# Effect of anticancer chemotherapy drugs on lung cancer cell line (COR-L23)

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## Abstract

The study includes anti-cancer chemotherapy drugs activity on (COR-L23 and COR-L23R) cell line in vitro , and effects of Cisplatin on cell growth and its apoptosis through treatment those cell with different time interevals(4, 6 and 18 hours) and different concentrations of Cisplatein and mixed chemotherapy: C1 (3000µM), C2 (300µM), C3 (30µM). With using plate of 24 wells, the apoptosis results showed 81.92% , 77.45% , 70.73% respectively and 68.92% control group for 4 hr. For 6 hr were 90.68% , 79.95% , 71.56% respectively and 70.99% control group .The results for 18 hr were 93.38% , 81.97% , 69.52% respectively and 64.56% control group .

The results for Cisplatin resistance showed 90.4%, 84.72%,83.39% respectively and 78.42% control group for 4hr,while the results for 6hr were 88.96% , 85.56% , 73.1% respectively and 64.21% control group ,and for 18hr were 89.27%,77.78%,75.1% respectively and 63.25% control group .While the results for mixed chemotherapy were 93.9%, 78.18%,63.38% respectively and 51.48% for control group for 4hr.The result for 6hr were 93.95%,82.18%,79.4% respectively and 58.52% ,while the results for 18hr were 95.4%,84.72%,83.39% respectively and 78.42% for control group .

## Conclusions

The results were detected of lung cancer cell line (COR-L23) affected differently during the treatment of anti-cancer drugs with increasing time and concentration .

**Key word:** Lung cancer, Chemotherapy , Apoptosis .

## Electrophoresis Introduction

Lung cancer is by far the leading cause of cancer-related death in the world. Several environmental factors associated with an increased risk of developing lung cancer have been identified, including mainstream cigarette smoking; exposure to radon, polycyclic aromatic hydrocarbons , nickel, chromate, arsenic, asbestos, chloromethyl ethers and ionizing radiation; and chronic obstructive pulmonary disease with airflow obstruction [1] .

Lung cancer causes more deaths than the sum of the next three leading cancers: breast, colon, and prostate. According to recent study . lung cancer can be classified into two main categories: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Non- small cell lung cancer can further be divided into sub-categories such as adenocarcinomas, squamous cell (epidermoid) carcinomas, brancho-alveolar carcinomas, and large cell carcinomas [2] .

The majority of chemotherapeutic agents elicit their anti-tumor effects by induction of apoptosis through irreversible DNA damage. Cisplatin and Carboplatin are two platinum based agents that form platinum adducts with DNA strands after passive diffusion into the cells, thereby preventing DNA replication and cell division [3] .

Other non-platinum based agents used for treatment of lung cancer, such as Gemcitabine (gemzar) employ a similar mechanism of action . Gemcitabine is a prodrug that must be phosphorylated into the active form upon entry into the cell [4] . Paclitaxel, originally isolated from yew trees, is a compound that acts on the microtubule environment of the cell. Paclitaxel is capable of stabilizing established microtubule environments as well as shifting soluble tubulin proteins into a stable microtubule polymer. Microtubules are in a dynamic state where tubulin is responsible for mitotic spindle formations, cell shape maintenance and cell motility [5].which reduces the capacity for PC activation . The other study shown increased tumor factor (TF) activity and enhanced thrombin generation on chemotherapy treated endothelial cells [6] . Previous in vitro studies have shown that treatment of endothelial cells Cisplatin and Gemcitabine results in a dose-dependent increase of TF activity [7].

## Aim of study

Lung cancer is the leading cause of cancer-related mortality worldwide. Approximately 85% of lung cancer cases are of the non–small cell type (NSCLC). Surgery, chemotherapy, and radiotherapy have been used in various combinations to improve survival and maximize the therapeutic benefit. Therefore, the present study aim to check the chemotherapy effect on lung cancer cell line COR-L23 (apoptosis). Comparing the apoptosis data between COR-L23 cell line and COR-L23 chemotherapy treatment .

## Material & Method

The practical part of this study carried out in Salford University/ UK .

Cell Culture: COR-L23 Cells were grown in culture media supplemented with (10% Fetal bovine serum (FBS), 2 mM L-glutamine, 100 µg/ml streptomycin) at 37°C in a humidified atmosphere containing 5%

$CO_2$. 25 ml flask was prepare from new vial of COR-L23 in 25 ml flask after 3 days make the sub culturing to Three 100 ml flasks at a density of $10^6$ cells for each flask and change the media after 4 days to get the good growth for cell to cover all area of down flask, then make subcalturing to prepare mor samples and apoptosis test .

**Table (1) :Show the chemotherapy concentration for mixed (C1,C2,C3) treated the COR-L23 cell line for different time (4,6,18) hours**

| Chemotherapy | C1 | C2 | C3 |
|---|---|---|---|
| Doxorubicin | 1 μM | 0.1 μM | 0.01 μM |
| Gemcitabine | 10 μM | 1 μM | 0.1 μM |
| Vinorelbine | 10 μM | 1 μM | 0.1 μM |
| Paclitaxol | 10 μM | 1 μM | 0.1 μM |
| Cisplatin | 1000 μM | 100 μM | 10 μM |

**Table (2): Show the Cisplatin concentration (C1,C2,C3) treated the COR-L23 and COR-L23Resestanse cell line for different time (4,6,18) hours**

| Cells type | C1 | C2 | C3 |
|---|---|---|---|
| COR-L23 | 3000 μM | 300 μM | 30 μM |
| COR-L23 R | 3000 μM | 300 μM | 30 μM |

The treated cells by chemotherapy divided into three samples; the first sample treated by mixed chemotherapy with using plate of 24 wells to apoptosis test .Count the cells as previously described and dilute to 1x $10^5$ per /ml. add 500 μL of cells solution per well (50000 cells/well). In a 24 well plate ,four samples can be accommodated ; sample 0 (a,b,c) ,sample C1(a,b,c), sample C2(a,b,c), sample C3(a,b,c) .Later the plates were placed in the incubator (humidified ,37 C° ,5% $CO_2$),then after 3 days the old media removed from each well of the incubated plate and add 500μl of media with drug to all wells ,as shown in table (1). Samples were harvested at 3 time interevals: 4, 6 and 18 hours.The treatment of, different concentration of mixed chemotherapy as shown in table (1) .

The second sample treated by three concentration of cisplatein as shown in table (2) used plate 24 wells to apoptosis test .Count the cells for COR-L23 and COR-L23R as previously described and dilute to 1x $10^5$ per /ml. add 500 μL of cells solution per well (50000 cells/well).

In a 24 well plate ,eight samples can be accommodated sample 0 (a,b,c) ,sample C1(a,b,c), sample C2 (a,b,c), sample C3(a,b,c) and sample 0R (a,b,c) ,sample C1R(a,b,c), sample C2R(a,b,c), sample C3R (a,b,c) ,after that place the plate in the incubator (humidified ,37 C° ,5% CO2) . Then after 3 days the old media were removed from each well of the incubated plate and 500μl of media with drug were added to all wells,as shown in table (2).Samples were harvested later after 3 time interevals: 4, 6 and 18 hours .

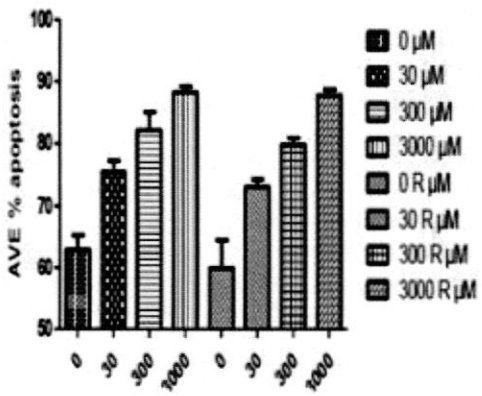
## Apoptosis tests by chemotherapy

Cell apoptosis DAPI Detection Kit (Cat.No.L00312) provides a rapid and convenient assay for apoptosis based upon fluorescent detection. 4, 6-Diamidino-2-phenylindole (DAPI) is a kind of specific dye for binding DNA. This dye is not completely permeable. Once it overpasses cell membranes of normal cells, the blue fluorescence will be observed by fluorescent microscopy.

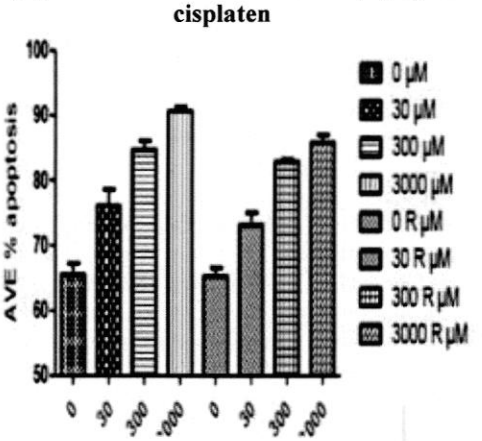
## Results and discussion

Oxidative stress and inflammatory events, in response to cigarette smoke, play an important role in airway and alveolar epithelium injury . In the present study, we investigated the effect of chemotherapy on cytotoxicity, apoptosis as well as increase or decrease of gene expression release in a variety condition (time & concentration) of alveolar epithelial cells (COR-L23), and compared the effect with control group (which is out of chemotherapy treatment) .

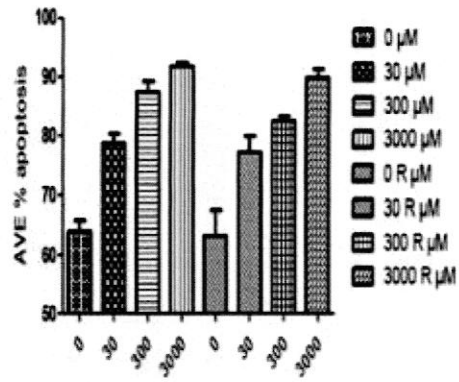
Chemotherapy differentially induced cytotoxicity in various epithelial cell lines in adose-dependent manner. Among the cell lines studied, lung epithelial cell lines (COR-L23) were found to be more sensitive to cisplatein when compared to both human lung epithelial cell lines (COR-L23) without chemotherapy and resistance ,as shown in figures (1,2) .




**I-Apoptosis in COR-L23 resistant (R) by 4hr cisplaten**




**II-Apoptosis in COR-L23 resistant (R) by 6hr cisplaten**




**III-Apoptosis in COR-L23 resistant (R) by 18hr cisplaten**

Figure (1) Graphs (I, II, III) showing apoptosis by Cisplatin in COR-L23 and COR-L23R Bars represent average % cells from 3 independent wells. Error bars represent SD .

This finding is supported by earlier studies showing that exposure of rat lung epithelial cells to lower concentrations of cisplatien resulted in a significant decrease in cell viability.

In our results high concentration of chemotherapy showed high sensitive of COR-L23 to transformed epithelial cell lines. We postulate that the CSE induced cytotoxic effects may be due the presence of highly reactive electrophilic compounds (aldehydes and quinones) present in CSE [8] .

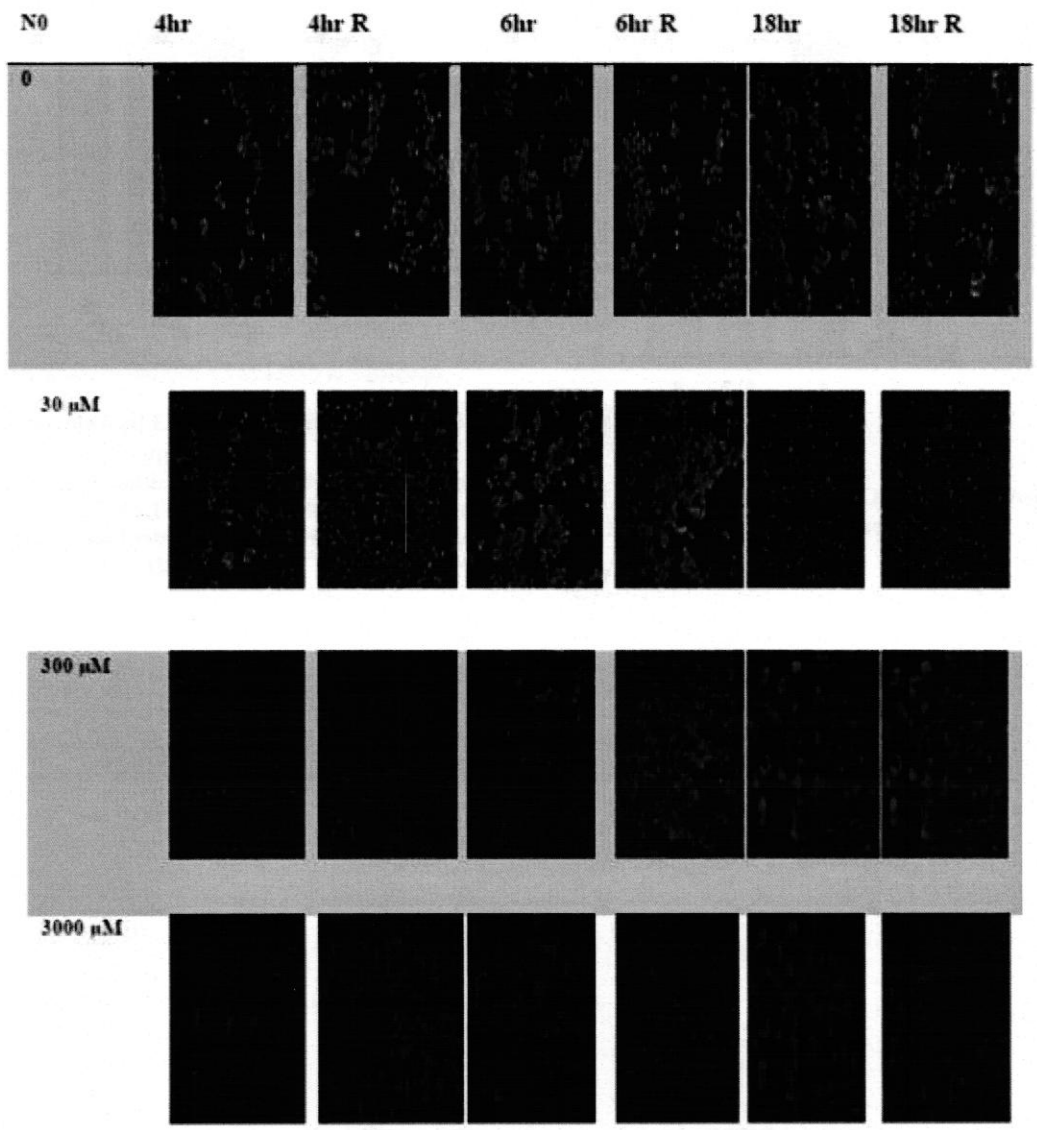
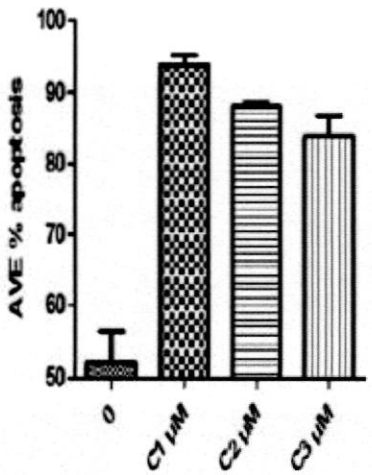
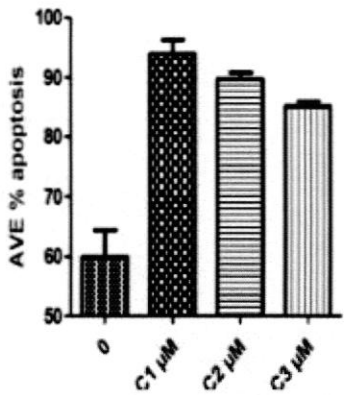



**Figure (2) : The florescence microscope images for the effect of different times (4hr,6hr,18hr) for (C1,C2,C3) for cisplaten concentration on apoptosis for COR-L23 and COR-L23 R cell line**

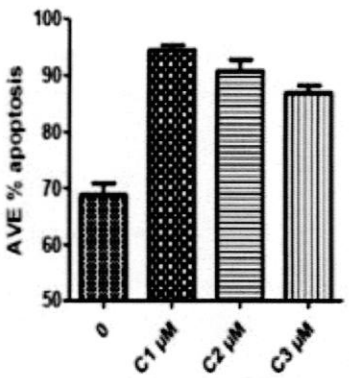
Apoptosis is a well-defined programmed response that results in characteristic morphologic changes, such as cell shrinkage, condensation and fragmentation of nuclear material . Necrosis on the other hand, is a passive response characterized by cytoplasmic swelling, rapid loss of plasma membrane integrity, and eventually cell lyses [9] . The cell line COR-L23, found to be more sensitive to mixed chemotherapy when compared to both human lung epithelial cell lines (COR-L23) without chemotherapy ,as shown in figures (3,4) . In other studies, showing that exposure of rat lung epithelial cells to lower concentrations of mixed chemotherapy resulted in a significant decrease in cell viability [10]. Our results also showed that were highly sensitive to high concentration compared with cisplatien .




**I-Apoptosis of COR-L23 by 4hr mixed chemotherapy**




**II-Apoptosis of COR-L23 by 6hr mixed chemotherapy**




Apoptosis of COR-L23 by 18hr mixed chemotherapy

**III-Apoptosis of COR-L23 by 18hr mixed chemotherapy**

**Figure (3): Graphs ( I, II, III ) showing apoptosis by mixed chemotherapy in COR-L23 Bars represent average % cells from 3 independent wells. Error bars represent SD**

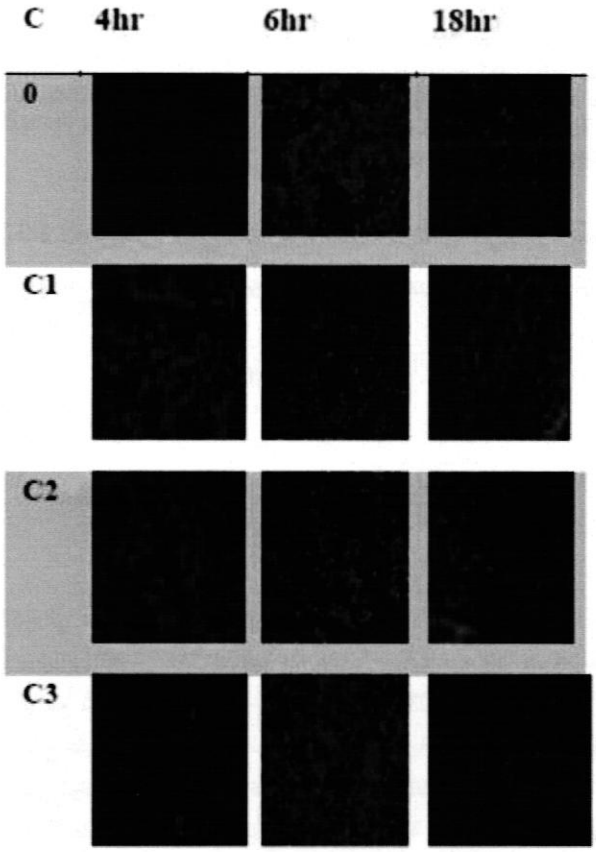



**Figure (4) : The florescence microscope images for the effect of different times (4hr,6hr,18hr)for (C1,C2,C3) concentration of chemotherapy mixed in apoptosis for COR-L23 cell line**

There was a significant variability in their sensitivity to different doses of chemotherapy. Our data showing time and concentration induced necrosis with no or little evidence of apoptosis is in contrast to previous studies in macrophages and endothelial cells [10] .As a complement to genetic approaches, cancer cell proteomics can be a powerful tool to get better insights into the complex mechanisms of chemo resistance and to identify candidate resistance biomarkers .

Chemotherapy has the ability to induce an increase in mutation frequency that is quantifiable and we believe that this increased mutation frequency can enhance that cell lines' ability to develop chemo resistance [11, 12] .

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# تأثير الأدوية المضادة للسرطان على خطوط خلايا سرطان الرئة (COR-L23)

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**الملخص**

تضمنت هذه الدراسة متابعة تأثير العلاج الكيميائي Cisplatein على خط الخلايا لسرطان الرئة (COR-L23 ,COR- L23R) ، وكذلك تأثير العلاج الكيميائي على نمو الخلايا ومراحل موتها ، وذلك من خلال معالجة هذه الخلايا في أوقات مختلفة وتراكيز مختلفة . حيث تم اختيار ثلاث فترات زمنيه للمعالجة (4 و6 و18 ساعة) مع ثلاث تراكيز مختلفة وهي C1(3000µM), C2 (300µM), C3 (30µM) من العلاج الكيميائي Cisplatein وكذلك خليط من العلاج الكيميائي ، وباستخدام لوحة الاختبار الحاوية على 24 حفره تقاس مراحل موت الخلايا و كانت النتائج 81.92٪ ، 77.45٪ ، 70.73٪ على التوالي مقارنة بمجموعة السيطرة 92،68٪ لفترة المعالجة 4 ساعات ، بينما كانت النتائج المعالجة لفترة 6 ساعات 68،90٪، 95،79٪، 56،71٪ على التوالي مقارنة ب 99،70٪ لمجموعة السيطرة . في حين بلغت نتائج المعالجة لفترة 18 ساعة هي 38،93٪، 97،81٪، 52،69٪ على التوالي مقارنة بـ56،64٪ لمجموعة السيطرة . اما نتائج المعالجة لخلايا COR- L23R فقد كانت 90.4٪ ، 72،84٪، 39،83٪ على التوالي و42،78٪ لمجموعة السيطرة لفترة 4 ساعات ، اما لفترة 6 ساعات فكانت 96،88٪ ، 56،85٪ ، 73.1٪ على التوالي و٪ 21،64 لمجموعة السيطرة ، في حين بلغت النتائج لفترة 18 ساعة 27،89٪ ، 77.78٪ ، 75.1٪ على التوالي و٪ 25،63 لمجموعة السيطرة . بينما كانت النتائج لخليط العلاج الكيميائي 93,9٪ ، 78٪ ،18، 63,38٪ على التوالي و 48،51٪ لمجموعة السيطرة لفترة4 ساعات . وكانت النتائج 95،93٪، 18،82٪، 4.79٪ على التوالي و٪ 52،58 لمجموعة السيطرة لفترة 6 ساعات ، في حين بلغت نتائج فترة 18 ساعة 95.4٪ ، 72،84٪ ، 39،83 ٪ على التوالي 42،78٪ لمجموعة السيطرة .

**الاستنتاجات :** اشارة النتائج الى انه خطوط خلاي سرطان الرئه (COR-L 23) تتاثر وبصوره متباينه خلال معاملتها بالادويه المضاده للسرطان بزيادة الوقت والتركيز .