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Dose Dependent Cytotoxicity Effect of Doxorubicin on Breast Cancer Cell Line (AMJ13) Proliferation: *in Vitro* Study

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Abstract

The purpose of the current investigation was to identify the dose-dependent effect of doxorubicin on the proliferation of the AMJ13 cell line. The AMJ13 breast cancer cell line was used to investigate the cytotoxicity of the medication doxorubicin. The median inhibitory concentration (IC_{50}) was calculated using the Methyl thiazolyltetrazolium (MTT) assay. Doxorubicin's IC_{50} value, which ranged from 162.2 to 308,3, was 223.6. Doxorubicin inhibited the proliferation of AMJ13 cells to a greater or lesser extent at concentrations of 1000, 500, 250, 125, 62.5, and 31.2 g/ml (58.8%, 46.4%, 32.3%, 23.8%, 11.3%, and 0.896%). respectively. The percentage of cytotoxicity (CT) After 72 hours of treatment, doxorubicin inhibited MCF7 cell growth in a dose-dependent manner, with a CT% of 90% at a dosage of 50 M. To sum up, doxorubicin displays strong cytotoxicity against the AMJ13 breast cancer cell line. It could be concluded that the effect of doxorubicin on the proliferation of the AMJ13 is dose dependent. In addition, morphological changes and apoptosis significantly enhance the inhibition of growth.

KEYWORDS
Iraq, Breast cancer, IC_{50}, DOX, MTT

INTRODUCTION

Cancer is a major public health issue around the world, and it is the second leading cause of death. Cancer is a category of diseases characterized by the uncontrollable growth and spread of abnormal cells. If the spread is not stopped, it can lead to death (Siegel and Miller, 2019).

Cancer therapy tries to destroy cancer cells while causing the least amount of harm to normal cells (Fournier and Schirrmacher, 2013). One of the most common cancers in women is breast cancer (Bray *et al*., 2018). After substantial research, the illness is still incurable and has a two-year survival rate (Tevaarwerk *et al*., 2013).

Surgery, chemotherapy, adjuvant hormone therapy, and radiotherapy are all options for treating breast cancer (Sankaranarayanan *et al*., 2013). Age, parity, family history of breast cancer, particularly in first-degree relatives, radiation exposure, smoking, and genetics of breast cancer (*BRCA1* and *BRCA2* genes mutations) are all associated risk factors for breast cancer in females (Bray *et al*., 2018).

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*., 2013).

Breast cancer is the most common form of malignant tumor in Iraqi women and the main reason why women die from malignant neoplasms (Alwan, 2017). After substantial research, the illness is still incurable and has a two-year survival rate (Tevaarwerk *et al*., 2013).

One of the well-known and frequently applied antineoplastic drugs used to treat a breast cancer Doxorubicin (Dox) is an efficient antineoplastic drug for many different types of cancer. Yet numerous systemic side effects limit its widespread usage in breast cancer treatment. Dox damages DNA because it inhibits the DNA topoisomerase II enzyme (Renu *et al*., 2018).

Doxorubicin is a broad-spectrum Anthracycline antibiotic that is frequently used in combination with other medications to treat various malignancies, such as solid tumors, lymphomas, and leukemia, and is widely used as a chemotherapy agent for the treatment of breast cancer (Humber *et al*., 2007; Sirwi *et al*., 2021)

Doxorubicin continues to be one of the most active and popular chemotherapy drugs for the treatment of both early-stage and advanced breast cancer. Doxorubicin's inhibitory efficacy may be altered by the slower growth rate of breast cancer cells in spheroid cultures because slower-growing cells may be more susceptible to its cytotoxicity than faster-growing cells (Middleton *et al* ., 2018).

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 on various cancer cells through different forms of cytotoxic effects without exhibiting considerable damage to normal cells (Katiyar *et al*., 2009).

The cytotoxic and ant proliferative effects of doxorubicin are exerted through several mechanisms, but the best known is the poisoning of topoisomerase II cleavage complexes (McClendon and Osheroff, 2007).

Other effects of DOX include its intercalation into DNA and the generation of free radicals causing DNA damage and lipid peroxidation (Minotti *et al*., 2004). Clonogenic experiments

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showed that DOX inhibited cell proliferation at higher doses because it can enter the cells thanks to its lipophilic characteristics, but it can also be easily extruded by the cells by the MDR mechanism (Kapse-Mistry *et al*., 2014).

The aims of this study were to investigate the cytotoxicity of Doxorubicin on AMJ13 (breast cancer cell), to explain how this drug either inhibits or increases apoptosis, and to explain the dose-dependent effect of doxorubicin on the growth and proliferation. of the AMJ13 breast cancer cell line.

MATERIALS AND METHODS

Maintenance of cell culture

AMJ13 breast cancer cell lines were supplied by the tissue culture unit of the ICCMGR (Iraqi Centre for Cancer and Medical Genetic Research), 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 units/ml penicillin, and 100 units/ml streptomycin and incubated at 37°C for 24 h to allow cell attachment, proliferation, and confluent monolayer achievement.
Doxorubicin was purchased from OncoAce, India.

Cytotoxicity Assays

To determine the cytotoxic effect of doxorubicin, a methyl thiazolyl tetrazolium (MTT) cell viability assay was conducted on 96-well plates (Adil *et al*., 2020). AMJ13 seeded at 1×10^4 cells/well. After 1 day, or when a confluent monolayer was achieved, cells were treated with doxorubicin 50mg. Viability was measured after 72 h of treatment by removing the medium, adding 28 µL of a 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 sulfoxide) followed by 37°C incubation for 15 min with shaking (Abdullah *et al*., 2020). The absorbency was determined on a microplate reader at 492 nm (the test wavelength); the assay was performed in triplicate.

The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation: (absorbance of treated cell / absorbance of untreated cell) *100. Cell viability: 100% cytotoxicity. GI% is calculated as follows: mean of control *100 * mean of treatment *100 (Al-Shammari *et al*., 2020).

Acridine orange- propidium iodide assay

The apoptotic responses in cell lines (treated and control) were assessed by using AO/PI. Following the seeding of 5000 cells per well in a plate, doxorubicin infection was performed for 24 hours in a 37°C incubator for the classic dual staining. Exact 50 l of the AO/PI stain mixture (at room temperature) were applied to the test wells for 30 seconds.

The stain was then eliminated. Utilizing a Leica fluorescence microscope, the pictures were captured. Fluorescence microscopy and ImageJ software were used to measure fluorescence intensity (Al-Shammari *et al*., 2020).

Statistical analysis

GraphPad Prism 6 was used to perform an inverted t-test statistical analysis on the collected data (Mohammed *et al*., 2019). The results were shown as the mean SD of measurements made in triplicate (Al-Ziaydi *et al*., 2020). Version 1 isobologram was used to compare the variations across groups under various circumstances. P value lower than 0.05 were deemed significant.

RESULTS

The results of the present study are shown in Figures 1–5, revealed that when the concentration of DOX increased, the cytotoxicity percentage (Growth inhibition percentage) was raised (Figure 1). The IC_{50} of Doxorubicin in different concentrations using the MTT assay and Graph Pad Prism software ranged from 162.2 to 308.3 (223.6).

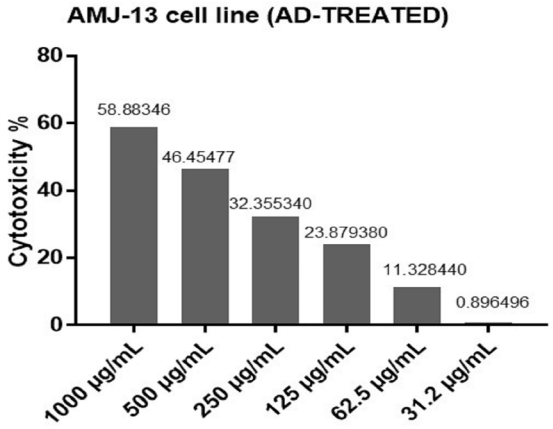

Fig. 1. Cytotoxicity effect (CT %) of DOX) in different concentration on AMJ13 cells.

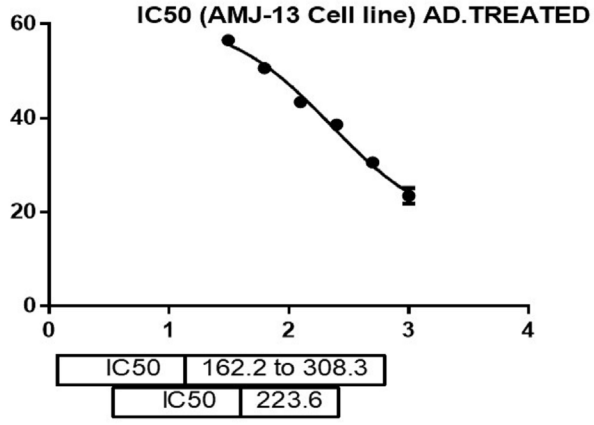

Fig. 2. Half maximal inhibitory concentration (IC_{50}) after exposure of the AMJ13 cell line to DOX in different concentrations using MTT assay and Graph Pad Prism software.

The optical density (OD) of DOX showed a non-significant (ns) effect in a lower concentration of DOX (31.25 µg/ml) and a significant effect in other concentrations. That is explained in Figure 3.

Following medication exposure, DOX for utilized concentrations (1000, 500, 250, 125, 62.5, and 31.2 µg/ml) turned into a single cell suspension, and the number of cells started to decline. That concentration of DOX depicts the reduction in cell number and killing impact of the graduate as the concentration of DOX increases (Figure 4).

To explain the consequences of apoptosis, all cells were dyed with AO/PI and examined under a fluorescence microscope. After receiving a dose of DOX for 72 h, the green color represents live cells, and the red color displays dead cells (Figure 5).

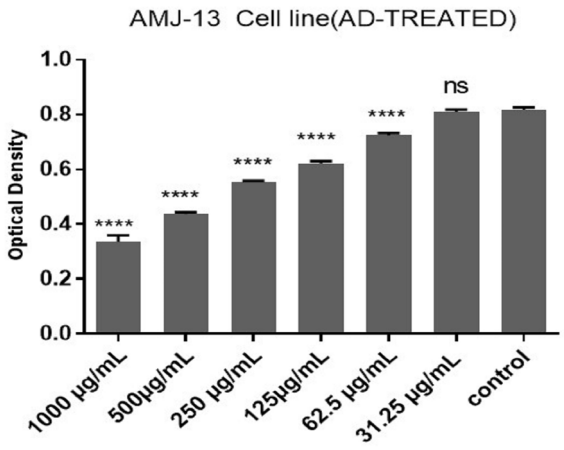

Fig. 3. optical density of DOX in different concentrations on AMJ13 cell line using (Graph Pad Prism software).

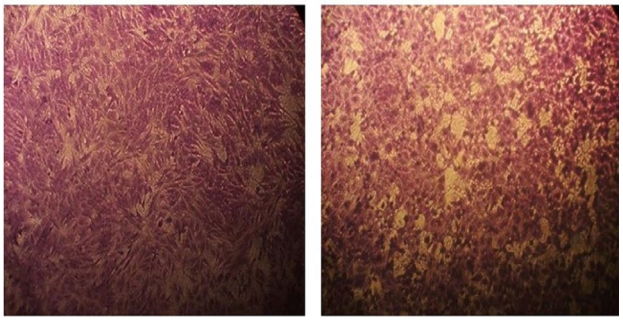

Fig. 4. Morphological pictures for AMJ13 cell line *in Vitro* before treatment were a full number of cells, monolayer cell shape (An inverted microscope, 10x).

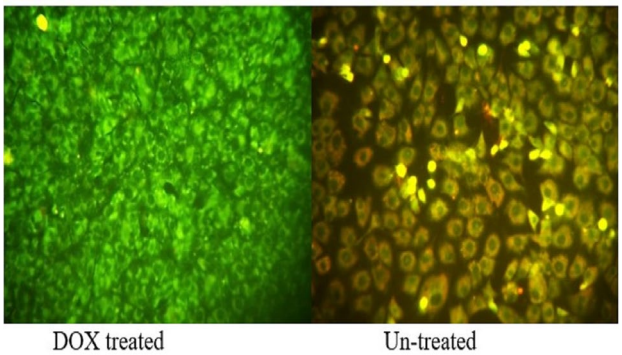

Fig. 5. Analysis of the effects of DOX treatment on AMJ13 cell lines, treated and control cells (fluorescence microscope 10X).

DISCUSSION

In this study, AMJ13 cell line was used, the first continuous breast cancer cell line from an Iraqi patient (Al-Shammari *et al*., 2015). It is treated with DOX in a dose dependent manner.

The cytotoxicity assay was assessed using different concentrations of (1000, 500, 250, 125, 62.5, 31.2 µg/ml) methotrexate and aspirin, and Co-treatment. According to these findings by MTT cytotoxicity, increasing the concentration of the inhibitor increases cytotoxicity or enhances growth inhibition. For breast cancer cell lines,

The half-maximum inhibitory concentration (IC_{50}) of a pharmacological inhibitor is a measure of its ability to inhibit AMJ13. The IC_{50} value is a quantitative measure that reveals how much of a specific inhibitory drug is present. As an anthracycline antibiotic, doxorubicin is effective against a variety of tumors; only a few cancer types are resistant to the medication. The list of cancers treated with doxorubicin includes Hodgkin's and non-Hodgkin's lymphoma, breast, ovarian, testicular, acute leukemia, soft tissue sarcoma, lung, bladder, gastric (stomach), thyroid, hepatoma, Wilm's tumor, and neuroblastoma (Chabner, 2001). Numerous studies (Pinto, *et al*., 2009) and the own recent study revealed that DOX and IND have cytotoxic effects on cancer cells are dose-dependent .

DOX is frequently used in clinical practice to treat a variety of tumors and has previously been administered to individuals with prostate cancer (Heidenreich, *et al*., 2004)

DNA damage is the main mechanism by which topoisomerase II inhibitors, such as DOX, cause cell death (Pommier, *et al*., 2010). Additionally, they are known to cause lipid peroxidation and free-radical DNA damage (Chatterjee, *et al*., 2010).

In the present study, apoptosis was visible as red cells in AO/PI-stained cells by using 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. 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)

Dox has been shown to have a pleiotropic effect on cells, affecting everything from cell proliferation to interacting with topoisomerase II (Fornari *et al*., 1994). to inducing apoptosis by triggering caspases (Inoue-Yamauchi *et al*., 2017). and causing mitochondrial membrane potential disruption (Gamen *et al*., 2000). The cultivated MCF7 cells displayed an elongated multipolar epithelial-like cell shape, with nuclear polymorphism and many nuclei in majority of the cells, expressing cell morphological traits and revealing many mitotic figure (Figs. 4-5). The morphological images for MCF7 *in Vitro* before treatment were full number of cells, monolayer cell shape. Following medication exposure, DOX for utilized concentrations were (1000, 500, 250, 125, 62.5, 31.2 µg/ml) turned into single cell suspension, and the number of cells started to decline. Figures 4-5 depicts the reduction in cell number and killing impact of graduate as the concentrations of methotrexate and aspirin and co-treatment are increased

DOX treatment alone resulted in a dose-dependent reduction in the size and quantity of MCF7 cells. The cells were smaller and uneven in form, and the number of cellular processes decreased.

CONCLUSION

The effect of doxorubicin on the proliferation of theAMJ13 is dose dependent. In addition, morphological changes and apoptosis significantly enhance the inhibition of growth.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

Abdullah, S.A., Al-Shammari, A.M., Lateef, S.A., 2020. Attenuated measles vaccine strain have potent oncolytic activity against Iraqi patient derived breast cancer cell line. Saudi Journal of Biological Sciences 27, 865-872.

Adil, B.H., Al-Shammari, A.M., Murbat, H.H., 2020. Breast cancer treatment using cold atmospheric plasma generated by the FE-DBD scheme. Clinical Plasma Medicine 19, 100103.

Al-Shammari, A.M., Alshami, M.A., Umran, M.A., Almukhtar, A.A., Yaseen,

N.Y., Raad, K., 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, 223-230.

Al-Shammari, A.M., Jalill, R.D.A., 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, 1691-1702.

Al-Ziaydi, A.G., Al-Shammari, A.M., Hamzah, M.I., Kadhim, H.S., Jabir, M.S., 2020. Newcastle disease virus suppress glycolysis pathway and induce breast cancer cells death. Virusdisease 31, 341-348.

Alwan, N.A., 2017. Family history among Iraqi patients diagnosed with breast cancer. International Journal of Science and Research 6, 869-873.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., 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, 394-424.

Chabner, B., 2001. Ryan DP, Paz-Ares L, Garcia-Carbonero R, and Calabresi P. Antineoplastic agents In: Goodman and Gilman's The Pharmacological Basis of Therapeutics, New York: McGraw Hill.

Chatterjee K.J., Zhang J., Honbo, N., Karliner J.S., 2010. Doxorubicin cardiomyopathy. Cardiology 115, 155–162.

Fornari, F.A., Randolph, J.K., Yalowich, J.C., Ritke, M.K., Gewirtz, D.A., 1994. Interference by doxorubicin with DNA unwinding in MCF-7 breast tumor cells. Molecular Pharmacology 45, 649-656.

Fournier, P., Schirrmacher, V., 2013. Oncolytic Newcastle Disease Virus as Cutting Edge between Tumor and Host. Biology 2, 936-975.

Gamen, S., Anel, A., Pérez-Galán, P., Lasierra, P., Johnson, D., Piñeiro, A., Naval, J., 2000. Doxorubicin treatment activates a Z-VAD-sensitive caspase, which causes ΔΨm loss, caspase-9 activity, and apoptosis in Jurkat cells. Experimental Cell Research 258, 223-235.

Guo, X., Zhang, X., Wang, T., Xian, S., 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, 37-43.

Humber, C., Tierney, J., Symonds, R., Collingwood, M., Kirwan, J., Williams, C., Green, J., 2007. Chemotherapy for advanced, recurrent or metastatic endometrial cancer: a systematic review of Cochrane collaboration. Annals of Oncology 18, 409-420.

Inoue- Yamauchi, A., Jeng, P.S., Kim, K., Chen, H.-C., Han, S., Ganesan, Y.T., Ishizawa, K., Jebiwott, S., Dong, Y., Pietanza, M.C., 2017. Targeting the differential addiction to anti-apoptotic BCL-2 family for cancer therapy. Nature Communications 8, 16078.

Kapse-Mistry, S., Govender, T., Srivastava, R., Yergeri, M., 2014. Nanodrug delivery in reversing multidrug resistance in cancer cells. Frontiers in Pharmacology 5, 159.

Katiyar, R., Singhvi, N., Kushwaha, R., Ramji, L., 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, 229-232.

Liu, X., Johnson, S., Liu, S., Kanojia, D., Yue, W., Singh, U.P., Wang, Q., Wang, Q., Nie, Q., Chen, H., 2013. Nonlinear growth kinetics of breast cancer stem cells: implications for cancer stem cell targeted therapy. Sci. Rep. 3, 2473.

McClendon, A.K., Osheroff, N., 2007. DNA topoisomerase II, genotoxicity, and cancer. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 623, 83-97.

Minotti, G., Menna, P., Salvatorelli, E., Cairo, G., Gianni, L., 2004. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacological reviews 56, 185-229.

Pommier, Y., Leo, E., Zhang, H., Marchand, C., 2010. DNA topoisomerases and their poisoning by anticancer and antibacterial drugs," Chemistry and Biology 17, 421–433.

Renu, K., Abilash, V., PB, T.P., Arunachalam, S., 2018. Molecular mechanism of doxorubicin-induced cardiomyopathy–An update. European Journal of Pharmacology 818, 241-253.

. Sankaranarayanan, R., Alwan, N., Denny, L., 2013. How can we improve survival from breast cancer in developing countries? Breast Cancer Management 2, 179-183.

Siegel, R.L., Miller, K.D., 2019. Cancer Statistics 2019. 69, 7-34.

Sirwi, A., Shaik, R.A., Alamoudi, A.J., Eid, B.G., Kammoun, A.K., Ibrahim, S.R., Mohamed, G.A., Abdallah, H.M., Abdel-Naim, A.B., 2021. Mokko lactone attenuates doxorubicin-induced hepatotoxicity in rats: Emphasis on Sirt-1/FOXO1/NF-κB axis. Nutrients 13, 4142.

Tevaarwerk, A.J., Gray, R.J., Schneider, B.P., Smith, M.L., Wagner, L.I., Fetting, J.H., Davidson, N., Goldstein, L.J., Miller, K.D., Sparano, J.A., 2013. Survival in patients with metastatic recurrent breast cancer after adjuvant chemotherapy: little evidence of improvement over the past 30 years. Cancer 119, 1140-1148.