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Combined anticancer effects of capsaicin and sodium selenite on PI3K/AKT/mTOR signaling pathway in MDA-MB-231 and L929 cell lines

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Capsaicin (CAP), a toxic alkaloid found in chili peppers, exhibits anticancer potential by inducing apoptosis in cancer cells, thereby inhibiting tumor growth. CAP exerts its effects through mechanisms such as increasing reactive oxygen species (ROS) production, preventing metastasis, and enhancing the efficacy of chemotherapeutic agents. Sodium selenite (SS), a widely used selenium compound, plays a crucial role in neutralizing hydrogen peroxide (H_2O_2) and lipid hydroperoxides, which can damage DNA. This study investigated their effects on MDA-MB-231 and L929 cell lines using MTT, Comet, apoptosis, malondialdehyde (MDA), and antioxidant enzyme activity assays. The LD_{50} value of CAP was determined to be 50 μM, with cytotoxicity analyses revealing a dose-dependent decrease in cell viability. Comet assay confirmed DNA damage, while the highest apoptotic cell count was observed at the combined concentration of 50 μM CAP and 5 nM SS. Immunohistochemical analysis identified the involvement of the PI3K/AKT/mTOR signaling pathway. Additionally, increased MDA levels and decreased antioxidant enzyme activity were observed. CAP and SS exhibited a synergistic effect, reducing proliferation rates in both cell lines, highlighting their potential as a therapeutic strategy in cancer treatment.

Keywords: Oxidative Stress, PI3K/AKT/mTOR, Apoptosis, Antioxidant enzyme activities, Apoptototic effect, Comet test, Immunohistochemical test

Cancer is the general name of diseases that occur as a result of the uncontrolled proliferation of damaged cells in any part of the body and their spread beyond that area[1]. Breast cancer remains the most common cancer among women in the U.S., with an estimated 316,950 new invasive cases in women in 2025. Although significant advances have been made in detection and treatment, 42,170 women are expected to die from breast cancer in 2025[2]. Although significant advances have been made in the diagnosis and treatment of breast cancer in recent years, some patients may experience recurrence and metastasis after treatment. However, the underlying mechanisms of breast cancer have not been fully elucidated. Therefore, the development of new strategies to prevent recurrence and metastasis of breast cancer is of critical importance to improve the survival and quality of life of patients[3,4].

Red pepper is in the genus Capsicum of the Solanaceae fam[5]. Capsaicin, the active ingredient in capsicum, prevents oxidation and provides antioxidant properties by protecting the cell against free radical damage[5]. In addition, capsaicin is a molecule that metabolically increases lipid peroxidation, reduces the amount of adipose tissue, lowers serum triglyceride levels and inhibits glycogen metabolism in skeletal muscles *in vitro*[6]. Capsaicin has been reported to have various pharmacological effects, especially analgesic and anticancer effects[7]. The pharmacological effects of capsaicin are quite complex due to its interaction with TRPV1 channels and its ability to influence gene transcription[8]. Herbal products can prevent cancer by inducing apoptosis through various signaling pathways. Natural products, such as capsaicin and ursolic acid, in particular, exhibit multi-targeted anticancer effects with high efficacy and low toxicity by regulating various signaling pathways[9,10].

Sodium selenite (SS) is an important compound that is mostly found in seafood, cereals, vegetables and meat in nature, is an important compound that protects the redox balance in the body, which is included in the structure of proteins that destroy DNA-damaging H_2O_2 and lipid hydroperoxides[11]. This element found in mammalian organisms is involved in the structure of many enzymes such as

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glutathione peroxidase (GPx), thiorethoxin reductase (TR) and deiodinases[12]. SS is an element that has anti-carcinogenic and antiviral effects and plays an important role in aging. In its deficiency, neurological diseases such as Alzheimer's disease, Parkinson's disease, increased infection in the body, decreased thyroid function and increased cancerous cell formation[13]. When SS deficiency is not corrected, the activity of the enzyme GPx is reduced in all body tissues in the long term. SS deficiency in the body increases the intensity of free radical processes. Under the influence of sodium selenite, it contributes to the increase in the content of lipid peroxidation products and the inhibition of antioxidant function by increasing the activity of glutathione-dependent enzymes in the cell[14]. Cell culture plant extracts, which were discovered long ago and are now the most widely used in cancer studies, are given *in vivo* to the determined cell culture lines[14]. In the light of all these developments, *in vitro* determination of the cytotoxic effects of active substances in foods has gained great importance in recent years[15].

In this study, increasing doses of capsaicin and SS were applied to L929 and MDA-MB-231 cell lines and a series of analyses were performed to evaluate the synergistic effect. In this context, MTT assay was used to evaluate cell viability, measurement of malondialdehyde (MDA) levels to determine oxidative stress, antioxidant activity to investigate antioxidant defence mechanisms of cells, comet assay to evaluate DNA damage, apoptosis assay to determine cell death mechanisms and immunohisto-chemistry test targeting PI3K, AKT and mTOR proteins to evaluate cell death. The aim of this study was to examine the biological effects of capsaicin and sodium selenite on cancer cells and to develop new therapeutic strategies, especially in the context of breast cancer cells with aggressive properties such as the MDA-MB-231 cell line. In this context, the study is designed to contribute to the advancement of basic scientific knowledge and inform the development of potential strategies for clinical applications.

The aim of this study is to investigate the anticancer effects of capsaicin and sodium selenite in combination on MDA-MB-231 and L929 cell lines using PI3K/AKT/mTOR, MTT, SOD, CAT, and GPX markers. Our goal is to reveal the apoptosis processes and determine whether the capsaicin–sodium selenite combination produces a selective and synergistic antitumor effect in cancer cells.

The H1 hypothesis of our study is that capsaicin and sodium selenite together strongly inhibit PI3K/AKT/mTOR pathway activity and reduce cell viability in MDA-MB-231 cells. The null hypothesis (H0) is that the combination of capsaicin and sodium selenite will not produce a significant difference in the PI3K/AKT/mTOR pathway or cell viability.

Materials and Methods

Cell culture

MDA-MB-231 and L929 cell lines were obtained from the American Type Culture Collection (ATCC) at the Global Bioresource Centre. The MDA-MB-231 cell line was grown in DMEM F12 and the L929 cell line was grown in RPMI medium incubated in a humidified environment at 37°C with 5% CO_2 and 95% air.

Preparation of capsaicin and sodium selenite stock solution

The capsaicin, which has a molecular weight of 305.41 g/mol, was dissolved in 0.061 g dimethyl sulfoxide and formed a stock solution. 2.39 mg of powdered sodium selenate in 100 mL of selenium prepared in water is the standard.

MTT cell proliferation assay

The most widely used tetrazolium salt (MTT) was used to measure cytotoxicity in cell culture. In this study, capsaicin doses between 5 μM-100 μM were applied to MDA-MB-231 cell line and L929 cell line by MTT method. After 24 h incubation, the colour change in the formazans in the added and dissolved MTT solution was measured at 570 nm absorbance value with a microplate reader spectrophotometer. In this study, 2.5, 5, 10 nM SS doses were applied to MDA-MB-231 cell line and L929 cell line by MTT method. To examine the effect of SS, 50 μM capsaicin dose was combined with 5 nM SS dose in MDA-MB-231 and L929 cell lines. At the end of 24 hours, MTT was added and the optical density of the cells was read at 570 nm wavelength in ELISA device[16].

Measurement of MDA level

Membrane lipid peroxidation was measured according to the Devasagayam and Tarachand method[17]. The cells were seeded in 96-well plates, 5 nM SS and 50 μM capsaicin doses were applied to the cells both individually and in combination to the treatment groups determined in MDA-MB-231 and L929 cells after 24 h of incubation. At the end of 24 h incubation, standards were first established according to the procedure provided with the kit and the

experiment was continued according to the procedure. At the end of the experiment, stop solution was added and measured at 450 nm in the spectrophotometer.

Determination of antioxidant enzyme activities

Determination of SOD, CAT, GPx enzyme activities in L929 and MDA-MB-231 cell lines were performed using 'Bioassay Technology Laboratory ELISA Kit'. In the treatment groups determined in MDA-MB-231 and L929 cells, 5 nM SS and 50 µM capsaicin doses were applied to the cells both individually and in combination. 24 h incubation was allowed. Results were defined as µmol/mg protein[18].

Comet test

MDA-MB-231 and L929 cells were detached from the flask surface using trypsin-EDTA solution and cells were counted. Cells were seeded in 6-well plates and incubated overnight at 37°C in a humidified atmosphere containing 5% CO_2. Subsequently, MDA-MB-231 and L929 cells were treated with 50 µM capsaicin and SS (5 nM). Both cell lines were exposed to these compounds individually and in combination for 24 h. After the treatment period, cells were mixed 1:1 with low melting point agarose (LMA), then transferred to slides and covered with coverslips. The slides were kept at 4°C for 20 min to solidify the gel. The preparations were then subjected to lysis, pre-electrophoresis and electrophoresis, followed by washing with dH_2O. Slides were dried for 15 min, stained with ethidium bromide (EtBr) and analysed using fluorescence microscopy to assess DNA damage[19].

Apoptosis detection with TUNEL asssay

In this study, 8-well culture slides were used for cell proliferation. Cells were incubated at 37 °C in 5% CO_2 medium. L929 and MDA-MB-231 cells were treated with 5 nM SS and 50 µM capsaicin concentrations both individually and in combination. Control groups were not treated with any substance. Cells were incubated for 48 h. For TUNEL assay, Thermo Scientific UltraVision Detection System Large Volume Anti-Polyvalent, HRP (RTU) kit was used according to the manufacturer's protocol. Nuclei were visualised using fluorescent DAPI (Invitrogen) staining, which emits blue and green wavelengths[20].

Investigation of PI3K, AKT and mTOR signalling pathways by ımmunocytochemistry

The experiment was performed using 8-well slides. Each well was seeded with 105 cells and then incubated at 37°C with 5% CO_2 for 24 h to facilitate cell adhesion to the slide. In the experiment, MDA-MB-231 and L929 cells were treated with 5 nM SS and 50 µM capsaicin both individually and in combination. After incubation, each well was washed with PBS buffer. Cells were then fixed using paraformaldehyde. After fixation, three washes were performed with PBS buffer. The wells were then incubated with cold triton-X for 10 min. After incubation, washing was performed with PBS buffer. Cells were then incubated with H_2O_2 for 3 min and washed three times with PBS after incubation. After these steps were completed, Large Volume Ultra V Block was applied followed by incubation with primary antibodies targeting PI3K, AKT and mTOR signalling pathways for 1 h. After incubation, cells were washed with PBS buffer and exposed to DAB (3,3′-diaminobenzidine) for 3 min. After incubation, cells were washed three times with dH_2O followed by application of a mounting medium. In the final step, the preparations were covered with long coverslips and the appearance of the cells was examined under a light microscope[21].

Statistical analyzes

All statistical analyzes were performed by Tukey test and one-way analysis of variance (ANOVA) using Windows SPSS 30.0 computer program. As a result, $P < 0.05$ was considered statistically significant.

Results

MTT cell proliferation assay

According to the results of MTT study, the LD_{50} value for capsaicin applied at different doses (5-100 µM) in L929 and MDA-MB-231 cell lines was 50 µM. According to the MTT test results, when the control group was considered 100% viable, capsaicin dose increase decreased the growth in L929 and MDA-MB-231 cell lines. While SS showed toxic effect at high doses, it was determined by repeated studies that it decreased the growth reduction rate in L929 and MDA-MB-231 cell lines and the LC_{50} was 5 nM (Fig. 1).

Comet test

According to the comet analysis performed for the detection of DNA damage in cells, DNA damage was detected in DNA tail percentage, DNA tail percentage, tail length and tail moment values in the control group, 50 µM capsaicin and 5 nM sodium selenite treated groups in MDA-MB-231 cell line and in the control group, 50 µM capsaicin and 5 nM sodium selenite treated groups in L929 cell line. In the light of these studies, capsaicin doses that would

cause toxic effects on L929 and MDA-MB-231 under *in vitro* conditions were determined within the scope of the studied parameters (Fig. 2, Table 1 and 2).

Measurement of MDA level

It has been determined that MDA causes cross-linking of compounds in the cell membrane, changes ion permeability and leads to negative results. In the study, an increase in MDA level was observed in L929 and MDA-MB-231 cells exposed to capsaicin and SS. The highest MDA level was observed in MDA-MB-231 and L929 cells exposed to a combination of 50 µM capsaicin and 5 nM sodium selenite (Fig. 3).

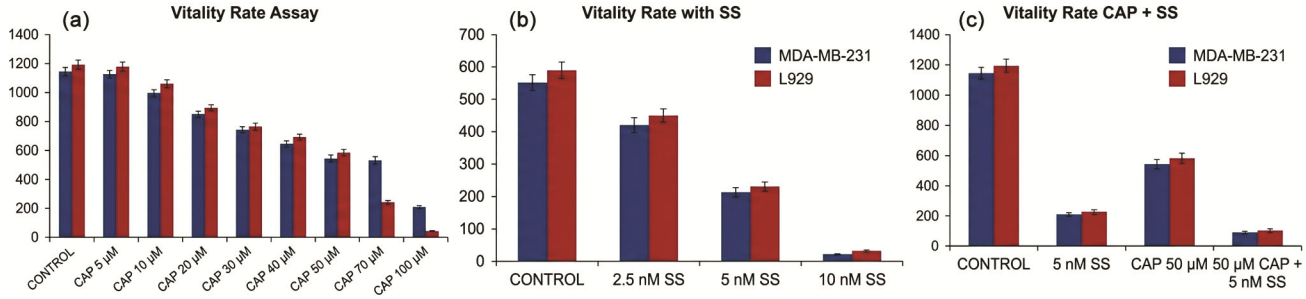

Fig. 1 — MTT assay results showing the effects of capsaicin (CAP) and sodium selenite (SS) on the viability of MDA-MB-231 and L929 cell lines. Data are presented as mean ± standard deviation ($P < 0.05$).

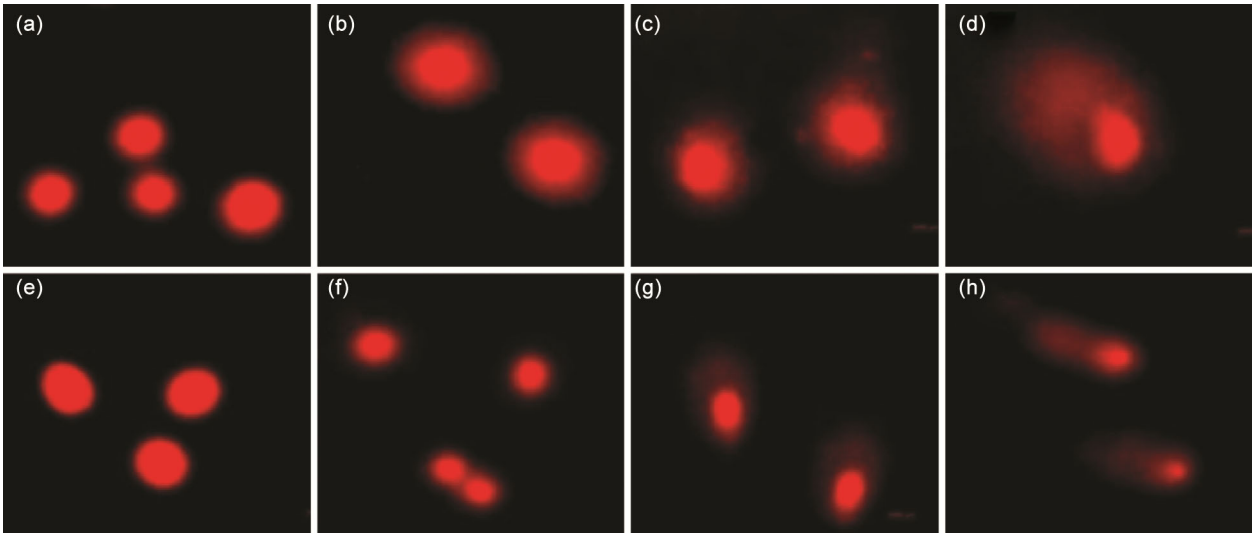

Fig. 2 — Comet assay results showing the effects of capsaicin (CAP) and sodium selenite (SS) on the viability of MDA-MB-231 and L929 cell lines. Data are presented as mean ± standard deviation ($P < 0.05$).

Table 1 — Comparing DNA tail lengths with comet analysis in the MDA-MB-231 cells.

Groups	Tail DNA % ± SD	Tail Length ± SD	Tail Moment ± SD
Control Group	38.52±3.46	2.48±0.11	0.95±0.003
5 nM Sodium Selenite	41.62±0.66	5.29±1.26	2.20±0.008
50 µM Capsaicin	56.43±3.63	25.03±2.56	14.2±0.09
50 µM Capsaicin +5 nM Sodium Selenite	66.57±2.98	31.67±1.44	21.08±0.04

Table 2 — Comparing DNA tail lengths with comet analysis in the L929 cells.

Groups	Tail DNA % ± SD	Tail Length ± SD	Tail Moment ±SD
Control Group	28.14±2.25	1.65±0.41	0.46±0.009
5 nM Sodium Selenite	39.21±3.63	1.25±2.56	0.49±0.09
50 µM Capsaicin	66.57±2.98	31.67±1.44	21.08±0.04
50 µM Capsaicin +5 nM Sodium Selenite	81.24±4.56	66.61±4.11	54.1±0.18

Determination of antioxidant enzyme activities

In our study, for MDA MB 231 and L929, a decrease in SOD enzyme activity was observed at 50 µM capsaicin and 5 nM sodium selenite concentrations (P<0.05) (Fig. 4). In terms of CAT enzyme activity, the lowest enzyme activity was observed at 50 µM capsaicin and 5 nM sodium selenite concentrations compared to the other groups (Fig. 4). In our study, as a result of GPx enzyme activity, it was observed that the enzyme activity decreased at 50 µM capsaicin and 5 nM sodium selenite concentrations in the groups with increased capsaicin dose compared to the control group (Fig. 4).

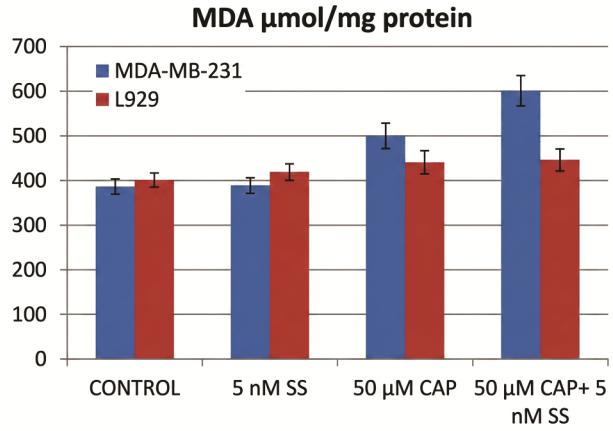

Fig. 3 — MDA levels in control and treatment groups with capsaicin and sodium selenite (P < 0.05).

Apoptosis detection with TUNEL assay

Increasing doses of CAP and SS caused a significant increase in the number of apoptotic cells after treatment, while SS positively affected this effect. The highest apoptotic-necrotic cell number was reached when 50 µM CAP and 5 nM SS concentrations were applied for MDA-MB-231 and L929 (Fig. 5 and 6).

Investigation of PI3K, AKT and mTOR signalling pathways by ımmunocytochemistry

Immunocytochemical staining was performed in MDA-MB-231 and L929 cell lines using primary

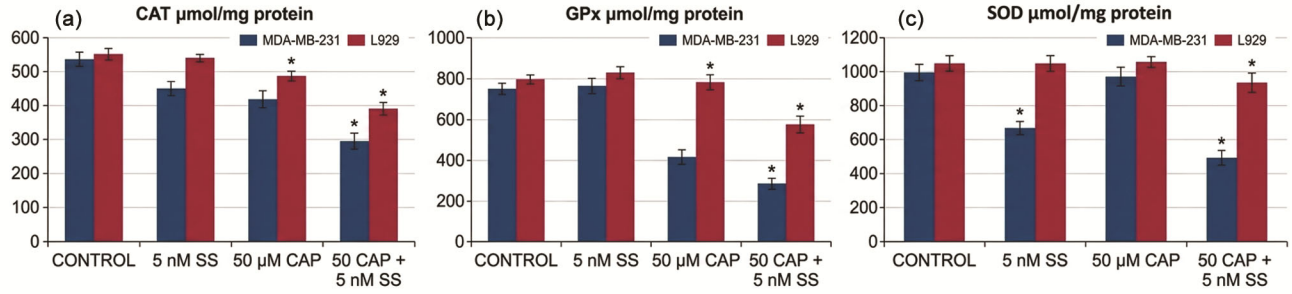

Fig. 4 — SOD, CAT and GPx enzyme activities in control and treatment groups with capsaicin and sodium selenite (P < 0.05).

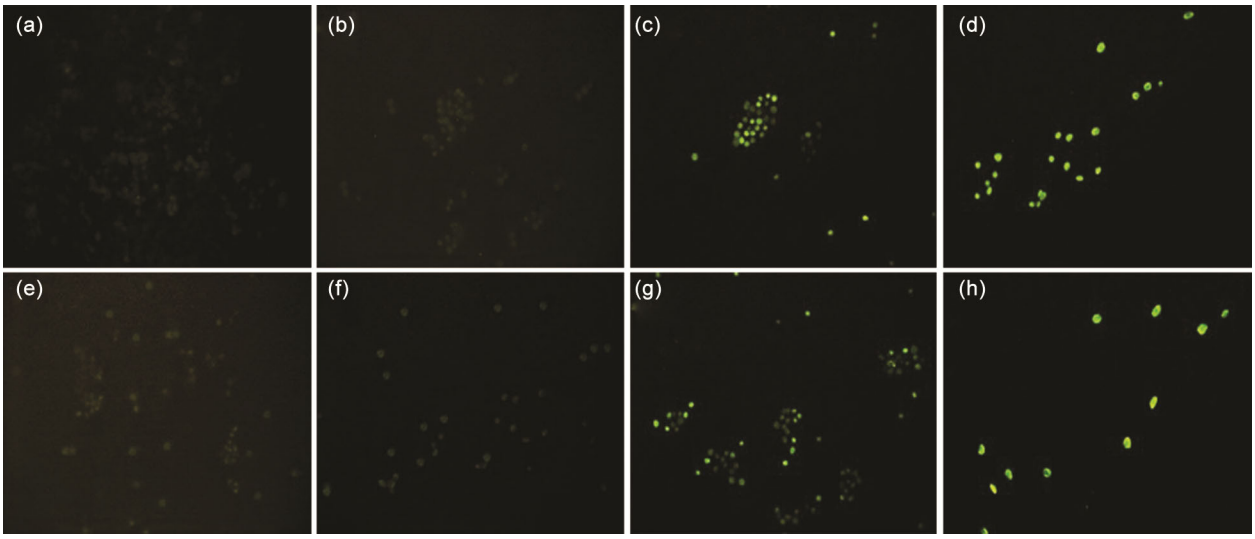

Fig. 5 — TUNEL assay microscopic images of apoptotic cells in MDA-MB-231 (a–d) and L929 (e–h) cell lines. MDA-MB-231 cells: (a) control group, (b) treatment with 5 nM sodium selenite (SS), (c) treatment with 50 µM capsaicin (CAP), and (d) combined treatment with 50 µM CAP and 5 nM SS. L929 cells: (e) control group, (f) treatment with 5 nM SS, (g) treatment with 50 µM CAP, and (h) combined treatment with 50 µM CAP and 5 nM SS.

antibodies targeting PI3K, AKT and mTOR proteins to evaluate cell death. Changes in the expression levels of PI3K, AKT and mTOR proteins in the stained cells were evaluated. These changes and the pathways associated with cell death were shown in Fig. 7.

Discussion

The effect of active ingredients in foods on cancer cells is a widely researched and studied subject today. The fact that the drugs used in cancer treatment are more specific and used together with alternative methods that will increase the effect will have a relaxing effect on both patients and the health system.

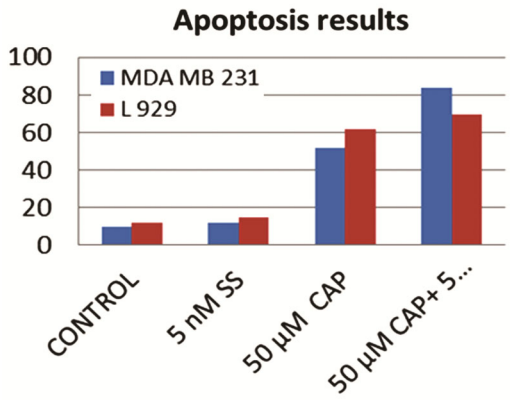

Fig. 6 — Cell death in MDA-MB-231 and L929 cell lines after 48-hour treatment with capsaicin (CAP) and sodium selenite (SS), depending on concentration. Data are presented as mean ± standard deviation ($P < 0.05$).

Although there are many studies on this subject, there are still many active substances and mechanisms to be discovered. For this purpose, different results have been shown in many studies with capsaicin and SS. In our study, the cytotoxic, oxidative, and signaling pathway-regulating effects of capsaicin and sodium selenite together on MDA-MB-231 and L929 cell lines were evaluated. The findings indicate that the LD50 level was reached at a 50 µM capsaicin concentration, with a significant decrease in cell viability and the triggering of apoptosis. MTT assay results were accepted as 100% viable and it was demonstrated that the results obtained as a result of the use of capsaicin with 5 nM SS, which decreased the growth in MDA-MB-231 and L929 cells, showed a synergistic effect and decreased the growth rate faster and decreased cell viability. These data are consistent with the toxicity ranges reported in the literature for MDA MB 231 and L929 cell lines[22-24]. In these studies, capsaicin was applied in the range of 10–200 µM, and a similar dose-dependent decrease in viability was reported in the follow-up of cytotoxic, oxidative, and signaling pathway modulators. Furthermore, the LD50 dose values applied to other cancer types are in a similar range[25].

The dose-dependent antiproliferative effect of capsaicin, particularly in triple-negative breast cancer, has been supported by numerous studies. Our study demonstrates that capsaicin disrupts cellular membrane integrity by increasing lipid peroxidation, as evidenced by the rise in MDA levels. Increased

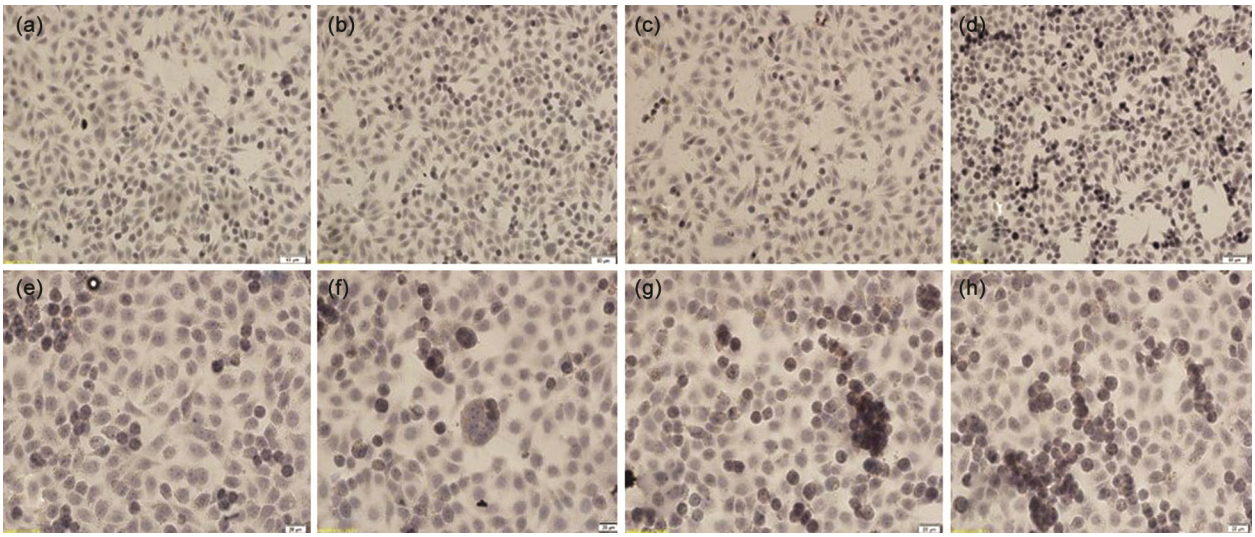

Fig. 7 — Immunohistochemistry results showing the identification of PI3K, AKT, and mTOR signaling pathways in MDA-MB-231 (a–d) and L929 (e–h) cell lines. MDA-MB-231 cells: (a) control group, (b) treatment with 5 nM sodium selenite (SS), (c) treatment with 50 µM capsaicin (CAP), and (d) combined treatment with 50 µM CAP and 5 nM SS. L929 cells: (e) control group, (f) treatment with 5 nM SS, (g) treatment with 50 µM CAP, and (h) combined treatment with 50 µM CAP and 5 nM SS.

lipid peroxidation leads to elevated ROS production, thereby rendering cancer cells more susceptible to the apoptotic process. The studies by Tang 2024 and Liu 2025 reported that the capsaicin-induced increase in ROS significantly enhances the cell death mechanism[26,27]. This result is consistent with our study and is an important indicator that capsaicin supports apoptosis.

Cancer cells are more sensitive to oxidative stress than normal cells. The decrease in SOD and CAT activities, which are antioxidant enzymes observed in our study findings, with the application of 50 μM capsaicin and 5 nM SS, reveals that oxidative balance is disrupted. Thus, the suppression of antioxidant defense accelerates cell death. This result is consistent with the findings reported by Mulè 2025 and Radhakrishna 2024. When the increase in MDA and the decrease in SOD–CAT are evaluated together in our study, it can be said that capsaicin acts as an agent that enhances the cell death mechanism by disrupting the ROS balance[28,29].

Immunohistochemical analysis findings revealed that capsaicin and sodium selenite inhibit the PI3K/AKT/mTOR signaling pathway, which sustains cell survival signals. Studies by Chen 2021 and Luján-Méndez 2023 have shown that capsaicin inhibits the same pathway, increasing caspase activation and weakening proliferation signals. Therefore, the apoptosis findings in our study appear to be related not only to oxidative stress but also to the disruption of cell survival signals. In the Şanlıer study, capsaicin was reported to affect breast cancer cell viability by inducing G2/M cell cycle arrest, reducing PI3K and Akt phosphorylation, and regulating the CDK8/PI3K/Akt/Wnt/β-catenin signaling pathway. The results of this study are consistent with our findings[22-30].

In the study by Mosqueda-Solís *et al*. it was reported that capsaicin inhibited migration of B16-F10 melanoma cells, and this effect was mediated by the suppression of the PI3-K/AKT/Rac1 signaling pathways. These findings suggest that capsaicin may have potential as an agent for cancer treatment and prevention[31]. In this study, the expression changes of PI3K, AKT, and mTOR proteins were examined through immunohistochemical staining during the evaluation of cell death. Significant changes in the expression levels of these proteins were observed in MDA-MB-231 and L929 cells following the application of capsaicin and SS.

The key point of our study is that the capsaicin molecule fits the double-edged sword concept. Luján-Méndez *et al.* 2023. While it exhibits antioxidant effects at low doses, it can have the opposite effect at high doses[30]. Accordingly, exceeding the LD_{50} value of 50 μM in our study supports that capsaicin significantly increases oxidative stress-mediated apoptosis at higher doses. The limitations of the study are that it is only *in vitro* and only the PI3K/AKT/mTOR pathway has been evaluated. When supported by more comprehensive studies, it may be possible to observe the effects of capsaicin on other apoptotic or necrotic signaling pathways. In future studies, a wider range of doses could be tested, *in vivo* models could be studied, and combinations of capsaicin with chemotherapeutic agents or other phytochemicals could be investigated. This would allow for the observation of more diverse therapeutic synergistic effects. In conclusion, this study demonstrated that capsaicin exerts a significant anticancer effect in MDA-MB-231 cells through increased oxidative stress, suppression of antioxidant defense, and inhibition of the PI3K/AKT/mTOR pathway. These findings indicate the potential for capsaicin to be used as a complementary or supportive agent in cancer treatment.

Selenium is an element known for its protective effect and is frequently used in drug combinations[32]. Selenium-containing compounds exhibit selective cytotoxicity against tumor cells. Epidemiological studies have shown that decreased serum selenium levels are significantly associated with the incidence of endometrial cancer and breast cancer[33]. At supranutritional doses, SS acts as a pro-oxidant, causing excessive ROS production in tumor cells, thereby altering redox homeostasis and exhibiting cytotoxic effects[34]. The induction of oxidative stress plays a critical role in the anti-tumor effects of SS[35].

A study conducted by Lv C. (2024) *et.al*. indicated that sodium selenite triggers autophagy and apoptosis in cervical cancer cells via the MPK/mTOR/FOXO3a pathway. According to this study, SS gradually reduced the viability of Hela and SİHA cell lines. They reported that this reduction in viability occurred via apoptosis and autophagy[36].

A study conducted by Chen *et.al*. (2022) indicated that glucose restriction in various cancer cell lines resulted in SS being more effective as a cytotoxic agent. They stated that SS causes the collapse of redox-dependent cancer cells by disrupting the redox

balance within the cell. Thus, when SS is taken in conjunction with intermittent fasting, tumor growth is suppressed with a treatment that keeps systemic toxicity low under metabolic stress, and this situation may be a protective treatment approach. Parallel to our study, the protective effect of SS has been demonstrated[37].

In a study conducted by Kim (2023), SS was used at high doses in cancer. This was found to reduce proliferation and increase cell death. While high doses of SS killed cancer cells, they had less effect on normal cells, highlighting its protective onco-therapeutic potential. These findings are consistent with our study[38].

In another study conducted by Cheng P. and colleagues, SS was used at different doses in five different thyroid cancer cell lines. The results showed that it induced apoptosis by arresting the cell cycle in the G1/G0 phase and suppressed the AKT/mTOR signaling pathway by increasing ROS levels. They suggested that SS could potentially be used as a protective agent in advanced thyroid cancer[39].

In conclusion, studies show that many plant extracts have proapoptotic, antiproliferative, antiangiogenic, and immunomodulatory effects. Therefore, they can be used as complementary and protective agents to conventional chemotherapies. However, testing these extracts on different cell lines and comparing the results is crucial in terms of sensitivity. To increase the success of cancer treatments, it is essential to discover new phytochemical/protective molecules, elucidate the mechanisms of these molecules on signaling pathways such as cell cycle, PI3K/AKT/mTOR, MAPK/ERK, and Wnt/β-catenin, and resolve issues related to the standardization, safety, and purity of plant extracts. – dose–purity issues of plant extracts is essential[40,41].

Conclusion

Breast cancer is the most common and widely discussed type of cancer among women. Cancer pathogenesis is influenced by endocrine factors such as age, family history, poor nutrition, lack of physical activity, and hormonal imbalance. In an organism, programmed cell death, known as apoptosis, is necessary for normal growth, but this process is induced in cancer cells. Therefore, ensuring apoptosis plays an important role in cancer treatment.

Numerous studies have shown that phytochemicals such as carotenoids specifically inhibit breast cancer growth and induce apoptosis. Secondary metabolites derived from plants are being extensively researched for their biological effects, such as triggering apoptosis. These phytochemicals play a key role in preventing cancer progression. Epidemiological studies and experimental results indicate that carotenoids can be used as dietary anticancer agents. Carotenoids can neutralize free radicals with their antiproliferative and antitumor activities.

Capsaicin exhibits antiproliferative (growth-inhibiting) and pro-apoptotic (cell death-inducing) effects in multiple types of cancer. It achieves these effects by arresting the cell cycle in the G0/G1 or G2/M phase, activating mitochondria-mediated apoptosis mechanisms, by increasing ROS (reactive oxygen species) production to create oxidative stress in cancer cells, and by inhibiting critical signaling pathways such as PI3K/AKT/mTOR, MAPK, STAT3, and NF-κB.

Consistent with findings from previous studies, this study underlines that the synergistic interaction between capsaicin and SS exhibits anticancer effects. Capsaicin was observed to induce significant cytotoxicity against the human breast cancer cell line MDA-MB-231 under *in vitro* conditions, highlighting its potential as a promising therapeutic agent. These results are not only in agreement with the existing literature, but also provide further evidence supporting the value of exploring combination therapies for cancer treatment.

Furthermore, this study contributes to the growing body of knowledge regarding the antitumour properties of natural and synthetic compounds, providing new perspectives on their application in targeted cancer therapies. By demonstrating the potential of these compounds in combination, this study lays the groundwork for future research aimed at improving treatment protocols, discovering underlying molecular pathways and evaluating their efficacy in clinical settings. It is expected that what is presented here will guide more comprehensive studies and ultimately advance the development of innovative therapeutic strategies to combat aggressive cancer types such as MDA-MB-231.

Funding information

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Data availability

The corresponding author will provide the datasets used and/or analyzed during the current work upon reasonable request.

Ethical Approval

Not applicable.

Consent to Participate

Not applicable.

Consent to Publish

All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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