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Selenium: The Cancer Shield

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Abstract

Introduction: Breast cancer is one of the most common types of cancer in the world and the treatments are being improved day by day. Although chemotherapy agents have begun to be used, side effects, resistance development and toxicity seen with these drugs are still steps that limit treatment. **Objective:** Our aim in this study is to show whether sodium selenate (NaS), which has different effects on many different cells, has antiproliferative and apoptotic effects on MCF-7 breast cancer cells, considering the dose-time relationship, and to reveal its effect on oxidant stress parameters. **Methods:** 10, 20, 30, 40 and 50 μM sodium selenate was applied to the cells for 24, 48 and 72 hours. MTT test was applied to show the proliferative effect. Apoptosis was also measured with Annexin V/7AAD in MCF7 cells. Malondaldehyde (MDA) and Glutathione (GSH) levels were studied to reveal the oxidant/antioxidant balance. **Results:** It has been shown that as the NaS dose increases in MCF-7 cells, cell viability decreases ($p < 0.001$). In HEK 293 embryonic kidney cells, NaS applied at increasing doses, compared with the control group, no statistical difference was detected at 10 and 20 μM doses ($p > 0.05$), but at all subsequent increasing NaS doses, viability was found statistically significant decreased (p: 0.001, $p < 0.001$ and $p < 0.001$, respectively). It was determined that there was a dose-dependent increase in the rate of apoptotic cells in MCF-7 cells. The highest apoptotic activity was measured as 76.14%. It was determined that MDA levels increased and GSH levels decreased in MCF-7 cells with increasing doses of NaS application ($p < 0.05$, $p > 0.05$, respectively). **Conclusion:** Considering that NaS has an antitumor effect on breast cancer, increases oxidative stress and increases apoptosis by supressing the antioxidant protection system, and does not have a toxic effect on normal body cells at certain doses. When all these features evaluated, we think, selenium should be considered as a natural option in the treatment of breast cancer.

Keywords

Sodium Selenite, Breast Cancer, Apoptosis, Oxidative Stress

1. Introduction

Cancer is a large group of diseases that tend to spread to neighboring tissues or distant organs of the body beyond their normal limits as a result of the uncontrolled growth of abnormal cells [1]. While cardiovascular diseases have been among the leading causes of death all over the world for many years, cancer has started to be among the leading causes of death in 57 countries in recent years [2]. In 2020, approximately 19.3 million new cancer cases were detected in the world and 9.9 million cancer-related deaths occurred worldwide. The most common cancer in both sexes is breast cancer with 11.7%, followed by lung cancer with 11.4%. The incidence of breast cancer in developed countries is 88% higher than in developing countries. However, death rates were found to be 17% higher in developing countries. According to GLOBOCAN data; while the average breast cancer incidence worldwide is 11.6%, the death rate is 6.9% in both sexes [2] [3]. In our country, breast cancer is the most common type of cancer seen in women. According to the data of Türkiye Ministry of Health, breast cancer incidence has increased 4.5 times since 2000 [4]. Although new and more effective chemotherapy agents are being used day by day in the treatment of breast cancer, serious side effects, resistance development and toxicity observed with these drugs are still steps that limit treatment. For this reason, the interest in the search for new treatments continues. The reason why we specifically chose breast cancer in our study is that it is the most common type of cancer in our country and in the world and has become a public health problem due to its increasing frequency and mortality rates.

Although the incidence of breast cancer is high, the exact cause is not fully understood. Researchers are currently conducting many studies to identify factors that contribute to breast cancer and prevention strategies. It is suggested that basically genetic, endocrine and environmental factors are effective in the development of breast cancer. Age, race, socioeconomic status, genetic factors, number of births, breastfeeding, lifestyle, daily dietary habits, physical activity, alcohol and smoking affect the development of breast cancer. While some of these cannot be changed, the majority of these factors can be changed [5]. While genetic factors are detected in approximately 5% - 10% of all diagnosed breast cancers, breast cancers that occur due to risk factors arising from environmental factors and lifestyle are more common in societies [3]. Changes in these environmental and lifestyle factors, which frequently cause breast cancer, are held responsible for the increases in both incidence rates and death rates in developing countries, including our country [6]. There are studies showing that lifestyle changes, especially changes in eating habits, in developing countries may be responsible for the increase in the incidence of breast cancer. Furthermore, inadequate fresh vegetable consumption and industrial fruit juices, soft drinks and sweets were found related with breast cancer development [7] [8]. In some studies it has been shown that the incidence of breast cancer increases with the adoption of Western-style eating habits in low- and middle-income countries

[9]-[11]. In addition, correcting the nutritional content in the right direction reduces the incidence of breast cancer and the risk of death due to it by one third. It has been shown that diets high in polyunsaturated fat have been reported to increase the occurrence of breast cancer in animal models [12] [13]. The Western diet mostly contains refined grains that are low in fiber, nutrients and minerals, excessive sugar, saturated fats and large amounts of red meat and processed meat products. However, the Mediterranean diet includes plenty of fresh vegetables and fruits, oilseeds, nuts such as hazelnuts and walnuts, fish, legumes, unrefined whole grains and olive oil. There are studies showing that the development of breast cancer increases with a Western diet [14]. An inverse relationship has been found between compliance with the Mediterranean type of nutrition and breast cancer. Studies have shown that the higher the compliance with the Mediterranean diet, the lower the risk of breast cancer [15] [16]. The Mediterranean diet is associated with higher consumption of fish, which are among the richest sources of dietary selenium. In addition, fresh vegetables and fruits, nuts such as walnuts and hazelnuts, and grains in the Mediterranean diet are also rich in Se [17] [18]. Se level in the human body is proportional to the amount of Se in soil and water, therefore it varies between countries depending on the soil and water structure. New Zealand, Finland, Australia, China and America soils are the poorest countries in terms of Se. Due to these differences, Se is not present in the diet of approximately 0.5 - 1 billion people in the world [19]. According to GLOBOCAN data, Australia, New Zealand, Asian countries and some Northern and Western European countries are at the top of the countries where breast cancer is most common [3]. In our opinion, breast cancer may be more common in these countries because the diet of almost all of these countries is based on the Western diet, which is poor in Se, and their soils are poor in Se. For this reason, the relationship between Se and breast cancer has attracted our attention.

Se is an important trace element of vital importance in human health. The main source of selenium in humans is food intake. For adults, a daily dietary intake of 55 μg of Se is recommended. The maximum tolerable daily dose has been determined as 400 μg, and it has been shown that toxic effects may occur above these doses [20]. Dietary Se is involved in many biological activities in human body. It exists in organic and inorganic forms. While the organic form is taken from plant and animal foods, the inorganic form is used to enrich nutrients and support diets. Sodium selenate (NaS) is the inorganic Se salt. Depending on the chemical form, concentration and metabolic activity of ingested Se, it has been shown to inhibit the proliferation capacity of some cancer cells, support apoptosis and regulate immune system responses [21] [22]. However, studies have shown that Se can act differently in different cells. Depending on the nature of the target cells, the chemical form and dose of Se, it may have prooxidant activity in some cells and opposite effects, such as antioxidant activity, in other cells [23] [24]. Studies have shown that Se intake is inversely proportional to cancer

development and that Se promotes cancer cell apoptosis by acting as a prooxidant in ovarian cancer cells [25] [26]. Additionally, NaS blocks harmful reactive oxygen radicals in lung and colon cancer cells. In human lung carcinoma cells, NaS modulated both the extrinsic and intrinsic apoptotic pathways, also increased autophagy which were both mediated by ROS. It has also been shown in other different studies that NaS activates many signaling pathways and triggers apoptosis by increasing harmful reactive oxygen species (ROS) in lung and colon cancer cells [27] [28].

Based on all these reasons, our aim in this study is to show whether selenium (Se) has antiproliferative and apoptotic effects on MCF-7 breast cancer cells, considering the dose-time relationship and to reveal its effect on oxidant stress parameters. To the best of our knowledge, this is the first study in the literature in which the effects of Se on MCF-7 breast cancer cells were compared with normal kidney epithelial cells in terms of antitumor, apoptotic and lipid peroxidation effects.

2. Methods and Experimental Details

2.1. Cell Culture and Drug Administration

Human breast cancer cell line (MCF-7; ATCC HTB-22, USA) and human embryonic kidney cell line (293T; ATCC CRL-3216, USA) were used from stock created by propagating cells purchased from the American Type Culture Collection. Cells were cultured in Dulbecco's modified medium (DMEM-HA) 88% from Eagle, 10% Fetal Bovine Serum (FBS; Biochrom, Germany), 1% Penicillin-Streptomycin and 1% Sodium Pyruvate (Capricorn, Ebsdorfergrund Germany) has been cultured. 75 cm^2 flasks were used to continue the culture of MCF-7 and HEK 293T cells, which were cultured in a 37˚C incubator containing 5% CO_2. The beginning of the culture of the cells was at a level that covered 50% of the flask, and when the density of the cells reached the range of 1×10^6 - 2×10^6, covering 70% of the flask, they were passaged 2 - 3 times a week to ensure culture continuity. Sodium selenite was purchased from Pharma Kimya (Türkiye).

2.2. Cell Viability Test with MTT (Methyl Diphenyl Tetrazolium) Assay

MTT test, which is performed to determine metabolic activity and cytotoxic effect, is a non-radioactive, spectrophotometric, colorimetric test based on the separation and reduction of tetrazolium salts by living cells. MCF7 and HEK 293T cell lines were seeded in 96-well plates at 1×10^4 cells. 10, 20, 30, 40 and 50 µM sodium selenite, prepared by dissolving it in water, was applied to the cells for 24, 48 and 72 hours. After drug application, 10 µL of 5 mg/mL MTT (St. Louis, Missouri, USA) solution was added to the cells in 100 µL of medium, and they were incubated for 4 hours at 37˚C in a humid environment containing 5% CO_2. Formazan crystals formed following incubation were dissolved with DMSO (dimethyl sulfoxide) and colorimetric detection was measured at a wave length of

570 nm. The obtained absorbance values were calculated using the origin graph program according to the formulation (% Viability = OD_{570}-Drug/OD_{570}-Control × 100).

2.3. Apoptosis Measurement with Annexin V/7AAD Assay

MCF7 cell line was seeded into a 48-well plate with 4×10^4 cells in each well. Sodium selenite (10, 20, 30, 40 and 50 μM) was applied to the cells for 48 hours. At the end of the period, after Trypsin-EDTA treatment, the cells were washed once with PBS (phosphate buffered saline), then 100 μl of Muse® Annexin V/Dead Cell kit solution was added and incubated in the dark for 20 minutes. After incubation, quantitative measurements of apoptosis and necrosis were made on the Muse® Cell Analyzer (Millipore, Austin, TX, USA). The Muse® Annexin V/Dead Cell Kit provides screening and detection of 4 different cell populations: dead cells, live cells, early and late apoptotic cells. As a result of the analysis, the total amount of apoptotic cells (sum of early and late apoptotic cells) was transferred to the graphs as a percentage.

2.4. Biochemical Analysis

MCF-7 cells were transferred to 12-well plates. After 10, 20, 30, 40 and 50 μM sodium selenite was applied to the cells, it was kept in an incubator with 5% CO_2 at 37˚C for 48 hours. After incubation period, the medium was aspirated from the cells and transferred to tubes. Precipitation was achieved by centrifugation in a rapidly cooled centrifuge at +4˚C and 1100 rpm for 5 minutes. The supernatant was transferred to a separate microcentrifuge tube and MDA and GSH tests were performed using the specified methods.

2.4.1. Malondialdehyde (MDA)

MDA production and lipid peroxidation were evaluated by the method of Ohkawa [29]. MDA forms a pink complex at high temperatures in the presence of thyobarbituric acid, and this color is read spectrophotometrically at a wave length of 532 nm. Results were given as micromol/mg protein.

2.4.2. Glutathione (GSH)

GSH levels were measured spectrophotometrically at 412 nm by the method of Beutler [30]. GSH solution was used as standard. The results were expressed as micromol/mg protein.

2.4.3. Statistical Analysis

Statistical analysis of the obtained data was carried out using the SPSS 21 package program. Comparisons between groups were made with Oneway ANOVA, one of the independent sample tests. Tukey tests were applied for multiple comparisons. Results are given as mean ± standard deviation (Mean ± SD). Statistically significant level was accepted as $p < 0.05$.

3. Results

In our study, increasing doses of 10, 20, 30, 40 and 50 μM NaS were administered to both MCF-7 breast cancer cells and HEK 293T human embryonic kidney cells and the viability percentages of these cells were evaluated at varying time intervals of 24, 48 and 72 hours. Table 1 shows the viability levels of the control group and all other groups. In the MCF-7 cell series, a statistically significant difference ($p < 0.001$) was detected in terms of viability levels when the control group data were compared with all other groups. Although it was observed that cell viability decreased as the NaS dose increased, no statistically significant difference was detected between the groups in increasing doses after the 20 μM NaS dose ($p > 0.05$). A statistically significant difference was detected between the 10 μM NaS breast cancer cell and 20 μM NaS breast cancer cell groups ($p < 0.05$). No statistically significant difference was detected between each 20 μM NaS and 30 μM NaS groups, between 30 μM NaS and 40 μM NaS groups, and between 40 μM NaS and 50 μM NaS groups ($p > 0.05$). As a result, although there were decreases in viability rates with increasing doses after the 20 μM NaS dose, it was not found to be statistically significant. In addition, when the viability rates at 24, 48 and 72 hours were compared between the groups, no statistically significant difference was observed ($p > 0.05$). While viability rates were found to be lower in almost all doses at the 48th and 72nd hours when compared with 24th hour data (Graphic 1).

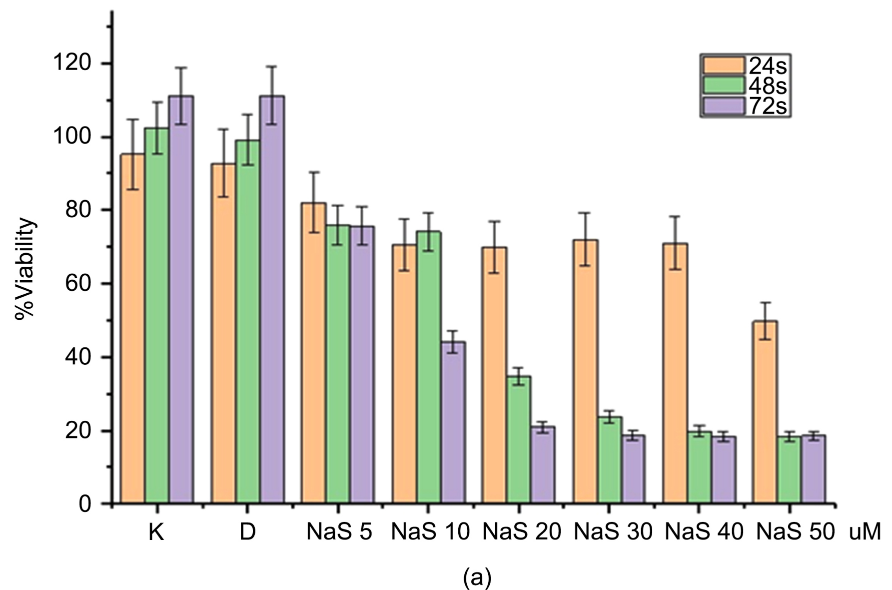
Table 1. The effect of NaS on viability of MCF-7ve HEK 293T cell lines.

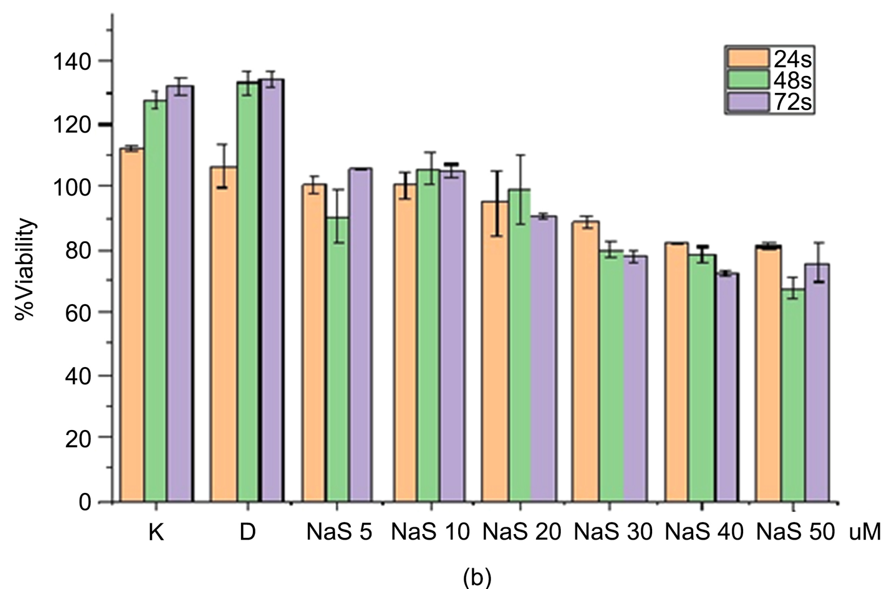
Group	MCF-7 Viability	HEK293T
Control	0.570 ± 0.044	1.237 ± 0.101
NaS10	0.338 ± 0.079*	1.208 ± 0.101
NaS20	0.201 ± 0.085*	1.133 ± 0.036
NaS30	0.140 ± 0.041*	0.966 ± 0.505**
NaS40	0.110 ± 0.007*	0.757 ± 0.284**
NaS50	0.106 ± 0.003*	0.676 ± 0.023**

All data were expressed as mean ± standars deviation. *Comparison of MCF-7 + NaS cell groups with MCF-7 control group using One-Way ANOVA, Tukey HSD multiple comparison test $p < 0.001$; **Comparison of HEK 293T + NaS cell groups with the control group using One-Way ANOVA, Tukey HSD multiple comparison test $p \leq 0.001$.

In order to observe the possible toxic and similar effects of NaS on normal body cells, NaS was also applied to the human embryonic kidney cell line (HEK 293T) at the same doses and durations and its effects on viability were observed (Table 1). The control group was compared with the NaS groups administered in increasing doses. There were no statistically significant difference between the 10 μM NaS and 20 μM NaS groups ($p > 0.05$). In other words, 10 and 20 μM NaS

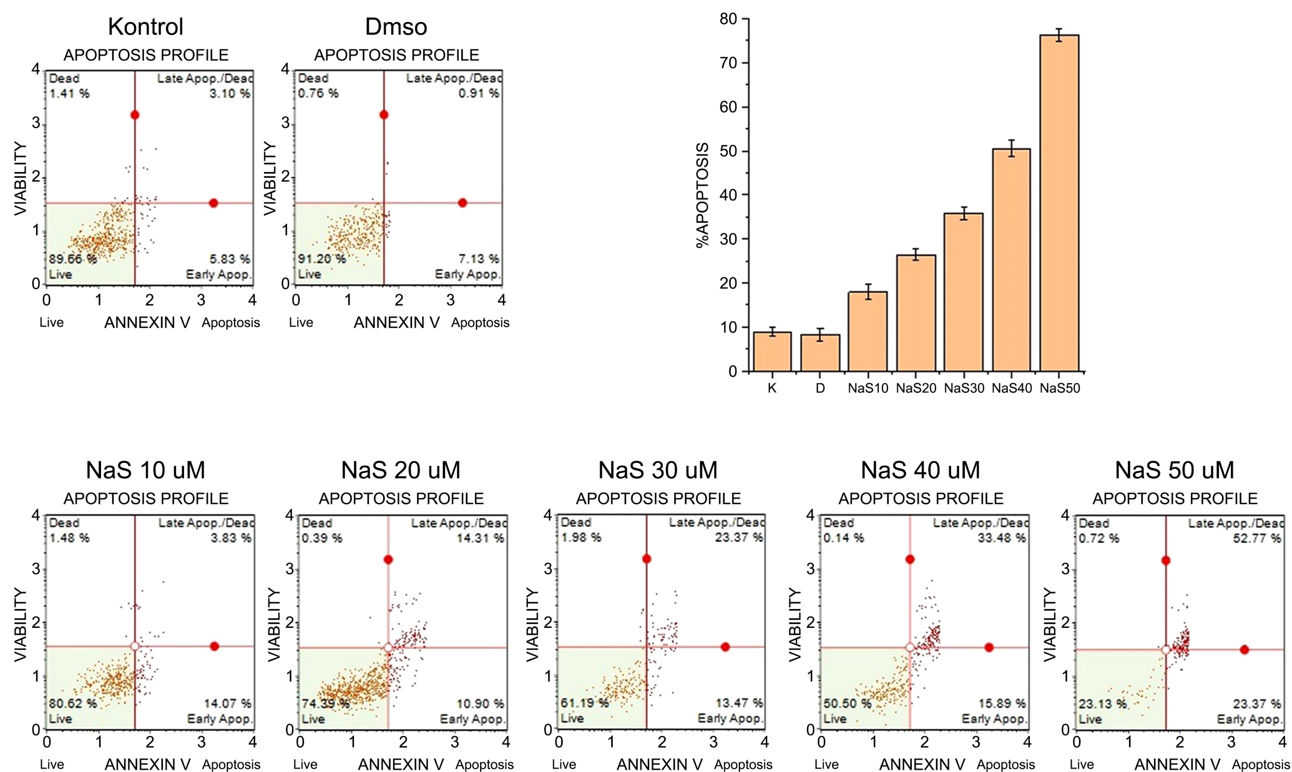
applications did not create any change on normal cell viability. However, statistically significant difference was detected between the 30 μM NaS and the control group, between 40 μM NaS and the control group, and between 50 μM NaS and the control group (p: 0.001, $p < 0.001$ and $p < 0.001$, respectively). These results show that increasing doses starting from 30 μM NaS, may cause toxic effects on cells by reducing the viability of normal body cells. No statistically significant difference was detected between the 10 μM NaS and the 20 μM NaS groups ($p > 0.05$). Furthermore, when the viability rates at 24, 48 and 72 hours were compared between all groups, no statistically significant difference was detected ($p > 0.05$) (**Graphic 1**).



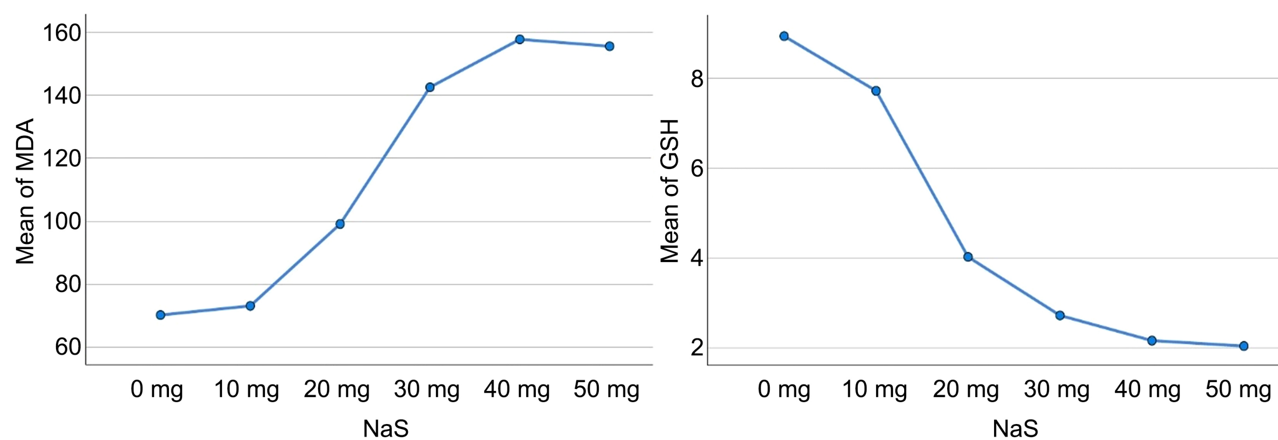


Graphic 1. (a) Viability rates of MCF-7 cell lines at varying NaS doses and times; (b) Viability rates of HEK 293T cell lines at varying NaS doses and times.

Additionally, the rates of apoptosis observed with the application of varying doses of NaS to the MCF-7 breast cancer cell groups were compared. It was determined that there was a dose-dependent increase in the rate of apoptotic cells in MCF-7 cells applied NaS for 48 hours ($p < 0.05$). The total apoptosis rates were calculated as 17.90% in the 10 μM NaS group, 25.21% in the 20 μM NaS group, 36.84% in the 30 μM NaS group and 49.34% in the 40 μM NaS group. The most effective apoptotic activity was measured as 76.14% in cells treated with 50 μM NaS (Graphic 2). It has been shown that increasing doses of NaS increase apoptosis rates.



Graphic 2. Muse Annexin V apoptosis profile of MCF-7 cells treated with increasing doses of NaS.



Graphic 3. MDA and GSH levels in MCF-7 cells applied increasing doses of NaS. MDA: Malondialdehyde, GSH: Glutathione.

MDA and GSH levels were studied in the supernatant portions of MCF-7 cells treated with varying doses of NaS for 48 hours in order to obtain information about the oxidant/antioxidant levels. As seen in **Graphic 3**, it was determined that with the application of increasing doses of NaS, MDA levels and therefore oxidant levels increased in MCF-7 cells, while GSH levels, namely antioxidant levels, decreased. However, when compared in terms of MDA levels, a statistically significant difference was detected between all groups ($p < 0.05$), although a decrease in GSH levels was shown in all groups, no statistically significant difference was detected ($p > 0.05$).

4. Discussion

Since the discovery of cancer, researchers have been searching for treatment methods that have less toxic effects on normal body cells and are more effective on cancer cells. Se, a micronutrient, plays a role in many biological activities in the body and is an important component of the Mediterranean diet, which is suitable for our country's diet. Some in vitro studies have shown that NaS shows antitumor activity in leukemia, lymphoma, stomach and colon cancers at doses ranging from 1 - 20 μM [31]-[33]. Although it is still unclear under what conditions the mechanism of action and selectivity of NaS in cancer cells change, it is thought that differences in the tumor microenvironment contribute to this situation [31]. The results of studies on breast cancer regarding Se species, which can show both oxidant and antioxidant properties in the body depending on metabolic processes, target cells, dose and the type of Se used, are contradictory. Therefore, in our study, we aimed to reveal the effects of NaS on proliferation, apoptosis and oxidant/antioxidant balance in breast cancer at varying doses and application times. In our study, it was revealed that the viability of MCF-7 breast cancer cells decreased statistically significantly with increasing doses of NaS, but there was no difference in cell viability at increasing doses after the 20 μM dose. In addition, in HEK 293T embryonic kidney cells, which are normal cell lines, the statistically significant decrease in cell viability when compared to the control group starting from the 30 μM dose supports that it may have a toxic effect on normal cells starting from this dose. In their study, Cao *et al.* showed that viability decreased in breast cancer cell lines with increasing doses of NaS and that increasing doses could have a toxic effect by having an antiproliferative effect on normal cells [34]. Da Costa *et al.* in their studies with different Se types, revealed that the highest antitumor activity among all Se types was provided by NaS at increasing doses [35]. Consistent with the literature, our study revealed that NaS reduces breast cancer cell viability and proliferation and does not have a toxic effect on normal healthy cells up to a certain dose.

Resistance to chemotherapeutic drugs used in breast cancer is one of the leading causes of treatment failure. One of the most basic mechanisms of this resistance is the blockage of apoptosis in tumor cells. There are data that Se promotes apoptosis in many different cancer cells such as lung and colon [27] [28]

[36]. In their study, Cao *et al.* showed that NaS increased apoptosis in breast cancer cells with increasing doses, but decreased the viability of normal cells above certain doses [34]. In our study, it was shown that apoptosis rates increased with increasing doses in MCF-7 cell lines treated with NaS. Although it was observed that the highest apoptosis effect was observed with 76.14% at the highest dose (50 μM), our study revealed that it had a toxic effect on normal cells at these high doses. However, the exact mechanisms by which Se-induced apoptosis occurs are still unclear.

Oxidative stress is an unstable condition caused by the imbalance between oxidants and antioxidants in cells, resulting in increased production of free oxygen species (ROS) in cells. Many vital cell functions such as cell metabolism, signaling pathways, gene expression, proliferation and apoptosis are affected by oxidative stress [37]. Lipid peroxidation is a cell damage mechanism used as an indicator of oxidative stress in cells and tissues. MDA, the end product of this mechanism, is also an indicator of oxidative stress occurring in tissues [38]. Cells have protection mechanisms against damage caused by oxidative stress and lipid peroxidation. Some enzymes with antioxidant effects can clean the cell from oxidative damage due to ROS. GSH is a cofactor that is involved in these enzymatic pathways with antioxidant effects and reacts with free oxygen radicals [39]. Oxidative stress in tumor cells is higher than in normal cells to enable rapid growth and rapid adaptation to the microenvironment. However, studies have revealed that oxidative stress is not always tumor-protective and can work in multiple ways. It has been shown that antioxidant enzymes will decrease in the face of increasing oxidative stress and the resulting lipid peroxidation activates pathways that trigger apoptosis in some cancer cells, leading to the death of tumor cells [34] [40]. There are studies indicating that antioxidant-effective GSH levels increase in many cancers such as breast, lung, head and neck, thus protecting the cancer cell from increased oxidative stress due to high metabolic processes [41] [42]. It has been shown in some studies that, depending on the type and dose of Se used, GSH levels in cells decrease and the cell becomes unprotected against oxidative stress and initiates programmed cell death [43]. It has been shown in some cancer cells that as GSH levels decrease, lipid peroxidation in the cell increases and cell death occurs [42]. Qi *et al.* showed in their study that NaS activates many pathways that trigger apoptosis by increasing oxidative stress in uterine cancer cells [24]. Similarly, Zhang *et al.* revealed that NaS in breast cancer cells caused an increase in ROS within the cell [36]. Based on all these data, in our study, when MDA and GSH levels were measured in order to reveal the oxidant/antioxidant balance and current situation in MCF-7 breast cancer cells; it has been shown that with increasing doses of NaS application, MDA levels increase statistically significantly, while GSH levels were found decreased. In other words, it has been revealed that oxidative stress increases and the antioxidant protection system weakens in MCF-7 cells with NaS application.

5. Conclusions

In conclusion, as revealed in our study consistent with the literature; we demonstrated that NaS has an antitumor effect by significantly reducing viability rates on breast cancer cell lines at increasing doses, but can have a toxic effect on normal body cells starting from a dose of 30 μM. In addition, we think that increasing doses of NaS increase oxidative stress in breast cancer cell lines, and with the depletion of GSH stores due to NaS leaves the cell vulnerable to oxidative stress, and this causes cell death by triggering different apoptosis pathways. According to our screenings, we think that our study may contribute to the literature as it is the first study in which all these steps were designed in a single study in the breast cancer cell line compared to the normal embryonic kidney cell line. Based on all these results, considering that NaS has an antitumor effect on breast cancer, increases oxidative stress and increases apoptosis by suppressing the antioxidant protection system, and does not have a toxic effect on normal body cells up to certain doses, selenium is a natural option both in the treatment of breast cancer and in the prevention of breast cancer.

In our study, more effective results could have been obtained if the NaS doses found to be effective on breast cancer cells in our study had been shown to have possible synergistic effects together with some chemotherapy drugs used in breast cancer treatment. Additionally, the molecular acting mechanisms of Se in cancer cells may be identified by further tests to strengthen the results. Apoptosis pathways and different oxidant/antioxidant balance indicators may be researced in future new studies. We think that planning such a research will give a new direction to breast cancer treatment in terms of shedding light on future research.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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