Article-Human and Animal Health

Angelica sylvestris and *Delphinium staphisagria* Extracts Induces Antiproliferation through Caspase-mediated Apoptosis on Human Cancer Cells

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HIGHLIGHTS

- Anticancer activities of ASE and DSE extracts were investigated.

- *Angelica sylvestris* have anticancer potential on HT-29 and HeLa cells.

- *Delphinium staphisagria* have anticancer potential on A549 and MCF-7 cells.

- ASE and DSE extracts induce apoptosis on cancer cells.

Abstract: *Angelica sylvestris* and *Delphinium staphisagria* are medicinal and aromatic herbs with a long history in medicine and food industry. In this study, we have investigated anti-cancer activity of *Angelica sylvestris* and *Delphinium staphisagria* extracts on various cell lines of lung (A549), breast (MCF-7), colon (HT-29), and cervix (HeLa) origin. Also, cytotoxicity was tested on human healthy bronchial epithelial (BEAS-2B) cells. In vitro experiments showed that plant extracts suppressed cell growth and proliferation at low concentrations by reducing cell viability on cancer cells in a time and concentration-dependent manner. It was observed that *Angelica sylvestris* was more effective in HT-29 and HeLa cells and *Delphinium staphisagria* in A549 and MCF-7 cells by suppressing cell proliferation and increasing cell death. Cell death mode (apoptosis/necrosis) was investigated via fluorescent imaging, caspase-cleaved cytokeratin 18, activated caspase-3, and cleaved-PARP (poly (ADP-ribose) polymerase). In order to evaluate the cell death

mode by plant extracts apoptotic markers were investigated by fluorescence staining. *Delphinium staphisagria* extract (50-200 µg/mL) caused a decrease in cell density in A549 and MCF-7 cells compared to untreated controls. A similar situation was observed in HT-29 and HeLa cell lines when treated with ASE. As a result, *Delphinium staphisagria* extracts induced apoptosis in A549 and MCF-7, while *Angelica sylvestris* extracts induced apoptosis in HT-29 and HeLa cancer cells.

Keywords: *Angelica sylvestris; Delphinium staphisagria;* cancer cells; cell death; apoptosis.

INTRODUCTION

Cancer, a general term used for a large group of diseases that can affect any part of the body is among the most important causes of morbidity and mortality in the world today according to GLOBOCAN data by the International Agency for Cancer Research (IARC) for 2018. It is estimated that there are 18.1 million new cancer cases and 9.6 million cancer-related deaths annually. Breast, lung, colorectal, and prostate cancers can be listed among the most common types of cancer diagnosed [1].

Increasing number of cancer deaths and furthermore high treatment costs increase the interest in new treatment approaches and the discovery of new chemopreventive agents from natural sources [2,3]. Natural products are important sources for cancer treatment. Moreover, the fact that new natural compounds obtained from different sources have a more effective/selective and less toxic effect reveals the importance of natural sources [4,5]. Natural compounds with various structural and bioactivity are obtained from different sources such as plants, marine organisms, and microorganisms. These cause the development of compounds with therapeutic potential as a result of molecular modifications, and some are used as medicines in the clinic [6]. Natural products or compounds derived from natural products have been included in the most effective cancer treatments to date. Even today, some natural compounds are known to have antioxidant, anti-proliferative, and pro-apoptotic effects in many cancers such as breast, colon, brain, melanoma, and pancreas [7].

Angelica sylvestris (Wild angel grass) and *Delphinium staphisagria* (Entele grass) are the plant species used in our study. These two types of plants grown in different regions of Turkey belongs to the Apiaceae [8] and Ranunculaceae [9] families respectively. Grown in the Northeast Anatolia region of Turkey *Angelica sylvestris*, it is a perennial plant with a maximum length of 2.5 meters. The root of the plant contains tannins and essential oils. In addition, the brown colour root has a special smell, as well as an acrid taste and a thick texture. The root of the plant is used as a soothing and digestive facilitating agent. *Delphinium staphisagria*, which grows in western and southern regions of Turkey, is a two-year herbaceous plant with a maximum length of 1.5 meters. This plant having a vertical, cylindrical, branched and hairy body, contains fixed and essential oils and alkaloids.

Although there have been several studies of Angelica species in the literature [10-12], we have not found any studies of the anticancer activity of *Angelica sylvestris*, which grows in Turkey. Again, in the literature review, although there are various studies related to *Delphinium staphisagria*, only one study sample related to anticancer activity was found. In this research, as a result of MTT viability analysis there were no positive results suggesting the anticancer activity of the plant [9]. Therefore, we performed the study with *Angelica sylvestris* and *Delphinium staphisagria* plant species.

This study was performed to investigate the cytotoxic and apoptotic activities of *Angelica sylvestris* and *Delphinium staphisagria* plant extracts (ASE and DSE respectively) on the human colon (HT-29), lung (A549), cervical (HeLa), and breast (MCF-7) cancer cell lines. Also, human healthy bronchial epithelial (BEAS-2B) cells were used as a control group.

MATERIAL AND METHODS

Extraction of plant samples

Angelica sylvestris root and *Delphinium staphisagria* seeds were purchased commercially from local herbal shop in Turkey. Thirty grams of plant samples were extracted in 200 mL ethanol, methanol, chloroform, hexane and water consecutively in a Soxhlet extractor for 24 hours. A rotary evaporator at 40 °C was used to concentrate the crude extracts. Lyophilized residues were stored at - 20 °C for later use.

Cell culture

Human colon (HT-29), lung (A549), cervical (HeLa), breast (MCF-7) cancer and human bronchial epithelial (BEAS-2B) cells were cultured in RPMI 1640 supplemented with penicillin G (100 U/mL), streptomycin (100 µg/mL), L-glutamine, and 10% fetal bovine serum (Invitrogen, Paisley, UK) at 37 °C in a humidified atmosphere containing 5% CO_2.

Lyophilized extracts of *Angelica sylvestris* root and *Delphinium staphisagria* seeds were prepared in DMSO (0.05 g/ 0.5 mL) for a stock solution and required concentrations for cell culture were prepared with the medium.

Cell proliferation assay

Cells (HT-29, A549, HeLa, MCF-7, and BEAS-2B) were seeded as 5×10^3 cells per well of a 96-well plate and treated with plant extracts (3.12-200 µg/mL) for 72 hours. Only cells cultured in medium and 0.1% DMSO was used as control groups. At the end of the period, 50 µL of 50% ice-cold TCA (w/v) was added to each well of the 96-well plate (when the final volume was 200 µL) to fix the cells for 1 hour. Then, 50 µL of SRB dye (in 1% acetic acid v/v) was added and incubated for 30 minutes. After this step, which determines the population density of the cell, the plate was washed with 1% acetic acid to remove unbound SRB dye ~~bound~~. At the end of washing, 10 mM tris base (150 µL/well) was added to dissolve the dye bound to the proteins. And finally, absorbances were measured spectrophotometrically at 564 nm (Biotek Microplate Readers, Winooski USA). The viability of untreated cells (control cells) was considered to be 100%, and the following formula (%) = [100 (sample Abs)/(control Abs)] was used to calculate the viability of cells treated with plant extract. In the experiment, each concentration was repeated in three different wells independent of each other.

Morphological Evaluation

Cytotoxic effects of plant extracts on A549, MCF-7, HT-29, and HeLa cells were also evaluated morphologically. The effect on HT-29, A549, HeLa, and MCF-7 cells at a concentration of 50, 100, and 200 µg/mL for 72 hours for each plant extract was morphologically evaluated under a phase-contrast microscope (Euromex, Nederland).

Fluorescence imaging

Hoechst 33342 and Propidium iodide (PI) fluorescent dyes were used to determine the mode of death (apoptosis/necrosis). Hoechst, which penetrates through the cell membrane and binds to DNA, paints the nuclei of all living and dead cells (apoptotic/necrotic). Propidium Iodide (PI), penetrates only membrane-damaged cells and stains all dead (necrotic) cells. In this method, Hoechst+/ PI- observed cells are considered alive and apoptotic, and Hoechst+/PI+ observed cells are considered necrotic. A549, HT-29, MCF-7 and HeLa cells were counted and planted to be 5×10^3 cells (in triplicate) in 96 well plate containing 100 µL of the medium. After 24 hours of incubation, 200, 100 and 50 µg/mL concentrations of plant extracts were applied on the cells, respectively, within 100 µL. 100 µL of the medium was removed from the untreated control wells and fresh medium was added in the same proportion. Then, after 72 hours of incubation, 50 µL of the medium was removed from all wells. Hoechst and PI dyes were prepared at 5 and 1 µg/mL concentrations, respectively, and applied to the cells. Cells were incubated for 30 minutes at room temperature and then viewed under a fluorescent microscope.

Caspase-cleaved cytokeratin 18 (M30) detection

Caspases, an enzyme group that is activated during the apoptosis process, cuts cytokeratin 18 (CK18) from ASP396 and converts it into fragmented CK18. In this way, the presence of caspase-mediated apoptosis is evidenced by the recognition of fragmented CK18 with M30. For this, a commercially available immunological test kit (M30-Apoptosense ELISA kit, Peviva AB, Sweden) was used. Cells (A549, MCF-7, HeLa, HT-29) were counted and seeded in a 96-well plate (in triplicate) containing 100 µL of medium with 5 x 10^3 cells per well. Cells were treated with 100 µg/mL plant extract (ASE and DSE) for 72 hours. Then 10% NP-40 was added to all wells to lyse cells. It was incubated for 10 minutes at room temperature in a shaker. The lysate in all wells was collected and centrifuged for 10 seconds at 2000 rpm. Supernatant and horseradish peroxidase conjugate were added to the wells covered with mouse monoclonal M30 antibody, which recognized CK18 surface and incubated for 4 hours in the shaker. After washing the wells with a

washing solution, 200 μL of TMB substrate was added. Plate was incubated at room temperature and in the dark for 20 minutes. The reaction was terminated via stop solution and the resulting colour intensity was measured at 450 nm (FLASH Scan S12, Eisfeld, Germany) with a spectrophotometer.

Measurement of Active Caspase-3

PARP acts as a marker in the apoptotic process like caspases. To perform these experiments, cells (A549, MCF-7, HeLa, HT-29) were and Cleaved PARP Levels counted and cultivated in 6-well plates (in triplicate) containing 15×10^4 cells per well and 2000 μL of the medium. 24 hours after the cells were plated, ASE (HT-29 and HeLa) and DSE (A549 and MCF-7) at concentrations of 50, 100, 200 μg/mL were applied to the relevant cells for 48 hours. The medium on the 6-well plate was collected in centrifuge tubes to obtain cell lysates. After the wells were washed with cold 1X PBS, the cells were removed from the surface using a scraper in 1X PBS and collected in the same centrifuge tube and centrifuged at 1000 g at + 4 °C for 5 minutes. After centrifugation, the supernatant was removed and the pellet was suspended with 70-100 μL of RIPA buffer for lysis, transferred to 1.5 mL centrifuge tubes, and kept on ice for 30 minutes. During this time, the mixture in the tubes was mixed by pipetting every 10 minutes. At the end of the period, the tubes were centrifuged at 13000 g for 15 minutes at + 4 °C. The supernatant containing the proteins were collected in 0.5 mL centrifuge tubes and was stored at - 20 °C until use. In 1 mL of RIPA buffer; 10 μL 200 mM PMSF, 10 μL 100 mM sodium orthovanadate, 20 μL protease inhibitor mixture was added. The active caspase-3 and cleaved PARP levels associated with apoptotic treatment after lysate was determined using the human caspase-3 (active) ELISA kit (Invitrogen Corporation, Camarillo, CA) and the PARP Cleaved [214/215] ELISA kit (Invitrogen, CA), according to the protocols described in the manufacturer's instructions.
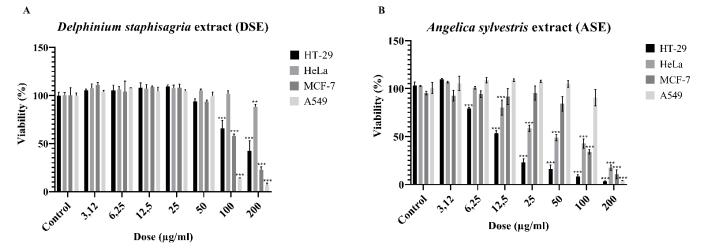
Statistical analysis

Statistical evaluations were carried out with the GraphPad program. All measurements made in the study were repeated three times and the results are given as mean ± standard deviation. One-way ANOVA variance analysis was used for comparisons between groups. $p < 0.05$, $p < 0.01$, $p < 0.001$ values were considered significant.
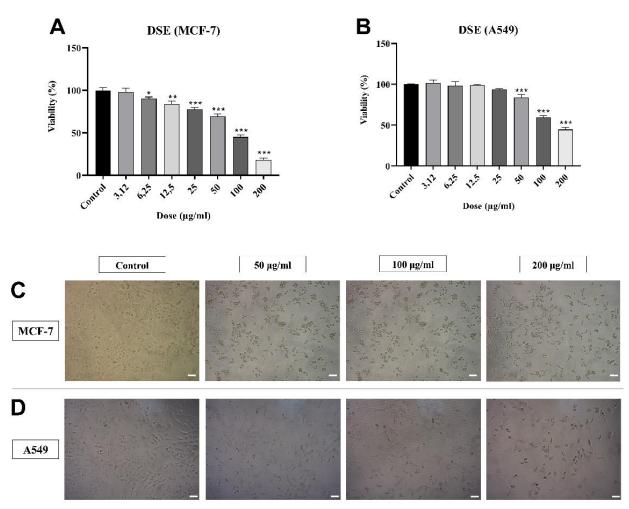
RESULTS

The anti-growth activity of plant extracts on cancer cells

Anti-growth activity of DSE and ASE were assessed on the human colon (HT-29), cervix (HeLa), lung (A549), breast (MCF-7) cancer and, human healthy bronchial epithelial cells (Beas-2B) by SRB viability test. Extracts were applied in different concentrations (3.12-200 μg/mL) for 72 hours on cells. According to the SRB results, it was determined that the DSE decreased cell viability in a concentration-dependent manner on MCF-7 and A549 cells compared to HT-29 and HeLa cancer cells (Figure 1A). It was observed that viability was significantly decreased especially at high concentration (200 and 100 μg/mL). When the SRB results of different concentrations (3.12-200 μg/mL) of ASE were examined, it was determined that it had a higher cytotoxic effect on HeLa and HT-29 cancer cells compared to A549 and MCF-7 cancer cells (Figure 1B). It was observed that this effect was concentration-dependent and at the concentration range of 12.5-200 μg/mL, viability was statistically decreased in HT-29 and HeLa cells (p< 0.001) after treatment with ASE.
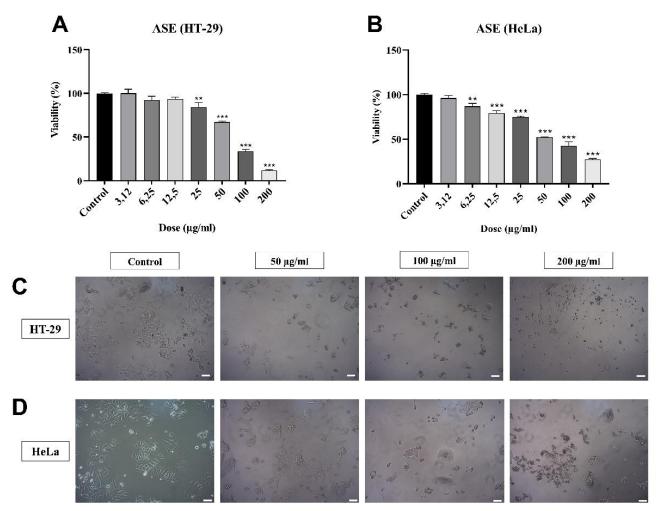
A

B

Figure 1. Cytotoxicity of the extracts of DSE (A) and ASE (B) by SRB viability assay. The effects of extracts were applied on the human colon (HT-29), cervix (HeLa), breast (MCF-7), lung (A549) cancer cells. *Denotes statically significant differences in comparison with control: **(p< 0,01) and ***(p< 0.001). Data are presented as mean ± SD (n= 3). (ASE: *Angelica sylvestris* extract, DSE: *Delphinium staphisagria* extract*).*

After determining the anti-proliferative effect of DSE and ASE on cells, cell viability was re-examined in cells where the extracts were effective. Viability in MCF-7 and A549 cells treated with 72 hours DSE (3.12-200 µg/mL) was determined by the SRB test. It was observed that cell viability was decreased with DSE on concentration-dependent manner (Figure 2A). The decrease in cell viability was statistically significant between 6.25-200 µg/mL concentrations compared to the control. Compared to A549 cells, DSE was observed to be more effective on MCF-7 cells. Cell viability in A549 cells was found to be significantly reduced at concentrations of 50, 100 and 200 µg/mL (Figure 2B). The images taken with phase contrast microscope also confirmed the cytotoxic effect results generated by SRB test (Figure 2C, 2D).

Figure 2. Determination anti-growth activity of DSE on MCF-7 (A) and A549 (B) cells by SRB viability assay. Morphological evaluation of MCF-7 (C) and A549 (D) cell viability after treatment of DSE. *Denotes statically significant differences in comparison with control: *(p< 0.05), **(p< 0.01) and ***(p< 0.001). Data are presented as mean ± SD (n= 3). Microscope objective magnification 10x. Scale bar 100 μm. (DSE: *Delphinium staphisagria* extract).
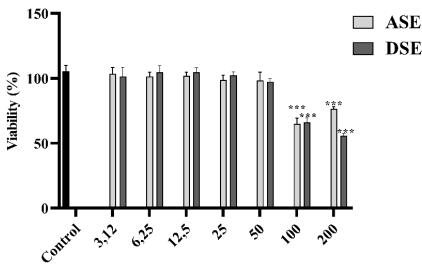
The effects of ASE examined on HeLa and HT-29 cells. It was observed that the results obtained were consistent with the previous analysis and the viability decreased depending on the concentration. A cytotoxic effect was observed in HT-29 cells from an initial concentration of 25 μg/mL (Figure 3A). Statistically significant changes were observed in HeLa cells between 6.25-200 μg/mL concentrations (Figure 3B).

Figure 3. Determination anti-growth activity of ASE on HT-29 (A) and HeLa (B) cells by SRB viability assay. Morphological evaluation of HT-29 (C) and HeLa (D) cell viability after treatment of DSE. *Denotes statically significant differences in comparison with control: *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$). Data are presented as mean ± SD (n= 3). Microscope objective magnification 10x. Scale bar 100 μm (ASE: *Angelica sylvestris* extract).

When the cytotoxic effects of DSE and ASE on healthy cells were evaluated, it was observed that cell viability decreased only at 100 and 200 μg/mL concentrations. These reductions were also found to be over 50%. It was found that DSE and ASE are not as effective on healthy cells as cancer cells (Figure 4).

BEAS-2B

Figure 4. Assessment of cytotoxicity of DSE and ASE on human healthy bronchial epithelial cells (BEAS-2B) by SRB viability assay. *Denotes statically significant differences in comparison with control: *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$). Data are presented as mean ± SD (n= 3) (ASE: *Angelica sylvestris* extract, DSE: *Delphinium staphisagria* extract).
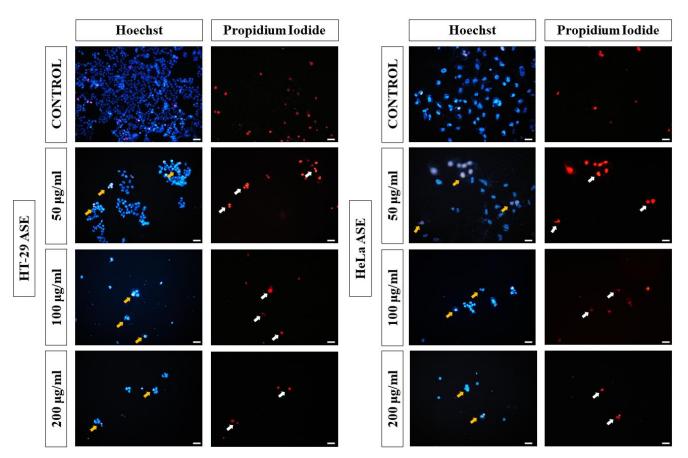
Apoptosis inducing effects of plant extracts

After the plant extracts were determined to kill cancer cells, fluorescent staining was performed to determine the mechanism death. Hoechst and PI dyes were used to stain the cells after plant extract treatment to evaluate the morphology and death mode via fluorescence microscopy. Nuclei of cells that die with apoptosis are smaller and brighter than normal cells, while cells that undergo necrosis are larger and less stained. When the fluorescent images were evaluated, it was confirmed by Hoechst staining that DSE decreased cell density in MCF-7 and A549 cells. In MCF-7 and A549 cells, some cells are smaller and brighter than control cells in Hoechst staining after DSE application, indicating that the cells die with apoptosis. In addition, PI dye positivity observed in some cells indicates that the cells die with secondary apoptosis (Figure 5).
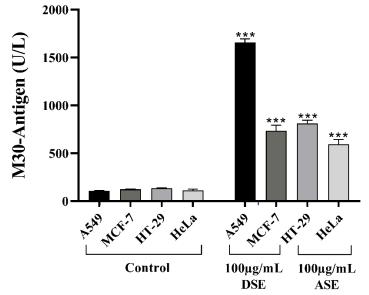
Figure 5. Hoechst and propidium iodide fluorescent staining image of DSE after 72 hours of cytotoxic effect on A549 and MCF-7 cells. Yellow arrows indicate pyknotic/fragmented nuclei in apoptotic cells, while white arrows indicate the PI image of pyknotic nuclei. Microscope objective magnification 20x. Scale bar 40 µm (DSE: *Delphinium staphisagria* extract).

The decrease in cell viability in HT-29 and HeLa cells treated with ASE was also supported by fluorescent microscopy images. It is observed with Hoechst staining that cell viability decreases depending on the concentration of plant extracts applied. In addition, nuclear shrinkage and brightness, which is a marker of apoptosis in some cells, is also noteworthy. Again, observation of PI dye in some cells suggests late-stage apoptosis in cells (Figure 6).
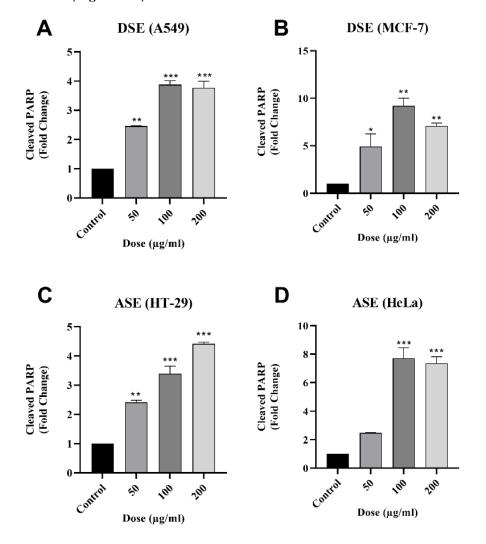
Figure 6. Hoechst and propidium iodide fluorescent staining image of ASE after 72 hours of cytotoxic effect on HT-29 and HeLa cells. Yellow arrows indicate pyknotic/fragmented nuclei in apoptotic cells, while white arrows indicate the PI image of pyknotic nuclei. Microscope objective magnification 20x. Scale bar 40 μm (ASE: *Angelica sylvestris* extract).

To confirm that the mode of cell death caused by ASE and DSE in the respective cells was apoptosis, the cells were treated with plant extracts at a concentration of 100 μg/mL for 72 hours, and then the caspase-cleaved cytokeratin-18 values were investigated by the M30 Elisa technique (Figure 7). A significant increase in caspase-cleaved cytokeratin-18 levels was observed in cells exposed to plant extracts compared to untreated cells. In the light of these results, it was once again confirmed that the mode of death created by DSE and ASE on their respective cell lines is apoptosis.
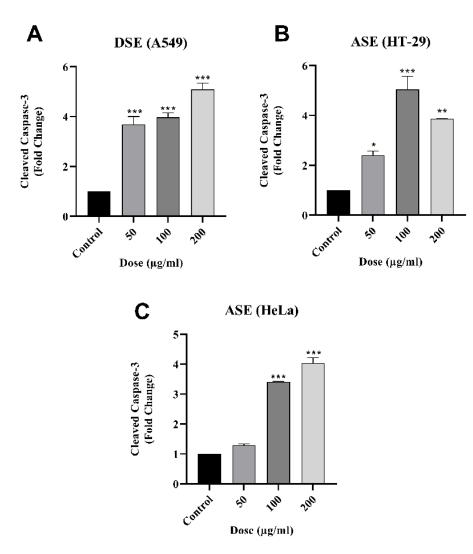

Figure 7. M30-Antigen levels findings. A549 and MCF-7 cells were treated with DSE for 72 hours while HT-29 and HeLa cells were treated with ASE. M30-antigen levels were measured by the ELISA test as described in the materials and methods section. *Denotes statically significant differences in comparison with control: ***(p< 0.001). Data are presented as mean ± SD (n= 3) (ASE: *Angelica sylvestris* extract, DSE: *Delphinium staphisagria* extract).

Next, in order to examine the apoptosis mechanisms in cells in more detail, the activities of the cleaved-PARP (Figure 8) and the cleaved-caspase 3 activity (Figure 9) were investigated. According to our results, the level of cleaved-PARP was significantly increased after DSE treatment in A549 and MCF-7 cells. It was observed that the level of cleaved-PARP in A549 cells was approximately 4 times higher than in the control group (Figure 8A). In MCF-7 cells, it was determined that the level of cleaved-PARP increased up to 10 times, especially after 100 µg/mL concentration (Figure 8B). In HT-29 and HeLa cells, the level of cleaved-PARP was increased as concentration dependent after ASE treatment. This increase was measured as approximately 5-fold at 200 µg/mL concentration in HT-29 cells (Figure 8C) and 8-fold at 100 µg/mL concentration in HeLa cells (Figure 8D).

Figure 8. After treatment with DSE in A549 (A) and MCF-7 (B) cells; similarly, the determination of cleaved PARP levels in HT-29 (C) and HeLa (D) cells after treatment with ASE. Both extracts were treated for 72 hours at different concentrations (50, 100, 200 µg/mL). *Denotes statically significant differences in comparison with control: **($p < 0.01$) and ***($p < 0.001$). Data are presented as mean ± SD (n= 3). (ASE: *Angelica sylvestris* extract, DSE: *Delphinium staphisagria* extract).

When cleaved-caspase 3 activity was evaluated, it was observed that cleaved-caspase 3 activity increased significantly as the dose increased in A549 cells after DSE treatment (Figure 9A). In HT-29 and HeLa cells, it was determined that ASE treatment increased the cleaved-caspase 3 activity in HT-29 cells especially approximately 6-fold at 100 µg/mL concentration (Figure 9B). In addition, cleaved-caspase 3 activity was raised approximately 5-fold at 200 µg/mL concentration in HeLa cells (Figure 9C).

Figure 9. After treatment with DSE in A549 (A) cells; similarly, determination of cleaved-caspase 3 levels in HT-29 (B) and HeLa (C) cells after treatment with ASE. Both extracts were treated at different concentrations (50, 100, 200 µg/mL) for 72 hours. *Denotes statically significant differences in comparison with control: *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$). Data are presented as mean ± SD (n= 3). (ASE: *Angelica sylvestris* extract, DSE: *Delphinium staphisagria* extract).

DISCUSSION

Cancer is a global health problem and treatment methods include many chemotherapy drugs or drugs developed for molecular targets. However, standard chemotherapy treatment still does not respond as expected. At this point, in addition to traditional treatment methods, the use of herbal or other natural products becomes important. Today, more than 20% of modern drugs are prepared directly or indirectly from traditional herbs. Moreover, herbal substances are used as drug active substances in the treatment of many diseases including cancer [13].

In this study, possible cytotoxic and apoptotic effects of ASE and DSE on different cell lines were evaluated. The cytotoxic effect of ASE and DSE (3.12-200 µg/mL, 72 hours) were evaluated by SRB analysis on different cancer cells (HT-29, HeLa, A549 and MCF-7) and human healthy bronchial epithelial cells (BEAS-2B). As a result of the SRB test, a decrease in cell viability percentages was observed parallel to the increase in concentration (Figure 1), while plant extracts did not appear to cause a significant decrease in cell viability when applied to human healthy bronchial epithelial cells (Figure 4). While DSE suppressed the growth in A549 and MCF7 cells (Figure 2), ASE shows the same effect on HT-29 and HeLa cells (Figure 3). The decrease in cell viability indicates that plant extracts have a selective effect on cancer cells and affect the different mechanisms, leading to death. Cheng and coauthors [10] have shown that *Angelica sinensis*, a different genus, inhibits proliferation in various human cancer cells (A549, HT-29, DBTRG-05MG, J5) and can induce apoptosis of cells. Their study shows that *Angelica sinensis* has an in vitro antitumor activity by inducing apoptosis. Lin and coauthors [14] have observed that the acetone extract of the *Angelica sinensis*

plant suppresses tumour cell proliferation and tumour cell apoptosis, according to findings from in vitro and in vivo studies. Since there are not enough studies on ASE, these results we obtained have similar results to the studies conducted with other species.

Apoptotic markers were investigated to determine the cell death mode triggered by plant extracts using fluorescent dyes. When the extract of the DSE was evaluated at concentrations of 50-200 µg/mL in A549 and MCF-7 cells, it was observed that cell density decreased significantly in both cell lines compared to control in Hoechst dye (Figure 5). A similar situation is observed in HT-29 and HeLa cell lines treated with ASE. When the extract of the ASE is evaluated at the same concentrations in these cell lines, it appears that the cell density in both cell lines is significantly reduced in cells stained with Hoechst dye (Figure 6).

In our study, caspase-cleaved cytokeratin 18 level and cleaved-PARP levels, which are the determinants of cell death mode (apoptosis/necrosis), detected in A549, HT-29, HeLa and MCF-7 cells treated with ASE and DSE. When the levels of cytokeratin 18 were examined with the M30 ELISA test (Figure 7), it was observed that while the increased M30-antigen levels were observed, there was also an increase in the cleaved-PARP level at the same concentrations (Figure 8). In order to support these results, caspase 3 activities in cells have also been investigated. Cleaved (active) caspases, which are markers in the determination of apoptosis, are activated when they receive an apoptotic signal and take a role in forming typical apoptosis characteristics by cutting many molecules such as DNA repair and replication proteins, cytoskeletal proteins. When looking at caspase 3 activity in cells treated with DSE and ASE, it is seen that there are significant increases compared to control (Figure 9). MCF-7 cells are unable to express caspase 3 due to a deletion mutation in exon 3 of the gene, and thus caspase 3 activity was not investigated in these cell lines [15,16]. Our results show that the anticancer effects of ASE and DSE are a combination of effects on inhibiting tumour cell growth and inducing tumour cell apoptosis involving the caspase-mediated mechanism.

To conclude, the plant extracts used in this study produced concordant results with the literature in terms of their anti-growth activity stemming from inhibition of cell proliferation and promotion of apoptosis in different cell lines in vitro. Further studies are needed for the use of ASE and DSE as drug active ingredients in cancer chemotherapy.

Conflicts of interest: The authors declare that there is no conflict of interest regarding the content of this article.

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