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Evaluation the Cytotoxic Effects of Cisplatin on a Human Lung Cancer Cell Line A549

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Abstract

Millions of people worldwide lose their lives to lung cancer, which is an international health issue. As a result, it has drawn the attention of several researchers from all over the world, and everything related to this condition in terms of changes, whether physiological or immunological, before and after therapy, has been studied. In the present investigation, six different chemotherapeutic doses were utilized, ranging from (31.25-1000 µg/ml) over (48) hours of exposure, to assess the cytotoxic effect of cisplatin on the human lung cancer cell line A549. In the results of the current study showed the highest rate of cell inhibition was at the concentration (1000) µg / ml, as it reached about (70.400), while at a concentration of (31.25) µg /ml, cell inhibition was at its lowest rate, which was (19.60), and the results also showed that cisplatin had anti-lung cancer activity at an inhibitory concentration of half the number of cells used in the experiment (IC50), as it was about (43.75) µg/ml. The results of the current study showed that there was a significant increase ($P<0.05$) in the six concentrations of cisplatin when compared with control cells.

Keywords: Cisplatin, Lung Cancer, A549

1. Introduction

The burden of cancer is steadily increasing worldwide. Lung cancer is one of the deadliest types of cancer, with unsettlingly high mortality rates (1 in 5 cancer deaths worldwide) [1]. In the last ten years, lung cancer, also known as human bronchial cancer has killed more people than any other type of cancer for both sexes worldwide, with more than 228,000 cases reported just in 2019, in Iraq, lung cancer is the second most common cancer after breast cancer, with more than 2000 cases reported in the previous year's [2] and according to world health organization, out of a total of 14,500 deaths from different other forms of cancer diseases in Iraq, lung cancer took the lives of more than 1,960 people, making it the disease with the highest death rate [3]. Environmental, lifestyle, and inherited genetic abnormalities can all contribute to lung cancer. Lung cancer is largely caused by environmental and lifestyle factors, with little contribution from inborn genetic flaws, some examples of environmental and lifestyle variables that cause lung cancer include asbestos, ionizing radiation, sulfur mustard, coal-tar pitch, and tobacco use, so according to reports, tobacco use is the leading risk factor for lung cancer in 90% of men and 80% of women, in which the international agency for research on cancer estimates that cigarette smoke comprises 4000 compounds that can be identified, of which more than 60 are carcinogenic [4]. Cisplatin (cis), also known as Cis-DiamineDichloroPlatine II (CDDP) [5], is a platinum-based chemotherapy used to treat malignancies linked to various types of cancer in many tissues [6], including the bladder, ovary, lung, testicles, esophagus, cervical, and breast cancers and it is frequently administered intravenously [7]. As soon as cisplatin enters the cytoplasm of cancer cells, it starts to work inside by creating covalent links with DNA; these connections inhibit cancer cells' ability to transcription and replicate DNA [8], therefore, the infected cells undergo programmed cell death as a result of the DNA repair mechanism being activated [9]. Cisplatin has cytotoxic effects on the human lung cancer cell line A549 *in vitro* by activating the tumor suppressor protein p53, which upon activation initiates the DNA disruption response, leading to cell cycle arrest and death in culture [10]. Consequently, cisplatin was regarded as one of the most chemotherapy drug using againstdifferent types of cancer, particularly lung cancer of both types (SCLC and NSCLC), as it demonstrated a significant benefit by increasing patients' chances of surviving for five years by (5.3%) after using it in treatment [11], so the aim of this study was to know the cytotoxic effects of cisplatin against the human lung cancer cell line A549.

2 Methods

2.1 Cell Culture

Cisplatin was obtained from (Santa Cruz Biotechnology company), with a weight of (100 mg) and a serial number (Sc-200896), then it was transferred to the laboratory of the Iraqi Biotechnology Company located in Baghdad / Al-Harithiya, to performed the tests on A549 cell line, after making six concentrations (1000, 500, 250, 125, 62.5, 31.2 mg/ml), it was prepared by dissolving (0.01 gm) of the powder in a media free serum. A549 cells, which are adenocarcinoma human alveolar basal epithelial cells, were first created in 1972 by (D. J. Giard, *et al*.), through the excision and culture of malignant lung tissue from the explanted tumor of a 58-year-old Caucasian male, the cells serve as models for the investigation of lung cancer and the creation of medication therapy [12]. MTT cell viability assays were run on 96-well plates to quantify the cytotoxic effect [13]. The number of cells per well for each cell line was 1×10^4. After 24 hrs, a Cells were treated with cisplatin after achieving confluent monolayer after 48 hours of treatment, and A549 Cell (untreated) considered a control. cell viability was assessed by removing the medium, adding 28 µL of an MTT solution containing 2 mg/mL, and incubating the cells for 1.5 hours at 37 °C. Following the MTT solution removal. By adding 130 µL of DMSO (Dimethyl Sulphoxide) and incubation at 37 °C for 15 min while shaking, the remaining crystals in the wells were solubilized [14]. The assay was carried out in triplicate, and the absorbency was measured on a microplate reader at 492 nm (the test wavelength). The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation [15]:

Cell viability % = (Absorbance of treated cell/ Absorbance of untreated cell) × 100

Cytotoxicity % = 100 - Cell viability

2.2 Statistical Analysis

The obtained data were statically analyzed using an unpaired t-test with Graph Pad Prism [16]. The values were presented as the mean ± SD of triplicate measurements [17].

3. Results

3.1 Cytotoxic Effect of Chemotherapy (Cisplatin) in Human Lung Cancer Cell Line A549

The results of the current study demonstrated the presence of cytotoxic effects of cisplatin in the human lung cancer cell line A549 after treatment for a period (48) hours, where the cytotoxicity was calculated based on the percentage rate of cell inhibition (Inhibition rate).The highest percentage of cell inhibition occurred at concentrations (1000, 500, 250) µg / ml, which were (70,400, 68,300, 64,500), respectively, while The lowest percentage of cell inhibition was at the concentration (31.2) µg/ml, when was (19.60), as shown in (Figure 1), also the results showed that the (IC50) concentration of cisplatin was (43.75) µg/ml, as it ranged between (24.77-77.28) µg/ml, as shown in (Figure 2), note that the increased cytotoxicity and consequent rise in anti-tumor activity result from the lower (IC50) concentration, and the image (1) shows the shape of A549 cancer cells under an inverted microscope, which were exposed to the (IC50) concentration of cisplatin, compared with control (untreated A549 cancer cells) shown in image (2).

As shown in Figure 3, the results of the present study revealed a significant increase ($P < 0.05$) in the six concentrations of cisplatin when compared with control cells (untreated cancer cells), these concentrations were 1000 (0.2663 ± 0.02876), 500 (0.2857 ± 0.00318), 250 (0.3193 ± 0.007839), 125 (0.458 ± 0.01473), 62.5 (0.582 ± 0.01617), and 31.25 (0.7243 ± 0.005207) compared with Control cells (0.9013 ± 0.02106).

The current study's findings, which were statistically analyzed using the Tukey's multiple comparisons test for cisplatin drug concentrations, revealed that there was a significant decrease ($P>0.05$) between the concentrations of 1000 and (500, 250), but a significant increase ($P< 0.05$) when comparing 1000 with the concentrations of 125, 62.5, and 31.25. In addition, there was a significant increase ($P<0.05$) when comparing 1000 with control cells. Additionally, there was a significant increase ($P<0.05$) when comparing the concentration (500) to the concentrations (125, 250, 62.5, 31.25), as well as when compared to control cells. According to the study's findings, there was also a significant increase ($P<0.05$) when comparing concentration (250) with each of the concentrations (125, 62.5, and 31.25), as well as when comparing the same concentration with control cells, and the findings demonstrated a statistically significant increase ($P<0.05$) between concentration (125) and concentrations (62.5, 31.25), as well as a statistically significant rise when compared to control cells, furthermore, there were significant changes between concentration (62.5) and control cells, with a significant increase ($P<0.05$) seen when comparing concentration (62.5) with concentration (31.25), and Comparing concentration (31.5) to control cells further shown a statistically significant increase (Table 1).

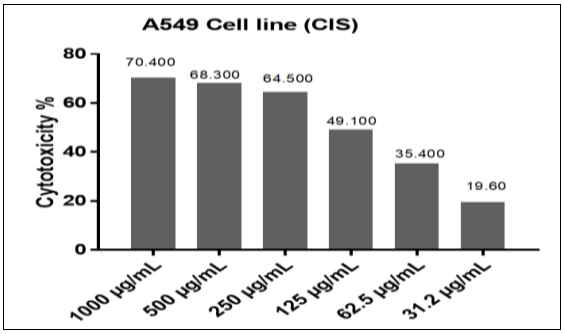

Fig 1: A curve showing the cytotoxicity effects in A549 cell line after treatment with six concentrations (3 replicates for each concentration) of the cisplatin, using the MTT assay *in vitro* for (48) hours

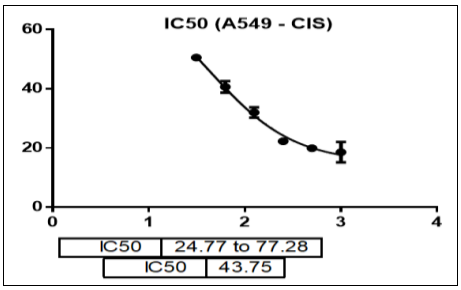

Fig 2: A curve showing the effect of the inhibitory half-cell concentration (IC50) of the cisplatin on the A549 cell line

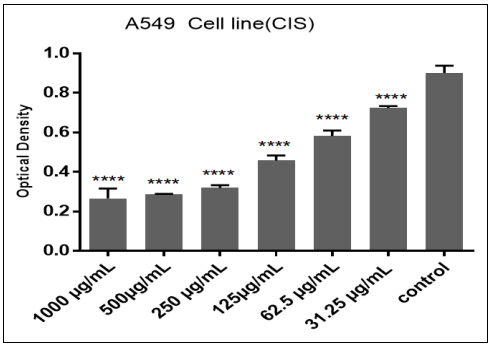

Fig 3: Showing significant differences between the effect of Chrysin concentrations on A549 cell line compared with control cells using MTT assay in laboratory for (48) hours, ***, ****: (P < 0.05), ns: non–significant, SE: standard error, (repeats=3)

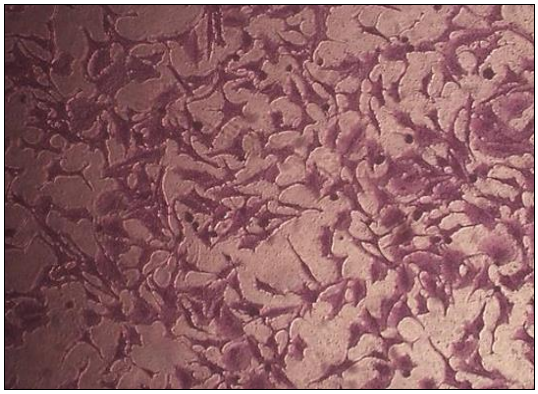

Image 1: Showing A549 cell line under an inverted microscope after being treated with concentration (IC50) of the cisplatin (Crystal violet stain, Magnifying power 100X)

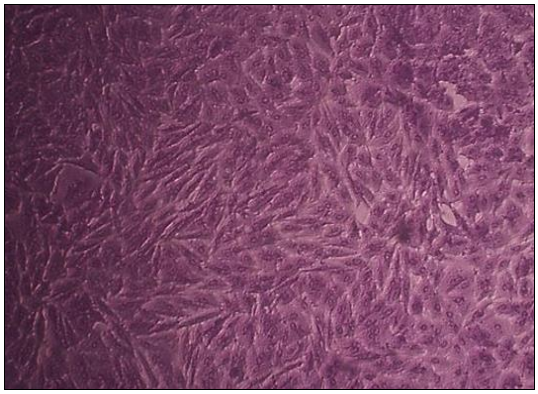

Image 2: Showing the control cells (untreated with cisplatin) A549 human lung cancer cell line (Crystal violet stain, Magnifying power 100X)

Table 1: Tukey's test for multiple comparisons between the concentrations of cisplatin with each other when used as an antitumor activity against human lung cancer cell line A549, in addition to comparing it with control cells

Tukey's multiple comparisons test	significances	P.value
1000 µg/mL vs. 500 µg/mL	ns	0.5406
1000 µg/mL vs. 250 µg/mL	ns	0.1500
1000 µg/mL vs. 125 µg/mL	****	0.0040
1000 µg/mL vs. 62.5 µg/mL	****	0.0007
1000 µg/mL vs. 31.25 µg/mL	****	< 0.0001
1000 µg/mL vs. control	****	< 0.0001
500 µg/mL vs. 250 µg/mL	ns	0.1640
500 µg/mL vs. 125 µg/mL	****	0.0003
500 µg/mL vs. 62.5 µg/mL	****	< 0.0001
500 µg/mL vs. 31.25 µg/mL	****	< 0.0001
500 µg/mL vs. control	****	< 0.0001
250 µg/mL vs. 125 µg/mL	***	0.0011
250 µg/mL vs. 62.5 µg/mL	****	0.0001
250 µg/mL vs. 31.25 µg/mL	****	< 0.0001
250 µg/mL vs. control	****	< 0.0001
125µg/mL vs. 62.5 µg/mL	**	0.0048
125 µg/mL vs. 31.25 µg/mL	****	< 0.0001
125 µg/mL vs. control	****	< 0.0001
62.5 µg/mL vs. 31.25 µg/mL	***	0.0011
62.5 µg/mL vs. control	****	0.0003
31.25 µg/mL vs. control	****	0.0012
****** ،*** ،**: (P < 0.05), ns: non–significant**		

4. Discussion

Several scientific papers have indicated to the cytotoxic effects of cisplatin on a wide range of cancer cell lines, as [18] showed during his study on the cytotoxicity of cisplatin in the human testicular cancer cell line (TGCT), that cisplatin It has cytotoxic effects on both cancer cell lines (NT2 / D1) and (833K) after treating them with concentrations ranging from (0.01 - 10 uM) for a period of (48) hours, as the results showed a significant decrease in cell viability in both cell lines. The results showed that the inhibitory concentration of half of the number of (IC50) cells in the cell line (NT2 / D1) was (0.3 uL), while the concentration was (1.02 uL) in the (833K) cell line, this is consistent with the results of the current study, which showed an increase in the cytotoxicity of cisplatin in A549 cell line after being treated with different concentrations for (48) hours, as the higher the concentration, the greater the cytotoxicity, therefore, the cell vitality decreases and the inhibition rate increases, [19] also mentioned that the mechanism of cisplatin's toxicity to cancer cells may be through inhibition of cell proliferation proteins such as, (CDK6 and CDK4), which leads to programmed cell death (apoptosis). In a study conducted by [10], on the cytotoxic effects of cisplatin on a human lung cancer cell line and a normal lung cell line, the results showed that it had a cytotoxic effect in the A549 cell line after being treated with different concentrations and for a periods (24, 48), in addition, the results showed that the (IC50) concentration differed according to the time of exposure to the treatment, as its percentage was (36.94 uL) after (48) hours, while was (6.59 uL) after (72) hours, and this is consistent with the results of the current study, which showed that the (IC50) of cisplatin was (43.75 µg/ml), as the lower the concentration of (IC50), the greater the cytotoxicity of the treatment, leads to decrease in cell viability, and the decrease in the value of (IC50) indicates to resistance of cancer cells to chemotherapy as a result of the increase in cytotoxicity, and vice versa, the higher of (IC50), indicates to the lower of cancer cell resistance to chemotherapy and, therefore decrease in cytotoxicity, and one of the reasons that lead to the resistance of lung cancer cells to chemotherapy is hypoxia, which leads to inhibition of the process of programmed cell death [20, 21] also indicated that the use of cisplatin against a squamous cell carcinoma cell line (SSC-25) showed a cytotoxic effect, as a decrease in cell viability was observed with increasing concentrations, as the concentrations ranged from (0.4 - 100 µg/ml), and the value of (IC50) was (12.56 µg/ml) after a period of exposure (24) hours, and this is consistent with the results of the current study, the purpose of determining the (IC50) concentration is to understand the biological and therapeutic properties of the drug used in the treatment [22], the current study's findings concurred with [23], which

demonstrated that cisplatin had a cytotoxic effects when utilized against the human glioblastoma cancer cell line (A1235) at different concentrations.

5 Conclusion

The research established that (1000 mg/mL) of cisplatin is the ideal concentration for inducing cytotoxicity in the A549 cell line, and (43, 75 mg/mL) of inhibitor was the appropriate dose for half of the cell population.

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