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Anti-cancer effect of camptothecin and chloroquine on the inhibition of lung cancer and breast cancer cell proliferation

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Abstract

Background:This research aimed to evaluate the toxic effects of chloroquine alone and in combination with camptothecin on two human cancer cell lines, namely, MCF-7 breast cancer cells and A549 lung cancer cells.
Objective: To determine the percentage inhibition of breast and lung cancer cells after 72 h of exposure to treatments utilizing (originally against C. trachomatis), chloroquine and camptothecin alone and in combination and to calculate the respective half-maximal inhibitory concentration (IC50) values. Methods: Exposure to varying amounts of chloroquine, camptothecin, and a mixture of these drugs was performed for 72 hours. The percentages of growth inhibition and IC_{50} were obtained to investigate the cytotoxic effects. Results: Single treatment with chloroquine strongly reduced cell viability in both models; however, it moderately inhibited (57 - 63%) MCF-7 cells and strongly inhibited (72 - 77%) A549 cells, suggesting that lung cancer cells are more sensitive to chloroquine. Dual treatment with chloroquine and camptothecin resulted in dose-related cytotoxic activity. The inhibition was 17 - 35% at lower doses, whereas a greater percentage of cell death occurred (52 - 67%) at higher doses. Analysis using the IC_{50} further confirmed the presence of an interaction between the two drugs. Conclusion: These findings indicate that chloroquine potentiates the antitumor effects of CPT, especially in A549 cells.

Keywords
Camptothecin, Chloroquine, lung cancer (A549), breast cancer (MCF-7), combination therapy

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Introduction

Combination therapy plays a vital role in modern cancer treatment, enhancing the use of multiple effective drugs and reducing the risk of treatment resistance. Unlike monotherapy, combination therapies target diverse molecular pathways, inhibit tumor growth, induce apoptosis, reduce the number of cancer stem cells, and limit their toxicity to healthy tissues [1,2].

Despite major advances in diagnosis and treatment over the past two decades, lung cancer remains the leading cause of cancer-related deaths [3]. Combination therapies have significantly improved survival rates, particularly when combined with biomarkers and personalized medicine strategies [4]. A well-studied example is camptothecin (CPT), a potent topoisomerase I inhibitor originally derived from Camptothecia acuminata, which has demonstrated potent antitumor effects in both clinical and preclinical models [5,6]. CPT works by stabilizing the DNA-topoisomerase I complex, leading to DNA strand breaks and apoptosis [7]. However, the clinical use of camptothecin is limited by its low solubility and dose-dependent toxicity [8,9].

Preclinical studies have demonstrated that CPT induces autophagy in lung cancer cells (such as H1299 and H460 cells), where autophagy functions as a survival mechanism [11]. Pharmacological inhibition of autophagy, such as by 3-methyladenine, enhances CPT-induced DNA damage, confirming that autophagy interferes with resistance in this context [11]. Therefore, combining CPT with autophagy inhibitors has emerged as a promising therapeutic strategy [12]. Chloroquine (CQ) is among the most studied inhibitors. It prevents the fusion of autophagosomes with lysosomes, induces lysosomal membrane permeabilization, stabilizes p53, and increases the susceptibility of cancer cells to apoptosis [13,14]. Preclinical and clinical data also suggest that chloroquine may act simultaneously as a chemo- and radiosensitizer, with potential benefits in small-cell lung cancer [15,16].

In breast cancer, especially in aggressive subtypes such as triple-negative breast cancer (TNBC), resistance to conventional chemotherapy remains a major therapeutic challenge. Combination therapy has received increasing attention. Therapeutic strategies that combine PARP inhibitors with DNA-damaging agents or immune checkpoint inhibitors with chemotherapy have shown improved efficacy. Furthermore, inhibition of autophagy has been linked to increased chemosensitivity in breast cancer cells, strengthening its role as a therapeutic enhancer [18,19].

Considering these factors together, the combination of cytotoxic agents and autophagy inhibitors represents a promising strategy in multiple tumor types and provides a rationale for further investigation of such therapeutic regimens in clinical settings.

Materials and methods

Cell viability and cytotoxicity

The human lung carcinoma cell line A549 and the human breast carcinoma cell line MCF-7 were provided by the Cell Bank Unit, Iraqi Center for Cancer and Medical Genetics Research, Mustansiriyah University. Both types of cell lines were grown in monolayer cultures in RPMI-1640 culture media supplemented with 10% fetal bovine serum (FBS), 100 µg of streptomycin, 100 IU of penicillin, and L-glutamine to create an optimal environment for growth. Periodic tests were performed to verify the viability, sterility, and morphology of A549 and MCF-7 cells.

Reagents

A stock solution of camptothecin (CAS 7689-03-4) (Santa Cruz, USA) and Chloroquine-d4 Diphosphate Salt (Santa Cruz, USA) was prepared. To evaluate the cytotoxic effects of camptothecin and chloroquine, A549 (lung cancer) and MCF-7 (breast cancer) cells were cultured in 96-well plates (1×10^4 cells/well) and treated after 24 h or upon reaching a confluent monolayer. Camptothecin stock solution (10 mM) in dimethyl sulfoxide was diluted to final concentrations of 0.1, 0.5, 1.0, and 5.0 µM, while chloroquine stock solution (1 mM) was diluted to concentrations of 5, 10, and 15 µM and dissolved in 1 mM DMSO. The final concentration of DMSO in all the wells did not exceed 0.1%.

Untreated cells were treated with RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). All experiments were performed in triplicate (four independent experiments, each with four technical replicates).

The cells were incubated at 37°C for 72 h. After they were incubated, the cells were rinsed with PBS and stained with 50 µl of 5 mg of crystal violet solution (BDH, England) for 30 min at 37°C. After the samples were washed three times with distilled water, the absorbance was measured at 540 nm using a microplate reader (Organon Teknika Reader 230S, Austria). Cell proliferation was estimated as the percentage absorbance compared with that of the untreated control wells, while cytotoxicity was calculated using the following formula:

(A-B)/Ax100, where A is the average absorbance of the control wells and B is the average absorbance of the treated control wells. The IC_{50} value was defined as the lowest concentration that reduced cell viability by 50%.

Image morphology and quantitative analysis

Four randomly selected fields were used to capture images of treated and untreated cells using a Leica Microsystems (Germany) color digital camera mounted on an inverted light microscope at 200× magnification.

Colony formation assay:

A blood cell counter was used, and the cells were cultured at a low density (500-1000 cells per well) in six-well plates. The cells were allowed to adhere overnight under standard culture conditions (37°C).

After adhesion, the cells were treated with the specified concentrations of chloroquine (CQ), camptothecin (CAMP), or a combination of both. After 24 hours of treatment, the medium was replaced with fresh, drug-free complete culture medium. The cells were then incubated for 10-14 days to allow colony formation, with the medium being replaced every 3-4 days.

After the incubation period, the cancer cell colonies were washed with PBS, fixed in 4% paraformaldehyde solution for 15 minutes and stained with 0.5% crystal violet solution for 20 minutes. The excess crystal violet dye was removed using distilled water, and the plates were left to dry under room conditions.

Colonies consisting of more than 50 cells were counted manually using an inverted microscope. The surviving fraction was calculated by normalizing the number of colonies formed after treatment to the plating efficiency of the untreated control group.

Plating efficiency (PE) = number of colonies in control group ÷ number of cells seeded

Surviving Fraction SF = Number of colonies after treatment ÷ (number of cells seeded × plating efficiency) [27].

Statistical analysis:

The data are shown as the means ± standard errors of the means. One-way ANOVA was performed, followed by Tukey's post hoc test. Analysis of variance was employed to compare the data dilution sequence. Differences were considered to be statistically significant when P was < 0.05. For this research, we used GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA), and IC_{50} values were calculated using nonlinear regression analysis (log inhibitor vs. response) with GraphPad Prism software.

Results

As shown in Figure 1 below, the toxic effect of camptothecin on MCF7 and A549 cells after 72 hours of exposure is presented. Camptothecin induced significant dose-related cytotoxicity in both cell lines (P < 0.001). In MCF7 cells, the cytotoxicity ranged from 26% at 0.1 µM to 38% at 5 µM, whereas in A549 cells, it ranged from 18% at 0.1 µM to 57% at 5 µM, indicating the sensitivity of A549 cells to the treatment.

In A549 cells, the compound exhibited dose-dependent inhibitory activity (ranging from 20% to 60%), with an IC50 value of approximately 5 µM, indicating a moderate cytotoxic effect compared with its weaker activity against MCF7 cells.

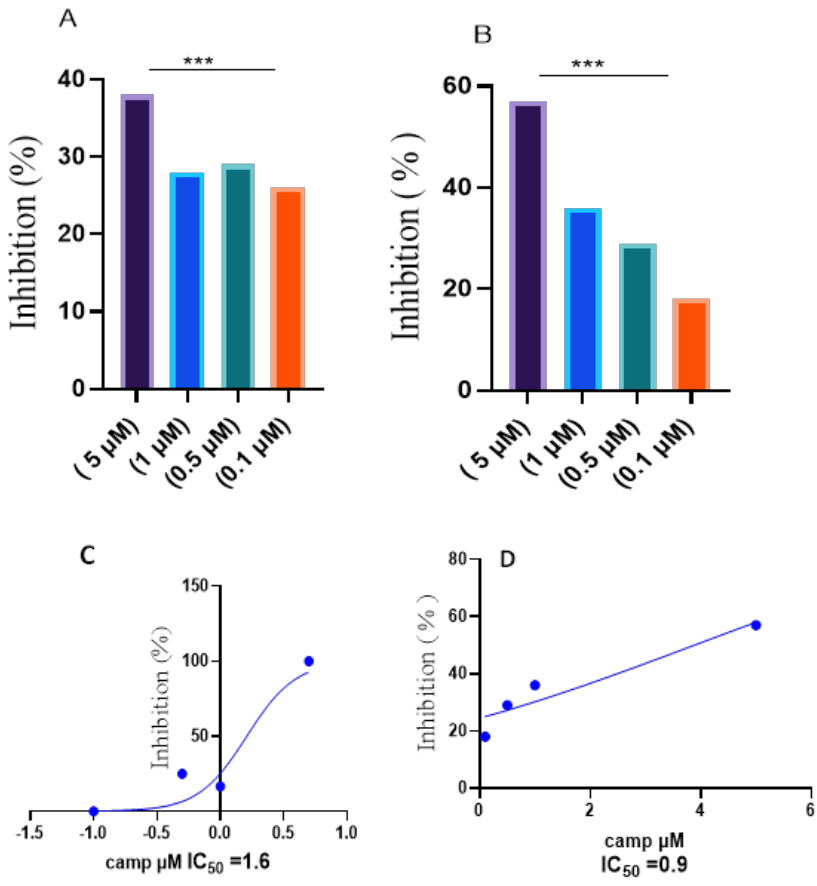

Figure 1: Single treatment with camptothecien after 72 hours in the (A) MCF7 cell line, (B) A549 cell line after 72 hours of treatment, (C) IC50=1.6 for the camptothecien in the MCF7 cell line, (D) IC50=0.9 for the camptothecien in the A549 cell line

As shown in Figure 2, treatment with chloroquine (CQ) for 72 hours induced significant cytotoxic effects in both the MCF7 and A549 cells ($P < 0.005$). In the MCF7 cells, the cytotoxicity ranged from 57% to 63%, whereas it was greater in the A549 cells, reaching 72% to 77%. Dose–response analysis revealed a clear concentration-dependent increase in inhibition, with A549 cells showing greater sensitivity to CQ than MCF7 cells did. These results indicate that compared with the control treatment, CQ has stronger inhibitory effects on lung cancer cells (A549) than on breast cancer cells (MCF7) under the tested conditions.

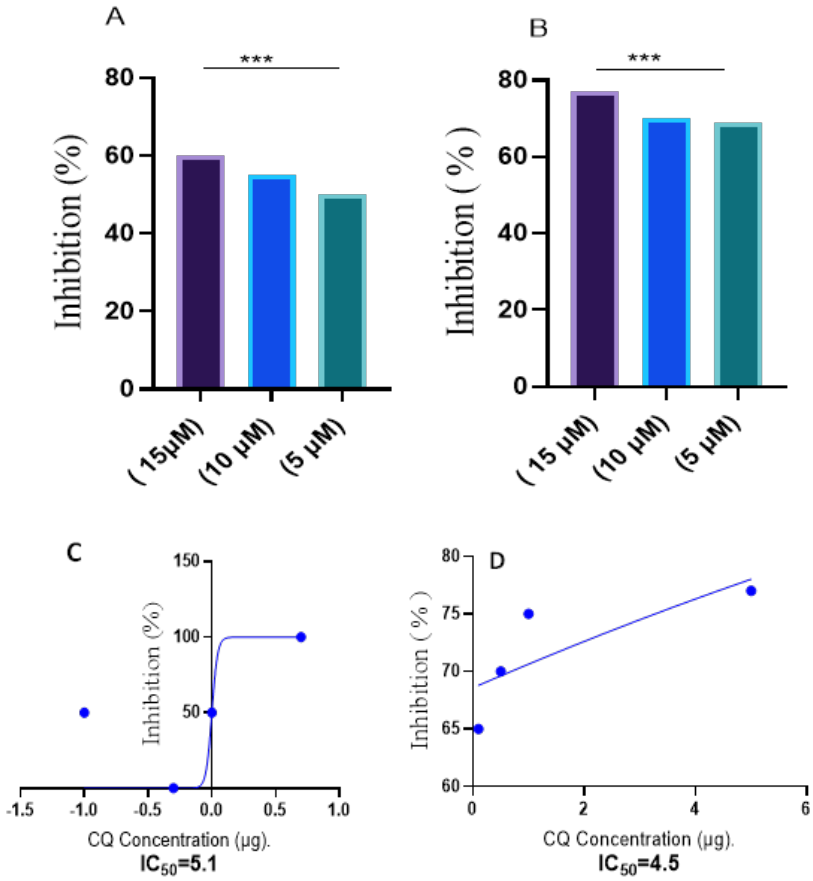

Figure 2: Single treatment with cloroquen after 72 hours in the (A) MCF7 cell line, (B) A549 cell line after 72 hours of treatment, (C) IC50=5.1 of CQ in the MCF7 cell line, (D) IC50 =4.5 of CQ in the A549 cell line

As shown in Figure (3) below, the combination treatment with 5 µM CQ and 1,0.5,0.1 µM Camp resulted in a dose-dependent cytotoxic effect in both MCF7 and A549 cells ($P < 0.001$). In MCF7 cells, the toxicity ranged from 17% to 52%, whereas in A549 cells, it ranged from 18% to 55%, with A549 cells showing slightly higher sensitivity. Dose–response and half-maximal inhibitory concentration (IC50) analyses further confirmed that the inhibitory activity of the CQ-Camp combination was greater than that of the individual treatments, suggesting that the combination treatment inhibited cancer cell growth.

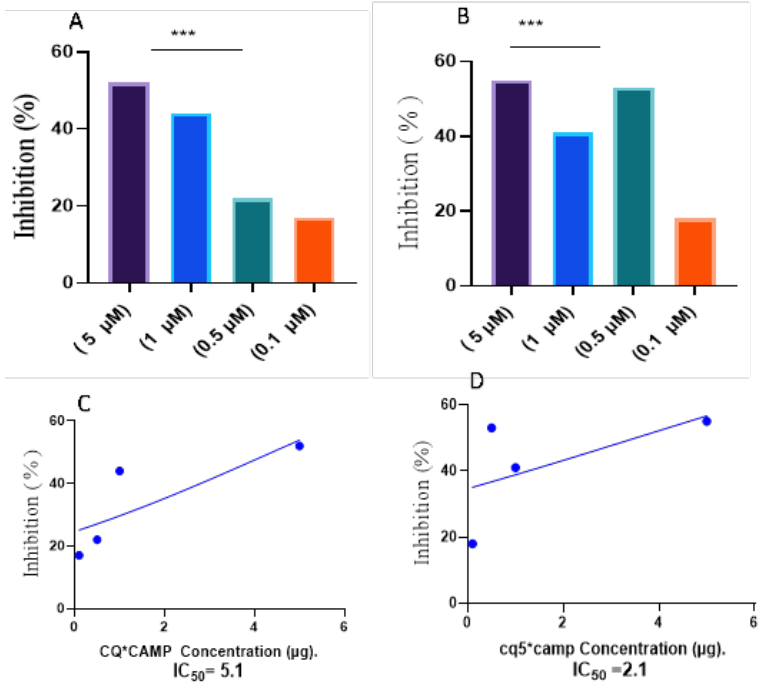

Figure 3: The cytotoxicity of combination therapy with cloroquien (5 µM) and camptothecien (1, 0.5, and 0.1 µM) after 72 hours in (A) the MCF7 cell line, (B) the A549 cell line, (C) the A549 cell line (IC50= 5.1), and (D) the A549 cell line (IC50=2.1).

The data in Figure (4) below explain that combination treatment with CQ (10 µM) and Camp (1, 0.5, and 0.1 µM) for 72 h significantly increased the cytotoxicity of both MCF7 and A549 cells in a dose-dependent manner ($p < 0.001$), with A549 cells showing slightly greater sensitivity. Dose–response and half-maximal inhibitory concentration (IC50) analyses revealed a rapid increase in inhibition at lower concentrations followed by a plateau, indicating that compared with single-agent treatments, the synergistic effects of CQ–Camp strongly inhibit potency and cytotoxic effects.

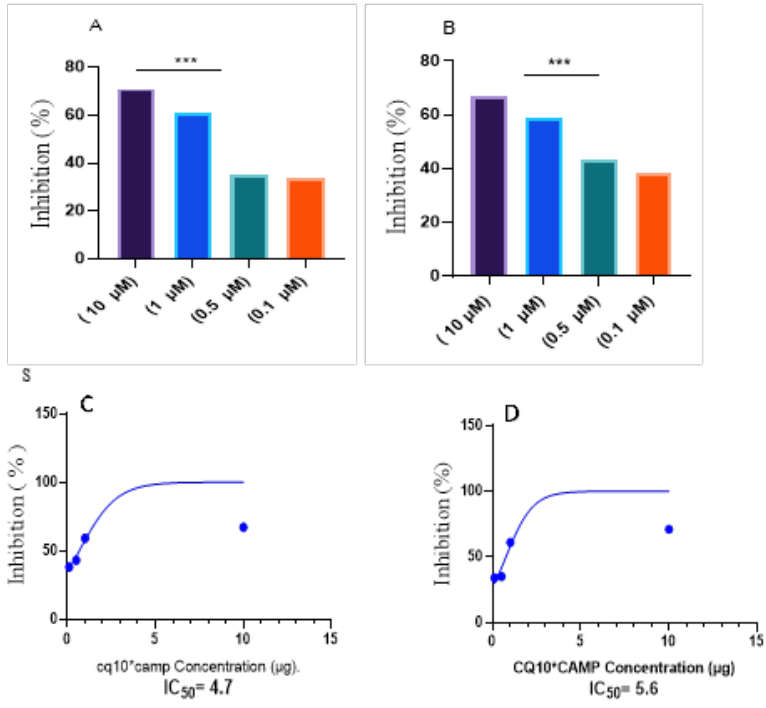

Figure 4: The cytotoxicity of combination therapy with cloroquien (1 µM) and camptothecien (1, 0.5, and 0.1 µM) after 72 hours in (A) the MCF7 cell line, (B) the A549 cell line, (C) the A549 cell line with an IC50 of 5.6 for combination therapy, and (D) the A549 cell line with an IC50 of 4.7.

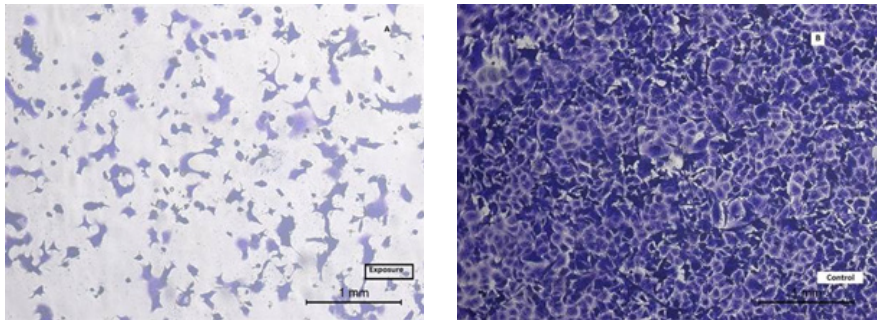

Figure 5: Microscopy image of the A549 cell line after 72 hours of the cytotoxicity assay. (B) Image control of the (A 549) cell line. (A) Image of (A 549) cell line inhibition at a concentration of CQ10* µM *1 µM Camp (microscope magnification power 200X).

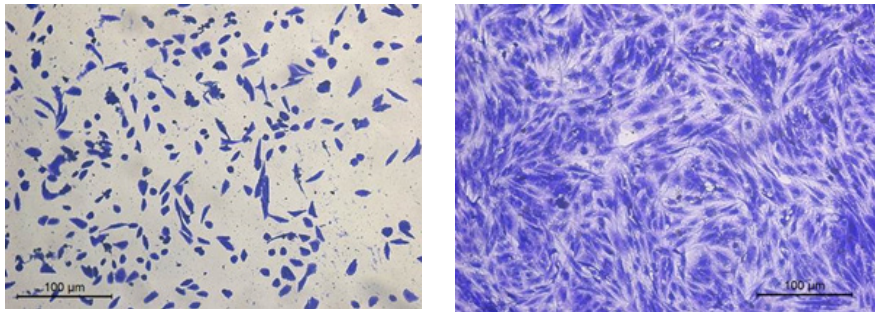

Figure 6: Microscopy image of the (MCF7) cell line after 72 h of the cytotoxicity assay. (B) Control image of the MCF7 cell line. (A) Image of (MCF7) cell line inhibition at a concentration of 5 µM CQ; *camp, 1 µM (200X magnification).

Combined treatment with chloroquine and camptothecin reduces colony formation

The surviving fraction (SF) of the MCF-7 and A549 cells was assessed using a colony formation assay. Untreated control cells exhibited high clonogenic survival, with SF values normalized to 1.00. Combined treatment with chloroquine (CQ) and camptothecin (CAMP) resulted in a marked reduction in the surviving fraction in both cell lines. In MCF-7 cells, the SF concentration decreased to approximately 0.55 following treatment with CQ (5 µM/ml) plus CAMP (1 µM) and further decreased to approximately 0.16 with CQ (10 µM) plus CAMP (1 µM). Similarly, A549 cells presented SF values of approximately 0.56 and 0.15 at the corresponding treatment concentrations. These results indicate a concentration-dependent suppression of clonogenic survival in both cell lines

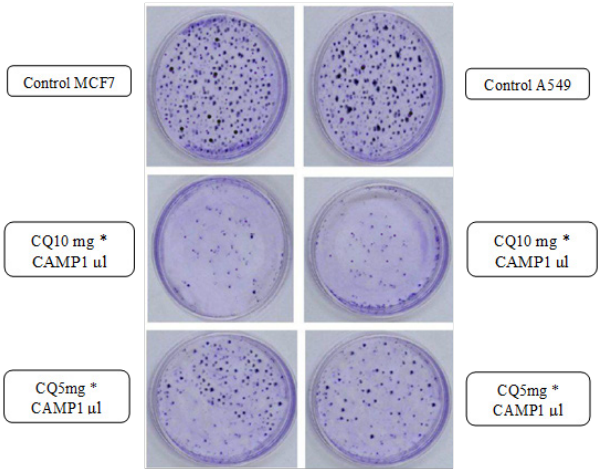

Figure 7: Photographic representation of the results of the colony formation assay in MCF-7 and A549 cells after treatment with the combination of chloroquine and camptothecin.

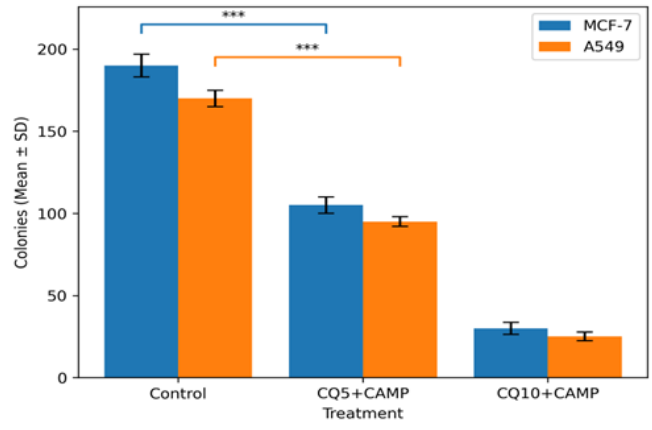

Figure 8. Colony formation in MCF-7 and A549 cells after treatment with chloroquine (CQ) and camptothecin (CAMP). The data are shown as the mean ± standard deviation. ***$p < 0.001$ compared with the control group.

Discussion

The results of the current study revealed that A549 lung cancer cells were more sensitive to chloroquine (CQ), whether it was used alone or in combination with camptothecin (CAMP), than MCF-7 breast cancer cells were. This difference was consistently observed in both short-term cytotoxicity tests and long-term colony-forming analysis, highlighting a clear variation in the cell response to treatment depending on the cell type. Similar observations have been reported in lung cancer models, which often show greater sensitivity to chemical stress than breast cancer cells do [20, 21].

The results of the cytotoxicity test revealed that the A549 cells had greater inhibition rates and smaller IC_{50} values than the MCF-7 cells did, indicating that the former were more sensitive to drug-induced inhibition. Large-scale analysis of the drug sensitivity of cancer cells revealed that cancer cells of different origins differ significantly in terms of their treatment response. In particular, breast cancer cells such as MCF-7 cells are relatively resistant to treatment [22].

The data obtained from the colony formation test further confirmed the observations above, with a significant reduction in both the number and size of the colonies produced by A549 cells following exposure to chloroquine, suggesting substantial inhibition of their proliferation potential in the long term. On the other hand, compared with control cells, MCF-7 cells still had some potential for producing colonies even following exposure to chloroquine, which suggests that certain cells were resistant to proliferation inhibition. The findings obtained from these experiments are in agreement with those of previous studies demonstrating that colony formation tests can be used to successfully detect growth inhibition [23-25].

Notably, the combination of chloroquine and CAMP led to a significant increase in the anticancer effects of the drugs in both types of cells. Compared with the individual application of each agent, the individual application of each agent significantly inhibited cancer cell growth and proliferation in terms of the viability and number of colonies. However, the difference between the groups was more noticeable when A549 cells were used, indicating that cancer cell proliferation nearly stopped. The observed results are in accordance with the data concerning the efficacy of combination therapy with DNA-damaging agents [26-28].

In summary, the more prominent reaction in A549 cells is attributed to inherent cellular properties that render lung cancer cells less capable of enduring repeated stress induced by drugs, whereas MCF-7 cells tend to have an increased level of resistance. Thus, the effectiveness of the treatment should be determined on the basis of both short- and long-term assessments. It is imperative that therapeutic interventions targeting cancer cells incorporate combinations that take into consideration their biological traits [20-28].

Conclusion

The presence of chloroquine (CQ) caused a significant reduction in the cell viability of MCF-7 breast cancer cells and A549 lung cancer cells, where A549 cells were more sensitive to the drug than MCF-7 cells were. The combination effect of chloroquine with camptothecin (CAMP) produced a dose-dependent increase in cell-killing activity and a reduction in the IC_{50} value compared with those of single drug use, which suggests that the effectiveness of anticancer treatment was enhanced. These findings suggest that chloroquine is an effective sensitizer for chemotherapy, especially in lung cancer cells [20-28].

Author Declarations

Acknowledgments

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Funding Statement

This research received no external funding.

Conflict of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

Ethics Statement

This study was conducted exclusively using established in vitro human cancer cell lines (MCF-7 and A549) obtained from the Cell Bank Unit of the Iraqi Center for Cancer and Medical Genetics Research, Mustansiriyah University. No human participants, patient samples, or live animals were involved. Therefore, formal ethical approval and informed consent were not required for this study.

Consent for Publication

Not applicable.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions

All authors contributed equally to this work and have read and approved the final version of the manuscript.

Supplementary Materials

No supplementary materials are associated with this manuscript.

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