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The effects of *Sideritis akmanii* on endoplasmic reticulum stress, inflammation, and DNA damage in experimentally ER-stress-induced MCF-7 cancer cells

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ABSTRACT

Cancer is one of the diseases that became a social problem that can happen with uncontrolled proliferation, growth, differentiation, and spread of cells in our body. Breast cancer, on the other hand, is one of the types of cancer with the highest incidence in women. In our study, endoplasmic reticulum (ER) stress is induced by thapsigargin (T) in MCF-7 cells and then, the effects of *Sideritis akmanii* acetone extract (SAE) on cell viability, ER stress, inflammation, and DNA damage were investigated. The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] test was used to determine the effect of SAE on cytotoxicity and the comet (SCGE; single-cell gel electrophoresis) assay was used for the effects on genotoxicity. Additionally, the mRNA expression levels of both ER stress parameters (ATF4: activating transcription factor 4, ATF6: activating transcription factor 6, PERK: protein kinase RNA-like ER kinase, GRP78: glucose-regulated protein 78) and inflammation-related parameters (TNF alpha: tumor necrosis factor-alpha, IFN-gamma: interferon-gamma, IL-6: interleukin-6, IL-8: interleukin-8, IL-12: interleukin-12) were determined by qPCR. The results showed that DNA damage levels increased as a result of T treatment, DNA damage caused by T decreased when a low dose of SAE was administered and a high dose of SAE further increased DNA damage levels. It was determined that SAE, administered in different doses with T or alone in experimental groups, increased mRNA expression levels of all ER stress and inflammatory genes compared to the control group. As a result, it has been determined that *S. akmanii*, especially at high doses, may exhibit anticarcinogenic effects through its effects on genotoxic, cytotoxic, and ER stress in MCF-7 cells.

1. Introduction

Cancer is a disease that is the second most cause of death after cardiovascular diseases both in the world and in our country. Cancer is caused by the uncontrolled proliferation, growth, differentiation, and spread of cells in our body. Breast cancer is one of the most common types of cancer among women (Ferlay et al., 2015). Breast cancer is a multifactorial disease in which there is a strong interaction between genetic and environmental factors (McPherson et al., 2000). Many studies have shown that oxidative stress and inflammation, which can become chronic under the influence of environmental factors, can be associated with the development and progression of cancer. Inflammation and oxidative stress can be effective in stimulating oncogenic factors through both DNA damage and endoplasmic reticulum (ER) stress. However, chemotherapeutic agents such as carboplatin, which have many anticarcinogenic effects, and bioactive substances of plants that have antioxidant effects can show anticarcinogenic effects by increasing oxidative stress, inflammation, and ER stress (Hazman et al., 2018).

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There is a linear relationship between the reactive oxygen species (ROS) that occur in cancer cells and the level of DNA damage caused by them and ER stress can occur due to DNA damage through ROS (Evyapan et al., 2019). Both DNA damage and ER stress-mediated programmed cell death (apoptosis) is effective in maintaining growth, development, and homeostasis in healthy individuals, while in cancer patients they can be used to eliminate cancer cells (Hazman et al., 2018).

ER has numerous important functions such as regulating intracellular calcium storage, post-translational modification, folding, and delivery of newly synthesized proteins (Mori, 2000). These functions of the ER are essential for the vital continuity of the cell. ER damage or a defect in ER functions causes errors in protein folding or intracellular calcium storage dysregulation, leading to a condition defined as ER stress (Sancho-Martínez et al., 2012). ER transmembrane proteins called IRE1 (inositol-requiring enzyme 1), PERK, and ATF6 which are stimulated as a result of ER stress would lead to the development of unfolded protein response (UPR) and activate signal transduction pathways that lead the cell to apoptosis (Fribley et al., 2009; Mori, 2000; Treiman, 2002). Normally, IRE1, PERK, and ATF6 proteins are bound by the ER chaperone protein named GRP78 (ER-chaperone glucose-regulated protein 78) and are in an inactive form. However, UPR, which develops due to protein folding suppression which happens due to the factors such as oxidative stress, DNA damage, and inflammation, which are caused by the effect of various factors in the tissues/cells in the organism, triggers apoptosis via caspase 12 by separating GRP78 from these 3 proteins (IRE1, PERK, and ATF6) localized in the ER transmembrane (Çelik et al., 2015; Hazman et al., 2018).

In this study, presented in this context, we investigated the effects of *Sideritis akmanii*, an endemic sage species, on ER stress and DNA damage in breast cancer (MCF-7) cells. For this purpose, *S. akmanii* acetone extract (SAE) was prepared first and then the cytotoxicity of this extract was determined. ER stress, inflammation, and DNA damage levels were detected after the application of different doses of SAE.

2. Materials and methods

2.1. Acetone extraction of S. akmanii

The plants were collected from the Kumalar plateau in the countryside of Suhut District of Afyonkarahisar province on July 16, 2015, when flowering was at its highest level. The plants dried in the shade were pulverized. 20 g of the plant material was weighed and extracted with 250 ml of acetone in the Soxhlet device for 24 hours. After the extraction process, acetone was evaporated with the help of a rotary and it was kept at +4 °C until it was used.

2.2. Determination of SAE cytotoxicity levels in MCF-7 cells

The MTT solution used in the study was prepared by dissolving the MTT salt (Sigma) in a phosphate buffer (pH: 7.4) at a concentration of 5 µg/ml. MTT solution was prepared fresh for each use.

$1x10^5$ MCF-7 cells were seeded into each well of the 24-well plate using the cell suspension, whose number of cells per ml was determined with the trypan blue method. The plates were incubated at 37 °C in the incubator (Panasonic) containing 5% CO_2 (carbon dioxide). When the density of the cells on the plate surface reached 50-60%, SAE doses were administered as 1000, 750, 500, 250, 100, 50, and 10 µg/ml. 1% DMSO was used as a control. The plates were placed into the incubator, which had 5% CO_2, to be incubated for 24 hours at 37 °C. After incubation, MTT solution with a temperature of 37 °C was added to each well at a rate of 10% of the well's volume and incubated again for 3 hours. At the end of the incubation, the formazan crystals in the wells of the plate were removed with an automatic pipette without damaging the cells. Subsequently, the formazan crystals remaining at the bottom of the wells on the plate were dissolved in 800 µl of DMSO added to each well (Hazman et al., 2021). Immediately after, absorbance values were determined at 540 nm with a microplate reader spectrophotometer (BioTek ELx-800). Cell viability of the control group which was not treated with SAE was considered 100%, and the effect of each dose on cell viability was calculated using the formula shown below (Günay et al., 2016; Ulasli et al., 2013). At least three repetitions were performed for each dose in the MTT analysis used to determine cell viability.

$$Cell\ viability\ (\%) = 100\ \mathrm{x}\frac{Absorbance\ of\ sample}{Absorbance\ of\ control}$$

2.3. Creation of experimental groups and experimental model

SAE doses to be applied to experimental groups were determined according to cytotoxicity analysis. To obtain cell lysates to be used as samples in the analysis, $5x10^5$ cells were seeded in 25 cm^2 flasks in a 5 ml medium. When the confluent ratio reached 50-70%, ER stress was induced by applying 50 µl 10-8 M T to each flask in the respective groups. SAE at doses of 10, 50, and 100 µg/ml was applied to flasks with and without ER stress. Experimental groups created in the study and applications used on experimental groups are presented in Table 1.

Table 1. The design of the experimental groups

Experimental groups*	Applications to cells
Group 1: Control	After the cells were seeded, no application was made other than medium.
Group 2: DMSO	DMSO, the solvent of T and SAE, was applied to the cells at a rate of 1%.
Group 3: Thapsigargin (T)	To create ER stress in cells, T was applied at a final concentration of 10^{-8} M.
Group 4: SAE_{10}	SAE was applied to the cells at a final concentration of 10 µg/ml.
Group 5: T + SAE_{10}	SAE at 10 µg/ml was applied to the cells together with T at 10^{-8} M concentration.
Group 6: SAE_{50}	SAE was applied to the cells at a final concentration of 50 µg/ml.
Group 7: T + SAE_{50}	SAE at 50 µg/ml was applied to the cells together with T at 10^{-8} M concentration.
Group 8: SAE_{100}	SAE was applied to the cells at a final concentration of 100 µg/ml.
Group 9: T + SAE_{100}	SAE at 100 µg/ml was applied to the cells together with T at 10^{-8} M concentration.

* Studies with experimental groups were carried out with at least 3 repetitions.

2.4. Determination of DNA damage

After SAE and T applications were performed on MCF-7 cells, DNA damage in samples was determined using the comet assay. 24 hours after the experimental groups mentioned in Table 1, the cells were collected from the base of the flask by trypsinization and detrypsinization. Cell suspensions belonging to experimental groups created in 1 ml of phosphate-buffered saline (PBS) were used in the

analysis. For this purpose, 20 µl of cell suspension was taken and mixed with 100 µl of low melting agarose (LMA) in eppendorf. All of the cell-LMA mixtures in the eppendorf were taken and prepared in slides by treating them with normal melting agarose (NMA) one day in advance. Following the stages of lysis and electrophoresis, each preparation was stained. The stained preparations were scored by counting 100 cells in a fluorescence microscope (Singh et al., 1988).

2.5. RNA isolation, cDNA synthesis, and gene expression levels

In MCF-7 cells, mRNA expression levels were determined by using total RNA isolated from experimental groups, and complementary DNA (cDNAs) was determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) method using a commercial cDNA synthesis kit (GeneMatrix). In the analyses, total RNAs belonging to MCF-7 cells were isolated using a commercial kit (GeneMatrix). The amount of RNAs obtained was determined through the optic density (OD_{260}/OD_{280}) in nanodrop (BioTek, Epoch 2). RNAs with an OD_{260}/OD_{280} ratio of 1.7-2.2 were used in the study (Hazman et al., 2021).

Syber Green PCR Master Mix (10 µl) and primer pair (oligonucleotide) were added to 1 µl of the cDNA of each sample obtained by following the manufacturer's protocols. Primers are specific for each transcription analysis and were determined using studies in the literature (Günay et al., 2016). The primers presented in Table 2 were used at the level of 100 ng in each RT-PCR reaction.

Table 2. Expression primers used in real-time PCR analysis

Genes	Primers	
B-actin	F: 5'-CACCCCAGCCATGTACGTTGC-3'	R: 5'-CCGGAGTCCATCACGATGCCA-3'
IFN-γ	F: 5'-GCAATCTGAGCCAGTGC-3'	R: 5'-TTTGGAAGCACCAGGCA-3'
TNF-α	F: 5'-GAGTGACAAGCCTGTAGCCCA-3'	R: 5'-GCTGGTTATCTCTCAGCTCCACG-3'
IL 12	F: 5'-GACCCAGGAATGTTCCCATGC-3'	R: 5'-TTTCTGGAGGCCAGGCAACTC-3'
IL-6	F: 5'-GGTACATCCTCGACGGCATCT-3'	R: 5'-GTGCCTCTTTGCTGCTTTCAC-3'
IL-8	F: 5'-GACAAGAGCCAGGAAGAAAC-3'	R: 5'-CTACAACAGACCCACACAATAC-3'
GRP78	F: 5'-GCCTGTATTTCTAGACCTGCC-3'	R: 5'-TTCATCTTGCCAGCCAGTTG-3'
PERK	F: 5'-CCCCAACAAGGCCAGCCTGG-3'	R: 5'-GGACAGCCAGCCGTGTTCCC-3'
ATF4	F: 5'-TCAAACCTCATGGGTTCTCC-3'	R: 5'-GTGTCATCCAACGTGGTCAG-3'
ATF6	F: 5'-CTCCGAGATCAGCAGAGGAA-3'	R: 5'-AATGACTCAGGGATGGTGCT-3'

Table 3. Determination of cytotoxicity levels of SAE in MCF-7 cells

Concentration (µg/ml)	MCF-7 cell viability levels (%)
1000	29.86 ± 3.44
750	32.29 ± 3.24
500	2986 ± 5.66
250	24.82 ± 1.65
100	20.65 ± 0.89
50	44.27 ± 0.69
25	81.07 ± 7.48
0	100.00 ± 8.41

The data are presented as mean ± standard deviation ($n \geq 3$). A group of cells without any SAE treatment (dose 0) were used as the control group.

Table 4. Lethal doses of SAE in MCF-7 cells

Lethal doses (LDs)*	Concentration (µg/ml)
LD_0	0.002
LD_5	0.036
LD_{10}	0.169
LD_{15}	0.479
LD_{50}	38.851

* Lethal doses were determined as a result of calculating % viability rates (Table 3) obtained from MTT analysis after applying different doses of SAE to MCF-7 cells through the probit analysis program.

In the qPCR analysis performed, the protocol was applied for the β-actin gene for 10 minutes at 95 °C, 1 minute at 94 °C, 1 minute at 61 °C and 1 minute at 72 °C. The application for ATF6, PERK, ATF4, and IL-6 was performed for 10 min at 95 °C, 1 min at 94 °C, 1 min at 60 °C and 1 min at 72 °C. The protocol applied is as follows: For GRP78, 10 minutes at 95 °C, 30 seconds at 95 °C, 1 minute at 58 °C, and 30 seconds at 72 °C; for TNF-α and IL-12 for 10 minutes at 95 °C, 30 seconds at 95 °C, 1 minute at 54 °C and 30 seconds at 72 °C; for TNF-α and IL-12 for 10 minutes at 95 °C, 30 seconds at 95 °C, 1 minute at 54 °C and 30 seconds at 72 °C. A total of 40 cycles were performed in all analyses. Gene expression values were calculated according to the formula 2-ΔΔCt. The calculation of the ΔΔCt values used in this study was performed with the formula presented below (Pfaffle, 2001).

$$\Delta\Delta Ct = (\text{Cttarget gene} - \text{Ctβactin})\text{experimental group} - (Cttarget\ gene - Ct\beta actin)control\ group$$

3. Results and discussion

3.1. Effect of SAE on cytotoxicity levels in MCF-7 cells

The viability rates (%) obtained as a result of MTT analysis performed to determine the cytotoxicity of SAE on the MCF-7 cell line and the SAE lethal doses calculated using these rates are presented in Table 3 and Table 4, respectively.

When the data in Table 3 and Table 4 are examined, it was seen that the toxicity of SAE in MCF-7 cells begins from low doses. In the study, 3 doses have been administered to MCF cells were determined by taking into account the cytotoxic doses of SAE (Table 3 and Table 4). The analyzes were continued by using 10 µg/ml of SAE extract, which is lower than the LD_{50} dose as a low dose, a dose close to the LD_{50} dose (50 µg/ml) as the medium dose, and 100 µg/ml SAE extract as the high dose.

3.2. Effect of SAE on DNA damage in MCF-7 cells

Statistical evaluation of the data obtained from comet assay is presented in Table 5. Compared to the control group, only T administration and high (50 and 100 µg/ml) doses of SAE applications were found to increase DNA damage scores in the experimental groups. It was observed that this increase was greater in the groups in which T was administered together with high doses of SAE. It was observed that the DNA damage did not change in the experimental groups in which a low dose of SAE was applied, compared to the control and DMSO groups.

Table 5. Effect of SAE on DNA damage in MCF-7 cells

Experimental groups	DNA damage levels
Control	9.67 ± 2.08^{a}
DMSO	14.33 ± 3.79^{ab}
T	45.00 ± 5.00^{c}
SAE_{10}	17.33 ± 5.77^{ab}
T + SAE_{10}	18.33 ± 7.64^{b}
SAE_{50}	68.67 ± 1.53^{d}
T + SAE_{50}	92.00 ± 3.00^{f}
SAE_{100}	82.00 ± 3.00^{e}
T + SAE_{100}	94.00 ± 5.29^{f}

The data are presented as mean ± standard deviation (n = 5). ^{a,b,c,d,e,f}: The difference between mean values carrying different exponential expressions on the same row is statistically significant ($p < 0.05$). Abbreviations: DMSO; dimethyl sulfoxide, T; Thapsigargin, SAE; *S. akmanii* acetone extract.

Table 6. The effect of SAE on mRNA levels of some genes related to ER stress and inflammation in MCF-7 cells

Experimental groups	Genes whose mRNA expression levels are determined								
	ATF6	ATF4	PERK	Grp78	IL-6	IL-8	IL-12	TNF-α	IFN-γ
*SAE_{10}	+ 0.43	+ 30.27	+ 0.71	+ 0.26	+ 4.29	+ 0.46	+ 0.23	+ 0.19	+ 0.27
*T + SAE_{10}	+ 0.53	+ 27.92	+ 0.68	+ 0.52	+ 1.60	+ 0.97	+ 0.52	+ 0.29	+ 0.51
*SAE_{50}	+ 5.72	+ 0.75	+ 0.75	+ 1.60	+ 12.20	+ 2.47	+ 0.17	+ 0.96	+ 1
*T + SAE_{50}	+ 1.61	+ 0.84	+ 0.84	+ 1.27	+ 1.31	+ 0.48	+ 0.38	+ 0.54	+ 0.68
*SAE_{100}	+ 0.28	+ 50.09	+ 0.56	+ 0.84	+ 6.16	+ 6.77	+ 0.14	+ 0.33	+ 0.38
*T + SAE_{100}	+ 0.23	+ 124.40	+ 1.24	+ 0.93	+ 16.14	+ 5.54	+ 0.65	+ 0.47	+ 0.53

* It shows the experimental groups whose stimulation and suppression conditions were expressed compared to the control group. Data expressed by '+' indicates that the data of the specified experimental group of the relevant gene is stimulated compared to the data of the control group of the relevant gene. Abbreviations: DMSO; dimethyl sulfoxide, T; thapsigargin, SAE; *S. akmanii* extract, ATF4; activating transcription factor 4, ATF6; activating transcription factor 6, PERK; protein kinase RNA-like ER kinase, GRP78; glucose regulating protein 78; TNF-α; tumor necrosis factor-alpha, IFN-γ; Interferon-gamma, IL-6; Interleukin-6, IL-8; Interleukin-8, IL-12; Interleukin-12.

3.3. Effects of SAE on ER stress and inflammation in MCF-7 cells

In the presented study as stated before, the expression levels of ATF6, ATF4, PERK, and GRP78 mRNA of the ER stress genes and expression levels of IL-6, IL-8, IL-12, TNF α, and IFN-γ mRNA of the inflammatory and pro-inflammatory genes were calculated by normalizing with the β-actin gene. The findings showed that SAE, administered to experimental groups with T or in different doses alone, increased mRNA expression levels of ER stress and inflammation-related genes compared to the control group. The findings are presented in Table 6.

We conducted this study using cell culture (MCF-7 cells) and included control groups in our experiments. The advantage of cell culture experiments is that they can promptly allow the observation of the biological effects of the test substance in question. Besides, the use of cells that grow unlimitedly in cell culture does not require the ethics committee report encountered when conducting studies with experimental animals. Therefore, cell culture studies are widely used in cancer research, biochemistry, cytogenetics, and molecular biology studies worldwide. In our study, the cytotoxic effects of SAE on MCF-7 cells were investigated by the MTT method. It was observed that the cytotoxic effect of SAE on MCF-7 cells increased depending on the increase in concentration. In 24-hour application, the LD_{50} dose of SAE was found to be 38.851 µg/ml. Due to low doses of the LD_{50} dose (38.851 µg/ml) of SAE, it may be recommended to investigate the anticarcinogenic activity of this sample in further studies. Similar to our study, Yumrutas et al. (2015), in their study on MCF-7 cells, found that 100 µg/ml dose of methanol extract of *S. syriaca* reduced cell viability in MCF-7 cells (54.91 % of the control group).

Loizzo et al. (2007) extracted the essential oils of *S. perfoliata* in their study and detected 15 monoterpenes, 17 sesquiterpenes, and 1 diterpene in the essential oil. In their cell culture studies with essential oils, they determined that essential oils showed high activity on human melanoma (C32) cells and kidney adenocarcinoma (ACHN) cells, and their IC_{50} values were determined as 98.58 and 100.90 µg/ml, respectively. In our study, it was determined that SAE, which is in the same family as *S. perfoliata*, decreased cell viability in MCF-7 cells depending on the increasing concentration.

Todorova and Trendafilova (2014) determined in their study with S. scardica that 100 µg/ml of the plant's diethyl ether extract decreased cell viability in B16 and HL-60 cells by 51.3% and 77.5%, respectively. They have shown that decreased cell viability is caused by flavonoids (apigenin, luteolin, and their glycosides). In line with this information, it is thought that flavonoids contained in SAE may cause cytotoxicity observed on MCF-7.

The comet assay is a widely used method for assessing DNA damage in vitro and in vivo. It is also known as single-cell gel electrophoresis (SCGE). It is based on a run with electrophoresis after the lysis of cells embedded in agarose gel. While the undamaged DNA remains intact in the cell nucleus during the electrophoresis process, breaks emerge as a result of DNA fragmentation. The fragments of broken DNA extend toward the anode, forming a comet-shaped structure. This comet-shaped DNA is then made visible using a DNA-binding dye. To assess the extent of DNA damage, the comet's shape, size, and amount of DNA in it is measured. Types of analysis protocols of the comet method are the measurement of DNA crosslinks (e.g. thymine dimers), and DNA single- and double-strand breaks. The advantages of the comet assay are that it requires a small number of

cells per sample and provides individual damage data per cell. Scoring comets in the comet assay can be done either visually or with image analysis software (Tice et al., 2000).

In our study, we aimed to investigate the possible genotoxic effects of SAE on MCF-7 cells. Compared to the control group, only T administration and high doses (50 and 100 µg/ml) of SAE applications were found to increase DNA damage scores in the experimental groups. It was observed that this increase was greater in the groups in which T was administered together with high doses of SAE. It was observed that the DNA damage did not change in the experimental groups in which low-dose SAE was applied, compared to the control and DMSO groups. These data indicate that low-dose SAE can be used for preventive and therapeutic purposes as it does not affect DNA damage, while high doses may show anticarcinogenic effects on cancer cells due to increased DNA damage.

In their study with flavonoids, Das et al. (2017) found that combined flavonoid applications induced DNA damage in human erythroleukemic cell line (K562) cells. For example, they have shown that the combination of quercetin and myricetin leads to maximum DNA damage. In our study, it is thought that the presence of combined flavonoids in SAE increases the DNA damage on MCF-7 cells depending on the increase in the dose.

Guo et al. (2016) showed that Jungermannenon A and B diterpenoids initiate ROS production on PC3 (prostate cancer), which leads to the induction of mitochondrial damage and permanent DNA damage. In our study, the combination of diterpenoids' presence in SAE may cause the induction of mitochondrial damage and permanent DNA damage by initiating ROS production in MCF-7 cells.

Chronic inflammation affects immune system components that are directly linked to cancer progression. Under normal conditions, immune cells, which include granulocytes, macrophages, mast cells, lymphocytes, dendritic cells, and natural killer cells, act as a preliminary defense against pathogens. When tissue damage occurs, macrophages and mast cells secrete cytokines and chemokines to direct circulating leukocytes to the damaged area to destroy pathogens (Samadi et al., 2015).

In our study, expression levels of IL-6 cytokine of SAE on MCF-7 cells increased in all application groups compared to the control. Gene expression levels of IL-8, a chemokine, were increased in SAE10, T+SAE10, and T+SAE50 applications, while they increased in other applications. The effect of SAE on the IL-12 and TNF-α genes was found to be similar and suppressed in all application groups. While the IFN-γ gene showed a similar effect with control at a concentration of 50 µg/ml, it was suppressed in other administration groups. In our study, it is thought that SAE has a similar effect on the effect of tissue damage by increasing the expression of IL-6 cytokine.

IL-8 greatly affects the tumor microenvironment. It is the chemokine that promotes the activation of endothelial cells, angiogenesis, neutrophil infiltration in the tumor site, and cancer development through increased autocrine signaling. Inhibition of IL-8 in cancer cells is crucial as it reduces the proliferation and progression of cancer cells. Especially in prostate cancer, IL-8 plays an important key role in the adhesion of cancer cells to the endothelium and extracellular matrix by increasing the risk of cancer and cell survival. Aqueous extract of *S. syriaca* was observed to significantly lower IL-8 levels on stimulated human colon cancer cell line 29 (HT29) and human prostate cancer cell line 3 (PC3) cells (Kogiannou et al., 2013). In our findings, IL-8 gene expression levels in MCF-7 cells of SAE were suppressed in SAE10, T+SAE10, and T+SAE50 applications, while they increased in SAE50, SAE100, and T+SAE100 applications. These results suggest that the consumption of *S. akmanii* as a tea by the people in Anatolia may be medicinally effective. It can be considered that it indirectly slows the development and progression of cancer in low doses when consumed by the public as tea.

ER is very sensitive to changes occurring at the physiological and biochemical level, such as ion balance in its lumen, and synthesis charge. Glucose deprivation, calcium depletion, exposure to free radicals, impairment of glycosylation, excessive cholesterol load and disruption of cell control mechanisms due to some pathological and physiological reasons associated with oxidative stress induction causes the accumulation of misfolded proteins and subsequently an increase in ER stress (Imai et al., 2016; Sozen & Ozer, 2017). The accumulation of misfolded proteins in the ER generates the UPR response as a protective reaction that reduces incipient protein synthesis, facilitates protein folding, and increases proteasomal degradation of misfolded proteins (Christen & Fent, 2016). UPR is primarily a survival response that activates protein misfolding mechanisms. However, prolonged ER stress conditions can disrupt cell life. The three pathways activated by ER stress are IRE1, PERK, and ATF6. In cases where there is no ER stress, these three pathways are suppressed by the GRP78/BIP chaperon (Kapuy et al., 2014).

Zhou et al. (2014), in their study, investigated the relationship between ER stress and apoptosis by inducing ER stress with T in MCF-7 cells. They showed that in MCF-7 cells induced by ampelopsin (an important bioactive component of *Ampelopsis grossedentata*), T increased the expression of the GRP78 gene. They also determined that T increased ampelopsin-induced cell growth inhibition and apoptosis. In our study, it was observed that the expression levels of the GRP78 gene, which plays a role in the UPR response in ER stress, in the SAE50 and T+SAE50 application groups increased compared to the control. It was observed that the PERK gene involved in UPR response in ER stress was suppressed in all application groups, but there was an increase in T+SAE100 administration compared to the control. Also, ATF6, which is involved in UPR response and is under GRP78 control in ER stress, was found to increase in SAE50 and T+SAE50 application groups such as GRP78. This suggests that GRP78 reacts to ER stress by activating ATF-6 in the case of ER stress. ATF4, which is under PERK control in the case of ER stress, was observed to have increased expression levels at low and high concentrations and expression levels were suppressed at a dose of 50 µg/ml SAE. This situation suggests that ATF4 is activated differently from PERK.

4. Conclusions

In line with the results obtained from this study, it was determined that SAE has genotoxic and cytotoxic effects on MCF-7 cells. As an endemic species, this plant was found to cause ER stress in MCF-7 cells and could be effective in inflammation. This study will help to investigate the apoptosis process associated with ER stress in the future. It is thought that this study can also lead the path of studies to explain the possible phytotherapeutic effects of *S. akmanii*.

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Conflict of interest

The authors confirm that there are no known conflicts of interest.

Statement of ethics

In this study, no method requiring the permission of the "Ethics Committee" was used.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Supplementary File

None.

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