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ORIGINAL ARTICLE

Aloe vera-induced apoptotic cell death through ROS generation, cell cycle arrest, and DNA damage in human breast cancer cells

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Abstract

This study aimed to evaluate the in vitro cytotoxicity of ethanolic extract of *Aloe vera* (AVE) against human cancer cell lines. Cytotoxic effect of AVE was evaluated by MTT and NRU assays in human breast (MCF-7) and lung (A-549) cancer cell lines. AVE-induced morphological changes were also visualized under phase contrast microscope. Further, intracellular reactive oxygen species (ROS) and mitochondrial membrane potential (MMP) levels were detected using DCF-DA and Rh-123 stains, respectively under a fluorescence microscope. Cell cycle progression was measured by flow cytometric analysis. DNA damage was detected by single cell gel electrophoresis (comet assay). The profile of genes related to apoptosis (p53, bax, bcl-2, caspase-3 and -9) was assessed by quantitative real time PCR. AVE exhibited significant cytotoxicity in a dose dependent manner to both MCF-7 and A-549 cells with an IC_{50} values of 195 µg/mL and 298 µg/mL, respectively. Moreover, AVE induces overproduction of ROS and decreases MMP level in MCF-7 cells. Flow cytometric analysis confirmed that AVE-induced SubG1 cell cycle arrest. The increased p53, bax, capsase-3 and -9 gene expression levels and decreased bcl-2 gene expression level positively correlated AVE-induced MCF-7 cell apoptosis. In conclusion, this study provides mechanistic details of anticancer potential of *A. vera.* This study also proved that AVE could be a promising anticancer agent in preventing and treating cancer diseases.

Keywords *Aloe vera* · Cytotoxicity · ROS generation · Cell cycle · DNA damage · Gene expression

Abbreviations

MCF-7	Human breast cancer cell line
A-549	Human lung cancer cell line
AVE	Ethanolic extract of *A. vera*
ROS	Reactive oxygen species
DCF-DA	2′,7′-dichrolofluorescin diacetate
Rh-123	Rhodamine-123
DNA	Deoxyribonucleic acid
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
MMP	Mitochondrial membrane potential
PBS	Phosphate buffer saline
$CaCl_2$	Calcium chloride
DMEM	Dulbecco's modified eagle's medium
RNA	Ribonucleic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2–5-diphenyltetrazolium bromide)
NRU	Neutral red uptake
DMSO	Dimethyl sulfoxide
FBS	Fetal bovine serum
LMA	Low melting agarose
NMA	Normal melting agarose
BC	Breast cancer

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Introduction

Nearly all types of cells can pledge cancer cell growth. Breast cancer (BC) is accounting for about 411,000 death per year, which is around 14% of all cancer associated death in female (Parkin et al. 2005). In the developing countries, breast cancer

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is extremely dominant and the number of cancer cases are expected to increase in coming years because of environmental contamination, tobacco use, occupational exposure, life style changes, and dietary factors (Momenimovahed and Salehiniya 2019). The risk of emerging BC cases in each woman is 12.8% (one in eight) in United States (Okano et al. 2021). Compared to developed countries, the moderately deprived survival of BC in developing countries has been observed due to inadequate screening, diagnosis, and fewer handiness of suitable treatment (Da Costa Vieira et al. 2017). Although cancer treatment and diagnosis has made countless steps in relation to mortality; still there is deficiency of effective treatment. Many studies have been focused on the development of chemotherapeutic agent to combat against cancer diseases (Chikara et al. 2018; Pucci et al. 2019). Inappropriately, compared to herbal medicines, the conventional chemical drug causes adverse side effects on normal cells/tissues (Buranrat et al. 2020). Plants are a rich source for new drug development against cancer diseases. The anticancer agents from plants have been proven to possess antiproliferative and proapoptotic properties with less side effects (Ahmad et al. 2020). In spite of progression in drug development, there is a still need to search for new drugs derived from plants. The name *Aloe L.* derived from "Alloeh" is an Arabic word and its meaning is "shining bitter substance" (Surjushe et al. 2008). The Aloe species are inhabitant of arid climates and are disseminated in Africa, India, and other dry areas. There are around 500 known Aloe species and the majority of them are originated in South Africa (Klopper and Smith 2007). They play essential part in the management of various ailments by moderating biological pathways (Rahmani et al. 2015). The Aloe plants are also known for various pharmacological and biological actions such as antioxidants (López et al. 2013), antibacterial, anti-inflammatory (Ndhlala et al. 2009), anti-fungal, antitumor, anti-arthritis (Cock 2015), anti-rheumatoid (Yagi 2015), antimutagenic (Lee et al. 2000a), antimicrobial (Dharajiya et al. 2017), antidiabetic (Kumar et al. 2011), and antiulcer (Reddy et al. 2016). Aloe plants are also reported for constipation cure (Wintola et al. 2010), reddening out toxins and waste from the body, gastrointestinal disorders (Qin et al. 2010), immune system deficiencies (Radha and Laxmipriya 2015), and detoxification (Koo et al. 2019). The potential anticancer activities of these plants have been associated with various bioactive compounds (Grimaudo et al. 1997; Kim et al. 1999; Lee et al. 2000b; Pecere et al. 2000; Kabbas et al. 2008; Pan et al. 2013). *A. vera* is an effective plant with so many health applications (Lanka, 2018). It is known to possess many therapeutic properties such as antioxidant, anti-inflammatory, antimicrobial, antiviral, anticancer, and would healing (Gao et al. 2019; Fatima et al. 2022). *A. vera* is also used to prevent many ailments for example diabetes, asthma, stress, heartburn, kidney stones, rheumatism, arthritis, AIDS, skin burn, pain, cancer, and digestion related problems (Pathak and Sharma 2017). Even though *A. vera* is reported as an important and resourceful medicinal plant, but cancer cell death mechanism(s) induced by AVE has not been described yet in details. The present investigation aimed to study cytotoxic, genotoxic, and apoptotic potential of ethanolic extract of *A. vera* on human breast carcinoma (MCF-7).

Materials and methods

Chemicals and reagents

Dulbecco's modified eagles' medium (DMEM), MTT, neutral red, dimethyl sulfoxide (DMSO), low melting (LMA) and normal melting (NMA) agarose, ethidium bromide, PBS, ethanol, $CaCl_2$, formaldehyde, rhodamine-123 (Rh-123), and 2′,7′-dichloroflourescin diacetate (DCFDA) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Fetal bovine serum (FBS) and streptomycin and penicillin were purchased from Gibco Thermo Fisher Scientific, USA. Culture flasks, multi-well plates, and other consumables were purchased from Nunc, Denmark.

Preparation of plant extract

A. vera plant was collected from a wild area of Riyadh, KSA during November month. The dried samples were made into powder form by fine grinding. The plant material (500 g) was then soaked in 2 L of hydroalcoholic solvent (95% ethanol: 5% water) at room temperature for 2 days. The solvent was then filtered through Whatman filter paper#1. The filtrate was dried using a rotary evaporator at 40 °C. The obtained concentrated yield of extract was named as AVE and used for further studies.

Cell viability assay (MTT)

AVE-induced cytotoxicity was assessed in MCF-7 and A-549 cell lines by MTT assay as defined in previous studies (Siddiqui et al. 2008). The cells were distributed in a flat bottom 96-well microtiter plate at a density of 1×10^4 cells/well. After overnight incubation, cells were exposed to various concentrations (1–200 µg/mL) of AVE followed by 24 h incubation. Then, 10 µL MTT solution (5 mg/mL) was added into wells four hours prior to end of incubation. The resulting formazan crystals were dissolved in DMSO.

Cell viability assay (NRU)

AVE-induced cytotoxicity was also measured by NRU assay (Siddiqui et al. 2010). The cells were distributed in a flat bottom 96-well microtiter plate at a density of 1×10^4 cells/well. After overnight incubation, cells were exposed to various concentrations (1–200 µg/mL) of AVE followed by 24 h incubation. After removal of medium, 100 µL NR solution

(50 µg/mL) was added to each well. The plate was then incubated for 3 h. After washing the wells, the dye was extracted by the solution (50% ethanol, 1% acetic acid, 49% water).

Morphological observation

AVE-induced morphological assessment was done in MCF-7 and A-549 cell lines. Before exposure to AVE (1–200 µg/mL), cells were grown in a 6-well culture plate at 1×10^5 cells/well. After 24 h exposure, cells were analyzed under an inverted microscope.

Determination of ROS

ROS was determined using DCF-DA dye. In brief, MCF-7 cells (2×10^4 cells/well) were distributed in a 24 well plate and grown for overnight. Next, cells were treated with 50–200 µg/mL of AVE for 24 h. The cells were then washed and incubated with DCF-DA for one hour in dark. The DCF fluorescence intensity was analyzed by fluorescence microplate reader and parallel set of cells were visualized under fluorescence microscope for qualitative analysis.

Mitochondrial membrane potential analysis

Membrane potential is an important parameter of mitochondrial function and was measured using Rh-123 dye. In brief, MCF-7 cells were grown in a 24 well plate. After exposure to AVE (50, 100, and 200 µg/mL), cells were washed and incubated with Rh-123 dye for one hour at 37 °C. The fluorescence was measured using fluorescence microscope and fluorescence images was grabbed under fluorescence microscope.

Cell cycle

Briefly, MCF-7 cells (2×10^4 cells) were distributed in 24 well plate and exposed to AVE (50–200 µg/mL). Untreated control set of cells were also run parallel. After incubation for 24 h, cells were harvested and washed. Cells were centrifuged and fixed in ethanol (70%) for 60 min. Later, cells were washed, centrifuged, and stained in 500 µL of ethidium bromide for 30 min. The cell cycle distribution was determined by acquiring 10,000 cells each time using flow cytometer.

Genotoxicity by comet assay

The genotoxicity of AVE was assessed by comet assay. The assay was achieved under alkaline condition, as earlier described method (Saquib et al. 2009). AVE-treated cells were collected and suspended in 1% LMA, layered on precoated slides with 1% NMA, lysed at 4 °C for 1 h. The electrophoresis was done for 30 min at 0.7 V/cm (300 mA). The slides were neutralized and stained with 20 µg/mL ethidium bromide. The comet slides were observed at 40x magnification using a fluorescence microscope connected with CCD camera.

Real-time PCR analysis

According to the RNAeasy mini kit protocol from Qiagen, total RNA from exposed and unexposed cells was isolated. Then, 1 µg RNA was transcribed into cDNA using MLV reverse transcriptase kit (GE Healthcare, UK). The detail protocol and sequences of specific sets of primers used has been described in our earlier publication (Al-Oqail et al. 2017). Real-Time quantitative PCR analysis was performed in 96-well plate on Light Cycler 480® instrument (Roche Diagnostics, Switzerland) using 2x SYBR Green I from Roche Diagnostics.

Statistical analysis

One-way analysis of variation (ANOVA) was used to analyze the significant difference between control and treated group. Analyses were performed with GraphPad Prism software version 5.0. p values of less than 0.05 were considered significant.

Results

Cytotoxicity assessment

The cytotoxic activity of AVE against both human cancer cell lines was investigated using MTT and NRU assays. These cell viability endpoints revealed that AVE decreased cell viability of MCF-7 and A-549 cells in a dose dependent way after 24 h of exposure. As observed by MTT assay and given in Fig. 1, AVE-treatment at 25, 50, 100, and 200 µg/mL decreased viability of MCF-7 cells by up to 12%, 27%, 38%, and 52%, respectively (Fig. 1a). However, the viability of A-549 cells was decreased by up to 8%, 14%, 19%, and 34% at 25, 50, 100, and 200 µg/mL of AVE, respectively (Fig. 1b). The results of NRU assay are given in Fig. 2. As shown in figure, the decrease of MCF-7 cell viability was recorded as 10%, 24%, 36%, and 49% at 25, 50, 100, and 200 µg/mL, respectively (Fig. 2a), whereas the reduction in the viability of A-549 cells was recorded as 6%, 10%, 17%, and 30% at 25, 50, 100, and 200 µg/mL of AVE, respectively (Fig. 2b). The lower concentrations (1, 5, and 10 µg/mL) of AVE did not produce any adverse effect on the viability of MCF-7 and A-549 cells. AVE showed better cytotoxic activity on MCF-7 and lesser cytotoxic activity on A-549 cells. Therefore, further

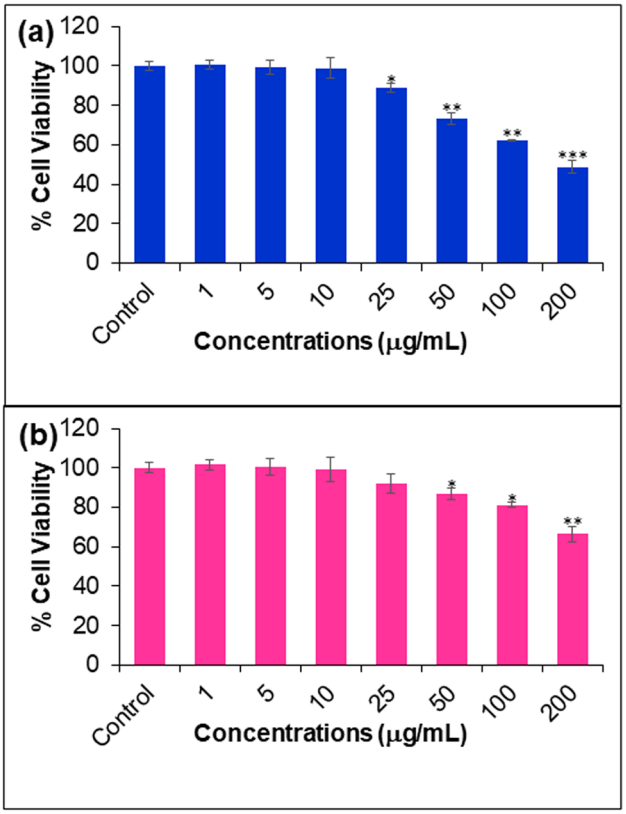

Fig. 1 Cytotoxic potential of *Aloe vera* extract (AVE) against human cell lines as measured by MTT assay. Both cell lines were exposed to different concentrations (1–200 µg/mL) of *A. vera* extract for 24. (**a**) Human breast cancer (MCF-7) and (**b**) Human lung adenocarcinoma (A-549) cells. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ vs control set

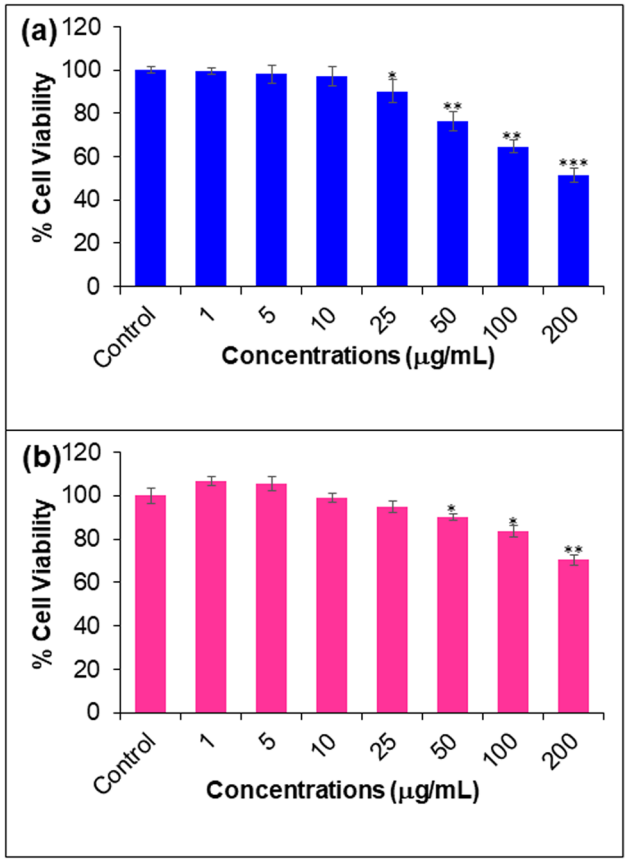

Fig. 2 Cytotoxic potential of *Aloe vera* extract (AVE) against human cell lines as measured by NRU assay (**a**) Human breast cancer (MCF-7) and (**b**) Human lung adenocarcinoma (A-549) cells. Both cell lines were exposed to different concentrations (1–200 µg/mL) of *A. vera* extract for 24 h. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ vs control set

mechanistic studies were conducted on MCF-7 cells using the cytotoxic concentrations (50–200 µg/mL) of AVE.

Morphological observation

Morphological changes of MCF-7 and A-549 cells after AVE exposure was assessed using light microcopy. As depicted in Fig. 3, in control cells, high density of monolayer cells with undamaged membrane was observed. Nevertheless, the cells showed morphological alterations such as decrease in cell volume upon the treatment of AVE. After 24 h treatment, features of apoptotic cells were clearly observed, where cells exhibited the creation of shrinking, membrane blebbing, and rounded cell bodies. The cells depicted the most prominent effects after the exposure to AVE. The microscopic observations showed that at higher concentrations (100 and 200 µg/mL), MCF-7 and A-549 cells become shrunken, rounded, and detached from the surface.

Effect of AVE on ROS production

ROS is involved under both pathological and physiological conditions. Therefore, elevated level of ROS was examined in AVE treated MCF-7 cells. The results showed that the intensity of green fluorescence in MCF-7 cells exposed to AVE at 50, 100, and 200 µg/mL was found to be significantly higher as compared to untreated control (Fig. 4a). The quantitative analysis also showed that ROS level in AVE treated MCF-7 cells was increased as compared to control ($p<0.01$). The level of ROS in MCF-7 cells were recorded as 131%, 175%, and 220% at 50, 100, and 200 µg/mL of AVE, respectively (Fig. 4b). The results showed that AVE treatment, dose dependently, increased ROS generation in MCF-7 cells.

Effect of AVE on MMP level

Rh-123 fluorescence dye was used to determine the mitochondrial membrane permeability in MCF-7 cells exposed to AVE. As shown in Fig. 5, AVE-treatment decreased the intensity of red fluorescence in MCF-7 cells. As observed by fluorescence microcopy, compared to control, AVE treatment dose dependently decreased the intensity of Rh-123 fluorescence in MCF-7 cells (Fig. 5a). After 24 h exposure,

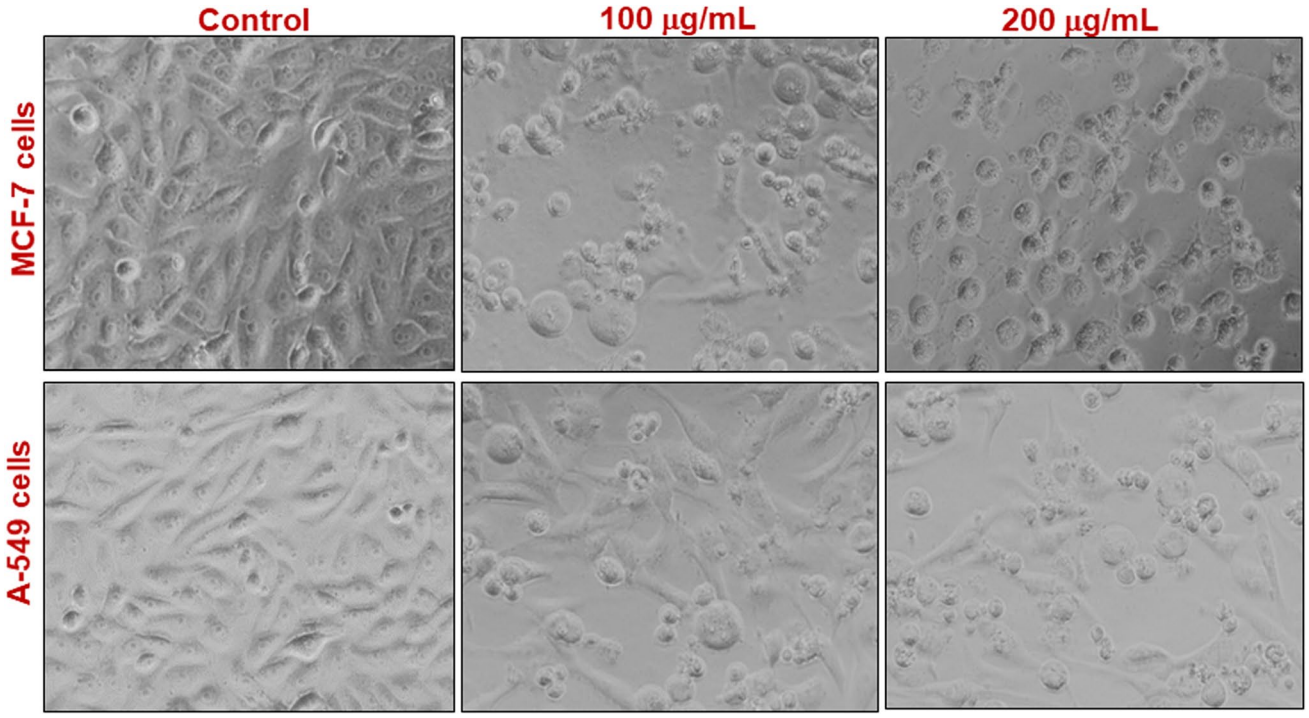

Fig. 3 Morphological changes of MCF-7 and A-549 cells after treatment with *Aloe vera* extract (AVE) by light microscopy technique

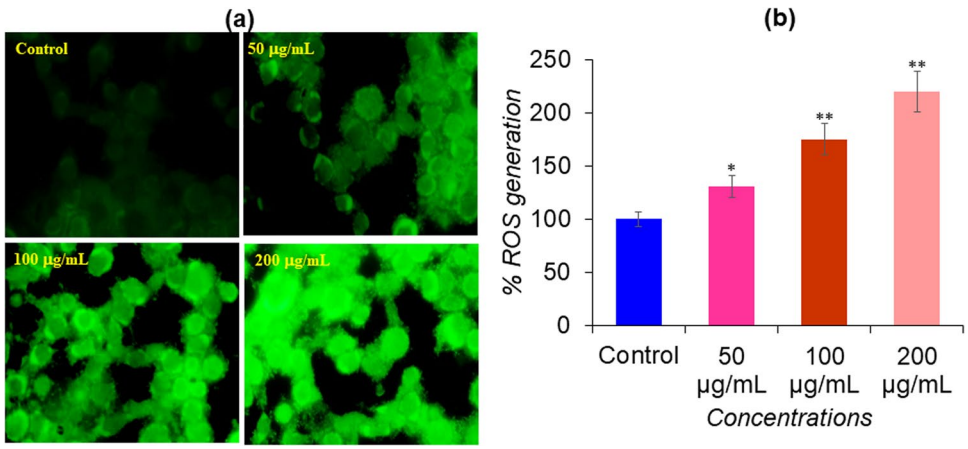

Fig. 4 AVE-induced intracellular ROS generation in MCF-7 cells. (**a**) The fluorescence images exhibiting ROS production in control and treated cells and (**b**) Percentage of ROS at 50, 100, and 200 µg/mL of AVE. *$p < 0.05$ and **$p < 0.01$ vs control

compared to control, AVE decreased the fluorescence of MMP by up to 21%, 39%, and 55% at 50, 100, and 200 µg/mL, respectively ($p < 0.01$) (Fig. 5b).

Effect of AVE on cell cycle progression

MCF-7 cells were treated with 50, 100, and 200 µg/mL of AVE to examine the effects of AVE on cell cycle progression by flow cytometry. The cell cycle phase distribution of MCF-7 cells treated with AVE at 50–200 µg/mL is depicted in Fig. 6. The cell cycle arrest induced by AVE was concentration dependent. After 24 h exposure of AVE, an increase in cell population of SubG1 phase was noted. The cell population in SubG1 phase was recorded as 15.3%, 29.3%, and 43.2% at 50, 100, and 200 µg/mL of AVE, as compared to 1.7% in control.

Effect of AVE on DNA

To confirm the induction of cell death, MCF-7 cells were exposed to various concentrations of AVE and DNA damage was assessed by comet assay. Figure 7, represents percentage of damaged nuclei in arbitrary units as measured by comet assay in MCF-7 cells exposed to AVE. As shown in comet images, it was found that AVE-induced significant upsurge in mean values of comet tail length at 50–200 µg/mL of AVE. After 24 h exposure, the percentage of tail length values were 13.9%, 19.5%, and

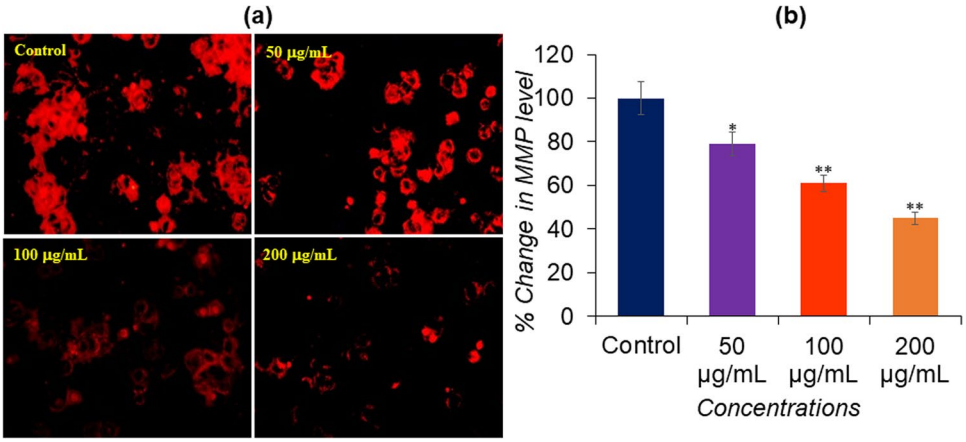

Fig. 5 Mitochondrial membrane potential analysis after the exposure of AVE at 50–200 µg/mL (**A**) The fluorescence images showing the intensity of Rh-123 dye in control and treated cells. (**B**) Percentage loss of MMP at 50, 100, and 200 µg/mL of AVE. *$p < 0.05$ and **$p < 0.01$ vs control

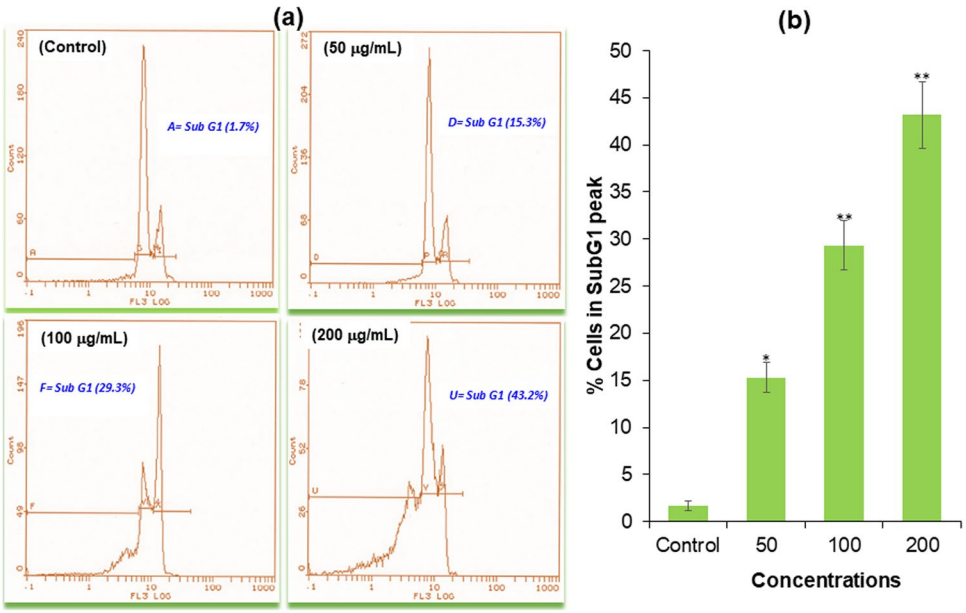

Fig. 6 Flow cytometric evaluation of cell cycle arrest post AVE treatment. (**a**) AVE caused an increase in SubG1 phase of cell cycle in dose dependent way. (**b**) Graph showing the percentage of cells in SubG1 phase. *$p < 0.05$ and **$p < 0.01$ vs control

27.8% at 50, 100, and 200 µg/mL of AVE, respectively compared to control (0.41%), hence providing a clear evidence that AVE causes DNA damage (Fig. 7b).

Effect of AVE on the expression of apoptotic marker genes

Real-Time qPCR was employed to evaluate the effect of AVE on the expression of the apoptotic marker genes (p53, bax, bcl-2, caspase-3 and -9). As shown in Fig. 8, AVE-treatment at 200 µg/mL significantly increased p53, bax, capsase-3 and -9 gene expression levels and decreased bcl-2 gene expression level in MCF-7 cells ($p < 0.05$). The results showed that p53 gene expression level was increased by up to 3.5-fold in MCF-7 cells exposed to AVE at 200 µg/mL. Moreover, caspase-3 and -9 genes were increased by up to 2.7- and 3.2-fold, respectively. However, expression level of bax gene was increased by up to 3.1-fold and bcl-2 gene was decreased by up to 1.4-fold as compared to control.

Discussion

Cancer leftovers one of the foremost causes of mortality and is speedily increasing globally (Sung et al. 2021). The developing trends indicate that cancer cases will increase due to the prediction of increasing population and occurrence in the number of new cases (Hortobagyi

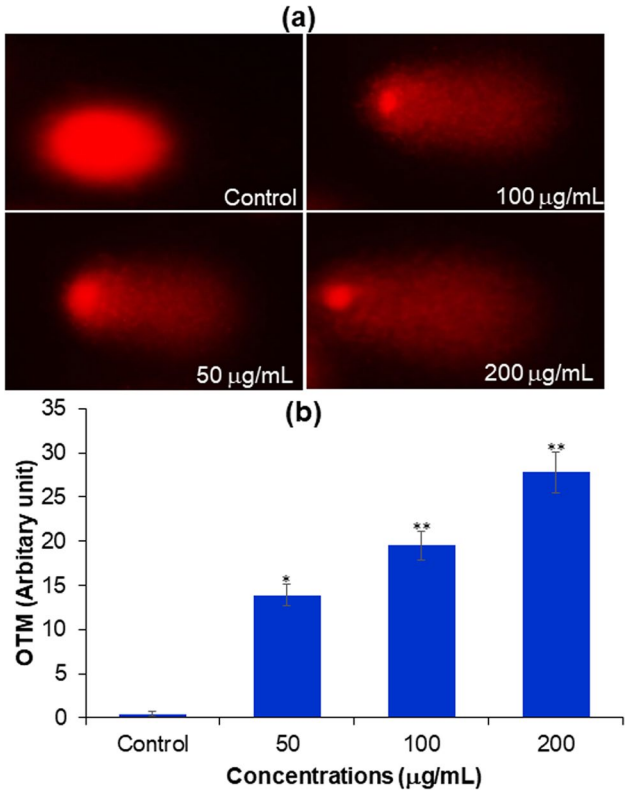

Fig. 7 AVE-induced DNA damage in MCF-7 cells. (**a**) Representative comet images exhibiting tail length in control and treated cells. (**b**) DNA damage in MCF-7 cells presented as % tail length measured by alkaline comet assay. *$p < 0.01$ and **$p < 0.001$ vs control

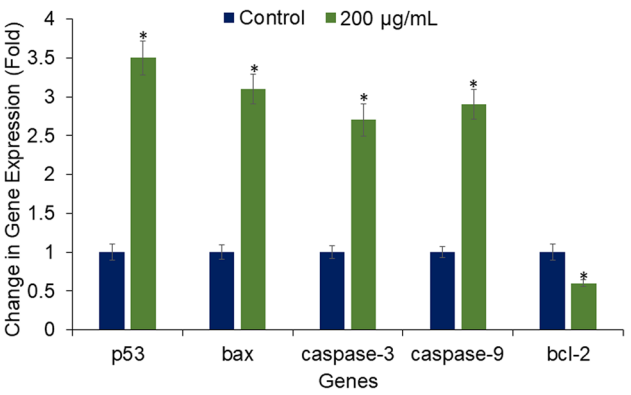

Fig. 8 Effect of AVE exposure on apoptotic marker genes (p53, bax, caspase-3, caspase-9 and bcl-2) analyzed by quantitative real time PCR. *$p < 0.01$ vs control

et al. 2005). Thus, searching the extracts from bioactive plants with their identified beneficial and therapeutic properties, are an important area of study for the management of cancer. A number of plant extracts have shown pharmacological potential against chronic diseases like cancer (Ahmad et al. 2020; Wang et al. 2019). *A. vera,* an oldest medicinal plant has been found effective against many diseases including cancer (Maan et al. 2018). Due to its many therapeutic properties (Gao et al. 2019), *A. vera* has expanded noteworthy consideration for searching the new chemotherapeutic agent. The cytotoxic potential of *A. vera* continues to be the interest of research from last many decade until now (Majumder et al. 2022). Studies showed that *A. vera* leaf extract is effective against various cancer cells, such as colorectal and melanoma (Laux et al. 2022; Tong et al. 2022). Consequently, this study aimed to evaluate the in vitro cytotoxicity of ethanolic extract of *A. vera* (AVE) against human cancer cell lines. The cytotoxic potential of AVE was assessed by MTT and NRU assays. These cytotoxicity assays with cultured cells are widely accepted methods for the assessment of cytotoxic potential of plant extracts because they are rapid, sensitive, simple, reliable, and inexpensive (Farshori 2021). MTT assay is based on the conversion of MTT into formazan crystal by mitochondria of viable cells, while NRU assay is used to monitor lysosomal integrity of viable cells (Al-Oqail 2021). The results showed that 24 h exposure of AVE induces a dose dependent cytotoxicity on both MCF-7 and A-549 cells. AVE showed strong cytotoxic activity on MCF-7 cells with the IC_{50} value of 195 µg/mL and comparatively lesser cytotoxic activity on A-549 cells with the IC_{50} value of 298 µg/mL. Therefore, further mechanistic studies were conducted on MCF-7 cells using the cytotoxic concentrations (50–200 µg/mL) of AVE. Previous results also illustrated the cytotoxic potential of *A. vera* extract against B16F10 melanoma cells in this concentration range (Chandu et al. 2012). The cytotoxic potential of methanolic leaf extract of *A. vera* in MCF-7 has also been defined with IC_{50} value of 74.33 µg/mL (Srihari et al. 2015). These findings were in agreement with the earlier reports that *A. vera* extracts or its components significantly inhibited the growth of human breast and cervical cancer cells in vitro (Hussain et al. 2015). *A. vera* was also reported to inhibit tumor growth (Tomasin and Cintra Gomes-Marcondes 2011). In addition to *A. vera,* other aloe genus such as *Aloe arborescens* have also been documented to be effective against cancer disease (Singab et al., 2015). Other studies also suggest that leaf extract of *A. vera* significantly induced cytotoxicity against human breast cancer cells (MCF-7) as compared to normal peripheral blood mononuclear cell (PBMC) (Basak et al. 2017). The therapeutic effects of bioactive compounds of *A. vera* have also been reported to be involved in cancer prevention and treatment (Majumder et al. 2019).

It is well known that oxidative stress produced by ROS, plays a key role in cancer cell death (Maqbool et al. 2020). Our results showed that AVE treatment dose dependently increased ROS generation in MCF-7 cells, as compared to control ($p < 0.01$). Many studies also confirm that plant

extracts induce ROS generation leads to cancer cell death (Vallejo et al. 2017; Ramalingam and Rajaram 2018). Aloe-emodin, isolated from *A. vera* extract has been demonstrated to induce anticancer effect against human squamous carcinoma cells through ROS production (Chiu et al. 2009). A number of other chemopreventive molecules such as doxorubicin, bleomycin, and platinum complexes are known to induce cellular ROS generation (Marullo et al. 2013). ROS is also involved in the mitochondrial depolarization and apoptosis progression (Redza-Dutordoir and Averill-Bates 2016). The loss of mitochondrial membrane potential is an early stage of apoptosis (Ly et al. 2003). Thus, the assessment of MMP depolarization is of importance for the assessment of apoptosis (Cui et al. 2007). Our results confirmed that AVE-treatment caused significant decline of MMP, interfering that AVE enhanced MCF-7 cell death along with loss of MMP. Other studies also revealed that plant extracts could induce cytotoxicity through mitochondrial depolarization (Al-Oqail et al. 2021). The increased level of ROS has been stated to cause DNA damage (Nita and Grzybowski 2016). Herein, we found that MCF-7 cells treated with AVE showed significant DNA damage in a dose dependent way as shown by comet assay. These findings confirmed that DNA damage caused by AVE could be because of ROS generation in MCF-7 cells. It was also reported that Aloe plants had genotoxic effects in mammalian test systems. The anticancer potential of *A. vera* extract have been demonstrated against hepatocellular carcinoma through DNA damage and gene expression (Shalabi et al. 2015).

We have further confirmed that AVE-treatment causes cell cycle arrest and tempted apoptosis in MCF-7 cells. The morphological observation documented that MCF-7 cells after 24 h exposure with AVE showed cellular shrinkage and rounded bodies, suggesting the initiation of apoptosis. This growth inhibitory effect may be due to the cell cycle arrest at SubG1 phase as confirmed by flow cytometric study (Fig. 6). AVE-treatment at 50, 100, and 200 µg/mL arrested the cells at SubG1 phase, suggesting that AVE may influence the cell cycle progression. Despite the fact that AVE inhibited cell growth, its blocked cell proliferation by cell cycle arrest at SubG1 phase in MCF-7 cells. Earlier, Hussain et al. (2015), reported that *A. vera* extract can inhibit MCF-7 and HeLa cancer cells proliferation via SubG1 arrest. It is reported that Aloe derived phytochemicals blocked cell cycle development in cancer cell lines (Du et al. 2019; Svitina et al. 2021). Similarly, Zhang et al. (2017) has reported that Aloesin from *A. vera* arrested cell cycle, inhibited the growth of cancer SKOV3 cells, and induced apoptosis. Apoptosis is supposed to be the reason of cell cycle arrest in subG1 phase. Therefore, to assess the mechanism, the expression profile of apoptosis related genes (p53, bax, bcl-2, caspase-3 and caspase-9) were studied by quantitative real time PCR. One characteristic properties of *A. vera* is its capacity to induce mitochondrial pathway of apoptosis in a number of cancer cells (Kumar et al. 2019). The mitochondrial apoptosis pathway is based on the activity of bcl-2 family and depends on interaction between anti- and pro-apoptotic genes. Our results indicate that treatment of MCF-7 cells with AVE results in changes in the expression of apoptosis related genes. Treatment of MCF-7 cells in the presence of 200 µg/mL of AVE for 24 h increased the expression level of proapoptotic genes (p53, bax, capsase-3 and -9), while the expression level of antiapoptotic gene bcl-2 was downregulated post 24 h exposure. These findings showed that MCF-7 cell apoptosis is connected with p53 and caspase activation. The results also suggest that caspase dependent apoptosis is linked with inflection of bax/bcl-2. Further, p53 interact with bcl-2 family and induces mitochondrial outer membrane depolarization and triggers the mitochondrial pathway of apoptosis. Previously, Shalabi et al. (2015) has shown that *A. vera* extract treatment upregulated p53 gene and downregulated bcl-2 in HepG2 cell death. Similarly, Jiang et al. (2020) described that Aloe-emodin, a major component of *A. vera* induces breast cancer cell death through mitochondria dependent and downregulating bcl-2 expression. Thus, the findings of present investigation established that AVE induces MCF-7 cell death via originating the apoptosis progression.

Conclusion

The present study suggests that *A. vera* extract has cytotoxic potential and inhibit the proliferation of cancer cells. Further, our results showed that AVE induced ROS production and mitochondrial membrane dysfunction in MCF-7 cells. We found that AVE inhibits MCF-7 cell growth, leading to DNA damage, and subsequently cell cycle arrest at SubG1 phase. Concurrently, AVE-induced cell apoptosis mediated through upregulation of p53, bax, capsase-3 and -9 gene expression levels and downregulation of bcl-2 gene. This study provides mechanistic details of anticancer potential of *A. vera.* This in vitro study proves that AVE could be a useful therapeutic agent for the management of cancer disease. Further studies are therefore needed on the molecular mechanisms of anticancer potential of AVE under in vivo condition.

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Author contributions Nida N. Farshori and Maqsood A. Siddiqui conceptualized, designed the experiments, and drafted the manuscript. Mai M. Al-Oqail and Ebtesam S. Al-Sheddi helped in experiments and analyzed the data. Shaza M. Al-Massarani revised and edited the manuscript. Quaiser Saquib and Javed Ahmad performed flow cytometry

and real time PCR experiments. Abdulaziz A. Al-Khedhairy provided resources.

Data availability Data and materials of this study are available on request.

Declarations

Ethical approval This paper does not contain any studies with human or animals.

Consent to participate Not Applicable.

Consent to publish The author approves processing of this manuscript for publication.

Conflict of interest The author declares that there is no conflict of interest.

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