https://doi.org/10.33380/2305-2066-2023-12-1-52-58
UDC 616-085

Research article / Оригинальная статья

Cytotoxic Effect of 6-Ethyl-Chenodeoxycholic Acid and Cabazitaxel on PC-3 Cells

Munaf H. Zalzala[1], Wrood S. Al-khfajy[2]✉, Raghad Abdulsalam Khaleel[3]

[1] Department of Pharmacology and Toxicology, University of Baghdad, College of Pharmacy, Baghdad-Iraq
[2] Department of Pharmacology and Toxicology, College of Pharmacy, Mustansiriyah University, Baghdad-Iraq
[3] Department of Pharmacology, College of Medicine, University of Al Iraqia, Baghdad-Iraq

✉ **Corresponding author:** Wrood S. Al-khfajy. **E-mail:** pharm.wroodsalim@uomustansiriyah.edu.iq

ORCID: Munaf H. Zalzala – https://orcid.org/0000-0003-0552-6469; Wrood S. Al-khfajy – https://orcid.org/0000-0003-4451-8939; Raghad Abdulsalam Khaleel – https://orcid.org/0000-0001-5757-5356.

Received: 15.08.2022 **Revised:** 11.01.2023 **Published:** 24.02.2023

Abstract

Introduction. Chemotherapy with Cabazitaxel (CBZ) is a typical first-line treatment option for naïive castration-resistant prostate cancer resistant to docetaxel. On the other hand, Cabazitaxel's therapeutic success is constrained by chemoresistance and side effects.

Aim. To assess whether 6 alpha-ethylchenodeoxycholic acid (6-ECDCA), a selective agonist for bile acid receptors will enhance the efficacy of CBZ in androgen-independent prostate cancer cells.

Materials and methods. The 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability assay was used to assess the cytotoxicity of 6-ECDCA and CBZ medicines or their combinations against the human prostate cancer cell line (PC-3). The combination outcome suggested by Chou TC et al. was then evaluated using the combination index (CI) to find out the nature of synergism, antagonism, and additive effect of the drug's combination. Furthermore, the Dose-Reduction Index (DRI) was determined to measure how many times the dose could be reduced for each drug in a synergistic combination.

Results and discussion. Analysis of the dose- effect curve showed that the treatment of PC-3 cells with CBZ alone or combined with 6-ECDCA for 48 h led to 50 % cytotoxicity of 20.5 nM and 4.7 nM, respectively. 6-ECDCA at 1.77 μM had an additive effect based on the CI value, which was 1.02, while at 21.02 μM, the CI was 0.54 which designates a strong synergistic effect. The combination of CBZ and 6-ECDCA at a submaximal lower dose by 6-folds of each one produced a 95 % cell death than treatment with either agent alone

Conclusion. The Combination index plot showed CI ≤ l for all combinations used in this study, which indicates additive and synergistic interactions between CBZ and 6-ECDCA. The significant impact of 6-ECDCA in combination with CBZ for treating androgen-independent prostate cancer cells was confirmed by this study to be preferred to the treatment with a single drug.

Keywords: 6 alpha-ethylchenodeoxycholic acid; Cabazitaxel; synergistic combination chemotherapy

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

Contribution of the authors. Wrood S. Al-khfajy and Munaf H. Zalzala – methodology; software, formal analysis; investigation. Raghada Abdalsalam Khaleel – review and editing. All authors have read and agreed to the published version of the manuscript.

Acknowledgment. The authors thank college of pharmacy / Mustansiriyah University for providing the facilities to perform this study.

For citation: Zalzala M. H., Al-khfajy W. S., Khaleel R. A. Cytotoxic effect of 6-ethyl-chenodeoxycholic acid and cabazitaxel on PC-3 cells. *Drug development & registration*. 2023;12(1):52–58. https://doi.org/10.33380/2305-2066-2023-12-1-52-58

INTRODUCTION

Cabazitaxel (CBZ) is an authorized first-line therapies in castration-resistant prostate cancer (CRPC) with a significant clinical outcome comparison with Docetaxel [1, 2]. These tubulin-active agents stabilize microtubules compositions, restraining cells in the late G_2–M phase of the cell cycle, resultant in cell death [2]. Nevertheless, survival of patients continues to be a challenge by the incidence of dose-dependent adverse side effects that were reported in 82 percent of patients and acquired resistance [3]. This highlighting a need to identify additional therapeutic targets to enhance chemotherapy based on taxanes. Finding compounds that, when used in conjunction with the present chemotherapeutic drugs, enable dose reduction without compromising efficacy, as well as the avoidance and/or overcoming of drug resistance, is therefore of great clinical significance. As a result, combination therapy, a form of cancer treatment that incorporates two or more therapeutic drugs, is increasing in importance [4, 5]. The 6 alpha-ethylchenodeoxycholic acid (6-ECDCA) is a potent, first class selective agonist for NR1H4 bile acid receptor derived from the primary human bile acid chenodeoxycholic acid

(CDCA) [6]. It is used clinically as an orally administered drug once daily to treat primary biliary cholangitis [7]. Bile acid signaling is involved in many physiological and pathological processes [8–10]. The 6-ECDCA has been shown to have protective antifibrotic effects in models of inflammatory bowel disease and diabetic nephropathy [8, 11]. Furthermore, new evidence confirms that it plays a pivotal role in carcinogenesis [12, 13]. Furthermore, recent results show that 6-ECDCA makes cells more sensitive to chemotherapeutic drugs [14]. Furthermore, the 6-ECDCA and the antiparasitic medication nitazoxanide (NTZ) worked together cooperatively to decrease tumor growth by preventing the expression of β-Catenin [15]. Moreover, Acyclic retinoid (ACR) agonist and the bile acid receptor GW4064 demonstrated synergistic inhibitory effects on the development of HCC at lower doses of each drug [16]. Furthermore, our previous recent study has shown that 6-ECDCA inhibits the proliferation, survival and migration of prostate cancer cells [17].

Therefore, the main objective was to investigate whether 6-ECDCA potentiates the cytotoxic and tumor-suppressive effects of Cabazitaxel and thus makes prostate cancer cells more chemosensitive to reduce the dose and toxicity and boost its efficacy in combating drug resistance. In addition, this study finds out the nature of action of drug combinations by assessment the CI and DRI.

MATERIALS AND METHODS

All the investigations conformed to the ethics of research and the study was approved by the Ethics Committee of the University.

Chemicals

6 alpha-ethylchenodeoxycholic acid (6-EDCA) and cabazotaxel (CBZ) were purchased from Medical Chemical Express (USA). MTT 3-(4,5-Dimethylthiazol-2-yl)-2, 5 Diphenyltetrazolium Bromide was purchased from Promega (USA). MTT was diluted in PBS at a rate of 5 mg/ml to create the MTT stock solution. Filtration was used with a 0.45 µm filter unit to sterilize this solution. DMSO solvent was used to solubilize the formazan crystals. Best-grade solutions and chemicals were used in this study.

Cell culture

The human androgen -independent prostate cancer cell line (PC-3) was obtained from the ATCC American Type Culture Collection ATCC (USA) and grown in RPMI 1640 medium complemented with 10 % fetal bovine serum (FBS), 2 µM of L-glutamine in the presence of 1 % penicillin/streptomycin. The cell culture was maintained in a 37 °C incubator with a humidified 5 % CO_2. Cells were kept in the logarithmic growth phase. Cells were regularly tested for mycoplasma contamination.

In vitro cytotoxicity

Using the 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) viability assay, the cytotoxicity of the medicines or their combinations was assessed [18]. PC-3 cells were seeded in 96-well plates at a density of 5000 cells per well and cultured in a CO_2 incubator for 24 hours. After 24 hours, PC-3 cells were treated with CBZ alone at different low concentrations 1.25, 2.5, 5, 10, 20, 40, 80 and 160 nM, and another 96 well PC-3 cultured plate was treated with both CBZ / 6-ECDCA at different low concentrations of 6-ECDCA (0.5, 1, 2, 4, 8, 16, 32 and 64 µM) at 1:400 ratio. The Cell viability was determined after 48 hours by reading the absorbance at 530 nm using a microplate reader (BioTEK, USA). The cell viability was calculated using the following formula: Cell viability (%) = {(absorbance of the treated cells-blank) / (absorbance of the untreated cells-blank)} × 100. The experiment was achieved with 3 replications. The IC_{50} (the concentration required for 50 % cell inhibition) was calculated using the GraphPad Prism 9.2 program (GraphPad Software Inc., USA).

Synergistic effects analysis

As previously mentioned, the software Compusyn was used to investigate synergism, and its analysis method was based on Chou and Talalay's principle of the median effect [19]. This statistical analysis used data from the cell viability inhibitory experiments mentioned above. The following formula is used to produce a numerical index called the combination index (CI): $CI = D_1/(Dx)_1 + D_2/(Dx)_2$, where D_1 and D_2 are the concentrations of reagent 1 and 2 used together to achieve x% of the total effect of the medication, and $(Dx)_1$ and $(Dx)_2$ are the concentrations of separate agents to achieve the same efficacy. The CI value ($CI > 1$, antagonism; $CI = 1$, additive; $CI^{\bullet} < 1$, synergism) is a mathematical and quantitative representation of the pharmacological interaction between two medications. Based on data obtained from the cytotoxicity experiment and electronic software, CI values were made from a collection of fraction affected (Fa) levels from 0.05 to 0.9 (i. e. 5 to 90 % growth suppression). The Fa-CI plot and Fa-log (CI) plot illustrate the actual CI values at diverse suppressive levels. Dm. (the median-effect dose that inhibits 50 % of the growth under study) also studied. DRI was also measured to calculate how much the folds in dose reduction for each drug in synergistic combination, $DRI = 1$ indicates no dose reduction, whereas $DRI > 1$ and $DRI < 1$ indicate favorable and unfavorable dose reduction, respectively.

Morphological changes study:

An inverted microscope (Optika, Italy) was used to examine the morphology of PC-3 cells. Cellular alteration was examined as follows: After confluence (70 to 80 %), PC-3 cells were cultivated in a 60 mm cell culture plate.

The cells were then treated for 48 hours with fixed concentrations of CBZ and/or 6-ECDCA at their IC_{50}. After 48 hours of incubation, apoptotic features were recognized by the manifestation of cell shrinkage and/or the presence of membrane-bound cellular bodies. The morphological changes of the apoptotic cells were examined under an inverted microscope at 40x magnifications and captured with a digital camera (Optika, Italy).

Statistical analysis

The mean and standard deviation were used to express the data of the MTT assay (SD). GraphPad Prism 9.2 was used to calculate the IC_{50} values for each medication (GraphPad Software Inc., USA). Using the parameters of the CompuSyn tool, the dose-effect curve, CI values, Fa-CI, and DRI-Fa plots were measured (Compusyn Inc, USA). Differences that had a p-value <0.5 were considered significant.

RESULTS

Combined treatment with Cabazitaxel and 6-ECDCA induced a higher Proliferation inhibitory effect than that with Cabazitaxel alone on the PC-3 cells

The basic requirement for the estimation of synergistic activity is to know the potency and slope of the concentration-response curves of each drug molecule. Then, we measured the IC_{50} of CBZ in androgen independent prostate cancer cells that had been exposed to serial dilutions ranging from 1.25–160 nM for 48 hours using the MTT cytotoxicity test. Depending on the data in (figure 1, A), CBZ inhibited PC-3 cell proliferation in a dose-dependent effect. IC_{50} was 20.01 nM for CBZ, corroborating previously published literature [20]. Previous our work showed that the IC_{50} for 6-EDCA in PC-3 cells was 8 μM at 48 hours incubation time [17]. As shown below, CBZ being more potent than 6-EDCA in prostate cancer cell line. The CBZ + 6-EDCA group had higher inhibitory rates of the prostate cell line than each drug when administered independently at all concentrations. With the increase in CBZ concentration, the proliferation inhibitory rates of PC-3 cells reached significantly 80 and 97.3 % in the CBZ + 6-EDCA group at 20 and 40 nM CBZ, respectively (figure 1, B).

Both 6-ECDCA and Cabazitaxel have different combinations of synergistic effects when administered concurrently in androgen-independent PC-3 Cells

In combination chemotherapy, the dosage and timing of administration are crucial since they can influence the effectiveness of the treatment. Combining cytotoxic substances can have a synergistic, additive, or antagonistic effect depending on the ratio and timing of administration. This led us to identify the drug ratio and dosage schedule that produce the greatest amount of synergy. To determine whether 6-ECDCA and CBZ could work together, cells were treated for 48 hours in a constant ratio of 1:400 of CBZ to 6-ECDCA and 6-EDCA. Then, other analytical techniques were used, including the isobologram analysis, curve-shift analysis, and combination index (CI). As shown in the figure 2, A, the Dose-response curves illustrated in (table 1, figure 2, A) showed that CBZ/6-ECDCA combination was significantly

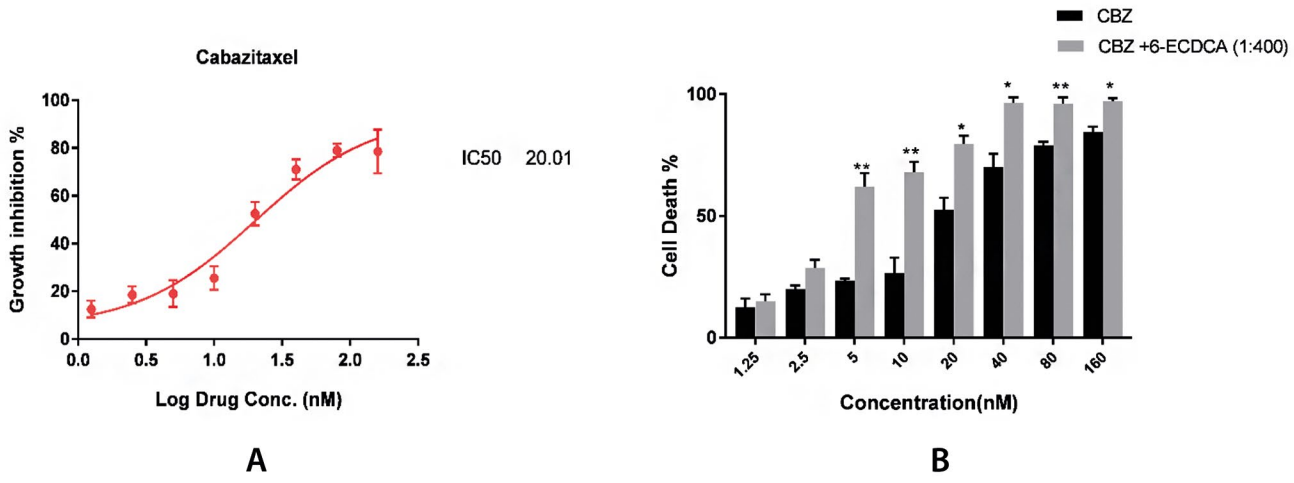
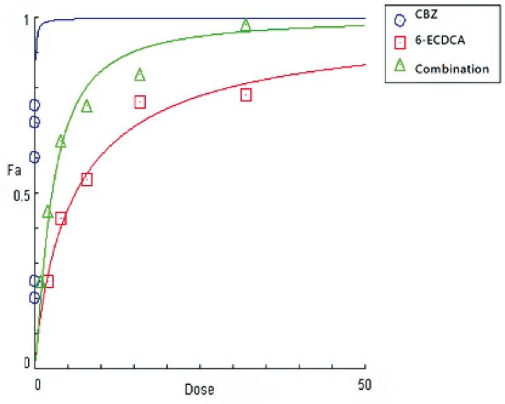


Figure 1. Combination treatment of Cabazitaxel (CBZ) with 6-ethylchenodeoxycholic acid (6-EDCA) resulted in a significantly higher dose-dependent reduction in prostate cancer cell viability than CBZ alone (A). Dose-response curve of Cabazitaxel (CBZ) on prostate cancer cell line. After being exposed to CBZ doses ranging from 0 M to 160 nM, the cells were tested for vitality; cells treated with vehicle served as controls (B). A representative histogram image demonstrating the effects of cabazitaxel alone or in combination with cells that had received a 1:400 constant ratio of CBZ: 6-ECDCA concurrently for 48 hours. Data points represent the average of three independent trials in triplicate. * p < 0.05; ** p < 0.01

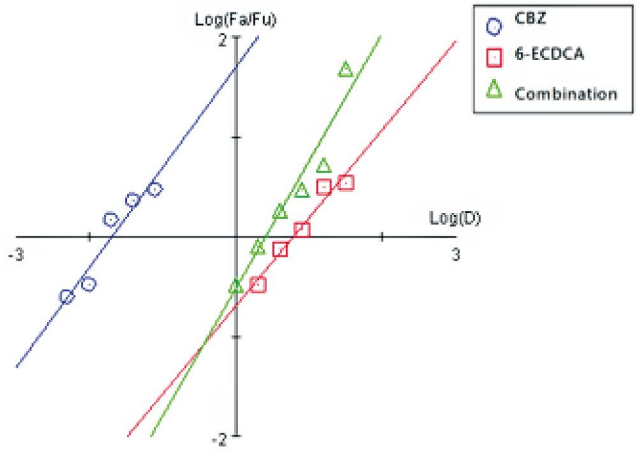
better than the single drug treatment confirming the favorable effect of 6-EDCA in combination with CBZ for treatment. The CBZ/6-EDCA group shows a steeper sigmoidal curve compared to CBZ or 6-ECDCA alone as shown by their slopes (m values). Both compounds have $R^2 > 0.96$ representing an excellent linear correlation, values of r were 0.95 or greater, demonstrating good conformity of dose-dependent effect data with respect to the median effect principle for in-vitro studies

Dose-dependent response curves represent a qualitative graphic illustration of the activity of combined drug, while CI presents a quantitative measure to assess the degree of drug combination. As shown in table 1, the CI value decreased as the concentration of CBZ combination increased. The CI value of the combination at 6.8 nM of CBZ was 1.025 that signifies slight additive action. While the CI value of 20.54 nM of CBZ and 61.78 nM were 0.74314 and 0.54078 respectively that indicates moderate synergistic action. These findings show that 6-ECDCA significantly and synergistically increased the sensitivity of prostate cancer cells to cabazitaxel therapy and decreased cell viability.

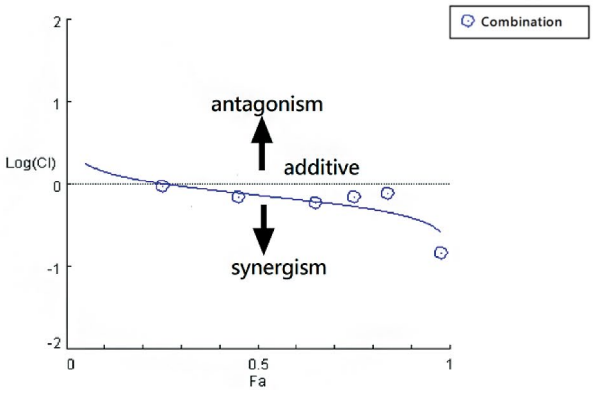


	m	Dm (μM)	R^2
CBZ	1	0.02	0.97
6-ECDCA	0.88	6.11	0.98
Combination(1:400)	1.28	2.61	0.97

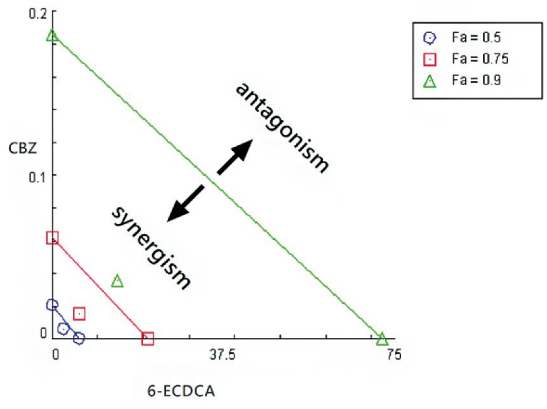
A



B



C



D

Figure 2. Individual and combination cytotoxicity of Cabazitaxel (CBZ) and 6-ethylchenodeoxycholic acid (6-ECDCA) in PC-3 prostate cancer cells (A). Concentration-dependent cytotoxicity of CBZ and 6-EDCA from MTT cytotoxicity test at 48 hours treatment (B). The CI plot of combinations treatment for 6-ECDCA and cabazitaxl drugs (C). Isobolograms showing the doses necessary for inhibition at 50 % (Fa 0.5), 75 % (Fa 0.75) and 90 % (Fa 0.9) for each drug separately. The dose pair is plotted as a point (symbol) beneath each Fa isobole or line to show synergism. CI values <1 are symbolic of synergistic effects; CI values >1 are symbolic of antagonistic effects; and CI values =1 are symbolic of additive effects, Fraction affected (FA) is a measure of the determined effect (cytotoxicity measured by an MTT assay). Slope (m), correlation coefficients (r), and median effect dosages (Dm) from median-effect plots.CI, combination index; Fa, fractional inhibition; CBZ, Cabazitaxel; 6-EDCA, 6-ethyle chenodeoxy acid. Data points are mean of three independent trials performed in triplicates. These were automatically generated using the CompuSyn software (Paramus, NJ, USA)

Table 1. Combination Index (CI) and Dose reduction index (DRI) to assess the degree of drug combination using the CompuSyn Software. The table lists the dose combinations that were evaluated along with the effects they had, affected fractions (Fa) and matching CI and DRI values

Effect (Fa)	Dose of CBZ (μM)	Dose of 6-ECDCA (μM)	CI Value	DRI for CBZ	DRI for 6-ECDCA
0.25	0.00683	1.77	1.02	2.474	1.608
0.45	0.01680	4.87	0.70	3.018	2.189
0.5	0.02054	6.11	0.74	3.155	2.346
0.75	0.06178	21.02	0.54	4.023	3.422
0.95	0.39293	167.58	0.32	6.051	6.452

The 6-ECDCA acts as an Adjuvant to reduce the effective dose of the chemotherapeutic potential of CBZ

After combining CBZ with 6-ECDCA, the Dose-Reduction Index (DRI), which assesses how many times a single drug's dose can be decreased when administered in combination, was determined. As shown in figure 3, the CompuSyn analyses for the prostate cancer cells line indicating that both CBZ and 6-ECDCA had an inhibitory effect, and that the combination treatment was superior to a single-drug treatment. Furthermore, the DRI plot revealed that both drugs had a DRI value over 1, However, the dosage reduction in CBZ was greater than that in 6-ECDCA, indicating that 6-ECDCA may help reduce the adverse effects of CBZ. Interestingly, the CBZ+ 6-EDCA group at all concentrations exhibit a higher dose-reduction index >1. For prostate cancer cells (PC-3 cells), single CBZ and 6-EDCA showed dose reductions of 2.47 and 1.6 times at Fa of 0.25. While at Fa = 0.5, the DRI were 3.1 and 2.3 for CBZ and 6-ECDCA, respectively (table 1).

Morphological Comparison between Prostate cancer Cells upon Exposure to 6-ECDCA, CBZ and 6-ECDCA /CBZ Co-Treatment

In the control group, a 0.5 % exposure to DMSO causes prostate cancer cells (PC-3 cells) to display a typical morphological pattern (figure 4, i). The CBZ group, on the other hand, was shown to negatively affect cells as evidenced by low density of the cells and shrinkage of the cells (figure 4, ii), whereas in the combined therapy of 8 μM 6-ECDCA and 20 nM CBZ substantially triggered cell death as evidenced by the rise in the number of dead and de-attached cells (figure 4, iv). Following the combined therapy, The PC-3 cells demonstrated the typical phenotypic signs of apoptosis, such as cell shrinkage, cellular blebbing, and the creation of apoptotic bodies (figure 4, iv).

DISCUSSION

The most effective anticancer drug for the treatment of naive and metastatic prostate cancer is Cabazitaxel [21]. Its effectiveness was however reduced by the rapid evolution of resistance and systemic toxicity [3]. Pre-

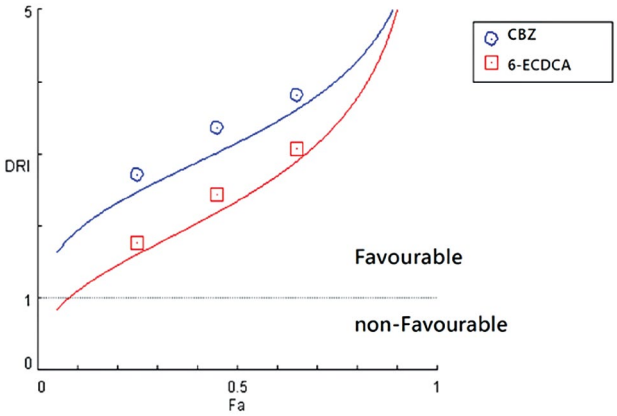


Figure 3. The DRI of the CBZ and 6-ECDCA drug combinations is illustrated, the DRI value >1 indicated favorable drug combinations, while DRI less than 1 indicating unfavorable combination. Data obtained via CompuSyn analysis

clinical findings suggest that combining innovative agent with conventional therapies proposes new advantages effective therapeutic approach for overcoming toxicity by lowering the effective dose and reduce or interruption the initiation of drug resistance [5, 22–24]. Based on effectiveness and acceptable toxicity, a number of promising agents are developing with a possible impact in Cabazitaxel-based combos for enhancing therapeutic outcomes [1, 4, 20]. In line with this, the present study was designed to investigate the cytotoxic effectiveness of the combination of the semisynthetic bile acid analogue 6-ECDCA and Cabazitaxel in Androgen independent prostate cancer cells (PC-3). Our study demonstrated that 6-ECDCA, when combined with Cabazitaxel, produce concentration dependent cytotoxic effect in the PC-3 cells. Indeed, combinations of Cabazitaxel with 6-ECDCA revealed CI values ≤1 (revealing of additive and synergistic interactions). Furthermore, Cabazitaxel alone did not totally caused complete PC-3 cell death even at concentrations greater than 8-folds above their corresponding IC_{50} values, while combinations of Cabazitaxel with 6-ECDCA caused complete eradication of PC-3 cells at much lower concentrations. Consistent with that, the combination of Cabazitaxel and 6-ECDCA at submaximal lower dose by 6- folds (DRI = 6) of each one produced a greater reduction in PC-3 cell viability (95 % cell death) than treatment with either agent alone. A DRI value of more than 1 is of important importance because it signifies therapeutic advantage under circumstances in which the dose of any particular component of combinative drugs is reduced to reduce the adverse effects caused by cytotoxicity in normal cells. According to morphological examination, 6-ECDCA and CBZ together triggered apoptotic- cell death in prostate cancer cells, which was evident by the presence of distinct apoptotic characteristic features such as cell shrinkage, cells de-attachment, and presence of apoptotic bodies [25, 26]. Theoretically, combination therapy with therapeutic agents that are found to be efficient as monotherapy in

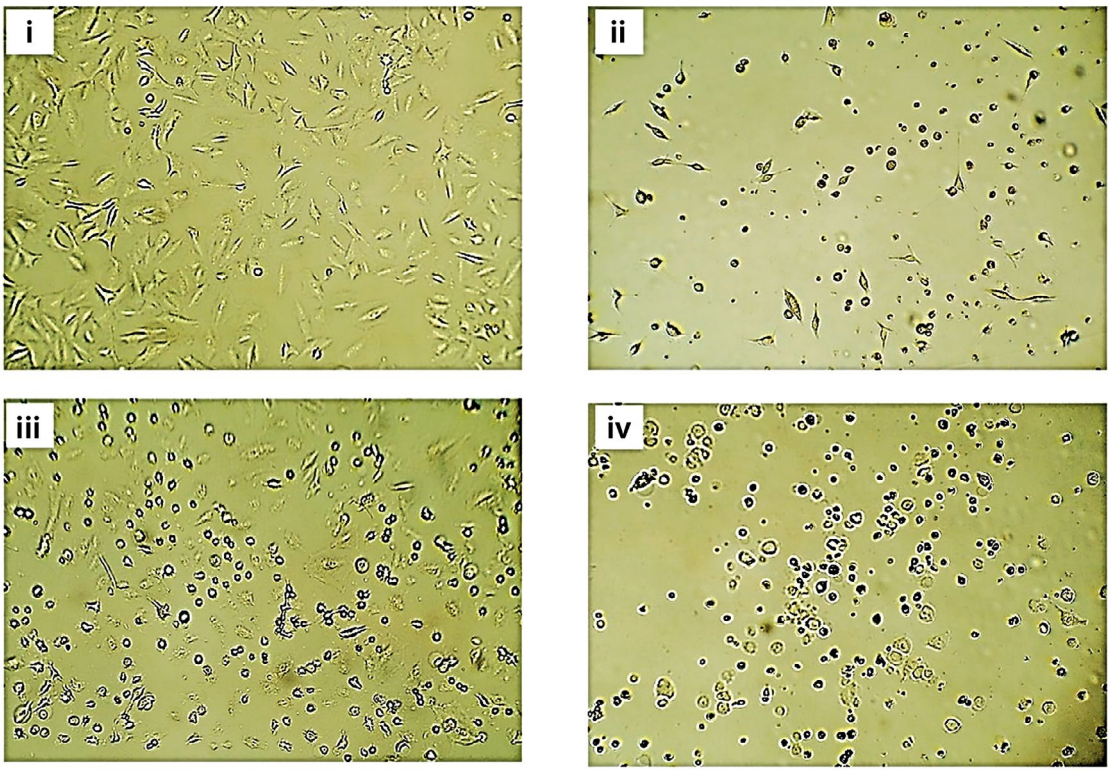


Figure 4. Effects of 6-ECDCA and CBZ alone or in combination on the morphology of prostate cancer cells (PC-3) (i) 0.05 percent DMSO is added to the culture medium, (ii) 20 nM CBZ, (iii) 8 nM 6-ECDCA, or (iv)20 nM CBZ plus 8 μM 6-ECDCA are all used to treat PC-3 cells. Phase contrast microscopy was used to evaluate the cellular morphology of PC-3 cells at a magnification of 40x (scale 80 μm)

the clinic should show good therapeutic effects without further toxicity at lower drug dosages [21]. One of the advantages of combination chemotherapy is improved patient compliance due to decrease in the number [27, 28] and resistance [29, 30]. The most interesting observation emerging from our data was 6-ECDCA which have been used safely in many patients against primary cholangitis [6, 31] it inhibited prostate cancer cell growth ,migration and induce apoptosis by inhibiting Cyclin D_1 and MMP-9 expression while it induced PTEN/PI3K/AKT signaling pathway [17]. According to a different study, 6-ECDCA had a strong inhibitory effect on the JAK2/STAT3 pathway by upregulating SOCS3 expression in colon cancer cells [32]. These findings confirm those of earlier studies, as ECDCA also found to inhibit the production of regulated gene products involved in angiogenesis (VEGF), invasion (MMP-9), proliferation (cyclin D1, c-Myc, and COX-2), and antiapoptosis (Bcl-2, Bcl-xL, and XIAP) [33–35]. A more recent study reported that enhancement of PI3K/AKT signaling was related to castration-refractory and Docetaxel resistant castration resistant prostate cancer [36–38]. From the literature, it can be resolved that, the anticancer effects of 6-ECDCA may also mediated by preventing the PI3K/AKT/ABCG2 signaling pathway- mediated chemotherapeutic resistance [39]. In further support of these findings, 6-ECDCA has previously been shown to as a suppressor for the epithelial to mesenchymal transition (EMT) in different cancer models [35, 40, 41]. Recent studies have reported that prostate cancer cells are more sensitive when the PI3K/AKT and/or EMT pathways are blocked [42, 43]. Taken together, these findings suggest that the 6-ECDCA resensitize prostate cancer cells that are resistant to CBZ and caused a highly decreases in cell viability.

CONCLUSION

In conclusion, our results show for the first time that 6-ECDCA increased cabazitaxel-mediated cytotoxicity. Combination index (CI) analysis showed that simultaneous administration of 6-ECDCA and Cabazitaxel was able to exert strong synergistic effect on PC-3 at all doses tested. This approach allows the use of a much less dose of cabazitaxel and might be a new therapeutic option for prostate cancer and possibly other types of cancer with perhaps lower chemoresistance potential and less side effects.

REFERENCES

1. Erdogan S., Serttas R., Turkekul K., Dibirdik I. The synergistic anticancer effect of salinomycin combined with cabazitaxel in CD44+ prostate cancer cells by downregulating wnt, NF-κB and AKT signaling. *Mol Biol Rep*. 2022;49(6):4873–4884.
2. Ebner B., Chaloupka M., Berg E., Stief C. G., Kretschmer A., Herlemann A. Prostate cancer: When to treat, which treatment options by stage? *MMW Fortschr Med*. 2021;163(7):36–40.
3. Vrignaud P., Semiond D., Benning V., Beys E., Bouchard H., Gupta S. Preclinical profile of cabazitaxel. *Drug Des Devel Ther*. 2014;8:1851–1867.

4. Mukhtar E., Adhami V. M., Siddiqui I. A., Verma A. K., Mukhtar H. Fisetin Enhances Chemotherapeutic Effect of Cabazitaxel against Human Prostate Cancer Cells. Mol Cancer Ther. 2016;15(12): 2863–2874.
5. Bansal D., Reimers M. A., Knoche E. M., Pachynski R. K. Immunotherapy and Immunotherapy Combinations in Metastatic Castration-Resistant Prostate Cancer. *Cancers (Basel)*. 2021;13(2).
6. Zhang Y., Jiang R., Zheng X., Lei S., Huang F., Xie G., Kwee S., Yu H., Farrar C., Sun B., Zhao A., Jia W. Ursodeoxycholic acid accelerates bile acid enterohepatic circulation. *Br J Pharmacol*. 2019;176(16):2848–2863. DOI: 10.1111/bph.14705.
7. Duan W. J., Jia J. D. Research advances in primary biliary cholangitis. *Zhonghua Gan Zang Bing Za Zhi*. 2017;25(11):801–804.
8. Di Ciaula A., Wang D. Q., Molina-Molina E., Lunardi Baccetto R., Calamita G., Palmieri V. O., Portincasa P. Bile Acids and Cancer: Direct and Environmental-Dependent Effects. *Ann Hepatol*. 2017;16(Suppl. 1: s3–105.):s87–s105. DOI: 10.5604/01.3001.0010.5501.
9. Liu X., Wang Y. An overview of bile acid synthesis and its physiological and pathological functions. *Yi Chuan*. 2019;41(5):365–374.
10. Garcia M., Thirouard L., Sedès L., Monrose M., Holota H., Caira F., Volle D. H., Beaudoin C. Nuclear Receptor Metabolism of Bile Acids and Xenobiotics: A Coordinated Detoxification System with Impact on Health and Diseases. *Int J Mol Sci*. 2018;19(11):3630. DOI: 10.3390/ijms19113630.
11. Pellicciari R., Fiorucci S., Camaioni E., Clerici C., Costantino G., Maloney P. R., Morelli A., Parks D. J., Willson T. M. 6alpha-ethyl-chenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. *J Med Chem*. 2002;45(17):3569–3572. DOI: 10.1021/jm025529g.
12. Cariello M., Peres C., Zerlotin R., Porru E., Sabbà C., Roda A., Moschetta A. Long-term Administration of Nuclear Bile Acid Receptor FXR Agonist Prevents Spontaneous Hepatocarcinogenesis in Abcb4. *Sci Rep*. 2017;7(1):11203. DOI: 10.1038/s41598-017-11549-7.
13. Di Ciaula A., Wang D. Q.-H., Molina-Molina E., Baccetto R. L., Calamita G., Palmieri V. O., Portincasa P. Bile Acids and Cancer: Direct and Environmental-Dependent Effects. *Ann Hepatol*. 2017;16(Suppl. 1: s3–105):s87–s105. DOI: 10.5604/01.3001.0010.5501.
14. Wang W., Zhan M., Li Q., Chen W., Chu H., Huang Q., Hou Z., Man M., Wang J. FXR agonists enhance the sensitivity of biliary tract cancer cells to cisplatin via SHP dependent inhibition of Bcl-xL expression. *Oncotarget*. 2016;7(23):34617–34629. DOI: 10.18632/oncotarget.8964.
15. Yu J., Yang K., Zheng J., Zhao W., Sun X. Correction: Synergistic tumor inhibition of colon cancer cells by nitazoxanide and obeticholic acid, a farnesoid X receptor ligand. *Cancer Gene Ther*. 2022;29(6):871–872.
16. Ohno T., Shirakami Y., Shimizu M., Kubota M., Sakai H., Yasuda Y., Kochi T., Tsurumi H., Moriwaki H. Synergistic growth inhibition of human hepatocellular carcinoma cells by acyclic retinoid and GW4064, a farnesoid X receptor ligand. *Cancer letters*. 2012;323(2):215–222. DOI: 10.1016/j.canlet.2012.04.015.
17. Al-khfajy W. S., Arif I. S., Al-sudani B. T. Synergistic effect of obeticholic acid and fasting-mimicking on proliferative, migration, and survival signaling in prostate cancer. *Pharmacia*. 2022;69(2):579–587.
18. Kumar P., Nagarajan A., Uchil P. D. Analysis of Cell Viability by the MTT Assay. *Cold Spring Harb Protoc*. 2018;2018(6).
19. Chou T.-C., Rideout D. C. Synergism and antagonism in chemotherapy. San Diego: Academic Press; 1991. xvi. 752 p.
20. Carbonetti G., Converso C., Clement T., Wang C., Trotman L. C., Ojima I., Kaczocha M.. Docetaxel/cabazitaxel and fatty acid binding protein 5 inhibitors produce synergistic inhibition of prostate cancer growth. *Prostate*. 2020;80(1):88–98. DOI: 10.1002/pros.23921.
21. Achard V., Putora P. M., Omlin A., Zilli T., Fischer S. Metastatic Prostate Cancer: Treatment Options. *Oncology*. 2022;100(1):48–59.
22. Lee S. T., Wong P. F., Hooper J. D., Mustafa M. R. Alpha-tomatine synergises with paclitaxel to enhance apoptosis of androgen-independent human prostate cancer PC-3 cells in vitro and in vivo. *Phytomedicine*. 2013;20(14):1297–1305.
23. Li X., Lao Y., Zhang H., Wang X., Tan H., Lin Z., Xu H. The natural compound Guttiferone F sensitizes prostate cancer to starvation induced apoptosis via calcium and JNK elevation. *BMC cancer*. 2015;15:254. DOI: 10.1186/s12885-015-1292-z.
24. Tran L. N. K., Kichenadasse G., Sykes P. J. Combination Therapies Using Metformin and/or Valproic Acid in Prostate Cancer: Possible Mechanistic Interactions. *Curr Cancer Drug Targets*. 2019;19(5):368–381.
25. Darzynkiewicz Z., Traganos F. Measurement of apoptosis. *Adv Biochem Eng Biotechnol*. 1998;62:33–73.
26. Martelli A. M., Zweyer M., Ochs R. L., Tazzari P. L., Tabellini G., Narducci P., Bortul R. Nuclear apoptotic changes: an overview. *J Cell Biochem*. 2001;82(4):634–646. DOI: 10.1002/jcb.1186.
27. Aljubran A., Leighl N., Pintilie M., Burkes R. Improved compliance with adjuvant vinorelbine and cisplatin in non-small cell lung cancer. Hematol Oncol Stem Cell Ther. 2009;2(1):265-71.
28. Lee J. J., Chu E. Adherence, Dosing, and Managing Toxicities With Trifluridine/Tipiracil (TAS-102). *Clin Colorectal Cancer*. 2017;16(2):85–92.
29. Pfeiffer P., Yilmaz M., Möller S., Zitnjak D., Krogh M., Petersen L. N., Poulsen L. Ø., Winther S. B., Thomsen K. G., Qvortrup C. TAS-102 with or without bevacizumab in patients with chemorefractory metastatic colorectal cancer: an investigator-initiated, open-label, randomised, phase 2 trial. *Lancet Oncol*. 2020;21(3):412–420.
30. Negri M., Gentile A., de Angelis C., Montò T., Patalano R., Colao A., Pivonello R., Pivonello C. Vitamin D-Induced Molecular Mechanisms to Potentiate Cancer Therapy and to Reverse Drug-Resistance in Cancer Cells. *Nutrients*. 2020;12(6):1798. DOI: 10.3390/nu12061798.
31. Nevens F., Andreone P., Mazzella G., Strasser S. I., Bowlus C., Invernizzi P., Drenth J. P. H., Pockros P. J., Regula J., Beuers U., Trauner M., Jones D. E., Floreani A., Hohenester S., Luketic V., Shiffman M., van Erpecum K. J., Vargas V., Vincent C., Hirschfield G. M., Shah H., Hansen B., Lindor K. D., Marschall H.-U., Kowdley K. V., Hooshmand-Rad R., Marmon T., Sheeron S., Pencek R., MacConell L., Pruzanski M., Shapiro D. A Placebo-Controlled Trial of Obeticholic Acid in Primary Biliary Cholangitis. *N Engl J Med*. 2016;375(7):631–643. DOI: 10.1056/NEJMoa1509840.
32. Li S., Xu Z., Guo J., Zheng J., Sun X., Yu J. Farnesoid X receptor activation induces antitumour activity in colorectal cancer by suppressing JAK2/STAT3 signalling via transactivation of SOCS3 gene. *J Cell Mol Med*. 2020;24(24):14549–14560.
33. Attia Y. M., Tawfiq R. A., Ali A. A., Elmazar M. M. The FXR Agonist, Obeticholic Acid, Suppresses HCC Proliferation & Metastasis: Role of IL-6/STAT3 Signalling Pathway. *Sci Rep*. 2017;7(1):12502.
34. Han C. Y. Update on FXR Biology: Promising Therapeutic Target? *Int J Mol Sci*. 2018;19(7):2069. DOI: 10.3390/ijms19072069.
35. Lv B., Ma L., Tang W., Huang P., Yang B., Wang L., Chen S., Gao Q., Zhang S., Xia J. FXR Acts as a Metastasis Suppressor in Intrahepatic Cholangiocarcinoma by Inhibiting IL-6-Induced Epithelial-Mesenchymal Transition. *Cell Physiol Biochem*. 2018;48(1):158–172. DOI: 10.1159/000491715.
36. Braglia L., Zavatti M., Vinceti M., Martelli A. M., Marmiroli S. Deregulated PTEN/PI3K/AKT/mTOR signaling in prostate cancer: Still a potential druggable target? *Biochim Biophys Acta Mol Cell Res*. 2020;1867(9):118731.
37. Kosaka T., Miyajima A., Shirotake S., Suzuki E., Kikuchi E., Oya M. Long-term androgen ablation and docetaxel up-regulate phosphorylated Akt in castration resistant prostate cancer. *J Urol*. 2011;185(6):2376–2381.
38. Yasumizu Y., Miyajima A., Kosaka T., Miyazaki Y., Kikuchi E., Oya M. Dual PI3K/mTOR inhibitor NVP-BEZ235 sensitizes docetaxel in castration resistant prostate cancer. *J Urol*. 2014;191(1):227–234.
39. Wang L., Lin N., Li Y. The PI3K/AKT signaling pathway regulates ABCG2 expression and confers resistance to chemotherapy in human multiple myeloma. *Oncol Rep*. 2019;41(3):1678–1690.
40. Mao J., Chen X., Wang C., Li W., Li J. Effects and mechanism of the bile acid (farnesoid X) receptor on the Wnt/β-catenin signaling pathway in colon cancer. *Oncol Lett*. 2020;20(1):337–345.
41. Peng Z., Chen J., Drachenberg C. B., Raufman J. P., Xie G. Farnesoid X receptor represses matrix metalloproteinase 7 expression, revealing this regulatory axis as a promising therapeutic target in colon cancer. *J Biol Chem*. 2019;294(21):8529–8542.
42. Duran G. E., Wang Y. C, Francisco E. B., Rose J. C., Martinez F. J., Coller J., Brassard D., Vrignaud P., Sikic B. I. Mechanisms of Resistance to Cabazitaxel. *Molecular Cancer Therapeutics*. 2015;14(1):193–201. DOI: 10.1158/1535-7163.MCT-14-0155.
43. Liu R., Chen Y., Liu G., Li C., Song Y., Cao Z., Li W., Hu J., Lu C., Liu Y. PI3K/AKT pathway as a key link modulates the multidrug resistance of cancers. *Cell Death & Disease*. 2020;11(9):797.