ORIGINAL ARTICLE

Synergistic effects of Zoledronic acid and Quercetin in vitro on breast cancer cells migration, proliferation and viability

LAILA ALSAWALHA[1], RANIA AL-GROOM[1], MAJDOLEN RAMMAHA[2], DIYA HASAN[1], MUHANNAD I. MASADEH[2], SARA ABAZA[1]

[1]Department of Allied Medical Sciences, Zarqa University College, Al-Balqa Applied University, Salt- Jordan; [2]Dept. Biology and Biotechnology, Faculty of Science, The Hashemite University, Zarqa-Jordan

ABSTRACT

Background and aim of the work: Quercetin: a natural bioactive molecule has showed promise in cancer prevention and treatment. It is used for various cancers including breast cancer. The bisphosphonate Zoledronic acid (ZA); has recently come into therapeutic use. Despite the introduction of several medicinal techniques, breast cancer remains the most common uncurable cancer in women. Given this, the current study aims to elucidate the synergistic antitumor interaction between quercetin and zoledronic acid; both of which have anti-proliferative and anti-metastatic capabilities, notably targeting fatty acid synthesis and cholesterol synthesis in breast cancer cells in vitro.

Research design and methods: Human breast cancer cell lines MCF-7 and MDA-MB-231 were treated with a quercetin, ZA, or their combination. Quercetin was applied at concentrations of 480, 240, 120, 60, 30, and 10 μM, and ZA at 360, 160, 80, 40, 20, and 10 μM. Combination treatments were applied using 80:80, 180:80, and 80:160 μM for MCF-7 cells, and 90:171, 180:171, 90:340 μM for MDA-MB-231 cells. Cell proliferation was assessed using the MTT cytotoxicity assay. RT-PCR was performed to analyze the expression of genes linked to apoptosis, clone creation, and cell migration.

Results: The quercetin-ZA combination induced Bcl-2-independent apoptosis and significantly inhibited cell growth. The combined treatment was more effective than either medication alone after 72 hours, with IC50 values of 80:160 μM for MCF7 (p=0.0021) and 90:171 μM for MDA-MB-231 cells (p=0.0161). Self-renewal and clonogenic tumor development were markedly inhibited after 3-hour combination treatment. Moreover, MCF7 and MDA-MB 231 cell invasiveness was significantly decreased from 24 to 72 h compared with untreated cells.

Received: 28 November 2025 | Accepted: 28 December 2025

Correspondence: Laila Alsawalha, Ph.D. / Department of Allied Medical Sciences, Zarqa University College, Al-Balqa Applied University, Salt- Jordan. Yusuf Abd alrasool Street, 11732, Amman, Jordán. / E-mail: lmsawalha@Bau.edu.jo
ORCID: 0000-0002-2034-4869

Conclusions: The quercetin-ZA combination synergistically suppressed MCF-7 and MDA-MB-231 breast cancer cells, reducing migration and self-renewal. These findings highlighted their potential adjuvant effects on conventional treatments of breast cancer. (www.actabiomedica.it)

Key words: breast cancer, zoledronic acid, quercetin, metastasis, apoptosis

Introduction

Breast cancer (BC) is the most prevalent malignancy and the leading cause of cancer-related mortality among women worldwide. Owing to advancements in imaging, surgery, and medical treatments, the detection and treatment of breast cancer (BC) have improved, with higher survival rates and reduced recurrence. However, 5% of patients with BC have metastases at the time of diagnosis, with bone being the most common site. Osteolytic bone metastases are the primary cause of bone lesions and resorption. Growth factors and cytokines in bone influence BC cell differentiation and proliferation, and bone metastasis accelerates tumour cell growth (1,2). In cancer research, combination therapies that target proliferation, angiogenesis, and oxidative pathways have shown promising outcomes (3,4). Zoledronic acid (ZA) is a nitrogen-containing bisphosphonate (BP) and bone-modifying drug that prevents metastatic disease progression. ZA has been shown to avoid cancer bone metastasis by delaying osteoclast recruitment, proliferation, and differentiation from preosteoclasts (5,6) by decreasing the osteolytic activity of mature osteoclasts (7). All nitrogen-containing BPs inhibit farnesyl pyrophosphate synthase in the mevalonate pathway (8), resulting in inhibition of farnesyl pyrophosphate and geranylgeranyl pyrophosphate. These isoprenoids are required for post-translational macromolecule modification (i.e., farnesylation and geranylgeranylation) of signalling GTPases, such as Ras, Rho, and Rac(9). Recently, natural compounds such as quercetin have gained increasing attention in treating breast cancer. Quercetin is a common dietary flavonoid. Its biological effects are complex and include antibacterial, anticarcinogenic, anti-inflammatory, and anti-diabetic activities. In plants, quercetin is predominantly present as a glycoside, which is converted to quercetin conjugates in the intestine. Its biphasic, dose-dependent chemopreventive activity makes it a promising candidate in oncology. Its anticancer effects include apoptosis, cell cycle arrest, mitotic process suppression, and reduced proliferation (10). Various studies have investigated the influence of quercetin on different forms of cancer and found that a high intake of quercetin is inversely associated with the risk of prostate, lung, stomach, and breast cancer (11) . Moreover, the antilipogenic effect of quercetin is strongly associated with its ability to arrest growth and induce apoptosis in prostate and breast cancer cells, indicating that FASN inhibition may be at least one of the mechanisms by which quercetin exerts its anti-carcinogenic effects (12). Combination therapy is an effective strategy for cancer treatment, as it targets multiple pathways to lower drug resistance and disrupt tumor homeostasis. To achieve the highest efficacy with the least toxicity, it is essential to understand cancer drug interactions (13). Recently, several studies have shown that quercetin may act synergistically, enhancing the toxicity of other treatments in different cancer cells (14). The present study aimed to investigate the effects ZA and quercetin on BC cells in vitro, focusing on cell migration, proliferation, and viability.

Methodology

Cell culture

Human MCF-7 cells and MDA-MB 231 triple-negative BC cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA,

USA), cultured in high-glucose DMEM (PAN-Biotech, Germany), both supplemented with 10% fetal bovine serum (FBS), 1% glutamine, and 1% penicillin-streptomycin. For subculturing, trypsin-EDTA as used for cell detachment. Cells were maintained at 37°C in a humidified environment of 5% CO_2 and 95 % air (15).

In vitro cytotoxicity (MTT) assay

Cell proliferation was evaluated using the MTT assay. Between 5,000 and 10,000 cells per well were seeded in 96-well plates containing 10% FBS-supplemented growth medium and allowed to grow for 24 h. Stock solutions of ZA (AK Scientific, USA), quercetin (AK Scientific), and their combinations were prepared in 10% of DMSO, with the final DMSO concentration in culture not exceeding 0.1%. Cells were treated with quercetin at different concentrations (480, 240, 120, 60, 30, and 10 µM) and ZA (360, 160, 80, 40, 20, and 10 µM) for 24, 48, and 72 h. Each well was then filled with freshly prepared MTT solution (3-(4,5-dimethylthiazol2yl)-2,5-diphenyltetrazolium bromide) (5 mg/mL; AK Scientific). After a 3-hour incubation, cells were examined under an inverted microscope to determine whether formazan crystals had formed. Each well was then treated with a 200 µL mixture of DMSO and isopropanol and incubated for 30-45 minutes; cell growth inhibition assay were performed in triplicate (16). Cell growth inhibition induced by quercetin and ZA was determined by measuring absorbance at 570 nm. The IC_{50} values were determined the concentration-dose response curves using linear interpolation on a semi-log plot (17). Depending on the cytotoxic effect of each compound separately, a second MTT assay was performed to evaluate combination therapy using three different combination concentrations, 80:80, 160:80, and 80:160 µM for MCF-7, and 90:171, 180:171, and 90:340 µM for MDA-MB-231, following the previous MTT test procedure. Cell viability (%) was calculated using the formula: Cell viability (%) = OD treated/OD control x 100%. Where OD treated is the absorbance of treated cells and OD control is the absorbance of untreated control cells.(15).

Preparation of quercetin stock solutions

Quercetin stock solutions were prepared at 1 mM and 0.1 mM to generate working concentrations of 480, 240, 120, 60, 30, and 10 µM. The 1 mM stock1 solution was prepared by dissolving 0.003 g of quercetin in 1 mL DMSO and 9 mL PBS. The stock2 solution (0.1 mM) was obtained by a 1:10 dilution of the stock1. Working concentrations were prepared by further dilutions, using the formula C1V1=C2V2.

Preparations of ZA stock solutions

ZA stock solutions were prepared at 1 mM and 0.1 mM to generate working concentrations of 360, 160, 80, 40, 20, 10, and 0 µM. The 1 mM stock1 solution was prepared by dissolving 0.0029 g of ZA in 1 mL DMSO and 9 mL PBS. The stock2 solution (0.1 mM) was obtained by a 1:10 dilution of the stock1(18). Working concentrations were prepared by further dilutions, using the formula C1V1=C2V2.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

qRT-PCR was used to quantify mRNA levels of apoptotic markers Bcl-2, Mcl-1, and Actin. Total RNA was extracted from control and treated cells (after 72 h treatment) using the GeneAll Hybrid-R™ (Korea), following the manufacturer's guidelines. cDNA was created using the EasyScript® First-Strand cDNA Synthesis SuperMix (TransGen Biotech, Korea). EasyScript® RT/RI Enzyme Mix and 2×ES Reaction Mix (Macrogen, South Korea) were used to amplify the target cDNA for Bcl-2, Mcl-1, and Actin (normalization gene). A total of 1 pg-1 µg of cDNA template, 10 µM forward and reverse primers, nuclease-free water, and 10 µL of TransStart® Green qPCR SuperMix (2X) were combined. Amplification was performed on a 7500 Real-Time PCR System (Thermo Fisher, SN 2750010686) with the following cycling conditions: Stage 1: Holding: 94°C for 30 s. Stage 2: Denaturation: 94°C for 5 s, Annealing: 60°C for 34 s, Elongation: 72°C for 34 s. Stage 3: Melting curve: 70°C for 34 s, 60°C for 1 min, 95°C for 30 s. The list of the forward and reverse primers used in Table 1. Experiments were performed in triplicate (19).

Table 1. lists the forward and reverse primers used for Bcl-2, Mcl-1, and Actin.

Gene	Forward primer	Reverse primer
Bcl2	ATCGCCCTGTGGATGACTGAGT	GCCAGGAGAAATCAAACAGAGGC
Mcl1	CCAAGAAAGCTGCATCGAACCAT'	CAGCACATTCCTGATGCCACCT
Actin	CACCATTGGCAATGAGCGGTTC	AGGTCTTTGCGGATGTCCACGT

Clonogenic assay

BC cells (100-200*10^3/well) were seeded in six-well plates. The following day, cells were treated with different concentrations of the studied drugs: 80:80, 160:80, 80:160 μM for MCF-7, and 90:171, 180:171, 90:340 μM for MDA-MB-231, for 3 h. The cells were then subcultured (1,000 cells/well, in duplicate) for 12 days. Subsequently, cells were fixed with 4% paraformaldehyde, stained with crystal violet, and photographed and counted under a microscope (20).

Wound healing assay

MCF-7 and MDA-MB-231 cells were seeded in 6-well plates and cultivated to 80% confluence. Wounds were created by scraping the monolayer with a 200 μL pipette tip, and non-adherent cells were removed by washing with PBS. Cells were then treated with the IC_{50} concentrations of each compound, the IC_{50} combination, or left untreated. Phase-contrast microscopy (10x objective) was used to capture images of treated and untreated control cells at 0, 24, 48, and 72 h after wound development. Cell migration into the wound gap was quantified using ImageJ. Experiments were performed in triplicate (21).

Statistical analysis

The obtained data were statistically evaluated using GraphPad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, CA, USA). One-way analysis of variance (ANOVA) in conjunction with Tukey's multiple comparisons test was used to analyze the effects of the treatment. Nonlinear regression (curve fit) was used to calculate drug 50% inhibitory concentration (IC50). The data are expressed as mean ± SEM for clonogenic tests and mean ± SD. For the wound healing assay, a two-way ANOVA was used to assess the interaction between different doses of combination ZA/Qr and time. Statistical tests were two-tailed, with a P-value < 0.05 indicating statistically significant differences. Data visualization and high-resolution 1200 DPI graphical renderings were created to verify the precision of error bar display.

Results

Anti-proliferative activity of quercetin and ZA

Cytotoxic assays were conducted using MCF7 and MDA-MB-231 cell lines to determine the optimum concentrations of quercetin and ZA. Seven different concentrations (480, 240, 120, 60, 30, 10, and 0 μM) for quercetin and six concentrations (360, 160, 80, 40, 20, 10, and 0 μM) for ZA were applied to the cells, separately. As shown in Figure 1 A-D, both quercetin and ZA, had a significant cytotoxic effect on both BC cell lines, dramatically reducing cell viability. The cytotoxicity of the quercetin and ZA combination was subsequently examined. Based on the individual IC_{50} values of quercetin and ZA (approximately 80-171 μM in both cell lines), combination treatments were prepared using 80:80, 180:80, 80:160 μM for MCF7 cells and 90:171, 180:171, and 90:340 μM for MDA-MB-231 cells. In MCF7 cells, the 80:160 μM quercetin-ZA combination reduced cell proliferation by 50% after 72 h (p= 0.0021). In MDA-MB-231 cells, the 90:171 μM combination suppressed proliferation by more than 80% (p= 0.0161). Overall, combined quercetin and ZA produced greater suppression of cell growth than either compound alone in both cell types. These findings implied that quercetin-ZA combination therapy significantly inhibits human BC cell proliferation (Figure 2 A-B).

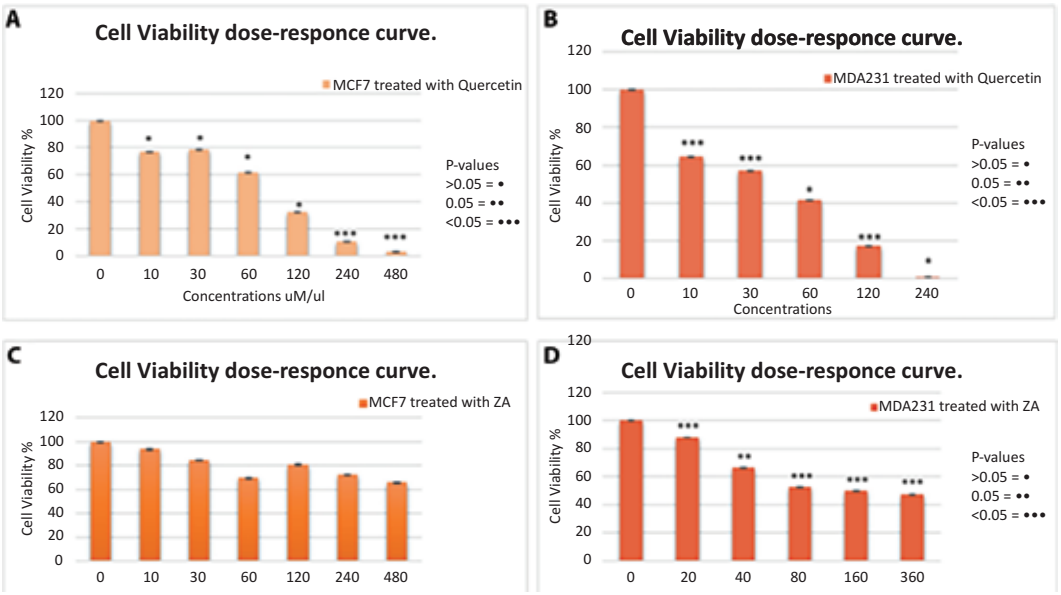

Figure 1. Effects of varying quercetin and ZA concentrations on MCF7 and MDA-MB-231 cell growth. (A and C) Proliferation of MCF7 cells treated with quercetin or ZA for 24, 48, and 72 h, with or without treatment. (B and D) Proliferation of MDA-MB-231 cells treated with quercetin or ZA for 24, 48, and 72 h, with or without treatment. For each time point, data are presented as the percentage of the treated group relative to the control group. Values represent the mean ± standard error of the mean (SEM) from three independent experiments.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

qRT-PCR analysis was performed to measure the expression of anti-apoptotic genes Bcl-2 and Mcl-1 in MCF7 and MDA-MB-231 cells treated with the quercetin-ZA combination at its IC_{50}. The mRNA levels of both genes were normalized to Actin expression (Figure 3 A-B).

Phase-contrast images showed that untreated MCF-7 cells formed large, densely packed, and well-defined epithelial-like colonies (Figure 4 B, A). In contrast, quercetin-ZA-treated cells exhibited marked reduced colony size, limited proliferation, and sparse distribution (Figure 4 A, B). Morphological changes in MDA-MB-231 cells after 12 days of treatment are shown in Figure 5B. Wild-type MDA-MB-231 cells displayed elongated, epithelial-like morphology and formed large, densely packed, well-defined colonies. In contrast, quercetin-ZA-treated cells exhibited markedly reduced colony size and number, with limited and sparse cell growth.

Wound-Healing Assay

The wound-healing test is an in vitro method for analyzing two-dimensional cell migration. A cell-free area was created in the confluent monolayer, which stimulates cell migration into this gap. A live-cell microscope was used to view the migration over several hours, showing the consistent movement of the cells. Compared to untreated cells, migration of both breast cancer cell lines (MCF7 and MDA-MB-231) was significantly decreased following treatment with the quercetin-ZA combination (80:160 μM for MCF7 and 90:171 μM for MDA-MB-231) from 24 to 72 h. In untreated cells, the wound created by scratching the monolayer was healed within 24 h, whereas the wound-healing process was considerably slowed in quercetin-ZA-treated cells, as shown in Figure 6.

Discussion

Many oncogenic signaling pathways are activated in the majority of malignancies. Consequently, the

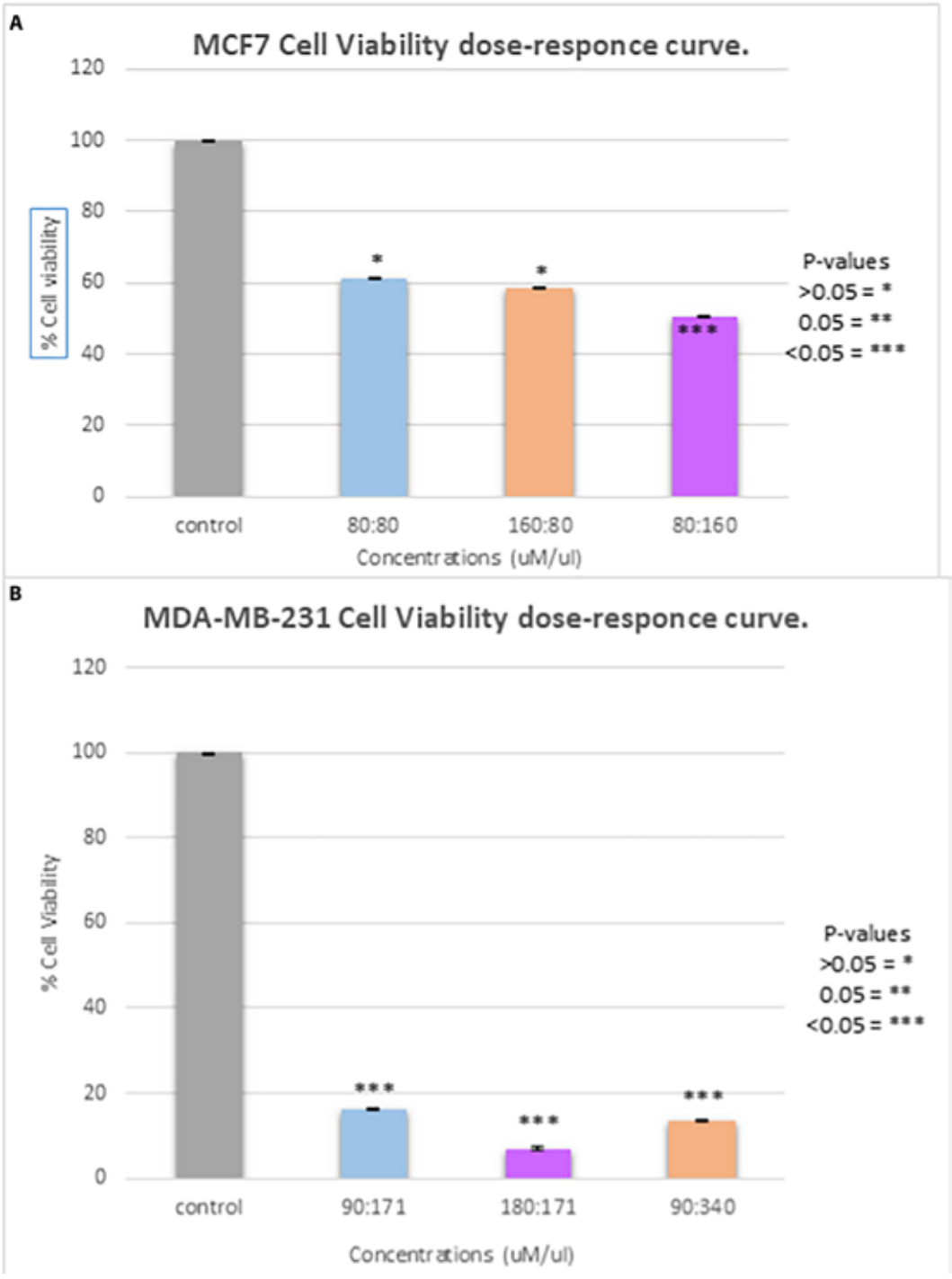

Figure 2. Effects of the quercetin-ZA combination on (A) MCF7 and (B) MDA-MB-231 cell growth. Cell proliferation was measured after 72 h of treatment. Results are presented as the percentage of treated cells relative to the control group. Each graph represents the mean of three independent experiments ± standard error of the mean (SEM).

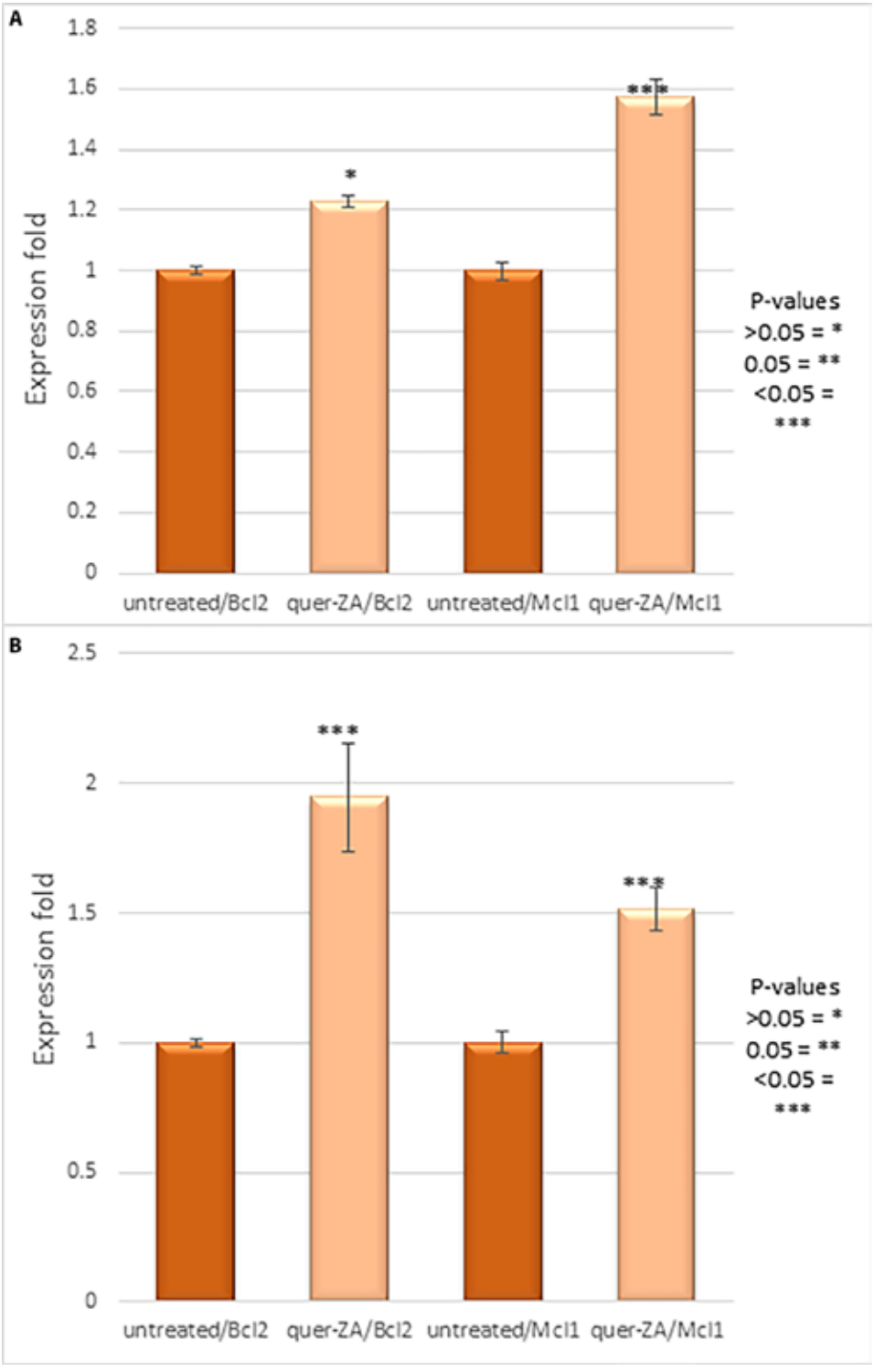

Figure 3. Effects of the quercetin-ZA combination on anti-apoptotic gene expression. (A) MCF-7 and (B) MDA-MB-231 cells were treated with the quercetin-ZA combination for 72 h. mRNA levels of Bcl-2 and Mcl-1 were normalized to β-actin. Each graph represents the mean of three separate experiments ± the standard error of the mean (SEM).

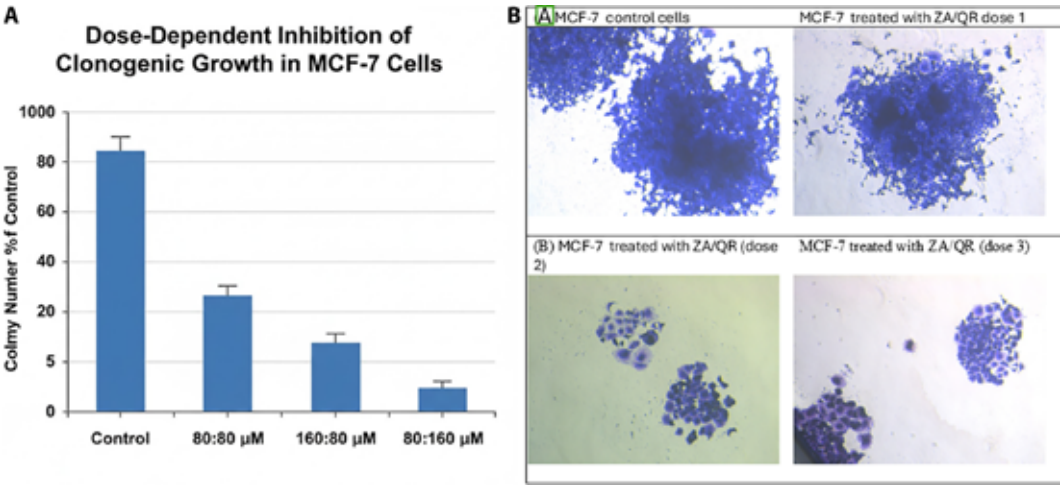

Figure 4. Effects of the quercetin-ZA combination on clonogenic growth in breast cancer cells. (A) Control MCF7 colonies and (B) MCF7 cells treated with increasing concentrations of the quercetin-ZA concentration for 3 h. Colonies were fixed and counted after 12 days. Colony number and size decreased as in a dose-dependent manner. Colonies were fixed and counted after 12 days. Colony number and size decreased as in a dose-dependent manner. A quantitative examination of colony survival for MCF-7 (dosages 80:80, 160:80, 80:160) and MDA-MB-231 (doses 90:171, 180:171, 90:340). Representative photos of untreated control colonies) indicate vigorous growth, whereas treated colonies; show significant reductions in size and quantity. The data are presented as mean ± SEM. The high-resolution (1200 DPI) format clearly depicts the greater inhibitory effect of increasing ZA ratios in both cell cultures.Bars represent the mean ± standard error of the mean (SEM) of three independent experiments performed in duplicate.

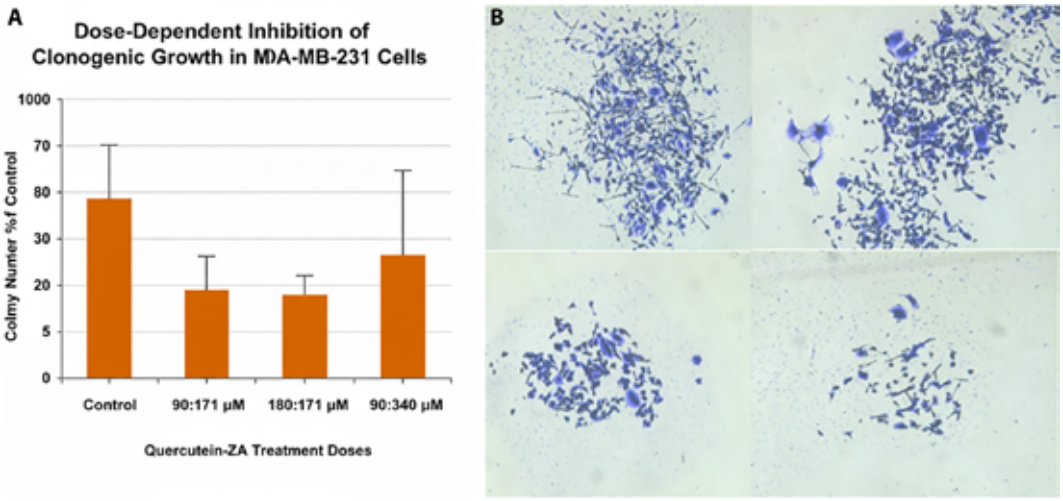

Figure 5. Effects of the quercetin-ZA combination on clonogenic growth in MDA-MB-231 cells. (A) Quantitative analysis of colony formation after treatment with three concentrations of the quercetin-ZA combination. and (B) MDA-MB-231 cells treated with increasing concentrations of the quercetin-ZA combination for 3 h. Colonies were fixed and counted after 12 days. Colony-number and size decreased in a dose-dependent manner. Bars represent the mean ± standard error of the mean (SEM) of three independent experiments performed in duplicate.

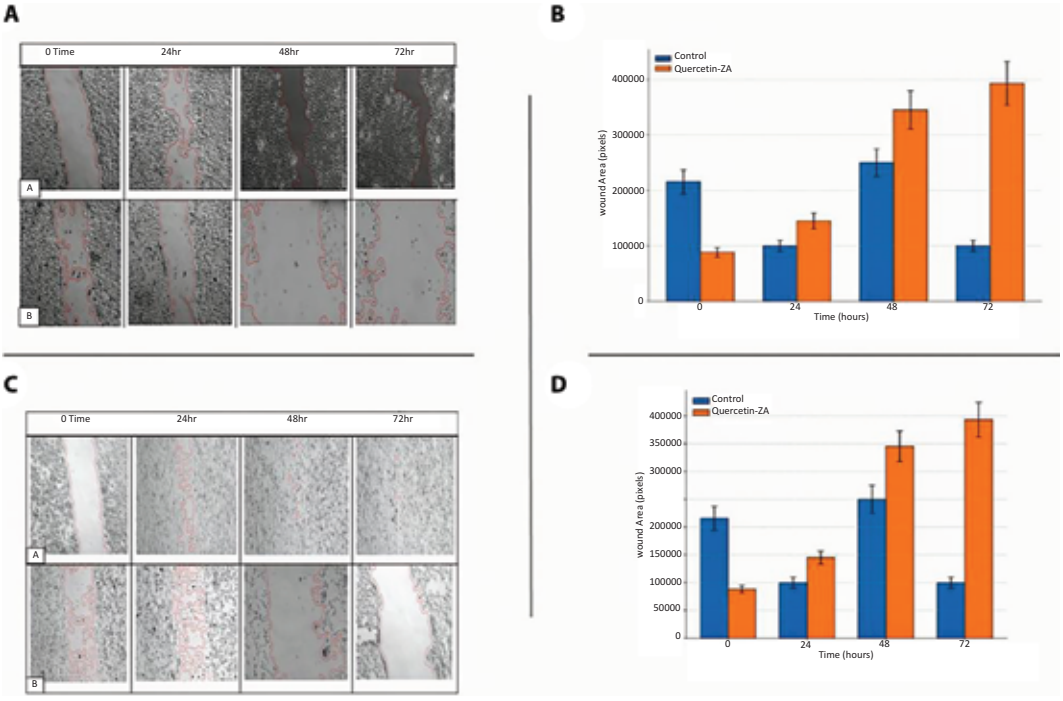

Figure 6. Effects of the quercetin–ZA combination on cell migration in the wound-healing assay. (A) Representative phase-contrast images of MCF7 cells at 0, 24, and 72 h after scratch formation. Cells were plated in 6-well plates, wounded with a p20 pipette tip, and monitored over time. (B) Quantitative analysis of wound closure in untreated and quercetin–ZA-treated MCF7 cells. (C) Representative phase-contrast images of MDA-MB-231 cells showing migration into the wound area in untreated and quercetin-ZA-treated conditions. Red lines indicate the initial wound borders. (D) Quantitative analysis of wound area (pixels). Data are expressed as mean ± SD of three independent experiments.

development of combination therapies using several anticancer drugs capable of blocking complex oncogenic pathways has been the focus of recent research. Different investigations into the therapeutic effects of quercetin and ZA have revealed their anticancer properties. Furthermore, several in vitro and in vivo studies have shown that quercetin and ZA can inhibit cell proliferation and tumor progression. Numerous prior studies have demonstrated quercetin's ability to work in combination with other compounds; however, to the best of our knowledge, no prior research has examined the combined anticancer effects of quercetin and ZA across multiple cancer cell lines. In the present study, we found that quercetin and ZA significantly suppressed the proliferation of human BC cells (MCF7 and the triple-negative MDA-MB-231 cell line). The combination of 80:160 μM quercetin-ZA, for MCF7 and 90:171 μM for MDA-MB-231 cells inhibited cell growth with greater efficiency than either drug alone, indicating that the combined treatment significantly reduced proliferation in both BC cell lines. To evaluated the extent of quercetin and ZA's effects, we selected both mildly malignant (MCF7) and highly malignant (MDA-MB-231) BC cells for this investigation. We used the MTT assay to determine the percentage of cell death and observed the cytotoxic effects of increasing quercetin and ZA doses. We found that quercetin and ZA significantly suppressed the proliferation of human BC cells (MCF7 and the triple-negative MDA-MB-231 cell line). The combination of 80:160 μM quercetin-ZA, for MCF7 and 90:171 μM for MDA-MB-231 cells inhibited cell growth with greater efficiency than either drug alone, indicating that the combined treatment significantly reduced proliferation in both BC cell lines. To evaluate the extent of quercetin and ZA's effects, we selected both mildly malignant (MCF7) and highly malignant (MDA-MB-231) BC cells for this investigation. We

used the MTT assay to determine the percentage of cell death and observed the cytotoxic effects of increasing quercetin and ZA doses. Our results showed that 72-hour treatment with quercetin and ZA dramatically decreased the viability of both MCF7 and MDA-MB-231 cells in a concentration-dependent manner, with half-maximal inhibitory concentrations (IC_{50}) ranging from 80 to 171 μM (Figure 1 A-D). Unexpectedly, MDA-MB-231 cells were more sensitive to the treatments than MCF7 cells. The combination of quercetin and ZA also demonstrated a synergistic lethal effect in both cell lines (Figure 2-A B). According to our findings, quercetin-ZA synergism caused apoptosis and inhibited the proliferation of these cancer cells. These findings are consistent with those of previous studies showing that quercetin and ZA have promising synergistic antiproliferative effects on the MCF-7 cell line. Similar observations have been reported by (23), where quercetin therapy combined with other treatments modulated tumor suppressor genes and exerted anticancer effects against triple-negative breast cancer (TNBC) cells. Quercetin and ZA exert their effects through various mechanisms, including the modulation of dysregulated signaling pathways such as PI3K/AKT, NF-κB, P53, Wnt/β-catenin, MAPK, JAK/STAT, and Hedgehog. Additionally, they also disrupt intracellular signaling molecules such as VEGF, caspases, Bax, Bcl-2, and TNF-α (24)(25). In contrast, multiple factors can contribute to intrinsic cancer cell resistance. Few studies have reported on ZA-resistant cell lines resulting from low-dose exposure. MCF-7 cells display an elevated Bcl2/Bax ratio and chemotherapeutic drug resistance. Resistance mechanisms involving enzymes such as farnesyl diphosphate synthase and proteins like heat shock protein-27 have also been observed in osteosarcoma cells (26). Therefore, quercetin and ZA exhibit both pro- and anti-apoptotic effects. Based on previous studies, we assumed that cell death caused by the quercetin-ZA combination results from targeting anti-apoptotic genes Bcl-2 and Mcl-1, which are important target genes and clinical diagnostic biomarkers for BC, especially in estrogen receptor (ER)-positive tumors. However, qRT-PCR results showed that the expression of these genes was not inhibited (Figure 3 A-B). This indicates that apoptosis occurred as a result of targeting other genes or pathways mentioned previously. Due to limited resources and financial support, we were not able to study these additional targets or determine the exact mechanism of cell death during the study period; however, these findings will allow us to conduct detailed research experiments to identify the targeted genes and the consequences of this targeting. Despite TNBC's potent tumor-initiating and self-renewing potential, there is currently no authorized targeted therapy for the disease, although chemotherapy remains the standard of care. For those afflicted with this biologically aggressive malignancy, identifying new molecular targeted therapies for TNBC would be extremely beneficial. Targeting the ability of cells to migrate and develop into secondary tumors is one way to reduce their aggressiveness. The ability of a single cell to proliferate into a colony is measured using clonogenic assays, which are frequently employed in vitro to examine the long-term survival of cells treated with chemotherapeutic drugs. As shown in Figure 4 A-C, quercetin-ZA combination treatment of both BC cell lines for 3 h inhibited the formation of clonogenic tumors, as predicted by the cell viability assay. Similar findings have been reported by (24). To evaluate the effect of quercetin-ZA on malignancy, we examined its impact on colony development. The group treated with quercetin had a much lower number of colonies than the control group. Phase-contrast microscopy revealed that the colonies in the quercetin group were noticeably smaller than those in the control group (Figure 6). Following quercetin-ZA administration, the number of colonies significantly decreased due to cell death. Consequently, quercetin-ZA inhibits the ability of cells to form secondary tumors, primarily by restricting their ability to move and metastasize. We observed that, after 24 h of treatment, cell migration in the quercetin-ZA-treated group was significantly reduced compared with the control group (Figure 6). According to our findings, combination therapy prevented BC cells from proliferating, clonally expanding, and migrating, consistent with previous reports (24). Regarding quercetin's possible enhancement of anticancer effects, one study showed that quercetin suppresses the production of MMP-2 and MMP-9 through the PI3K/Akt signaling pathway, hence preventing cell migration and invasion in HCCLM3 cells. Previous research

has also demonstrated that quercetin inhibits glioma and BC progression by downregulating MMP-9 and p-Akt (25). In a different investigation, ZA was shown to inhibit migration and invasion in resistant cell lines, suggesting its potential to prevent tumor metastasis. These findings indicate that ZA may serve as an adjuvant therapy for patients with endocrine-resistant BC (26).

Conclusion

In conclusion, we showed that quercetin-ZA combination synergistically suppressed MCF-7 and MDA-MB-231 breast cancer cells, reducing migration and self-renewal. To translate these findings into clinical practice, comprehensive studies are required to assess the safety, efficacy, and optimal combined formulations.

Ethics approval: No humans or animal subjects were used during this study

Conflicts of interest: Each author declares that he or she has no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangement, etc.) that might pose a conflict of interest in connection with the submitted article.

Authors contribution: Conceptualization, L.S; methodology, MR and L.S ;software, L.S; validation, L.S, RQ and D.H ; formal analysis,; MR ,DH and L.S ; investigation, L.S; R.Q , D. H; resources, L.S, S.A; data curation, L.S; M.M; writing—original draft preparation, L.S ; writing—review and editing, L.S, R.Q, D.H, S.A, M.R, M.M; visualization, M.A and L.S ;supervision, M.M ; project administration, L.S, R.Q,M.M ; All authors have read andagreed to the published version of the manuscript

Declaration on the use of AI: None

Acknowledgments: The authors would like to thank the scientific staff of Prpf Malik Zuhluf at the University of Jordan for facilitating the use of their instruments and laboratory.

Funding: This research received no external funding.

References

1. Burguin A, Diorio C, Durocher F. Breast cancer treatments: Updates and new challenges. J Pers Med. 2021;11(8):758. doi: 10.3390/jpm11080808
2. Song X, Wei C, Li X. The Signaling Pathways Associated With Breast Cancer Bone Metastasis. Front Oncol. 2022;12:864501. doi: 10.3389/fonc.2022.855609
3. Qiao H, Wang TY, Yan W, Qin A, Fan QM, Han XG, et al. Synergistic suppression of human breast cancer cells by combination of plumbagin and zoledronic acid in vitro. Acta Pharmacol Sin. 2015;36(9):1085-98. doi: 10.1038/aps.2015.42
4. Massi A, Bortolini O, Ragno D, Bernardi T, Sacchetti G, Tacchini M, et al. Research progress in the modification of quercetin leading to anticancer agents. Molecules. 2017;22(8):1273. doi: 10.3390/molecules22081270
5. Wang B, Zhan Y, Yan L, Hao D. How zoledronic acid improves osteoporosis by acting on osteoclasts. Front Pharmacol. 2022;13:1019825. doi : 10.3389/fphar.2022.961941
6. Ibrahim T, Mercatali L, Sacanna E, Tesei A, Carloni S, Ulivi P, et al. Inhibition of breast cancer cell proliferation in repeated and non-repeated treatment with zoledronic acid. Cancer Cell Int. 2012;12:48. doi: 10.1186/1475-2867-12-48
7. Ulukaya E, Karakas D, Dimas K. Tumor chemosensitivity assays are helpful for personalized Cytotoxic treatments in cancer patients. Medicina (Kaunas). 2021;57(2):131. doi: 10.3390/medicina57060636
8. Reyes-Farias M, Carrasco-Pozo C. The anti-cancer effect of quercetin: Molecular implications in cancer metabolism. Int J Mol Sci. 2019;20(13):3134. doi: 10.3390/ijms20133177
9. Lupu R, Menendez J. Pharmacological Inhibitors of Fatty Acid Synthase (FASN)-Catalyzed Endogenous Fatty Acid Biogenesis: A New Family of Anti-Cancer Agents? Curr Pharm Biotechnol. 2006;7(6):483-94. doi: 10.2174/138920106779116928
10. Lupien LE, Dunkley EM, Maloy MJ, Lehner IB, Foisey MG, Ouellette ME, et al. An inhibitor of fatty acid synthase thioesterase domain with improved cytotoxicity against breast cancer cells and stability in plasma. J Pharmacol Exp Ther. 2019;370(2):147-58. doi: 10.1124/jpet.119.258947
11. Mokhtari RB, Homayouni TS, Baluch N, Morgatskaya E, Kumar S, Das B, et al. Combination therapy in combating cancer. Oncotarget. 2017;8(23):38022-43. doi: 10.18632/oncotarget.16723
12. Li N, Sun C, Zhou B, Xing H, Ma D, Chen G, et al. Low concentration of quercetin antagonizes the cytotoxic effects of anti-neoplastic drugs in ovarian cancer. PLoS One. 2014;9(7):e100367. doi: 10.1371/journal.pone.0100314
13. Weiskirchen S, Schröder SK, Buhl EM, Weiskirchen R. A Beginner's Guide to Cell Culture: Practical Advice for Preventing Needless Problems. Cells. 2023;12(5):682. doi: 10.3390/cells12050682
14. Jhamb SK, Goyal A, Pandey A, Bhowmik A. A review of cytotoxicity testing methods and in vitro study of biodegradable Mg-1%Sn-2%HA composite by elution method.

J Mater Sci Mater Med. 2025;36(1):15. doi: 10.1007/s10856-025-06951-x
15. Bechtold B, Clarke J. Multi-factorial pharmacokinetic interactions: unraveling complexities in precision drug therapy. Expert Opin Drug Metab Toxicol. 2021;17(4):397-412. doi: 10.1080/17425255.2021.1867105
16. Rouhrazi H, Turgan N, Oktem G. Zoledronic acid overcomes chemoresistance by sensitizing cancer stem cells to apoptosis. Biotech Histochem. 2018;93(2):77-88. doi: 10.1080/10520295.2017.1387286
17. Haller F, Kulle B, Schwager S, Gunawan B, Von Heydebreck A, Sültmann H, et al. Equivalence test in quantitative reverse transcription polymerase chain reaction: Confirmation of reference genes suitable for normalization. Anal Biochem. 2004;335(1):1-9. doi: 10.1016/j.ab.2004.08.024
18. Brix N, Samaga D, Hennel R, Gehr K, Zitzelsberger H, Lauber K. The clonogenic assay: robustness of plating efficiency-based analysis is strongly compromised by cellular cooperation. Radiat Oncol. 2020;15(1):248. doi: 10.1186/s13014-020-01697-y
19. Yarrow JC, Perlman ZE, Westwood NJ, Mitchison TJ. A high-throughput cell migration assay using scratch wound healing, a comparison of image-based readout methods. BMC Biotechnol. 2004;4:21. doi: 10.1186/1472-6750-4-21
20. Rhman MA, Devnarain N, Khan R, Owira PMO. Synergism Potentiates Oxidative Antiproliferative Effects of Naringenin and Quercetin in MCF-7 Breast Cancer Cells. Molecules. 2022;27(13):4214. doi: 10.3390/nu14163437
21. Kundur S. Synergistic anticancer action of quercetin and curcumin against triple-negative breast cancer cell lines. J Cell Physiol. 2019;234(7):11103-18. doi: 10.1002/jcp.27761
22. Asgharian P, Tazekand AP, Hosseini K, Forouhandeh H, Ghasemnejad T. Potential mechanisms of quercetin in cancer prevention: focus on cellular and molecular targets. Cancer Cell Int. 2022;22(1):257. doi: 10.1186/s12935-022-02677-w
23. Kars MD, İşcan ÖD. In Vitro Evaluation of Zoledronic Acid Resistance Developed in MCF-7 Cells. Anticancer Res. 2007;27(6B):4031-7. PMID: 18225567
24. Wang R, Yang L, Li S, Ye D, Yang L, Liu Q, Zhao Z, Cai Q, Tan J, Li X. Quercetin Inhibits Breast Cancer Stem Cells via Downregulation of Aldehyde Dehydrogenase 1A1 (ALDH1A1), Chemokine Receptor Type 4 (CXCR4), Mucin 1 (MUC1), and Epithelial Cell Adhesion Molecule (EpCAM). Med Sci Monit. 2018 Jan 21;24:412-420. doi: 10.12659/msm.908022. PMID: 29353288; PMCID: PMC5788241
25. Lu J, Wang Z. Quercetin Inhibits the Migration and Invasion of HCCLM3 Cells by Suppressing the Expression of p-Akt1, Matrix Metalloproteinase (MMP). Med Sci Monit. 2018;24:2583-9. doi: 10.12659/MSM.906172
26. Adchariyasakulchai P, Sakunrangsit N, Chokyakorn S, Suksanong C, Ketchart W. Anticancer effect of zoledronic acid in endocrine-resistant breast cancer cells via HER-2 signaling. Biomed Pharmacother. 2024;171: 116142. doi: 10.1016/j.biopha.2024.116142