Egyptian Journal of Medical Human Genetics

RESEARCH

Open Access

Effect of *Dioscorea* extract on *Bax* and *Bcl-2* gene expression in MCF-7 and HFF cell lines

Fatemeh Bojar Doulaby[1], Mahsa Kavousi[1*] and Faranak Jamshidian[1]

Abstract

Background In cancer cells, the balance between proliferation and apoptosis is disturbed. There is a direct relationship between gene expression and the process of apoptosis. The two genes involved in apoptosis are *Bax* and *Bcl-2*, and it is now well established that some plant compounds can alter the expression of genes. The aim of this study is to determine the rate of change in the expression of these genes in the cell line MCF-7 treated with *Dioscorea* extract for 24, 48 and 72 h. For this purpose, the plant extract was prepared by Soxhlet method and diluted in different concentrations. MCF-7 and HFF cell lines were treated in three replicates with different concentrations of the extract at intervals of 24, 48, and 72 h. To evaluate the toxicity of the extract, the MTT assay was performed and the IC_{50} value was calculated. Both cell types were cultured at IC_{50} concentration with three treatments and three replicates. RNA extraction, cDNA synthesis and real-time PCR were then performed. Flow cytometry was performed to further confirm apoptosis.

Results MTT results showed that 72 h treatment with *Dioscorea* extract in IC_{50} concentration had the greatest effect on the death of MCF-7 cancer cells, while the cells of the control cell line remained healthy. The results of the study of gene expression changes showed that when treated with the plant extract for 24 h, the increase in *Bax* gene expression and the decrease in *Bcl-2* gene expression were not statistically significant. At 48-h treatment, the decrease in *Bcl-2* expression was not statistically significant, whereas the increase in *Bax* expression, which was 2.1 times, was statistically significant. When treated with the plant extract for 72 h, *Bax* expression increased 2.72 times and *Bcl-2* gene expression decreased 0.67 times. Flow cytometry showed that 72-h treatment with plant extract at a concentration of 438.35 µg/ml was the most effective treatment for MCF-7 cancer cell death.

Conclusions The expression ratio of *Bax* gene to *Bcl-2* is equal to 4.06, which indicates the induction of more apoptosis by treatment with plant extract.

Keywords Apoptosis, *Bax*, *Bcl-2*, *Dioscorea*, MCF-7 cell line

Background

Breast cancer accounts for about one-third of all cancers that affect women. Worldwide statistics show that 2.3 million new cases of breast cancer are diagnosed each year. It is the leading cause of death after lung cancer [1] and that too in Iranian women [2, 3]. In breast cancer, cancer cells enter in the lymphatic vessels. They migrate to the lymph nodes and spread to other parts of the body through the lymphatic system [4]. Oncogenes were the first tumorigenic genes to be identified. In fact, proto-oncogenes regulate normal cell division, and mutation turns them into oncogenes [5]. *Bcl-2* is located on chromosome 18 and encodes a 24-kDa protein found in the nuclear membrane, endoplasmic reticulum, and mitochondrial membrane. The *Bcl-2* gene is involved in the inhibition of apoptosis and increases cancer cell survival. In contrast, expression of the *Bax* gene triggers apoptosis and death of cancer cells [6]. Studies have shown

*Correspondence:
Mahsa Kavousi
mkavosi@yahoo.co.uk
[1] Department of Biology, East Tehran Branch, Islamic Azad University, Tehran, Iran

Springer Open

© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

that plants are effective in treating gastrointestinal cancer, skin cancer, oral cancer, and breast cancer [7]. Plants play an important role in the prevention and treatment of cancer. In recent years, the use of natural plant products has increased due to the side effects of chemotherapeutic agents [8, 9]. Different species of *Dioscorea* are used as food and medicine. The substances contained in this plant such as flavonoids, diosgenin, dioscorin, tannins, saponins, and phenols are valuable foods with beneficial effects [10]. A study was conducted on the presence of diosgenin in white yam. This is because the tubers are the staple food of hundreds of thousands of indigenous people in Africa, especially in Nigeria. [11]. The results of another study conducted in Japan to evaluate the effect of *Dioscorea* extract on reducing cancer risk were satisfactory. The antioxidants in *Dioscorea* can scavenge free radicals [12]. A study shows that ethanolic extracts of *D. rotundata* can be used to effectively treat breast cancer tumors [13]. In 2021, a study was conducted on the hemolytic activity of the extract and the cytotoxic effect of *D. bulbifera* on MDA-MB-231 cell line. The results of the 24-, 48-, and 72-h treatments showed that an increase in estrogen levels increased the risk of breast cancer. It was also shown that MDA-MB-231 was reduced by lowering serum estrogen levels [14]. The results of a study on a variety of chemicals from *D. bulbifera* suggest that this plant may be preferred for contraceptive capsules and pills to cure diseases [15]. The results of another study showed that *Dioscorea* is converted into medicines and used as a valuable diet and prevention of diseases [16]. In 2019, Li showed the beneficial clinical effect of a modified *Dioscorea* tablet in the treatment of diseases associated with chronic cerebral hypoperfusion [17]. In 2018, a study was conducted on the effect of *Dioscorea Opposit* extract on MCF-7 cell line. This Chinese herb has no side effects and could become a new drug for the treatment of premenopausal syndrome [18]. In 2010, a study was conducted on the effect of dioscorealide B extract on MCF-7 cell line and the changes of *Bcl-2* and *Bax* gene expression. The results showed that dioscorealide B treatment decreased the expression of the anti-apoptotic protein Bcl-2 and increased the expression of the pre-apoptotic proteins Bax and Bak. As a result, caspase 9 and 7 were activated, respectively. Therefore, this study provided a molecular basis for the further development of dioscorealide B as a new chemotherapeutic agent for the treatment of breast cancer [19]. In 2010, another study was conducted on the effect of caspase-9 inhibitor on MCF-7 cell line. The results showed that treatment with dioscorealid B extract increased the gene expression of *Bax* and decreased the gene expression of *Bcl-2* [20]. In 2016, a study was conducted on the effect of *D. membranacea* extract on apoptosis of L929 cancer cell line. The L929 cancer cell line increases *Bax* expression and decreases *Bcl-2* gene expression after treatment with the extract. Thus, it is involved in the apoptosis of L929 cancer cells [21]. In 2019, a study was conducted on MDA-MB -231 and MCF-7 cells with two treatments of 24 and 48 h. In this study, DiosR and DiosB containing diosgenin were applied to cancer cells. Comparison of 24- and 48-h treatments with DiosB and DiosR showed that 48-h treatments with plant extracts were more effective than 24-h treatments in the MDA-MB-231 cell line. In addition, both extracts were more effective in the cancer cell line MCF-7 than in the cell line MDA-MB -231. The greatest effect on MCF-7 cells compared to MDA-MB -231 was seen with the 48-h treatment [22]. In 2021, Mainasara et al. showed that the methanolic extract of *D. bulbifera* exhibited ant proliferative activity compared with a standard drug. The antioxidant activity of the methanol, ethyl acetate, and hexane extracts of the plant was significant compared to ascorbic acid, indicating the potential of the leaves of this species as natural antioxidants. Thus, the ant proliferative activities shown by the leaf extracts confirm the ancient use of this plant against various breast cancers [14].

Since it is important to investigate the effect of phytochemicals on cancer cell growth, in the present study, *Dioscorea* extract was investigated on MCF-7 cell line as cancer cell line and HFF fibroblast cell line as control cell line.

Methods

Dioscorea was obtained from China and MCF-7 and HFF cell lines from the National Genetic Resources Center of Iran. For every 10 g of plant powder, 100 cm^3 of 70% ethanol was used as solvent. The solution was boiled at 90 °C for 10 min and filtered through a sieve. The extract was then transferred to the micro tube and DMEM culture medium was added. MCF-7 Cells were cultured with DMEM and stored in a 37° CO_2 incubator. When the cell density reached 80%, cell passage was performed. MCF-7 cells at a density of 20,000 cells and HFF cells at a density of 20,000 cells were cultured in 96-house plates. The *Dioscorea* extract plate was then added to the first row, and dilution was performed in each row with each pipette. These steps were performed for 24, 48, and 72 h with three replicates. The concentrations used in this study are 2000, 1000, 500, 250, 125, 62.5, 31.25 µg/ml, respectively.

MTT assay technique

The MTT method is based on mitochondrial activity in living cells. This method is based on the reduction and

disruption of yellow crystals (tetrazolium) by the enzyme mitochondrial succinate dehydrogenase and the formation of purple crystals.

After the treatment time, 20 µl of MTT dye was added to the wells containing the cells and extract, and the plate was incubated for 4 h. Then, 100 µl of isopropanol reagent was added to the wells and incubated for 15 min. The plate was placed in an ELISA instrument to determine the absorbance at a wavelength of 570 nm. Then, the IC_{50} concentration was determined for each treatment in each cell line.

Evaluation of apoptosis induced by flow cytometry method

Flow cytometry was used to determine the degree of apoptosis and necrosis induced by 24-, 48-, and 72-h treatment with plant extracts in the MCF-7 and HFF cell lines. Cells were stained with Annexin-v and propidium iodide (PI). Data analysis was performed using instrument software in 4 areas of the curve Q1 to Q4. After staining cells together with PI and annexin-v, necrotic cells are stained with PI and young apoptotic cells are stained with annexin-v and old apoptotic cells are stained with PI and annexin-v.

Real-time PCR technique

RNA was extracted from cells treated with IC_{50} extract for 24, 48, and 72 h. The fermentase kit was used for RNA extraction. A Nanodrop device (generator) was used before performing real-time PCR to ensure optimal quality of RNA. A mixture of 20 µl, including 10 µl of master mix green, 2 µl of primer for each gene (Table 1), and 6 µl of distilled water were added to the micro tube. Then, the real-time PCR reaction (Table 2) was performed using the instrument (Cycler96-roche). The changes in gene expression were measured at $2^{-\Delta\Delta Ct}$. Changes in the expression of *Bax* and *Bcl-2* genes compared with the reference gene *β-actin* were examined by real-time PCR.

Table 2 PCR steps and repeat cycle

Stages	Temperature (°C)	Time (s)	Number of cycles
Denaturation	95	10	40
Annealing	55	20	
Extension	72	20	

Result

Result of extraction

The extraction of the *Dioscorea* plant was carried out by the Soxhlet method. After determining the concentration of 667 mg/ml extract, the desired concentrations were prepared using DMEM extract and culture medium and by sequential dilution.

Evaluation of biological percentage of MCF-7 cell line under the influence of different concentrations of *Dioscorea* extract after 24, 48 and 72 h

After determining the optimal cell number, 20,000–30,000 cells were cultured in each well.

Figure 1 shows that the plant extract did not cause healthy cells to die in the HFF cell line. As the concentration of the extract increases, the survival rate of cancer cells decreases. This indicates that the plant extract can be used to kill cancer cells without harming healthy cells. This condition was also observed in the 48- and 72-h treatments. In the 24-h treatment of cancer cells with the plant extract, the IC_{50} was reached at a concentration of 1178.95 µg/ml.

When treated with the plant extract for 48 h, it was found that the plant extract had no lethal effect on the HFF cell line. As the concentration of the extract increases, the survival rate of the cancer cell lines decreases. In the 48-h treatment with the plant extract, the IC_{50} was determined at a concentration of 568.25 µg/ml (Fig. 2).

Table 1 Sequence of primers used in this research

Gene	Primer pair sequences	Tm (°C)	GC%	Accession N
β-actin (F)	5′-TCCTCCTGAGCGCAAGTAC-3′	59.11	57.89	NM-011001.5
β-actin (R)	5′-CCTGCTTGCTGATCCACATCT-3′	60.41	52.38	NM-011001.5
Bax (F)	5′-GAGCTGCAGAGGATGATTGC-3′	59.05	55.00	NM-138764.5
Bax (R)	5′-AAGTTGCCGTCAGAAAACATG-3′	57.65	42.86	NM-138764.5
Bcl-2 (F)	5′-ATTGGGAAGTTTCAAATCAGC-3′	54.98	38.10	NM-000657.3
Bcl-2 (R)	5′-CAGTCTACTTCCTCTGTGATGTTG-3′	58.83	45.83	NM-000657.3

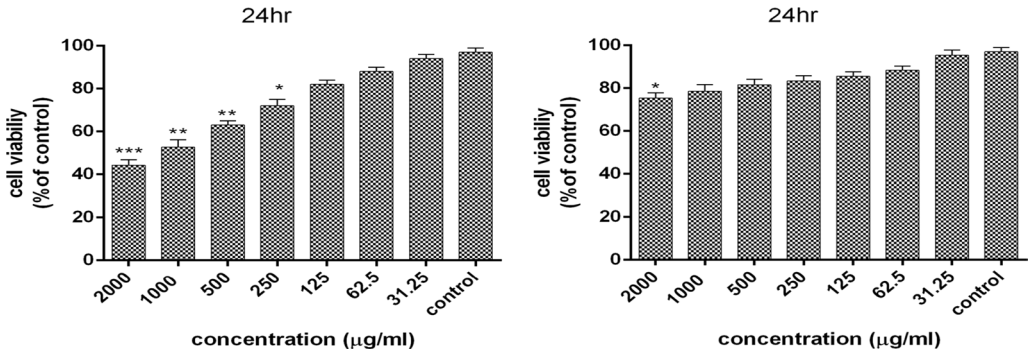

Fig. 1 Percentage of survival of MCF-7 cells (right) and normal cells (left) to different concentrations of *Dioscorea* extract after 24 h by colorimetric method (*p value < 0.05, **p value < 0.01, ***p value < 0.001)

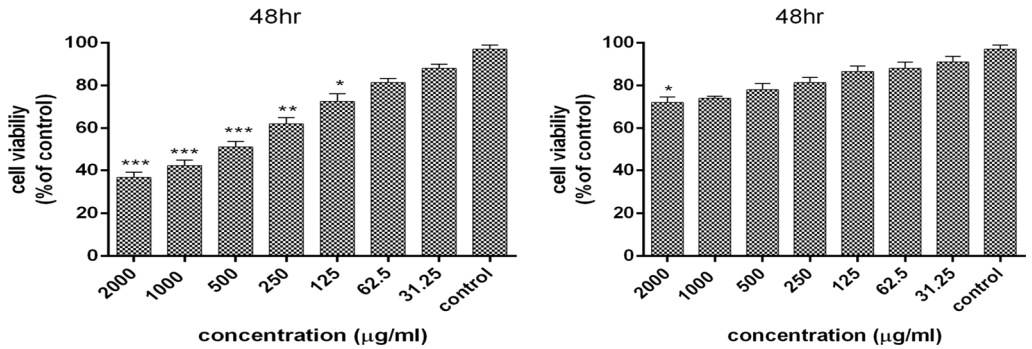

Fig. 2 Percentage of survival of MCF-7 cells (right) and normal cells (left) to different concentrations of *Dioscorea* extract after 48 h by colorimetric method (*p value < 0.05, **p value < 0.01, ***p value < 0.001)

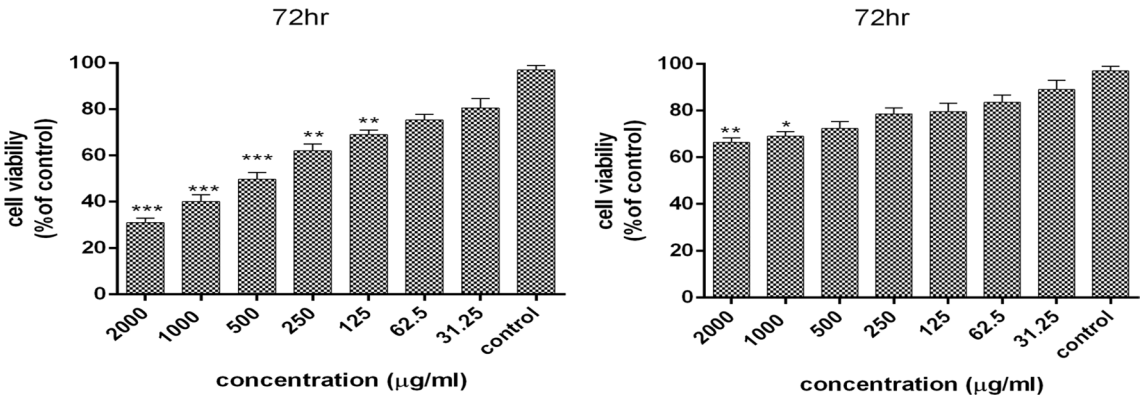

Fig. 3 Percentage of survival of MCF-7 cells (right) and normal cells (left) to different concentrations of *Dioscorea* extract after 72 h by colorimetric method (*p value < 0.05, **p value < 0.01, ***p value < 0.001)

Table 3 IC_{50} value of MCF-7 cell line (right) and normal cell line (left)

Time (h)	IC_{50} (µg/ml)	Time (h)	IC_{50} (µg/ml)
24	1178.95	24	4678.43
48	568.25	48	4167.76
72	438.35	72	3654.21

After 72 h of treatment with the plant extract, it was found that the plant extract had no lethal effect on the HFF cell line. During the 72-h treatment with the plant extract, the IC_{50} value was 438.35 µg/ml (Fig. 3).

Table 3 compares the IC_{50} results obtained by treatment with the plant extract in the cancer cell line and the control. The results show that at the IC_{50} concentrations

obtained for the cancer cell line, the control cells remained healthy, but the cancer cells died.

The statistical studies on the 24-, 48-, and 72-h treatments with the plant extract with p value < 0.01 are shown in Tables 4 and 5. According to this table, the survival rate of MCF-7 cells is highest at a concentration of 31.25 µg/ml. The viability of MCF-7 cells decreases with increasing concentration.

The result of the quantified RNA extract

After the amount of RNA extracted from the cells of the cell line was evaluated using the Nanodrop device at an absorbance ratio of 260 to 280 nm, the RNA concentration was 480.5 ng/µl (Fig. 4).

Table 4 Mean bioavailability of MCF-7 cell line with different concentrations of *Dioscorea* extract in 24, 48 and 72 h by MTT assay

Time (h)	Concentration (µg/ml)						
	2000	1000	500	250	125	62.5	31.25
24	44.4 ± 0.28	52.5 ± 0.28	63 ± 0.62	72.25 ± 0.46	82.75 ± 0.32	88.75 ± 0.32	94.52 ± 0.28
48	36.1 ± 0.36	42 ± 0.36	51.2 ± 0.58	62 ± 0.28	72.75 ± 0.78	81.75 ± 0.78	88.5 ± 0.88
72	31.75 ± 0.47	39.75 ± 0.47	47.75 ± 0.38	60.6 ± 0.62	69 ± 0.47	75 ± 0.47	80.25 ± 0.35

Table 5 Mean bioavailability of normal cells with different concentrations of *Dioscorea* extract in 24, 48 and 72 h by MTT assay

Time (h)	Concentration (µg/ml)						
	2000	1000	500	250	125	62.5	31.25
24	75.5 ± 0.25	78.5 ± 0.33	81.4 ± 0.35	83.5 ± 0.33	85 ± 0.55	88.25 ± 0.4	94.6 ± 0.37
48	72.66 ± 0.31	74.33 ± 0.29	78.1 ± 0.31	81 ± 0.4	86.66 ± 0.6	88 ± 0.33	91.33 ± 0.73
72	66.6 ± 0.38	69.8 ± 0.33	72.75 ± 0.44	78.6 ± 0.43	79.75 ± 0.43	83.6 ± 0.58	89 ± 0.55

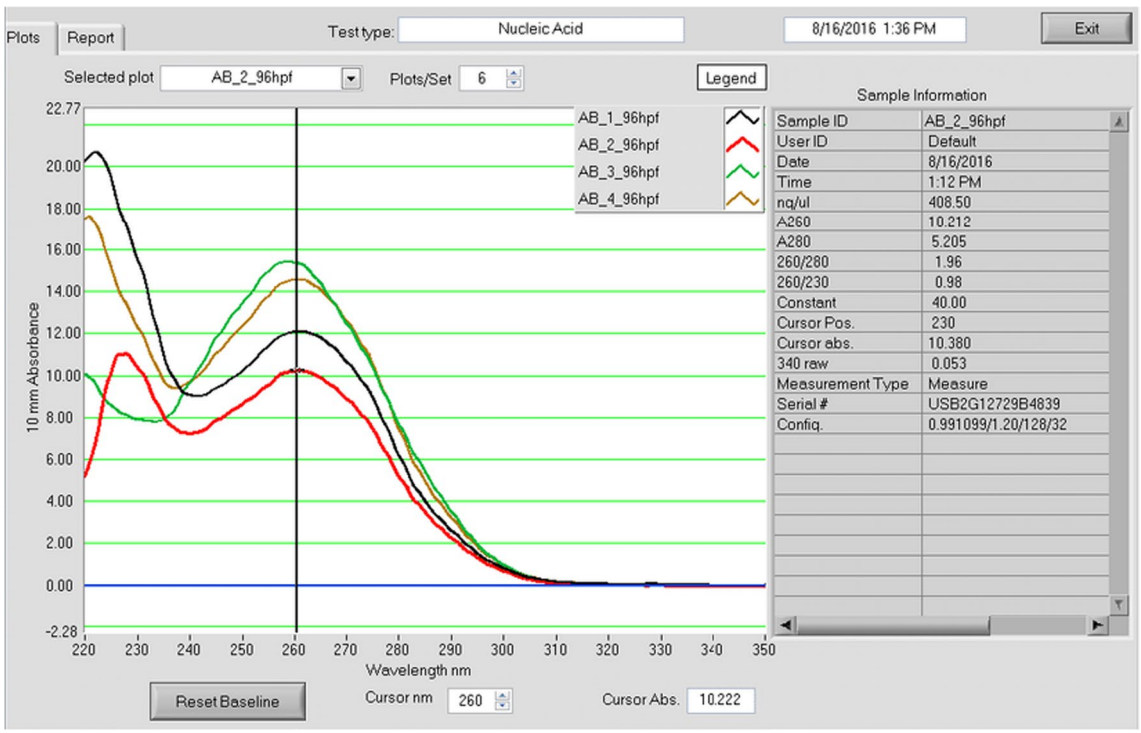

Fig. 4 The results of quantitative analysis of RNA concentrations extracted from the MCF-7 breast cancer cell line using Nanodrop

Table 6 Ct values and fold changes in genes expression

	24-h					48-h					72-h				
	Ct- *Bcl-2*	**Ct-*Bactin***	**Fold change**	**Ct- *Bax***	**Fold change**	**Ct- *Bcl-2***	**Ct-*Bactin***	**Fold change**	**Ct- *Bax***	**Fold change**	**Ct- *Bcl-2***	**Ct-*Bactin***	**Fold change**	**Ct- *Bax***	**Fold change**
Control	24.56	20.43	1	24.76	1	24.78	20.43	1	25.76	1	25.21	20.43	1	24.89	1
Extract	24.91	20.65	0.91383145	24.29	1.613283518	25.21	20.65	0.864537231	24.78	2.265767771	25.99	20.65	0.678302164	23.82	2.445280555
Extract	25.72	21.32	0.829319546	25.11	1.453972517	26.14	21.32	0.721964598	25.63	2.02791896	26.67	21.32	0.673616788	24.21	2.969047141

Real-time PCR results

The melting curve shows that specific amplification of the desired gene fragments occurred well, and the absence of priming and nonamplification of nonspecific fragments for each gene indicates that there is no contamination in the samples. *β-actin* was considered as a control. After cDNA synthesis, a real-time PCR reaction was performed using primers for the *Bax* and *Bcl-2* genes. Ct values were determined based on the amplification plot. Subsequently, gene expression changes were calculated using the Livak method (Table 6).

Expression profile of the studied genes in normal and cancer cells treated with *Dioscorea* extract

Using the Ct values from the real-time PCR reaction of the treated cells and the *β-actin* gene, gene expression analysis was performed using the Livak method. In Table 7 and Fig. 5, the fold change and the changes in the expression of *Bax* and *Bcl-2* genes were calculated, respectively, compared with the reference gene and the control cell. The greatest decrease and increase in gene expression was seen at the 72-h treatment.

The expression of *Bcl-2* and *Bax* genes in 72 h after treatment with the extract has a significant decrease of 0.67 (p value < 0.05) and a significant increase of 2.72 (p value < 0.001), respectively.

The expression of *Bax* and *Bcl-2* genes at 24, 48 and 72 h and the expression of *Bax* and *Bcl-2* genes in MCF-7 cell lines treated with *Dioscorea* extract are shown in Table 7 and Fig. 6.

Results of induced apoptosis at 24, 48 and 72 h

Based on the quadrant plots, it can be seen that the apoptosis induced by the studied plant extract increased in 48 h compared to the control group. After 48 h, the apoptosis induced by IC_{50} dilution of the plant extract was statistically significant in the cell line compared to the control group (p value < 0.001) (Table 8). The difference with the control group was not significant. After 72 h, more primary apoptosis was observed than delayed apoptosis and necrosis (Table 9).

Flow cytometry results in Fig. 7 show the rate of induced apoptosis in HFF cells. The results show that 99.8% of the control cells survived because they did not become apoptotic as a result of treatment with the plant extract. The number of cells showing necrosis during the collection and processing phase is very low, 0.032%. The percentage of cells with primary apoptosis is 0.074% and the percentage of cells with delayed apoptosis is 0.064% (Fig. 7). The results of induction of apoptosis with three replicates in MCF-7 cells treated with IC_{50} dose for 24 h are shown in Fig. 7. The results show that at the IC_{50} concentration of the plant extract (1178.95 µg/ml), 92.9% of the cancer cells remained healthy. In this study, we used two different assays on separate instruments to measure two different parameters. It is important to note that it is very unlikely to get identical results, especially when comparing the percentage of cell populations to the relative absorbance.

The MTT assay was used to assess the metabolic activity of the cells, whereas the annexin V/PI (FACS) assay was used to measure the cell populations stained with a DNA antibody, which allowed the assessment of apoptosis induction and progression. The results do not have to match exactly; rather, it is critical that a similar trend be observed.

From the results of the MTT and FACS cytotoxicity assays, it can be concluded that the cytotoxicity of the extract increases with time. The tendency to cytotoxicity depends on duration, as the degree of cytotoxicity increases with time.

The results of induction of apoptosis with three replicates in MCF-7 cells treated with IC_{50} dose for 48 h are shown in Fig. 8. The results show that at IC_{50} concentration of the plant extract (568.25 µg/ml), 84.6% of cancer cells survived. The percentage of cells affected by necrosis in the collection and preparation phase was 1.12%, the percentage of cells with primary apoptosis was 4.51%, and the percentage of cells with delayed apoptosis was 9.77%. The results of apoptosis induction upon 72-h treatment with the plant extract are shown in Fig. 8. MCF-7 Cells were treated with an IC_{50} concentration of 438.35 µg/ml for 72 h. The results show that 77.5% of the cancer cells survived. Comparison of the three treatments shows that the 72-h treatment has the greatest effect on cancer cell death. The percentage of cells affected by necrosis was 1.94%, and the percentage of cells with primary and delayed apoptosis was 11.2% and 9.29%, respectively.

The amount of primary and delayed apoptosis in the 24-h treatment compared to the control group was 6.63% and the rate of necrosis was 0.477%, which is statistically significant with a p value < 0.001. In the 48-h treatment with the plant extract, the amount of primary and delayed apoptosis compared to the control group was 14.28% and the rate of necrosis was 1.12%, which is statistically significant (p value < 0.001). In the 72-h treatment with the plant extract, the percentage of primary and late apoptosis compared to the control group is 20.49% and the percentage of necrosis is 1.94%, which is statistically significant (p value < 0.001) (Table 8). Therefore, it can be concluded that 72 h treatment with a plant extract in IC_{50} concentration has the greatest effect on killing MCF-7 cancer cells. The rate of primary apoptosis, delayed apoptosis, and necrosis for all three treatments can be seen in Fig. 9 and Table 9.

Table 7 Comparison of *Bax* and *Bcl-2* gene expression in MCF-7 cancer cell lines treated with *Dioscorea* extract at 24, 48 and 72 h

Gene	Time (h)			
	24	48	72	Control
Bax	1.53 ± 0.54 *p value* = 0.064	2.1 ± 0.35 *p* value < 0.01	2.72 ± 0.43 *p* value < 0.001	1.011 ± 0.33
Bcl2	0.87 ± 0.24 *p value* = 0.094	0.79 ± 0.21 *p value* = 0.076	0.67 ± 0.22 *p* value < 0.05	1.018 ± 0.28

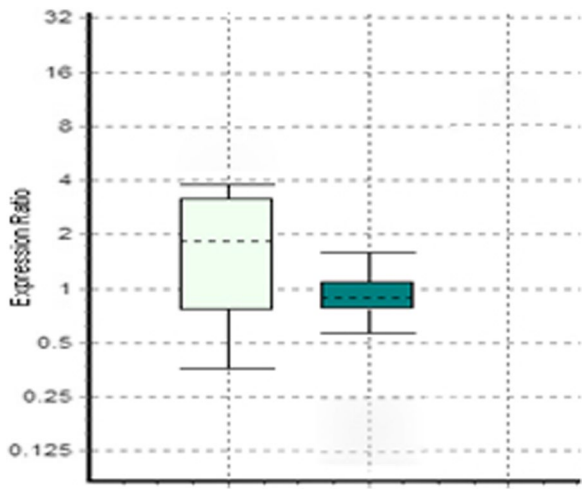

Fig. 5 Fold change of *Bax* (right) and *Bcl-2* (left) genes in 72-h treatment

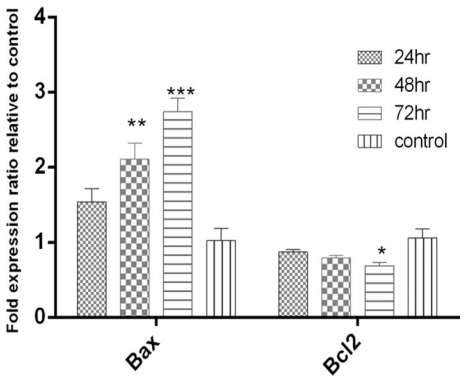

Fig. 6 Expression rate of *Bax* and *Bcl-2* genes in 24-, 48- and 72-h treatment (*p value < 0.05, **p value < 0.01, ***p value < 0.001)

As shown in Table 9, the amount of primary apoptosis and necrosis was not significant at 24- and 48-h treatment. Delayed apoptosis was significant at 24- and 48-h treatment with the plant extract (p value < 0.001). In 72-h treatment with the plant extract, the amount of primary and delayed apoptosis was significant with p value < 0.001, while the amount of necrosis was not statistically significant. Therefore, it can be said that the rate of necrosis is not statistically significant in any of the three treatments. Among the three treatments of 24, 48 and 72 h with the plant extract, the 72-h treatment with the plant extract at a concentration of (IC_{50}) 438.35 µg/ml has the best effect on inducing apoptosis in MCF-7 cell line cells.

Table 8 Comparison of induced apoptosis with necrosis by plant extract in 24-, 48- and 72-h treatment on MCF-7 cell line

	IC50 (24 h)	IC50 (48 h)	IC50 (72 h)
Apoptotic cells	6.63 ± 0.34	14.28 ± 0.29	20.49 ± 0.39
Necrotic cells	0.477 ± 0.31	1.12 ± 0.36	1.94 ± 0.35
p value	*p* value < 0.001	*p* value < 0.001	*p* value < 0.001

Discussion

When treated with the plant extract for 72 h, *Bax* gene expression increased by 2.72, which is statistically significant (p value < 0.001). Also, *Bcl-2* gene expression decreased 0.67-times, which is significant at a p value < 0.05. Therefore, it can be concluded that 72 h treatment with the plant extract is the most effective treatment for the death of MCF-7 cancer cells. The ratio of *Bax* gene expression to *Bcl-2* is 4.06.

The flow cytometry results also show that the 72-h treatment with the plant extract is the most effective treatment. The amounts of apoptosis induced at 24, 48, and 72 h are 6.63, 14.28, and 20.49, respectively. The amounts of necrosis induced in these treatments are 0.477, 1.12, and 1.94, respectively. In the healthy cell line, the values of apoptosis induced and necrosis induced are 0.318 and 0.032, respectively. This means that the treatment with the plant extract induced apoptosis and necrosis in cancer cells but had no effect on healthy cells. Therefore, 72-h treatment with a plant extract at a concentration of 438.35 µg/ml is the most effective treatment for the death of MCF-7 cancer cells, and the amount of primary and delayed apoptosis induced was significant (p value < 0.001).

In the following, we compare the results of the present study with those of previous studies.

Our results are in agreement with those of Saekoo et al. [19] and Saekoo et al. [20]. In the present study, *Bax* gene expression increased 2.72-times and *Bcl-2* gene expression decreased 0.67-times under the influence of 72-h treatment with the plant extract.

The results of this study are consistent with those of Jeonghun et al. [22]. In the present study, the expression of *Bax* gene increased and the expression of *Bcl-2* gene

Table 9 Evaluation of apoptosis induced by plant extract in 24-, 48- and 72-h treatment on MCF-7 and HFF cell lines

	Control	24 (h)	48 (h)	72 (h)
Early Apoptosis	0.074 ± 0.18	1.29 ± 0.23 *p* value = 0.089	4.51 ± 0.32 *p* value = 0.12	11.2 ± 0.38 *p* value < 0.001
Delayed Apoptosis	0.064 ± 0.23	5.34 ± 0.27 *p* value < 0.001	9.77 ± 0.35 *p* value < 0.001	9.29 ± 0.31 *p* value < 0.001
Necrosis	0.032 ± 0.25	0.477 ± 0.29 *p* value = 0.067	1.12 ± 0.26 *p* value = 0.087	1.94 ± 0.27 *p* value = 0.095

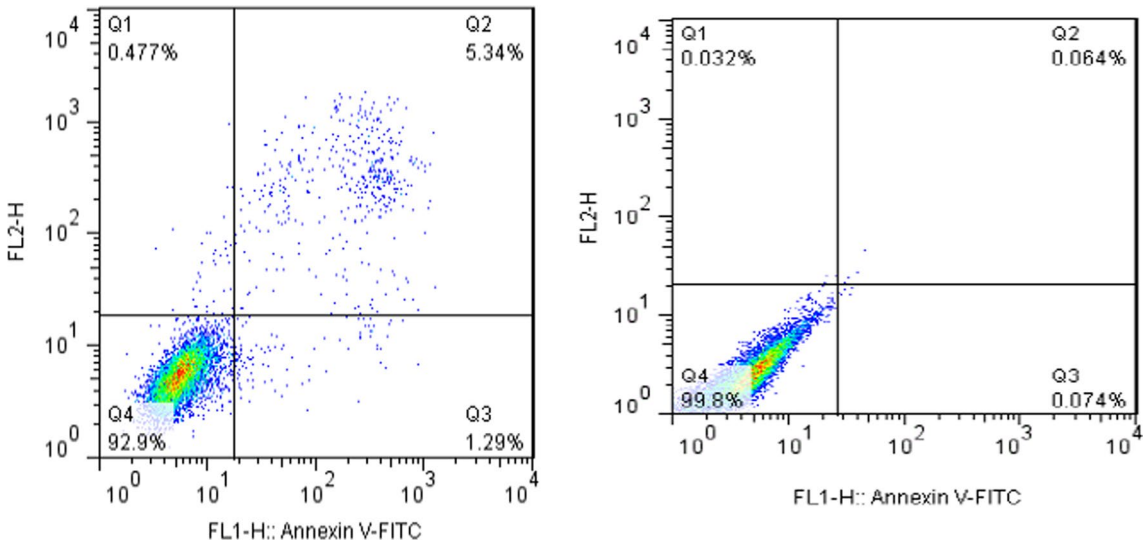

Fig. 7 Results from the study of induction of apoptosis in control cells (left side) and in MCF-7 cells treated with IC_{50} concentration of *Dioscorea* extract in 24 h (right side)

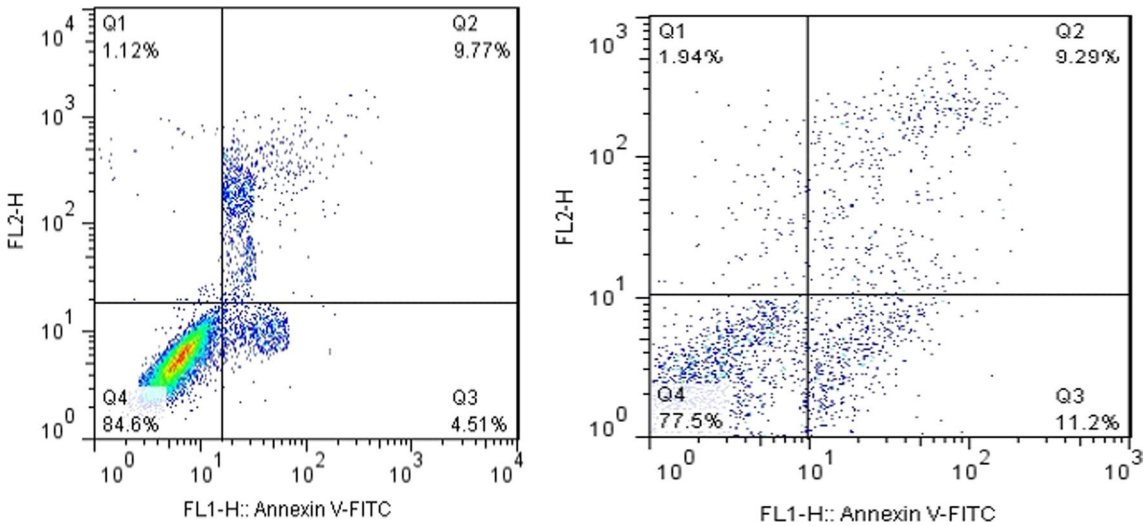

Fig. 8 Results of apoptosis induction in MCF-7 cells treated with IC_{50} concentration of *Dioscorea* extract in 48 h (right side) and 72 h (left side)

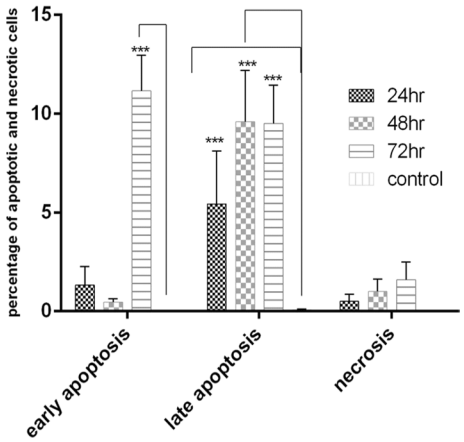

Fig. 9 Comparison of delayed apoptosis, primary apoptosis and necrosis in 24-, 48- and 72-h treatment with plant extract (*p value < 0.05, **p value < 0.01***, p value < 0.001)

decreased under the influence of 72-h treatment with the plant extract.

Our results are in agreement with those of Mainasara et al. [14]. In the present study, apoptosis was shown to be induced in MCF-7 cells treated with the extract. The results of this study are also in agreement with those of Nantana et al. [21]. In the present study, after 72 h of treatment with the plant extract, the expression of *Bax* gene increases and the expression of *Bcl-2* gene decreases.

Conclusion

According to the obtained results, *Dioscorea rhizoma* extract can be proposed as a therapeutic agent in cancer treatment, which of course requires further studies.

Acknowledgements
The authors of the article thank everyone who contributed to the research.

Author contributions
FB conceptualized and wrote the manuscript. MK wrote the manuscript and did the necessary editing and supervised. FJ helped in the editing of the manuscript. All authors read and approved the final manuscript.

Funding
Not applicable.

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
The ethics ID of this research is IR.IAU.SRB.REC.1399.121.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 28 November 2022 Accepted: 6 October 2023
Published online: 01 November 2023

References

1. Lukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A (2021) Breast cancer-epidemiology, risk factors, classification, prognostic markers, and current treatment strategies: an updated review. Cancers (Basel) 13(17):4287. https://doi.org/10.3390/cancers13174287
2. Kazeminia M, Salari N, Hosseinian-Far A, Akbari H, Bazrafshan MR, Mohammadi M (2022) The prevalence of breast cancer in Iranian women: a systematic review and meta-analysis. Indian J Gynecol Oncolog 20:14. https://doi.org/10.1007/s40944-022-00613-4
3. Alizadeh M, Ghojazadeh M, Piri R, Mirza-Aghazadeh-Attari M, Mohammadi S, Naghavi-Behzad M (2021) Age at diagnosis of breast cancer in Iran: a systematic review and meta-analysis. Iran J Public Health 50(8):1564–1576
4. American Cancer Society. Breast Cancer Facts & Figures 2019–2020. Atlanta: American Cancer Society, Inc. (2019). https://www.google.com/amp/s/amp/.cancer.org/research/cancer-facts-statistics/breast-cancer-facts-figures.html
5. Brown G (2021) Oncogenes, proto-oncogenes, and lineage restriction of cancer stem cells. Int J Mol Sci 22(18):9667. https://doi.org/10.3390/ijms22189667
6. Qian S, Wei Z, Yang W, Huang J, Yang Y, Wang J (2022) The role of BCL-2 family proteins in regulating apoptosis and cancer therapy. Front Oncol 12(12):985363. https://doi.org/10.3389/fonc.2022.985363
7. Dehghani Nazhvani A, Sarafraz N, Askari F, Heidari F, Razmkhah M (2020) Anti-cancer effects of traditional medicinal herbs on oral squamous cell carcinoma. Asian Pac J Cancer Prev 21:479–484. https://doi.org/10.31557/APJCP.2020.21.2.47.2.479
8. Ali M, Wani SUD, Salahuddin M, Sn M, Mruthunjaya K, Dey T, Zargar MI, Singh J (2023) Recent advance of herbal medicines in cancer: a molecular approach. Heliyon 9(2):e13684. https://doi.org/10.1016/j.heliyon.2023.e13684
9. Noorbazargan H, Amintehrani S, Dolatabadi A, Mashayekhi A, Khayam N, Moulavi P, Naghizadeh M, Mirzaie A, Mirzaei Rad F, Kavousi M (2021) Anti-cancer & anti-metastasis properties of bioorganic-capped silver nanoparticles fabricated from *Juniperus chinensis* extract against lung cancer cells. AMB Express 11(1):61. https://doi.org/10.1186/s13568-021-01216-6
10. Obidiegwu JE, Lyons JB, Chilaka CA (2020) The *Dioscorea* Genus (Yam): an appraisal of nutritional and therapeutic potentials. Foods 9(9):1304. https://doi.org/10.3390/foods9091304
11. Kanu AN, Ezeocha CV, Nwamaka P (2018) A review on bioactive compounds of yam varieties for human disease management. Asian Food Sci J 1(4):1–10. https://doi.org/10.9734/AFSJ/2018/40473
12. Zeng M, Ren Y, Zhang B, Wang S, Liu M, Jia J, Guo P, Zhang Q, Zheng X, Feng W (2021) In vitro non-small cell lung cancer inhibitory effect by new diphenylethane isolated from stems and leaves of *Dioscorea oppositifolia* L. via ERβ-STAT3 pathway. Front Pharmacol 12:622681. https://doi.org/10.3389/fphar.2021.622681
13. Odimegwu JI, Odukoya OA, Español A, Sales ME (2020) Human breast tumour cells viability effect of African *Dioscorea rotunata* tuber extravts in MCF-7 and MDA-MB231 cell lines. BioRxiv. https://doi.org/10.1101/2020.05.08.084269
14. Mainasara MM, Abu Bakar MF, Md Akim A, Linatoc AC, Abu Bakar FI, Ranneh YKH (2021) Secondary metabolites, antioxidant, and antiproliferative activities of *Dioscorea bulbifera* leaf collected from Endau Rompin, Johor, Malaysia. Evid Based Complement Altern Med 11(2021):8826986. https://doi.org/10.1155/2021/8826986
15. Trimanto TA, Hapsari L (2015) Diversity and utilization of *Dioscorea* Spp. tuber as alternative food source in Nganjuk Regency. East Java. Agrivita 37(2):97–107. https://doi.org/10.17503/agrivita.v37i2.504
16. Salehi B, Sener B, Kilic M, Sharifi-Rad J, Naz R, Yousaf Z, Mudau FN, Fokou PVT, Ezzat SM, El Bishbishy MH, Taheri Y (2019) Dioscorea plants: a genus rich in vital nutra-pharmaceuticals: a review. Iran J Pharm Res 18(1):68–89. https://doi.org/10.22037/ijpr.2019.112501.13795
17. Li HB (2019) Restorative effect of modified dioscorea pills on the structure of hippocampal neurovascular unit in an animal model of chronic

cerebral hypoperfusion. Heliyon 5:e01567. https://doi.org/10.1016/j.heliyon.e01567
18. Zeng M, Zhang L, Li M, Zhang B, Zhou N, Ke Y, Feng W, Zheng X (2018) Estrogenic effects of the extracts from the Chinese Yam (*Dioscorea opposite* Thunb.) and its effective compounds in vitro and in vivo. Molecules 23(2):11. https://doi.org/10.3390/molecules23020011
19. Saekoo J, Dechsukum C, Graidist P, Itharat A (2010) Cytotoxic effect and its mechanism of dioscorealide B from *Dioscorea membranacea* against breast cancer cells. J Med Assoc Thai 93(suppl.7):S277–S282
20. Saekoo J, Graidist P, Leeanansaksiri W, Dechsukum C, Itharat A (2010) Dioscorealide B from the traditional Thai medicine Hua-Khao-Yen induces apoptosis in MCF-7 human breast cancer cells aviation of Bax, Bak and bcl2 protein expression. Nat Prod Commun 5(12):1921–1926. https://doi.org/10.1177/1934578X1000501217
21. Nantana J, Parisatcha S (2016) Antiproliferation of fibrosarcoma cell line (L929) by Hua-Khao-Yen-Tai Thai medicinal plant (*Dioscorea membranacea* Pierre). J Biol Sci 16:236–241. https://doi.org/10.3923/jbs.2016.236.241
22. Jeonghun H, Wanyoung L, Dueun Y (2019) Chemoresistance in the human triple-negative breast cancer cell line MDA-MB-231 induced by doxorubicin gradient is associated with epigenetic alterations in histone deacetylase. J Oncol. https://doi.org/10.1155/2019/1345026

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen journal and benefit from:

► Convenient online submission
► Rigorous peer review
► Open access: articles freely available online
► High visibility within the field
► Retaining the copyright to your article

Submit your next manuscript at ► springeropen.com