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Anticancer Effects of Cisplatin and 6-Gingerol Co-Treatment on MDA-MB-231 Breast Cancer Cells

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Abstract

Breast cancer is known to be a common cancer diagnosed by people around the world, and a high number of breast cancer cases are recorded annually. Triple-negative breast cancer (TNBC), the most aggressive form of breast cancer, is often difficult to treat as it lacks expression of estrogen, progesterone and human epidermal growth factor receptors that are often targeted for other breast cancer subtypes. Hence, chemotherapy remains the main option to treat TNBC. Cisplatin, a powerful anticancer drug, is commonly used as a chemotherapeutic agent for TNBC treatment. Nevertheless, the development of chemoresistance and adverse side effects observed in clinics limit the use of cisplatin in treating cancer. Various studies have shown that cisplatin, in combination with plant-based compounds, has successfully reduced its toxicity as well as tumour resistance to its treatment. Recent findings have shown that 6-gingerol, the major phytochemical in ginger, possesses excellent anticancer properties. Therefore, this study reports the anticancer effects of cisplatin, 6-gingerol and cisplatin-6-gingerol co-treatment on the cell viability and changes in morphology of MDA-MB-231 breast cancer cells, which represents the TNBC subtype. Alamar Blue assay was performed to study cell viability while morphological changes in cells before and after treatments were observed via microscopy imaging. In addition, the drug-drug interaction was studied via the CompuSyn software. The IC_{50} concentrations of cisplatin, 6-gingerol and combined cisplatin-6-gingerol treatments were achieved at 8.08μM, 80.24μM and 58.53μM, respectively. In the combined therapy, a reduced cisplatin concentration (5μM) was used with 0-100 μM 6-gingerol.The combination therapy required a lower dosage of both agents (5μM cisplatin in combination with 58.53μM 6-gingerol) to inhibit 50% of MDA-MB-231 cell growth. Both solo and combined treatments reduced the cell viability of MDA-MB-231 effectively in a dose-dependent manner but with different capacities. Morphological changes of MDA-MB-231 cells upon each treatment suggested that each treatment could induce different cell fate. However, the analyzed drug-drug interaction between 5 μM cisplatin and 0-100 μM 6-gingerol on MDA-MB-231 cells revealed that the combined treatment exerted an antagonistic effect. It is presumed that the cisplatin concentration used counteracted with the effect of 6-gingerol, consequently affecting the overall inhibitory effect in MDA-MB-231 cells. In conclusion, the present study shows that solo treatment of cisplatin and 6-gingerol, respectively, had a more significant inhibitory effect compared to the co-treatment on MDA-MB-231 breast cancer cells. However, further studies are required to examine the combined effect of cisplatin and 6-gingerol using different combination strategies for more promising outcomes that can provide valuable knowledge in contributing to TNBC treatment management.

Keywords Cisplatin, 6-gingerol, combination therapy, triple-negative breast cancer

1.0 INTRODUCTION

Cancer significantly impacts mortality rates and life expectancy worldwide. In 2020, GLOBOCAN reported 2.3 million new female breast cancer cases, making it the most diagnosed cancer (Sung et al., 2021). According to WHO, breast cancer is the most diagnosed cancer and the second leading cause of cancer-related death in Malaysia. Breast cancer is classified into four subtypes based on hormone receptor expression: Estrogen Receptors (ER), Progesterone Receptors (PR), and Human Epidermal Growth Factor Receptors 2 (HER2) (Orrantia-Borunda et al., 2022). Triple Negative Breast Cancer (TNBC) lacks all these receptors, making it the most aggressive subtype with high metastasis and recurrence risks, worsened by the presence of cancer stem cells (CSCs) population within the tumor, leading to poor treatment and prognosis (Zhang et al., 2023). The CSCs have similar characteristics as healthy stem cells, which possess self-renewable capability and multi-lineage differentiation (Bisht et al., 2022, Meng, 2023).

Although some targeted and immunotherapies are currently available, the more common TNBC treatment options are surgery, radiotherapy, and chemotherapy (Yao et al., 2019, Furlanetto et al., 2020). Due to the lack of prognostic biomarkers and limited therapeutic targets, chemotherapy remains the most common treatment for early-stage TNBC (Gupta et al., 2020), which can cause apoptosis and DNA strand break (Yu et al., 2020). Cisplatin, a platinum-based anticancer drug, is effective for cancer tissues with defective DNA repair mechanisms, such as those with BRCA mutations often found in TNBCs (Yu et al., 2020). Although TNBC cells are sensitive to cisplatin treatment, cisplatin-treated TNBC cells face limitations such as chemoresistance (Ghosh, 2019) and adverse side effects (Singh et al., 2018).

Since the beginning of human civilization, medicinal plants have been used as therapeutic agents. Among the various types of bioactive compounds found in Zingiber officinale (ginger), 6-gingerol stands out for its significant anticancer potential by producing greater anti-angiogenic and apoptotic effects (Shahshahan et al., 2023). It has been shown to inhibit tumor growth in breast (Ibrahim et al., 2014), ovarian (Shahshahan et al., 2023), colon (Garg, 2023), and other cancers while enhancing the efficacy of chemotherapy agents like cisplatin and reducing their toxicity (Sp et al., 2021). Studies have demonstrated 6-gingerol's ability to inhibit proteosomal activity, induce DNA damage, arrest cell cycles, and regulate apoptotic markers such as PARP and cleave caspase-3 expression, underscoring its molecular role in cancer treatment (Sp et al., 2021).

Despite its promising benefits, 6-gingerol's therapeutic potential is constrained by its poor bioavailability and water solubility, which leads to poor oral absorption (Wang et al., 2018). Its rapid metabolism has also significantly limited its clinical applications (Xu et al., 2016). Current research has been focusing on combination therapy, using multiple drugs targeting different molecular pathways to increase cancer cells' susceptibility to treatments. Studies have shown encouraging results when 6-Gingerol was co-administered with cisplatin in treating ovarian and gastric cancers (Shahshahan et al., 2023) (Yang et al., 2019). Therefore, the present study was conducted and reports the anticancer effects of cisplatin and 6-gingerol on cell viability and morphological changes in MDA-MB-231 breast cancer cells.

2.0 EXPERIMENTAL

2.1 Cell Culture

MDA-MB-231 cells were cultured in T25 flasks using Dulbecco's Modified Eagle's Medium (DMEM) enriched with 10% Fetal Bovine Serum (FBS), and 1% antibiotic (100U/mL penicillin, 100µg/ mL streptomycin). The culture was incubated and maintained at 37°C and 5% of CO_2. The cells were serially passaged 3 times a week at 60-70% cell confluency.

2.2 Cell Viability Assay

Cells were trypsinized, harvested, centrifuged, and resuspended in a complete medium. Cell viability was assessed using Trypan blue and a hemocytometer, with viable cells remaining unstained and nonviable cells stained blue. Cell counts were recorded, and the concentration of viable cells was calculated. For drug treatment, 3000 cells/ well were seeded in a 96-well plate and incubated overnight until it reached 70% confluency. Cells were treated with cisplatin (MedChem Express, USA) at various concentrations ranging from 0 to 50 µM and 6-gingerol (ChemFaces, China) from 0 to 150 µM. For co-treatment, cisplatin was fixed at 5 µM and combined with varying concentrations of 6-gingerol (0-100 µM). Cells were treated in triplicate and incubated for 24 hours.

AlamarBlue™ Cell Viability Reagent (Thermo Fisher Scientific, USA) was used to determine MDA-MB-231 cell viability in response to the cisplatin and 6-gingerol treatments. Reading was taken at 570 nm to quantify cell viability, with the amount of formazan dye proportional to the number of live cells. After incubation, 10 µL of Alamar Blue reagent was added to each well and incubated for 4 hours. Absorbance was measured using a microplate reader (BMG Labtech, Germany) to determine the inhibitory effect of cisplatin and 6-gingerol as well as the half-maximal inhibitory concentration (IC_{50}) concentrations that induced a 50% reduction in MDA-MB-231 cells viability. Concurrently, morphological changes induced by these treatments were observed using an inverted microscope (Nikon, Japan).

2.3 Drug-drug Interaction

The CompuSyn (CompuSyn, Inc., Paramus, NJ, USA) software was used to construct the isobologram to identify the combination index (CI) between the interaction of cisplatin and 6-gingerol. The combination index of more than 1 (CI > 1) indicates an antagonistic effect of cisplatin and 6-gingerol while a combination index equal to 1 (CI=1) indicates an additive effect, and a combination index of less than 1 (CI < 1) indicates a synergistic effect (Rodea-Palomares et al., 2015).

2.4 Statistical Analysis

Microsoft Excel and GraphPad Prism 7 (GraphPad Software, Inc.) were used to perform the statistical analysis of data obtained in this study. Data obtained were displayed in a two-tailed distribution curve to compare the negative control (untreated MDA-MB-231 cells) with the cisplatin, 6-gingerol, and combined cisplatin-6-gingerol treated MDA-MB-231 cells. Lastly, data obtained from the Alamar Blue assay for cell viability was presented as mean ± SEM. The significance is shown as: *ns* $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$. All experiments were conducted in triplicates in three independent experiments.

3.0 RESULTS AND DISCUSSION

3.1 Cisplatin and 6-Gingerol Reduced Cell Viability of MDA-MB-231 Cells

The anticancer effect of solo cisplatin and 6-gingerol treatment, as well as combined cisplatin-6-gingerol treatment on the MDA-MB-231 breast cancer cell viability, were observed after 24 hours of treatment, respectively. According to Figure 1A, the MDA-MB-231 cell viability decreased slightly upon 2.5-5.0 μM cisplatin between 8.08% to 20.37%. However, the cell viability showed significant reduction when treated with 7.5 μM of cisplatin with 48.29% followed by 61.47%, 74.65%, 81.59%, 87.95%, 89.78%, 95.27%, 97.08% and 98.4% ranging from 10-50.μM respectively. As the concentration of cisplatin increased, the cell viability also gradually decreased from 38.53% (10μM) to 1.57% (50μM). In parallel to the reduction in cell number upon increasing cisplatin concentration, a change in morphology of the remaining MDA-MB-231 cells was observed as well (Figure 2).

In contrast to cisplatin treatment, Figure 1B reveals that a higher 6-gingerol concentration range was required to inhibit the growth of MDA-MB-231 cells. It is visible that no significant reduction in cell viability was observed upon 0-40 μM 6-gingerol (0% - 18.59%) while 23.01%-47.72% between 50-80 μM and more than 50% reduction at the subsequent higher 6-gingerol concentrations between 90-150 μM (54.53-92.9%). A prominent inhibitory effect and change in cell morphology in MDA-MB-231 cells ideally were observed upon 70 μM 6-gingerol onwards. Both cisplatin and 6-gingerol inhibited MDA-MB-231 cell viability in a dose-dependent manner but with distinct cytotoxicity concentrations.

As bioaccumulation of cisplatin in cells has been proven to cause multiple organ toxicity despite its significant anticancer activity in TNBC patients, various studies on cisplatin co-treatment with phytochemicals, including 6-gingerol are ongoing currently (Dasari et al., 2022). 6-gingerol and other ginger phytochemicals possess the ability to protect cells from oxidative damage caused by cisplatin and have been shown to decrease hepatotoxicity in animal models as well [6,18]. Therefore, in this study, the concentration of cisplatin was fixed at a concentration (5 μM) lower than the IC_{50} concentration (8.08 μM) and combined with varying concentrations of 6-gingerol (0-100 μM). According to Figure 1C, the cell viability of MDA-MB-231 gradually decreased from 0μM (100%) to 100 μM (21.95%). About 50% reduction in the number of viable cells was observed at 5μM cisplatin in combination with 60μM 6-gingerol (51.85%) compared to solo 5 μM cisplatin (79.63%) and 60μM 6-gingerol (75%) treatments respectively in MDA-MB-231 cells (Figure 1C). This indicates that the combining of two different therapeutic agents such as cisplatin and 6-gingerol, with each at a lower concentration, could alleviate the cell growth inhibitory effect in MDA-MB-231 cells compared to solo cisplatin and 6-gingerol treatments, respectively. The combined cisplatin-6-gingerol effect most likely contributed to MDA-MB-231 cell sensitization towards each treatment (Salari et al., 2023). The findings correlated well with morphological changes observed under an inverted microscope, supporting the enhanced performance of cisplatin-sensitized MDA-MB-231 cells towards 6-gingerol (Salari et al., 2023).

The IC_{50} of each treatment was determined through non-linear regression analysis. According to Figure 1D, solo cisplatin achieved an IC_{50} of 8.08 μM (95% CI: 7.68 μM - 8.48 μM), solo 6-gingerol achieved an IC_{50} of 80.24 μM (95% CI: 77.05 μM - 83.4 μM), and the combination of 5 μM cisplatin and 6-gingerol achieved an IC_{50} of 58.53 μM (95% CI: 53.24 μM - 64.33 μM). This indicates that adding 5μM cisplatin reduced the IC_{50} concentration of 6-gingerol by 21.71μM. The cell viability of MDA-MB-231 was further reduced in the combined cisplatin-6-gingerol treatment as compared to the solo drug treatments respectively. A similar trend in inhibitory effect was reported by Kapoor and colleagues whereby apoptosis in human oral cancer cells (SCC4 and KB) and cervical cancer cells (HeLa) was significantly induced upon treatment with 6-gingerol in combination with wortmannin, rapamycin, and cisplatin (Kapoor et al., 2016). Several other studies have also shown that the combination of cisplatin with 6-gingerol can sensitize the malignant cells towards the treatment and promote early apoptosis following 24 hours of treatment (Sp et al., 2021, Rastogi et al., 2015).

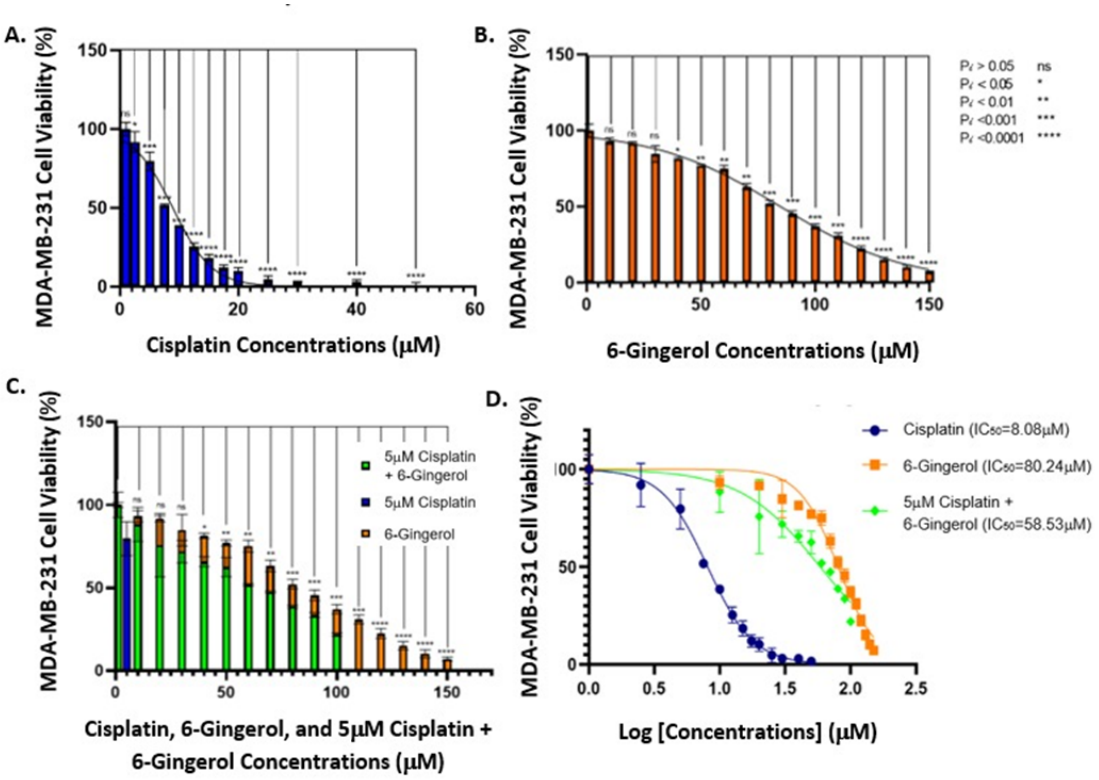

Figure 1 Effect of (A.) Solo cisplatin treatment (0, 2.5, 5, 7.5,10, 12.5, 15, 17.5,20, 25, 30, 40 and 50 μM); (B.) Solo 6-gingerol treatment (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140 and 150 μM) and (C.) 5 μM cisplatin+6-gingerol (0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μM) co-treatment respectively on MDA-MB-231 cell viability and dose-response curve: IC_{50} concentrations achieved via cisplatin, 6-gingerol and 5 μM cisplatin+6-gingerol co-treatments (D.) The significance is shown as: *ns* p> 0.05; * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001; **** p ≤ 0.0001. All experiments were conducted in triplicates in three independent experiments.

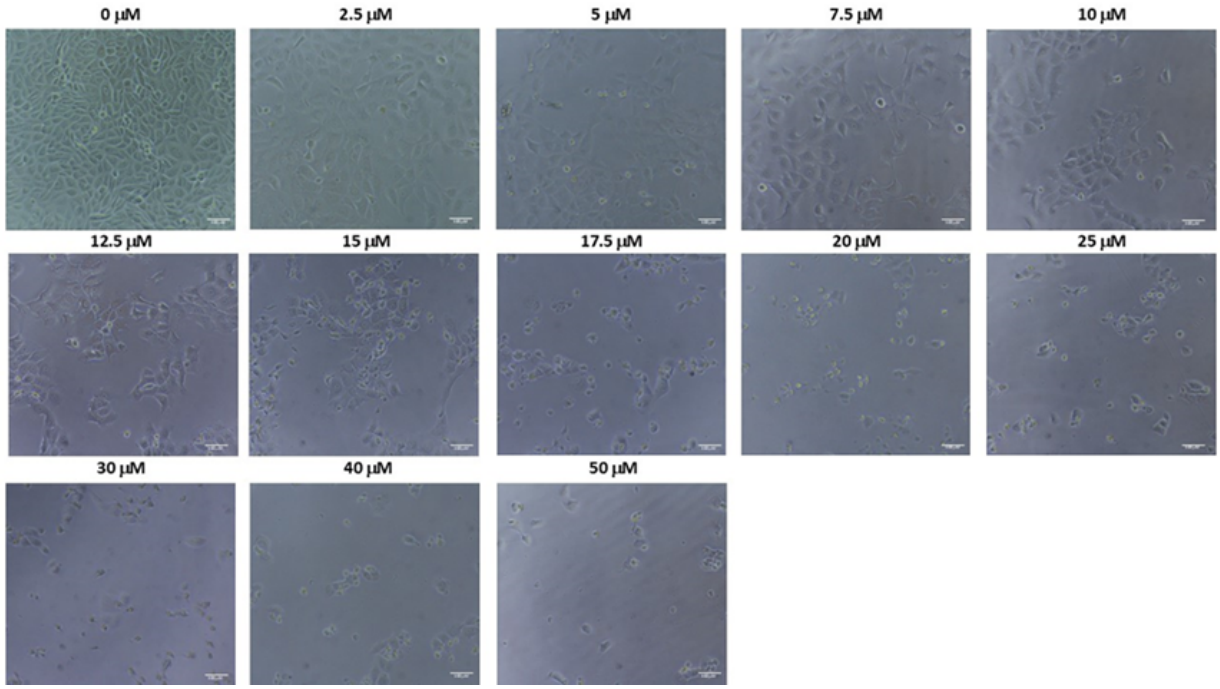

Figure 2 Microscopic images of untreated (control) and treated MDA-MB-231 cells upon solo cisplatin treatment (0, 2.5, 5, 7.5,10, 12.5, 15, 17.5,20, 25, 30, 40 and 50 μM) post 24 hours at 10X magnification.

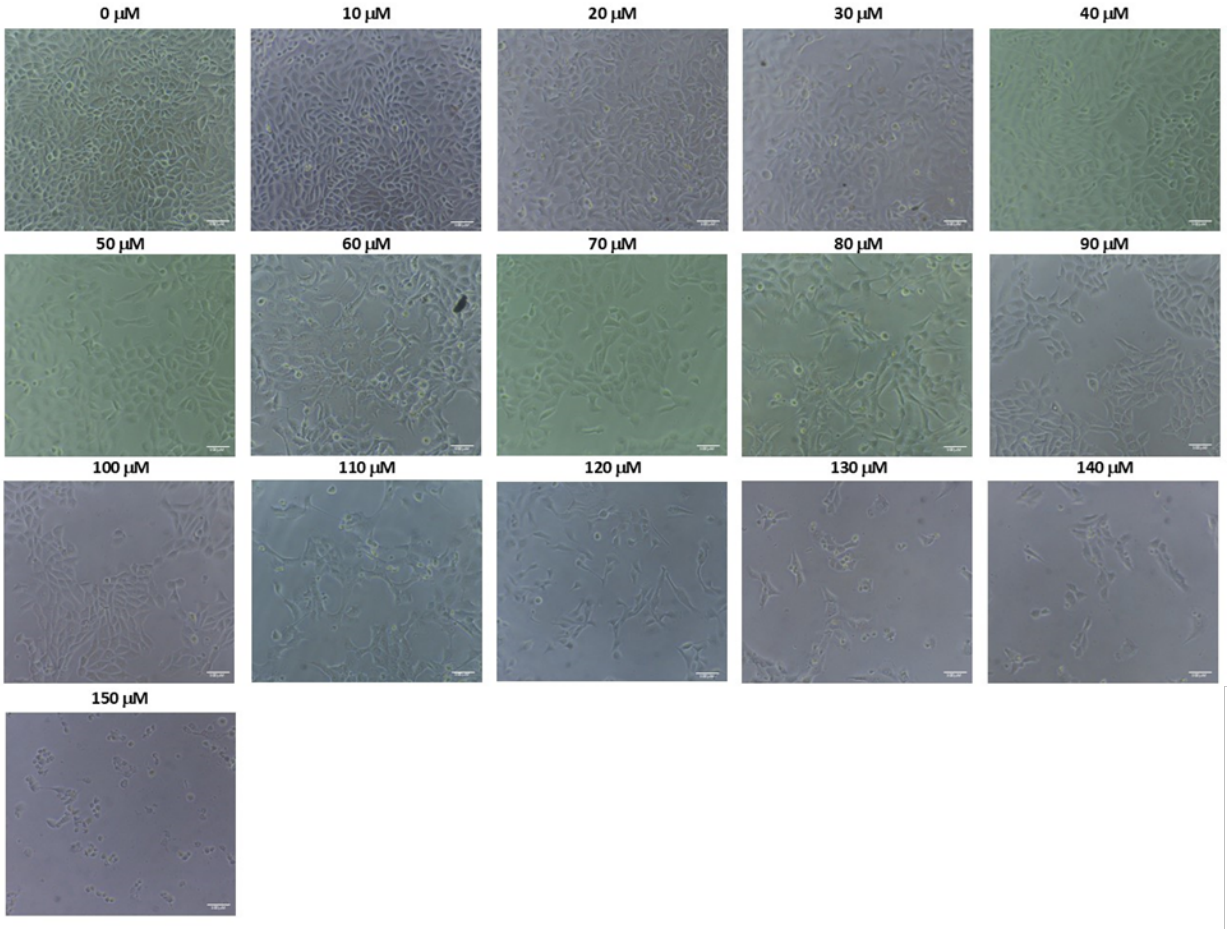

Figure 3 Microscopic images of untreated (control) and treated MDA-MB-231 cells upon solo 6-gingerol treatment (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140 and 150 μM) post 24 hours at 10X magnification.

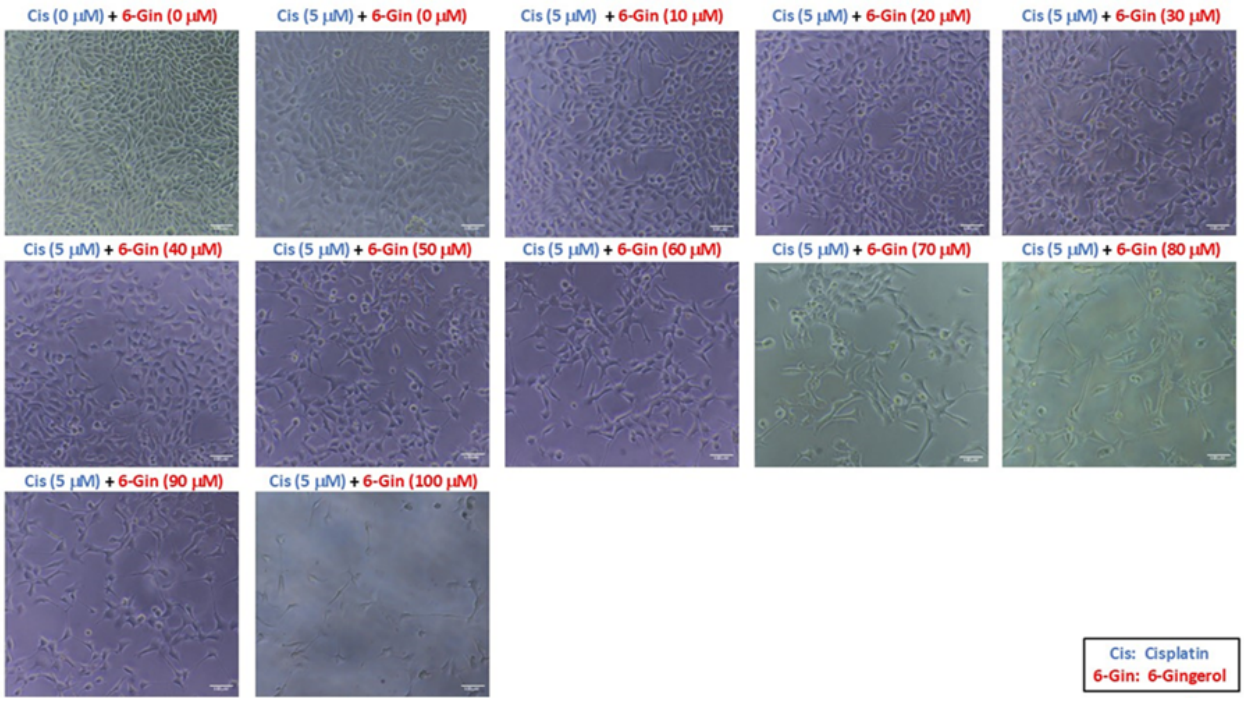

Figure 4 Microscopic images showing untreated (control) and treated MDA-MB-231 cells upon 5 μM cisplatin+6-gingerol co-treatment (0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μM) post 24 hours at 10X magnification.

3.2 Cisplatin, 6-Gingerol and Cisplatin-6-Gingerol Co-treatment Induced Morphological Changes in MDA-MB-231 cells

Upon 24 hours of cisplatin, 6-gingerol and combined cisplatin-6-gingerol treatment in MDA-MB-231 cells, the change in cell morphology was observed under the microscope and compared to the untreated MDA-MB-231 cells (Figure 5). It can be observed that the viable cell number reduced with increased concentration with each treatment respectively in a dose-dependent manner, which correlates well with the cell viability percentage reduction shown in Figure 1 A-C. MDA-MB-231 cells undergo morphological changes based on the possible underlying mechanism exerted by each treatment between low and high concentrations of solo cisplatin, solo 6-gingerol, and co-treatment. MDA-MB-231 cells upon 2.5-5μM appeared slightly enlarged. In contrast, some remaining cells appeared more enlarged, and some elongated upon 7.5 μM treatment, followed by fewer viable cells but with thinner and elongated appearance upon 10 μM cisplatin (Figure 2). Most cells were smaller and rounded and appeared apoptotic, like those found upon 12.5-50 μM cisplatin (Figure 5).

In contrast to cisplatin treatment, MDA-MB-231 cells upon 6-gingerol treatment didn't seem to show any visible change in morphology and size up to 50 μM treatment. However, upon 60-70 μM 6-gingerol, cells appeared slightly elongated, followed by a smaller, thinner, and more elongated appearance in the remaining cells upon 80-100 μM treatment. MDA-MB-231 cells treated with 110-140 μM 6-gingerol, on the other hand, clearly reduced the number of viable cells. In contrast, the remaining cells appeared smaller and elongated, irregular in shape and dying, while at 150 μM, the remaining number of cells was significantly less, mostly small, rounded, and fragmented cells (Figure 3 and Figure 5). This finding aligns with a previous study done by Sp and colleagues, where it was reported that 6-gingerol induced cell death both in MDA-MB-231 and MCF-7 breast cancer cells (Sp et al., 2021).

As for the combined 5 μM cisplatin with 10-100 μM 6-gingerol treatment, MDA-MB-231 cells generally appeared small, elongated, and stiffened (Figure 4 and Figure 5) in comparison to the untreated, as well as solo cisplatin and 6-gingerol treated cells. Upon co-treatment of 5 μM cisplatin and 60-70 μM 6-gingerol, MDA-MB-231 cells appeared more elongated and neural-like (Figure 4). Meanwhile, cisplatin co-treatment with 80-100 M 6-gingerol displayed a neural-like cell appearance along with some rounded apoptotic-like cells (Figure 4). These varied morphological changes in MDA-MB-231 cells upon solo cisplatin, 6-gingerol, as well as combined cisplatin-6-gingerol treatments, may be correlated with the possible mode of the action exerted, respectively. The enlarged cell upon cisplatin treatment is most likely due to cisplatin-induced chromatin repulsion osmotic pressure and membrane tension as a result of DNA damage via its cytotoxic adduct formation (Kim et al., 2023, Yu et al., 2020). In contrast, the small, elongated, stiffened, and neural-like cell phenotypes, which are prominent in the combined treatment, could be the result of decondensed chromatin (Meng, 2023). These cell phenotypes are known to represent the differentiated state in which cells are less proliferative but still viable (Bakar et al., 2023).

Based on these observations, it can be concluded that MDA-MB-231 cells were highly sensitive towards cisplatin, with a significant reduction in viable cells and growth inhibition at very low concentrations presumed to be induced by DNA damage. However, the remaining viable and resistant cells upon cisplatin treatment could be in a less proliferative state and undergo cell death upon higher cisplatin concentration, as seen in Figure 2. Figure 3, on the other hand, suggests that MDA-MB-231 cells were less sensitive towards 6-gingerol treatment as a higher percentage of cells appeared still viable up to 100 μM. However, these viable cells were more differentiated in phenotype, so one can presume that 6-gingerol could induce the differentiation of MDA-MB-231 cells (Prabhakaran et al., 2013). The fact that MDA-MB-231 cells are enriched with cancer stem cells (CSCs), which possess similar characteristics as normal stem cells, may explain the potential for multi-lineage differentiation upon cisplatin and 6-gingerol treatment leading to limited cell proliferation (Kim et al., 2023, Meng, 2023). These cells are not killed; however, they have limited proliferative capacity but eventually undergo cell death as the 6-gingerol concentration is significantly increased. Cell nucleus fragmentation, as well as irregular and rounded apoptotic bodies observed at high concentrations, is indicative of an active apoptosis process possibly induced by ROS and DNA double-strand breaks (Sp et al., 2021, Balvan et al., 2015) (Figures 3 and 5). As for the combined treatment, the presence of 5 μM cisplatin in addition to 6-gingerol is presumed to have sensitized MDA-MB-231 cells by further limiting the proliferative capacity and making them more susceptible to cell death. This can be correlated with the decrease in 6-gingerol IC_{50} concentration (Figure 3D). Significant studies have shown that the MDA-MB-231 belongs to the Claudin-Low TNBC subtype, which is the least differentiated, highly associated with breast cancer stem cells, highly proliferative, and often results in poor prognosis (Lucero et al., 2020, Prat et al., 2010). Hence, combined cisplatin-6-gingerol treatment could be a potential mode of treatment for TNBC as it can limit proliferation and induce apoptosis (Bakar et al., 2023, Salari et al., 2023), possibly preventing metastasis in patients. Regardless of the potential of combined cisplatin-6-gingerol treatment presented in this study, more comprehensive studies are required to confirm the possible mechanism proposed.

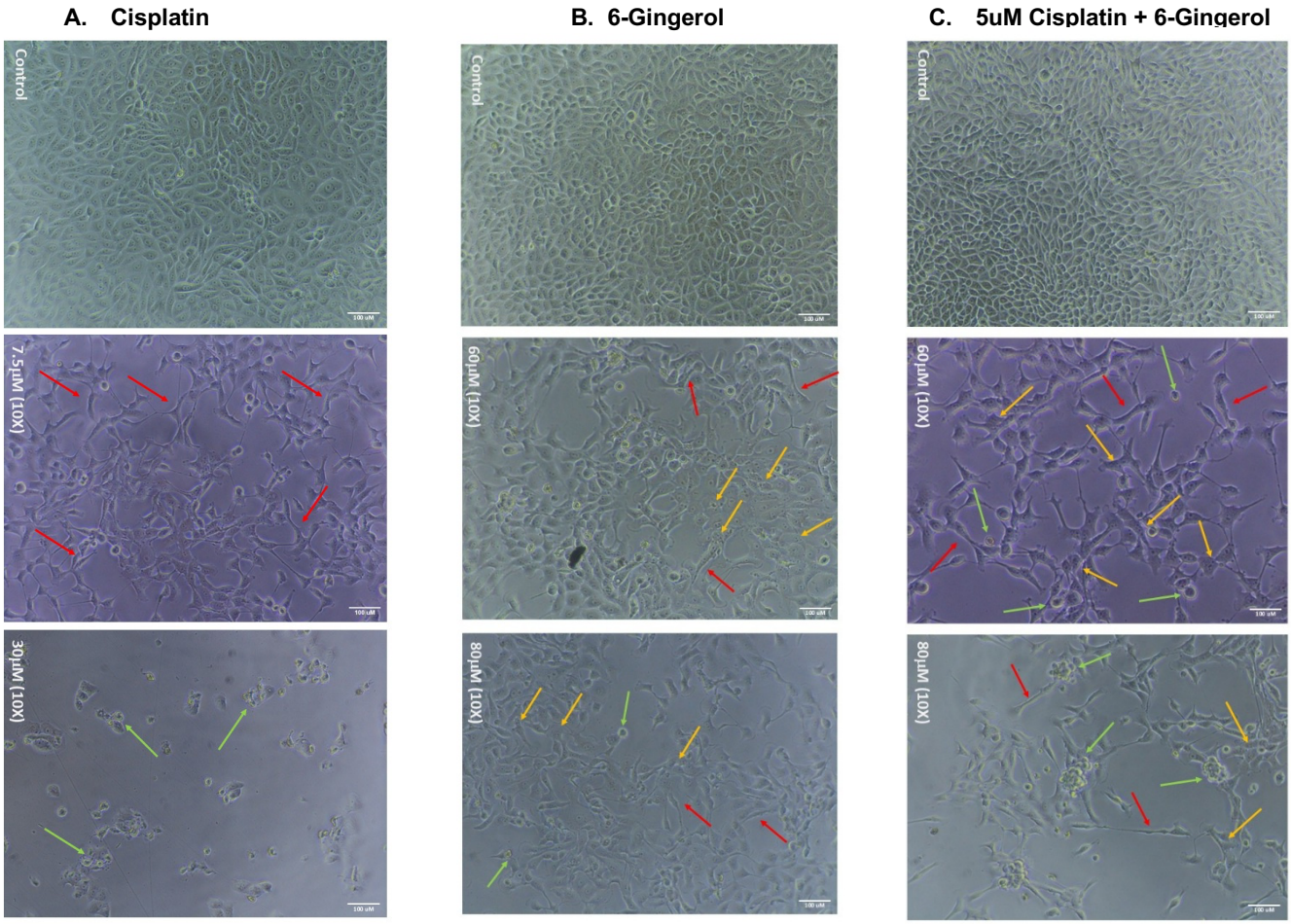

Figure 5 Morphological changes of untreated (control) and treated MDA-MB-231 upon solo cisplatin, solo 6-gingerol, and 5 μM cisplatin+6-gingerol treatments at 10X and 20X magnification. [The red arrow (→) indicates elongated cells; The yellow arrow (→) indicates fragmented nucleus; The green arrow (→) indicates the apoptotic bodies formation.

3.3 Drug-drug Interaction of Cisplatin and 6-Gingerol Co-treatment

To further study the effectiveness of combining cisplatin and 6-gingerol against MDA-MB-231, a drug-drug interaction analysis was performed. A fa-CI plot and isobologram were generated using CompuSyn software to analyze the interaction. Figure 6A illustrates the combination index (CI) versus the fraction affected (Fa) by a particular dose. The Fa-CI plot is an effect-oriented graphic where if the CI=1 indicates the combination of drugs has additive effects, CI < 1 has a synergistic effect, and CI > 1 have an antagonistic effect (Rodea-Palomares et al., 2015). By referring to Figure 6B, interestingly, the combination between cisplatin and 6-gingerol exhibited an antagonistic effect except for the combination of 5 M cisplatin with 100 M 6-gingerol, which exhibited an additive effect.

The isobologram generated shows the equi-effective Cartesian plane for the combination of 5 μM cisplatin and 6-gingerol at fa=1. According to theory, any mixture ratio (A: B) should give an effect located on the straight transversal line (the additivity line). Suppose the doses required for A + B to achieve the desired effect are less than the sum of their individual effects. In that case, this indicates a synergistic effect, with coordinates below the additive line and vice versa, which is an antagonistic effect (Rodea-Palomares et al., 2015). The isobologram was normalized to single 6-gingerol concentrations (Figure 6C), and most combinations were above the additivity line, indicating the 5 μM cisplatin with 6-gingerol co-treatment resulted in an antagonistic effect against MDA-MB-231, showing insignificant reduction in cell viability and requiring higher cisplatin concentration. This finding suggests that the cisplatin concentration used in the co-treatment with 6-gingerol was not sufficient, hence leading to an incomplete mechanism of action and consequently not being able to eradicate the cells. Nevertheless, despite the insignificant increase in viable cell percentage, the insufficient concentration of cisplatin affected the MDA-MD-231 TNBC cells by limiting their proliferation. It contributed to the sensitization of cells towards 6-gingerol treatment (Figure 1B-C). Similar findings by other studies were reported in which combined treatments of cisplatin with other drugs presented degrees of antagonism (Cesna et al., 2018, Salari et al., 2023). Additionally,the antagonism presented could also be

attributed to 6-gingerol that may interfere with signaling pathways activated by cisplatin and vice-versa, potentially reducing the overall efficacy of the combination therapy, where similar findings have been reported in studies investigating the combined effects of cisplatin and shogaol in head and neck cancers (Kotowski et al., 2017). To c, the concentrations of the two agents used in combination may need to be further optimized by testing with various other cisplatin doses below the IC_{50} concentration obtained in the solo treatment to achieve synergistic effects. Elevated doses of either cisplatin or 6-gingerol could induce cytotoxicity that counteracts the therapeutic effects of each other, consequently canceling off and limiting significant inhibitory effects in MDA-MB-231 breast cancer cells.

Despite the antagonistic effect observed, the combinative therapy of cisplatin with 6-gingerol should be further researched as many recent studies have shown encouraging results in treating various cancers such as ovarian and gastric cancer. In addition, a recent study also claimed that combined cisplatin and 6-gingerol treatment in ovarian cancer cells *in vitro* and *in vivo* managed to reduce adverse side effects of cisplatin treatment despite the antagonistic effect posed by cisplatin-6-gingerol (Salari et al., 2023). This suggests that 6-gingerol might have the potential to protect against cisplatin-induced multiple organ toxicity and thereby improve the quality of life for chemotherapy patients. A further comprehensive study on the various fixed cisplatin concentrations in combination with various 6-Gingerol concentrations is crucial to identify the potent cisplatin-6-Gingerol combined therapy.

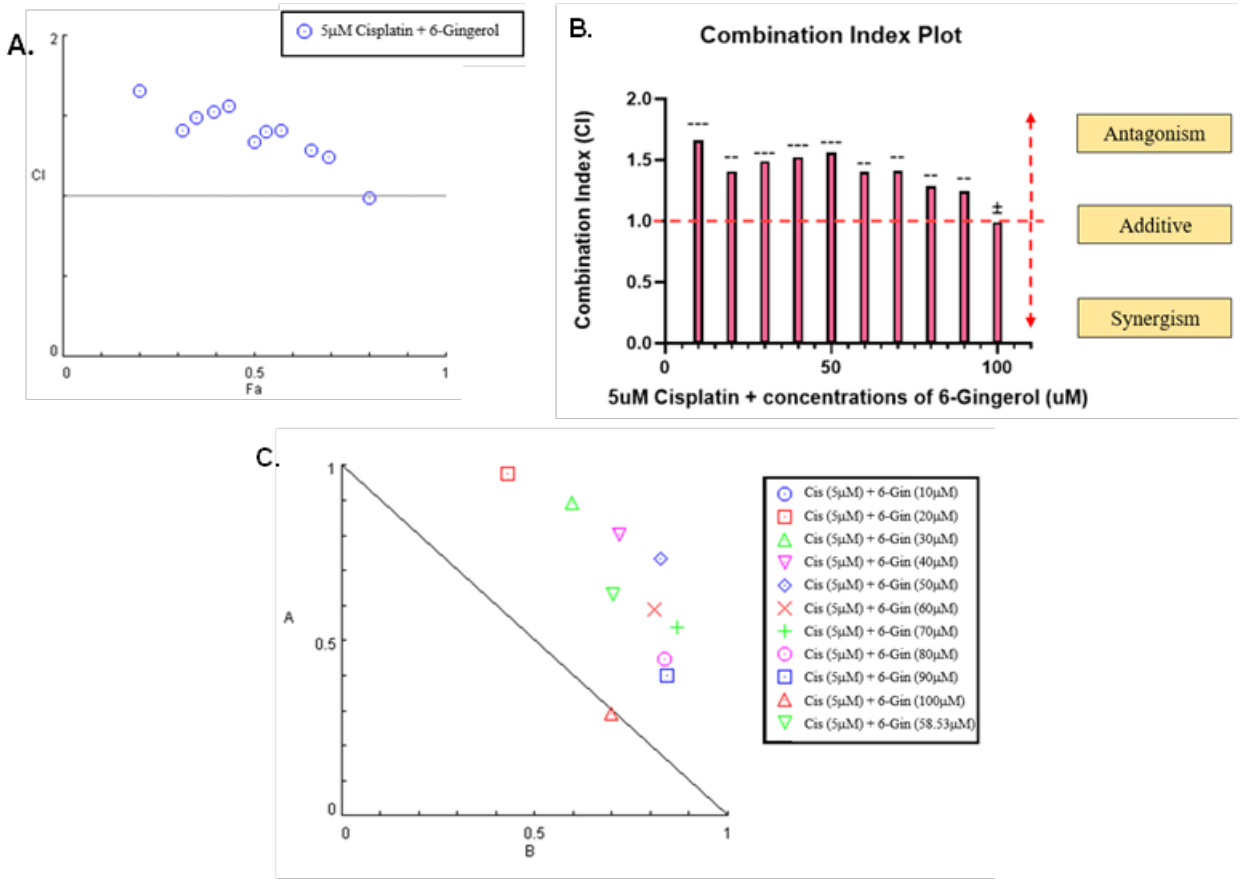

Figure 6 A. Fa-CI Plot; B. Combination Index (CI) Plot of cisplatin-6-gingerol co-treatment; C. Normalized isobologram for 5μM cisplatin+6-gingerol co-treatment

4.0 CONCLUSION

In summary, the findings from this present investigation provide us with valuable fundamental data that propose that cisplatin, 6-gingerol, and combined cisplatin-6-gingerol treatments significantly reduced MDA-MB-231 cell viability and induced cell morphological changes in a dose-dependent manner but with different concentration efficiency respectively. MDA-MB-231 cells were most sensitive towards solo cisplatin treatment, followed by combined 5μM cisplatin-6-gingerol and finally solo 6-gingerol treatment with IC_{50} concentrations of 8.08 μM, 58.24 μM, and 80.24 μM achieved, respectively. Although a reduction in the IC_{50} concentration of 6-gingerol and distinct morphological changes were observed upon co-treatment with cisplatin, the drug interaction study exhibited an antagonistic effect on MDA-MB-231 cells. Further studies looking into the different cisplatin-6-gingerol concentrations and treatment duration on MDA-MB-231 cells can be conducted to rule out possible synergistic interaction between cisplatin and 6-gingerol. Furthermore, the changes in cell morphology upon each treatment correlated with

the effect of each therapy respectively on the cell viability of MDA-MB-231 cells. The correlation between cell viability and change in cell morphology forms the basis for predicting the possible mechanism of action exerted upon the solo and combined treatments, respectively. The findings show that although cells were still viable upon combined cisplatin-6-gingerol treatment, the appearance of the remaining cells suggests that the cells were physiologically affected and had limited proliferative capacity. It is crucial to conduct a further comprehensive investigation to identify the mechanism of action of the combined cisplatin-6-gingerol treatment on MDA-MB-231 and other TNBC-related cells. The findings could contribute to the management of TNBC patients.

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References

Bakar, N. F. a. B. A., Yeo, Z. L., Hussin, F., Madhavan, P., Lim, V., Jemon, K., & Prabhakaran, P. (2023). Synergistic effects of combined cisplatin and Clinacanthus nutans extract on triple negative breast cancer cells. *Journal of Taibah University Medical Sciences*, *18*(6), 1220–1236. https://doi.org/10.1016/j.jtumed.2023.04.003

Balvan, J., Krizova, A., Gumulec, J., Raudenska, M., Sladek, Z., Sedlackova, M., Babula, P., Sztalmachova, M., Kizek, R., Chmelik, R., & Masarik, M. (2015). Multimodal Holographic Microscopy: Distinction between Apoptosis and Oncosis. *PLoS ONE*, *10*(3), e0121674. https://doi.org/10.1371/journal.pone.0121674

Bisht, S., Nigam, M., Kunjwal, S. S., Sergey, P., Mishra, A. P., & Sharifi-Rad, J. (2022). Cancer Stem Cells: From an Insight into the Basics to Recent Advances and Therapeutic Targeting. *Stem Cells International*, *2022*, 1–28. https://doi.org/10.1155/2022/9653244

Cesna, V., Sukovas, A., Jasukaitiene, A., Naginiene, R., Barauskas, G., Dambrauskas, Z., Paskauskas, S., & Gulbinas, A. (2018). Narrow line between benefit and harm: Additivity of hyperthermia to cisplatin cytotoxicity in different gastrointestinal cancer cells. *World Journal of Gastroenterology*, *24*(10), 1072–1083. https://doi.org/10.3748/wjg.v24.i10.1072

Dasari, S., Njiki, S., Mbemi, A., Yedjou, C. G., & Tchounwou, P. B. (2022). Pharmacological Effects of Cisplatin Combination with Natural Products in Cancer Chemotherapy. *International Journal of Molecular Sciences*, *23*(3), 1532. https://doi.org/10.3390/ijms23031532

E, E. S., Z, E., A, H. A., N, M., M, E., & E, O. (2020). Possible Protective Role Of Sodium Salicylate Nanoemulsion And Ginger On Cisplatin-Induced Hepatotoxicity In Rats (Biochemical And Histopathological Study). *International Journal of Current Pharmaceutical Research*, 133–139. https://doi.org/10.22159/ijcpr.2020v12i3.38323

Furlanetto, J., & Loibl, S. (2020). Optimal systemic treatment for early Triple-Negative breast cancer. *Breast Care*, *15*(3), 217–226. https://doi.org/10.1159/000508759

Garg, A. P. (2023). Identification of Gingerol (6-Gingerol) as Humming Inhibitor of Cancer through Docking Analysis. *www.jscimedcentral.com*. https://doi.org/10.47739/1091

Ghosh, S. (2019). Cisplatin: The first metal based anticancer drug. *Bioorganic Chemistry*, *88*, 102925. https://doi.org/10.1016/j.bioorg.2019.102925

Gupta, G. K., Collier, A. L., Lee, D. H., Hoefer, R., Zheleva, V., Van Reesema, L. L. S., Tang-Tan, A. M., Guye, M. L., Chang, D. Z., Winston, J. S., Şamli, B., Jansen, R., Petricoin, E. F., Goetz, M. P., Bear, H. D., & Tang, A. (2020). Perspectives on Triple-Negative Breast Cancer: current treatment strategies, unmet needs, and potential targets for future therapies. *Cancers*, *12*(9), 2392. https://doi.org/10.3390/cancers12092392

Ibrahim, A. S., Sobh, M., Eid, H. M., Salem, A., Elbelasi, H. H., ElNaggar, M. H., Abdelbar, F. M., Sheashaa, H., Sobh, M., & Badria, F. A. (2014). Gingerol-derivatives: emerging new therapy against human drug-resistant MCF-7. *Tumor Biology*, *35*(10), 9941–9948. https://doi.org/10.1007/s13277-014-2248-7

Kapoor, V., Aggarwal, S., & Das, S. N. (2016). 6-Gingerol Mediates its Anti Tumor Activities in Human Oral and Cervical Cancer Cell Lines through Apoptosis and Cell Cycle Arrest. *PTR. Phytotherapy Research/Phytotherapy Research*, *30*(4), 588–595. https://doi.org/10.1002/ptr.5561

Kim, C., Gonye, A. L., Truskowski, K., Lee, C., Cho, Y., Austin, R. H., Pienta, K. J., & Amend, S. R. (2023). Nuclear morphology predicts cell survival to cisplatin chemotherapy. *Neoplasia*, *42*, 100906. https://doi.org/10.1016/j.neo.2023.100906

Kotowski, U., Kadletz, L., Schneider, S., Foki, E., Schmid, R., Seemann, R., Thurnher, D., & Heiduschka, G. (2017). 6-shogaol induces apoptosis and enhances radiosensitivity in head and neck squamous cell carcinoma cell lines. *Phytotherapy Research*, *32*(2), 340–347. https://doi.org/10.1002/ptr.5982

Lucero, M., Thind, J., Sandoval, J., Senaati, S., Jimenez, B., & Kandpal, R. P. (2020). Stem-like Cells from Invasive Breast Carcinoma Cell Line MDA-MB-231 Express a Distinct Set of Eph Receptors and Ephrin Ligands. *Cancer Genomics & Proteomics*, *17*(6), 729–738. https://doi.org/10.21873/cgp.20227

Meng, L. (2023). Chromatin-modifying enzymes as modulators of nuclear size during lineage differentiation. *Cell Death Discovery*, *9*(1). https://doi.org/10.1038/s41420-023-01639-z

Orrantia-Borunda, E., Anchondo-Nuñez, P., Acuña-Aguilar, L. E., Gómez-Valles, F. O., & Ramírez-Valdespino, C. A. (2022). Subtypes of breast cancer. In *Exon Publications eBooks* (pp. 31–42). https://doi.org/10.36255/exon-publications-breast-cancer-subtypes

Prabhakaran, P., Hassiotou, F., Blancafort, P., & Filgueira, L. (2013). Cisplatin induces differentiation of breast cancer cells. *Frontiers in Oncology*, *3*. https://doi.org/10.3389/fonc.2013.00134

Prat, A., Parker, J. S., Karginova, O., Fan, C., Livasy, C., Herschkowitz, J. I., He, X., & Perou, C. M. (2010). Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. *Breast Cancer Research*, *12*(5). https://doi.org/10.1186/bcr2635

Rastogi, N., Duggal, S., Singh, S. K., Porwal, K., Srivastava, V. K., Maurya, R., Bhatt, M. L., & Mishra, D. P. (2015). Proteasome inhibition mediates p53 reactivation and anti-cancer activity of 6-Gingerol in cervical cancer cells. *Oncotarget*, *6*(41), 43310–43325. https://doi.org/10.18632/oncotarget.6383

Ray, R., Khashali, H. A., Haddad, B., Wareham, J., Coleman, K., Alomari, D., Ranzenberger, R., Guthrie, J., Heyl, D., & Evans, H. G. (2022). Regulation of cisplatin resistance in lung cancer cells by nicotine, BDNF, and a B-Adrenergic receptor blocker. *International Journal of Molecular Sciences*, *23*(21), 12829. https://doi.org/10.3390/ijms232112829

Rehman, M. U., Ahmad, B., Arif, A., Rasool, S., Farooq, A., Razzaq, R., Bhat, S. A., Bashir, S., Shabir, O., Amin, I., Masoodi, M., Mir, M. U. R., & Shah, M. Y. (2015). Zingerone protects against cisplatin-induced oxidative damage in the jejunum of Wistar rats. *Oriental Pharmacy and Experimental Medicine/Oriental Pharmacy and Experimental Medicine*, *15*(3), 199–206. https://doi.org/10.1007/s13596-015-0187-5

Rodea-Palomares, I., González-Pleiter, M., Martín-Betancor, K., Rosal, R., & Fernández-Piñas, F. (2015). Additivity and interactions in ecotoxicity of pollutant mixtures: Some patterns, conclusions, and open questions. *Toxics*, *3*(4), 342–369. https://doi.org/10.3390/toxics3040342

Salari, Z., Khosravi, A., Pourkhandani, E., Molaakbari, E., Salarkia, E., Keyhani, A., Sharifi, I., Tavakkoli, H., Sohbati, S., Dabiri, S., Ren, G., & Shafie'ei, M. (2023). The inhibitory effect of 6-gingerol and cisplatin on ovarian cancer and antitumor activity: In silico, in vitro, and in vivo. *Frontiers in Oncology*, *13*. https://doi.org/10.3389/fonc.2023.1098429

Shahshahan, Z., Khosravi, A., Pourkhandani, E., Molaakbari, E., Salarkia, E., Keyhani, A., Sharifi, I., Tavakkoli, H., Sohbati, S., Dabiri, S., Ren, G., & Shafie'ei, M. (2023). The inhibitory effect of 6-gingerol and cisplatin on ovarian cancer and antitumor activity: In silico, in vitro, and in vivo. *Frontiers in Oncology*, *13*. https://doi.org/10.3389/fonc.2023.1098429

Singh, L., Aldosary, S., Saeedan, A. S., Ansari, M. N., & Kaithwas, G. (2018). Prolyl hydroxylase 2: a promising target to inhibit hypoxia-induced cellular metabolism in cancer cells. *Drug Discovery Today*, *23*(11), 1873–1882. https://doi.org/10.1016/j.drudis.2018.05.016

Sp, N., Kang, D. Y., Lee, J., Bae, S. W., & Jang, K. (2021). Potential antitumor effects of 6-Gingerol in P53-Dependent mitochondrial apoptosis and inhibition of tumor sphere formation in breast cancer cells. *International Journal of Molecular Sciences*, *22*(9), 4660. https://doi.org/10.3390/ijms22094660

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209–249. https://doi.org/10.3322/caac.21660

Wang, Q., Wei, Q., Yang, Q., Cao, X., Li, Q., Shi, F., Tong, S., Feng, C., Yu, Q., Yu, J., & Xu, X. (2018). A novel formulation of [6]-gingerol: Proliposomes with enhanced oral bioavailability and antitumor effect. *International Journal of Pharmaceutics*, *535*(1–2), 308–315. https://doi.org/10.1016/j.ijpharm.2017.11.006

Xu, Y., Wang, Q., Feng, Y., Firempong, C. K., Zhu, Y., Omari-Siaw, E., Zheng, Y., Pu, Z., Xu, X., & Yu, J. (2016). Enhanced oral bioavailability of [6]-Gingerol-SMEDDS: Preparation, in vitro and in vivo evaluation. *Journal of Functional Foods*, *27*, 703–710. https://doi.org/10.1016/j.jff.2016.10.007

Yang, L., Zha, L., Luo, L., Chen, X., Zhang, Q., Gao, C., Zhuang, X., Yuan, S., & Qiao, T. (2019). [6]-Gingerol enhances the cisplatin sensitivity of gastric cancer cells through inhibition of proliferation and invasion via PI3K/AKT signaling pathway. *Phytotherapy Research*, *33*(5), 1353–1362. https://doi.org/10.1002/ptr.6325

Yao, Y., Chu, Y., Xu, B., Hu, Q., & Song, Q. (2019). Radiotherapy after surgery has significant survival benefits for patients with triple-negative breast cancer. *Cancer Medicine*, *8*(2), 554–563. https://doi.org/10.1002/cam4.1954

Yu, K., Ye, F., He, M., Fan, L., Ma, D., Mo, M., Wu, J., Li, G., Di, G. H., Zeng, X., He, P., Wu, K., Hou, Y., Wang, J., Wang, C., Zhuang, Z., Song, C., Lin, X., Toss, A., . . . Shao, Z. (2020). Effect of adjuvant paclitaxel and carboplatin on survival in women with Triple-Negative breast Cancer. *JAMA Oncology*, *6*(9), 1390. https://doi.org/10.1001/jamaoncol.2020.2965

Zhang, L., Chen, W., Liu, S., & Chen, C. (2023). Targeting breast cancer stem cells. *International Journal of Biological Sciences*, *19*(2), 552–570. https://doi.org/10.7150/ijbs.76187