Available online on 15.02.2024 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Research Article

Gallic Acid and Doxorubicin Treatment HeLa cell lines by MTT assay

Serap Mutlu ÖZÇELIK OTÇU *, Murat AKKUŞ

MD, Dicle University, Medical Faculty, Department of Histology and Embryology, Diyarbakır, Turkey

Article Info:

Article History:

Received 24 Dec 2023
Reviewed 17 Jan 2024
Accepted 03 Feb 2024
Published 15 Feb 2024

Cite this article as:

Özçelik Otçu SM, Akkuş M, Gallic Acid and Doxorubicin Treatment HeLa cell lines by MTT assay, Journal of Drug Delivery and Therapeutics. 2024; 14(2):247-251

DOI: http://dx.doi.org/10.22270/jddt.v14i2.6459

*Address for Correspondence:

Serap Mutlu ÖZÇELİK OTÇU, Dicle University, Medical Faculty, Department of Histology and Embryology, Diyarbakır, Turkey

Abstract

Objective: Cervical cancer, as it is medically known, occurs in the cells in the lower part of the uterus called the cervix and is one of the most common gynecological cancers in the world. Hormonal and genetic factors play a major role in the development of endometrial cancer. Chemotherapeutic drugs are very effective in treatment. However, serious side effects and the development of drug resistance limit the use of these drugs. In our study, the cytotoxic effects of Gallic acid (GA), which is found in many popular foods, on HeLa cells were investigated.

Methods: In our study, different doses of GA and Dox were applied to the cells for 48, 72 h and cytotoxicity levels were determined by the MTT method. All results were analyzed statistically.

Results: It was observed that cytotoxicity by MTT was at the highest level in the GA and Dox administered group. IC50 was determined and it was found that GA IC50 was 242.4 and Dox IC50 was 124.6 for 48 h.

Conclusion: As a result of the study, it was shown that the highest cytotoxic effect occurred with GA application and this may support Dox application with a synergistic effect.

Keywords: Servics cancer, Gallic acid, Doxorubicin, MTT

INTRODUCTION

Cervical cancer is an important health problem that threatens the lives of approximately 500,000 women worldwide every year. Smoking, exposure to human papillomavirus and immune system disorders are among the risk factors for cervical cancer. Although people with cervical cancer can be treated when the tumor is in its early stages, long-term morbidity from treatment is common.[1]

Cervical cancer is one of the most common malignant tumors affecting women.[2] Cervical cancer is a significant cause of morbidity and mortality in women and is currently the second most common malignant disease in women worldwide.[3] Increasing routine screening of the cervix plays a significant role in improving the disease prognosis by enabling the diagnosis of a large number of early-stage cervical cancer patients.[4] In patients diagnosed with cervical cancer that has progressed to an inoperable stage or has recurred in other organs, commonly used anti-cancer chemotherapy, cisplatin-based chemotherapy drugs, is used.[5] For this reason, there is a trend towards the discovery of new and more effective agents for patients with cervical cancer, as in many types of cancer.

Gallic acid is a trihydroxybenzoic acid that can be found in a variety of herbal medicines, foods, and beverages. A number of studies have demonstrated the potential anticancer activity of Gallic acid and its derivatives both in vivo and in vitro. In addition to its antitumor potential against cancer, it also plays a functional role in diabetes, hypercholesterolemia, cardiac hypertrophy, fibrosis and hypertension due to its excellent oxidation resistance.[6,7]

Doxorubicin (Dox), sold under the trade name Adriamycin, is a chemotherapy drug. Doxorubicin is known as an inhibitor that prevents DNA synthesis by interfering with DNA strands. Although doxorubicin is toxic to both cancer and normal cells, the mechanism of cell death may not be similar in both cells.[8] At the same time, Dox binds to the cell membrane and changes the physical properties of the membrane, thus impairing the membrane function of the cell.[9]

In this study we conducted with the HeLa cell line obtained from cervical cancer cells, the effects of Doxorubicin and Gallic acid in cell proliferation/migration and possible synergistic or antagonist effects were investigated.

MATERIAL AND METHODS

Cell Culture

10, 25, 50, 75, 100, 250, 500, 750 and 1000 µM/nM concentrations of GA/Dox applied to HeLa cell line and human skin keratinocyte cell line HaCat as healthy cell line were used. Dox and GA were obtained commercially and their concentrations were prepared in the appropriate solvent and applied to HeLa and HaCat cells.

MTT assay

In order to determine the IC50 doses in the groups where we will apply Doxorubicin and Gallic acid, HeLa and HaCaT cell lines were inoculated with the help of automatic multipipettes in 96-well culture dishes at a cell count of 3000-5000/well, respectively. After approximately 16 hours, serial dilutions were made in the dose range of doxorubicin 10-1000 nM and Gallic acid 10-1000 µM and incubated in plates with 9 different concentrations for 48, 72 h. When we analyzed cell viability in

the MTT test, the outer wells of the culture dishes were excluded to reduce trial error. Each agent and vehicle control group was set to consist of 6 wells. MTT test was applied to analyze the cells remaining alive after incubation. According to the results of the MTT analysis, the effects of GA and Dox at different concentrations were calculated by using the SPSS 20 statistical package program and probit analysis for the IC50 values for each tumor cell line and chemotherapy agent in the control and experimental groups.

Statistical analysis

In the study, the difference between the live cell ratios determined by the MTT test. In the comparison of the two groups, depending on the homogeneity of the data, the independed sample T test or the Mann Whitney U test were used. Analyzes were made with SPSS 20, (IBM, USA) program, and $p \leq 0.05$ was used.

RESULTS

After HeLa and HaCat cells grown in T75 flasks became 90% confluent, they were treated with trypsin and removed and inoculated into 96-well culture dishes at 3000 cells/well to perform MTT analyses. In order to determine the IC50 doses of GA and Dox, 10-1000 µM amounts of GA and 10-1000 nM Dox were applied to these cultures and incubated for 48 hour. At the end of the incubation period, drugs were removed from the cultures by pipetting and spectrophotometric measurement was performed according to the ratio of tetrazolium salts formed by adding MTT solution. 48 hours of Dox application, the IC50 value was found to be 124.6 nM. Significant decreases in cell proliferation were observed as the dose increased. In the application that started with 100,000 cell cultivation, the average number of cells was obtained as 65,000 for all time periods in the Dox application at a concentration of 1000 nM. As a result of 48 and 72 hours of treatment, the number of cells were the lowest with 74.79 and 68.39. After the IC50 value was found as a result of the statistical analysis, it was determined that the cell viability decreased significantly after 25 nM concentration Dox application compared to the vehicle group (Table 1).

Table 1: Dox application to HeLa cells for 48, 72 hours.

HeLa-3-Dox		N	Cell viabilty (%)	Std.dev.	Std. error	95% confidence interval		Min.	Max
						Under line	Upper line		
48 h	Vehicle	6	100,0000	6,56478	2,88063	94,2120	106,7880	92,56	105,61
	10,00	6	97,4424	4,09363	1,77167	93,1492	100,7435	92,30	100,73
	25,00	6	92,1621	2,36872	0,38744	90,5245	95,6011	89,81	97,42
	50,00	6	74,7989	2,52741	1,13140	76,1476	82,4502	76,85	84,83
	75,00	6	72,0333	5,76647	2,33904	63,0206	77,0460	61,96	77,67
	100,00	6	63,9512	1,18259	0,48751	60,6954	68,2069	65,24	67,85
	250,00	6	28,5875	1,14197	0,49498	17,3923	20,7828	17,64	19,81
	500,00	6	11,9202	0,84545	0,33360	11,8970	14,6635	12,92	13,98
	750,00	6	9,6684	0,27619	0,12908	10,9280	10,4888	9,73	11,44
	1000,00	6	7,8696	0,92252	0,36620	8,6040	9,4353	7,56	9,06
72 h	Vehicle	6	100,0000	4,37747	1,78709	95,4061	104,5939	94,86	107,21
	10,00	6	107,0373	4,13062	1,68632	102,7025	111,3721	103,38	112,73
	25,00	6	97,2106	2,87885	1,17528	94,1894	100,2318	92,40	101,40
	50,00	6	68,3932	4,37524	1,78618	63,8016	72,9847	62,97	74,03
	75,00	6	53,2594	4,57535	1,86788	48,4579	58,0609	47,23	60,32
	100,00	6	46,0141	2,57403	1,05085	43,3129	48,7154	43,39	49,24
	250,00	6	11,4848	0,85459	0,34888	10,5880	12,3817	10,28	12,53
	500,00	6	6,0946	0,30940	0,12631	5,7699	6,4192	5,66	6,37
	750,00	6	4,2396	0,33113	0,13519	3,8921	4,5871	3,79	4,76
	1000,00	6	3,5575	0,21186	0,08649	3,3351	3,7798	3,28	3,79

As a result of the data obtained by applying MTT test on HeLa cell series after GA application, % cell viability and IC50 value calculated using probit analysis and statistical data compared to the control are given in Table 2. On the other hand, a decreased cell viability was detected in the HaCaT cell line only as the GA dose increased. The survival rate of the HeLa cell line after GA application was found to be 70 on average compared to all times. The survival rate of the HaCaT cell line after GA application. Significant differences were detected between GA and HaCaT and HeLa cell lines. The IC50 value was obtained as 242.4 µM/L in the 48 h and 236.4 µM/L in the 72 h for GA application (Table 2).

Table 2: GA application to HeLa cells for 48, 72 hours.

HeLa-GA		N	Cell viabilty (%)	Std.dev.	Std. error	95% confidence interval		Min.	Max
						Under line	Upper line		
48 h	Vehicle	6	100,000	3,8846	1,6116	96,828	100,231	90,07	100,24
	10 uM	6	95,597	6,7870	2,8414	93,239	98,928	90,13	102,3
	25 uM	6	94,209	5,6378	2,2743	90,324	96,090	89,61	100,8
	50 uM	6	92,356	5,5622	1,8847	90,417	100,458	87,49	99,7
	75 uM	6	89,716	4,9203	2,0301	85,500	92,834	82,0	99,6
	100 uM	6	75,394	4,1242	2,1850	68,807	77,985	72,8	88,4
	250 uM	6	58,418	6,1733	2,8126	47,995	60,840	44,7	70,6
	500 uM	6	19,278	0,6629	0,1969	19,792	17,769	16,6	22,7
	750 uM	6	12,789	0,5252	0,2235	13,234	11,345	12,8	18,2
	1000 uM	6	10,593	0,5245	0,2127	10,054	10,137	10,6	12,4
72 h	Vehicle	6	100,0000	5,69610	2,32542	94,0223	105,9777	92,53	109,29
	10 uM	6	105,9298	5,84306	2,38542	99,7979	112,0617	97,97	112,29
	25 uM	6	108,7830	5,26179	2,14812	103,2611	114,3050	102,29	116,86
	50 uM	6	102,9636	3,86716	1,57876	98,9052	107,0219	96,69	106,80
	75 uM	6	99,2039	2,87622	1,17421	96,1855	102,2223	94,66	102,65
	100 uM	6	86,5115	3,29477	1,34508	83,0538	89,9691	82,71	91,04
	250 uM	6	48,4630	9,13771	3,73046	38,8736	58,0525	34,66	62,31
	500 uM	6	10,3778	0,44761	0,18274	9,9081	10,8475	9,82	10,94
	750 uM	6	8,3180	0,34747	0,14185	7,9534	8,6827	7,76	8,72
	1000 uM	6	9,4031	0,29955	0,12229	9,0887	9,7174	9,14	9,98

Dox and GA were also applied to the healthy cell series and their cytotoxic effects were analyzed. After the application of both agents, the % cell viability resulting from the MTT test in the HaCaT cell series and the IC50 values calculated using probit analysis and the statistical analyzes obtained when compared to the control are given in Table 3 and 4. IC50 value could not be obtained by applying Dox to the HaCaT cell line for 48, 72 hours. When compared to the vehicle control group, it was determined that significance was achieved after 48, 72 hours of Dox application and 50 nM concentration.

Table 3: Dox application to HaCaT cells for 48, 72 hours.

HaCaT-Dox		N	Cell viabilty (%)	Std.dev.	Std. error	95% confidence interval		Min.	Max
						Under line	Upper line		
48 h	Vehicle	6	100,00	5,037	2,120	94,65	100,35	90,3	104,71
	10 nM	6	96,92	5,897	2,229	90,64	101,21	87,51	102,54
	25 nM	6	91,98	7,365	3,753	82,18	101,77	82,85	100,52
	50 nM	6	85,13	4,568	2,654	81,38	96,09	76,13	90,26
	75 nM	6	84,22	6,411	3,126	72,43	92,01	70,62	90,43
	100 nM	6	82,00	5,291	2,276	70,34	84,66	70,60	82,67
	250 nM	6	75,98	5,424	3,533	70,25	82,72	66,64	84,55
	500 nM	6	76,59	7,086	3,603	70,06	85,06	65,28	80,18
	750 nM	6	69,97	5,769	2,459	66,85	80,00	63,46	82,62
	1000 nM	6	49,0831	2,18907	0,89368	46,7858	51,3804	46,25	52,24
72 h	Vehicle	6	100,0000	4,54117	1,85393	95,2343	104,7657	93,69	106,54
	10 nM	6	131,0009	19,06617	7,78373	110,9921	151,0096	99,73	152,39
	25 nM	6	91,1959	29,30584	11,96406	60,4413	121,9505	69,17	146,61
	50 nM	6	96,1419	19,76228	8,06792	75,4026	116,8811	78,33	134,18
	75 nM	6	84,1246	9,59846	3,91856	74,0517	94,1976	70,22	96,70
	100 nM	6	81,8479	9,19165	3,75248	72,2018	91,4939	71,52	95,58
	250 nM	6	73,2264	4,71484	1,92482	68,2784	78,1743	67,84	80,40
	500 nM	6	72,0880	4,37986	1,78807	67,4916	76,6843	63,97	76,70
	750 nM	6	60,1290	3,45439	1,41025	56,5038	63,7542	54,97	63,92
	1000 nM	6	49,0831	2,18907	0,89368	46,7858	51,3804	46,25	52,24

As a result of GA application, it was observed that there was a decrease in HaCaT cells depending on the dose increase. In the application that started with 100,000 cell cultivation, the number of cells was found to be 42.81 in the 48-hour GA

application at a concentration of 750 µM and 7 in the 72-hour application (Table 4). As a result of statistical analysis, it was determined that cell viability decreased significantly after 500 µM GA application (Table 4).

Table 4: GA application to HaCaT cells for 48, 72 hours.

HaCaT-GA		N	Cell viabilty (%)	Std.dev.	Std. error	95% confidence interval		Min.	Max
						Under line	Upper line		
48 h	Tasıt	6	100,00	9,990	5,483	90,46	108,54	96,12	111,84
	10 uM	6	100,32	2,710	2,510	100,46	104,27	100,97	118,32
	25 uM	6	98,93	5,515	1,667	100,01	100,75	104,86	110,83
	50 uM	6	96,55	4,440	2,401	101,90	100,19	100,11	114,54
	75 uM	6	100,44	5,579	1,254	100,61	102,28	105,32	110,84
	100 uM	6	92,31	3,989	1,246	98,26	110,46	107,41	112,54
	250 uM	6	96,77	2,942	1,322	96,66	112,88	115,66	114,74
	500 uM	6	97,10	7,999	3,547	98,63	104,58	96,53	106,56
	750 uM	6	42,81	1,739	2,116	36,90	24,72	20,32	23,95
	1000 uM	6	34,76	3,867	1,275	24,76	20,76	20,43	20,12
72 h	Tasıt	6	100,0000	6,16275	2,51593	93,5326	106,4674	93,20	106,89
	10 uM	6	122,4544	13,17698	5,37948	108,6261	136,2828	110,63	145,18
	25 uM	6	124,4985	14,14616	5,77515	109,6531	139,3440	109,62	145,84
	50 uM	6	133,1994	18,45010	7,53222	113,8372	152,5615	106,66	152,78
	75 uM	6	122,2516	11,34963	4,63347	110,3409	134,1623	105,33	139,16
	100 uM	6	108,2568	5,12892	2,09387	102,8743	113,6392	102,27	115,41
	250 uM	6	104,2145	4,93256	2,01371	99,0381	109,3909	96,62	111,12
	500 uM	6	9,4473	0,36882	0,15057	9,0602	9,8343	9,07	10,01
	750 uM	6	7,6749	0,16072	0,06561	7,5063	7,8436	7,49	7,92
	1000 uM	6	8,3869	0,26002	0,10615	8,1141	8,6598	8,06	8,77

DISCUSSION

This study also showed that Gallic acid and Doxorubicin significantly suppressed the proliferation of HeLa cells and accelerated their apoptotic processes, which is consistent with the results of previous studies. Previous studies have revealed that GA inhibits cell proliferation and invasion of different tumors such as small cell lung cancer.[10]. It has played an important role especially in the treatment of breast and colon cancer. The occurrence of colon cancer is closely associated with the abnormal expression of multiple genes. Therefore, regulating the expression of certain key genes such as SRC during tumorigenesis to inhibit malignant transformation is an effective tool to control tumor growth. Although chemotherapeutic drugs are very effective, serious side effects and the development of drug resistance limit the use of these drugs. The use of natural products with anticancer activity may help partially overcome these problems. A number of studies investigated the cytotoxic effect of GA in various cell lines and showed that IC50 for Calu-6, A 549 and HeLa.[11] These results indicate that Gallic acid induces cell death in tumor cells with relatively high selectivity. In our study, it was observed that gallic acid treatment decreased the growth of HeLa cells in a dose-dependent manner with the IC50 value in 48 hours. The strong effect of gallic acid treatment seen in our study is similar to that reported by Sánchez-Carranza et al. [12] They reported that gallic acid enhanced the cytotoxic effect of Paclitaxel in A2780 cells and A2780AD cells through treatment. They argued that the inhibition of proliferation and the arrest of the G2/M phase might be due to the ROS-mediated inhibition of kinases regulated by excessive ROS production by Gallic acid and by extracellular signals triggered by Paclitaxel.[12]

Chemotherapeutic drugs have a very important place in cancer treatment and although they are very effective in treatment, the use of these drugs is limited due to their side effects such as damage to vital organs such as the heart and liver, and the emergence of drug resistance over time. For this reason, every natural product with anticancer activity is the focus of attention and it is thought that it can help to overcome these problems, albeit partially. In this study, the ability of Gallic acid to be an alternative to chemotherapeutic agents such as doxorubicin in human HeLa cells.

Natural compounds such as flavonoids are recognized as important agents for cancer prevention and treatment because of their potential therapeutic effects and limited toxicity to healthy cells. In carcinogenesis, flavonoids interfere with intracellular signal transduction pathways, suppress proliferation, angiogenesis, metastasis, and increase apoptosis.[13,14] In this study, GA and DOX treatment decreased the growth of HeLa cells. The cytotoxic effect of GA can be explained by its pro-oxidant property, which is accepted as an apoptosis inducer in different cancer cell lines, especially HeLa cells.[15,16] The different IC50 values of GA on HeLa cell in our study can be explained by the fact that although MTT assay has been used in cancer research for 30 years,[15] it rarely gives a consistent IC50 value for a particular chemical. He et al. attributed this problem to differences between manufacturers and to formulas used by different laboratories.[17]

Searches continue in different sources for the effects of substances obtained from natural sources such as Gallic acid. Moss and marine algae are popular targets. Studies have shown that subjects given algae and caraway extracts increase dregeneration in vital organs such as liver, kidney and pancreas.[18,19]. In an in vitro study using gallic acid, it was reported thatcell viability decreased in cervical cancer cells.[20] It was alsoreported that natural antioxidants inhibited cell proliferation indifferent cancer cell lines (ovarian, breast, prostate, stomach, colon, nerve). In our gallic acid study, findings that will support the studies were obtained. Its effective role on the signaling mechanisms of both cell viability and proliferation has been demonstrated.[20-24]

CONCLUSİON

As a result, the cytotoxic effect of GA in cervical cancer was determined by the cell viability analysis test MTT. The findings were parallel to the anticancer findings of GA. In the HeLa cell line, the combination with the chemotherapy agent Dox caused cell death, and in addition to the antioxidant effect, it produced positive results. Also such as GA alternative antioxidants have a number of advantages such as lower dose and fewer advers effects. It is important to bring natural substances with antioxidant and anticancer activity, such as GA, to the clinic.

Acknowledgement: This study was a part of doctorate thesis of Serap Mutlu ÖZÇELİK OTÇU

Conflict of interest: No potential conflict of interest was reported by the author(s).

Ethic: Ethical approval is not required because commercially available cell lines are used as an in vitro study.

Financial Support: This study was founded by Dicle University Department of Scientific Research Projects (DÜBAP) with Project number (funding no: TIP. 23.027)

REFERENCES

1.Herzog TJ, Wright JD. The impact of cervical cancer on quality of life--the components and means for management. Gynecol Oncol.2007;107(3):572-577. https://doi.org/10.1016/j.ygyno.2007.09.019 PMid:179638262.

2. Franco EL, Schlecht NF, Saslow D. The epidemiology of cervical cancer. Cancer J. 2003;9(5):348-59. PMid:146903093 https://doi.org/10.1097/00130404-200309000-00004.

3. Ginsburg O, Bray F, Coleman MP, Vanderpuye V, Eniu A, Kotha SR, etal. The global burden of women's cancers: a grand challenge inglobal health. Lancet. 2017;389(10071):847-860. https://doi.org/10.1016/S0140-6736(16)31392-7PMid:278149654.

4. da Costa AM, Hashim D, Fregnani JHTG, Wiederpass E. Overall survival and time trends in breast and cervical cancer incidence and mortality in the Regional Health District (RHD) of Barretos,São Paulo, Brazil. BMC Cancer 2018;18: 1079. https://doi.org/10.1186/s12885-018-4956-7 PMid:30404614PMCid:PMC62230735.

5. Kumar L, Harish P, Malik PS, Khurana S. Chemotherapy and targeted therapy in the management of cervical cancer. Curr Probl Cancer 2018;42(2):120-128. https://doi.org/10.1016/j.currproblcancer . PMid:295303936.

6. Başaran SÖ, Kaplan Ö, Aşır F. Effect of Gallic Acid on distant organ stomach in ıntestinal ıschemia reperfusion ınjury. Journal of Drug Delivery and Therapeutics, 2023;13(5): 17-21. https://doi.org/10.22270/jddt.v13i5.60497.

7. Han D, Zhang QY, Zhang YL, Han X, Guo SB, Teng F, et al. Gallic acid ameliorates angiotensin II-induced atrial fibrillation by inhibiting immunoproteasome- mediated PTEN degradation in mice. Front Cell Dev Biol. 2020;8:594683. PMCid:PMC76734428. PMid:33251220 https://doi.org/10.3389/fcell.2020.594683

8. Takemura G, Fujiwara H. Doxorubicin-induced cardiomyopathy fromthe cardiotoxic mechanisms to management. Progress incardiovascular diseases, 2007;49(5):330-352. https://doi.org/10.1016/j.pcad.2006.10.002 PMid:173291809.

9. Alves AC, Magarkar A, Horta M, Lima J, Bunker A, Nunes C, Reis S. Influence of doxorubicin on model cell membrane properties: insights from in vitro and in silico studies. Scientific reports. 2017;7(1):6343. https://doi.org/10.1038/s41598-017-06445-zPMid:28740256 PMCid:PMC552471410 .

10. Phan AN, Hua TN, Kim MK, Vo VT, Choi JW, Kim HW, et al. Gallic acid inhibition of SRC-STAT3 signaling over comes acquiredresistance to EGF receptor tyrosine kinase inhibitors in advanced non-small cell lung cancer. Oncotarget. 2016;7(34):54702-13. https://doi.org/10.18632/oncotarget.10581 PMid:27419630PMCid:PMC534237411.

11. Park WH. Gallic acid induces HeLa cell death via increasing GSH depletion rather than ROS levels. Oncol Rep. 2017;37(2):1277-83. https://doi.org/10.3892/or.2016.5335 PMid:2803541712.

12. Sánchez-Carranza JN, Díaz JF, Redondo-Horcajo M, Barasoain I,Alvarez L, Lastres P, et al. Gallic acid sensitizes paclitaxel-resistant human ovarian carcinoma cells through an increase in reactive oxygen species and subsequent down regulation of ERK activation. Oncol Rep. 2018;39(6):3007-14. PMid:2969318913. https://doi.org/10.3892/or.2018.6382

13. Srivastava S, Somasagara RR, Hegde M, Nishana M, Tadi SK, Srivastava M, et al. Natural flavonoid interacts with DNA, arrests cell cycle and causes tumor regression by activating mitochondrial pathway of apoptosis. Sci Rep. 2016;(12)6:24049. https://doi.org/10.1038/srep24049 PMid:27068577PMCid:PMC482864214.

14. Ravishankar D, Rajora AK, Greco F, Osborn HMI. Flavonoids as prospective compounds for anti-cancer therapy. Int J Biochem Cell Biol. 2013;45:2821-2831. PMid:2412885715. https://doi.org/10.1016/j.biocel.2013.10.004

15. Barltrop JA, Owen TC, Cory AH, Cory JG. 5-(3-Carboxymethoxyphenyl)- 2-(4,5-dimethylthiazolyl)-3-(4-sulfophenyl) tetrazolium, inner salt (MTS) and related analogs of3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT) reducing to purple water-soluble formazans as cell viability indicators. Bioorg Med Chem Lett. 1991;1:611-4. https://doi.org/10.1016/S0960-894X(01)81162-816.

16. Umut S, Afşin Y, Özdemir İ, Özçelik SM, Öztürk Ş. Anticancer effect of Gallic acid against HeLa cervical cancer cell line. Dicle Medical Journal. 2023:365-73. https://doi.org/10.5798/dicletip.1360684 .

17. He Y, Zhu Q, Chen M, Huang Q, Wang W, Li Q, Huang Y, Di W. The changing 50% inhibitory concentration (IC50) of cisplatin: a pilot study on the artifacts of the MTT assay and the precise measurement of density-dependent chemoresistance in ovarian cancer. Oncotarget. 2016;7(43):70803-21. PMid:27683123 PMCid: PMC5342590117. https://doi.org/10.18632/oncotarget.12223

18. Irkin LC and Öztürk Ş, 2022. Ameliorative effects of Ulva rigida (C.Agardh, 1823) on cadmium-induced nephrotoxicity in Wistar albino rats. Pak Vet J, 42(3): 419-423. http://doi.org/10.29261/pakvetj/2022.051 .

19. Özturk Ş, Yayıntaş ÖT. Investigation of hepatotoxic effect of bryophytes (Homalothecium sericeum (HEDW) Schimp.) on rat liver. Fresenius Environmental Bulletin. 2021;30(2):1134-1146.

20. Özdemir İ, Ekinci C. Effect of Thymoquinone on ovarian carcinoma cell viability (OVCAR-3). Journal of Drug Delivery and Therapeutics. 2023;13(10):76-81. https://doi.org/10.22270/jddt.v13i10.6263 .

21. Sönmez PK, Albayrak G, Özkut M, Şen B, Toros P, Öztürk Ş, et al. Antiproliferative and apoptotic effects of the medicinal plants on breast cancer cell lines. Proceedings. 2017;1:1022. https://doi.org/10.3390/proceedings1101021 .

22. Toros P, Şen B, Sönmez PK, Özkut M, Öztürk Ş, et al. The Effect of herbal medicine on prostate cancer cells in culture. Proceedings.2017;1:1034. https://doi.org/10.3390/proceedings1101034 .

23. Özkut M, Albayrak G, Sönmez PK, Şen B, Toros P, Öztürk Ş, et al. Anticancer effects of oleocanthal and Pinus pinaster on breast cancer cell in culture. Proceedings. 2017;1:1020. http://doi:10.3390/proceedings1101020.

24. Toros P, Şen B, Sönmez PK, Özkut M, Öztürk Ş, Çöllü F, et al. The Effect of herbal medicine on colon cancer cells in culture. Proceedings. 2017;1:1033. https://doi.org/10.3390/proceedings1101033 .