



Research Article

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Synergistic Cytotoxic and Clonogenic Effects of Paclitaxel and Allicin in MDA-MB-231 Triple-Negative Breast Cancer Cells

Sajad Qasim Shnain^{1*}, Yassir Mustafa Kamal Al-Mulla Hummadi¹, Afrah Muhammed Hassan Salman¹, Anas Sattar²¹Department of Pharmacology and Toxicology, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq; ²School of Health, Sports and Biosciences, University of East London, London, UK

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Abstract

Background: Triple-negative breast cancer (TNBC) lacks validated molecular targets and relies on cytotoxic chemotherapy limited by toxicity. Combining paclitaxel with the redox-active garlic compound allicin may permit dose reduction while preserving activity. **Objective:** To define the in vitro cytotoxic profiles of paclitaxel and allicin, quantify their interaction, and assess the durability of the effect in MDA-MB-231 cells. **Methods:** Cytotoxicity was measured by MTT assay after 24, 48, and 72 h, and IC₅₀ values were derived by non-linear regression. The interaction was quantified by the combination index (CI) and dose-reduction index (DRI) using the Chou–Talalay method (CompuSyn). Long-term viability was assessed by colony formation. **Results:** The 72-h IC₅₀ values were 60 nM (paclitaxel) and 170 μM (allicin). The interaction was effect-dependent: synergism (CI<1.0) occurred at low-to-intermediate effect levels (CI=0.649, 0.776, and 0.880 at fractions affected of 0.452, 0.650, and 0.820), with antagonism at higher levels (CI=1.062 and 1.249). The DRI exceeded unity for both agents across the synergistic range (up to 2.82-fold for paclitaxel and 3.39-fold for allicin). In the colony assay, the combination gave the lowest surviving fraction (0.3094 vs. paclitaxel and 0.5353 for allicin; $p<0.0001$). **Conclusions:** In MDA-MB-231 cells, paclitaxel and allicin act synergistically at low-to-intermediate effect levels and durably suppress clonogenic survival in vitro, supporting further mechanistic evaluation of allicin as a potential dose-sparing adjunct to paclitaxel in this model.

Keywords: Allicin; Chou–Talalay; Colony formation; Drug synergy; Paclitaxel; Triple-negative breast cancer.

التأثيرات التآزرية السمية الخلوية والمُثبِّطة لتكوين المستعمرات للباليكيتاكسيل والأليسين في خلايا سرطان الثدي ثلاثي السلبية MDA-MB-231

الخلاصة

الخلفية: يفتقر سرطان الثدي ثلاثي السلبية (TNBC) إلى أهداف جزيئية مُثبِّتة ويعتمد على العلاج الكيميائي السام للخلايا المحدود بسبب سُمِّيَّته. وقد يسمح الجمع بين الباليكيتاكسيل ومركب الأليسين الثوموي النشط أكسدة واختزالاً بتقليل الجرعة مع الحفاظ على الفعالية. **الهدف:** تحديد الخصائص السمية الخلوية للباليكيتاكسيل والأليسين مختبرياً، وقياس التفاعل بينهما، وتقييم ديمومة التأثير في خلايا MDA-MB-231. **الطرائق:** قُيِّمت السمية الخلوية باختبار MTT بعد 24 و 48 و 72 ساعة، واستُخرجت قيم التركيز المثبِّط النصفية (IC₅₀) بالانحدار غير الخطي. وحُدِّد التفاعل كميًا بمؤشر التوليفة (CI) ومُؤشر تقليل الجرعة (DRI) باستخدام طريقة تشو-تالاي (CompuSyn). وقُيِّمت الحيوية طويلة الأمد باختبار تكوين المستعمرات. وفُورنت المجموعات بتحليل التباين أحادي الاتجاه مع اختبارات توكي البعدية؛ واعتُبرت قيمة P الأقل من 0.05 ذات دلالة إحصائية. **النتائج:** كانت قيم IC₅₀ عند 72 ساعة 60 نانومولار (للباليكيتاكسيل) و 170 مايكرومولار (للأليسين). وكان التفاعل معتمداً على مستوى التأثير، إذ حدث التآزر (CI<1) عند مستويات التأثير المنخفضة إلى المتوسطة (CI=0.649 و 0.776 و 0.880) عند نسب تأثر بلغت 0.452 و 0.650 و 0.820، مع تضادٍ عند المستويات الأعلى (CI=1.062 و 1.249). وتجاوز مؤشر تقليل الجرعة (DRI) الواحد الصحيح لكلا العاملين عبر النطاق التآزري (حتى 2.82 ضعفاً للباليكيتاكسيل و 3.39 ضعفاً للأليسين). وفي اختبار تكوين المستعمرات، أعطت التوليفة أدنى كسر بقاء (0.3094) مقارنةً بالباليكيتاكسيل (0.5353) والأليسين ($p<0.0001$). **الاستنتاجات:** في خلايا MDA-MB-231، يعمل الباليكيتاكسيل والأليسين بشكل تآزري عند مستويات التأثير المنخفضة إلى المتوسطة، ويُثبِّتان بشكل دائم بقاء الخلايا المُكوِّنة للمستعمرات مختبرياً، مما يدعم إجراء تقييم آلي إضافي للأليسين بوصفه عاملاً مساعداً محتملاً مُثبِّلاً للجرعة مع الباليكيتاكسيل في هذا النموذج.

*Corresponding author: Sajad Q. Shnain. Department of Pharmacology and Toxicology, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq: sajadqasim70@uomustansiriyah.edu.iq**Article citation:** Shnain SQ, Al-Mulla Hummadi YMK, Salman AMH, Sattar A. Synergistic Cytotoxic and Clonogenic Effects of Paclitaxel and Allicin in MDA-MB-231 Triple-Negative Breast Cancer Cells. *Al-Rafidain J Med Sci.* 2026;11(2):53-59. doi: <https://doi.org/10.54133/ajms.v11i2.3168>© 2026 The Author(s). Published by Al-Rafidain University. This is an open access journal issued under the CC BY-NC-SA 4.0 license (<https://creativecommons.org/licenses/by-nc-sa/4.0/>).

INTRODUCTION

Breast cancer is the most frequently diagnosed malignancy in women worldwide and a leading cause of cancer-related death [1]. Triple-negative breast cancer (TNBC), defined by the absence of estrogen and progesterone receptor expression and the lack of human epidermal growth factor receptor 2 (HER2) overexpression, accounts for approximately 15–20% of breast cancers but contributes disproportionately to mortality because of its aggressive biology, early visceral recurrence, and poorer survival than other subtypes [2,3]. Unlike hormone receptor-positive or HER2-positive disease, TNBC lacks established molecular targets, so cytotoxic chemotherapy remains the mainstay of systemic treatment, with recent

additions such as immune checkpoint inhibitors and poly(ADP-ribose) polymerase inhibitors benefiting only biomarker-selected subgroups [4,5]. MDA-MB-231 is a widely used, aggressive, mesenchymal-like (claudin-low) TNBC cell line that harbors a mutant tumor-suppressor background and is broadly resistant to many cytotoxic agents, which makes it a stringent model for evaluating combination strategies [6]. Paclitaxel, a taxane that stabilizes microtubules and arrests cells in mitosis, is among the most active agents in TNBC; however, its clinical use is constrained by dose-limiting toxicities such as neuropathy and myelosuppression and by the development of resistance [7,8]. A widely pursued preclinical strategy to mitigate these limitations is the

rational combination of paclitaxel with a second agent that enhances its cytotoxicity, with the aim of reducing the dose of each drug [9,10]. Rigorous, theory-based methods such as the Chou–Talalay median-effect approach are required to distinguish true synergy from a merely additive effect and to quantify the dose-sparing potential of a combination [9,11]. Allicin (diallyl thiosulfinate), the principal thiosulfinate of freshly crushed garlic, is a thiol-reactive organosulfur compound with antiproliferative, pro-oxidant, and pro-apoptotic activity reported across diverse cancer cell types [12]. Its chemistry and broad biological actions, including the depletion of intracellular glutathione and the elevation of reactive oxygen species, are well characterized [13]. This redox-modulating activity, together with the modulation of apoptosis-related and survival signaling, provides a plausible biochemical basis for sensitizing cancer cells to a co-administered cytotoxic agent [14]. Garlic-derived organosulfur compounds, including allicin, have also been reported to augment the activity of conventional chemotherapeutic drugs in preclinical models [15]. In breast cancer specifically, several natural compounds act synergistically with paclitaxel and lower its effective concentration *in vitro* [16], including agents tested in the MDA-MB-231 model [17]. Despite this rationale, the quantitative pharmacological nature of the paclitaxel–allicin interaction in TNBC has not been rigorously characterized, and it is not known whether any combined benefit is durable. The present study was therefore designed to determine the individual *in vitro* cytotoxic profiles of paclitaxel and allicin in MDA-MB-231 cells, to quantify their interaction across a range of effect levels using the Chou–Talalay combination index method, and to establish whether any combined benefit translates into a durable suppression of long-term reproductive viability assessed by the colony formation assay [18]. This study aims to define the *in vitro* cytotoxic profiles of paclitaxel and allicin, quantify their interaction, and assess the durability of the effect in MDA-MB-231 cells.

METHODS

Cell line and culture conditions

The human triple-negative breast adenocarcinoma cell line MDA-MB-231 was maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin at 37°C in a humidified atmosphere containing 5% CO₂. Cells in the exponential growth phase were used for all experiments.

Drugs and reagents

Paclitaxel (≥98% purity, HPLC; powder) and allicin (≥96% purity; liquid) were obtained from Sigma-Aldrich (USA). A paclitaxel stock solution (5 mM) was prepared in dimethyl sulfoxide (DMSO), sterilized by passage through a 0.22 µm filter, aliquoted, and stored at –20°C protected from light, the stock concentration being selected so that the final

DMSO content in every well remained at or below 0.1% (v/v) across the entire range of concentrations tested. Allicin, supplied as a pure liquid, was not stored as a frozen DMSO stock; instead, it was drawn directly from the neat liquid and prepared fresh on the day of each experiment, a working concentration of 2000 µM being made up in complete medium containing 0.1% (v/v) DMSO to match the vehicle conditions and limit degradation. The vehicle-control wells likewise received complete medium containing 0.1% (v/v) DMSO, and all results were normalized to this vehicle control.

MTT cell viability assay

Cells were seeded in 96-well plates at 3×10^3 cells per well and allowed to adhere overnight. Paclitaxel (7.8125–4000 nM) and allicin (3.9–2000 µM) were each applied as ten serial two-fold dilutions, and viability was assessed after 24, 48, and 72 h using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) [19]. At the end of each incubation period, 12 µL of MTT solution (5 mg/mL in PBS, freshly prepared) was added to each well (final concentration ≈ 0.536 mg/mL), and the plates were incubated for a further 3 h at 37°C in the dark. The medium was then aspirated, 100 µL of DMSO was added per well to solubilize the formazan, and the plates were shaken for 10 min at room temperature. Absorbance was measured against a reagent blank at 560 nm using a microplate reader (Promega, USA). Viability was expressed relative to vehicle-treated controls, and IC₅₀ values were determined from sigmoidal dose–response curves using a four-parameter log(inhibitor) versus normalized-response model with variable slope in GraphPad Prism (version 10.4.1). The 72-h IC₅₀ of each agent was used as the reference single-agent concentration for the subsequent combination and colony formation experiments. Each concentration was tested in technical triplicate, and the entire experiment was repeated in three independent biological replicates.

Combination index analysis

The pharmacological interaction between paclitaxel and allicin was quantified by the Chou–Talalay method, which is based on the median-effect principle of the mass-action law [9]. A fixed-ratio (constant-ratio) design was used, anchored to the 72-h IC₅₀ of each agent (paclitaxel : allicin = 60 nM : 170 µM). Five equipotent combination levels (0.25×, 0.5×, 1×, 2×, and 4× of the IC₅₀-equivalent concentration of each drug) were prepared, together with matched dilution series of each single agent, and viability was assessed after 72 h of exposure. The fraction affected (Fa) at each level was entered into CompuSyn software (version 1.0; ComboSyn Inc., USA), which computes the combination index as $CI = (D)_1/(Dx)_1 + (D)_2/(Dx)_2$, where (D)₁ and (D)₂ are the doses of the two drugs in combination required to produce a given Fa, and (Dx)₁ and (Dx)₂ are the doses of each drug alone required to produce the same Fa. CI < 1, CI = 1, and CI > 1 denote synergism, additivity, and antagonism, respectively. The dose-reduction index

(DRI) was computed for each agent at each effect level. A linear correlation coefficient (r) > 0.90 for the median-effect plots was set as the minimum acceptance threshold [11].

Colony formation assay

Cells were seeded in 6-well plates at 500 cells per well and allowed to adhere for 24 h. The medium was then replaced with fresh medium containing vehicle control, paclitaxel alone (60 nM), allicin alone (170 μ M), or the combination (60 nM paclitaxel + 170 μ M allicin), applied simultaneously as a 72-h pulse. Treatments were then removed and replaced with drug-free medium, and plates were incubated undisturbed for 10 days, with gentle medium renewal every 4 days. Colonies were fixed with cold methanol for 15 min, stained with 0.5% (w/v) crystal violet for 30 min, and colonies of ≥ 50 cells were counted under an inverted microscope by an observer blinded to treatment identity. The plating efficiency (PE) and surviving fraction (SF) were calculated as $PE = (\text{number of colonies counted} / \text{number of cells seeded}) \times 100$ and $SF = PE \text{ of the treated condition} / PE \text{ of the vehicle control}$ [18].

Ethical considerations

This study used a commercially available, established human cancer cell line and did not involve human participants, identifiable human material, or animals; institutional ethical approval was therefore not required.

Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD) of three independent biological replicates ($n = 3$). Differences among groups were assessed by one-way analysis of variance (ANOVA) followed by Tukey post-hoc multiple-comparison tests, after confirming homogeneity of variances with the Brown–Forsythe test. Analyses were performed in GraphPad Prism (version 10.4.1), and a p -value below 0.05 was considered statistically significant. Exact p -values are reported where available; $p < 0.0001$ was used for values below the software reporting threshold. Given the small number of replicates ($n = 3$), individual data points are overlaid on all bar graphs.

RESULTS

Paclitaxel reduced the viability of MDA-MB-231 cells in a concentration- and time-dependent manner (Figure 1). The cytotoxic effect was greatest after 72 h

of exposure, with an IC_{50} of 60 nM (95% CI, 30.02–84.13 nM; $R^2 = 0.9852$). Potency decreased at shorter exposures, the IC_{50} rising to 715.8 nM at 48 h ($R^2 = 0.9779$) and to 2391 nM at 24 h ($R^2 = 0.9934$) (Table 1). The progressive fall in IC_{50} with exposure time is consistent with the cell-cycle-dependent, cumulative mechanism of taxane-induced cytotoxicity. Allicin produced a comparable concentration- and time-dependent reduction in viability, although at concentrations approximately 2,800-fold (nearly three orders of magnitude) higher than paclitaxel (Figure 2).

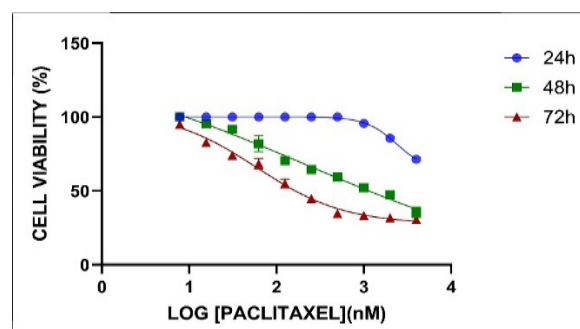


Figure 1: Dose–response curves of paclitaxel on MDA-MB-231 cell viability at 24, 48 and 72 h, fitted using a four-parameter non-linear regression model with variable slope. Cells were exposed to ten serial two-fold dilutions of paclitaxel (7.8125–4000 nM), and viability was determined by MTT assay. Data are mean $\pm$ SD of three independent biological replicates ($n = 3$).

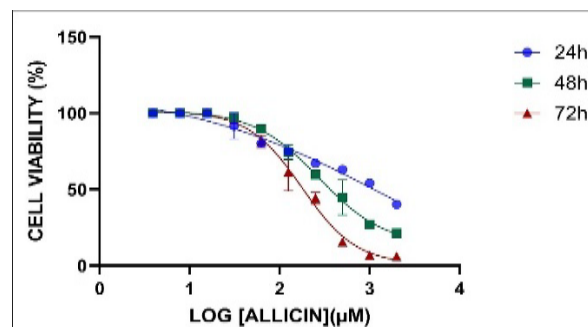


Figure 2: Dose–response curves of allicin on MDA-MB-231 cell viability at 24, 48 and 72 h, fitted using a four-parameter non-linear regression model with variable slope. Cells were exposed to ten serial two-fold dilutions of allicin (3.9–2000 μ M), and viability was determined by MTT assay. Data are presented as mean $\pm$ SD of three independent biological replicates ($n = 3$).

The 72-h IC_{50} was 170 μ M (95% CI, 152.7–209.2 μ M; $R^2 = 0.9870$), with an excellent fit of the dose–response model to the experimental data and increased to 273.6 μ M at 48 h ($R^2 = 0.9842$) (Table 1).

Table 1: Half-maximal inhibitory concentrations (IC_{50}), 95% confidence intervals (CI), goodness-of-fit (R^2) and Hill slope for paclitaxel and allicin in MDA-MB-231 cells (MTT assay)

Agent/time	IC_{50}	95% CI	R^2	Hill slope	Reliability
Paclitaxel – 24 h	2391 nM	2114 – 2971 nM	0.9934	–2.413	Reliable
Paclitaxel – 48 h	715.8 nM	530.7 – 1203 nM	0.9779	–1.206	Reliable
Paclitaxel – 72 h	60 nM	30.02 – 84.13 nM	0.9852	–0.8631	Reliable
Allicin – 24 h	314.7 μ M	198.7 – 1030 μ M	0.9780	–0.8592	Reliable
Allicin – 48 h	273.6 μ M	206.2 – 424.5 μ M	0.9842	–1.021	Reliable
Allicin – 72 h	170 μ M	152.7 – 209.2 μ M	0.9870	–1.446	Reliable

IC_{50} , half-maximal inhibitory concentration; CI, confidence interval; R^2 , coefficient of determination.

At 24 h, allicin was less potent, with an IC_{50} of 314.7 μ M ($R^2 = 0.9780$), confirming that a 24-h exposure

was insufficient to elicit substantial cytotoxicity at the concentrations tested. When combined at a fixed ratio

derived from their 72-h IC_{50} values, paclitaxel and allicin produced a dose–effect curve that was shifted to the left of either single agent, indicating enhanced potency at lower combined doses (Figure 3A). Importantly, the interaction was not uniform across the dose range but was effect-level dependent (Table 2; Figure 3B). At low-to-intermediate effect levels, the interaction was clearly synergistic, with CI values of 0.649 at $F_a = 0.452$ and 0.776 at $F_a = 0.650$, and remained synergistic, although weaker, at $F_a = 0.820$ ($CI = 0.880$). At higher effect levels the CI exceeded unity, indicating a transition toward a nearly additive interaction at $F_a = 0.910$ ($CI = 1.062$) and moderate

antagonism at $F_a = 0.959$ ($CI = 1.249$). These higher-effect levels correspond to the upper, steeper portion of the dose–response curve, where CI estimates are inherently more sensitive to small variations in the fraction affected and should therefore be interpreted with greater caution than the low-to-intermediate values. The conservative isobologram was consistent with these findings, with the low-to-intermediate effect points falling below the line of additivity (Figure 3C). Across the synergistic range, the dose-reduction index remained above unity for both agents (Table 2; Figure 3D).

Table 2: Combination index (CI) and dose-reduction index (DRI) values for the paclitaxel–allicin combination at each fixed-ratio combination level in MDA-MB-231 cells

Combination level	Paclitaxel dose (nM)	Allicin dose (μ M)	F_a	CI	DRI-PAC	DRI-ALC	Interpretation
0.25×	15	42.5	0.452	0.649	2.82	3.39	Synergism
0.5×	30	85	0.650	0.776	2.38	2.81	Moderate synergism
1×	60	170	0.820	0.880	2.12	2.45	Slight synergism
2×	120	340	0.910	1.062	1.78	2.00	Nearly additive
4×	240	680	0.959	1.249	1.52	1.69	Moderate antagonism

F_a : fraction affected; CI: combination index; DRI: dose-reduction index; PAC: paclitaxel; ALC: allicin. Combination levels are expressed as multiples of the IC_{50} -equivalent concentration of each agent. $CI < 1$, synergism; $CI \approx 1$, additivity; $CI > 1$, antagonism.

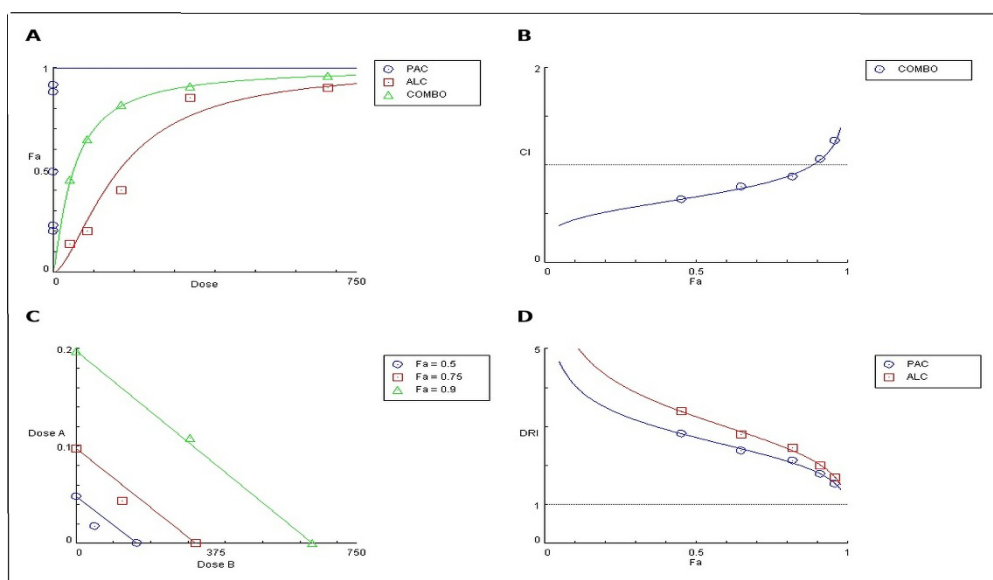


Figure 3: Chou–Talalay analysis of the paclitaxel–allicin combination in MDA-MB-231 cells. (A) Dose–effect curves of paclitaxel, allicin and their combination derived from CompuSyn. (B) Combination index (CI) versus fraction affected (F_a); the horizontal line at $CI = 1$ denotes additivity, values below denote synergism and values above denote antagonism. (C) Conservative isobologram. (D) Dose-reduction index (DRI) for each agent across effect levels. Synergism was observed at $F_a = 0.452$, 0.650 and 0.820, with a transition toward antagonism at $F_a > 0.90$.

The dose-sparing effect was greatest at the lowest effect level, where the combination corresponded to a 2.82-fold reduction in the paclitaxel concentration and a 3.39-fold reduction in the allicin concentration relative to the single-agent concentrations producing the same effect, and declined progressively to 1.52-fold and 1.69-fold, respectively, at $F_a = 0.959$. The median-effect parameters for each single agent and for the combination are summarized in Table 3. The high linear correlation coefficients ($r = 0.9635$ for paclitaxel, 0.9688 for allicin, and 0.9999 for the combination) confirm conformity to the median-effect model and validate the CI and DRI calculations. In the colony formation assay, all three treatments significantly reduced long-term reproductive viability relative to the vehicle control [$F(3, 8) = 162.5$, $p < 0.0001$] (Figure 4).

Table 3: Median-effect parameters (D_m , m and linear correlation coefficient r) for paclitaxel, allicin and their combination, derived from the Chou–Talalay analysis (CompuSyn)

Agent/Combination	D_m	m	r
Paclitaxel	0.04791	1.5504	0.9635
Allicin	162.49	1.61375	0.96879
Paclitaxel + allicin	49.9577	1.20962	0.99987

D_m : median-effect dose (μ M; for the combination, D_m is expressed on a total-dose basis); m : Hill-type slope coefficient; r : linear correlation coefficient of the median-effect plot. All r values exceeded the 0.90 acceptance threshold.

The mean surviving fractions were 0.6561 for allicin, 0.5353 for paclitaxel, and 0.3094 for the combination, the last corresponding to a reduction of clonogenic survival to approximately 31% of control. The combination produced a significantly lower surviving fraction than either paclitaxel alone ($p = 0.0005$) or allicin alone ($p < 0.0001$), and paclitaxel was significantly more potent than allicin ($p = 0.0224$).

Tukey post-hoc analysis assigned the four conditions to distinct groups. These results indicate that the combination produces a durable suppression of reproductive viability; they do not, however, independently establish mechanistic synergy, which was quantified separately by the Chou–Talalay analysis of the MTT data.

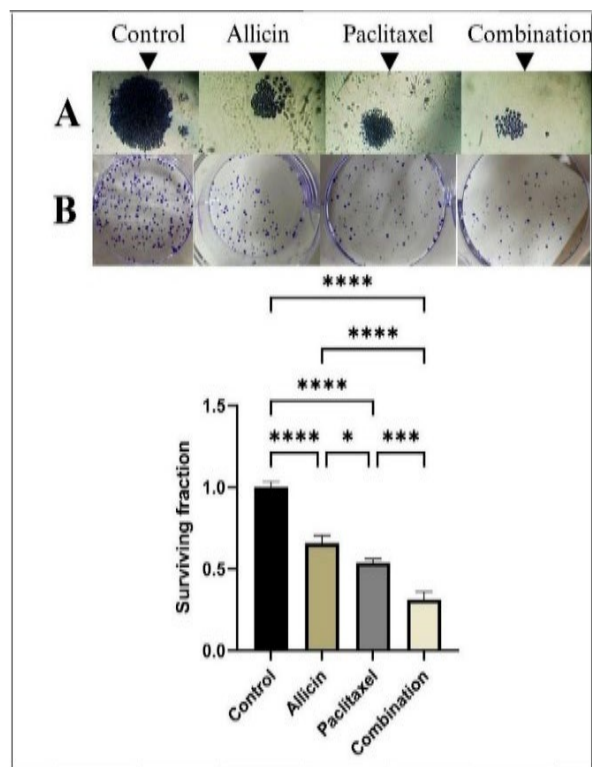


Figure 4: Effect of paclitaxel (60 nM), allicin (170 μ M) and their combination on long-term reproductive viability of MDA-MB-231 cells (colony formation assay). (A, B) Representative photographs of stained 6-well plates and colonies, captured at equal magnification. (C) Surviving fraction normalized to vehicle control. By Tukey post-hoc test, the combination differed significantly from paclitaxel alone ($p = 0.0005$) and from allicin alone ($p < 0.0001$), and paclitaxel differed from allicin ($p = 0.0224$). Data are presented as mean $\pm$ SD of three independent biological replicates ($n = 3$).

DISCUSSION

This study provides a quantitative pharmacological characterization of the interaction between paclitaxel and allicin in MDA-MB-231 TNBC cells and examines whether the combined effect is durable. Three principal findings emerged. First, both agents were independently cytotoxic in a concentration- and time-dependent manner, providing experimentally useful reference concentrations for subsequent combination testing. Second, the interaction was synergistic but strictly effect-level dependent: synergy ($CI < 1$) was confined to the low-to-intermediate effect range and gave way to a nearly additive and then antagonistic interaction at higher effect levels. Third, at the IC_{50} -based doses, the combination produced a durable suppression of clonogenic survival that significantly exceeded that of either single agent *in vitro*. The individual cytotoxicity profiles are consistent with the known pharmacology of the two agents. The steep decline in the paclitaxel IC_{50}

between 24 and 72 h reflects the cell-cycle-dependent mechanism of taxanes, which stabilizes microtubules and disrupts normal mitotic progression, with cell death arising from aberrant, frequently multipolar, division and chromosome mis-segregation rather than from sustained mitotic arrest alone; longer exposure therefore captures a greater fraction of cells passing through these defective divisions [7]. Allicin was active in the micromolar range, in agreement with previous reports of its antiproliferative activity in multiple tumor models, where concentrations of tens to low hundreds of micromolar are typically required [12]. The difference in molar potency of nearly three orders of magnitude between the two agents reflects their distinct mechanisms—targeted microtubule stabilization for paclitaxel versus broad thiol-reactive, redox-based actions for allicin—and underlines the value of a fixed-ratio design anchored to the IC_{50} of each drug when their interaction is analyzed [11]. The effect-level dependence of the combination index is the central pharmacological finding of this study and is frequently overlooked when synergy is reported as a single summary value. The Chou–Talalay framework makes explicit that the nature of an interaction can change across the dose–response curve [11]. The present data illustrate this clearly: the same drug pair that is synergistic at modest levels of growth inhibition becomes antagonistic when each agent is driven toward its maximal effect. This pattern is mechanistically plausible. At low-to-intermediate doses, allicin-mediated depletion of glutathione and elevation of reactive oxygen species may lower the threshold at which paclitaxel-induced mitotic stress commits a cell to death, producing a cooperative effect [14]. At high doses, by contrast, each agent may approach saturation of its own cytotoxic pathway, leaving little capacity for further cooperative enhancement. The data therefore suggest that any cooperative effect of this combination is confined to the lower-to-intermediate effect range rather than to maximal-dose exposure. The dose-reduction index is consistent with this interpretation. Across the synergistic range, the CI analysis indicated that equivalent *in vitro* cytotoxicity could be achieved at more than two-fold lower concentrations of both agents. This finding is a pharmacological, *in vitro* observation rather than a demonstration of clinical dose reduction; nonetheless, because paclitaxel toxicity is dose-related [8], a favorable DRI provides a quantitative rationale for the further preclinical evaluation of allicin as a possible dose-sparing partner, an approach that parallels other natural-compound–paclitaxel combinations reported in breast cancer models [10]. Comparable observations have been reported for paclitaxel combined with an *Echinacea angustifolia* extract [20] and with oleuropein [21], both of which enhanced paclitaxel cytotoxicity in breast cancer cells, while a luteolin–paclitaxel combination enhanced apoptosis specifically in MDA-MB-231 cells [22]. A key consideration for translational relevance is that the allicin concentration used here (170 μ M) is high, and allicin is chemically unstable and rapidly degraded in

solution and in biological systems. The concentrations required for activity in vitro may therefore not be readily achievable or sustained in vivo, and the dose-reduction benefit—rather than the absolute concentration—is the more meaningful pharmacological outcome. Any translational interpretation of these results would require dedicated formulation and stability studies (for example, stabilized or encapsulated allicin preparations) together with pharmacokinetic characterization [12,14]. The colony formation results add a dimension that metabolic viability assays cannot capture. The MTT assay reports short-term metabolic activity and has recognized limitations, including sensitivity to metabolic perturbation independent of cell number [19]. The colony formation assay, by contrast, measures the capacity of single cells to survive and proliferate into colonies and is regarded as the gold-standard measure of reproductive cell death after cytotoxic treatment [18]. The finding that the combination reduced the surviving fraction to approximately 31% of control, significantly below either monotherapy, indicates that the enhanced effect extends beyond an acute reduction in metabolic activity to a durable loss of reproductive capacity, which is relevant to the prevention of disease recurrence and is a particular concern in TNBC given its propensity for early relapse [2,3]. It should be emphasized, however, that the colony assay supports durable suppression of reproductive viability and does not by itself prove mechanistic synergy; the concordance between the MTT-based interaction analysis and the clonogenic results nonetheless strengthens confidence that the combined effect is biologically meaningful rather than an artifact of a single assay format.

Study Limitations

Several limitations of this study should be acknowledged. It was conducted in a single TNBC cell line; although MDA-MB-231 is a widely used, aggressive mesenchymal-like model, the generalizability of the findings requires confirmation in additional TNBC cell lines of differing molecular background. The colony formation assay was performed at the fixed IC₅₀-based doses, which correspond to the upper part of the synergistic range, so this experiment characterizes the magnitude of the combined effect at a single dose rather than redemonstrating synergy across all effect levels. The Chou–Talalay method itself assumes that the dose–response relationships follow the median-effect equation and does not account for time-dependent or pharmacokinetic interactions [11]. Finally, no selectivity assessment in non-malignant cells and no direct mechanistic confirmation of the basis of synergy were performed in this study; establishing a selectivity index, defining the molecular mechanism across the synergistic range, characterizing allicin stability and formulation, and validating the combination in vivo are the principal directions for future work.

Conclusion

In MDA-MB-231 triple-negative breast cancer cells, paclitaxel and allicin interact synergistically at low-to-intermediate effect levels in vitro, with the interaction becoming antagonistic at high effect levels, and the CI analysis indicates that equivalent cytotoxicity can be achieved at reduced concentrations of both agents. This combination also produces a durable in vitro suppression of clonogenic survival that exceeds single agent. These findings support further mechanistic evaluation of allicin as a potential dose-sparing adjunct to paclitaxel in MDA-MB-231 TNBC cells and should be confirmed in additional cell lines, with formulation/stability and in vivo studies, before any translational conclusions are drawn.

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Conflict of interests

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Data sharing statement

The data that supports the findings of this study are available from the corresponding author upon a reasonable request.

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