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Original scientific article

Solanum pseudo-capsicum EFFECTS ON *Bax* AND *Bcl-2* GENE EXPRESSION AND APOPTOSIS IN MCF-7 CELL LINE

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In the present study, the effect of *Solanum pseudo-capsicum* extract on MCF-7 cell line was investigated. Control cell line in this study was HFF skin fibroblast cell line. Cells have been exposed to the treatment for 24h, 48h and 72 hours respectively. Then, the viability of dual cell lines was assessed using MTT assay. Its results showed that 72-hour treatment with plant extract at IC_{50} concentration had the greatest effect on cancer cell death. Flow cytometry results showed that 48 hours of treatment with plant extract at IC_{50} concentration, the highest rate of induced apoptosis occurs. Then, Real time PCR test was performed to measure changes in the expression of *Bax* and *Bcl-2* genes The reference gene in this study was *β-actin*. The results of this study showed that in cancer cells treated with the plant extract, the expression of *Bax* pro-apoptotic gene increased and the expression of anti-apoptotic gene *Bcl-2* decreased. Therefore, 72-hour treatment had the greatest effect on increasing *Bax* gene expression and decreasing *Bcl-2* gene expression. Due to the lower side effects of plants compared to conventional chemical drugs used in the treatment of cancer, it is possible to take an effective step in the treatment of this disease by using new methods in identifying effective plant compounds and purifying them.

Keywords: Solanum pseudo-capsicum, MCF-7, *Bax* and *Bcl-2*, MTT assay, Real time PCR, Flow cytometry.

INTRODUCTION

Cancer is a multi-stage process that has a long onset and is rapidly progressing. This fatal lesion is caused by the gradual occurrence of several mutations in genes that control vital cell pathways, including growth, development, and apoptosis. These mutations cause the production of tumor cell masses. By creating traits such as escaping apoptosis and metastasis, the ability to reproduce spontaneously and ignore cancer growth factors is created (DEMBIC, 2020). Apoptosis is the genetically programmed death of a cell that plays an important role in many

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physiological and pathological conditions. *Bax* (pro-apoptotic gene) and *Bcl-2* (anti-apoptotic gene) genes are important genes involved in the mitochondrial pathway of apoptosis. There is a direct relationship between changes in the expression of these genes and the cancerogenesis. In apoptosis induced by various factors in the internal pathway of apoptosis, Bax protein is a key protein. Bcl-2 has an anti-apoptotic effect in response to various apoptotic stimuli by preventing the release of cytochrome C from the mitochondria (KORDEZANGENEH *et al*., 2015; SHARMA *et al*., 2018). In cancer cells, the balance between proliferation and apoptosis is lost. Cancer cells inhibit apoptosis for their abnormal growth and proliferation (ZHANG *et al*., 2018). There have been many advances in cancer treatment recently, but cancer treatment still remains an important challenge. Significant evidence from chemical studies and cell culture research shows that some herbal supplements may slow or prevent cancer in the early prevention (KAVOUSI *et al*., 2019; KAVOUSI *et al*., 2020; NOORBAZARGAN *et al*., 2021). Solanaceae plant extract has antitumor effects and induction of apoptosis. Solanine is one of the compounds of plants of this family, a kind of alkaloid. Alkaloids have an inhibitory effect on tumor cell growth due to their cytotoxic effects (SUN *et al*., 2014). Another compound in plants of the family Solanaceae is solamargin, which has been shown to play an effective role in increasing apoptosis in cancer cells in various studies (ZHANG *et al*., 2018; BURGER *et al*., 2018). Recent studies have shown that the composition of Canavanine in the root of *Solanum pseudo-capsicum* acts as an inhibitor for nitric oxide synthase in mammals and it is also considered as a factor that changes the concentration of nitric oxide in plants. Canavanine increases RNA and protein levels and its high concentrations causes DNA fragmentation. This compound also causes reactive oxygen species to accumulate in the cells (KRASUSKA *et al*., 2016). One of the factors involved in the development of cancer is a disorder in the apoptotic pathway. On the other hand, the widespread use of common anticancer drugs also causes therapeutic resistance so that it limits other treatment options. Research has shown that compounds of plant origin are of particular importance due to their low side effects (ZHANG *et al*., 2018; HUANG *et al*., 2018). Therefore, the study of changes in gene expression due to treatment with plant extracts can be one of the therapeutic in cancer studies. In this study, the expression of *Bax* and *Bcl-2* genes in MCF-7 cell line was changed by treatment with *Solanum pseudo-capsicum* extract by Real time PCR. Then the induced apoptosis and necrosis were evaluated by flow cytometry. It seems that no study has been done on the effect of *Solanum pseudo-capsicum* extract on MCF7 cell line in the world.

MATERIALES AND METHODS

Extraction method

Solanum pseudo-capsicum plant was prepared from the plant bank of Iran Biological Resources Center with each barium number 1342. To prepare the extract, the shoots of *Solanum pseudo-capsicum* were first blown into air and then dried in the shade. The leaves were thoroughly pulverized by an electric grinder and stored in glass containers. The prepared powder was used for extraction by Soxhlet method. In order to prepare the extract, first 10 g of *Solanum pseudo-capsicum* leaf powder is added to 100 cc of 70% ethanol and after stirring, it is boiled for 90 minutes at 90° C. The filtered extract is kept in a watch glass container and in an incubator to remove the remaining alcohol. In this way, the extract is obtained. From the extract obtained using PBS, the concentrations required for the treatment of the cells used are prepared.

Cell line preparation and cell culture

In this study, breast cancer cell line (MCF-7) with IBRC number C10071 and control cell line (HFF skin fibroblast) were prepared from the National Center for Genetic and Biological Resources of Iran and RPMI1640 medium was used for cell culture. Culture of two cell lines were performed at 24, 48 and 72 hours in three plates. Three replicates were considered in each plate for each concentration. A 96-well plate is used for cell culture. The cells were separated from the bottom of the flask using trypsin (Similar to the steps of cell passage). At the end, instead of using culture medium containing 10% FBS, one milliliter of culture medium containing five percent FBS was added to the cell sediment and pipetted. 100 microliters of 5% FBS culture medium containing 10,000 cells was poured into each well. The plate was placed in a CO_2 incubator at 37° C for 24 hours so that the cells adhere to the bottom of the wells.

Add the plant extract to the cell culture

As mentioned before, different concentrations were prepared by serial dilution method. Culture of two cell lines were performed at 24, 48 and 72 hours in three plates. After 24 hours of incubation, three plates were taken out of the incubator. The culture medium was emptied. Then, 100 microliters of the concentrations made with three repetitions were added to the wells. Then the plate was incubated for 24, 48 and 72 hours and the effect of cytotoxicity was measured after the treatment.

MTT assay

To prepare MTT solution with a concentration of 5 mg/ml, 50 mg of MTT powder was dissolved in 10 ml of PBS. When used in staining, it was diluted 10 times with PBS to obtain a MTT solution of 0.5 mg/ml. At this stage, 100 microliters of the prepared solution were added to each well. The plate was placed in a CO_2 incubator at 37° C for three hours. After leaving the incubator, the solution was emptied. Then, 100 µl of DMSO was added to each well to dissolve the produced formazan crystals and the plate was placed at room temperature for 30 minutes to dissolve the formazan well. Then the absorbance (OD) was measured by Elisa Reader at a wavelength of 570 nm. In this assay, the lower the read uptake compared to the control, the more it can be concluded that the number of viable cells is reduced and growth is more inhibited. In this assay, the lower the read uptake compared to the control state, it can be concluded that the number of viable cells is reduced and growth is more inhibited. Then the IC_{50} (represents the concentration at which a substance exerts half of its maximal inhibitory effect) value was determined in three treatments.

RNA quantification and cDNA synthesis

The amount and concentration of RNA produced were evaluated by nanodrop (NanoDrop Technologies, Wilmington, DE, USA). The Revert AidTM First Strand cDNA Synthesis Kit (Fermentas) was used to make the cDNA.

Evaluation of changes in expression of Bax and Bcl-2 genes

The primer sequence of the studied genes is shown in Table 1. Real Time PCR was used to evaluate the expression of *Bax* and *Bcl-2* genes relative to *β-actin* reference gene. Real

Time PCR reaction was performed with Bioneer exicycler 96 according to the following protocol. Primary and Secondary denaturation was performed in 95° C for 10 minutes and 20 seconds, respectively. Annealing was performed in 57° C for 40 seconds. The data were then analyzed using the device's own software to examine gene expression.

Table 1. Sequence of used primers

Gene	Primer pair sequences	Amplicon length (bp)	TM (ºC)	%CG	Accession N
B-actin	F: 5′-TCCTCCTGAGCGCAAGTAC-3′	19	59.11	57.89	N.M_001101.5
	R: 5-′CCTGCTTGCTGATCCACATCT-3′	21	60.41	52.38	
Bax	F: 5-'GAGCTGCAGAGGATGATTGC-3′	20	59.05	55.00	N.M_138764.5
	R: 5-'AAGTTGCCGTCAGACATG-3'	21	57.65	42.86	
Bcl-2	F: 5-'ATTGGGAAGTTTCTCAGC-3′	21	54.98	38.10	N.M_000657.3
	R: 5-'CAGTCTACTTCCTCTGTGATGTTG-3'	24	58.83	45.83	

Evaluation of apoptosis by flow cytometry by Annexin-V kit

The Annexin V-FITC kit (eBioscience, USA Affymetrix) was used for this purpose. Annexin-V was used as a marker to identify apoptotic cells in the present study. Therefore, the rate of apoptosis and necrosis of two cancer cell lines and control treated with plant extract of *Solanum pseudo-capsicum* and with two colors Annexin-V and propidium iodide (PI) were compared. The cells were treated with IC_{50} concentration of plant extract in treatments of 24, 48 and 72 hours for flow cytometry.

Statistical analysis of data

Statistical calculation of this study was performed using SPSS 16 software and the results were analyzed by one way ANOVA. The difference in expression of target genes between control and treated samples was calculated. Information was considered as mean ± standard deviation (SD) and P-value <0.05 was considered significant. After performing Real Time PCR reaction using Ct obtained using Rest 2009 software method, ΔΔCt of each sample was calculated.

RESULTS

Extraction

Extraction of *Solanum pseudo-capsicum* was done by Soxhlet method. After determining the concentration of the extract and the determined concentrations were prepared using RPMI 1640 culture medium and dilution.

Treatment with plant extract for 24, 48 and 72 hours

Examination of Figures 1, 2 and 3 show that the plant extract did not cause the death of healthy cells in the control cell line. As the concentration of the extract increases, the survival rate of cancer cell cells decreases. In MCF-7, the IC_{50} concentration of the plant extract in the 24-hour treatment was 432.5 µg/ml (Figure 1). In MCF-7, the IC_{50} value was obtained at 48

hours in MCF-7 cancer cell line at a concentration of 186.4 µl/ml (Figure 2). The IC_{50} value was obtained in the 72-hour MCF-7 treatment at 102 µl/ml (Figure 3).

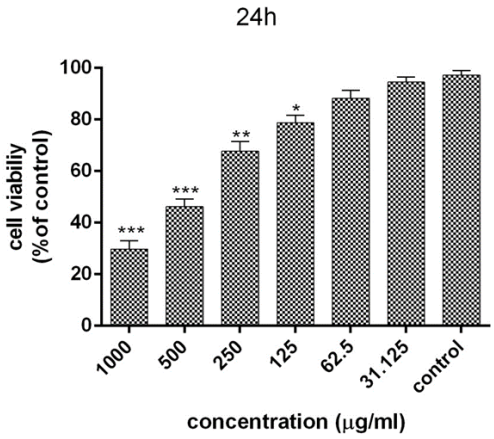

Figure 1. Effect of *Solanum pseudo-capsicum* leaf extract on cell viability at different concentrations in 24 hours treatment (P-value<0.05 * ،P-value<0.01** ،P-value<0.001 ***)

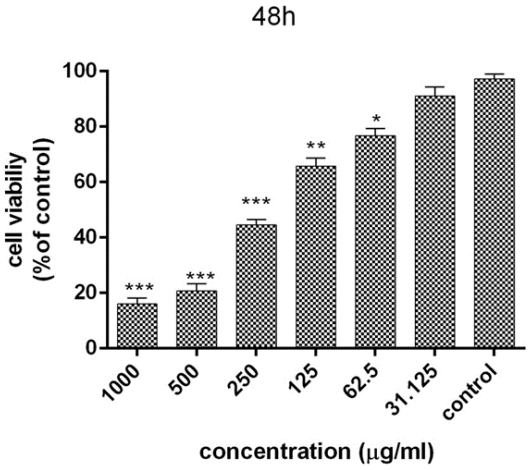

Figure 2. Effect of *Solanum pseudo-capsicum* leaf extract on cell growth at different concentrations in 48 hours treatment (P-value<0.05 * ،P-value<0.01** ،P-value<0.001 ***)

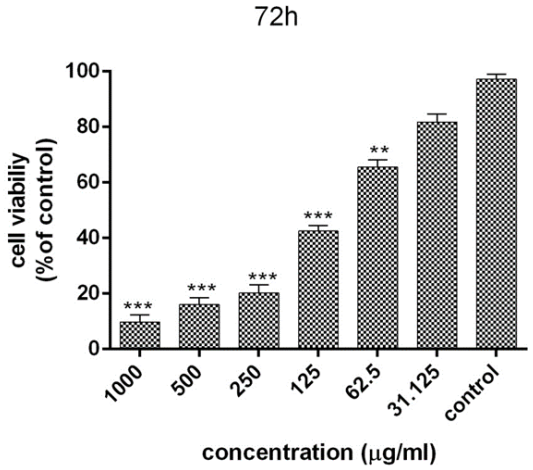

Figure 3. Effect of *Solanum pseudo-capsicum* leaf extract on cell growth at different concentrations in 72 hours treatment (P-value<0.05 * ،P-value<0.01** ،P-value<0.001 ***)

According to Table 2, it can be seen that 72-hour treatment with *Solanum pseudo-capsicum* extract has the greatest effect on MCF-7 cell line cell death.

Table 2. IC_{50} concentrations in three treatments of 48, 24 and 72 hours with plant extract

Duration of treatment (h)	IC_{50} (µg/ml)
24	432. 5
48	186.4
72	102

Table 3. Cell viability following application of different extract concentrations, after 24,48 and 72 hours

Concentration µg/ml / Duration of treatment h	31.25	62.5	125	250	500	1000
24	95.25±0.28	88.75±0.32	78.25±0.46 P-value<0.05	67±0.62 P-value<0.01	47.5±0.28 P-value<0.001	29.4±0.28 P-value<0.001
48	92.5±0.88	77.75±0.78 P-value<0.05	63±0.28 P-value<0.01	44.2±0.58 P-value<0.001	21±0.36 P-value<0.001	16.1±0.36 P-value<0.001
72	81.25±0.35	66±0.47 P-value<0.01	42.6±0.62 P-value<0.001	21.75±0.38 P-value<0.001	17.75±0.47 P-value<0.001	10.75±0.47 P-value<0.001

Statistical studies related to 24-, 48- and 72-hour treatments with plant extract P-value <0.01 are shown in Table 2. According to Table 3, at a concentration of 31.25 µg / ml in 24-, 48- and 72-hour treatments, 95.25%, 92.25% and 81.25% of the cells are alive, respectively

which are not statistically significant. MCF-7 cell viability decreases with increasing concentration. Reduction of cell viability at 62.5 µg/ml concentration in 24 hours treatment is statistically significant and in the other two treatments is significant. But in all three treatments at higher concentrations, the reduction in cell viability was significant.

Flow cytometry in treatment of 48 and 72 hours

Flow cytometry results in Figure 4 show that 96% of HFF cell line cells are viable. Because most of them did not give apoptosis due to treatment with plant extract. The number of cells that underwent necrosis due to collection and preparation steps is 3.73% Which is insignificant compared to the percentage of living and healthy cells. The number of cells with primary and delayed apoptosis is 0.189% and 0%, respectively. In 48-hour treatment at a concentration of 186.43 µg /ml of IC_{50} plant extract in MCF-7 cancer cell line compared to healthy HFF cell line, the rate of induced apoptosis was statistically significant (P-value <0.001). But the ratio of necrosis in MCF-7 cancer cell line was not significant compared to healthy HFF cell line. In 48-hour treatment, more primary apoptosis was seen than delayed apoptosis and necrosis (Figure 5). Also, comparison of induced apoptosis with necrosis showed that there was a significant increase in induction of apoptosis compared to necrosis in 48 hours (P-value <0.01).

Flow cytometry results in Figure 6 show the expected survival of 96.1% of HFF cell line cells. Because most of them are not involved in apoptosis. The percentage of cells with primary and delayed apoptosis is 0.189% and 0%, respectively. 3.73% of cells have necrosis due to collection and preparation steps, which is insignificant compared to healthy and living cells.

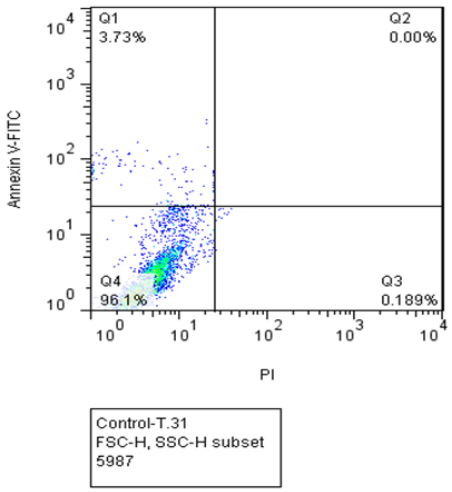

Figure 4. Induction of induced apoptosis in control cells

For 72 hours of treatment with plant extract, a concentration of 102 µg/ml was used. The rate of apoptosis and necrosis induced by the studied plant extract in 72 hours in MCF-7 cancer cell line compared to healthy HFF cell line showed a significant increase (P-value

<0.001) (Figure 7). Results of induction of apoptosis after three replications in MCF-7 cell line cells treated with IC_{50} dose for 72 hours, shown in Figure 7. After 72 hours of treatment with IC_{50} concentration of plant extract, the percentage of cells that have necrosis in the collection and preparation stages. 14.4%, the number of cells with primary apoptosis is 17.1% and the number of cells with delayed apoptosis is 23.5%. The survival rate of MCF-7 cells after 72 hours of treatment with IC_{50} concentration of plant extract is 45.2%.

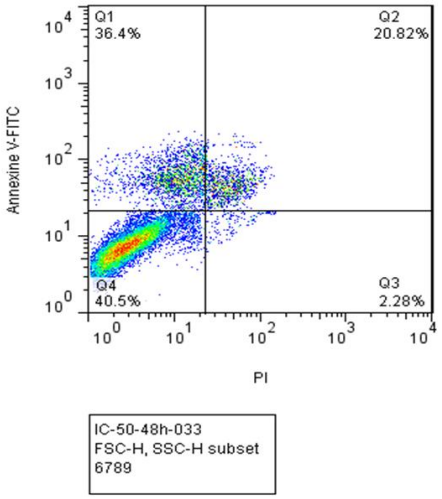

Figure 5. Induction of apoptosis in MCF-7 cells treated with plant extract at IC_{50} concentration in 48 hours

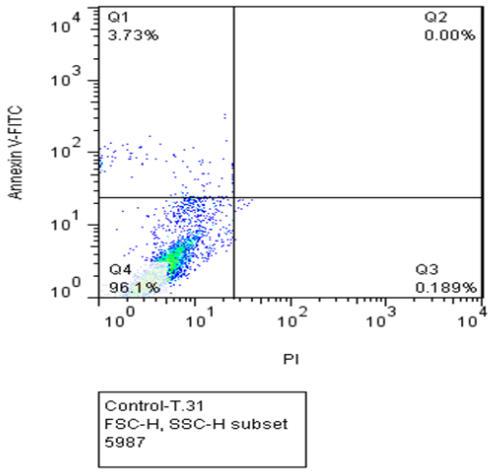

Figure 6. Induced apoptosis in control cells in 72 h treatment

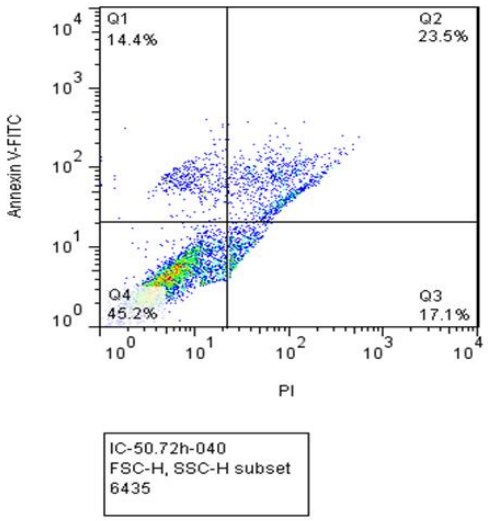

Figure 7. Induction of apoptosis in MCF-7 cell line cells treated with plant extract at IC_{50} concentration for 72 hours

As shown in Figure 8 and Table 4, a significant increase in apoptosis was observed compared to the control group. So that the rate of primary apoptosis in 48 hours compared to the time of 72 hours and the control group showed a significant difference (P-value<0.001). Delayed apoptosis at 72 hours compared to 48 hours and the control group showed a significant difference (P-value<0.001) and necrosis was significantly different in 72 hours compared to 48 hours in the control group (P-value<0.001). Finally, it can be said that the most apoptosis was induced in 48 hours of treatment with plant extract and the most induced necrosis occurred in 72 hours of treatment with plant extract. Therefore, the best time to induce apoptosis is 48 hours and the best time to induce lethality is 72 hours.

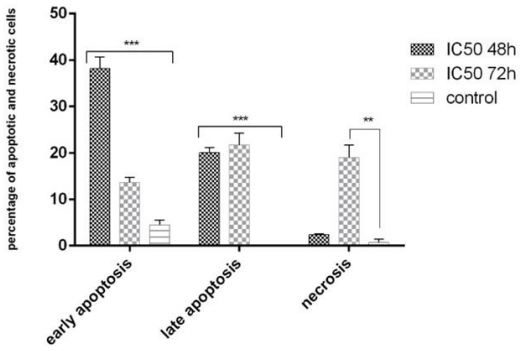

Figure 8. Comparison of necrosis and apoptosis induced by plant extracts at 48 and 72 hours

Table 4. Comparison of delayed apoptosis, primary apoptosis and necrosis in 48- and 72-hours treatment with plant extract

	Control	IC_{50} (48 h)	IC_{50} (72 h)	P- Value
Early apoptosis	4.48±1.06	38.15±2.47	13.6±1.13	<0.001
Late apoptosis	0.12±0.08	20.05±1.08	21.75±2.47	<0.001
Necrosis	0.71±0.73	2.39±0.163	19.00±2.68	<0.01

The amount of primary and delayed apoptosis in 48-hour treatment with plant extract at IC_{50} concentration, compared with the control group, is statistically significant (P-value < 0.001). The graph shows that the amount of primary apoptosis is about 38% and the amount of delayed apoptosis is 20%. The amount of necrosis caused by 48-hour treatment with plant extract at IC_{50} concentration was not significant compared to the control group.

The amount of primary and delayed apoptosis in 72-hour treatment with plant extract at IC_{50} concentration, compared with the control group, had a significant relationship (P-value <0.001). Figure 8 shows that the amount of primary apoptosis is about 14% and the amount of delayed apoptosis is about 22%. The amount of necrosis in 72-hour treatment with plant extracts at IC_{50} concentrations was significant compared to the control group and was about 18% (Figure 8).

Real Time PCR

The amount of increased *Bax* gene expression showed a significant difference compared to the control cell line that the difference was obtained for 24 hours treatment (P-value <0.05) and for 48- and 72-hours treatments (P-value <0.01). Expression of *Bcl-2* anti-apoptotic gene in treatments performed at 48 and 72 hours showed decreased expression and in the period of 72 hours after treatment, the lowest expression was observed. Also, the amount of reduction was significant compared to the control cell line, which was significant in 48 hours (P-value <0.05) and 72 hours (P-value <0.01). Also, the amount of reduction was significant compared to the control cell line, which was significant in 48 hours (P-value <0.05) and 72 hours (P-value <0.01) (Table 5).

Table 5. Results of comparing the effect of plant extract on the expression of Bax and Bcl-2 genes in cancer and control cells

Treatment h / Gene	24	48	72	Control
Bax	1.76±0.85 P-value<0.05	2.46±0.18 P-value<0.01	2.89±0.24 P-value<0.05	1.011±0.33
Bcl2	0.88±0.24 P-value=0.087	0.65±0.21 P-value<0.05	0.59±0.22 P-value<0.01	1.018±0.28

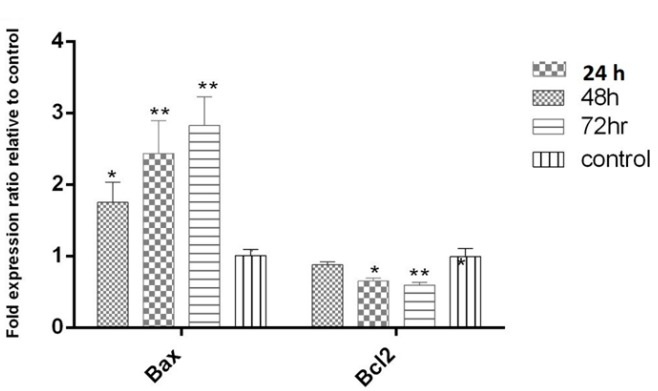

Figure 9. Expression of *Bcl-2* anti-apoptotic gene and *Bax* apoptotic gene in 24-, 48- and 72-hour treatments with plant extract (P-value<0.01** P-value<0.05 *)

According to Figure 9, it can be said that the expression level of the reference gene is always constant and equal to 1. In 24-hour treatment with plant extract, *Bax* gene expression increased by about 1.8 times and *Bcl-2* gene expression decreased by about 0.9 times. In 48-hour treatment with plant extract, *Bax* gene expression increased about 2.5 times and *Bcl-2* gene expression decreased about 0.65 times. In 72-hour treatment with plant extract, *Bax* gene expression increased by about 3.9 times and *Bcl-2* gene expression decreased by about 0.6 times, which are statistically significant. If changes in *Bax* and *Bcl-2* gene expression ratio are considered in 24-, 48- and 72-hour treatments with *Solanum pseudo-capsicum* extract, it turns out that this ratio increases with increasing hours of treatment with plant extract at IC_{50} concentration. In 24-hour treatment with plant extract at IC_{50} concentration, the ratio of *Bax* gene expression to *Bcl-2* is equal to 2. In 48-hour and 72-hour treatment with plant extract at IC_{50} concentration, this ratio is 3.84 and 4.67, respectively. Comparing this value, it can be concluded that 72-hour treatment has the greatest effect on increasing *Bax* expression and decreasing *Bcl-2* expression.

DISCUSSION

According to the results obtained in this study, *Solanum pseudo-capsicum* extract has the effect of apoptosis on MCF-7 cell line and increases the expression of apoptotic gene and decreases the expression of anti-apoptotic gene. The results of this study are compared with similar previous research. Ding et al. examined induction of apoptosis in human hepatoma SMMC-7721 cells by solamargine from *Solanum nigrum*. SM significantly inhibited the growth of SMMC-7721 and HepG2 cells and induced cell apoptosis. Cell cycle analysis revealed that SM caused cell cycle arrest at the G2/M phase. Moreover, SM could up-regulate the expression of *caspase-3* (Ding et al., 2013). In the present study, the highest rate of apoptosis was observed in MCF-7 cancer cells under 48-hour treatment with *Solanum pseudo-capsicum* extract at IC_{50} concentration and they had the highest rate of necrosis after 72 hours of treatment with this extract. Hawary et al. investigated the effect of solasudin combination from *Solanum mauritianum*. After performing cytotoxic reactions, the researchers concluded that increasing the

concentration of the extract reduced the viability of MCF-7 cells. Flow cytometry results showed that the highest rate of apoptosis was observed in 48-hour treatment (HAWARY *et al*., 2015). Lee et al. studied saponins from *Solanum torvum* and *S. macaonense* and their cytotoxic and anti-allergic effects. Spirostanol saponins, 1−6 and 18−19 (normal-type F ring), isolated from *Solanum torvum* and *S. macaonense* showed anti-metastatic and anti-inflammatory effects, especially 2 and 18 could be chosen as the promising candidates against inflammation-associated tumors (LEE *et al*., 2015). The results of these researchers are consistent with the present results due to the effect of the plant extract in changes in the expression of genes effective in apoptosis and reduced cell survival. Popova et al. studied phytochemical composition of leaves and stems of *Solanum nigrum* L. and *Solanum dulcamara* L. The results of their study showed that this plant can be used as a medicine due to its valuable medicinal compounds (POPOVA *et al*., 2017). Yu et al. studied the effect of *Solanum incanum* extract, SR-T100, on melanoma cancer. They showed that the plant extract has the ability to induces apoptosis in cancer cells. The results of their study introduced SR-T100 as a new compound with the ability to eliminate regional and metastatic melanoma. The results of these two studies are consistent. JAYAKUMAR *et al*. (2017) extracted an extract from the unripe fruit of *Solanum mauritianum* and investigated the effect of different concentrations of the extract on different cancer cells. Solasodine treated MCF-7 cells showed significant decline in the expression of *Bcl-2* and *Bcl-xL* after 48 h, with an increase in *Bax* and *Bak* expression (JAYAKUMAR *et al*., 2017). The results of this study are consistent with the results of the present study due to increased apoptosis and increased gene expression. ZHANG *et al*. (2018) investigated the effect of solamargine extract extracted from *Solanum nigrum* on the induction of apoptosis in QBC939 cholangiocarcinoma cells. The results of this study showed that solamargine reduced the viability of QBC939 cells and it can also induce apoptosis and alter mitochondrial permeability. Quantitative analysis of PCR results showed that solamargine decreased *Bcl-2*, *PAPAR* and increased *Bax* expression (ZHANG *et al*., 2018). The results of this study are consistent with the results of the present study. *Bax* gene increases and *Bcl-2* gene expression decreases. RAJABI *et al.* (2018) studied the effect of herbs on different cancer cell lines and measured the rate of apoptosis in cancer cells. They examined the expression of different genes in cell lines treated with plant extracts. With treatment with plant extracts, the expression of apoptotic genes increased and the expression of anti-apoptotic genes decreased (RAJABI *et al*., 2018). The result of the present study is consistent with the result of the research of RAJABI *et al*. (2018). BINBING *et al*. (2019) investigated the effect of solamargin extract from *Solanum nigrum* on human cholangiocarcinoma cells. The results showed that this combination increased the expression of *Bax* gene by 3.8 times and decreased the expression of *Bcl-2* gene by 0.63 times (BINBING *et al*., 2019). The results of this study are consistent with the results of the present study due to the effect of the extract in terms of treatment time and concentration required for apoptosis. In another research, Kumar et al. examined changes in gene expression in HepG2 and human immune cell treated with *Solanum nigrum* extract. Results indicated improved cytotoxicity with expanding concentrations of extract. The ethanolic extract of *S. nigrum* was found to have high cytotoxic effect on the liver cancer cell line HepG2. The extract enhances the proliferation of PBMC when compared with HepG2 cells. Thus the *S. nigrum* is a potent antitumor agent in the context of liver cancer (KUMAR *et al*., 2020). The results of these two studies are consistent in proving the effect of the extract on gene expression.

CONCLUSION

The results showed that in 24-, 48- and 72-hours treatments with *Solanum pseudo-capsicum* extract at IC_{50} concentration, the expression of *Bax* gene to *Bcl-2* increased 2, 3.84 and 4.67 times, respectively. Therefore, 72-hour treatment had the greatest effect on increasing *Bax* gene expression and decreasing *Bcl-2* gene expression. According to promising results of this in vitro study, there are some potential applications of *Solanum pseudo-capsicum* in the future, but more investigations are certainly needed

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Solanum pseudo-capsicum EFFEKTI NA *Bax* I *Bcl-2* EKSPRESIJU GENA I APOPTOZU U MCF-7 ĆELIJSKOJ LINJI

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Izvod

U ovoj studiji istražen je efekat ekstrakta *Solanum pseudo-capsicum* na ćelijsku liniju MCF-7. Kontrolna ćelijska linija u ovoj studiji bila je ćelijska linija fibroblasta kože HFF. Ćelije su bile izložene tretmanu 24h, 48h i 72h respektivno. Zatim je procenjena viabilnost dvostrukih ćelijskih linija korišćenjem MTT testa. Rezultati su pokazali da je 72-časovni tretman biljnim ekstraktom u koncentraciji IC_{50} imao najveći efekat na smrt ćelija raka. Rezultati protočne citometrije su pokazali da se nakon 48 sati tretmana biljnim ekstraktom u koncentraciji IC_{50} javlja najveća stopa indukovane apoptoze. Zatim je izvršen RT PCR test da bi se izmerile promene u ekspresiji *Bax* i *Bcl-2* gena. Referentni gen u ovoj studiji je bio *β-actin*. Rezultati ovog istraživanja pokazali su da je u ćelijama raka tretiranim biljnim ekstraktom povećana ekspresija *Bax* pro-apoptotičkog gena i smanjena ekspresija anti-apoptotičkog gena *Bcl-2*. Stoga je 72-časovni tretman imao najveći efekat na povećanje ekspresije *Bax* gena i smanjenje ekspresije gena *Bcl-2*. Zbog nižih neželjenih efekata biljaka u poređenju sa konvencionalnim hemijskim lekovima koji se koriste u lečenju raka, moguće je preduzeti efikasan korak u lečenju ove bolesti korišćenjem novih metoda u identifikaciji efikasnih biljnih jedinjenja i njihovom prečišćavanju.

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