* (c) in MDA-MB-231 cells after treatment for 24, 48, and 72 hr. Data are presented as mean ± SEM (n = 3) and were analyzed using two-way ANOVA followed by Tukey's test (*p<0.05, **p<0.01, ***p<0.001 versus control).

Scratch assay

To investigate the effect of SeNPs and UA on cell migration, the scratch assay was performed. As shown in Figure 4, the motility of treated cells with 150 µg/mL SeNPs, 20 µg/mL UA, and the combination of them were reduced, according to the density of cells that filled the gap, compared to the control group. In addition, the combination of 150 µg/mL SeNPs plus 20 µg/mL UA demonstrates the lower invasion rate rather than single UA treated cells. It was also obtained that all treated groups exhibited the highest inhibition of cell motility after 72 hr of incubation.

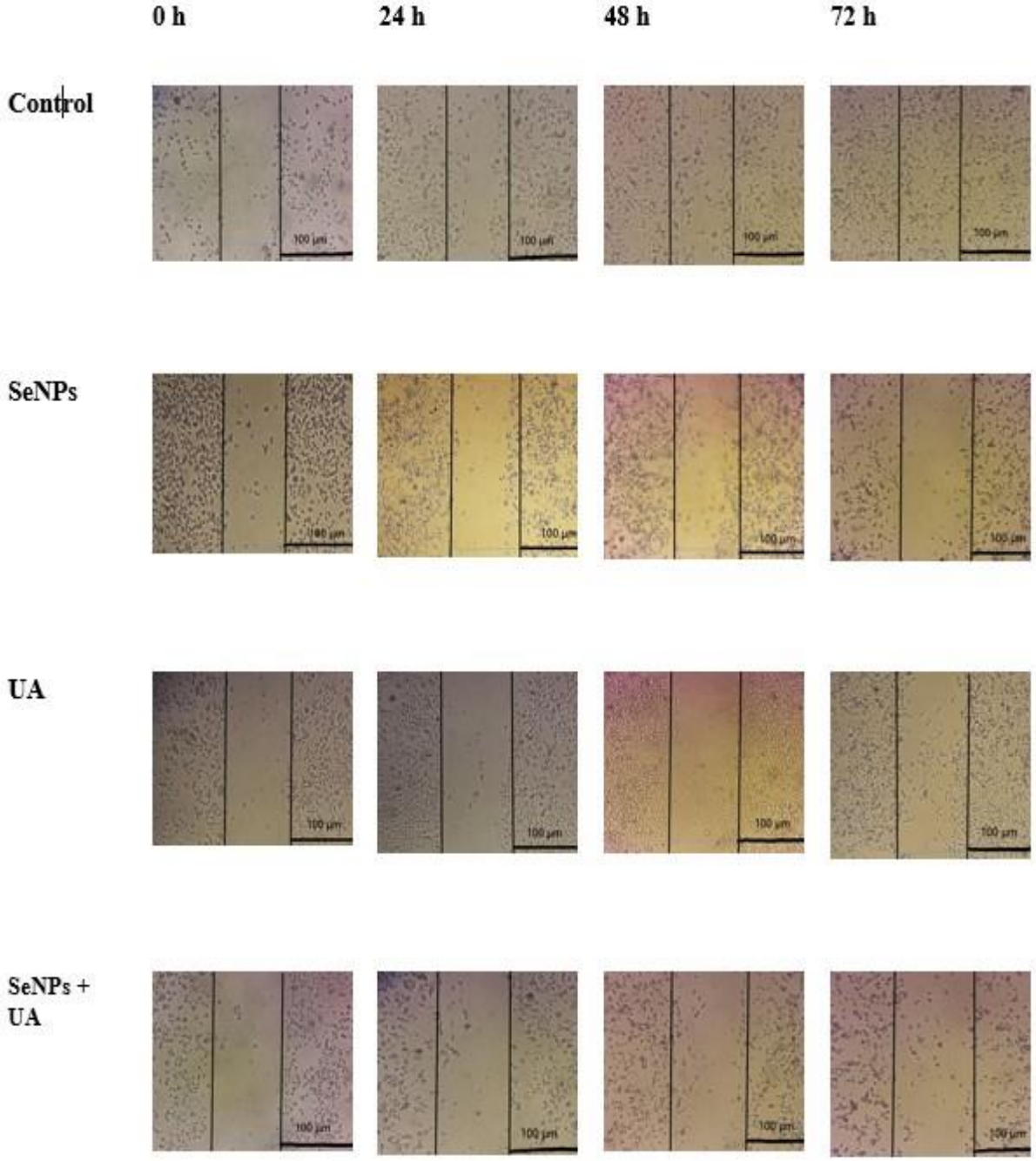

Figure 4. Effects of 150 µg/mL SeNPs, 20 µg/mL ursolic acid (UA), and combination of them after 24, 48, and 72 hr on migration in MDA-MB-231 cells using scratch assay. Scale bar:100 µm

Discussion

Although advancements in cancer therapies have improved outcomes for some subtypes, metastasis in TNBC remains a critical challenge, necessitating innovative approaches, as this study explores. Current conventional cancer therapies such as paclitaxel and doxorubicin, are associated with substantial systemic toxicity. These chemotherapeutic agents can cause cardiotoxicity, myelosuppression, peripheral neuropathy, and alopecia, often necessitating dose reductions or treatment interruptions that compromise efficacy. In addition, TNBC frequently develops drug resistance, reducing long-term effectiveness. Such limitations underscore the urgent need for safer, more tolerable adjuvant therapies that can reduce adverse effects while maintaining anticancer activity (Obidiro et al. 2023; Song et al. 2025).

Cancer metastasis requires a cascade of events such as cell adhesion, ECM degradation, and invasion. Our findings confirm that *MMP-9* and *MMP-2*, well-established drivers of cancer invasion through ECM degradation, were significantly downregulated by the combination of SeNPs and UA, highlighting their potential as therapeutic targets. In the tumor microenvironment of the highly metastatic carcinomas, *MMP-9* and *MMP-2* are up-regulated, which leads to Epithelial-mesenchymal transition (EMT) events and metastasis (Creighton et al. 2010; Li et al. 2017). Furthermore, tumor cells must reduce cell adhesion to promote migration. *ICAM-1*, a glycoprotein belonging to the immunoglobulin superfamily and involved in a variety of immune and inflammatory responses, lymphoid trafficking, and several malignancies, including TNBC, has the potential to be positively correlated with tumor metastasis and invasion by modulating cell-cell adhesion. Also, *ICAM-1* has the potential to serve as a molecular target for treating patients with TNBC (Aysola et al., 2013). Although we did not directly investigate upstream signaling pathways such as NF-κB, Akt/PI3K, or JAK/STAT in this study, existing evidence strongly suggests that they play important roles in regulating the genes we measured. NF-κB activation has been shown to drive the expression of adhesion molecules, including *ICAM-1*, through promoter binding and transcriptional regulation (Zhou et al. 2007). NF-κB also mediates the expression of MMPs such as *MMP-2* and *MMP-9*, in response to inflammatory stimuli, thereby linking inflammatory signaling to extracellular matrix remodeling. Upstream of NF-κB, Akt/PI3K signaling can enhance its activation via phosphorylation-dependent degradation of IκB, providing a route through which survival and stress pathways may influence pro-inflammatory gene expression (Mao et al. 2024). In addition, JAK/STAT signaling has been implicated in endothelial inflammatory responses and can interact with both NF-κB and PI3K/Akt pathways to modulate pro-inflammatory mediators (Groten et al. 2025). While these findings provide a plausible mechanistic context for our observations, directly examining these pathways remains an important avenue for future studies.

According to above, the combination influences the transcriptional expression of metastasis-related genes, including *MMP-2*, *MMP-9*, and *ICAM-1*, suggesting a possible anti-metastatic effect.

Nanoparticle-sized selenium showed the most significant anti-proliferative effect on MDA-MB-231 cells at the concentration of 150 µg/mL, rather than other treatment and control groups (Figure 2a). UA inhibited cell proliferation significantly at the dose of 20 µg/mL in comparison to different doses of UA and the control group (Figure 2b). Interestingly, the combination of 150 µg/mL SeNPs and 20 µg/mL UA indicated a significant effect on reducing cell viability compared with other mixed doses and control group (Figure 2c). Results of gene expression level assay by Real-time PCR (Figure 3) showed a time-

dependent reduction in the expression level of *MMP-9* and *MMP-2* after treatment with 150 μg/mL SeNPs, 20 μg/mL UA, and their combination at mentioned doses. The expression of *MMP-2* was decreased by SeNPs, UA, and the combination group by about 0.4, 0.5, and 0.6, respectively, compared with the control group, and the reduction of *MMP-9* was about 0.5, 0.6, and 0.7, respectively. The outcomes indicated that the combination treatment with SeNPs and UA resulted in the greatest decrease in *MMP-9* and *MMP-2* gene expression levels, in contrast to applying each of them separately. Thus, based on the data obtained, it can be concluded that SeNPs and UA possess the potential to induce reductions in cell viability and motility. This improvement allows for lower effective doses of both agents, minimizing side effects while enhancing the efficacy. Selenium nanoparticles, due to their nanoscale size, large surface area, and superior cellular membrane penetration, significantly improve bioavailability and targeted drug delivery. This allows effective therapeutic action at reduced dosages, potentially minimizing toxicity (Bisht et al. 2022). Furthermore, *ICAM-1* expression was significantly reduced with UA by about 0.3-fold and 0.5-fold with a combination of SeNPs and UA, while administration of 150 μg/mL SeNPs alone indicated the lowest effect on *ICAM-1* expression with less than 0.2-fold reduction, which was not statistically significant. This observation highlights the fact that UA can target multiple molecular pathways involved in cancer progression including proinflammatory transcription factors, cell cycle proteins, growth factors, kinases, cytokines, chemokines, adhesion molecules, and inflammatory enzymes. This broad targeting can inhibit cancer initiation, promotion, and metastasis. The scratch assay was carried out to clarify the potential relationship between cell migration and metastasis with the downregulation of MMPs and *ICAM-1* on treated cells. As shown in Figure 4, all treated cells demonstrated lower mobility than the control group, which exhibits that migration in drug-exposed cells has occurred at a lower rate. As concluded in this study, although applying 150 μg/mL SeNPs and 20 μg/mL UA separately revealed more cell inhibition rate, a combination group of SeNPs and UA not only decreases the viability of cancer cells in comparison to the control group but also harbors beneficial effects in reducing the migration and metastasis of MDA-MB-231 cells by their most effective downregulation of *MMP-9*, *MMP-2*, and *ICAM-1* expression. Also, previous studies proved that the high expression of these markers is positively associated with aggressive tumor phenotypes and a lower prognosis of breast cancer (Guo et al. 2014). A study shows that UA alone reduces *MMP-2* and *MMP-9* expression, suppressing migration and invasion of MDA-MB-231 cells (Zhang et al. 2021). Another study shows that SeNP formulations significantly reduce viability and promote apoptotic pathways in MDA-MB-231 cells, reinforcing the utility of SeNPs as effective nanocarriers in TNBC therapeutic strategies (Abdul-Razek et al. 2025). These promising findings position the combination of SeNPs and UA as a novel therapeutic strategy for TNBC. However, further validation in preclinical models is essential to unravel the precise mechanisms and evaluate clinical applicability.

We acknowledge several limitations in this study. While qRT-PCR allowed assessment of gene expression, it does not necessarily reflect protein abundance or activity; protein-level validation, such as Western blot, is needed in future work to confirm modulation of *MMP-2*, *MMP-9*, and *ICAM-1*. Cytotoxicity was evaluated using MTT assays against negative controls only, without a positive anticancer reference, which limits direct potency comparisons. Incorporating protein assays and standard positive controls in follow-up studies will provide a more comprehensive

understanding of efficacy, dosing, and underlying mechanisms.

Conflicts of interest

The authors declare no conflicts of interest regarding this manuscript.

Authors' Contributions

F.A. performed the experiments and prepared the original draft. A.SH. supervised the preparation of the nanoparticles. H.SH. and M.S. revised the manuscript. M.Y. was responsible for the study conception, design, and supervision of all parts of the study. All the authors reviewed the manuscript.

Abbreviations

TNBC: triple-negative breast cancer, SeNPs: selenium nanoparticles, UA: Ursolic acid, MMP: matrix metalloproteinases, *ICAM-1*: Intercellular Adhesion Molecule 1, ECM: extracellular matrix, mTOR: mammalian target of rapamycin, Wnt :Wingless-related integration site, NF-κB: Nuclear Factor kappa-light-chain-enhancer of activated B cells, PI3K/Akt: Phosphoinositide 3-kinase/Protein kinase B, ERK: Extracellular signal-regulated kinase, PARP: Poly(ADP-ribose) polymerase, qPCR: Quantitative Polymerase Chain Reaction, DMSO: dimethyl sulfoxide, FBS: fetal bovine serum, IRBC: Iranian Biological Resource Center, DMEM: Dulbecco's modified Eagle medium, ERs: Estrogen Receptors, PRs: Progesterone Receptors, HER2: Human Epidermal Growth Factor Receptor 2

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