# Investigation of the Anti-proliferative and Apoptotic Effects of *Aloe vera* Extracts on HL60 Human Acute Myeloid Leukemia and MCF-7 Breast Cancer Cell Lines

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## Abstract

Many of the anti-cancer compounds which are currently used have an origin in natural sources including plants. *Aloe vera* is one of the plants that has been used in traditional medicine for centuries to treat a variety of diseases and cancer prevention. In this study, the cytotoxic effect of *Aloe vera* crude extract on tumor cell lines including HL60 human acute myeloid leukemia and MCF-7 breast cancer cells was studied by cell viability assay, changes in cell morphology, and apoptosis analysis. According to the results, the treated cells with *Aloe vera* extract in comparison to the untreated cells exhibited significant decline in viability in a time and dose dependent manner. MTT assay showed that $IC_{50}$ value of *Aloe vera* extract on MCF-7 cells was 0.5 mg/ml during the first 24 hours, while in this time $IC_{50}$ of extract on HL 60 cells was 1 mg/ml. Also, morphological characteristics of treated MCF-7 & HeLa cells showed typical features of cell death at the morphological level such as rounding off of cells, cell shrinkage and detachment from the substrate, thus indicating that *Aloe vera* extract induces cell death by apoptosis. Cell death mediated by through the apoptotic pathway was also confirmed by TUNEL assay. Interestingly, *Aloe vera* extract did not have any significant cytotoxicity towards normal cells. Therefore, the difference in sensitivity to the *Aloe vera* extract between MCF-7 and HL60 cancer cells and normal cells suggested that *Aloe vera* extract can be used as a chemotherapeutic drug for treatment of human acute myeloid leukemia and breast cancer.

**Keywords**: *Aloe vera* Extract, Anti-cancer, Breast Cancer, Human Acute Myeloid Leukemia, Cytotoxicity, Apoptosis

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## Introduction

Cancer as a major public health problem is responsible for 13% (7.6 million deaths) of all deaths in the world. According to the latest reports provided by the Ministry of Health and Medical Education (MOHME), cancer is the third cause of death in Iran after coronary heart disease and accidents. However, with the birth rate decline and aging population and also reducing the number of death caused by accidents, it is expected that cancer is becoming the second cause of death in Iran [1]. According to the estimates more than 90,000 of new cancer cases occur annually in Iran, and it is envisaged this number will double by the year 2020. The most common cancer among men and women are stomach cancer (20.6%) and breast cancer (28.1%), respectively, and the most common childhood cancer is leukemia [2]. Breast cancer (BC) is the most common cancer and the main cause of female mortality in the world which represents about 25% of all cancers in women. BC occurrence rate in Iranian women is 24 case per 100,000, which is lower than of high income countries [3, 4]. Breast cancer is a multi-factorial disease and epidemiological studies have shown that the hormonal factors, genetic and environmental exposures are associated with incidence of BC [5]. Leukemia is also a metastatic and malignant cancer for blood-forming organs which is resulted due to incomplete evolution and uncontrolled proliferation of white blood cells (WBCs). Leukemia is responsible for about 8% of all cancers and involves all age groups with different prevalence and incidence rate in Iran and whole the world [6]. This cancer is classified to main four categories with different clinical symptoms including acute lymphatic leukemia (ALL), chronic myeloid leukemia (CML), chronic lymphatic leukemia (CLL), and acute myeloid leukemia (AML). AML is the most common malignancy in children and consists about 30% of total adults' leukemia cases [7, 8].

Generally, cancer treatment is as a major problem and success in treating it is one of the most important medical challenges. For a long time it has been proven that natural products are a significant source of chemical compounds which provide the basis for identification of novel anti-cancer drugs [9]. Accordingly, many of the drugs which used as anti-cancer have an origin in natural sources including plants [10]. *Aloe vera* is one such plant being studied extensively for its medical therapeutic benefits such as cancer prevention. More than 75 active components with medicinal value have been identified in *Aloe vera* which includes a range of biological activities such as antimicrobial effect, immunostimulatory, wound healing, anti-inflammatory, and anti-cancer [11]. Many studies demonstrated that *Aloe vera* has remarkable preventive and therapeutic potential against various cancers. The bio-

active constituents of *Aloe vera* with chemopreventive potential such as lectin, aloin, barbaloin, aloe-emodin and aloesin have shown to possess immune-potentiating, anti-mutagenic, apoptosis-inducing, anti-proliferative, anti-metastatic, and anti-oxidant potential [12]. Therefore, due to the multifaceted approach of *Aloe vera* towards targeting cancer cells and less undesirable side effects, the compounds of this plant can be a suitable candidate for cancer treatment [13]. Based on this topic, the present study was designed to evaluate the anti-proliferative and apoptotic effects of *Aloe vera* extract on HL60 h-AML and MCF-7 breast cancer cell lines by cell viability assay, changes in cell morphology, and apoptosis analysis

## Materials and Methods
### Preparation of Aloe vera extract
The alcohol (ethanol) extract was prepared from fresh *Aloe vera* leaves. Briefly, 100 g of freshly cut leaves of *Aloe vera* was mixed with 500 ml of 70% ethanol and crushed in a blender, agitated on a shaker incubator at 4ºC for 72 hours [14]. In the following obtained solution was filtered through filter paper (Albert®, Pore 7–11, size 185 mm, England) and then lyophilized in a lyophiliser (Vacuum Gauge, USA). The yield was 1.5%. The extract was stored in tightly sealed dark containers in a freezer at −20°C for later use.

### Preparation of drug solutions
A serial dilution was made from the stock in DMEM medium (Gibco, USA) to required concentrations between 0.5-2 mg/ml for the treatment of cells.

### Cell lines
The effect of *Aloe vera* extract was investigated on MCF-7 human breast carcinoma and HL60 human acute myeloid leukemia cell lines obtained from Iranian Biological Resource Center (IBRC), Tehran, Iran. Also, L929 fibroblast cells were used as control. The cells were maintained in DMEM supplemented with 10% FBS (Gibco, USA), penicillin (100 units/ml), and incubated at 37°C in a humidified atmosphere with 5% $CO_2$.

### Cell viability assay
The cell viability and the anti-proliferative activity of *Aloe vera* extract on MCF-7, HL60, and L929 cells was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) metabolic activity assay. Briefly, the cells were plated at a density of ~$1\times10^4$ cells/well in 96-well plates in triplicates. After overnight, the culture medium was removed and replaced by 100 µl of DMEM culture medium containing 0.5-2 mg/ml concentrations of *Aloe vera* extract. Then, plates were incubated in a humidified incubator at 37°C for 24, 36 and 48 h, respectively. In the following, the culture medium in each well was removed and replaced by 100 µl of MTT (5 mg/ml in PBS) solution (Sigma, USA) and incubated for 3-4 h. MTT solution was removed and DMSO (Sigma, USA) was added to dissolve formazan crystals. The absorbance was recorded on a microplate reader at the wavelength of 570 nm (Bio-Rad Laboratories, Hercules, USA). The viability percentage was calculated as AT/AC x 100; where AT and AC are absorbance of treated and control cells, respectively [15].

### Detection of apoptotic effect of Aloe vera extract on MCF-7 and HL60 cells
#### Microscopic examination
Morphological changes in MCF-7 and HL60 cells were observed after treatment with *Aloe vera* extract at $IC_{50}$ concentrations for 24 and 48 h using a normal inverted microscope (Nikon, Japan). The untreated cells were used as negative control.

#### TUNEL assay for detection of DNA fragmentation
DNA fragmentation was detected by terminal deoxy transferase (TdT)-mediated dUTP nick- end labeling (TUNEL) with the In situ Direct DNA Fragmentation Kit (Abcam, USA) according to the manufacturer's protocol. Briefly, $1.5\times10^5$ cells (MCF-7, HL60) were cultured into chamber slides and incubated for 24 h at 37°C. Then cells were treated with *Aloe vera* extract at concentration required for 50% inhibition of cell growth ($LC_{50}$) for 24 h. After treatment, the cells were fixed with paraformaldehyde 4% (w/v) in PBS (pH 7.4) for 1 h at room temperature and rinsed twice with PBS. Subsequently, the fixed cells were incubated with blocking solution (3% $H_2O_2$ in methanol) for 10 min and rinsed with PBS. In the following, the cells were incubated in permeabilisation solution (0.1% Triton X-100 in 0.1% sodium citrate) for 2 min on ice. Then, 50 µl of reaction mixture containing nucleotide and TdT enzyme was added to the cells and incubated for 1 h at 37°C. The slides were washed three times with PBS and stained with 20 µl propidium iodide (10 µg/ml) for 5 min, and rinsed three times with PBS. Finally, the stained cells were analyzed with the fluorescence microscopy (Nikon, Japan).

### Statistical analysis
Statistical analysis was accomplished by SPSS software. The data were a representative of three independent experiments expressed as the mean ± standard deviation (SD). The significance level was determined $p < 0.05$.

## Results
### Cytotoxic effects of Aloe vera extract on MCF-7 and HL60 cells
The cytotoxic effects of *Aloe vera* extract on the growth of MCF-7 and HL 60 cells which was evaluated by MTT are shown in Figure 1a and 1b. Also, L929 cells were used as control (Figure 1c). As shown in Figure 1 and 2, the treated cells with *Aloe vera* extract in comparison to the untreated cells exhibited significant decline in viability in a time and dose dependent manner. Accordingly, in the higher concentration of the *Aloe vera* extract (2 mg/ml) and the longer time of treatment (48 h), the more significant cytotoxicity was achieved. So that after 48 hours, cells that were treated with 2 mg/ml concentration of extract about 90% of them were dead (Fig. 1 & 2). Data analysis of MTT assay showed that $IC_{50}$ (dose required for 50% inhibition) of *Aloe vera* extract on MCF-7 cells was 0.5 mg/ml during the first 24 hours, while in this time IC50 of extract on HL60 cells was 1 mg/ml (Fig. 1a & 1b). The $IC_{50}$ values strongly indicated that during the first 24 hours the Aloe vera extract has a higher cytotoxicity effect on MCF-7 cells compared to HL60. Also, we evaluated cytotoxicity effect of the *Aloe vera* extract on non-cancer

L929 fibroblast cells which compared to cancer cells, the normal cells were not influenced by extract (Figure 1c). Although a reduction in cell viability was observed during the first 24 h but over time, cell proliferation led to an increase in the number of cells.

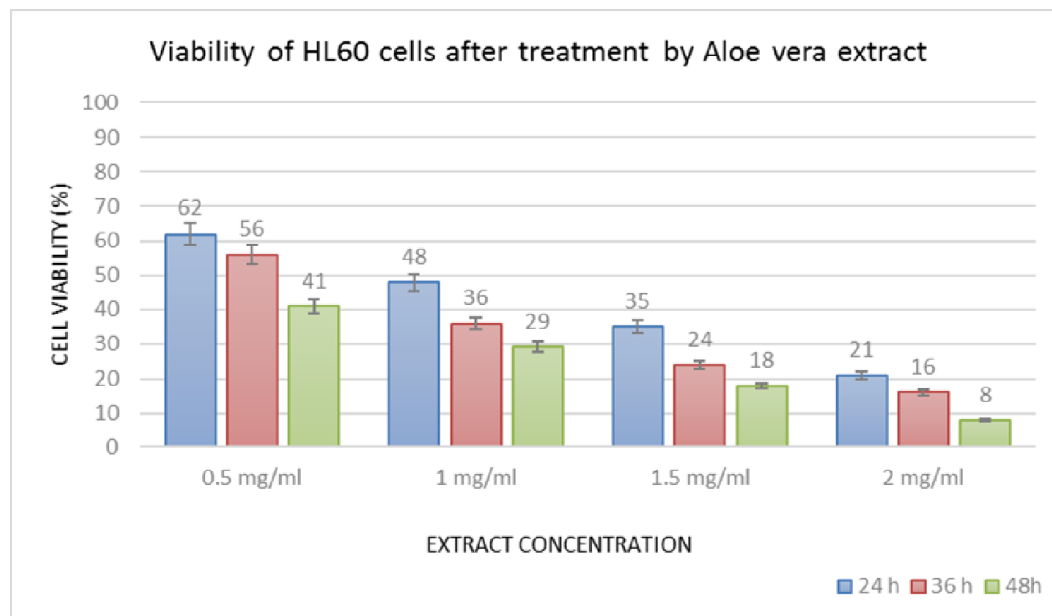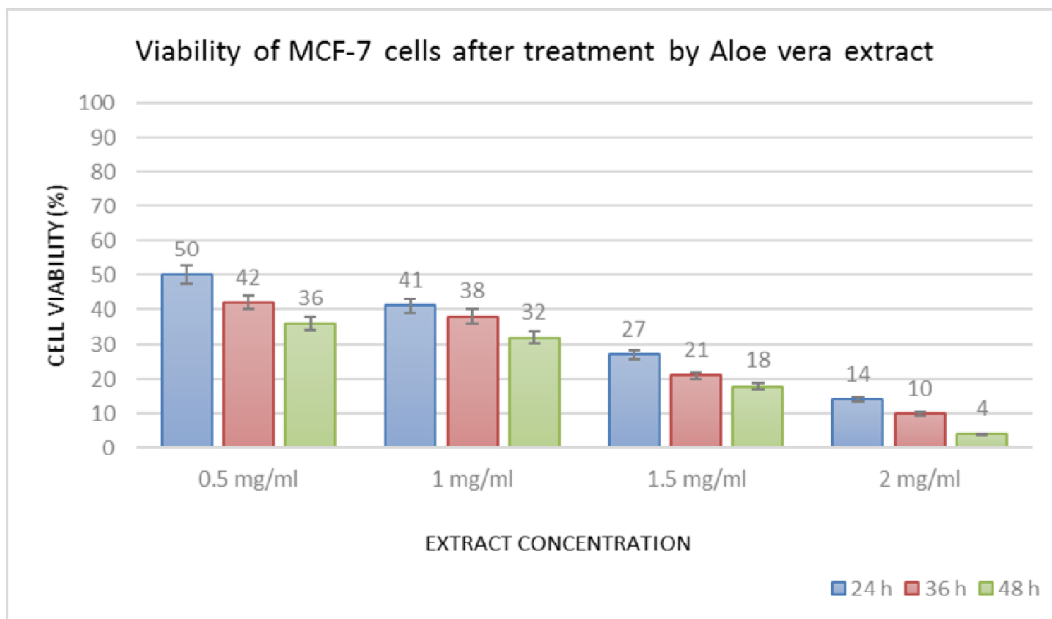### Apoptotic effect of Aloe vera extract on MCF-7 and HL60 cells

*Morphological examinations*

Morphological characteristics of treated MCF-7 & HL60 cells were visualized by an inverted microscope for 24 and 48 h, respectively. Compared to untreated cells, treated cells by *Aloe vera* extract showed typical features of cell death at the morphological level such as rounding off of cells, cell shrinkage and detachment from the substrate which was exacerbated in a dose and time-dependent manner, thus indicating that Aloe vera extract induces cell death by apoptosis (Figure 2).

*Detection of DNA fragmentation by TUNEL assay*

Since DNA fragmentation is as a marker for programmed cell death or apoptosis, in the current study, we used TUNEL assay to confirm the presence of nucleosomal DNA fragments in treated cells with *Aloe vera* extract. As shown in Figure 5, after the treatment of MCF-7 and HL60 cells with $IC_{50}$ concentration of extract which was obtained in the first 24 h, the apoptotic cells produced a green fluorescence color, whereas the nonapoptotic cells were not stained with similar observation (Figure 3).
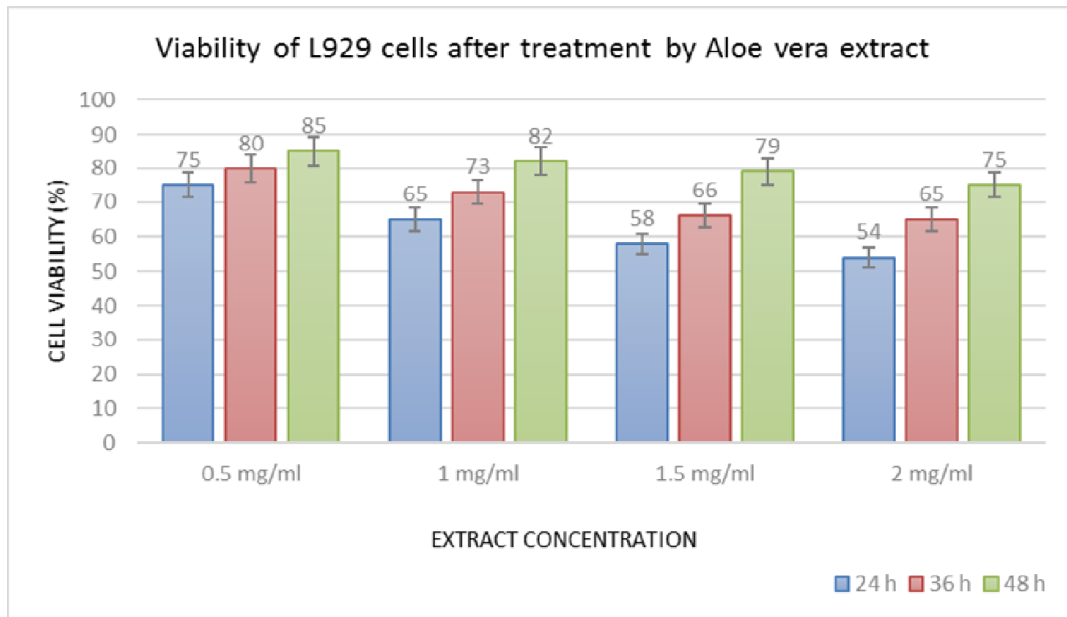
**Figure 1**. The cytotoxicity effect of the *Aloe vera* extract on MCF-7 (A) and HL60 (B) cancer cell lines and normal L929 fibroblast cells (C), as control, using MTT assay. Cells were cultured for 24, 36, and 48 h with the indicated concentrations of extract. The cytotoxicity of the *Aloe vera* extract was determined based on percentage of viable cells in culture after the scheduled times by measuring absorption of cell cultures at 540 nm. The data in each column represents three independent experiments ($p < 0.05$).
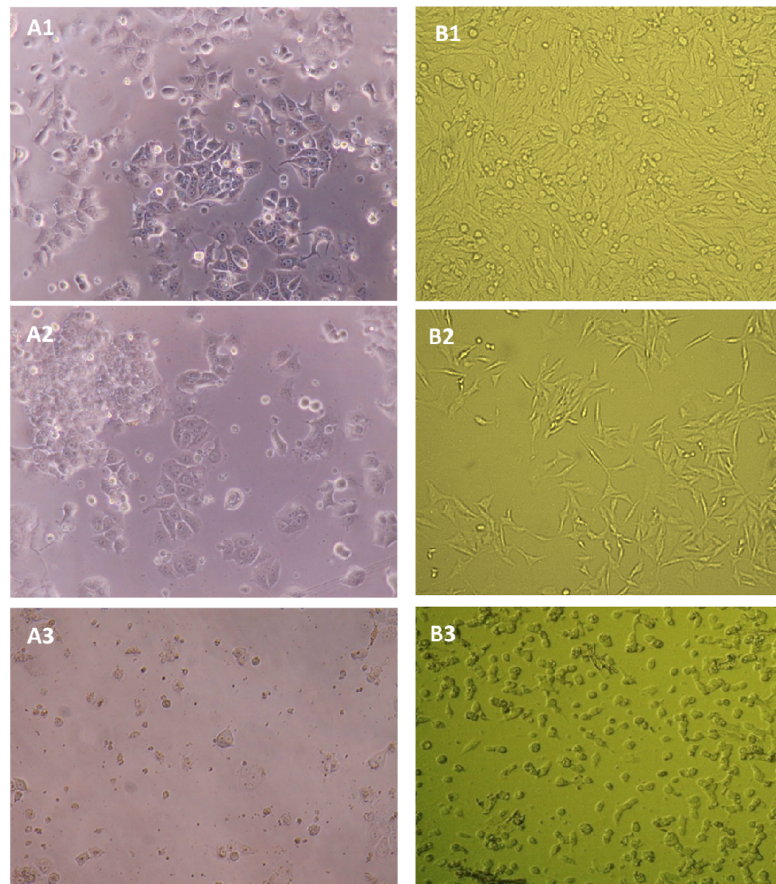


**Figure 2**. Treatment of MCF-7 (A) and HL60 cells (B) by *Aloe vera* extract at $IC_{50}$ concentrations leads to morphological changes typically associated with apoptosis. A1 and B1: untreated cells; A2 and B2: treated cells by *Aloe vera* extract after 24 h; A3 and B3: treated cells by *Aloe vera* extract after 48 h. Characteristics of rounding off and substrate detachment of treated cells compared to untreated cells are visible in a time dependent manner (Magnification 40X).
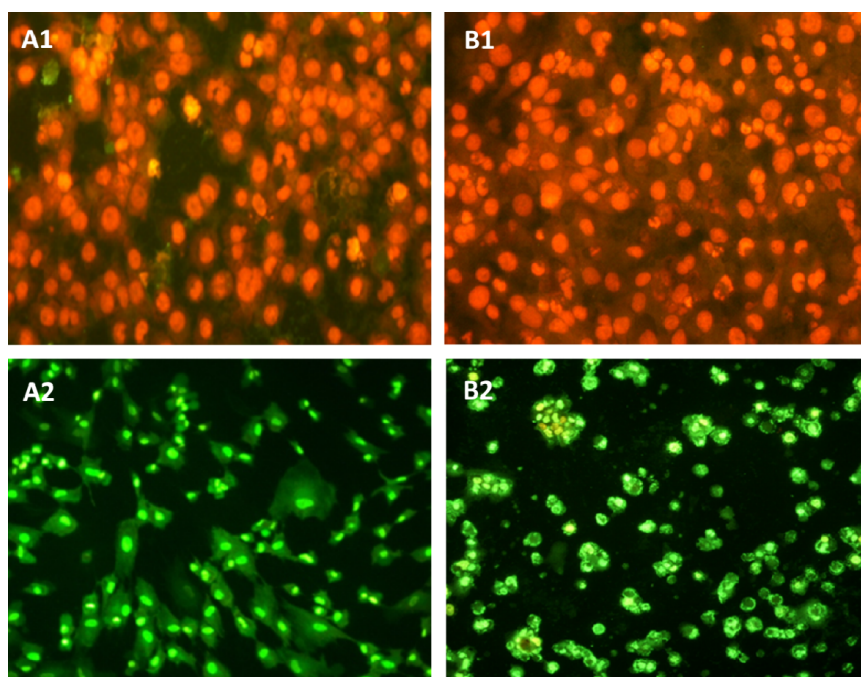
**Figure 3**. Apoptotic DNA fragmentation in MCF-7 (A) and HL60 (B) cells was evaluated with TUNEL staining after 24 h administration of the *Aloe vera* extract at $IC_{50}$ concentrations. A1 and B1: PI staining that shows DNA condensation; A2 and B2: green fluorescence staining that shows apoptotic cells (Magnification 40X).

**Discussion**

The most prominent feature of achieving anticancer drugs is the killing of cancer cells without causing excessive side effects to normal cells. This remarkable and ideal factor is achievable by apoptosis induction in cancer cells [16, 17]. Many studies demonstrated that plants are as an important source of bioactive agents that can induce apoptosis in cancer cells [18, 19]. *Aloe vera* is one of the plants that has been used in traditional medicine for centuries to treat a variety of diseases such as diabetes, asthma, and herpes. In recent years many evidence has shown that crude extracts from *Aloe vera* can act as anti-cancer alone or synergistically with chemotherapeutic drugs [20, 21]. For instant, aloe-emodin, an anthraquinone compound present in the *Aloe vera* leaves, has exhibited anticancer activity against esophageal, colon, and pancreatic cancer cells and many other types of cancers. It seems that anti-cancer activity of the aloe-emodin is by down regulating many key cancer promoting molecules without cytotoxic effects [13]. Accordingly, in the present study, anti-cancer property, the cytotoxic and apoptotic activity of *Aloe vera* extract on MCF-7 and HL60 cancer cells were evaluated.

The results showed that *Aloe vera* extract has strong anti-cancer activity and inhibited the growth of MCF-7 and HL60 cells through the induction of programmed cell death or apoptosis. The $IC_{50}$ values indicated that the *Aloe vera* extract had a more potent cytotoxic effect on MCF-7 cells compared to HL60 cells. During the first 24 h, 0.5 mg/ml of the extract leads to death about 50% of the MCF-7 cells while $IC_{50}$ concentration for HL60 cells was 1 mg/ml. Generally, our findings showed that anti-cancer activity of the *Aloe vera* extract is exacerbated in a dose and time-dependent manner.

Moreover, in comparison with cancerous cells, *Aloe vera* extract did not inhibit L929 normal cells. Although, during the first 24 h and in a dose-dependent manner, decrease in cell viability was observed but after 24 h the growth of the cells was normal.

The difference in sensitivity to the *Aloe vera* extract between MCF-7 and HL60 cancer cells and normal cells suggested that *Aloe vera* extract can be used as a chemotherapeutic drug for treatment of human acute myeloid leukemia and breast cancer. On the other hand, in cancer therapy killing cancerous cells through apoptosis is as ideal cell death. In this regard, morphological characteristics of treated cells and analysis of DNA fragmentation by TUNEL assay revealed that *Aloe vera* extract induced the apoptosis in a dose dependent manner. Therefore, current results show the potential of the *Aloe vera* extract as a basic anti-cancer agent to inhibit tumor cell growth and trigger apoptosis.

**Conclusion**

Finally, our results demonstrated the in vitro cytotoxic and apoptotic activity of the *Aloe vera* extract against breast carcinoma and acute myeloid leukemia cells, thus possibly suggesting a new potential anti-cancer agent for the treatment of these cancers and are not deleterious towards non-cancerous cells.

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