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Original Article

Alpha-mangostin and nab-paclitaxel in breast cancer cell models: improved antitumor efficacy through combination therapy

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Abstract

In the pursuit of more effective breast cancer therapies, the investigation of interactions between novel compounds and established chemotherapeutics has become increasingly important. This study investigates the combinatory effects of alpha-mangostin (α-MG) and nab-paclitaxel on MCF-7 and MDA-MB-231 cell lines, utilizing the xCELLigence RTCA system for continuous real-time cellular analysis, BrdU incorporation assays for proliferation assessment, and the quantification of mitotic activity and caspase-3/7 levels to elucidate apoptotic mechanisms. Our findings demonstrate that both α-MG and nab-paclitaxel independently induce significant inhibition of cellular proliferation and modulate cell cycle dynamics over a 24 to 72-hour period. Notably, when combined, these agents exhibit a pronounced enhancement of cell cycle inhibition and apoptosis, surpassing the effects observed with monotherapy. This potentiation effect suggests that α-MG augments the therapeutic efficacy of nab-paclitaxel, potentially allowing for reduced dosages in clinical applications. The study underscores the potential of α-MG as an adjuvant in breast cancer treatment, offering a promising strategy to optimize therapeutic regimens, minimize adverse effects, and improve patient outcomes in clinical oncology.

Keywords: MCF-7, Nab-paclitaxel, MDA-MB-231, Alpha-Mangostin, Cell Line, Breast cancer, Therapeutic optimization.

1. Introduction

Breast cancer ranks as the predominant malignancy diagnosed among women globally, with an approximate annual incidence of 2.3 million new cases leading to 685,000 deaths [1]. The structural heterogeneity of breast cancer may necessitate the utilization of diverse treatment approaches to achieve success and reduce the risk of recurrence. Despite the application of diverse approaches including surgery, radiotherapy, chemotherapy, endocrine therapy, and immunotherapy, addressing unintended side effects and overcoming drug resistance continues to pose significant challenges. Additionally, high mortality and recurrence rates persist as challenges in the realm of breast oncology [2].

The molecular classification of breast cancer closely parallels its clinical categorization. Breast tumors are genetically classified into main categories such as basal-like, Luminal-A, Luminal-B, human epidermal growth factor receptor 2 (HER2)-positive/enriched with HER2/overexpressing HER2 breast cancer, and normal-like tumors. These genetic subtypes often exhibit heterogeneity and carry significant implications for prognosis and treatment decisions. The Luminal (A and B) subtypes which are predominantly estrogen and progesterone receptor-positive and HER2-negative cells, account for the majority of breast tumors (90-95%). Variations in these subtypes influence individuals prognosis and treatment selection [3].

In cancer treatment, chemotherapy targets trigger apoptosis in cancer cells and restrict their growth by disrupting cellular structures and hindering cell migration. Instability in molecules forming extracellular connections also hinders cancer cell decrease and migration. Antimitotic agents disrupt intracellular microtubule conformation, leading cells toward apoptosis [4]. The apoptotic mechanism is one of the processes that become dysregulated in breast cancer. Apoptosis can occur through extrinsic pathways mediated by receptors or intrinsic pathways dependent on mitochondrial processes. In the intrinsic mechanism, Bcl-2-associated proteins function as regulators, leading to the translocation of cytochrome c into the cytosol. This event

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subsequently activates caspases, which drive the cell towards apoptosis [5].

Paclitaxel, a taxane commonly employed in breast cancer treatment, was first isolated from the bark of Taxus brevifolia in 1971 [6]. Taxanes are used clinically as chemotherapeutic agents in malignancies such as metastatic breast cancer, where they exert anti-mitotic effects by inhibiting microtubule depolymerization, thus stabilizing microtubules [7] and blocking cells in the G2 and M phases, resulting in cellular apoptosis. Furthermore, taxanes block the pro-survival effects of Bcl-2 family members and enhance TP53 gene activation [8]. The fact that paclitaxel is not water-soluble imposes some limitations on its use [9]. For clinical use, it has been combined with less hydrophobic compounds. However, this can lead to various adverse side effects [10]. Nab-paclitaxel, or Abraxane®, is an innovative formulation produced by combining paclitaxel with human serum albumin using high-pressure processing [11]. Compared to conventional paclitaxel, nab-paclitaxel demonstrates a lack of solvent-related toxicity such as hypersensitivity reactions and neutropenia, the elimination of the need for premedication with prophylactic steroids due to hypersensitivity reactions, and provides 33% more tumor penetration compared to regular paclitaxel and slower paclitaxel absorption [9,12]. Nonetheless, dose-dependent side effects of nab-paclitaxel such as neutropenia and peripheral neuropathy, remain concerns [12].

Alpha-Mangostin (α-MG), a xanthone compound derived from the fruit of Garcinia mangostana L., is noted for its wide-ranging pharmacological properties, including anti-inflammatory, antidiabetic, cardioprotective, antibacterial, antiallergic, antifungal, antioxidant, and anticancer activities, with high tolerability [13]. α-MG exerts its antiproliferative effects on cancer cells by engaging different targets and signaling pathways [14]. Specifically, in breast cancer cells, α-MG inhibits fatty acid synthase (FAS), reduces intracellular fatty acid levels, promotes apoptosis, blocks anti-apoptotic Bcl-2 proteins and enhances the tumor-suppressive activity of p53 by preventing its binding to MDM2. Additionally, α-MG suppresses the PI3K/Akt pathway, increases intracellular accumulation of antitumor drugs by inhibiting ABCG2, increases ROS levels within the cell and impairs mitochondrial integrity by inducing mitochondrial membrane potential disruption, enhancing Bax and cytochrome c release, and decreasing Bcl-2/Bcl-xL levels. This disruption leads to the induction of caspase-9 and caspase-3, ultimately triggering apoptosis [14,15,16].

In this study, the effects of nab-paclitaxel and α-MG on cellular kinetics, mitotic activity, cellular growth, and apoptotic processes were investigated in two distinct breast cancer cell lines. We aimed to assess the potential of α-MG to enhance the efficacy of nab-paclitaxel therapy and to indirectly reduce the adverse effects associated with breast cancer treatment through dose reduction in two different breast cancer cell line models. MCF-7 cells are estrogen receptor (ER)-positive, progesterone receptor (PR)-positive, and HER2-negative, making them suitable for modeling luminal A-type breast cancer. These cells are generally more sensitive to hormone therapies. On the other hand, MDA-MB-231 cells are triple-negative breast cancer (TNBC) cells, lacking expression of ER, PR, and HER2. These cells are more aggressive, resistant to treatment, and associated with poor prognosis, making them a valuable model for investigating new therapeutic strategies [17].

2. Materials and methods

2.1. Cell culture

The MDA-MB-231 metastatic breast carcinoma cell line and the MCF-7 human breast epithelial cell line derived from ductal carcinoma in situ were procured from the European Cell Culture Collection (CCL) for this investigation. Chemicals were procured from Sigma-Aldrich (St. Louis, MO, USA) unless specified differently. The cell cultures were prepared in Dulbecco's Modified Eagle Medium (DMEM, high glucose), supplemented with 10% fetal bovine serum (Gibco, USA), streptomycin (Ulugay, Türkiye), penicillin (Pfizer, USA), and amphotericin B at 37°C under 5% CO2 in an environment with controlled humidity. The pH of the cell culture media was adjusted to 7.2 with NaHCO3.

2.2. Dose usage

The αMG, with a purity greater than 98%, was purchased from Tocris Bioscience UK, while nab-paclitaxel, with a purity exceeding 99.18%, was obtained from Selleckchem. Alpha-MG (15 µM, 20 µM, and 25 µM) and nab-paclitaxel (5 nM, 10 nM, and 15 nM) were used to determine cell kinetics. The minimum dosage for nab-paclitaxel (5 nM) was selected for the combination dose experiments for both cell lines. In MCF-7 cells, 5 µM α-MG is used for combination dose experiments, while in MDA-MB-231 cells, 1 µM α-MG is used for combined dose experiments.

2.3. Cell index

The cytotoxic effects of nab-paclitaxel and α-MG were assessed using xCELLigence DP (Acea Biosciences, Inc.). Prior to measurement, 100 µl of cell suspension was dispensed into 16-well E-plates, each containing 100 µl of culture medium. The seeding densities were set at 10,000 cells/well for MCF-7 cell line and 5,000 cells/well for MDA-MB-231 cells. The E-plates were incubated at 25°C under aseptic conditions for 20 minutes before being transferred to the xCELLingence system. The cells were then sustained at 37°C with 5% CO2 and 100% relative humidity, and cell index measurements were recorded at 15-minute intervals. After overnight incubation, the drug treatments were applied, and subsequent observations were recorded at 24, 48, and 72-hour intervals.

2.4. BrdU labelling index (BrdU LI)

The BrdU labeling index was employed to evaluate cell division rate, proliferation, and the impact on the cell cycle. Experiments were conducted using nab-paclitaxel and α-MG at optimal combination concentrations, adhering to the protocol provided in the BrdU Cell Proliferation Assay Kit (cat. no. 2750; EMD Millipore).

2.5. Mitotic activity

Mitotic activity was assessed by seeding 20,000 cells per well in a 96-well cell culture plate, according to the protocol provided in the Mitotic Assay Kit (cat. no. 18021; Active Motif). The effect of nab-paclitaxel and α-MG, applied at optimal combination concentrations, on mitotic activity was subsequently evaluated.

2.6. Caspase activity

Caspase 3 and 7 activities were quantified using the CaspaTag Caspase 3, 7 In Situ Assay Kit (cat. no. APT403; EMD Millipore) following the kit instructions.

2.7. Statistical analysis

Cellular dynamics were evaluated across different experimental groups. Statistical analyses were performed using one-way ANOVA, followed by Dunnett's post-hoc test for subsequent comparisons. Cell proliferation indices were analyzed via the xCELLigence system with additional parameters assessed using Dunnett's test. All statistical analyses were validated through replication, with experiments conducted in triplicate. Statistical computations were made using SPSS Statistics software (version 22.0; IBM), with a significance level set at $P < 0.05$.

3. Results

3.1. Cell index analysis

Real-time monitoring of cell index values demonstrated that the combination of nab-paclitaxel and α-MG significantly reduced cell proliferation and induced noticeable alterations in cellular morphology compared to the control groups in both cell lineages.

In Figure 1-A, the cell index of MCF-7 cells is depicted for approximately 90 hours. A comparison is made between the control group and three different concentrations of α-MG (15 µM, 20 µM, and 25 µM). Time is measured in hours, ranging from 20 to 90 hours. The cell index serves to evaluate cell proliferation, adhesion, and morphological changes. α-MG inhibits the proliferation of MCF-7 cells in a manner correlated with dosage. Higher concentrations of α-Mangostin (20 µM and 25 µM) notably reduce the cell index, indicating a substantial impact on cell viability and proliferation. Particularly, the concentration of 25 µM demonstrates the most pronounced inhibitory effect, suggesting its potential efficacy in diminishing the viability of MCF-7 cells. In Figure 1 B, the following analysis examines the impact of varying concentrations of nab-paclitaxel (5 nM, 10 nM, and 15 nM) on the cell index of MCF-7 cells for approximately 100 hours. The results highlight the differential impacts of nab-paclitaxel on MCF-7 cells at various concentrations: At 5 nM, nab-paclitaxel initially induced DNA damage, leading to the cessation of proliferation, but did not result in cell death. The cells have potentially entered a state of growth arrest or are in a repair phase. 10 nM nab-paclitaxel exhibits pronounced antimitotic effects, preventing successful cell division and leading to a gradual decline in cell index, indicative of cell death or senescence over time. At 15 nM, nab-paclitaxel exhibited potent cytostatic effects, significantly inhibiting cell proliferation and resulting in a marked reduction in cell index. In Figure 1 C, Cell index plot illustrating MCF-7 cells subjected to combined dosages (5 nM nab-paclitaxel+1 µM α-MG, 5 nM nab-paclitaxel+5 µM α-MG, 5 nM nab-paclitaxel+10 µM α-MG). It was determined that all combined doses exhibited cytotoxic effects, and 5 nM nab-paclitaxel with 5 µM α-MG was selected as the appropriate dose for the MCF-7 cells.

In Figure 2 D, cell index graph of MDA-MB-231 cells treated with 15 µM, 20 µM and 25 µM α-MG (α-MG). 15 µM α-Mn was cytostatic, 20 µM αMn was antimitotic, and 25 µM αMn caused DNA damage. In Figure 2 E, Cell index chart depicting MDA-MB-231 cells exposed to 5 nM,

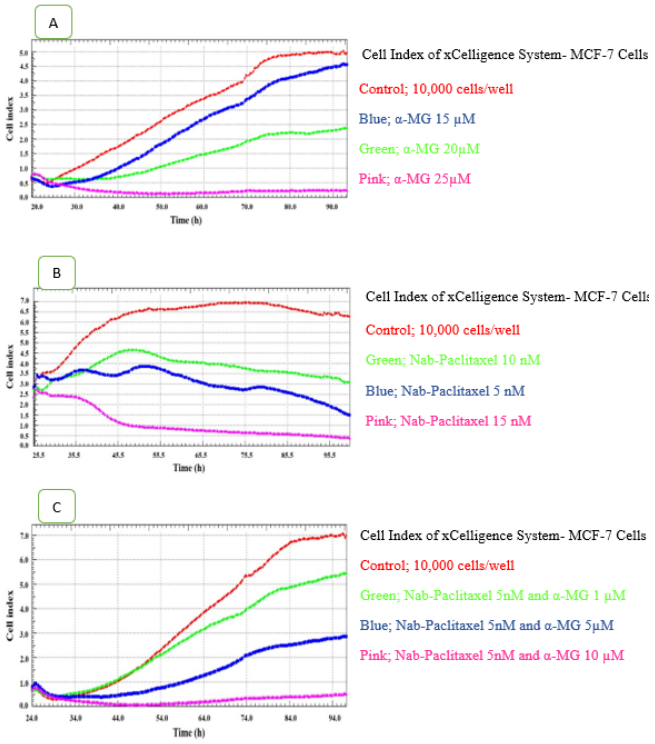

Fig. 1. A: Cell index graph of MCF-7 cells treated with α-MG at concentrations. All concentrations exhibited cytostatic effects (*p<0.05). B: Cell index graph of MCF-7 cells treated with nab-paclitaxel at concentrations 5 nM nab-paclitaxel (DNA damage); 10 nM nab-paclitaxel (Antimitotic); 15 nM nab-paclitaxel (Cytostatic) (*p<0.05). C: Cell index graph of MCF-7 cells treated with combined concentrations of nab-paclitaxel and α-MG. All combined treatments exhibited cytostatic effects (*p<0.05).

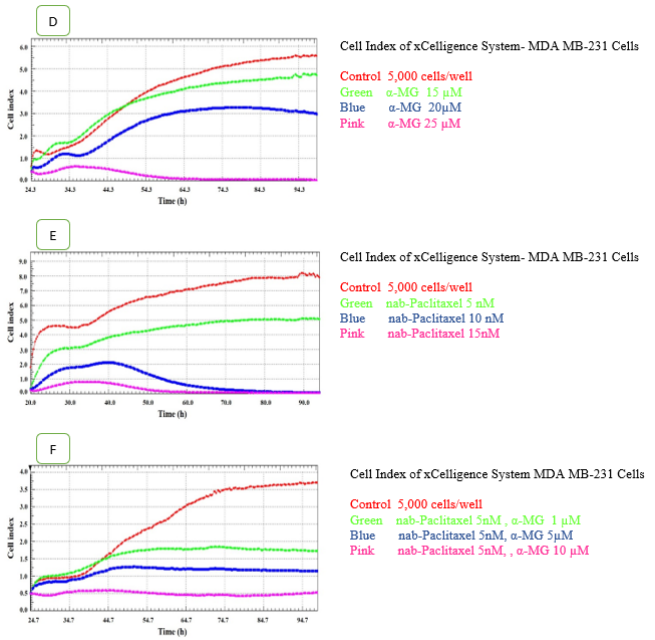

Fig. 2. D: Cell index graph of MDA-MB-231 cells treated with α-MG at concentrations. 15 µM α-MG (Cytostatic); 20 µM α-MG (Antimitotic);25 µM α-MG (DNA Damage). (*p<0.05) E: Cell index graph of MDA-MB-231 cells treated with nab-paclitaxel at concentrations. 5 nM nab-paclitaxel (Antimitotic); 10 nM nab-paclitaxel (Antimitotic); 15 nM nab-paclitaxel (Antimitotic). (*p<0.05) F: Cell index graph of MDA-MB-231 cells treated with combined doses of nab-paclitaxel and α-MG. 5 nM nab-paclitaxel + 1 µM α-MG (Cytostatic), 5 nM nab-paclitaxel + 5 µM α-MG (Cytostatic) and 5 nM nab-paclitaxel + 10 µM α-MG (Cytostatic) (*p<0.05).

10 nM and 15 nM nab-paclitaxel. All doses exhibited anti-mitotic effects. Figure 2 F represents the cell index chart depicting MDA-MB-231 cells exposed with combined concentrations. The doses of 1, 5, and 10 µM α-MG were tested in combination with 5 µM of nab-paclitaxel, and all doses exhibited cytostatic effects. It was determined that combined doses of 5 µM nab-paclitaxel with 1 µM α-MG were selected as the appropriate dose for the MDA-MB-231 cells.

3.2. BrdU labelling index

The BrdU incorporation rate exhibited a significant reduction in DNA synthesis in cells subjected to a combined treatment of nab-paclitaxel and α-MG, indicating a suppression of cellular proliferation. (Table 1. and Fig.3).

For MCF-7 cells, a combination treatment of 5 nM nab-paclitaxel (Abraxane) and 5 µM α-MG was administered, while MDA-MB-231 cells were exposed to 5 nM nab-paclitaxel (Abraxane) and 1 µM α-MG. The table presents the absorbance values corresponding to the BrdU labeling index, indicating DNA synthesis rates in response to the combination treatments for 0-72 hours. Statistical significance was determined with *p < 0.05.

3.3. Mitotic activity

Mitotic activity assays revealed a substantial decrease in the number of mitotic cells upon treatment with nab-paclitaxel and α-MG, indicating a significant disruption of the cell cycle.

Table 2 presents the absorbance values of MCF-7 cells for 72 hours, comparing the control group with those exposed to a combination of nab-paclitaxel and α-MG. The pairing of nab-paclitaxel and α-MG substantially reduced the viability of MCF-7 cells over time, as evidenced by lower absorbance values in the treated group relative to the controls. This reduction in absorbance was sustained and increased from 24 to 72 hours, suggesting that the combination therapy effectively inhibits cell proliferation and induces cell death.

These data highlight the potential of using a combination of nab-paclitaxel and α-MG as an effective therapeutic strategy for reducing breast cancer cell viability.

Table 2 also displays the absorbance values of MDA-MB-231 cells over the same 72-hour period. The table compares the control group with the group administered a combination of nab-paclitaxel and α-MG. The treated

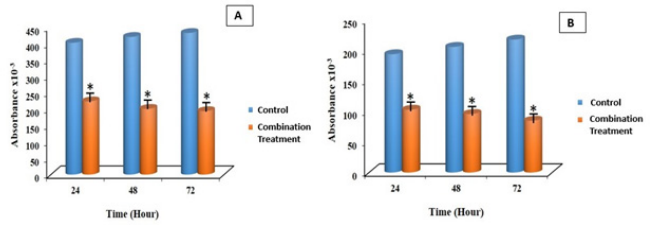

Fig. 3. Absorbance Values of MCF-7 (A) and MDA-MB-231 (B) Cells Following Combined Doses ($p < 0.05$). The blue bars represent the control groups, while the orange bars indicate cells treated with combined doses of 5nM Nab-paclitaxel (Abraxane) and 5 µM α-MG for MCF-7 cells, and 5nM Nab-paclitaxel (Abraxane) and 1 µM α-MG for MDA-MB 231 cells. Data highlight the effects of the combined treatments on DNA synthesis in both cell lines.

Table 1. BrdU Labeling Index Absorbance Values of MCF-7 and MDA-MB-231 Cells for Control and Combination Treatment Groups Over 0 to 72 hours (*p < 0.05).

Time (Hours)	Absorbance Values (450-655 nm) (MCF-7)	
	Controls	Combination Treatment (5 nM Abraxane + 5 µM α-Mangostin)
24	$403x10^{-3}$ ±0.002	$224x10^{-3*}$ ±0.002
48	$421 x10^{-3}$ ±0.003	$201x10^{-3*}$ ±0.002
72	$433 x10^{-3}$ ±0.004	$194x10^{-3*}$ ±0.003
Time (Hours)	**Absorbance Values (450-655 nm) (MDA-MB-231)**	
	Controls	**Combination Treatment (5 nM Abraxane + 1 µM α-Mangostin)**
24	$234x10^{-3}$ ±0.003	$117 x10^{-3*}$ ±0.002
48	$247x10^{-3}$ ±0.002	$109 x10^{-3*}$ ±0.003
72	$256x10^{3}$ ±0.003	$89 x10^{-3*}$ ±0.001

Table 2. Mitotic Activity Absorbance Values of MCF-7 and MDA-MB-231 Cells for Control and Combination Treatment Groups Over 0 to 72 hours (*p <0.05).

Time (Hour)	Absorbance Values (450-655 nm) (MCF-7)	
	Control	Combination Treatment (5 nM Abraxane+ 5 µM α-Mangostin)
24	$303 x10^{-3}$ ±0.002	$155x10^{-3*}$ ±0.002
48	$313 x10^{-3}$ ±0.004	$143x10^{-3*}$ ±0.003
72	$324 x10^{-3}$ ±0.003	$117 x10^{-3*}$ ±0.001
Time (Hour)	**Absorbance Values (450-655 nm) (MDA-MB-231)**	
	Control	**Combination Treatment (5 nM Abraxane+ 1 µM α-Mangostin)**
24	$194 x10^{-3}$ ±0.001	$103x10^{-3*}$ ±0.001
48	$206 x10^{-3}$ ±0.003	$96x10^{-3*}$ ±0.002
72	$218 x10^{-3}$ ±0.004	$85 x10^{-3*}$ ±0.001

group exhibited a sustained decrease in absorbance values over time relative to the control group, significantly reducing the viability of MDA-MB-231 cells. This reduction persisted from 24 to 72 hours, indicating that the combination treatment effectively suppresses cell proliferation and promotes apoptosis in the cells (Fig. 4).

3.4.Caspase activity

Caspase activity levels were investigated in cells exposed to the combined treatment of nab-paclitaxel and α-MG at various time points. Table 3 presents caspase activity in MCF-7 cells over 72 hours comparing the control group with the group receiving combined treatment. At 24 hours, caspase activity in the combination treatment group is significantly higher in comparison with the control group. This trend continued at 48 hours, where the combined treatment group exhibited further increased caspase activity, suggesting sustained apoptotic activity. By 72 hours, caspase activity reached its peak in the combination treatment group, showing the highest levels compared to earlier time points and the control group.

The accompanying bar chart displays caspase activity in MDA-MB-231 over the same 72-hour period. At 24 hours, the combination treatment group showed increased caspase activity relative to the control group. This elevation continued at 48 hours, where caspase activity further escalated in the combination treatment group indicating ongoing apoptotic signaling. By 72 hours, caspase activity peaks in the combination treatment group, surpassing both earlier time points and in controls.

The combination of nab-paclitaxel and α-MG significantly elevated caspase 3 and 7 activities, indicating enhanced induction of apoptosis. (Table 3). This sustained increase in caspase 3 and 7 activities from 24 to 72 hours highlighted the potential efficacy of the combination therapy in promoting cellular apoptosis in mammary carcinoma cells, as demonstrated across our cell lines (Figure 5).

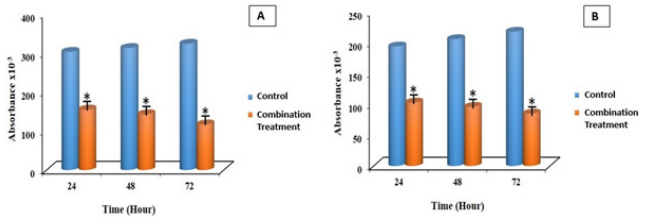

Fig. 4. Mitotic Activity Results: Absorbance Values of MCF-7 cells (A) and MDA-MB-231 cells (B) Treated with Combined Concentrations Over 24, 48 and 72 hours ($p < 0.05$). The blue bars represent the control group, and the orange bars represent the combined treatment group. For MCF-7 cells (A), the combined treatment consisted of 5nM Nab-paclitaxel (Abraxane) and 5 μM α-MG, and for MDA-MB-231 cells (B), the combination included 5nM Nab-paclitaxel (Abraxane) and 1 μM α-MG. Absorbance values, corresponding to mitotic activity, were measured at 24, 48 and 72 hours to assess the impact of the treatment on cell proliferation.

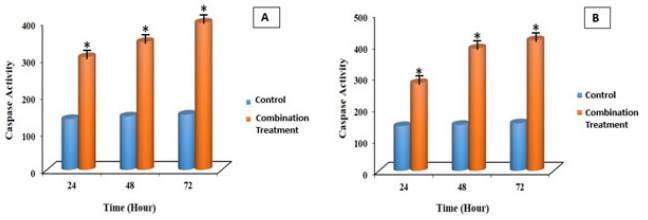

Fig. 5. Caspase 3-7 Activity Results: Fluorescence Values of MCF-7 (A) cells and MDA-MB-231 (B) Cells Treated with Combined Treatment Over 0-72 hours ($p < 0.05$). Panel A: the fluorescence values for MCF-7 cells treated with combined treatment of 5nM Nab-paclitaxel (Abraxane) and 5 μM α-Mangostin. Panel B: the fluorescence values for MDA-MB-231 cells treated with combined treatment of 5 nM nab-paclitaxel (Abraxane) and 1 μM α-MG. *$p < 0.05$.

4. Discussion

In breast cancer treatment, while radiological, chemotherapeutic, or surgical methods offer substantial benefits, they also present various adverse effects [18]. Therefore, the need for additional support in halting cancer progression, promoting healthy cellular development, or enhancing the effectiveness of chemotherapeutics used to combat drug resistance underscores the necessity to scientifically determine the efficacy of the adjunctive agents utilized.

In our study, we aimed to investigate the cell kinetics in MCF-7 and MDA-MB-231 cells when nab-paclitaxel, which is reported to cause fewer side effects compared to paclitaxel, is used in combination with α-MG. We also evaluated the activation of apoptotic pathways and analyzed cell cycle dynamics by determining BrdU (bromodeoxyuridine) incorporation rates and mitotic index values. BrdU is a nucleoside that integrates into DNA during DNA synthesis, indicating active cell proliferation. The mitotic index was analyzed in two different breast cancer cell

Table 3. Caspase 3-7 Activity Levels of MCF-7 and MDA-MB-231 Cells for Control and Combination Treatment Groups Over 0 to 72 Hours; Fluorescence Values at 24, 48, and 72 hours (*$p < 0.05$).

Time (Hour)	Fluorescence Values (450-655 nm) (MCF-7)	
	Control	Combination Treatment (5 nM Abraxane+ 5 μM α-Mangostin)
24	137 ±10	304* ±12
48	144±11	345* ±14
72	149 ±11	397* ±13
Time (Hour)	**Fluorescence Values (450-655 nm) (MDA-MB-231)**	
	Control	**Combination Treatment (5 nM Abraxane+ 1 μM α-Mangostin)**
24	142 ±11	279* ±14
48	146±12	389* ±13
72	151 ±11	415* ±13

lines treated with our combination doses. The increased mitotic index demonstrated that the nab-paclitaxel and α-MG combination affected the mitotic phase. In our experiments, we aimed to highlight the potential of reducing side effects and enhancing therapeutic efficacy by using nab-paclitaxel, a drug commonly used in advanced stages of breast cancer, in combination with α-MG. This combination might reduce side effects by allowing dose reduction due to the lower side-effect profile of nab-paclitaxel.

Based on the analysis of our results, the combination dose activates apoptotic pathways. The increase in the levels of apoptotic caspases such as caspase-3 and caspase-7 was a significant indicator of apoptosis activation. Caspase-3 and caspase-7 are key enzymes involved in the execution of cell death. Although the increase in caspase-3 and caspase-7 levels in our experiments suggests the activation of the apoptosis process, we emphasize that the molecular pathways involved in apoptosis mechanisms should be thoroughly analyzed for our combination treatment. Specifically, the expression levels of apoptosis markers such as Bax, p53, cytochrome c, PARP, and Bid, for both individual and combination treatments, as well as the analysis of healthy control cell lines, should be further evaluated.

In our experiments, the doses of α-MG and nab-paclitaxel used for the cell lines were as follows: MCF-7 cells: 5 nM nab-paclitaxel + 5 μM α-MG combination. MDA-MB-231 cells: 5 nM nab-paclitaxel + 1 μM α-MG combination. These doses were selected based on observations that they significantly affected cell viability, proliferation, and apoptosis, without causing excessive toxicity. In clinical settings, the standard dose of nab-paclitaxel is typically 260 mg/m² every 3 weeks [19], while the doses of α-MG used in experimental stages vary widely, generally ranging from 1–10 μM. The doses of α-MG and nab-paclitaxel chosen in our study, although lower than clinical doses, were effective in reducing cell proliferation and enhancing apoptosis in the in vitro setting. This suggests that lower combination doses may offer a promising option to reduce systemic toxicity in patients.

The current literature reports various α-MG concentrations that produce different effects. Scolamiero and colleagues found that when α-MG concentrations ranged from 0.1 to 30 μg/ml, they affected MDA-MB-231 and MCF-7 cells in distinct ways. The study showed that the IC50 values for MDA-MB-231 cells ranged from 0.70 to 1.25 μg/ml, and doses of α-MG between 5 and 15 μg/ml increased cell size, induced apoptosis, and inhibited cellular migration. In MCF-7 cells, however, α-MG concentrations between 0.1 and 30 μg/ml led to bimodal changes in cellular volume [20].

In another study, See and colleagues reported that α-MG exhibited strong cytotoxicity against MCF-7 and MDA-MB-231 cells. The IC50 values were found to be 4.43 μM and 3.59 μM after 24 hours, and 2.74 μM and 2.60 μM after 48 hours, respectively [21]. Similarly, another investigation highlighted that α-MG induced apoptosis and that its cytotoxic effects varied depending on both the treatment duration and concentration [22]. Additionally, the stronger effect of α-MG on MCF-7 cells has been associated with estrogen receptor signaling. In this cascade, α-MG activates caspase-8, caspase-9, and caspase-7, increases Bax, p53, and cytosolic cytochrome c levels, induces PARP cleavage, downregulates Bid and Bcl-2, and promotes the translocation of AIF from the mitochondria to the cytosol. Importantly, α-MG was more effective at lower concentrations in MDA-MB-231 cells after 48 hours [15].

αMG has been investigated in various cancer cell lines. Markowicz et al. reported that αMG had IC50 values below 5 μg/mL in squamous carcinoma cells and glioblastoma multiforme cells demonstrating the anti-tumoral effects [23]. Another study found that αMG induced a 50% growth inhibition at doses above 30 μM in MCF-7, MDA-MB-231, NCI-H-460, and SF-268 cells [24]. α-MG has been observed to suppress cell migration by disrupting the actin network in comparison to normal bronchial diploid cells (CCD-14Br) and small cell lung cancer cells (A549) [25]. In myelomonocytic KG-1 cells, α-MG reduced cellular surface stiffness and disrupted microvillus arrangement [26]. Additionally, in melanoma cells (A375, B16F10, M14, and SK-MEL-2) α-MG suppressed PI3K phosphorylation, inhibited cell growth, movement, and infiltration and induced G0/G1 phase arrest, mitochondrial swelling and membrane depolarization [27].

Our findings demonstrate that the combination of α-MG with nab-paclitaxel significantly increased caspase 3-7 levels in both cell lines used in our study, indicating the activation of apoptotic pathways. This is consistent with literature suggesting that α-MG inhibits FAS, suppressing anti-apoptotic Bcl-2 family proteins and enhancing caspase activity. Additionally, studies have shown that α-MG inhibits the PI3K/Akt pathway and ABCG2, which may increase the intracellular uptake of nab-paclitaxel. Furthermore, α-MG has been reported to increase intracellular reactive oxygen species, alter mitochondrial permeability, and promote the release of cytochrome c into the cytosol, thereby activating effector caspases [14, 15, 16].

The anti-carcinogenic effects of α-MG have been shown to be significant, with its activity demonstrated in various cell types, and its antitumor effects associated with multiple molecular mechanisms [28]. However, due to α-MG's poor water solubility, its use in cancer therapy may require high concentrations to achieve adequate bioavailability and efficacy. This poses challenges for bioavailability and clinical application [29]. Considering the side effects of high-dose chemotherapeutics and solubility issues, our study focused on investigating the efficacy of minimal dose combinations in our cell lines. The combination doses applied in our in vitro breast cancer cell lines showed promising results in terms of potential advantages for proliferation control.

As a p53-MDM2 inhibitor, α-MG may reduce the impact of chemotherapy on healthy cells by halting the cell cycle. Our study also demonstrated that the combination of α-MG with nab-paclitaxel altered cell kinetics in breast cancer cell lines, exhibiting antiproliferative, antimitotic, and apoptosis-inducing properties. The properties of α-MG, such as inhibiting metastasis and angiogenesis, triggering apoptotic pathways, inducing cell cycle arrest, and its anti-inflammatory and cytotoxic effects, as well as its selectivity toward cancer cells, underscore its high therapeutic potential [30, 32].

5. Conclusion

In conclusion, while various formulations of α-MG are still under development [33, 14], ongoing research into nab-paclitaxel, particularly in advanced-stage breast

cancer and paclitaxel-resistant cases [34], highlights the significance of these therapeutic approaches. The combination of nab-paclitaxel with adjunctive agents like α-MG may offer substantial therapeutic benefits. However, comprehensive clinical and preclinical studies are required to fully understand these interactions, optimal dosing regimens, and potential adverse effects.

Conflict of Interests
The authors have no conflict of interest to declare.

Consent for publications
Not applicable.

Ethics approval and consent to participate
No human or animal ethics approval was required.

Informed consent
Not applicable.

Availability of data and material
The authors declare that all data are embedded in the manuscript.

Authors' contributions
O.O., I.C., F.A., and M.R.T. conceived and designed the project. O.O. and I.C. performed the experiments and analyzed the data. O.O. and I.C. drafted the manuscript. F.A. and M.R.T. critically revised the manuscript. All authors approved the final version of the manuscript.

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