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ORIGINAL ARTICLE

Screening for antitumor activity of various plant extracts on HeLa and C 4-I cell lines

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Summary

Purpose: *Cancer is a long process that leads the organism to death and is associated with the normal cells acquiring the ability to divide permanently. Nowadays, the use of natural products in cancer therapy has a great importance. In addition, working with plants that are endemic to Turkey and determining the biological activities of these plant extracts, is extremely important due to the potential for new drug development. There is no comparative study available in the literature on the antitumor effects of Colchicum sanguicolle, a new found species of the genus Colchicum in Turkey, Crateagus microphylla, of the genus Crateagus and Centaurea antiochia of the genus Centaurea. In this study, we tried to demonstrate the antitumor effect of these plant extracts on HeLa and C 4-1 cells.*

Methods: *Five different doses (0.001, 0.01, 0.05, 0.25 and 0.5 mg/ml) of the three plant types were prepared and applied for 24, 48 and 72 hrs on the cervical cancer derived cell lines. Subsequently, the growth rate was evaluated with the mitochondrial dehydrogenase enzyme method.*

Results: *Colchicum sanguicolle extracts showed the most effective antitumor activity. For the Colchicum sanguicolle extract, the IC_{50} dose for HeLa cells was 0.01 mg/ml at 48 hrs, while for the C-4 I cells it was 0.001 mg/ml at 48 hrs. These results showed that C-4 I cells were more sensitive to the Colchicum sanguicolle extracts.*

Conclusion: *The results of from this study regarding the antitumor effect of plant extracts of endemic varieties of Turkey may have an important place in design and development of anticancer drugs and would make contributions to other studies to be conducted in this area.*

Key words: *antitumor, Centaurea antiochia, Colchicum sanguicolle, Crateagus microphylla, C 4-I, HeLa*

Introduction

Cancer can be defined as a genomic disease that appears as a result of dynamic changes of the DNA of an organism's cells during its life time. Chemotherapy and/or radiotherapy and surgical methods are used in the treatment of different cancer types. It is important to develop new approaches to increase the antitumor effects against cancer cells in order to increase the efficacy of cancer treatments [1,2]. Up to now there are many drugs known to have non-steroidal anti-inflammatory, antiviral and antitumor effects. Today, the use of natural products to treat cancer are very common, particularly in underdeveloped or developing countries. However, some plants have not yet been scientifically proven to exert antitumor activity. Therefore it is very important to study endemic/non-endemic plant extracts with unknown biological activities to possibly develop

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new drugs that will guide the intentional use of natural medicines [3]. So far there have not been any studies investigating the antitumor effects of the new found endemic species of *Colchicum sanguicolle, Crateagus microphylla* species of the genus *Crateagus* and *Centaurea antiochia* var. praelta.

Liliaceae (Colchicaceae) obtained from the *Colchicum* species of plants belonging to the colchicine family, are now among natural products. Colchicine is an agent with antitumor properties used in chemotherapy, and it is classified as a mitotic inhibitor [4,5] acting as a tubulin 2 polymerization inhibitor. Changes are observed in the structure of mitochondria due to the inhibition of tubulin. Additionally, formation of the spindle apparatus is suppressed, release of hemotactic factors decreases, and an increase in cyclic AMP can be seen in tubulin inhibition. The agents that harm the function of microtubules *in vitro*, arrest the cells in the mitotic phase, resulting in cell death by necrosis or apoptosis [6].

Tropolone alkaloid components of the different species belonging to the genus *Colchicum* have been shown to be different according to the different regions and growth stages [7]. In this study, a new species of this genus (*Colchicum sanguicolle*) was scrutinized.

Centaurea L. (Compositae) species, is one of the many representatives of the genus and has an expansive area of habitation in Turkey. 123 of the total 192 species found in Turkey are endemic to Turkey's flora [8]. Among the public, *Centaurea* is commonly referred to as "Cornflower" and is widely known to have species that are used as a tonic, for constipation, appetizer, chest softener, as antipyretic etc [9,10]. In this study, extracts prepared from the above-ground parts of the plant *Centaurea antiochia* Wagenitz, an endemic species, were used. Species belonging to the genus *Centaurea* include catechin [11].

Crataegus microphylla species belonging to the *Rosaceae* family is an endemic species in Turkey. It contains several kinds of polyphenols such as chlorogenic acid, vitexin and derivatives, routine, hiperocid, isoquercitrin and quercetin.

In this study, our first aim was to demonstrate the antitumor effect of these plant types on various cancer cell lineages. The data obtained from this study intended to show to what extent antitumor effects intersect with different cell types and explain at what level they differ. We hope that the results of this study will play an important role in designing and developing natural anticancer drugs and will make contributions to other studies that might be conducted in this area.

Methods

Collection of plants

Part of the tubers of the endemic plant *Colchicum sanguicolle* selected for this study were collected around Antalya, Akdag Yesilgol, while fruity and were registered to The Herbarium of the Faculty of Pharmacy of Istanbul University (ISTE) with No. 48868. *Centaurea antiochia* Boiss. var. praealta (Boiss. & Honey.) plants were collected from Icel, Mersin, Turkey between June and September and were registered with ISTE No. 98247. *Crataegus microphylla* C.Koch plant leaves were collected from Bolu and registered with ISTE No. 76223.

Preparation of extracts

The fresh tubers of the plant *Colchicum sanguicolle* were sliced, flaked, dried out in the oven at 60 °C and pulverized. Extraction was performed using Merck methanol Soxhlet, after *Colchicum Sanguicolle* was condensed and then the extract was lyophilized. Seeds were powdered after drying, extracted initially using petroleum ether, afterwards exposed to methanol extraction and then lyophilized.

Above-ground parts of plants *Centaurea antiochia* Boiss. var. praealta (Boiss. & Bal.) Wagenitz were dried in the shade at room temperature. They were then powderized with methanol and consumed in the percolator. *Centaurea Antiochia* was condensed in the percolate rotovapor at temperatures not exceeding 50 °C, resulting in an intensely lyophilized extract.

Crataegus microphylla C. Koch leaves were dried out in the shade at room temperature. They were then powderized with methanol and consumed in the percolator. *Crataegus microphylla* was condensed in the percolate rotovapor at temperatures not exceeding 50 °C, resulting in an intensely lyophilized extract.

Antitumor activity

Cell lines: The C-4 I cell line used in the experiment is derived from cervical carcinoma. These cells were regularly passaged twice a week in our laboratory in standard tissue culture medium consisting of Waymouth medium (W1625, Sigma) containing 10% fetal bovine serum (FBS, Gibco Lab.), 100 IU/ml penicillin (Pronap by Pfizer) and 100 mg/ml streptomycin (streptomycin sulfate, Ulugay IE).

The cells were incubated in suspension culture form containing 5% CO_2 and 95% humid air at 37 °C.

HeLa cells are another tumor cell lineage derived from human cervical cancer and have a different sensitivity to agents than C-4 I cells. HeLa cells were regularly passaged in standard tissue culture medium consisting of MEM (minimum essential medium, Sigma) containing 10% FBS (Gibco Lab.), 100 IU/ml penicillin

(Pronap by Pfizer) and 100 mg/ml streptomycin (streptomycin sulfate, Ulugay IE) and were incubated at 37 °C in 5% CO_2 and 95% humid air, forming monolayer cultures.

The cells used in the experiments were grown in plastic tissue culture containers. Passage started when the surface of flask was covered by the cells. For this purpose, first cells non-adherent to the surface were placed into a centrifuge tube, then cells adhering to the surface were washed out with HBSS (Hank's Balanced Salt Solution, Sigma) by pipetting. Cells still adherent to surface were removed from the surface with trypsin and they were suspended after being transferred to the same centrifuge tube. Centrifugation was performed at 1500 rpm for 3 min. After centrifugation, the supernatant was discarded. Medium specific to the cultured cell type was added to stir and resolve the precipitate and the required number of cells were placed in cell culture dishes for each experiment.

Determination and preparation of the different concentrations of extracts

Doses of the extracts used in the experiments were determined based on ethnobotanical information and previous studies. Stock solutions were prepared as 5 mg/ml concentration in sterilized medium (MEM) for HeLa, and Waymouth for C 4-1 without FBS. Then, 5 different doses (0.001, 0.01, 0.05, 0.25 and 0.5 mg/ml) of plant extracts were prepared by diluting stock solution with medium (MEM) for HeLa, and Waymouth for C 4-1 containing 10% FBS and each of these different doses was applied for 24, 48 and 72 hrs on the cells. The proliferation rates and related graphs for the cell lines used in these experiments were determined for each dose and hour respectively.

Mitochondrial dehydrogenase activity

The proliferation rates of the experimental group were determined by cell viability assay (MTT: 3-(4,5-dimethylyhiazol-2-il)-2,5-diphenyl tetrazolium bromid) and cytotoxicity test [12,13]. At the end of the experimental period, 20 µl of MTT solution and 200 µl (MEM for HeLa and Waymouth for C 4-I) were added into 96-well plates containing cells and incubated for 4 hrs at 37 °C. At the end of this period, DMSO was added and the cells were left on the shaker overnight. Afterwards, the samples were read using the ELISA Reader Plate at 570 nm wavelength, with the reference wavelength at 690 nm.

Morphological evaluation

Morphological changes that occurred in the cells were examined by phase contrast microscopy (10x20 and 10x40 magnification) and variations of cytotoxicity based on dose and time were photographed. Morphological changes in the nucleus and in the cytoplasm were investigated.

Statistics

One way ANOVA test was applied to all experimental groups, and Dunnett's test was also applied to determine whether the experimental groups showed a significant difference from the control group. A p value <0.05 was considered as statistically significant. All analyses were carried out using GraphPad Prism version 4.00 (GraphPad Software. San Diego California, USA).

Results

Detailed results of this study are displayed in Tables 1-6. A significant decrease or increase of proliferation rates of HeLa and C 4-I cells was shown in comparison to the control group ($p<0.01$). Viability graphs based on the absorbance values for HeLa cells are shown in Figures 1-3, and C-4 I cells related graphs are shown in Figures 4-6. As seen in the Figures, the antiproliferative effect increased in parallel to the dose and time of exposure of extracts for both HeLa cells and C 4-I.

As illustrated in Figure 1, when *Colchicum sanguicolle* extract was applied to HeLa cell cultures at 0.001 mg/ml, cytotoxicity appeared and

Table 1. Absorbance values of *Colchicum sanguicolle* extracts against HeLa cells at 24, 48 and 72 hrs treated with 5 different doses (D1: 0.001 mg/ml, D2: 0.01 mg/ml, D3: 0.05 mg/ml, D4: 0.25 mg/ml, D5: 0.5 mg/ml) by MTT assay

	Absorbance values (570 – 690 nm). Mean ±SD		
Treatment groups	*24hrs*	*48hrs*	*72hrs*
Control	557.4×10^{-3} ±0.02	448.3×10^{-3} ±0.02	836.4×10^{-3} ±0.03
D1	536.3×10^{-3} ±0.03*	555.4×10^{-3} ±0.02*	471.3×10^{-3} ±0.03*
D2	459.4×10^{-3} ±0.03*	238.1×10^{-3} ±0.02*	145.3×10^{-3} ±0.02*
D3	397.0×10^{-3} ±0.02*	250.4×10^{-3} ±0.02*	132.3×10^{-3} ±0.01*
D4	406.3×10^{-3} ±0.03*	269.7×10^{-3} ±0.03*	137.4×10^{-3} ±0.01*
D5	438.4×10^{-3}±0.02*	274.4×10^{-3} ±0.02*	143.5×10^{-3} ±0.01*

*significant ($p<0.01$) compared with control

Table 2. Absorbance values of *Centaurea antiochia* extracts against HeLa cells at 24, 48 and 72 hrs, treated with 5 different doses (D1: 0.001 mg/ml, D2: 0.01 mg/ml, D3: 0.05 mg/ml, D4: 0.25 mg/ml, D5: 0.5 mg/ml) by MTT assay

	Absorbance values (570 – 690 nm). Mean ±SD		
Treatment groups	*24hrs*	*48hrs*	*72hrs*
Control	338.1×10^{-3} ±0.03	631.5×10^{-3} ±0.01	789.4×10^{-3} ±0.02
D1	576.5×10^{-3} ±0.03*	801.5×10^{-3} ±0.04*	840.5×10^{-3} ±0.03*
D2	504.3×10^{-3} ±0.03*	697.5×10^{-3} ±0.03*	905.3×10^{-3} ±0.02*
D3	427.0×10^{-3} ±0.03*	807.3×10^{-3} ±0.03*	924.1×10^{-3} ±0.04*
D4	483.2×10^{-3} ±0.03*	700.3×10^{-3} ±0.03*	930.3×10^{-3} ±0.04*
D5	451.3×10^{-3}±0.03*	626.1×10^{-3} ±0.03	786.5×10^{-3} ±0.02

*significant (p<0.01) compared with control

Table 3. Absorbance values of *Crateagus microphylla* extracts against HeLa cells at 24, 48 and 72 hrs, treated with 5 different doses (D1: 0.001 mg/ml, D2: 0.01 mg/ml, D3: 0.05 mg/ml, D4: 0.25 mg/ml, D5: 0.5 mg/ml) by MTT assay

	Absorbance values (570 – 690 nm). Mean ±SD		
Treatment groups	*24hrs*	*48hrs*	*72hrs*
Control	$1{,}113.2\times10^{-3}$ ±0.04	800.3×10^{-3} ±0.03	$1{,}058.5\times10^{-3}$ ±0.04
D1	830.2×10^{-3} ±0.03*	773.3×10^{-3} ±0.03	696.5×10^{-3} ±0.04*
D2	924.5×10^{-3} ±0.04*	831.3×10^{-3} ±0.03	765×10^{-3} ±0.03*
D3	954.3×10^{-3} ±0.01*	851.1×10^{-3} ±0.02*	789.2×10^{-3} ±0.04*
D4	$1{,}059.3\times10^{-3}$ ±0.01*	$1{,}250.1\times10^{-3}$ ±0.03*	948.7×10^{-3} ±0.03*
D5	$1{,}387.4\times10^{-3}$ ±0.01*	$1{,}411.5\times10^{-3}$ ±0.04*	$1{,}223.4\times10^{-3}$ ±0.03*

*significant (p<0.01) compared with control

Table 4. Absorbance values of *Colchicum sanguicolle* extracts against C-4 I cells at 24, 48 and 72 hrs, treated with 5 different doses (D1: 0.001 mg/ml, D2: 0.01 mg/ml, D3: 0.05 mg/ml, D4: 0.25 mg/ml, D5: 0.5 mg/ml) by MTT assay

	Absorbance values (570 – 690 nm). Mean ±SD		
Treatment groups	*24hrs*	*48hrs*	*72hrs*
Control	859.3×10^{-3} ±0.02	$1{,}653.8\times10^{-3}$ ±0.24	$1{,}475.4\times10^{-3}$ ±0.06
D1	789.5×10^{-3} ±0.03*	500.6×10^{-3} ±0.16*	281.1×10^{-3} ±0.05*
D2	541.8×10^{-3} ±0.03*	354.6×10^{-3} ±0.17*	233.2×10^{-3} ±0.06*
D3	610.8×10^{-3} ±0.02*	452.9×10^{-3} ±0.16*	245.9×10^{-3} ±0.06*
D4	581.8×10^{-3} ±0.03*	315.2×10^{-3} ±0.10*	323.3×10^{-3} ±0.06*
D5	609.8×10^{-3} ±0.02*	299.5×10^{-3} ±0.11*	324.8×10^{-3} ±0.07*

*significant (p<0.01) compared with control

Table 5. Absorbance values of *Centaurea antiochia* extracts against C-4 I cells at 24, 48 and 72 hrs, treated with 5 different doses (D1: 0.001 mg/ml, D2: 0.01 mg/ml, D3: 0.05 mg/ml, D4: 0.25 mg/ml, D5: 0.5 mg/ml) by MTT assay

	Absorbance values (570 – 690 nm). Mean ±SD		
Treatment groups	*24hrs*	*48hrs*	*72hrs*
Control	890.5×10^{-3} ±0.16	$1{,}602.2\times10^{-3}$ ±0.28	$1{,}433.9\times10^{-3}$ ±0.04
D1	$1{,}064.1\times10^{-3}$ ±0.30*	$1{,}436.4\times10^{-3}$ ±0.20*	$1{,}630.2\times10^{-3}$ ±0.06*
D2	$1{,}051.6\times10^{-3}$ ±0.16*	$1{,}605.0\times10^{-3}$ ±0.16	$1{,}630.6\times10^{-3}$ ±0.08*
D3	$1{,}022.7\times10^{-3}$ ±0.24*	$1{,}685.5\times10^{-3}$ ±0.20	$1{,}624.4\times10^{-3}$ ±0.08*
D4	$1{,}050.5\times10^{-3}$ ±0.20*	$1{,}497.1\times10^{-3}$ ±0.26*	$1{,}711.2\times10^{-3}$ ±0.09*
D5	$1{,}114.7\times10^{-3}$ ±0.21*	$1{,}661.3\times10^{-3}$ ±0.17	$1{,}706.3\times10^{-3}$±0.11*

*significant (p<0.01) compared with control

Table 6. Absorbance values of *Crateagus microphylla* extracts against C-4 I cells at 24, 48 and 72 hrs, treated with 5 different (D1: 0.001 mg/ml, D2: 0.01 mg/ml, D3: 0.05 mg/ml, D4: 0.25 mg/ml, D5: 0.5 mg/ml) doses by MTT assay

	Absorbance values (570 – 690 nm). Mean ±SD		
Treatment groups	*24hrs*	*48hrs*	*72hrs*
Control	487.8×10^{-3} ±0.06	771.5×10^{-3} ±0.08	$1{,}176\times10^{-3}$ ±0.19
D1	442.6×10^{-3} ±0.04*	$1{,}051\times10^{-3}$ ±0.17*	$1{,}525\times10^{-3}$ ±0.08*
D2	544.6×10^{-3} ±0.08*	$1{,}110\times10^{-3}$ ±0.10*	$1{,}589\times10^{-3}$ ±0.13*
D3	427.3×10^{-3} ±0.07*	$1{,}115\times10^{-3}$ ±0.12*	$1{,}504\times10^{-3}$ ±0.19*
D4	618.0×10^{-3} ±0.09*	914.4×10^{-3} ±0.12*	$1{,}651\times10^{-3}$ ±0.18*
D5	724.5×10^{-3} ±0.06*	$1{,}150\times10^{-3}$ ±0.25*	$1{,}460\times10^{-3}$ ±0.09*

*significant (p<0.01) compared with control

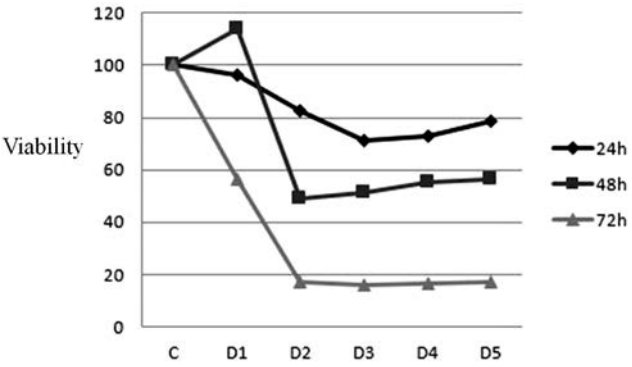

Figure 1. Percent viability values of HeLa cells which were treated with 5 different doses (D1: 0.001 mg/ml, D2: 0.01 mg/ml, D3: 0.05 mg/ml, D4: 0.25 mg/ml, D5: 0.5 mg/ml) of *Colchicum sanguicolle* (C: Control). (p<0.01).

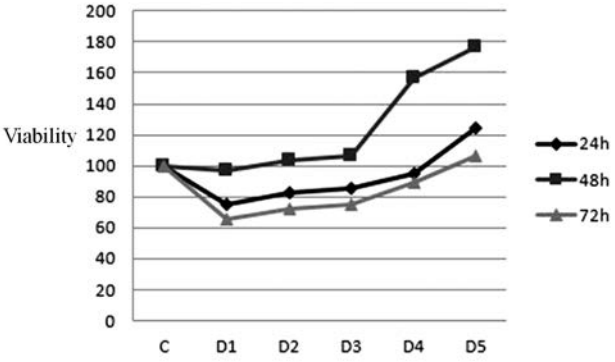

Figure 3. Percent viability values of HeLa cells which were treated with 5 different doses (D1: 0.001 mg/ml, D2: 0.01 mg/ml, D3: 0.05 mg/ml, D4: 0.25 mg/ml, D5: 0.5 mg/ml) of *Crateagus microphylla* (C: Control). (p<0.01).

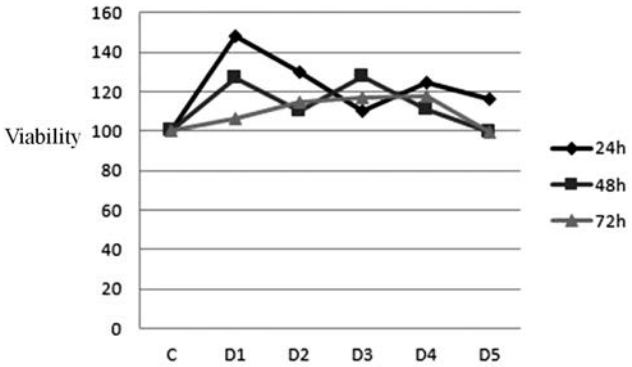

Figure 2. Percent viability values of HeLa cells which were treated with 5 different doses (D1: 0.001 mg/ml, D2: 0.01 mg/ml, D3: 0.05 mg/ml, D4: 0.25 mg/ml, D5: 0.5 mg/ml) of *Centaurea antiochia* (C: Control). (p<0.01).

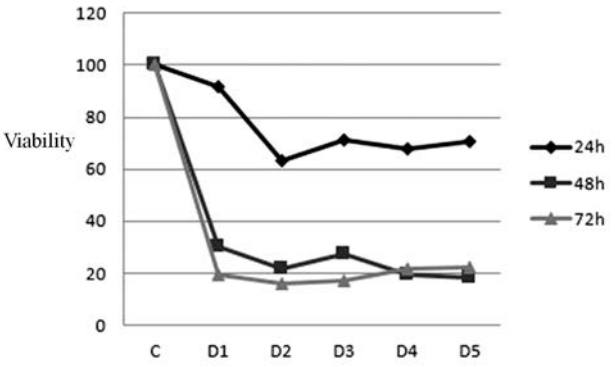

Figure 4. Percent viability values of C-4 I cells which were treated with 5 different doses (D1: 0.001 mg/ml, D2: 0.01 mg/ml, D3: 0.05 mg/ml, D4: 0.25 mg/ml, D5: 0.5 mg/ml) of *Colchicum sanguicolle* (C: Control). (p<0.01).

continued to increase dependent on time of exposure from 48 hrs to 72 hrs. For 0.01 mg/ml and other doses cytotoxicity appeared after the first 24 hrs and increased in parallel with dose and time of exposure. IC_{50} dose for the *Colchicum sanguicolle* extract applied to HeLa cell cultures was 0.01 mg/ml at 48 hrs.

As seen in Figure 2, HeLa cell cultures treated with *Centaurea antiochia* extract were conducted with 5 different doses and showed no significant toxic effects. Also for the 0.001 mg/ml dose, cell proliferation increased during the first 24 and 48 hrs, but later it was found to be parallel to the increase of the control group.

As seen in Figure 3, HeLa cell cultures that were handled with *Crateagus microphylla* extracts in 5 different doses, 0.001, 0.01 and 0.05 mg/ml reduced viability during the 24 and 72 hrs when compared with the control group.

As seen in Figure 4, C-4 I cells treated with *Colchicum sanguicolle* extract with 5 different doses, toxic effects appeared after 24 hrs. For all dos-

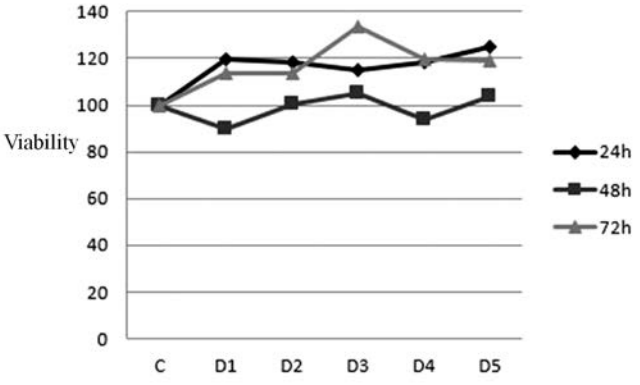

Figure 5. Percent viability values of C-4 I cells which were treated with 5 different doses (D1: 0.001 mg/ml, D2: 0.01 mg/ml, D3: 0.05 mg/ml, D4: 0.25 mg/ml, D5: 0.5 mg/ml) of *Centaurea antiochia* (C: Control). (p<0.01).

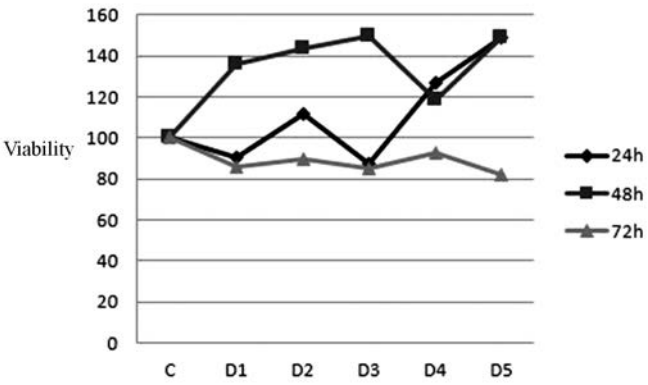

Figure 6. Percent viability values of C-4 I cells which were treated with 5 different doses (D1: 0.001 mg/ml, D2: 0.01 mg/ml, D3: 0.05 mg/ml, D4: 0.25 mg/ml, D5: 0.5 mg/ml) of *Crateagus microphylla* (C: Control). (p<0.01).

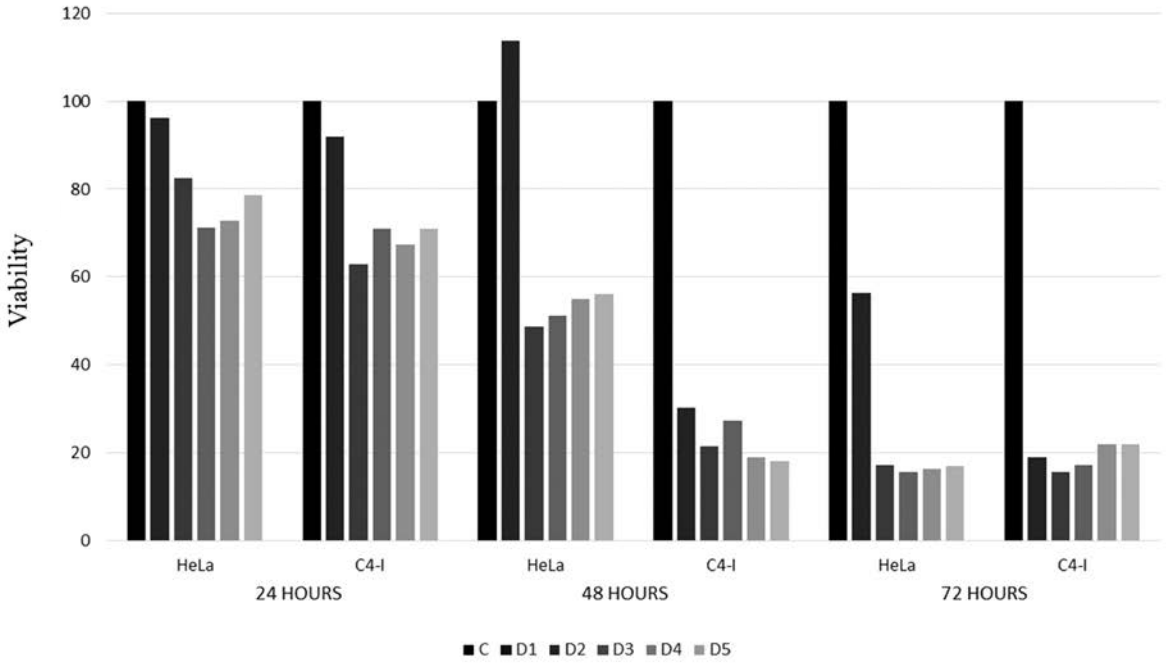

Figure 7. Percent viability values of HeLa and C-4 I cells treated with *Colchicum sanguicolle* at 0-72 h (C: Control D1: 0.001 mg/ml, D2: 0.01 mg/ml, D3: 0.05 mg/ml, D4: 0.25 mg/ml, D5: 0.5 mg/ml) (p<0.01).

es, the maximum toxic effect was the same for the 48 and 72-hr treatments. The IC_{50} dose for C-4 I cells was 0.001 mg/ml at 24 hrs.

Figure 5, showing C-4 I cell cultures treated with *Centaurea antiochia* extract, reveals no toxic effects.

Figure 6 reveals that C-4 I cell cultures treated with 5 different doses of *Crateagus microphylla* extract showed significant increase in viability only with 0.25 and 0.5 mg/ml after 24 hrs. After 72 hrs all doses showed viability by 10% when compared with the control group.

Colchicum sanguicolle appeared to be the most effective extract among the three extracts applied to the cells. For HeLa cells the IC_{50} dose for *Colchicum sanguicolle* extract was 0.01 mg/ml at 48 hrs and for C-4 I cells it was 0.001 mg/ml at 48 hrs.

Figure 7 shows both cell lineages when treated with *Colchicum sanguicolle* extract. It can be seen that C-4 I was more sensitive to the treatment with *Colchicum sanguicolle* extract.

HeLa and C-4 I cells were viewed by phase contrast microscopy for cytotoxicity and morphological changes at the IC_{50} dose of treatment with *Colchicum sanguicolle* extract at the end of 48 hrs incubation. There were no instances of granulation, vacuoles, nuclear condensation and apoptotic bodies in the control group of the two cell lineages. For HeLa cells, granulation, vacuoles, nuclear condensation and apoptotic bodies increased after

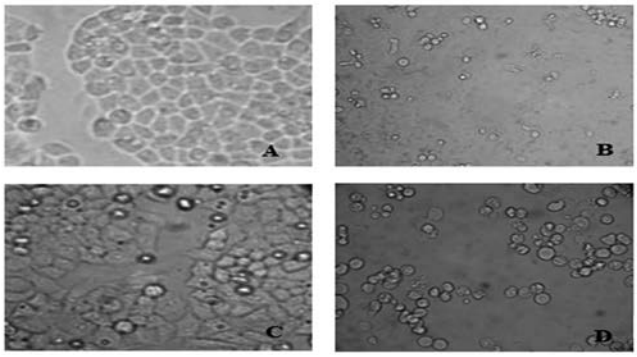

Figure 8. Phase contrast microscopy of HeLa and C-4 I cells which were treated with *Colchicum sanguicolle* for 48 hrs (**A:** HeLa control, **B:** HeLa 0.05 mg/ml, **C:** C-4 I control, **D:** C-4 I 0.001 mg/ml).

48-h incubation in a dose-dependent manner (Figure 8). Similarly, for C-4 I cells, granulation, vacuoles, nuclear condensation and apoptotic bodies increased after 48-h culture in a dose-dependent manner (Figure 8).

Discussion

Although cancer is often seen as a single disease, it is actually a complex group of diseases that affect the cells and tissues. Cancer cell genotypes collectively point to the growth of malignant cells and include eight fundamental changes to cell physiology: self sufficiency in growth signals, insensitivity to growth inhibitory signals, escape from programmed cell death (apoptosis), unlimited proliferation potential, sustaining angiogenesis, tissue invasion, metastasis, and the reorganization of the energy metabolism and immune suppression [14].

Nowadays, it is important to develop new strategies that will suppress the proliferation of cancer cells in order to increase the effectiveness of cancer treatment. Until today there are many drugs known for their antitumor activities. However, the use of natural products, especially in recent years, has become a widespread research topic [15,16]. Colchicine is derived from *Colchicum* belonging to the *Liliaceae (Colchicaceae)* family, and is among the natural active substances. Colchicine is a mitotic inhibitor acting as a tubulin 2 polymerization inhibitor. Changes are observed in the structure of the mitochondria due to the inhibition of tubulin, the formation of the spindle apparatus is suppressed, release of hemotactic factors decreases, and an increase in cyclic AMP can be seen. The agents that harm the function of microtubules *in vitro*, halt the cells in the mitotic phase, resulting in cell death by necrosis or apoptosis. [6,17,18].

Plant extracts for HeLa and C-4 I cell cultures during 24,48 and 72 hrs were evaluated for antiproliferative effects. Among the extracts, colchicum was shown to be most effective.

The control group for HeLa cultures was accepted as 100% and the viability with 0.001, 0.01, 0.05, 0.25 and 0.5 mg/ml at the end of 24 hrs was 96, 82.4, 71.27, 72.89 and 78.63%, respectively. Viability values for the same doses at the end of 48 hrs were 113.72, 48.77, 51.23, 55.12 and 56.15%, respectively. At the end of 72 hrs 56.33, 17.34, 15.79, 16.39 and 17.1% values were obtained.

Assuming the control group for C-4 I cells of *Colchicum sanguicolle* to have 100% viability, values of 0.001, 0.01, 0.05, 0.25 and 0.5 mg/ml at the end of 24 hrs were 91.85, 63, 71, 67.5 and 70.9%, respectively. The viability values for the same doses were 30.3, 21.45, 27.4, 19.1 and 18.1% after 48 hrs. After 72 hrs, the values were 9.1, 15.8, 17.35, 21.9 and 22% for each dose, respectively. According to these results the antiproliferative effects of the *Colchicum sanguicolle* extract on HeLa and C-4 I cells increased depending on time and dose.

For *Colchicum sanguicolle* extract, the IC_{50} dose of HeLa cells at 48 hrs was 0.01 mg/ml, while for the C-4 I cells it was 0.001 mg/ml. According to these results, C-4 I cells were more sensitive to *Colchicum sanguicolle* extracts and showed consistency with other studies of *Colchicum sanguicolle* extract.

In a study conducted by Pirildar et al. [4] tropolone alkaloid components of different species belonging to the genus *Colchicum* were shown to be different according to different regions and growth stages. Also, *Colchicum baytopiorum* CD Brickell methanol extracts when applied to HL60 (IC_{50}: 6.5– < 0.1 μg/mL) and K562 cell lines (IC_{50}: > 500–44 μg/mL) were shown to have cytotoxic activity. In our study the new species of this genus *Colchicum sanguicolle* K.Persson [7] was used. In a study by Artun et al. [19] *Colchicum sanguicolle* K.Persson extract showed high cytotoxic effect on HeLa cells.

Centaurea L. (Compositae) species in Turkey, is one of the many representatives of the genus and has an expansive area of habitation. Of the total 192 species found in Turkey, 123 are endemic to Turkey's flora [8]. Among the public, *Centaurea* is commonly referred to as "Cornflower" and is widely known to have species that are used as a tonic, for constipation, appetizer, chest softener, as antipyretic etc [9]. In experiments conducted on rats, catechins have been shown to shrink tumors

by binding to urokinase which is involved in tumor growth and development [20].

In this study a species of the endemic plant *Centaurea antiochia* Boiss. var. *praealta* (Boiss. & Bol.) Wagenitz was used. Its extract was prepared using the above-ground parts. Species belonging to the genus *Centaurea* contains sesquiterpene lactone and flavonoid compounds [21]. Another plant used in this study was *Crataegus microphylla* C. Koch plant belonging to the *Rosaceae* family, and its extract was prepared from the leaves. Flavonoids in extract were previously isolated in the lab and the structure was determined as hesperetin, eriodictyol, luteolin, quercetin, luteolin 7-O-glucoside, hyperoside, vitexin, viteksin 4'-O-rhamnoside [22].

Centaurea antiochia extract applied on HeLa and C-4 I cells did not have a pronounced cytotoxic effect. Only HeLa cells of 0.001 mg/ml implementation and at 24 and 48 hrs were found to have increased proliferation. These results are in agreement with other studies. However, this suggests that for improved cytotoxic activity of the *Centaurea antiochia* extract higher doses need to be implemented.

In a study by Baykan Erel et al. [23] *Centaurea aphrodisea* Boiss. *Centaurea athoa* DC., *Centaurea hyalolepis* Boiss., *Centaurea iberica* Trev. and *Centaurea polyclada* DC all belonging to the genus *Centaurea (Asteraceae)* analyzed the phenolic contents of these plants. The highest flavonoid content was in the *C. hyalolepis* plant extract (QUE 34.866mg/ml). Also the cytotoxic activity of 5 plants was examined for n-hexane, chloroform and methanol extracts on SK-MEL (malignant melanoma), KB (oral epidermal carcinoma), BT-549 (breast carcinoma), SK-OV-3 (ovarian carcinoma), Vero (normal kidney fibroblasts) and LLC- PK1 (kidney epithelial) cell lines. Only chloroform extracts were observed to have inhibitory effects on cells. *C. polyclada* chloroform extract was shown to have the strongest inhibitory effect on BT-540, KB and SK-OV-3 cell lines (30,33 and 47mg/ml). *C.athoa* chloroform extract had cytotoxic effects on all cell lines (BT-540, BT-549, KB, SK-OV-3, VERO, KB, SK-MEL and LLC-PK1) (40-56 mg/ml). *C. aphrodisea*, *C. hyalolepis* and *C. iberica* chloroform extracts were found to have an intermediate inhibitory effect on BT-549, SK-OV-3 cell lines.

In our experiments the effect of *Crateagus microphylla* extract on HeLa cells was implemented at 5 different doses and 0.001, 0.01 and 0.05 mg/ml were determined to reduce proliferation especially at 24 and 72 hrs. The dose of 0.25 and 0.5 mg/ml significantly increased viability for 48 hrs. In C-4 I cell line 5 different doses were applied and significantly increased viability was observed after 48 hrs. C-4 I cells also showed similar viability results compared to HeLa cells. However, similar to their sensitivity to other extracts, C-4 I cells were again more sensitive to *Crateagus microphylla* extracts than HeLa cells. For this extract pronounced cytotoxic activity was observed for higher doses.

In a study conducted by Min et al., [24] uvaol, ursolic acid and 3-oxo-ursolic acid cytotoxic ursane-tip triterpenes were isolated from *Crataegus pinnatifida* species. In order to determine the cytotoxic activity of these three compounds, the authors applied them on the murine cancer cell line L1210 and human cancer cell lines A549, SK-OV-3, SK-MEL-2, XF498 and HCT15. Uvaol and ursolic acid had an intermediate effect on L1210 cells, while they had low cytotoxic effect on the other cancer cell lines. However, 3-oxo-ursolic acid had an effective influence on cytotoxicity on both L1210 and A549, SK-OV-3, SK-MEL-2, XF498 and HCT15 cell lineages.

Svedström et al. [25] isolated epicatechin units from the leaves of the *Crataegus leavigata* plant and flowers. In a study done by Jankun et al. [26] a derivative of catechin (epigallocatechin-3 gallate) was shown to inactivate urokinase, commonly seen in different types of cancer in humans. Experiments conducted on rats have shown catechins to shrink tumors by binding to urokinase which is involved in tumor tissue growth and development. Saekia et al. [27], have shown that catechin compounds induce apoptosis in U937 histiocytic lymphoma cells. Also, Luo et al. [28] used epigallocatechin-3-gallate on MCF-7 cells and inhibited proliferation as well as cell growth.

The results of the present study revealed the most effective dose and exposure duration of plant extracts and/or fractions for antitumor activity. They also provided information about the molecules responsible for this activity and their mechanisms of action, paving thus the way to develop new herbal medicines and forming the foundation for future studies.

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Conflict of interests

The authors declare no confict of interests.

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