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Role of novel anticancer drug Roscovitine on enhancing radiosensitivity in carcinoma cell lines

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ABSTRACT

The present study was conducted to evaluate the radiosensitization effect of Roscovitine (cyclin dependent kinase inhibitor) in carcinoma cell lines. Three cell lines are used, liver carcinoma cell line (HepG2), brain carcinoma cell line (U251), Lung carcinoma cell line (H460) in this study cells were treated with Roscovitine in different concentrations ranging from 0.1 μM to 100 μM before exposure to radiation doses ranging from 0.5 Gy to 20 Gy according to each experiment. The cell viability by MTT assay, the cell cycle analysis by flow cytometry and DNA fragmentation repair mechanism by diphenylamine were measured after Roscovitine treatment with or without radiation exposure to explore the sensitization effect of Roscovitine. The present study conclude that Roscovitine a good candidate as radiosensitizer for modifying the ionizing radiation (IR) response in cancer cells, beside its cyclin dependent kinase inhibitor function, roscovitine can generate DNA Double strand Breaks and cooperate to enhance IR induce DNA damages. Roscovitine is currently in clinical trials, although our findings suggest that the combination of Roscovitine with IR appears to be a very promising especially for liver, brain and lung cancer treatment, further investigation is needed to evaluate the therapeutic index before tested in clinical trials .

INTRODUCTION

Cancer has become an important topic in medicine since it is a major cause of death in both developed and developing countries and it is secondary to that of myocardial infraction (1). Chemotherapy and radiotherapy are mainstays of cancer treatment, primarily by affecting dividing cells. In recent years the development of drugs targeting specific proteins, which interfere in signaling pathways, has advanced tremendously.

Targeted cancer therapy can be designed to interfere with a specific molecular pathway important in the genesis and/or maintenance of the malignant phenotype. Equally an improved strategy for cancer treatment would be to use small molecules to selectively differentiate cancerous cells into normal cells (2). This contrasts with traditional anti-cancer agents, which interfere with some aspect of the global cellular machinery that is shared by malignant and non-malignant cells. The term "molecular targeted" has been used to refer to agents that target pathways, which are activated in cancer cells including those regulating growth, survival and apoptosis. The promise of targeted therapy is that it will more efficiently eradicate malignant cells while leaving normal host cells largely unaffected (3, 4).

Several cancerous cells have corrupted control of the cell cycle and various Cyclin dependent Kinases (Cdks) are activated in many tumors compared to normal cells; targeting Cdks would be an intelligent strategy to block and interfere with tumor cell proliferation as an alternative to classical cytotoxic drugs (5). Cyclin dependent Kinase inhibitor are potent inhibitors of cell cycle progression that can be used to treat proliferative disorders. Cdk inhibitors may be very useful as antitumor drugs, either alone or as part of combined therapies. Purines are considered a family of Cdk inhibitors and Roscovitine is considered a member of this family, it is a potent and selective inhibitor of Cdk (6).

Immunosuppressive effect and myelotoxicity associated with purine analogs are limiting factors for the use of these agents. The treatment with purine analogs may cause high frequency of serious infections with unusual pathogens (7). Studies concerning the severe prolonged immunosuppression induced by several purine analogs in cancer patients have been reported (8). Roscovitine (olomucine related purine) has no significant immunosuppression effect (5). Several strategies to circumvent toxicity to normal tissues in cancer patients treated with chemotherapy, radiotherapy and purines analogs have been reported. Dose individualization, combination therapy are mainly used to avoid both toxicity and relapse risk (9). Little is known about the biological effects of the cyclin dependent kinase inhibitor compounds when combined with Ionizing radiation in human carcinoma. (10)

The aim of the present work was to investigate the in vitro cytotoxic activity of Roscovitine when combined with ionizing radiation against a panel of human cancer cell lines, including those of the liver (HepG2), lungs (H460) and brain (U251), in an attempt to minimize the side effects of Roscovitine and ionizing radiation, and to decrease the cancer cell ability to develop resistance for these modalities.

MATERIALS AND METHODS

Materials:

The Semi Synthetic Compound Tested (Roscovitine): **Roscovitine (CYC202):** Chemical name: 2-(R)-(1-Ethyl-2-hydroxyethylamino)-6-benzylamino-9-isopropylpurine

Human tumor cell lines:

Three human carcinoma cell line were used in this study, Hep-G2 (Liver carcinoma cell line), U251 (Brain Carcinoma cell line) and H460 (Lung carcinoma cell line). They were obtained frozen in liquid nitrogen (-180^{o}C) from the American Type Culture Collection. The tumor cell line was maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing.

Chemicals and Reagents:

All chemicals used in the present study are analytically pure and their sources and uses are displayed as mentioned in the text.

Radiation source:

Cells were treated with radiation by using **Cobalt-60** machine, at the National Cancer Institute, as a source of γ rays in a dose rate 0.5 Gy/min. Cells treated with different doses of radiation according to the cell type and the type of experiment ranging from 0.5Gy to 20Gy.

Methods:

Cells and culture conditions:

Three human carcinoma cell lines were used in this study Hep-G2, U251and H460 they are grown as monolayer culture in RPMI-1640 medium supplemented with 10% FBS and 100 units/ml penicillin and 2 mg/ml streptomycin. The cell lines were incubated at 37^{o}C in 5% CO_2-95% air in a high humidity atmosphere in the water-jacketed incubator (Revco, GS laboratory equipment, RCO 3000 TVBB, USA.). They were regularly subcultured to maintain them in the exponential growth phase. The sterile conditions were strictly attained by working under the equipped laminar flow (Micro flow Laminar flow cabinet, MDH limited, Hampshire SP 105AA, U.K.).

Anti-tumor activity against human Carcinoma cell lines:

The MTT Cell Viability Assay:

The method was carried out according to **Hansen *et al* (11) and Vistica *et al*(12).** Cytotoxicity was measured using the MTT Cell Viability Assay. MTT (3-[4,5-dimethyl thiazole-2-yl]-2,5 Diphenyl tetrazolium bromide) (Sigma, St Luis, USA), The extent of the reduction of MTT is quantified by measuring the absorbance at 570 nm **(12)**. Human carcinoma cell lines ($0.5X10^5$cells/well) in serum-free medium were plated in a flat bottom 96-well microtiter plate. U251 and HepG2 were treated with Roscovitine in different concentrations ranging from 0.1 to 100µM and with or without radiation exposure at doses of 2, 4, and 6Gy, H460 were treated with same concentration of Roscovitine and with or without radiation exposure at doses of 0.5, 1, 2, 4, and 6Gy. Cells incubated for 48 h at 37°C, in a humidified 5 % CO_2 atmosphere. After incubation, medium was removed and 50 µl MTT solution/well was added and incubated for an additional 4 h. Followed by photometric determination of the absorbance at 570 nm using microplate ELISA reader (Meter tech. Σ 960, USA). Control cells were treated with vehicle alone. Data was expressed as the percentage of relative viability compared with the untreated cells compared with the vehicle control, with cytotoxicity indicated by <100% relative viability. Percentage of relative viability was calculated using the following equation: **[Absorbance of treated cells / Absorbance of control cells] X 100.** Then the half maximal inhibitory concentration IC_{50} was calculated from the equation of the dose response curve.

Flow cytometric cell cycle analysis:

The method was carried out according to that of **Rigg et al (13) and Givan, (14).** Briefly, cell lines ($5x10^5$cells/well) were plated in 6 well microplates. Cells were collected after treatment with Roscovitine, exposed to irradiation and left for incubation as mentioned in viability assay. Then they were washed with PBS, re-suspended in 1ml of PBS, and fixed with 7 ml of ice-cold 70% ethanol, then cells were centrifuged, the ethanol was removed and washed once in PBS. The cell pellets were then re-suspended in 1 ml of PI /Triton X-100 staining solution (0.1% Triton X-100 in PBS, 0.2 mg/ml RNase A, and 10 mg/ml PI) and were incubated for 30 min. at room temperature. The stained cells were analyzed using a MoFlo flow cytometer (MoFlo, DakoCytomation, Denmark).

DNA fragmentation assay.

Fragmented double-stranded DNA can be separated from chromosomic DNA upon centrifugal sedimentation, precipitation, hydrolysis and colorimetrical quantitation upon staining with Diphenyl amine, which binds to deoxyribose. In the present study, cells were exposed to radiation at a dose of 20 Gy in case of HepG2 and U251cell lines and 10 Gy in case of H460 cell line. This high dose of radiation is used to make high percent of fragmentation to study the inhibition of DNA fragmentation repaired after treatment of cells by Roscovitine before the exposure to the radiation dose. The methods briefly, Cell lines ($5x10^5$cells/well) were plated in 6 well microplates. After treatment with Roscovitine in different concentrations (2.5, 5, 10 and 20 µM) with or without exposure to radiation dose (20Gy) in HepG2, U251 and (10Gy) in H460, cells incubated at 37°C, in a humidified 5 % CO_2 atmosphere. Then cells were collected after 1h and 24h. Cells were washed twice with ice–cold PBS then DNA fragment was determined according to methods of **Sellins and Cohen (15)** and **Taylor(16)**.

RESUITS

Cell Viability Assay

Cytotoxicity was measured by the MTT method and expressed as the survival fraction compared with untreated control cells. Three human carcinoma cell lines (H460, HepG2 & U251) were treated with Roscovitine in different concentrations ranging from 0.1 to 100 µM and revealed dramatically inhibited the cell growth in dose dependent manner (Table 1).

Table 1: Different cancer cell lines are represented for their mean % survival compared to untreated controls in presence of different drug concentrations

Cell line Dose (µ M/ml)	HepG2	U251	H640
	Mean % Survival ±SE		
0.1	74.6±4.3	98.2±4.1	95.7± 7.1
1.0	77.1±9.8	90.8±3.8	90.6±13.4
2.5	65.9±2.0	86.2±8.6	84.2±8.3
5.0	61.7±7.6	94.9±7.9	78.0±10.2
10.0	55.1±4.9	71.5±4.5[a]	70.4±5.6
20.0	35.0±8.5[a]	36.0±3.1[b]	32.0±3.5[b]
50.0	2.4±2.7[c]	00.0±0.0[c]	00.0±0.0[c]
100.0	0.6±1.8[c]	00.0±0.0[c]	00.0±0.0[c]

**Mean of three repeated experiments.*
Significant change from untreated corresponding contro a ($p<0.05$)
b at ($p<0.01$) and c at ($p< 0.001$).

From the results given in **Figures 1**, we find that the effect of radiation increased when combined with Roscovitine doses in the **HepG2** cell line, the viability of the cells were significantly lower in doses ranging from 0.1-20 µM when compared to the other cell lines radiated without Roscovitine**,**

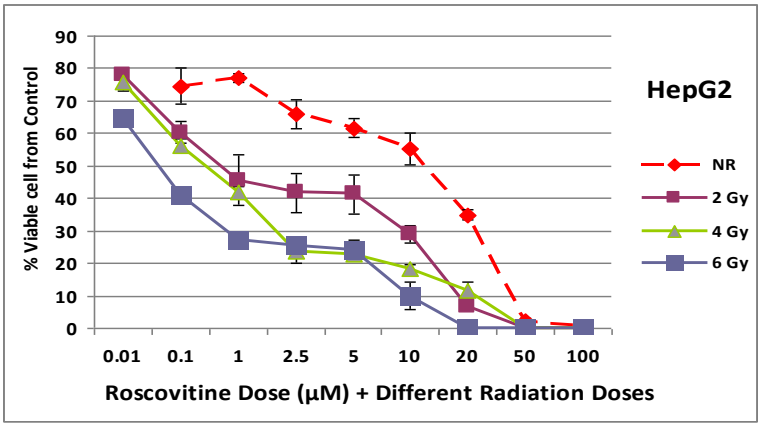

Fig 2: Cell survival experiments represent relative mean % viability of HepG2 cell line when treated with Roscovitine alone and different radiation doses.

From the results given in **figure 2,** the effect of radiation increased when combined with Roscovitine doses in the **U251** cell line, the viability of the cells were significantly lower in doses ranging from 0.1-20 µM when compared to the other cell lines radiated without Roscovitine.

From the results given in **Figure 3,** the effect of radiation at the lowest dose (2Gy) on cell viability was significantly decrease in **H460** cell line for this result we treated H460 with 0.5,1 Gy to measure the sensitization effect Roscovitine, in H460 cells more sensitive to radiation doses when compared with HepG2 and U251cells lines. The viability of the cells was significantly lower in doses ranging from 0.1-20 µM when compared to the other cell lines radiated without Roscovitine.

For example 2Gy gave 95% viable cells but when combined with 5 µM Roscovitine gave 45% viable cells in case of HepG-2 cell line **(Fig 1)** and depressed from 95% to 45% when U251 cell line treated with 5 µM pre exposed to 2 Gy **(Fig 2**), while, H460 cell line needed to 0.1 µM of Roscovitine concentration pre-irradiation to decrease the viability percent from 83.4 to 68% **(Fig 3).** Thus Roscovitine is considered good candidate as Radiosensitizer in U251 cell line. As shown in figure1, 2 and 3 Roscovitine is considered a good candidate as Radiosensitizer in HepG2, U251 and H460 cell lines. IC50 of different radiation doses were showed in **Table 2.**

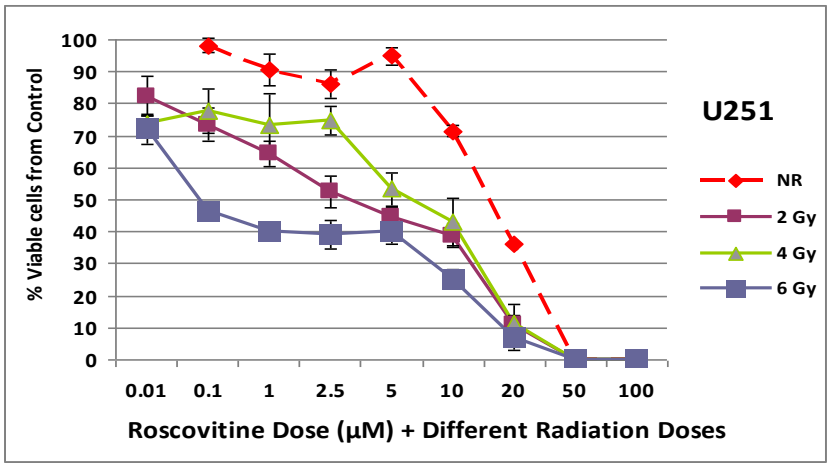

Fig 3: Cell survival experiments represent relative mean % viability of U251 cell line when treated with Roscovitine alone and different radiation doses.

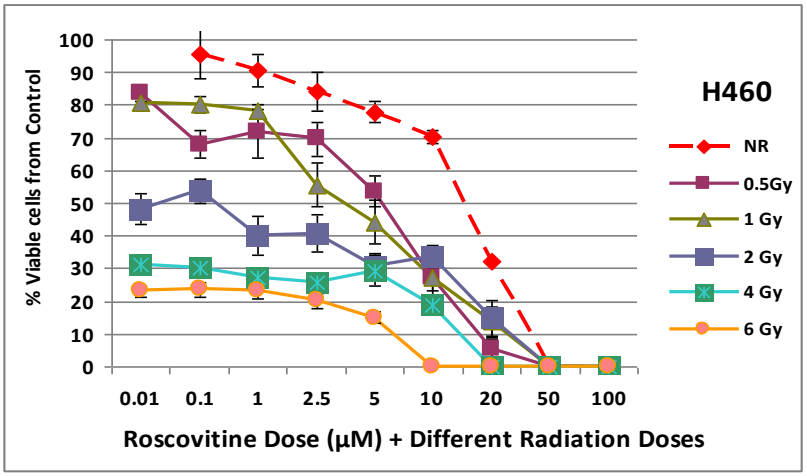

Fig 3.2: Cell survival experiments represent relative mean % viability of H460 cell line when treated with Roscovitine alone and different radiation doses.

Table 2 : The IC50 values for different treatment on the three carcinoma cell lines.

Type of treatment	HepG2	U251	H460
Drug alone	9.00 µM	--------	15.0 µM
Drug+0.5 Gy	----------	--------	3.75 µM
Drug+1 Gy	----------	--------	5.00 µM
Drug+2 Gy	0.70 µM	2.5 µM	0.2 0µM
Drug+ 4 Gy	0.50 µM	5.0 µM	---------
Drug+ 6 Gy	0.05 µM	0.1 µM	----------

2) Flow cytometric cell cycle analysis

To investigate further the nature of growth inhibition by Roscovitine and radiation in HepG2, U251 and H460 cell lines, flow cytometric DNA ploidy analysis was performed. Histogram of DNA per cell was obtained for each treated specimen and represented in the following tables as % of cells in each phase of the cell cycle, flow cytometric DNA analysis is performed to determine the presence of diploidy cells in a population, and to determine the percentage of cells in each phase of the cell cycle and estimate the growth fraction of the population.

From the results given in **Table 2**, we find that the different drug doses and radiation doses had no effect in diploid cell phases in HepG2. None of the cells (0%) was found in the S phase. In the mean time 67.2% of cells were in the G2/M phase, which was double the number of those cells in the G0/G1 phase (33.6%). The remaining cells were found in the G2/G1 phase (<2%). These results indicate that this type of treatment; whether Roscovitine alone or with radiation exposure, had no effect on the diploid tumor cells. Applying these experiments on the other cell lines (H460 & U251) gave the same results, thus they were not illustrated.

Table 3: Cell cycle modification for human carcinoma cell lines (H460) in untreated specimen (control), different drug doses, and radiation dose 2Gy in diploid cells.

Cell line types	HepG-2			
Groups	G0/G1	S	G2/M	G2/G1
Control	33.60±2.4	0	67.20±1.2	1.67±0.060
5 µM	32.98±3.1	0	65.95±2.1	1.06±0.050[b]
10 µM	32.76±1.4	0	65.52±2.1	1.72±0.030
20 µM	32.81±3.3	0	65.63±3.4	1.60±0.010
2Gy	32.77±3.1	0	65.55±5.1	1.70±0.010
5µM+2Gy	32.97±2.1	0	65.80±2.9	1.00±0.003[b]
10µM+2Gy	33.05±2.5	0	66.10±2.9	0.90±0.001[b]
20µM+2Gy	32.90±1.1	0	65.85±3.7	1.00±0.001[b]

**Mean of three repeated experiments.*

Significant change from untreated corresponding control b at (p<0.01).

Changes in the cell cycle phases of the aneuploid cells of HepG2 after different treatments are given in **Tables (3).** From the results**,** it was found that 25% of the untreated HepG2 (control) cells were found in the G0/G1 phase. Treatment of cells with roscovitine alone caused an increase in the number of cells at the G0/G1phase, thus increase was correlated with the drug dose reaching 33.2% at 20µM Roscovitine.

The radiosensitizing effect of the drug was furtherly observed where 2Gy radiation gave 26.5% of the cells in the G0/G1 phase, by combining different doses of Roscovitine with radiation dose 2Gy the % of cells in the G0/G1 increased giving maximum value at 20µM Roscovitine and 2Gy (37%), this increase in the G0/G1 phase was accompanied by a decrease in the S phase, starting with 28% of cells in the S phase for control, reaching 3.4% for cells treated with 20µM and 2Gy. In the mean time % of cells found in the G2/M phase increased in a non- homogenous way giving different patterns with different Roscovitine concentration.

Combining of Roscovitine with 4Gy and 6Gy radiation gave a similar pattern as that found with 2Gy, experiment showing a remarkable arrest at the G2/M and decrease in the S phase cells reaching 0% for cells treated with 10, or 20 µM Roscovitine and 4Gy, or 6Gy (**Tables 3**).

From the results given in **Tables (4) also**, it was found that 18.7% of the untreated **U251** (control) cells were found in the G0/G1 phase. Treatment of cells with roscovitine alone caused an increase in the number of cells at the G0/G1phase, thus increase was correlated with the drug dose reaching 26 % at 20µM Roscovitine. The radiosensitizing effect of the drug was furtherly observed where 2Gy radiation gave 14.5% of the cells in the G0/G1 phase, by combining different doses of Roscovitine with radiation dose 2Gy the % of cells in the G0/G1 increased giving maximum value at 20µM Roscovitine and 2Gy (36 %), this increase in the G0/G1 phase was accompanied by a decrease in the S phase, starting with 33.2% of cells in the S phase for control, reaching 11.3% for cells treated with 5µM and 2Gy. In the mean time % of cells found in the G2/M phase increased in a non-homogenous way giving different patterns with different Roscovitine concentration. Combining of Roscovitine with 4Gy and 6Gy radiation gave a similar pattern as that found with 2Gy, experiment showing a remarkable arrest at the G2/M and decrease in the S phase cells reaching 0% for cells treated with 10, or 20 µM Roscovitine and 4Gy, or 6Gy

Changes in the cell cycle phases of the aneuploid cells of H460 after different treatments are given in **Tables (5).** it was found that 28% of the untreated **U251** (control) cells were found in the G0/G1 phase. Treatment of cells with roscovitine alone caused an increase in the number of cells at the G0/G1phase, thus increase was correlated with the drug dose reaching 33.5 % at 20µM Roscovitine. The radiosensitizing effect of the drug was furtherly observed where 0.5Gy radiation gave 30.6% of the cells in the G0/G1 phase, by combining different doses of Roscovitine with radiation dose 0.5Gy the % of cells in the G0/G1 increased giving maximum value at 20µM Roscovitine and 0.5Gy (37 %), this increase in the G0/G1 phase was accompanied by a decrease in the S phase, starting with 21.5% of cells in the S phase for control, reaching 0.5% for cells treated with 5µM and 0.5Gy. In the mean time % of cells found in the G2/M phase increased in a non-homogenous way giving different patterns with different Roscovitine concentration. Combining of Roscovitine with 1Gy and 2Gy radiation gave a similar pattern as that found with 0.5Gy, experiment showing a remarkable arrest at the G2/M and decrease in the S phase cells reaching 0% for cells treated with 5,10, or 20 µM Roscovitine and 1Gy, or 2Gy.

Table 4: Cell cycle modification for human carcinoma cell lines (HepG2) & (U251) in untreated specimen (control), different drug doses, and radiation dose 2Gy in aneuploid cells.

Cell line types	HepG-2				U251			
Groups	G0/G1	S	G2/M	G2/G1	G0/G1	S	G2/M	G2/G1
Control	25.0±1.1	27.97±2.3	46.23±3.9	0.8±0.01	18.7±0.9	33.2±1.4	46.5±2.1	1.5±0.02
5 µM	26.31±2.2	28.39±2.1	43.88±3.2	1±0.01^{c}	20.3±1.9	33.5±4.1	45.0±3.7	0.85±0.01^{c}
10 µM	30.19±1.2^{a}	17.8±2.1^{a}	50.9±2.9	1±0.02^{b}	20.7±1.7	34.4±2.3	43.7±3.3	1.0±0.01^{c}
20 µM	33.17±1.1^{a}	19.1±1.6	46.9±3.2	0.8±0.01	26.1±1.7^{a}	33.5±2.4	39.3±3.1	0.85±0.01^{c}
2Gy	26.5±2.2	14.3±1.6^{a}	58.5±2.3	0.6±0.02^{b}	14.5±2.1	34.8±2.6^{b}	49.6±3.6	0.9±0.01^{c}
5µM+2Gy	35.3±3.4	12.5±1.4^{b}	51.3±3.1	0.7±0.01^{b}	24.6±2.4	11.3±3.2^{a}	61.3±3.1^{a}	2.4±0.02^{c}
10µM+2Gy	33.8±1.1^{a}	7.1±0.4^{b}	58.19±5.1	0.8±0.01	35.8±1.2^{b}	12.2±4.1^{b}	50.1±2.9	1.8±0.02^{b}
20µM+2Gy	36.96±1.4^{b}	5.39±0.1^{b}	56.8±3.4	0.8±0.01	35.7±2.4^{b}	12.9±1.1	49.6±2.6	1.7±0.01^{c}
4Gy	34.31±1.5^{a}	14.88±1.5^{a}	50.16±2.3	0.6±0.01^{c}	21.3±3.1	36.0±2.3	41.4±5.1	1.1±0.01^{a}
5µM+4Gy	35.1±2.1^{a}	0.067±0.01^{c}	57.3±4.9	0.8±0.01	21.89±3.2	30.5±1.9^{c}	46.0±4.2	1.5±0.02
10µM+4Gy	39.7±2.5^{a}	0.0±0.0^{c}	59.2±4.9	0.9±0.02^{a}	35.0±2.5^{b}	0.0±0.0^{c}	62.7±5.1	1.9±0.03^{b}
20µM+4Gy	38.4±2.3^{a}	0.0±0.0^{c}	60.8±5.1	0.8±0.01	36.0±3.4^{a}	0.0±0.0^{c}	62.2±2.6^{a}	1.7±0.04^{a}
6Gy	31.68±3.6	13.4±1.5^{a}	52.27±3.6	2.6±0.04^{c}	23.8±2.7	19.7±1.3^{b}	55.5±5.3	0.9±0.02^{c}
5µM+6Gy	38.3±1.0^{b}	1.57±0.03^{b}	59.4±4.1	0.6±0.02^{b}	33.3±1.9^{b}	11.9±1.1^{b}	53.8±4.3	0.9±0.01^{c}
10µM+6Gy	40±1.1^{b}	0.0±0.0^{c}	59.1±1.9	0.9±0.03	42.8±4.6^{a}	0.0±0.0^{c}	56.5±4.6	0.7±0.01^{c}
20µM+6Gy	37± 3.2^{a}	0.0±0.0^{c}	61.9±5.1	1.1±0.05^{a}	47.0±2.5^{b}	0.0±0.0	52.3±2.9	0.7±0.01^{c}

**Mean of three repeated experiments.*
Significant change from untreated corresponding control, a ($p<0.05$) b at $p<0.01$) and c at ($p< 0.001$).

Table 5: Cell cycle modification for Brain carcinoma cell line (H460) in untreated specimen (control), different drug doses, and radiation dose 2Gy in aneuploid cells.

Cell cycle phases / Groups	*G0/G1*	*S*	*G2/M*	*G2/G1*
Control	28±3.4	21.5±2.1	49.5±2.6	0.95±0.01
5 µM	28±2.1	21.9±2.2	49.3±3.4	0.80±0.01[b]
10 µM	29.4±1.4	23.3±2.7	46.5±2.8	0.70±0.01[c]
20 µM	33.5±2.1	19.3±2.3	46.2±3.9	0.96±0.03
0.5Gy	30.6±2.1	7.90±1.7	60.7±4.8	0.75±0.02[b]
5µM+0.5Gy	30.1±2.3	0.5±0.01[a]	68.3±4.6[a]	1.0±0.01[a]
10µM+0.5Gy	35.7±3.6	0.66±0.01[b]	62.8±5.1	0.80±0.02[b]
20µM+0.5Gy	37.3±2.9	0.80±0.01[b]	60.7±6.1	0.80±0.03[a]
1Gy	35.2±3.8	6.2±0.8[b]	57.7±2.9	0.80±0.02[a]
5µM+1Gy	38.9±3.2	0.0± 0.0[b]	59.6±3.9	0.80±0.01[b]
10µM+1Gy	38.2±4.2	0.8±0.01[b]	59.9±5.2	0.80±0.03[a]
20µM+1Gy	36.8±2.1	1.4±0.12[b]	60.6±4.1	0.80±0.04[a]
2Gy	33.9±2.7	6.7±0.34[b]	58.6±3.9	0.80±0.01[b]
5µM+2Gy	37.7±2.5	0.0±0.0[b]	61.5±5.1	0.80±0.02
10µM+2Gy	36.8±4.2	0.0±0.0[b]	62.3±3.7	0.87±0.03[b]
20µM+2Gy	40.5±2.1	0.0±0.0[b]	58.5±4.9	0.80±0.02

Mean of three repeated experiments.

Significant change from untreated corresponding control a ($p<0.05$) b at $p<0.01$) and c at ($p< 0.001$).

DNA index

The DNA content of cells is measured by the ability of propidium iodide to bind to DNA under appropriate staining conditions. DNA index (DI) defined as the difference in DNA content ratio of tumor/standard DNA fluorescence. An increase in DNA index has long been known to be associated with malignancy. Aneuploid carcinomas tended to have lower DI whereas aneuploid populations within multiploid carcinomas tended to have higher DI.

From the results given in **Table 6,** we find that the combination of radiation and roscovitine caused significant decrease in the DNA index when compared with cells treated with radiation alone in HepG2, U251 and H460 cells line.

One tumor characteristic that might help both the understanding of the fundamental aspects of tumor growth and the selection of best treatment is tumor ploidy. Ploidy refers to the chromosomal organization of cells. In normal human cells, the chromosomes are organized in pairs; this is called diploid. Any other organization is abnormal, and is called aneuploid. Evidence is accumulating to indicate that for many types of cancer, aneuploid cells indicate a more aggressive tumor and a poorer prognosis.

Table 6: DNA index modification for human carcinoma cell lines (HepG2), (U251) and (H460) in untreated specimen (control), different drug doses, and radiation doses.

Types of cell line	HepG2	U251	H460
Drug & radiation dose	DNA index		
Control	1.60±0.03	1.90±0.04	20±0.03
5 µM	1.70±0.05	1.40±0.02[b]	1.9±0.04
10 µM	1.60±0.03	1.07±0.02[c]	1.7±0.05[a]
20 µM	1.13±0.02[c]	1.23±0.04[b]	1.7±0.04[b]
2Gy	1.80±0.04[a]	1.30±0.02[c]	1.2±0.01[c]
5µM+2Gy	1.10±0.01[c]	0.70±0.02[c]	1.4±0.02[c]
10µM+2Gy	1.00±0.03[c]	0.80±0.01[c]	1.3±0.04[c]
20µM+2Gy	0.86±0.01[c]	0.74±0.01[c]	1.2±0.03[c]
4Gy	1.67±0.02	1.04±0.03[c]	1.6±0.03[b]
5µM+4Gy	1.13±0.01[c]	0.70±0.01[c]	1.3±0.01[c]
10µM+4Gy	0.80±0.01[c]	0.80±0.02[c]	1.2±0.02[c]
20µM+4Gy	0.80±0.02[c]	0.70±0.02[c]	1.0±0.01[c]
6Gy	1.19±0.03[b]	1.70±0.03[a]	1.6±0.01[b]
5µM+6Gy	1.20±0.04[b]	1.60±0.03[b]	0.9±0.01[c]
10µM+6Gy	0.90±0.02[c]	1.32±0.04[b]	0.8±0.01[c]
20µM+6Gy	0.66±0.01	0.6±0.01[c]	1.0±0.02[c]

**Mean of three repeated experiments.*

Significant change from untreated corresponding control a ($p<0.05$) b at ($p<0.01$) and c at ($p< 0.001$).

The rate of change in the number of aneuploid cells to diploid cells in **HepG2** cell line after treatment with different doses of roscovitine alone or combined with different doses of Radiation was determined by flow cytometrical analysis, results are given in **Table 7**

From the results given in **Table 7**, we find that at the beginning of the experiment the untreated **HepG2** cells (control) showed a 97.7% aneuploidy with only 2.3% diploidy. Roscovitine alone caused nearly 11% changes with the 20µM Roscovitine dose. The radiosensitizing effect of the drug was furtherly demonstrated where cells treated with maximum dose of Roscovitine and radiation revealed only 38.9% aneuploidy with an increase in the number of diploid cells reaching to 61%. Results revealed that the rate of change from aneuploid to diploid increased with the increase in Roscovitine doses combined with increase in radiation doses. The rate of change in the number of aneuploid cells to diploid cells in U251 cell line after treatment with different doses of roscovitine alone or combined with different doses of Radiation was determined by flow cytometrical analysis, results are given in **Table 7**.

Table 7: Changes in the aneuploid cells to diploid ones after treatment of human carcinoma cell lines, (HepG2), (U251) and (H460) with different doses of Roscovitine& radiation alone or combined.

Types of cell lines	*(HepG2)*		*(U251)*		*(H460)*	
Concentration of Drug & Radiation	*% of cells*		*% of cells*		*% of cells*	
	Diploid	*aneuploid*	*Diploid*	*Aneuploid*	*Diploid*	*aneuploid*
Control	2.27±0.01	97.73±5.1	4.0±0.02^{c}	96.0±4.0	3.69±0.01	96.31±6.9
5 µM	3.18±0.01^{c}	96.82±5.2	7.3±0.02^{c}	92.7±4.3	10.8±0.20^{c}	89.2±5.8
10 µM	4.79±0.02^{c}	95.21±3.8	8.1±0.01^{c}	91.9±4.9	12.1±0.31^{c}	87.9±3.9
20 µM	13.96±0.12^{c}	86.04±5.2	10.3±0.03^{c}	89.68±3.2	15.5±0.22^{c}	74.5±4.6
2Gy	8.1±0.31^{c}	91.9±7.1	11.1±0.11^{c}	88.9±5.2	4.79±0.02^{a}	95.21±3.8
5µM+2Gy	10.8±0.42^{c}	89.2±8.1	17.1±0.98^{c}	82.9±4.2	22.51±0.42^{c}	77.49±4.4
10µM+2Gy	17.03±0.90^{c}	82.97±3.2	22.8±0.13^{c}	77.2±4.7^{a}	30.73±0.78^{c}	69.27±3.2^{a}
20µM+2Gy	22.51±0.99^{c}	77.49±6.1	28.1±0.51^{c}	71.9±3.9	40.89±1.9^{c}	59.11±2.9^{a}
4Gy	12.5±0.43^{c}	87.5±5.8	14.05±0.21^{b}	85.95±4.1^{a}	20.1±2.1^{a}	79.9±2.8
5µM+4Gy	15.0±0.21^{b}	85.0±5.6^{a}	30.5±2.80^{b}	69.5±4.1^{b}	30.5±2.3^{b}	69.5±4.8^{b}
10µM+4Gy	25.0±3.40^{b}	75.0±4.3^{a}	40.1±3.1^{b}	59.9±3.8^{b}	45.0±3.2^{c}	55.0±3.4^{b}
20µM+4Gy	30.73±2.51^{b}	69.27±3.1^{a}	46.3±3.6^{a}	53.7±3.7^{a}	55.2±3.8^{c}	44.8±4.4^{b}
6Gy	24.0±2.11^{b}	76.0±3.6^{a}	20.5±3.1^{c}	79.5±3.2^{b}	40.0±3.1^{b}	60.0±4.8^{b}
5µM+6Gy	40.7±2.12^{c}	59.3±2.4^{b}	42.7±2.5^{c}	57.3±5.1^{b}	58.03±2.9^{c}	41.97±4.0^{b}
10µM+6Gy	55.83±3.11^{c}	44.17±3.1^{c}	53.87±2.4^{c}	46.13±2.2^{b}	64.5±3.9^{c}	35.5±3.3^{b}
20µM+6Gy	61.06±3.45^{c}	38.94±2.2^{c}	59.06±5.3^{b}	40.94±2.1^{b}	71.5±4.5^{c}	28.5±2.1^{b}

**Mean of three repeated experiments.*
Significant change from untreated corresponding control, a ($p<0.05$) b at $p<0.01$) and c at ($p< 0.001$).

From the results given in **table 7**, we find that at the beginning of the experiment the untreated **U251** cells (control) showed a 96.0% aneuploidy with only 4.0% diploidy. Roscovitine alone caused nearly 6% changes with the 20µM Roscovitine dose. The radiosensitizing effect of the drug was furtherly demonstrated where cells treated with maximum dose of Roscovitine and radiation revealed only 40.9% aneuploidy with an increase in the number of diploid cells reaching to 59.1%. Results revealed that the rate of change from aneuploid to diploid increased with the increase in Roscovitine doses combined with increase in radiation doses. The rate of change in the number of aneuploid cells to diploid cells in **H460** cell line after treatment with different doses of Roscovitine alone or combined with different doses of Radiation was determined by flow cytometrical analysis, results are given in **Table 7**

From the results given in **table 7**, we find that at the beginning of the experiment the untreated **U251** cells (control) showed a 96.0% aneuploidy with only 4.0% diploidy. Roscovitine alone caused nearly 6% changes with the 20µM Roscovitine dose. The radiosensitizing effect of the drug was furtherly demonstrated where cells treated with maximum dose of Roscovitine and radiation revealed only 40.9% aneuploidy with an increase in the number of diploid cells reaching to 59.1%.

Results revealed that the rate of change from aneuploid to diploid increased with the increase in Roscovitine doses combined with increase in radiation doses.

The rate of change in the number of aneuploid cells to diploid cells in H460 cell line after treatment with different doses of Roscovitine alone or combined with different doses of Radiation was determined by flow cytometrical analysis, results are given in **Table 7.**

3)DNA Fragmentation Assay.

Cancer cells are capable to repair DNA fragmentation, a process that enables them to escape apoptosis and prolong their survival .Treatment of cells with Ionizing radiation can lead to the creation of Double-strand breaks. Double-strand breaks are often rapidly repaired by a simple mechanism. In this experiment we examined the radiosensitizing effect of Roscovitine for intensifying the radiation effect by inhibiting the DNA repair mechanism for the different cancer cell lines.

Cells were treated with Roscovitine doses (2.5, 5, 10, and 20 μM) before its exposure to radiation dose (20 Gy) in **HepG2** and **U251**, and (10Gy) in **H460** and incubated for 1hr and 24 hrs, and then subjected to diphenylamine (DPA) colorimetric assay to evaluate the % of fragmentation repair.

From the results given in **Tables (8),** we find that the combination of radiation and roscovitine was caused an intense decrease in DNA fragmentation repair mechanism when compared with cells radiated alone without Roscovitine in HepG2 cells, 24hrs After exposure of cells to radiation 70% of fragmented DNA repaired in the absence of Roscovitine, in the presence of roscovitine <35% was repaired.

From the results given in **Tables (8),** we find that the combination of radiation and roscovitine was significantly decrease DNA fragmentation repair mechanism when compared with cells radiated alone without Roscovitine in H460 cells, 24hrs After exposure of cells to radiation 66% of fragmented DNA repaired in the absence of Roscovitine, in the presence of roscovitine <39% was repaired. The best effect (highest % of DNA fragmentation) was observed in the group of H460 cells treated with the highest dose (20μM**)** of roscovitine and radiation after 1hr, descending with the decrease in roscovitine doses.

From the results given in **Tables (8)** we find that the combination of radiation and roscovitine was caused an intense decrease DNA fragmentation repair mechanism when compared with cells radiated alone without Roscovitine in **U251** cells, 24hrs After exposure of cells to radiation 60% of fragmented DNA repaired in the abscence of Roscovitine, in the presence of roscovitine <32% was repaired

The best effect (highest % of DNA fragmentation) was observed in the group of **U251** cells treated with the highest dose (20μM) of roscovitine and radiation after 1hr, descending with the decrease in roscovitine doses.

Table 8: % of DNA fragmentation in human carcinoma cell line HepG2, U251 and H460 in control cells and treated cells with 20Gy radiation dose and 2.5µM Roscovitine dose

Type of treatment % of DNA fragmentation (Mean ±S.E)			
Types of cell line	HepG2	U251	H460
Groups	Dose of Roscovitine (2.5 µ M)		
Control	5.2 ±0.26	2.5 ±0.15	3.1 ±0.13
2.5µM	8.8 ±0.79^{c}	4.6 ±1.6	10.5 ±1.5^{a}
Rad after 1h	59.5 ±3.0^{c}	40.3 ±1.1^{c}	70.7 ±6.0^{b}
Rad after 24h	18.1 ±2.8^{c}	16.0 ±1.9^{b}	23.0 ±3.8^{a}
Rad after 1h +2.5µM	62.2 ±3.3^{c}	45.1 ±1.0^{c}	73.8 ±4.9^{c}
Rad after 24h +2.5µM	40.8 ±5.2^{c}	31.4 ±4.0^{b}	45.3 ±4.8^{b}
	Dose of Roscovitine (5 µ M)		
Control	5.2 ±0.26	2.5 ±0.15	3.1 ±0.13
5µM	9.1 ±0.55^{c}	6.5 ±1.4	13.7 ±1.4
Radiation after 1h	59.5 ±3.0^{c}	40.3 ±1.1^{c}	70.7 ±6.0^{c}
Radiation after 24h	18.1 ±2.8^{c}	16.0 ±1.9^{b}	23.0 ±3.8
Rad after 1h +5 µM	66.8 ±2.5^{c}	50.5 ±2.9^{c}	78.1 ±6.7^{c}
Rad after 24h + 5µM	52.3 ±6.7^{c}	43.4 ±4.3^{b}	60.6 ±3.0^{c}
	Dose of Roscovitine (10 µ M)		
Control	5.2 ±0.26	2.5 ±0.15	3.1 ±0.13
5µM	11.1±0.9^{a}	7.7 ±1.7	14.5 ±3.0
Radiation after 1h	59.5 ±3.0^{a}	40.3 ±1.1^{c}	70.7 ±6.0^{c}
Radiation after 24h	18.1 ±2.8^{a}	16.0 ±1.9^{b}	23.0 ±3.8
Rad after 1h +10 µM	68.3±3.04^{a}	46.8 ±1.5^{c}	81.5 ±5.2^{c}
Rad after 24h + 10µM	57.7±3.0^{a}	40.1 ±1.2^{c}	70.9 ±7.0^{c}
	Dose of Roscovitine (20 µ M)		
Control	5.2 ±0.26	2.5 ±0.15	3.1 ±0.13
5µM	18.1±1.1^{a}	14.4 ±2.9^{a}	18.1 ±2.7
Radiation after 1h	59.5 ±3.0^{a}	40.3 ±1.1^{c}	70.7 ±6.0^{b}
Radiation after 24h	18.1 ±2.8^{a}	16.0 ±1.9^{b}	23.0 ±3.8^{c}
Rad after 1h +10 µM	71.3±4.9^{a}	63.3 ±1.6^{c}	83.4 ±6.6^{c}
Rad after 24h + 10µM	63.8±6.7^{a}	58.3 ±3.0^{c}	70.3 ±7.8^{c}

**Mean of three repeated experiments.*

Significant change from untreated corresponding control, a ($p<0.05$) b at $p<0.01$) and c at ($p< 0.001$).

From the above results we find that the inhibitory effect of radiation observed after 1hr was more potent than that obtained after 24hrs for all cell lines. We also found that the effect was correlated with roscovitine dose. **H460** cell line was considered more sensitive for this modality of treatment (reaching 83.4% fragmentation with 10Gy) than **HepG2** followed by the **U251** (reaching 71.3% & 63.3 fragmentation respectively with 20 Gy).

The best effect (highest % of DNA fragmentation) was observed in the group of **HepG2** cells treated with the highest dose (20µM**)** of roscovitine and radiation after 1hr, descending with the decrease in roscovitine doses.

DISCUSSION:

One strategy in the development of anticancer therapeutics has been to arrest malignant proliferation through inhibition of the enzymatic activity of cyclin-dependent kinases (Cdks), which are key regulatory molecules of the cell cycle **(3)**. This generated a new category of compounds named small molecule Cdk inhibitors, which can directly antagonize the action of Cdks **(17)**. However, several cyclin-CDK complexes have been found to be dispensable for cell proliferation owing to functional redundancy, promiscuity, and compensatory mechanisms. Although these issues have hampered their progress into the clinic, several novel compounds are currently being preclinically and clinically evaluated but have not as of yet resulted in a drug approval **(18)**.

Compounds that inhibit Cdk activity and are currently in clinical trials, include flavopiridol, *R*-Roscovitine (CYC-202), UCN-01 (7-hydroxystaurosporine).

Purines are considered a family of Cdk inhibitors and Roscovitine is considered a member of this family, it is a potent and selective inhibitor of Cdk **(6)**. Immunosuppressive effect and myelotoxicity associated with purine analogs are limiting factors for the use of these agents **(7, 8).**

Although Roscovitine has no significant immuno-suppression effect **(5)**, dose individualization, and combination therapy are mainly used to avoid both toxicity and relapse risk **(9)**. In the mean time little is known about the biological effect of Roscovitine when combined with ionizing radiation in human carcinomas **(10)**.

The aim of the present work was to investigate the in vitro cytotoxic activity of Roscovitine when combined with ionizing radiation against a panel of human cancer cell lines, including those of the liver (HepG2), lungs (H460) and brain (U251).

The HepG2 cell line was chosen as a model of hepatocellular carcinoma (HCC). Hepatocellular carcinoma is the third most common cause of cancer death in the world **(19)**. In Egypt; recent studies reported that the burden of HCC has been increasing with a doubling in the incidence rate in the past 10 years **(20, 21)**. This has been attributed to several biological; e.g. hepatitis B and C virus infection **(22, 23)** and environmental factors; e.g. aflatoxin, AF **(24)**.

The second carcinoma cell line chosen for the present study was the lung H460 cell line. The growth of lung cancer may lead to metastasis, invasion of adjacent tissue and infiltration beyond the lungs. The vast majority of primary lung cancers are carcinomas of the lung, derived from epithelial cells. In epithelial cells, the major regulatory checkpoint is the transition from G_1 to the S phase. This transition is characterized by the phosphorylation of retinoblastoma protein (pRb), and is catalyzed by the Cdk4-D1 enzyme complex **(25)**.

Brain carcinoma cell line (U251), was the third line used in the present study. Brain tumors are the leading cause of cancer mortality in children and remain difficult to cure despite advances in surgery and adjuvant therapy **(26).** Despite the potential importance of the cell cycle and apoptosis pathways in brain tumor etiology, little has been published regarding brain tumor risk associated with common gene variants in these pathways **(27).**

In the present study, Roscovitine inhibited cell proliferation of the three carcinoma cell lines tested (HepG2, H460 and U251), in a dose response curve, which revealed IC50 values for HepG2, H460, and U251 of 9, 15 and 16 µM respectively. The maximum effect was found on the HepG2 cell line at the minimum doses when compared to the other cell lines ($p<0.001$). However at the higher doses the three cell lines gave indistinguishable results. The effect of Roscovitine on radiated cells was more intense. Similar results were reported in the literature for different tumors with diverse interpretations. **Meijer *et al*. (28)** revealed that Roscovitine inhibited the kinase activities of Cdc2/cyclin B, Cdk2/cyclin A, and Cdk2/cyclin E complexes, mutually with the proliferative activities of human breast epithelial cells, lung cancer cells, and gastric cancer cells. They stated that micromolar concentrations of Roscovitine were able to prevent the cell cycle progressions.

Similarly, **Mihara and his coworker (6)** found that Roscovitine inhibited the cell proliferation rate in a dose dependent manner in head and neck squamous cell carcinoma cell lines, Likewise, **Wu et al.,(29)** reported that Roscovitine inhibited proliferation of rabbit retinal pigment epithelial cells in a dose-dependent manner.

In the present study, ionizing Radiation inhibited cell proliferation of all carcinoma cell lines tested. Similar results were given by **Vucic *et al*. (30)**, who recorded that cell growth was significantly reduced after exposure to ionizing Radiation treatment and this reduction was time and dose dependent**.**

The radiosensitizing effect of Roscovitine was verified in the present work, where the combination of minimally toxic concentrations of both Roscovitine and ionizing radiation significantly inhibited the cell proliferation when compared to ionizing radiation alone.

Our results are in agreement with **Maggiorella *et al.* (31)** who reported that at low doses of Roscovitine, a potentialization in the inhibition of cell proliferation was found when it was added to ionizing Radiation. Equally, **Zhang and his coworker (10)** found that pretreatment with minimally toxic concentration of Roscovitine significantly radiosensitized human non-small cell lung cancer cells by inhibiting colony formation.

Tumor ploidy is a tumor characteristic that might help both the understanding of the fundamental aspects of its growth and the selection of best treatment **(32)**. Polyploidy, an increased number of chromosome sets, is common phenomenon in nature. In humans, polyploidy often occurs in specific tissues as part of terminal differentiation.

The exact role of aneuploidy in tumorigenesis is still not clear **(33)**, where it has long been debated, whether aneuploidy directly contributes to tumorigenesis or reflects nonspecific changes during tumor progression. However, **Weaver and Cleveland (34)** found that aneuploidy promotes tumorigenesis in some contexts and inhibits it in others.

Several mechanisms are thought to be responsible for the generation of aneuploid sets of chromosomes: these comprise failure in cell division, such as defective chromosome separation caused

by compromised mitotic checkpoint signaling or centrosome aberrations **(35)**. More than a few reports have shown that cells with a defective mitotic checkpoint are more resistant to several types of anticancer drugs, and that the specific physiological changes that are triggered by polyploidization might be used as novel targets for cancer therapy **(36)**.

In the present work, all of the three cell lines investigated were aneuploid (0% diploid cells) before treatment. On treatment with Roscovitine and/or radiation the percentage of diploid cells increased accompanied by a drop in the percentage of aneuploid cells, indicating that cells began to undergo differentiation and behave as normal cells **(32)**.

Checkpoints are the pathways that halt the progression of cell cycle in response to cellular stress. The targets on checkpoint pathways are potential anticancer strategies because abrogation of checkpoint function drives tumor cells toward apoptosis and enhances the efficacy of oncotherapy. Several cellular stresses may trigger checkpoint pathways, leading to cell-cycle arrest at G_1 and G_2 phase **(37)**.

In the present study, Roscovitine significantly retarded the growth of the three carcinoma cell lines; HepG2, U251, and H460 cells, which was mainly due to cell cycle arrest at the G1 and G2 phases, as perceived from the flow cytometric charts. A decrease in the S phase population was observed, indicating that the cells were prevented from entering the S phase. On the other hand, inactivation of cyclin kinases by Roscovitine led to accumulation of the cells in the G2/M phase of the cell cycle.

Similar observations were given by **Vucic *et al.* (30)** and **Wu *et al*. (29).** The present results also concur with a number of studies on different tumors with assorted interpretations.

Ljungman *et al.* (38) postulated that Roscovitine induces the accumulation of the tumor suppressor p53, to arrest cells in the G1and G2/M phases of the cell cycle, and to induce apoptosis in human cells. Even if these cellular effects are thought to be caused directly by Roscovitine specific inhibition of cyclin-dependent kinases, other mechanisms may contribute as well.

And his coworker **(6)** pointed out that Roscovitine blocked the functional activities of Cdk2 and Cdc2, which led to a decrease in the S phase population, indicating that the cells were prevented from entering the S phase. On the other hand, inactivation of Cdc2 by Roscovitine led to accumulation of the cells in the G2/M phase of the cell cycle.

According to **Soo *et al.* (39**) irradiation significantly retards the growth of cultured cells, which is not due to induction of apoptosis but mainly due to cell cycle arrest at G1 and G2 phase. The delay can give the cell enough time to repair DNA damage. If DNA damage happened in G1 phase, the G1/S arrest will allow the cancer cell time to repair the damage before entry into S phase and replicate the damaged DNA. Similarly, the G2/M arrest can enable the cell to repair DNA damage before cell division. The S phase arrest occurs only after relatively high dose radiation **(40).**

In the present study, it was found that H460 was more radiosensitive than HepG2 and U251, where detection of cell cycle phases revealed that H460 had lower percent of S phase and higher percent of G2/M than the other cell lines. These elucidations were built on the results of a number of previous studies.

Quiet *et al*. (41) investigated two cell lines of different radiosensitivity and found that the radioresistant cell line had twice the number of cells in S-phase than the more sensitive cell line. **Liu**

***et al*. (42**) indicated that cells are most radiosensitive in M and G2 phases and most radioresistant in S phase, while for cells with long cycle time, another peak of resistance is observed in early G1.

The radiosensitizing effect of Roscovitine was evident in the three carcinoma cell lines selected for the present study. We found that the combination of radiation and Roscovitine caused an increase in the number of cells in the G0/G1and G2/M, accompanied by a decrease in the S phase; reaching to 0% at the higher radiation doses (at 2 Gy for H640, and 4 Gy for HepG2 & U251 cell lines), as compared to cells treated with radiation alone. Similarly, **Maggiorella *et al.* (31**) found that maximum G2/M checkpoint arrest was found in cells treated with Roscovitine accompanied with 4 Gy dose exposure with an 82% of the cell population blocked in G2 phase, which was associated with a pronounced decrease in DNA synthesis**.**

The DNA content of cells was measured by the ability of propidium iodide to bind to DNA under appropriate staining conditions. DNA index (DI) is defined as the difference in DNA content ratio of tumor/standard DNA fluorescence. An increase in DNA index has long been known to be associated with malignancy **(43)**.

In our study, DNA index was significantly decreased in the carcinoma cell lines after combining Roscovitine with radiation exposure, indicating a decrease in the number of cancer cells.

According to **Chen *et al*. (44)** many studies have reported that DNA index (DI) in relation to S-phase is independent factor for diagnosis in different types of tumors, where they defined diploid tumors as having a DI of (0.9 -1.1) and an aneuploid tumors with DI of >1.1.

Measurement of DNA fragmentation with diphenylamine (DPA) colorimetric assay is preferentially used to evaluate late apoptosis **(16)**. Evasion of DNA damage-induced cell death is a key step toward malignant transformation and therapeutic resistance **(45)**. Ionizing radiation can lead to the creation of single-strand breaks and double-strand breaks. Double-strand breaks are often rapidly repaired in cancer cells by the simple mechanism of joining free ends, a significant source of DNA mutations, which occur due to the non-homologous end joining pathway (NHEJ) **(64)**. The NHEJ pathway was found to be predominantly activated in cancer cells for repairing IR- induced DNA damage. Consequently hindering the DNA repair processes has the potential to inhibit recovery of mildly damaged tumor cells after radiation treatment, and also to increase their susceptibility to chemotherapy **(47)**.

We investigated the restraining effect of Roscovitine on DNA repair process, where the % of DNA fragmentation was measured after 1h and 24h of radiation. It was found that radiation produced high percent of DNA fragmentation in the three panels of carcinoma cell lines treated with 20Gy dose (in HepG2 & U251), and 10 Gy dose (H460). Maximum % fragmentation was observed after 1 hour of radiation with the higher Roscovitine doses, in H640 followed by HepG2 and U251 cell lines. In the mean time we found a decrease in the DNA % fragmentation after 24 hours of radiation indicating a repair in the DNA in all the carcinoma cell lines, we also found that in the combination treatment roscovitine blocked DNA repair process after 24 hours.

A similar observation was reported by **Maggiorella *et al.* (31**). **Zhang *et al* (10)** found that combination treatment of Roscovitine blocked DNA repair process after IR, while the singly used treatment did not. In addition, **Crescenzi *et al.*(48)** indicated that Roscovitine, by hindering DNA repair processes, has the potential to inhibit recovery of mildly damaged tumor cells after doxorubicin

treatment and to increase the susceptibility of tumor cells to chemotherapy, and that these data indicate a novel mechanism underlying combined chemotherapy, which may have wide application in treatment of carcinomas.

CONCLUSSION:

It could be concluded that micromolar concentrations of Roscovitine were able to prevent the cell cycle progressions and it also significantly radiosensitized the three carcinoma cell lines under investigation. The maximum effect was on the HepG2 cell line at the Roscovitine minimum doses.

Also, Roscovitine and/or radiation caused a drop in the percentage of aneuploid cells and a rise in the diploid ones, indicating that cells began to undergo differentiation and behave as normal cells. Roscovitine significantly retarded the growth of HepG2, U251, and H460 cells, which was mainly due to cell cycle arrest at the G1 and G2 phases, and its radiosensitizing effect was evident in the three carcinoma cell lines studied. Roscovitine blocked DNA repair process after 1 hour radiation in the three carcinoma cell lines studied, showing a better effect on the H460 carcinoma cell line.

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دور العقار الجديد المضاد للسرطان(روسكوفيتن) في زيادة حساسية الخلايا السرطانية المعالجة بالإشعاع

ايمـــــان نعمـــــان و امينة مدحت و نادية مرقص و هيام مصطفى

مرض السرطان من أهم المشكلات الصحية على مستوى العالم حيث يعتبر من المسببات الرئيسية للوفاة في الدول النامية والدول المتقدمة بعد الذبحة الصدرية. وبالرغم من نجاح الجراحة في استئصال بعض الأورام إلا أن انتشار المرض أو ظهوره مرة أخرى مشكلة توجد في معظم الأورام.

أثبتت الدراسات السابقة أن زيادة نشاط أنزيم السيكلين كينيز المعتمد (Cdk) الذي يحفز الخلايا على الانقسام له علاقة مباشرة بالتكونات السرطانية. يعتبر الروسكوفيتن مثبط للسيكلين بروتين كينيز حيث انه ينافس جزيء ATP في الإتحاد مع هذا البروتين مما يؤدى إلى تثبيط انقسام للخلية. إلى جانب ذلك التأثير فهو يؤدى إلى تكسير الحمض النووي الـ (دنا) DNA. تهدف هذه الدراسة الى معرفة مدى تأثير الروسكوفيتن ، احد المركبات الواعدة ، على زيادة حساسية الخلايا السرطانية للعلاج بالإشعاع وذلك في محاولة لتقليل الآثار الجانبية لكل منها وكذلك لتقليل مقاومة الخلايا السرطانية للعلاج بالإشعاع. ولقد تمت الدراسة على ثلاثة أنواع من الخلايا السرطانية المنماة خارج الجسم وهى خلايا المخ السرطانية الآدمية (U251) وخلايا الكبد السرطانية الآدمية(HepG2) وخلايا الرئة السرطانية الآدمية (H460). وتضمنت خطة البحث تحديد حساسية الخلايا للمركب وحساسية الخلايا للإشعاع لكل منهم على حدا وكذلك تأثيرهم المشترك على نمو الخلايا باستخدام اختبار ال (تى أم تى) ودراسة هذا الجمع بين العقار والإشعاع على دورة انقسام الخلايا السرطانية باستخدام جهاز التدفق الخلوي. دراسة تأثير الروسكوفيتن كمثبط لإصلاح الخلية بعد تكسير الـ DNAالناتج عن التعرض للإشعاع بواسطة اختبار ال(دى فينيل أمين). أظهرت النتائج البحثية أن الروسكوفيتن يعتبر محفزا جيدا لزيادة حساسية الخلايا السرطانية للعلاج بالإشعاع. و عند دراسة تأثير الجمع بين المركب والإشعاع على دورة انقسام الخلايا السرطانية ثبت توقف دورة حياة الخلية في المرحلتين G1/S و G2/M وكذلك وجد ان تأثير الجمع بين المركب والإشعاع على نمو الخلايا وجد انه يزيد من تثبيط نمو الخلايا أكثر من كلا منهم على حدا و لدلك ثبت ان عقار الروسكوفيتين يعتبر مثبط لإصلاح الخلية بعد تكسير الـ DNA حيث أدى إلى تقليل نسبة إعادة إصلاح الـ DNAعند مقارنتها بالخلايا التي تعرضت للإشعاع بدون الروسكوفيتن ومن هذه الدراسة يثبت أهمية استخدام الروسكوفيتن كأحد المحفزات الجديدة لتفادى مقاومة الخلايا السرطانية للعلاج بالإشعاع.