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Investigation of Carmofur-Derived Growth Supression in Lung Cancer Cell

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***Abstract* —** Lung cancer is a malignant form of uncontrolled cell growth in lung tissues with high incidence of mortality in the world. Despite the rise in survival ratios in recent five years lung cancer patients survived relatively poor because of the treatment limitations. Chemotherapeutics have biggest handicap in their usage for cancer treatment that cancer cells develop resistance to them. Herein, carmofur, a form of pyrimidine analogue was used for stimulating cell death in lung cancer cells based on its anti-proliferative potency in many cancer cells. Cytotoxic activity was tested via MTT assay. In addition, ultrastructural and morphological changes were evaluated via transmission and confocal microscopies. The IC50 value was determined to be 23 µM for 24 hours. Morphological and ultrastructural analyses imply to clear apoptosis. Consecutively, carmofur might be suggested for deeper examinations in the manner of improvement of the apoptosis triggering potency for designing a florouracil-derived permanent antineoplastic agent.

Keywords— Carmofur, Lung cancer, Apoptosis, Cancer treatment, Cytotoxicity

I. INTRODUCTION

Cancer has become serious health problem worldwide due to the high morbidity and mortality levels. Cancer is difficult to defeat in entire living systems. In spite of trial at pre-clinical and clinical levels to treat the cancer during last decades, a cure for certain types of cancer still remain unravelled and cancer incidence continues to increase. As one of that forms of cancer, has been reported to be main reason of death both for men and women. It is the most frequent diagnosed malignancy in the world and both its annual incidence and mortality rates have augmented over the past 25 years [1]. The growth of neoplastic cells was suggested to be related to the intracellular ceramide levels [2]. Ceramide levels of normal cells is higher than that of some tumor cells in humans that is attributed to the inverse correlation of ceramide with the malignancy developing processes [3],[4]. Based on this, recently drugs that increase the intracellular ceramide levels are used in experimental and clinic cancer researches for cell growth suppression or triggering apoptotic cell death [5]. Consequently, stimulating the ceramide production in cells has become one of the main strategies of inducing apoptotic cell death in a variety of tumors [3],[4]. These approaches indicate to intracellular enzyme pathways for controlling the levels of ceramides of cells as new targets for cancer therapy [6]. Human ceramidase is one of the enzymes that reduce the ceramide level in the cell [7],[8]. Increased ceramidase activity in cancer cells cause an decrease in ceramide levels as well as increase the sphingosine-1-phosphate levels of the cells thus supress apoptosis and trigger the proliferation of cancer cells [7],[8]. Carmofur or 1-Hexylcarbamoyl-5-fluorouracil (HCFU) has an antineoplastic influence derived from its potency on inhibiting the human ceramidase activity and is used to treat patients with many types carcinomas [9],[10]. In addition, it is identified as first low-dose inhibitor of aCDases in the cells thus recently is used in the clinic to threat the colorectal cancer [11]. The ceramidase inhibitory activity of carmofur is determined in genetically modified human embryonic kidney 293 (HEK 293) cells overexpressing acid ceramidases and non-modified HEK 293 cells by Realini et al [10]. They showed that by short-term carmofur application for 24 hours in AC-overexpressing HEK 293 cells ceramide levels significantly decreased and half maximal inhibitory concentration of carmofur for modified cells detected to be 50-fold higher then that of non-modified HEK 293 cells. On other hand this finding refer to the importance of supressing ceramidase activity as a tool for cancer treatment [10]. Moreover, the anti-cancer abilities of carmofur imply to its property to generate 5-FU, a pyrimidine analogue that hinder DNA synthesis in cancer cells by supressing thymidylate synthase [10]. In the structure of this antimetabolite agent/carmofur a hexylcarbamoyl substituent as a transport form is present and it helps 5-FU in penetration into the cells [12],[13]. Based on this property, carmofur has been used for cancer therapy in human for many years. Furthermore, the anti-proliferative activity of carmofur under cancer cells has been declared as a separate property of 5-FU generating ability [11]. For these reasons, it appears to be an important tool for in vitro and in vivo studies on cancer research and consequently, it supports to the idea of inhibiting ceramidases for medical purpose [11].

In this study it was investigated the versatile cytotoxic activities of carmofur in A549 human alveolar basal epithelial adenocarcinoma cells including with its anti-proliferative and apoptotic effects in the morphology and ultrastructure of the cells in the manner of providing and alternative option for designing and usage of anti-cancer agents.

II. RELATED WORK

Studies performed prior to our study focused mainly on metabolites of sphingolipid pathway that are closely related with cell death mechanisms. The investigations of these studies indicated to the role of ceramidase enzymes on cancer treatment [10],[11]. The first nanomolar inhibitor of ceramidase enzymes-carmofur has not been investigated for its potency to inhibit non-small cell lung adenocarcinoma (A549) cells. Consequently, the purpose of this study was to investigate the anti-proliferative and apoptotic efficacies of carmofur including with the morphological and ultrastructural changes on A549 cells.

III. METHODOLOGY

Materials

A549 (ATCC® CCL-185™) cells were purchased the American Type Culture Collection (Manassas, USA). Carmofur, fetal bovine serum, penicillin streptomycin, dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl-2H-tetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St. Louis, USA) and Roswell Park Memorial Institute medium (RPMI-1640) was from GIBCO (Grand Island, USA).

Cell culture and MTT colorimetric assay

A549 cells were grown in RPMI-1640 medium containing penicillin–streptomycin (100 units/mL-100 µg/mL) and fetal bovine serum (10%) at 37°C and 5% CO_2 in humidified incubator until they become confluent. In all of the experiments were used cell culture flasks with at least 85% confluency and each experiment was prepared in triplicate. For MTT assay, a stock solution of carmofur (in DMSO) was prepared and further dilutions of the solution were made with fresh culture medium (RPMI-1640) till the final concentration of DMSO per well not exceed 0.1-0.2%. After the adjustment of the carmofur concentrations in the range of 5-100 µM in 96-well plates, A549 cells were seeded 1×10^3/well. Treated cells were allowed to incubation for 24 hours under the same culture conditions. At the end of the incubation 20 µL/well of MTT solution (5 mg/mL) was added. Cells were incubated with MTT solution at a cell culture incubator for 3 hours. Following this period the growth media in the well were aspirated than DMSO (200 µL/well) was added in each well. The plates were read on an ELISA reader (EL × 808, BioTek, USA) at a wavelength of 540 nm (n=3). Viability percentages were calculated compared to the control group and the half maximal inhibitory concentration (IC_{50}) of carmofur for 24 hours under A549 cells was detected.

Analysing the carmofur-derived morphological changes in A549 cells

Confocal microscopic evaluation was performed in order to detect the morphological changes of carmofur treated A549 cells. In brief, A549 cells plated onto cover slips in six-well cell culture plates were incubated at 37 °C in a cell culture incubator with the IC_{50} value of carmofur for 24 hours. A group of untreated cells were grown under the same conditions. After incubation period, growth medium of treated and untreated cells was distanted, and the cells were washed with phosphate buffered saline (PBS, Invitrogen, USA). Washed cell samples were fixed in 2% glutaraldehyde for 15 minutes at room temperature. Then, cells were washed again and stained in Alexa fluor-488 phalloidine and acridine orange. All samples were imaged under a Leica ICS-SP5 II confocal microscope supplemented with an adequate software (Leica Confocal Software Version 2.00, Leica, Germany).

Analysing the ultrastructure of carmofur treated A549 cells

For analysing the ultrastructural changes was used a transmission electron microscope (TEM). In this manner A549 cells were treated with IC_{50} concentration of carmofur for 24 hours. Untreated and carmofur treated A549 cells were fixed in glutaraldehyde (2.5%) and post-fixed with osmium tetroxide (2%). Fixed cell samples were dehydrated in ethanol then embedded in Epon 812 epoxy. Embedded samples were polymerized for 48 hours. Obtained blocks were sectioned by a maximum thickness of 100 nm in ultramicrotome (Leica EMUC6). The thin sections were placed in copper grids and stained with uranyl acetate and lead citrate. Stained samples were images by using a TEM (FEI Tecnai BioTWIN).

Flow Cytometric Analyses of Cell Death in carmofur treated A549 cells

Cell death mode of carmofur treated and untreated A549 cells was analysed by using annexin V-FITC and PI technique on a flow cytometer. Briefly, A549 cells were seeded (5x105cells/well) in six-well culture plates and exposed to IC_{50} concentration of carmofur for 24 hours at 37°C in a 5% carbon dioxide incubator. Untreated A549 cells were grown at the same conditions and used as control cells. After the incubation, untreated and treated cells were harvested by trypsinization and added to separate tubes. Cells in each tube were washed in PBS. After the washing cells were resuspended and 100µL of each sample was transferred to separate tubes. 100µL of annexin-V reagent was added per tube and incubated for 20 minutes in the dark at room temperature according to the user manual of Muse® Annexin-V and Dead Cell Assay Kit. The analyse of the stained cells were performed by using Muse™ Cell Analyzer (Merck, Millipore, Hayward, California, USA).

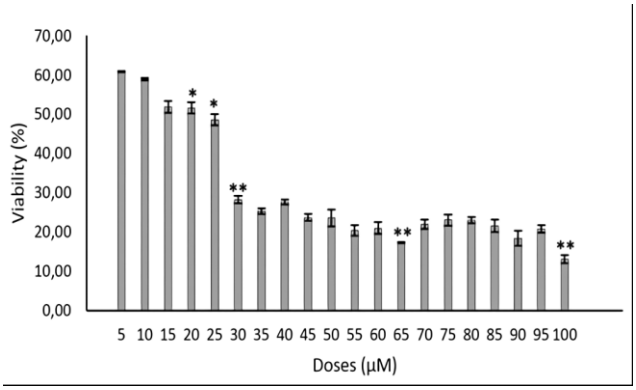
Statistical Analysis

The statistical analyses of the results were performed by using one way variance analysis for multiple comparisons of GraphPad Prism 6.0 for Windows.

IV. RESULTS AND DISCUSSION

MTT Results

It was detected that as the carmofur concentrations applied to A549 cells increased, the reduction in the percentage of viability of the cells occurred after 24 hours when compared to the control group. The viability inhibition curve (Figure 1) of A549 cells exposed to carmofur concentrations for 24 hours was prepared with the Microsoft Office Excel program and the IC_{50} value was determined as 23 µM for this application period. The decrease detected on treated cells was statistically significant in comparison with the obtained viability percentage of control cells. These values imply to the cytotoxicity of carmofur on A549 cells in dose dependent manner.



*p<0.05; **p<0.01

Figure 1. Viability percentages of A549 cells exposed to different carmofur concentrations ranging from 5-100 µM for 24 hours.

Confocal Microscopic Results

Confocal images of acridine orange and phalloidine stained A549 cells treated with IC_{50} value of carmofur for 24 hours showed highly changed morphology when compared with the untreated A549 cells as shown in figure 2A and B. Untreated cells were with fusiform morphology and compact cytoskeleton and nuclei (Figure 2A). Whereas carmofur treated cells showed chromatin condensation, holes on cytoskeleton and circular cell shape due to the shrinkage caused by carmofur (Figure 2B). The detected morphological changes were evaluated as apoptotic signs in the accordance with our ultrastructural analysis results.

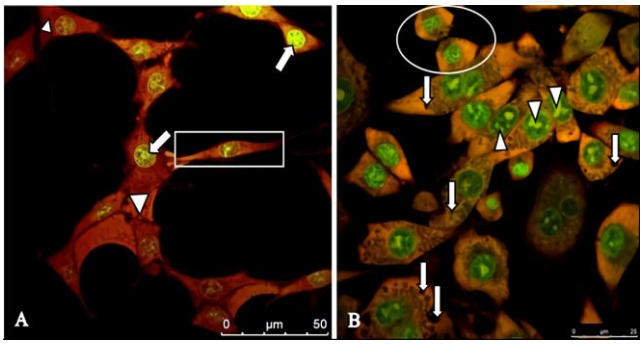


Figure 2. Confocal images of the untreated A549 cells (A: Arrow-normal nuclei, Arrowhead-normal cytoskeleton, Rectangle-fusiform cell shape) and carmofur treated A549 cells (B: Arrowhead-Chromatin condensation, Arrow-Holes on cytoskeleton, Circle-Shrunken cells).

Transmission Electron Microscopic Results

TEM results were evaluated compared to the micrographs of the untreated A549 cells prossessed in the same conditions with the treated cells (Figure 3A). The fine structural changes caused in A549 cells by 24 hours application of the IC_{50} concentration of carmofur (23 µM) determined in TEM micrographs were holes in cytoskeleton, chromatin condensation, granulated cytoskeleton and circular shaped shrunken cells (Figure 3B and C). Furthermore, ring shaped chromatin condensation, ondulation (shrinkage) in nuclear membrane were determined in the same group of cells (Figure 3D). Similar findings were recorded on confocal microscopy results. These ultrastructural changes were considered as indicators of programmed cell death.

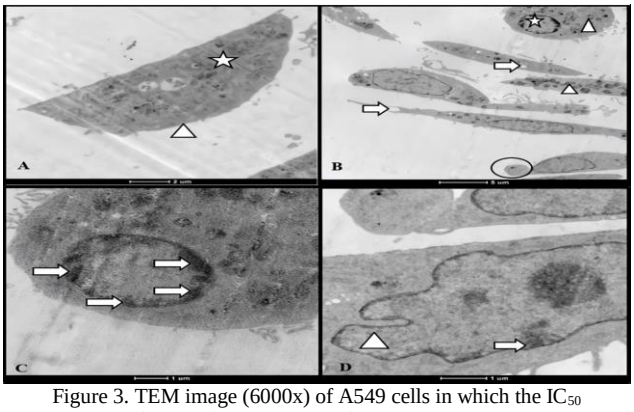


Figure 3. TEM image (6000x) of A549 cells in which the IC_{50} concentration of Carmofur was applied for 24 hours. A. Ultrastructure of untreated A549 cells; Asterisk-normal cytoskeleton, Arrowhead-normal cell membrane. B. Arrow-Holes in cytoskeleton, Asterisk-Chromatin condensation, Arrowhead-granulated cytoskeleton, Circle-shrunken cell. C. Arrow; ring-shaped chromatin condensation formation. D. Arrow; chromatin condensation, Arrowhead; Ondulation (shrinkage) in nuclear membrane.

Flow cytometry results

Apoptotic profiles of untreated and carmofur treated A549 cells were determined by annexin-V analyses in a flow cytometer. The untreated A549 (control) cells were found to be 95.25% viable. In this group of cells 4.07% were dead and 0.68% total apoptotic cells. In carmofur treated A549 cells 78.24% of cells were alive. Whereas, 19.86% of the treated cells were total apoptotic and 1.90% of total cell count were dead. Our annexin-V analysis results underlined carmofur treated cell population undergone apoptosis.

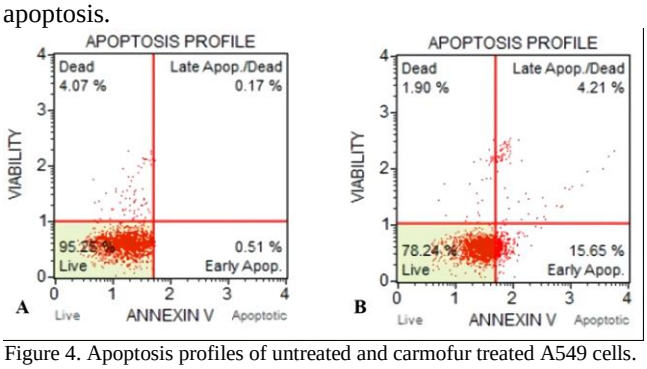


Figure 4. Apoptosis profiles of untreated and carmofur treated A549 cells. A. Untreated A549 cells; Late necrotic, necrotic or dead cells (0.17%), Apoptotic/dead cells (4.07%), Live cells (95.25%) and Apoptotic/early apoptotic cells (0.51%). B. Carmofur treated A549 cells; Late necrotic, necrotic or dead cells (4.21%), Apoptotic/dead cells (1.90%), Live Cells (78.24%) and Apoptotic/early apoptotic cells (15.65%).

Discussion

Recent research on the cancer therapy field are increasingly focused on intracellular targets of antineoplastic agents by cell death triggering character. Cancer therapy studies indicate some key strategies to treat the disease. Preventing cancer disease before its occurrence has been indicated as one of that strategies. Another is preventing cancer cells proliferation or triggering cancerous cell death in low doses in comparison with healthy cells [14]. Cancerous cells are uncontrolled proliferating cells. Uncontrolled proliferation has many pathways at the cellular level. The use of antineoplastic agents and ceramidase inhibitors are indicated as supressing strategies of these pathways, recently [14]. In this study carmofur, indicated in many studies as a ceramidase inhibitor [10],[12],[14] and that has good potency of being anticancer agent for several cancer types, has been investigated on A549 cells in the respect of examining its cytotoxic, anti-proliferative and pro-apoptotic activities for short-time application of 24 hours. On the basis of our research, it is showed that the viability of A549 cells exposed to different carmofur concentrations was decreased in a dose-dependent manner. The half maximal inhibition concentration of carmofur on A549 cells was detected to be low (23 µM) for short-term applications (Figure 1). Similar findings were detected by other researchers12 in human neuroblastoma SH-SY-5Y cells. They have found that carmofur showed weak cytotoxicity on SH-SY-5Y cells with an IC_{50} of 76 µM [12]. In comparison, our value has at about two times more extended cytotoxicity. In the same study, the carmofur treatment of MH-22A mouse hepatoma cells was indicated to cause an IC_{50} of 43 µM [3] that is too high compared to our IC_{50} value of 23 µM. The difference between the concentrations might be depended to the cell type or the age of used cells as well as the intracellular ceramide/ceramidases levels of the used cells. Since, carmofur has been declared as a good inhibitor of human ceramidases [10]. Growth inhibition recorded in our research caused by carmofur might also be attributed to the same characteristic but deeper examinations are required. Nonetheless, carmofur exhibited its toxicity in quite low doses on A549 cells in this study.

Survival permission during its life time and abnormal proliferation are two of the main characteristics of the cancer cells [15]. There is a good deal of evidence for ceramidases involved in regulation of cell survival/death and for the reqruiting potency of their inhibitors in the treatment of varying types of cancers [11]. Notably, cancer therapy approaches involving cytotoxic drugs that cause cell death in the cells with high proliferation and regeneration levels. Recently, targeting mechanisms related with uncontrolled cell growth increasingly has become a standard of treatment for cancer sufferers [15]. In this study, carmofur as a ceramidase inhibitor, efficiently triggered cell death in A549 cells when applied for 24 hours at its IC_{50} value of 23 µM. In our confocal (Figure 2) and TEM (Figure 3) findings strong hallmarks of apoptotic cell death are detected. In our results of confocal evaluation, the fusiform morphology of the A549 cells was converted to circular after the carmofur treatment. Furthermore, the chromatin of the treated cells was highly condensed as well as hole were formed in the cytoskeleton of the same cell group. In the carmofur treated cell's ultrastructure were recorded pivotal changes implying apoptosis. Main alterations in these cells detected under TEM were ring shaped chromatin condensation, ondulation (shrinkage) in nuclear membrane, perforated and granulated cytoskeleton (Figure 3). Our confocal and TEM results were strongly supported by the results of [16] that indicate apoptosis in carmofur treated human hepatocarcinoma HepG2 cells based on the morphological changes recorded under confocal microscopy as chromatin condensation, membrane blebbings and confocal and pyknotic nucleus [16]. Our findings refer the main purpose of cancer therapy that aims at inhibiting cell proliferation and involve apoptotic cell death of cancer cells [16]. Apart from all morphological and ultrastructural hallmarks another specific examination of cell death mode was performed by annexin-V analyses. This dye attach to the phosphatidylserines (PS, membrane phospholipids) that are transferred to the outer layer of the cell membrane during the apoptosis. Our flow cytometry findings the percentage of cells underwent apoptosis after the carmofur treatment was significantly increased (Figure 4B). In the accordance with our findings detected total apoptotis in carmofur treated HepG2 cells for 24 hours. Moreover, our findings are supported by our previous study with another ceramidase inhibitor on A549 cells [17].

V. CONCLUSION AND FUTURE SCOPE

In conclusion, starting with the aim of the recent cancer research to stimulate apoptosis in cancer cells, in our study were showed considerably apoptosis triggering, anti-proliferative and cytotoxic efficacies of carmofur on A549 cells. As it was stated in other studies sphingolipid pathway is one of the tools for cancer therapy. Notably, carmofur has been indicated as a strong potent inhibitor of the ceramidases, key members of sphingolipid pathway involved in balancing the sphingosine-1-phosphate/ceramide ratio that is decisive for cell survival/death. All taken together, our cytotoxicity, pro-apoptotic findings can be related to the ceramidases inhibitory action of carmofur but deeper investigations need to be performed for unravel the mechanism of action for the agent on the A549 cells. Under any circumstances, in our study carmofur was determined as an agent with good potency to inhibit cancer cell growth by stimulating apoptosis that is preferred cell death mode without inflammation in cancer therapy.

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REFERENCES

[1] M.A. Phanse, P.D. Chaudhari, S. Birambole, S.R. Pattan, M.J. Patil, R.S. Dhikale, ''Cytotoxic Assays for Lung and Colon Cancer,'' Pharmacologyonline, Vol. 1, pp.979-995, 2010.

[2] H. Furuya, Y. Shimizu, T. Kawamori, ''Sphingolipids in cancer,'' Cancer Metastasis Reviews, Vol. 30, pp.567, 2011.

[3] L. Riboni, R. Campanella, R. Bassi, R. Villani, S.M. Gaini, F. Martinelli-Boneschi, P. Viani, G. Tettamanti, ''Ceramide Levels are Inversely Associated with Malignant Progression of Human Glial Tumors,'' Glia, Vol. 39, Issue.2, pp.105-13, 2002.

[4] B. Ogretmen, Y.A. Hannun, ''Biologically Active Sphingolipids in Cancer Pathogenesis and Treatment,'' Nature Reviews Cancer, Vol. 4, Issue.8, pp.604-616, 2004.

[5] R. Kolesnick, ''The Therapeutic Potential of Modulating the Ceramide/Sphingomyelin Pathway,'' Journal of Clinical Investigation, Vol. 110, Issue.1, pp.3-8, 2002.

[6] D. Canals, D.M. Perry, R.W. Jenkins, Y.A. Hannun, ''Drug Targeting of Sphingolipid Metabolism: Sphingomyelinases and Ceramidase,'' British Journal of Pharmacology, Vol. 163, Issue.4, pp.694-712, 2011.

[7] X. Liu, J.C. Cheng, L.S. Turner, S. Elojeimy, T.H. Beckham, A. Bielawska, T.E. Keane, Y.A. Hannun, J.S. Norris, ''Acid Ceramidase Upregulation in Prostate Cancer: Role in Tumor Development and Implications for Therapy,'' Expert Opinion on Therapeutic Targets, Vol. 13, Issue.12, pp.1449–1458, 2011.

[8] A.E.M. Mahdy, J.C. Cheng, L. Jun, ''Acid Ceramidase Upregulation in Prostate Cancer Cells Confers Resistance to Radiation: Ac Inhibition, A Potential Radiosensitizier,'' Molecular Therapy, Vol. 17, Issue.3, pp.430–438, 2009.

[9] P. Zhang, S. Liu, ''Solvent Effects on the UV Absorption Spectrum of Carmofur,'' Scientific Research Publishing Inc, Vol. 4, pp.25-31, 2016.

[10] N. Realini, C. Solorzano, C. Pagliuca, D. Pizzirani, A. Armirotti, R. Luciani, M.P. Costi, T. Bandiera, D. Piomelli, ''Discovery of Highly Potent Acid Ceramidase Inhibitors With in Vitro Tumor Chemosensitizing Activity,'' Scientific Reports, Vol.3, pp.1035, 2012.

[11] E.M. Saied, C. Arenz, ''Small Molecule Inhibitors of Ceramidases,'' Cellular Physiology and Biochemistry, Vol. 34, Issue.1, pp.197-212, 2014.

[12] I. Domracheva, R. Muhamadejev, M. Petrova, E. Liepinsh, A. Gulbe, I. Shestakova, G. Duburs, P. Arsenyan, ''1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC) Increases Carmofur Stability and In Vitro Antiproliferative Effect,'' Toxicology Reports, Vol. 2, pp.377–383, 2015.

[13] M. Furukawa, T. Karakama, M. Kaji, S. Gomita, M. Yoshimura, N. Watanabe, ''Collaborative Work to Evaluate Toxicity on Male Reproductive Organs by Repeated Dose Studıes in Rats 16) Effects Of Short-Term Administration of Carmofur on Spermatogenesis,'' The Joumal Of Toxicological Sciences, Vol. 25, pp.163-171, 2000.

[14] O.A. Greer, N. Amoyal, L. Nisotel, J.N. Fishbein, J. Macdonald, J. Stagl, I. Lennes, J.S. Temel, S.A. Safren, W.F. Pirl, ''A Systematic Review of Adherence to Oral Antineoplastic Therapies,'' The Oncologist, Vol. 21, Issue.3, pp.354-76, 2016.

[15] M.A. Feitelson, A. Arzumanyan, R.J. Kulathinal, S.W. Blain, R.F. Holcombe, J. Mahajna, M. Marino, M.L. Martinez-Chantar, R. Nawroth, I. Sanchez-Garcia, D. Sharma, N.K. Saxena, N. Singh, P.J. Vlachostergios, S. Guo, K. Honoki, H. Fujii, A.G. Georgakilas, A. Bilsland, A. Amedei, E. Niccolai, A. Amin, S.S. Ashraf, C.S. Boosani, G. Guha, M.R. Ciriolo, K. Aquilano; S. Chen, S.I. Mohammed, A.S. Azmi, D. Bhakta, D. Halicka, W.N. Keith, S. Nowsheen, ''Sustained Proliferation in Cancer: Mechanisms and Novel Therapeutic Targets,'' Seminars in Cancer Biology, Vol. 35 Suppl, 25-54, 2015.

[16] G. Kuş, ''Cytotoxic and Apoptotic Function of Carmofur in Hepatocellular Carcinoma,'' Kocatepe Medical Journal, Vol. 18, pp.55-60, 2017.

[17] E. Çömlekçi, C. Vejselova Sezer, H.M. Kutlu, ''Handling a Novel Nanoscale D-Erythro-Mapp in Lung Cancer Treatment: an in Vitro Cytotoxicity Research,'' International Journal of Scientific Research in Biological Sciences, Vol.7, Issue.4, pp.14-18, 2020.

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