Original Article Open Access

Archives Of Breast Cancer

DOI: 10.32768/abc.20229183-95

Targeting Mitochondrial-Nuclear Apoptotic Signaling Pathways, Metabolic Pathways and Reversal of Doxorubicin (DOX) Cell Resistance in MCF-7 and MDA-MB-231 Cancer Cell Lines by Sambucus Nigra (SNA)

Ali Ghanbari[a], Shiva Roshankhah[a], Mohammad Reza Salahshoor[a], Cyrus Jalili[a], Mojtaba Esmaeli*[a]

[a]*Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran*

ARTICLE INFO

Received:
22 September 2021
Revised:
20 November 2021
Accepted:
23 November 2021

Keywords:
Breast cancer,
Doxorubicin,
Drug resistance,
Sambucus nigra

ABSTRACT

Background: The study aimed to assess the effect of anti-tumor and anti-proliferative properties of Sambucus nigra (SNA) on *MCF-7* and *MDA-MB-231* breast tumor cell lines.

Methods: The cytotoxicity of SNA was assessed based on dose/time by the MTT assay. Also, the influence of SNA on apoptotic pathways, cellular and metabolic resistance in these cell lines was examined by real-time PCR, lipid peroxidase was measured by malondialdehyde (MDA) and the effect of apoptosis and necrosis was determined by flow cytometry.

Results: Our data showed that DOX, SNA, and DOX + SNA treatment induced the expression of *p53*, *Bax*, *Bcl-2*, *Caspase-3*, and 8 levels involved in the apoptotic pathways. ATP binding cassette subfamily B member 4 *(ABCB4)* gene expression was decreased in *MDA-MB-231* breast cancer cells compared to *MCF-7*. Also, we observed that DOX, SNA, and DOX + SNA treatment induced expression of Monocarboxylate transporters (MCTs) metabolic pathways such as MCT1 and MCT4.

Conclusion: Overall, the outcomes of this investigation show that the combination of SNA-Doxorubicin (DOX) in different groups of these cancer cells, especially in the MDA-MB-231 cell lines synergistically intensified the induction of apoptosis in them. SNA enhances the anti-cancer effects of DOX to induce cellular apoptosis, alter metabolic pathways, and reduce cellular resistance. The research highlights the promising use of SNA as a chemosensitizer in the chemotherapy.

INTRODUCTION

Cancers are one of the main concerns in the world, and in a report published by the World Health Organization, the number of patients suffering from it will increase significantly in the coming years.[1] Currently, chemotherapy, radiotherapy, and surgery strategies are used to treat cancer patients.[2] However, there is no useful medicine for invasive breast cancer.[1]

Doxorubicin (DOX) is one of the chemotherapy drugs, inhibiting cell proliferation and preventing metastasis of this cancer by binding to DNA.[3] However, this drug, like other chemotherapy drugs, is not able to completely cure breast cancer due to cellular resistance.[3,4] On the other hand, intravenous injection of this drug causes problems.[5] Studies show that the combination of this drug with other drugs and chemotherapeutic agents reduces its side effects and increases its effectiveness, and that it is necessary to increase its anti-cancer function and reduce its anti-toxicity

***Address for correspondence:**
Mojtaba Esmaeli, PhD
Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
Tel: +98 9132224474
Email: mojtaba.esmaeily@bums.ac.ir

through co-delivery systems.[6,7] However, most combination therapies with conventional chemo-therapy drugs are not optimal for the treatment of invasive breast cancers and have not been widely used, and, as a result, have not been able to make significant improvements in patients' recovery compared to single-dose treatment. In addition, the combined use of drugs often leads to an overlap of their toxicity in the body and leads to tissue damage.[8] Therefore, adopting an appropriate combination dose during treatment increases its therapeutic effectiveness, overcomes the side effects of its monotherapy, and provides a wide range of polytherapy.[9,10]

According to research conducted by the International Agency for Research on Cancer on anti-cancer and anti-tumor properties of plant extracts, on the one hand, and, on the other hand, the significant contribution of plants to the structures of chemotherapy drugs, many researchers in recent years have sought to use these compounds in their anti-cancer research.[11-15]

Sambucus nigra (SNA) is one of these plant compounds that has been used in traditional and medical medicine since ancient times. Different sections of this herb such as flowers, leaves, fruits, roots, and bark have been used in many types of research.[16] The herb is found in abundance in humid and temperate regions of northern Iran.[17] It contains a variety of active ingredients, including ribosome-inactivating proteins or ebulin,[18] with anticancer properties, immunotoxins substances, flavonoids, cardiac glycosides, and tanins.[19,20] Anthocyanidin compounds in this plant such as Cyanidin-3-O-glucoside in anti-cancer research have inhibited the growth of cancer cells.[20] Other compounds of this plant are polyphenolic substances that have antioxidant properties preventing tissue damage due to oxidative stress.[21,22] Many studies have shown that ethyl acetate in this plant has very effective anticancer properties on cancer cell lines.[19,23]

In recent years, synergistic combination chemotherapy using plant compounds has been considered a promising strategy to overcome the above limitations. SNA can be a good candidate, but despite extensive research on the antioxidant properties and other properties of this plant species, no research has been found on the effect of the SNA extract on the side effects of doxorubicin chemotherapy drugs in *MCF-7* and *MDA-MB-231* cell lines so far. The main purpose of this study was to show the effects of SNA and DOX separately and their combination on the expression of genes involved in apoptotic, metabolic, and cellular resistance pathways in human breast cancer cell lines and to investigate the occurrence of apoptosis and necrosis in them by cellular and molecular methods, to achieve effective chemotherapy methods and reduce their side effects.

METHODS

Cell culture

MDA-MB-231 (ATCC number: HTB-26) and *MCF-7* (ATCC number: HTB-22) cancer cell lines studied in this study were purchased from Pasteur Institute in Tehran. These cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich) medium containing 10% fetal bovine serum (Sigma-Aldrich), 100µg/ml streptomycin and 100IU/ml penicillin, amphotericin B (Sigma, USA) and 2mM glutamine. The cells were incubated at 37°C, with 95% humidity, and 5% CO_2.

Drug sensitivity assay

The sensitivity of the cancer cell lines to DOX (Sigma-Aldrich) and SNA (Sigma-Aldrich) was determined by 3-(4, 5-dimethylthiazol-2-yl) test -2, 5-diphenyl-tetrazolium bromide (MTT). For this purpose, 2×10^4 cell densities were seeded into a 96-well plate and were incubated for 24 hours. Then, they were treated with DOX and SNA in various concentrations (0, 2.5, 5, 10, 20, 50, and 100µM) and a combined dose for them with various concentrations (0µM DOX + 0µM SNA, 1.25µM DOX + 1.25µM SNA, 2.5µM DOX + 2.5µM SNA, 5µM DOX + 5µM SNA, 10µM DOX + 10µM SNA, 20µM DOX + 20µM SNA, and 50µM DOX + 50µM SNA) with serum-free culture medium for 60 minutes and then replaced with the usual culture medium containing FBS. After 72 hours, the cell culture mediums were removed and 20µL of MTT solution (5mg/ml) was added to them, which was incubated for 4 hours under the same culture conditions. The medium was displaced, and 200µL of DMSO was added to each well and was maintained for 2 hours. Finally, the plates were then read using an ELISA plate reader (Bio-tek Instruments, USA) at 570nm. Three replications were performed for each sample. The obtained values were used to plot IC_{50} (inhibitory concentration to produce 50% cell death) curves based on dose-response curves.

Treatment groups

Based on the results of the MTT assay, cells were divided into 8 groups.

1-MCF-7 cell line control group

MCF-7 cells were cultured for 24 hours in a 96-well plate. Then their culture medium was drained and washed with PBS. The cells were then exposed to 0.001% DMSO + DMEM for 1 hour. Then, their culture medium was drained and washed with PBS. Then, a culture medium containing DMEM +10% FBS+ 1% pen/strep was added to each well and incubated for 72 hours.

2-Doxorubicin (DOX) group of MCF-7 cell line

In this group, MCF-7 cells were cultured for 24 hours under the same conditions. Then their culture medium was drained and washed with PBS. In the next

step, the cells were exposed to 10µM DOX+DMEM for 1 hour. Then, their culture medium was drained and washed with PBS. Then, a culture medium containing DMEM+10% FBS+1% pen/strep was added to each well and incubated for 72 hours.

3-Sambucus nigra (SNA) group of MCF-7 cell line

In this group, *MCF-7* cells were cultured for 24 hours under the same conditions. Then, their culture medium was drained and washed with PBS. In the next step, the cells were exposed to 10µM SNA+DMEM for 1 hour. Then, their culture medium was drained and washed with PBS. A culture medium containing DMEM+10% FBS+1% pen/strep was then added to each well and incubated for 72 hours.

4-Sambucus nigra (SNA) + Doxorubicin (DOX) group of MCF-7 cell line

In this group, *MCF-7* cells were cultured for 24 hours under the same conditions. Then their culture medium was drained and washed with PBS. In the next step, the cells were exposed to 2.5µM SNA+2.5µM DOX+DMEM for 1 hour. Their culture medium was drained and washed with PBS. Then, a culture medium containing DMEM+10% FBS+1% pen/strep was added to each well and incubated for 72 hours.

5-MDA-MB-231 cell line control group

MDA-MB-231 cells were cultured for 24 hours in 96-well plate wells. Then, their culture medium was drained and washed with PBS. The cells were then exposed to 0.001% DMSO+DMEM for 1 hour. Their culture medium was drained and washed with PBS. Then, a culture medium containing DMEM+10% FBS+1% pen/strep was added to each well and incubated for 72 hours.

6-Doxorubicin (DOX) group of MDA-MB-231 cell line

In this group, *MDA-MB-231* cells were cultured for 24 hours under the same conditions. Then, their culture medium was drained and washed with PBS. In the next step, the cells were exposed to 20µM DOX+DMEM for 1 hour. Then, their culture medium was drained and washed with PBS. A culture medium containing DMEM+10% FBS+1% pen/strep was added to each well and incubated for 72 hours

7-Sambucus nigra (SNA) group of MDA-MB-231 cell line

In this group, *MDA-MB-231* cells were cultured for 24 hours under the same conditions. Then, their culture medium was drained and washed with PBS. In the next step, the cells were exposed 10µM SNA+DMEM for 1 hour. Then, their culture medium was drained and washed with PBS. A culture medium containing DMEM+10% FBS+1% pen/strep was then added to each well and incubated for 72 hours.

8-Sambucus nigra (SNA)+Doxorubicin (DOX) group of MDA-MB-231 cell line

In this group, *MDA-MB-231* cells were cultured for 24 hours under the same conditions. Their culture medium was drained and washed with PBS. In the next step, the cells were exposed to 5µM SNA+5µM DOX+DMEM for 1 hour. Then, their culture medium was drained and washed with PBS. Then, a culture medium containing DMEM+10% FBS+1% pen/strep was added to each well and incubated for 72 hours.

Determination of malondialdehyde (MDA)

The level of lipid peroxidation in cell membranes was measured by measuring malondialdehyde (MDA) levels. For this purpose, 2×10^4 *MDA-MB-231* and *MCF-7* from each breast cancer cell line were cultured in a 96-well plate. After 24 hours, these cell lines were treated based on the dose obtained from IC_{50}. Then, by changing the medium after 1 hour and 72 hours after incubating the cells, it was used to measure the amount of MDA according to the protocol included in the brochure recommended in the MDA Assessment Kit (Kia-Zist, IRI). Finally, the concentration of MDA at 515nm was measured by an ELISA plate reader (Biotek Instruments, USA). MDA levels were calculated according to the standard curve. The test was replicated three times for each group.

Real-time quantitative PCR

Total cellular RNAs were extracted from cancer cell lines using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). After examining the purity and concentration of the extracted RNAs, the first cDNA strand was synthesized according to the manufacturer's instructions using Revert AidTM H Minus First Strand cDNA Synthesis Kit (Takara Bio, Shiga, Japan). The Real-time PCR technique was performed using the SYBR Green reaction kit (Takara Bio, Shiga, Japan). The primers and sequence of the selected genes are shown in Table 1. PCR programs were performed as follows: 95°C for 30s, followed by 40 cycles of 95°C for 5s and 60°C for 30s. *GAPDH* was used as an internal control gene. Relative measurements of the relevant genes were analyzed using the $2^{-\Delta\Delta ct}$ method as a relative ratio of *GAPDH* expression in each sample.[24]

Apoptosis Assay by Flow Cytometry

Apoptosis evaluation was carried using the flow cytometry method to detect apoptotic cells with annexin V/propidium iodide (annexin V/PI) apoptosis assay kit (Roche Company, Switzerland). For this purpose, after culturing and treating cells and separating them, supernatant was isolated from them and the cells were washed twice with cold PBS. A 1xbinding buffer (100µl) was used to suspend the cells. Then, 5µl of annexin and 10µl of propidium iodide were added to them for staining. Next, the cells were examined by flow cytometry (Becton Dickinson

Facscalibur USA) and the percentage of kind of cell death in them was determined.

Statistical Analysis

In this study, the data were analyzed using SPSS, version 23. For statistical analysis of quantitative data, one-way ANOVA and Tukey post hoc test were used. Also, the data in the results section were considered as Mean±SEM and the level of significance between the groups as $P<0.05$. These results were associated with three independent replications for each sample.

Table 1. Primer sequences used in Real-Time PCR

Gene name		Primer sequence
BAX	forward	5′-GCGAGTGTCTCAAGCGCATC-3′
	reverse	5′-CCAGTTGAAGTTGCCGTCAGAA -3′
BCL-2	forward	5′-ATGTGTGTGGAGAGCGTCAACC-3′
	reverse	5′-GCATCCCAGCCTCCGTTATC-3′
P53	forward	5′-CCTCAGCATCTTATCCGAGTGG-3′
	reverse	5′-TGGATGGTGGTACAGTCAGAGC-3′
GAPDH	forward	5′-GGAGCGACATCCGTCCAAAAT-3′
	reverse	5′-GGCTG TGTCAATCTTCTCATGG-3′
Caspase-3	forward	5'-CAGAACTGGACTGTGGCATTG-3'
	reverse	5'–GCTTGTCGGCATACTGTTTCA-3'
Caspase-8	forward	5′-CATCCAGTCACTTTGCCAGA-3′
	reverse	5′-GCATCTGTTTCCCCATGTTT-3′
ABCB4	forward	5′-GCAGACGGTGGCCCTGGTTGG-3′
	reverse	5′-GGATTTAGCGACAAGGAAA-3′
MCT1	forward	5′-GTGGCTCAGCTCCGTATTGT-3′
	reverse	5′-GAGCCGACCTAAAAGTGGTG-3′
MCT4	forward	5′-CCATGCTCTACGGGACAGG-3′
	reverse	5′-GCTTGCTGAAGTAGCGGTT-3′

RESULTS

Cell viability and IC_{50} values

The cytotoxic effect of the SNA, DOX, and SNA+DOX on *MCF-7* and *MDA-MB-231* cells was determined using MTT assay. As shown in Figure 1:A, concentrations above 20µM SNA in both *MDA-MB-231* and *MCF-7* cancer cell lines significantly reduced their survival. Therefore, the IC_{50} value for both cell lines under SNA treatment was determined to be 10µM. Also, the IC_{50} value of cells treated with DOX showed that *MCF-7* and *MDA-MB-231* cells treated with concentrations higher than 20 and 50µM were associated with a sharp decrease in survival, respectively (Figure 1:B). Therefore, IC_{50} values for *MCF-7* and *MDA-MB-231* cancer cell lines were selected at 10 and 20µM, respectively. Finally, the combined effect of SNA and DOX on the survival of these cells was investigated, which showed that the IC_{50} value for DOX and SNA in *MCF-7* cell lines was 5µM (2.5µM DOX+2.5µM SNA) and that this value in *MDA-MB-231* cell lines was 10µM (5µM DOX+5µM SNA) (Figure 1:C).

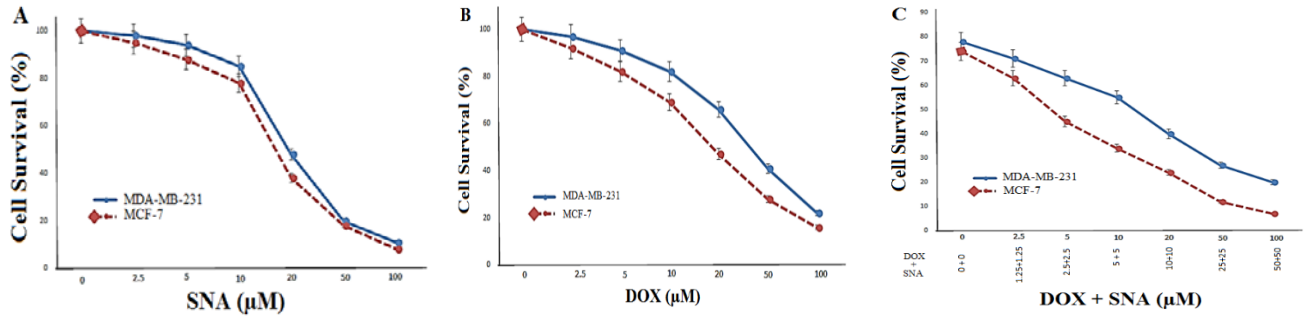

Figure 1. Evaluation of IC_{50} (µM) and survival rate of MCF-7 and MDA - MB-231 breast cancer cells exposed to different concentrations of SNA (Sambucus nigra) (0-100µM), DOX (Doxorubicin) (0-100µM), and DOX + SNA (Doxorubicin + Sambucus nigra) (0µM DOX + 0µM SNA, 1.25µM DOX + 1.25µM SNA, 2.5µM DOX + 2.5µM SNA, 5µM DOX + 5µM SNA, 10µM DOX + 10µM SNA, 20µM DOX + 20µM SNA and, 50µM DOX + 50µM SNA) (Figures A, B and C, respectively). IC_{50} and the survival rate of these cells were determined by the MTT assay within 72 hours. The results are as mean ± SD and at least three replications were performed for each group.

The IC_{50} value was obtained for different concentrations of SNA (Sambucus nigra), DOX (Doxorubicin), and DOX+SNA (Doxorubicin +Sambucus nigra) after 72 hours of incubation (mean±SD, n=3), as shown in Table 2.

Table 2. The IC_{50} value was obtained for different concentrations of SNA (Sambucus nigra), DOX (Doxorubicin), and DOX+SNA (Doxorubicin+Sambucus nigra) after 72 hours of incubation (mean ± SD, n=3).

Cell line	IC_{50} rate (μM)			CI[a] value
	SNA (μM)	DOX (μM)	SNA + DOX (μM)	
MCF-7	10±2.6	10±0.3	2.5±1.0+2.5±0.6	.5±0.51
MDA-MB-231	10±1.8	20±1.3	5±1.2+5±0.4	.75±0.85

a: combination index.
CI<1 indicates synergistic effect, CI=1 indicates additive effects and CI >1 indicates antagonistic effect.

Lipid peroxidation levels

The present study showed that the level of MDA in the treated groups in both cell lines was significantly increased compared to the control group (Figure 2). This increase was higher in the *MDA-MB-231* cancer cell line compared to the *MCF-7* cancer cell line. Also, the level of *MDA* in both cell lines treated with DOX+SNA was higher compared to the other groups and showed a significant difference with them (P<0.05).

Real-Time polymerase chain reaction expression of apoptotic genes

In this study, the relative expression levels of genes associated with apoptosis such as *p53, Bax, Bcl-2, Caspase-3, and 8* in *MDA-MB-231* and *MCF-7* cells were analyzed. Figure 3: A, shows the *p53 gene* expression changes. In *MCF-7* cancer cell lines, *p53 gene* expression was significantly higher in the treated groups compared to *MDA-MB-231* cancer cell lines. However, the expression of this gene in the untreated group in both cell lines was negligible. Also, *p53 gene* expression in the DOX+SNA treated group in both *MCF-7* and *MDA-MB-231* cancer cell lines showed high expression compared to the other groups and was significantly different from them (*P*<0.05).

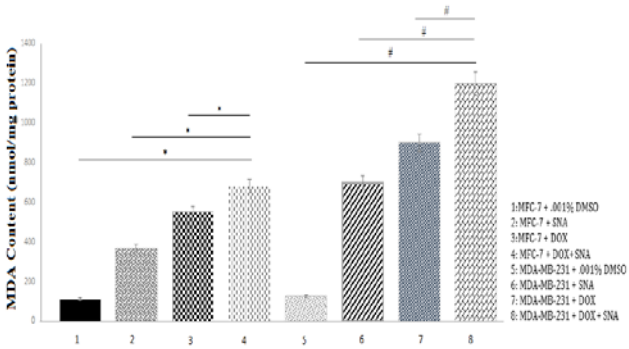

Figure 2. Induction of lipid peroxidase damage in MCF-7 and MDA-MB-231 cancer cell lines treated with SNA (Sambucus nigra), DOX (Doxorubicin), and DOX + SNA (Doxorubicin + Sambucus nigra) and control group After 72h by measuring the concentration of malon-dialdehyde (MDA). The results are shown as mean values ± SD for three replications for each group. The symbols * and # indicate the significant (*P<0.05, #P<0.05) relationship as compared with the DOX + SNA group.

Based on Figure 3: B-C, the expression of *Caspase-3 and 8* levels were significantly higher in treated *MCF-7* and *MDA-MB-231* cancer cell lines than in untreated cells. Also, there was a significant (P<0.05) difference in the *Caspase-3 and 8 genes* expressed in both types of cancer cells *MCF-7* and *MDA-MB-231* cells.

As anticipated, the changes in *Bax* and *Bcl-2* genes expression were seen when *MCF-7* and *MDA-MB-231* cells were treated with DOX, SNA, and DOX+SNA. According to Figure 3: D, the *Bcl-2* gene expression in untreated groups in *MCF-7* and *MDA-MB-231* cancer cell lines compared to different groups treated in these cancer cell lines was significantly higher. Our results showed that DOX and SNA reduced *Bcl-2* gene expression in these two cancer cell lines, but when the DOX+SNA combination was used in the *MCF-7* and *MDA-MB-231* cancer cell lines, a significant decrease in *Bcl-2* gene expression was shown among other groups, which was significantly different from other groups (P<0.05). This result suggests that the combination of SNA with DOX significantly induced apoptosis by reducing *Bcl-2* gene expression. The levels of the Bax gene expression levels were significantly (p<0.05) higher in the DOX and DOX+SNA groups than in the control groups (Figure 3: E). The expression of this gene was higher in *MDA-MB-231* compared to *MCF-7* cell lines.

Our results also showed a significant increase in *Bax/Bcl-2* ratio in both *MCF-7* and *MDA-MB-231* cancer cell lines in the DOX+SNA-treated group (p<0.05). The highest *Bax/Bcl-2* ratio (more than 15 times) was observed in the *MDA-MB-231* cancer cell line treated with DOX+SNA (Figure 3: F).

Expression of ABCB4 gene

The results of *ABCB4 gene* expression by RT-PCR are shown in Fig. 3: G. The *ABCB4* gene expression was markedly (p<0.05) higher in the DOX and DOX+SNA groups than in the control groups. The highest expression of the *ABCB4* gene was in *MCF-7* cancer cell lines so that it was decreased in the group treated with DOX+SNA compared to the groups treated with DOX. However, the expression of the *ABCB4* gene in the DOX-treated group had the highest expression compared to the DOX+SNA or SNA-treated groups. The expression level of the *ABCB4* gene in *MDA-MB-231* cancer cell lines was also significant (P<0.05) in the group treated with DOX compared to the groups treated with DOX+SNA or SNA. However, in the group treated with DOX+SNA, the expression of the *ABCB4* gene was significantly (P<0.05) reduced compared to the groups treated with DOX, and DOX + SNA.

Expression of MCT1 and MCT4 genes

We determined the effects of DOX, SNA, and the combined therapy on the expression of *MCT1* and *MCT4* (Figure 3: H-I). The expression level of *MCT1* and *MCT4* genes significantly (*P*<0.05) decreased after treatment of cells by DOX, SNA, and DOX + SNA. We found that *MCT1* and *MCT4* gene expression was high in both untreated breast cancer cell lines. However, in both *MCF-7* and *MDA-MB-231* cell lines, the highest decrease in the expression of these genes was observed in the DOX+SNA-treated groups compared to the SNA or DOX-treated groups. These results suggest that SNA has been able to reduce the expression of *MCT1* and *MCT4* genes, and its use with DOX showed a significant decrease in expression of these genes in *MCF-7* and *MDA-MB-231* breast cancer cell lines (P<0.05).

Induction of SNA (Sambucus nigra)-influenced apoptosis in MCF-7 and MDA-MB-231 breast cancer cell lines

To evaluate the incidence of apoptosis in the cancer cell lines, treated with SNA, DOX, DOX+SNA, and control group, flow cytometry was applied with Annexin V/PI double staining (Figure 4). Cells stained with Annexin V/PI were divided into 4 groups. For each cancer cell line in different groupings, four panels are presented. In order to provide a convenient comparison between the percentage of normal cells, early apoptosis, late apoptosis, and necrosis in different groups in both *MCF-7* and *MDA-MB-231* cancer cell lines, the results of flow cytometric data analysis are presented in Tables 3 and 4.

The results of the analysis of flow cytometry data indicated that the *MCF-7* and *MDA-MB-231* cancer cell lines treated with SNA like DOX caused apoptosis in them, such that in the *MCF-7* cancer cell lines, this result was more consistent (Tables 3 and 4). The results obtained in *MCF-7* cancer cell lines treated with SNA or DOX showed that the highest and lowest types of cell death were related to necrosis and early apoptosis. Also, in the group treated with DOX+SNA, the most common type of cell death was observed as late apoptosis and necrotic (Table 3).

In the *MDA-MB-231* cancer cell line, the observed results were similar to the results for the *MCF-7* cancer cell lines, and that in the SNA group, the highest and lowest type of cell death was seen as early apoptosis and necrotic, which was similar to the DOX group (Table 4). In the DOX+SNA group, no significant necrotic death was seen, and the most common type of cell death in this group was observed as early and late apoptosis (Table 4).

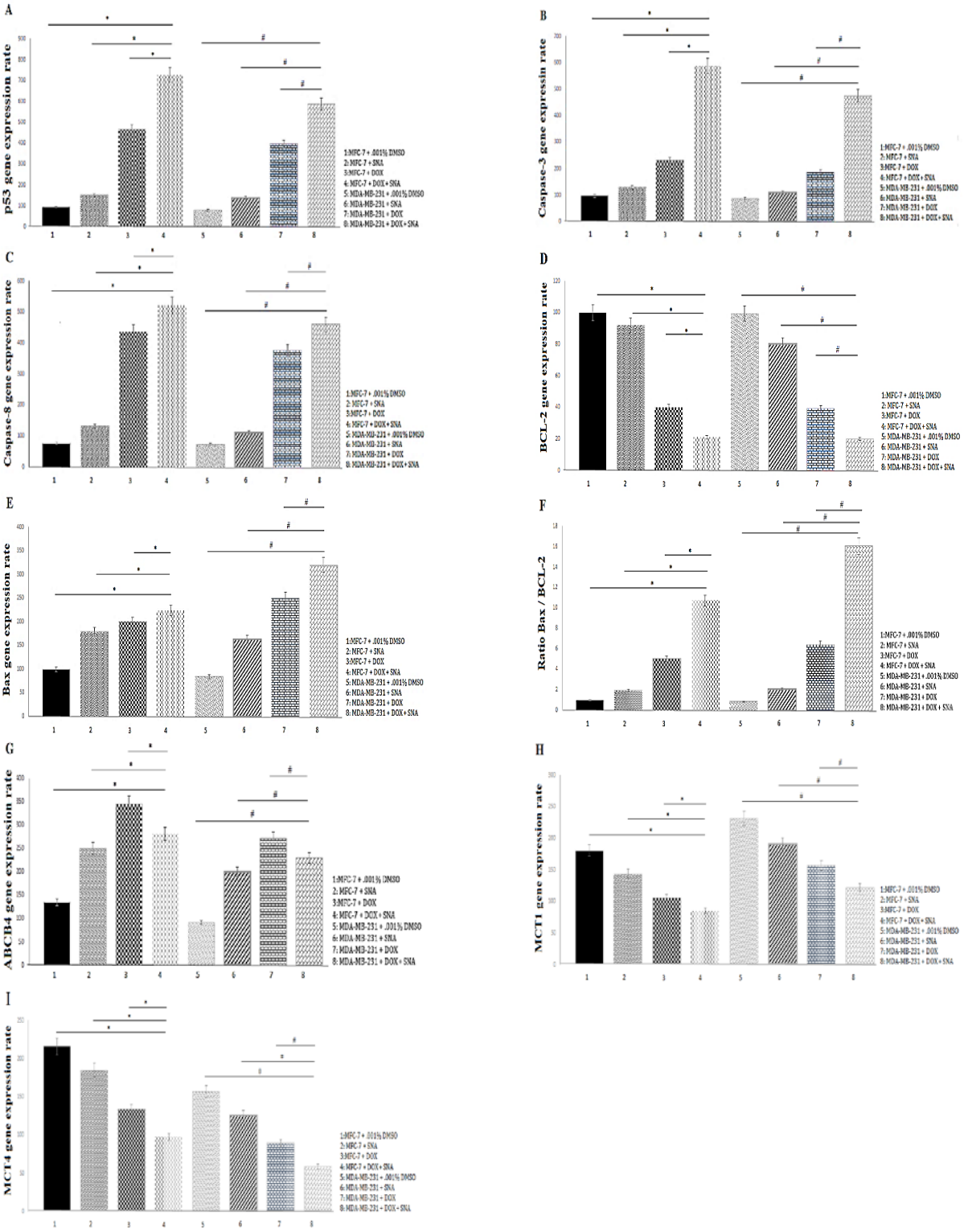

Figure 3. Real-time PCR (Real-time polymerase chain reaction) results for apoptotic genes P53, Bax, Bcl-2, Caspase-3, and 8 (A-F), toxicity-resistant ABCB4 gene (G), MCT1 and MCT4 metabolic pathway genes (H-I), in different groups MCF-7 and MDA-MB-231 cancer cell lines treated with SNA (Sambucus nigra), DOX (Doxorubicin), and DOX+SNA (Doxorubicin+Sambucus nigra) 72 hours after incubation. The values presented are shown as mean ± standard deviation for three replications for each group. The symbols * and # indicate the significant (*P<0.05, #P<0.05) relationship as compared with the DOX+SNA group.

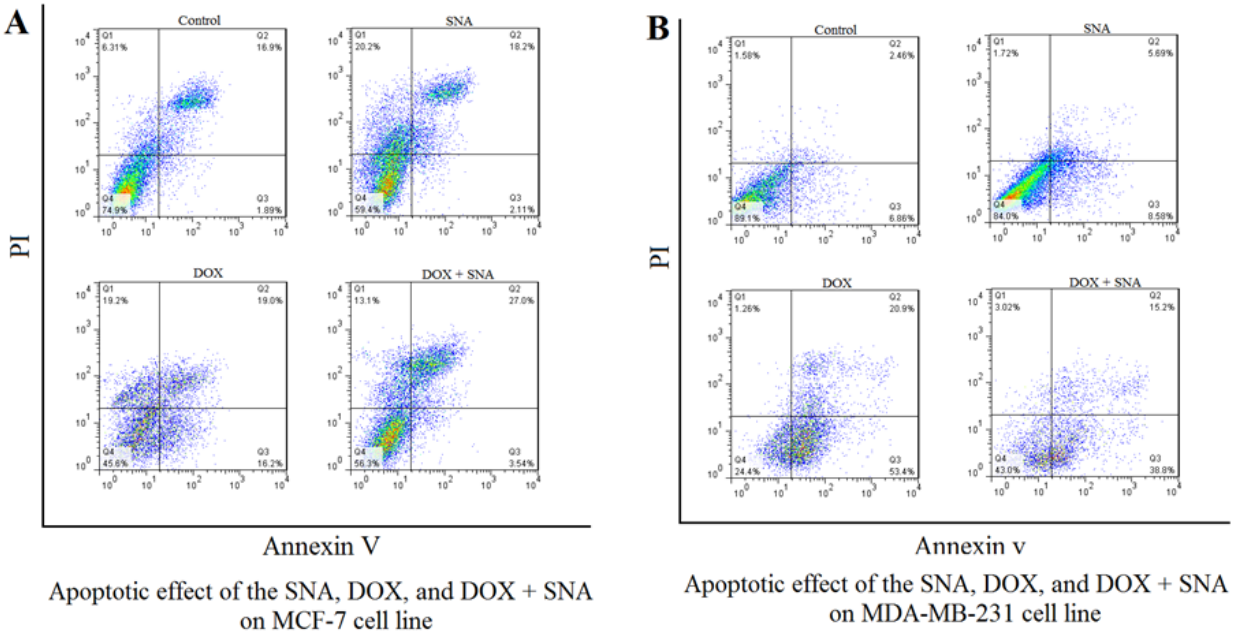

Figure 4. Induction of apoptosis in MCF-7 (A) and MDA-MB-231 (B) breast cancer cell lines affected by the apoptotic activities of SNA (Sambucus nigra), DOX (Doxorubicin), and SNA+DOX (Sambucus nigra + Doxorubicin) within 72 hours. Flow cytometric analysis using Annexin V/PI staining.

Table 3. Percentage of normal cells, early apoptosis, late apoptosis and necrotic cells in MCF7 cell lines after 72 hours in treated SNA (Sambucus nigra), DOX (Doxorubicin) and SNA+DOX (Sambucus nigra + Doxorubicin) and control groups (mean ± SEM, n=3).

Cell line	Drug	Normal (alive) cells	Early Apoptosis	Late Apoptosis	Necrotic
	control	74.9±1.58	1.89±0.595	16.9±1.235	6.31±1.1
	SNA	59.4±0.73	2.11±0.917	18.2±0.265	20.2±0.85
MCF7	DOX	45.6±0.12	16.2±0.308	19.0±1.690	19.2±1.87
	SNA + DOX	56.3±0.630	3.54±0.654	27.0±2.32	13.10±0.941

Table 4. Percentage of normal cells, early apoptosis, late apoptosis and necrotic cells in MDA-MB-231cell lines after 72 hours in treated (SNA (Sambucus nigra), DOX (Doxorubicin) and SNA+DOX (Sambucus nigra + Doxorubicin) and control groups (mean ± SEM, n=3).

Cell line	Drug	Normal (alive) cells	Early Apoptosis	Late Apoptosis	Necrotic
	control	89.1± 1.618	6.86± 1.153	2.46± 1.027	1.58± 0.550
	SNA	84.0± 2.862	8.58± 1.311	5.69±1.274	1.72± 1.868
MDA-MB-231	DOX	24.4± 0.441	53.4± 1.068	20.9± 1.040	1.26±.0398
	SNA + DOX	43.0± 1.020	38.8± 0.852	15.2± 0.929	3.02±0.715

DISCUSSION

IC_{50} values and analysis of MTT data clearly showed that treatment of *MCF-7* and *MDA-MB-231* breast cancer cell lines with a combination of SNA and DOX led to synergistic and dose-dependent effects in these cell lines, inhibiting the proliferative activity in them. We also showed that the IC_{50} dose of DOX was almost quadrupled in the DOX-treated groups compared with the DOX+SNA-treated groups. The therapeutic value of DOX in the treatment of cancers is limited due to its side effects on cardiac tissue,[25] and

the emergence of drug resistance in treated cancer cells.[26,27] In contrast, SNA is a non-toxic substance whose anti-cancer activity has been proven against various cancers.[28,29] The concentration ratio of SNA to DOX has played an important role in determining the degree of synergistic effects; for example, in this study, the differences observed in different concentrations of DOX and SNA in different compounds were evaluated.

Flow cytometry analysis for breast cancer cell lines, which were treated and untreated, was performed in vitro to determine whether growth inhibitory effects in DOX+SNA-treated groups can be attributed to apoptosis or not. The results showed that these effects and reactions can be attributed to the function and structures of DOX and SNA inside cells.

Many studies have shown that the causes of cancer cells' resistance to clinical treatment with DOX and other chemotherapeutic drugs are changes in the glycosylation of cancer cells and the release of these cells from the mechanisms of cell death, making their treatment difficult.[30,31] Different levels of expression of glycans on the surface of cancer cells have caused differences in their metastasis.[32,33] Numerous studies on glycoproteins, especially α2, 6-linked Sias, have shown that the survival rate of cancer patients and their prognosis are closely related to this glycoprotein.[33] Therefore, with sufficient information about the properties of glycan epitopes, it can be used to target proteins and glycans. For this reason, the most important treatment challenge for cancer cells is the lack of apoptosis. Therefore, targeting and inducing apoptosis in cancer cells is one of the treatment strategies. Many studies have been shown that lectins induce apoptosis in a variety of cancer cells.[34-36] A study on SNA has shown that this plant exactly matches the mentioned method in inducing apoptosis.[37]

A study of SNA on ovarian cancer has shown that this plant compound has an effect on mitochondria, causing cancer cell death through intrinsic apoptotic pathways.[37] Other studies have suggested that cancer cells can be treated by disrupting mitochondrial cell membrane function and inducing apoptosis.[38] Changes in the morphology of the mitochondrial cell membrane indicate the amount of energy it provides to the cell.[39]

Decreased mitochondrial energy supply causes the mitochondrial cell membrane to rupture, releasing cytochrome-c in its membrane into the mitochondrial cytosol, which then activates the caspase cascade, leading to cell death.[40] Studies have shown that SNA induces mitochondrial membrane permeability and, along with activation of the caspase cascade and release of cytochrome-c into the mitochondrial cytosol, leads to apoptosis.[37]

In some studies on SNA, it has been shown that this substance has played a significant role in altering the cellular respiration pathway and has been able to increase the production of ATP and basal *OCR*.[37] Using this method, SNA is able to prevent cancer cells from metastasizing and by disrupting the energy supply of cancer cells, it stops the cell cycle in G2/M phase.[37]

Numerous studies on SNA have shown that its anti-cancer activity relates to the structures of this compound.[41-43] This compound acts on nuclear transcription factors through action and causes apoptosis in a variety of cancer cells.[44] Another compound in SNA reported to have anti-cancer properties is ribosome-inactivating proteins (RIPs), which interfere with the synthesis of proteins by their enzymatic properties through the activity of N-glycosidase on the large RNA of ribosomes.[45-47] Various studies have also shown that RIPs in SNA affect DNA and polynucleotides.[48] The results of our study on SNA and breast cancer cell lines *MCF-7* and *MDA-MB-231* showed that, according to the mechanisms mentioned above, it can have positive effects comparable to using DOX, and can increase the induction effects of apoptosis.

Studies have shown that overexpression of ABC transporters has been directly linked to resistance to chemotherapy drugs.[49] These studies have shown that the *ABCB4* transporter acts as an out-of-cell transmitter for drugs such as digoxin, paclitaxel, daunorubicin, vinblastine, and ivermectin, preventing them from accumulating in the cell.[50] Although *ABCB4* gene expression was increased in DOX-treated groups, these results were similar to the results of other studies,[51-55] although this gene expression was reduced in SNA-treated groups. On the other hand, in the groups treated with DOX+SNA, the synergistic effects of SNA showed that the expression of the *ABCB4* gene in these groups significantly decreased. These results were similar to other studies with DOX in combination with other plant extracts.[54]

In this study, it was shown that in the groups treated with DOX+SNA, the expression of genes of the *MCT1* and *MCT4* significantly decreased, possibly due to the presence of effective compounds in SNA such as Flavonoids.[56] Numerous studies have shown that different compounds of Flavonoids had different functions in these transporters.[57,58]

In the present study, *MCF-7* and *MDA-MB-231* breast cancer cell lines showed high expression of the *p53* gene after treatments with DOX and SNA. These findings are in agreement with the results obtained in similar studies.[59] The results of real-time PCR analysis showed an increase in *Bax* gene expression and a decrease in *Bcl-2* gene expression in *MCF-7* and *MDA-MB-231* breast cancer cell lines. These results indicated that SNA had an inhibitory effect on cell proliferation and induced apoptosis. Thus, increasing *Bax* expression and decreasing *Bcl-2* expression may

enhance the antitumor effects of SNA in chemotherapy treatments.

In confirmation of the above, we also assessed the level of lipid peroxidase by measuring MDA. In our study, the highest level of MDA was observed in both *MCF-7* and *MDA-MB-231* cell lines in the DOX+SNA groups. These results indicated that SNA and DOX, in combination with each other, have increased lipid peroxidase levels in these cell lines. On the other hand, many studies have shown that increasing the level of MDA disrupts the structure and function of vital molecules, which in turn leads to mitochondrial dysfunction and eventually apoptosis in cells.[60] In addition, many studies have shown that another reason for the increase in MDA levels in cells is the effect of reactive oxygen species (ROS) on them.[61] Therefore, the function of SNA, DOX, and their combination in *MCF-7* and *MDA-MB-231* cell lines can be a confirmation of these hypotheses, which requires extensive research in this field

CONCLUSION

The results presented showed that SNA with its anti-tumor properties had an effect on breast cancer cell lines. SNA induced apoptosis through the expression of *p53, Bax, Caspase-3, and 8* genes and early apoptotic cell death. The compound also inhibited cell proliferation by reducing *Bcl-2* gene expression, decreased cellular resistance by decreasing *ABCB4* gene expression and interfered with metabolic pathways through *MCT1* and *MCT4* gene expression. Expression of the studied genes suggests that SNA has synergistic effects with chemotherapy drugs.

ACKNOWLEDGEMENTS

All the authors would like to thank the Faculty of Medicine of Kermanshah University of Medical Sciences and the Department of Anatomy for their cooperation in implementing this project.

FUNDING

The financial support for this investigation was provided by Kermanshah University of Medical Sciences, Iran.

CONFLICT OF INTEREST

No conflicts of interest have been reported by the authors.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2018;68(6):394-424. doi: 10.3322/caac.21609.
2. Williams DH. Fertility preservation in the male with cancer. Current urology reports. 2013;14(4):315-26. doi: 10.1007/s11934-013-0345-6.
3. Liu B, Zhang X, Li C, He F, Chen Y, Huang S, et al. Magnetically targeted delivery of DOX loaded Cu 9 S 5@ mSiO 2@ Fe 3 O 4-PEG nanocomposites for combined MR imaging and chemo/photothermal synergistic therapy. Nanoscale. 2016;8(25):12560-9. doi: 10.1039/C5NR06322A.
4. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: an evolving paradigm. Nature Reviews Cancer. 2013;13(10):714-26. doi: 10.1038/nrc3599 .
5. Dayton A, Selvendiran K, Meduru S, Khan M, Kuppusamy ML, Naidu S, et al. Amelioration of doxorubicin-induced cardiotoxicity by an anticancer-antioxidant dual-function compound, HO-3867. Journal of Pharmacology and Experimental Therapeutics. 2011;339(2):350-7. doi: 10.1124/jpet.111.183681.
6. Randall ME, Filiaci VL, Muss H, Spirtos NM, Mannel RS, Fowler J, et al. Randomized phase III trial of whole-abdominal irradiation versus doxorubicin and cisplatin chemotherapy in advanced endometrial carcinoma: a Gynecologic Oncology Group Study. Journal of clinical oncology. 2006;24(1):36-44. doi:10.3410/f.14006.471513.
7. Thigpen JT, Brady MF, Homesley HD, Malfetano J, DuBeshter B, Burger RA, et al. Phase III trial of doxorubicin with or without cisplatin in advanced endometrial carcinoma: a gynecologic oncology group study. Journal of clinical oncology. 2004;22(19):3902-8. doi:10.1200/JCO.2004.02.088.
8. Miles D, von Minckwitz G, Seidman AD. Combination versus sequential single-agent therapy in metastatic breast cancer. The oncologist. 2002;7:13-9. doi:10.1634/theoncologist.2002-0013.
9. Lee JH, Nan A. Combination drug delivery approaches in metastatic breast cancer. Journal of drug delivery. 2012;2012. doi: 10.1155/2012/915375.
10. Mokhtari RB, Homayouni TS, Baluch N, Morgatskaya E, Kumar S, Das B, et al. Combination therapy in combating cancer. Oncotarget. 2017;8(23):38022. doi: 10.18632/oncotarget.16723.
11. Moyad MA, Carroll PR. Lifestyle recommendations to prevent prostate cancer, part I: time to redirect our attention? Urologic Clinics. 2004;31(2):289-300. doi: 10.1016/j.ucl.2004.03.006.
12. Kruk J. Physical activity in the prevention of the most frequent chronic diseases: an analysis of the recent evidence. Asian Pacific Journal of Cancer Prevention. 2007;8(3):325. doi: Not Available.

13. Dossus L, Kaaks R. Nutrition, metabolic factors and cancer risk. Best practice & research clinical endocrinology & metabolism. 2008;22(4):551-71. doi: 10.1016/j.beem.2008.08.003.
14. Naji Reyhani Garmroudi S, Karimi E, Oskoueian E, Homayouni-Tabrizi M, Iranshahi M. Ferutinin: A phytoestrogen from ferula and its anticancer, antioxidant, and toxicity properties. Journal of biochemical and molecular toxicology. 2021;35(4):e22713. doi: 10.1002/jbt.22713.
15. Zarei M, Karimi E, Oskoueian E, Es-Haghi A, Yazdi MET. Comparative study on the biological effects of sodium citrate-based and apigenin-based synthesized silver nanoparticles. Nutrition and cancer. 2021;73(8):1511-9. doi: 10.1080/01635581.2020.1801780.
16. Sidor A, Gramza-Michałowska A. Advanced research on the antioxidant and health benefit of elderberry (Sambucus nigra) in food–a review. Journal of Functional Foods. 2015;18:941-58. doi: 10.1016/j.jff.2014.07.012.
17. Ebrahimzadeh MA, Mahmoudi M, Karami M, Saeedi S, Ahmadi AH, Salimi E. Separation of active and toxic portions in Sambucus ebulus. Pakistan journal of biological sciences: PJBS. 2007;10(22):4171-3. doi: 10.3923/pjbs.2007.4171.4173.
18. Jiménez P, Tejero J, Cordoba-Diaz D, Quinto EJ, Garrosa M, Gayoso MJ, et al. Ebulin from dwarf elder (Sambucus ebulus L.): a mini-review. Toxins. 2015;7(3):648-58. doi: 10.3390/toxins7030648.
19. Mikulic-Petkovsek M, Schmitzer V, Slatnar A, Todorovic B, Veberic R, Stampar F, et al. Investigation of anthocyanin profile of four elderberry species and interspecific hybrids. Journal of Agricultural and Food chemistry. 2014;62(24):5573-80. doi: 10.1021/jf5011947.
20. Ding M, Feng R, Wang SY, Bowman L, Lu Y, Qian Y, et al. Cyanidin-3-glucoside, a natural product derived from blackberry, exhibits chemopreventive and chemotherapeutic activity. Journal of Biological Chemistry. 2006;281(25):17359-68.doi: 10.1074/jbc.M600861200.
21. Kiselova Y, Ivanova D, Chervenkov T, Gerova D, Galunska B, Yankova T. Correlation between the in vitro antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2006;20(11):961-5. doi: 10.1002/ptr.1985..
22. Ebrahimzadeh MA, Enayatifard R, Khalili M, Ghaffarloo M, Saeedi M, Charati JY. Correlation between sun protection factor and antioxidant activity, phenol and flavonoid contents of some medicinal plants. Iranian journal of pharmaceutical research: IJPR. 2014;13(3):1041.
23. Saravi SS, Shokrzadeh M, Shirazi FH. Cytotoxicity of Sambucus ebulus on cancer cell lines and protective effects of vitamins C and E against its cytotoxicity on normal cell lines. African Journal of Biotechnology. 2013;12(21).doi: 10.5897/AJB09.1577.
24. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods. 2001;25(4):402-8. doi:10.1006/ meth.2001.1262.
25. Sung J-M, Cho H-J, Yi H, Lee C-H, Kim H-S, Kim D-K, et al. Characterization of a stem cell population in lung cancer A549 cells. Biochemical and biophysical research communications. 2008;371(1):163-7. doi:10.1016/j.bbrc.2008.04.038.
26. Schuurhuis G, Broxterman H, De Lange J, Pinedo H, Van Heijningen T, Kuiper C, et al. Early multidrug resistance, defined by changes in intracellular doxorubicin distribution, independent of P-glycoprotein. British journal of cancer. 1991;64(5):857-61. doi: 10.1038/bjc.1991.413.
27. Sung J-M, Cho H-J, Yi H, Lee C-H, Kim H-S, Kim D-K, et al. Characterization of a stem cell population in lung cancer A549 cells. Biochemical and biophysical research communications. 2008;371(1):163-7. doi: 10.1016/j.bbrc.2008.04.038
28. Thole JM, Kraft TFB, Sueiro LA, Kang Y-H, Gills JJ, Cuendet M, et al. A comparative evaluation of the anticancer properties of European and American elderberry fruits. Journal of medicinal food. 2006;9(4):498-504. doi: 10.1089/jmf.2006.9.498.
29. Olejnik A, Olkowicz M, Kowalska K, Rychlik J, Dembczyński R, Myszka K, et al. Gastrointestinal digested Sambucus nigra L. fruit extract protects in vitro cultured human colon cells against oxidative stress. Food chemistry. 2016;197:648-57. doi: 10.1016/j.foodchem.2015.11.017.
30. Häuselmann I, Borsig L. Altered tumor-cell glycosylation promotes metastasis. Frontiers in oncology. 2014;4:28.doi:10.3389/fonc.2014.00028
31. Fulda S. Tumor resistance to apoptosis. International Journal of Cancer. 2009;124(3):511-5. doi: 10.1002/ijc.24064.
32. Arnal-Estapé A, Nguyen DX. Sweets for a bitter end: lung cancer cell–surface protein glycosylation mediates metastatic colonization. Cancer discovery. 2015;5(2):109-11. doi: 10.1158/2159-8290.cd-15-0013.
33. Reticker-Flynn NE, Bhatia SN. Aberrant glycosylation promotes lung cancer metastasis through adhesion to galectins in the metastatic niche. Cancer discovery. 2015;5(2):168-81. doi: 10.1158/2159-8290.
34. Shi Z, An N, Zhao S, Li X, Bao J, Yue B. In silico analysis of molecular mechanisms of legume lectin-induced apoptosis in cancer cells. Cell Proliferation. 2013;46(1):86-96. doi: 10.1111/cpr.12009.
35. Savanur MA, Eligar SM, Pujari R, Chen C, Mahajan P, Borges A, et al. Sclerotium rolfsii lectin induces stronger inhibition of proliferation in human breast cancer cells than normal human mammary epithelial cells by induction of cell apoptosis. PloS one. 2014;9(11):e110107. doi: 10.1371/journal.pone.0110107.
36. Tatsuta T, Hosono M, Takahashi K, Omoto T, Kariya Y, Sugawara S, et al. Sialic acid-binding lectin (leczyme) induces apoptosis to malignant mesothelioma and exerts synergistic antitumor

effects with TRAIL. International journal of oncology. 2014;44(2):377-84. doi:10.3892/ijo.2013.2192.
37. Chowdhury SR, Ray U, Chatterjee BP, Roy SS. Targeted apoptosis in ovarian cancer cells through mitochondrial dysfunction in response to Sambucus nigra agglutinin. Cell death & disease. 2017;8(5):e2762-e. doi: 10.1038/cddis.2017.77.
38. Henry-Mowatt J, Dive C, Martinou J-C, James D. Role of mitochondrial membrane permeabilization in apoptosis and cancer. Oncogene. 2004;23(16):2850-60. doi: 10.1038/sj.onc.1207534.
39. Westermann B. Bioenergetic role of mitochondrial fusion and fission. Biochimica et Biophysica Acta (BBA)-Bioenergetics. 2012;1817(10):1833-8. doi: 10.1016/j.bbabio.2012.02.033.
40. Youle RJ, Karbowski M. Mitochondrial fission in apoptosis. Nature reviews Molecular cell biology. 2005;6(8):657-63. doi: 10.1038/nrm1697.
41. Zhang Y, Vareed SK, Nair MG. Human tumor cell growth inhibition by nontoxic anthocyanidins, the pigments in fruits and vegetables. Life sciences. 2005;76(13):1465-72. doi: 10.1016/j.lfs.2004.08.025
42. Chemler JA, Leonard E, Koffas MA. Flavonoid biotransformations in microorganisms. Anthocyanins: Springer; 2008. p. 191-255. doi: 10.1007/978-0-387-77335-3_7.
43. Lazzè MC, Savio M, Pizzala R, Cazzalini O, Perucca P, Scovassi AI, et al. Anthocyanins induce cell cycle perturbations and apoptosis in different human cell lines. Carcinogenesis. 2004;25(8):1427-33. doi: 10.1093/carcin/bgh138.
44. Santen RJ. Benign Breast Disease in Women. [Updated 2018 May 25]. In: Feingold KR, Anawalt B, Boyce A, et al., editors: Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.;2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK278994/
45. Girbés T, Ferreras JM, Arias FJ, Stirpe F. Description, distribution, activity and phylogenetic relationship of ribosome-inactivating proteins in plants, fungi and bacteria. Mini reviews in medicinal chemistry. 2004;4(5):461-76. doi: 10.2174/1389557043403891.
46. Stirpe F. Ribosome-inactivating proteins. Toxicon. 2004;44(4):371-83. doi: 10.1016/j.toxicon.2004.05.004.
47. Ng T, Wong JH, Wang H. Recent progress in research on ribosome inactivating proteins. Current protein & peptide science. 2010;11(1):37-53. doi: 10.2174/138920310790274662.
48. Arias F, Rojo M, Iglesias R, FERRERAS JM, Girbes T. Vicia sativa L.'run-off'and purified ribosomes: polyphenylalanine synthesis and molecular action of ribosome-inactivating proteins. Journal of experimental botany. 1993;44(8):1297-304.doi: 10.1093/jxb/44.8.1297.
49. Chen Z, Shi T, Zhang L, Zhu P, Deng M, Huang C, et al. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family in multidrug resistance: A review of the past decade. Cancer letters. 2016;370(1):153-64. doi: 10.1016/j.addr.2012.09.027.
50. Smith AJ, van Helvoort A, van Meer G, Szabó K, Welker E, Szakács G, et al. MDR3 P-glycoprotein, a phosphatidylcholine translocase, transports several cytotoxic drugs and directly interacts with drugs as judged by interference with nucleotide trapping. Journal of Biological Chemistry. 2000;275(31):23530-9. doi: 10.1074/jbc.M909002199.
51. Němcová-Fürstová V, Kopperová D, Balušíková K, Ehrlichová M, Brynychová V, Václavíková R, et al. Characterization of acquired paclitaxel resistance of breast cancer cells and involvement of ABC transporters. Toxicology and applied pharmacology. 2016;310:215-28. doi:10.1074/jbc.M909002199.
52. Hontecillas-Prieto L, Garcia-Dominguez DJ, Vaca DP, Garcia-Mejias R, Marcilla D, Ramirez-Villar GL, et al. Multidrug resistance transporter profile reveals MDR3 as a marker for stratification of blastemal Wilms tumour patients. Oncotarget. 2017;8(7):11173. doi: g/10.18632/oncotarget.14491.
53. Januchowski R, Wojtowicz K, Andrzejewska M, Zabel M. Expression of MDR1 and MDR3 gene products in paclitaxel-, doxorubicin-and vincristine-resistant cell lines. Biomedicine & Pharmacotherapy. 2014;68(1):111-7. doi: 10.1016/j.biopha.2013.09.004.
54. Wen C, Fu L, Huang J, Dai Y, Wang B, Xu G, et al. Curcumin reverses doxorubicin resistance via inhibition the efflux function of ABCB4 in doxorubicin-resistant breast cancer cells. Molecular medicine reports. 2019;19(6):5162-8. doi: 10.3892/mmr.2019.10180.
55. Huang J-F, Wen C-J, Zhao G-Z, Dai Y, Li Y, Wu L-X, et al. Overexpression of ABCB4 contributes to acquired doxorubicin resistance in breast cancer cells in vitro. Cancer chemotherapy and pharmacology. 2018;82(2):199-210. doi: 10.1007/s00280-018-3603-y.
56. Way T-D, Lee H-H, Kao M-C, Lin J-K. Black tea polyphenol theaflavins inhibit aromatase activity and attenuate tamoxifen resistance in HER2/neu-transfected human breast cancer cells through tyrosine kinase suppression. European Journal of Cancer. 2004;40(14):2165-74. doi: 10.1016/j.ejca.2004.06.018.
57. Jones RS, Parker MD, Morris ME. Quercetin, morin, luteolin, and phloretin are dietary flavonoid inhibitors of monocarboxylate transporter 6. Molecular pharmaceutics. 2017;14(9):2930-6. doi: 10.1021/acs.molpharmaceut.7b00264.
58. Shim CK, Cheon EP, Kang KW, Seo KS, Han HK. Inhibition effect of flavonoids on monocarboxylate transporter 1 (MCT1) in Caco-2 cells. Journal of pharmacy and pharmacology. 2007;59(11):1515-9. doi: 10.1211/jpp.59.11.0008.
59. El-Far AH, Darwish NH, Mousa SA. Senescent colon and breast cancer cells induced by doxorubicin exhibit enhanced sensitivity to curcumin, caffeine, and thymoquinone. Integrative Cancer Therapies.

2020;19:1534735419901160. doi: 10.1177/1534735419901160.
60. Maridonneau-Parini I, Harpey C. Effect of trimetazidine on membrane damage induced by oxygen free radicals in human red cells. British journal of clinical pharmacology. 1985;20(2):148-51. doi: 10.1111/j.1365-2125.1985.tb05047.x.
61. Selvi MT, Thirugnanasampandan R, Sundarammal S. Antioxidant and cytotoxic activities of essential oil of Ocimum canum Sims. from India. Journal of Saudi Chemical Society. 2015;19(1):97-100. doi: 10.1016/j.jscs.2011.12.026.

How to Cite This Article

Ghanbari A, Roshankhah Sh, Salahshoor MR, Jalili C, Esmaeli M. Targeting Mitochondrial-Nuclear Apoptotic Signaling Pathways, Metabolic Pathways and Reversal of Doxorubicin (DOX) Cell Resistance in MCF-7 and MDA-MB-231 Cancer Cell Lines by Sambucus Nigra (SNA). Arch Breast Cancer. 2022; 9(1):83-95.
Available from: https://www.archbreastcancer.com/index.php/abc/article/view/465