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Curcumin-Celecoxib: a synergistic and rationale combination chemotherapy for breast cancer

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Abstract. – OBJECTIVE: Over-expression of COX-2 has been linked with various molecular signaling such as carcinogenesis, invasiveness, and malignant tumour metastasis. Besides, the use of celecoxib is also related to lowering the risk of breast cancer. This study therefore designed to explore the synergistic inhibitory effect of the combination of curcumin and celecoxib on the growth of human breast cancer cells.

MATERIALS AND METHODS: In our investigation, we treated MDA-MB-231 cancer cells with different concentrations of curcumin and celecoxib. The enzyme-linked immunoassay was used to measure the COX-2 expression levels. MDA-MB-231 growth was examined by MTS cell viability assay, and synergy detection was carried out using combination index approaches. The drug-likeliness of the tested drugs (curcumin and celecoxib) were computed and predicted ADME pharmacokinetic parameters by in silico. Further, we have conducted BOILED-Egg plot and bioavailability radar analysis for the curcumin and celecoxib.

RESULTS: The result of the physicochemical and ADMET/pharmacokinetic properties showed that these two drugs have good oral and optically bioavailable absorption. The present in silico study could offer a reliable theoretical basis for future structural modification of these compounds to treat breast cancer. The in vitro results suggested that curcumin and celecoxib individually inhibited the growth of MDA-MB-231 cells in a dose-dependent manner. The effect was synergistic for MDA-MB-231 cells relative to the two compounds individually. The synergistic growth inhibitory effect was mediated by a mechanism that possibly involves inhibition of the COX-2 pathways.

CONCLUSIONS: Our findings show the prominent anti-proliferative effects of celecoxib and/or curcumin on MDA-MB-231 cells, providing a rationale for further detailed preclinical and potential clinical studies of this combination for breast cancer therapy. Further, these computed parameters suggested that curcumin possesses a high tendency to act as an adjuvant drug with celecoxib in the treatment of breast cancer.

Key Words:
MDA-MB-231, Human breast cancer, Celecoxib, Curcumin, COX-2, Adjuvant molecule, Cytotoxicity, ADME/T.

Introduction

Breast tumor is one of the most world's common malignant cancers and the leading cause of cancer death among women[1]. Although several anticancer drugs have been clinically used for the treatment of breast cancer, there is still a lack of safe and target specific potential treatment available for the breast tumor[2]. Besides, the efficacy chemotherapeutic strategy currently available for breast cancer therapy is limited due to its drug-resistance, target-specific, and undesirable adverse effects[3]. Therefore, the invention and development of novel chemotherapeutic agents and/or synergistic regimen are immediately required to minimize the adverse effects and to enhance anticancer effect against breast cancer. To overcome these serious issues, the combination of conventional therapeutic agents with naturally isolated bioactive phytochemical compounds is an ideal approach, likely to enhance the effectiveness, provide synergistic antitumor efficacy and clinical outcomes, as well as to reduce the

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potential adverse events during the chemotherapy of breast cancer.

Prostaglandins are signaling lipophilic molecules and their abnormal expression has been linked with the inflammation process and cancer development[4]. The results of substantial clinical and experimental studies reveal that this inflammatory process plays a vital role in the development of various common cancers. Celecoxib is one of the specific inhibitors of cyclooxygenase-2 (COX-2) and has been commonly used in the clinics. The utilization of a novel NSAID is associated repeatedly with the decreased risk of the incidence and development of numerous types of cancer, mainly breast and colon cancers[5]. Subsequently, COX-2 is regularly overexpressed in premalignant stages, specific COX-2 inhibitors have been explored as potential chemopreventive agents. Human trials with targeted inhibitors of selective COX-2 will be important in exploring if COX-2 is a possible signaling target in breast cancer therapy. Various scientific data from the *in vitro* cell culture experiments and animal models have well demonstrated that celecoxib may suppress various cancer cells[6-8]. Nevertheless, its chemopreventive role in the response of tumour cells to anticancer agents and the associated molecular mechanism is still yet to be investigated.

Curcumin (diferuloylmethane) is a natural polyphenolic compound isolated from the dietary spice of turmeric rhizomes (*Curcuma longa*, Family: Zingiberaceae). It has been scientifically documented to reduce the activation of NF-κB transcription factor induced by various inflammatory conditions[9,10]. Remarkably, it was proven to possess significant pharmacological effects on various molecular pathways of the tumour, which has been associated with cancer chemotherapy[10-13]. Hence, curcumin is one of the most promising phytoconstituent which target diverse cancers and inflammation-associated diseases[14]. The safety and toxicity of curcumin have been investigated in numerous animal experiments which revealed that curcumin had several advantages over classical chemotherapeutic agents, including broad-spectrum of pharmacological activities and least toxic adverse outcomes.

The Phase-I investigation indicated the efficacy, safety and tolerability of the synergistic combination of docetaxel and curcumin therapy in metastatic and advanced breast tumor patients[15]. Selective inhibitors of COX-2 have revealed their efficacy in several animal cancer models and limited clinical trials in humans. Evans et al[16] found that the overexpression of COX-2 is positively associated with the p170 level, indicating a potential for specific COX-2 inhibitors in the cytotoxicity regulation of anticancer agents. However, the molecular mechanisms underlying the anticancer effect of combined treatment of celecoxib and curcumin are complex and worth investigating and further developing to an advanced level of breast cancer research. Based on the scientific literature of celecoxib and curcumin, we have hypothesized that curcumin would improve the chemotherapeutic outcome through potentiating the proliferation inhibitory properties of celecoxib on human breast carcinoma cells.

Materials and Methods

Cells and Cell Culture

Human breast cancer cell line (MDA-MB-231) and fetal bovine serum (FBS) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Curcumin (98% purity) and celecoxib were purchased from Sigma-Aldrich Co. LLC (Sigma-Aldrich, St. Louis, MO, USA). The cell lines were grown and maintained in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Grand Island, NY USA). All other supplies used in the experiments were of analytical grade and obtained from VWR (VWR Scientific, West Chester, PA, USA).

Cell Viability Assay

The MDA-MB-231 cells were cultured in DMEM with 10% FBS and 1% penicillin-streptomycin antibiotics. Cells were incubated at 37°C in a 5% CO_2 atmosphere. MTS cell proliferation assay is a colourimetric assay that is used to determine the viability of cells in proliferation or cytotoxic assays. This assay is composed of a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS] and an electron coupling reagent (phenazine ethosulfate; PES). PES was added to increase the stability of the solution. Assays were performed by adding the CellTiter 96® AQueous One Solution Reagent directly to 96-well plates and incubating them for 4 hours and then measured the absorbance at 490 nm with a 96-well plate reader. Overall, the quantity of formazan product as measured by the amount of 490 nm absorbance was directly proportional to the number of living cells in cul-

ture[17]. Viability of the MDA-MB-231 cells was measured using the MTS assay (CellTiter 96, Promega, Madison, WI, USA). In brief, MDA-MB-231 breast cancer cells were seeded in a 96-well plate using DMEM media supplemented with 10 % FBS and 1 % penicillin-streptomycin at 37ºC with 5% CO_2 at a density of $1.5x10^4$ cells/well. Celecoxib and curcumin were dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution. The cells were treated with four different concentrations of celecoxib and curcumin (10 µM, 15 µM, 20 µM and 25 µM) and the DMSO in the samples were kept at a concentration not greater than 0.2 %. The control group was treated with vehicle (2.5 % polyethylene glycol and 0.2 % DMSO). Choosing these concentrations was consistent with other studies that have been done with other cell lines. The cells were incubated with the agents for 72 hours. Then, the MTS reagent was added, and cells were incubated further for 2 hours. Using a spectrophotometer (Synergy HT Multi-Mode Microplate Reader, BioTek Instruments, Winooski, VT, USA,), the cell viability was determined by measuring the absorbance at 490 nm. Each measurement was done in triplicate.

Measuring Total Cyclooxygenase-2 (COX-2) in Whole Cells

Cell-based ELISA has all the materials required to run an ELISA using fluorogenic substrates to measure total cyclooxygenase-2 (COX-2) in the whole cells[18]. This kit was used to investigate the inhibitory effects of curcumin and celecoxib on COX-2 expression. MDA-MB-231 cells were grown in 96-well microplates and stimulated with different concentrations of curcumin, celecoxib or in combination for 24 hours. After stimulation, MDA-MB-231 cells were fixed and permeabilized in the wells using 4% formaldehyde. The breast cancer cells were simultaneously incubated with two primary antibodies: one antibody specific for COX-2 and the other one normalization antibody that is specific for GAPDH, a housekeeping protein. Two secondary antibodies recognizing the different species are labelled with either horseradish-peroxidase (HRP) or alkaline phosphatase (AP), and two spectrally distinct fluorogenic substrates for either HRP or AP are used for detection. The fluorescence of the target protein is normalized to that of the housekeeping protein in each well for the correction of well-to-well variations.

Computational Protocols

ADME Prediction

QikProp of Schrodinger Maestro-18 (2018) running on an Intel CORE i7 based HP Z230 workstation with Microsoft Windows 10 OS was employed for the computational work to predict drug-likeliness and various pharmacokinetic parameters of curcumin and celecoxib. The drug-likeliness is mainly based on the Lipinski's rule of five which constitutes the molecular weight, partition coefficient (QPlogPo/w), number of hydrogen bond- donors and acceptors. On the other hand, crucial pharmacokinetic ADME properties such as QPlogS, QPlogHERG, QPPCaco, QPPMDCK, QPlogKhsa, and % human oral absorption were computed using QikProp tools. Before the submission of structures to the QikProp tools, the structures of curcumin and celecoxib were sketched using 2D built panel of LigPrep module of Maestro. LigPrep is a utility of the Schrodinger software suite that combines tools for generating 3D structures from 1D (Smiles) and 2D (SDF) representations, searching for tautomers, steric isomers and perform a geometry minimization of the ligands. By employing the Ligprep protocol, the ligands were prepared using OPLS3 with default settings.

BOILED-Egg Pot and Bioavailability Radar Analysis

BOILED-Egg plot is essential for drug discovery and development and performed for curcumin and celecoxib using the Swiss ADME online server[19]. Bioavailability Radar is used to predict drug-likeness and lead optimization for drug discovery projects[20]. 2D structures of curcumin and celecoxib were sketched using 2D built panel of ChemBioDraw Ultra version 14.0 (Cambridge Soft Corporation) and saved the files individually in mol2 format. Then, the 2D structure of curcumin and celecoxib were imported to the SwissADME server and converted to SMILES formula. Upon running the program, the results of BOILED-Egg plot and bioavailability radar plot were obtained.

Statistical Analysis

Data are reported as mean ± SD. Comparisons between multiple groups were performed with one-way analysis of variance (ANOVA) and Tukey's paired test using SigmaPlot® software. Results with a *p*-value of less than 0.05 were considered statistically significant. Each

Table I. The classification of the combination index (CI).

Synergism		Antagonism	
CI Value	**Synergy category**	**CI Value**	**Antagonism category**
0.85-0.9	Slight synergy	1.1-1.2	Slight Antagonism
0.7-0.85	Moderate synergy	1.2-1.45	Moderate Antagonism
0.3-0.7	Synergy	1.45-3.3	Antagonism
0.1-0.3	Strong synergy	3.3 - 10	Strong Antagonism
< 0.1	Very strong synergy	> 10	Very Strong Antagonism

experiment was conducted as three independent experiments. Analysis of the effects of the drug combination was performed using the CompuSyn synergism analysis software (CompuSyn, Inc.). This method, proposed by Chou (2010) was used to determine the nature of the drug and drug interaction such as synergism, additivity or antagonism[21]. This method, using the combination index (*CI*) equation (Equation I), allows classifying the antitumor activity of the drug combination (Table I).

$$CI = \frac{(D)^1}{(D_x)^1} + \frac{(D)^2}{(D_x)^2}$$

Equation I

Where $(D)_1$ is the dose of drug 1 and $(D)_2$ is the dose of drug 2 which are required to produce ×% effect in combination. $(D_x)^1$ and $(D_x)^2$ are the doses of two different drugs required to produce ×% effect individually.

Results

Effect on Cell Growth

The effect of celecoxib and curcumin on tumor cell proliferation has been assessed in combination and alone. The treatment of MDA-MB-231 cells to different concentrations (10, 15, 20 and 25 μM) of curcumin caused in a significant decrease of the cell viability in a dose-dependent way in comparison to the control cells as revealed in Figure 1A. These results confirm the growth inhibitory effects of curcumin which have been studied in various cell lines. The anti-proliferative effect was shown when treating cells with 15, 20, 25 μM of celecoxib as shown in Figure 1B. As shown in Figure 1A and 1b, MDA-MB-231 cell growth was individually inhibited by both curcumin and celecoxib in a dose-dependent manner. Exposure of MDA-MB-231 cells with 10 μM curcumin and 10 μM celecoxib reduced the viability by 31% and 10%, respectively. Cell viability decreased significantly by 56 % when cells were treated

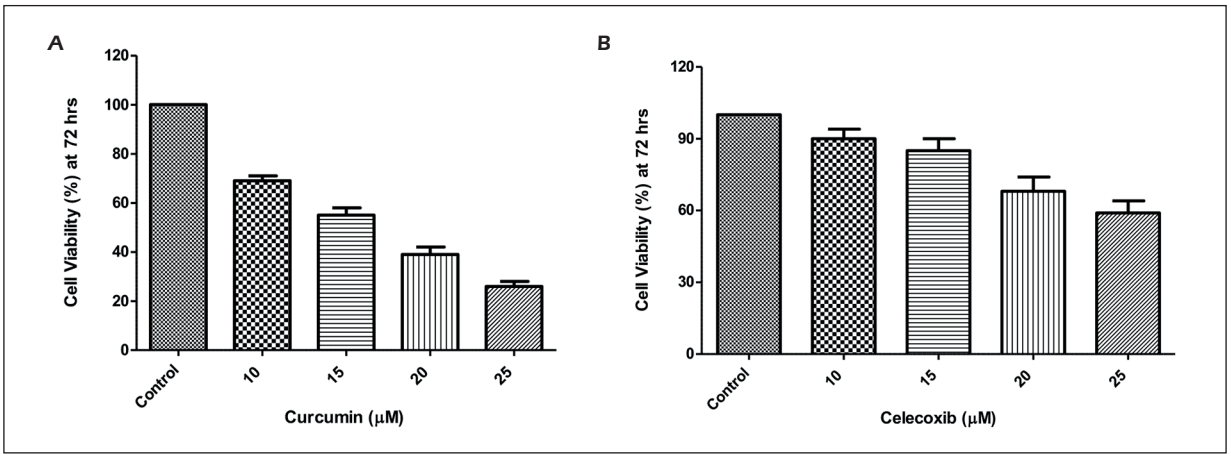

Figure 1. MTS cell viability assay in the presence of different concentrations of curcumin **(A)** and celecoxib **(B)** for 72 hours. The assay was performed to evaluate the induction of cell death in MDA-MB-231 cell line in the presence of curcumin after 72 hours exposure. Data = mean ± SD, n=3, *p<0.05 compared to control, one-way ANOVA test and Tukey paired test were used to compare the results.

at the same concentrations with a combination of curcumin and celecoxib (Figure 2A). Similar growth inhibitory effects (85%) were observed when treating MDA-MB-231 cells with 20 µM curcumin and 25 µM celecoxib combined (Figure 2B and 2C). These data indicated that the effects of curcumin or celecoxib alone on breast cancer cells are minimal. On the other hand, following treatment with curcumin and celecoxib together at different concentrations, the viability of MDA-MB-231 cells is synergistically reduced. The detailed CI values with combined treatments of curcumin and celecoxib on the growth of MDA-MB-231 cells are given in supporting information (**Supplementary Table I**).

Effect on COX-2 Expression Levels

Compared to the control, curcumin showed a dose-dependent reduction of the COX-2 levels as shown in Figure 3A. This finding suggests curcumin acts by downregulating the COX-2 protein. Celecoxib alone, however, showed an increase in COX-2 expression compared to the control which suggests that celecoxib may upregulate COX-2 or inhibit degradation of protein synthesis (Figure 3B). In a synergistic combination, curcumin and celecoxib down-regulates the expression of COX-2 in MDA-MB-231 cells as acquired with the human/mouse COX-2 cell-based ELISA assay. Curcumin at a concentration of 10 µM in combination with various concentrations of celecoxib including 10, 15, 20, 25 µM exhibited a synergistic reduction in the COX-2 expression as compared to each concentration alone that was observed through CI values (Figure 4A). Furthermore, 15 µM curcumin in combination with various concentrations of celecoxib at 10, 15, 20, and 25 µM synergistically reduced the expression of

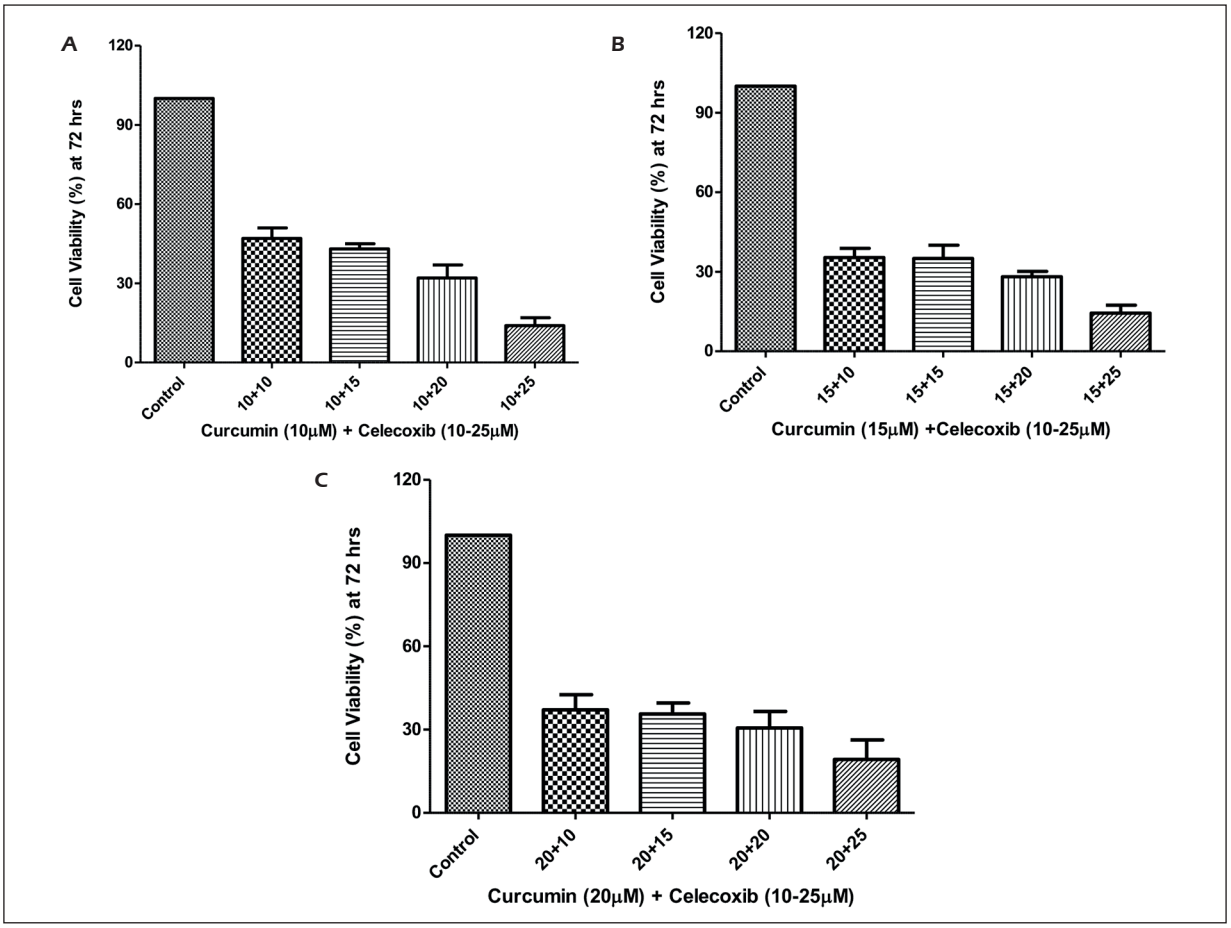

Figure 2. MTS cell viability assay in the presence of a different concentration of celecoxib with 10 µM **(A)**, 15 µM **(B)** and 20 µM **(C)** curcumin for 72 hours. The assay was performed to evaluate the induction of cell death in MDA-MB-231 cell line in the presence of the combination of celecoxib + curcumin after 72 hours exposure. Data = mean ± SD, n=3. CI<1 indicates synergism, CI=1 indicates additive and CI>1 indicates antagonism effect.

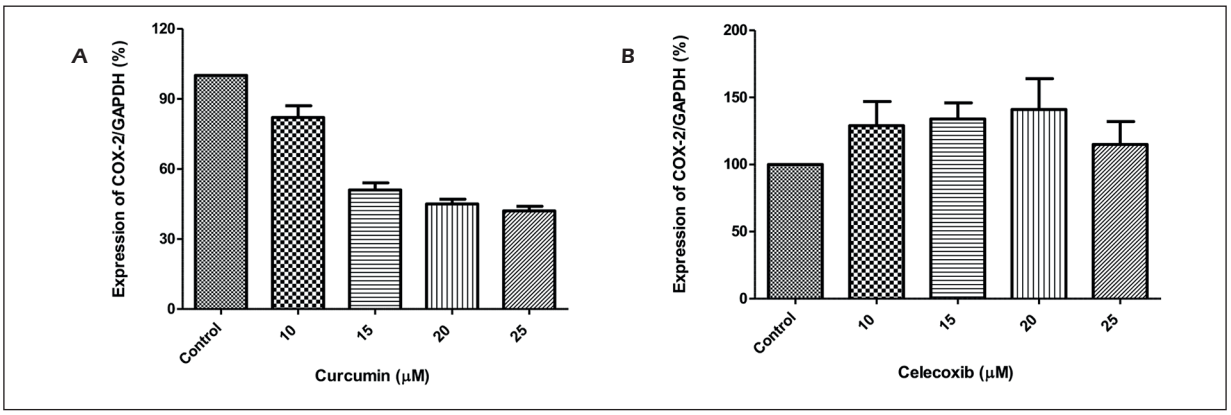

Figure 3. COX-2 expression in the presence of a different concentration of curcumin **(A)** and celecoxib **(B)** for 24 hours. The assay was performed to evaluate the expression of COX-in MDA-MB-231 cell line in the presence of curcumin after 24 hours of exposure. Data = mean ± SD, n=2, *p<0.05 compared to control, one-way ANOVA test and Tukey paired test were used to compare the results.

COX-2 compared with each concentration alone (Figure 4B). Besides, a synergistic reduction of COX-2 levels when cells were treated with 20 μM of curcumin with different celecoxib concentrations indicated by the CI value (Figure 4C). The detailed CI values with combined treatments

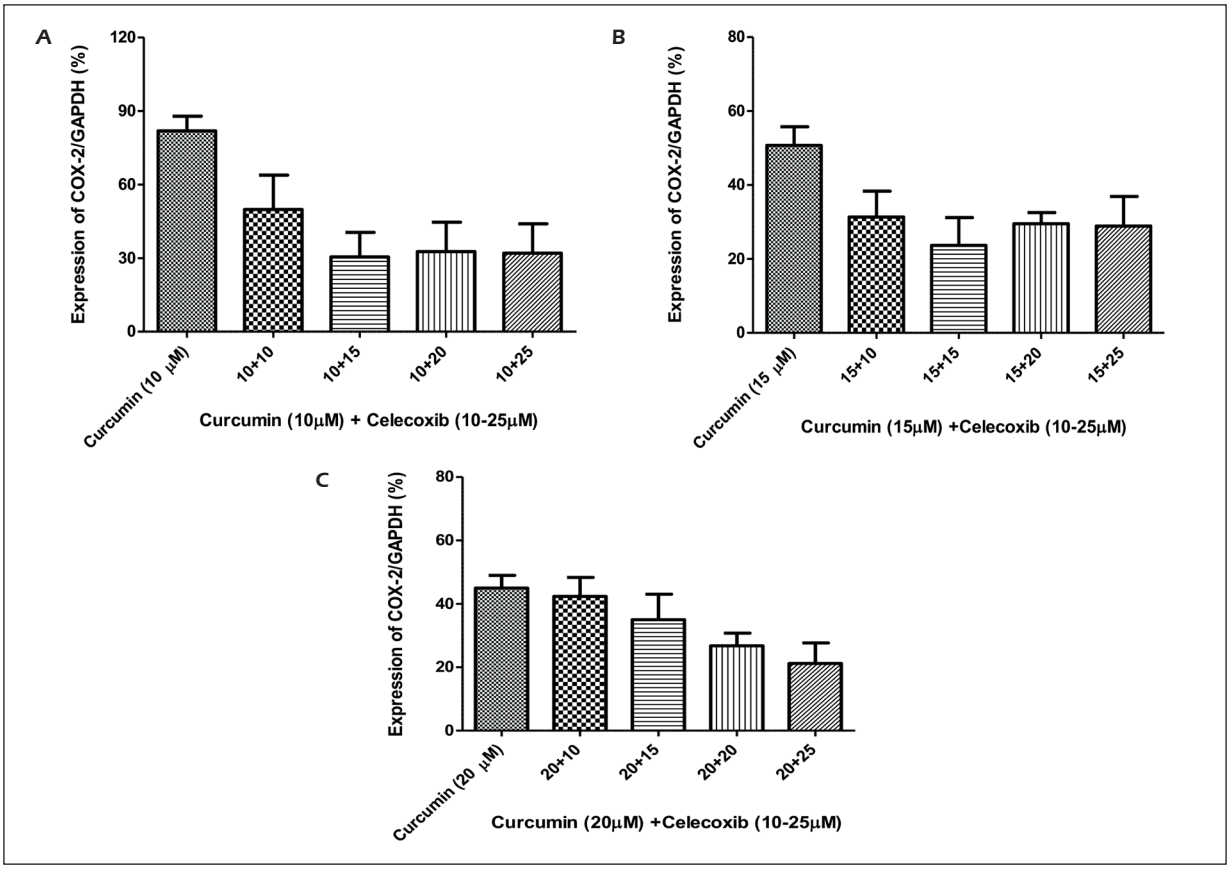

Figure 4. COX-2 expression in the presence of a different concentration of celecoxib with 10 μM **(A)**, 15 μM **(B)** and 20 μM **(C)** curcumin for 24 hours. The assay was performed to evaluate the expression of COX-2 in MDA-MB-231 cell line in the presence of the combination of celecoxib + curcumin after 24-hour exposure. Data = mean ± SD, n=2. CI<1 indicates synergism, CI=1 indicates additivity and CI>1 indicates antagonism effect.

of curcumin and celecoxib on COX-2 expression levels of MDA-MB-231 cells are given in supporting information (**Supplementary Table II)**.

ADME Prediction

To describe the drug-likeliness of curcumin and celecoxib (Figure 5), we have screened them for Lipinski's rule of five, wherein the molecular properties will be correlated with the oral bioavailability of the respective molecules. QikProp of Schrodinger Maestro-18 was employed for the computation of various parameters and the results are summarized in Table II.

BOILED-Egg Plot Analysis

BOILED-Egg plot is essential for drug discovery and development and performed for curcumin and celecoxib, individually using the Swiss ADME online server[19]. The BOILED-Egg plot of curcumin and celecoxib was presented in Figure 6 and collected the results in Table III.

Bioavailability Radar Analysis

Bioavailability radar is used to predict drug-likeness and accomplished for curcumin and celecoxib, individually using the Swiss online server[19]. Six physicochemical properties were taken into account: lipophilicity, size, polarity, solubility, flexibility and saturation. Figure 7 represents the bioavailability radar analysis of curcumin and celecoxib. The detailed results are given in supporting information (**Supplementary Figures 1 and Supplementary Figures 2**).

Discussion

Considering the significant anticancer effect of curcumin in humans, we designed this study to explore the synergistic potential of curcumin in the MDA-MB-231cells along with a Cox-2 selective inhibitor (celecoxib). In the treatment of various types of cancer, combinations of natural products have been used successfully to gain a higher therapeutic effect with the lower dosage and to reduce drug resistance and/or side effects[22,23]. Curcumin, an active polyphenolic compound, and well-studied *Curcuma longa's* phytoconstituent, has helped to support its many health benefits. It is derived from an edible plant and has been consumed by humans for centuries, suggesting low or negligible toxicity. It is well recognized as a cancer preventive drink in Japan and is currently under development for cancer treatment in countries such as Asia, USA, and Europe[24]. Also, the concept of combining COX-2 inhibitors with natural chemotherapeutic agents was intensively investigated in recent years using various *in vitro* and *in vivo* animal models[25].

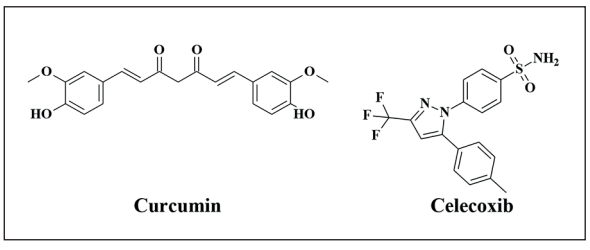

Figure 5. 2D Chemical structures of curcumin and celecoxib.

Several epithelial tumors, including breast cancer, contain over-expressed COX-2 levels. Inhibition of COX-2 reduces tumor occurrence and the development of various invasive tumors in animal models. Numerous investigations have revealed an association between COX-2 expression and aggressive breast cancer parameters[26]. Several preclinical studies on *in vivo* models of breast cancer have shown that enhanced expression of COX-2 is directly related to the pathogenesis of mammary tumors that are susceptible to specific COX-2 inhibitors[27]. Scholars[28,29] indicated that there is a positive correlation between expression COX-2 and sensitivity to the COX-2 inhibitor's apoptotic effects. Studies[30,31] have shown the potential of natural products as a novel chemotherapy adjuvant that effectively improves the anticancer effect of chemotherapeutic drugs.

The administration of dietary curcumin to a human xenograft model in the nude mice model reduced the breast cancer metastasis to the lung, supported by the diminished expression of matrix metallopeptidase-9, NF-κB, and COX-2[32]. Curcumin is also found to decrease the 5-Fluorouracil (5-FU) induced cytotoxicity in human breast cancer cell lines, as demonstrated by an increased LD value of 5-FU[33]. Thus, the potentially synergistic combination of COX-2 inhibitors and natural products is expected to play an important role in future of breast cancer treatment[34]. Moreover, the results revealed that when these two drugs were combined, the COX-2 level could be reduced and found to be more advantageous than monotherapeutic drugs. COX-2 overexpression can confer resistance to apoptosis induction by various anticancer agents, and the upregulation of Bcl-2 by COX-2 could provide a potential mechanism for this reduced apoptotic susceptibility[35,36].

Table II. The drug likeliness and *in silic*o ADME properties of curcumin and celecoxib calculated using QikProp.

Drugs	Drug likeliness (Lipinski's rule of five)					*In silico* ADME by QikProp						
	Molecular weight	QPlogP O/W[a]	H-bond donor	H-bond acceptor	Violation of Lipinski's rule	QPlogS[b]	QPlogHERG[c]	QPPCaco[d]	QPPMDCK[e]	QPlogKhsa[f]	% of human oral absorption	Violation of rule of three
Curcumin	368.38	2.92	2	6	0	-3.2	-5.939	1795.302	941.784	0.297	67	0
Celecoxib	381.37	4.21	1	7	0	-5.106	-5.997	1815.931	942.77	0.286	62	0

[a]Predicted octanol/water partition co-efficient log p (acceptable range from -2.0 to 6.5). [b]Predicted aqueous solubility in mol/L (acceptable range: -6.5 to 0.5). [c]Predicted IC_{50} value for blockage of HERG K+ channels (concern below -5.0). [d]Predicted Caco-2 cell permeability in nm/s (acceptable range: < 25 is poor and > 500 is good). [e]Predicted apparent MDCK cell permeability in nm/s (acceptable range: < 25 is poor and > 500 is good). [f]Prediction of binding to human serum albumin (acceptable range: -1.5 to 1.5). [g]Percentage of human oral absorption (< 25% is poor and > 80% is good).

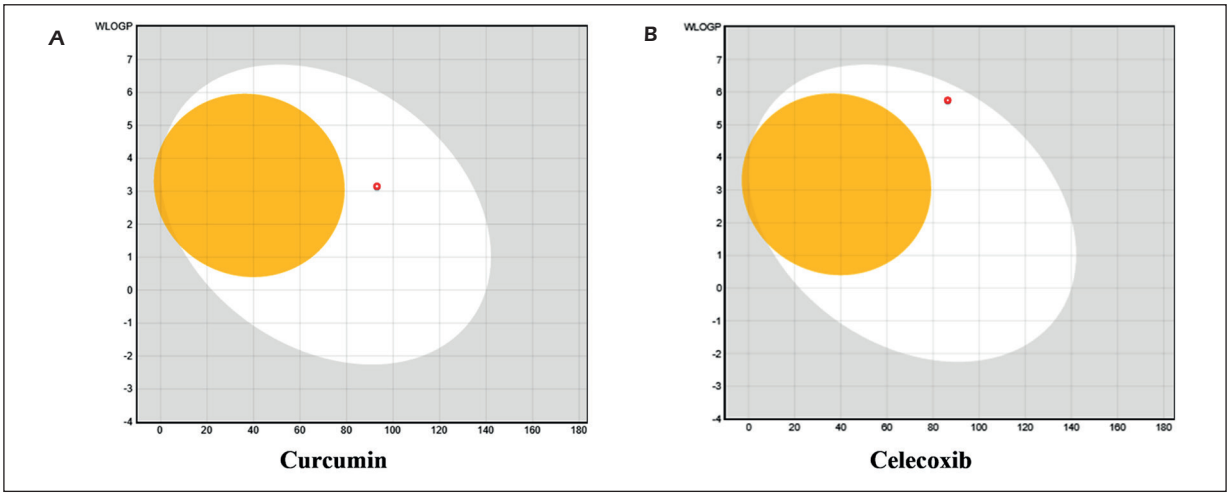

Figure 6. BOILED-Egg plot of curcumin and celecoxib.

Table III. Results of BOILED-Egg analysis and bioavailability radar analysis.

Drugs	Key Parameters of BOILED-Egg					
	MW	TPSA	XLOGP3	MLOGP	GI absorption	BBB permeation
Curcumin	368.38	93.06 Å^2	3.20	1.47	High	No
Celecoxib	381.37	86.36 Å^2	3.40	2.65	High	No

Results from earlier *in vitro* and *in vivo* studies have shown that the elevated expression of COX-2 thus counteracts the apoptosis activated by the increased Bcl-2 expression[37,38].

Curcumin combined with numerous anticancer drugs has been reported to decrease the expression of multiple apoptosis-related genes[39]. It is our first research showing that COX-2 protein expression may be further lowered with the combined use of curcumin and celecoxib compared to the individual administration of these drugs. Furthermore, this combination treatment has caused a dose-dependent reduction of COX-2 protein in the growth of MDA-MB-231 cells. Indeed, by inhibiting the COX-2 pathway, the synergistic antiproliferative effect of curcumin and celecoxib

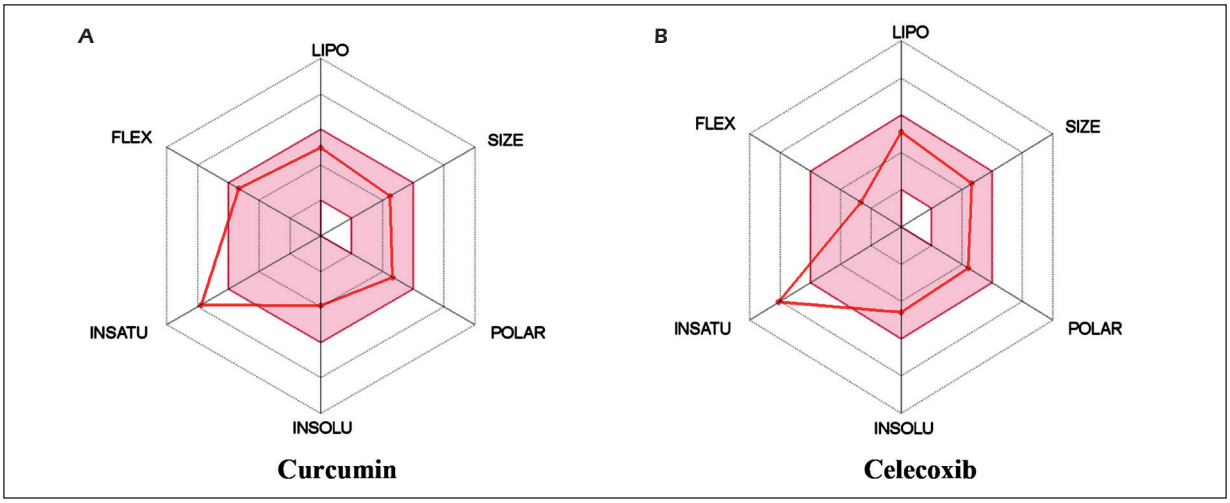

Figure 7. Bioavailability radar analysis of curcumin and celecoxib.

was found in the colorectal cancer cells[40]. These findings confirmed that this potential combination could modulate the pathways of apoptosis, and beyond the principal action on COX-2.

The pharmacokinetic ADME properties play significant roles in the determination of the safety and efficacy of drug-like compounds. Human intestinal absorption (HIA) and Caco-2 permeability (QPPCaco) parameters are the best markers of the absorption of the drug in the intestine and Caco-2 monolayer penetration, respectively. HIA data are the sum of bioavailability and absorption evaluated from the ratio of excretion or cumulative excretion in urine, bile, and feces[41]. Moreover, QPPCaco permeability parameter acts as a crucial feature regulating the metabolism of drugs[42]. The predicted percentages of human oral absorption for these drugs were found to be more than 60% and QPPCaco values were >500. The partition coefficient (QPlogPo/w) and water solubility (QPlogS) are important parameters for the absorption and distribution of the drugs[43]. QPlogPo/w was determined to be 2.92 and 4.21 respectively for curcumin and celecoxib. Further, QPlogS values were computed as -3.2 and -5.106 and fall within the acceptable ranges. Thus, QikProp predicted the physicochemically crucial descriptors and pharmaceutically relevant properties, all of which established that these drugs confer good properties (Table II) and can be considered as synergistic to each other.

Lipinski rule-of-five offers physicochemical boundaries outside of which the likelihood for a drug molecule to become an oral drug is less. Besides the ADME prediction, the efficacy of small molecules may become a failure due to unfavorable pharmacokinetic properties. To assess the predictive power of the model for passive gastrointestinal absorption, and to complement it with the prediction for brain access by passive diffusion, the BOILED-Egg (Brain Or IntestinaL EstimateD permeation predictive model) plot was employed in our study[44]. This study also provided information about the polarity and lipophilicity of drugs and it affords a unique statistical plot to support the prediction of physicochemical properties like gastrointestinal absorption. From the results, it was determined that curcumin and celecoxib have high gastrointestinal absorption as the drugs are observed inside the white zone of the egg than the yellow zone. A physicochemical range on each axis was defined with a fixed descriptor range. The pink area in Figure 3 indicates the radar plot of the molecule has to fall entirely to be considered drug-like. The pink area also represents the optimal range for each property (lipophilicity: XLOGP3 between −0.7 and +5.0, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 Å^2, solubility: log *S* not higher than 6, saturation: the fraction of carbons in the sp3 hybridization not less than 0.25, and flexibility: no more than 9 rotatable bonds). Both curcumin and celecoxib predicted that the saturation is not bioavailable because it's too much flexible. Further other physicochemical properties suggested for optimal bioavailability. Moreover, these *in silico* predictive results should be confirmed by *in vivo* pharmacological assay for the treatment of breast cancer.

Conclusions

The effectiveness of chemotherapy is limited in cancer treatment due to the frequent drug resistance, lack of therapeutic selectivity, and undesirable side effects. This study demonstrated that curcumin, a naturally occurring triterpenoid, can enhance the antitumor effect of celecoxib on MDA-MB-231 cell through the downregulation of COX-2, and the consequent may be due to the induction of apoptosis. Thus, down-regulation of COX-2 and the consequent activation of apoptosis is a way to sensitize breast cancer cells by this rational combination therapy. Curcumin is a boon in the phase of new drug discovery as it can specifically target cancer cells and is nontoxic to other body cells. This combination of curcumin and celecoxib likely to increase the effectiveness of entire chemotherapy as well as to reduce the adverse outcomes. Although curcumin has not been approved for clinical application, it is an effective and low-toxic adjuvant for celecoxib in the treatment of breast cancer.

In the new drug development process, pharmacokinetic (ADME) and bioavailability investigation played crucial roles in drug-likeness properties. This investigation also demonstrated (ADMET)/pharmacokinetics and bioavailability properties of curcumin and celecoxib. From this study, it was observed that curcumin and celecoxib have high gastrointestinal absorption as these drugs are observed inside the white zone of the egg than the yellow zone of the plot. Both curcumin and celecoxib predicted that the saturation is not bioavailable because it's too flexible. The *in-silico* analysis of curcumin-celecoxib combination may lead to the development

of potential drug-like candidates with favorable safety profiles followed by suitable pharmacological evaluation. In short, these bioavailability and pharmacokinetics predictions could improve the success rate for cancer therapy through this synergistic combination of curcumin and celecoxib. These are also beneficial for obtaining safer and more potent anticancer drugs for the treatment of breast cancer.

Thus, the prospect of the research with better mechanisms of utilizing curcumin as an adjuvant with celecoxib will become a mainstream drug combination against breast cancer.

This experimental study establishes that curcumin, a naturally occurring curcuminoid, can augment the anticancer potential of celecoxib on breast cancer cells through the downregulation of COX-2. The synergistic combination treatment of curcumin with COX-2 inhibitors could be another encouraging regimen in the impending management of breast cancer. However, additional studies are warranted to confirm the inhibition of COX-2 expression leading to apoptosis in cancer cells by stimulating pro-apoptotic proteins. Taken together, a better understanding of the implication and underlying mechanisms of action of combined curcumin and celecoxib to the chemotherapy may provide a useful approach to combat cancer, effectively.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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