Istanbul J Pharm 50 (1): 42-48
DOI: 10.26650/IstanbulJPharm.2019.0039
Original Article

Investigation of aloe-emodin and *Aloe vera* gel extract on apoptosis dependent pathways in leukemia and lymphoma cell lines

Serap Erdem Kuruca[1], Eda Çandöken[2], Nuriye Akev[2]

[1]Istanbul University, Istanbul Faculty of Medicine, Department of Physiology, Istanbul, Turkey
[2]Istanbul University, Faculty of Pharmacy, Department of Biochemistry, Istanbul, Turkey

ORCID IDs of the authors: S.E.K. 0000-0001-7878-9994; N.A. 0000-0003-1804-7774

Cite this article as: Erdem Kuruca, S., Candoken, E., & Akev, N. (2020). Investigation of aloe-emodin and *Aloe vera* gel extract on apoptosis dependent pathways in leukemia and lymphoma cell lines. *İstanbul Journal of Pharmacy, 50* (1), 42–48.

ABSTRACT

Background and Aims: The present study was designed to evaluate the mechanism of cytotoxic effects of aloe-emodin relative to *Aloe vera* gel extract (AVG) on chronic myelogenous leukemia K562 and Burkitt's lymphoma P3HR-1 cell lines.
Methods: Cytotoxicity tests were assessed by tetrazolium bromide (MTT) assay. Annexin V-FITC/PI labelling was used for apoptosis/necrosis evaluation and western blotting for apoptotic molecules measurement.
Results: It was shown that, AE has cytotoxic activity againts K562 and P3HR-1 cells with an IC_{50} value of 60.9 µM and 28 µM, respectively. AVG was found cytotoxic on K562 cells with an IC_{50} value of 243.2 µg/mL and ineffective on P3HR-1 cells. The ratio of apoptotic cells (46.7%) was high in only K562 cells after AVG treatment. The percentage of apoptotic cells (K562=34.1, P3HR-1=38.8) was higher than necrotic cells (K562=11.9, P3HR-1=16.6) after AE treatment. The main apoptosis pathway in both cell lines was found to be through caspase-3 and caspase-9 activations after AE treatment, caspase-8 was also activated in K562 cells but suppressed in P3HR-1 cells. Likewise caspase-9 was activated in K562 cells after AVG treatment.
Conclusion: These results suggest that AE and AVG realized cell death activating apoptotic mechanisms in K562 and P3HR-1cells through extrinsic and intrinsic pathways.

Keywords: *Aloe vera*, aloe-emodin, cytotoxicity, leukemia, lymphoma, apoptosis, caspases

INTRODUCTION

As cancer continues to be one of the devastating illnesses of our century, the plant kingdom, with its diversity, is also researched in regard to cytotoxic and anticancer agents. It has been well-established that many of the naturally occurring phytochemicals can target multiple pathways involved in cancer cells and are considered as promising candidates for anticancer drug development. Several *in vivo* and *in vitro* studies were conducted with the *A. vera* leaf and gel extracts regarding their antitumour effects (Saito 1993; Tsuda et al., 1993; Corsi, Bertelli, Gaja, Fulgenzi & Ferrero, 1998; Akev et al., 2007). Aloe-emodin (AE) is an anthraquinone derivative purified from *A. vera* leaves, which, in addition to its well established laxative effect, has been reported to exhibit antiviral, antimicrobial, hepatoprotective and anticancer properties (Pecere et al., 2000; Mijatovic et al., 2004; Lu, Lin, Yang, Leung & Chang, 2007; He, Yan, Mo & Liang, 2008; Harlev, Nevo, Lansky, Ofir & Bishayee, 2012). Considerable attention has been given recently to the possibility of utilizing AE as a chemotherapeutic drug for the treatment of various types of cancers (Chen, Hsieh, Chang &, Chung, 2014). In a previous study undertaken in our laboratory, the anticancer effects of *Aloe vera* leaf skin and gel extracts, as well as aloe-emodin, were evaluated against B16F10 murine melanoma cells (Çandöken, Erdem Kuruca & Akev, 2017).

Address for Correspondence:
Nuriye AKEV, e-mail: nakev@istanbul.edu.tr

Submitted: 08.07.2019
Revision Requested: 05.11.2019
Last Revision Received: 20.11.2019
Accepted: 05.12.2019
Published Online: 05.03.2020

The present study was undertaken in order to determine and to compare the cytotoxic effects of *A. vera* leaf gel extract (AVG) and AE on human chronic myelogenous leukemia K-562, human promyelocytic leukemia HL-60 and Burkitt's lymphoma P3HR-1 cell lines, and to understand pathways molecular of cell death mechanisms.

Usually, the stimuli which induces cell death *in vitro* is dose-dependent. At low doses, a variety of injurious stimuli such as heat, radiation, hypoxia and cytotoxic anticancer drugs can induce apoptosis, but these same stimuli can result in necrotic cell death, and be undesirable because it generates inflammation response and effect damaging neighboring cells, at higher doses. Consequently, an evaluation of cell death type is important as determination of IC_{50} values, that provide 50% inhibition of cell growth.

MATERIALS AND METHODS

Cell lines and cell culture

Chronic myelogenous leukemia K-562, acute promyelocytic leukemia HL-60 and Burkitt's lymphoma P3HR-1 cell lines were purchased from the American Type Culture Collection (ATCC). The cells were cultured in RPMI (Roswell Park Memorial Institute Medium; Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS; Capricorn FBS-12A), 100 units/mL penicillin and 100 µg/mL of streptomycin (Gibco 15140-122), in a humidified incubator containing 5% CO_2 at 37°C. The cells were sub-cultured every 2 or 3 days.

Preparation of extracts

Fresh leaves of *A. vera* (L.) Burm. f. (Xanthorreaceae) (ISTE No. 65118), cultivated in the Greenhouse of Istanbul University Alfred Heilbronn Botanical Garden, were used.

The activity of four types of extracts was investigated throughout the study. These were *A. vera* fresh leaf skin aqueous extract, *A. vera* leaf gel extract (AVG), *A. vera* fresh leaf skin methanolic extract and *A. vera* dried leaf skin methanolic extract. The preparation of the extracts was described previously (Çandöken et al., 2017).

Preparation of test materials and reference drugs

Aloe-emodin (AE; 1,8-dihydroxy-3-[hydroximethyl]-anthraquinone) was purchased from Sigma-Aldrich (St Louis, MO, cat no. A7687). *A. vera* extracts (10 mg/mL) and AE (20 mM) stock solutions were prepared in dimethyl sulfoxide (DMSO). Imatinib, which was used as a reference chemotherapeutic drug, was purchased from Santa Cruz.

MTT colorimetric assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay was undertaken for cytotoxic activity (Mosmann 1983), as previously described (Çandöken et al. 2017). Varying concentrations of *A. vera* extracts, and as positive controls: AE (1.25 – 200 µM) and IM (Imatinib) (0.1 – 50 µM) were used in 10 µL/well volumes, and the assay was done in a total volume of 100 µL in microplates.

An ELISA microplate reader (Rayto RT-2100C) was used for absorbance measurement, and the percentage of viable cells (VI) was determined using the equation:

VI = (Absorbance of the treated cells ÷ Absorbance of the control cells) ×100

The cytotoxic concentrations of extracts that provide 50% inhibition of cell growth (IC_{50}) were calculated from a dose-response curve. The cytotoxic effects of *A. vera* extracts and reference drugs were evaluated by comparing the IC_{50} values of cell lines.

Flow cytometry analysis

An Annexin V-FITC/propidium iodide (PI) assay kit (Millipore) was used in order to determine normal, apoptotic, and necrotic cells as previously described (Çandöken et al. 2017).

Western blot analysis

For the apoptic protein expression analysis, cells (45 mL; 10^5 cells/mL culture medium) were seeded in 75 cm^2 cell culture treated flasks. After AE or AVG treatment, the cells were harvested and pelleted according to a previously described method. The pellets were washed with PBS, then resuspended in a 100 µl lysis buffer (1mM EDTA, 10 mM Tris-HCL, 0.5% Triton X-100, pH 8)with the addition (1:1000) of 100 nM phenylmethylsulfonyl fluoride (PMSF) followed by incubation on ice for 60 min by vortexing every 10 min. The cell lysate was centrifuged at 14 000 rpm for 10 min, and the supernatant was collected as total protein extract. The protein concentration was detected using the Bradford method. All the samples were mixed with Laemli sample buffer (2X) (Biorad), then transferred to a 80°C water bath for 15 min and stored at −80°C for later use. 10-20 µg of protein was separated on 15% SDS-PAGE gel performed at 200 mA. PVDF blotting membranes (0.45 micron) were activated in methanol for a few seconds, after electrophoresis. Blotting was performed overnight at 40 mA at 4°C using Towbin reagent, Wet/Tank Blotting Systems, BIORAD. The membranes were blocked with 5% skimmed milk powder in TBS (Sigma) containing 0.05% Tween-20 (Santa Cruze) for 1 h at RT, and then incubated with primary antibodies overnight at 4°C on a shaker at low speed. Antibodies directed against the following proteins were used in this study: actin (1:1000, Santa Cruz Biotechnology, sc-1616, goat polyclonal IgG), caspase-3 (1:200, Santa Cruz Biotechnology, sc-7148, rabbit polyclonal IgG), caspase-8 (1:200, Santa Cruz Biotechnology, sc-56070, mouse monoclonal IgG_1), caspase-9 (1:200, Santa Cruz Biotechnology, sc-7885, rabbit polyclonal IgG). The secondary antibodies used were goat anti-rabbit IgG-HRP (HRP: Horseradish Peroxidase, Santa Cruz Biotechnology, sc-2030) for caspase-3 and caspase-9, and goat anti-mouse IgG-AP (AP: Alkaline Phosphatase, Santa Cruz Biotechnology, sc-2008) for caspase-8. The fluorescent bands were visualized with the KETA Wealtec Chemiluminescence Imaging System with Magic-Chemi software, and AP detection was performed using the Novex® AP Chromogenic Substrate (Invitrogen, WP20001).

Statistical analysis

The results were statistically analyzed using the independent Student's *t*-test. Data were represented as means ± standard deviation (S.D.) and at least in triplicate. Results were considered significant with $P<0.05$ (*), $P<0.01$ (**) ve $P<0.001$ (***).

RESULTS

Cytotoxic activity

The cytotoxic effects were evaluated by comparing the IC_{50} values of all of the cell lines. Cytotoxic activity results obtained from MTT are summarized in Table 1. The data showed that AE and *A. vera* extracts had potential selective cytotoxic activity against the cells investigated. Further studies were carried out using IC_{50} concentrations as described (Mahbub et al. 2013).

The cytotoxic effects of *A. vera* extracts (62.5, 125 and 250 µg/mL) on K-562, HL-60 and P3HR-1 cells given as percentage of viable cells are shown in Figure 1. The cytotoxic efficacy was as follows: AVG >*A. vera* dried leaf skin methanolic extract > *A. vera* fresh leaf skin methanolic extract > *A. vera* fresh leaf skin aqueous extract for K-562 cells, *A. vera* fresh leaf skin methanolic extract >*A. vera* dried leaf skin methanolic extract > AVG > *A. vera* fresh leaf skin aqueous extract for HL-60 cells, whereas, P3HR-1 human lymphoma cells showed resistance to *A. vera* extracts (Table 1).

The cytotoxic effects of AE on K-562, HL-60 and P3HR-1 cells given as percentage of viable cells are shown in Figure 2. The results showed that the IC_{50} value of AE against K-562 was 60.98±0.90 µM AE. Contrarily, AE showed lower IC_{50} at 20.93±1.96 µM and 28.06±1.69 µM AE concentration against HL-60 and P3HR-1 respectively (Table 1). Anticancer drugs with limited side effects, inducing apoptosis and targeting selective cytotoxicity to the cancer cells are the drugs of choice.

The effects of IM on K-562, HL-60 and P3HR-1 cells given as percentage of viable cells are shown in Figure 3. It was shown

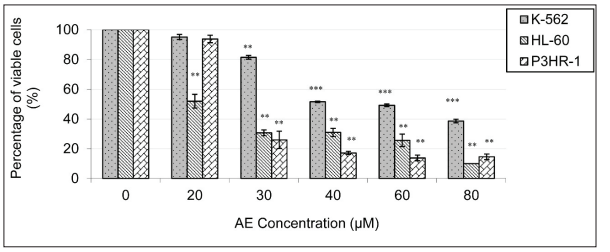

Figure 2. The effects of AE on K-562, HL-60 and P3HR-1 cells. Proliferation suppression appears to be dose dependent. Reported mean±S.D. values are from a representative trial out of two or more trials. Significant differences are indicated by *(P<0.05), **(P<0.01) ve ***(P<0.001), compared with control cells.

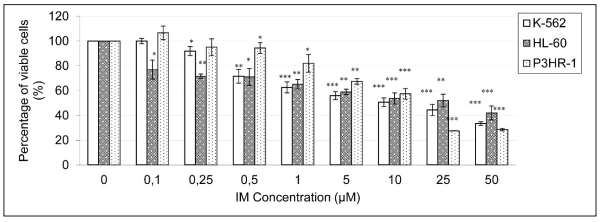

Figure 3. The effects of *Imatinib* on K-562, HL-60 and P3HR-1 cells. Proliferation suppression appears to be dose dependant. Reported mean±S.D. values are from a representative trial out of two or more trials. Significant differences are indicated by *(P<0.05), **(P<0.01) ve ***(P<0.001), compared with control cells.

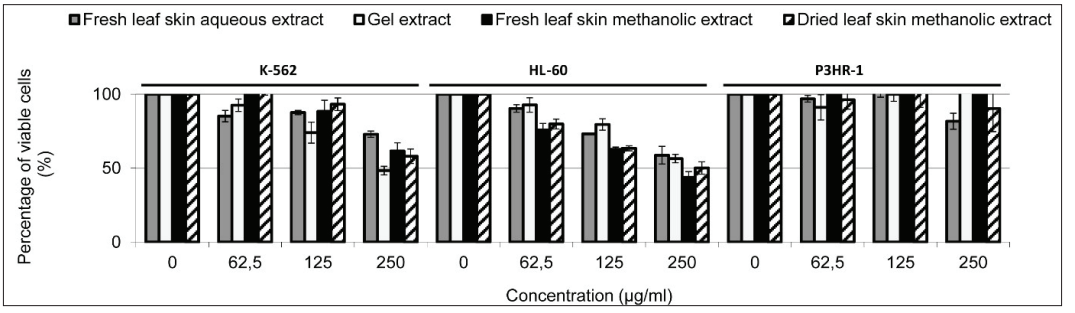

Figure 1. The effects of *A. vera* extracts on K-562, HL-60 and P3HR-1 cells. Proliferation suppression appears to be dose dependent. Reported mean±S.D. values are from a representative trial out of two or more trials.

Table 1. IC_{50} values of AE, *A. vera* extracts and IM for leukemia and lymphoma cell lines.

	IC_{50}±S.D.					
Cell line	AE (µM)	Fresh leaf skin aqueous extract (µg/mL)	Gel extract (µg/mL)	Fresh leaf skin methanolic extract (µg/mL)	Dried leaf skin methanolic extract (µg/mL)	IM (µM)
K-562	60.98±0.90	485.53±83.47	243.21±19.22	336.39±78.67	323.80±54.41	10±0.34
HL-60	20.93±1.96	290.22±47.41	289.42±18.17	204.84±2.31	229.34±20.13	25±1.28
P3HR-1	28.06±1.69	>250	>250	>250	>250	15±0.55

AE: Aloe-emodin; IM: Imatinib. K-562 human chronic myelogenous leukemia; HL-60 human acute promyelocytic leukemia; P3HR-1 human Burkitt's lymphoma.

that among leukemia cells, IM has selective cytotoxic effect on HL-60 cells at 10±0.34 µM, while it has no effect on HL-60 cells in the same concentrations with an even higher IC_{50} level such as 25±1.28 µM. The IC_{50} value of IM against P3HR-1 was 15±0.55 µM at the same time.

Annexin-V/PI apoptosis assay

To corroborate that the cytotoxic effect of K-562, HL-60 and P3HR-1 cells treated with IC_{50} values of 60 µM, 20 µM and 28 µM AE for 72 h, respectively were associated with the induction of apoptosis, an Annexin-V-FITC/PI double-staining assay was used to evaluate the percentage of apoptotic and necrotic cells. AE have apoptotic and necrotic effects on K-562 (33.99% apoptosis and 11.99% necrosis) and P3HR-1 (38.85% apoptosis and 16.66 % necrosis) cells, while treatment with AE in HL-60 cells did not induce apoptosis and necrosis in cells (Figure 4). Apoptosis and necrosis were not detected as possible mechanisms of cytotoxicity of AE in HL-60 cells.Annexin V-propidium iodide double-staining demonstrated AE potentiate apoptosis rather than necrosis in K-562, HL-60 and P3HR-1 cells.

As shown in Figure 5, AVG have apoptotic and no necrotic effects on K-562 (46.75% apoptosis and <1% necrosis) and P3HR-1 (21.49% apoptosis and <1% necrosis) cells with IC_{50} values of 250 µg/mL and 290 µg/mL AVG respectively. AVG was not assessed on the P3HR1 cells with the Annexin V-FITC/PI assay because it was ineffective.

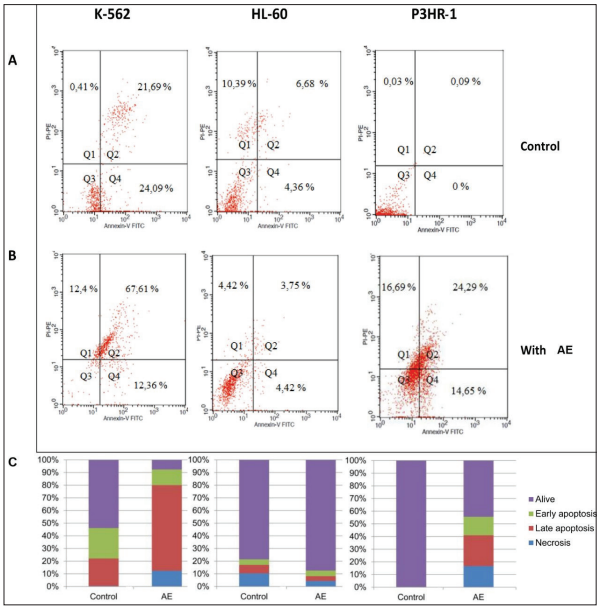

Figure 4. Flow Cytometry of K-562, HL-60 and P3HR1 cells treated with annexin V/PI. The cells after treatment with IC_{50} concentration of AE for 72 h, stained with FITC-annexin V/PI and analyzed by FACScan flow cytometer marked for apoptosis/necrosis. Q1: Annexin V negative/PI positive; Q2: Annexin V/PI positive; Q3: Annexin V positive/PI negative; Q4: Annexin V/PI negative. (**A**) Control cells without the presence of aloe emodin; (**B**) Cells incubated with aloe emodin, The analysis of data from flow Cytometry was performed using the FlowJo software (**C**) Statistical analysis of alive, early apoptosis, late apoptosis and necrosis phenomena observed in aloe emodin treatment and non-treatment groups.

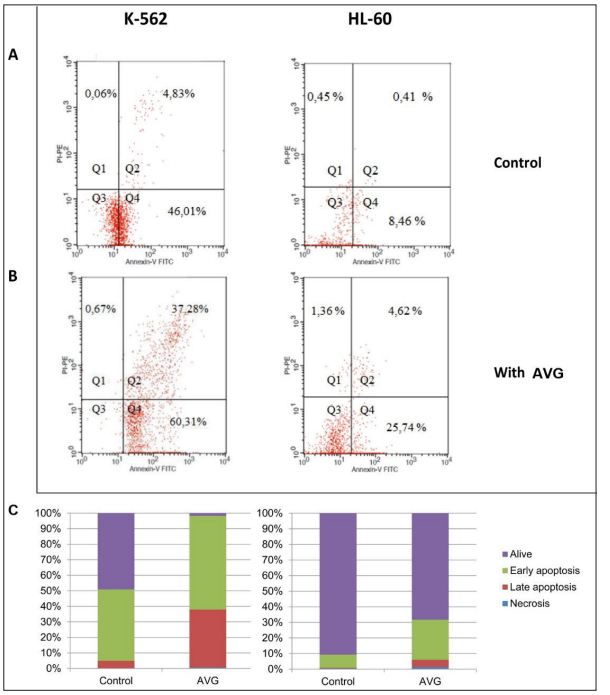

Figure 5. Flow Cytometry of K-562 and HL-60 cells treated with annexin V/PI. The cells after treatment with IC_{50} concentration of *A. vera* gel (AVG) for 72 h, stained with FITC-annexin V/PI and analyzed by FACScan flow cytometer marked for apoptosis/necrosis. Q1: Annexin V negative/PI positive; Q2: Annexin V/PI positive; Q3: Annexin V positive/PI negative; Q4: Annexin V/PI negative. (**A**) Control cells without the presence of AVG; (**B**) Cells incubated with AVG, The analysis of data from flow Cytometry was performed using the FlowJo software (**C**) Statistical analysis of early and late apoptosis phenomena observed in AVG treatment and non-treatment groups.

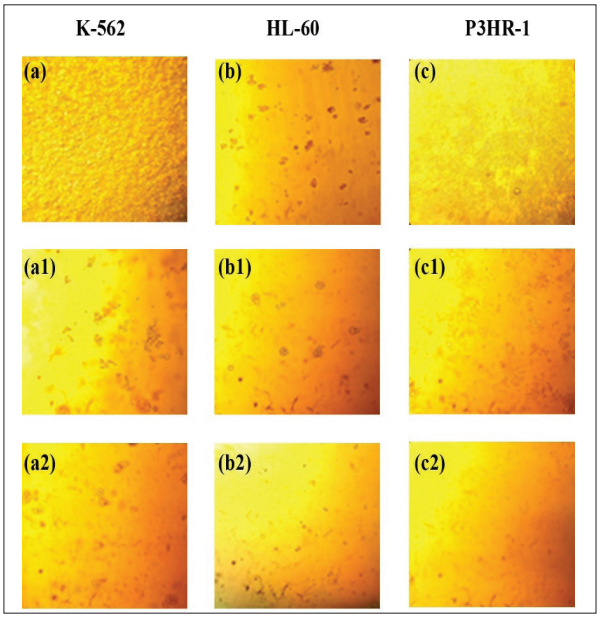

Figure 6. Inverted microscope images(X10), after treatment with the IC50 concentration for AE and AVG for 72 h. (a): K-562 cells, (b): HL-60 cells, (c): P3HR-1 cells, (a1): K-562 cells treated with AE for 72 h, (b1) HL-60 cells treated with AE for 72 h, (c1) P3HR-1 cells treated with AE for 72 h, (a2) K-562 cells treated with AVG for 72 h, (a2) HL-60 cells treated with AVG for 72 h, (b2) P3HR-1 cells treated with AVG for 72 h.

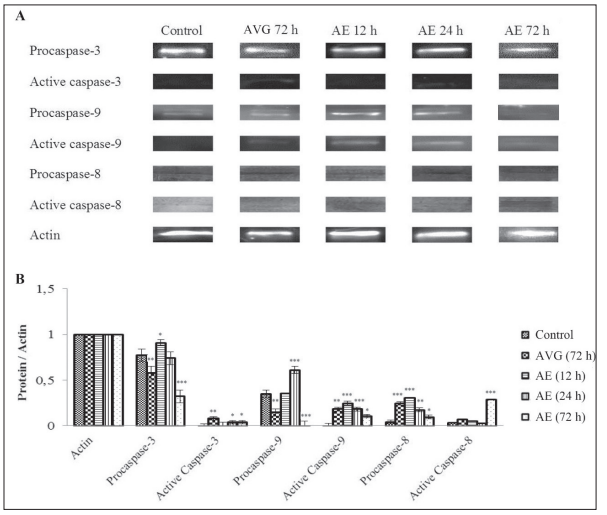

Figure 7. Western blot analysis of caspase proteins expression levels after AVG and AE treatment of K562 cells. (**A**): The K562 cells after treatment with IC_{50} concentration of AVG for 72 h and AE for 12 h, 24 h, 72 h, equal amounts of total protein were examined by western blot analysis with indicated antibodies. Actin was used as a loading control. (**B**): Band intensity was analyzed by densitometry. Fold change of protein expression levels was calculated after bands were normalized to Actin. Representative data from three independent experiments are shown. Values represent mean±SD from three independent experiments. Significant differences are indicated by *(P<0.05), **(P<0.01) ve ***(P<0.001), compared with control cells.

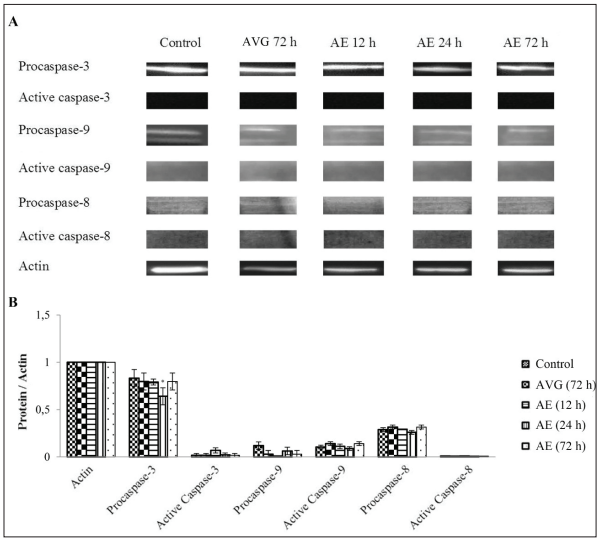

Figure 8. Western blot analysis of caspase proteins expression levels after AVG and AE treatment of HL-60 cells. (**A**): The HL-60 cells after treatment with IC_{50} concentration of AVG for 72 h and AE for 12 h, 24 h, 72 h, equal amounts of total protein were examined by western blot analysis with indicated antibodies. Actin was used as a loading control. (**B**): Band intensity was analyzed by densitometry. Fold change of protein expression levels was calculated after bands were normalized to Actin. Representative data from three independent experiments are shown. Values represent mean±SD from three independent experiments. Significant differences are indicated by *(P<0.05), **(P<0.01) ve ***(P<0.001), compared with control cells.

The morphological assessment of the effect of AE and AVG on cellular proliferation of cells was also demonstrated by Inverted microscope images (Figure 6).

Expression of apoptotic-related proteins in AVG treated K-562 cells

To understand the underlying molecular mechanisms of AE and AVG induced apoptosis in K-562 cells, several molecular markers critical for the apoptosis process were analyzed through western blotting. After exposure of K-562 cells to AE for 12, 24 and 72 hours, as shown in Figure 7, the active caspase-9 and procaspase-8 expression increased in the first 12 hours, the procaspase-8 protein was cleaved to the corresponding active forms in the 24 hours and the caspase-3 active form increased in the 72 h. The AVG treatment induces apoptotic death in K-562 cells through a caspase-dependent pathway likewise AE in the 72 hours.

As shown in Figure 8, no significant changes were observed in procaspase protein expression nor in the amount of active caspase. The AVG treatment did not induce apoptotic death in HL-60 cells through a caspase-dependent pathway likewise AE in the 72 hours.

After the P3HR-1 cells were exposed to AE for 12, 24 and 72 hours, as shown in Figure 9, procaspase-3, -9, -8 protein were

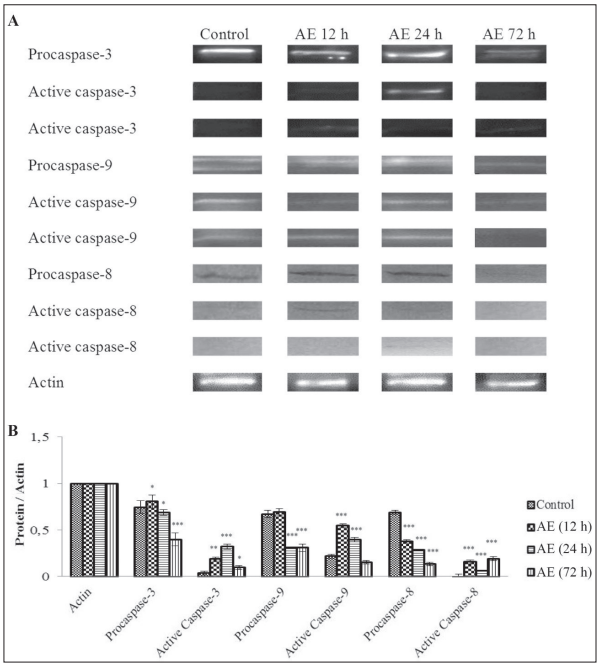

Figure 9. Western blot analysis of caspase proteins expression levels after AE treatment of P3HR-1 cells. (**A**): The P3HR-1 cells after treatment with IC_{50} concentration of AVG for 72 h and AE for 12 h, 24 h, 72 h, equal amounts of total protein were examined by western blot analysis with indicated antibodies. Actin was used as a loading control. (**B**): Band intensity was analyzed by densitometry. Fold change of protein expression levels was calculated after bands were normalized to Actin. Representative data from three independent experiments are shown. Values represent mean±SD from three independent experiments. Significant differences are indicated by *(P<0.05), **(P<0.01) ve ***(P<0.001), compared with control cells.

cleaved to the corresponding active forms in the first 12 hours. These results indicate that AE treatment induces apoptotic death in P3HR-1 cells through a caspase-dependent pathway.

DISCUSSION

Among the various known therapeutic effects of *Aloe vera,* studies have shown that preparations of the plant leaves have the ability to prevent the growth or to regress certain tumours (Akev et al., 2015). Considerable attention has been given recently to the possibility of utilizing AE, an anthraquinone purified from *A. vera* leaves, as a chemotherapeutic drug (Chen et al., 2014). Some researchers claim that the synergistic effect of the compounds contained in plant extracts should be preffered to purified compounds (Williamson, 2001).

A number of studies have demonstrated that AE is capable of inducing apoptotic cell death in various cancer cells such as neuroectodermal cancer cells (Pecere et al., 2000), hepatoma (Kuo, Lin & Lin, 2002), gastric cancers (Chen, Lin, Chang, Fang & Lin, 2007; Qin et al., 2010), bladder carcinoma (Lin et al., 2006), glioma cells (Acevedo-Duncan, Russel, Patel & Patel, 2004) or squamous cell cancer (Chiu et al., 2009). Some of the anticancer mechanisms were also related to cell cycle arrest (Chen et al., 2004; Guo et al., 2007; Chiu et al., 2009) or DNA damage through oxidative stress (Lee, Lin, Yang, Leung & Chang, 2006).

There are also a few studies undertaken on leukemia cells (Tabolacci et al., 2011). The IC_{50} value of 29 µM was found for aloe-emodin in human K562 leukemia cell line (Harlev et al., 2012). Mahbub et al. (2013) found an IC_{50} value of 500 µM for emodin in K562 cells. In our study, this value was found to be 60.98±0.90 µM. Chen et al. (2004) reported an IC_{50} value of AE for HL-60 / ADR of 5.79 µmol / L. In our study, the IC_{50} value of AE for HL-60 was found to be 20.93±1.96 µM. No literature was found for AE treatement with P3HR-1 human lymphoma cells. In our study, the IC_{50} value of AE for P3HR-1 human lymphoma cells was 28.06±1.69 µM.

Imatinib mesylate (IM), a specific Bcr-Abl tyrosine kinase inhibitor, has been highly successful in the treatment of chronic myelogenous leukemia (Gu, Santiago & Mitchell, 2005). Therefore, it was chosen as a reference drug in the present study. The IC_{50} value of IM was reported to be 10.49±1.24 µM in K562 human chronic myeloid leukemia cells (Wu, Huang & Ma, 2013). Similarily, in our study, this value was found to be 10±0.34 µM. for the same cell line. The IC_{50} value of IM for HL-60 human promyelocytic leukemia cells was > 8 µM for 48 h (Gu et al. 2005). In our study, the IC_{50} value of IM for HL-60 human promyelocytic leukemia cells was found to be 25±0.51 µM. There has been no published literature on the IC_{50} value of IM for P3HR-1 human lymphoma cells. In our study, the IC_{50} value of IM for P3HR-1 cells was found to be 15±5.5 µM.

Induction of apoptosis is an important mechanism of chemoprevention and chemotherapy for cancer. The variety of injurious stimuli such as heat, radiation, hypoxia and anticancer drugs can induce apoptosis, but these same stimuli can result in necrotic cell death, undesirable because it generates inflammation response. The apoptotic and necrotic cell death are directly related to the drug concentration because drug treatment in high concentration causes necrosis, and also low concentration stimulates apoptosis. To determine whether the inhibition of cell proliferation by extracts from *A. vera* was due to the induction of apoptosis, we assessed the latter with the Annexin V-FITC/PI method. Therefore, we preferred the percentage of apoptosis as a criterion in evaluation of cell death type. IC_{50} values (concentration that provide 50% inhibition of cell growth) were used to assess the cytotoxic effects of extracts and comparing to reference drugs. Among all the extracts tested, *A. vera* gel extract was found to be the most effective on the tested cells, with the lowest IC_{50} values. Therefore, apoptosis and necrosis were measured in cells treated with AVG or AE in the doses according to IC_{50} values. Flow Cytometry images clearly showed apoptosis and necrosis changes in the treated cells.

Chen et al., (2004) reported that aloe-emodin inhibited cell proliferation in human promyelocytic leukemia HL-60 cells, caused cell cycle involvement in G2 / M phase and showed apoptotic activity. They found that morphological changes in the cells were observed in 10 - 25 µM aloe-emodin applications. In the determination of the apoptotic pathway, the effect of 10 µM aloe-emodin dose on the expression of caspase-3, caspase-8 and caspase-9 expressions in cells at different times (12, 24, 48, and 72 h) was determined (Chen et al., 2004).

In a study conducted by Mahbub et al. (2013), the apoptotic effect of AE on HL-60 and K562 cells was performed by Hoechst 33342 with DNA staining and caspase-3 activity by Flow Cytometry. HL-60 cells were found to be more sensitive than K562 cells to AE treatment and also the dose of 50 µM AE induced significant induction of caspase 3 activity in both cell lines. Contrary to this study, in our study, Western Blot studies conducted with AE at IC_{50} doses on HL-60 cells, did not show similar AE-induced caspase-3 activity. This result was found to be compatible with our Annexin V / PI test of AE for HL-60 cells test results (0.54% apoptosis). The apoptotic effect of *A. vera* gel (21.49% apoptosis) observed in the Annexin V / PI test for HL-60 cells could not be shown in Western Blot experiments. *A. vera* gel treatment did not induce apoptotic death in HL-60 cells through a caspase-dependent pathway likewise AE in the 72 hours. Here, the mechanism is caspase-dependent apoptosis, whereas caspase-independent apoptosis is also known. Accordingly, the apoptosis observed in HL60 cells of *A. vera* gel may be caspase-independent. Whereas IC_{50} doses of 60 µM AE treatment induce apoptotic death in K-562 cells through a caspase-dependent pathway likewise AVG in the 72 hours.

Cytotoxicity and Western Blot studies with *A. vera* extracts or aloe-emodin for P3HR-1 Burkitt's lymphoma cells were not found in the literature. In our study, the Annexin V / PI study with AE for P3HR-1 cells concluded that the apoptotic protein expression (increased active caspase-3 expression) was compatible with the end result (38.85% apoptosis for aloe- emodin). Our results suggested that caspase-3 was at the top of the hierarchy of the caspase cascade and is an initiator caspase to be evoked. This activation occured as early as 12 h af-

ter AE treatment in P3HR-1 cells, whereas caspase-9 showed activation after 24 h. There was no significant change in the expression of active caspase-8 until the end of 72 h, whereas an increase in active caspase-9 was observed in the 24 h experiment.

As a conclusion we can say that their selective cytotoxic and apoptotic effects make *A. vera* gel and aloe-emodin promising drugs for alternative cancer therapy.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- N.A., S.EK., E.C.; Data Acquisition- E.C., S.E.K.; Data Analysis/Interpretation- N.A., S.EK., E.C.; Drafting Manuscript- N.A., S.EK., E.C.; Critical Revision of Manuscript- N.A., S.E.K.; Final Approval and Accountability- N.A., S.EK., E.C.; Technical or Material Support- N.A., S.EK., E.C.; Supervision- N.A., S.E.K.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This study was funded by Scientific Research Projects Coordination Unit of Istanbul University. Project number: 8775.

REFERENCES

- Acevedo-Duncan, M., Russell, C., Patel, S. & Patel, R. (2004). Aloe–emodin modulates PKC isozymes, inhibits proliferation, and induces apoptosis in U-373MG glioma cells. *International Immunopharmacology 4 (Special Issue: Future Trends in Aloe Therapeutics),*1775–1784.
- Akev, N., Can, A., Sütlüpınar, N., Çandöken, E., Özsoy N, Yılmaz Özden, T., … Üzen, E. (2015). Twenty years of research on *Aloe vera*. *Journal of the Faculty Pharmacy Istanbul University, 45*,191–215.
- Akev, N., Turkay, G., Can, A., Gurel, A., Yıldız, F., Yardibi, H., … Uzun, H. (2007). Effect of *Aloe vera* leaf pulp extract on *Ehrlich ascites* tumours in mice. *European Journal of Cancer Prevention, 16*, 151–157.
- Chen, H. C., Hsieh, W. T., Chang, W. C. & Chung, J. G. (2004). Aloe-emodin induced in vitro G2/M arrest of cell cycle in human promyelocytic leukemia HL-60 cells. *Food & Chemical Toxicology, 42,* 1251–1257.
- Chen, R., Zhang, J., Hu, Y., Wang, S., Chen, M. & Wang, Y. (2014). Potential antineoplastic effects of Aloe-emodin: a comprehensive review. *The American Journal of Chinese Medicine, 42*, 275–288.
- Chen, S.-H., Lin, K.-Y., Chang, C.-C., Fang, C.-L., & Lin, C.-P. (2007). Aloe-emodin-induced apoptosis in human gastric carcinoma cells. *Food & Chemical Toxicology, 45*' 2296–2303.
- Chiu, T.-H., Lai, W.-W., Hsia, T.-C., Yang, J.-S., Lai, T.-Y., Wu, P.-P., … Chung, J.-G. (2009). Aloe-emodin induces cell death through S-phase arrest and caspase-dependent pathways in human tongue squamous cancer SCC-4 cells. *Anticancer Research, 29*, 4503–4512.
- Corsi, M. M., Bertelli, A. A., Gaja, G., Fulgenzi, A. & Ferrero, M.E. (1998). The therapeutic potential of *Aloe vera* in tumor-bearing rats. *International Journal on Tissue Reactions, 20*,115–118.
- Çandöken, E., Erdem Kuruca, S. & Akev, N. (2017). Evaluation of the anticancer effects of *Aloe vera* and aloe emodin on B16F10 murine melanoma and NIH3T3 mouse embryogenic fibroblast cells. *Istanbul Journal of Pharmacy 47*, 77–83.
- Gu, J. J., Santiago, L., & Mitchell, B. S. (2005). Synergy between imatinib and mycophenolic acid in inducing apoptosis in cell lines expressing Bcr-Abl. *Blood, 105*, 3270–3277.
- Guo, J. M., Xiao, B. X., Liu, Q., Zhang, S., Liu, D. H. & Gong, Z. H. (2007). Anticancer effect of aloe-emodin on cervical cancer cells involves G2/M arrest and induction of differentiation. *Acta Pharmacologica Sinica, 28*, 1991–1995.
- Harlev, E., Nevo, E., Lansky, E. P., Ofir, R. & Bishayee, A. (2012). Anticancer potential of aloes: antioxidant, antiproliferative, and immunostimulatory attributes. *Planta Medica, 78*, 843–852.
- He, T.-P., Yan, W.-H., Mo, L.-E. & Liang, N.-C. (2008). Inhibitory effect of aloe-emodin on metastasis potential in HO-8910PM cell line. *Journal of Asian Natural Products Research, 10*, 383–390.
- Kuo, P. L., Lin, T. C. & Lin, C. C. (2002). The antiproliferative activity of aloe-emodin is through p53-dependent and p21-dependent apoptotic pathway in human hepatoma cell lines. *Life Sciences, 71*, 1879–1892.
- Lee, H. Z., Lin, C. J., Yang, W. H., Leung, W.C. & Chang, S. P. (2006). Aloe-emodin induced DNAdamage through generation of reactive oxygen species in human lung carcinoma cells. *Cancer Letters, 239*, 55–63.
- Lin, J. G., Chen, G. W., Li, T. M., Chouh, S. T., Tan, T. W. & Chung, J. G. (2006). Aloe-emodin induces apoptosis in T24 human bladder cancer cells through the p53 dependent apoptotic pathway. *Journal of Urology, 175*, 43–47.
- Lu, G. D., Shen, H. M., Ong, C. N. & Chung, M. C. (2007). Anticancer effects of aloe-emodin on HEPG2 cells: Cellular and proteomic studies. *Proteomics. Clin Applications, 1*, 410–419.
- Mahbub, A. A., Le Maitre, C. L., Haywood-Small, S. L., McDougall, G. J., Cross, N. A. & Jordan-Mahy, N. (2013). Differential effects of polyphenols on proliferation and apoptosis in human myeloid and lymphoid leukemia cell lines. *Anti-Cancer Agents in Medicinal Chemistry, 13*, 1601–1613.
- Mijatovic, S., Maksimovic-Ivanic, D., Radovic, J., Popadic, D., Momcilovic, M., Harhaji, L., … Trajkovic, V. (2004). Aloe-emodin prevents cytokine-induced tumor cell death: the inhibition of autotoxic nitric oxide release as a potential mechanism. *Cellular and Molecular Life Sciences, 61*, 1805–1815.
- Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods, 65*, 55–63.
- Pecere, T., Gazzola, M. V., Mucignat, C., Parolin, C., Vecchia, F. D., Cavaggioni, A., … Palu, G. (2000). Aloe-emodin is a new type of anticancer agent with selective activity against neuroectodermal tumors. *Cancer Research, 60*, 2800–2804.
- Qin, H. B., Zhu, M., Du, T. K., She, Q.-Y., Yang, J.-A., Zhu, X.-L., … Zhang, J.-W. (2010). Effects of aloe-emodin on proliferation and migration of human gastric cancer cell line BGC-823. *Acta Anatomica Sinica, 41*, 909–911.
- Saito, H. (1993). Purification of active substances of *Aloe arborescens* Miller and their biological and pharmacolgical activity. *Phytotheropy Research, 7*, S14–S19.
- Tabolacci, C., Oliverio, S., Lentini, A., Rossi, S., Galbiati, A., Montesano, C., … Baninati, S. (2011). Aloe-emodin as antiproliferative and differentiating agent on human U937 monoblastic leukemia cells. *Life Sciences, 89*, 812–820.
- Tsuda, H., Matsumoto, K., Ito, M., Hirono, I., Kawai, K., Beppu, H. … Nagao, M. (1993). Inhibitory effect of *Aloe arborescens* Miller var. *natalensis* Berger (Kidachi aloe) on induction of preneoplastic focal lesions in the rat liver. *Phytotherapy Research, 7*, S43–S47.
- Williamson, E. M. (2001). Synergy and other interactions in phytomedicines. *Phytomedicine, 8*, 401–409.
- Wu, R. J., Huang, Y. Q. & Ma, X. D. (2013). [Effect of phenylhexyl isothiocyanate on drug-resistance to imatinib in K562/G01 cell line]. *Zhonghua Xue Ye Xue Za Zhi, 34*, 149–152.