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Dose-dependent cytotoxicity effect of Aspirin on MCF7 cell line proliferation

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ABSTRACT

Aspirin, acetylsalicylic acid is a nonsteroidal anti-inflammatory drug (NSAID). Because aspirin nonselectively blocks COX-1 and COX-2 via irreversible acetylation. COX-2 regulates many functions such as augmentation of apoptosis, inhibition of angiogenesis, and it has anticancer effect. The aim of the present study was to detect Dose dependent cytotoxicity effect of Aspirin on MCF7 cell line proliferation and activity. Aspirin concentrations (1000, 500, 250, 125, 62.5, 31.2µg/ml). aspirin, was tested for cytotoxicity against the breast cancer cell line MCF7 using the MTT assay. The results showed that aspirin has cytotoxicity against MCF7 cells. the IC50 of the aspirin dose dependent treated range (from 243.3 to 888.6 µg/ml was (465 µg/ml). reduction in the growth, viability, proliferation, and change in morphology of cancer cells and apoptosis, all this effect increased with the increasing concentration of aspirin. Growth inhibition (GI) at highest concentration (1000µg/ml).is 35.5 %.and in lowest concentration (31.2µg/ml) is 4.8 %. In conclusion, aspirin has anticancer cytotoxicity effect.

1. Introduction

Cancer is A significant public health concern because it has been the second-highest cause of mortality in the world. It was characterized by uncontrolled cell proliferation and spread. Mortality could occur if the growth was not stopped **[1]**. Cancer treatment aims to eliminate cancer cells while causing the fewest number of adverse effects on healthy cells. a cancer therapy, localized and/or systemic treatments are used to lessen uncomfortable symptoms, combined with complementary therapies **[2]**. The most frequent malignancy in women was breast cancer (24.2%) and affects 6.6 percent of them, and the most prevalent cancer in Iraqi women. **[3]**.

The prognosis for individuals with breast cancer had been significantly improved due to breakthroughs in cancer therapy, but it was still not completely adequate. The heterogeneity of the disease known as breast cancer was influenced by genetic, epigenetic, and environmental variables. Significant development has occurred in recent years in understanding the mechanisms that govern how breast cancer develops and spreads. To fully comprehend the novel pathogenic genes and cancer-causing pathways, more study is still needed **[4]**.

Aspirin might be used in conjunction with conventional chemotherapeutic or radiotherapy treatment. On the other hand, researchers found that individuals on a constant dosage of MTX who were also taking nonsteroidal anti-inflammatory medicines (NSAIDs) had a lower rate of kidney elimination of the drug **[5, 6]**.

Aspirin is metabolized to salicylic acid via carboxylesterase, which has many therapeutic reactions with various medications such as charcoal, methotrexate, and antacids. The majority of such reactions arise due to aspirin's

capacity to move medicines from albumin binding. Because it is metabolized in various ways, it may interfere with the metabolism of many other medicines. Whenever a high dosage of aspirin is combined with additional medicine, the level of the other drug may rise or reduce. Whereas if pharmacodynamics and pharmacokinetics of the medication are understood **[7]**..

The aim of the present investigation was to assess the effect of aspirin on MCF7 breast cancer cell proliferation.

2. Materials and methods

Maintenance of cell culture

MCF7 obtained from Iraqi Center for Cancer and Medical Genetic Research, Baghdad, Iraq. (ICCMGR) is breast cancer cell line, maintenance in Roswell Park memorial institute (RPMI) 1640 medium supplemented with 10% Fetal bovine, 100 units/ml of penicillin, and 100 g/ml of streptomycin. Trypsin-EDTA was used to passage the cells, and they were then reseeded at 50% confluence twice weekly and cultured at 37 °C.**[8]**

Cytotoxicity Assays

The MTT (**Methyl thiazolyl tetrazolium)** cell viability assay was conducted on 96-well plates. Cell lines were seeded at 1 $\times 10^4$cells/well. When a confluent monolayer was achieved, cells were treated with ASA. 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 **[9].** The absorbency was determined on a microplate reader at 492 nm (test wavelength); the assay was performed in triplicate. The percentage of cytotoxicity was calculated by equation. The formula for calculating cell viability is (absorbance of treated cell / absorbance of untreated cell) * 100. Cell viability - 100% cytotoxicity**.**

GI% is calculated as follows: mean of control *100 * mean of treated * 100. y **[8]**

Acridine orange Propidium iodide test (AO\PI)

The apoptotic rates in cell lines (treated and untreated) were assessed by (AO/PI), for 24 hrs in an incubator set at 37 °C, 5000 cells per well were infected with (gold N.P). for the classic dual staining. Exactly 50 µl of the AO/PI stain mixture (at room temperature) was applied to each test well for 30 sec. The stain was then eliminated. Leica fluorescence microscope was used to capture the photographs that show the apoptosis in the cell line. [8].

Statistical analysis

T-test with Graph Pad Prism 6 was used to statistically evaluate the data. **[10]**. The results of triple measurements were provided as the mean± SD. **[11].** Isobologram version 1 was used to appear the difference result under different concentration. (1000, 500, 250, 125, 62.5, 31.2µg/ml).

3. Results

CYTOTOXICITY

Cytotoxicity inhibited MCF7 cell growth

The cytotoxicity was assessed using different concentrations of aspirin (samara, Iraq) (1000 ,500 ,250 ,125 ,62.5 ,31.2 µg/ml) by MTT cytotoxicity assay. According to these findings, increasing the concentration of the inhibitor increases cytotoxicity or enhances growth inhibition. For MCF7, there is a statistically significant difference between inhibition by aspirin as shown in Figures (1). The Minimal Essential Medium (MEM) is used as a positive control for comparing the dose dependent effects of aspirin.

Dose Asp	**1000 µg/ml**	**500 µg/ml**	**250 µg/ml**	**125 µg/ml**	**62.5 µg/ml**	**31.2 µg/ml**
Growth inhibition%	**%35.5**	**%27.3**	**%18.8**	**%12.4**	**%7.2**	**%4.8**

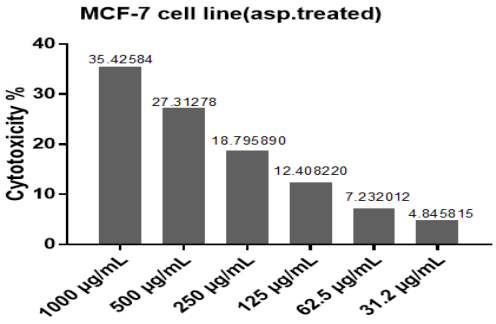


Figure 1: Cytotoxicity effect (CT %) of aspirin in different concentration on MCF7 cells.

when the concentration of aspirin increased the cytotoxicity percentage (Growth inhibition%) a raised

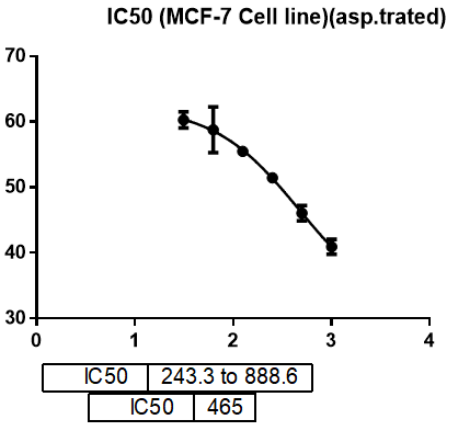


Figure 2: half maximal inhibitory concentration (IC50) after exposure the mcf7 cell line to aspirin in different concentration using MTT assay and GraphPad Prism software.

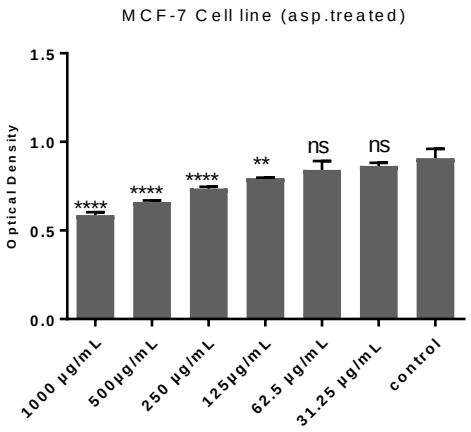


Figure 3: cell density (optical density) of aspirin in different concentrations on MCF7 cell line. the OD non-significant (ns) in fewer concentration of ASA, and significant in a higher other concentration of ASA using GraphPad Prism software.

Morphology of cell cultures

The cultured MCF7cells 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 (4). The Morphological pictures for MCF7 *in vitro* un-treated before was full number of cells, monolayer cell shape. After drugs exposure of aspirin for used concentrations were (1000,500,250,125,62.5,31.2 µg/ml) turn into single cell suspension, the number of cells began to decrease. the figures (4) refer to graduate decreased in cell number and killing effect of graduate when increase of concentration of aspirin.

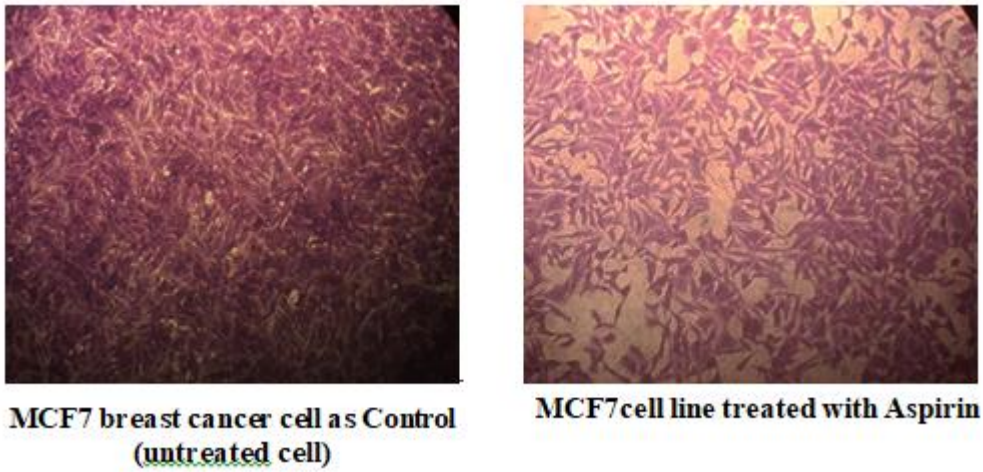
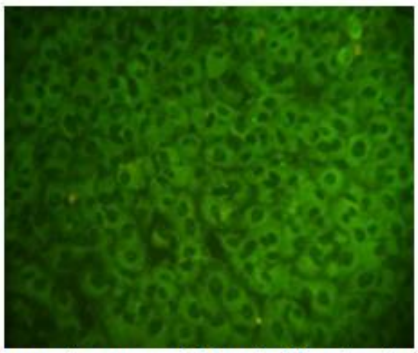


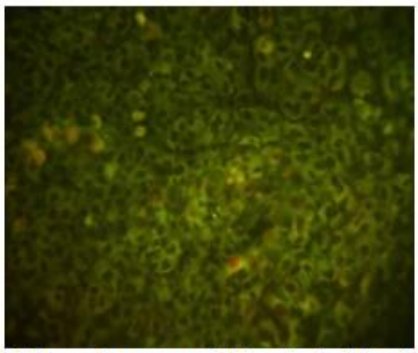
Figure 4: The morphological images of MCF7 treated by aspirin in vitro .

The morphological images for MCF7 in vitro before treatment were full number of cells, monolayer cell shape. Following medication exposure, aspirin, for utilized concentrations were (1000, 500, 250, 125, 62.5, 31.2µg/ml) turned into single cell suspension, and the number of cells started to decline. That concentration of, ASA, depicts the reduction in cell number and killing impact of graduate as the concentration of, ASA, increase. that viewed with an inverted microscope, 10x.

Stained cells- AO/PI results



MCF7 breast cancer cell Control (untreated).



MCF7 breast cancer cell treated with Aspirin

Figure 5: Analysis of the effects of aspirin on MCF7 cell line.

Analysis of the effects of aspirin on MCF7 cell line, of treated and untreated (control) cells. To explain the consequences of apoptosis, all cells were dyed with AO/PI and examined under a fluorescence microscope (10X). After receiving a dose of aspirin for 72 hours, the green color represents live cells, and the red color displays dead cells Figure (5).

Discussion

Interest in the pharmacological effects of bioactive chemicals on the treatment and prevention of cancer has grown during the past two decades. It has been demonstrated to have several anti-cancer properties in a variety of cancer cells through distinct cytotoxic effects without significantly harming normal cells [12, 13].

A pharmacological inhibitor's capacity to inhibit MCF7 is quantified by the half-maximum inhibitory concentration (IC50). A quantitative method for estimating the concentration of an inhibiting drug is the IC50 value. It was demonstrated that aspirin, and had a distinct impact on breast cancer cell lines [9].

Aspirin is a nonsteroidal anti-inflammatory medication with anti-cancer effects. Aspirin may reduce tumor development by modifying cell growth and death, mostly by suppressing native prostaglandin synthesis through reduction of cyclooxygenase (COX) enzyme function, especially COX-2. Persuasive experiments has arisen demonstrating that COX-2 was upregulated in breast cancer yet not in healthy breast tissue, making aspirin a possible anticancer drug [14].

Aspirin and other nonsteroidal anti-inflammatory medications (NSAIDs) may also suppress cancer cell growth and cause apoptosis, according to (Maniewska and Jeżewska [15]. The capacity of these medications to suppress cyclooxygenase-2 (COX-2) and other inflammatory pathways, according to the scientists, may mediate their anti-cancer actions.

The in vitro results of this study revealed that increasing the concentrations of ASA in MCF7 increases cytotoxicity and improves anti proliferation against MCF7. These results are consistent with prior research on the impact of these medicines on apoptosis in cancer cells. According to [16], aspirin may promote apoptosis in cancer cells by inhibiting COX-2 and other inflammatory pathways. ASA showed inhibitory rates in its cytotoxicity activity on cell lines. These results showed that the six concentrations used on MCF7 cell lines had a gradual effect. When compared to past research, our findings are comparable with those of Hashiguchi et al. [17] and Jiang et al.[16], who discovered that ASA may cause apoptosis in cancer cells. However, our work is unusual in that we looked at how these medicines affected apoptosis in MCF7 cells, which are a kind of human mammary epithelial cell line often utilized in breast cancer research.

In our investigation, we discovered that ASA caused considerable apoptosis in MCF7 cells. The presence of condensed and fragmented nuclei revealed a significant rise in the number of cells undergoing apoptosis. These results imply that ASA may have anticancer action due

to their capacity to trigger apoptosis in cancer cells.

Razak et al. [18] and Guo et al. [19] discovered that aspirin suppressed the development of MCF7 cells in a dose-dependent manner, with a cytotoxicity (CT%) of 25% after 72 hours of treatment at a dosage of 1 mM. Aspirin suppressed the development of MCF7 cells in a dose-dependent manner, with a CT% of 60% at a dosage of 5 mM after 72 hours of treatment, according to Ranjbarnejad *et al* [20].

ASA for 72 hours influences the morphological changes and apoptosis shown after treatment with aspirin. In AO/PI-dyed and treated cells, apoptosis was obvious as red cells, whereas healthy cells were green. The natural process of planned cell death known as apoptosis is carefully regulated by the organism and can be brought on by a variety of physical and chemical factors. Despite the fact that apoptosis is regulated by three main signaling pathways (mitochondrion, death receptor, and endoplasmic reticulum signaling pathways), The mitochondrial level is typically where apoptotic signals is combined and intensified[21]. The current study used the inhibitor ASA to find a complementary or supportive treatment for chemotherapy or other traditional treatments. Additionally, apoptotic induction resulted in anti-tumor efficiency and the inhibition of cancer cell development.

Conclusion

In vitro study on MCF7 cell line, investigate the ASA decreased cell growth, reduced optical density, decreased in cell number, increased killing impact, caused cytotoxicity in addition to morphological alterations and apoptosis in MCF7.

Recommendations

1- using other cancer cell line types are recommended.

2- the in vivo study to show the impact of treatment on cancer cells and on physiological parameters in lab animals.

Acknowledgment

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Ethical approval

This study proposal approved by faculty of veterinary medicine, Tikrit university, Iraq.

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تأثير السمية الخلوية المعتمدة على الجرعة للأسبرين على خط خلايا MCF7 وتكاثر الخلايا السرطانية

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الملخص

الأسبرين، حمض أسيتيل الساليسيليك هو دواء مضاد للالتهابات غير الستيرويدية .(NSAID) لأن الأسبرين يمنع بشكل غير انتقائي COX-1وCOX-2 عن طريق أستلة لا رجعة فيها. ينظم COX-2 العديد من الوظائف مثل زيادة موت الخلايا المبرمج، وتثبيط تكوين الأوعية الدموية، وله تأثير مضاد للسرطان. كان الهدف من هذه الدراسة هو الكشف عن تأثير السمية الخلوية المعتمدة على الجرعة للأسبرين على تكاثر خط خلايا MCF7 ونشاطه. تركيزات الأسبرين (1000 ، 500 ، 250 ، 125، 62.5 ، 31.2 ميكروغرام / مل). الأسبرين تم اختباره للسمية الخلوية ضد خط خلايا سرطان الثدي MCF7 باستخدام مقايسة MTT. أظهرت النتائج أن الأسبرين له سمية خلوية ضد خلايا MCF7. كان IC50 من النطاق المعالج المعتمد على جرعة الأسبرين (من 243.3 إلى 888.6 ميكروغرام / مل (465 ميكروغرام / مل). انخفاض في النمو، والقدرة على البقاء، والانتشار، والتغيير في مورفولوجيا الخلايا السرطانية وموت الخلايا المبرمج ، كل هذا التأثير زاد مع زيادة تركيز الأسبرين. تثبيط النمو (GI) عند أعلى تركيز (1000 ميكروغرام / مل) هو 35.5٪ وفي أدنى تركيز (31.2 ميكروغرام / مل) هو 4.8٪. في الختام، الأسبرين له تأثير السمية الخلوية المضادة للسرطان....