Original Research Article

Anticancer effect of the fruit and seed extracts of *Momordica charantia* L. (Cucurbitaceae) on human cancer cell lines

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Abstract

Purpose: To investigate anticancer effects of Momordica charantia L. (M. charantia) fruit and seed extracts on some cancer cell lines.

Methods: Human cancer cell lines, including lung cancer (A549), breast cancer (MCF-7), chronic myeloid leukemia (K562) and T cell leukemia (Jurkat) were incubated with the extracts (0 - 0.8 mg/mL) for 72 h. The cytotoxic effects of the extracts were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-dipenyltetrazolium bromide (MTT) assay. A549 and MCF-7 cells were treated with the ethanol fruit extract (FE) for 24 h and stained with propidium iodide (PI) for the analysis of cell cycle arrest using flow cytometry. Annexin V-FITC/PI staining along with flow cytometry analysis and caspase-3 assays were carried out to determine the apoptosis of the cells treated with FE extract for 24 h. Vascular endothelial growth factor (VEGF) secretion of the cells exposed to FE extract for 1 h was determined using enzyme-linked immunosorbent assay (ELISA). Cell invasion assay was applied to detect cell migration after treatment with FE extract for 48 h.

Results: Ethanol fruit extract (FE) resulted in 90, 92, 85 and 87 % cytotoxicity against K562, A549, MCF-7 and Jurkat cell lines, respectively. However, ethanol seed extract of seed (SE) was less effective (≤42 %) on cytotoxicity against cancer cells. Acetone fruit extract (FA) caused 82, 75 and 59 % cytotoxicity on MCF-7, Jurkat and K562 cells, respectively, whereas 20 % cytotoxicity was observed on A549 cells. Dose analyses of FE extract indicated that K562 cells had the lowest IC50 value (0.082 mg/mL). In addition, FE extract treatment caused accumulation of A549 and MCF-7 cells in the S phase of the cell cycle. Moreover, apoptotic cell death was observed in A549 or MCF-7 cells treated with the FE extract. While the treatment of A549 cells with LPS for 24 h resulted in 19-fold increase in VEGF secretion, combination of FE with LPS caused 9.6-fold decrease in VEGF secretion, indicating the antiangiogenic activity of FE extract. Furthermore, FE extract treatment led to a significant decrease in the invasive properties of A549 and PC-3 cells when compared to untreated cells.

Conclusion: Among the M. charantia extracts, FE extract displayed the highest anticancer potency against cancer cell lines, indicating that M. charantia FE extract may be a potential source for development of anticancer compounds in future.

Keywords: Momordica charantia, Anticancer, Cytotoxicity, Apoptosis, VEGF, Cell invasion

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INTRODUCTION

Cancer is a malignant disease and it is one of the main reasons of death worldwide. External and internal factors contribute to initiate the disease. Even though tremendous progress has been made to improve chemotherapeutic anticancer agents, most of them have severe toxic side effects. Due to their lack of selectivity against cancer cells, many anticancer agents exhibit narrow therapeutic potency [1]. Therefore, substantial research has been carried out the world over to discover new anticancer agents that selectively kill malignant tumor cells but not normal cells. Plants are good sources for development of both effective and safe drugs against cancer. In fact, plants have been used as a remedy in cancer treatment for centuries cancer and more than 3000 plant species have been listed [2].

Momordica charantia L. is a bitter melon and it belongs to Cucurbitaceae family and it is known as bitter melon, *karele* and *pare*. It grows in tropical areas of the Amazon, Asia, Middle East, India and South America. This plant has long been used as both food and medicine [3]. In traditional medicine, different parts of the plant including seeds, fruits, leaves and roots have been used for treatments of microbial infections, wounds inflammation, fever and hypertension [4]. In clinical practice, *M. charantia* extracts from fruits have been used for diabetes, dyslipidemia and microbial infections [5-7].

Momordica charantia contains biologically active phytochemicals including momordin, charantin, triterpenes, proteins, steroids, alkaloids, saponins, flavonoids, fatty acids and vitamins such as A, E, C, B_{12} and folic acid [8]. The blood sugar lowering property of bitter melon is due to chemicals known as charantins, insulin-like peptide and alkaloids. Several proteins in bitter melon have been identified as anticancer substances. For example, momordin was shown to have anticancer activity against Hodgkin's lymphoma in animal models. Additionally, α and β momorcharin and cucurbitacin B exhibit anticancer effects. Indeed, an analog of these proteins was chemically synthesized and named as "MAP-30".

Although clinical trials have not been conducted using *M. charantia* extracts in cancer patients, *in vitro* studies indicated that *M. charantia* extracts exhibited antitumor activities against certain cancer cell lines [9]. Purified compounds from *M. charantia* such as cucurbitane-type triterpenoids, MAP-30 and conjugated fatty acids exerted *in vitro* anti-tumor activities against cancer cell lines. Additionally, several studies indicated that crude extracts of *M. charantia* are more potent than individual compounds [10]. However, the anticancer effects of crude *M. charantia* extracts against certain cancer cell lines have not been fully elucidated.

Therefore, this study investigates the anticancer activities of crude extracts from fruit and seed of *M. charantia* against A549, MCF-7, K562 and Jurkat cell lines based on cytotoxicity, cell cycle arrest, apoptosis, angiogenesis and cell invasion.

EXPERIMENTAL

Preparation of *M. charantia* extracts

The plant *M. charantia* was cultivated and harvested in the Edirne province of Turkey. The aerial parts of the plant were air-dried. Dried fruit and seed of the plant were separately ground into powder and extracted with 96% ethanol or acetone by shaking at 53-55°C. After filtration, the solvent was removed using a rotary evaporator (IKA, RV 10, USA) at 42-43°C. The extracts were then lyophilized and stored at -20 °C until being used. A stock solution was obtained by dissolving the extract in 10 % DMSO. Working dilutions of the extract contained DMSO less than 1 % and it showed no contribution on the experimental parameters.

Cell lines and culture conditions

Cancer cell lines A549, MCF-7, K562, normal human bronchial epithelium (BEAS-2B), normal human embryonic kidney (HEK293) were originally purchased from the American Type Culture Collection (ATCC) and kind gifts from Dr. Yusuf Baran (İzmir Institute of Technology, Turkey). Jurkat cell line (Clone E6-1 ATCC) was a kind gift from Dr. Mehtap Kılıç Eren (Adnan Menderes University, Turkey). The cells were grown in RPMI 1640 medium containing stable L-glutamine; 10 % fetal bovine serum (FBS); penicillin (100 U/mL) and streptomycin sulphate (100 mg/mL) (Biochrom, Germany) at 37°C in a humidified incubator with 5% CO_2.

Cytotoxicity assay and selectivity index

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-dipenyltetrazolium bromide] assay was carried out to detect the cytotoxic activities of the extracts. Reduction of MTT salt by living cells indicates the function of mitochondria and cell viability [11]. Cells at a density of 2×10^4 cells/mL were seeded in 96-well plates. Following incubation for 24 h, the cells were treated with the extract (0-0.8 mg/mL) for 72 h. Then, 10 µL

of MTT reagent (5 mg/mL) was added into the wells and incubated for 4 h. After discarding the medium, 100 μL DMSO was added to dissolve formazan crystals. Absorbance was measured at 540 nm with a spectrophotometer (Thermo Scientific, Multiskan FC, USA).

Selectivity index (SI) of a crude extract is expressed as in the previous report [12] using Eq 1.

$$SI = \frac{LC_{50} \text{ of CENC}}{LC_{50} \text{ of CECC}} \cdots\cdots\cdots (1)$$

where LC_{50} = concentration required to kill 50 % of the cell population, CENC= crude extract in a normal cell line, CECC= crude extract in cancer cell line.

Cell cycle analysis

Cancer cell lines at 5×10^5 cells were seeded in a 6-well plate and treated with the extracts (0.2, 0.4 and 0.8 mg/mL) for 24 h at 37°C. Cells were washed and centrifuged at 1200 rpm for 10 min. Resuspended cells were fixed in absolute ethanol and kept at -20°C for 48 h. After that, cells were washed with PBS and centrifuged at 1200 rpm for 10 min. The cells were resuspended in 1 ml PBS containing 0.1% Triton X-100 and 100 μL of RNase A (200 μg/mL) and then incubated at 37°C for 30 min. After adding 100 μL of PI (1 mg/mL), cells were incubated for further 15 min in the dark. Finally, cells were analyzed by flow cytometry (BD FACSCanto) using the ModFit LT 3.0 software.

Flow cytometry assay

Exponentially growing cells were cultured at 5×10^5 cells in 6-well plates (Greiner, Germany) and allowed to attach for 24 h at 37°C. The cells were exposed to the extracts at 0.2, 0.4 and 0.8 mg/mL for 24 h. Annexin V-FITC (fluorescein isothiocyanate)/PI (propidium iodide) co-staining assay was carried out according to the manufacturer's Annexin V-FITC Apoptosis Detection Kit (eBioscience, USA) protocol.

The cells treated with plant extracts were washed with PBS, tripsinized, washed and resuspended in a binding buffer. After adding 5 μL of Annexin V-FITC and 10 μl PI (20 μg/mL) into each cell suspension, the cells were incubated for 15 min in the dark. After adding binding buffer (500 μL), 10,000 cells for each group were analyzed by flow cytometry (BD FACSCanto A, BD Biosciences) using the BD FACSDiva software v6.13.

Caspase-3 activity assay

Caspase-3 activity assay was performed according to the instructions of manufacturer of a colorimetric assay kit (Abcam, Cambridge, UK). After treatment of cells with the plant extract at 0.8 mg/mL for 36 h, the cells were washed, harvested, lysed and centrifuged. Total protein amount was determined by the Bradford assay [13]. Then, 200 μg of protein from each sample was assayed for caspase-3 activity with a specific substrate DEVD-*p*-NA. Absorbance of free p-nitroanilide (p-NA) produced by the cleavage of the substrate by activated caspase 3 was measured at 405 nm in a plate reader.

Determination of human VEGF

A549 cells were cultured at 2×10^5 cells in a 6-well plate and incubated at 37 °C for 1 h. Then, the cells were exposed to the plant extract at 150 μg/mL and incubated for 12, 24, 48 or 72 h at 37°C. After centrifugation, the supernatants were collected and stored at -20°C. Untreated cells served as control. The concentrations of VEGF in the supernatants were determined by ELISA as described in the manufacturer's procedure for the VEGF ELISA kit (Boster Biological Technology, USA). The absorbance was measured at 450 nm in a plate reader. The VEGF concentrations of cell culture supernatants were calculated from a standard curve of VEGF.

Cell invasion assay

Cancer cell invasion analysis was carried out according to the protocol described in the Cell Invasion Assay Kit (Chemicon, USA). Extracellular matrix (ECM) was rehydrated by adding warm serum-free medium to the upper inserts for 1 – 2 h at room temperature. After removing the medium, 500 μL of medium containing 10 % FBS was added to the lower chamber. A549 cells or PC-3 cells were seeded at 3×10^5 cells in 300 μL of serum-free medium in an invasion chamber (upper insert) and treated with the plant extract at a 150 μg/mL concentration for 48 h at 37°C in a CO_2 incubator. After incubation, cells were gently removed using a cotton-tipped swab. The invasive cells on the surface of lower membrane were stained by dipping the insert in the staining solution (500 μL) for 20 min. Inserts were rinsed with water, air-dried and counted in 3 randomly selected fields using an inverted microscope (Olympus, E330, Japan). The percentage of invasion (PI) was calculated using Eq 2.

$$PI = \frac{MNTCOM}{MNUTCOM} \times 100 \cdots\cdots\cdots (2)$$

where MNTCOM = mean number of treated cells on the membrane, MNUTCOM = mean number of untreated cells on the membrane

Statistical analysis

Each result was presented as the mean ± SD. The data were analyzed using GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, CA). Comparison of treatments among the groups was performed using one-way ANOVA and post-hoc analysis. Significance was *at p* < 0.01 and < 0.0001.

RESULTS

Cytotoxicity of plant extracts

Ethanol extract of fruit (0.8 mg/mL) caused cytotoxicity more than 80 % on all cell lines that were tested (Figure 1). Acetone extract of fruit (FA) displayed 82% and 78% cytotoxicity on MCF-7 and Jurkat cell lines whereas 25% and 60% cytotoxicity were observed on A549 and K562 cells. On the other hand, both ethanol (SE) and acetone (SA) extracts of seed (SA) resulted in cytotoxicity levels of lower than 42 % in all cell lines that were tested. These findings show that, unlike seed extracts, fruit ethanol extract exerted a high level of cytotoxicity on A549, MCF-7, K562 and Jurkat cell lines.
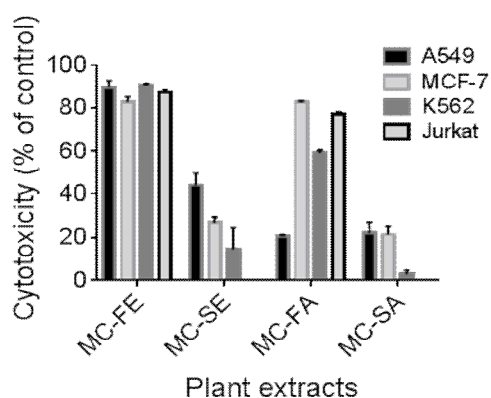

Figure 1: Cytotoxic effects of plant extracts on different cancer cell lines. Cells were treated with extracts (0.8 mg/mL) or left untreated for 72 h. Extracts; MC, *M. charantia*; FE, ethanol extract of fruit; SE, ethanol extract of seed; FA, acetone extract of fruit; SA, acetone extract of seed. Data are means (±SD) of three independent experiments

Dose- and time- dependence of cytotoxicity

Ethanol extract of fruit (FE) exerted cytotoxic activity on A549, MCF-7 and K562 cells in a dose- and time-dependent manner (Figure 2). The percentage of cell death at all doses gradually increased for 24, 48 and 72 h. A549 cells displayed 90 % cytotoxicity even at 0.2 mg/mL for 72 h (Figure 2 a), whereas MCF-cells presented 80 and 40 % cytotoxicity at 0.4 and 0.2 mg/mL for 72 h (Figure 2 b). Additionally, K562 cells exhibited 90 % cytotoxicity even at 0.1 mg/mL for 72 h (Figure 2 c). Moreover, the extract at 0.2 mg/mL caused 78 % cytotoxicity on Jurkat cells (Figure 2 d). According to the IC_{50} values, K562 was the most sensitive cell line because it had the lowest IC_{50} value (0.082 mg/mL) compared to the other cell lines.

One of the most important aspect of an extract is selective cytotoxicity against cancer cells compared to normal cells. Therefore, the cytotoxic effect of the FE extract was tested on normal cell lines BEAS-2B and HEK293, as well. Although the extract at 0.2 mg/mL caused 90 % cytotoxicity on A549 cells at 72 h, 50 % cytotoxicity was observed in BEAS-2B (Figure 2 e). In addition, HEK293 cells exhibited cytotoxicity less than 30 % at all doses tested (Figure 2 f). These results indicate the selectivity of the FE extract against the cancer cells.
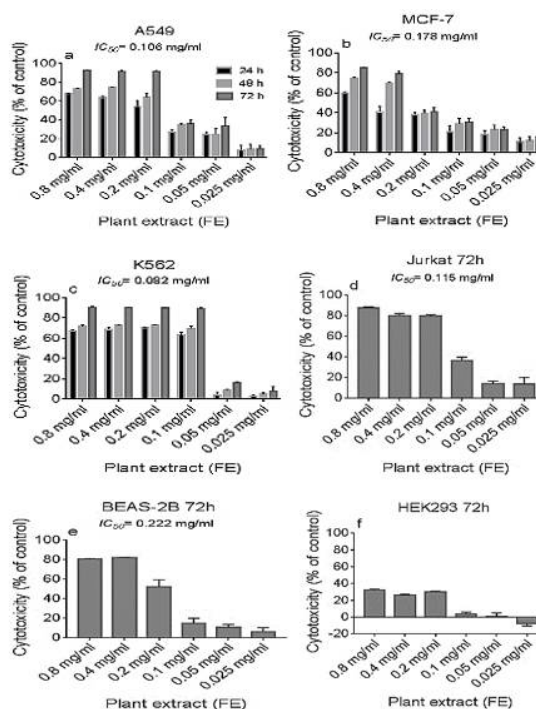

Figure 2: Dose- and time-dependent effects of FE extract

As acetone extract of fruit (FA) exerted the most effective cytotoxic effect on MCF-7 and Jurkat cells (Figure 1), time- and dose-dependent response of MCF-7 cells and dose-dependent response of Jurkat cells were tested. The highest cytotoxicity was observed after 72 h of incubation for all doses (Figure 3 a). While MCF-7 cells

exhibited 80 % cytotoxicity at 0.2 mg/mL, Jurkat cells displayed 38 % cytotoxicity at 72 h (Figure 3 a, b). Based on the IC_{50} values, MCF-7 cells were more sensitive to the extract than Jurkat cells. However, normal cell line HEK293 displayed cytotoxicity less than 30 % after treatment with the FA extract at all doses (Figure 3 c), indicating the selectivity of the extract.
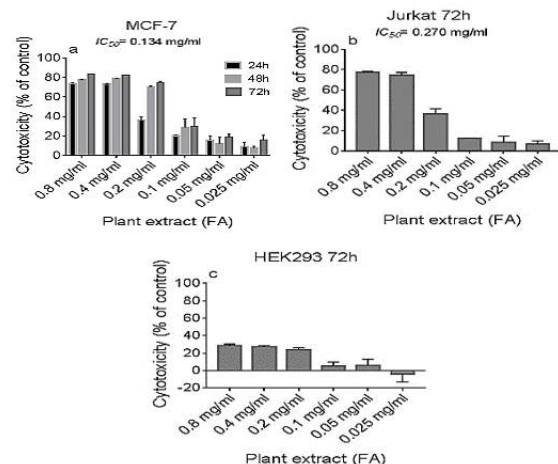

Figure 3: -Dose and time- dependent effects of FA extract

Effects of FE extract on cell cycle distribution of cancer cells

A549 and MCF-7 cells were treated with the FE extract at 0.2, 0.4, 0.8 mg/mL for 24 h to see if progression of the cells through the cell cycle was arrested. Extract at 0.2 mg/mL displayed no significant effect on the G_1, S and G_2 phases of A549 cells in comparison to the untreated control cells (Figure 4A a,b,e). However, the extract at 0.4 and 0.8 mg/mL caused an increase in the number of cells at the S phase to 30 and 61 %, respectively (Figure 4A a,b,c and Figure 5 a). Additionally, the percentage of cells at the G_2 phase gradually increased in control to 18.7 % in A549 cells treated with the extract at 0.4 mg/mL. However, the percentage of cells was 0.4 at the G_2 phase of cells treated with the extract at 0.8 mg/mL (Figure 4A a,b,c and Figure 5 a). These results indicate that, when A549 cells were treated with the extract at 0.4 and 0.8 mg/mL, they were arrested at the S phase of the cell cycle.

The cell cycle distribution of MCF-7 cells are presented in Figure 4B. Similar to A549 cells, MCF-7 cells exhibited cell cycle arrest at the S phase of the cell cycle after treatment with the extract at all concentrations that were tested (Figure 4 B, a,b,c,d, and Figure 5 b).
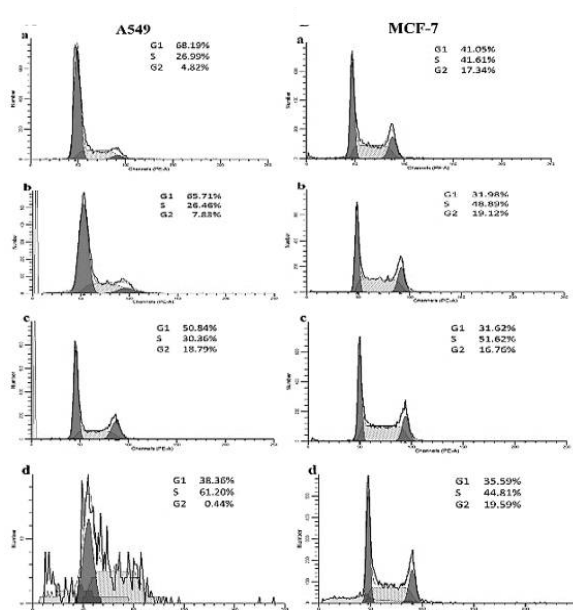

Figure 4: Cell cycle analysis of cancer cell lines treated with FE extract of *M. charantia*. Treated cells were stained with propidium iodide and DNA content was determined using flow cytometry. Histograms present cell cycle distribution of A549 (A) and MCF-7 (B) cells after treatment with no extract (a), 0.2 mg/mL extract (b), 0.4 mg/mL extract (c) 0.8 mg/mL extract for 24 h
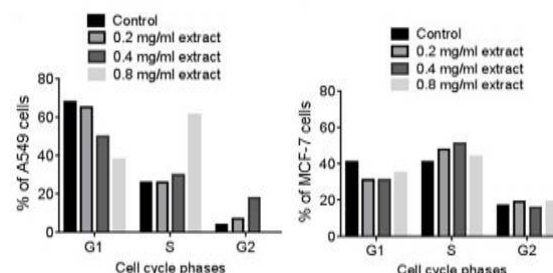

Figure 5: Proportion of cells (%) at G_1, S and G_2 phases of the cell cycle are shown in a bar diagram

Effect of FE extract on apoptosis of cancer cells

Disruption of cell cycle progression may induce apoptotic/necrotic cell death. Therefore, flow cytometric analysis was carried out to determine if apoptosis was induced in A549 and MCF-7 cells after treatment with the extract (0.2, 0.4, and 0.8 mg/mL). The extract at 0.2 mg/mL did not cause an increase in the percentage of apoptotic cells in comparison to the untreated cells (Figure 6A a,b, and Figure 7 a). On the other hand, the percentage of apoptotic A549 cells increased from 3.7 % (untreated) to 6 and 39.5 % after treatment with the extract at doses of 0.4 and 0.8 mg/mL, respectively (Figure 6A a,c,d and Figure 7 a). When the apoptotic cells were examined in MCF-7 cells, the percentage of

apoptotic cells increased from 2 % (untreated) to 19.9 and 35.5 % in the cells treated with the extract at 0.4 and 0.8 mg/ml, respectively (Figure 6B a,c,d and Figure 7 b). These results indicate that the FE extracts at doses of 0.4 and 0.8 mg/mL promoted apoptosis in both A549 and MCF-7 cells. These findings support the data observed in cell cycle analysis, especially in the cells treated with 0.8 mg/mL extract.
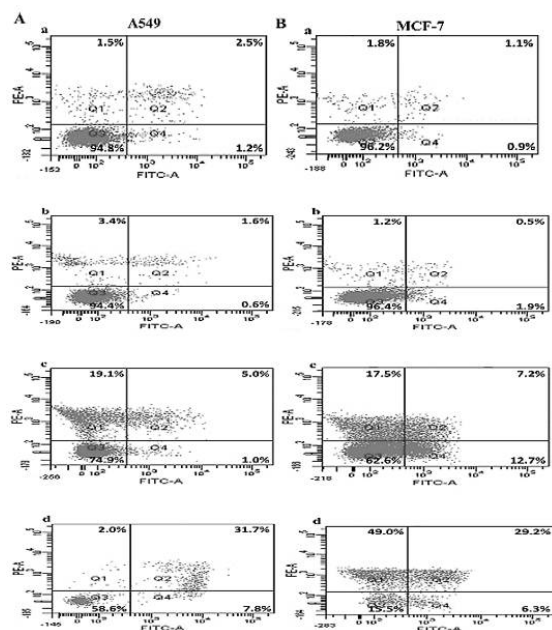

Figure 6: Flow cytometric analysis of apoptosis in cancer cells treated with FE extract. A549 (A) and MCF-7 (B) cells were treated with no extract (a), 0.2 mg/mL (b), 0.4 mg/mL (c) and 0.8 mg/mL extract (d) for 24 h. Cells were distributed into four quadrants: viable cells (Q3), early apoptotic cells (Q4), late apoptotic cells (Q2) and necrotic cells (Q1)
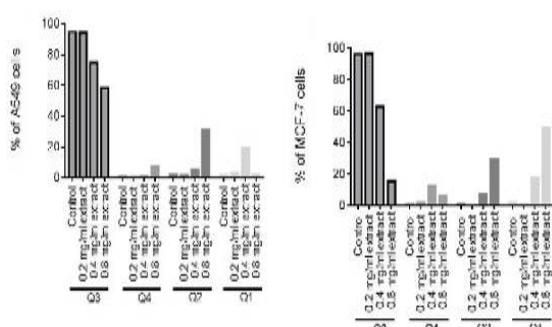

Figure 7: Percentages of apoptotic cells are presented as a bar diagram

Effect of FE extract on caspase-3 activation

Caspases involved in apoptosis are caspase-3,-6,-7,-8,-9. Caspase-3 is one of the caspases with roles in execution of apoptosis in a cell so that the cleavage of an apoptotic substrate by activated caspase-3 is a good indicator of the presence of apoptosis. In this study, A549 cells were treated with the FE extract at 0.8 mg/mL for 36 h to see if caspase-3 was activated. The results of three independent experiments showed that the extract caused 15-fold increase in caspase-3 activity of treated A549 cells in comparison to untreated control cells ($p<0.0001$). This result is consistent with the data obtained from the flow cytometric analysis.

Effect of extracts on VEGF secretion

Numerous phytochemical compounds from plant extracts may influence the angiogenic activity of various cell types. Airway epithelial cancer cell line A549 releases the VEGF protein constitutively [14]. Therefore, the angiogenic effect of the FE extract of *M. charantia* at 0.15 mg/mL was examined on A549 cells. VEGF secretions of A549 cells treated with the FE extract at different time points (12, 24, 48 and 72 h) were respectively 3.6, 2.8, 2.1, and 1.4-fold higher than those of untreated control cells (Figure 8). Similar results were obtained with cells treated with the FA extract. Additionally, VEGF secretion of A549 cells treated with lipopolysaccharide (LPS, 10 µg/mL) for 24 and 48 h were 19- and 17-fold higher than those of untreated cells. However, combination of the FE extract with LPS resulted in 9.6 and 4.6-fold decrease in VEGF secretion after 24 and 48 h of treatment, respectively (Figure 8). These findings indicate that *M. charantia* FE and FA extracts have significant effects on induction of angiogenesis when compared to untreated A549 cells. However, the FE extract in combination with LPS resulted in a significant decrease in VEGF secretion when compared to that observed in the cells treated with LPS alone.
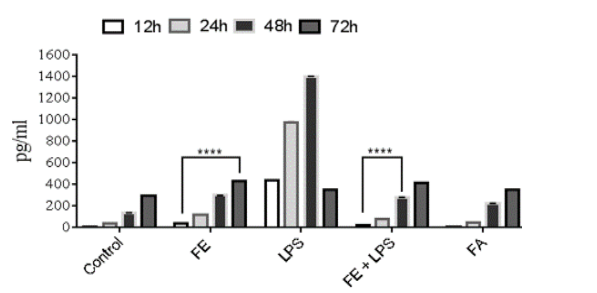

Figure 8: Effect of the extracts on VEGF secretion of A549 cells. Cells were left untreated or treated with 150 µg/mL extract for different time points, and VEGF concentration in supernatants was detected by ELISA. Data are mean ± SD of triplicate samples. ****$p < 0.0001$

Effect of FE extract on cell invasion

One of the most important steps in tumor metastasis is correlated with cell invasion that

involves cellular migration and interaction with the microenvironment. Cell invasion assay was carried out to see if *M. charantia* FE extract has an effect on invasion of cancer cells. Lung cancer A549 and prostate cancer PC-3 cells were treated with the extract at 0.1, 0.2, and 0.4 mg/mL for 24 h. Invasive cells passing through the membrane were counted, and percentages of invasion were determined in comparison to the control. A549 cells treated with the FE extract at 0.1, 0.2, and 0.4 mg/mL showed 1.05, 1.06 and 1.1-fold decreases in invasion in comparison to the control (Figure 9). Similar to A549 cells, PC-3 cells also exhibited 1.14, 1.13, and 1.11-fold decreases in terms of invasion. As a result, *M. charantia* FE extract displayed a significant decrease in the invasive properties of A549 and PC-3 cancer cells, compared to untreated cells.
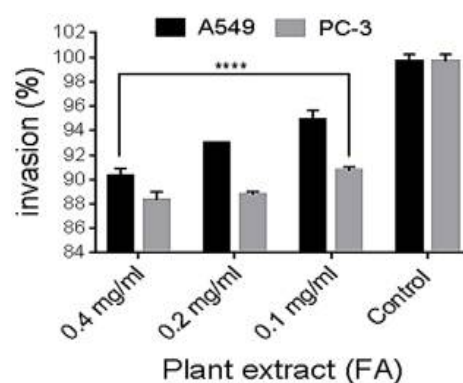

Figure 9: Effect of FE extract on invasion potential of A549 cells. Cells were treated with extract for 24 h. Data are mean ± SD of three independent experiments. ****: *p* < 0.0001

DISCUSSION

Extensive research had been carried out for the isolation of active components from *M. charantia*, and analysis of their pharmacological properties [15]. However, the mechanism of action of phytochemicals from this plant is poorly understood. In the light of the medicinal values of *M. charantia,* this study was designed to investigate its cytotoxic, apoptotic, angiogenic and invasive effects against A549, MCF-7, K562 and Jurkat cancer cell lines, as well as normal healthy cell lines BEAS-2B or HEK293.

Ethanol extract of fruit led to cytotoxicity by more than 82 % on four cancer cell lines. However, the FA extract caused different levels of cytotoxicity ranging from 20 % to 82 % against cancer cells. Extraction of different phenolic compounds requires various solvents with differing polarities [16]. It seems that phenolic compounds extracted by ethanol are more effective on cytotoxic activity against cancer cells than those extracted by

acetone. In contrast, seed extracts (SE and SA) were not as effective as fruit extracts on cytotoxic activity against the cancer cell lines. This could be due to the different kinds and levels of secondary metabolites that were present in the seed. Each part of a plant contains different secondary metabolites in different constituents [17]. Indeed, the phytochemical contents of seeds were found to be very limited when compared to the fruits [10]. Therefore, these results suggest that the fruit part of *M. charantia* should be used for development of a therapeutic agent against these cancer cells.

Development of a therapeutic drug against cancer requires selectivity between cancer cells and normal cells. If the selectivity index (SI) value of a compound is higher than 2, it is considered as high selectivity [12]. After treatment with the FE extract, A549 and K562 cells displayed SI values of 2 and 2.7 indicating that FE extract is more selective on K562 cells than it is on A549 cells. In contrast, the SI value of MCF-7 and Jurkat cells were 1.2 and 1.9. The fact that the greater the SI value is, the more selective it is and SI value less than 2 indicates general toxicity [12], it can be suggested that FE extract of *M. charantia* may be a promising therapeutic candidate in patients with lung cancer and chronic myeloid leukemia.

Cell cycle progression from the G_0 to M phase is mainly regulated by cyclins and cyclin-dependent kinases (CDKs). Inhibition of cell cycle progression is an effective strategy for development of a novel cancer drug. Cell cycle analysis of the FE-treated A549 cells revealed that the FE extract induced S phase cell cycle arrest at 24 h in this study. Similarly, another study reported cell cycle arrest at the S phase after treatment with *M. charantia* extracts [18]. This indicates that the FE extract might interfere with initiation of DNA replication. Arrest at the S phase occurs through direct binding of a suppressor to replication machinery or phosphorylation of a critical element of replication control [19]. Agents that induce expression of cell cycle inhibitory regulators such as INK4 and Cip/Kip family members may be beneficial in control of malignant formations. From this finding, it appears that FE extract causes cell cycle arrest at the S phase by mechanisms yet to be revealed.

Induction of the apoptotic pathway within cancer cells is a main target for development of a therapeutic drug. Apoptosis was determined using simultaneous staining of cells with FITC-Annexin-V/PI. In early apoptosis, membrane phosphatidylserine (PS) is translocated from the

inner to the outer surface of the cytoplasmic membrane. Translocated PS may be detected and quantified using flow cytometry. In this study, dose dependent increase in early and late apoptosis suggests that induction of apoptosis takes place through more than one pathway which is probably due to many compounds in the FE extract. In addition, apoptosis was confirmed by observation of raising activity of caspase-3 in FE-treated cells. Activation of executer caspase-3 can be mediated by different caspase activities [20]. Therefore, evaluation of different caspase activities in FE treated cells will reveal the pathway responsible for caspase-3 activation.

Angiogenesis is defined as formation of new blood vessels, and it plays an important role in metastatic spread of tumor cells. Vascular endothelial growth factor (VEGF) and its receptor are major mediators of angiogenesis. Lipopolysaccharide (LPS) is a strong stimulator of inflammation, and it can also lead to carcinogenesis and promotes angiogenesis [21]. After treatment of A549 cells with the FE extract, a slight increase in VEGF secretion was observed However, LPS treatment of cells led to a major elevation of VEGF levels in comparison to untreated cells. Interestingly, this elevation decreased sharply in the cells treated with LPS plus the extract, suggesting anti-angiogenic function of the FE extract of *M. charantia* in A549 cells. Similarly, Xu *et al* [22] reported that sesamin resulted in a reduction of LPS-induced invasion and VEGF secretion of PC-3 cells. Elucidation of the molecular mechanism involved in an anti-angiogenic effect may improve the potential of the extract to be used as a new therapeutic agent for treatment or prevention of lung cancer.

Malignant tumor development depends on invasive and metastatic capacity of tumor cells. In this study, the FE extract did not increase the invasive properties of A549 and PC-3 cells, but it significantly decreased invasion of the cells. Invasion and migration of cancer cells are regulated by multiple molecular mechanisms. For example, matrix metalloproteinases (MMPs) are involved in tumor invasion and metastasis [23]. Both MMP-2 and -9 play a role in degradation of type-I and type-IV collagens and the extracellular matrix (ECM). However, tissue inhibitors of metalloproteinases (TIMPs) form complexed with MMPs and prevent their proteolytic activities. Therefore, overexpression of TIMPs may prevent invasion and metastasis in several cancer cells. Future studies may elucidate possible signaling pathways involved in inhibition of MMP-2 and MMP-9 expression and stimulation of TIMP1 and

TIMP2 in A549 and PC-3 cancer cells after treatment with the FE extract of *M. charantia*.

CONCLUSION

The findings of this study show that the ethanol fruit extract of *M. charantia* has the highest cytotoxic activities of all the extracts examined (> 80 %) against A549, MCF-7, K562 and Jurkat cell lines. This extract causes cell cycle arrest at the S phase and apoptotic cell death in A549 and MCF-7 cells, as well as inhibition of invasion and LPS-induced angiogenesis of A549 and/or PC-3 cells. These findings suggest that FE extract may be a promising tool for the development of anticancer drugs against lung cancer, breast cancer, chronic myeloid leukemia and T cell leukemia. Future studies should attempt to elucidate the molecular mechanisms regulating the extract-mediated cellular signaling in these cancer cell lines.

DECLARATIONS

Acknowledgement

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was carried out by the authors named in this article, and all liabilities pertaining to the claims related to the content of this article will be borne by the authors. HG designed the experiments. MA, NÇ and HG performed the experiments. HG, MA and NÇ analyzed the data. HG wrote the manuscript.

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