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ORIGINAL ARTICLE

Assessment of Cytotoxicity Profiles of Different Phytochemicals: Comparison of Neutral Red and MTT Assays in Different Cells in Different Time Periods

Fenoliklerin Sitotoksisite Profillerinin Değerlendirilmesi: Farklı Hücrelerde Farklı Zaman Aralıklarında Nötral Kırmızı ve MTT Yöntemlerinin Karşılaştırılması

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ABSTRACT

Objectives: Phenolic compounds exhibit several health protective properties. Galangin, curcumin, pycnogenol, puerarin and ursolic acid are commonly used plant phenolics in folk medicine. The aim of our study was to evaluate the difference between neutral red uptake (NRU) and MTT assays using different plant phenolics (galangin, curcumin, pycnogenol, puerarin and ursolic acid) in healthy and cancer cells in different time periods.

Materials and Methods: In this study, the cytotoxic effects of these phenolic compounds were investigated by NRU and MTT assays in healthy (V79, Chinese hamster fibroblast cell line) and cancer [human cervix epithelial adenocarcinoma cell line Henrietta Lacks (HeLa) and human mammary carcinoma cell line (BT-474)] in 18, 24 and 48 h incubation periods.

Results: Our results demonstrated that galangin, curcumin, pycnogenol, puerarin and ursolic acid decreased cell viability of V79, HeLa and BT-474 cells in a dose-dependent manner in 18, 24 and 48 h incubation periods. However, the cell survival rate was much lower in 48 h incubation period. There was no difference between the results from NRU and MTT assays.

Conclusion: To decide which incubation period and which cytotoxicity study to be used, the cytotoxicity mechanism of the compound must be known.

Key words: MTT, neutral red, plant phenolics

ÖZ

Amaç: Fenolik bileşikler sağlığı koruyucu farklı özellikler gösterir. Galangin, kurkumin, piknogenol, puerarin ve ursolik asit halk tıbbında yaygın olarak kullanılan bitkisel fenoliklerdendir. Bu çalışmanın amacı, nötral kırmızı alım (NKA) ve MTT yöntemleri arasındaki farkı sağlıklı hücreler ve kanser hücrelerinde farklı bitkisel fenoliklerin (galangin, kurkumin, piknogenol, puerarin ve ursolik asit) farklı zaman aralıklarında farklı zaman aralıklarında belirlemektir.

Gereç ve Yöntemler: Bu çalışmada, bu fenolik bileşiklerin sitotoksik etkileri sağlıklı hücreler (Çin hamster fibroblast hücre hattı, V79) ve kanser [insan serviks epitelyal adenokarsinoma hücre hattı, Henrietta Lacks (HeLa) ve insan meme karsinoma hücre hattı (BT-474)] hücrelerinde 18, 24 ve 48 saatlik inkübasyon sürelerinde NKA ve MTT yöntemleriyle değerlendirilmiştir.

Bulgular: Bulgularımız galangin, kurkumin, piknogenol, puerarin ve ursolik asitin V79, HeLa ve BT-474 hücre canlılıklarını 18, 24 ve 48 saatlik inkübasyon sürelerinde doza bağımlı olarak azalttığını göstermiştir. Ancak en az hücre canlılık oranı 48 saatlik inkübasyon sonrası görülmüştür. NKA ile MTT yöntemlerinin sonuçları arasında fark görülmemiştir.

Sonuç: Sitotoksisite analizinde kullanılacak yöntem ve inkübasyon süresinin belirlenmesi için maddelerin sitotoksisite mekanizması bilinmelidir.

Anahtar kelimeler: MTT, nötral kırmızı, bitkisel fenolikler

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INTRODUCTION

Consumption of great amounts of fruits and vegetables rich in phenolic compounds has been associated with health benefits such as anti-artherogenic, antiinflammatory, anti-microbial, antioxidant, anti-thrombotic, and cardioprotective effects.[1,2] Due to the cytotoxicity profile of many phenolic compounds, it is suggested that these compounds can inhibit the survival of cancer cells. But the data about the cytotoxicity of these compounds in healthy cells are limited.

Galangin (3,5,7-trihydroxyflavone), is present at high concentrations in propolis and in an Indian root, *Alpinia officinarum*, which is a common spice in Asia.[3] It is suggested that galangin has antioxidant, antimutagenic, antiinflammatory, antiviral and anticancer properties.[4,5]

Curcumin (diferuloyl methane), the major yellow pigment from the rhizomes of turmeric (*Curcuma longa L.*), have gained increasing interest because of its chemopreventive properties against human cancers.[6] Turmeric, the powdered rhizome is commonly used as an antiseptic, antidote for poisoning, for treating respiratory disorders, some skin diseases, and as a household remedy for treating sprains and swellings caused by injury.[7,8]

Pycnogenol (PYC) is a standardized natural plant extract obtained from the bark of the French maritime pine *Pinus pinaster* (formerly known as *Pinus maritime*).[9] PYC has been used in European countries as a dietary food supplement. It has strong antioxidant activity and capacity to efficiently scavenge reactive oxygen and nitrogen species.[10]

Puerarin (daidzein-8-C-glucoside) is the main isoflavone derived from the root of *Pueraria lobata* (kudzu root).[11] In experimental models it is also suggested to be used in the prevention and treatment of cardiovascular diseases, diabetes, cancer and osteoporosis.[12]

Ursolic acid (3β-hydroxy-urs-12-en-28-oic acid) is a pentacyclic triterpenoid obtained from plants. It has long been used in traditional Chinese medicine because of its anti-inflammatory, anti-arthritic, cytostatic and anti-proliferative, hepatoprotective effects.[13]

Cytotoxicity assays are widely used in toxicology studies. The NR uptake (NRU) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assays are commonly used cytotoxicity assays to determine the cytotoxic properties of compounds. NRU assay has been used as an indicator of cytotoxicity in cultures of primary hepatocytes[14] and other cell lines.[15] Living cells take up the neutral red, which is concentrated within the lysosomes of cells.[16] MTT, a water soluble tetrazolium salt, is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by succinate dehydrogenase within the mitochondria. The formazan product is impermeable to the cell membranes and therefore it accumulates in healthy cells.[17]

The aim of our study was to evaluate the difference between NRU and MTT assays using different plant phenolics (galangin, curcumin, PYC, puerarin and ursolic acid) in healthy (V79, Chinese hamster fibroblast cell line) and cancer [human cervix epithelial adenocarcinoma cell line (HeLa) and human mammary carcinoma cell line (BT-474)] cells in different time periods (8, 24 and 48 h).

MATERIALS AND METHODS

Chemicals

The chemicals used in the experiments were purchased from the following suppliers: fetal calf serum (FCS), trypsin-EDTA, penicillinstreptomycin, from Biological Industries (Kibbutz Beit-Haemek, Israel), minimum essential medium (MEM), dimethyl sulfoxide, Triton X-100, phosphate buffered saline (PBS), ethanol, NR, MTT, galangin, curcumin (97%, purity), ursolic acid from Sigma (St Louis, USA), puerarin from Fluka (St. Gallen, Switzerland). PYC®, a registered trade mark of Horphag Research Ltd., (Geneva, Switzerland), was provided by Henkel Corporation (La Grange, IL, U.S.A.).

Cell culture

V79, HeLa and BT-474 cells were seeded in 75 cm^2 flasks in 20 mL MEM supplemented with 10% FCS and 1% penicillin-streptomycin and then grown for 24 h in an incubator at 37°C in an atmosphere supplemented with 5% CO_2.

Determination of cytotoxicity by NRU assay

The cytotoxicity of phenolic compounds was performed in V79, HeLa and BT-474 cell lines by NRU assay following the protocols described by Di Virgilio et al.[18] and Saquib et al.[19]. Following disaggregation of cells with trypsin/EDTA and resuspension of cells in the medium, a total of 10^5 cells/well were plated in 96 well tissue-culture plates. After 24 h incubation, the different concentrations of galangin, curcumin, PYC, puerarin and ursolic acid in medium were added. The cells were incubated for 18, 24 and 48 h at 37°C in 5% CO_2, then the medium was aspirated. The cells were then incubated for an additional 3 h in the medium supplemented with NR (50 µg/mL). The absorbance of the solution in each well was measured in a microplate reader at 540 nm and compared with the wells containing untreated cells. Results were expressed as the mean percentage of cell growth inhibition from three independent experiments. Cell viability was plotted as the percent of control (assuming data obtained from the absence of phenolic compounds as 100%).

Determination of cytotoxicity by MTT assay

MTT assay was performed by the method of Mosmann[17] with the modifications of Holst-Hansen and Brünner[20] and Kuzma et al.[21]. A total of 10^5 cells/well were plated in 96 well tissue-culture plates. After 24 h incubation, cells were exposed to the different concentrations of galangin, curcumin, PYC, puerarin and ursolic acid in medium for 18, 24 and 48 h at 37°C in 5% CO_2 in air. Then, the medium was aspirated and MTT (5 mg/mL of stock in PBS) was added (10 µL/well in 100 µL of cell suspension), and cells were incubated for an additional 4 h with MTT dye. At the end of incubation period, the absorbance of the solution in each well was measured in a microplate reader at 570 nm. Results were expressed as the mean percentage of cell growth from three independent experiments. Cell viability was

plotted as the percent of control (assuming data obtained from the absence of phenolic compounds as 100%).

RESULTS

Determination of cytotoxicity in V79 cell line

A concentration dependent decrease was seen in the survival of cells exposed to galangin, curcumin, PYC, puerarin and ursolic acid in all time periods in both cytotoxicity assays. But in 48 h incubation period, the cell survival is found much lower (Table 1) (Figure 1, 2).

Determination of cytotoxicity in HeLa cell line

A concentration dependent decrease was seen in the survival of cells exposed to galangin, curcumin, PYC, puerarin and ursolic acid in all time periods in both cytotoxicity assays. But in 48 h incubation period, the cell survival is found much lower (Table 2) (Figure 3, 4).

Determination of cytotoxicity in BT-474 cell line

A concentration dependent decrease was seen in the survival of cells exposed to galangin, curcumin, PYC, puerarin and ursolic acid in all time periods in both cytotoxicity assays. But in 48 h incubation period, the cell survival is found much lower (Table 3) (Figure 5, 6).

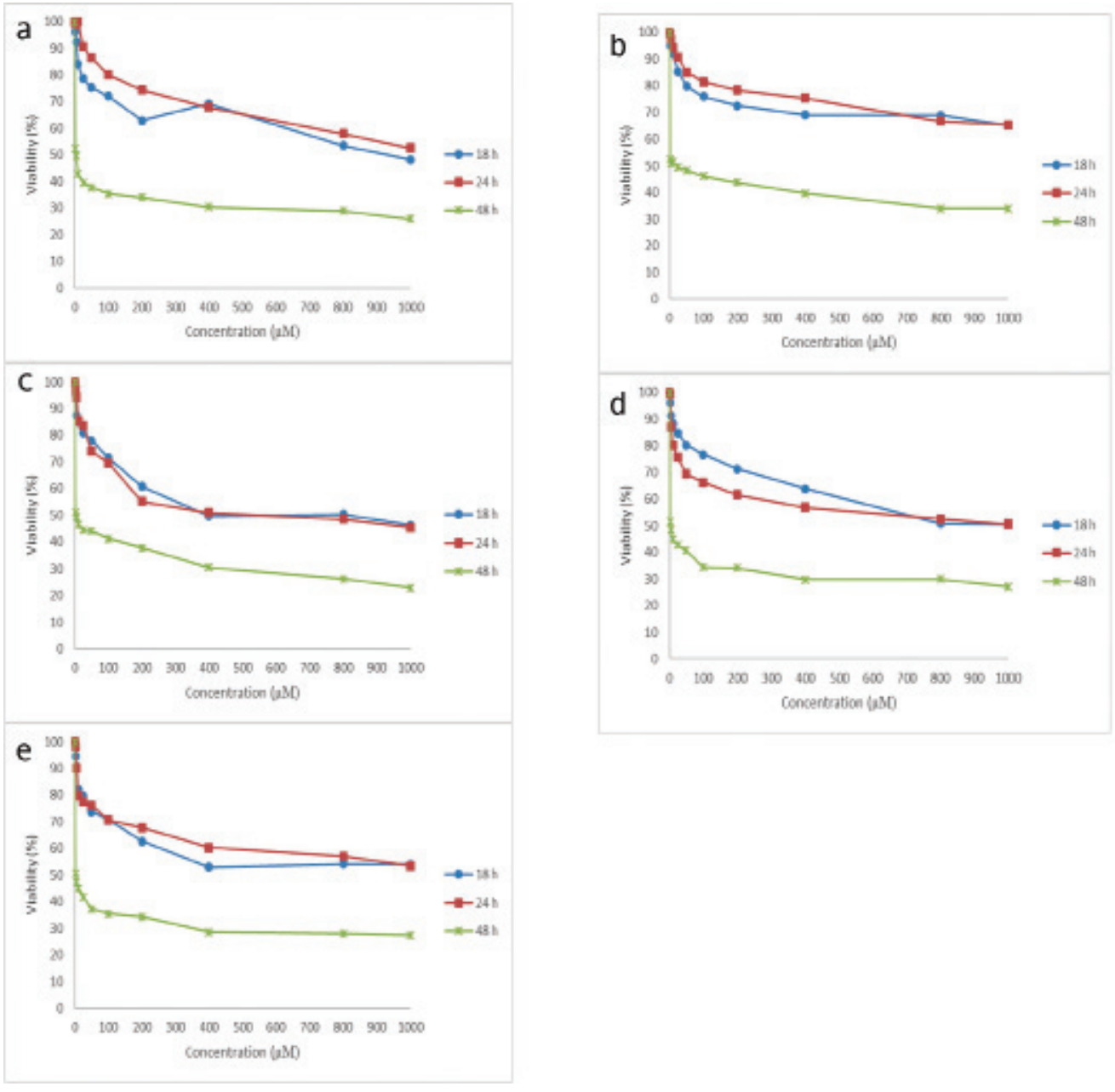

Figure 1. Cytotoxic effects of a) galangin, b) curcumin, c) pycnogenol, d) puerarin and e) ursolic acid in V79 cells by neutral red uptake assay

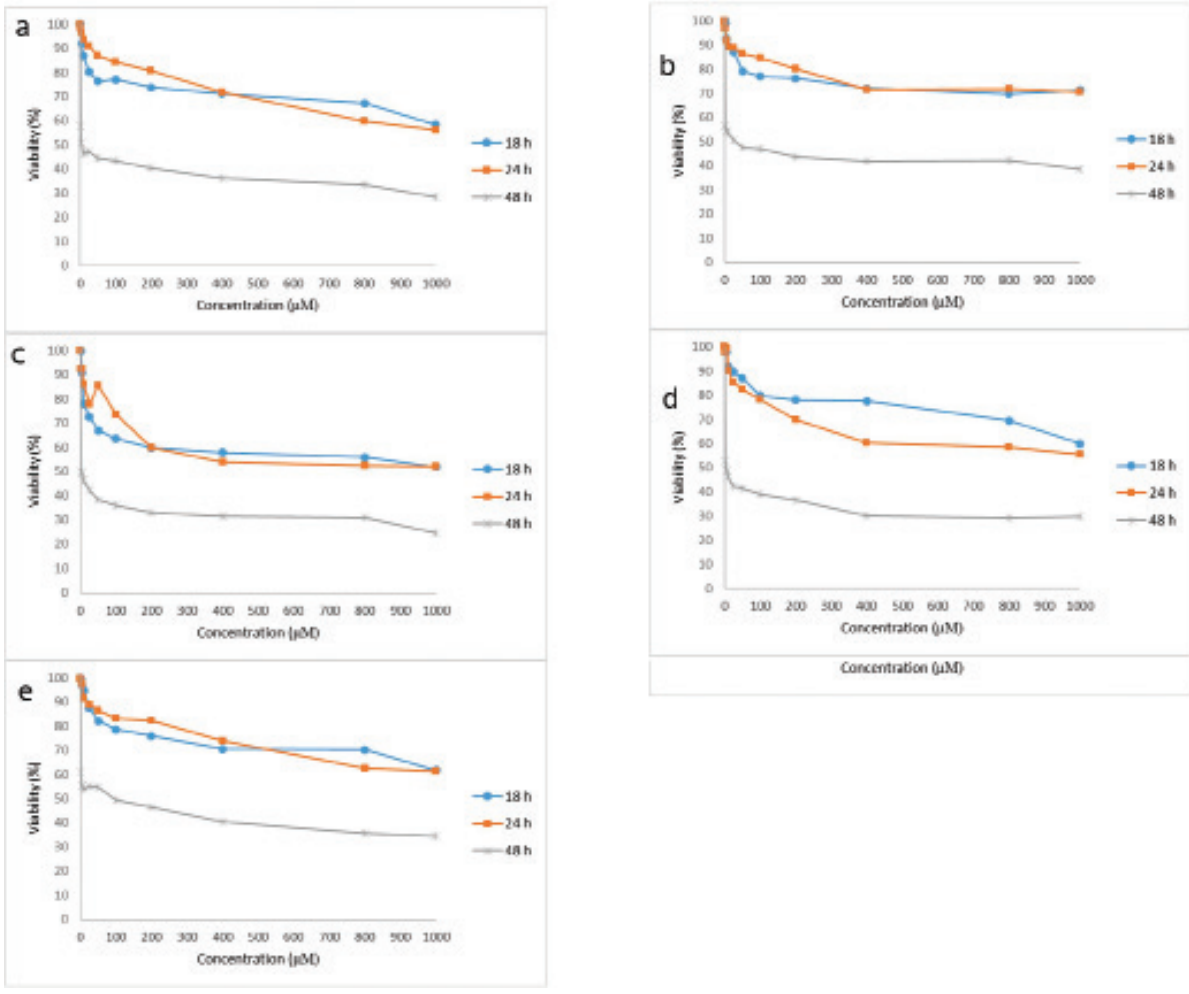

Figure 2. Cytotoxic effects of, a) galangin, b) curcumin, c) pycnogenol, d) puerarin and e) ursolic acid in V79 cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide assay

DISCUSSION

The cytotoxic effects of galangin, curcumin, PYC, puerarin and ursolic acid were investigated by NRU and MTT assays in V79, HeLa and BT-474 cells in 18, 24 and 48 h incubation periods. This is the first study about cytotoxic effects of these phenolics in healthy and cancer cell lines with two different assays and different incubation periods. Our results demonstrated that both galangin, curcumin, PYC, puerarin and ursolic acid decreased cell viability of V79, HeLa and BT-474 cells in a dose dependent manner in 18, 24 and 48 h incubation periods. But the cell survival rate was much lower in 48 h incubation period.

In SNU-484 cells, galangin has shown cytotoxic effect in a dose dependent manner and IC_{50} value of galangin in this cell line has found 100 µM.[22] In an another cytotoxicity study with galangin, it has shown that the cytotoxic effect has increased in a dose dependent manner on HepG2 cells.[23] As a result of the small number of studies carried out that galangin has no cytotoxic activity under 100 µM in different methods and different cell lines. Lantto et al.[24] have studied cytotoxicity of curcumin in two different cell lines [neuroblastoma (SH-SY5Y) and fibroblast (CV1-P) cells] by MTT and lactate dehydrogenase (LDH) leakage assays and their results have indicated that curcumin significantly decreased the metabolic activity of these cells.[24] Also, Mehta et al.[25] have showed anti-proliferative effect of curcumin on human breast tumor cell lines BT-20, T-47D, SKBR3 and MCF-7 by MTT assay. The effects of curcumin on the viability of human leukemia cell lines (U937 and Molt4) by MTT assay were also determined and dose dependent cytotoxic effects of curcumin were found.[26] Taner et al.[27] demonstrated the cytotoxic profile of PYC in healthy CHO cells. In this study, PYC has not showed cytotoxic effects at the concentrations of up to 150 µg/mL in CHO cells during 24 h exposure but above this concentration the cytotoxicity of PYC has started and the cell viability was decreased below 50% at 300 µg/mL.[27] There is limited study about cytotoxicity of puerarin. In a single study, it is demonstrated that puerarin has shown cytotoxic effects on HT-29 cells in a dose and time dependent manner.[28] In CaCo-2 cells, the viability of cells has decreased at concentrations higher than 100 µM with ursolic acid exposure for 48 h[29,30] have demonstrated that ursolic acid decreased the cytotoxic effects of ultraviolet B on lymphocytes in trypan blue and MTT methods.

It has been reported that different cytotoxicity assays can give

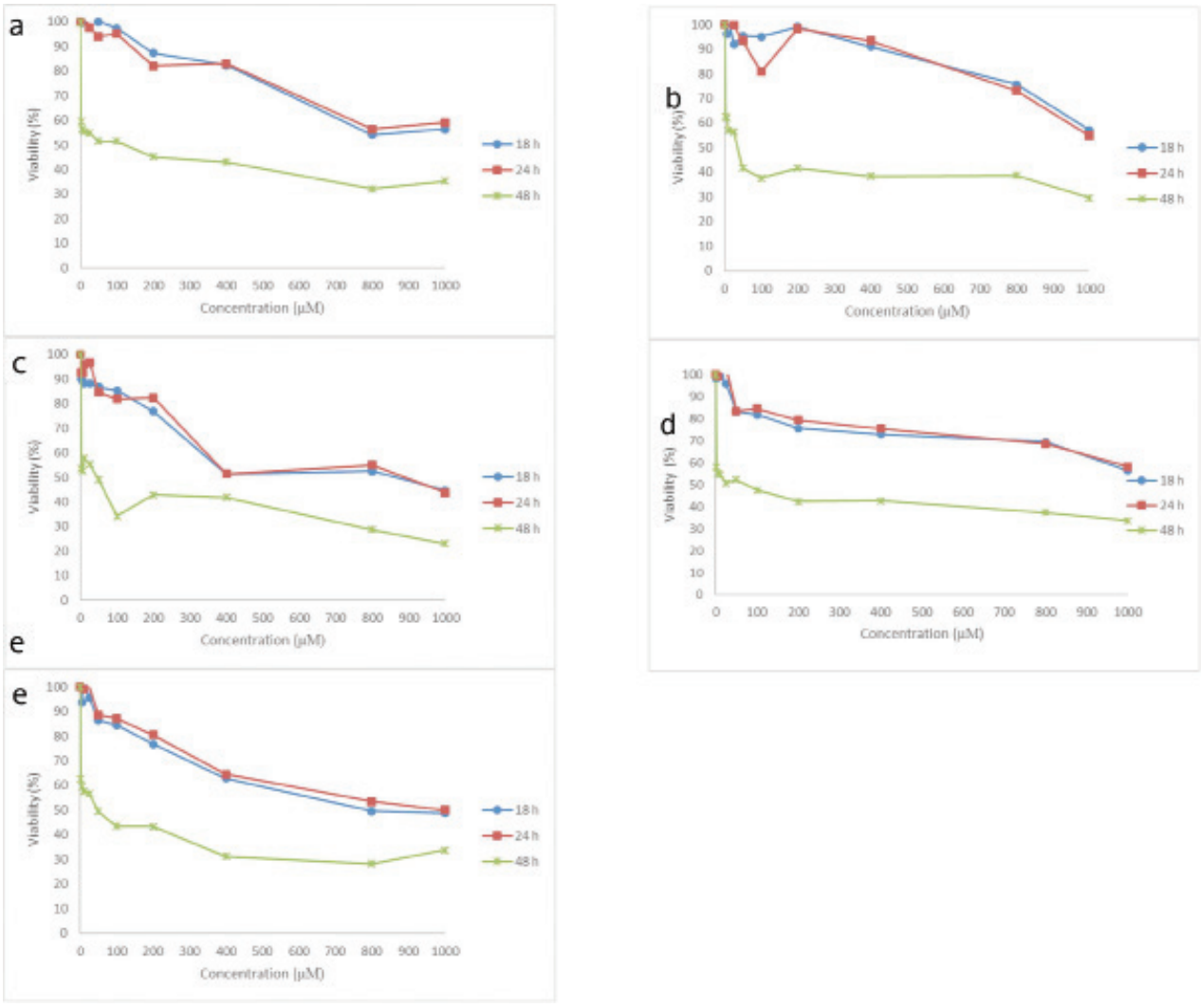

Figure 3. Cytotoxic effects of a) galangin, b) curcumin, c) pycnogenol, d) puerarin and e) ursolic acid in HeLa cells by neutral red uptake assay

different results due to the chemical and the cytotoxicity assay employed.[31] Fotakis and Timbrell[16] have compared four different cytotoxicity assays (LDH, a protein, NRU and MTT assays). Different sensitivity was observed for each assay. The NRU and the MTT assays were found to be the most sensitive in detecting cytotoxic events. Putnam et al.[32] have also studied cytotoxicity of cigarette smoke condensate with eight different (NRU, LDH release, kenacid blue binding, MTT, XTT, acid phosphatase activity, sulforhodamine B binding and resazurin binding) cytotoxicity assays. Four of the more widely used cytotoxicity assays (NRU, MTT, kenacid blue and LDH) were also evaluated at 3, 6, 12 and 18 h time points in this study. They have concluded that assays that measure membrane integrity (LDH) are useful for short exposure times (1 h), NRU assay was the most sensitive for moderate (3-6 h) exposure times; and assays that measure total cell number (NRU and kenacid blue) were more sensitive for longer exposure times (12, 18 and 24 h).[32] But in our study, both phenolics showed similar cytotoxicity profile in NRU and MTT assays in all exposure times.

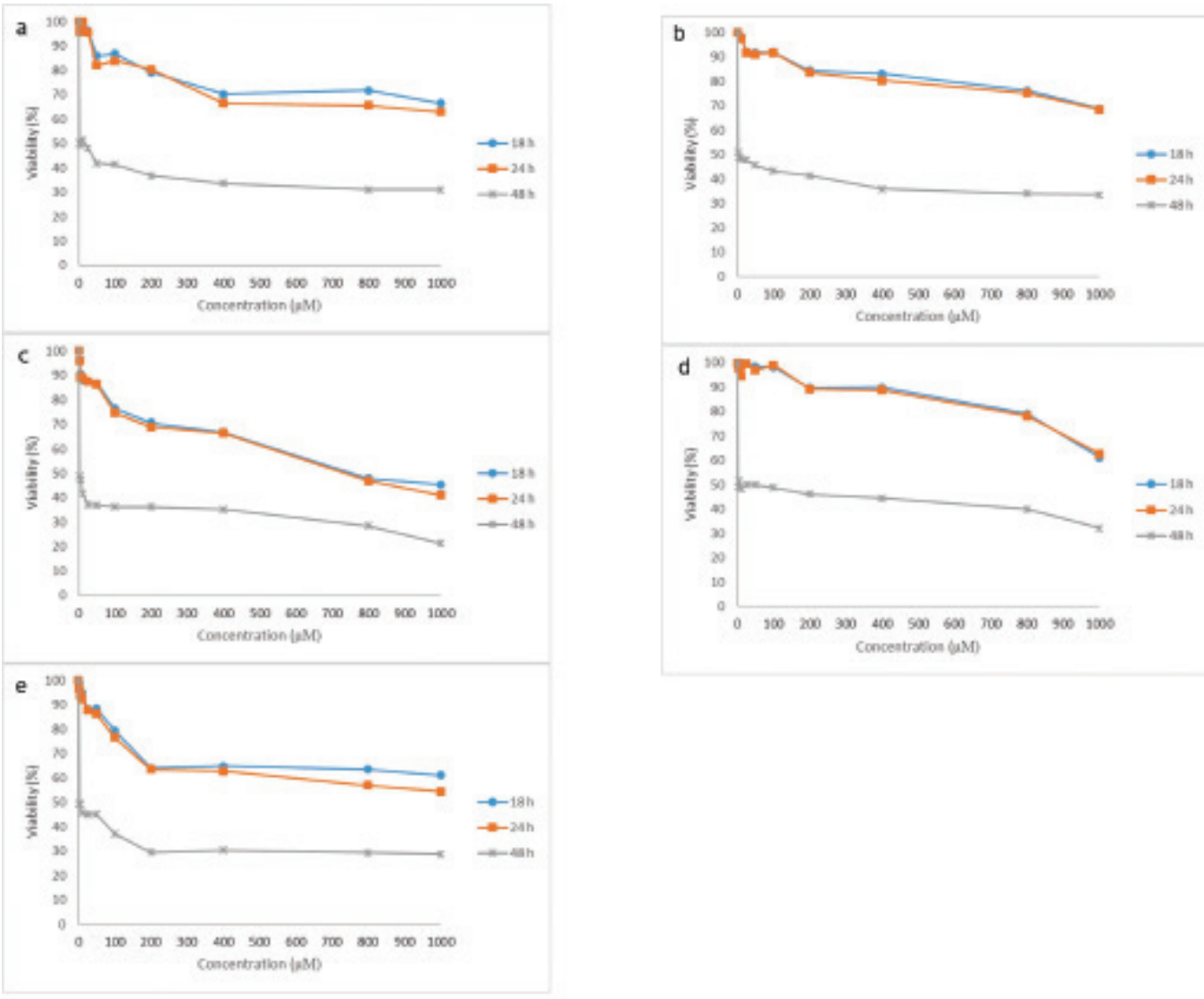

Figure 4. Cytotoxic effects of a) galangin, b) curcumin, c) pycnogenol, d) puerarin and e) ursolic acid in HeLa cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide assay

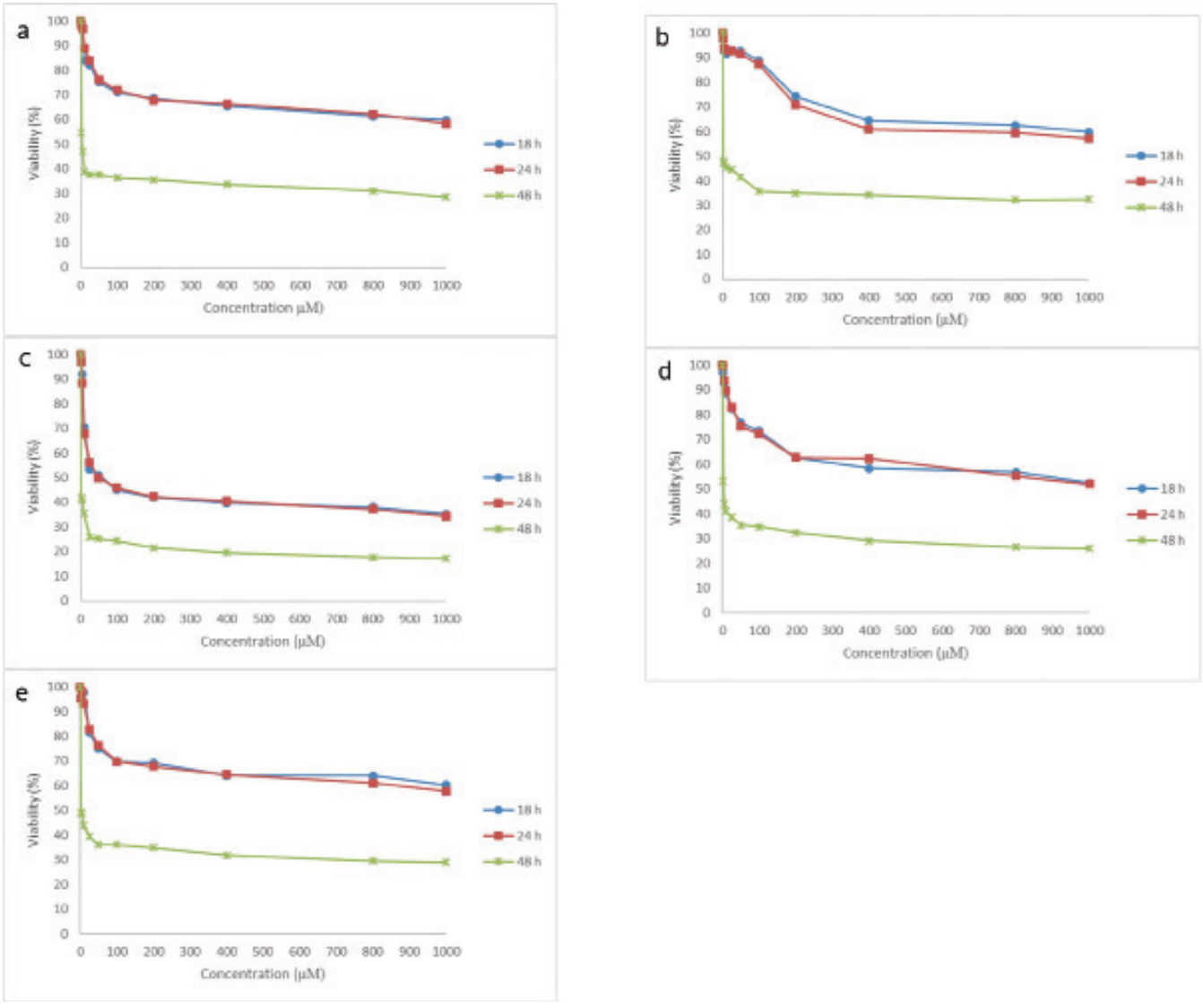

Figure 5. Cytotoxic effects of a) galangin, b) curcumin, c) pycnogenol, d) puerarin and e) ursolic acid in BT-474 cells by neutral red uptake assay

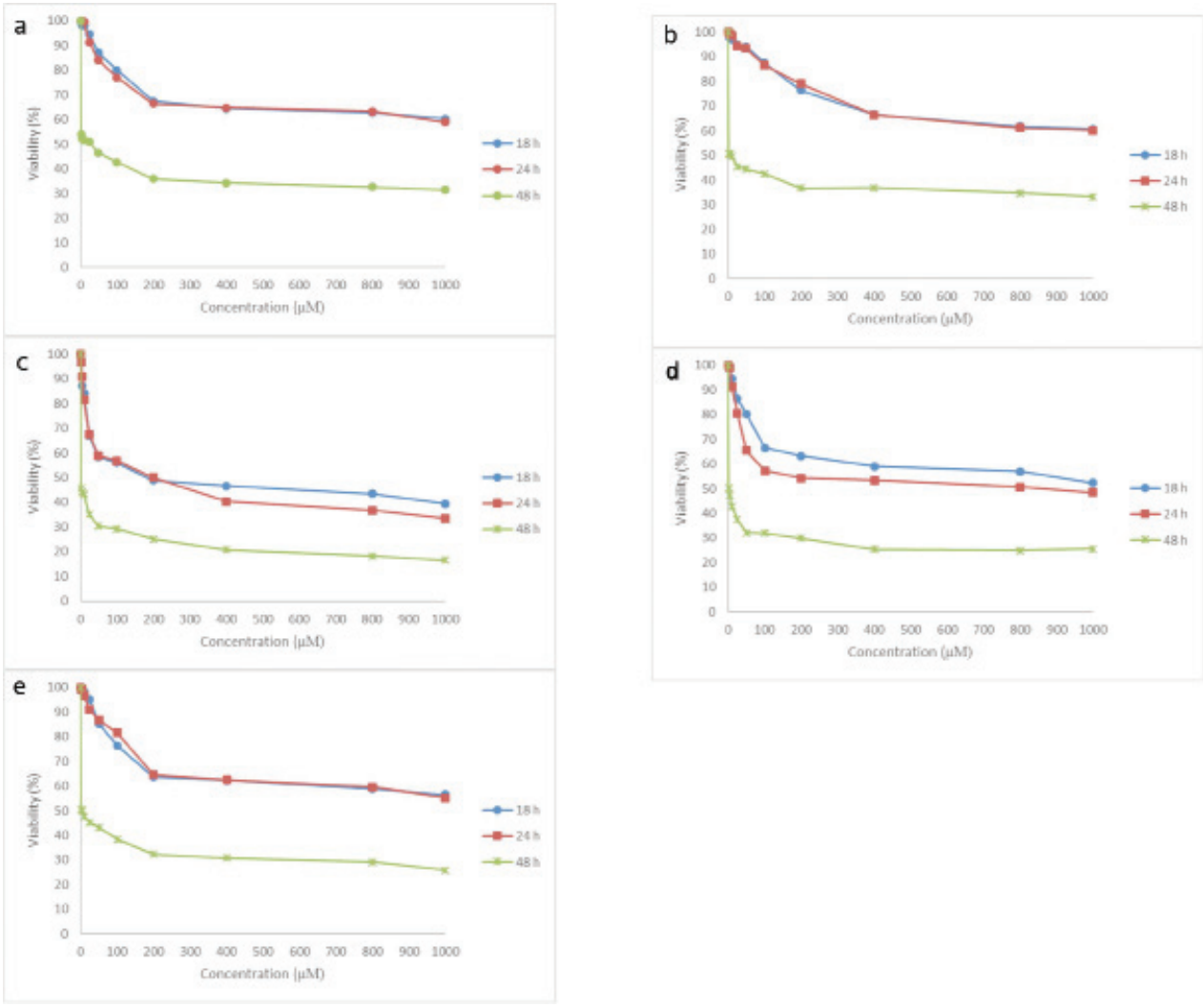

Figure 6. Cytotoxic effects of a) galangin, b) curcumin, c) pycnogenol, d) puerarin and e) ursolic acid in BT-474 cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide assay

Table 1. Viability (%) of V79 cells exposed to galangin, curcumin, pycnogenol, puerarin and ursolic acid

	18 h NRU (%)	18 h MTT (%)	24 h NRU (%)	24 h MTT (%)	48 h NRU (%)	48 h MTT (%)
Negative control	100.00	100.000	100.00	100.00	100.00	100.00
1000 μM galangin	48.263	58.388	52.579	56.372	25.898	28.426
800 μM galangin	53.444	67.179	57.996	59.919	28.986	33.411
400 μM galangin	69.153	71.308	67.857	71.913	30.428	36.102
200 μM galangin	62.689	73.759	74.404	80.607	34.105	40.276
100 μM galangin	72.019	77.114	80.059	84.335	35.405	43.396
50 μM galangin	75.271	76.319	86.388	87.156	37.802	44.670
25 μM galangin	78.559	80.393	90.595	90.845	39.569	47.389
10 μM galangin	83.979	86.929	99.880	93.649	42.799	46.417
5 μM galangin	92.412	92.023	99.107	96.603	49.563	50.841
2 μM galangin	96.160	99.959	99.503	98.343	52.244	57.574
1000 μM curcumin	65.148	71.208	65.297	70.663	33.942	38.498
800 μM curcumin	68.951	69.821	66.726	71.680	34.044	42.232
400 μM curcumin	69.098	72.047	75.396	71.555	39.609	41.973
200 μM curcumin	75.950	76.251	81.527	80.240	46.008	43.764
100 μM curcumin	72.478	76.954	78.373	84.750	43.753	46.957
50 μM curcumin	79.845	79.184	85.158	86.513	48.263	47.626
25 μM curcumin	85.430	87.181	90.674	88.853	49.421	50.540
10 μM curcumin	92.100	89.417	94.246	89.146	51.635	53.518
5 μM curcumin	95.002	92.465	97.579	91.759	50.903	54.381
2 μM curcumin	95.241	98.774	99.503	96.821	52.447	56.517
1000 μM pycnogenol	46.298	51.899	45.396	52.039	23.014	24.638
800 μM pycnogenol	50.374	55.883	48.571	52.449	26.203	30.837
400 μM pycnogenol	49.770	57.770	50.972	53.896	30.550	31.506
200 μM pycnogenol	60.812	59.709	55.079	59.941	37.863	33.060
100 μM pycnogenol	71.596	63.510	69.642	73.577	41.174	35.952
50 μM pycnogenol	77.916	66.986	73.948	85.599	43.956	38.261
25 μM pycnogenol	80.984	72.618	83.551	77.793	44.322	42.188
10 μM pycnogenol	85.118	77.989	85.436	86.266	46.597	46.310
5 μM pycnogenol	87.304	90.645	94.424	92.311	49.461	49.396
2 μM pycnogenol	95.352	99.739	97.420	92.460	51.046	49.979
1000 μM puerarin	50.560	59.885	50.521	55.571	27.117	29.802
800 μM puerarin	50.909	69.520	52.480	58.528	29.920	29.258
400 μM puerarin	63.935	77.557	56.805	60.408	29.839	30.082
200 μM puerarin	71.284	78.059	61.567	69.788	34.125	36.750
100 μM puerarin	76.759	79.807	66.230	78.271	34.470	38.995
50 μM puerarin	80.323	87.101	69.464	82.279	40.706	41.368

Table 1. Continue						
25 µM puerarin	84.751	89.632	75.674	85.421	42.758	42.447
10 µM puerarin	88.444	91.722	80.257	90.123	44.891	46.289
5 µM puerarin	91.163	97.930	87.142	99.554	48.446	48.856
2 µM puerarin	96.215	98.212	99.285	97.664	51.635	52.698
1000 µM ursolic acid	54.032	61.724	53.392	61.457	27.483	34.463
800 µM ursolic acid	54.143	70.263	56.984	62.659	28.031	35.607
400 µM ursolic acid	52.958	70.540	60.317	73.896	28.639	40.527
200 µM ursolic acid	62.704	75.929	67.797	82.222	34.348	46.396
100 µM ursolic acid	70.825	78.549	70.615	83.284	35.445	49.288
50 µM ursolic acid	73.764	82.182	76.190	86.534	37.314	54.834
25 µM ursolic acid	79.809	87.348	77.718	88.743	41.765	55.028
10 µM ursolic acid	82.179	94.796	79.781	91.589	44.972	53.949
5 µM ursolic acid	90.685	97.046	90.000	97.643	47.328	55.309
2 µM ursolic acid	94.543	99.417	98.075	99.405	50.741	60.898

NRU: Neutral red uptake assay, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide

Table 2. Viability (%) of HeLa cells exposed to galangin, curcumin, pycnogenol, puerarin and ursolic acid

	18 h NRU (%)	18 h MTT (%)	24 h NRU (%)	24 h MTT (%)	48 h NRU (%)	48 h MTT (%)
Negative control	100.000	100.000	100.000	100.000	100.000	100.000
1000 µM galangin	58.388	66.607	56.372	63.003	28.426	31.157
800 µM galangin	67.179	71.725	59.919	65.681	33.411	31.117
400 µM galangin	71.308	70.253	71.913	66.483	36.102	33.633
200 µM galangin	73.759	79.332	80.607	80.417	40.276	36.840
100 µM galangin	77.114	86.896	84.335	84.080	43.396	41.520
50 µM galangin	76.319	86.006	87.156	82.111	44.670	41.832
25 µM galangin	80.393	96.149	90.845	95.513	47.389	48.074
10 µM galangin	86.929	101.936	93.649	99.980	46.417	51.415
5 µM galangin	92.023	95.589	96.603	98.088	50.841	49.454
2 µM galangin	99.959	98.790	98.343	96.028	57.574	50.343
1000 µM curcumin	71.208	68.823	70.663	68.510	38.498	33.537
800 µM curcumin	69.821	76.277	71.680	75.226	42.232	33.942
400 µM curcumin	72.047	83.150	71.555	80.417	41.973	35.971
200 µM curcumin	76.251	84.454	80.240	83.619	43.764	41.342
100 µM curcumin	76.954	92.018	84.750	91.786	46.957	43.303
50 µM curcumin	79.184	91.823	86.513	91.292	47.626	45.616
25 µM curcumin	87.181	92.153	88.853	91.797	50.540	47.782
10 µM curcumin	89.417	98.279	89.146	97.770	53.518	48.094
5 µM curcumin	92.465	103.645	91.759	100.508	54.381	49.164

Table 2. Continue

2 μM curcumin	98.774	106.660	96.821	101.788	56.517	51.059
1000 μM pycnogenol	51.899	45.356	52.039	41.157	24.638	21.395
800 μM pycnogenol	55.883	47.984	52.449	46.920	30.837	28.616
400 μM pycnogenol	57.770	66.816	53.896	66.505	31.506	35.315
200 μM pycnogenol	59.709	70.663	59.941	69.048	33.060	36.327
100 μM pycnogenol	63.510	76.332	73.577	74.542	35.952	36.367
50 μM pycnogenol	66.986	86.626	85.599	86.178	38.261	37.132
25 μM pycnogenol	72.618	87.489	77.793	87.498	42.188	37.366
10 μM pycnogenol	77.989	88.233	86.266	88.757	46.310	41.721
5 μM pycnogenol	90.645	90.548	92.311	89.291	49.396	47.381
2 μM pycnogenol	99.739	96.153	92.460	95.739	49.979	48.830
1000 μM puerarin	59.885	61.129	55.571	62.729	29.802	32.204
800 μM puerarin	69.520	79.070	58.528	78.287	29.258	40.004
400 μM puerarin	77.557	89.926	60.408	88.790	30.082	44.395
200 μM puerarin	78.059	89.734	69.788	89.364	36.750	46.133
100 μM puerarin	79.807	98.265	78.271	99.198	38.995	48.719
50 μM puerarin	87.101	98.341	82.279	96.858	41.368	50.011
25 μM puerarin	89.632	99.822	85.421	99.691	42.447	50.123
10 μM puerarin	91.722	99.897	90.123	94.659	46.289	48.228
5 μM puerarin	97.930	100.123	99.554	97.615	48.856	51.415
2 μM puerarin	98.212	99.808	97.664	98.940	52.698	49.053
1000 μM ursolic acid	61.724	61.261	61.457	54.489	34.463	28.750
800 μM ursolic acid	70.263	63.663	62.659	56.967	35.607	29.343
400 μM ursolic acid	70.540	64.994	73.896	62.824	40.527	30.443
200 μM ursolic acid	75.929	64.107	82.222	63.668	46.396	29.507
100 μM ursolic acid	78.549	79.582	83.284	76.726	49.288	37.129
50 μM ursolic acid	82.182	88.356	86.534	86.300	54.834	45.019
25 μM ursolic acid	87.348	88.331	88.743	87.812	55.028	45.086
10 μM ursolic acid	94.796	94.991	91.589	92.253	53.949	45.621
5 μM ursolic acid	97.046	95.286	97.643	94.307	55.309	49.189
2 μM ursolic acid	99.417	99.359	99.405	96.942	60.898	49.610

NRU: Neutral red uptake assay, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide

Table 3. Viability (%) of BT-474 cells exposed to galangin, curcumin, pycnogenol, puerarin and ursolic acid

	18 h NRU (%)	18 h MTT (%)	24 h NRU (%)	24 h MTT (%)	48 h NRU (%)	48 h MTT (%)
Negative control	100.000	100.000	100.000	100.000	100.000	100.000
1000 µM galangin	59.776	60.333	58.272	58.989	28.589	31.454
800 µM galangin	61.264	62.857	62.119	63.301	31.015	32.675
400 µM galangin	65.408	64.506	66.390	64.763	33.596	34.328
200 µM galangin	68.512	67.449	67.759	66.337	35.500	35.949
100 µM galangin	70.992	79.984	71.915	77.078	36.271	42.579
50 µM galangin	75.184	87.068	76.121	84.103	37.640	46.423
25 µM galangin	82.016	94.622	84.026	91.434	37.514	50.948
10 µM galangin	83.872	98.075	88.900	99.630	38.725	51.458
5 µM galangin	97.472	98.159	96.675	99.958	47.191	52.227
2 µM galangin	98.784	98.915	98.142	100.325	54.603	53.811
1000 µM curcumin	59.824	60.786	57.164	60.100	32.415	33.252
800 µM curcumin	62.490	61.786	59.511	61.181	32.179	34.705
400 µM curcumin	64.496	66.611	60.831	66.434	34.209	36.809
200 µM curcumin	74.351	76.456	70.905	79.126	34.949	36.725
100 µM curcumin	88.653	87.527	87.074	86.588	35.767	42.567
50 µM curcumin	92.603	94.293	91.263	93.445	41.342	44.433
25 µM curcumin	92.960	95.013	92.616	94.414	44.516	45.530
10 µM curcumin	91.536	97.031	93.017	98.698	45.460	49.585
5 µM curcumin	92.544	97.831	93.871	99.296	46.814	50.497
2 µM curcumin	97.648	99.064	97.702	99.930	47.939	50.923
1000 µM pycnogenol	35.200	39.491	34.349	33.512	17.120	16.513
800 µM pycnogenol	38.042	43.412	37.200	36.704	17.589	18.185
400 µM pycnogenol	39.680	46.636	40.456	40.307	19.544	20.785
200 µM pycnogenol	41.920	48.808	42.282	50.040	21.526	25.140
100 µM pycnogenol	44.944	56.079	45.933	56.811	24.312	29.349
50 µM pycnogenol	51.088	58.468	49.845	58.898	25.193	30.355
25 µM pycnogenol	53.408	66.865	56.007	67.550	25.854	35.147
10 µM pycnogenol	70.544	84.243	67.710	81.613	35.484	43.310
5 µM pycnogenol	91.888	87.303	88.443	90.637	40.897	44.223
2 µM pycnogenol	98.240	97.328	96.952	96.720	42.014	45.532
1000 µM puerarin	52.384	52.263	51.964	48.410	25.885	25.584
800 µM puerarin	56.882	57.004	55.175	50.662	26.467	24.906
400 µM puerarin	58.336	59.103	62.184	53.354	29.064	25.390
200 µM puerarin	62.653	63.222	62.642	54.300	32.305	29.887
100 µM puerarin	73.376	66.454	72.290	57.206	34.729	31.926
50 µM puerarin	76.768	80.315	75.240	65.467	35.374	32.109

NRU: Neutral red uptake assay, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide

CONCLUSION

In conclusion, in this study, the cytotoxic effects of galangin, curcumin, PYC, puerarin and ursolic acid were examined in different cell lines by NRU and MTT assays in 18, 24 and 48 h periods. All of the studied phenolics were decreased the cell viability of both cells with increasing dose. But the cytotoxic effects of phenolics were found more in 48 h incubation period. There is no difference between the results from NRU and MTT assays. Further investigation such as using more cell lines and different reliable cytotoxicity assays and incubations with various concentrations at many time points should be performed to corfirm beneficial and toxic effects of phenolics.

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