https://doi.org/10.31925/farmacia.2026.2.18 **ORIGINAL ARTICLE**

ANTICARCINOGENIC POTENTIAL OF HIGH DOSE BORIC ACID ON MCF-7 CELLS: EVALUATION OF OXIDATIVE STRESS AND GENOTOXICITY-INDUCED APOPTOSIS

AHMET BÜYÜKBEN [1], ÖMER HAZMAN [2], İBRAHİM HAKKI CİĞERCİ [3], MEHMET FATİH BOZKURT [4], SEFA ÇELİK [5], LAÇİNE AKSOY [2]*

[1]*Program of Chemistry Technology, Cay Vocational School, Afyon Kocatepe University, Afyonkarahisar, Turkey*
[2]*Department of Chemistry, Faculty of Science and Arts, Afyon Kocatepe University, Afyonkarahisar, Turkey*
[3]*Department of Molecular Biology and Genetics, Faculty of Science and Arts, Afyon Kocatepe University, Afyonkarahisar, Turkey*
[4]*Department of Veterinary Pathology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey*
[5]*Faculty of Medicine, Department of Medical Biochemistry, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey*

**corresponding author: lacinetur@aku.edu.tr*

Manuscript received: January 2026

Abstract

This study determined the IC_0 and IC_{50} doses of boric acid in the MCF-7 cell line, the extent to which boric acid inhibits proliferation at low doses, and the extent to which it affects gene expression in apoptosis. Total antioxidant and oxidant status, the effect on DNA damage, and genotoxicity were determined. The mRNA expression levels of *Bcl-2*, *p53*, *TRAIL2*, and *Bax* genes related to apoptosis and proliferation, were analysed by RT-PCR. Estrogen receptor α positive (ERα+) and Caspase 3+ cells were analysed by immunocytochemical methods. The obtained data showed that the IC_0 and IC_{50} doses of boric acid increased oxidative stress, with statistical significance ($p < 0.05$). The IC_{50} dose also had genotoxic effects. It was determined that the IC_0 dose suppressed *Bcl-2*, *p53*, and *TRAIL2*, and the IC_{50} dose suppressed *Bax*. It was found that the ERα+ and Caspase 3+ cell numbers in all groups were higher than in the control. This shows that all applied doses increased the activation of Caspase 3, an apoptotic marker. It can be said that boric acid is also anticarcinogenic at high doses.

Rezumat

Acest studiu a determinat dozele IC_0 și IC_{50} ale acidului boric pe linia celulară MCF-7, măsura în care acidul boric inhibă proliferarea la doze scăzute, precum și efectele acestuia asupra expresiei genice implicate în apoptoză. Au fost evaluate statusul antioxidant și oxidant total, efectul asupra leziunilor ADN-ului, precum și genotoxicitatea. Nivelurile de expresie a ARNm ale genelor *Bcl-2*, *p53*, *TRAIL2* și *Bax*, implicate în apoptoză și proliferare, au fost analizate prin RT-PCR. Celulele pozitive pentru receptorul estrogenic α (ERα+) și pentru caspaza 3 (Caspase 3+) au fost evaluate prin metode imunocitochimice. Datele obținute au evidențiat faptul că dozele IC_0 și IC_{50} de acid boric au determinat o creștere semnificativă din punct de vedere statistic a stresului oxidativ ($p < 0,05$). Doza IC_{50} a prezentat, de asemenea, efecte genotoxice. S-a constatat că doza IC_0 a suprimat expresia genelor *Bcl-2*, *p53* și *TRAIL2*, în timp ce doza IC_{50} a suprimat expresia genei *Bax*. Numărul celulelor ERα+ și Caspase 3+ în toate grupurile experimentale a fost mai ridicat comparativ cu grupul martor. Acest rezultat indică faptul că toate dozele aplicate au amplificat activarea caspazei 3, un marker apoptotic. Se poate afirma, astfel, că acidul boric prezintă potențial anticarcinogen inclusiv la doze mari.

Keywords: breast cancer, MCF-7, boric acid, apoptosis, Caspase 3

Introduction

Boron is an important trace element found in nature as borax, boronatocalcite, and boric acid, and it plays a role in cell membranes, mineral and hormone metabolism, and enzymatic reactions. Boron exists as boric acid (BA) at physiological pH. Studies have shown that the antioxidant and anti-osteoporotic effects of boron can alter the metabolic processes of various enzymes and minerals, as well as immune system regulation. Since it increases the amount of reduced glutathione in the body, reduces oxidative damage and prevents the production of other reactive oxygen species (ROS) products, it has therapeutic applications, especially in diseases characterised by oxidative stress in their pathophysiology (1-3). There are also studies showing that it suppresses prostate, cervical, lung, and breast cancer (4-7). Carcinogenesis is a multifactorial process primarily driven by genetic predisposition and environmental factors. Evading apoptosis, unlimited division capacity, enhanced angiogenesis, resistance to anti-growth signals and metastasis, as well as one's own growth signals, are the main mechanisms that enable the progression of carcinogenesis. According to 2020

data, breast cancer is currently one of the most commonly diagnosed cancers, with an estimated 2.3 million new cases worldwide. Breast cancer is the most common cancer among women (8). Surgical and chemotherapeutic methods that allow the removal of breast cancerous tissues are applied in the treatment. After surgery and/or chemotherapy, radiotherapy is also applied to ensure that all cancer cells are destroyed and to minimise the possibility of breast cancer recurrence. Endocrine therapy is effective in cases of breast cancer recurrence or metastasis. Due to the expression of estrogen receptors (ERs), which are very common in breast cancer patients, blocking them through hormonal therapy is widely used as one of the potential treatment methods. Endocrine therapy aims to reduce estrogen levels or prevent breast cancer cells from being stimulated by estrogen (9-12).

Boron and its compounds are frequently used in many industries. It has recently spread widely due to its technological applications. Boron compounds, which are used in paints to protect the surface of the material and prevent factors such as rust, contamination, corrosion, etc., are used in antiseptics, boron alloys, fire retardants, nylon production, photography, textile industry, glass and glass fibre production, enamel and glaze. In recent years, it has also been used as a super lubricant. Many antioxidant agents, such as boric acid, are known to have anticarcinogenic effects at low doses (13). Boric acid was used as a material in this study because boron compounds are produced in excess of 1,000,000 metric tons *per* year, their use is widespread, and there is a high potential for human exposure at high doses both orally and dermally. In the presented study, it was determined what the IC_0 and IC_{50} doses of boric acid are in the MCF-7 cell line, to what extent boric acid inhibits proliferation in MCF-7 cells according to low dose, IC_0 and IC_{50} doses, and to what extent it affects *p53*, *Bcl-2, Bax* and *TRAIL2* gene expressions in the apoptosis of these cells. In addition, the effect of boric acid application on MCF-7 cells on ERα+ and Caspase 3+ cell numbers was demonstrated immunohistochemically. The aim was to determine the effects of different doses of boric acid application on oxidative stress levels and genotoxicity.

Materials and Methods

The study was started by determining the cytotoxic doses of boric acid in MCF-7 cells. After determining the boric acid doses to be applied to the experimental groups, the experimental groups were formed. In the next stage, cells were grown in a minimum of 5 flasks *per* group, and the determined boric acid doses were applied to the experimental groups. After cell lysates were obtained, oxidative stress, genotoxicity, apoptosis and immunochemical analyses were performed to determine the effects of boric acid on the MCF-7 cell line.

Preparation of the cell line and medium used in the study

MCF-7 cells were used to determine the effects of BA on oxidative stress, genotoxicity, and Caspase 3+ cell numbers, apoptosis, and proliferation in breast cancer. MCF-7 cells used in the study were produced from American Type Culture Collection (ATCC-Manassas, VA, USA) stocks. The medium prepared for the propagation of MCF-7 cells contains fetal bovine serum, penicillin, streptomycin, sodium pyruvate, L-glutamine and high glucose Dulbecco's Modified Medium (DMEM, Sigma Aldrich, USA). After the cells were inoculated, all incubations were carried out in a sterile environment at 5% CO_2 and 37°C in an incubator (Panasonic MCO-18AC, Japan).

Determination of cytotoxicity levels and experimental groups

Cytotoxic effects were measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The method is based on the reduction of yellow MTT by dehydrogenases in metabolically active cells, which results in the formation of purple formazan (14). For the multiplication of MCF-7 cells, cells were grown in flasks in medium containing 10% FBS, 100 units/mL penicillin, 100 µg/mL streptomycin, 1 mM sodium pyruvate, 2 mM L-glutamine and DMEM. Sufficiently proliferated cells were seeded into 96-well plates at 10^4 cells *per* well. They were incubated for 1 day to allow the cells to adhere to the bottom of the container. After the wells were at least 50% confluent, boric acid was applied to each well at a concentration of 20-150 mM. MTT dye dissolved in phosphate-buffered saline (PBS) at pH 7.4 was added to the wells at 10%. The cells were incubated for another 4 hours at 37ºC. At the end of the period, the MTT dye was removed from the cells, and 200 µL of DMSO was added to each well, which was then incubated for another 10 minutes. The colour change was determined at 540 nm wavelength in the ELISA plate reader (ELx800, Biotek, USA). The cell viability rate of the experimental group (Control Group) that did not receive BA was accepted as 100%, and the effect of each dose on cell viability was calculated as:

$$(\%)\ Cell\ Viability = \left[\left(100 * \frac{Absorbance_{sample}}{Absorbance_{control}}\right)\right]$$

Cell viability at each dose was expressed as a percentage. As a result of MTT applications, the BA dose that reduced cell viability by 50% compared to the Control Group was accepted as the IC_{50} dose (101.19 mM). The BA dose that did not affect cell viability compared to the Control Group was accepted as the IC_0 dose (1.27 mM) and used in the studies. The study groups were formed as follows.

Control Group (n = 5): 2 mL DMEM was applied. Low Dose BA Group (n = 5): 0.127 mM BA (1/10 ratio of IC_0 dose) was applied by dissolving in 2 mL DMEM. BA IC_0 Group (n = 5): 1.27 mM BA (IC_0) was applied by dissolving in 2 mL DMEM. BA IC_{50} Group (n = 5): 101.19 mM BA (IC_{50}) was applied by dissolving in 2 mL DMEM.

Preparation of lysates and lysate protein levels

The experimental groups were incubated for 24 hours after application for lysate preparation. Cells that adhered to the flask bottom during incubation were removed by trypsinisation/detrypsinization processes. Phosphate buffer solution containing Triton X-100 and a protease inhibitor cocktail was added to the cells. After sonication was applied to the cells, intracellular and organelle fluids were transferred to the lysis buffer. Total antioxidant capacity (TAS) and total oxidant capacity (TOS) were measured in supernatants obtained by centrifugation. Cell lysate protein levels used in biochemical analysis were measured spectrophotometrically using a commercial ELISA kit (51254, Fluka, USA) based on the Bradford method (15).

Total antioxidant capacity, total oxidant capacity and oxidative stress index

TAS and TOS levels were determined in cell lysates to determine the effect of boric acid on MCF-7 cells. Lysis buffer was used as a blank. TAS and TOS were measured as oxidative stress markers using commercial kits (Rell Assay, Gaziantep, Turkey) that employ spectrophotometric methods. The oxidative stress index (OSI) levels were calculated according to the formula given below, in accordance with the kit protocol (16, 17), using TAS and TOS levels. Biochemical analysis results were reported as ratios relative to total protein.

$$OSI = \left[\frac{TOS}{TAS}\right]$$

Determination of DNA damage and genotoxicity

Comet and micronucleus methods were used to determine the effects of BA on genotoxicity in MCF-7 cells. The Comet technique is a sensitive, rapid, and reliable method used to detect single- and double-strand DNA breaks caused by various agents. It is based on the principle that DNA molecules with different molecular weights and charges migrate differently in an electric field at alkaline pH. First, 20 µL of cellular suspension was mixed with low-melting-point agarose (LMA). Then, the cell-LMA mixture was prepared on slides with normal melting point agarose (NMA). The preparations were placed on ice cassettes to freeze. Following cover glass removal, cell lysis was performed in a solution containing 10 mM Tris (pH 10), 100 mM EDTA, and 2.5 M NaCl for 1 hour. After lysis, the slides were rinsed with redistilled water and transferred to a horizontal electrophoresis chamber containing cold electrophoresis buffer (pH 13, 300 mM NaOH, 1 mM EDTA). The slides were incubated at 4°C for 20 minutes to allow DNA unwinding. Electrophoresis was carried out at 20°C (25 V and 300 mA) for 20 minutes. The slides were neutralised by washing with Tris buffer (0.4 M, pH 7.5) after post-electrophoresis. All steps were carried out under dim lighting to avoid additional DNA damage. Finally, the stained slides (0.1 mg/mL ethidium bromide, 70 diluted 1:4) were observed by a fluorescence microscope (Olympus BX 51).

For each experimental sample, three slides were prepared, and 100 cells *per* slide were analysed to assess DNA damage. The degree of DNA damage was classified into five categories: Type 0 (Undamaged), Type 1 (minimal damage), Type 2 (moderate damage), Type 3 (severe damage), and Type 4 (extensive damage). Genetic damage index (GDI) and damaged cell percentage (DCP) were calculated for DNA damage (18).

$$DNA\ Damage\ scores = AU = 0 \times Type\ 0 + 1 \times Type\ 1 + 2 \times Type\ 2 + 3 \times Type\ 3 + 4 \times Type\ 4$$

$$100\ Damaged\ Cell\ Percentage = Type\ 2 + Type\ 3 + Type\ 4$$

Micronuclei are formations originating from complete/acentric chromosome fragments that are not included in the main nucleus and are usually caused by deficiencies in genes controlling the cell cycle, defects in the mitotic spindle, kinetochore or other parts of the mitotic apparatus and chromosomal damage. The increase in the number of micronuclei is considered an indirect indicator of chromosome irregularities caused by various agents in cells and genomic instability in somatic cells (19). Cells in groups were spread on clean slides and fixed in ethanol for the micronucleus test. The slides were air-dried and exposed to 5% giemsa stain to identify cell nuclei. For micronucleus testing, cytokinesis blockade was not used. Micronucleus frequencies were determined by counting the nuclei (10^3 cell nuclei/ for each slide) in the preparations obtained using a light microscope (20).

Molecular analyses

Total RNAs to be used in gene expression analyses were isolated from cell lysates using the Reverse Transcription-Polymerase Chain Reaction (RT-PCR) method using a commercial kit (Gen Matrix, EURx, Poland). The amount and purity of the total RNA obtained were determined by measuring their optical density in a NanoDrop (Epoch 2, BioTek, USA). RNAs with an OD260/280 ratio between 1.7 and 2.2 were used in the study. To be used as templates in the PCR reaction, total RNA from each sample was taken, and complementary DNA (cDNA) was synthesised using a commercial cDNA synthesis kit (ThermoFischer Scientific, USA) using

reverse transcriptase (RT). 1.5 µl of cDNA from each sample was used, and Sybr Green PCR Master Mix (12.5 µl) and the primer pair (oligonucleotide) were added in amounts as specified by the protocol. Primers were specific to each transcription analysis and were selected based on studies in the literature (21). Based on the cycle threshold (Ct) values of the amplification curves obtained after the amplification process consisting of three steps, denaturation, primer annealing and chain extension, the relative changes in mRNA expression levels of target genes were calculated using the $2^{-\Delta\Delta Ct}$ method (22). The mRNA expression level was determined as a fold change. Calculations were performed using REST 2009 (Relative Expression Software Tool, Version 1, Qiagen, Germany). The *β-actin* gene was used as the endogenous control, and correction (normalisation) was applied to the expression levels of other genes according to the *β-actin* gene level of each sample. While analysing the anticarcinogenic effects of BA in MCF-7 cells, mRNA expression levels of *p53, Bcl-2, Bax* and *TRAIL2* were analysed using RT-PCR (CFX-96, Bio-rad, USA) device using total RNA isolated from Control and treatment Groups and subsequently synthesised cDNAs.

Immunocytochemical records

Cells were fixed in 5% buffered neutral formaldehyde solution, pelleted using the cytoblock application, and routine tissue follow-up was performed. Cells were blocked in paraffin, and sections were taken using a microtome. Rabbit Anti-estrogen α receptor and rabbit anti-caspase-3 antibodies were dropped onto the samples and incubated. Peroxidase enzyme conjugated streptavidin was treated (Standard Vectastain Elite ABC Kit, PK-6100, Vector Laboratories Inc, CA, USA). Incubated for 30 minutes and washed with PBS. The peroxidase substrate 3-amino-9-ethylcarbazole (AEC) was applied to colour the reaction. All samples were examined with a light microscope (Zeiss Axio Lab. A1 Microscope – AxioCam ICC 5 Camera). Zeiss-ZEN 2 imaging and ImageJ analysis software were used to analyse each section. Four photographs of each sample were taken at the highest magnification. All cells were counted, and the percentage of positive cells was calculated (14).

Statistical analysis

Data obtained from biochemical and genotoxicity analyses were evaluated using SPSS 18. One-way analysis of variance (ANOVA) was used for parametric tests with normally distributed data, and the Duncan test was used as a post hoc test. The Kruskal-Wallis test, a nonparametric test, was applied to the analysis results that did not show normal distribution to determine whether there was a statistical difference. For the parameters determined to have statistical differences, which groups differed from the Control Group was determined using the Mann-Whitney U test. Statistical evaluation of the data obtained from molecular analyses was performed using REST 2009 (Relative Expression Software Tool, Version 1, Qiagen, Germany). The acute toxic dose of BA was determined by applying the EPA Probit Analysis Program (Version 1.5). Dose–response curves were calculated using a linear regression model. The resulting IC_{50} value was reported, along with its 95% confidence interval.

Results and Discussion

Breast cancer is the most common malignant tumour in women worldwide. With 0.5 million deaths and more than two million new cases in 2020, it is the second leading cause of death in women worldwide. According to the International Agency for Research on Cancer, the number of new cases is expected to exceed three million by 2040 (23).

Boron is widely used in agriculture, industry, and the cosmetic sector, as well as in traditional medicine. Boron is mainly taken in the form of BA and circulates in the body in this form. Dietary intake significantly affects metabolic and physiological systems in humans and animals. Boron was first used for cancer treatment with “boron neutron capture therapy”. This treatment targets cancer cells with minimal damage to healthy cells and is used especially in gliomas and metastatic brain tumours. BA-induced boron neutron capture therapy induced DNA double-strand breaks and apoptosis. It reduces cell proliferation, migration and invasion in breast cancer. It is also known that BA and calcium fructoborate inhibit cell proliferation in MDA-MB-231 cancer cell lines. Recent studies on breast cancer and BA suggest BA as a chemical protective agent (24).

Epidemiological and experimental studies have shown that boron has positive effects on various types of cancer. It has been reported that individuals who follow a boron-rich diet and live in areas with boron-rich water and soil have a lower risk of developing certain types of cancer, such as prostate, cervix and lung. BA has been shown to have potential cancer-preventing properties and suppresses cancer cell proliferation in many cancer cell lines (25).

The study used the triple-positive, luminal-like adenocarcinoma cell line MCF-7, which is the most preferred in breast cancer research, in terms of estrogen and progesterone receptors. Apart from the MCF-7 cell line, studies conducted on other cell lines, such as HL60, Sertoli, and TM3 Leydig, have shown that cell viability decreases with increasing doses of low-concentration BA over a wide range (26, 27). In addition, it was reported that although a similar decrease was observed in MCF-10A cells treated with 0.1-1 mM BA, the decrease was not statistically significant (28). It is also noteworthy that the viability

in these studies was greater than 50% and that the effects of BA were particularly evident at low doses. We based our dosage design on the literature of Acerbo *et al*. (2015), where com-parable high millimolar concentrations (up to 50 mM) were utilised to evaluate the apoptotic resp-onses of cancer cell lines (29). In addition, recent studies have also achieved IC_{50} values in the 75-100 mM range (30) and at ~50 mM concentrations in different cell lines (31). In the presented study, cytotoxicity analysis was performed by applying 20-150 mM BA and MTT analysis was performed. According to this analysis, cell viability decreased with increasing concentration (Figure 1). Consistent with the literature, increasing doses of BA increased toxicity, accelerating cell destruction.

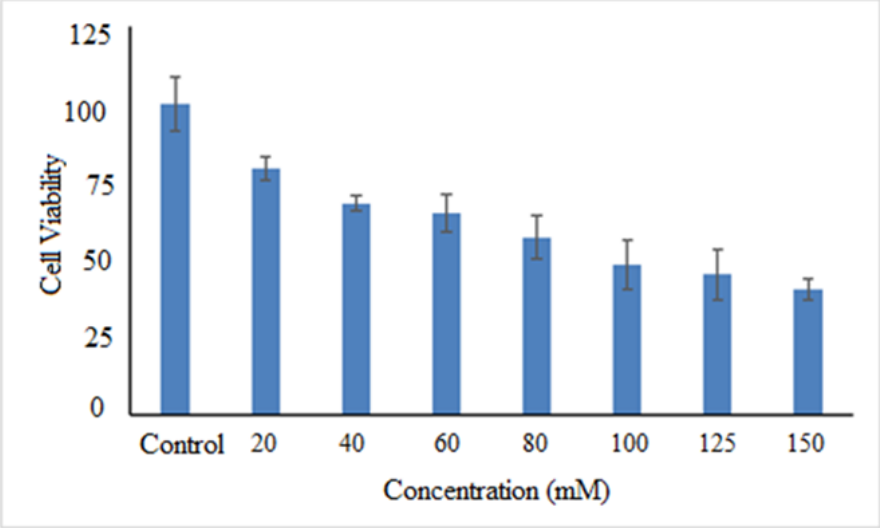

Figure 1.
MCF-7 cell viability of various BA concentrations

Data obtained from the cytotoxicity analysis of BA were determined by applying the EPA Probit Analysis Program (Version 1.5) to determine the acute toxic dose (IC_{50}) of BA as 101.19 mM and the IC_0 dose (IC_0) as 1.27 mM. Other BA doses for MCF-7 cells were calculated as IC_5: 4.579 mM, IC_{10}: 9.067 mM, and IC_{15}: 14.385 mM. Experimental groups were determined based on these doses. Low-dose BA Group was created using 1/10 of the IC_0 dose of BA (0.127 mM), IC_0 Group using the IC_0 dose (1.27 mM) and high-dose BA Group using the IC_{50} dose (101.19 mM). Table I shows the inhibition concentrations of BA in MCF-7 cell lines.

Table I
BA inhibition concentrations on MCF-7 cell lines

	Exposure Concentration (mM)	95% Confidence Limits	
		Low	High
IC_0	**1.269**	**0.241**	**3.189**
IC_5	4.576	1.456	8.675
IC_{10}	9.067	3.789	14.835
IC_{15}	14.385	7.205	21.369
IC_{50}	**101.190**	**84.094**	**1854.447**

Effects of BA on oxidative stress and genotoxicity

Oxidative stress is generally defined as a situation in which the balance between oxidants and antioxidants is disrupted. The main source of oxidative stress in living organisms is reactive oxygen species, which are molecules such as hydrogen peroxide, superoxide and hydroxyl radicals derived from oxygen and are highly reactive to biomolecules. One of the most harmful cons-equences of oxidative stress is the formation of DNA lesions (32, 33). High levels of oxidative stress are associated with the pathological origins of many diseases, especially cancer, as well as Alzheimer's, inflammation, and cardiovascular diseases. The concentration of these species increases in the cell during oxidative stress, driven by an increase in the ROS system, which has oxidant properties. While the ROS level in normal cells is around 0.02 µM, cancer cells continuously produce ROS at levels ranging from 100 µM to 1 mM. Therefore, oxidative stress is an important parameter in evaluating the extent of cancer in cells (34). Total antioxidant capacity reflects the combined effects of all antioxidants in intracellular and extracellular fluids, and total oxidant capacity reflects the combined

effects of all oxidants. The measurement of TAS and TOS can provide more valuable information than the separate measurement of antioxidants and oxidants. The OSI, calculated as the ratio of TOS to TAS, is an important indicator of oxidative stress in the examined sample (16). It has been reported that BA increases antioxidant activity and reduces oxidative stress at low doses (35, 36). The effects of dietary BA and borax supplementation on antioxidant activity and DNA damage in rats were investigated, and it was reported that it reduced the damage and supported the antioxidant defence mechanism (37). In addition, in a study examining the effect of BA against ethanol-induced kidney damage, it was observed that BA did not change the TAS, TOS and OSI values (35, 38) in a study examining the effect of high concentration boron and its compounds on nephrolithiasis and oxidative stress, there was no difference in TAS, TOS and OSI levels (39), in another study examining the effectiveness of BA in the treatment of osteomyelitis caused by methicillin-resistant *Staphylococcus aureus*, TAS levels decreased with BA application (40). In literature studies, it is seen that low-dose BA can support the antioxidant system and reduce oxidative stress, while in some studies where high-dose BA was applied, it was observed that it either did not change oxidative stress or increased it. In the present study, the TAS value of BA decreased in all study groups compared with the Control Group (Table II). While the low-dose TOS values were the same as the control, the TOS values were statistically significantly higher in the IC_0 and IC_{50} Groups. Therefore, the OSI values in these groups were also higher than those in the Control Group. It can be thought that lipid peroxidation and DNA damage may occur due to increased oxidative stress. Although the extent of oxidative stress in the IC_{50} Group was compared to the IC_0 Group, there was no statistical difference between these two groups. The highest level of oxidative stress was observed in the IC_{50} Group. Therefore, it can be said that the increased BA dose on increasing oxidative stress.

DNA damage refers to physical or chemical changes in DNA in cells that can affect the interpretation and transmission of genetic information. DNA can be damaged by a variety of exogenous and endogenous insults, including chemicals, radiation, free radicals, and topological changes, each of which causes different forms of damage. Depending on the nature of the DNA lesion, specific pathways are activated to facilitate the identification and repair of damaged regions (41, 42). Although DNA is a stable structure, it can undergo spontaneous oxidative damage under physiological conditions throughout life. Excessive amounts of oxidants can directly damage DNA and affect the activities of repair enzymes, leading to destruction. Comet assays are one of the standard methods for basic applications related to DNA damage and repair and for the assessment of DNA damage (43), while micronucleus is a biomonitoring method that can be useful in investigating the genotoxic and carcinogenic potential of many materials, especially chemicals, to which living beings are exposed, in estimating cancer risk and in following up developing cancer. If DNA damage is low, it is efficiently repaired by DNA repair mechanisms. Severe damage causes cell death by stimulating apoptotic mechanisms. Comet Assay is a method that allows the identification of all single- and double-strand breaks. It is a frequently preferred method for DNA damage measurement because it is simple, fast, sensitive, applicable to different cell types and types of DNA damage, and does not require radioactive labelling. DNA breaks formed by apoptosis-mediated cytotoxicity result in increased DNA migration during electrophoresis. Double-strand breaks are formed. Apoptotic cells can be detected by electrophoresis in neutral or alkaline media (44, 45).

Cytogenetic methods are widely used for *in vivo* genotoxicity testing (on animals) and for *in vitro* genotoxicity testing of drug or food chemical compounds (on cell lines). Micronuclei are small, round-shaped bodies containing chromatin that can be seen in the cytoplasm of cells and are thought to result from DNA damage or genomic instability. Micronuclei can occur as a result of natural pro-cesses such as metabolism or ageing, or can be triggered by many environmental factors, dangerous habits and different diseases (46).

Comet values and micronucleus fractions (MN) of the groups are also given in Table II. It is observed that both parameters in the high-dose IC_{50} Group are statistically significantly higher than those in the Control and Low-dose Groups. In the study, it was observed that the OSI value increased in the IC_0 and IC_{50} Groups, and oxidative stress increased. MN levels and tail moments detected by comet analysis also increased in the IC_{50} Groups and caused damage to DNA (Table II). In particular, the difference between the tail moments and micronucleus frequencies of the IC_{50} Group, where the highest oxidative stress was observed, and the Control Group was statistically significant ($p < 0.05$). This situation explains the relationship between increasing BA dose, oxidative stress, and subsequent DNA damage.

Table II

Effects of BA applied at different doses to MCF-7 cells on total antioxidant status, total oxidant status, oxidative stress index, comet analysis and micronucleus frequency

	Group 1-Control	Group 2-Low Dose	Group 3-IC_0	Group 4-IC_{50}
TAS (mmol Trolox Equiv/g protein)	0.356 ± 0.058^{b}	0.248 ± 0.036^{a}	0.217 ± 0.052^{a}	0.240 ± 0.031^{a}
TOS (µmol H_2O_2 Equiv/g protein)	1.690 ± 0.354	1.045 ± 0.076	$2.94 \pm 0.841^{a,b}$	$3.985 \pm 1.298^{a,b}$
OSI (AU)	5.01 ± 1.82	4.28 ± 0.65	$14.41 \pm 5.72^{a,b}$	$17.19 \pm 7.16^{a,b}$
DNA damage scores (AU)	4.33 ± 0.57	4.67 ± 1.53	5.33 ± 2.31	$7.67 \pm 1.15^{a,b}$
Micronucleus (% frequency)	0.00333 ± 0.0006	0.00333 ± 0.0006	0.00667 ± 0.0006	$0.01667 \pm 0.0015^{a,b}$

Data are given as mean ± standard deviation (n = 5). a, b: The difference between means with different exponents in the same row is statistically significant ($p < 0.05$). a: represents groups that are statistically different from the Control Group; b: represents groups that are statistically different from the Low-dose BA Group.

Effect of BA on apoptosis and proliferation

Much information on breast cancer has been obtained from *in vivo* studies and *in vitro* studies using breast cancer cell lines. Breast cancer cell lines are widely used for breast cancer modelling, covering a panel of diseases with different phenotypic associations. Despite the advantages of providing homogeneous material for cancer studies and being easy to apply, these cell lines can also accumulate some mutations in the first and subsequent cultures (47). In MCF-7, which has an epithelial structure and can also regulate insulin-like growth factor-binding proteins, HER2 expression is normal, while ER and PR are positive (48). Apoptosis affects both the development and the maintenance of the organism's internal balance. If conditions such as increased stress at the cellular level, increased levels of reactive nitrogen and reactive oxygen species are not controlled, genetic disorders and cancer can occur. Apoptosis ensures the elimination of cells that exhibit this process and protects the organism's health. While pro-apoptotic factors trigger apoptosis and initiate the pathway, anti-apoptotic factors prevent apoptosis and inhibit the pathway. Signal pathways that trigger apoptosis can be of internal or external origin. The apoptosis pathway triggered by extrinsic stimuli is triggered by the binding of TNF, FAS-L or TRAIL death ligands to their receptors on the cell membrane of another cell; when conditions such as DNA damage or stress occur, the apoptosis pathway triggered by intrinsic stimuli is activated by a cascade regulated by members of the *BCL-2* Group (49). Proteases (Caspases) and the *BCL-2* gene family play important roles in regulating apoptosis in cells. If the homeostatic balance between cell division and apoptosis is disrupted, cancer develops, and mutations in pro-apoptotic and anti-apoptotic genes that regulate apoptosis are implicated in this process (50). In MCF-7 lines, Caspase 6, Caspase 7, Caspase 9, and *Bcl-2* expressions are good, while *p53* and *p21* gene expressions are normal. In addition, estrogen-induced proliferation and increased estrogen expression contribute to the line's proliferation (51). In the study, mRNA expression levels of the *Bcl-2*, *p53*, *TRAIL2*, and *Bax* genes, which are related to apoptosis and proliferation, were determined. Expression gene ratios of the group are shown in Figure 2. It was determined that low-dose BA applied to MCF-7 cells did not significantly affect gene expression levels. It was determined that BA applied at an IC_0 dose in MCF-7 cells suppressed *Bcl-2*, *p53* and *TRAIL2* mRNA expression levels. It was determined that BA, applied at an IC_{50} dose, suppressed mRNA expression of *Bax*, a proapoptotic gene, in MCF-7 cells.

These data weaken the idea that BA at LD_0 and LD_{50} doses affects MCF cells *via* the canonical apoptosis pathway described in previous sections, which consists of two main mechanisms (intrinsic (mitochondrial) and extrinsic (death receptor) pathways) and is coupled with other apoptotic mechanisms in the caspase cascade. However, it should be noted that many proapoptotic molecules in the canonical mechanism are not included in the parameters used in our study. Among these proapoptotic molecules, cytochrome c and apoptosis inhibitor proteins (IAPs), which mediate apoptosis through mitochondrial dysfunction, can be mentioned (52). In addition, BA may have stimulated the caspase cascade by affecting molecular mechanisms in which calcium ions and phospholipase C pathway ligands are more effective (Calpains and Calcium Signalling by IP3 Receptor pathway) (53, 54). It should also be noted that DNA damage in MCF-7 cells, driven by increased oxidative stress, can trigger caspase-mediated apoptosis *via* PARP (Poly(ADP-ribose) polymerase) activation (55). Considering that BA increases oxidative stress and causes DNA damage, *Cas3* may have been stimulated *via* PARP activation. Besides the canonical pathway, another mechanism for *Cas3* stimulation could be *via* endoplasmic reticulum stress. In MCF-7 cells, ER stress is a pathological condition characterised by the accumulation of unfolded proteins in the ER lumen, which activates the cell's survival or apoptotic mechanisms. BA may have induced the formation of unfolded or misfolded proteins in MCF-7 cells, leading to the activation of molecular chaperones, such as GRP78, which can stimulate apoptosis. These chaperones, in turn, can activate proteins such as ATF4, CHOP, and IRE1,

thereby stimulating the mitogen-activated protein kinase (MAPK) pathway and leading to apoptosis (56). *Cas12* generally mediates the activation of *Cas3* in this signalling pathway. In light of this information, the study presented shows that high doses of BA are cytotoxic, as indicated by MTT data. This toxicity may be due to apoptosis, which is independent of *Cas3*, or to non-apoptotic mechanisms such as autophagy and necroptosis, or to necrosis (57).

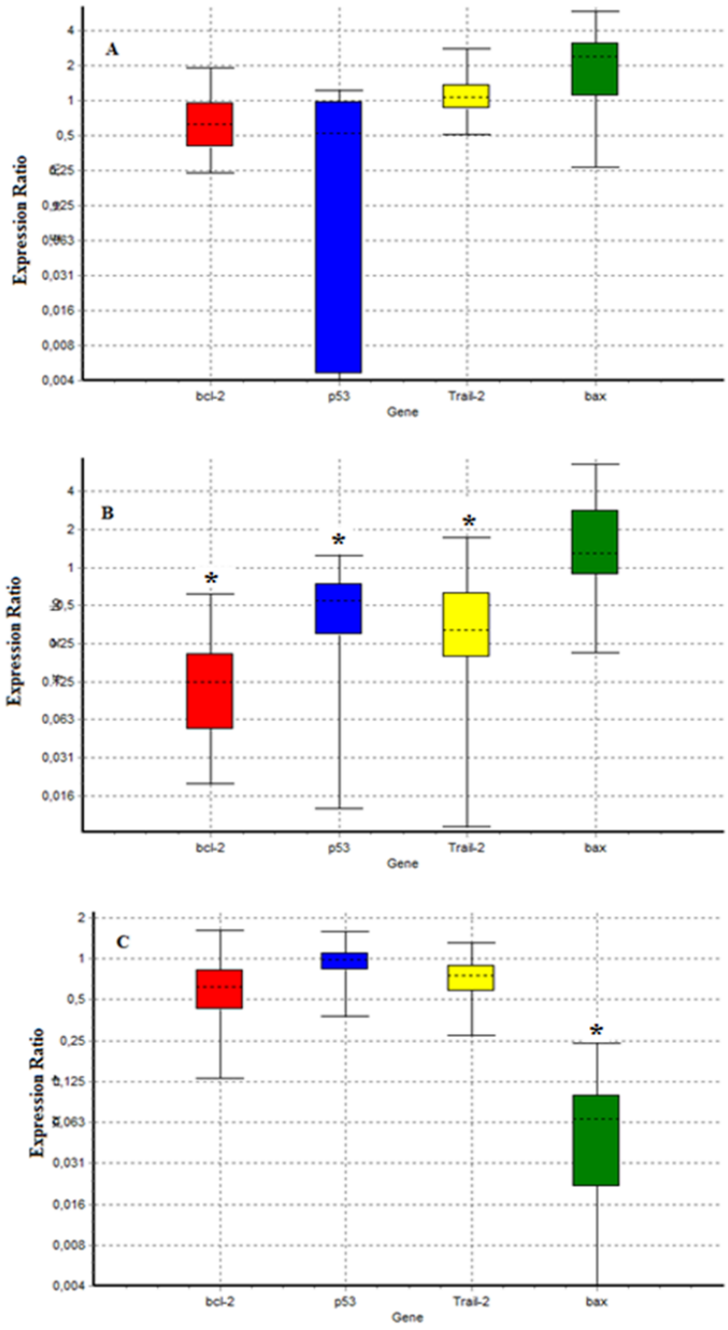

Figure 2.
Effect of BA on apoptosis and proliferation in MCF-7 cells. A: Comparison of the Control Group and the Low dose Group, B: Comparison of the Control Group and the IC_0 Group, C: Comparison of the Control Group and the IC_{50} Group are shown

Effect on caspase 3-positive and ER-positive cell numbers

MCF-7 cancer cell line has complex morphological and molecular features. An important prognostic marker is the presence of ER. Approximately 60% of breast cancer cells overexpress ER-α, which plays an important role in the transcription of nuclear DNA required for mammary gland development and

is essential for the breast cancer signalling network. ER-α also regulates cell proliferation and differentiation through paracrine mechanisms. These findings support the usefulness of ER-α as a potential molecular target to inhibit the progression of malignant cells. The development of molecularly targeted anticancer drugs is the dominant focus of current research efforts (58). Estrogen, which stimulates cellular proliferation in breast tissue, exerts its effects through receptors. ERs can be found as nuclear receptors inside the cell or conjugated with G proteins in the cell membrane. Estrogen stimulates gene transcription *via* the nuclear receptors ERα and ERβ, increasing proliferation in breast cells (59, 60). In a study in the literature, it was determined that BA (4-75 mg/kg) induced uterine growth in ovariectomized rats, increased epidermal cell height and proliferating cell nuclear antigen expression, and was reported to have estrogen-like effects *in vivo*. In the same study, BA was applied to MCF-7 cells at various doses (10^{-2}-10^{-8} mol/L), and proliferation indices were measured; no proliferative effect was observed at these doses (61). In the study, ERα+ and caspase 3+ cell numbers in MCF-7 cells after different doses of BA are shown in Table III. The number of ERα+ cells increased significantly in all groups compared with the Control Group. It can be assumed that all applied BA doses may trigger proliferation in MCF-7 cells. The literature indicates that high expression of ERα is closely associated with cellular proliferation in breast cancer; however, the precise molecular mechanisms by which ERα controls breast cancer cell proliferation are not clearly understood. It has been reported that ERα may exert a proliferative effect by regulating the cell cycle, particularly by suppressing *p53*/*p21* and stimulating proliferative cell nuclear antigen (PCNA) and proliferation-associated Ki-67 antigen (Ki-67) (62). Another study indicated that ERα was suppressed while genotoxicity, oxidative stress, and inflammation-mediated apoptosis were induced in MCF-7 cells (14). In light of this information, it can be concluded that there is an inverse correlation between apoptosis induction and ERα induction. However, the study presented here shows that both ERα and apoptosis increased simultaneously, but proliferative features (lack of Bcl-2 stimulation) were not observed in MCF-7 cells (Figure 2). This situation can be explained by the cell cycle being shaped by the balance between cell death and proliferation. It is possible that proliferation is suppressed in cases where cell death is excessively stimulated through non-apoptotic mechanisms, such as apoptosis or necroptosis, or through different mechanisms, such as the necrosis pathway (57). In this case, the proliferative effect of ERα, which normally promotes proliferation in MCF-7 cells, may have been suppressed. The apoptotic mechanism can be induced by extracellular or intracellular signals. Both provide activation of a protease cascade called caspases as part of the apoptotic signalling cascade. Initiator caspases (Caspase 2, 8, 9 and 10) activate effector caspases (Caspase 3, 6 and 7) upon activation by pro-apoptotic signals. Caspase 3 is responsible for nuclear changes in apoptosis and is considered the main effector caspase because both the extrinsic and intrinsic pathways lead to its activation. Caspase 3 is the most downstream enzyme in the apoptosis-inducing protease pathway and is probably the enzyme most clearly associated with cell death because it cleaves essential proteins in the cell repair process. In the presented study, MCF-7 cells were determined to be *Cas3*+ using immunostaining methods. This was surprising, as studies in the literature report that Caspase-3 expression is absent in MCF-7 cells (63). However, studies also show that *Cas3* is present in MCF-7 cells and can be stimulated by various factors. In one such study investigating the role of ubiquitin-specific peptidase 42 (USP42) in regulating apoptosis during breast cancer proliferation, the presence of *Cas3* and denatured *Cas3* in MCF-7 cells, and especially the stimulation of denatured *Cas3* by USP42, was demonstrated by western blot analysis (64). From this perspective, some information in the literature contradicts itself. This may be related to the different antibodies used to detect the presence or absence of *Cas3*, or to determine whether *Cas3* is suppressed or stimulated. In our study, a rabbit polyclonal anti-Caspase-3 antibody (ab13847) was used. The supplied antibody recognizes a cleaved form of Caspase 3 (~17 kDa) in wild-type cells after apoptosis is induced, but not in Caspase 3 knockout cells. Given that the MCF-7 cells we used were wild-type and that, based on our results, wild-type MCF-7 cells possess cleaved *Cas3*, it can be concluded that wild-type MCF-7 cells possess cleaved *Cas3*. Indeed, one of the first studies on this subject reported that although there was no major gene alteration in Caspase-3 in MCF-7, a 125-nucleotide deletion resulted in a frameshift at the translation level (65). In this context, even though *Cas-3*-dependent apoptosis does not occur in MCF-7 cells, further scientific studies are recommended to assess the validity of inducing apoptosis in these cells *via* the *Cas-3* cleavage product.

Microscopic images of Caspase 3+ cells and ERα+ cells are given in Figure 3. In the study, the number of Caspase 3+ cells increased in the IC_0 and IC_{50} Groups compared with the Control Group (Table III). This increase indicates that the *Caspase 3* pathway is triggered by oxidative stress and DNA damage. The Caspase 3 pathway is stimulated by increased *p53* expression and a subsequent increase in the *Bax/Bcl-2* ratio, which triggers apoptosis (14, 66). Oxidative stress can also activate extrinsic pathways. In particular, it can activate TRAIL-R1/2, FAS and TNF-R1 death receptors, and apoptosis

occurs *via* the Caspase 3 pathway following the activation of Caspase 8 (67, 68). In this study, *p53* expression decreased at the IC_0 dose, but did not change at the IC_{50} dose. The *Bax*/*Bcl-2* ratio decreased at the IC_{50} dose and partially increased at the IC_0 dose. In addition, *TRAIL2* expression decreased at the IC_0 dose, while there was no difference at the IC_{50} dose. In addition, ERα+ levels increased significantly in all groups compared to the Control Group, suggesting that these doses can trigger proliferation. However, an increase in the number of Caspase 3+ cells and induced apoptosis is also seen at all applied BA doses. This shows that BA induces apoptosis in breast cancer cell lines *via* extrinsic pathways of oxidative stress, particularly *via* Caspase 3, which is triggered by activation of FAS-L and TNF receptors, rather than TRAIL receptors.

Table III

Effects of BA application at different doses on ERα+ cell numbers and *Caspase 3*+ cell numbers in MCF-7 cells

Group	Total Caspase 3+ cell numbers in 20 mm^2	Number of ERα+ cells *per* 1000 cells
Group 1-Control	22 ± 3^a	205 ± 12^a
Group 2-Low Dose	54 ± 5^b	318 ± 18^b
Group 3-IC_0	70 ± 3^c	316 ± 11^b
Group 4-IC_{50}	74 ± 3^c	260 ± 14^c

Data are given as mean ± standard deviation (n = 5). a, b, c: The difference between means with different exponents in the same column is statistically significant ($p < 0.05$).

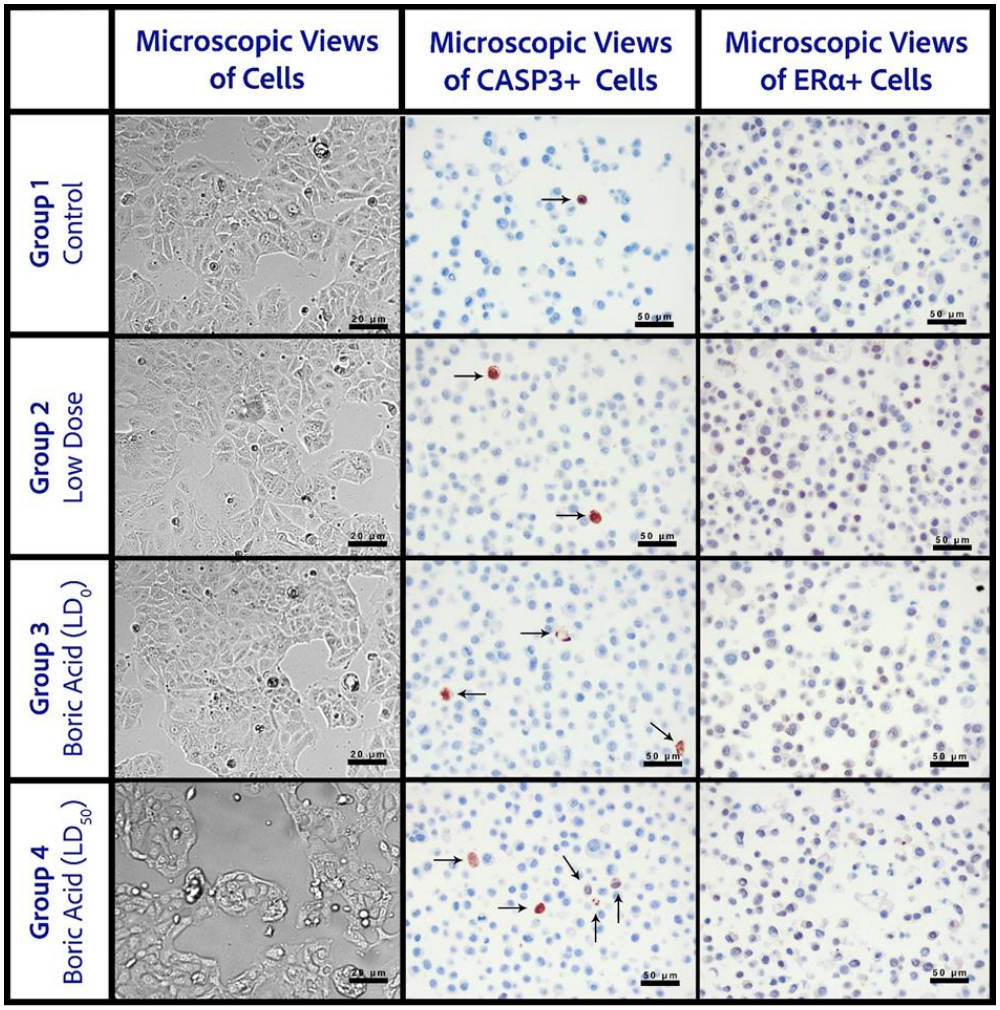

Figure 3.

Microscopic images of MCF-7 cells. A microscopic image of a slide used in the analysis of the experimental groups is included. Arrows (→) indicate ERα+ and Caspase 3+ cells

While this study provides valuable insights into the cytotoxic and apoptotic effects of BA on cancer cell lines, certain limitations must be acknowledged. Primarily, the calculated IC_{50}-equivalent concentration of BA (~101 mM) is notably higher than the standard physiological range. At such elevated millimolar concentrations, it is difficult to definitively exclude the possibility of non-specific cytotoxic artefacts,

such as osmotic imbalance, culture medium acidification, or generalised metabolic stress. Although comparable high concentrations have been utilized in previous *in vitro* models (29) to evaluate cellular responses, these physicochemical alterations may confound the precise interpretation of the targeted anticancer mechanisms. Therefore, future *in vivo* and *in vitro* investigations should incorporate rigorous monitoring of medium pH and osmolarity, and critically focus on expanding the dose-response analysis to include lower concentrations within a physiologically relevant range to validate the specific molecular pathways.

Conclusions

There is a need for non-toxic, non-resistant, effective agents due to their side effects in all types of cancer. In this study, the anticarcinogenic effects of different doses of BA on MCF-7 cells and the mechanisms underlying these effects were determined. It was observed that high-dose BA application increased oxidative stress and caused genotoxicity. While low-dose BA application did not have an effect on apoptotic and proapoptotic gene expressions in MCF-7 cells, it was determined that the IC_0 dose suppressed the *Bcl-2*, *p53*, and *TRAIL2* genes, the IC_{50} dose suppressed the *Bax* gene, and the ERα+ and Caspase 3+ cell numbers in all groups were higher than the control. This situation shows that the cell death signal may occur through DNA damage, oxidative stress, or extrinsic pathways. It is thought that apoptosis occurs through the activation of cytochrome C, which is released from the mitochondria into the cytoplasm, and Caspase 3. In this study, it was determined how the anticarcinogenic effects of the three different BA doses used varied in MCF-7 cells, and that low doses of BA had no cytotoxic effects.

Acknowledgement

Not applicable.

Funding

This work is supported by the Scientific Research Project Fund of Afyon Kocatepe University (AKU-BAP) under the Project number 17.KARIYER.108.

Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Ethics approval and consent to participate

Not applicable.

AI use disclosure

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

References

1. Cabus U, Secme M, Kabukcu C, Cil N, Dodurga Y, Mete G, et al. Boric acid as a promising agent in the treatment of ovarian cancer: Molecular mechanisms. Gene. 2021;796-797:145799.
2. Geyikoglu F, Koc K, Colak S, Erol HS, Cerig S, Yardimci BK, et al. Propolis and its combination with boric acid protect against ischemia/reperfusion-induced acute kidney injury by inhibiting oxidative stress, inflammation, DNA damage, and apoptosis in rats. Biol Trace Elem Res. 2019;192(2):214-221.
3. Timoshnikov VA, Slepneva IA, Chinak OA, Selyutina OY, Polyakov NE. Cytotoxic and ROS generation activity of anthraquinones chelate complexes with metal ions. Biometals. 2024;37(6):1643-1656.
4. Yildirim C, Cevik S, Yamaner H, Orkmez M, Eronat O, Bozdayı MA, et al. Boric acid improves the behavioral, electrophysiological and histological parameters of cisplatin-induced peripheral neuropathy in rats. J Trace Elem Med Biol. 2022;70:126917.
5. Barranco WT, Eckhert CD. Cellular changes in boric acid-treated DU-145 prostate cancer cells. Br J Cancer. 2006;94(6):884-890.
6. Korkmaz M, Uzgören E, Bakırdere S, Aydin F, Ataman OY. Effects of dietary boron on cervical cytopathology and on micronucleus frequency in exfoliated buccal cells. Environ Toxicol. 2007;22(1):17-25.
7. Mahabir S, Spitz MR, Barrera SL, Dong YQ, Eastham C, Forman MR. Dietary boron and hormone replacement therapy as risk factors for lung cancer in women. Am J Epidemiol. 2008;167(9):1070-1080.
8. Łukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast cancer—epidemiology, risk factors, classification, prognostic markers, and current treatment strategies—an updated review. Cancers (Basel). 2021;13(17):4287.
9. Morrow M, White J, Moughan J, Owen J, Pajack T, Sylvester J, et al. Factors predicting the use of breast-conserving therapy in Stage I and II breast carcinoma. J Clin Oncol. 2001;19(8):2254-2262.
10. Rouzier R, Perou CM, Symmans WF, Ibrahim N, Cristofanilli M, Anderson K, et al. Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res. 2005;11(16):5678-5685.
11. Yang TJ, Ho AY. Radiation therapy in the management of breast cancer. Surg Clin North Am. 2013;93(2):455-471.
12. Lumachi F, Luisetto G, Basso SM, Basso U, Brunello A, Camozzi V. Endocrine therapy of breast cancer. Curr Med Chem. 2011;18(4):513-522.
13. Yurtalan N, Geyikçi F, Uğuz G. Effect of boric acid addition on antibacterial dye production. J Turk Chem Soc Sect B: Chem Eng. 2024;7(2):175-182.
14. Hazman Ö, Sarıova A, Bozkurt MF, Ciğerci İH. The anticarcinogen activity of β-arbutin on MCF-7 cells: Stimulation of apoptosis through estrogen receptor-α signal pathway, inflammation and genotoxicity. Mol Cell Biochem. 2021;476(1):349-360.

15. Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-254.
16. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem. 2004;37(2):112-119.
17. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005;38(12):1103-1111.
18. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res. 1988;175(1):184-191.
19. Stopper H, Müller SO. Micronuclei as a biological endpoint for genotoxicity: A minireview. Toxicol In Vitro. 1997;11(5): 661-667.
20. Fenech M. The *in vitro* micronucleus technique. Mutat Res. 2000;455(1-2):81-95.
21. Günay E, Celik S, Sarinc-Ulasli S, Özyürek A, Hazman Ö, Günay S, et al. Comparison of the anti-inflammatory effects of proanthocyanidin, quercetin, and damnacanthal on benzo(a)pyrene exposed A549 alveolar cell line. Inflammation. 2016;39(2):744-751.
22. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001;29(9):e45.
23. El-Gamal R, Elfarrash S, El-Nablaway M, Salem AA, Zaraei SO, Anbar HS, et al. Anti-proliferative activity of RIHMS-Qi-23 against MCF-7 breast cancer cell line is through inhibition of cell proliferation and senescence but not inhibition of targeted kinases. BMC Cancer. 2023;23(1):1053.
24. Sevimli TS, Ghorbani A, Gakhiyeva F, Cevizlidere BD, Sevimli M. Boric acid alters the expression of dna double break repair genes in MCF-7-derived breast cancer stem cells. Biol Trace Elem Res. 2024;202(9):3980-3987.
25. Kahraman E, Göker E. Boric acid exert anti-cancer effect in poorly differentiated hepatocellular carcinoma cells *via* inhibition of AKT signaling pathway. J Trace Elem Med Biol. 2022;73:127043.
26. Canturk Z, Tunalı Y, Korkmaz S, Gulbaş Z. Cytotoxic and apoptotic effects of boron compounds on leukemia cell line. Cytotechnology. 2016;68(1):87-93.
27. Lu L, Zhang Q, Ren M, Jin E, Hu Q, Zhao C, et al. Effects of boron on cytotoxicity, apoptosis, and cell cycle of cultured rat sertoli cells *in vitro*. Biol Trace Elem Res. 2020;196(1):223-230.
28. Bradke TM, Hall C, Carper SW, Plopper GE. Phenylboronic acid selectively inhibits human prostate and breast cancer cell migration and decreases viability. Cell Adh Migr. 2008;2(3):153-160.
29. Acerbo AS, Miller LM. Assessment of the chemical changes induced in human melanoma cells by boric acid treatment using infrared imaging. Analyst. 2009;134(8):1669-1674.
30. Sevimli M, Bayram D, Özgöçmen M, Armağan I, Semerci Sevimli T. Boric acid suppresses cell proliferation by TNF signaling pathway mediated apoptosis in SW-480 human colon cancer line. J Trace Elem Med Biol. 2022;71:126958.
31. Çil N, Önder E, Damar AN, Tabatabaei S, Çabuş Ü, Mete GA. *In vitro* cytotoxic and apoptotic effects of boric acid on endometrial adenocarcinoma cell lines (HEC-1B and Ischikawa). Med Oncol. 2025;42(3):79.
32. Aksoy L, Alper Y. The effects of royal jelly on oxidative stress and toxicity in tissues induced by malathion, an organophosphate insecticide. J Hellenic Vet Med Soc. 2019;70(2):1517-1524.
33. Aguiar PHN, Furtado C, Repolês BM, Ribeiro GA, Mendes IC, Peloso EF, et al. Oxidative stress and DNA lesions: The role of 8-oxoguanine lesions in trypanosoma cruzi cell viability. PLoS Negl Trop Dis. 2013;7(6):e2279.
34. Ruan C, Liu L, Wang Q, Chen X, Chen Q, Lu Y, et al. Reactive oxygen species-biodegradable gene carrier for the targeting therapy of breast cancer. ACS Appl Mater Interfaces. 2018;10(12):10398-10408.
35. Sogut I, Paltun SO, Tuncdemir M, Ersoz M, Hurdag C. The antioxidant and antiapoptotic effect of boric acid on hepatoxicity in chronic alcohol-fed rats. Can J Physiol Pharmacol. 2018;96(4):404-411.
36. Türkez H, Geyikoğlu F, Tatar A, Keleş S, Ozkan A. Effects of Some Boron Compounds on Peripheral Human Blood. Z Naturforsch C J Biosci. 2007;62(11-12):889-896.
37. İnce S, Kucukkurt I, Cigerci IH, Fidan AF, Eryavuz A. The effects of dietary boric acid and borax supplementation on lipid peroxidation, antioxidant activity, and DNA damage in rats. J Trace Elem Med Biol. 2010;24(3):161-164.
38. Cikler-Dulger E, Sogut I. Investigation of the protective effects of boric acid on ethanol induced kidney injury. Biotech Histochem. 2020;95(3):186-193.
39. Ergul AB, Kara M, Karakukcu C, Tasdemir A, Aslaner H, Ergul MA, et al. High doses of boron have no protective effect against nephrolithiasis or oxidative stress in a rat model. Biol Trace Elem Res. 2018;186(1):218-225.
40. Güzel Y, Golge UH, Goksel F, Vural A, Akcay M, Elmas S, et al. The efficacy of boric acid used to treat experimental osteomyelitis caused by methicillin-resistant *Staphylococcus aureus*: an *In Vivo* Study. Biol Trace Elem Res. 2016;173(2):384-389.
41. Sancar A, Lindsey-Boltz LA, Ünsal-Kaçmaz K, Linn S. Molecular mechanisms of mammalian dna repair and the dna damage checkpoints. Annu Rev Biochem. 2004;73:39-85.
42. Goldstein M, Kastan MB. The DNA damage response: Implications for tumor responses to radiation and chemotherapy. Annu Rev Med. 2015;66:129-143.
43. Collins AR. The comet assay for DNA damage and repair: Principles, applications, and limitations. Mol Biotechnol. 2004;26(3):249-261.
44. Green MH, Lowe JE, Delaney CA, Green IC. Comet assay to detect nitric oxide-dependent DNA damage in mammalian cells. Methods Enzymol. 1996;269:243-266.
45. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, et al. Single cell gel/comet assay: Guidelines for *in vitro* and *in vivo* genetic toxicology testing. Environ Mol Mutagen. 2000;35(3):206-221.
46. Sommer S, Buraczewska I, Kruszewski M. Micronucleus assay: The state of art, and future directions. Int J Mol Sci. 2020;21(4):1534.

47. Riaz M, Van Jaarsveld MT, Hollestelle A, Smissen WJC, Heine AAJ, Boersma AW, et al. miRNA expression profiling of 51 human breast cancer cell lines reveals subtype and driver mutation-specific miRNAs. Breast Cancer Res. 2013;15(2):R33.
48. Dai X, Cheng H, Bai Z, Li J. Breast cancer cell line classification and its relevance with breast tumor subtyping. J Cancer. 2017;8(16):3131-3141.
49. Jürgensmeier JM, Xie Z, Deveraux Q, Bredesen D, Reed JC. *Bax* directly induces release of cytochrome c from isolated mitochondria. Proc Natl Acad Sci USA. 1998;95(9):4997-5002.
50. Moll UM, Zaika A. Nuclear and mitochondrial apoptotic pathways of p53. FEBS Lett. 2001;493(2-3):65-69.
51. Botos J, Barhoumi R, Burghardt R, Kochevar DT. Rb localization and phosphorylation kinetics correlate with the cellular phenotype of cultured breast adenocarcinoma cells. In Vitro Cell Dev Biol Anim. 2002;38(4):235-241.
52. Mustafa M, Ahmad R, Tantry IQ, Ahmad W, Siddiqui S, Alam M, et al. Apoptosis: A comprehensive overview of signaling pathways, morphological changes, and physiological significance and therapeutic implications. Cells. 2024;13(22):1838.
53. Sorimachi H, Ishiura S, Suzuki K. Structure and physiological function of calpains. Biochem J. 1997;328(Pt 3):721-732.
54. Johnson DE. Noncaspase proteases in apoptosis. Leukemia. 2000;14(9):1695-1703.
55. Mooney LM, Al-Sakkaf KA, Brown BL, Dobson PR. Apoptotic mechanisms in T47D and MCF-7 human breast cancer cells. Br J Cancer. 2002;87(8):909-917.
56. Park IJ, Kim MJ, Park OJ, Choe W, Kang I, Kim SS, et al. Cryptotanshinone induces ER stress-mediated apoptosis in HepG2 and MCF7 cells. Apoptosis. 2012;17(3):248-257.
57. Jan R, Chaudhry GE. Understanding apoptosis and apoptotic pathways targeted cancer therapeutics. Adv Pharm Bull. 2019;9(2):205-218.
58. Darwati D, Safitri AN, Ambardhani N, Mayanti T, Nurlelasari N, Kurnia D. Effectiveness and anticancer activity of a novel phenolic compound from *garcinia porrecta* against the MCF-7 breast cancer cell line *in vitro* and *in silico*. Drug Des Devel Ther. 2021;15:3523-3533.
59. Gompel A, Somaï S, Chaouat M, Kazem A, Kloosterboer HJ, Beusman I, et al. Hormonal regulation of apoptosis in breast cells and tissues. Steroids. 2000;65(10-11):593-598.
60. Bean LA, Ianov L, Foster TC. Estrogen Receptors, the hippocampus, and memory. Neuroscientist. 2014;20(5):534-545.
61. Wang Y, Zhao Y, Chen X. Experimental study on the estrogen-like effect of boric acid. Biol Trace Elem Res. 2008;121(2):160-170.
62. Liao X, Lu D, Wang N, Liu L, Wang Y, Li Y, et al. Estrogen receptor α mediates proliferation of breast cancer MCF–7 cells *via* a p21/PCNA/E2F1-dependent pathway. FEBS J. 2014;281(3):927-942.
63. Tian T. MCF-7 cells lack the expression of Caspase-3. Int J Biol Macromol. 2023;231:123310.
64. He C, Chen J, Tian R, Yang L, He W, He L, et al. Deubiquitinating enzyme USP42 promotes breast cancer progression by inhibiting JNK/p38-mediated apoptosis. Sci Rep. 2025;15(1):36751.
65. Kurokawa H, Nishio K, Fukumoto H, Tomonari A, Suzuki T, Saijo N. Alteration of *caspase-3* (CPP32/Yama/apopain) in wild-type MCF-7, breast cancer cells. Oncol Rep. 1999;6(1):33-37.
66. Arslan-Acaroz D, Bayşu-Sozbilir N. Ameliorative effect of boric acid against formaldehyde-induced oxidative stress in A549 cell lines. Environ Sci Pollut Res Int. 2020;27(4):4067-4074.
67. Selvakumar K, Krishnamoorthy G, Venkataraman P, Arunakaran J. Reactive oxygen species induced oxidative stress, neuronal apoptosis and alternative death pathways. Adv Biosci Biotechnol. 2013;4(1):14-21.
68. Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta. 2016;1863(12):2977-2992.