Benha University

Faculty of Science

Zoology Department

Carbon Quantum Dots (CQDs) in the targeting and treatment of tumours

Research Project

Submitted in Partial Fulfilment of the Requirements for the Award of the Degree of Bachelor of Science (B.Sc..) in Zoology and Chemistry

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2024/2025

Acknowledgment

﴿ وَإِذْ تَأَذَّنَ رَبُّكُمْ لَئِن شَكَرْتُمْ لَأَزِيدَنَّكُمْ ﴾ سورة إِبْرَاهِيمَ – الآية 7

﴿ الْحَمْدُ لِلَّهِ الَّذِي هَدَانَا لِهَٰذَا وَمَا كُنَّا لِنَهْتَدِيَ لَوْلَا أَنْ هَدَانَا اللَّهُ ﴾ سورة الأَعْرَافِ – الآية 43

“Gratitude turns what we have into enough.”

First and foremost, I express my heartfelt gratitude to Allah, whose endless mercy and guidance granted me the strength and determination to complete this research.

I would like to extend my deepest appreciation to my supervisor, **Dr. Marwa Saad Mohamady Mahmoud**, whose encouragement, insightful feedback, and unwavering support were a beacon throughout this journey. Her belief in my potential often reignited my own confidence during moments of doubt.

I am sincerely thankful to **Prof. Dr. Nasrallah Abdelhamid**, Head of the Zoology and Chemistry Department, for his continuous encouragement and for fostering an environment that nurtured my academic growth.

To my family, whose endless love and prayers formed the invisible foundation of my perseverance, and to my friends who offered me support in their own quiet ways — your presence made this path lighter and brighter.

I would also like to thank my professors and teaching assistants at the Faculty of Science for their invaluable knowledge and guidance over the past four years.

Finally, this research does not mark an end, but rather the beginning of a lifelong journey of discovery, learning, and contribution to the scientific community

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Introduction:

Cancer is one of the common diseases that leads to death in many places due to metastasis. Cancer cells are abnormal cells that grow and divide uncontrollably, invading surrounding tissues and sometimes spreading to all of the body. There are many types of cancer including breast, ovarian, liver, cervical and so on. The mutation and environmental factors like smoking are related to all cancers. The developed countries are more affected than developing countries in this disease.

The most common among women is breast cancer **(Xia *et al.*, 2022)**. Breast cancer can lead to a high rate of death due to mutations in genes or other factors **(Mahdavi *et al.*, 2019; Vatankhah *et al.*, 2023; Buist *et al.*, 2018; Hu *et al.*, 2022)**. This cancer is related to the same mutation of genes that leads to ovarian cancer **(Hu *et al.*, 2022)**. Cervical cancer is more common in developing countries than in developed countries against other types **(Zhang *et al.*, 2020; Prabhu M *et al.*, 2016)**. Cancer Therapy by chemicals is more dangerous as it affects normal cells like cancer cells which leads to a high rate of death. Scientists are interested in diagnosing and treating this disease without side effects so they are heading towards nanotechnology.

Nowadays, the whole world is heading towards nanotechnology. CQDs are used as nanoparticles in diagnosis and target treatment. CQDs can penetrate cancer cells easily. Cancer can be diagnosed early by biopsy, computerized tomography scan (CT), magnetic resonance image (MRI), endoscopy and x-rays. However, these tools lead to high costs, lack of awareness and unnecessary treatment. The conventional cancer treatments are surgery, chemotherapy and radiotherapy but there are side effects. There is a need for non-invasive and reliable treatment. CQDs

were used to detect cancer cells by imaging and therapy. So, Nanotechnology has an efficient role in cancer theranostics.

1. Carbon Quantum Dots (CQDs)

1.1 Properties and usage: -

In recent years, Nanotechnology has been used to fight many diseases. Carbon quantum dots, in their nanoscale below 10 nm, are used in many fields, including cell imagining **(Shi *et al.*, 2019)**, drug delivery **(Su *et al.*,2020)**, and targeting tumor cells (Fig. 1). They are used in cancer therapy due to their properties like biocompatibility, tinny molecules, non-toxicity, and fluorescence. CQDs are synthesised from natural, eco-friendly precursors **(Naik *et* al.,2022)**.

1.2 The precursors for synthesis: -

These precursors are biomolecules or medicinal plants (Fig. 2). The biomolecule precursors are proteins and amino acids that are biocompatible and abundant including albumin like bovine serum albumin with formic acid to produce nitrogen-doped CQDs with less toxicity and high fluorescence **(Tan *et al.*,2015)**, carbohydrates like glucose and biomass that contains lipids **(Qiao *et al.*, 2018 and Gusain *et al.*, 2021)**, nucleic acid by linking between the two strands of DNA to form DNA-CQDs that lead to carbonization, dehydration, condensation, and polymerisation. The cytosine base in DNA is used to synthesise CQDs by heat at 160°C. These CQDs were used in imaging cells and sensing due to their photostability, biocompatibility, and wide-range pH **(Luo *et al.*,2018)**.

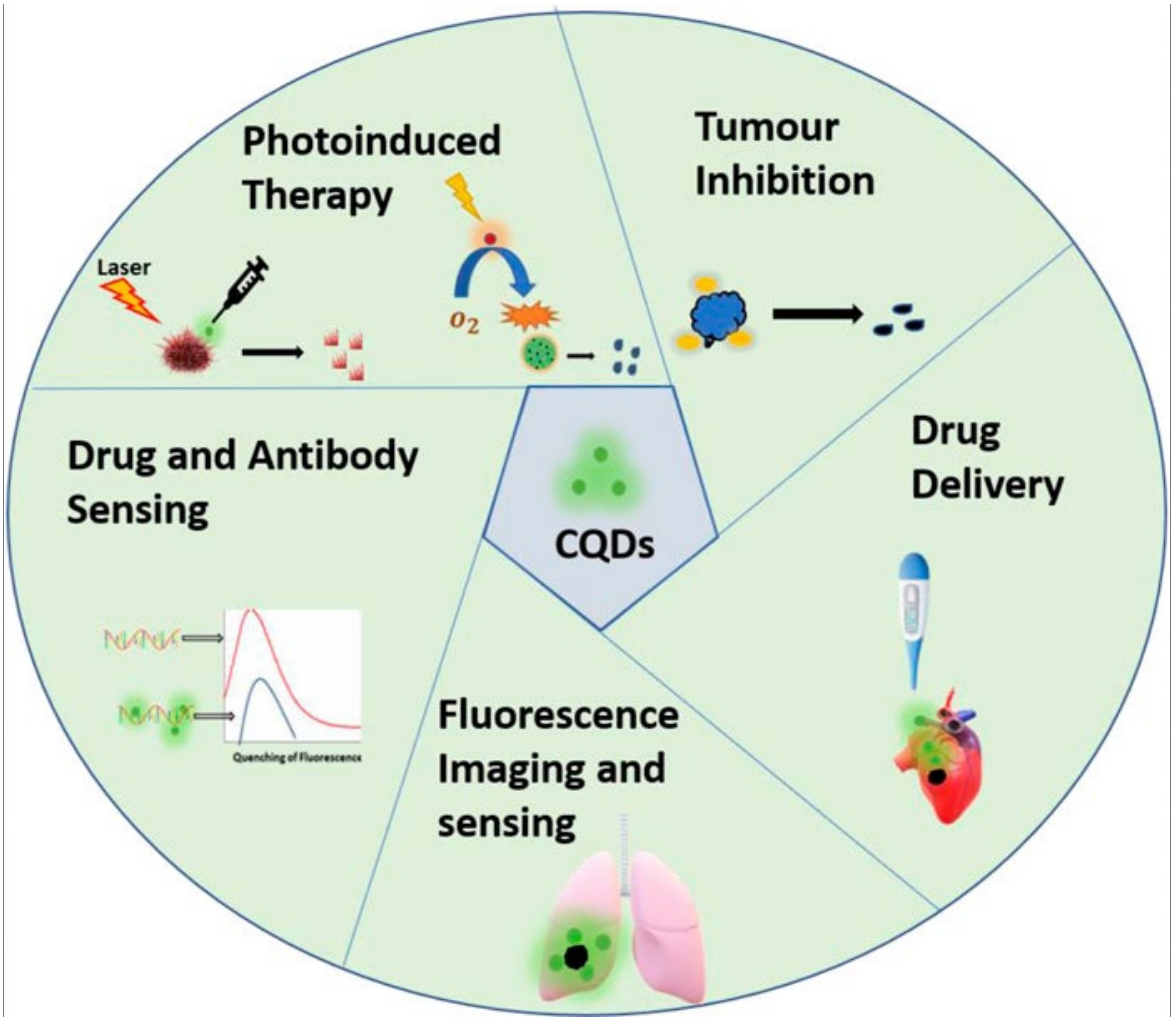

Figure 1: CQDs in cancer diagnosis and treatment. **(Naik *et* al.,2021)**

In the last studies, medicinal plant-based CQDs have drawn attention **(Naik *et al.*,2021)** due to their medicinal properties, such as anticancer properties, which make them useful in cancer treatment **(Park *et al.*, 2017)**. These CQDs are efficient in this field due to their photostability, high photoluminescence (PL), water solubility and nontoxicity **(Naik *et al.*,2021)**. Some of these plants used to synthesise CQDs are green tea **(He *et al.*, 2021)**, Ginger juice **(Naik *et* al.,2022)**, and walnut oil **(Arkan *et al.*, 2018)**.

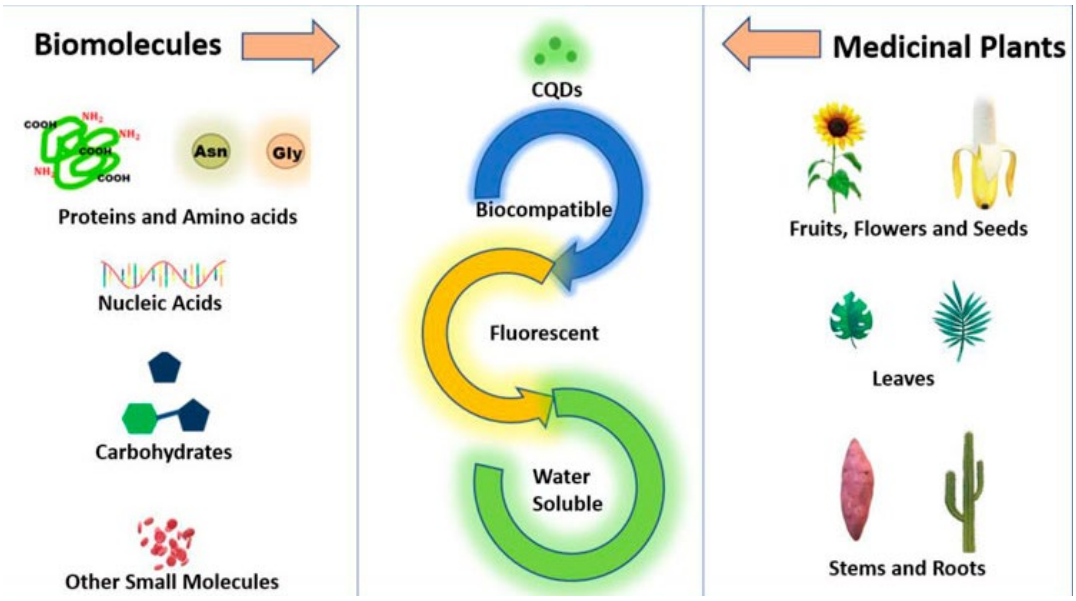

Figure 2: The synthesis of CQDs from biomolecules and medicinal plants. **(Naik *et al.*,2021)**

1. Breast Cancer

Breast cancer has become the main reason women die **(Obeagu *et al.*, 2024)**. Although BC is an issue for global public health, China ranks first globally due to its large population and high cancer rate **(Xia *et al.*, 2022)**. BC can be determined by a nipple discharge, breast shape or size change, or the discovery of breast lumps or mastalgia, even though breast examination is essential **(Heer *et al.*, 2020)**. It is from a mutation in breast cells. It has some types, including ductal, lobular **(Edward *et al.*, 2021 and Obeagu *et al.*, 2023)**, triple-negative breast cancer, breast Paget disease, inflammatory breast cancer, and soft tissue sarcoma **(Jagsi *et al.*, 2022)**. The most common cancers are ductal and lobular, which are present in location and still in situ or spread in adjacent tissue **(Iatrakis *et al.*, 2021)**.

BC is a multifactorial disease that comes with some risk factors, including diet, obesity, genetics, smoking, drinking, and cosmetics that contain estrogen

(Mahdavi *et al.*, 2019). Despite this, breast cancer can develop without any risks in women than men because of exposure to estrogen and progesterone. According to this, late menopause leads to BC due to exposure to estrogen and progesterone **(Vatankhah *et al.*, 2023)**. The ductal or lobular carcinoma in situ can appear again in women due to their personal history, which leads to increasing the risk of this disease **(Buist *et al.*, 2018)**. The increasing number of injuries by BC in close blood relations is related to family history **(Maio *et al.*, 2021)**. Mutations in BRCA genes, including BRCA1 & BRCA2, lead to uncontrolled growth in BCs and tumor cells. The presence of 1 or 2 copies of the mutated gene leads to a 50 % chance of inheriting in the family, and these genes can also lead to ovarian cancer **(Hu *et al.*, 2022)**. Early pregnancy decreases the rate of chance injury by BC **(Garnæs *et al.*, 2022)**.

2. 1 The CQDs in targeting tumor cells:

Methotrexate, MTX, is a drug used as an anticancer but non-targeting for tumor cells. That leads to some side effects in the body **(Zuber *et al.*, 2021 and Poursadegh *et al.*, 2024).** FA-CQDs with Ex sourced from BC cells were used as targeted MTX to avoid that (**Yang *et al.*, 2023 and Tiwari *et al.*, 2024)**. Ex derived from cancer cells and used as a targeted drug in a range from 40 to 100 nm in size due to their membrane composition, bioavailability, and diminished off-target cytotoxicity **(Gilligan *et al.*,2017 and Tiwari *et al.*, 2023)** and these vesicles don't affect normal cells **(Saw *et al.*, 2019 and Zhu *et al.*, 2021)**. There are folate receptors in the malignant cells **(Prieto *et al.*, 2020 and Khoshnood *et al.*, 2023)** that lead to using it in treating cancer cells when attached to CQDs **(Sattariazar *et al.*, 2023 and Zahed *et al.*, 2024)**.

2. 2 Drug loading and cell viability

Drug loading and cellular uptake study using CQDs, MTX-CQDs and Ex@MTX-CQDs in cancer cells (Fig. 3).

The release of MTX from Ex@MTX-CQDs at conditions pH 5 and 7.4 indicates that 65% of MTX was released in acidic conditions like the tumor microenvironment, and around 10% was released in neutral environments like normal cells. That leads to low toxicity in normal cells and high targeting (Fig. .4 A).

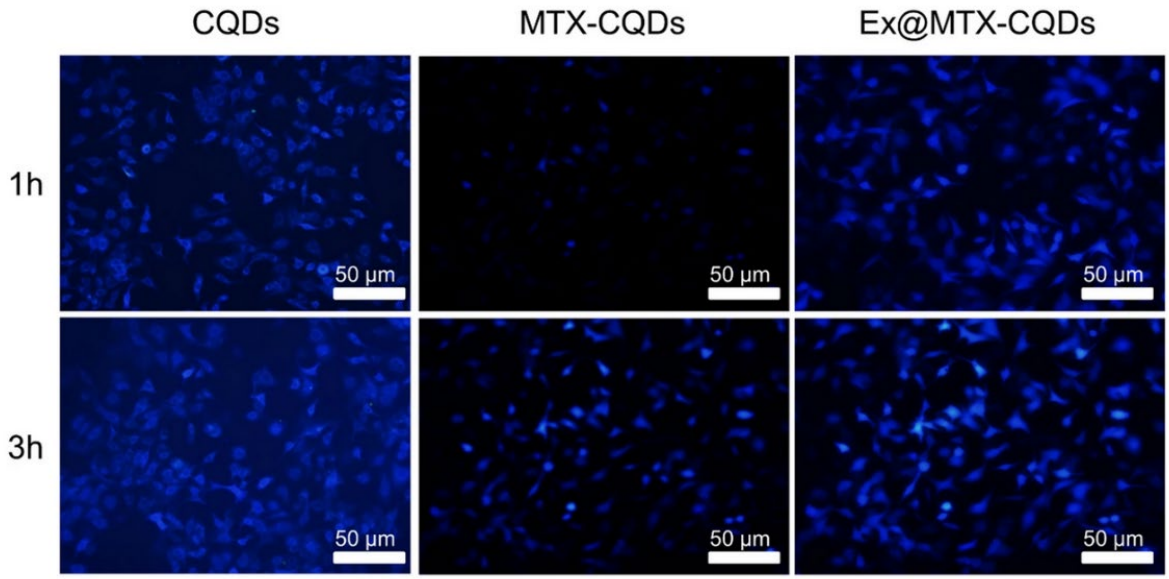

Figure 3: The cellular uptake images of CQDs, MTX-CQDs, and Ex@MTX-CQDs. **(Kazeminava *et al.*, 2024)**

The MTT assay measured the cell viability in the MCF-7 cell line. The effect of Ex@MTX-CQDs leads to less toxicity against breast cancer than CQDs, MTX, Ex, or MTX-CQDs. Ex@MTX-CQDs marked cytotoxicity against the MCF-7 cell line (Fig. 4 B).

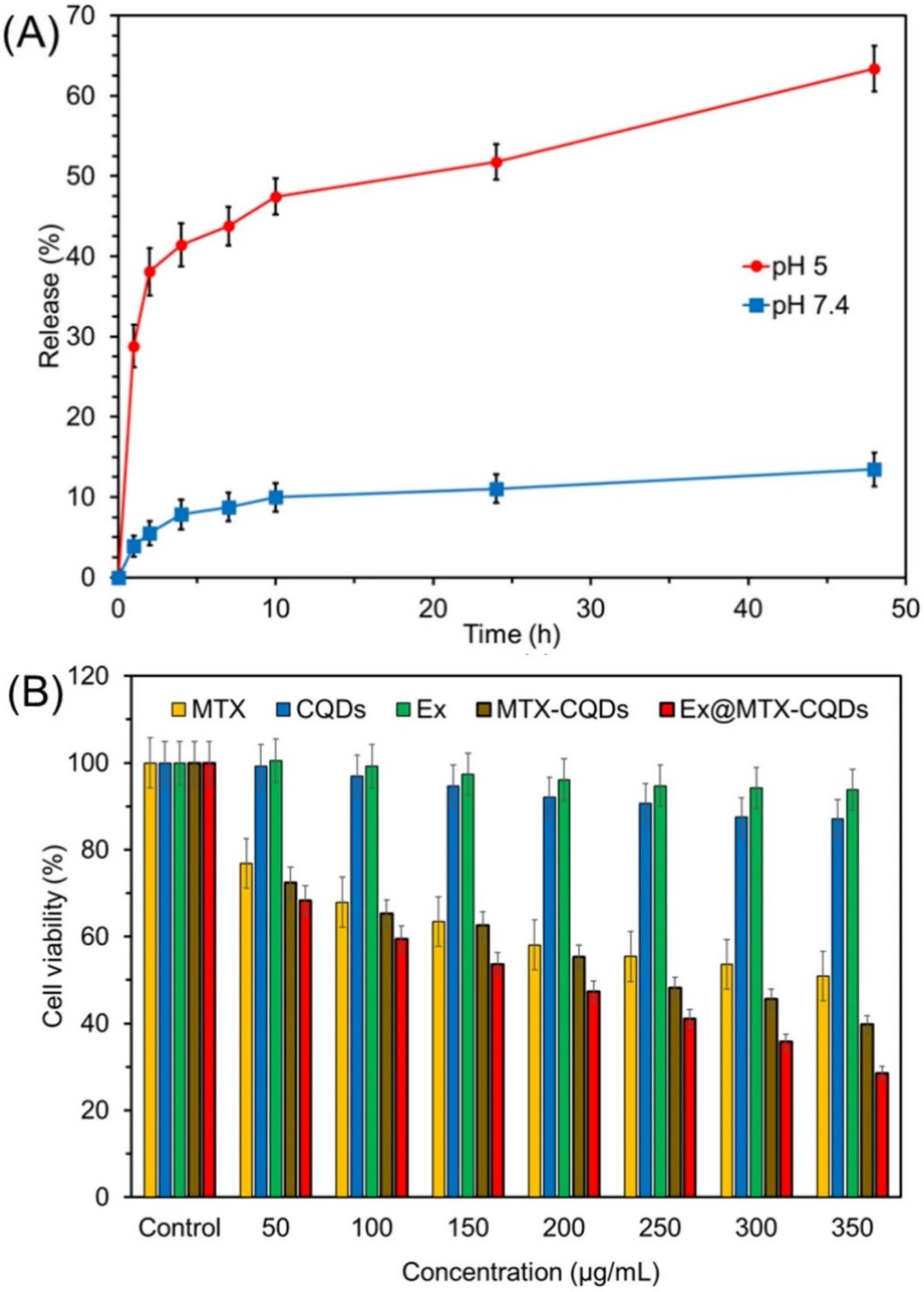

Figure 4: MTX release profile of Ex@MTX-CQDs (**A**) and the cell viability study of MCF-7 cell after incubation at various concentrations (mg/mL) of samples 48 h (**B**). **(Kazeminava *et al.*, 2024)**

2. Ovarian Cancer

Ovarian cancer is a lethal disease that leads women to die at an older age **(Feeney *et al.*, 2020 and Siegel *et al.*, 2020).** In the 21st century, the incidence of ovarian cancer has decreased in Northern America and Western Europe **(Siegel *et al.*, 2020)**. Despite the decreasing incidence rate, ovarian cancer is the most dangerous and leads to 2-thirds of deaths because of being diagnosed at later stages **(Aus *et al.*, 2020)**. The WHO has classed ovarian cancer into five major types: mucinous carcinoma, endometrioid carcinoma, high-grade serous carcinoma, low-grade carcinoma, and clear cell carcinoma due to differences in cell origin **(Kobel *et al.*, 2022)**. Women between 60 and 65 years old have the highest rate of ovarian cancer in developed countries **(Siegel *et al.*, 2020)**. The rate of ovarian cancer has increased because of the Western lifestyle, family size, feeding babies milk formula and a decrease in the rate of pregnancy **(Ali *et al.*, 2018)**. The survival rate has decreased at advanced stages like (III, IV) **(Timmermans *et al.*, 2018)** and varies due to different disease histotypes **(Zhou *et al.*,2021)**. An earlier diagnosis of breast cancer leads to an earlier diagnosis of ovarian cancer **(Ali *et al.*, 2023)**.

Ovarian cancer includes epithelial tumors, germ cell tumors and sex cord stromal tumors that are heterogeneous histologic types. When 10-15 % of ovarian cancer is due to genetics, there are multiple factors because of the complex and heterogeneous epithelial cells **(Janardhan *et al.*, 2015)**. Surface epithelial cells lead to 90% of ovarian cancer **(Ali *et al.*, 2023)**. Developed countries have a higher rate of ovarian cancer than developing countries because of increasing life expectancy, decreasing fertility rate, feeding babies in milk formula, western lifestyle, and increased daily intake of fatty diet and dense caloric food **(Rice *et al.*, 2020)**. Around 75% of ovarian cancer in older women is diagnosed after menopause **(Bandera *et al.*, 2016)** that's because of poor prognosis **(Ali *et al.*, 2020)**.

Ovarian cancer affects white American women more than African American women **(Bandera *et al.*, 2016)** because of BRCA mutations. Although BRCA1 and BRCA2 have the same functions in DNA repair, they affect cancer differently by their mutations. BRCA1 mutations are affected two to threefold compared with BRCA2 mutations. The injured first or second-degree relatives lead to BRCA1 & 2 mutations that affected ovarian cancer by 84% from BRCA1 mutations and 16% from BRCA2 mutations **(Ali *et al.*, 2023)**. A recent study shows that BRCA1 mutations are more aggressive than BRCA2 mutations because they lead to reprogramming in tumor cells **(Bruand *et al.*, 2021)**.

3. 1 The CQDs in targeting tumor cells:

CQDs are highly toxic to tumor cells. Copper metal is used to treat SKOV3 cells. Cu-based nanomaterials affect cell cycle regulation more than other nanomaterials related to carcinogenic processes **(Seong *et al.*, 2015 and Chen *et al.*, 2021)**.

The CQDs/Cu2O have higher toxicity against SKOV3 cells than CQDs or Cu2O, and the copper has high stability **(Chen *et al.*, 2021)**. The green nucleus indicates the controlled healthy cell, the orange nucleus indicates the late stage of apoptosis and the necrotic cells with uniformly red nuclei (fig .5).

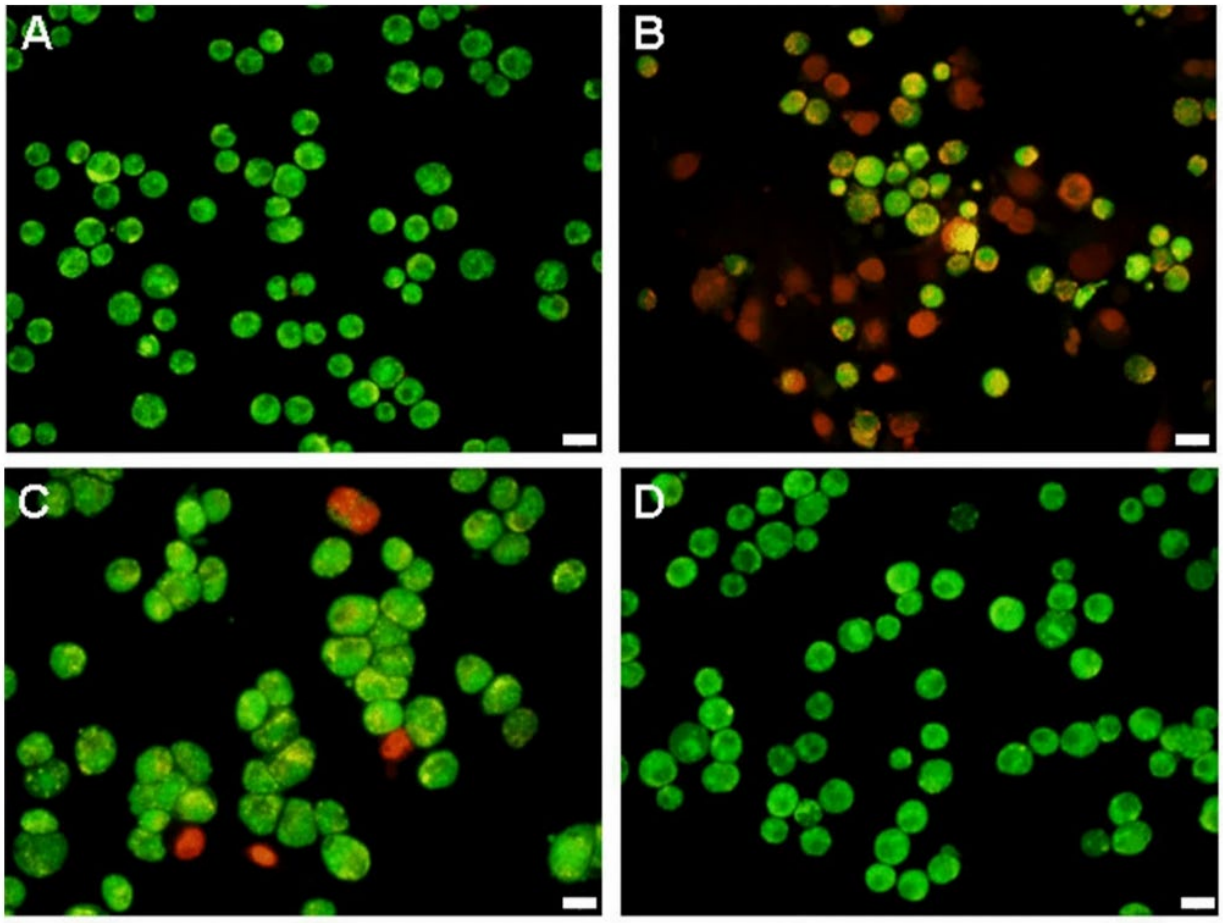

Figure 5: Fluorescence photomicrographs changes by AO/EB staining of SKOV3 cells after treatment with CQDs/Cu_2O (**B**), CQDs (**C**), or Cu_2O (**D**) for 24 h compared to controls (**A**), respectively. Scale bar 20 μm. **(Chen *et al.*, 2021)**

The CQDs/Cu2O also have higher toxicity in cancer cells than OXA and ART. The IC50 of CQDs/Cu2O in SKOV3 cells is less than the IC50 of each of OXA and ART **(WangJ *et al.*, 2016)**.

3. 2 The effect of CQDs/Cu2O:

The effect of CQDs in SKOV3 cells was non-toxic and biocompatible **(Shereema *et al.*,2015)**. In the MTT assay, a dependent concentration of CQDs/Cu2O led to cytotoxicity and the SKOV3 cells had a higher inhibition rate between other cancer

cell lines like (Hela, A549, HT-29, HCT116, BABL-3T3, HEK293T, J224AI) that indicate the CQDs/Cu2O lead to inhibit the growth of SKOV3 cells (fig. 6).

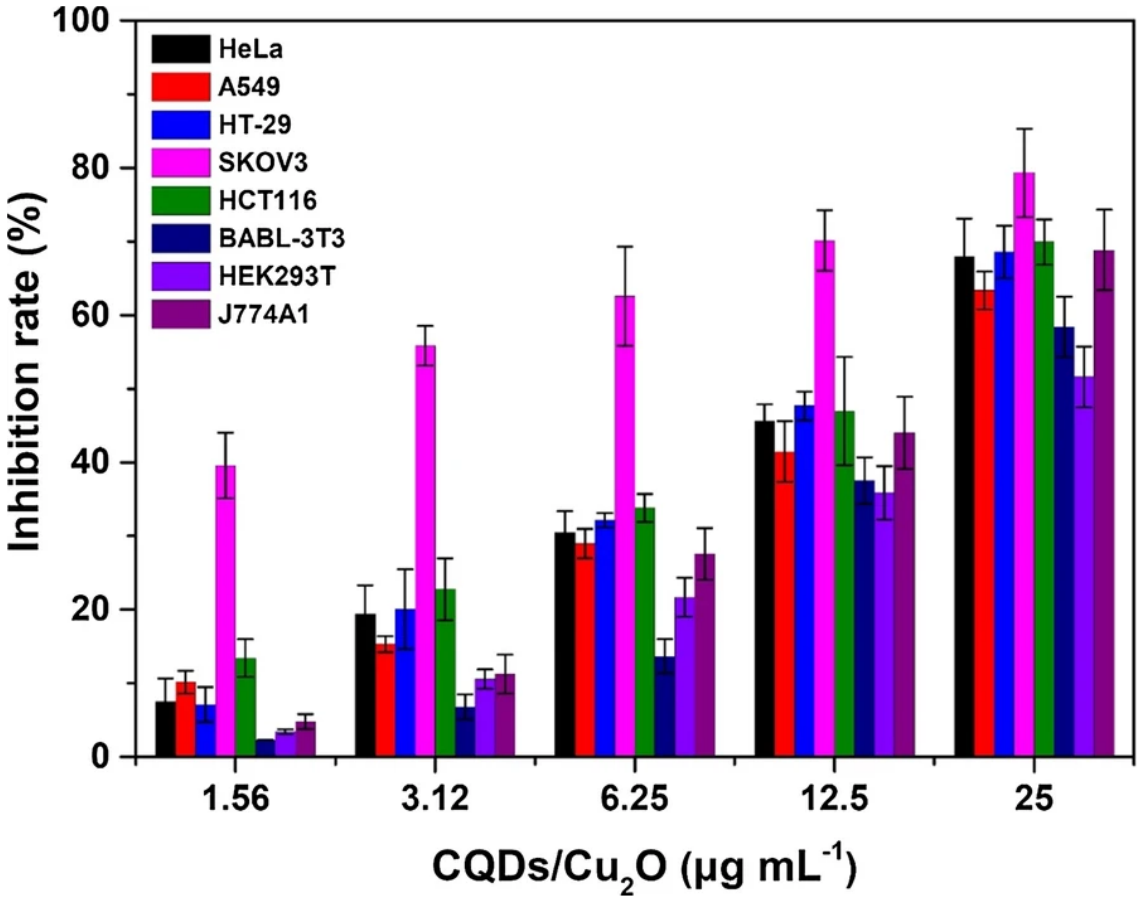

Figure 6: Differential cytotoxicity of CQDs/Cu_2O in cancer cells (HeLa, A549, HT-29, SKOV3, HCT116) and normal cells (BABL-3T3, HEK293T, J774A1) by the MTT assay for 24 h. **(Chen *et al.*, 2021)**

The WST assay shows the results from the MTT assay by the same range of inhibitory concentration. A low concentration of the CQDs/Cu2O leads to the differentiation between SKOV3 cancer and other cells associated with anticancer drugs **(WangJ *et al.*, 2016)**.

3. Liver cancer

Liver cirrhosis is a dangerous disease that spreads worldwide. The hepatic stellate cells have a role in this field and can develop cancer. It can be developed by the potential polarization of CAFs (cancer-associated fibroblasts), TAMs (tumor-associated macrophages) and TECs (tumor endothelial cells) **(Ma *et al.*, 2019)**. There are two types of liver cancer (Hepatocyte cancer and Cholangiocyte cancer), and they lead to differences in histology and molecular features. Features of HCCs are vascular and stromal invasion, thick cell trabeculae, increased cell density, loss of the reticulin framework, and a pseudo-glandular pattern **(Cogliati *et al.*, 2023)**. The iCCA differentiates according to the affected bile duct **(Vijgen *et al.*, 2017 and Kendall *et al.*, 2019)**. The HCC contains two phenotype classes: proliferation and non-proliferation **(Zucman-Rossi *et al.*, 2015 and Ally *et al.*, 2017)**, and the iCCA includes proliferation and inflammation **(Cogliati *et al.*, 2023)**. The HCC has unique molecular and histological features in the proliferation that lead to HBV infection, poor differentiation, pro-proliferative pathways, a worse prognosis, and increased rate of TP53 mutations, and the non-proliferation leads to a better outcome and WNT signalling due to CTNNB1 mutations **(Ally *et al.*, 2017)**. iCCA is similar to HCC and leads to common IDH1/2 mutations and FGFR2 fusions **(Sia *et al.*, 2015 and Nakamura *et al.*, 2015)**.

HBV and HCV can induce immunity to promote or suppress carcinogenesis. HBV can inhibit the effect of T-cells on cancer cells as the T_{reg} is highly immunosuppressive **(Pallett *et al.*, 2015; Tan *et al.*, 2019 and Lim *et al.*, 2019)**. HCV can promote cancer cells by loss of IL-2 secreting $CD4^+$ T-helper cells and the presence of exhaustion markers like TIM3, PD1 and galectin 9 that secrete from monocytes and promote T_{reg} cell expansion **(Hofmann *et al.*, 2021)**. During NASH, there is a crosstalk between immune cells and hepatocytes that leads to the

development of cancer like $CD8^{+}PD1^{+}T$ that induces liver damage in preclinical mouse models because of metabolic stimuli and $CD^{+}IL\text{-}17A^{+}T$ helper 17 cells that induces white adipose tissue neutrophil infiltration that mediates insulin resistance and fatty acid release **(Gomes *et al.*, 2016; Ma *et al.*, 2016; Pfister *et al.*, 2021 and Dudek *et al.*, 2021)**.

Cancer-associated fibroblasts (CAFs) can promote tumor cells by ligand-receptor interactions, deposition of ECM components and release of growth factors and inflammatory cytokines **(Sahai *et al.*, 2020; Donne *et al.*, 2023 and Llovet *et al.*, 2023)**. CAFs can mediate the secretion of collagen, proteoglycans and fibronectins with the crosslinking enzymes that promote tumor cell growth and increase tissue stiffness that activates integrins, SRC family kinases, FAK and YAP-TAZ signaling that leads to increased cancer cells by its pro-migratory and pro-proliferative in both cancer and stromal cells **(Cogliati *et al.*, 2023)**. For example, YAP-TAZ signaling is used as an anti-apoptotic protein in cancer cells, leading to tumor proliferation **(Stein *et al.*, 2015 and Chang *et al.*, 2015)**.

4. 1 CQDs and Huh7 cell line:

The CQDs were used with tryptophan and sorbitol for high stability and optical properties. The TC-WS-CQDs lead to cell toxicity up to a certain concentration, which leads to targeting tumor cells. The B -, G -, and R-WS-CQDs indicated the endocytosis of the TC-WS-CQDs in tumor cells after incubation. The nystatin and chlorpromazine incubate the CQDs to detect the location of tumor cells (Fig. 7).

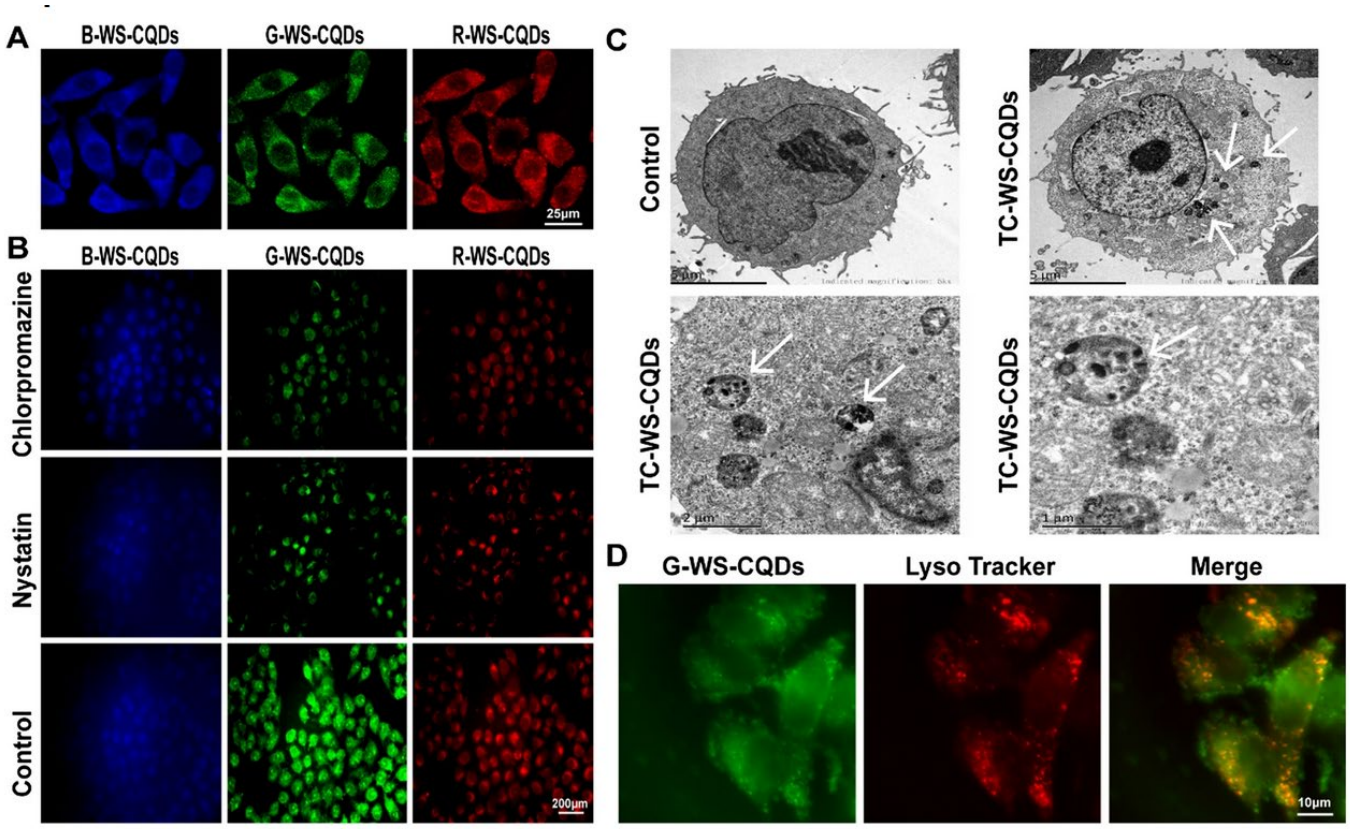

Figure 7: (**A)** LSCM images of Huh7 cells with TC-WS-CQDs incubation for 6 h. (**B)** Huh7 cells were treated with TC-WS-CQDs alone and co-treated with Nystatin (30 μM) or Chlorpromazine (20 μM) for 6 h. (**C)** TEM assessed the intracellular localization of TC-WS-CQDs in Huh7 cells. Arrows indicate intracellular vesicles engulfing TC-WS-CQDs in the cytoplasm. (**D)** G-WS-CQDs co-localise with lysosomes as tracked by LysoTracker. **(Wang *et al.*, 2022)**

4. 2 Treating by CQDs:

The CQDs induce high radiation therapy, decreasing the tumour cells' weight but not affecting the healthy cells (Fig. 8).

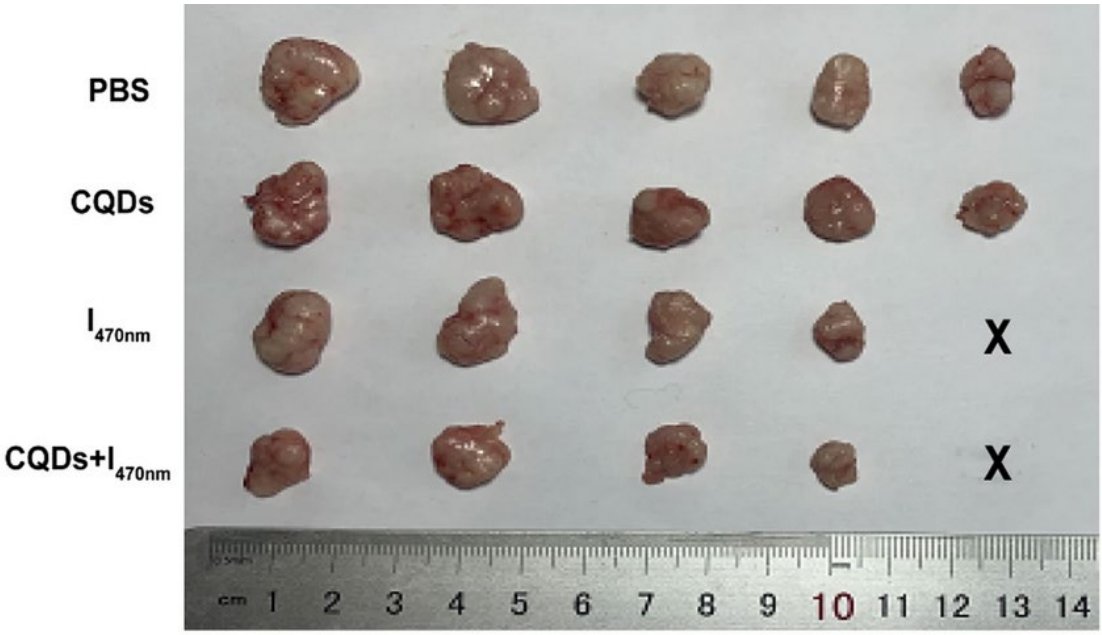

Figure 8: The effect of CQDs, I_{470nm} and CQDs+I_{470nm} on the size of tumor cells. **(Wang *et al.*, 2022)**

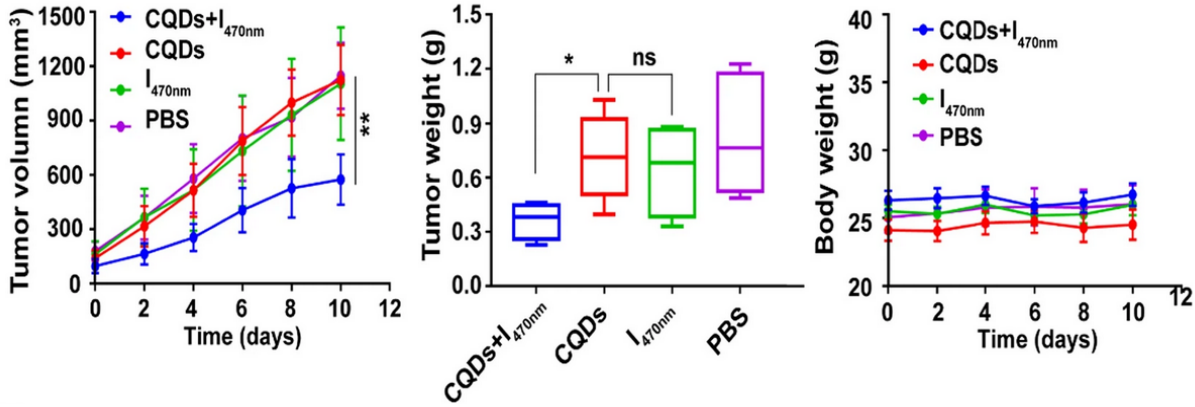

Figure 9: The effect of CQDs, I_{470nm} and CQDs+I_{470nm} on the tumor volume (mm^3), tumor weight (g) and body weight (g). **(Wang *et al.*, 2022)**

4. Cervical cancer

Cervical cancer is the second most common cancer **(Zhang *et al.*, 2020)** that leads to death **(Mattiuzzi *et al.*, 2020)**. This cancer has a higher rate in developing countries than in developed countries **(Zhang *et al.*, 2020)**. The low and middle-income individuals in developing countries have a higher rate of death from this cancer than in wealthier countries **(Prabhu *et al.*, 2016)**. The percentage of infection rate varies according to HPV genotype **(Zhang *et al.*, 2020)**. Women with HPV have a high risk of incidence of cervical intraepithelial neoplasia (CIN) and HPV types **(Stelzle *et al.*, 2021 and Yuan *et al.*, 2021)**.

HPV have many genotypes, there are around 13 of these that lead to cervical cancer **(Perkins *et al.*, 2023)**. The percentage of cancer changes according to the genotype of HPV that is associated with alpha-9 and so on (Table. 1). The high-risk or oncogenic HPV types, including HPV16 and HPV18, can lead to cervical cancer **(Cohen *et al.*, 2019)**. Sexual behaviour affects the incidence of HPV. HPV can spread after sexual intercourse, leading to cervical intraepithelial neoplasia (CIN) **(Zhang *et al.*, 2020)**.

Table 1: Indicate the percentage of cancer cells according to the genotype of HPV. **(Bedell *et al.*, 2020)**

Carcinogenic human papillomavirus (HPV) type	Proportion of cervical cancers, %	9-Year risk of progression of incident HPV infection to cervical intraepithelial neoplasia grade 3 or worse (CIN3+)	HPV species	Risk group	Included in 9-valent vaccine
16	60.3	6.3	Alpha-9	Highest	Yes
18	10.5	3.0	Alpha-7	High	Yes
45	6.1	2.2	Alpha-7	High	Yes
33	3.7	4.5	Alpha-9	Medium	Yes
31	3.6	2.2	Alpha-9	Medium	Yes
52	2.7	2.2	Alpha-9	Medium	Yes
58	2.2	1.9	Alpha-9	Medium	Yes
35	2.0	2.8	Alpha-9	Medium	No
39	1.6	1.1	Alpha-7	Lower	No
51	1.2	1.1	Alpha-5	Lower	No
59	1.1	0.9	Alpha-7	Lower	No
56	0.9	0.8	Alpha-6	Lower	No
68	0.6	1.0	Alpha-7	Lower	No

Multiple sexual partners and early age at first intercourse cause a high risk of cervical cancer **(Liu *et al.*, 2015)**. Oral contraceptive pills (OC) are associated with cervical cancer as the increase in the use of OC leads to an increase in the risk of cervical cancer **(Zhang *et al.*, 2020)**.

5. 1 CQDs against cervical cancer:

Nowadays, cervical cancer has spread among females in low and middle-income nations **(Bentivegna *et al.*, 2016)**. The World Health Organization (WHO) reports that cervical cancer is the fourth cancer spread among women **(Alam *et al.*, 2022)**. CQDs face this cancer because of their properties, including photostability, low toxicity and biocompatibility **(Singh *et al.*, 2018)**. CQDs are synthesised from chlorophyll to be non-toxic and don't negatively affect the body **(Devi *et al.*, 2019 and Unnikrishnan *et al.*, 2020)**. Chl-CQDs were used in the diagnostics and

therapeutics of tumor cells due to their long absorption wavelength **(Wu *et al.*, 2021)**.

5. 2 Chl-CQDs suppress the cell viability of SiHa cells:

MTT assay indicates that the Chl-CQDs cause inhibition in SiHa cancer and do not affect the normal kidney cells HEK-293. The viability of SiHa cells decreased by increasing the concentration of the drug. (fig. 10)

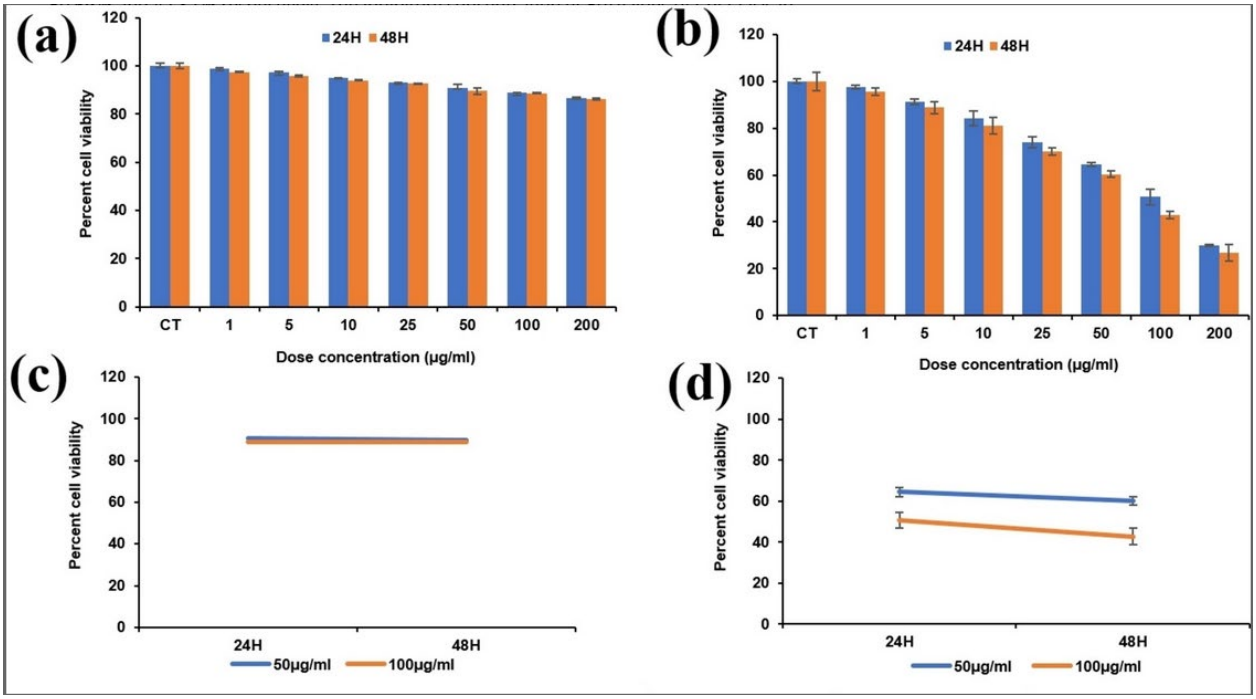

Figure 10: The effect of Chl-CQDs on the proliferation of cervical cancer cell line. HEK-293 and SiHa cells were exposed to indicated concentrations of doses and the cell viability was assayed using MTT as a substrate by taking absorbance at 570 nm. **(Alam *et al.*, 2022)**

5. 3 Chl-CQDs promote morphological changes in SiHa cells:

Chl-CQDs lead to uneven form, cell shrinkage and separation from the surface in SiHa cells. CQDs also lead to SiHa cell apoptosis, which was indicated by DAPI staining or by using AOPI staining. CQDs show the SiHa cells died by red

fluorescence, and the non-died or normal cells of the kidney appear with green fluorescence (Fig. 11).

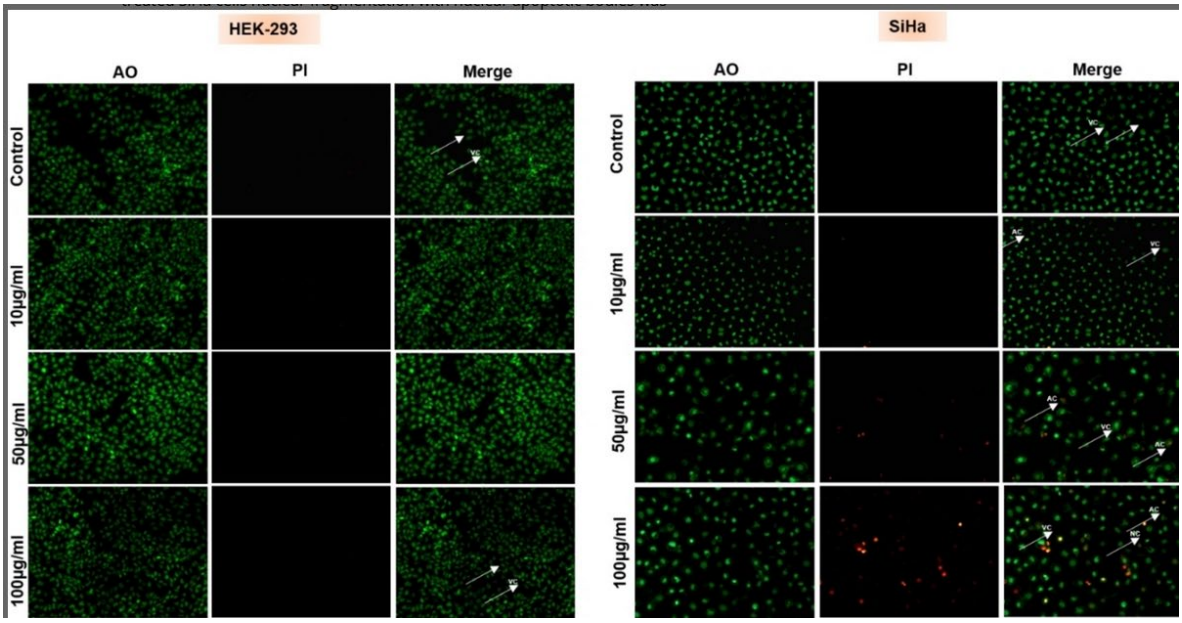

Figure 11: The effect of AOPI on SiHa cell and HEK-293 cell. **(Alam *et al.*, 2022)**

Chl-CQDs lead to the damage plasma membrane in tumor cells and don't affect normal cells and that was indicated by LDH release from the plasma membrane (Fig. 12).

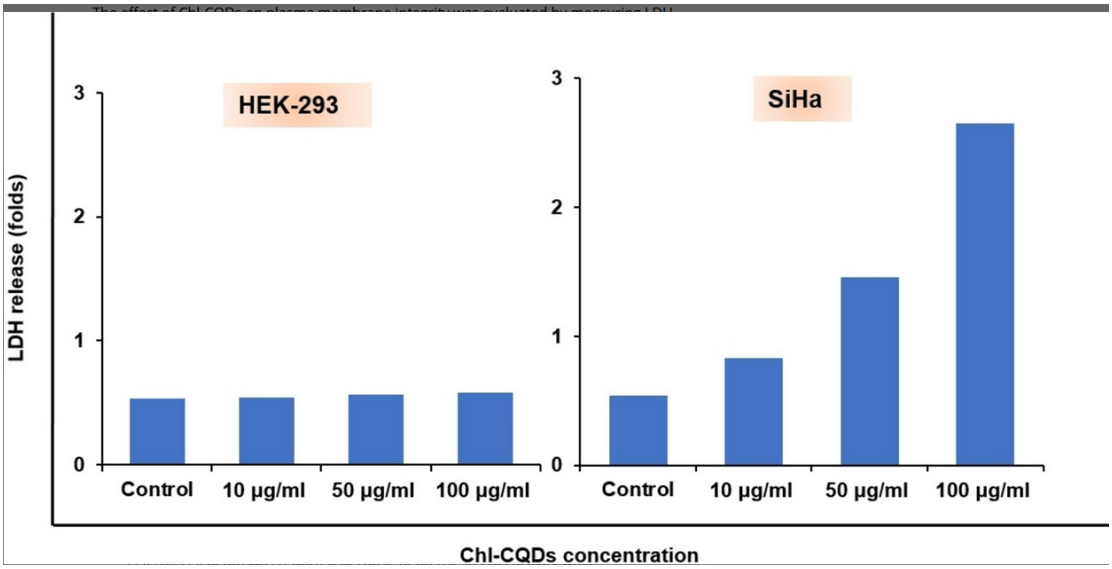

Figure 12: The effect of concentration of Chl-CQDs on releasing LDH (folds). **(Alam *et al.*, 2022)**

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