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Anticancer Effects of Combined Cisplatin and Ginger Extract on Triple-Negative Breast Cancer Cells

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Abstract
Triple-negative breast cancer (TNBC) is a diverse disease that typically affects younger women and has a higher rate of motility than other types of breast cancer. The purpose of this study was to determine the anticancer effects of combined cisplatin and ginger extract (CIS+GE) on MDA-MB-231 cells. Cell Titre-Glo assay, Caspase 3/7 assay, and isobologram analysis were used to investigate the growth inhibitory effect of CIS+GE, the ability of CIS+GE to induce apoptosis, and the interaction between cisplatin and GE. On MDA-MB-231 cells, cisplatin (5µM-100µM), GE (50µM-300µM), and CIS+GE (10µM +50µM- 10µM +300µM) concentrations were tested. Cisplatin (2.16% -63.4%), GE (29.59% -82.28%), and CIS+GE (40.82% -97.83%) inhibited cell viability in a dose-dependent manner. The phenotype of solo cisplatin-treated cells was differentiated, whereas the phenotype of GE-treated cells was apoptotic. When compared to single dosages, the combination of CIS+GE (10µM +50µM, 10µM +120µM) enhanced apoptosis considerably, with apoptotic rates of 1.52% and 1.77%, respectively. In addition, the combination of CIS+GE had a strong synergistic impact on MDA-MB-231 cells. Finally, these findings imply that the CIS+GE therapy strategy could be an effective anticancer agent for TNBC.
Keywords: TNBC; Cisplatin; Ginger extract

Introduction

Triple-negative breast cancer (TNBC) is the most aggressive and lethal type of breast cancer which has aggressive behavior molecular heterogeneity and accounts for 15-20% of all breast cancer cases (Lin et al., 2018). TNBC has a high local recurrence and distant metastasis compared to other forms of breast tumors. In addition, patients with TNBC have the worst outcome with a dismal prognosis. Currently, chemotherapy is the only treatment option for TNBC patients (Chan et al., 2022).

Cisplatin is a potent metal-based anticancer drug used to treat many types of solid cancers. However, drug resistance and adverse effects are the two drawbacks of cisplatin that limited its use as an anticancer drug (Poggio et al., 2018). As a result, combination therapy is utilized to reduce side effects and the problem of drug resistance, as it has been shown to be more effective in treating cancer. Combination therapy is a significant part of cancer treatment because it targets key pathways in a way that is either synergistic or additive (Zhu et al., 2022). This strategy has the potential to diminish drug resistance while also delivering therapeutic anti-cancer effects such as lowering tumor growth and metastatic potential, stopping mitotically active cells, reducing cancer stem cell populations, and inducing apoptosis. Therefore, many malignancies are treated with cisplatin-based combination chemotherapy.

Ginger on the other hand has shown efficient anticancer activities against TNBC cells (Mahomoodally et al., 2021). There are several studies on the anticancer effects of ginger and cisplatin on TNBC cells; however, the effects of combined cisplatin-ginger on TNBC cells have not been reported yet. Therefore, further assessment on the anticancer effect and synergistic effect of this co-treatment on TNBC cells was the focus of the research.

Materials and methods

Reagents and cell culture. Ginger (McCormick, Australia) was obtained from a local store, cisplatin was purchased from Tocris Bioscience, Caspase-Glo® 3/7 Assay, and Cell Titer96® Aqueous One Solution Cell Proliferation Assay was acquired from Promega. MDA-MB-231 (Faculty Science, UTM) breast cancer cells were maintained in Dulbecco's modified eagle's medium (DMEM)/F12+ glutamax^{TM} (Gibco by life science) enriched with 10% FBS (Gibco), and 1% antibiotic (100U/Ml penicillin, 100µg/ mL) (Gibco), and incubated in a humidified environment at 37˚C and 5% CO2. Then, at 60 -70% confluency, it was serially passaged.

Cell viability assay was used to evaluate the growth inhibitory effects of cisplatin, ginger extract (GE), and the combination of CIS+GE on MDA-MB-231 cells. $3.5x10^3$ cells/well were seeded in a 96-well plate for 24 hours. Then cells were treated with different concentrations of cisplatin, GE, and combined CIS+GE. After 24 hours 20µl of MTS reagent was added and after 1–4-hour incubation absorbance was recorded by Elisa microplate reader at 570nm.

Caspases 3/7 assay was performed on MDA-MB-231 cells to see if cisplatin, GE, or a combination of CIS+GE induce apoptosis. 3500cells/well were seeded in a 96-well plate. The cells were treated with cisplatin (10µM, IC50), GE (50µM, IC50), and combined CIS+GE (10µM+50µM, 10µM+120µM). Due to the limited volume of caspase 3/7 reagent, another concentration of drugs was not investigated. Caspase- Glo 3/7 reagent was prepared and added with the ratio of 1:1 to each sample, gently mixed for 30 seconds, then incubated for two hours. Lastly, the luminescence was recorded by Elisa microplate reader.

CompuSyn software (ComboSyn. Inc., Paramus, NJ. USA) was used to determine the interaction between cisplatin and GE. Dose and effects of cisplatin, GE, and combined CIS+GE which were achieved from MTS assay, entered the software and the result was achieved.

Results and discussion

MDA-MB-231 cells were treated with various concentrations of cisplatin, GE, and combined CIS+GE, and it was revealed that each drug could indeed reduce the MDA-MB-231 viability in a dose-dependent manner but with differing potency.

In mono-treatment, cisplatin reduced the viability of MDA-MB-231 by 0-30.67% at lower concentrations (0-25µM). At higher concentrations (50µM -100µM), the viability of MDA-MB-231 reduced approximately 49.51%-63.4%. GE mono-treatment, on the other hand, drastically reduced the viability of TNBC cells by 20.59% – 82.28% at concentrations ranging from 50µM to 300µM. Cisplatin and GE have previously been shown to reduce the viability of MDA-MB-231 cells in a dose-dependent manner, which is consistent with our findings (Nedungadi et al., 2021; Zhang & Xie, 2020).

In the combination therapy, MDA-MB-231 cells were treated with cisplatin and GE at the same time and combined CIS+GE suppressed the viability of the MDA-MB-231 cells more effectively than either cisplatin or GE alone. MDA-MB-231 cells were treated with cisplatin at a fixed concentration and GE at variable concentrations. Cisplatin has dose-dependent side effects and usually leads to drug resistance over time (Manohar & Leung, 2018). As a result, a low dose of cisplatin was administrated to minimize cisplatin-induced toxicity and maximize its anticancer efficacy by combining it with GE. The concentration of cisplatin used in this experiment was 10µM. The result of the MTS assay revealed that combined CIS+GE had a strong growth inhibitory effect and caused up to 97% reduction of TNBC cells at 10 µM +300 µM concentration (figure 1. A). Therefore, it can be concluded that when the different concentration of GE was combined with 10µM of cisplatin, it potentiated the growth inhibitory effect of cisplatin on MDA-MB-231 cells. The possible reason can be the cytotoxic effects of ginger's bioactive compounds. For example, 6- gingerol which is one of the most pungent components of ginger could inhibit metastasis of MDA-MB-231 cells by decreasing the expression of MMP-2 and MMP-9 (Kiyama, 2020; Lee et al., 2008). Moreover, 6-shogaol which is a dehydrated form of gingerol and a major bioactive compound of ginger inhibited autophagy and suppressed the proliferation of breast cancer cells by down-regulating the notch signaling system, and inducing apoptosis (Bawadood et al., 2020).

According to earlier studies, the ethanol extraction method has higher efficiency and is a superior way to obtain a significant amount of metabolite in ginger. Due to its polarity, ethanol appears to be able to extract more metabolites from ginger (Malmir et al., 2020; Shukla et al., 2019). Because ethanol was utilized as a solvent in this work, GE is likely to include all the relevant bioactive components. As a result, it is thought that bioactive components in ginger contributed to cisplatin's growth inhibitory effects and increased its anticancer effects at low concentrations (10µM).

In addition, cisplatin, GE, and CIS+GE were found to elicit a dose-dependent alteration in cell morphology. The cells in untreated wells retained their cohesiveness and original epithelial shape, but the cells in treated wells lost their epithelial shape and died as concentration increased. Microscopic examination revealed morphological alterations such as rounding and shrinkage. Even though the round form was the most common, additional morphologies such as cell blebbing, cytoplasmic vacuolation, irregular structure, and distribution were also observed. The same result was observed when MDA-MB-231 cells were treated with extracts of *Asplenium polyploidies Blume* and *A. dalhousie Hook* (Khattak et al., 2020), *Ficus carica*, and *Ficus salicifolia* latex (AlGhalban et al., 2021), which supports our findings. MDA-MB-231 cells treated with cisplatin, on the other hand, showed a more differentiated phenotype and they appeared to be longer and wider. This is in good agreement with prior research that found cisplatin induces differentiation in MDA-MB-231 cells by up-regulating differentiation markers and down-regulating cancer stem cell markers (Prabhakaran et al., 2013). Based on this finding, it could be suggested that Cisplatin potentially caused MDA-MB-231 cells to differentiate and become susceptible to GE apoptosis instead of causing apoptosis on its own accord.

Furthermore, CompuSyn analysis demonstrated that there was synergistic interaction between cisplatin and GE for the MDA-MB-231 cell line. As shown in table 1, the combination index (CI) values were always lower than 1.0 (CI<1) when cells were treated with varying concentrations of GE and 10µM of cisplatin, demonstrating that the two medicines had synergistic effects on MDA-MB-231 cells. As the concentration of GE increased, so did the range of synergism, which increased from moderate to very strong. As a result, it may be concluded that higher GE concentrations result in greater synergy.

Table 1: Combination Index (CI) value for MDA-MB-231 cells at a fixed concentration of cisplatin (10) with varying concentrations of GE. C<0.1 is very strong synergism, C=0.1-0.3 is strong synergism, C=0.3-0.7 is synergism, C=0.7-0.85 is moderate synergism, C=0.85-0.90 is slight synergisms, C=0.90-1.00 is nearly additive, C=1 is additive, and C>1 is antagonism (Chou, 2018).

Cisplatin	GE	IC	effect
10 µM	50 µM	0.83	Moderate synergism
10 µM	100 µM	0.98	Nearly additive
10 µM	150 µM	0.84	Moderate synergism
10 µM	200 µM	0.74	Synergism
10 µM	250 µM	0.53	Synergism
10 µM	300 µM	0.15	Very strong synergism

The goal of effective cancer therapy is to promote the death of cancer cells while causing no harm to normal cells. As a result, most cancer drugs are strategically designed to work in two ways: by inducing apoptosis and by inducing direct cytotoxicity (Chen et al., 2018). Apoptosis can be detected through morphological changes as well as biochemical analysis. The morphology of MDA-MB-231 after treatment with GE and CIS+GE confirmed apoptosis activity. MDA-MB-231 cells exhibited cell shrinkage, plasma membrane blebbing, cytoplasmic vacuolation, and round and irregular shape. These morphological changes are common in cells that have died apoptotically (Nedungadi et al., 2021). According to the findings, cisplatin and GE had minimal apoptotic effects at low concentrations (cisplatin 10µM, GE 50 µM). However, caspase3/7 activity increased considerably at IC50 concentrations of cisplatin (50µM) and GE (120µM). Furthermore, compared to the positive control (Taxol), cisplatin, and GE single doses, apoptotic activity was enhanced significantly when 10µM cisplatin was combined with 50 µM and 120 µM of GE.

Thus, it is thought that GE sensitized the apoptotic effect of cisplatin. Cisplatin did not affect caspase3/7 activity at low concentrations, however, the combination of GE+CIS dramatically promoted apoptosis and caspase3/7 activity in MDA-MB-231. Cisplatin, as well as combinations of cisplatin with tetrandrine (Bhagya et al., 2020), cisplatin plus lenalidomide (Yin et al., 2018), also induced apoptosis in MDA-MB-231 cells via the caspases pathway, which was in good agreement with our findings.

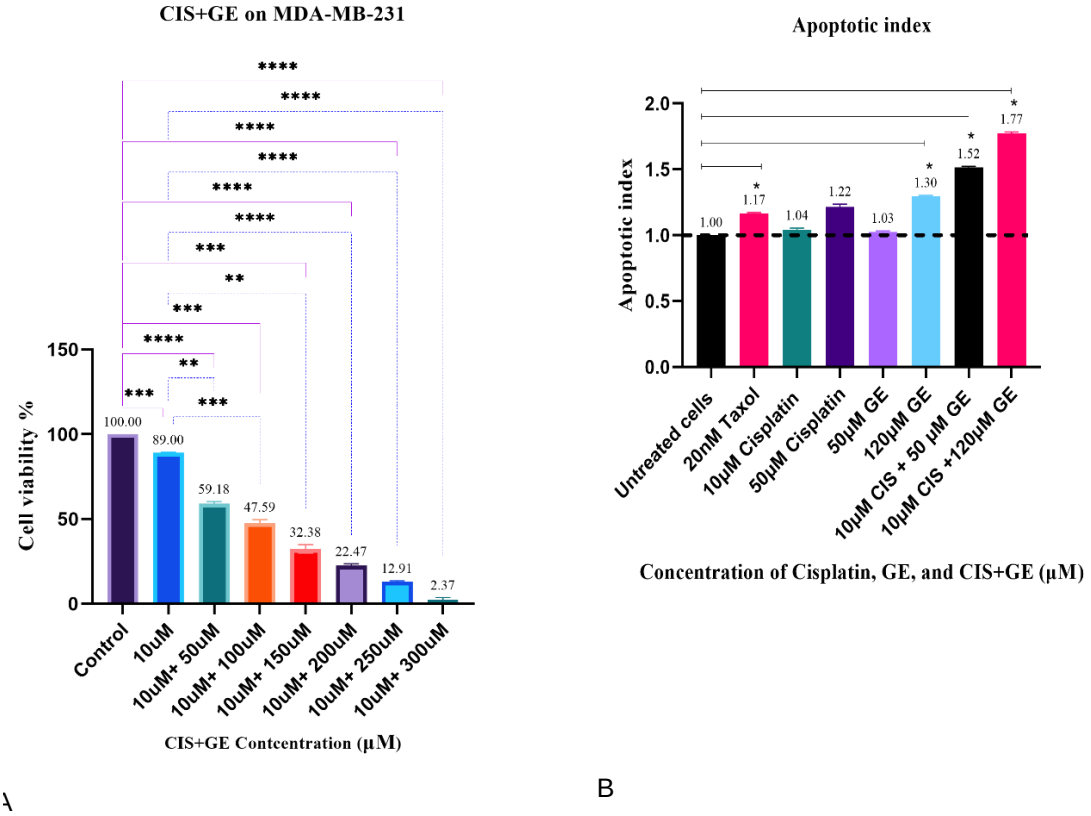

Figure 1 A) Growth inhibitory effect of combined CIS+GE on MDA-MB-231 cells. The vitality of the cells was altered in a dose-dependent way, and the data are presented as means ±SD. Purple lines (——) show the significant difference ($P<0.05$) between control and treated groups and dotted lines (_____) show a significant difference between 10µM and other treated groups. B) Apoptotic effect of cisplatin (10µM, 50µM), GE (50µM, 120µM), and combined of CIS+GE (10µM +50µM, 10µM +120µM) on MDA-MB-231 cells. All treatment groups were compared to the negative control or untreated cells and normalized to apoptotic index=1. Experiments were repeated two times and the results were expressed as means ± SD

Conclusion

In conclusion, both cisplatin and GE were potent anticancer drugs that can induce differentiation and limit the growth of MDA-MB-231 cells in a single treatment while inducing cell death via apoptosis upon combined treatment. Besides, a synergistic interaction between cisplatin and GE was proven with reducing the CI value. Furthermore, a higher apoptotic index was exhibited in combined treatment compared to a single treatment.

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