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MCF-7 MEME KANSERİ HÜCRE HATTINDA *LEUCOJUM AESTIVUM*'DAN ELDE EDİLEN GALANTAMİNİN ANTİANJİOJENİK VE APOPTOTİK ETKİLERİNİN ARAŞTIRILMASI

INVESTIGATION OF ANTIANGIOGENIC AND APOPTOTIC EFFECTS OF GALANTAMINE OBTAINED FROM *LEUCOJUM AESTIVUM* ON MCF-7 BREAST CANCER CELL LINE

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ÖZET

AMAÇ: Mevcut çalışma, MCF-7 meme kanseri hücrelerinde galantaminin antianjiojenik ve apoptotik etkilerini araştırmayı ve literatüre yeni bir bilgi kazandırmayı amaçlamaktadır.

GEREÇ VE YÖNTEM: Bu çalışmada, MCF-7 meme kanseri hücre dizisi kullanılmış ve galantamin, Leucojum aestivum'dan HPLC yöntemi ile elde edilmiştir. Galantaminin hücre canlılığı üzerindeki etkisi, kontrol grubu dahil 9 farklı dozda (kontrol, 10, 40, 70, 100, 130, 160, 190, 210 µg/ml) CCK-8 tahlili ile belirlendi. Daha sonra galantaminin etkisini anlamak için diğer analizler (toplam antioksidan seviyesi (TAS), toplam oksidan seviyesi (TOS), poli-ADP riboz polimeraz (PARP) ve vasküler endotelyal büyüme faktörü (VEGF) seviyeleri) 3 grupta incelendi; kontrol, LD50 (100 µg/ml) ve yüksek doz (210 µg/ml) grubu. Ardından oksidatif stres endeksi (OSI) hesaplandı.

BULGULAR: Galantamin hücre canlılığını azalttı. LD50 dozu 100 µg/ml olarak belirlendi. VEGF düzeylerinde herhangi anlamlı bir değişim olmadığı belirlendi. PARP düzeylerinde doza bağlı olarak anlamlı bir azalma olmuştur (Kontrol: 2.78667±0.155392, LD50: 1.51000±0.107145, Yüksek doz: 1.01000±0.054772 ng/L, p=0.000). Kontrol grubuna kıyasla TAS verilerinde doza bağlı anlamlı bir azalma (Kontrol: 0.09633±0.002658, LD50: 0.06283±0.002317, Yüksek doz: 0.04050±0.001871, p=0.000) ve TOS verilerinde doza bağlı anlamlı bir artış vardı (Kontrol:0.12500±0.010488, LD50: 0.21667±0.015055, Yüksek doz: 0.31833±0.021370, p=0.000). OSI verilerinde doza bağlı anlamlı artış bulundu. (Kontrol: 129.964±13.018, LD50: 345.161±26.480, Yüksek doz: 788.485±78.575, p=0.000).

SONUÇ: Galantaminin herhangi bir dozda VEGF düzeyleri baz alınarak anjiyogenezde anlamlı bir etkisinin olmadığı belirlendi. Galantaminin MCF-7 hücrelerine belirli dozlarda hücre canlılığına negatif etki yapmıştır ve oksidatif stresi artırdığı bulunmuştur. PARP seviyelerindeki düşüş hücrelerin apoptotik süreçle sonuçlanabileceği ihtimalini göstermektedir. Bu bulgular, kanser araştırmalarında galantamin kullanımına farklı bir yaklaşım getirmek için faydalı olabilir.

ANAHTAR KELİMELER: Galantamin, Oksidatif stres, Poli (ADP-riboz) polimeraz, Vasküler endotelyal büyüme faktörü, MCF-7 hücreleri.

ABSTRACT

OBJECTIVE: The current study aims to investigate the antiangiogenic and apoptotic effects of galantamine in breast cancer cells and to add new information to the literature.

MATERIAL AND METHODS: In this study, MCF-7 breast cancer cell line was used and galantamine was obtained from Leucojum aestivum by HPLC method. The effect of galantamine on cell viability was determined by CCK-8 assay at 9 different doses (control, 10, 40, 70, 100, 130, 160, 190, 210 µg/ml) including the control group. Then, to understand the effect of galantamine, other assays (total antioxidant status (TAS), total oxidant status (TOS), poly-ADP ribose polymerase (PARP), and vascular endothelial growth factor (VEGF) levels) were examined in 3 groups; control, LD50 (100 µg/ml) and high dose (210 µg/ml) group. The oxidative stress index (OSI) was then calculated.

RESULTS: Galantamine decreased cell viability. The LD50 dose was determined as 100 µg/ml. There was no significant change in VEGF levels. There was a significant dose-dependent decrease in PARP levels (Control: 2.78667±0.155392, LD50: 1.51000±0.107145, High dose: 1.01000±0.054772 ng/L, p=0.000). Compared to the control group, there was a significant dose-related decrease in TAS data (Control: 0.09633±0.002658, LD50: 0.06283±0.002317, High dose: 0.04050±0.001871, p=0.000) and a significant dose-related increase in TOS data (Control: 0.12500±0.010488, LD50: 0.21667±0.015055, High dose: 0.31833±0.021370, p=0.000). A significant dose-related increase in OSI data was found. (Control: 129.964±13.018, LD50: 345.161±26.480, High dose: 788.485±78.575, p=0.000).

CONCLUSIONS: It was determined that galantamine had no significant effect on angiogenesis at any dose based on VEGF levels. Galantamine had a negative effect on cell viability and proliferation at certain doses to MCF-7 cells and was found to increase oxidative stress. The decrease in PARP levels indicates the possibility that cells may result in an apoptotic process. These findings may be useful to take a different approach to the use of galantamine in cancer research.

KEYWORDS: Galantamine, Oxidative stress, Poly (ADP-ribose) polymerase, Vascular endothelial growth factor, MCF-7 cells.

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INTRODUCTION

Breast cancer is the most common and has the highest mortality rate among women in the world (1). The onset of breast cancer in women reduces the quality of life with effects such as stress, pain, social relations, depression, complex treatments, and economic burden (2). The main therapeutic options for breast cancer are radiotherapy, surgery, chemotherapy, and hormone therapy (2, 3). Various drugs have been developed for the treatment of breast cancer (4). Surgical treatment is a good option for local breast cancer, but the entire tumor may not be removed. At this stage, it is aimed to solve the problem with methods such as radiotherapy and chemotherapy. However, there are some side effects, the tumor may recur, drug resistance may develop, or it may have toxic effects on other healthy cells. In such cases, the use of natural components such as alkaloids is seen as an alternative strategy (5). Increasingly popular alkaloids have anticancer properties and various therapeutic potentials (3, 5).

Many alkaloids have angiogenesis inhibitory properties (6). Angiogenesis, meaning the increased formation of blood vessels, is essential for breast tumor growth to provide adequate oxygen and nutrition. Breast cancer cells, like all other cells, need continuous nutrition and oxygen support through the vascular network of the capillaries in the system. The mechanism of angiogenesis in all human tumor types is a unique target. Vascular endothelial growth factor (VEGF), a pro-angiogenic factor, stimulates angiogenesis through vascular endothelial growth factor receptors (VEGFR) and ligands. Signaling of intracellular VEGF and VEGFR can be inhibited by anti-angiogenesis therapy, and the development of tumor vessels can be inhibited (7).

One of the developed treatment methods is to cause cell death by interfering with the DNA repair mechanism of cancerous cells. The poly (ADP-ribose) polymerase (PARP) protein can be targeted in this direction. PARP inhibitors were developed mainly in the late 1990s to increase the anticancer activity of ionizing radiation and chemotherapy drugs (8). The base excision repair mechanism cannot function without PARP enzymes. When poly (ADP-ribose) polymerase-1 (PARP1) binds to the broken DNA strand, Nicotinamide Adenine Dinucleotide (NAD+) can bind to its active site. Single-strand DNA repair effectors are attracted by a process termed PARylation in which NAD+ transfers ADP-ribose moieties to certain proteins. When PARP1 auto-PARylates, it is released from DNA and returns to a catalytically inactive state. PARP inhibitors bind to PARP and inhibiting PARylation. They also trap inactive PARP on DNA, which prevents replication forks from forming, causing them to collapse and creating double-strand breaks (9).

The current study investigated the antiangiogenic and apoptotic effects of galantamine, an Amaryllidaceae alkaloid derived from *Leucojum aestivum (L. aestivum)*, on the human breast cancer cell line MCF-7, based on the therapeutic potential of the alkaloids.

L. aestivum, belonging to the Amaryllidaceae family, is a bulbous plant and is 30-60 cm tall. *L. aestivum* is known as "summer snowflake" in English. It blooms in March and June. Species usually have 1-5 flowers, which are bell-shaped. There are 10 species of the genus *Leucojum L*. in the world. Only *L. aestivum* species belonging to this genus grow naturally in Turkey. These species are endangered and have an economic value in Turkey (10).

The Summer snowflake is a very important medicinal plant and also a valuable ornamental plant. Both the bulbs and fresh green leaves of the species contain many important chemicals, primarily the galantamine alkaloid (11).

In the history of the development of pharmaceutical compounds, the plant has been recognized as a key factor in mimicking nature and isolating medicinal materials. Research to date has focused on secondary metabolites such as flavonoids and phenolics, which are widely used as biologically active metabolites. Because galantamine is positive for the long-term treatment of Alzheimer's disease, most studies in *L. aestivum* have been conducted in a small number of specific areas containing galantamine (10).

Galantamine is a tertiary alkaloid and a reversible, competitive inhibitor of the enzyme acetylcholinesterase (AChE), a widely studied therapeutic target for treating Alzheimer's disease (12). For this reason, galantamine is

known to treat neuronal, cognitive, and behavioral disorders related to the brain. There are findings on the effects of the lycorine component on apoptotic processes, which can also be obtained from *Leucojum L.* plant (13).

In this study, we aimed to contribute to the literature for future studies by investigating the antiangiogenic and apoptotic effects of the galantamine alkaloid found in *L. aestivum* on the MCF-7 breast cancer cell line. After obtaining galantamine by the HPLC method, in this regard, cck-8 assay was performed to analyze cell viability and proliferation. The levels of vascular endothelial growth factor (VEGF), which is associated with angiogenesis, and Poly (ADP-ribose) polymerase (PARP) protein, which has a role in DNA damage repair and cell fate, were investigated. Total antioxidant status (TAS) and total oxidant status (TOS) analyzes were performed. Oxidative stress index (OSI) was calculated.

MATERIALS AND METHODS

Cell Material And Cell Culture

The MCF-7 human breast cancer cell line used in this study was supplied from Afyon Kocatepe University Biochemistry Department Prof. Dr. Ömer Hazman. The main content of the medium used in the culture of MCF-7 cells is RPMI 1640, and it contains 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cell culture was carried out by incubating under sterile conditions in a carbon dioxide incubator providing 5% CO_2 and 37°C temperature conditions in T75 flasks containing sterile medium. Passaging was performed when the cells being replicated reached a density that covered approximately 85% of the culture flask. Cells were used in experiments when they reached a sufficient number.

Galantamine Extraction From Bulbs of *L. aestivum* L.

1.0 g of *L. aestivum* L. bulb sample was weighed into a 100 ml of Erlenmeyer flask. 40 ml of demineralized water at 80°C was added. For standard ultrasonic conditions, Erlenmeyer flasks were placed in the ultrasonic bath. The solvent level in the Erlenmeyer and the water level in the ultrasonic bath were kept the same. The temperature of the ultrasonic bath was set to 80°C and the time was set to 10 min. After extraction, the extracts were filtered through Whatman filter paper.

Quantification Of Galantamine By High Performance Liquid Chromatography

HPLC analyzes were performed with an Agilent 1260 system (Palo Alto, CA, USA) consisting of a quaternary pump, autosampler, UV detector, and ChemStation software. For the quantification of galantamine in plant materials, new selective, quick, precise, and accurate spectrophotometric and chromatographic procedures were developed and validated. Chromatographic separation was performed on an Agilent Extend C18 (250 × 4.6 mm, 5 µm) column. Ultrapure water containing 0.1% TFA and acetonitrile (85/15, v/v) were used as mobile phase at a flow rate of 1.0 ml min-1. Eluent detection was performed at a wavelength of 288 nm using a UV detector. The injection volume was 20 µl. Analytical method has been validated in order to meet the standards of the International Conference on Harmonisation (ICH) and the results showed that liquid chromatographic methods were linear, precise, accurate, rugged and robust. The limit of detection and limit of quantification values for the chromatographic method were calculated as 0.70 and 2.10 µg ml-1, respectively. Under these chromatographic conditions, the retention time for galantamine was determined to be 4.04 min.

Cell Counting Kit-8 (CCK-8) Cell Viability Assay

MCF-7 cells were seeded in 90 µl of RPMI medium containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in a 96-well plate at a volume of 5×10^5 cells/ml and incubated in a 5% CO2 and 37°C incubator. The wells were divided into 9 (control, 10, 40, 70, 100, 130, 160, 190, 210 µg/ml) groups, including the control, and 10 µl of galantamine was administered. Three repetitions of each group were performed. After 48 h of the incubation period, 10 µl of CCK-8 solution was added. It was incubated for 2 h. After the incubation period, the shaking was done for 10 s, and measurements were taken with a plate reader at a wavelength of 450 nm. Other analyzes were made on the results obtained.

Experimental Groups In The Study

In this study, there were 3 groups: control, LD50, and high dose (210 µg/ml) for PARP, VEGF, TAS, and TOS assays. These groups were determined according to the CCK-8 assay results. The control group is the group in which the same volume of medium was applied to this group when galantamine was applied to other groups since galantamine was dissolved in the medium.

Analysis Of Total Antioxidant Status (TAS) Levels

In this study, a commercial kit (Rell Assay, Gaziantep, Turkey) was used to measure the total effect of antioxidants in an environment. This kit works on the principle of determining the antioxidant capacity of the sample by following the reduction/inactivation process of a reactive species added to the medium by the antioxidants in it. Using the kit's standards, a standard solution was made at 5 different concentrations (0.125, 0.25, 0.5, 1, 2.5 mmol/mol Trolox). The calibration curve was then created, and the TAS levels in the samples were calculated. TAS levels (mmol Trolox Equivalent/L) were divided by total protein levels in each sample and represented as mmol Trolox Equivalent/g protein.

Analysis Of Total Oxidant Status (TOS) Levels

To determine the TOS levels in the samples, a commercial kit (Rell Assay, Gaziantep, Turkey) was used to determine the overall effect of the species that could cause oxidative stress. After performing the applications stated in the kit protocol, the absorbance values of the samples and the standard supplied with the kit were determined. TOS levels in the samples were calculated using the formula; [TOS=(Abs$_{sample}$/Abs$_{standard}$)×20]provided in the kit protocol and these absorbance values. The determined TOS results were found as µmol hydrogen peroxide equivalent liters (µmol H_2O_2 Equivalent /L). The calculated results were normalized by dividing the samples by their total protein levels. TOS levels were expressed as µmol H_2O_2 Equivalent/g protein.

Determination Of Oxidative Stress Index (OSI)

After determining the TOS and TAS in the environment, these values can be proportioned to calculate the OSI. Oxidative stress levels in samples were estimated by dividing TOS levels by TAS levels in the current study [OSI=((*TOS/TAS*)×100)] (14,15).

VEGF and PARP Assays

For PARP and VEGF analysis, cells in T75 flasks were detached with the help of trypsin. Then the medium was added to counteract the effect of trypsin. After this step, it was centrifuged, the supernatant was discarded, and the resulting pellet was suspended by the addition of medium. After the cells covered the bottom of the flask, 1×10^5 cells were inoculated into each well of the 24-well plate used in the experiment. After 24 h, the determined doses of galantamine were added to the cultivated cells and allowed to incubate for 48 h. After incubation, the medium was removed and the cells were washed with 700µl cold PBS. After washing, the PBS was removed. Cells were dissociated by the addition of 200µl trypsin. To eliminate the effect of trypsin, the cells transferred to the eppendorf containing the medium were centrifuged at 15°C, 240 g for 15 min. After centrifugation, the supernatant was discarded. These procedures were applied exactly for VEGF and PARP analyses. Then, after adding medium to Eppendorf and shaking a little, they were made ready for VEGF and PARP analysis. Disposable antibody-loaded plates in VEGF and PARP kits were cultivated and kit procedures were performed. For VEGF and PARP, Elisa kits (HUMAN VEGF / PARP ELISA Kit Lot No: 201911) were used. In the application methods, the appropriate procedural instructions in the ELISA kits were applied.

Statistical Analysis

For statistical analysis, the IBM-SPSS 22 (Armonk, NY, USA) program was used. First, it was checked whether the obtained data showed a normal distribution. Then, parametric or non-parametric tests were performed according to the normal distribution status. $P<0.0001$ was considered significant. The data in the tables are presented as mean ± SD.

RESULTS

Cell Viability

The CCK-8 assay results are given in Figure 1. In 8 (10, 40, 70, 100, 130, 160, 190, 210 µg/

ml) different doses of galantamine, the LD50 dose was determined as 100 µg/ml **(Figure 1)**.

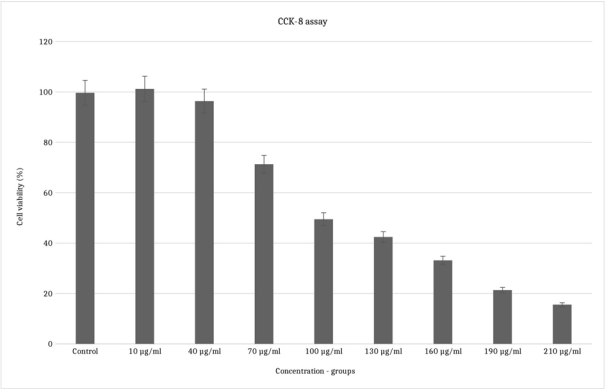

Figure 1: CCK-8 cell viability results of different doses of galantamine treatment on MCF-7 cell line.

Experimental Analysis Results

Table 1 shows the analysis results of TAS, TOS, VEGF, and PARP levels obtained in the experiment. There are six samples in each group. Statistical calculations that were made based on the findings was shown in **Table 1**.

Table 1: Experimental analysis results

Experimental Groups	Sample Number	TAS (mmol trolox equivalent/g protein)	TOS (µmol H2O2 equivalent/g protein)	PARP (ng/L)	VEGF (ng/L)
Control	1	0.098	0.13	2.77	0.010
	2	0.095	0.13	2.56	0.020
	3	0.093	0.12	2.66	0.030
	4	0.094	0.14	2.87	0.012
	5	0.099	0.11	2.88	0.013
	6	0.099	0.12	2.98	0.013
LD50 (100 µg/ml)	1	0.065	0.22	1.34	0.012
	2	0.066	0.23	1.55	0.015
	3	0.063	0.19	1.65	0.011
	4	0.060	0.23	1.50	0.023
	5	0.062	0.22	1.57	0.020
	6	0.061	0.21	1.45	0.011
High Dose (210 µg/ml)	1	0.040	0.33	1.11	0.012
	2	0.043	0.32	1.01	0.011
	3	0.039	0.35	1.02	0.012
	4	0.042	0.29	0.99	0.015
	5	0.038	0.32	0.98	0.012
	6	0.041	0.30	0.95	0.121

TAS: total antioxidant status; TOS: total oxidant status; PARP: poly (ADP-ribose) polymerase; VEGF: vascular endothelial growth factor. There are six samples in each group.

Statistical Analysis Results

TAS-TOS and OSI Statistical Analyzes Results

Table 2 shows the statistical analysis data of TAS and TOS. TAS (mmol trolox equivalent/g protein) control, LD50(100µg/ml) and high dose(210µg/ml) groups were calculated as 0.09633±0.002658, 0.06283±0.002317,0.04050 ±0.001871, respectively. TOS (mmol troloxequivalent/g protein) control, LD50(100µg/ml) and high dose(210µg/ml) groups were calculated as 0.12500±0.010488, 0.21667±0.015055, 0.31833±0.021370, respectively. There is a statistically significant dose-dependent decrease in the total antioxidant level compared to the control group. A statistically significant dose-dependent increase was found in the level of total oxidant compared to the control group. TAS and TOS data showed normal distribution and homogeneity among variances, and one-way ANOVA test was applied. Statistical differences were determined based on the p<0.0001 value. Post-hoc Duncan analysis test was used to determine the differences between the groups. There is considerable significance between groups denoted by different superscript letters in the same column. There is no significant difference between the same letters in the same column. To evaluate and analyze oxidative stress, values for OSI (oxidative stress index) are given in **(Table 2)**.

Table 2: TAS-TOS statistical analysis results

Experimental Groups	TAS (mmol trolox equivalent/g protein)	TOS (µmol H_2O_2 equivalent/g protein)	OSI (arbitrary unit)
Control	0.09633±0.002658^a	0.12500±0.010488^a	129.964±13.018^a
LD50 (100 µg/ml)	0.06283±0.002317^b	0.21667±0.015055^b	345.161±26.480^b
High Dose (210 µg/ml)	0.04050±0.001871^c	0.31833±0.021370^c	788.485±78.575^c
p value	0.000*	0.000*	0.000*

* (p<0.0001)
TAS: total antioxidant status; TOS: total oxidant status; OSI: oxidative stress index. Data are expressed as mean ± SD. Different superscripts in the same column indicate a significant difference.

OSI (arbitrary unit) control group was calculated as 129.964±13.018, LD50(100µg/ml) 345.161±26.480, and high dose(210µg/ml) 788.485±78.575. There is a statistically significant increase in the OSI data calculated according to the TAS and TOS test analysis results compared to the control group. It was determined that there was a normal distribution between the groups, but there was no homogeneity between the variances. When analyzed according to the p<0.0001 conditions, the Games-Howell test was applied to determine whether there were statistical differences and between which groups these differences were, because homogeneity between variances was not achieved. There is no significant difference between groups with the same superscript letters, but there is significance in groups with different superscript letters Table 2.

PARP and VEGF Statistical Analysis Results

Statistical analysis data of PARP and VEGF levels are as in Table 3. PARP control, LD50 (100µg/ml) and high dose(210µg/ml) groups were calculated as 2.78667±0.155392ng/L, 1.51000±0.107145n/L, 1.01000±0.054772ng/L, respectively. VEGF control, LD50(100µg/ml)

and high dose (210µg/ml) groups were calculated as 0.0163±0.0075ng/L, 0.0153±0.0050ng/L, 0.0305±0.0443ng/L, respectively. There is a significant decrease in the amount of PARP protein depending on the dose increase compared to the control group. There is no significant dose-related difference in VEGF amounts compared to the control group.

Different superscripts indicate that the data is significantly different from each other. One-way ANOVA-Duncan ($p<0.0001$) analysis method was applied because PARP data provided normal distribution and homogeneity. Since the VEGF data did not show a normal distribution, the non-parametric Kruskal-Wallis H test was applied. VEGF levels were based on $P<0.05$ significance value. The same superscript indicates no significant difference **(Table 3)**.

Table 3: PARP and VEGF statistical analysis results

Experimental Groups	PARP (ng/L)	VEGF (ng/L)
Control	2.78667±0.155392[a]	0.0163±0.0075[a]
LD50 (100 µg/ml)	1.51000±0.107145[b]	0.0153±0.0050[a]
High Dose (210 µg/ml)	1.01000±0.054772[c]	0.0305±0.0443[a]
p value	0.000*	0.752**

* (p<0.0001)
** (p<0.05)
PARP: poly (ADP-ribose) polymerase; VEGF: vascular endothelial growth factor. The same superscripts in the same column indicate no significant difference. Data are expressed as mean ± SD.

DISCUSSION

Galantamine is known as an AChE inhibitor. It has been proven by many studies to be effective in neurodegenerative diseases. There are very few studies showing the positive effects of some Amaryllidaceae alkaloids and AChE inhibitors on some cancer cells. In the current study, we tried to explain what kind of effects galantamine exerts on MCF-7 breast cancer cells. Galantamine is generally referred to as a reliable AChE inhibitor in the treatment of Alzheimer's disease. However, it is also known to have antidiabetic, anti-inflammatory, and antioxidant activity (16, 17). Some Amaryllidaceae alkaloids have antitumor activities. The most widely known alkaloids in this regard are lycorine, hemanthamine, and phenanthridone (18). Among the 13 Amaryllidaceae alkaloids, including galantamine, it has been revealed that hemantamine, hemantidine, and licorin exhibit the most potent cytotoxic activity against p53 mutant gastrointestinal cancer cells (19). It has also been determined that lycorine partially induces caspase-dependent apoptosis in MCF-7 cancer cells (20). It has been observed that Donepezil, an AChE inhibitor, ameliorates cardiotoxicity caused by a chemotherapeutic, doxorubicin, by reducing reactive oxygen species, mitochondrial damage, apoptosis, and necrosis (21). It has been stated that long-term (≥731 days) use of AChE inhibitors such as galantamine may be associated with the risk of age-related lung cancer (22). It has also been suggested that the decrease or inhibition of AChE and butyrylcholine esterase (BUChE) will cause the release of acetylcholine, which binds to nicotinic and muscarinic receptors, and that its increase will increase the proliferation and spread of lung cancer and will not be beneficial in suppressing carcinogenesis (23). In a study investigating the molecular, genetic, and pharmacological connections of 22 cancer types, especially breast cancer, Alzheimer's, and Parkinson's diseases, it is suggested that drugs used in neurodegenerative diseases such as galantamine may reduce the risk of cancer (24). It has been suggested that galantamine has potential as a chemotherapeutic molecule (25). Based on these data and our study, it shows that galantamine may also have important effects on breast cancer.

We tested it to learn how galantamine acts on PARP, which has an important role in DNA damage repair. Poly (ADP-ribosyl)ation is the cellular repair response to DNA damage and is catalyzed by poly (ADP-ribose) polymerase-1 (PARP1), the most abundant of the 18 different PARP isoforms. Upon detection of DNA strand damage, PARP1 binds to DNA, cleaves NAD+ between nicotinamide and ribose, and then replaces DNA nuclear acceptor proteins by forming a bond between the protein and the ADP-ribose residue. This process produces ribosyl-ribosyl bonds that act as a signal for other DNA repair enzymes and DNA base repair. Too much DNA damage in cells results in excessive PARP activation, resulting in cellular depletion of NAD+, thus slowing glycolysis, mitochondrial electron transport, and ATP formation in cells, which can lead to necrosis (26). When caspase-3 is activated in the cell's pathway to apoptosis, it breaks down some regulatory proteins, including PARP

(27). PARP inhibitors are currently used as drugs in the treatment of breast cancer (28). Galantamine treatment at a dose range of 0.1 - 10 µM on SH-SY5Y neuroblastoma cells did not affect cell viability according to MTT analysis data. In the same study, it was observed that neural apoptosis was observed in cells treated with 30 µM Aβ1-42 and decreased Aβ1-42-induced apoptosis and PARP cleavage after 1 µM galantamine treatment (29). On PC12 cells, a 10 µM dose of galantamine treatment was observed to significantly reduce Aβ25-35-induced apoptosis. In the same study, it was observed that galantamine reduced Aβ25-35-induced oxidative stress in PC12 cells and suppressed the activity of caspase-3, which has a role in PARP cleavage (30). In our study, galantamine application to MCF-7 cells affected cell viability, and the amount of LD50 was determined by CCK-8 analysis. A dose-dependent decrease was observed at the various concentrations we applied. As a result of the analysis, LD50 (100µg/ml) was found. It was observed that galantamine decreased the amount of PARP dose-dependently (LD50 and high dose). This reduction in PARP levels suggests the possibility that cell death may be associated with apoptosis.

It is known that excess free radicals in the environment can cause oxidative stress, resulting in DNA, protein, or lipid degradation, and if these effects cannot be corrected by some cellular mechanisms, they can cause cancer or cell death (31). Measurement of oxidative stress is therefore widely applied in studies. In a study investigating the effect of galantamine on colon cancer in albino Wistar rats, it positively regulated dimethylhydrazine-induced oxidative stress (32). In addition, it was stated that galantamine has antioxidant activity and that this activity is lost with the conversion of the enol group in galantamine to the carbonyl group (33). By measuring TAS and TOS levels and determining the OSI from these measurements, it can be determined what kind of effect the active substance has against oxidative stress. According to the TAS data in this study, galantamine significantly decreased antioxidant levels at 100 µg/ml and 210 µg/ml. On the contrary, in TOS data, oxidant levels increased significantly depending on the dose. Galantamine generally appears in studies with its antioxidative effect or oxidative stress regulating features. But in our study, galantamine increased oxidative stress on MCF-7 cancer cells in line with OSI data. The increase in oxidative stress may cause various damage to these cells.

VEGF is a very important factor in processes such as angiogenesis, anti-apoptotic, and cell migration, and anti-VEGF drugs are being developed against cell spread in anticancer studies (34). The effect of galantamine on the mRNA levels of some growth factors (BDNF, NGF, VEGF, FGF2, IGF1, and IGF2) in the hippocampus and prefrontal cortex of mice was investigated. 3 mg/kg of galantamine did not affect mRNA levels in the prefrontal cortex. It significantly affected BNDF and FGF2 mRNA levels at 3 h and IGF2 mRNA levels at 3, 6, and 12 h in the hippocampus. There was no change in the mRNA level of VEGF in either region (35). It was observed that VEGF release occurred in C2C12 mouse myoblast cells after 100 µM galantamine treatment in 24 h and increased further in 48 h (36). VEGF-A secretion, which reached high levels in rats with adjuvant arthritis, was significantly reduced after galantamine treatment. Galantamine has been reported to have an angiogenic and proliferative effect in its anti-arthritic role (37). In our study, we tested the angiogenic effect of galantamine in MCF-7 breast cancer cells. According to the data obtained, it was determined that galantamine did not have a significant effect on VEGF levels at any dose compared to the control group. Galantamine is mainly used in studies in the field of neurology. It has been shown that galantamine affects the DNA repair mechanism due to PARP reduction in MCF-7 cancer cells.

Galantamine increased oxidative stress in MCF-7 cells. According to the statistical results of VEGF levels in cells, galantamine did not show an angiogenesis inhibitory or supportive effect on MCF-7 cells. Considering the effects of galantamine on MCF-7, it shows that it has potential. however more studies on the effects of galantamine on different types of cancer are needed before this can be definitive or strong.

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