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COWPEA MOSAIC VIRUS (CPMV) AS A CARRIER FOR NEW CHLOROQUINE DERIVATIVES AS ANTICANCER AGENTS, MODIFICATION AND APPLICATION[a]

VIRUS DEL MOSAICO DEL CAUPÍ (CPMV) COMO PORTADOR DE NUEVOS DERIVADOS DE LA CLOROQUINA COMO AGENTES ANTICANCERÍGENOS, MODIFICACIÓN Y APLICACIÓN

KHALDA MOHEE HBEB[b], RANA ABD AL-ALY KHAMEES AL-REFAI'A[c] *, ZAINAB SHAKIR ABDULLAH AL-ALI[d]

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ABSTRACT: Plant virus nanoparticles (VNPs) such as Cowpea Mosaic Virus (CPMV) can be used for a broad range of medical applications because they are inexpensive to produce, safe, biodegradable, and efficacious as treatments. Additionally, they can be easily modified chemically and genetically. Thus providing an efficient drug delivery platform can target specific cells and tissues. This paper explores the use of CPMV as epitope-carrying nanoparticles for two new chloroquine derivatives and as a new tool in breast cancer therapy. Two derivatives derived from the reaction of 4,7-dichloroquine with (doxorubicin and docetaxel) which were synthesised and fully characterized in previous work to produce (CQ-DOX and CQ-DOC) were conjugated to the external carboxylates of CPMV. The number of each derivative has been calculated by using a florescent dye to be 87 ± 2 and 79 ± 1, respectively. The effectivity of attached and unattached CQ-compounds to the CPMV,s surface was investigated by MTT assay and ADPI loaded stain, and the IC50 for each CQ-derivative with and without conjugation with CPMV was evaluated to be 70.395μg/ml for CQ-DOX and 14.384μg/ml for CQ-DOC before modification while, cytotoxic activity enhanced after modification to be 0.015 nM and 0.038 nM respectively.

KEYWORDS: Plant virus; chloroquine; drug delivery; cancer.

RESUMEN: Las nanopartículas de virus vegetales (VNP), como el virus del mosaico del caupí (CPMV), se pueden utilizar para una amplia gama de aplicaciones médicas porque son económicas de producir, seguras, biodegradables y eficaces como tratamientos. Además, pueden modificarse química y genéticamente fácilmente. De este modo, proporcionar una plataforma eficiente de administración de fármacos puede apuntar a células y tejidos específicos. Este artículo explora el uso de CPMV como nanopartículas portadoras de epítopos para dos nuevos derivados de cloroquina

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[b]Ministry of Interior, Iraq
[c]University of Babylon, Department of Chemistry, Iraq
[*]Corresponding author: sci.ranaabdul@uobabylon.edu.iq
[d]University of Basrah, Department of Chemistry, Iraq

y como una nueva herramienta en la terapia del cáncer de mama. Dos derivados de la reacción de 4,7- dicloroquina con (doxorrubicina y docetaxel) que fueron sintetizados y completamente caracterizados en trabajos anteriores para producir (CQ-DOX y CQ-DOC) se conjugaron con los carboxilatos externos de CPMV. El número de cada derivado se calculó utilizando un tinte fluorescente siendo 87 $\pm$ 2 y 79 $\pm$ 1, respectivamente. La efectividad de los compuestos CQ adheridos y no adheridos a la superficie de CPMV se investigó mediante ensayo MTT y mancha cargada con ADPI, y se evaluó que la CI50 para cada derivado de CQ con y sin conjugación con CPMV era 70,395 μg/ml para CQ -DOX y 14,384 μg/ml para CQ-DOC antes de la modificación, mientras que la actividad citotóxica mejoró después de la modificación hasta ser 0,015 nM y 0,038 nM, respectivamente.

PALABRAS CLAVE: Virus vegetal; cloroquina; administración de fármacos; cáncer.

1. INTRODUCTION

Nowadays, cancer assumes the position as one of the major threats to survival on a global basis; it is the second leading cause of death in high-income nations, with roughly 21 million people expected to be affected by 2030.(Cao *et al.*, 2018; Bray *et al.*, 2018). Cancer is caused by various endogenous and exogenous variables, including genetics, lifestyle, and living situations; these risk factors can act in concert or sequence (Bayraç *et al.*, 2018). Cancerous cells no longer perceive signals that control cell proliferation, differentiation, and survival. Angiogenesis and apoptosis inhibition in these aberrant cells can lead to metastasis into distant tissues and the potential death of the organism. Despite the medication's unfavorable side effects, chemotherapy is the most widely accepted traditional cancer treatment method (Qin *et al.*, 2017; Sanmartín *et al.*, 2019).

Anti-cancer immunotherapies are classed as passive or active depending on their ability to re-activate the host immune system against cancerous cells (Banerjee *et al.*, 2018).

A key component of cancer therapy is combination therapy, a mode of care that combines two or more therapeutic drugs. The combinations of anti-cancer medications improve efficacy in comparison to monotherapy because they target critical pathways in a manner that is typically additive or synergistic. This approach may also minimize medication resistance and therapeutic anti-cancer effect, including reducing tumor growth, stopping the mitotic activity of cells, lowering cancer stems to cell populations, and inducing apoptosis. Most metastatic tumors still have low 5-year survival rates, and creating a new anti-cancer medicine is expensive and time-consuming. New tactics are therefore being proposed that focus on the survival pathways that deliver efficient and effective outcomes at a reasonable cost. Repurposing prescription drugs eventually prescribed that will someday be used as a strategy to treat illnesses other than cancer (Zhang *et al.*, 2021; Kimura *et al.*, 2013; Gurel-Gurevin *et al.*, 2018).

In this work chloroquine (CQ) was chosen to combine with traditional anti-cancer drugs such as doxorubicin and docetaxel due to its ability to show cytotoxicity effects against a variety of humanity's cancers

by inducing cell cycle arrest, inhibiting autophagy, plus ultimately inducing death in tumor cells. It also can increase sensitivity to numerous therapeutic types used in chemotherapy for cancer. Moreover, CQ has a powerful cancer-specific chemosensitizer when used with other chemotherapies as it has been described to enhance the intracellular marks of the medications through inhibitory degradation of autophagy cargo (Gasiorkiewicz *et al.*, 2021; El-Gowily *et al.*, 2021).

To maximize efficiency by limiting off-target effects, CPMV has been used as a delivery vehicle to increase therapeutic efficacy while reducing systemic toxicity. Because plant viruses have developed transport cargos toward host cells efficiently, they are an excellent starting point for creating specialized drug delivery vehicles (Duval *et al.*, 2020; Patel *et al.*, 2018).

The Cowpea mosaic virus (CPMV) is a VNP that has shown the ability to stimulate an anti-cancer immune response; specifically, CPMV VNPs have demonstrated the ability to improve preclinical outcomes by overcoming tumor-based immune suppression in local tumor situations (Hoopes *et al.*, 2018; Al-Kafage & Al-Refai'a, 2022).
Additionally, systemically administered CPMV can stimulate an additive therapeutic immune response following treatment of the tumor with a conventional agent such as radiation.

Here CPMV has been modified chemically with each CQ-derivative to display multiple copies of each one and then tested as anti-cancer agents against MCF-7 cells.

2. EXPERIMENTAL SECTION

2.1. Chemical synthesis

2.1.1. General procedure for the synthesis of compound CQ-DOX

The mixture of 4,7-dichloroquinoline (0.5 g, 2.5 μmol) and doxorubicin (0.0460g, 84 μmol) with normal saline were mixed before being heated at 120-130°C for 22 hours with stirring. The reaction was emptied into 100 mL of water and filtered after cooling, and the solid was recrystallized from 100 mL ethyl acetate before being heated; the precipitate was washed with chloroform, and the crude was purified by TLC (DCM/MeOH, 5/2) to give the chloroquinoline amino acid compound as a reddish solid to give yield (o.6181 g, 81%). Elemental analysis expected C: 62.75%, H: 4.83%, N: 4.07%: found C: 62. 09%, H: 7.45%, N: 4.12% for a chemical formula C36H33ClN2O10 with a molecular weight of 689.11(Al-Kafage & Al-Refai'a, 2022).

2.1.2. General procedure for the synthesis of compound CQ-DOC

The mixture of 4,7-dichloroquinoline (0.5 g, 2.5 μmol) and docetaxel (0.08 g, 98 μmol) were mixed before heating at 120-130°C for 23 hours with stirring. The reaction was emptied into 100 mL of water and filtered

after cooling, and then the solid was recrystallized in 100 mL of ethyl acetate before being heated; the precipitate was washed with chloroform, and the crude was purified by TLC (DCM/MeOH, 5/2) to give the chloroquinoline amino acid compound as an oily compound with dark brown color to give a yield of (0.78g, 64%). Elemental analysis expected C:, 64.11%, H:; 5.80%, N:; 2.93%: found C:; 64. 46%, H:; 7.28%, N:; 2.90% for a chemical formula C51H55ClN2O14 with a molecular weight of 955.44 (Al-Kafage & Al-Refai'a, 2022).

2.2. Chemical modification of CPMV

2.2.1. The modification of CPMV's external carboxyl

Freshly prepared (EDC) (0.1 g, 580 μmol) in 10.0 mL sodium phosphate buffer pH7.1, that was used in a1000, molar excess, with NHS (0.1g, 860 μmol) in 5Ml DMSO in 4000 molar excess were added to 200 μL CPMV (3 mg/mL), the reaction mixture was then allowable run for two hours with gentle stirring. Then 250 μL of CQ-DOX (0.01 g, 14.5 μmol) in 5 mL DMSO was added in 6000 molar excess, and 306 μL of CQ-DOC (0.01 g, 10.4 μmol) in 5 mL DMSO in the same molar excess were added separately. The reaction mixture's final volumetric concentration of DMSO was used at 20%. After stirring for 20h at room-temperature, each sample was centrifuged (14000 r.p.m) for 10 min. At the same temperature, 100 kDa cut-off columns were used to separate virus particles, they were washed three- times with sodium phosphate buffer (80 μL). The modified virions were re-suspended inside (100 μL) of 10 mM sodium phosphate PH 7.1. Recovery of the virus was 76.6% for CQ-DOX and 72.2% for CQ-DOC, according to the initial concentration of CPMV (Al-Refai'a, 2019).

2.2.2. Quantification of CQ -derivatives on external carboxyl using fluorescent dye

CF-488A dye (0.028 g, 43.5 μmol) in 2000 molar excess was liquefied within dry DMSO (2 ml), then mixed with 100 μL of 0.5 mg /ml CPMV, CPMV-CQ-DOX and CPMV-CQ-DOC particles in 0.01 M sodium phosphate buffer (PH 7.1). The final DMSO content was reduced to 20 percent (vol/vol). Each mixture was incubated for 2 h at room temperature before being allowed to continue for 22 hours at a temperature of 4°C for 22 hours while stirring.

2.3. Practical purification

2.3.1. Ultrafiltration

Ultrafiltration was employed to purify and concentrate virus particles coated with chloroquine derivatives. Samples were purified using (100 μL - 1 mL) of 100 kDa cut-off columns (Microcon, Amicon Millipore) and centrifuged. While buffer solution and tiny impurities can be passed through the membrane, and particles are retained on the filter.

2.3.2. Dialysis

This approach of nanoparticle purification is quite successful. The semipermeable membrane keeps particles inside while allowing tiny molecules to pass through. Centrifugal concentrators can immediately concentrate the sample if the protein concentration is too low for further processing or analysis. Float-A-Lyze tubing 100KD has been used for dialysis.

2.3.3. Electrophoresis

Modified and unmodified CPMV samples were taken in (17μL) from each sample and added separately to 3 μL of loading dye (Coomassie Staining Solu.) and loaded or going on 1.2% agarose gel in 50mL TBE buffer by spending an electric field between 1 to 5 Vcm^{-2} for 1.5 hours. For the staining of ethidium bromide (Et.Br) (nucleic Acid staining), 0.10 μg per mL (Et.Br) (5.0 μL) in 1.0 $\times$ TBEbuffer was mixed with a gel before running. Each particle was visualized on a UV-transilluminator at 302nm using an E-GRAPH device. The gel was stained with Coomassie staining solution overnight for coat protein visualization. A camera or scanner was used to capture gel images (Al-Refai'a, 2019).

2.4. Cell Viability Assay

The Pasteur Institute (located in Tehran, Iran) provided the MCF-7 cell line. Cells were grown and maintained at 37°C in a humidified incubator with 5% CO2 in air in Dulbecco's Modified Eagle Medium (D.M.E.M; Gibco;, Life. Technologies,; Waltham, M.A, U.S.A) was supplemented within 10% fetal-bovine-serum (F.B.S; Bio-West S.A.S, Nuaille,; France) and 1% P.S.F (antibiotic- antimycotic solution, Sigma-Aldrich, St.. Louis;, M.O, U.S.A). Cells were detached at 37°C using 0.250% trypsin (Gibco,; Invitrogen,; Waltham;, M.A, U.S.A) and 0.10% ethylene diamine tetraacetic acid (Merck,; Darmstadt, Germany) in phosphate-buffered -saline (PBS) after reaching 75% confluency. The cells were then re-suspended in DMEM with 10% FBS and 1% PSF. Before the trials. At a density of 5000 cells per-well, cells were then seeded onto 96-well.

Plates, and incubated for 24 hours. Then they were rinsed with PBS pH 7.4 (phosphate-buffered- saline) before incubating for 72 hours in a fresh medium having samples at various concentrations (20, 40, 80, 160, and 320 times dilutions). 3-(4, 5dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide The assay of (MTT) dye was used to evaluate cell viability and materials cytotoxicity effect at varying concentrations. After 72 hours incubation (37C°, and5 percent CO2 in a steamy atmosphere), MTT in (0.50 mg. ml-1 in PBS) was added to each well. At the same time each plate was then incubated for an additional 4 hours at the same temperature. At 37°C, the formazan was liquefied with 100 μL of DMSO with gently shaking. An ELISA reader was used to determine the absorbance at 570 nm. The average of three separate experiments was used to present the results. Then, the concentrations of substances (i.e., IC50values) that caused a 50% reduction in cell viability were determined.

2.4.1. 4,6-Diamidino-2-phenylindole dihydrochloride (DAPI) nuclear staining

After being given the treatment as mentioned earlier for 72 hours, MCF-7 cells were fixed with methanol and acetic acid in (3:1, v/v) and then rinsed with PBS buffer. Cells were stained for 20 minutes in the dark with 1 mg.ml-1 of DAPI stain after washing. A fluorescent microscope with a suitable excitation filter was used to record the stained pictures (Alkan *et al.*, 2022).

3. RESULTS AND DISCUSSION

CQ-derivatives were synthesized previously and fully characterized (Al-Kafage & Al-Refai'a, 2022). After that, each CQ derivative was conjugated with external carboxylates of CPMV. CPMV as an NPs could shield medications from being recognized and rejected by P-glycoprotein (P-gp), giving them a chance to be transported by the autophagy process after being taken up by cells. So that, DOX and DOC, would not be affected by autophagy, CQ might exert its effectiveness and block autophagy during the migration of each loaded derivative (Sun *et al.*, 2018).

The addressable carboxylate on the outer surface of CPMV was modified with EDC/NHS together to form CPMV-NHS-ester, CQ-DOX and CQ-DOC were also conjugated to the external surface of CPMV to form CPMV-CQ-DOX and CPMV-CQ-DOC. Addressable carboxyl is produced from acidic amino acids (aspartic and glutamic acids) on the exterior surface of wt CPMV, producing CPMV-CQ-DOX conjugates in 76.6 % recovered yield depending on the virus's starting concentration. Similarly, the exterior surface of wt CPMV was modified with CQ-DOC in 77 % recovery yield according to the initial virus concentration, as presented in Figure 1. After modification, the number of each CQ derivative was determined from the absorbance at 490-494 nm to be 87$\pm$ 2 and 79$\pm$ 1, respectively, from 180 chemical addressability carboxylates on the surface of CPMV. In the below Scheme there are two possibilities to conjugate compound R1 with CPMV and both can be conjugatedto the external carboxylate.

Successfully conjugation of each CQ derivative was monitored using agarose gel electrophoresis. In the electric field, modified and unmodified CPMV move toward the anode. CQ-DOX and CQ-DOC conjugated CPMV-CQ-DOX and CPMV-CQ-DOC can be visualized in the gel under UV light based on the intrinsic fluorescence of each CQ, when stained with ethidium bromide (EtBr stains the encapsulated CPMV genome) or Coomassie Blue (stains the coat proteins), the modified and unmodified CPMV can be identified. For modified particles (Figure 2A,2B), the mobility of CPMV toward the anode slows down upon conjugation of NHS-linkers to external carboxylates, and further slows down upon each CQ derivative conjugation as a consequence of a combination of charge and mass effects.

Results from the modification of CPMV with CF-488A dye are shown in (Figure 2C) indicating clear cleavage of dye-modified CPMV and modified CPMV on agarose gel after 8h dialysis; this may be derived from the cleavage of 24 amino acids terminus on the small subunits (Al-Refai'a, 2018).

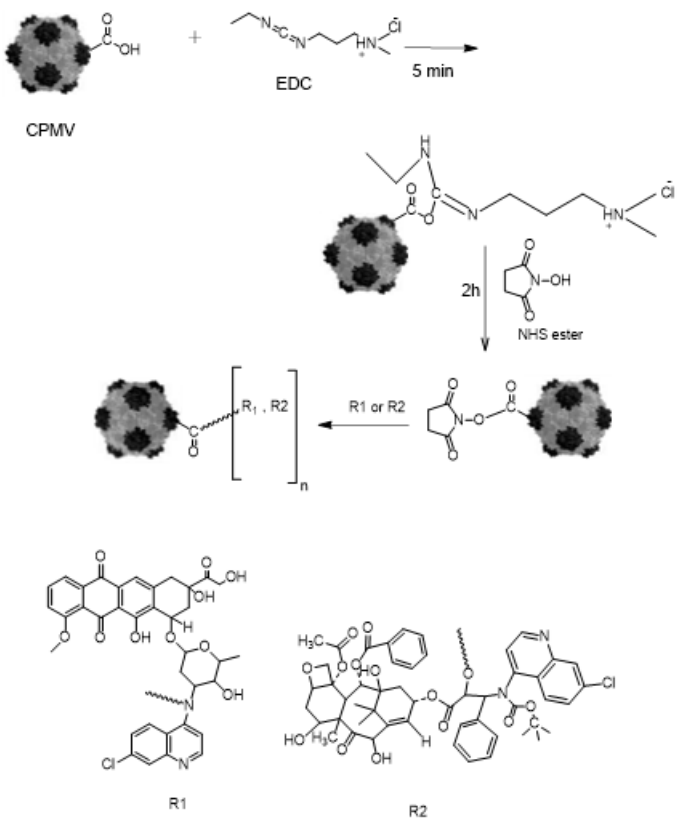

Figure 1: The modification of CPMV with each CQ-derivative using the EDC/NHS method. Source: Author's Own Work.

The 1% AgNO3 stained transmission electron micrographs show that the virus particles retain intact with no aggregation when CQ-DOX and CQ-DOC are bound and are monodisperse with an average diameter of 30.5 nm; this is consistent with dynamic light scattering measurements in solution (Figure 3).
The cell viability and drug efficacy of conjugated and unconjugated CQ derivatives (CQ-DOX and CQ-DOC) were evaluated using tissue culture techniques. Native CPMV nanoparticles are biocompatible and do not show any apparent cytotoxicity. CPMV-CQ-DOX and CPMV-CQ-DOC conjugates showed effective cell killing (Figures 4, 5), respectively. We observed significant differences in the cellular toxicity patterns comparing un-labeling CQ derivatives and labeling ones. The efficiency of each conjugated CQ-derivative to CPMV was essential to increase the effectiveness of small molecule chemotherapeutic drug (SMCD) and moderate its toxic and side effects.

The effect of each CQ-derivative against MCF-7cell was investigated, MCF-7cell cancer lines were treated with CQ-DOX first using a range of concentrations (6.25-100 μg.ml) in two-fold dilutions. Next, (IC50) was calculated for this derivative before conjugation with CPMV to be 70.375μg/ml and 0.051nM after conjugation according to the conjugated number of CQ-DOX on the CPMV surface. These results indicated

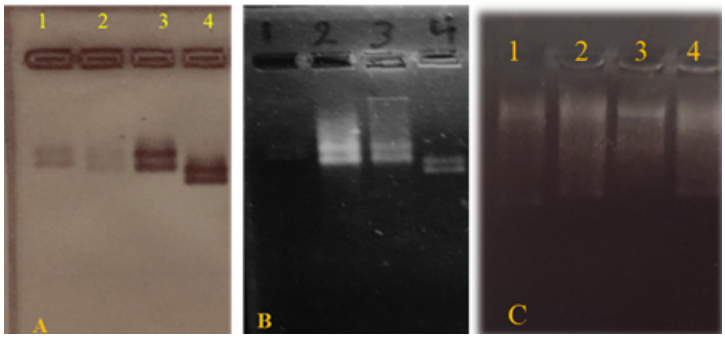

Figure 2: Agarose gel electrophoresis of modified and unmodified CPMV (A) Coomassie Blue stained (B) ethidium bromide staining. Lane 1- CPMV-CQ-DOC, lane 2- CPMV-CQ-DOX , lane 3- CPMV- NHS-ester and lane 4- wt CPMV. (C) ethidium bromide stained. Lane 1-CPMV, Lane 2- CPMV-dye, Lane 3- CPMV-CQ-DOC-dye, Lane 4- CPMV-CQ- DOX dye. Source: Author's Own Work.

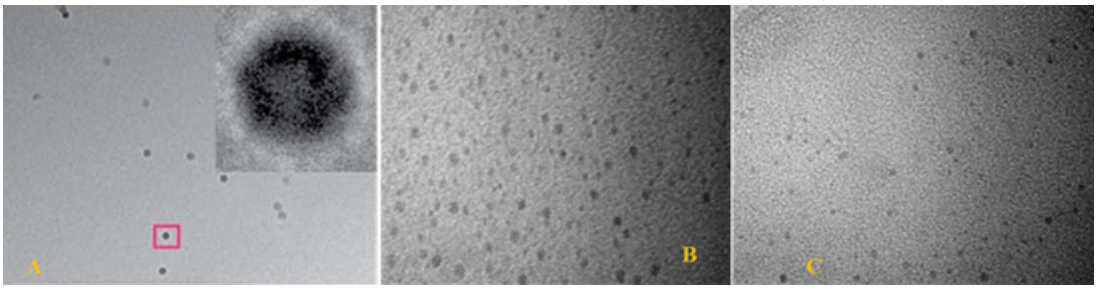

Figure 3: Transmission electron micrographs of 1 % AgNO3 solution with a scale of 100nm (A) unmodified CPMV (Aljabali *et al.*, 2012), (B) CPMV-CQ-DOX, and (C) CPMV-CQ-DOX. Source: Author's Own Work.

a remarkably significant illumination of the living cells and improved the vital role of the delivery system in increasing drug efficacy and activity. (Figure 4)

Similarly, IC50 values were obtained for CO-DOC before and after conjugation at 14.384μg/ml and 0.038 nM, respectively. (Figure 5)

It was reported that the combination of traditional anti-cancer drugs (DOX or DOC) plus CQ was more effective than DOX and DOC alone due to increasing the cytotoxic effect of these drugs by CQ on MCF7 cell lines (Madden *et al.*, 2017).

It can easily recognize the differences, cell viability was decreased much more significantly after using CQ derivatives with their carrier (CPMV) compared to CQ-DOX and CQ-DOC alone.
The uptake of CPMV is mediated by specific interaction with a surface-displayed form of the cytoskeletal protein vimentin. Surface vimentin expression has been detected in endothelial cells in vivo as well as in activated macrophages, and the ability of cells to internalize CPMV is correlated with the presence of surface vimentin in these cell types. CPMV accumulates within the tumor margin. This feature helped the drug

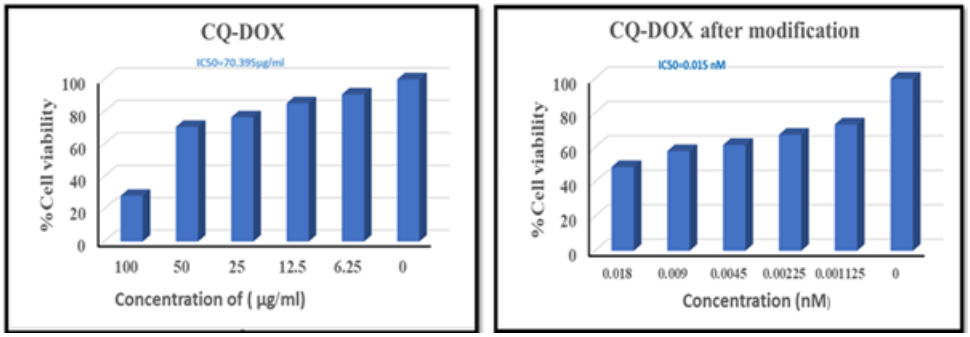

Figure 4: The effects of CQ-DOX on MCF-7 cell viability before and after labeling with CPMV. Source: Author's Own Work.

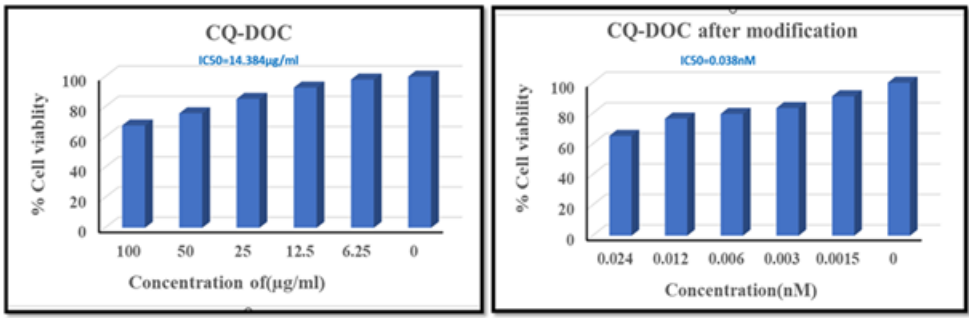

Figure 5: The effects of CQ-DOC on MCF-7 cell viability before and after labeling with CPMV. Source: Author's Own Work.

reach the tumor areas without affecting other parts of the body (Agrawal *et al.*, 2012).

At low dosages about (0.001-0.018 nM loaded CQ-DOX) and (0.001-0.014 nM loaded CQ-DOC), CPMV-CQ-DOX and CPMV-CQ-DOC were found to be more effective in conferring cytotoxicity. All had good conjugation effectiveness and loading capacity on CPMV particles, but CPMN-CQ-DOX showed the highest in vitro cytotoxicity and was eliminated against malignancy cells after 72 h.

On the other hand, DAPI staining revealed that MCF-7 cells treated with CQ-derivatives had considerably more apoptosis than the control, which revealed a significant percentage of cell death. DAPI staining results showed that CQ derivatives could successfully trigger apoptosis in MCF-7 cells due to illumination and morphology changing of malignant cells. (Figure 6)

The vimentin-specific uptake may render CPMV advantageous over other VNP systems due to cargo loading capacity, native tropism towards vimentin on endothelial cells and their native immunostimulatory effect within solid tumor cancer models (Beatty & Lewis, 2019).

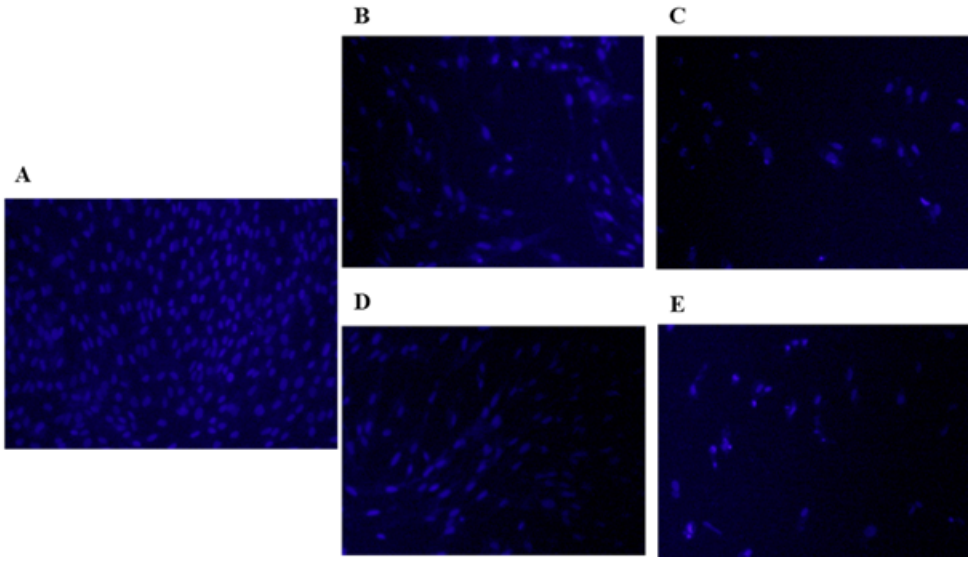

Figure 6: MCF-7 cancer cell staining. Cell nuclei stained with DAPI (blue). (A) Fluorescence signal from cancer cells without medication. (B) Fluorescence signal from cells incubated with CQ-DOX. (C) Fluorescence signal from cells incubated with CPMV-CQ-DOX.(D). Fluorescence signal from cells incubated with CQ-DOC and (E) Fluorescence signal from cells incubated with CPMV-CQ-DOC. Source: Author's Own Work.

4. CONCLUSION

In conclusion, the activity of each CQ-derivative as an anti-cancer agent using DAPI and MTT assay before modification with CPMV has shown more than a 50 % reduction in cell viability. In contrast, the results have shown much more growth inhibition after modification with great and unique IC50 values. The CPMV nanoparticles can carry a payload of molecules like drugs or dyes to tumor cells. Thus, drugs toxic to the healthy and the cancerous host cells can be preferentially released inside the cancerous cells, thereby mitigating off-target toxicity.

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Competing interests statement

The authors declare no competing financial or non-financial interests.

Authors contributions

Khalida Mohee AL Kafage performed the lab experiments.
Rana Abd Al-Aly Khamees Al-Refaia designed the work, analysed the data and wrote the article.
Zainab Shakir Abdullah Al-Ali involved in planning the work and added some comments on the manuscript.

References

Agrawal, Arpita & Marianne Manchester (2012). Differential uptake of chemically modified cowpea mosaic virus nanoparticles in macrophage subpopulations present in inflammatory and tumor microenvironments. *Biomacromolecules*, 13(**10**), 3320-3326.

Aljabali, Alaa A. A., Elaine J. Barclay, Nicole F. Steinmetz, George P. Lomonossoff & David J. Evans (2012). Controlled immobilisation of active enzymes on the cowpea mosaic virus capsid. *Nanoscale*, 4(**18**), 5640-5645.

Al-Kafage, K. M. & Al-Refai'a, R. A. K. (2022). New Synthesis of Chloroquinoline Derivatives as Anti-Cancer Agents. *HIV Nursing*, 22(**2**), 892-894.

Alkan, Hatice, *İ*brahim H. C., Muhammad Muddassir Ali, Omer Hazman, Recep Liman, lorica Col*ă* & Elena Bonciu (2022). Cytotoxic and genotoxic evaluation of biosynthesized silver nanoparticles using Moringa oleifera on MCF-7 and HUVEC cell lines. *Plants*, 11(**10**), 1293.

Al-Refai'a, Rana (2018). Modified Cowpea Mosaic Virus as a Carrier of Antimalarial Drugs. Diss. University of Hull.

Al-Refai'a, Rana (2019). Cowpea Mosaic Virus (CPMV) as a Carrier Vehicle for Antimalarial Drugs, Modification, and Application. *International Journal of Drug Delivery Technology*, 39(**3**),490-495.

Banerjee Kasturi, Sushil Kumar, Kathleen A. Ross, Shailendra Gautam, Brittany Poelaert, Mohd Wasim Nasser, Abhijit Aithal, Rakesh Bhatia, Michael J. Wannemuehler, Balaji Narasimhan, Joyce C. Solheim, Surinder K. Batra & Maneesh Jain (2018). Emerging trends in the immunotherapy of pancreatic cancer. *Cancer letters*, 417, 35-46.

Bayraç, Abdullah Tahir, Oya Ercan Akça, Füsun Inci Eyidoğan & Hüseyin Avni Öktem(2018). Target-specific delivery of doxorubicin to human glioblastoma cell line via ssDNA aptamer. *Journal of biosciences*, 43, 97-104.

Beatty, Perrin H. & Lewis, J. D. (2019). Cowpea mosaic virus nanoparticles for cancer imaging and therapy. *Advanced drug delivery reviews*, 145, 130-144.

Bray, Freddie, Jacques Ferlay, Isabelle Soerjomataram, Rebecca L. Siegel, Lindsey A. Torre & Ahmedin Jemal(2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.*CA: a cancer journal for clinicians*, 68(**6**), 394-424.

Cao, Bochen, Bray F., Ilbawi A. & Soerjomataram I. (2018). Effect on longevity of one-third reduction in premature mortality from non-communicable diseases by 2030: a global analysis of the Sustainable Development Goal health target. *The Lancet Global Health*, 6(**12**), e1288-e1296.

Duval, Kayla E. A., Robert J. Wagner, Veronique Beiss, Steven N. Fiering, Nicole F. Steinmetz & P. Jack Hoopes (2020). Cowpea mosaic virus nanoparticle enhancement of hypofractionated radiation in a B16 murine melanoma model. *Frontiers in Oncology*, 10, 594614.

El-Gowily, Afnan H., Samah A. Loutfy, Ehab MM Ali, Tarek M. Mohamed & Mohammed A. Mansour (2021). Tioconazole and chloroquine act synergistically to combat doxorubicin-induced toxicity via inactivation of PI3K/AKT/mTOR signaling mediated ROS-dependent apoptosis and autophagic flux inhibition in MCF-7 breast cancer cells. *Pharmaceuticals*, 14(**3**), 254.

Gasiorkiewicz, Bartosz Mateusz, Paulina Koczurkiewicz-Adamczyk, Kamil Piska & Elżbieta P?kala (2021). Autophagy modulating agents as chemosensitizers for cisplatin therapy in cancer. *Investigational new drug*, 39 (**2**), 538-563.

Gurel-Gurevin, Ebru, Hulya Tuba Kiyan, Osman Behzat Burak Esener, Seyma Aydinlik, Ayca Uvez, Engin Ulukaya, Konstantinos Dimas & Elif Ilkay Armutak (2018). Chloroquine used in combination with chemotherapy synergistically suppresses growth and angiogenesis in vitro and in vivo. *Anticancer research*, 38(**7**), 4011-4020.

Hoopes, P. Jack, Wagner R. J., Duval K., Kang K., Gladstone D. J., Moodie K. L., Crary-Burney M., Ariaspulido H., Veliz F. A., Steinmetz N.F. & Fiering S. N.(2018). Treatment of canine oral melanoma with nanotechnology-based immunotherapy and radiation. *Molecular pharmaceutics*, 150(**9**), 3717-3722.

Kimura, Tomonori, Yoshitsugu Takabatake, Atsushi Takahashi & Yoshitaka Isaka (2013). Chloroquine in cancer therapy: a double-edged sword of autophagy. *Cancer research*, 73(**1**), 3-7.

Madden, Andrew J., Oberhardt, B., Lockney, D., Santos, C., Vennam, P., Arney, D., Franzen, S., Lommel, S. A., Miller, C. R., Gehrig, P. & Zamboni, W. C. (2017). Pharmacokinetics and efficacy of doxorubicin-loaded plant virus nanoparticles in preclinical models of cancer. *Nanomedicine*, 12(**20**), 2519-2532.

Patel, Ravi, Anna E. Czapar, Steven Fiering, Nancy L. Oleinick & Nicole F. Steinmetz (2018). Radiation therapy combined with cowpea mosaic virus nanoparticle in situ vaccination initiates immune-mediated tumor regression. *ACS omega*, 3(**4**), 3702-3707.

Qin, Si-Yong, Ai-Qing Zhang, Si-Xue Cheng, Lei Rong & Xian-Zheng Zhang(2017). Drug self-delivery systems for cancer therapy. *Biomaterials*, 112, 234-247.

Sanmartín, O., Beato, C., Suh-Oh, H.J ., Aragón, I., España, A., Majem, M., Segura, S., Gúrpide, A., Botella, R. & Grávalos, C. (2019). Clinical management of cutaneous adverse events in patients on chemotherapy: a national consensus statement by the Spanish Academy of Dermatology and Venereology and the Spanish Society of Medical Oncology. *Actas Dermo-Sifiliográficas* (English Edition), 110(**6**), 448-459.

Sun, Jia-Hui, Chong Ye, En-He Bai, Ling-Ling Zhang, Shao-Jie Huo, Hao-Han Yu, Su-Yun Xiang & Shu-Qin Yu (2018). Co-delivery nanoparticles of doxorubicin and chloroquine for improving the anti-cancer effect in vitro. *Nanotechnology*, 30(**8**), 085101.

Zhang, Kai, Zhang, Kai, Jingjing Li, Xiaofei Xin, Xiaoqing Du, Di Zhao, Chao Qin, Xiaopeng Han, Meirong Huo, Lei Yang & Lifang Yin (2021). Dual targeting of cancer cells and MMPs with self-assembly hybrid nanoparticles for combination therapy in combating cancer. *Pharmaceutics*, 13(**12**), 1990.