Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 3, Issue 10, Oct., 2022

THE ANTI- CANCER SYNERGISTIC EFFECT OF CHLOROQUINE ON CANCER CELLS PROLIFERATION (IN VITRO STUDY)

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Abstract Objective:
This study aimed to investigate the possible in vitro cytotoxicity of Chloroquine .

Methods:
The dried leaves powder was extracted with Chloroquine. evaluated by MTT assay for cytotoxicity against AMJ13, breast cancer cell lines.

Results:
Chloroquine extract has potent cytotoxicity in a dose dependent manner, against AMJ13 cell where (IC50 6.982) .The cytotoxicity of extract with Chloroquine showed a synergistic activity on AMJ13 cell lines).

Conclusion:
Chloroquine extract has potent cytotoxicity against breast cancer cell lines.

Introduction

Cancer can be defined as a disease in which a group of abnormal cells grow uncontrollably by disregarding the normal role of cell division. normal cells are constantly subject to signals that dictate whether the cell should divide, differentiate into another cell or die.
Cancer is currently defined as a disease caused by changes or mutations in the cell genome. These modifications (DNA mutation) produce proteins that break the biological equilibrium between cell division and quiescence, resulting in cancerous cells that continue to divide. Cancer is currently thought

ISSN: 2776-1010 Volume 3, Issue 10, Oct., 2022

to be a multi-gene, multi-step illness that begins with a single aberrant cell (clonal origin) with an altered DNA sequence (mutation). The uncontrolled multiplication of these defective cells is followed by a second mutation, which results in the slightly aberrant stage. A tumor mass is formed as a result of a series of mutations and selective proliferation of these cells (Hejmadi , 2014).

Europe accounts for 23.4% of all cancer cases and 20.3% of all cancer deaths worldwide. America has 13.3% of the global population and is responsible for 21.0% of global incidence and 14.4% of global mortality.

Cancer mortality rates in Asia and Africa (57.3% and 7.3%, respectively).

Breast cancer in women (6.6 percent), Breast cancer is the most prevalent cancer diagnosed in women (24.2 percent). Breast cancer is the most frequent female tumor in Iraq, with the most new cases in 2018 (Bray et al., 2018).

In general, breast cancer affects more women than any other type of cancer, and it is especially prevalent in Iraq, where 23% of all female cancer cases worldwide were caused by breast cancer (Coleman et al., 2008). Cell lines are an important experimental tool in cancer research because they provide an infinite supply of a relatively homogeneous cell population capable of self-replication that can be widely distributed to facilitate comparative studies (Pandrangi et al., 2014). Cancer drug resistance is a well-known phenomenon that results when cancer becomes tolerant to pharmaceutical treatment (Wang et al., 2019) It considers as the biggest challenge in cancer treatment (Vasan et al., 2019). Furthermore, 90 percent of chemotherapy failures during cancer invasion and metastasis are due to drug resistance (Bukowski et al., 2020; Mansooriet et al., 2017).

The development of drug resistance in tumors counteracts the therapeutic effects of chemotherapeutic drugs, resulting in more aggressive tumor recurrence and worse prognoses for cancer patients (Liu et al., 2020).

Autophagy is an effective intracellular catabolic process that uses lysosomal degradation to degrade aberrant cellular protein aggregates and damaged organelles. However, it is required for cellular homeostasis and renovation (Wang et al., 2019). The autophagy process begins with the formation of the phagophore and concludes with the death of the autophagosome. However, cell biologists have been interested in the cellular and molecular mechanisms of this pathway since the late 1950s (Y) (Yu et al., 2017).

Methods

AMJ13 Ahmed Mortada jabria 2013 breast cancer cell line has been established from an Iraqi breast cancer patient. was established from the primary tumor of a 70-year-old Iraqi woman with a histological diagnosis of infiltrating ductal carcinoma (Al-Shammari et al., 2015).

Maintenance of cell cultures

AMJ13 was obtained from the Iraq biotech Cell Bank Unit and maintained in RPMI-1640 supplemented with 10% Fetal bovine, 100 units/ml penicillin, and 100 µg/ml streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 50% confluence twice a week, and incubated at 37 °C .

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Cytotoxicity Assays

To determine the cytotoxic effect, the MTT cell viability assay was conducted on 96-well plates. Cell lines were seeded at 1 ×10⁴cells/well. After 24 hrs. or a confluent monolayer was achieved, cells were treated with tested compound. Cell viability was measured after 72 hrs of treatment by removing the medium, adding 28 µL of 2 mg/ml solution of MTT (and incubating the cells for 1.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking (Al-Shammari et al., 2016). The absorbency was determined on a microplate reader at 492 nm (test wavelength); the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation:- (Al-Shammari et al., 2020).

Apoptosis Estimation (propidium iodide/Acridine orange assay):

The apoptotic attentions in cell lines (infected and control) were measured using (AO/PI). 5000 cells/well were seeded in plate, next infected with (gold N.P) for 24 hours in a 37 °C incubator. For traditional dual staining. The tested wells received exactly 50µl of the AO/PI stain mixture (at room temperature) for 30 seconds. After then, the stain was removed. The images were taken using a Leica fluorescent microscope (Al-Shammari et al., 2020).

Statistical analysis:

The obtained data were statically analyzed using an unpaired t-test with Graph Pad Prism 6 (Mohammed et al., 2019) . The values were presented as the mean ± SD of triplicate measurements (Al-Ziaydiet al., 2020).

Cell Lines Breast cancer

Cell lines (AMJ13) cell line were supplied by tissue culture unit / ICCMGR (Iraqi Centre for Cancer and Medical Genetic Researches), Baghdad, Iraq (Al-Shammari et al., 2015). These cells were maintained in RPMI-1640 media (Roswell Park Memorial Institute -1640 medium) with fetal bovine serum (FBS), 100 unit's/ml penicillin, and 100 unit's/ml streptomycin, and incubated at 37°C for 24 hrs to allow cell attachment, proliferation, and confluent monolayer achievement. These cells were regularly assessed for standard growth characteristics and regularly authenticated. in the humid atmosphere of 5% CO2 . 3-(4, 5-dimethylthiazol-2-yl)- 2, 5- diphenyltetrazolium Bromide (MTT) Assay.

cell lines AMJ13 were detached from their flasks when they reached the subconfluent monolayer by trypsinization. Culture medium (20 ml) and 10% serum were added to the falcons and mixed gently with cells to prepare cell suspension. The cell suspension in the culture flask was poured aseptically into a sterile beaker. Using a multi micropipette, 200µ1 of the cell suspension was transferred into each well in a 96-well microplate, and the plate was covered with a sterile adhesive film, The absorbance reading was taken at 492 nm by using micro-plate reader (Freshney, 2015) . The absorbance of cells cultured in control media was taken to represent 100% viability. The viability of treated cells was determined as a percentage of that for the untreated control. Each concentration was tested in triplicate, and the experiment was repeated twice.

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The concentration of the cells in each well was 1x104 . The percentage of cell line inhibition was determined as the mean ± SD using the following equation.

Result:

The Effect of Chloroquine on the Cell Number AMJ13 in Vitro

The cytotoxicity of AMJ13 cell lines was studied using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay. The cytotoxicity ratio was determined after cell lines were treated with Chloroquine to determine the concentration of inhibition that kills 50% of cells Median inhibitory conctration (IC50). The IC50 for Chloroquine was calculated using Graph Pad Prism (version 7). (Gao et al., 2003). After IC50 was measured, the cells were treated with selected different concentrations of Chloroquine (meark ,germany) (100 ,50 ,25 ,12.5 ,6.25, 3.12 µg/ml)**.**
depending on the IC50 values, after which they were incubated at 37°C for 72 hrs. Triplicates were used for each concentration of each treatment modality. (Al-shammari et al., 2016) After that, the media in 96- well microplate was discarded and 50 µl of serum free media (SFM) and 50 µl of MTT dye (yellow solution 2 mg/ml) were added into each well and incubated at 37°C for 3 hrs. MTT dye solution was discarded, the crystals remaining in the wells were solubilized by the addition of 100 µl of Dimethyl Sulphoxide (DMSO) (99.9%) (Santa Cruz Biotechnology, USA) into each well to dissolve the MTT-Formosan crystals, and the plate was wrapped with parafilm, rocked, and incubated for 15 min. The optical density value for treated and untreated cells was measured at 492 nm using the ELISA plate reader (Freshney, 2015), The endpoint parameter that was calculated for each cell line included percentage of cell growth, and the inhibition of cell growth (G.I) or percentage of cytotoxicity (CT %) which was calculated as:

Cell viability % =(absorbance of treated cell / absorbance of non- treated cell)*100
cytotoxicity % =100- cell viability
GI % = mean of control – mean of treted / mean of control *100

Where OD control represents the mean optical density of untreated wells and OD Sample represents the optical density of treated wells (Jabir et al., 2019). In a study on the viability of tumor cells, MTT was used to determine the effect of colchicines, as well as their combination. This assay is based on the metabolic reduction of colorless tetrazolium salt in viable cells by mitochondrial enzyme activity. Because of its specificity for living cells, it is particularly useful for assaying cell suspensions. (Mosmann, 1983).
Cytotoxicity against AMJ13 Cell Line Figure(2) shows the in vitro cytotoxicity of Colchicines extract on AMJ13 cells, The results
showed a dose dependent inhibition on the cell growth after 72hr. The extract concentrations used were (100, 50, 25, 12.5, 6.25, and 3.125µg/ml), with each concentration in triplicate and the experiments were repeated twice. The data is represented as the mean ±SD.

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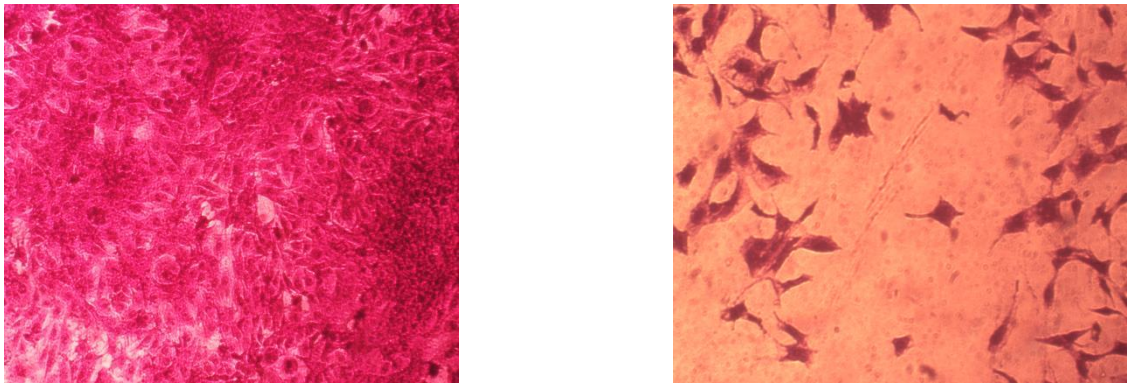
The percentages of AMJ13 cell growth inhibition were Chloroquine (GI%) were (72.7 % ,69.9 %, 66.1 % ,52.6 % , 24 % , and 16.7 %). at each mentioned concentration respectively.

The Effect of Chloroquine on the morphology of AMJ13 in Vitro

The cultured AMJ13 cells had an elongated multipolar epithelial-like cell shape, with nuclear polymorphism and multiple nuclei in most of the cells, which expressed the characteristics of cell morphology ,as well as showing many cells with mitotic figures (1). The Morphological pictures for AMJ13 in vitro un-treated before was full number of cell, monolayer cell shape. After drugs exposure each of Choloroquine for used concentrations were (100,50,25,12.5,6.25,13.2 µg/ml) turn into singale cell suspension, the number of cell began to decrease. the figures (1) refers to graduate decreased in cell number and killing effect of graduate when increase of conctration of Choloroquine.

Result



Un-treated CQ.

Figure (1), Morphological pictures for AMJ13 cell line in vitro un-treated (as control cells) and Cytotoxicity under an inverted microscope, (10x).

The Inhibiting Effect of Chloroquine on AMJ13 Growth Rate in Vitro

The cytotoxicity was assessed using different concentrations of Choloroquine (100 ,50 ,25 ,12.5 ,6.25 ,3.12 µg/ml) .by MTT cytotoxicity assay. According to these findings, increasing the concentration of the inhibitor increases cytotoxicity or enhances growth inhibition . For AMJ13, there is a statistically significant difference between inhibition by Choloroquine as shown in Figures (2) . the RPMI-1640 medium used as a positive control for comparing the effects of Choloroquine.

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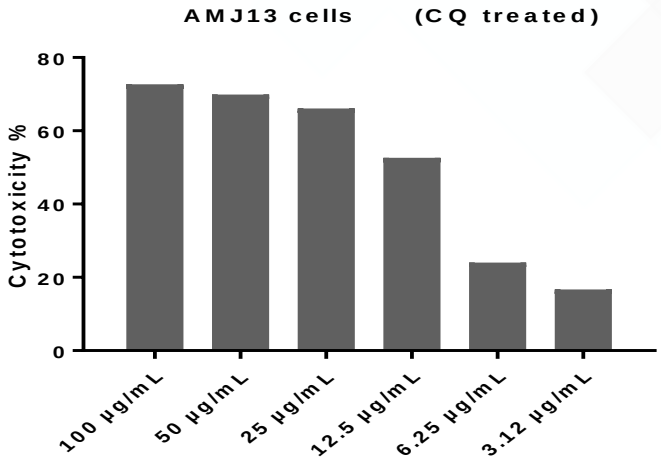

Dose	100	50	25	12.5	6.25	3.12
Growth inhibition	72.7	69.9	66.1	52.6	24	16.7

Figure (2) Cytotoxicity effect (CT %) after treatment of cell lines with Chloroquine to AMJ13 cells .

Estimation of Chloroquine (IC50) in AMJ13 in vitro

The IC50 value in breast cancer cell lines was determined to evaluate the effect of each treatment on cell growth.The result shown that breast cancer cells are effectively infected and destroyed by live attenuated AMJ13 and it caused a significant cytopathic effect in the infected cell lines after 72 h of infection with remarkable effect on AMJ13 cells IC50 of Chloroquine treated range(2.696 to 10.9 was 5.42) for AMJ13. Breast cancer treatment induces apoptosis significantly in breast cancer cell lines compared with control cells.

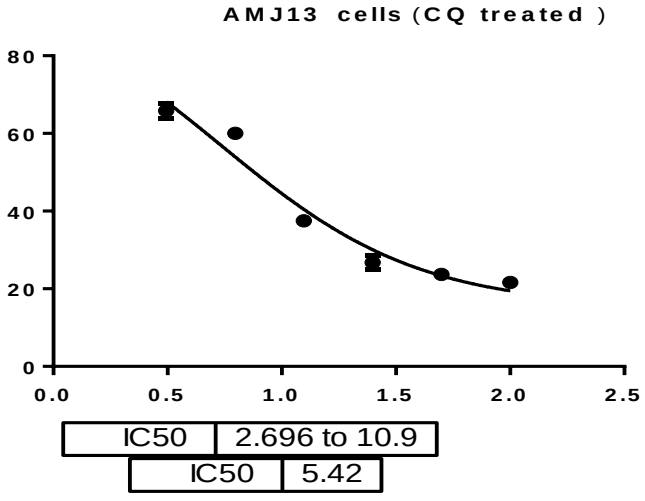

Figure (3): IC50 to exposure of Colchicines to cell lines: IC50 values after AMJ13 cell lines were treated with Chloroquine by Graph Pad Prism software.

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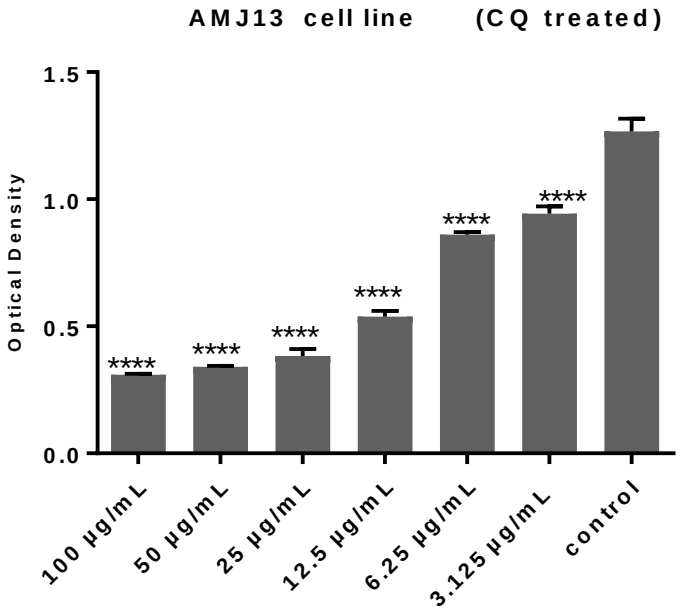
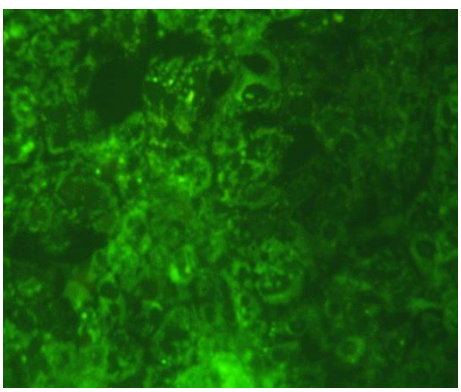
The Effect ofCholoroquine on AMJ13 Optical Density

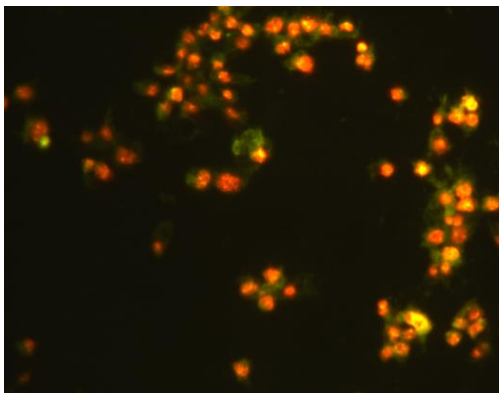
Optical Density is reflected of cell density, decreased in cell density having decreased optical density , refers to graduate decreased in cell number and killing effect of graduate when increase of concentration The Optical Density was assessed using different concentrations of Chloroquine (100 ,50 ,25 ,12.5 ,6.25 ,3.12 µg/ml).



Figure(4): Optical Density is reflected of cell density, this figure showing Optical Density of Chloroquin.



Un - treated



CQ.

Figure (5):Analysis of apoptosis in AMJ13 cells using AO/PI. Showing massive number apoptotic cells While there were no effect against control cells(X10).

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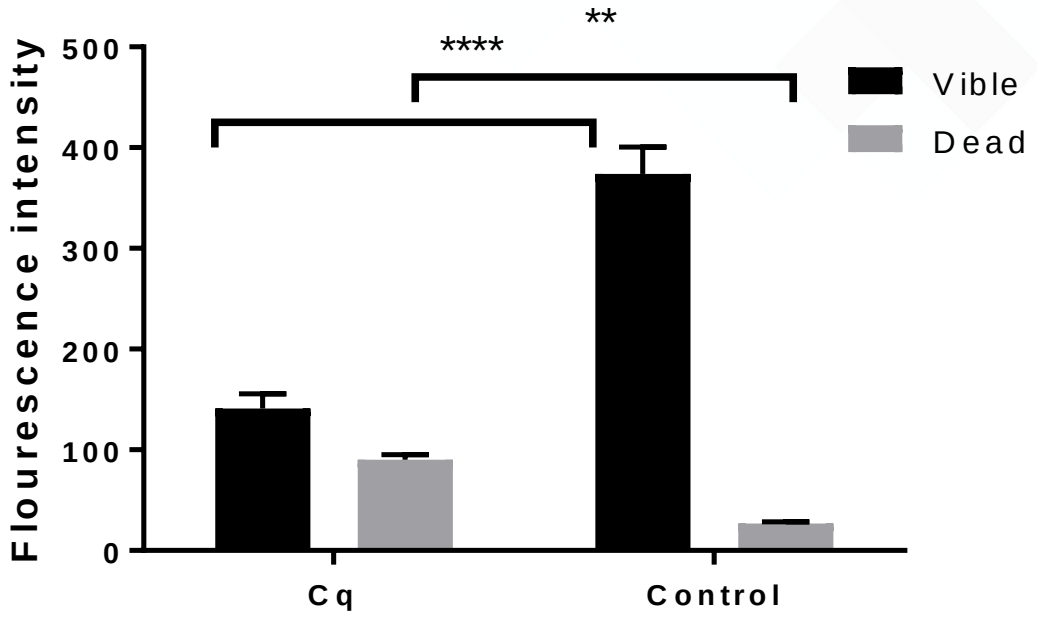

Figure (6) : Fluorescent intensity to calculate apoptosis in treated cells confirms that ensure apoptosis as demonstrated by red stained cells (treated cells) and green fluorescence in untreated control cells.

Discussion

The AMJ13 cell line, the first continuous breast cancer cell line from an Iraqi patient, is described here. The AMJ13 cell line was derived from an Iraqi patient with histologically diagnosed poorly differentiated infiltrative ductal carcinoma (Al-Shammari, et al., 2015). Over the last two decades, there has been a rise in interest in the pharmacological effects of bioactive compounds on cancer treatment and prevention. It has been shown to possess numerous anti-cancer activities in various cancer cells through different forms of cytotoxic effects without exhibiting considerable damage to normal cells (Katiyar et al., 2009; Mantena et al., 2006).

The half maximum inhibitory concentration (IC50) of a pharmacological inhibitor is a measure of its ability to inhibit AMJ13. The IC50 value is a quantitative measure that reveal show much of a specific inhibitory drug (Choloroquine) is required to prevent the spread of breast cancer proliferation. This is because the effect of chemotherapy on cells differs according to type, and this is related to changes in cancer cells After being treated with drugs. The effect of (Choloroquine) on breast cancer cell lines was shown to be very clear .

The results implied that synergism inhibitor had more effect in inhibition of proliferation, anticancer growth action, and caused increase in cytotoxicity and lead to induced morphological changes and apoptosis.

Apoptosis was visible as red cells in AO/PI stained cell by useing fluorescent microscopy and treated cells, while healthy cells were green. Apoptosis is a natural process of programmed cell death that can be triggered by a range of physical and chemical causes and is precisely managed by the organism.

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Although there are three major signaling channels in apoptosis (mitochondrion, death receptor, and endoplasmic reticulum signaling pathways), apoptotic signaling is frequently integrated and amplified at the mitochondrial level (Guo et. al, 2016). Other studies have employed various strategies, one by halving the provided dose of a chemotherapeutic drug (rituximab or doxorubicin) to lessen chemotherapy toxicity (Al shammari et al., 2016). Tamoxifen, an anti-estrogen medication, is currently used widely in the prevention and treatment of estrogen receptor positive breast cancer (Lazarus et al., 2009). However, many patients acquire tamoxifen resistance and suffer from severe adverse effects (Lazarus et al., 2009).

REFERENCES

1. Hejmadi, M., 2014. Introduction to cancer biology. Bookboon.
2. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A. and Jemal, A., 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), pp.394-424.
3. Coleman, M.P., Quaresma, M., Berrino, F., Lutz, J.M., De Angelis, R., Capocaccia, R., Baili, P., Rachet, B., Gatta, G., Hakulinen, T. and Micheli, A., 2008. Cancer survival in five continents: a worldwide population-based study (CONCORD). The lancet oncology, 9(8), pp.730-756.
4. Pandrangi, S.L., Raju Bagadi, S.A., Sinha, N.K., Kumar, M., Dada, R., Lakhanpal, M., Soni, A., Malvia, S., Simon, S., Chintamani, C. and Mohil, R.S., 2014. Establishment and characterization of two primary breast cancer cell lines from young Indian breast cancer patients: mutation analysis. Cancer cell international, 14(1), pp.1-20.
5. Liu, L., Yan, L., Liao, N., Wu, W.Q. and Shi, J.L., 2020. A review of ULK1-mediated autophagy in drug resistance of cancer. Cancers, 12(2), p.352.
6. Wang, X., Zhang, H. and Chen, X., 2019. Drug resistance and combating drug resistance in cancer. Cancer Drug Resistance, 2(2), p.141.
7. Vasan, N., Baselga, J. and Hyman, D.M., 2019. A view on drug resistance in cancer. Nature, 575(7782), pp.299-309.
8. Bukowski, K., Kciuk, M. and Kontek, R., 2020. Mechanisms of multidrug resistance in cancer chemotherapy. International journal of molecular sciences, 21(9), p.3233.
9. Mansoori, B., Mohammadi, A., Davudian, S., Shirjang, S. and Baradaran, B., 2017. The different mechanisms of cancer drug resistance: a brief review. Advanced pharmaceutical bulletin, 7(3), p.339.
10. Yu, Q., Liu, L., Wang, P., Yao, Y., Xue, Y. and Liu, Y., 2017. EMAP-II sensitize U87MG and glioma stem-like cells to temozolomide via induction of autophagy-mediated cell death and G2/M arrest. Cell Cycle, 16(11), pp.1085-1092.
11. Al-Shammari, A.M., Alshami, M.A., Umran, M.A., Almukhtar, A.A., Yaseen, N.Y., Raad, K. and Hussien, A.A., 2015. Establishment and characterization of a receptor-negative, hormone-nonresponsive breast cancer cell line from an Iraqi patient. Breast Cancer: Targets and Therapy, 7, p.223.

ISSN: 2776-1010 Volume 3, Issue 10, Oct., 2022

12. Al-Shammari, A.M., Salman, M.I., Saihood, Y.D., Yaseen, N.Y., Raed, K., Shaker, H.K., Ahmed, A., Khalid, A. and Duiach, A., 2016. In vitro synergistic enhancement of Newcastle Disease Virus to 5-fluorouracil cytotoxicity against tumor cells. Biomedicines, 4(1), p.3.
13. Al-Shammari, A.M., Al-Saadi, H., Al-Shammari, S.M. and Jabir, M.S., 2020, March. Galangin enhances gold nanoparticles as anti-tumor agents against ovarian cancer cells. In AIP Conference Proceedings (Vol. 2213, No. 1, p. 020206). AIP Publishing LLC.
14. Al-Shammari, A.M., Jalill, R.D.A. and Hussein, M.F., 2020. Combined therapy of oncolytic Newcastle disease virus and rhizomes extract of Rheum ribes enhances cancer virotherapy in vitro and in vivo. Molecular Biology Reports, 47(3), pp.1691-1702.
15. Mohammed, M.S., Al-Taee, M.F. and Al-Shammari, A.M., 2019. Caspase dependent and independent anti-hematological malignancy activity of AMHA1 attenuated newcastle disease virus. International Journal of Molecular and Cellular Medicine, 8(3), p.211.
16. Al-Ziaydi, A.G., Al-Shammari, A.M., Hamzah, M.I., Kadhim, H.S. and Jabir, M.S., 2020. Newcastle disease virus suppress glycolysis pathway and induce breast cancer cells death. Virusdisease, 31(3), pp.341-348.
17. Al-Shammari, A.M., Alshami, M.A., Umran, M.A., Almukhtar, A.A., Yaseen, N.Y., Raad, K. and Hussien, A.A., 2015. Establishment and characterization of a receptor-negative, hormone-nonresponsive breast cancer cell line from an Iraqi patient. Breast Cancer: Targets and Therapy, 7, p.223.
18. Freshney, R.I., 2015. Culture of animal cells: a manual of basic technique and specialized applications. John Wiley & Sons.
19. Gao, S., Yu, B.P., Li, Y., Dong, W.G. and Luo, H.S., 2003. Antiproliferative effect of octreotide on gastric cancer cells mediated by inhibition of Akt/PKB and telomerase. World journal of gastroenterology, 9(10), p.2362.
20. Al-Shammari, A.M., Salman, M.I., Saihood, Y.D., Yaseen, N.Y., Raed, K., Shaker, H.K., Ahmed, A., Khalid, A. and Duiach, A., 2016. In vitro synergistic enhancement of Newcastle Disease Virus to 5-fluorouracil cytotoxicity against tumor cells. Biomedicines, 4(1), p.3.
21. Jabir, M.S., Taha, A.A., Sahib, U.I., Taqi, Z.J., Al-Shammari, A.M. and Salman, A.S., 2019. Novel of nano delivery system for Linalool loaded on gold nanoparticles conjugated with CALNN peptide for application in drug uptake and induction of cell death on breast cancer cell line. Materials Science and Engineering: C, 94, pp.949-964.
22. Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods, 65(1-2), pp.55-63.
23. Katiyar, R.S., Singhvi, N.R., Kushwaha, R.V., Ramji, L. and Suryanarayana, N., 2009. VA-mycorrhizal association in arjuna and jamun trees in forest of Bhandara region, Maharashtra, India. International Journal of Plant Sciences (Muzaffarnagar), 4(1), pp.229-232.

ISSN: 2776-1010 Volume 3, Issue 10, Oct., 2022

24. Mantena, S.K., Sharma, S.D. and Katiyar, S.K., 2006. Berberine, a natural product, induces G1-phase cell cycle arrest and caspase-3-dependent apoptosis in human prostate carcinoma cells. Molecular cancer therapeutics, 5(2), pp.296-308.
25. Applegate, C.C. and Lane, M.A., 2015. Role of retinoids in the prevention and treatment of colorectal cancer. World journal of gastrointestinal oncology, 7(10), p.184.
26. Guo, X., Zhang, X., Wang, T., Xian, S. and Lu, Y., 2016. 3-Bromopyruvate and sodium citrate induce apoptosis in human gastric cancer cell line MGC-803 by inhibiting glycolysis and promoting mitochondria-regulated apoptosis pathway. Biochemical and biophysical research communications, 475(1), pp.37-43.
27. Lazarus, D.B., Kotrc, B., Wulf, G. and Schmidt, D.N., 2009. Radiolarians decreased silicification as an evolutionary response to reduced Cenozoic ocean silica availability. Proceedings of the National Academy of Sciences, 106(23), pp.9333-9338.