ORIGINAL ARTICLE

Apoptosis and anti-migratory effects of Methyl gallate combined with Cisplatin in human breast cancer (MCF-7) cells

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ABSTRACT

Introduction: Breast cancer is among the leading causes of cancer-related death in women worldwide. Cisplatin is a widely used chemotherapeutic drug because of its strong efficacy in killing various cancer cells. However, it presents several toxicity effects and development of drug resistance. Looking at these shortcomings, there is growing interest in combining conventional chemotherapeutic drugs with natural compounds. Therefore, a natural phenolic compound, methyl gallate, known to have strong anticancer and antioxidant properties, is a potential candidate for enhancing the therapeutic efficacy and reducing the side effects of current chemotherapy drugs.

Materials and Methods: This study aims to determine the antiproliferative effect of methyl gallate and its combination with cisplatin on MCF-7 cells using MTT assay. The combination effect was evaluated using MTT assay and then analysed using CompuSyn software. Besides, this study was done to evaluate the morphology of apoptotic cells using AO/PI stain and to determine the ability of the combination treatment to inhibit the migration of MCF-7 cells using wound healing assay.

Results: The cell viability was inhibited dose-dependently with IC_{50} values of methyl gallate and cisplatin were 20.21 µg/mL and 13.38 µg/mL respectively. Methyl gallate at fixed concentration combined with the lowest concentration of cisplatin inhibited greater MCF-7 cell growth compared to cisplatin alone with a combination index value of less than 1 indicating the synergistic effect. The MCF-7 cells treated with single and combination drugs exhibited several apoptotic characteristics. Migration of MCF-7 cells was also significantly inhibited by single and combination treatments. Conclusion: In conclusion, this study suggested that methyl gallate in combination with cisplatin has a good potential to be developed as a chemotherapeutic agent for breast cancer.

KEYWORDS:
Apoptosis, migration, cisplatin, methyl gallate, combination treatment, synergistic effect

INTRODUCTION

Breast cancer is the most commonly diagnosed cancer among women in 2022 where it accounts for an estimated 2.29 million new cases globally. Furthermore, breast cancer has become the leading cause of cancer death with approximately 665,684 deaths.[1] Meanwhile, with 34.1% of all cancers among women, breast cancer ranks as the first most common cancer in Malaysia. There are estimated 21,634 cases of female breast cancer were diagnosed from 2012 to 2016.[2]

There are different treatment options for breast cancer depending on the type of breast cancer and the severity such as its stage and cancer cells size. The available breast cancer treatments include chemotherapy, radiotherapy, hormonal therapy, and immunotherapy. Breast cancer is strongly associated with females, late menopause, older age, early menarche, nulliparity, positive family history and lack of breastfeeding.[3] Chemotherapy is the most common cancer therapy that uses drugs to destroy the cancer cells. It functions by inhibiting and slowing the growth of cancer. Unfortunately, chemotherapy has a lot of unfavorable side effects despite its effectiveness against cancer cells. It destroys the nearby healthy cells in addition to the cancerous ones. Because multiple drugs kill cancer cells in different ways, chemotherapy is now often administered as a combination of agents rather than a single agent.[4]

Cisplatin is one of the well-known platinum-based chemotherapeutic drugs used to treat numerous human malignancies including those of the bladder, head and neck, lung, ovarian, and testicular cancers. The ability of this compound to crosslink with purine bases on DNA has been connected to its mode of action. This causes DNA damage, disrupts DNA repair processes, and ultimately triggers apoptosis in cancer cells.[5] Nevertheless, cisplatin treatment has inherent limitations that hinder its efficacy. These limitations include the development of dose-limiting toxicities due to drug accumulation in non-targeted areas of the body. Several non-cancerous sites vulnerable to cisplatin toxicity include the kidneys, auditory system and brain.[6]

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Methyl gallate (MG) is a gallate ester derived from formal condensation of gallic acid. It is a polyphenolic compound that is commonly present in natural plants such as Meliaceae species, *Galla rhois*, and seed coats of *Givotia rottleriformis* Griff. MG has been extensively studied due to its numerous biological activities such as antiplatelet activity, ability to protect DNA damage from oxidative stress, ability to reduce lung injury caused by phosgene, ability to attenuate diabetic and antiapoptotic activity.[7] Moreover, MG has exhibited significant anticancer activity on various cancer cells such as liver, skin, cervix, lung, brain, and lymph nodes.[8] MG has been shown to induce apoptosis through a variety of mechanisms, including the production of reactive oxygen species (ROS), regulation of apoptotic and anti-apoptotic proteins, suppression and promotion of oncogenes, inhibition of matrix metalloproteinases (MMPs), and cell cycle arrest.[9]

Combination chemotherapy is one of the strategies to increase the efficacy of anticancer drugs by combining and administering multiple drugs. The combination of chemotherapy may enhance the efficiency of the drug since it impacts the cancer cells through different stages of the cell cycle.[10] The synergistic effect of the combination may increase the tumor responses, thereby overcoming the side effects by lowering the drug dosage. The combination therapy of cisplatin with other anticancer agents has been proven to be more effective as it will produce fewer toxic effects and prevent cancer cells from developing resistance.[11] The use of natural products, such as plants, herbs, and other substances derived from nature, for their potential anti-cancer properties has been a subject of interest and research. Numerous clinical trials are being conducted to evaluate the effectiveness and safety of using natural remedies to cure or prevent cancer.[12] Hence, combining MG which is known to have high antioxidant capacity, with cisplatin is a proposed alternative to enhance the cytotoxicity effect on MCF-7 cells.

To the best of our knowledge, there is no study has reported the combination of MG and cisplatin on MCF-7 cells. Hence, in this current study, MG in combination with cisplatin was investigated for its antiproliferative effects on MCF-7 cells. The apoptosis features and anti-migratory effect of MCF-7-treated cells by single and combination compounds were also evaluated through AO/PI staining and wound healing assay to postulate its potential role as an anticancer agent for breast cancer.

MATERIALS AND METHODS

Chemicals

Cisplatin, MG and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Fetal bovine serum, Dulbecco's modified Eagle's medium (DMEM), streptomycin and penicillin and trypsin were purchased from Gibco Thermo scientific. 3-(4,5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide (MTT) was purchased from Nacalai Tesque, Inc. (Japan).

Cell culture

MCF-7 cells were obtained from the stock culture at the School of Health Sciences, Universiti Sultan Zainal Abidin. The cell lines were grown in a humidified atmosphere at 370C with 5% (v/v) CO_2. The complete medium containing DMEM, 10% FBS, and 1% penicillin-streptomycin was used to culture cells.

Determination of cell viability and IC_{50}

The MTT assay was conducted to determine the IC_{50} values of cisplatin and MG. MCF-7 cells (4×10^3 cells/ml) were seeded in 96-well plates and incubated for 24 hours in a CO_2 incubator at 37°C. Upon reaching 80-90% confluency, the used medium was discarded. Subsequently, the cells were treated with different concentrations of cisplatin and MG, with 1% DMSO that served as the negative control. The treated cells were incubated for 72 hours in a CO_2 incubator.

Following the treatment period, 10 µl of MTT solution (5 mg/ml in PBS) were added to each well. The plate was covered with aluminum foil and placed in a humidified incubator with 5% CO_2 at 37°C for 4 hours. After this incubation period, the contents in the well were discarded, and 100 µl absolute DMSO was added to dissolve the blue formazan crystals produced by viable cells. Then, the absorbance (OD) of each well was measured using a microplate reader at a wavelength of 570 nm. The percentage of cell viability (%) was calculated using the formula:

$$Percentage\ of\ cell\ viablity\ (\%) = \frac{Absorbance\ in\ a\ well}{Absorbance\ of\ negative\ control} \times 100$$

The IC_{50} values of cisplatin and MG were determined from the dose-response curve, generated by plotting the percentage of cell viability (%) against log10 concentration (µg/ml) using GraphPad Prism software. These IC_{50} values were used in the combination treatment of MCF-7 cells.

Combination treatment

A non-constant combination of compounds MG and cisplatin method was applied to determine the combination effects of these two compounds. MCF-7 cells were treated with MG at a constant dose of IC_{50} (20.21µg/mL) combined with cisplatin at 5 serial concentrations starting range from its IC_{50} value, (13.38 to 6.69, 3.35, 1.67 and 0.84 µg/mL) using two-fold dilution. The MTT assay was used to calculate the percentage of cell viability (%). CompuSyn software was then used to calculate the combination effect. The pharmacological interaction of drug combinations was evaluated using a mathematical analysis and reported as a combination index (CI), The CI values can be expressed as additive (CI=1) synergism (CI<1) and antagonism (CI>1). The best combination was chosen to be used for treatment in the subsequent assays.

AO/PI staining

The morphological changes in apoptotic nuclei of MCF-7 treated cells were evaluated using Acridine Orange/ Propidium Iodide (AO/PI) staining. 2×10^5 MCF-7 cells per well were seeded into 6-well plates. The cells were incubated in a humidified incubator with 5% CO_2 at 37°C for 24 hours. Before treatment, the confluency of the seeded cells was checked to ensure it was 80-90% in each well. The cells were then treated with MG and cisplatin at their respective IC_{50} concentrations and a combination treatment. The cells were incubated again in a humidified incubator with 5% CO_2 at

Table I: Combination index for combination of cisplatin and MG on MCF-7 cells

Dose Cis	Dose MG	Effect	CI
13.38	20.21	0.62	2.42340
6.69	20.21	0.55	1.95718
3.35	20.21	0.45	1.85391
1.67	20.21	0.42	1.29350
0.84	20.21	0.4	0.93842

37°C for 72 hours. The cells were detached using trypsin and were transferred to the 15 mL tube used before and centrifuged at 1500 rpm for 10 minutes. After centrifugation, the supernatant was discarded, and 20 µL of AO solution was added to the tube and mixed with the cell pellet, followed by 20 µL of PI solution. The mixed solutions were immediately cooled by placing them in an icebox. A 10 µL of the cell solution was placed onto a clean slide and covered with a coverslip. The cells were quickly viewed under a fluorescence microscope to observe and identify apoptotic, condensed, and fragmented nuclei, distinguished by green and orange fluorescence. The entire procedure was performed without light exposure.

Wound healing assay

The determination of migratory inhibition of MCF-7 cells was performed using wound healing assay. MCF-7 cells were seeded at density 2.5 $\times 10^5$ per well into a 6-well plate and the plate was maintained in a humidified incubator with 5% CO_2 at 37°C for 24 hours or until they reached 100% confluency. After 24 hours, a sterile SPLScar™ Scratcher and its lid were used to create a straight scratch, or wound, across the center of each well. The wound area was observed and captured starting from 0 hours using an inverted microscope equipped with a digital camera. The cells were then treated with single and combination treatment. The cells were incubated again in a humidified incubator with 5% CO_2 at 37°C. The wound area was observed and captured at 0, 12, and 24 hours. The captured images were analyzed using, Image J to measure the wound width. The percentage of wound closure were calculated using the formula:

$$\text{Percentage of wound closure} = \frac{(\text{space at time 0} - \text{space at (t) hours}) \times 100}{\text{Space at time 0}}$$

Statistical analysis

Statistical analysis was performed using GraphPad Prism (version 10) statistical software. All data were presented as the mean ± standard deviation (SD) from triplicate experiments. The combination effects of the compounds were determined using CompuSyn software. The statistical significance of the data between the three groups was determined using one-way ANOVA with Turkey post hoc test where $p<0.05$ was considered statistically significant.

RESULTS

Antiproliferative effect of cisplatin and MG

The antiproliferative effects of cisplatin and MG on MCF-7 cells were assessed using MTT assay after 72 hours of incubation treatment with different concentrations of cisplatin and MG ranging from 100 µg/mL to 6.25 µg/mL. Figure 1 illustrates the antiproliferative effects of both compounds on MCF-7 cells after the treatment. The results showed that cisplatin and MG inhibited MCF-7 cell growth in a dose-dependent manner where the higher the concentration led to the greater cell inhibition. The IC_{50} values of cisplatin and MG on MCF-7 cells obtained from the dose-response curve of percentage of cell viability (%) against log10 concentration were 13.38 µg/mL and 20.21 µg/mL, respectively.

Combination effect of cisplatin and MG

The analysis of the combination effects using CompuSyn software was reported as combination indexes (CI) and expressed as synergistic, antagonistic or additive effects. Table I shows the CI values of different combinations of cisplatin and MG. Based on this result, the CI values of the combination of MG with cisplatin at concentrations 13.38 µg/mL, 6.69 µg/mL, 3.35 µg/mL and 1.67 µg/mL were more than 1, indicating antagonistic effects. However, the combination with cisplatin at 0.84 µg/mL showed the lowest CI which was less than 1. Thus, this combination reaction is considered to have a synergistic effect. Hence, it was chosen as the best combination and was used for the treatment in further assay.

Morphological observation of apoptotic cells (AO/PI staining)

The morphological changes and apoptotic features of cells treated with single and combination drugs was evaluated using Acridine Orange/ Propidium Iodide (AO/PI) staining. This staining method allows visualization of viable and non-viable cells using green and orange/ red fluorescent dyes. Viable cells appear with a green and uniform nucleus, while apoptotic cells exhibit an irregular bright green nucleus or irregular orange fluorescence. After 72 hours, the observation revealed that MCF-7 cells treated with the IC_{50} concentrations of cisplatin, MG and a combination of cisplatin and MG (CIS-MG) showed significant apoptotic characteristics compared to the untreated control cells. As shown in Figure 2, typical features of apoptotic cells, such as irregular or fragmented nuclei, nuclear condensation, irregular cell shapes and membrane blebbing, were visible in the treated cell groups (B-D), marked by bright green and orange dyes. Several dead cells also present in these treated groups marked by red dyes. In contrast, the untreated control cells (A) maintained its oval shape with an intact nucleus uniformly stained with green fluorescence.

Anti-migratory effect of CIS-MG on MCF-7 cells

MCF-7 cell migratory inhibition after treatment was evaluated using wound healing or scratch assay. The results of the wound healing assay on MCF-7 cells (Figure 3) demonstrated marked differences in cell migration between

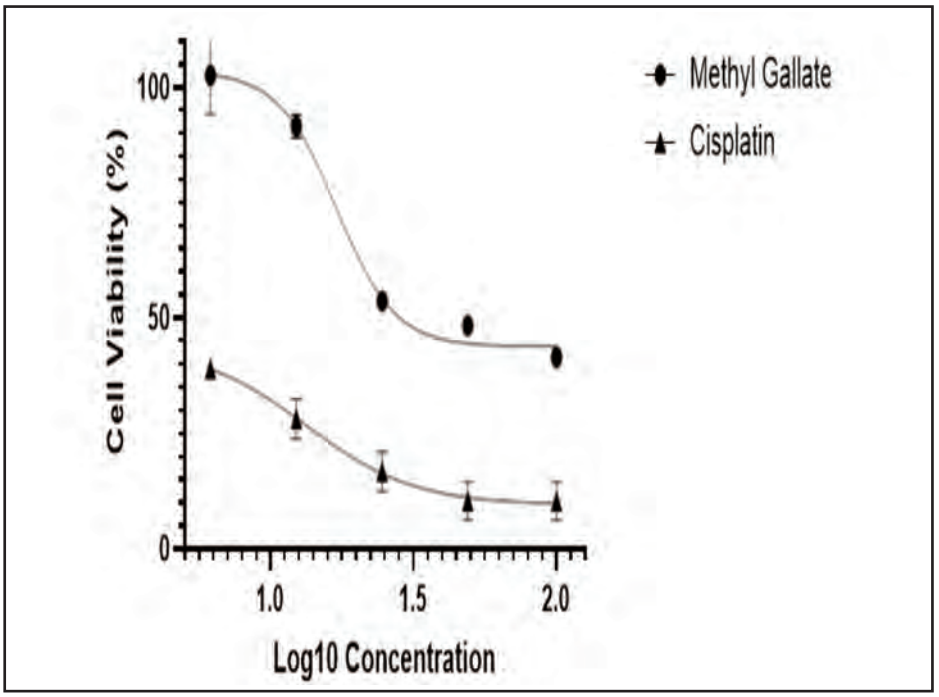

Fig. 1: Antiproliferative activity of cisplatin and MG on MCF-7 cells

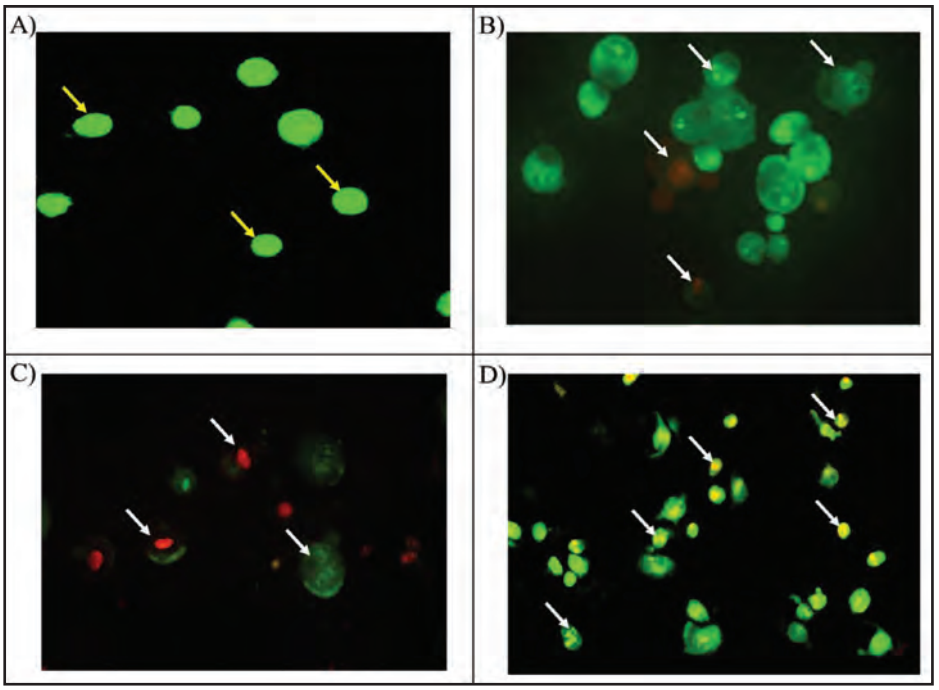

Fig. 2: The morphology of apoptotic cells with irregular or fragmented nucleus, nuclear condensation, irregular cell shape, membrane blebbing and apoptotic bodies characteristics (white arrow) presented in B) cisplatin, C) MG and D) CIS-MG groups, stained by AO/PI staining after 72 hours incubation. The oval shape and uniform nucleus maintained (yellow arrow) in A) untreated control cells. The images of cells were captured under 200x and 400x magnification using a fluorescence microscope

untreated and treated groups over time. As presented in Figure 4, the percentages of wound closure in cisplatin (70.1 ± 4.97), MG (58.58 ± 2.20) and CIS-MG (59.56 ± 0.58) were significantly lower than in untreated cells (79.87±5.35) at 12 hours of incubation treatment, indicating that all the treatments demonstrated migration-inhibitory effects on MCF-7 cells. At 24 hours of incubation, untreated cells and cells treated with cisplatin and MG alone were completely closed. Interestingly, only cells treated with CIS-MG were not completely closed with 73.74 ± 3.48% of wound closure. This finding indicated that CIS-MG has a higher ability in inhibiting and slowing MCF-7 cell migration.

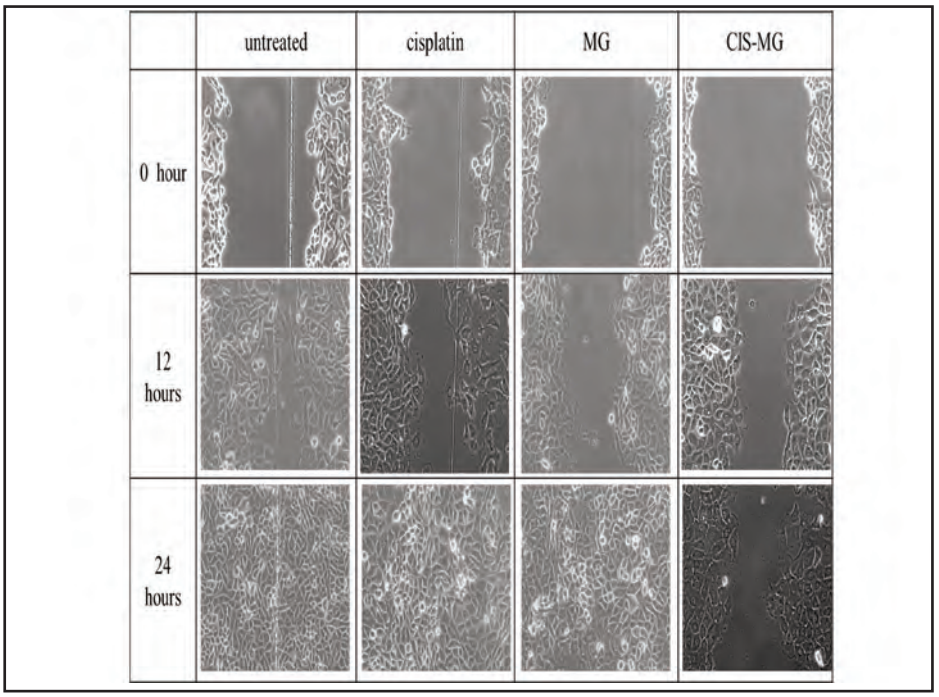

Fig. 3: The effect of single and combination treatments of MG with CIS on MCF-7 cell migration. The wound area of the cells was photographed at 0, 12 and 24 hours (40x original magnification). The wound area at 24 h for untreated, cisplatin and MG showed complete closure compared to cells treated with CIS-MG

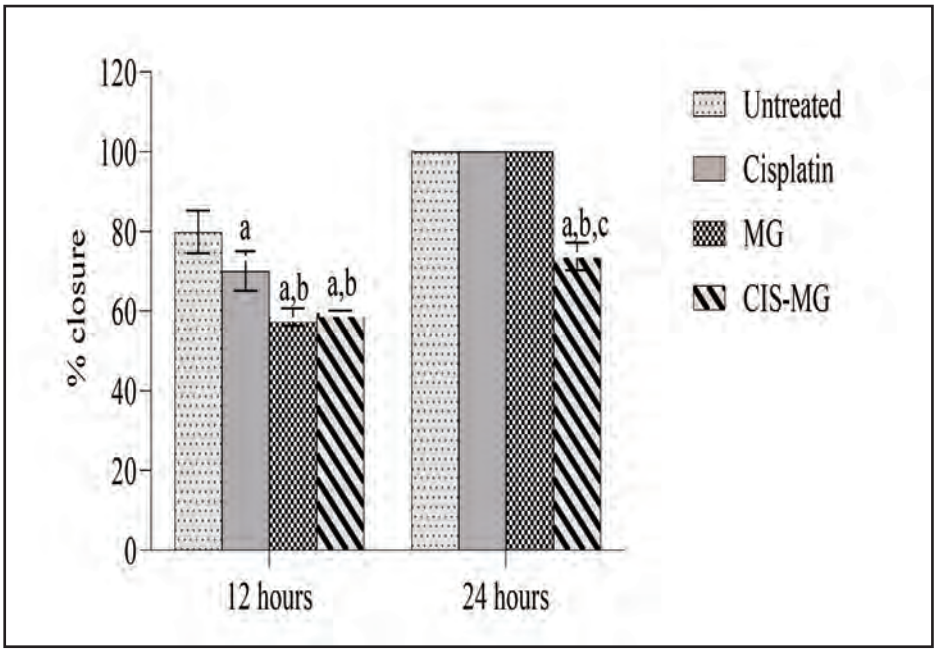

Fig. 4: Relative wound closure after 24 hours of incubation treatment. Results are presented as mean ± SD from three separate experiments. a: p<0.05 as compared to the untreated group, b: p<0.05 compared to cisplatin, c: p<0.05 compared to MG alone

DISCUSSION

Antiproliferative effect of Methyl Gallate on MCF-7 cells

Breast cancer is the most frequently diagnosed cancer and the second leading cause of cancer-related deaths among women. The rising number of cases highlights the urgent need for more effective treatments. Many conventional chemotherapeutic drugs face challenges such as drug resistance, cancer relapse, and significant side effects. Consequently, researchers are increasingly exploring natural compounds for the treatment and prevention of breast cancer. These natural compounds, derived from living organisms, can promote apoptosis and inhibit metastasis, thereby preventing cancer growth. Therefore, they have the potential to suppress cancer progression, improve patient survival rates, and reduce the number of deaths associated with breast cancer.[13]

MG, a natural phenolic compound, commonly found in plants such as maple leaves and *Camellia sinensis*[14] demonstrates various biological activities. These include antioxidant, anti-inflammatory, antimicrobial, and antitumor properties.[15] Based on the MTT assay results of cell viability, it was observed that both MG and cisplatin inhibited the proliferation of MCF-7 cells. Cell viability decreased in a dose-dependent manner compared to the untreated group, demonstrating a positive antiproliferative effect on MCF-7 cells. Both MG and cisplatin exhibited low IC_{50} values indicating their efficiency in inhibiting proliferation of MCF-7 cells. Despite its effectiveness on cancer cells, cisplatin presents several toxicity issues as it causes harm to normal cells. However, MG was shown to have cytoselective effects on cancer cells as it was reported to exhibit low cytotoxic effects on HaCat normal skin and Vero cells[16] and MDCK cells.[17]

The antiproliferative activity of MG on other cells has been reported previously. Treatment with MG on HeLa cells after 72 hours demonstrated a good cytotoxicity effect with IC_{50} values of 11.00 ± 0.58 µg/mL.[16] Similarly, a study by Mamat et al. showed the same antiproliferative effect of MG and cisplatin with IC_{50} values of 16.55 µg/mL.[18] MG, a major compound in *Syzygium coriaceum* leaf extract inhibited the proliferation of human hepatocellular carcinoma cells (HepG2) with an IC_{50} value of 24.2 ± 2.8 µg/mL.[19] On the other hand, another study found that MG significantly reduced the proliferation of Hep3B, Mahlavu, and HepJ5 Hepatocellular carcinoma (HCC) cells in a dose-dependent manner. The 50% inhibitory concentrations were >40 µg/ml for Hep3B, ~40 µg/ml for Mahlavu, and ~20 µg/ml for HepJ5 at 48 hours, indicating significant inhibition of cells.[17] Moreover, a study on melanoma mouse models and B16F10 cells also demonstrated the antitumor effect of MG.[20]

Combination effect of Cisplatin and Methyl gallate

Conventional cancer therapy offers clear benefits, yet despite the development of new anticancer agents, chemotherapy often has limitations including chemotherapy-induced toxicity, adverse reactions, inadequate target specificity, and, most critically, drug resistance during cancer progression. Combination therapy has emerged as a promising strategy for addressing these issues. By simultaneously delivering two or more therapeutic agents, such as chemotherapeutic drugs and phytochemical compounds, different signaling pathways in cancer cells can be targeted, leading to synergistic effects, improved targeting specificity, enhanced therapeutic outcomes, and overcoming multidrug resistance (MDR).[21] Due to the minimal side effects of natural compounds and their availability, there is a growing interest in pursuing combination therapy as a new strategy.[22]

In this study, the combination effects of cisplatin and MG on MCF-7 cells varied according to the different concentrations of cisplatin, the combination indexes were identified as synergistic and antagonistic reactions. The lowest concentration of cisplatin at 0.84 µg/mL, combined with MG in this study presents a CI value of less than 1 indicating a synergistic effect. This low CI value provided evidence that MG can enhance the cytotoxicity effects of cisplatin, even at the lowest concentration. Hence, this synergistic interaction shows that the combination treatment is more effective in killing cancer cells or inhibiting their cell growth compared to drug alone. This obtained result may suggested that when the drugs work better together, only lower doses of each drug are required to achieve the desired therapeutic effect and the risk of toxicity associated with higher doses of chemotherapy can be reduced. Hence, the combination of MG with lowest concentration of cisplatin has been used for further assays.

In a study conducted on cervical cancer cells (HeLa), increased inhibition of the cells was observed when combining MG with cisplatin, indicating that this combination can enhance cisplatin's efficacy. CI values of less than 1 suggest that MG works synergistically with cisplatin to inhibit HeLa cell proliferation. These combinations may cause DNA damage and activate the intra-S-phase checkpoint, which inhibits DNA synthesis and results in apoptosis or cellular senescence.[18] Moreover, MG has demonstrated synergistic interaction and stronger anti-cancer effects on EL4 murine lymphoma in combination with cisplatin compared to MG or cisplatin alone. The study suggests that MG inhibited Treg cell migration, thus enhancing the antitumor immune response and also caused no renal toxicity even in combination with cisplatin.[23]

Morphological observation of apoptotic cells (AO/PI staining)

Apoptosis, or programmed cell death, is an active physiological process resulting in cellular self-destruction. In cancer therapy, inducing apoptosis in cancer cells is a crucial strategy to eliminate malignant cells and prevent tumor progression effectively. Apoptosis can be initiated through two main pathways: the intrinsic and extrinsic pathways. The intrinsic pathway involves the release of cytochrome c from mitochondria, leading to the formation of the apoptosome and activation of caspases that execute cell death. The extrinsic pathway involves the formation of the death-inducing signaling complex (DISC) and the activation of initiator caspases, which also lead to apoptosis.[24] Apoptotic cell death is typically marked by chromatin condensation, DNA fragmentation, cell shrinkage, membrane blebbing, and detachment from the extracellular matrix.[25]

AO/PI staining was performed to evaluate cell nuclei and demonstration of apoptosis after treatment with the specific compounds. This dual staining technique is essential for distinguishing between live, apoptotic, and necrotic cells based on their membrane integrity and chromatin condensation. AO, a nucleic acid-binding dye, stains all nucleated cells green, allowing for the visualization of both live and apoptotic cells. PI, on the other hand, penetrates only cells with compromised membranes, which are characteristic of late apoptotic or necrotic cells, staining them red.[26]

In this study, there were various apoptotic characteristics in MCF-7 cells after being treated with single and combination treatments compared to untreated cells. These treated cells displayed several morphological changes such as nuclear irregularities and fragmentation, loss of cellular membrane integrity, nuclear condensation, damaged cell membranes and membrane blebbing indicated by green and orange staining. While the untreated cells remain healthy, viable

cells with a green, intact nuclear structure. These results suggested that the combination of cisplatin and MG may demonstrate its cytotoxicity effect on MCF-7 cells through the induction of apoptosis.

The apoptosis induction of MG has been reported previously on various cancer cell lines. Treatment with MG has induced apoptosis in human epidermoid carcinoma (A431) skin cancer cells by upregulating caspase-3 and upregulating bcl-2 expressions.[27] Another study by Abdullah et al. revealed that MG significantly inhibited the growth of HeLa cells by inducing apoptosis through upregulation of p53 and Bax, while Bcl-2 was significantly downregulated.[16] Moreover, MG also triggered apoptosis in B16F10 cells by elevating the expression of cleaved caspase-3.[20]

Anti-migratory effect of combination treatment on MCF-7 cells

One of the main causes of morbidity and mortality in solid cancer is metastases. The migration of cancer cells is one of the most important contributors to cancer metastasis. Therefore, inhibiting migration is an important approach for preventing or slowing cancer metastases.[28] Therefore, anti-cancer medications should be able to prevent cancer cells from penetrating the extracellular matrix (ECM) and developing into secondary tumors. Since these processes differ, it is crucial to differentiate between antiproliferative approaches and those that target motility, migration, invasion, and metastasis when concentrating on drug development efforts.[29]

The wound healing assay was conducted in this study to evaluate the migration inhibitory effect of single and combination treatments on MCF-7 cells. This study showed that the migration of MCF-7 cells was inhibited by single and combination treatments. Untreated MCF-7 cells, as expected, showed rapid cell migration, with high percentages of wound closure at 12 hours and complete closure within 24 hours. This result is consistent with previous reports, indicating that MCF-7 cells possess a very high migratory capacity, which forms the basis of their invasive and metastatic potential.[30]

In contrast, wound closure at 12 hours in cells treated with cisplatin, MG, and CIS-MG were much lower indicating their inhibitory effects on MCF-7 cell migration. Interestingly, CIS-MG demonstrated the most prolonged wound area for 12 and 24 hours. The combination treatment showed a greater inhibition effect on MCF-7 cell migration than the single treatment, indicating its synergistic potential. This finding aligns with previous studies that demonstrated the migration and invasion inhibitory on other cancer cell lines. MG has been reported to inhibit the migration and invasion of migration of hepatocellular carcinoma cells (BEL-7402) invitro and in vivo through the AMPK/NF-κB signaling pathway.[31]

CONCLUSION

In conclusion, MG demonstrated growth inhibition on breast cancer (MCF-7) cells with an IC_{50} value of 20.21 µg/mL. Moreover, a greater inhibition was observed when MG was combined with cisplatin. MG combined with the lowest concentration of cisplatin synergistically inhibited the growth of MCF-7 cells. Cells treated with combination treatment exhibited several apoptotic features thus suggesting its apoptosis-inducing effect. Furthermore, the combination also demonstrated anti-migratory effect on MCF-7 cells. This discovery might suggest that MG has potential as a natural source candidate to be combined with cisplatin in developing a novel therapeutic medication for breast cancer treatment. The findings may provide the basis for further studies in elucidating the underlying mechanism of synergistic reaction of the combinations on MCF-7 cells.

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