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Effects of Taxol and Rosmarinic Acid on MCF-7 Breast Cancer Cells

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Abstract
Breast cancer is one of the leading causes of cancer-related deaths among women worldwide. This study investigates the antiproliferative effects of Taxol, a standard chemotherapeutic agent, and rosmarinic acid (RA), a natural polyphenolic compound, on MCF-7 human breast cancer cells. Cell viability was assessed using the Alamar Blue assay following 24 hours of treatment. Taxol exhibited a potent cytotoxic effect with an IC_{50} of 8.620 nM, while RA showed moderate cytotoxicity with an IC_{50} of 59.04 µM. Morphological observation under light microscopy revealed that RA induced cellular elongation and structural alterations suggestive of apoptosis, whereas taxol treatment led to mitotic arrest and characteristic apoptotic features. The mechanisms of action are attributed to Taxol's microtubule stabilization and induction of apoptosis through p53 activation and ROS generation, while RA likely modulates oxidative stress and interferes with tumour-promoting signalling pathways. These findings indicate that both agents demonstrate significant antiproliferative effects, with RA showing potential as a complementary therapeutic due to its natural origin and reduced toxicity.
Keywords: Breast cancer; taxol; rosmarinic acid; MCF-7 cells

Introduction

Breast cancer is one of the most commonly diagnosed cancers and a leading cause of cancer-related mortality among women worldwide. In Malaysia, it ranks among the top causes of death in both private and government hospitals (Ministry of Health Malaysia, 2023). Globally, breast cancer accounted for approximately 670,000 deaths in 2022 (WHO, 2024). It is a heterogeneous disease comprising multiple subtypes, with luminal A being the most prevalent, representing 56.1% of diagnosed cases (See et al., 2023). This subtype is characterized by strong hormone receptor expression (ER and PR), negative HER2 status, and a low Ki-67 proliferation index (Li & Ma, 2020). Risk factors include age, hormonal exposure, reproductive history, and genetic predisposition, such as BRCA1 and BRCA2 mutations (Momenimovahed & Salehiniya, 2019; Venkitaraman, 2019).

Current treatment options for breast cancer include surgery, chemotherapy, hormone therapy, radiotherapy, and targeted therapy (Tan et al., 2020). Despite advancements, patients with metastatic breast cancer often face poor outcomes due to chemoresistance and adverse drug effects (Burguin et al., 2021). Taxol (paclitaxel) is a widely used synthetic chemotherapeutic drug known to induce apoptosis by stabilizing microtubules and arresting cell division (Sousa-Pimenta et al., 2023). However, long-term use of Taxol may lead to resistance and side effects such as hair loss, joint pain, and muscle pain (Mehraj et al., 2021). As an alternative, plant-based compounds like rosmarinic acid (RA), a natural polyphenol with antioxidant, anti-inflammatory, and antiproliferative properties, are being explored. RA has shown promising effects in reducing the viability, proliferation, migration, and invasion of cancer cells (Messeha et al., 2020; Huang et al., 2021).

Although Taxol is an effective chemotherapeutic agent, resistance to it remains a significant challenge, especially in luminal A breast cancer, which shows limited response to paclitaxel and doxorubicin (Cardoso et al., 2023). Furthermore, Taxol's toxicity can severely affect patients' quality of life. On the other hand, despite RA's known anticancer effects, there are limited studies directly comparing its cytotoxic and morphological effects with Taxol on luminal A breast cancer cells, such as MCF-7. Additionally, the pharmacological potential of many plant-derived compounds remains

underexplored (Yuan et al., 2023). Therefore, a comparative study between these two agents is necessary to explore RA's potential as a complementary or alternative therapeutic approach in breast cancer treatment.

The objective of this study is to evaluate the effects of Taxol and rosmarinic acid on MCF-7 breast cancer cells.

Materials and methods

MCF-7 human breast cancer cells were first cultured using a complete medium prepared with DMEM supplemented with L-glutamine, 10% fetal bovine serum (FBS), and 1% antibiotic solution containing penicillin (100 U/mL) and streptomycin (100 µg/mL). The cells were maintained in T25 flasks at 37°C in a humidified atmosphere with 5% CO_2 until they reached 70–80% confluency. Regular subculturing was done to maintain cell health and optimal growth conditions.

To prepare the drug treatments, Taxol and rosmarinic acid (RA) were obtained in powder form and dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions. For Taxol, a main stock solution was first prepared by dissolving 20mg of Taxol in DMSO. For RA, 20 mg of powder was dissolved in DMSO and then further diluted to the main stock. Stock concentration of Taxol was 1µM while RA was 5mM. The stock solutions were diluted into various concentrations by using complete media. The treatment concentration of Taxol ranged from 0-20nM with an interval of 2.5nM. The treatment concentration of RA ranged from 0 to 100 µM with an interval of 10 µM. These stock solutions were covered with aluminum foil to protect them from light and were stored at -80°C. This helped to prevent phytochemical decomposition for the stock solutions prepared.

To assess cell viability, the Alamar Blue assay was employed, ensuring each sample was tested in triplicate for reliable results. MCF-7 cells were harvested using trypsin, and a small aliquot (100µL) was counted using a hemocytometer to achieve a concentration of 5×10^3 cells per 100µL. These cells were then seeded into a 96-well plate and incubated for 24 hours. Following incubation, varying concentrations of taxol (0-20nM with an interval of 2.5nM) and rosmarinic acid (0-100µM with an interval of 10µM) were added to individual wells. After an additional 24-hour incubation period, 20 µL of alamarBlue Cell Viability Reagent was added to each well and incubated for an additional 4 hours at 37°C. To measure cell viability, the reduced resazurin dye was quantified using a Synergy HTX Multi-Mode microplate reader set to excite at 570nm. This method allowed for an accurate assessment of metabolic activity, reflecting cell viability based on the fluorescence intensity of the reduced dye.

To observe morphological changes, treated cells were examined under a light microscope. Images were captured at 10x and 20x magnifications, focusing on characteristics such as cell rounding, elongation, and neural-like appearances.

Statistical analysis was conducted using Microsoft Excel and GraphPad Prism 7 software. The objective was to compare cell viability between control samples and those treated with Taxol and rosmarinic acid (RA). A one-way ANOVA distribution was used to assess the significance of differences in cell viability between the control group and each treatment group (Taxol and RA). This statistical approach helped determine whether observed changes in cell viability due to treatment were statistically significant compared to untreated controls. The results were presented in the format of mean $\pm$ SD for cell viability measurements across all experimental conditions. The significance was indicated by a p-value $\leqslant 0.05$, indicating statistical significance.

Results and discussion

A set of taxol treatments ranging from 0 to 20 nM was carried out. According to Figure 1, the percentage of viable MCF-7 cells decreased from 100% to 85%, 75%, 60%, 50%, 30%, 20%, 10% and 5% as the concentration of Taxol increased from 0nM to 20nM, respectively.

The graph in Figure 2 depicts the relationship between Taxol concentration and cell viability. The calculated IC_{50} (half-maximal inhibitory concentration) concentration of Taxol achieved is approximately 8.620nM, indicating the concentration of Taxol required to reduce cell viability by 50%. This value reflects the ability of Taxol in inhibiting the growth of MCF-7 cells. The steep slope of the curve suggests

that small changes in taxol concentration can result in significant changes in cell viability, highlighting the agent's potency.

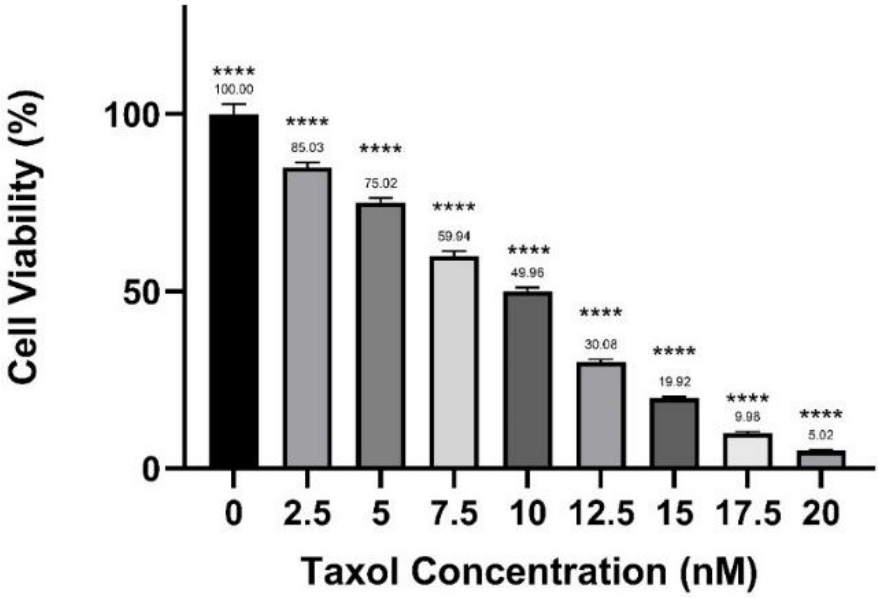

Figure 1 Effect of Taxol on MCF-7 cell viability, varying Taxol concentration (nM) after 24 hours of incubation. The graph above is a result of three sets of experimental data, each conducted in triplicate. The levels of statistical significance are as follows: ****P≤0.0001

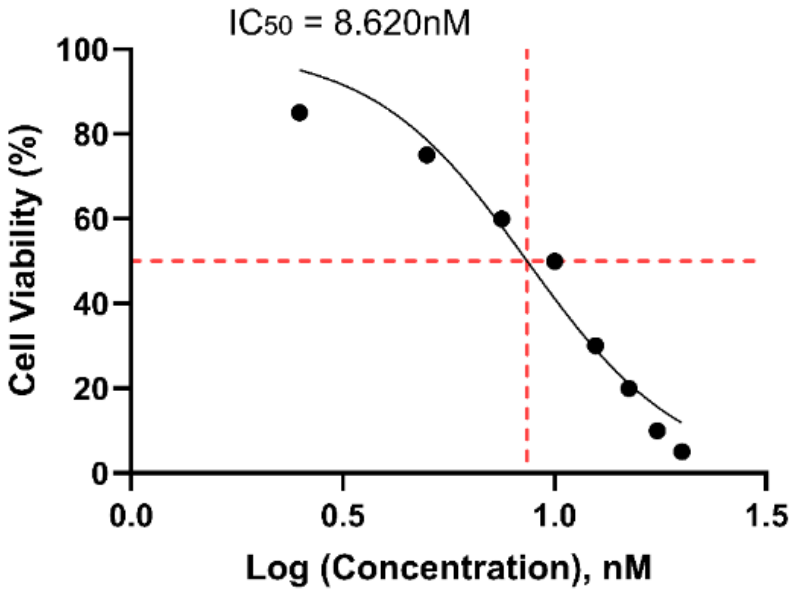

Figure 2 Graph of cell viability (%) by Log (Taxol concentration) (nM)

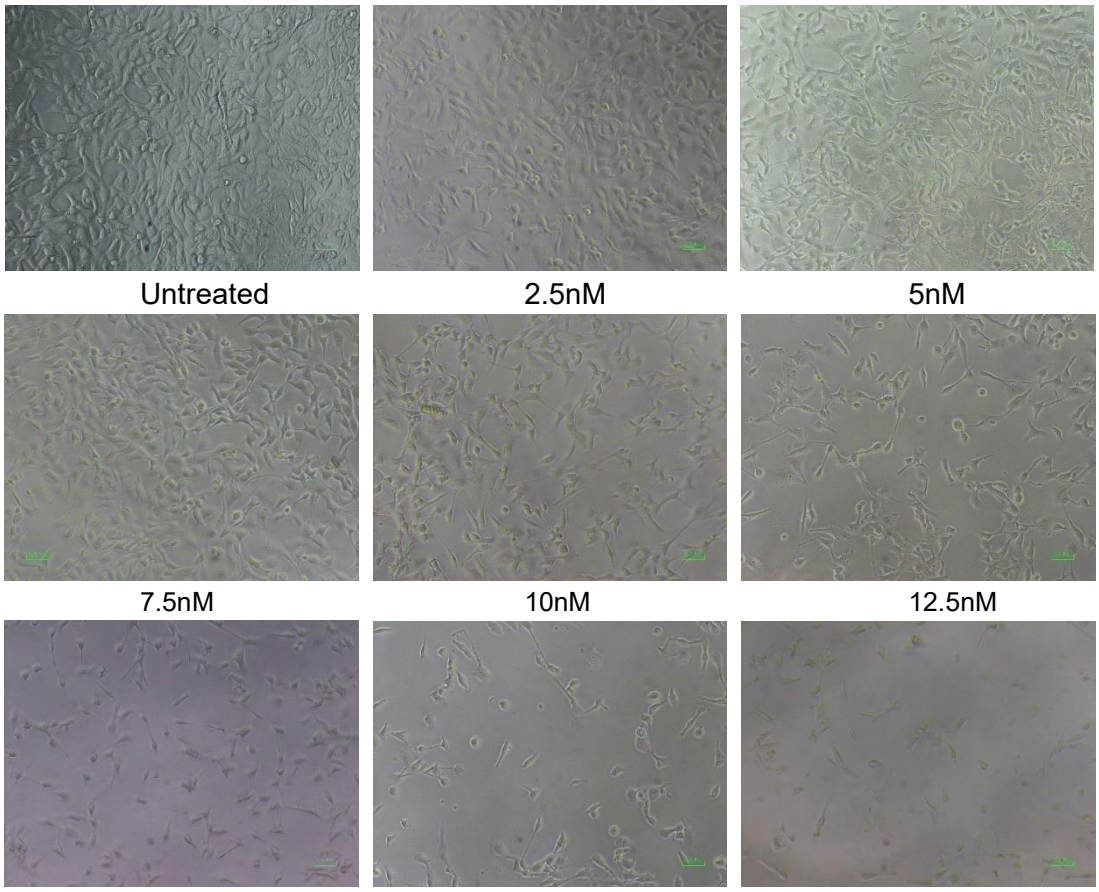

Figure 3 Microscopy images (10x) of untreated and 24-hour Taxol-treated MCF-7 cells at various concentrations

The microscopy image shown in Figure 4.3 illustrates the reduction in viable cells, which correlates with the cell viability percentage shown in Figure 4.1. The percentage of viable MCF-7 cells decreased from 100% to 85%, 75%, 60%, 50%, 30%, 20%, 10% and 5%, corresponding to the Taxol concentrations. In summary, as Taxol concentration increased, the number of viable cells decreased. Figure 3 shows that the untreated MCF-7 cells exhibited an epithelial-like cell shape with a distinct cell membrane and approximately 100% confluency. However, morphological changes were also noted as the concentration increased, which appeared elongated and rounded, indicative of potential apoptotic characteristics (Abdelaziz et al., 2022). Most cells showed neural-like morphology, suggesting they were unhealthy. The gaps between cells increased as the concentration of Taxol increased. Some of the cells appeared viable but showed reduced proliferative capacity.

Microscopic analysis showed that MCF-7 cells underwent noticeable morphological changes with increasing concentrations of Taxol, supporting its apoptotic effect. A higher number of cells appeared rounded at higher doses, a typical sign of apoptosis (Abdelaziz et al., 2022). This suggests cytoskeletal disruption and detachment from the surface (Hevia & Fanarraga, 2020). Some cells also showed elongated or neural-like shapes, likely due to Taxol's interference with normal microtubule structure (Vennila et al., 2012). In addition, greater gaps between cells and reduced confluency were observed, indicating lower cell-to-cell contact and reduced proliferation. These changes align with Taxol's known mechanism of stabilizing microtubules, which blocks cell division and leads to arrest at the G2/M phase, ultimately triggering apoptosis (Vennila et al., 2012). Together, the reduction in viability and the altered cell morphology confirm that Taxol induces apoptosis in MCF-7 cells in a dose-dependent manner.

Another set of rosmarinic acid treatments, ranging from 0 to 100 μM, was carried out. The cytotoxic effect of rosmarinic Acid on MCF-7 breast cancer cells was evaluated using the Alamar Blue

assay after 24 hours of treatment. As illustrated in Figure 4, RA significantly reduced cell viability in a dose-dependent manner across the concentration range of 0 to 100μM. As the concentration of rosmarinic acid increased, the percentage of viable cell decreased from 100% to 90%, 95%, 70%, 65%, 60%, 50%, 40%, 20% 10% and 7%.

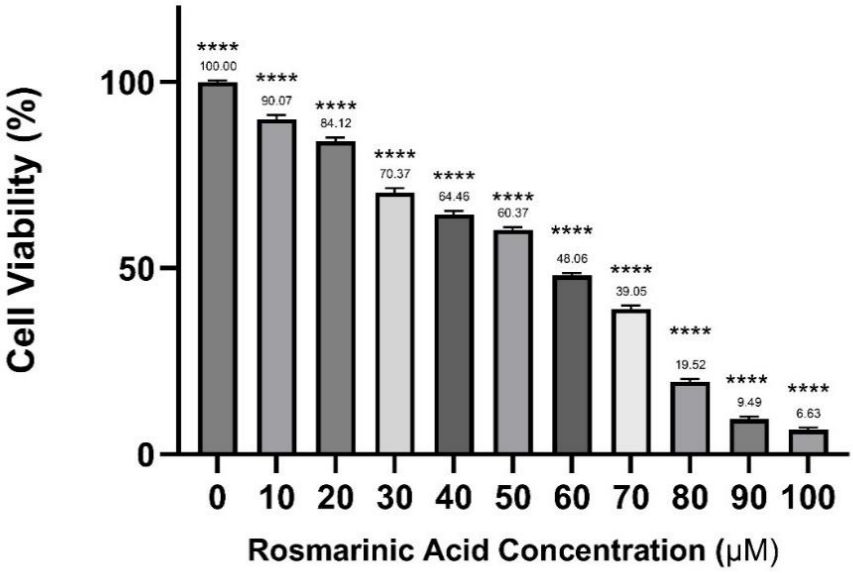

Figure 4 Effect of rosmarinic acid on MCF-7 cell viability, varying rosmarinic acid concentration (μM) after 24 hours of incubation. The graph above represents the results of three sets of experimental data, each conducted in triplicate. The levels of statistical significance are as follows: ****P ⩽ 0.0001

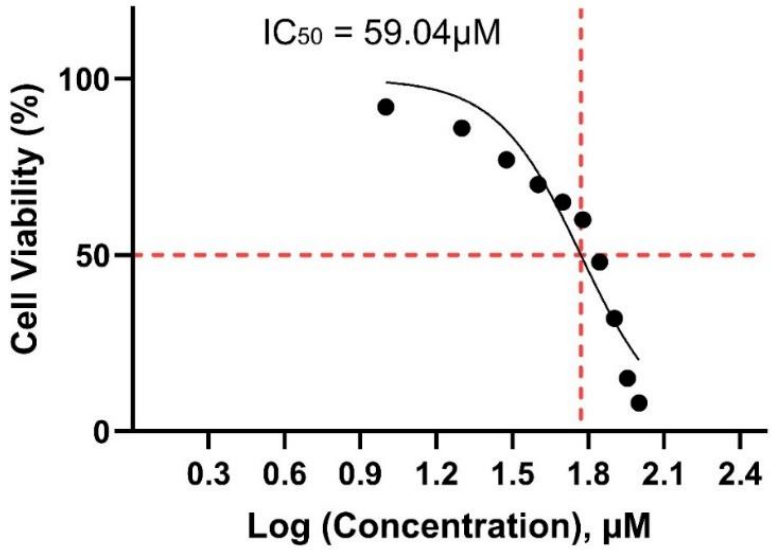

Figure 5 Graph of cell viability (%) by Log (Rosmarinic Acid concentration) (μM)

From the fitted curve in Figure 5, the IC_{50} value of rosmarinic acid was calculated to be 59.04 μM, indicating the concentration at which rosmarinic acid reduced MCF-7 cell viability by 50%. This IC_{50} value is consistent with the dose-dependent decrease in viability observed in the graph shown in Figure 4.

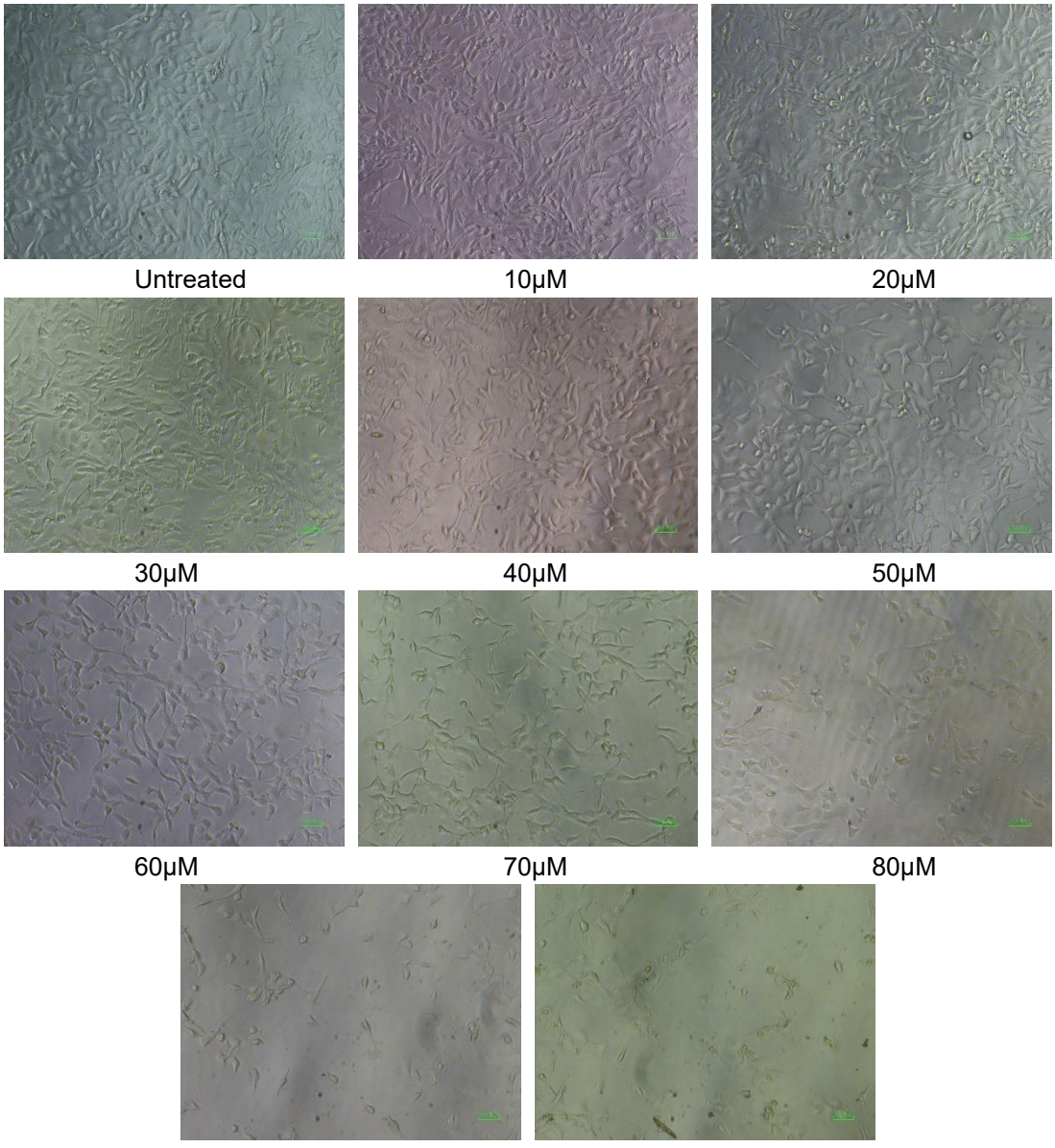

Figure 6 Microscopy images (10x) of untreated and 24 hours rosmarinic acid-treated MCF-7 cells at various concentrations

The morphology of MCF-7 cells was observed under an inverted microscope. As shown in Figure 6, cells treated with rosmarinic acid appeared rounded. However, most cells maintained an elongated morphology, especially at 60μM. This elongation may suggest cellular stress (Liu et al., 2021). A study by Berdowska et al. (2013) demonstrated a relatively high cytotoxic effect of RA on MCF-7 cells, which may be attributed to its chemical structure. RA possesses four free hydroxyl groups and two carbonyl functions. These functional groups are capable of producing reactive oxygen species (ROS), such as hydroxyl radicals (Berdowska et al., 2013). These radicals are capable of damaging cellular components, contributing to cell death. At 100μM, the round and floating cells were clearly observed, indicating apoptotic bodies. The detachment and reduced confluency characteristics commonly indicate apoptosis (Rezano et al., 2021). Moreover, the morphological changes appeared to correlate with increasing concentrations of rosmarinic acid. Compared to untreated cells, which maintained a typical adherent and epithelial-like shape, RA-treated cells exhibited progressive signs of damage at concentrations above 60 μM. At high concentrations of RA-treated cells, the observable rounded cells and reduction in cell viability may be caused by a toxic level of ROS that induces oxidative stress in MCF-7 cells (Zhao et al., 2023).

Rosmarinic acid has been shown to reduce the expression of the MDM2 gene (Juskowiak et al., 2018), a gene that encodes a protein capable of regulating the tumour suppressor p53. When p53 levels rise, it can stimulate the death of cancer cells. In breast cancer, overexpression of MDM2 leads to the degradation of p53 (tumour suppressor gene). With the low level of p53, cancer cells resist cell apoptosis. In addition, hypermethylation in cancer leads to the development of cancer cells by silencing the tumour suppressor gene. Up to 88% of DNA methyltransferase activity is inhibited by rosmarinic acid (Shumaila et al., 2023). This could aid in suppressing the tumour cells and reactivating the tumour suppressor gene that has been inactive.

Conclusion

This study investigated the antiproliferative effects of taxol and rosmarinic acid (RA) on MCF-7 human breast cancer cells. Both compounds significantly reduced cell viability in a dose-dependent manner, with taxol exhibiting strong cytotoxicity and RA showing moderate effects. Taxol's mechanism involves mitotic arrest and apoptosis via microtubule stabilization, while RA, as a natural polyphenol, likely acts through antioxidant activity and modulation of tumor-related signaling pathways. Microscopic examination further confirmed morphological changes associated with apoptosis in treated cells. Although taxol was more potent, RA demonstrated promising potential as a naturally derived compound with lower toxicity. These findings suggest the value of exploring RA as a complementary or alternative therapeutic agent in breast cancer treatment, contributing to the development of more effective and safer strategies.

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