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INVESTIGATION OF POTENTIAL ANTI-CANCER AND ANTI-INFLAMMATORY EFFECTS OF *DATURA STRAMONIUM* ETHANOLIC EXTRACTS AGAINST SELECTED HUMAN CANCER CELL LINES

Mehlika Alper*

Department of Molecular Biology and Genetics, Faculty of Science, Mugla Sitki Kocman University, Mugla, Turkey

ABSTRACT

In this study, the different biological properties particularly associated with anti-cancer and anti-inflammatory effects of the *D. stramonium* extracts from Edirne (Turkey) were examined for the first time.

The ethanolic extracts of leaves (DSL) and seeds together with flowers (DSSF) from *D. stramonium* L. were used. The cytotoxic activity was determined using MTT assay. Analysis of the cell cycle distribution and the cell death were performed using flow cytometry. The levels of the VEGF and the pro-inflammatory cytokines (IL-1α, IL-6 and TNF-α) were determined by ELISA.

DSL extract was more cytotoxic than DSSF extract. The DSL extract at 72 h exhibited selective cytotoxicity against HeLa cells with IC_{50} value of 265.7 µg/mL and MCF-7 cells with IC_{50} value of 272.5 µg/mL compared to healthy BEAS-2B cells with IC_{50} value of 622 µg/mL. The effect of the DSL extract on the cell cycle progression changed according to the type of cell lines. The DSL extract at 800 µg/mL was found to induce necrotic cell death in all the cancer cell lines tested. The VEGF secretion was determined to reduce by 36% in A549 cells and by 12% in PC-3 cells after treatment with 300 µg/mL DSL extract. The levels of pro-inflammatory cytokines (IL-6, TNF-α) secretion varied according to cell lines.

The findings of the present study suggest that *D. stramonium* may be an important source for novel anti-cancer agents.

KEYWORDS:
Datura stramonium, anti-cancer, anti-inflammatory, cancer cell lines

INTRODUCTION

Cancer is one of the most common causes of death in the World and continues to be a growing major health problem [1]. In cancer treatment, there are various procedures like immunotherapy, stem cell transplantation, surgery, radiotherapy, chemotherapy and they can cause serious side effects such as limited bioavailability, toxicity and nonspecificity [2]. Moreover, chemotherapy which is one of the major strategies of cancer treatment is usually associated with the development of multidrug resistance [3]. Therefore, recent researches focus on overcoming the side effects of chemotherapy and developing new anticancer agents with no toxic effects [4].

More than 60% of the currently used anticancer agents were originated from natural resources. Plants have historically been the main resources of natural products. Also, the currently used effective cancer chemotherapeutics such as vincristine, etoposide, paclitaxel (Taxol ®), docetaxel, topotecan, and irinotecan are plant-derived agents [5].

There are more than 3000 plants with anticancer properties in the world [6]. The secondary metabolites of the plants can play the significant role as both potential anticancer agents and cancer chemoprevention [7,8]. Environmental factors were reported to be one of the important determinants of the difference in secondary metabolites of plant species [9]. Therefore, the screening of therapeutic potential of plants from different environmental and ecological conditions may contribute to developing pharmacologically active compounds for cancer treatment.

Datura stramonium L. (DS) belonging to Solanaceae family is a widespread annual plant. This plant is commonly known as Angel's trumpet, Locoweed, Jimson weed, Datura or thorn apple [10, 11]. It is also named in our country such as "şeytan elması, boru çiçeği, abu zambak, cin otu, tatula" [12]. All parts of the plant are poisonous. On the other hand, the parts of the plant are practically used in indigenous and traditional folk medicine [13]. In different studies, the plant has been stated to possess medicinal values such as antiasthmatic, antimicrobial, acaricidal, anti-cancer, anti-inflammatory, antifungal, nematicidal activities and has phytochemical contents including different compounds like alkaloids, saponins, tannins, glycosides with various biological activities [10, 13, 14, 15]. However, the anti-cancer and anti-inflammatory properties of *D. stramonium* has been poorly evaluated.

Therefore, the present study for the first time aimed to investigate the various biological properties particularly associated with anti-cancer and anti-inflammatory effects of ethanolic extracts from *D. stramonium* collected from Edirne province (Turkey) on different cell lines

MATERIALS AND METHODS

Collection of plant species. *D. stramonium* (DS) specimens were collected from Edirne province in Turkey in September 2015, and authenticated by Dr. Fatma Güneş at the Department of Pharmaceutical Botany, Trakya University, Edirne. The voucher specimen was deposited at Trakya University Herbarium.

Preparation of plant extracts. Aerial parts of the plant were washed, air-dried under shade at room temperature and separated into two parts: leaves (DSL) and seeds together with flowers (DSSF). Each 10 gr of pulverized parts of DS were extracted with absolute ethanol (Merck, USA) in a shaking water bath (Stuart, SBS40, UK) for 6 at 53°C at least twice. The extracts were filtered using Whatman filter paper no. 1 and concentrated in a vacuum rotary evaporator (IKA, RV 10, USA) at 42–49°C. The crude extracts were stored at -20°C and protected from light until use. The stock solutions of the extracts were prepared in 10% DMSO (Applichem, USA) and serial dilutions were freshly performed with the growth medium. The final concentration of DMSO in cells tested did not exceed 0.1%.

Cell culture conditions. MCF-7 (breast cancer cell line), A549 (non-small-cell lung cancer cell line), HeLa (cervical cancer cell line), PC-3 (prostate cancer cell line), Daudi (Burkitt's lymphoma, CCL-213) and BEAS-2B (bronchial epithelial cell line) human cell lines used in this study were obtained from ATCC. The cell lines were cultured in RPMI-1640 medium (Biochrom, Germany) with stable L-glutamine (Biochrom, Germany) containing 10% heat-inactivated fetal bovine serum (FBS) (Biochrom, Germany), 100 units/mL penicillin and 100 mg/mL streptomycin (Biochrom, Germany) at 37°C in a humidified incubator with 5% CO_2 and 95% air. When the cells reached 80% confluence, they were passaged and used for the assay.

Cell cytotoxicity assay. The MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium Bromide) (Applichem, USA) assay [16] was used to evaluate the cytotoxic effects of the extracts against different cell lines. In brief, the cell lines at a density of $2x10^4$ cells/well were added into 96-well microplates (Greiner, Germany) in triplicate and incubated for 24 h. To screen the cytotoxic effects of the extracts, the cell lines were exposed to the extracts at 800 µg/mL for 72 h. For the dose and time course, experiments were performed for six different concentrations (800-25 µg/mL) of the extract for 24, 48 and 72 h under the same conditions. The untreated cells served as a control. At the end of incubation time, the medium in each well was discarded and replaced with 100 mL of fresh growth medium. Then, 10 µL of MTT (5 mg/mL) in phosphate-buffered saline (PBS) was added into each well and the microplate was incubated for a further 4 h at 37°C. After the medium with MTT was removed, the blue formazan crystals were dissolved in 100 mL of DMSO (Applichem, USA). The optical density of each well was read with a microplate reader (Thermo Scientific, Multiscan FC, USA) at 540 nm. The percentage of the viable cells was calculated using the following equation:

Cell viability % = (Mean Abs of treated cells/Mean Abs of untreated cells)x100 (1)

Analysis of cell cycle distribution. The percentages of MCF-7, A549, HeLa and PC-3 cells in each cell cycle phase were determined by propidium iodide (PI) (Sigma-Aldrich, USA) staining. Briefly, the cells were plated at $5x10^5$ cells/well in 6 well-plates. After 24 h incubation, the cells were treated with 800 µg/mL DSL extract for 24 h. The control cells were exposed to DMSO at 1% final concentration. Then, the cells were collected by trypsinization, washed with cold PBS and fixed in absolute ethanol. After 48 h of storage at -20°C, the cells were centrifuged at 1200 rpm for 10 min at 4°C and cell pellets were washed with cold PBS. Later, the cells were resuspended in 1 mL PBS containing 0.1% (v/v) Triton X-100 (Amresco, USA) and incubated with 100 µL of RNase A (200 µg/mL) (Applichem, USA) at 37°C for 30 min. Subsequently, each cell suspensions was treated with 100 µL PI (1mg/mL) and incubated at room temperature for 15 min in the dark. These stained cells were analyzed by BD FACSCanto flow cytometry (BD Biosciences, San Jose, CA) using Modfit LT 3.0 software to detect the cell percentage in each phase of the cell cycle.

Analysis of cell death. An annexin V-FITC apoptosis detection kit (eBioscience, BMSF500FI/100) was used for detection the extent of the cell death in treated and control cells, according to the manufacturer's directions. In brief, MCF-7, A549, HeLa and PC-3 cells were cultured at $5x10^5$ cells/well in 6-well plates and treated with the extract according to the method of cell cycle analyses described above. The cells treated with DMSO at 1% final concentration served as the control. After treatment, the cells were collected from each well by trypsinization and washed with cold PBS twice. Cell suspensions were centrifuged at 800 rpm for 5 min at 4°C. After careful removing supernatants, cells were resuspended in 190 µL binding buffer and 5 µL

Annexin V-FITC was added to each cell suspension. Cells were incubated at room temperature for 10 min in the dark and then centrifuged. Cells were again dissolved in 190 µL binding buffer. Then, each cell suspension was stained with 10 µL PI (20 µg/mL) at room temperature in the dark and analyzed with BD FACSDiva software v6.13 using BD FACSCanto flow cytometry (BD Biosciences, San Jose, CA).

Enzyme-linked immune sorbent assay (ELISA). The supernatants of the cell cultures were used for quantification of VEGF (vascular endothelial growth factor) in A549 and PC-3 cells, and for quantification of the pro-inflammatory cytokines (IL-1α, IL-6, and TNF-α) in A549 and Daudi cells by using human ELISA kits (Boster Biological Technology, USA). Briefly, cells were seeded at $2x10^5$ cells/well in a 6-well plate and incubated for 1 h. After treatment with 300 µg/mL (approximately lC_{50} value for cancer cells tested except for Daudi) DSL extract for 6 h, the supernatants of treated and untreated (control) cell cultures were collected and centrifuged at 14000 rpm for 30 second. These supernatants were aliquoted and stored at -20°C until the time of testing. A 100 µL of supernatant was then assayed for detection of the level of VEGF or inflammatory each cytokine produced through ELISA kits following the manufacturer's protocol. The absorbance of each well was measured at 450 nm by the usage of a microplate reader (Thermo Scientific, Multiscan FC, USA).

Statistical analysis. The data were presented as the mean ± standard error (SE). GraphPad Prism software version 7.0 (GraphPad Software Inc., San Diego, CA, USA) was used to perform statistical analyses and to calculate IC_{50} values.

RESULTS

The cytotoxic effects of the plant extracts in several cell lines. The cytotoxic effects of the crude extracts of the DS against MCF-7, A549, HeLa, PC-3, and BEAS-2B cells were determined by the MTT assay. Firstly, the ethanolic DSL and DSSF extracts at 800 µg/mL were separately tested for 72 h on the cancer cell lines used for MTT assay to detect the effective extract. According to the results, DSL extract exhibited more than 65% cytotoxicity on all cancer cell lines whereas DSSF extract cause less cytotoxicity than DSL extract (Figure 1). So, dose and time course experiments for 24, 48 and 72 h were performed for DSL extract in concentration range between 800 µg/mL to 25 µg/mL. In addition, BEAS-2B healthy cells were treated with same DSL extract concentration only for 72 h.

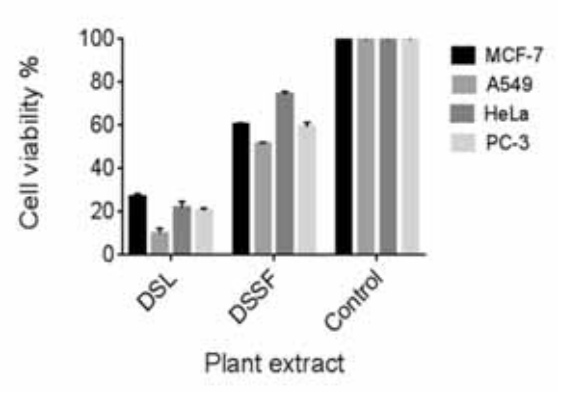

FIGURE 1
Screening of the cytotoxic effects of the extracts obtained from *D. stramonium* against cancer cell line.
The percentage of the cell viability in the cells treated with ethanolic DSL or DSSF extracts at 800 µg/mL concentration for 72 h were determined using MTT assay. Data is presented as mean± standard error (SE) from three independent experiments. DSL: leaves extract of *D. stramonium*, DSSF: seeds together with flowers extract of *D. stramonium*.

As shown in Figure 2, the DSL extract generally decreased the cell viability in a time- and concentration dependent manner. This extract was also found to be effective to different extent according to the cell lines tested. The most statistically significant cytotoxic effects in BEAS-2B were seen at concentrations of only 800 and 400 µg/mL for 72 h in contrast to the cancer cells. The approximate IC_{50} values (50% inhibitory concentrations) of the DSL extract against the cell lines tested were summarized in Table 1. The DSL extract at 24 h did not demonstrate more than 50% cytotoxicity on cancer cells except for PC-3 cells. At 72 h, DSL extract showed the highest cytotoxicity against HeLa cells with IC_{50} value of 265.7 µg/mL and MCF-7 cells with IC_{50} value of 272.5 µg/mL compared to normal BEAS-2B cells with IC_{50} value of 622 µg/mL. The IC_{50} value of this extract at 72 h were found to be 361 µg/mL and 369 µg/mL for A549 and PC-3 cells, respectively. These findings revealed that the most sensitive cells to the DSL extract at 72 h were HeLa and MCF-7.

Flow cytometric analysis of the cell cycle distribution. The cell cycle distribution in cells treated with 800 µg/mL DSL extract or with only DMSO at 1% final concentration (control) for 24 h were investigated using flow cytometry (Figure 3). There were increases in the percentage of the MCF-7 cells in the S phase from 41.61% to 45.08% and G2 phase from 17.34% to 25.06% after treatment, accompanied by a decrease in the percentage of MCF-7 cells in G1 phase. Treatment with this extract caused 6.31% and 15.50% increases of A549 cells in the S and G2 phase in comparison with control cells, respectively

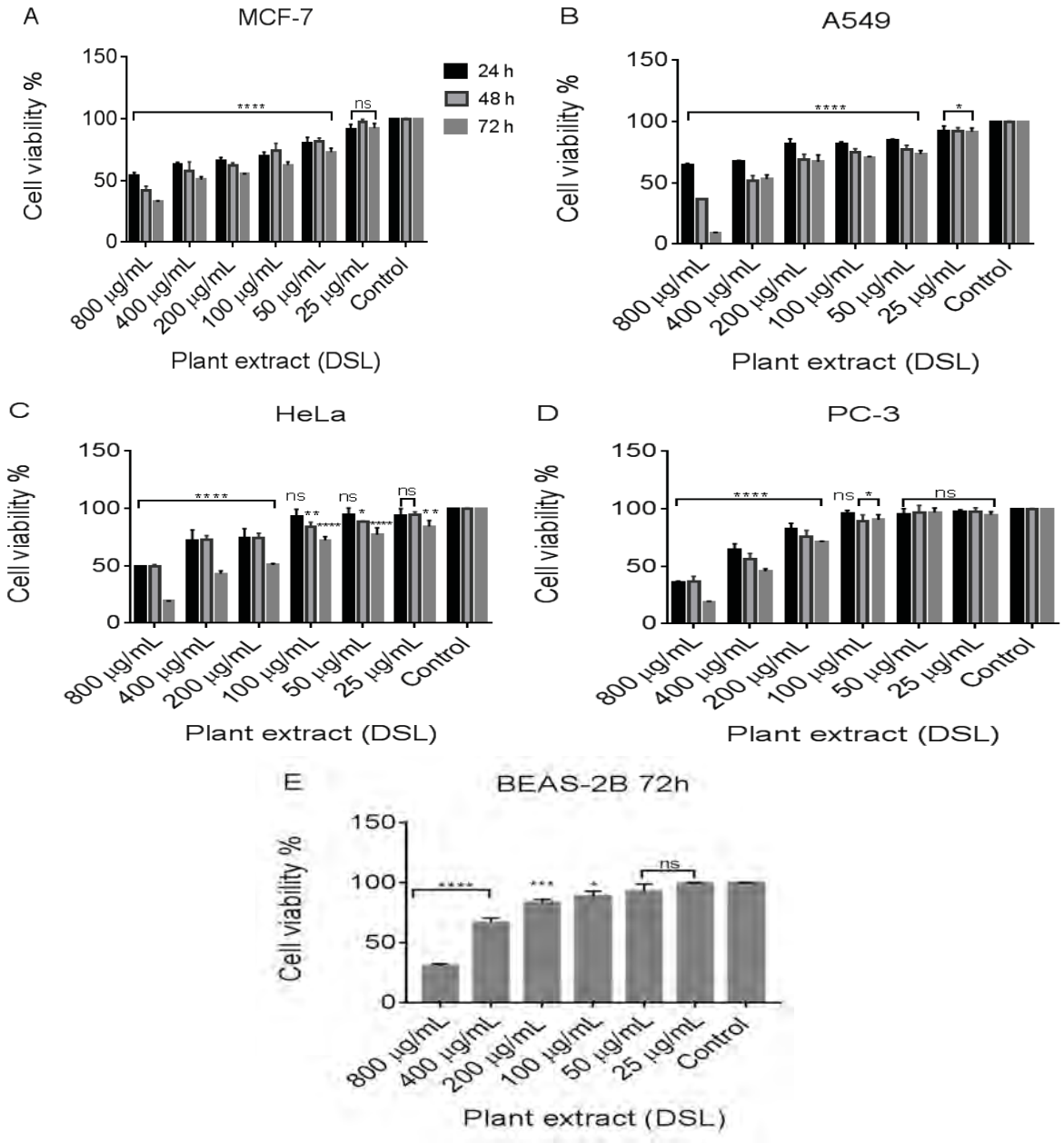

FIGURE 2

The cytotoxic effects of ethanolic extract of leaves of *D. stramonium* against MCF-7 (A), A549 (B), HeLa (C), PC-3 (D) cancer cell lines and BEAS-2B (E) normal cell line in different concentrations.

Cell viabilities of cancer cells were determined using MTT assay for 24, 48 and 72 h. Data was presented as mean± standard error (SE) from three independent experiments. Asterisks indicate statistical significance in cell viability of treated cells in comparison with untreated cells (control). ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05 and ns: non significant (P> 0.05).

TABLE 1

Approximate IC_{50} values of ethanolic extract of leaves of *D. stramonium* against different cell lines based on time course

	IC_{50} value±SE (µg/mL)		
Cell lines	24 h	48 h	72 h
MCF-7	---[a]	436.3±0.24	272.5±0.16
A549	---[a]	410±0.35	361±0.13
HeLa	---[a]	664±0.29	265.7±1.05
PC-3	629±0.12	528±1.03	369±1.05
Beas-2B	---[b]	---[b]	622±1.06

Note: [a]: Value was not calculated because there was no more than 50% cytotoxic effect. [b]: Value was not calculated for BEAS-2B cells

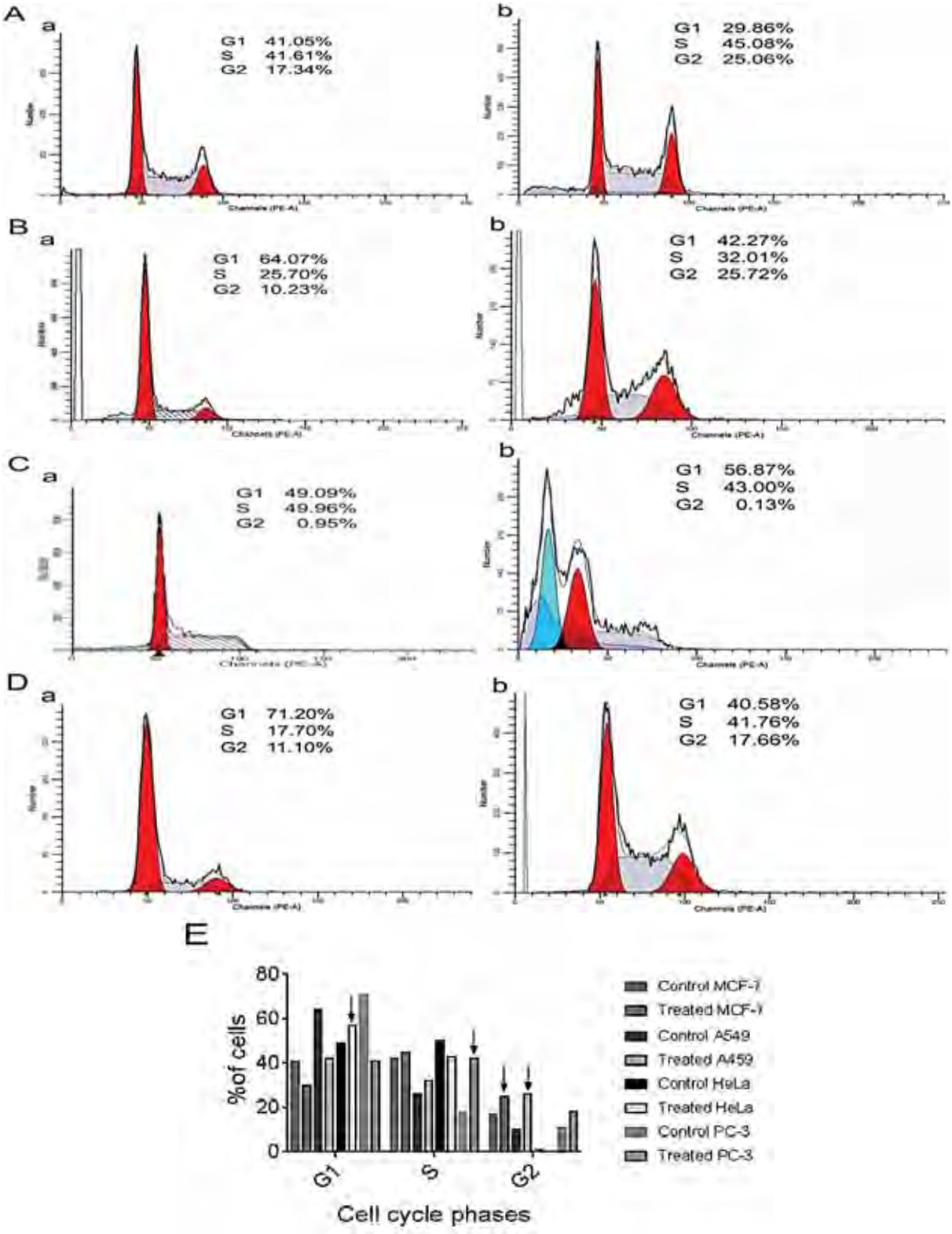

FIGURE 3
Analysis of cell cycle distributions by flow cytometry.
MCF-7 (A), A549 (B), HeLa (C), PC-3 (D) cells were either treated with 1% DMSO (control) (a) or treated with DSL extract at 800 µg/mL (b) for 24 h. The percentages of distribution of cells in the different phases of the cell cycle were shown as a bar graph (E).

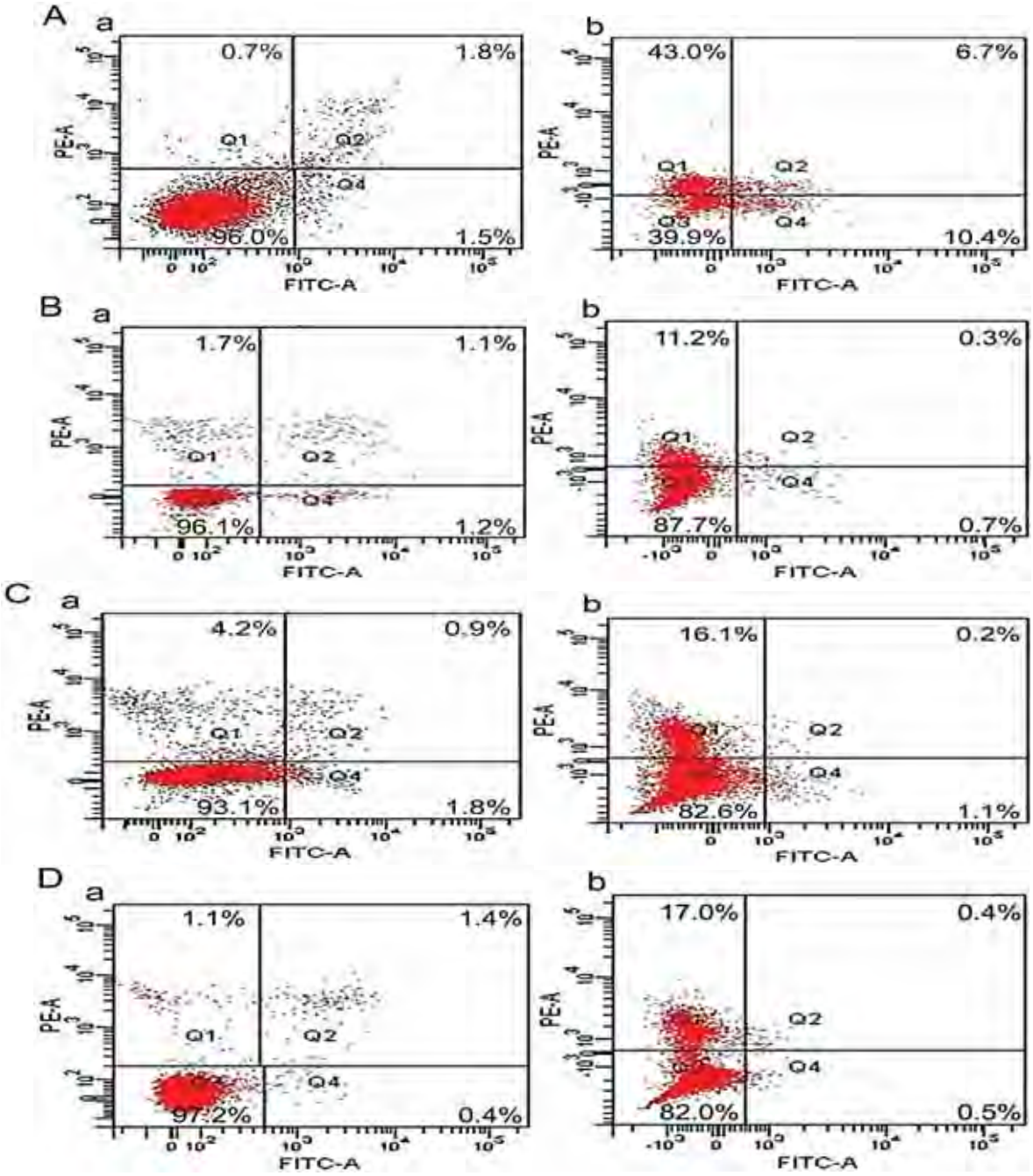

FIGURE 4

Analysis of cell death by flow cytometry. MCF-7 (A), A549 (B), HeLa (C), PC-3 (D) cells were either treated with 1% DMSO (control) (a) or treated with DSL extract at 800 µg/mL (b) for 24 h.

Cells were distributed into four quadrants: quadrant 3 (Q3) shows viable cells, quadrant 4 (Q4) shows early apoptotic cells, quadrant 2 (Q2) shows late apoptotic cells and quadrant 1 (Q1) shows necrotic cells.

and concomitant decrease in the percentage of the cells in G1 phase. In addition, treated PC-3 cells were arrested especially in S phase (from 17.70% to 41.76%) and G2 phase (from 11.10% to 17.66%) compared to control cells with a parallel decrease of the cells in G1 phase. However, an increase in the percentage of the HeLa cells was detected in G1 phase (from 49.09% to 56.87%). The data obtained from this assay clearly indicated that the DSL extract was likely changing the cell phase distribution according to the cell lines used.

Flow cytometric analysis of the cell death. The percentage of apoptosis and necrosis in the cells were analyzed using an annexin V-FITC apoptosis detection kit by flow cytometry for 24 h. As shown in Figure 4, DSL extract at 800 µg/mL was found to induce the apoptosis and necrosis in MCF-7 compared with the control cells. After treatment, the percentages of the apoptotic (quadrant 2 and 4) and necrotic (quadrant 1) MCF-7 cells increased from 3.3% to 17.1% and from 0.7% to 43%, respectively. In addition, the percentage of necrosis in other treated

cells increased from 1.7% to 11.2% for A549 cells, 4.2% to 16.1% for HeLa cells and 1.1% to 17.0% for PC-3 cells compared to control cells. Therefore, these results show that the DSL extract induces necrotic cell death in almost all cell lines tested, indicating that the extract at 800 µg/mL is toxic to the cells. These findings were consistent with cell cycle analysis, because disruption of cell cycle progression may ultimately lead to apoptotic/necrotic death [17].

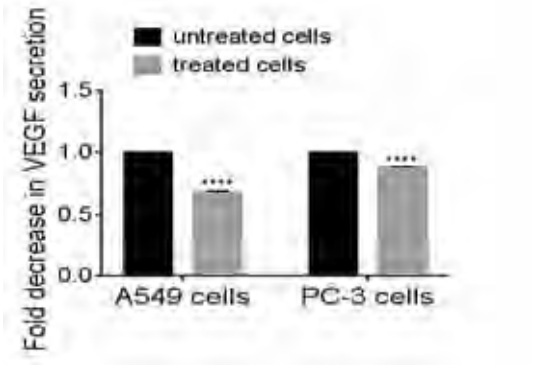

FIGURE 5
Effects of DSL extract on VEGF secretion of A549 and PC-3 cells.
The VEGF concentration in supernatants of the cells treated with the extract at 300 µg/mL for 6 h was determined by ELISA. The values represent the mean of three independent experiments ± SE. The concentration of VEGF in control (untreated) cells was taken as 1-fold. ****P<0.0001, versus control.

Detection of the VEGF level. It was reported that VEGF expression was determined in A549 [18] and PC-3 [19] cell lines. In this study, the VEGF levels in supernatants of the A549 and PC-3 cell cultures also investigated using human VEGF ELISA assay. These findings showed that VEGF secretion was reduced by 36% in A549 cells treated with 300 µg/mL DSL extract compared to untreated cells. However, the level of secreted VEGF in PC-3 cells was decreased by 12% after treatment (Figure 5). These results might indicate that the DSL extract has antiangiogenic properties, because the VEGF is known as an angiogenic factor [20].

Detection of the IL-1α, IL-6, and TNF-α levels. The levels of the pro-inflammatory cytokines IL-1α, IL-6, and TNF-α were assessed in the treated (with 300 µg / ml DSL extract) and untreated A549 and Daudi cell lines using ELISA. As shown in Figure 6, the DSL extract increased the level of the TNF-α (about 20%) in A549, and IL-6 (about 19%) in Daudi cells compared to untreated cells. However, this extract did not significantly inhibit IL-6 and TNF-α secretion in A549 and Daudi cells (about 4% inhibition for both cells), respectively. Similarly, it was observed a slight decrease in the level of the IL-1α release in both A549 and Daudi cells. Thus, the DSL extract may affect the levels of cytokine secretion according to the type of cell lines tested.

DISCUSSION

The investigation of new agents with selective and low side-effects continues to be an important case in cancer research. Natural products are one of the main sources of the potential pharmaceutical agents used for the prevention and treatment of cancer [5]. Plants have a strong potential for development of new anticancer agents to be used clinically due to their phytochemical properties [21]. It is known that the various bioactive phytochemicals exhibit anti-cancer activity by serving as anti-inflammatory agents, the regulator of cell cycle and apoptosis and the like [2]. So, the evaluation of the potential anti-cancer properties of the plants can provide a significant contribution to studies about cancer therapy. Although previous studies showed the various pharmacological activities of *D.*

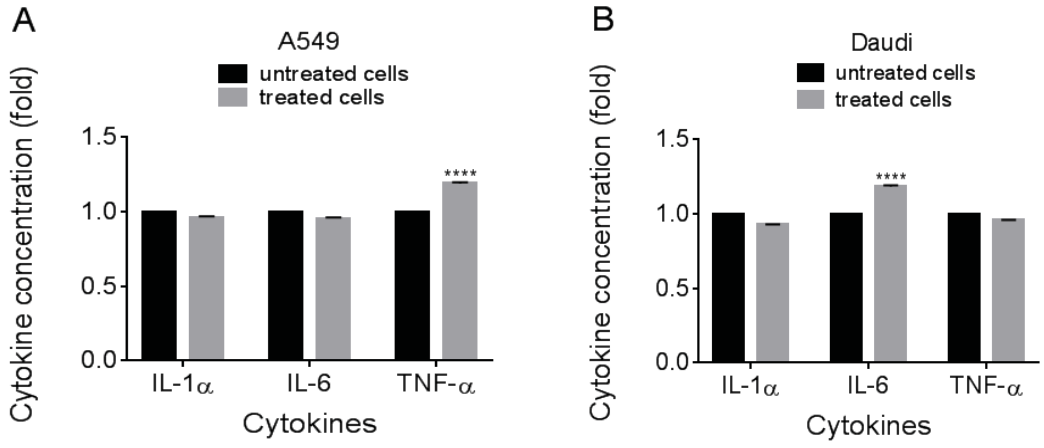

FIGURE 6
Effects of DSL extract on IL-1α, IL-6, and TNF-α secretion of A549 (A) and Daudi (B) cells.
The pro-inflammatory cytokines concentration in supernatants of the cells treated with the extract at 300 µg/mL for 6 h were determined by ELISA. The values represent the mean of three independent experiments ± SE. The concentrations of each cytokine in control (untreated) cells was taken as 1-fold. ****P<0.0001, versus control.

stramonium [reviewed by 11, 13, 14], there is not much research on the anti-cancer and anti-inflammatory effects of this plant in the literature.

In the present study, the ethanolic extracts of leaves (DSL) or seeds together with flowers (DSSF) of *D. stramonium* collected from Turkey were separately tested on different cell lines for cytotoxic effects using MTT assay for the first time according to the available literature. When the cytotoxic effect of the extracts at 800 µg/mL was screened for 72 h, it was observed that DSL extract showed more than 65% cytotoxicity whereas DSSF extract caused less than 25% cytotoxicity. Based on these results, the DSL extract was selected for further dose and time course experiments, the analyses of the cell cycle distribution, apoptosis, as well as ELISA.

The DSL extract generally increased cytotoxicity in a dose and time-dependent manner. It was observed that DSL extract at 72 h exhibited the high cytotoxic effects against HeLa cells with an IC_{50} value of 265.7 µg/mL and MCF-7 cells with an IC_{50} value of 272.5 µg/mL compared to healthy BEAS-2B cells with an IC_{50} value of 622 µg/mL. Also, this extract was detected to be relatively cytotoxic on A549 (IC_{50} value of 361 µg/mL) and PC-3 cells (IC_{50} value of 369 µg/mL). Based on the results, the DSL extract appears to exhibit the selective cytotoxicity to HeLa and MCF-7 cancer cells relative to BEAS-2B normal cells. Unlike the present study, Sasaki et al. expressed that *D. stramonium* agglutinin inhibited the proliferation of four human glial tumour cells, U251 (glioblastoma), SF-539 (gliosarcoma), SNB-75 (astrocytoma), and SNB-78 (astrocytoma) [22]. The therapeutic dose of *D. stramonium* as an Ayurvedic anti-cancer herb was reported to be 0.05–0.1 g along with some side effects such as vomiting, hypertension, loss of consciousness [23]. *D. stramonium* aqueous leaf extract was demonstrated to induce oxidative stress in different human cancer cell lines [24]. Merza et al. investigated the activity of the acetonic extract of *D. stramonium* seeds on cancer cell lines. These researchers reported that the extract exhibited cytotoxic effect by decreasing the viability of AMN3 (mammary adenocarcinoma) (42.91%) and brain cell lines (32.79%) and caused little effect on the viability of normal cell line Ref3 (rat embryonic fibroblast) [25]. Khalighi-Sigaroodi et al. stated the first report on cytotoxicity and antioxidant activity of five plant species collected from different regions of Iran. When these species were tested by brine shrimp lethality assay, methanol extracts and chloroform fractions of *D. stramonium* leaves were reported to show the highest cytotoxic activities with LC_{50} values of 21.66 µg/mL and 4.29 µg/mL, respectively [26]. In addition, methanolic seeds extract of *D. stramonium* collected from Konya caused a reduction in the cell proliferation of human lymphocytes [27]. In another study, it was declared that the EC_{50} value of fraction-10 from *D. stramonium* leaves for the human peripheral blood mononuclear cells (PBMC) was 19.1 µg/mL and fraction-10 pretreated PBMC exhibited enhanced cytotoxicity against A549 and MCF-7cell lines [28]. The CTC_{50} value (inhibit cell growth by 50%) of the compound isolated from the ethyl acetate fraction of *D. stramonium* flowers for liver cancer HePG2 cell lines was found to be 131.53 µg/mL [29]. Iqbal et al. explained that methanol extract of seeds of *D. stramonium* on MCF-7 cells showed increasing cytotoxicity with increasing concentrations of the extract and its IC_{50} value was 113.05 µg/mL [30]. All these findings support that the level of cytotoxicity may alter depending on the location and the part of the plant harvested as well as the selected solvent for extraction and the cell lines used. These results also confirm that there may be present potential anti-cancer substances in *D. stramonium*.

One of the common features of cancer is the alteration of cell cycle progression [31]. It has been emphasized that the targeted the regulation of the cell cycle is important for the treatment of cancer [32]. Also, various natural compounds can inhibit cancer cell growth as inhibitors of cell cycle progression [33]. Although it was declared that the ether extract of *D. stramonium* seeds has the ability to inhibit mitosis in cancer cells [34], the effect of the DSL extract from Edirne (Turkey) on cell cycle progression was investigated for the first time in the present study. The DSL extract treatment at 800 µg/mL for 24 h caused a significant cell cycle arrest at the S phase in PC-3 cells (about 24%) and the G2 phase in A549 cells (about 15.50%). The percentage of MCF-7 and HeLa cells after the treatment was increased by about 8% in the G2 and G1 phases of the cell cycle, respectively, compared to normal cells. Based on these results, it may be suggested that the DSL extract induced the cell cycle arrest and the cytotoxicity observed against cancer cell lines may be originating from this property of the extract.

The flow cytometry analyses showed that the percentage of necrotic cells was higher than that of apoptotic cells among all cells treated with DSL extract at 800 µg/mL for 24 h. So, the research result suggests that the main cell death pathway in treated cancer cells is necrosis. Unlike this study, Akal et al. displayed that methanolic seeds extract of *D. stramonium* exhibited apoptotic effect according to TUNEL assay results [27]. Karmakar et al. stated that alkaloids—scopolamine, trigonelline and tyramine compounds obtained from activity-guided fractionation of the MeOH extract of *D. stramonium* leaves exhibited TRAIL (a potent inducer of apoptosis in most cancer cells)-resistance overcoming activity at 50, 150, and 100 µM, respectively in TRAIL-resistant AGS cells [35]. Although it is expressed that plant-derived anti-cancer drugs can induce non-apoptotic cell deaths [36], it is still unclear whether necrosis plays an important role in the formation of the tumor. In addition, it has been stated that the necrosis of tumor cell can create an

inflammatory response, and an immune response to potentially malignant cells, so it may prohibit development of the tumor [37]. Necrosis has long been regarded as an uncontrolled, non-programmed form of cell death. However, the necrotic cell death was interestingly noted to be controllable [38]. Thus, the findings obtained in the present study may provide a significant contribution to the literature.

The development of new blood vessels from preexisting ones is called angiogenesis and this process is important in the growth and metastasis of cancer tissue [39]. VEGF is known as a key regulator of angiogenesis in cancer [40]. So, VEGF is considered as a rational target for anti-cancer therapy**.** The extract of seeds of *D. innoxia* was reported to inhibit angiogenesis by suppressing VEGF in cervical cancer HeLa cells by Pandey et al [41]. Also, Khoshkhoo et al. presented the anti-angiogenesis properties of *D. innoxia* [42]. In the present study, the effect of the DSL extract on the secretion of VEGF in A549 and PC-3 cells for 6 h were investigated. Based on the literature, this study revealed for the first time that the treatment with the DSL extract at 300 µg/mL reduced the level of VEGF in A549 and PC-3 cells by 36% and 12%, respectively, compared to untreated cells. So, these findings suggest that DSL extract has a potential antiangiogenic effect.

The pro-inflammatory cytokines like IL-1, IL-6, and TNF may enhance tumor cell survival [43]. Therefore, the DSL extract at 300 µg/mL was tested for its anti-inflammatory activity using A549 and Daudi cells for 6 h in this present study. For the purpose, relative levels of the secreted pro-inflammatory cytokines (IL-1α, IL-6, and TNF-α) in treated cells were determined by comparing those with the untreated cells. The data herein showed for the first time that the DSL extract at 300 µg/mL caused an increase not exceeding 20% in the level of secreted TNF-α and IL-6 in A549 and Daudi cells, respectively. Also it was observed that the level of IL-6 and TNF-α did not significantly alter in A549 and Daudi cells, respectively compared to untreated cells. In addition, the level of IL-1α in treated cells was found to slightly decrease in comparison to untreated cells. In a previous study, the leaf or whole plant of *D. stramonium* has been stated to use in Madhya Pradesh as anti inflamatuar and antispasmodic [44]. Unlike present study, Khahali et al. investigated the anti-inflammatory effect the alcoholic extract of seed of *D. stramonium* on inflammation and reported that the extract has the competence to markedly decrease the amount of inflammation in particular the acute form [45]. Also, Sonika et al. stated that the ethanolic extracts of fruits of *Coriandrum sativum*, leaves of *D. stramonium* and *Azadirachta indica* exhibited significant anti-inflammatory activity comparable to the standard drug Diclofenac sodium against carrageenan induced rat paw edema method [46]. In addition, the chemical composition and anti-inflammatory property of *D. stramonium* oil growing in Nigeria was firstly reported by Aboluwodi et al. [47]. These findings may suggest that the anti-inflammatory effect of *D. stramonium* may change based on the organisms, the cell type and the testing method chosen.

As a result, the ethanolic extracts of leaves of *D. stramonium* caused cytotoxic effects against different cancer cell lines, and exhibited the selective cytotoxicity to HeLa and MCF-7 cancer cells relative to healthy BEAS-2B, and arrested the cell cycle progression, and induced the necrosis, and reduced the level of VEGF secretion, and affected the level of pro-inflammatory cytokines (IL-6, TNF-α) secretion.

CONCLUSION

In conclusion, the leaves of *D. stramonium* may be an effective source for potential novel agents in the treatment of cancer. Further studies are necessary for isolation and identification of biologically active substances from the leaves of the plant.

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CORRESPONDING AUTHOR

Mehlika Alper
Department of Molecular Biology and Genetics,
Faculty of Science,
Muğla Sıtkı Koçman University,
Kötekli Campus, 48000 Muğla – Turkey

e-mail: mehlikaalper@mu.edu.tr