Anti-cancer and anti-inflamatory effects of *Centaurea solstitialis* extract on human cancer cell lines

İnsan kanser hücre hatları üzerinde *Centaurea solstitialis* özütünün anti-kanser ve anti-inflamatuar etkileri

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Abstract

Objectives: Natural products originating from plants have been used for many years in the treatment of various diseases including cancer. *Centaurea solstitialis* subsp. *solstitialis* is used in Turkish folk medicine. This study was the first to determine *in vitro* biological effects of ethanolic extract from flowering parts of *C. solstitialis* L. ssp. *solstitialis* collected from Mugla province.

Materials and Methods: Cytotoxic effect was evaluated against Daudi, A549 and HeLa cancer cells and one normal BEAS-2B cell line using MTT assay. Flow cytometric analysis and caspase-3 activity assay were performed to detect apoptotic cell death. Angiogenic factor (VEGF) secretion and the release of IL-1α, IL-6 and TNF-α by the cells treated with the extract were measured using ELISA assay.

Results: The extract exhibited cytotoxic effects against the all cancer cell lines used but HeLa and Daudi were the most sensitive cells with IC_{50} values of 63.18 µg/ml and 69.27 µg/ml, respectively. Selective cytotoxicity was observed between HeLa and normal BEAS-2B cell lines. The extract arrested the cell cycle at the S and G2 phases. In addition, apoptotic cell death was detected in HeLa and A549 cells. Moreover, the plant extract caused a significant decrease in VEGF secretion of A549 cells and a fluctuation in IL-1α, IL-6 and TNF-α secretion of A549 and Daudi cells.

Conclusion: These observations suggest that flowering part of *C. solstitialis* may be a potential source in the development of natural drugs for the treatment of cancer and modulation of cytokine secretion.
Key Words: *Centaurea solstitialis*, cancer cell lines, anti-cancer, anti-inflammatory

Öz

Amaç: Bitkilerden elde edilen doğal ürünler, kanser dahil çeşitli hastalıkların tedavisinde uzun yıllardır kullanılmaktadır. *Centaurea solstitialis* subsp. *solstitialis* in Türk geleneksel tıppında yeri olduğu bilinmektedir. Bu araştırma, Muğla ilinden toplanan *C. solstitialis*'in çiçekli kısımlarından elde edilen etanolik özütün in vitro biyolojik etkilerini belirleyen ilk çalışmadır.

Gereç ve Yöntemler: Özütün Daudi, A549 ve HeLa kanser hücrelerine ve normal BEAS-2B hücre hattına karşı sitotoksik etkisi MTT testi ile belirlendi. Apoptotik hücre ölümü akış sitometri analizi ve kaspaz-3 aktivite deneyleriyle araştırıldı. Özüt ile muamele edilen hücreler tarafından üretilen anjiyojenik faktör VEGF salınımı ve sitokinlerden IL-lα, IL-6 ve TNF-α'nın salınımı ELISA testleriyle ölçüldü.

Bulgular: Bitki özütü kullanılan tüm kanser hücre hatlarına karşı sitotoksik etki gösterirken, özüte karşı en duyarlı hücrelerin 63.18 µg/ml and 69.27 µg/ml olan IC_{50} değerleri ile sırasıyla HeLa ve Daudi hücreleri olduğu gözlendi. Seçici sitotoksisite HeLa ve normal BEAS-2B hücre hatları arasında tespit edildi. Bitki özütü S ve G2 fazlarında hücre döngüsü arrestine yol açtı. Buna ilave olarak, HeLa ve A549 hücrelerinde apoptotik hücre ölümü kaydedildi. Ayrıca bitki özütü A549 hücrelerinin VEGF salgılamasında anlamlı bir düşüşe yol açarken, A549 ile Daudi hücrelerinin IL-lα, IL-6 ve TNF-α salgılamasında önemli değişime neden oldu.

Sonuç: Bu bulgular kanser tedavisi ve sitokin salgısının modülasyonunda gerekli olan doğal ilaçların geliştirilmesi için *C. solstitialis*'in çiçekli kısımlarının potansiyel bir kaynak olabileceğini göstermektedir.

Anahtar kelimeler: *Centaurea solstitialis*, kanser hücre hatları, anti-kanser, anti-inflamatuar

INTRODUCTION

Cancer is one of the most common diseases in both developed and developing countries. Plant products have been used throughout the history to treat and prevent diseases because of their large number of different phytochemicals with different biological activities.[1] In fact, the compounds derived from plants play an important role in the development of anticancer agents to be used in clinical practice.[2] Since substantial evidince have proved that plant secondary metabolites are a potential source of anticancer compounds and cancer cells may develop resistance to existing drugs, today extensive research have being carried out all over the world to discover new plant species with anticancer properties.

Cancer is a multistage disease. Angiogenesis defined as formation of new blood vessels is an essential process in tumor development and prevention of tumor vasculation is a crucial strategy in cancer treatment.[3,4] Even though there are a variety of angiogenic factors, vascular endothelial growth factor (VEGF) is a key regulator of angiogenesis. Inreased VEGF secretion promotes invasion and metastasis so that targetting of VEGF is pivotal in the prevention of tumor metastasis. Therefore, discovery of a new plant extract with anti-angiogenic activity is necessary to serve an alternative to toxic chemotherapatics.

Inflammation is a common reason of many diseases and alone it is not sufficient to cause cancer, but epigenetic events and mutations caused by environmental exposure or immune mudulation contribute significantly to the cancer process.[5] In addition, the cytokine release-mediated inflammatory mechanisms were reported to facilitate cancer metastasis.[6] Various plants or their bioactive compounds can inhibit or stimulate different enzymes associated with inflammatory and immune response regulating pathways.[7] Because antiinflamatory drugs may be effective in cancer therapy or prevention,[8] it is important to evaluate antiinflamatory potential of plant extracts as well.

The genus *Centaurea* L. belonging to *Asteraceae* family is the third largest genus in Turkey.[9] Some *Centaurea* species are used as remedy against various disease in the Turkish folk medicine.[10] *Centaurea solstitialis* is known with its Turkish name “gelin

dikeni" and it has been used against hemorrhoids, pecticulcers, common colds,[11,12] malaria,[13] and herpes infections around the lips of children.[14] Previous studies examined the pharmacological and biological properties of *Centaurea* species and some *C. species* exhibited cytotoxic effects against some of the cancer cell lines.[15] Major constituents of *Centaurea* L. species were reported to be sesquiterpene lactones, flavonoids and fatty acids.[16,17] Nevertheless, to our knowledge there were no adequate reports about anti-cancer and anti-inflammatory effects of *C. solstitialis*. Therefore, this present study has scientific importance for the anti-cancer and anti-inflammatory potential of ethanolic extract from flowering parts of *C. solstitialis* collected from Mugla.

MATERIALS AND METHODS

Plant material

The plant *C. solstitialis* was collected during the flowering period from June to July 2015 from Muğla in the south west of Turkey. The plant species was identified in Herbarium Laboratory, Department of Biology, Mugla Sıtkı Koçman University.

Plant extraction

Flowering parts of *C. solstitialis* were washed with distilled water and air-dried under shade for about 15 days. Air-dried flowers were miled into a powder in a porcelain muller. The powder (10 g) was soaked in absolute ethanol (96^0, Merck, USA) and placed in soxhlet apparatus for 10 hr to obtain ethanolic extract. After filtration of the extract using Whatman filter paper no.1, ethanol was removed using a rotary evaporator (IKA, RV 10, USA). Solvent was evaporated by keeping the extracts at 37°C for 7 days. The powdered crude extract was stored at 4°C in an air-tight containers until used. The extract was dissolved in 10% DMSO as stock solution and furher diluted to obtain working solutions. DMSO in the final concentrations of the extract was less than 1% and showed no contribution on examined parameters

Cell lines and culture conditions

Daudi (Burkitt's lymphoma, CCL-213), A549 (lung carcinoma), HeLa (cervix adenocarcinoma) and BEAS-2B (normal bronchial epithelium) cell lines were originally obtained from ATCC. The cells were maintained in RPMI 1640 medium pre-mixed with stable L-glutamine (Biochrom, Germany) and supplemented with 10% heat inactivated fetal bovine serum (FBS) (Biochrom, Germany), penicilin (100U/ml) and strepromycin sulphate (100 mg/ml) (Biochrom, Germany). All cell lines were incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37 ^{0}C.

In vitro cytotoxicity assay

The cytotoxic effects of ethanolic extracts from flowering parts of *C. solstitialis* on Daudi, A549, HeLa and Beas-2B were determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- dipenyltetrazolium bromide) assay. In this assay, the reduction of yellow soluble MTT to insoluble blue formazan crystal by mitochondrial dehydrogenase reflects the cell viability.[18] A total of $4x10^3$ cells/well were seeded in 96-well plates (Greiner, Germany) as triplicate and incubated for 24 h. Plant extracts were added into the wells at 7 different final concentrations between 1000 µg/ml and 15.625 µg/ml and incubated for 72 h. Then, 10 µL of 5 mg/ml MTT reagent (Applichem, USA) in PBS was added into each well. After 4 h incubation, the medium was gently discarded and 100 µl pure DMSO was added into each well to dissolve formazan blue crystals formed in the cells. Absorbance of reduced MTT in each well was measured at 540 nm using a microplate reader (Thermo Scientific, Multiskan FC, USA).The cytotoxic effects of the extracts were determined by comparing the optical density of treated cells against the that of untreated cells.

Cell cycle analysis

Cells at $5x10^5$/well were seeded in 6 well-plates and treated with plant extracts at 500 µg/ml and 200 µg/ml for 24 h. After treatment, cells were washed with ice-cold PBS, fixed in 4 ml absolute ethanol and stored at -20 °C for 48 h. After that, cells were centrifuged at 1200 rpm for 10 min at 4 °C and cell pellets were washed in ice-cold PBS. Cells were resuspended in 1 ml PBS contaning 0.1% (v/v) Triton X-100 (Amresco, USA) and then 100 µl of RNase A (200 µg/ml) (Applichem, USA) was added to each of cell suspensions. After the incubation for 30 min at 37°C, 100 µl PI (1 mg/ml in ddH_20) was added to each cell suspension and cells were incubated in the dark for 15 min at room temperature. Cells were analyzed by BD FACSCanto flow cytometer using ModFit LT 3.0 software for cell cycle phases.

Apoptosis assay

Exponentially growing A549 and HeLa cells were cultured at $5x10^5$ cells/well in 6-well plates (Greiner, Germany) and incubated for 24 h. The cells were treated with plant extract at final concentrations of 200 µg/ml and 500 µg/ml for 24 h. Annexin V-FITC/propidium iodide (PI) staining was carried out using Annexin V-FITC Apoptosis Detection Kit (eBioscience, USA) protocol. Briefly, treated cells were washed with PBS, tripsinized, washed and resuspenden in binding buffer. Five µl of Annexin V-FITC and 10 µl prododium iodine (PI) at 20 µg/ml were added to each cell suspention and cells

were incubated for 15 min in the dark. After adding 500 µl binding buffer, ten thousand cells per group were analysed by flow cytometry (BD FACSCanto A, BD Biosciences) using BD FACSDiva software v6.13.

Caspase-3 activity assay

Caspase-3 activity of cell lysates was determined by colorimetric assay kits (Abcam, Cambridge, UK). A549 and HeLa cells were plated at 2×10^6 cells/well in 6-well plates and incubated for 24 h. Then, cells were treated with plant extract at 500 µg/ml for 36 h. After centrifugation, cells were resuspended in 50 µL of cell lysis buffer and incubated on ice for 10 min. Cell lysates were centrifuged at 10,000 x g for 1 min and protein concentration of each cell lysate was determined by Bradford assay (1976). Later, 200 µg of protein from each sample was mixed with 50 µL of 2X reaction buffer containing 10 mM DTT and 5 µL of the caspase-3 substrate (4 mM DEVD-*p*-NA) and incubated at 37°C for 2 h. Absorbance of p-NA light emission was read at 405 nm in microplate reader. Fold-increase in caspase 3 activity was determined by comparing the absorbance of p-NA from an apoptotic sample with that of untreated control cells.

Quantitative detection of human VEGF by enzyme-linked immunosorbent assay (ELISA)

To determine the effect of plant extract on VEGF secretion, A549 cells were cultured at a density of 2×10^5 cells/well in a 6-well plate and incubated for 1 h. Then, cells were treated with plant extract at 200 µg/ml and incubated for 6 h. Supernatants were collected after centrifugation and stored at -20°C until analysis. The untreated cells served as control. The concentrations of VEGF in cell culture supernatants were detected by ELISA assay as described in manufacturer's procedure (VEGF ELISA kit; Boster Biological Technology, USA). The absorbance of each well was measured using a microplate reader at 450 nm within 30 min. The VEGF concentrations of cell culture supernatants were interpolated from the standard curve.

Quantitative detection of human IL-1α, IL-6 and TNF-α by ELISA

In order to examine the effects of plant extract on inflamation, $2x10^5$ cells/well of A549 or Daudi cells were plated as triplicate in 6-well plates. Cells were treated with 200 µg/ml of *C. Solstitialis* extract for 6 h or left untreated to serve as control. Supernatants were collected and 100 µl of the supernatant was tested for inflamatory cytokine production by ELISA assay based on manufacturer's instructions using commercial human ELISA kits for IL1α, IL-6 and TNF-α (Boster Biological Technology, USA). The

amount of each cytokine in the supernatants was calculated from the formula of calibration curve of standard cytokine.

Statiscal analysis

Data were analyzed using GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, CA). Comparisions of treatments among groups were performed using one-way or two-way ANOVA and post-hoc analysis. Significance was presented as ***($P < 0.01$) and ****($P< 0.0001$). Data are mean ±SD of three replicates.

RESULTS

Cytotoxic activity of plant extract on different cancer cell lines

Cytotoxicity of the crude ethanolic extract from flowering parts of *C. solstitialis* at seven different concentrations were investigated on A549, Daudi, HeLa and Beas-2B cell lines to determine the IC_{50} value (µg/ml) that causes a 50% cell death. The results demonstrated that percentage of viable cells changed according to the cell lines used (Figure 1). The viability of all the cancer cells was significantly reduced by the extract in a concentration-dependent manner. However, the extract at concentrations of 15.6 and 31.2 µg/ml did not caused significant cytotoxicity on normal BEAS-2B cell line, indicating the selectivity of the extract against cancer cells (Figure 1D). The highest cytotoxicity with an IC_{50} value of 63.18 µg/ml was observed against HeLa cells (Figure 1C.) whereas IC_{50} values of A549 and Daudi cells were 252.5 µg/ml, 69.27 µg/ml, respectively (Figure 1 A.B). On the other hand, the extract exhibited a lower cytotoxic effect on normal BEAS-2B cells with IC_{50} value of 75.25 µg/ml when compared with the effects on HeLa and Daudi cancer cell lines (Figure 1). In other words, in respect to cytotoxicity HeLa and Daudi cells were the most sensitive cell lines against the extract.

The effect of plant extract on cell cycle distribution

Since the plant extract at 250 µg/ml was cytotoxic against all the cell lines tested, the rest of the analyses for diferent parameters were permorfed with the extract at 200 µg/ml and 500 µg/ml. The changes of cell-cycle progression of the HeLa and A549 cancer cells after treatment with plant extracts at 200 µg/ml and 500 µg/ml for 24 h were analyzed using flow cytometry with PI staining. Plant extract at 200 µg/ml showed virtually no effect in the cell cycle phases of HeLa cells (Figure 2A a,b,d). At 500 µg/ml, there was a slight increase in the percentage of HeLa cells in the G2 phase and it was accompanied by a decrease in the percentage of cells in the G1 phase from 48.97%

to 41.57% (Figure 2A a,c,d). In addition, treatment of A549 cells with plant extracts at 200 and 500 µg/ml for 24 h resulted in 7.1% and 12.5% increase of cells in the S phase, respectively, and it caused a concomitant decrease in the percentage of cells in the G1 phase (Figure 2B a,b,c,d). Furthermore, percentage of G2 phase cells increased from 4.8 to 15.6 and 9.6% in A549 cells treated with the extract at 200 and 500 µg/ml, respectively. These results suggest that the plant extract might inhibit the cell proliferation by arresting both cells especially in the G2 phase.

Apoptotic effect of plant extract

As cell cycle regulation and apoptosis are closely related, disruption of cell cycle progression may result in apoptotic/necrotic death.[20] Therefore, it was investigated whether or not apoptosis was initiated in cells treated with plant extracts for 24 h. As shown in Figure 3A, the percent of apoptotic HeLa cells (quadrant 2 and 4) increased from 1% to 9.3% and to 11.8% after treatment with the extract at 200 and 500 µg/ml, respectively (Figure 3A, a,b,c,d). In addition, the percent of apoptotic A549 cells (quadrant 2 and 4) increased from 3.7% to 4.5% and to 31.8% after treatment with 200 and 500 µg/ml extract, respectively (Figure 3B a,b,c,d). These findings demonstrate that the plant extract at 500 µg/ml induce apoptosis in both cells, especially in A549. These data were consistent with the results obtained from the cell cycle analysis.

Caspase 3 activation

Caspases play an important role in mediating various apoptotic signaling pathways. In the present study, we analyzed the activity of caspase 3 in A549 and HeLa cells treated with the extract at 500 µg/ml for 36 h. As shown in Figure 4, the extract increased caspase-3 activity about 1.65 and 1.5 fold compared to the control in HeLa and A549 cells, respectively. These results indicate that the plant extract induces apoptosis in both HeLa and A549 cells.

VEGF secretion of A549 cells

The VEGF is a potent cytokine produced by many cell types including most cancer cells and it has critical roles in physiological and pathological angiogenesis.[21] Because VEGF protein expression was determined in airway epithelial cancer cell line A549 by Koyama et al.[22], VEGF secretion of A549 cells was investigated after treatment with the extract at 200 µg/ml using human VEGF ELISA assay. The plant extract caused 2.5 fold decrease in VEGF secretion of A549 cells compared to untreated control cells (Figure 5), indicating the antiangiogenic function of the extract.

Effect of plant extract on IL-1α, IL-6 and TNF-α secretion

It has been known that different cytokines and growth factors may contribute cancer progression.[23] In this study, the IL-1α, IL-6 and TNF-α concentrations in A549 and Daudi cell culture supernatants after treatment with plant extract at 200 µg/ml was determined. The effect of plant extract on cytokine secretion varied according to the cell lines used. The highest level of inhibition on the release of cytokines was observed in A549 for IL-6 and Daudi for IL-1α compared to untreated control cells (Figure 6). In contrast, there was a slight increase in the release of IL-1α and TNF-α in A549 cells and IL-6 in Daudi cells. In other words, the plant extract caused a significant change in the cytokine levels of cancer cells.

DISCUSSION

Cancer is one of the major causes of death in the world.[24] It has been known for the centuries that plants have anticancer properties and they are important resources for the new anti-cancer drugs.[25] The genus *Centaurea* has been a subject of many phytochemical and biological research because of its wide spread application in folk medicine to treat various diseases. Different biological activities such as antioxidant,[26] antimicrobial,[27] anti-pyretic[28] and anti-ulcerogenic functions[29] were reported for *C. solstitialis* species. However, to our knowledge not much information is avaliable about anti-cancer and anti-inflamatory activities of *C. solstitialis* in the literature. Therefore, such biological activities of ethanolic extract from flowering parts of *C. solstitialis* extract were examined in this present study.

The investigation of cytotoxic effect of a plant extract against cancer cells is an important step for development of plant based drug for cancer treatment. Likewise, cytotoxic effect of ethanolic extract from flowering part of *C. solstitialis* on different cancer cell lines was tested. The findings indicated that plant extract showed cytotoxic effects at different levels according to the type of cell lines used. The extract exhibited the highest cytotoxicities on HeLa cells with IC_{50} value of 63.18 µg/ml and Daudi cells with IC_{50} value of 69.27 µg/ml whereas the IC_{50} value in BEAS-2B normal cell line was found to be 75.25 µg/ml. However, plant extract showed the lowest cytotoxic effect against A549 cells (IC_{50} value of 252.5 µg/ml). Erenler et al.[30] investigated antiproliferative activities of methanol extract of root, stem and flower parts of the *Centaurea solstitialis* L. ssp. *solstitialis* on C6 cells and HeLa cells *in vitro* and found that the methanol extract of stem exhibited the most antiproliferative activity. In contrast to their study, our previous investigation demonsrated that flowers were the most effective plant part compared to the stem (unpublished data) and so the ethanolic

extract only from flowering part was used in this present study. Reason for this may be due to the type of solvent used for extract preperation. In fact, different solvents results in extraction of chemical compounds at different scale.

Similar to this study, there are publications related to different *Centaurea* species that have cytotoxic effects against the A549 and HeLa cell lines. Artun et al.[31] reported that among the 14 plant extracts, the methanol extract of *Centaurea nerimaniae* exhibited the highest cytotoxic effect against the Vero normal cell line and methanolic extract of endemic *Centaurea antiochia* Boiss. var. *praealta* showed selective cytotoxic effect against the HeLa cell line with IC_{50} value of 427± 3.06 µg/ml. In another study, chloroform extracts of *Centaurea cadmea* showed the most inhibitory activities against HeLa (IC_{50}: 14.24 µg/ml), A549 (IC_{50}: 35.00 µg/ml) and U20S (IC_{50}: 43.10 µg/ml) human cancer cell lines and 293HEK (IC_{50}: 23.50 µg/ml) non-cancer cell line.[32] In addition, Zater et al.[33] represented that chloroformic extract of *C. diluta* Ait. subsp. *algeriensis* exhibited more significant cytotoxic effects on cancer cells A549, MCF-7 and U373 than the isolated pure compounds. Taken together, these studies indicate that cytotoxicity level changes depending on different *Centaurea* species and solvent used for extract preparation and type of cell lines used for in vitro cytotoxicity test.

Because cell cycle inhibition is a main target in the development and discovery of a drug against cancer, the effect of plant extract on cell cycle progression of HeLa and A549 cell lines after 24 h treatment was investigated in this study. Results indicated that the plant extract blocked the cancer cell proliferation by arresting both cells especially in the G2 phase of the cell cycle**.** In contrast to our results, Ghantous et al.[34] expressed that the inhibition of cell proliferation of papilloma and squamous cell carcinoma (SCC) cell lines by crude extract of *Centaurea ainetensis* and salograviolide A compound isolated from this plant was due to G0/G1 cell cycle arrest. Other researchers demonstrated that *Centaurea ainetensis* crude extract induced a progressive increase in the proportion of sub-G1 cells in HCT-116 cell line.[35]

Apoptosis is an important physiological process that play a critical role in the development and homeostasis in normal tissues; however, the balance between cell division and apoptosis is lost in cancer.[36,37] Therefore, targeting apoptosis in cancer treatment is crucial. In the cells undergoing apoptosis, phosphatidylserine (PS) translocates toward the extracellular side of membrane. Annexin-V is a phospholipid-binding protein so that translocation of PS to the outside of the membrane is detected

by Annexin V staining and it shows early stage apoptosis.[38] In the literature, only two studies investigated the apoptotic effects of extracts from *Centaurea ainetensis*[34] and *Centaurea fenzlii* Reichardt[39] on different cancer cell lines and showed the presence of apoptotic cell death. In the present study, Annexin V staining along with flow cytometryc analysis were carried out to reveal the mechanism in the cytotoxicicity of plant extract on A549 and HeLa cancer cells. Similar to previous studies, treatment of HeLa and A549 cells with *C. solstitialis* extract induced apoptosis and increased apoptotic cell number in a dose dependent manner (Figure 3).

Caspases, a family of proteases, play an essential role in apoptic pathway and become activated during early stages of apoptosis.[40] Because elavation in the caspase 3 activity is regarded as an apoptic marker, caspase 3 activity in treated and untreated cancer cell lines was examined. Results indicated that ethanolic extract of flowering parts of *C. solstitialis* caused an increase in caspase 3 activity in both HeLa and A549 cell lines (Figure 4). In addition, Yırtıcı et al.[39] reported that dichloromethane extracts-ethyl acetate fractions (CFDCMEAF) from *C. fenzlii* Reichardt exhibited an apoptotic effect on MCF-7 cells using flow cytometry and western blot analysis of an apoptosis related protein, PARP.

Angiogenesis is defined as formation of new microvessels from preexisting ones and required for tumor growth and distribution of tumor cells to distant locations.[41] Vascular endothelial growth factor (VEGF) has been known to be one of the most potent angiogenic factors. Previous studies indicated that inhibition of VEGF secretion supresses tumor growth, tumor invasion and metastasis.[41] A549, an airway epithelial cancer cell line, releases VEGF constitutively.[22] Therefore, an angiogenic potential of the extract on A549 cell line was investigated by measuring VEGF secretion after 6 h treatment. A significant inhibition of VEGF secretion in A549 cells implies that the plant extract has a potential as an anti-angiogenic agent in cancer therapy.

Inflammatory cytokines play a role in different stages of tumor development and many cytokines such as TNF, IL 1 and IL-6 can be induced by hypoxia, one of the well known properties of cancer cells.[42,43] Here, we tested effect of plant extract on secretion of IL-1α, IL-6 and TNF-α in A549 and Daudi cells. The plant extract at 200 µg/ml could not decrease TNF-α production in both cells (Figure 6). The plant extract significantly inhibited release of IL-6 in A549 and release of IL-1α in Daudi cells. According to a previous study, production of angiogenic factors such as VEGF could be induced by TNF, IL-1, and IL- 6.[42] A decrease in VEGF production in A549 cell may

be associated with decreased IL-6 production in A549 cells in this present study. Similar to our result, Talhouk et al.[44] reported that water extract of *C. ainetensis* inhibited IL-6 production in a dose-dependent manner. In addition, *in vivo* antiinflammatory effects of some *Centaurea* species were reported as well by Erel et al.[45] and Koca et al.[46] This present study indicates that induction or inhibition of inflamatory cytokines by ethanolic extract of *C. solstitialis* is cell type dependent.

Study Limitations:

Crude ethanolic extract from flowering parts of *C. solstitialis* were investigated for anti-cancer and anti-inflamatory potentials. Isolation of pure compounds in a future study will show if each constituent alone or with different combinations may exhibit increased anti-cancer or anti-inflamatory activities.

Conclusion:

Ethanolic extract from flowering parts of *C. solstitialis* showed a significant anti-cancer and anti-inflamatory potentials against different cancer cell lines indicating that flowering part of *C. solstitialis* is a potential source of active compounds for the development of natural drugs against cancer disease.

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Conflict of Interest:

No conflict of interest was declered.

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FIGURE LEGENDS

Figure 1. Cytotoxic activity of plant extract against different cancer cell lines. Human cancer cells A549 (A), Daudi (B), HeLa (C) and Beas-2B (D) were treated with ethanolic extract from flowering parts of *C. solstitialis* for 72h. Cell viability was determined based on MTT assay. Data are means (±SD) of three independent experiments. ****: P< 0.0001 compared with untreated cells.

Figure 2. Effect of plant extract on cell cycle distribution of cancer cells. Histograms present a cell cycle distrubition of HeLa (A) and A549 (B) cells after treatment with no extract (a), 200 µg/ml (b) and 500 µg/ml (c) extract for 24 h. The percentages of cells at different cell cycle phases are shown (d).

Figure 3. Plant extract induces apoptosis in cancer cells. HeLa (A) and A549 (B) cells were treated with no extract (a), 200 µg/ml (b) and 500 µg/ml extract (c) for 24 h. Cells were distributed into four quadrants: viable cells (Q3), early apoptotic cells (Q4), late apoptotic cells (Q2) and necrotic cells (Q1). The percentage of apoptotic cells (d).

Figure 4. Caspase 3 activity in HeLa and A549 cells after treatment with the plant extract. Cells were treated without or with the extract at 500 µg/ml for 36 h. Caspase 3 activity in untreated cells was taken as 1-fold and the change in the treated cells was expressed by comparing untreated cells. The results are means (±SD) of three independent experiments. ****: *P*<0.0001.

Figure 5. Effects of plant extract on VEGF secretion of A549 cells. Cells were treated with 200 µg/ml extract for 6 hr and VEGF concentration in supernatants was detected by ELISA. Results are presented as fold of change in relation to the control cells.
Data are means (±SD) of three independent experiments.

Figure 6. Effects of plant extract on cytokine secretion. A549 cells (A) and Daudi cells (B) were treated plant extract at 200 µg/ml for 6 h. The concentrations of IL-1α, IL-6 and TNF-α in supernatants of cancer cells were detected by ELISA. Results are presented as fold of change in relation to the control cells. Data are means (±SD) of three independent experiments.****: *P*<0.0001, ***: P<0.01, ns: non significant.

Figure 1.

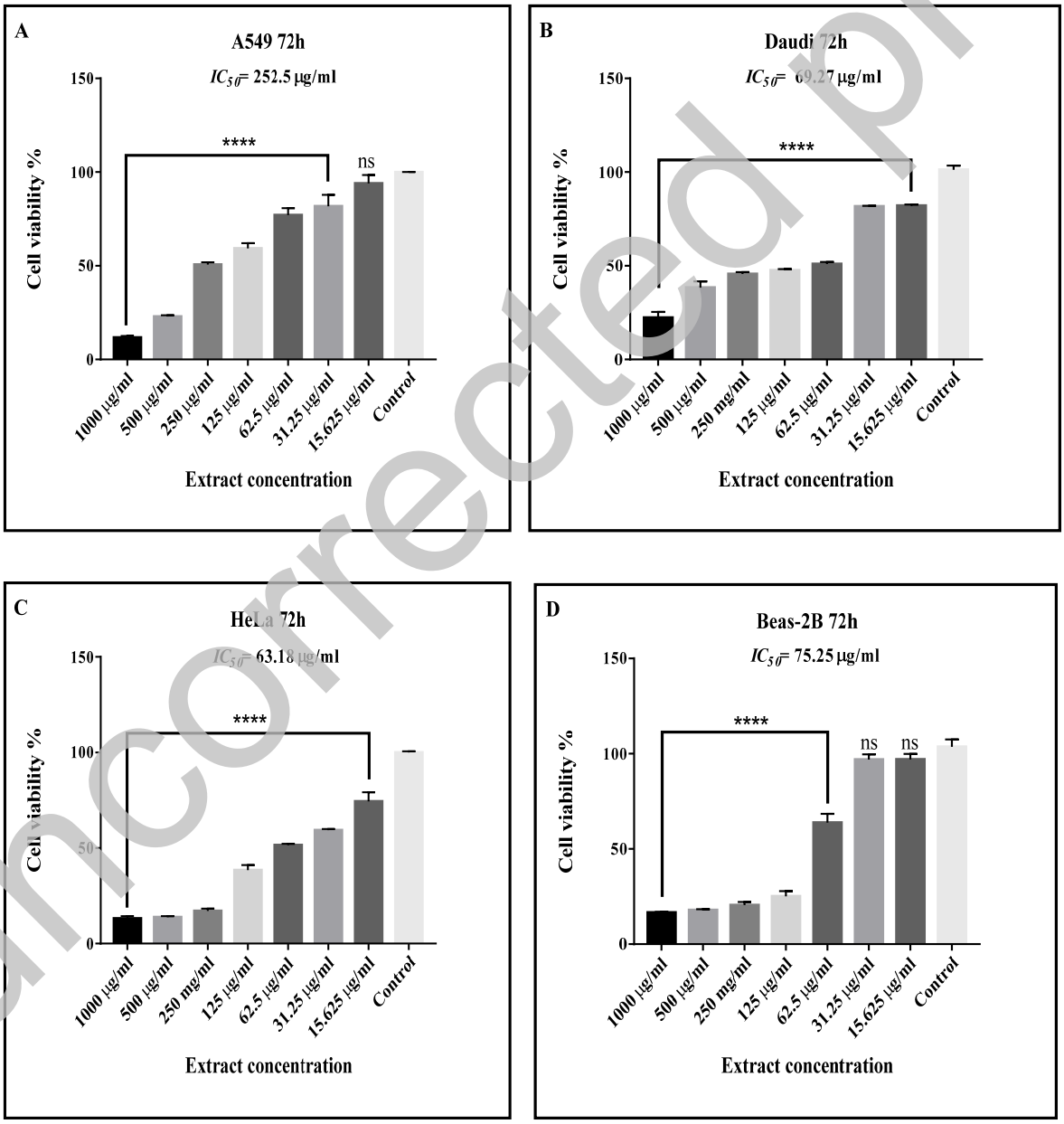
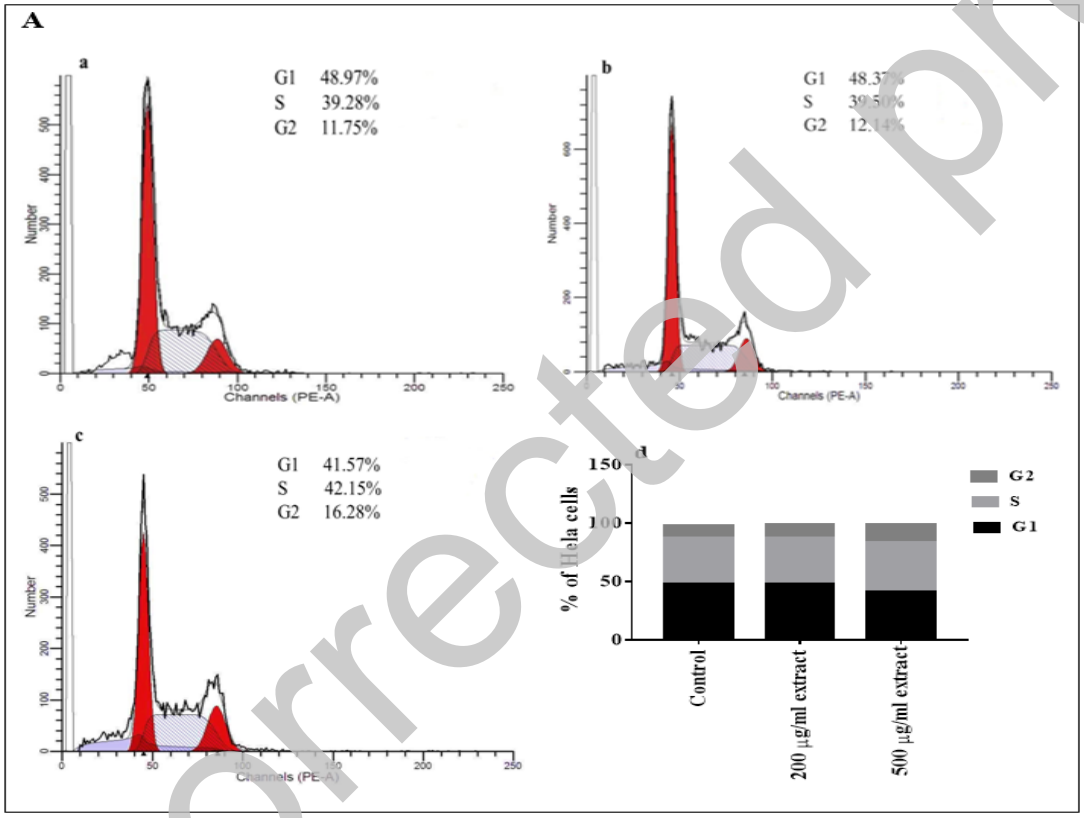


Figure 2



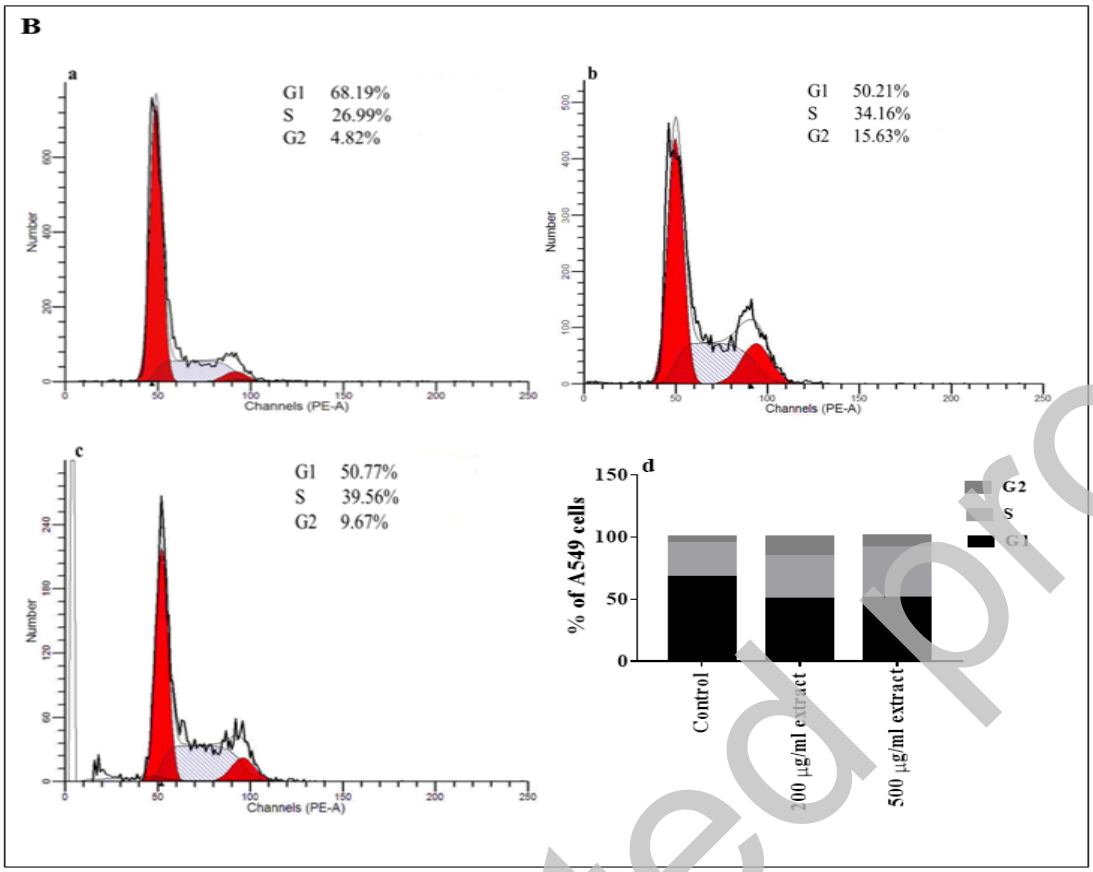
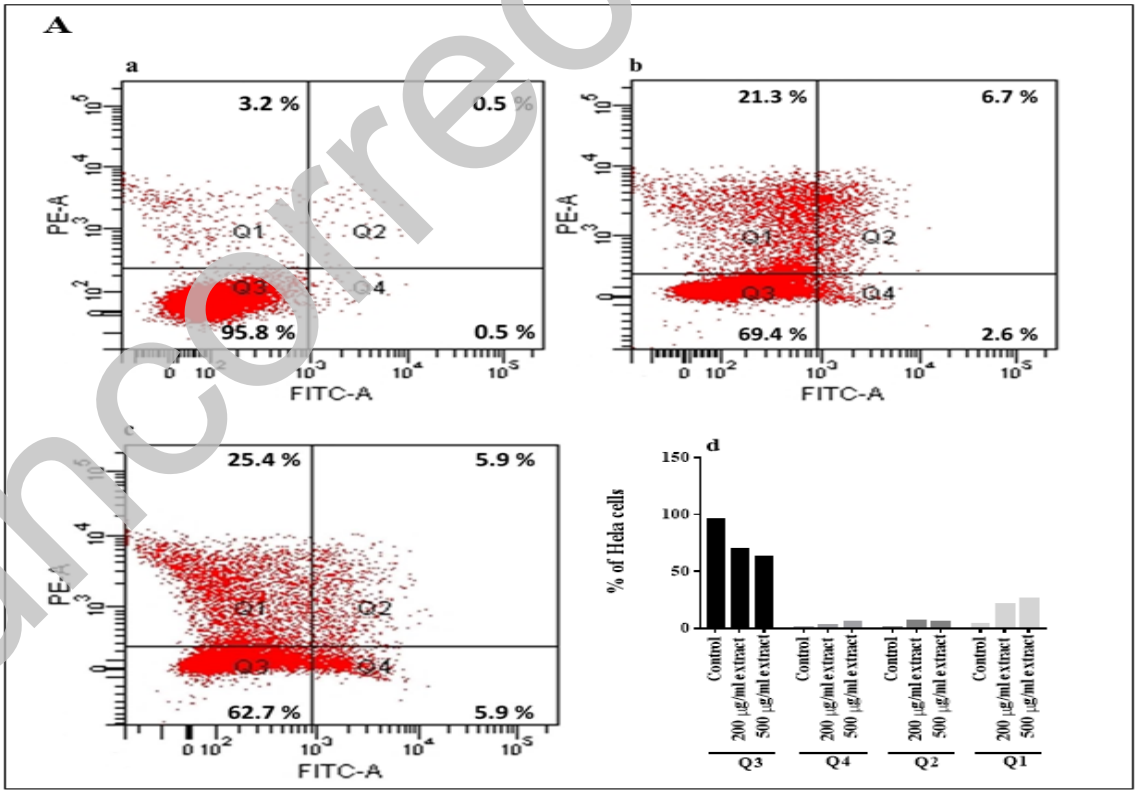


Figure 3



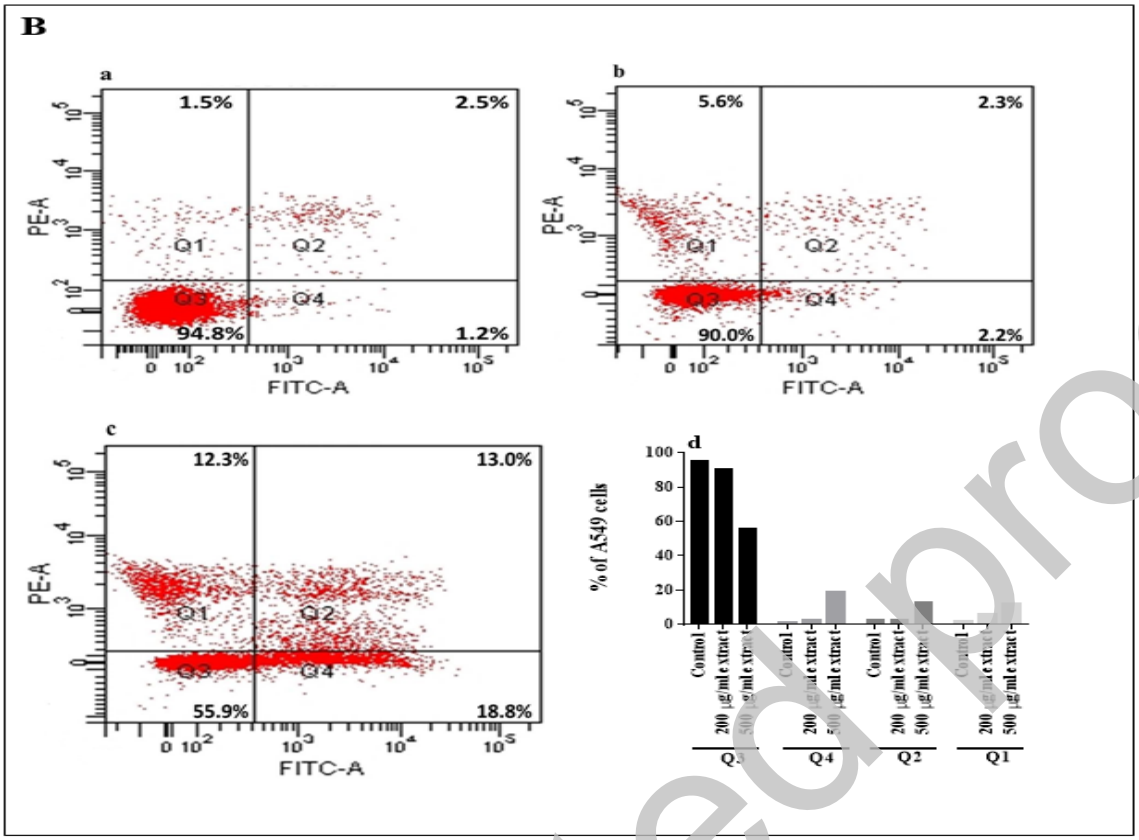


Figure 4

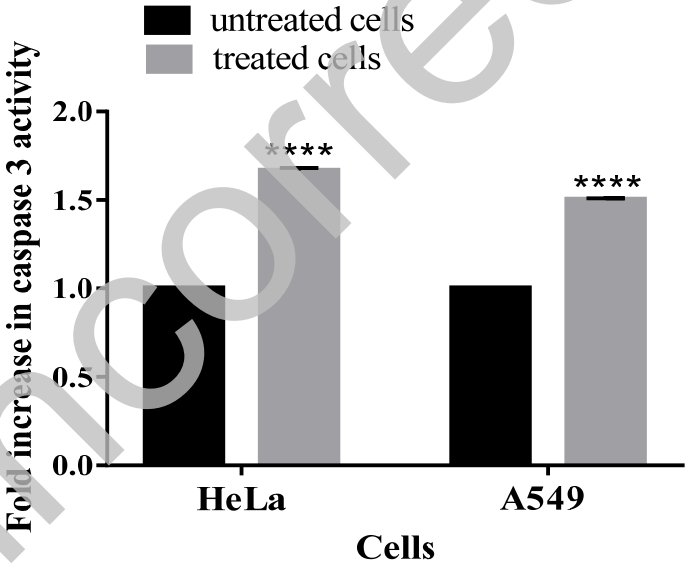


Figure 5

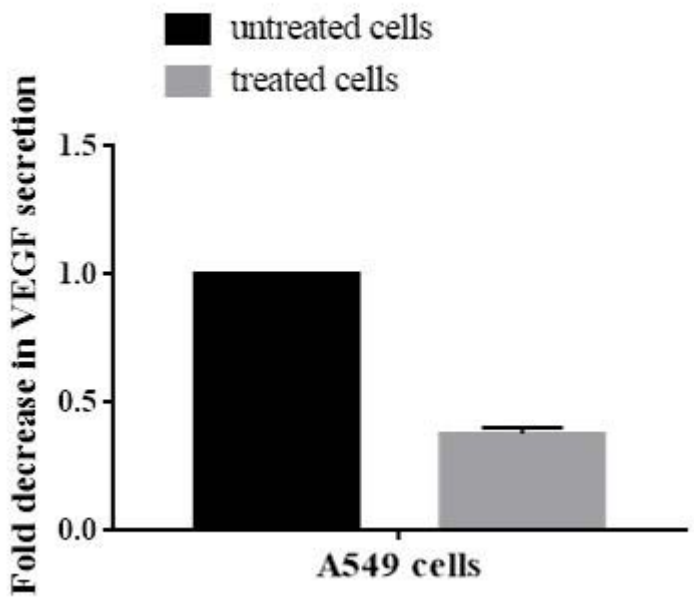


Figure 6