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EVALUATION OF CYTOTOXICITY OF SOME AGENTS AS A SCREENING TEST IN ANTITUMORAL ACTIVITY USING VERO, HE-LA AND HEP-2 CELL CULTURES

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Abstract

Tissue culture tests which presents in the in-vitro antitumoral activity tests that permit to investigate thoroughly the different effects of the compounds in specific doses which interacts with DNA have been researched by many authors.

In this study, from the antitumoral and antifungal agents namely, bleomicyn, mitomycin C, daunorubicin, cyclophosphamide, iphosphamide, vinorelbine, vinblastine, gemcitabine, cytarabine, carboplatin, cisplatin, flukonazole, and terbinafine were investigated by using Vero, He-La and Hep-2 cell cultures. The cytopathogenic effects of tested materials were evaluated by cell culture microscopy.

While the maximum cytotoxicity was determined in daunorubicin in minimal dose at 0.098µµg/ml, lower effect was determined in vincristine, mitomycin C, bleomicyn, gemcitabine, carboplatin, cytarabine, cisplatin, and vinblastine, and cytarabine in Vero, He-La and Hep-2 cell line cultures. No activities were observed with antifungal agents.

When the results were compared with the reports, the different cytotoxic activity in different cells and different doses may result to miss the possible antitumoral effect. For this reason, several cell line cultures have to be used. Cell culture tests can be used as a screening test in antitumoral activity screening for the small quantity of synthesized and expensive materials.

Key words: *Antitumoral activity, Cytotoxicity, Vero, He-La , Hep-2*

Antitumoral Aktivite Tarama Testi Olarak, Bazıı Ajanların Vero, He-La ve Hep-2 Hücre Kültürlerinde Sitotoksisitelerinin Değerlendirilmesi

DNA ile etkileşen bileşiklerin belirli dozlarda gösterdiği farklı etkilerin çok yönlü incelenmesine olanak tanıyan in-vitro antitumoral aktivite testleri arasında yer alan hücre kültürü testleri; birçok araştırıcı tarafından denenmektedir.

Çalışmada; antitumoral ve antifungal ajanlardan bleomisin, mitomisin, daunorubisin, siklofosfamid, ifosfamid, vinblastin, vinorelbin, gemistabin, sitarabin, karboplatin, sisplatin, flukonazol ve terbinafin, Vero, He-La ve Hep-2 hücre kültürlerinde araştırılmıştır. Sitopatojenik etki doku kültürü mikroskobu ile değerlendirilmiştir.

Vero, He-La ve Hep-2 hücre kültüründe en düşük dozda 0.098µg/ml'de en yüksek sitotoksisite daunorubisinde görülürken, bununla kıyasla daha az etki vinkristin, mitomisin C, bleomisin, gemsitabin, karboplatin, siderabin, cisplatin, vevinblastin' de görülmüştür. Antifungal ajanlarda sitotoksik etki görülmemiştir.

Yapılmış araştırmalarla kıyaslanan sonuçlarda maddelerin farklı hücrelerde farklı dozlarda sitotoksik etki göstermesi, olası antitumoral etkinin gözden kaçmasına neden olabileceğini ortaya koymuştur. Bu nedenle çeşitli hücre kültürlerinin kullanılması gereği görülmektedir. Miktarı az ve maliyeti yüksek olan maddeler için aynı zamanda hassas sonuçlar vermesi nedeniyle hücre kültürü testi antitumoral aktivite taramalarında ön tarama testi olarak seçilebilir.

Anahtar kelimeler: *Antitumör aktivite, Sitotoksisite, Vero, He-La, Hep-2*

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Introduction

The evaluation of the samples, such as cytotoxicity on tumor cells, necessitates screening of a great number of newly synthesis compounds or biologically active natural products. Animal models have always played an important role in drug evaluation, but with the development of a large number of cytotoxic drugs, animals models are too costly and the delay is too long for these models to be used for large-scale screening (1-3).

Major efforts were dedicated to the development of in-vitro assays based on a large panel of human cell cultures representing various tumor types. The requirement using this in-vitro system was first that the assay gives reproducible dose-response curves over a concentration range that includes the probable in-vivo effect of the drugs. Cell line culture tests with suitable tumor models which presents the in-vitro antitumoral activity tests that permit to determine the different effects of the compounds in specific doses which interacts with DNA have been researched by many authors (4-9).

In this study the cytotoxicity of bleomicyn, mitomycin C, daunorubicin, cyclophosphamide, iphosphamide, vinblastine, vinorelbine, gemcitabine, cytarabine, carboplatin, cisplatin, which are commonly used antineoplastic agents, and the antifungal agents of fluconazole, terbinafine were evaluated by using Vero, He-La and Hep-2 cell cultures.

Experimental

Tests Materials

In this study, bleomicyn, mitomycin C, daunorubicin, cyclophosphamide, iphosphamide, vinblastine, vinorelbine, gemcitabine, cytarabine, carboplatin, cisplatin, flukonazole and terbinafine were tested. Stock solutions were dissolved in dimethylsulphoxide (DMSO) at a final concentration of 50 µg/ml, and sterilized by filtration using 0.22 µm membrane (Sartorius, Germany).

Cell line and growth condition

He-La (Human cervix epithelial carcinoma), Hep-2 (Human larynx epidermidis carcinoma) and Vero (African green monkey kidney) cell lines used in this study were obtained from University of Ankara Faculty of Veterinary Department of Virology.

The cultures of the cells were grown in EMEM (Eagle's Minimal Essential Medium) enriched with 10% fetal calf serum (FCS) (Biochrom, Germany), 100 mg/ml streptomycin and 100 IU/ml penicillin in a humidified atmosphere of 5% CO_2 at 37 °C. The cells were harvested using Trypsin (Bibco Life Technologies, UK) solution.

Cytotoxicity Test

Media (EMEM) was placed into each 96 wells of the microplates (Greiner^{R}, Germany). Compound solutions were added first raws of the microplates and two-fold dilutions of the compounds were made by dispensing the solutions to the remaining wells. Two-fold dilution of each material was obtained according to Log_2 on the microplates.

The cytotoxicity of tested materials were determined by cell culture microscopy (X400) based on cellular morphologic alteration. Several concentrations of each sample were placed in contact with confluent cell monolayers and incubated in 5% CO_2 at 37 °C for 48 hours. After the end of this time the cells were evaluated and cytopathogenic effect (CPE) determined by comparing treated and controlling untreated cultures using cell culture microscope(10).

Staining of the cells

After content of the microplates were removed, the cell lines were lavaged with DMEM. Then 50μl methanol was added to each well and incubated for 30 seconds for fixation of the cells. After incubation period methanol was removed and 50μl Giemsa was added to the wells. After 2 minutes the stain was removed and the wells were lavaged with PBS and evaluated and photographed by cell culture microscope.

Results and Discussion

The doses of cytotoxicity determined by in-vitro cell culture method using Vero, He-La and Hep-2 cell culture of tested compounds are presented in Table I. While the maximum cytotoxicity was determined in daunorubicin in minimal dose at 0.098 μg/ml, lower effect was determined in vincristine, mitomycin C, bleomicyn, gemcitabine, carboplatin, cytarabine, cisplatin, and vinblastine in Vero, He-La and Hep-2 cell cultures.

In He-La and Hep-2 cell culture, bleomicyn, vinblastine, vincristine, gemcitabine, cytarabine, carboplatin, and cisplatin, were found cytotoxic at 0.049-0.098 μg/ml dosages, respectively. Also, mitomycin C was found cytotoxic at 0.195-0.39 μg/ml dosages. Cytotoxicity for cyclophosphamide at 12.5 μg/ml, and for iphosphamide at 3.125, 6.25 μg/ml dosages were determined. No cytotoxicity were observed with antifungal agents fluconazole and terbinafine at applied dosages.

TABLE 1. The Vero, He-La and Hep-2 cell cultures the maximum cytotoxic dosages (μg/ml) of tested compounds.

Materials	Vero	He-La	Hep-2
Bleomicyn	1.56	0.049	0.098
Mitomycin C	1.56	0.195	0.39
Daunorubicin	0.098	0.098	0.098
Cyclophosphamide	12.5	12.5	12.5
Iphosphamide	6.25	3.125	6.25
Vinblastine	0.195	0.049	0.098
Vincristine	0.098	0.049	0.098
Gemcitabine	1.56	0.049	0.098
Cytarabine	0.78	0.049	0.098
Carboplatin	1.56	0.049	0.098
Cisplatin	0.39	0.049	0.098
Fluconazole	-	-	-
Terbinafine	-	-	-

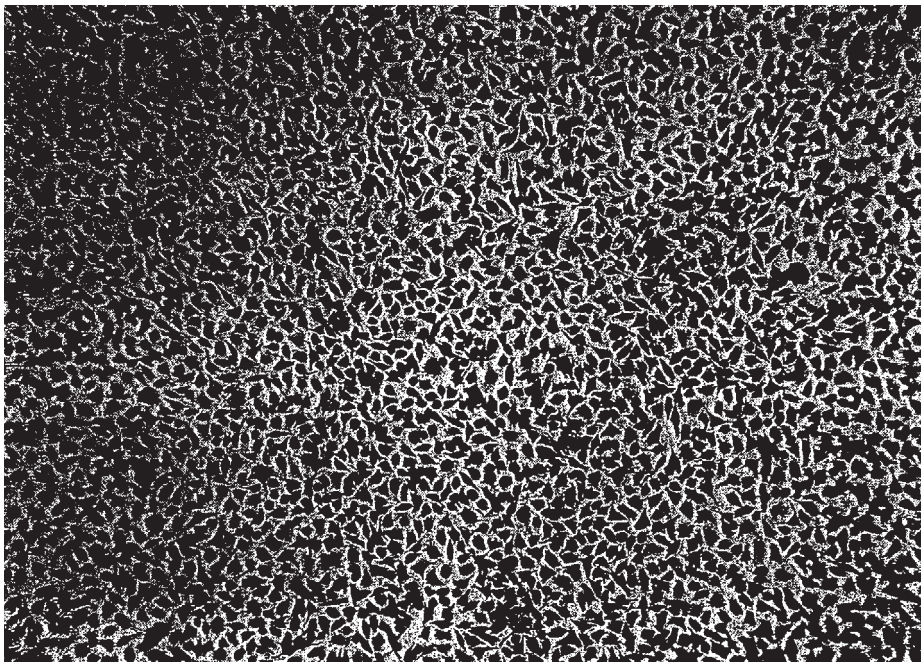

Figure 1. Giemsa stained He-La cell culture (x400)

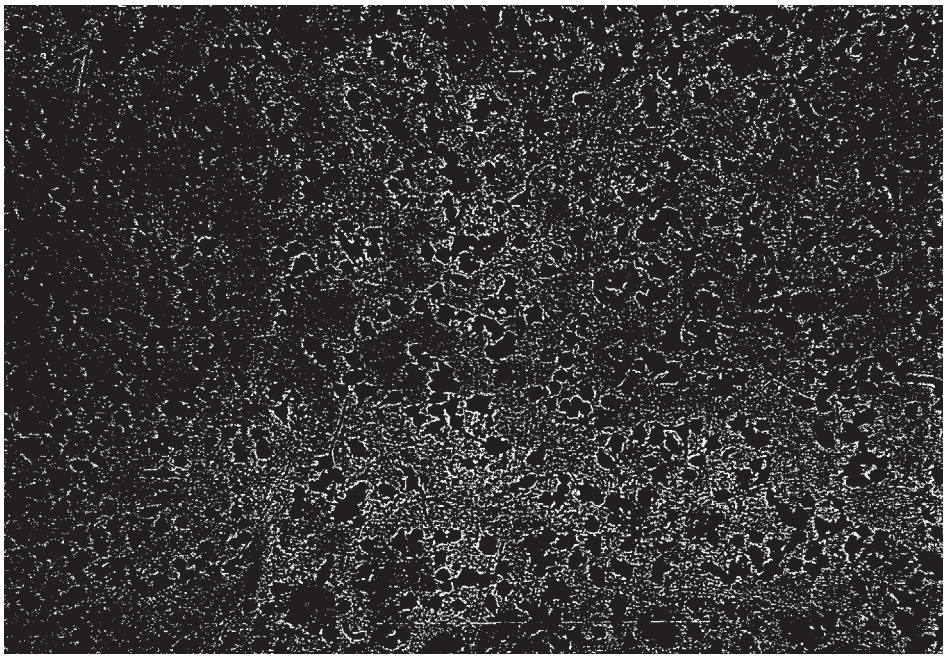

Figure 2. CPE of He-La cell culture with mitomycin C at 0.049 µg/ml dosage, after 24h incubation (Giemsa stained, x400)

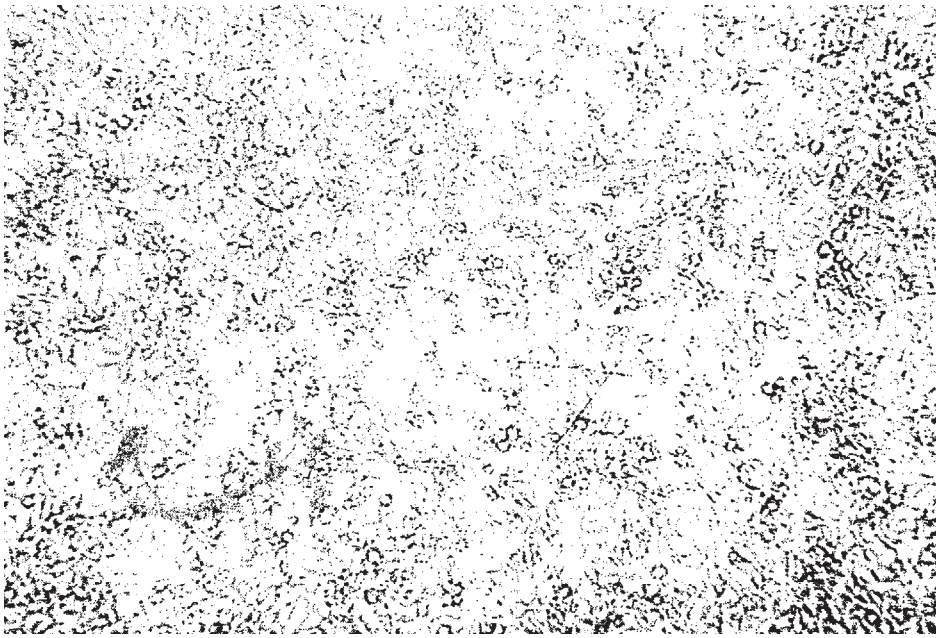

Figure 3. Vero cell culture

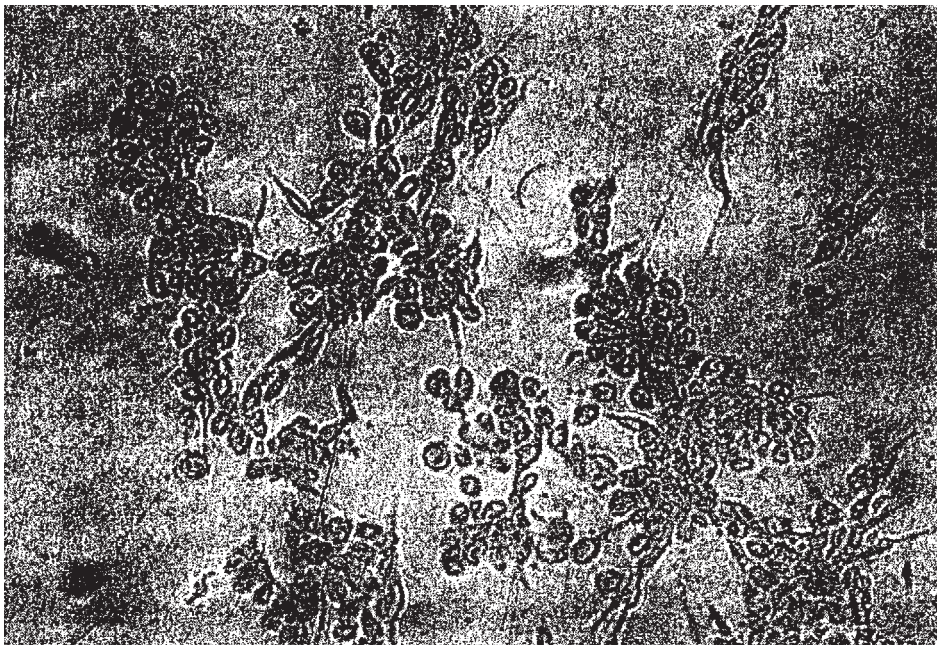

Figure 4. CPE of Vero cell culture with daunorubicin at 0.098 μg/ml dosage, after 24h incubation (x400)

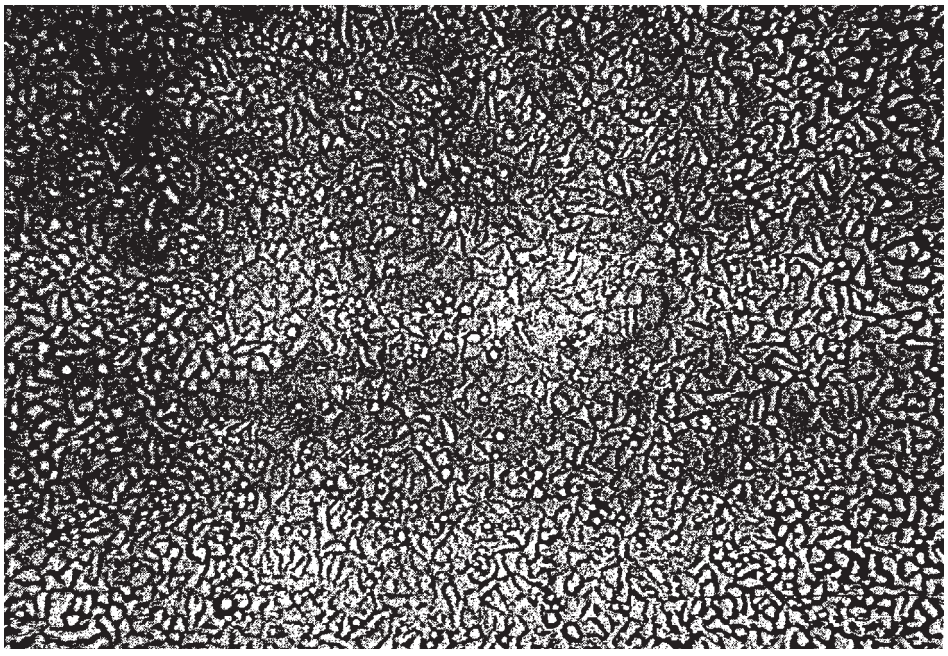

Figure 5. Hep-2 cell culture

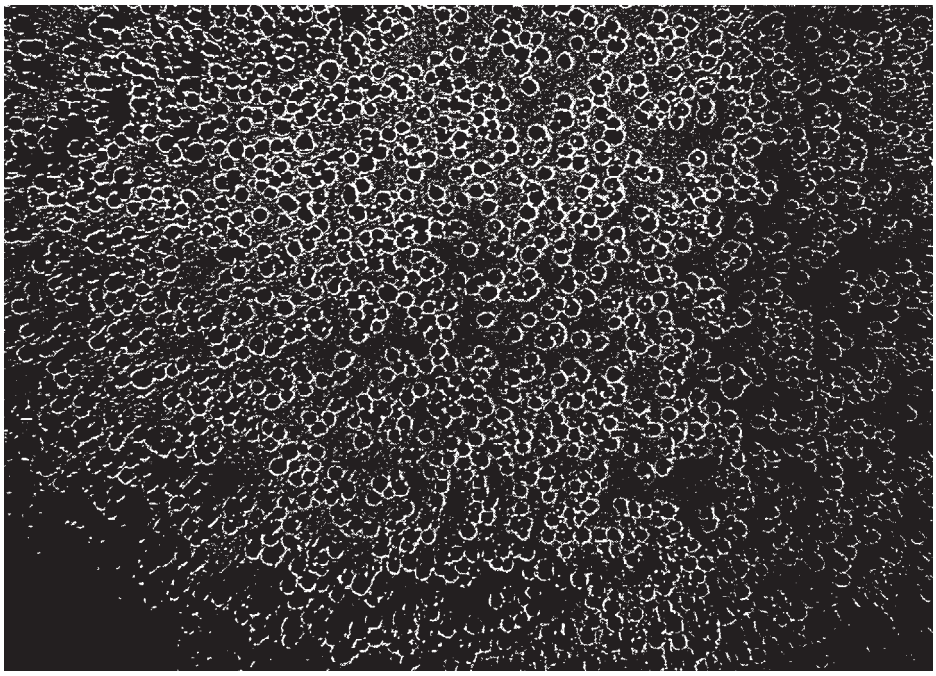

Figure 6. CPE of Hep-2 cell culture with bleomicyn at 0.098 μg/ml dosage, after 24h incubation (x400)

Cell line culture tests which presents in the in-vitro screening tests that permit to determine the different effects of the compounds in specific doses which interacts with DNA have been researched by many authors. The advantages like; repeatability, permission of control during every steps, ability of parametric comparisons of the materials, cost effectivity and no harmful results on livinthings like animal experiments are the reasons for preference of these tests (1).

Jacqueline, C., et al.(7) have determined different cytotoxicity for bleomicyn in different doses in their study with lymphocytes, keratinocytes and oral fibroblasts cell cultures. In our study, different cytotoxicity for bleomicyn and mitomycin C, in different doses were determined in He-La and Hep-2 cell cultures. The results of the different doses indicate that the difference in cell culture depends on the genetical factors of the cell culture type and the behavior of the compounds in that cell lines. Likely to this report Crook, T.R., et al.(9) have researched the different effect of cyclophosphamide at different times and concentrations in K562 human chronic myeloid cell cultures and it was determined that the cyclophosphamide effect was much higher in the hepatocyte added cell cultures. Cooper, J.K., et al.(11) who have also researched the cyclophosphamide cytotoxicity in human fibroblasts cell cultures and in addition they have used the hepatocyte mycrozomes and cofactor added test systems. Cytotoxicity doses were determined after a longer time incubation in the cell lines that does not consist additional activators.

However, Westwrdorf, J., et al.(12) have reported that the activity decreases with the addition of hepatocytes in V79 hamster cell cultures in their study with adriamicin and daunorubicin that have effective cytotoxicity. In agreement with this study, the maximum cytotoxicity was determined in daunorubicin in Vero, He-La, and Hep-2 cell culture. Pools, B.L., et al. (13) have indicated that the test systems should be designed according to the metabolic properties of the compounds that need metabolic activators. In our study, the lower doses than expected for cyclophosphamide and iphosphamide are also in agreement with the prior studies indicating that the test conditions, which need metabolic activators, should not be ignored. Therefore with the idea that applying this for all the tested compounds is not cost effective, it seems necessary to consider the compounds origins to choose the test systems.

Kruczynski, A., et al. (14) have used the pre-clinical tumor models to determine the antimicotic activity of vinfluinin which is a newly synthesized compound of vinorelbin. For vinflunin, higher activity doses were determined than the doses for vinblastine and vinorelbin. While similar toxicity doses were determined for vinblastin and vincristine, the cytotoxicity was much lower for vinblastine.

In another research, Krett, N.L., et al. (15) were tested cytarabine and gemcitabine which are nucleoside analogs in multiple myeloma cell cultures. The greater cytotoxicity of gemcitabine was determined in multiple myeloma cell cultures and the results suggested that gemcitabine is a potent nucleoside analog in multiple myeloma cell cultures.

Lidor, Y.J., et. al.(16) have researched the cytotoxicity of platinum coordination complexes in four different cell line (OVCA 420, 429, 432, 433) series and determined that cisplatin was mostly sensitive to OVCA 432 cell cultures. In our study, for carboplatin and cisplatin cytotoxicity was determined in He-La and Hep-2 cell cultures at similar dosages.

It was also reported by Su, W., et al. (17) that carboplatin required a 10 times higher drug concentration than cisplatin to induce a similar degree of growth inhibition on leukemic cell cultures (CEM, HL60, K562 and U937). However, cytotoxicity for cisplatin and carboplatin were determined at the same applied dosages on hematopoietic progenitor cell cultures. Cytotoxicity observed with identical dosages of carboplatin and cisplatin of the different cell cultures in our study. This would suggest similarities in the mechanisms of the action of the compounds.

Consequently, it was determined that, in tested materials the compounds have shown different activity doses in different cell cultures. But if the aim was to consider a general data about the activities of the tested compounds, the first screening could be searched with one cell line series and different cell line series should be used for further studies and it is not necessary to use different cell cultures when studying with the structure relationship of compounds are known.

In in-vitro antitumoral activity researches besides having reports about the activities of the compounds in different chemical structures, the increase and decrease in the activity by adding

different chemical groups may also be detected. For this purpose, it is known that many in-vivo and in-vitro studies have been studying. Most of the recent studies have been in research for the increasing of the sensitivity of these test systems.

Conclusion

When the results were compared with the reports, the different cytotoxic activity in different cells and different doses may result to miss the possible antitumoral effect. For this reason, several cell line cultures have to be used. Cell cultures test can be used as a screening test in cytotoxic activity screening for the small quantity of synthesized and expensive materials.

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