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CURCUMIN BOOSTS DOXORUBICIN CYTOTOXICITY IN BREAST CANCER CELLS

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Background. *Breast cancer remains the leading cause of cancer mortality in women due to the resistance to chemotherapy and severe side effects of doxorubicin (Doxo). One of the approaches to overcome this problem is to search for phytocompounds that can enhance the efficacy of chemotherapy and prevent the development of side effects.* ***Objective.*** *This work aimed to investigate in vitro whether the polyphenol curcumin (Cur) from turmeric (Curcuma longa) in combination with the commercial drug Doxo-HCl can enhance the cytotoxic effect of Doxo on breast cancer cell lines MDA-MB-231 and MCF-7 and to exhibit chemoprotective activity against normal HEK-293 cells.* ***Methods.*** *Cell viability was assessed with MTT assay, apoptosis induction by flow cytometry with Annexin V–eGFP and PI, reactive oxygen species (ROS) generation by the DCFH-DA probe. The combination index (CI) calculation method and CompuSyn, Biosoft software were used to determine the synergism or antagonism of the components.* ***Results.*** *The study revealed dose-dependent cytotoxicity, increased ROS formation, and increased morphological aberrations in cells when using the combination of Cur with Doxo-HCl compared to Doxo-HCl. Cur acted as a chemosensitizer, which synergistically enhanced the antitumor activity of Doxo-HCl while simultaneously reducing its cytotoxic effects in normal cells by reducing ROS production.* ***Conclusions.*** *The use of Cur in combination with Doxo-HCl will likely reduce the effective therapeutic dose of Doxo and increase the effectiveness of breast cancer chemotherapy.*

K e y w o r d s : breast cancer cell lines, curcumin, doxorubicin, combined treatment, combination index, chemosensitizer, cell viability, oxidative stress, apoptosis.

Breast cancer is the most frequent cancer and the primary cause of cancer death in women worldwide. The capacity of breast cancer cells' heterogeneous population to proliferate and invade is linked to increased aggressiveness and poor clinical outcomes. According to the WHO statistical analysis in 2022, breast cancer caused an estimated 670,000 deaths globally in 2022 in 185 countries [1]. Current breast cancer therapies focus on receptor targeting, including endocrine and anti-HER-2 mAbs approaches, as personalized treatment strategies [2]. In addition to chemotherapy and radiotherapy, these therapeutic approaches are associated with significant adverse effects, and patients may develop resistance to them. Furthermore, triple-negative breast cancer (TNBC) lacks standardized treatment protocols. Accounting for 15–20% of breast cancers, TNBC is characterized by aggressive biological behavior, including elevated proliferative indices, high histological grade, and early dissemination to distant organs such as the brain and lungs, which collectively contribute to poor clinical prognosis [3]. Treatment strategies for TNBC are restricted, with cytotoxic chemotherapy, predominantly anthracy-

clines and taxanes, serving as the mainstay of therapy. Thus, deep insight into developing specific and effective therapies for each breast cancer subgroup is crucial.

Doxorubicin (Doxo) exhibits significant antineoplastic activity and is recognized as one of the most effective FDA-approved chemotherapeutic drugs for managing breast cancer, various carcinomas, sarcomas, and hematological malignancies [4]. Doxo was first isolated from the bacterium *Streptomyces peucetius* var. *caesius* in the 1960s by Italian researchers and quickly became a medical outpost [5]. Despite extensive clinical utilization, the mechanisms of action of Doxo remain under intense debate. Doxo is a double-edged sword: while effective against cancer, its off-target toxicity limits dosing and reduces patient quality of life [6]. The mechanisms underlying the effects on cardiac tissues and systemic cytotoxicity have been intensively investigated [7]. Cardiac tissue is especially vulnerable, though the brain, kidneys, and liver are also affected. Furthermore, like other chemotherapeutic agents, cancer cells are capable of acquiring multidrugdrug resistance (MDR), which significantly limits the effectiveness of Doxo [8].

To combat MRD, combination regimens of DNA-incorporating agents such as anthracyclines, methotrexate, cyclophosphamide, and 5-FU are employed to enhance cytotoxic efficacy, even at reduced concentrations. Molecules capable of enhancing tumor cell susceptibility to conventional therapies and reducing cell survival under these conditions are referred to as chemosensitizers [9].

Curcumin (diferuloylmethane) is a naturally yellow-orange polyphenolic compound found in the rhizome of turmeric (*Curcuma longa* L.), a plant of the ginger family (*Zingiberaceae*). Turmeric is cultivated primarily in India, China, and other Southeast Asian countries, and it is traditionally used as a culinary spice (the main ingredient in curry powder), a dye, and a medicine. Over 4,000 preclinical *in vitro* and animal studies have documented the antioxidant, anti-inflammatory, antiviral, and antitumor activities of Cur [10]. Mechanistic investigations demonstrate that Cur suppresses proliferation, invasion [11], angiogenesis, and metastasis across diverse cancers by modulating multiple cell signaling pathways [12]. A lot of experimental studies have highlighted curcumin's potential to inhibit chemoresistance through the downregulation of oncogenic signaling pathways, including MMP-2, TGF-β, EMT, PI3K/Akt, NF-κB, and AP-1 [13–15]. These findings suggest its potential utility in the prevention of malignancies such as multiple myeloma, colorectal, pancreatic [16], breast [17], prostate, and lung cancers in both animal models and humans.

This study focuses on cell death in breast cancer cell lines after the combined use of Doxo and Cur to evaluate the potential synergistic action and cytotoxicity, and the chemosensitizing effect of Cur, which may increase the effectiveness of conventional anthracycline-mediated anticancer therapy.

Materials and Methods

Models, reagents, and solutions. MDA-MB-231 ($ER_{(-)}$, $PR_{(-)}$, HER-2/$neu_{(-)}$), MCF-7 ($ER_{(+)}$, $PR_{(+)}$, HER-2/$neu_{(-)}$, Ki-67^{low})and HEK-293 (human embryonic kidney) cell lines were obtained from the Bank of Cell Lines from Human and Animal Tissues of the R.E. Kavetsky Institute of Experimental Pathology, Onco-logy and Radiobiology of the NAS of Ukraine (Kyiv, Ukraine) RPMI-1640, Trypsin-EDTA (0.25%) and qualified FBS were from Sigma-Aldrich and Gibco. Propidium iodide (P1304MP, ThermoFisher), Cur (C1386, Sigma-Aldrich) and Doxo-HCl (Arterium TM, Kyivmedpreparat) were used. Genetic constructions encoding recombinant Annexin-V-eGFP were obtained earlier in our lab and have been employed in comparable research [18, 19].

Cell culture maintenance. MDA-MB-231, triple-negative breast cancer cells -, MCF-7 breast cancer cells, and HEK-293 cell lines were sub-culturing and passaging as monolayers in cell culture flasks (Greiner Bio-One, Germany) maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2, in RPMI-1640 media supplemented with heat inactivated 10% FBS and antibiotics: 100 mg/l streptomycin, 10 000 U/l penicillin G and 250 mg/l amphotericin B. Cells were harvested for further experiments using 0.25% trypsin-EDTA or 30 mM EDTA in PBS when the cells reached ~80% confluence.

Preparation of drug combination. Powdered Cur (C1386, Sigma-Aldrich) and Doxo-HCl (Arterium TM, Ukraine) were dissolved in 0.5% DMSO and stored at 4°C. The combination of Cur and Doxo-HCl was made using the combination index (CI) method according to [20] using formula (1):

$$CI = \frac{IC50amix}{IC50a} + \frac{IC50bmix}{IC50b} .$$

Obtained CI values equal, smaller, or greater than 1.0 indicate an additive, synergistic, or antagonistic effect, respectively [20].

CompuSyn (Biosoft, Cambridge, UK) software is also used to automatically determine the synergism or antagonism of multiple drugs at any effective combination dose.

Cell viability assay. The cytotoxic and anti-proliferative effects of Cur, Doxo-HCl, and their combination were evaluated in MDA-MB-231, MCF-7, and HEK-293 cells using the MTT assay. Cells ($1{\times}10^4$/ml) in 100 µl were seeded into 96-well (F-bottom) plates and incubated overnight at ~70-80% confluency before drug treatment. Cur (10–100 µM) and Doxo-HCl (0.25–5 µM) were applied at varying concentrations to assess dose- and time-dependent viability (24 and 48 h). Combination doses were determined based on IC_{50} values using Quest Graph™ IC_{50} Calculator (AAT Bioquest Inc., USA). Results of cell viability are expressed as a percentage, based on the ratio of the absorbance of treated cells to that of the controls (100%) and calculated using formula (2):

$$\%\,Cell\ viability\ = \frac{OD\ of\ test\ average}{OD\ of\ control\ average} * 100\,.$$

Cell viability was quantified by adding MTT (3-(4,4-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) dye (5 mg/ml in RPMI-1640 medium free from phenol red), which is reduced by mitochondrial oxidoreductases to purple formazan crystals. Absorbance was measured at 570 and 630 nm using a Multiscan FC microplate reader (Thermo Fisher Scientific, USA).

Cell morphology assay. Cell morphology was examined under the Inverted Biological Microscope LB-11IVBM (Labotronics Scientific, USA), and images were captured and analyzed with NIS-Elements and ImageJ software.

Measurement of oxidative stress. Intracellular level of reactive oxygen species (ROS) generation was measured using the cell-permeable DCFH-DA dye (5-(-6)-carboxy-2,7-dichlorofluorescein diacetate). DCFH-DA is a non fluorescent probe that, upon deacetylation in viable cells, is converted into dichlorofluorescein (DCF) through the action of ROS. After 24 h of Cur, Doxo-HCl, and combined drug mix treatment, the control and treated cells MDA-MB-231, MCF-7, and HEK-293 were incubated with DCFH-DA (10 µM in PBS) for 30 min at RT (25°C) in the dark. Fluorescence was captured using a DxFLEX Flow Cytometer, FITC-A channel (Beckman Coulter, USA), and processed with Kaluza Analysis Software.

Determination of apoptosis stages. The percentage of viable, early and late apoptotic, and dead cells at different drug doses was quantified using a flow cytometer by Annexin V-eGFP and PI double staining system. After 48 h of treatment, the treated cells were stained with Annexin V-eGFP (2.5 µg) for 30 min and PI (1 µl) for 5 min at 4°C (dark), following they were analyzed by flow cytometry, with each determination based on the acquisition of 10,000 events.

Statistical data analysis. Data were analyzed using conventional methods of variation statistics, with the calculation of the $M \pm m$. One-way ANOVA followed by post hoc Tukey's multiple comparison test to evaluate differences between groups (e.g., control vs. Cur, control vs. Doxo-HCl, and Doxo-HCl vs. Cur+Doxo-HCl). For pairwise comparisons where appropriate, a Student's *t*-test was applied. Statistical calculations were done, and the results presented in the graphs were processed using MS Excel Software. Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

Cur and Doxo-HCl combination treatment affects cell morphology and viability. Graphical representations of dose dependent responses were evaluated following treatment with curcumin (Cur) and doxorubicin (Doxo-HCl) (Fig. 1). MTT assays revealed a progressive reduction in cell viability over 24 and 48 h, with the 48-hour exposure chosen for IC_{50} determination and subsequent morphology observation and PI/AnV-eGFP staining analyses. The IC_{50} values were calculated as 50 µM for Cur and 2.25 µM for Doxo-HCl for MDA-MB-231 cells and 55 µM for Cur and 2.0 µM for Doxo-HCl for MCF-7 cells (Table 1).

Synergistic interactions were further calculated and confirmed using CompuSyn software and the SynergyFinder+ tool, both of which are often -used in biomedical research. CI, which analyzed the combined drug concentration against MDA-MB-231 cells, was evaluated.

Remarkably, when the Cur was added at 32.8 µM in combination with Doxo-HCl (0.3 µM), a dramatic decrease in malignant cells' survival was observed; thus, a synergistic effect was evident (CI = 0.79, CI < 1 – synergism, according to formula 1). Therefore, in these cases, the Cur enhanced

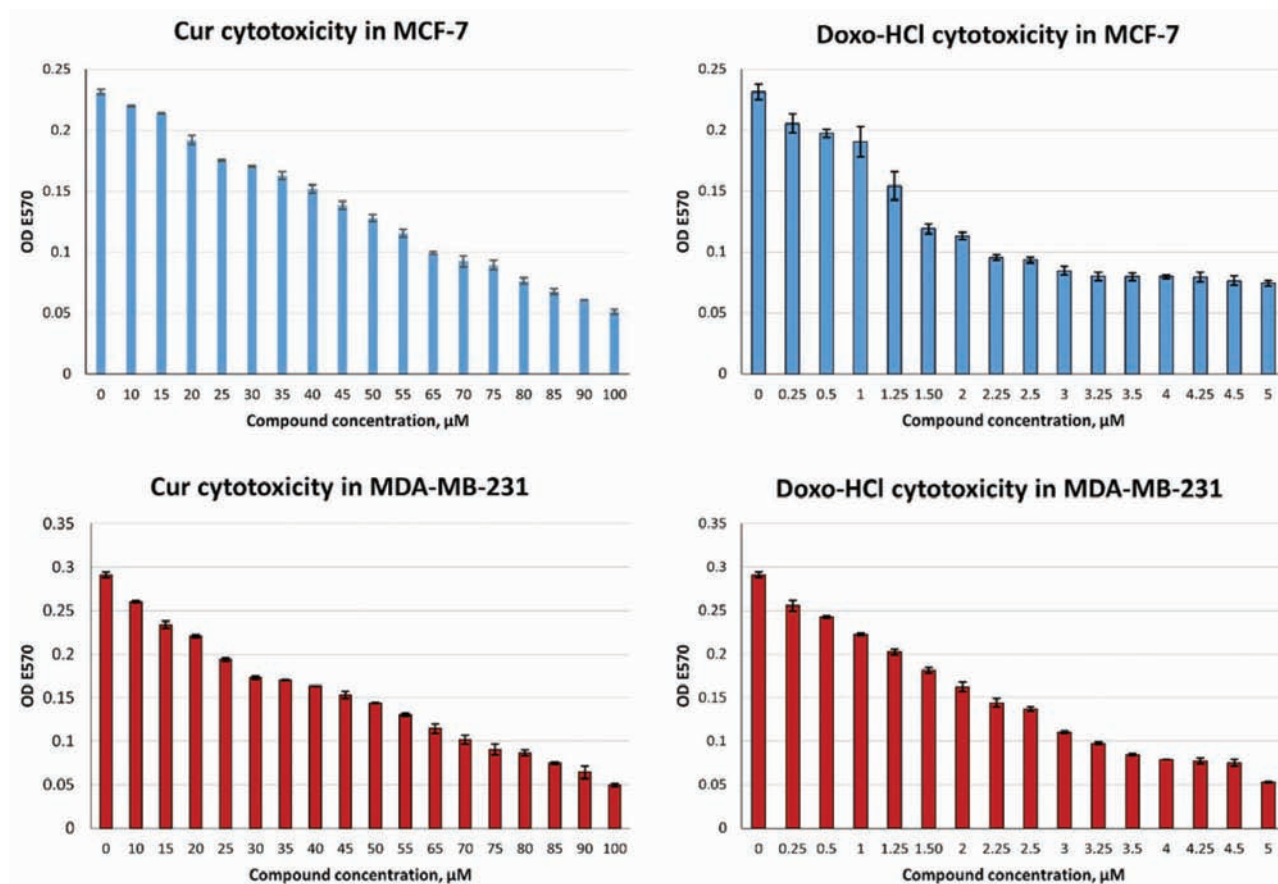

Fig. 1. Evaluation of Doxo-HCl and Cur cytotoxicity against MCF-7 and MDA-MB-231 breast cancer cells according to MTT results (48 h). M ± SD, n = 12 for each concentration, P < 0.05 compared to control (0 µM)

Table 1. IC_{50} and combined CI concentration values for analyzed substances that affect MCF-7 and MDA-MB-231 cell viability as single compounds and in mixture

Substance	IC_{50}, µmol/l	
	MDA-MB-231	MCF-7
Doxo-HCl (*a*)	2.25 ± 0.008	2.0 ± 0.049
Cur (*b*)	50.0 ± 0.024	55.0 ± 1.510
Doxo-HCl:Cur (*amix*)	0.3	n/a*
Doxo-HCl:Cur (*bmix*)	32.8	n/a*

Note. *Effective combined concentrations for MCF-7 and their CI were not calculated.

the effects of Doxo-HCl, leading to greater toxicity in tumor cells while decreasing Doxo-HCl concentration. This synergistic effect of 32.8 µM Cur and 0.3 µM Doxo-HCl is supported by the cell viability MTT assay results, which demonstrated their significant cytotoxicity for both cancer cell lines compared to a Single Doxo-HCl dose (Fig. 2).

Morphological alterations observed after 48 h of single or combined treatment are presented in Fig. 3(*A*, *B*), where untreated cells retained normal adherence and surface features. In contrast, MDA-MB-231 cells treated with IC_{50} of Cur and Doxo-HCl exhibited rounded morphology, while the highest proportion of non-adherent, rounded, and floating MDA-MB-231 cells appeared under the combination of 32.8 µM Cur and 0.3 µM Doxo-HCl, consistent with apoptotic induction, including cell shrinkage and plasma membrane blebbing, which appeared within 12 h and culminated in complete cell death by 64 h. Notably, HEK-293 cells exposed to the

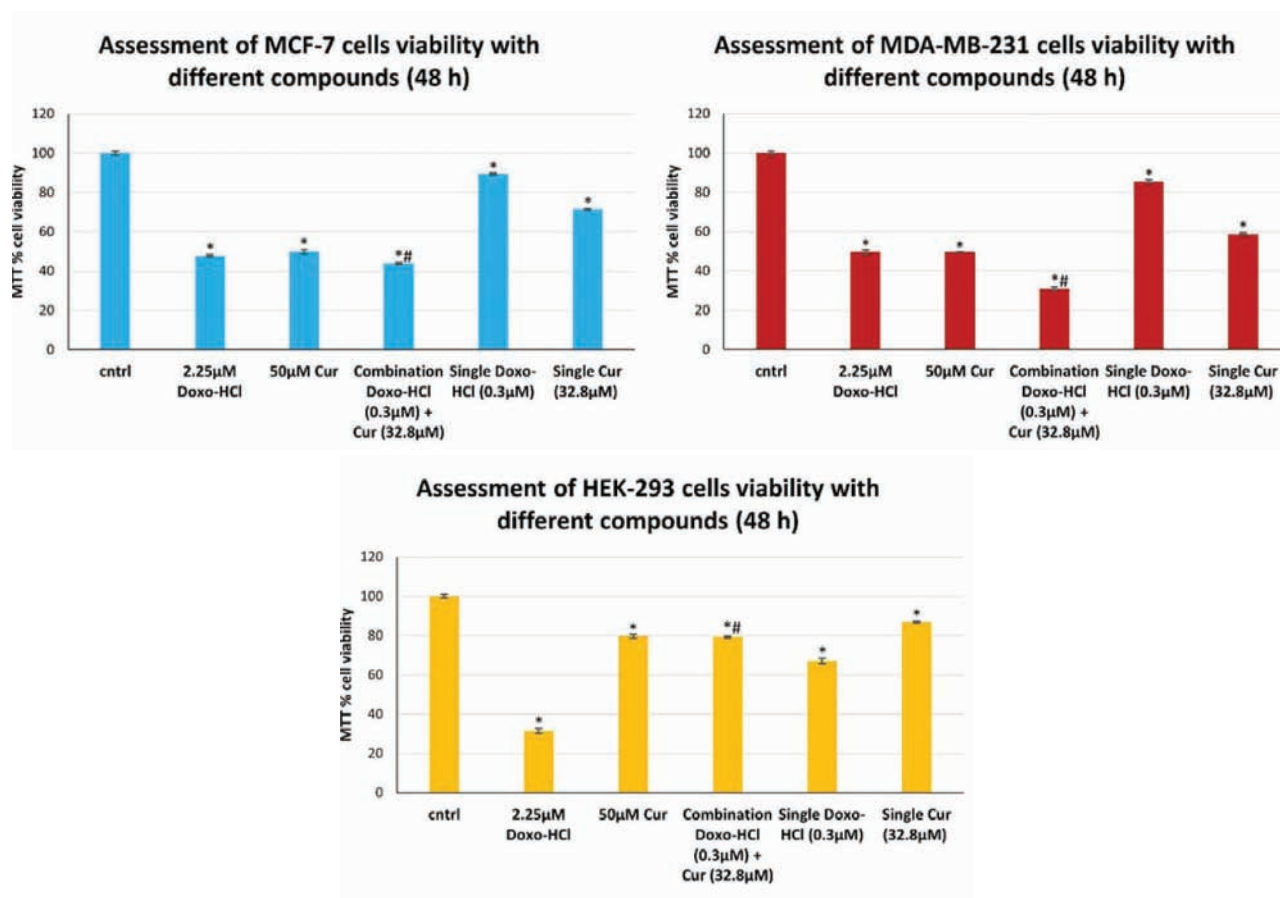

*Fig. 2. Cell viability assessment of MCF-7, MDA-MB-231 and HEK-293 cells with different drug doses (48 h); *P < 0.05 vs. contrl, #P < 0.05 vs. Single Doxo-HCl, n = 12*

drug combination exhibited no marked changes in morphology (Fig. 3, *B*) or viability, underscoring the chemoprotective role of Cur against Doxo-HCl-induced toxicity.

Cur exerts chemoprotective efficacy on non-malignant cells in combined Cur and Doxo-HCl treatment. Firstly, the loading of Cur and the Doxo derivative Doxo-HCl at a concentration equal to IC_{50} for MDA-MB-231 cells vs. against HEK-293 was assessed using the MTT assay. A general reduction in cell viability and a decrease in the number of live cells were observed for Doxo-HCl. Therefore, this chapter aimed to investigate whether the antioxidant Cur can reduce Doxo-induced ROS generation and cell damage in normal cells.

Despite Cur enhances Doxo-induced apoptosis in MDA-MB-231 cells, evidence of chemoprotective activity was further established in the normal embryonic kidney HEK-293 cell line (Fig. 2).

Cur and Doxo-HCl co-treatment independently stimulates ROS generation in cancer cells. To evaluate the effects of pro(anti)oxidant Cur on oxidative stress in MDA-MB-231 cells induced by Doxo-HCl in MDA-MB-231 cells, the fluorescent dye, DCFH-DA, was used to measure intracellular ROS generation. TNBC MDA-MB-231 cells were treated with a combination of pro(anti)oxidant Cur and Doxo-HCl simultaneously. Results showed that Doxo-HCl cotreated with Cur (32.8 μM) for 24 h significantly increased intracellular ROS levels compared with control cells (Fig. 4). Fluorescent right shift in flow cytometry data, indicating highly elevated intracellular ROS in combination dose-treated MDA-MB-231 cells (24 h), was evaluated. Moreover, Doxo-HCl and Cur applied simultaneously at chosen doses showed more ROS production than when using the singleeither Doxo-HCl or Cur at a concentration equal to IC_{50}.

However, increases in ROS production induced by Doxo-HCl with Cur for 24 h of treatment were significantly reduced in HEK-293 cells. In contrast, Cur failed to prevent ROS generation in BC cells treated with Doxo-HCl, which indicates Cur's dichotomous properties.

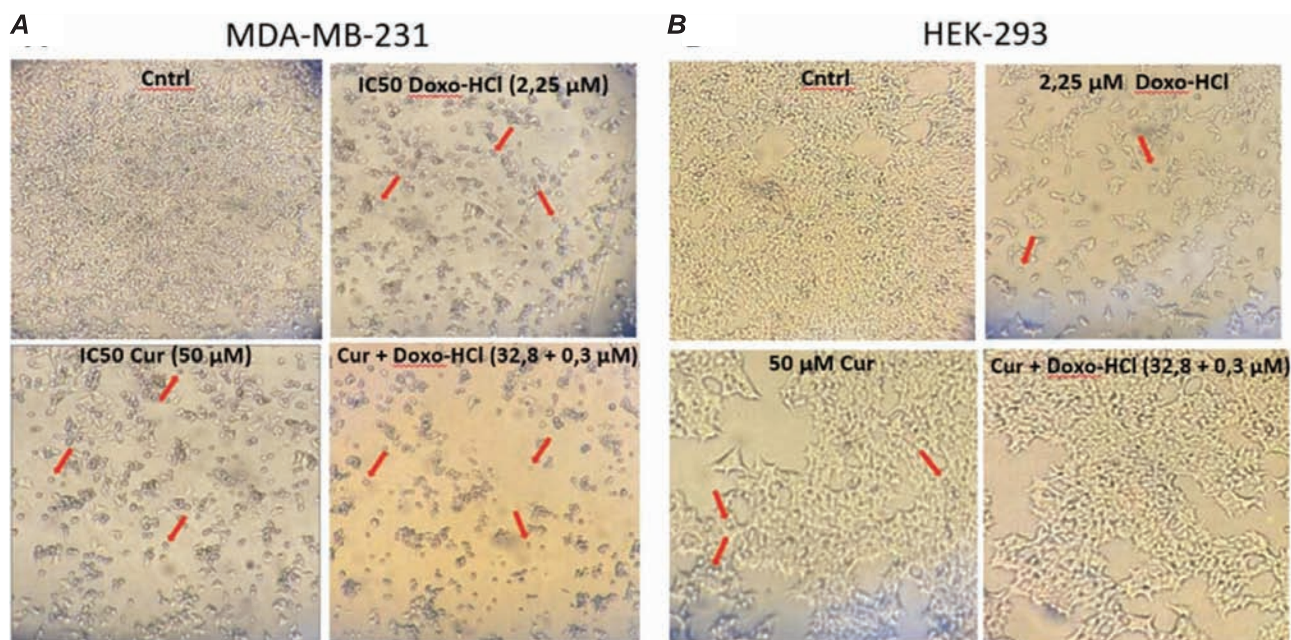

*Fig. 3. **A** – Microphotographs illustrating the synergistic cytotoxicity of combined Cur (32.8 µM) and Doxo-HCl (0.3 µM) co-treatment in MDA MB 231 cells after 48 h. **B** – Microphotographs demonstrating the chemoprotective effect of Cur in the same combination treatment against HEK-293 cells after 48 h. Red arrows indicate apoptotic or dead cells*

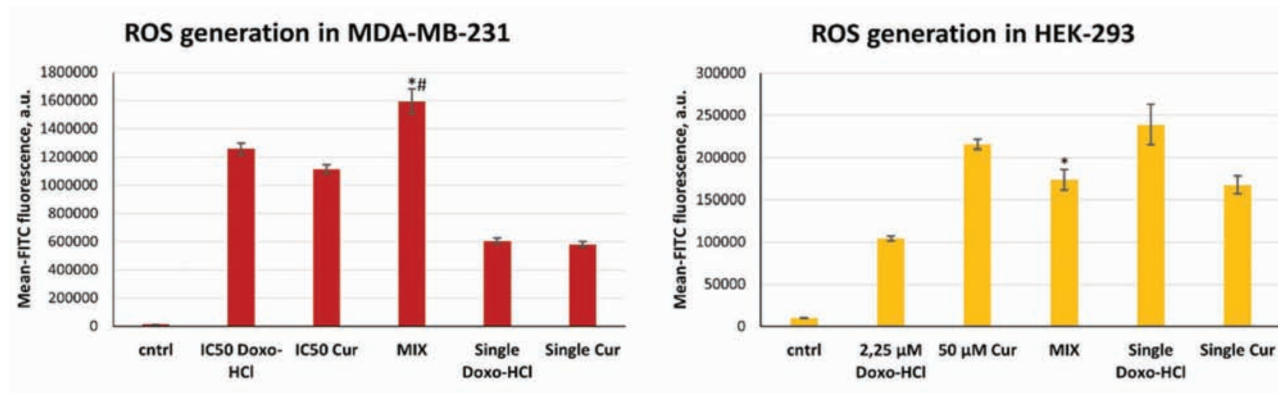

*Fig. 4. Graphical representation of mean DCFH-DA fluorescence in MDA-MB-231 and HEK-293 cells subjected to varying treatment. Data are presented as M ± SD, n = 12; *P < 0.05 vs. Single Dox, $^{\#}P < 0.05$ vs. Single Cur*

Quantitative assessment of apoptotic phases in TNBC cell line MDA-MB-231. To calculate the number of events corresponding to each cell subset and apoptotic stage, specific cell populations were gated on a bivariate dot plot corresponding to the PI to DNA in nuclei intercalation in damaged cells versus annexin V-eGFP binding to phosphatidylserine exposed on the outer membrane of apoptotic cells (Fig. 5).

The Cur (32.8 µM) and Doxo-HCl (0.3 µM) co-treatment (MIX) caused a significant ($P < 0.05$) increase in AnV/PI positive cells at 48 h post-exposure (70.93%), which was more than when using Single Dox (22.66%) or Single Cur (32.98%). Moreover, the drug combination increased cancer cell death byat 14.32 and 41.44% compared to the IC_{50} Doxo-HCl and IC_{50} Cur, respectively, while decreasing the Doxo-HCl concentration by 7.5 times.

Aggressive biological behavior of some breast cancer subtypes (TNBC, etc.) aggressive biological behavior is a major determinant of the limited survival period after diagnosis. The exploration of

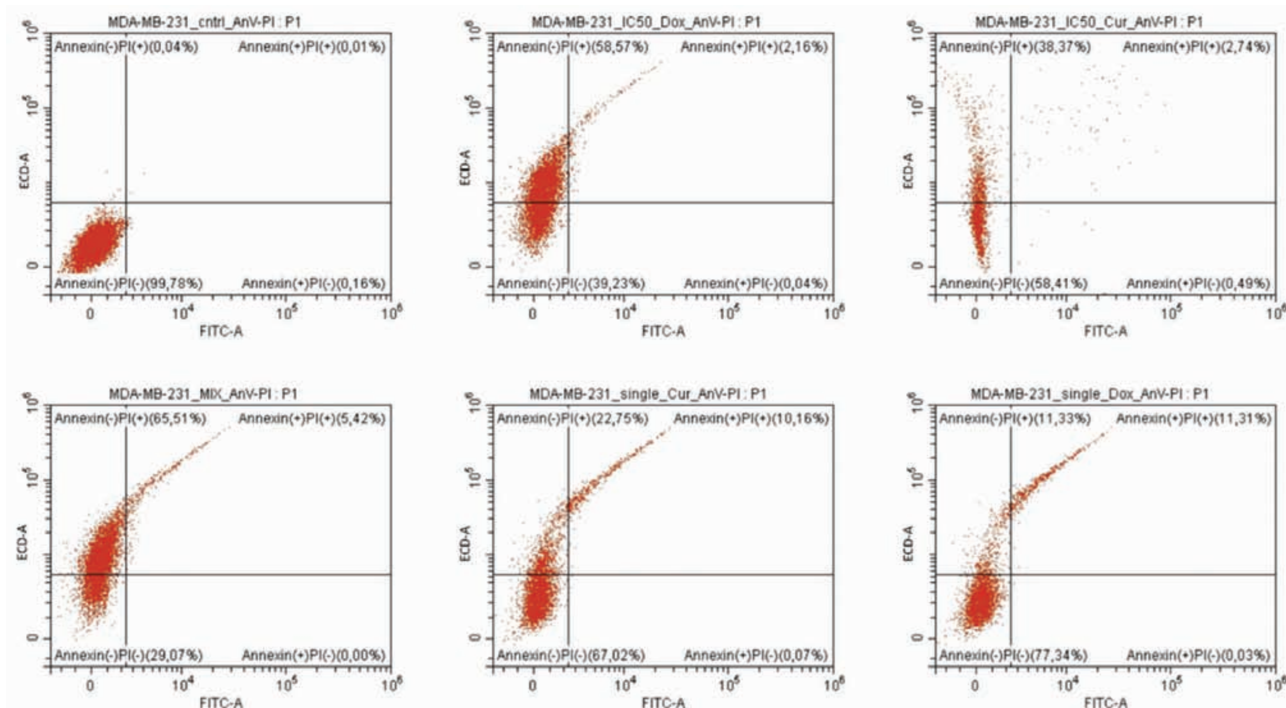

Fig. 5. Annexin V-eGFP/PI distribution in TNBC MDA-MB-231 cells with varying treatment. Cell distribution assay was performed by flow cytometry (48 h)

natural products continues to provide vital leads for anticancer drug development. This study was designed to investigate the impact of the well-known plant-based polyphenolic compound Cur as a complementary and chemosensitizer compound on the TNBC MDA-MB-231 and $ER_{(+)}PR_{(+)}$ MCF-7 cell lines. Among the two cancer cell lines studied, the MCF-7 cell line is considered less invasive. In contrast, MDA-MB-231 is more aggressive and invasive than other breast cancer subtypes. Furthermore, the pharmacological interactions between Cur derivative and standard chemotherapeutic agent Doxo were examined to evaluate their potential utility in the treatment of TNBC or hormone therapy-sensitive BC patients. The therapeutic benefit of plant polyphenols in cancer treatment lies in their capacity to suppress tumor growth and mitigate side effects and drug resistance when combined with conventional chemotherapeutics [21].

Cur is widely recognized for its cytotoxic effects on certain cancer cell types [22, 23]. Nevertheless, its relatively low efficacy has limited direct therapeutic application, prompting ongoing investigations aimed at improving its utility through strategies such as enhancing bioavailability and structural modification [24].

Doxo is effective against various cancers, but its systemic toxicity restricts limits its therapeutic application, and certain antioxidant agents may be beneficial in alleviating its toxicity. In this context, Cur and its analogues need not exhibit strong standalone cytotoxicity; rather, they should act on specific molecular targets within cancer cells, particularly those associated with MDR.

In our study, both Cur and Doxo-HCl have shown high cytotoxic and anti-proliferative dose-dependent effects in MCF-7 and MDA-MB-231 cells. Further studies on the Cur's effect on the non-malignant HEK-293 cells did not reveal a dramatic decrease in cell viability, which reached 20% when MDA-MB-231 cells were exposed to the Cur IC_{50} concentration. In contrast, Doxo-HCl, which was used as a primary chemotherapeutic compound, showed the same cytotoxicity against both normal and cancerous cells.

Taking all this evidence, in the presented study, we characterized the potential anticancer activity of a Cur + Doxo-HCl combination on breast cancer cell lines. Regarding the activity of individual compounds against BC *in vitro*, the results obtained in the co-treatment assay showed that Cur (32.8 μM) has a higher efficacy than its IC_{50} dose (50 μM)

against the same cells. Combination treatment with lower doses but simultaneously action of Cur and Doxo-HCl decreases cell viability byin 1.63 and 2.04 times for MCF-7 and 1.9 and 2.76 times for MDA-MB-231, respectively, than single individual treatment doses of Cur and Doxo-HCl . Moreover, Cur (32.8 μM) and Doxo-HCl (0.3 μM) co-treatment by doses calculated by CompySun software and SynergyFinder + tool, synergistically affects both cancer cell line subtypes, and CI was was equal to 0.79 (CI < 1 – synergism). It should be emphasized that combined treatment by Cur (32.8 μM) and Doxo-HCl (0.3 μM) increases cancer cells' death in both 1.6 times vs. IC_{50} (2.25 μM) Doxo-HCl and IC_{50} (50 μM) Cur for MDA-MB-231, while this negligible or lower effect (1.08 vs. IC50 Doxo-HCl and 1.13 times vs. IC_{50} Cur) is observed for MCF-7. Taking into account the stronger synergistic action of the drug combination precisely toward MDA-MB-231, it is an aggressive behavior of this type of cancer with limited treatment options, significant challenges due to the lack of specific target receptors, high recurrence rates, and pronounced molecular heterogeneity [25], only TNBC was chosen for further DCFH-DA and AnV/PI staining analysis.

An additional finding was the inverse relationship between the generation of ROS and the viability of MDA-MB-231 cells under combined treatment. Doxo-HCl was found to enhance the ROS production in both HEK-293 and MDA-MB-231 cells.

Combined drug application demonstrated higher efficacy in ROS generation byin 2.63 and 2.75 times vs. Single Dox and Single Cur, respectively, in MDA-MB-231 cells. Those effects were also compared with the IC_{50} of Doxo-HCl and Cur drug doses ROS yielding, where higher cellular oxidative stress loading was still observed under the combined action of the drugs. Interestingly, co-treatment of HEK-293 cells with pro(anti)oxidants Cur and Doxo-HCl for 24 h showed a decrease in Doxo-HCl-induced ROS levels. So, Cur may serve as a potential protective agent against Dox-induced toxicity through a mechanism that involves it ROS-scavenging properties and playsand plays a role in preventing cellular damage caused by ROS. Our findings indicate that Cur exerts both pro and antioxidant properties.

To investigate in more detail the effects of the Cur + Doxo-HCl combination on cell apoptosis, a confirmatory flow cytometry assay was performed. The effect on the cells' morphology in the 6-well plate was observed after 12 h of incubation with the compounds and their combination, while the late apoptotic effect was more pronounced after 48 h, particularly with the drug combination. Indeed, after 64 h, almost the entire population was detached from the TC plastic. A remarkable increase in cellular apoptosis was observed in the combination treatment, where only 29.07% of cells were found viable, 5.42% cells demonstrated morphological features consistent with late apoptosis, and the remaining 65.51% were dead cells. In the single doses of Cur and Doxo-HCl, a reduction in apoptosis was visible. In addition, longer incubation times led to complete cell death. Evaluation of the combination dose in HEK-293 cells revealed minimal toxicity, confirming Cur's chemoprotective role against Doxo-HCl.

Combination therapies involving Cur with other chemotherapeutic agents, such as Doxo-HCl, allowed for the sensitization of BC cells in vitro and the reduction in drug doses, thereby minimizing anthracycline antibiotic side effects.

Conclusions. In conclusion, our study demonstrates the benefits of combined treatment with the pro(anti)oxidant Cur and the conventional chemotherapeutic agent Doxo-HCl: reducing systemic cytotoxicity caused by Doxo-induced ROS and synergistic anticancer effects (CI < 1 according to the combined effect). Cur is a useful modulator to enhance the antitumor activity of Doxo-HCl while reducing the adverse effects of cytotoxicity by decreasing ROS production in normal untransformed cells. We expect that the addition of Cur may enable a reduction in the effective dose of Doxo and enhance the quality of life in patients with breast cancer. In the future, we hope that this pro(anti)oxidant and its derivatives (curcuminoids) can serve as potential complementary or alternative medicine for the integrative cancer therapies.

Study limitations. This study has several methodological constraints. First, the experiments were conducted exclusively *in vitro*, which does not fully replicate the tumor microenvironment or systemic drug metabolism. Second, while MCF-7 and MDA-MB-231 represent luminal and triple negative breast cancer subtypes, they do not capture the full heterogeneity of breast cancer, and HEK 293 cells, though widely used as a non malignant comparator, are not tissue matched to breast epithelium. Finally, the study focused on viability, morphology, apoptosis, and ROS generation, without exploring deeper molecular mechanisms such as signaling pathways or gene expression changes. Moreover, the clinical

application of curcumin is hindered by its limited bioavailability.

Author contributions. AAS coordinated and conducted the experiments, researched data, analyzed the results, software, drafted the manuscript, paper conceptualization; MIL. conducted the experiments, validation, drafted the manuscript; SIR – participated in interpreting the results, resources, visualization, review and editing; AJL – analyzed the results, visualization, review and editing; I-MMK – conducted the experiments, statistical data analysis, drafted the manuscript; OSM – analyzed the results, review, and editing; DVK coordinated project funding, study conceptualization, supervision, review, and editing. All authors read and approved the final manuscript.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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КУРКУМІН ПОСИЛЮЄ ЦИТОТОКСИЧНІСТЬ ДОКСОРУБІЦИНУ В КЛІТИНАХ РАКУ МОЛОЧНОЇ ЗАЛОЗИ

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Вступ. Рак молочної залози залишається провідною причиною смертності жінок від злоякісних новоутворень через резистентність до хіміотерапії та важкі побічні ефекти доксорубіцину (Doxo). Одним із підходів до подолання цієї проблеми є пошук фітосполук, здатних підвищити ефективність хіміотерапії та запобігти розвитку небажаної дії ліків. **Мета.** Метою цієї роботи було дослідити *in vitro*, чи може поліфенол куркумін (Cur) з куркуми (*Curcuma longa*) у поєднанні з комерційним препаратом Doxo-HCl посилювати цитотоксичний вплив Doxo на клітинні лінії раку молочної залози MDA-MB-231 і MCF-7 та проявляти

хемопротекторну активність щодо нормальних клітин HEK-293. **Методи.** Життєздатність клітин оцінювали за допомогою МТТ-тесту, індукцію апоптозу – за допомогою проточної цитометрії з Annexin V-eGFP і PI, генерацію активних форм кисню (ROS) – за допомогою зонду DCFH-DA. Для визначення синергізму або антагонізму компонентів використовували метод розрахунку індексу комбінованої дії (CI) та програмне забезпечення CompuSyn, Biosoft. **Результати.** Під час дослідження виявлено дозозалежну цитотоксичність, підвищене утворення ROS та посилення морфологічних аберацій клітин при використанні комбінації Cur з Doxo-HCl у порівнянні з Doxo-HCl. Cur діяв як хемосенсибілізатор, який синергійно підсилював протипухлинну дію Doxo-HCl і водночас зменшував його цитотоксичні ефекти у нормальних клітинах завдяки зменшенню продукування ROS. **Висновки.** Ймовірно, що використання Cur у поєднанні з Doxo-HCl дозволить зменшити ефективну терапевтичну дозу Doxo та підвищить ефективність хіміотерапії раку молочної залози.

Ключові слова: клітинні лінії раку молочної залози, куркумін, доксорубіцин, комбінована дія, індекс комбінованої дії, хемосенсибілізатор, життєздатність клітин, оксидативний стрес, апоптоз.

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