Investigate The Different Effect Of Nicotine On H460 And H441 Lung Cells Viability

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ABSTRACT

Background: Nicotine is the foremost chemical constituent responsible for addiction in tobacco products, in the non-ionized condition can be easily absorbed via epithelial tissue of the lung, the mouth, the nose and across the skin

Objective:The study examines the harmful effect of the nicotine which is an important component of cigarette *in vitro*.

Type of the study: Cross-sectional study.

Methods: Examines the harmful effect of the nicotine which is an important component of cigarette *in vitro* by using two types of lung cancer cell lines (H460 *TP53*+/+, H441 *TP53*-/-).

Results: The results showed the total count of H460(*TP53*+/+) cancer lung cell lines was (5.2 $\times 10^6$ cells /ml) , the number (4.9 $\times 10^6$ cells /ml) of them were alive and (3.6$\times 10^5$ cells /ml) of them were dead, with percentage of viability (93.15%), while the total count of H441(*TP53*-/-) lung cancer cells was (5.1 $\times 10^6$ cells /ml), the number (4.1 $\times 10^6$ cells /ml) of them were alive, and the number (9.9$\times 10^5$ cells /ml) of them were dead with percentage of viability (80.55%). And it revealed that the nicotine inhibited viability of H460 lung cancer cell lines in all concentrations (1, 10, 500 and 1000 µ M) and the cells were completely abolished by treatment with (1000 µ M) when the viability reached to minimum percentage (3.43%), while nicotine induced proliferation in H441 lung cancer cell lines even in lowest concentration (1 µ M), whereas the viability reached to maximum percentage (41.04%) at concentration (1000 µ M). Also it noticed that the treatment with nicotine at concentrations (1,10, 500 and 1000 µ M) for 24 and 48 hr induced the apoptosis but not necrosis in H460 lung cancer cell lines, especially at the highest concentrations (1000 µ M) for 48hr when the percentage of apoptosis reached to the maximum value (55.5%), however it was induced proliferation in H441 lung cancer cell lines with highest proliferation at concentration (1000 µ M), when the percentage apoptosis value reached to the minimum percentage (1.5%) when compared to un treated cells (control).

Conclusions: the cells lack to *TP53*(H441 *TP53*-/-) will proliferate when exposed to nicotine and some of these cells will suffer from necrosis as a replacement of apoptosis.

Keywords: Nicotine, H460 *TP53*+/+, H441 *TP53*-/-, Viability, Apoptosis , Necrosis .

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Nicotine is the foremost chemical constituent responsible for addiction in tobacco products, in the non-ionized condition can be easily absorbed via epithelial tissue of the lung, the mouth, the nose and across the skin(1) Nicotine, has been shown to induce tumor growth by promoting cell-cycle progression, and avoidance apoptosis in different systems (2). Many studies have shown that nicotine can provoke lung cells propagation sequenced by incidence cancers (3,4) .In addition several researches reported that *TP53* modifications are the common genetic activities in tumor progress (5 ,6) because *TP53* regulates many cellular events as DNA replication by inducing cell cycle arrest and apoptosis (7). Investigations conferred that stimulate or repressor of *TP53* causes apoptosis or cell proliferation. In the present study we have tried to indicate the effect of four concentrations of nicotine (1,10, 500 and 1000 µ M.) on lung cells viability, apoptosis and necrosis when the cells have wild type of p53 (H460 *TP53*+/+) and prove that *TP53* plays an essential role in regulating apoptosis depending of nicotine stimulation . Additionally we tried to indicate that the cells lack to *TP53*(H441 *TP53*-/-) will proliferate when exposure to nicotine and some of these cells will suffer from necrosis as a replacement of apoptosis.

Methods : All experiments were done at Biomaterial Laboratory/University of Missouri/Columbia/USA. Nicotine was purchased from Sigma /USA. Lung cancer cell-lines (H460 *TP53*+/+, H441 *TP53*-/-) were obtained from American Type Culture Collection (ATCC), cells were cultured in RPMI 1640 culture medium (Sigma, USA) supplemented with 10% fetal bovine serum (Sigma, USA).

Determination of Cell Viability by Trypan blue exclusion assay: Cells concentration and viability were determined by using countess (Sigma, USA) and (0.4%) trypan blue dye (Thermo fisher, USA) as described by (8).

Determination of cell cytotoxiticy by MTT assay: Determination of cell cytotoxiticy was done by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay according to (9) after treated H460 and H441cells with nicotine (1 µ M,10 µ M, 5 00 µ M and

1000 µ M) concentrations, the absorbance was read using a 96-well plate reader(Biocompare, USA) at a wave length of 570 *nM*.

Evaluation apoptotic and necrotic cells: Apoptotic and necrotic cells were measured by apoptosis assay using Annexin V- apoptosis kit (Abnova, Taiwan) and flow cytometery (Thermo fisher, USA) as mentioned by (10) after treated H460 and H441cells with nicotine at (1 µ M,10 µ M, 5 00 µ M and 1000 µ M) concentrations, untreated cells were established as control.

Results analysis: Analyzing and graph the data by Microsoft Excel 2007 software program version 2007.

Results: The result of H460 cancer lung cell lines showed that the total count was (5.2 $\times 10^6$ cells /ml) , a number (4.9 $\times 10^6$ cells /ml) of them were alive and (3.6$\times 10^5$ cells /ml) of them were dead, with percentage of viability (93.15%), while the result of H441 lung cancer cells appeared a total count (5.1 $\times 10^6$ cells /ml), the number (4.1 $\times 10^6$ cells /ml) of them were alive, and the number (9.9$\times 10^5$ cells /ml) of them were dead with percentage of viability (80.55%).

Estimation of the cell toxicity: The effect of Nicotine on H460 and H441cell lines were estimated through cell toxicity using the MTT assay, analyzing and graphing of the data was carried out by Microsoft Excel 2007 software. The results showed that nicotine inhibited the viability of H460 cells (which have wild type of *TP53*) in a all concentrations. It was noticed that the cells was completely abolished by treatment with (1000 µ M) of nicotine and viability reached to minimum percentage (3.43%) as shown in figure (1).

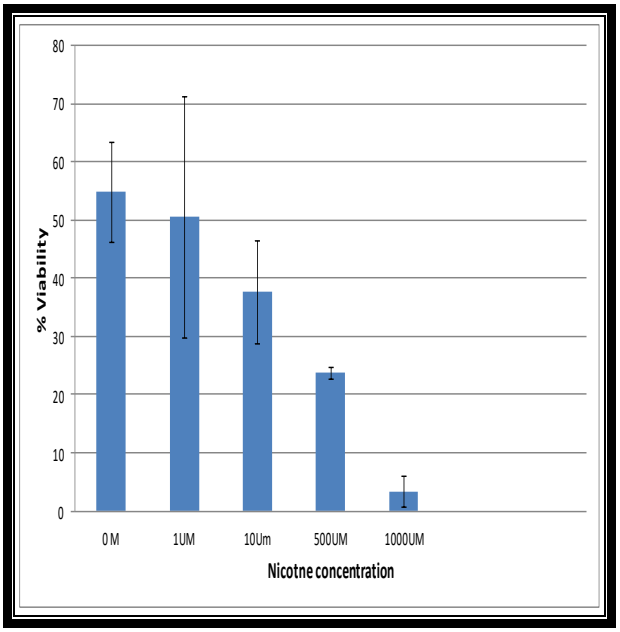

Figure 1: Cytotoxic effects of nicotine on H460 lung cancer cells by MTT assay with wave length of 570 nM.

While nicotine induced proliferation in lung cancer cells lacking *TP53* (H441) even at lower concentration (1 µ M), it was noticed that the viability reached maximum percentage (41.04%) at concentration (1000 µ M) as shown in figure (2).

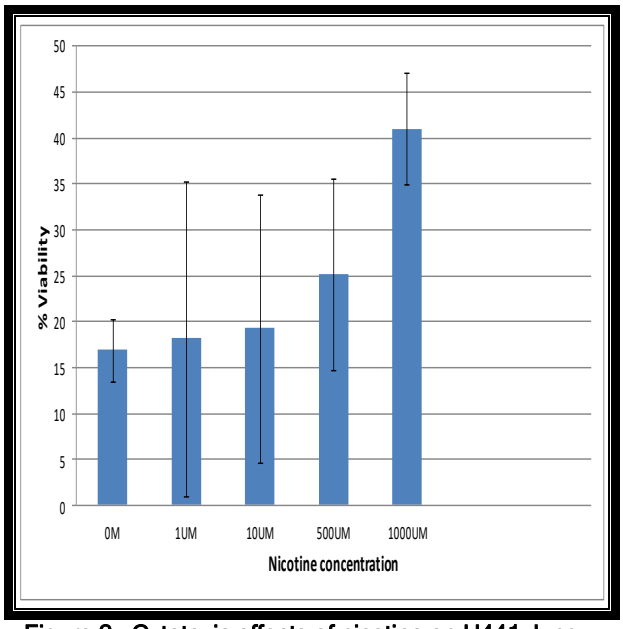

Figure 2 : Cytotoxic effects of nicotine on H441 lung cancer cells by MTT assay with wave length of 570 nM.

Determination the cell apoptosis and necrosis: To determine whether the growth inhibition in H460 cells and proliferation in H441 cells by nicotine were associated with the induction of apoptotic and necrotic cell. The apoptosis and necrosis index were evaluated by AnnexinV-FITC staining method using flow cytometry. According to this method, the results showed that the treatment with nicotine in concentrations (1 µ M,10 µ M, 500 µ M and 1000 µ M) for (24 hr) induced the apoptosis in H460 cells in all concentrations when compared with untreated cells (as control) especially at highest concentrations (1000 µ M), when apoptosis reached to a maximum value (55.5%), also it was noticed that the nicotine did not enhance necrosis in H460 cells, just as it did in apoptosis, which reached to minimum necrosis value (3.5%). However, the nicotine induced proliferation in H441 lung cancer cells and the highest proliferation at (1000 µ M) concentration which apoptosis value reached to minimum percentage (1.5%), and at the same time it was noticed the necrosis value reached to (20.7%) (Figures 3, 4 and 5). Also current findings showed that the treatment with nicotine at all concentrations (1 µ M,10 µ M, 500 µ M and 1000 µ M) for (48 hr) induced a high rate of apoptosis in H460 lung cancer cell , which reached to maximum value (57.5%) at highest concentrations (1000 µ M), when compared with the treatment for (24 hr), while the necrosis value was (13%), that may be happened due to exposure to

nicotine for longer time, even though induced proliferation of H441 lung cancer cells has been reported in the findings above, but it was observed that the treatment with nicotine for (48 hr) enhanced more proliferation chiefly at highest concentrations (1000 µ M) which reached to minimum value of apoptosis (1.35%), also H441 cells suffered from necrosis more than the H460 (20%). This perhaps attributed to lacking of the H441 lung cancer cell to *TP53*gene which responsible for the apoptosis therefore the cell death cause by necrosis and not by apoptosis as shown in figures (6, 7and 8).

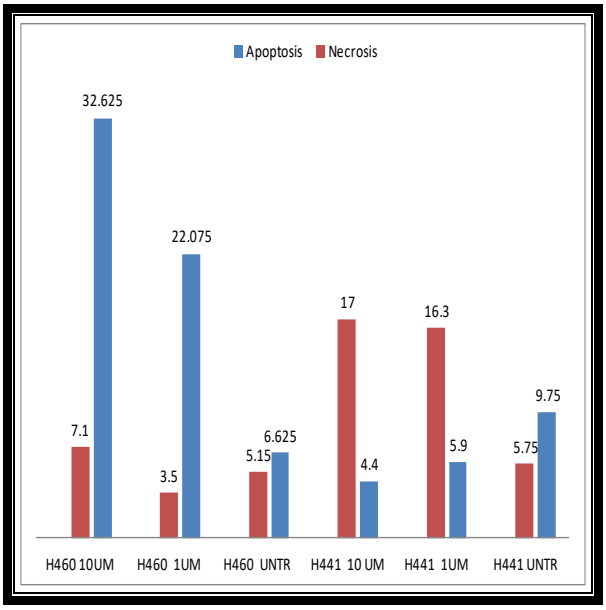

Figure 3 : Apoptosis and necrosis percentages after 24 hr of nicotine treatment with concentrations (1 µ M and 10 µ M) in H460 and H441 lung cancer cell.

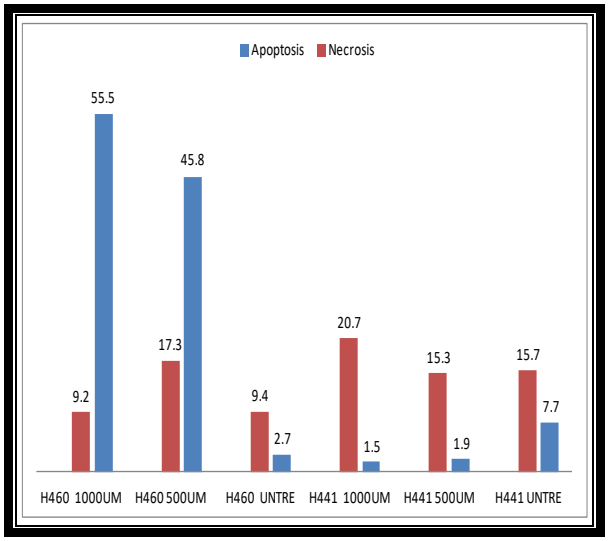

Figure 4 : Apoptosis and necrosis percentages after 24 hr of nicotine treatment with concentrations (500 µ M and 1000 µ M) in H460 and H441 lung cancer cell.

It seems that the viability, apoptosis and necrosis indexes of nicotine in various concentrations are different in the lung cancer cell lines H441(*TP53*-/-) and H460(*TP53*+/+) which indicates that the prompting *TP53* by nicotine may be different in cells with and without *TP53*. Many efforts have been devoted by different finding groups to determine the role of nicotine in the process of carcinogenesis by tobacco. It has been concerned as a significant risk factor in the proliferation of different cancer cells (11 , 12) and has been found to induce enhanced tissue perfusion and angiogenesis (13 , 14) Moreover, it was convincingly revealed that nicotine induces proliferation in lung cancer cells

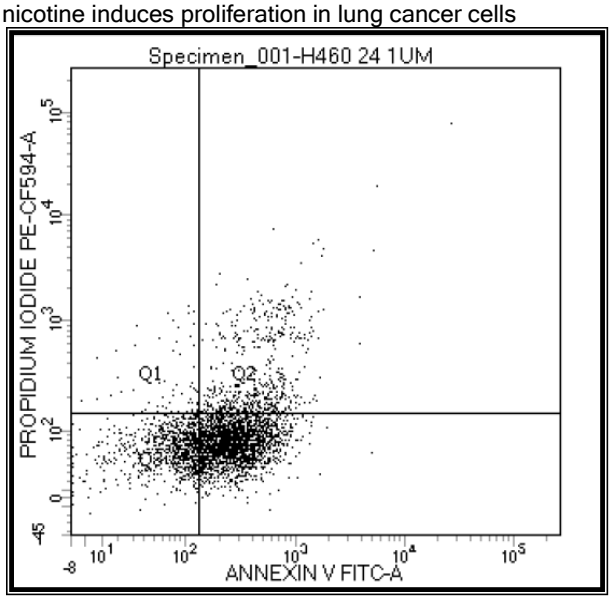

Figure 5 : Apoptosis and necrosis after 24 hr of nicotine treatment with concentration (1 µM) in H460 lung cancer cells by flow cytometry.

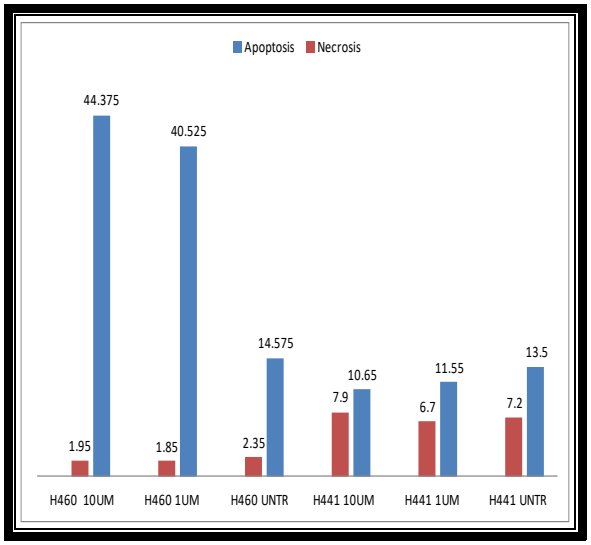

Figure 6 : Apoptosis and necrosis percentages after 48 hr of nicotine treatment with concentrations (1 µ M and 10 µ M) in H460 and H441 lung cancer cells.

particularly in those with lacked *TP53* state, in addition to that, the occurrence of necrosis in some of these cells. In this regard, the ability of nicotine to increase adherence-independent proliferation of tumor cells is well accepted (3), and nicotine has been revealed to induce proliferation in lung cancer cells, but the regulatory role of *TP53* on

nicotine- induced proliferation has not yet been confirmed(13).

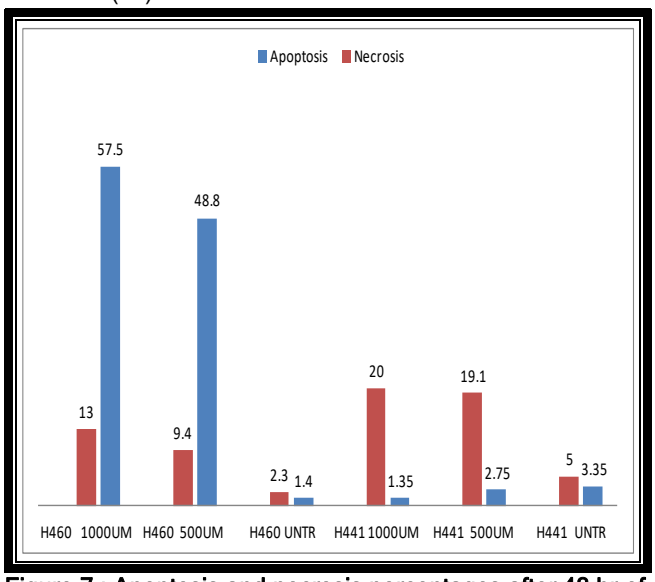

Figure 7 : Apoptosis and necrosis percentages after 48 hr of nicotine treatment with concentrations (500 µ M and 1000 µ M) in H460 and H441 lung cancer cells.

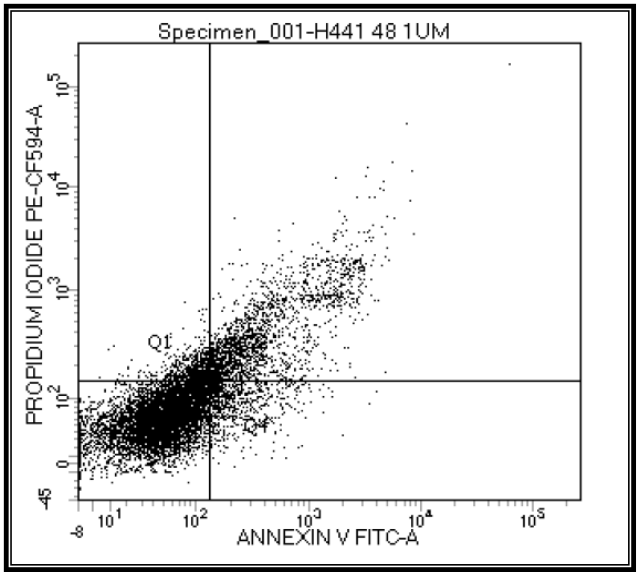

Figure 8 : Apoptosis and necrosis after 48 hr of nicotine treatment with concentration (1 µM) in H441 lung cancer cells by flow cytometry.

A significant increase was observed in the proliferation of lung cancer cells lacking TP53, at same time increased apoptosis and decreased necrosis in cells have wild type of TP53, which depending on the concentration of nicotine, revealed a detrimental role of nicotine especially in lung cancer cells, further validated that the treatment with nicotine in lacked TP53 status led to proliferation and induction necrosis in lung cancer cells lack TP53 (H441) and led to apoptosis in present of TP53 in cells carrying wild type TP53 (H460) . This established a conviction to the role of TP53 in the induction of various survival signals by nicotine .People with TP53 mutations who smoke might be more likely to get other cancer, as TP53mutations are related with a many of tobacco-associated tumors including lung, head and neck, and bladder cancers (15) .

Effects of nicotine have been confirmed for a many of tumor cells, such as breast, colon, and lung (16,17). Furthermore, many studies have shown that the nicotine promoted endothelial cell migration and proliferation (18, 19). Also (20) provided a new information about, how cigarette smoke exposure can induced TP53 expression related with lung cell apoptosis.

The mechanisms responsible of the genotoxic effects resulting by nicotine have not been proven yet, but it is of importance, that effects are noticed at concentrations of nicotine not much higher than those found in blood of smokers (21).

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