Biological Trace Element Research
https://doi.org/10.1007/s12011-021-02647-9

ORIGINAL ARTICLE

Therapeutic Effects of Newly Synthesized Boron Compounds (BGM and BGD) on Hepatocellular Carcinoma

Meliha Koldemir Gündüz[1] · Melda Bolat[2] · Güllü Kaymak[3] · Derya Berikten[1] · Dursun Ali Köse[4]

Received: 14 January 2021 / Accepted: 17 February 2021
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC part of Springer Nature 2021

Abstract

Boron has an important potential for facilitating biological activity and for use in pharmaceutical drug design. Boron glycine monoester (BGM) and boron glycine diester (BGD) compounds containing boron atoms were synthesized and investigated their cytotoxic, oxidative stress, and antimicrobial activities on the HepG2 cancer cell line. The cytotoxic activity of newly synthesized boron compounds on hepatocellular carcinoma was determined by the MTT method for 48 h. Antioxidant (CAT, GSH), lipid peroxidation (MDA), and enzyme activity (ACP, ALP) analyses were determined by spectrophotometric methods in HepG2 cells. Antimicrobial activity was determined by the disk diffusion method. After 48 h of BGM and BGD application to HepG2 cells, we found the IC_{50} values as 9.9 mM and 24 mM, respectively. While CAT and ACP enzyme activities decreased in all groups compared to the control, ALP enzyme activity did not change in the BGM group but increased in the BGD group. It was determined that the GSH level did not change in all groups, while the MDA level increased. It has been stated that these IC_{50} doses of BGM and BGD have antibacterial effects on *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922. Newly synthesized boron compounds, particularly BGM, with their cytotoxic, oxidative stress, and antimicrobial effects, could provide a new therapeutic approach for the treatment of hepatocellular carcinoma.

Keywords Boron glycine monoester · Boron glycine diester · HepG2 · Oxidative stress · Antimicrobial

Introduction

Hepatocellular carcinoma is the fourth leading cause of death among cancer types worldwide. Globally, the incidence and mortality rate of liver cancer, which ranks sixth in cancer incidence, vary by region [1]. While the incidence is low in North America and Europe, the incidence of death is very high in Japan and China. The difference in the incidence of death by region is due to changes in exposure to risk factors, including chronic hepatitis C or hepatitis B virus infection, heavy alcohol consumption, diabetes, obesity, and nonalcoholic fatty liver disease (NAFLD) [2, 3]. With the treatment approaches applied after the cancer diagnosis, the life span is limited to a maximum of 5 years. Since procedures such as resection and liver transplantation cannot be applied in the treatment of hepatocellular carcinoma, chemotherapy has special importance [4, 5]. As the most widely used chemotherapeutic drugs are known to cause side effects, there is a need for the development of effective and safe agents for the prevention and treatment of hepatocellular carcinoma.

Reactive oxygen species (ROS) are produced by cellular enzymes or as a result of a decrease in single or multi-electron oxygen in the mitochondrial airway. Although the increase in intracellular ROS level causes oxidative stress and DNA damage, the effects of ROS are generally balanced by enzymatic (catalase (CAT), superoxide dismutase (SOD)) or non-enzymatic antioxidants (glutathione (GSH), ascorbic acid, uric acid). Oxidative stress inducibility can be determined by directly measuring intracellular ROS, or indirectly measuring damage to biomolecules, including proteins, DNA, RNA, and lipids, or by measuring antioxidant levels. When it was discovered in 1981 that insulin increases intracellular H_2O_2

✉ Meliha Koldemir Gündüz
meliha.koldemirgunduz@ksbu.edu.tr

[1] Training and Research Center, Kütahya Health Sciences University, Kütahya, Turkey

[2] Department of Property Protection and Safety, Technical Sciences Vocational School, Hitit University, Çorum, Turkey

[3] Simav Vocational School of Health Services, Kütahya Health Sciences University, Kütahya, Turkey

[4] Department of Chemistry, Faculty of Arts and Sciences, Hitit University, Çorum, Turkey

Published online: 26 February 2021

levels and increases tumor cell proliferation, the role of ROS in cancer cells is still not fully proven, although it is thought that there may be a link between ROS and cellular transformation. Cancer cells use large amounts of ATP as "fuel" for uncontrolled proliferation [6]. ROS accumulation, which increases with this uncontrolled energy use, must be prevented by counter mechanisms to ensure cell survival. Cancer cells have higher ROS levels than normal cells due to glycolytic metabolic adaptations and oncogenic transformations that accelerate metabolism [7]. The high ROS level in tumor cells makes the cells more susceptible to the harmful effects of increased oxidative stress caused by drug therapy [8]. Determining ROS production in different conditions in cancer cells will help to evaluate the conditions under which ROS are oncogenic and tumor suppressor.

Boron is one of the rare elements with biological functions in living things [9]. Nutritional boron is mainly obtained from plant-based boron chemicals and boric acid dissolved in water [10–12]. It has been shown that boron is essential as a trace element in the life cycle of some higher animals, and plays an important role in the proliferation and development of cells. Low levels of dietary boron impair bone health, brain function, plasma lipid profiles, and immune response [11, 13, 14]. In animal studies, boron has also been found to have a protective effect against liver injury [12, 15, 16]. Recent studies with boron are generally in the direction of investigating the anticancer activity, antimicrobial effect, drug administration, and protein-biomolecular interactions of newly synthesized molecules containing boron [17–21]. In animal studies, it has been observed that the boron taken into the body is hydrolyzed into boric acid in the small intestine, and most of it is rapidly excreted in the urine before being metabolized. A small amount may be involved in body tissues such as bone and spleen [22–24]. Boron is often found in supplements such as vitamin D, calcium, magnesium, soy isoflavones, chondroitin sulfate, glucosamine, curcumin, boswellia, gelatin, and ipriflavone, which combine various nutrients. The known boron supplement forms (boric acid, borax, etc.) began to be questioned after a while due to reasons such as accumulation in tissues instead of bone marrow. Thereupon, boron esters were synthesized as an upper form of boron. Little is known about boron esters. Recent studies are trying to overcome this deficiency in the literature [25–28].

It has been determined that physiologically active boron has the following properties [29]:

(i) a cell-signaling molecule; (ii) the co-factor of the enzymes it regulates; (iii) a non-enzymatic co-factor; (iv) both structural and functional roles, including electron transfer, redox sensing, and structural modules; and (v) a role in the cytoskeletal structure.

Although boron has not yet been shown to be an important nutrient in animal cells, more data will likely support such a role in the future. It has been suggested that the complexation of borate via organic *cis*-diol groups is the most likely chemical mechanism for this element to participate in the evolution of the living world [9].

Calcium fructoborate (Ca-FB) is one of the first boron ester compounds isolated from natural resources of plants and whose structural properties have been illuminated [30]. In plant metabolism, the transport of boron (B) to cells is facilitated by passive diffusion (via NIPs protein) facilitated through the channel through membranes and outflow through energy-dependent active transport (via BORs protein) [31, 32]. With regard to animal metabolism, a specific boron transporter has not been precisely defined, although such a transporter has previously been claimed. It has been shown in previous studies that the claimed boron carrier NaBC1 pumps [33, 34] actually carry NH_3 and not B [35]. It is highly probable that the transport of boron ester compounds (BEC) across the cell membrane by free diffusion and an aquaporin-like protein carrier for boric acid (BA) is highly probable [36, 37]. According to the available literature, the actual BA and BEC transport mechanisms in animal and human cells are still not fully elucidated [38]. Fructoborates and glucoborates, which are herbal BEC complexes, can be transported via active sugar transporters, because these carriers have low permeability compared to boron compounds that do not contain chelate ester bonds [39, 40]. However, the question of whether the boron ester compounds fructoborate and glucoborates are bioavailable as intact molecules or in fragments (for example, sugar-borate or boric acid and sugar) remains unanswered [41, 42]. Therefore, the positive effects of boron in cells can be highlighted and increased. It is absolutely necessary to obtain complex structures with -C-O-B-O- ester bond formed by esterification reaction similar to that of Ca-FB structure. Compared to boric acid (BA), boron-glycine monoester (BGM) and boron-glycine diester molecules synthesized by us are transported to the cells, and the degree of hydrolysis in the aqueous environment will be decisive in the comparison of BGM and BGD molecules. The hydrolysis of the diester-like BGD molecules will be slower, so their activities will be higher compared to BGM molecules. However, the amount of boron carried by the mass is at a lower level compared to BGM molecules will remain. As the rapid occurrence of hydrolysis will increase the formation of tetrahydroxy borate [$B(OH)_4^-$] anion, it will provide a mechanism similar to the decomposition product of boric acid. Therefore, hydrolysis can be seen as the determining factor for the activation of boron ester compounds [25, 43].

The HepG2 cell line is highly valuable for screening cytotoxicity in the early stages of pharmaceutical studies. Some compounds that produce reactive and toxic metabolites are classified as toxic in HepG2 cells [44]. In our study, boron glycine monoester (BGM) and boron glycine diester (BGD) molecules were synthesized and investigated for the treatment of hepatocellular carcinoma. This study aims to investigate the

anticancer, antioxidant, and antimicrobial effects of newly synthesized boron compounds on HepG2 cells.

Materials and Methods

Synthesis of Boron Derivative Compounds

Glycine (Sigma-Aldrich) was dissolved in a mixture of distilled water and methanol (Sigma-Aldrich). Solid H_3BO_3 (Sigma-Aldrich) was added to the mixture, and the solution was stirred on a magnetic stirrer for about 1 h at room temperature; some of the solvents were removed in the evaporator until the density of the solution was thick and left to crystallize at room temperature. The product obtained was dried in a vacuum oven at 50 °C. Then it was characterized by using elemental analysis, FT-IR spectroscopy, melting point determination, thermal analysis methods [45].

HepG2 Cell Culture

HepG2 (ATCC® HB-8065 ™) hepatocellular carcinoma cell line to be used in the study was commercially available from the American Type Culture Collection (ATCC) (Manassas, USA). Before starting the cell culture study, the medium containing EMEM (Eagle's Minimum Essential Medium; ATCC, USA) + 10% fetal bovine serum (FBS; ATCC, USA) + penicillin/streptomycin (100 μg/ml; Gibco, US) was prepared in a UV cabinet under sterile conditions.

Application of Boron Compounds to HepG2 Hepatocellular Carcinoma Cells

BGM and BGD solutions were prepared mechanically in the medium. Boron compounds of 50 mM, 25 mM, 10 mM, 1 mM, and 500 μM were added to HepG2 hepatocellular carcinoma cells, and the cells were incubated for 48 h. Only the culture medium was added to the control cells.

Application of MTT Test to HepG2 Hepatocellular Carcinoma Cells

By using the MTT [3- (4,5-dimethyldiazol-2-yl) -2,5 diphenyl tetrazolium bromide] method, the proportion of living cells in the cell population was determined colorimetrically. The test was carried out according to the principle of transformation of the yellow MTT dye, which is released by the disintegration of the tetrazolium ring, into a dark blue-purple formazan product. Cells grown in flasks were inoculated in 96-well microplates as 10,000 cells/200 μl medium 24 h before the application and left for incubation. Different doses of boron compounds were applied to the cells. MTT analysis was performed according to the method of Yerlikaya et al. [46]. Data was analyzed with the GraphPad Prism 5.0 program (GraphPad Software, Inc., La Jolla, CA, USA) and a graph was generated. To calculate the IC50 value, the data were normalized by nonlinear regression analysis using the GraphPad Prism 5.0 program.

Cell Viability Analysis

Viability rates of cells after BGM and BGD treatment were calculated compared to untreated control cells. The viability of untreated cells was considered 100%, and the viability percentages of cells were calculated as follows:

$$\%\text{Viability} : (\text{treated cell}/\text{untreated cell}) \times 100$$

Oxidative Stress Analysis in HepG2 Cells

HepG2 cells were trypsinized from the flask and homogenized with a homogenate buffer at the end of BGM and BGD applications. All analyses were done without breaking the cold chain. After being homogenized, it was centrifuged at 4 °C at 10,000 rpm for 20 min; then the pellet part was discarded and the supernatant was used in experiments.

Determination of Total Protein It is based on the principle of measuring the absorbance at 595 nm of the colored solutions formed as a result of binding the Coomassie Brilliant Blue (G-250) dye to proteins [47].

Determination of Malondialdehyde It is the spectrophotometric evaluation of the absorbance of the pinkish color formed as a result of the reaction between MDA, a lipid peroxidation product, and thiobarbituric acid (TBA) [48].

Determination of Total Glutathione The colored product formed as a result of the reaction of Ellman reagent, DTNB (5-5 ′dithiobis 1-2 nitrobenzoic acid), and sulfhydryl groups are evaluated spectrophotometrically [49].

Catalase Enzyme Activity Determination CAT enzyme catalyzes the conversion reaction of H_2O_2 to H_2O. This transformation can be followed by a decrease in absorbance at 240 nm. A decrease in absorbance in 1 min is an indicator of catalase activity [50].

Determination of Alkaline Phosphatase Enzyme Activity ALP enzyme hydrolyzes *p*-nitrophenyl phosphate used as a substrate to *p*-nitrophenol depending on the pH of the

environment. The absorbance given by the resulting product at 405 nm is evaluated spectrophotometrically [51].

Determination of Acid Phosphatase Enzyme Activity ACP enzyme hydrolyzes *p*-nitrophenyl phosphate used as a substrate to *p*-nitrophenol depending on the pH of the environment. The absorbance given by the resulting product at 405 nm is evaluated spectrophotometrically [51].

Antimicrobial Activity

BGM and BGD solutions were tested for in vitro growth inhibitory activities against Gram-positive bacteria *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212) and Gram-negative bacteria *Pseudomonas aeruginosa* (ATCC 277853), *Escherichia coli* (ATCC 25922), and *Candida parapsilopsis* (ATCC 22019) as yeast (obtained from Kütahya Health Sciences University Faculty of Medicine) which have pathogenic effects in humans. Vancomycin (30 μg) for bacteria and amphotericin-B (100U) for yeast were used as control drugs.

Disk diffusion testing was performed according to the Clinical and Laboratory Standards Institute [52]. Bacteria and yeast suspensions with turbidity adjusted to McFarland 0.5 (~ 108 cfu/ml) in physiological saline solution were inoculated on the Mueller-Hinton agar (Merck) surface for bacteria and on the Sabouraud dextrose agar (Conda) surface for yeast using the spread plate method. Then, sterile commercial blank disks (Oxoid, 6 mm in diameter) soaked with 15 μl (IC50 dose/disk) BGM and BGD solutions and control drug disks were placed on the agar surface. The test plates were incubated at 37 °C for 24 h for bacteria and 35 °C for 48 h for yeast. The zones formed after the incubation periods were measured and inhibition zone diameters greater than 7 mm were recorded. All analyses were carried out in triplicate.

Statistical Analysis

Cell viability MTT analysis was performed using GraphPad Prism 5 (GraphPad Software, Inc.). Statistical analysis of oxidative stress parameters was performed using the IBM SPSS Statistic 23 computer program. Study findings were expressed as mean ± standard error (SD). Comparisons between two groups are by parametric Student's *t*-test and nonparametric Mann-Whitney *U* test in unequal variances; comparisons between more than two groups are one-way ANOVA; Tukey's post hoc test was conducted to compare the significant difference between groups following ANOVA in statistically significant results. In all statistical comparisons, those with a significance level of less than $p < 0.05$ were considered significant.

Results

Synthesis of Glycine Boron Esters

Boric acid gives an esterification reaction with compounds containing more than one hydroxyl group (one of which is acidic) (*cis*-diol) or a carboxyl group in a sterical position that does not interfere with switchgear and forms tetrahedral anionic ester complex structures [53–59]. Partial esterification with these compounds can form mono-chelate (1:1 complex) or bis-chelate (1:2 complex) structures [60]. The complexation reaction is simply based on the esterification reaction between the –OH groups of the $B(OH)_4^-$ anion formed as a result of the dissolution of boric acid in the aqueous medium and the –OH groups at the *cis*-diol position of the organic ligand by eliminating water. Metal cations are used as counter-ions to stabilize these anionic ester structures due to the negative charge of the boron center [45, 61]. $NaHCO_3$ was used as the source of metal cation. First, the glycine ligand was converted into Na-glycinate salt with sodium bicarbonate (Scheme 1); The HCO_3^- anion released was converted into carbon dioxide and water. While preparing the monoester boron-glycine, boric acid was added to the Na-glycinate solution at a stoichiometric ratio of 1:1. According to the reaction in Scheme 2, one of the free electron pairs in the structure of carbonyl oxygen was given to the empty "p" orbital of the boron atom to form a coordination bond. The acidic oxygen group in the form of sodium salt enters the esterification reaction with one of the –OH groups of boric acid and causes $Na+$ cation and $–OH^-$ to be released in the aqueous environment. The basic $–OH^-$ anion in the environment caused a proton to separate from the $–NH_2$ group of the glycine ligand which remained acidic, and consequently, the double bond of the carbonyl group shifted onto the –NH group. The main reason for this can be shown by the reduction of electrons on the carbonyl oxygen that provides coordination by giving a pair of electrons to the boron atom. The diester structure is similar to the monoester structure, and the stoichiometrically added second glycine ligand gives the esterification reaction with the remaining two –OH groups of boric acid (Scheme 3). Then, cold acetone was added to the solutions of the ester compounds whose solvent was removed in the evaporator to precipitate them. The precipitate/crystals are immediately taken to the desiccator to prevent moisture absorption after vacuum filtration. Ultrapure water is used for boron ester solutions. Ultrapure water was left at boiling temperature for 2 h, and nitrogen gas was passed. The chemical composition analyses of the ester compounds are given in Table 1.

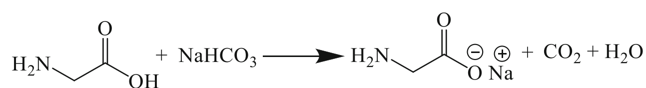
Since crystals suitable for single-crystal structure form analysis could not be obtained, the structural characterization was made by comparing the infrared spectrum and thermal analysis curves with the data of the crystal analyzed boron

Table 1 Elemental analysis results of boron-glycine ester compounds

Complexes	MW (g/mol)	Yield	Content (%) Exp. (Teo.)			Color	MP (°C)
			C	H	N		
$Na[B(OH)_2(C_2H_3NO_2)].H_2O$ $C_2H_5BNNaO_4$	140.86	68	17.57 (17.05)	4.02 (3.58)	9.81 (9.94)	White	137
$Na[B(C_2H_3NO_2)_2].H_2O$ $C_4H_8BN_2NaO_5$	179.90	74	23.92 (26.71)	4.32 (3.36)	14.03 (15.57)	White	205

esters available in the literature. Accordingly, the most important evidence supporting the formation of ester compounds is the peaks attributed to the $\nu_{asym}(BO)/BO_4$ & ν(C-O) ve $\nu_{sym}(BO)/BO_4$ stress vibrations observed in the infrared spectra. It was observed that the characteristic asymmetric asym $\nu_{asym}(BO)/BO_4$ stretch appearing in the 1220 cm^{-1} region in boric acid shifted to the 1273 cm^{-1} region in the monoester compound and to the 1266 cm^{-1} region in the diester compound. Again, shifts of the symmetrical $\nu_{asym}(BO)/BO_4$ stretch observed in the 834 + 815 cm^{-1} region in boric acid to 999 + 941 cm^{-1} regions in the monoester compound and 994 + 937 cm^{-1} regions in the diester compound were observed. It is thought that the flat and strong $–OH_{H2O}$ peak observed in the diester compound is due to the water remaining in the molecular cavities due to the sample not being fully dried. These other important peak values summarized in Table 2 are also compatible with the literature. As the proof of the transformation of the acidic $–NH_2$ group in the glycine ligand to –NH after esterification, it was determined that the twin –NH stretch peaks observed in 2891 and 2808 cm^{-1} regions in glycine shifted to the 2911 cm^{-1} region in ester compounds. In the diester molecule, the –NH stretch below the peak of the broad and strong aqua ligand was observed in the form of the shoulder (Fig. 1).

The details of the decomposition steps are summarized in Table 3 by recording the thermal analysis curves (TGA/DTA/DrTGA) of the boron-glycine ester compounds (Fig. 2). Molecules that were found to not contain hydrated water in their structures trapped moisture and water in their molecular cavities due to their inability to dry them completely. The first step belongs to the removal of water from this moisture. In the monoester compound, in the next step, two –OH groups attached to the boron atom are removed from the structure by one water elimination. It was determined that the glycine –NH group was removed before the dehydrated compound and



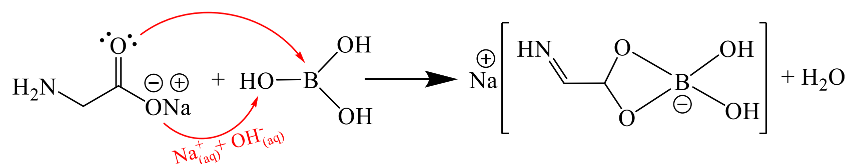
Scheme 1 Glycine ligand conversion into Na-glycinate salt with sodium bicarbonate

finally the organic residue was burned. In the diester compound, after the moisture water, two degradation steps attributed to the removal of –NH groups were observed. Then, thermal decomposition was completed with the burning of organic residue. Thermal decomposition of both ester molecules resulted in $NaBO_2$ residue remaining in the reaction vessel. The presence of the residual product of interest was identified by infrared spectroscopy. The harmony of the theoretical and experimental results of all decomposition steps was seen. The experimental amount of the final residue product is more than the theoretical amount is attributed to the thermal decomposition in an inert nitrogen atmosphere. Accordingly, due to the lack of sufficient oxygen, some carbonized carbon, which cannot be fully combusted, accumulates on the final residue product and causes the expected white color to turn black.

Cytotoxic Effects of BGM and BGD on HepG2 Hepatocellular Carcinoma Cells

Cells were cultured in a standard medium for 24 h before applying boron compounds to HepG2 cells. The cell culture medium was treated with control and BGM, BGD at concentrations of 50 mM, 25 mM, 10 mM, 1 mM, and 500 μM, and then incubated for 48 h. Only the culture medium was added to the control cells. As a result of the application of boron compounds, MTT test was performed on the cells. Statistical analysis of the results obtained with the MTT test was performed with the GraphPad Prism 5.0 program. According to the statistical analysis after the application of 50 mM, 25 mM, 10 mM, 1 mM, and 500 μM BGM to HepG2 cells for 48 h, the IC50 value of the boron-glycine monoester at the 48th hour was calculated as 9.9 mM. According to statistical analysis after 48 h of 50 mM, 25 mM, 10 mM, 1 mM, and 500 μM BGD applications to HepG2 cells, the IC50 value of boron-glycine diester at the 48th hour was calculated as 24 mM. This result shows that boron compounds have a cytotoxic effect on HepG2 cells.

In order to analyze the survival rate of HepG2 cells as a result of the application of different concentrations, 50 mM, 25 mM, 10 mM, 1 mM, and 500 μM BGM, BGD, and control



Scheme 2 A coordination bond

were applied to the cells grown until they reach the logarithmic phase in 96 microplates. Subsequently, a MTT cytotoxicity test was performed (Fig. 3). The cell viability was determined as 20%, 28%, 42%, 52%, and 73%, respectively, when 50 mM, 25 mM, 10 mM, 1 mM, and 500 µM BGM application was compared with the control. The 50 mM and 25 mM BGM were found to have a lethal effect on HepG2 cells. It was determined that 10 mM and 1 mM BGM application had an antiproliferative effect in HepG2 cells. The application of 500 µM BGM had no proliferative effects on HepG2 cells (Fig. 3). The cell viability was determined as 30%, 80%, 82%, 86%, and 88%, respectively, when 50 mM, 25 mM, 10 mM, 1 mM, and 500 µM BGD application results were compared with the control. While 50 mM BGD had a lethal effect on HepG2 cells, other doses were found to have no proliferative effects in HepG2 cells (Fig. 3). As a result of the application of glycine to the cells, it was determined that as the dose amount increased, the vitality rate increased.

Effects of BGM and BGD on Oxidative Stress Parameters in HepG2 Hepatocellular Carcinoma Cells

Some enzyme activities were investigated in hepatocellular carcinoma cells to determine the oxidative effect of newly synthesized boron compounds. The catalase enzyme is the best known among enzymatic antioxidants. ALP and ACP enzymes are widely found in nature. It exists in all species, from humans to bacteria. This indicates that these enzymes are important in biochemical reactions. Catalase (CAT) enzyme activity decreased in HepG2 cells where IC50 doses of both BGM and BGD were applied compared to the control ($p < 0.01$) (Fig. 4a). Acid phosphatase (ACP) enzyme activity was observed to be decreased in the groups in which both boron compounds were applied compared with the control ($p < 0.05$) (Fig. 4b). The decrease in alkaline phosphatase (ALP) enzyme activity in the BGM group was not statistically significant when compared to the control ($p > 0.05$). However, there is a statistically significant increase in the BGD group ($p < 0.05$) (Fig. 4c).

The amount of cellular glutathione is important in maintaining cellular functions and can vary in the case of detoxification and oxidative stress. The increase in total glutathione (GSH) in BGM and BGD groups was not statistically significant ($p > 0.05$) (Fig. 5a). The increase in the amount of malondialdehyde whose lipid peroxidation is a metabolite ($p < 0.05$) indicates an increase in lipid peroxidation in both groups (Fig. 5b).

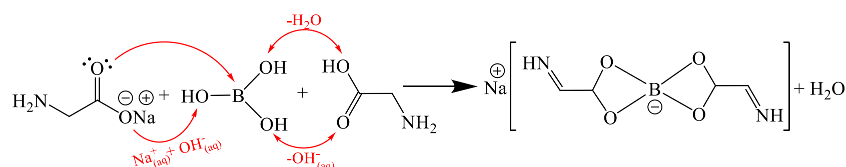
Antimicrobial Effect of BGM and BGD

Antimicrobial activity results determined by the disk diffusion method are given in Table 4. According to these results, it was determined that the IC50 dose (9.9 mM) of the boron-glycine monoester determined according to the MTT test results had an antibacterial effect against *S. aureus* ATCC 29213 (13 mm) and *E. coli* ATCC 25922 (16 mm). It was observed that BGM has no antifungal effect against *C. parapsilopsis* ATCC 22019 strain. Similarly, the IC50 dose (24 mM) determined according to the MTT test results of BGD was found to have an antibacterial effect against *S. aureus* ATCC 29213 (7 mm) and *E. coli* ATCC 25922 (11 mm), but it has no antifungal effect against *C. parapsilopsis* ATCC 22019 strain.

Discussion

Although there are many studies on cancer treatments today, the number of effective treatments is very low. Therefore, new drugs with low toxicity should be developed. Boron atoms interact with proteins by forming hydrogen and covalent bonds in living things and show antibacterial, antiviral, and anticancer properties [65]. Since cancer cells are constantly dividing, their metabolic activities are high and they need more energy than healthy cells. Boron compounds affect biochemical cycles that produce energy in cancer cells and cause less energy to be produced. Thus, the proliferation of cancer cells is negatively affected.

Tombuloglu et al. [13] in an in vitro study investigated the effects of a semi-maximal inhibitory concentration of boric



Scheme 3 Esterification reaction with the remaining two –OH groups of boric acid

Table 2 Some important infrared peaks of boron-glycine ester compounds.

Molecules	ν(O-H)	ν(N-H)	ν(C=O)	ν(COO)	ν_a(BO)/BO_4 and ν(C-O)	ν_s(BO)/BO_4
Glycine [62]	-	2891, 2808	1605	1498	- and 1385	-
Boric acid [63, 64]	~3300		-	-	1220 and -	834 + 815
BGM	3359	2911	-	1472	1108 and 1273	999 + 941
BGD	3382	~2912	-	1474	1102 and 1266	994 + 937

acid on HepG2 cells, increasing boric acid concentrations (0.5, 1, 5, 10, 15, 20, 25, 30, and 40 mM). They determined the IC50 value per hour as 24 mM. In their study, they reported that 5 mM and above boric acid application prevented growth on HepG2 cells and observed a gradual decrease in the viability of cells [13]. In a study investigating the effect of borax, which is known to inhibit the growth of tumor cells, on HepG2 cells, it was found that borax has an antiproliferative effect on cells [14]. Wei et al. [14] showed that borax reduced the proliferation of the HepG2 cell line in vitro depending on the dose (0.5, 1, 2, 4, 8, and 16 mM) and time (6, 12, 24, 48, and 72 h) [14]. Meiyanto et al. [66] investigated the effect of the new synthesized CCB-2 compound containing boron on different cancer cells. They determined the IC50 value of the new compound with boron in MCF-7/HER-2, MCF-7, RAW 264.7, and 4T1 cells of 12 µM, 54 µM, 26 µM, and <10 µM, respectively [66]. This result indicates that the development of CCB-2 compound as an anticancer agent is promising [66]. According to the statistical analysis performed after the administration of 50 mM, 25 mM, 10 mM, 1 mM, and 500 µM BGM and BGD compounds to HepG2 cells for 48 h, the IC50 value was calculated as 9.9 mM and 24 mM, respectively. In another study, it was shown that the application of 1 mM boron for 48 h to HL-60 cells reduced cell viability by 50% [67]. According to a study investigating the 24-h effect of boric acid on human prostate cancer cell DU-145, cell viability rates were 45.8%, 61%, 84.3%, 78.5, 84.4%, 89.0%, and 87.8%, respectively, in the application of 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, and 0.19 mM boric acid [68]. In our study, we determined the survival rate in 50 mM, 25 mM, 10 mM, 1 mM, and 500 µM BGM HepG2 cells as 20%, 28%, 42%, 52%, and 73%, respectively. Cell viability was determined as 30%, 80%, 82%, 86%, and 88%, respectively, after applying 50 mM, 25 mM, 10 mM, 1 mM, and 500 µM BGD to HepG2 cells. These results show the inhibitory effects of newly synthesized boron compounds in HepG2 cells. It shows that BGM in particular can serve as a candidate therapeutic agent for hepatocellular carcinoma.

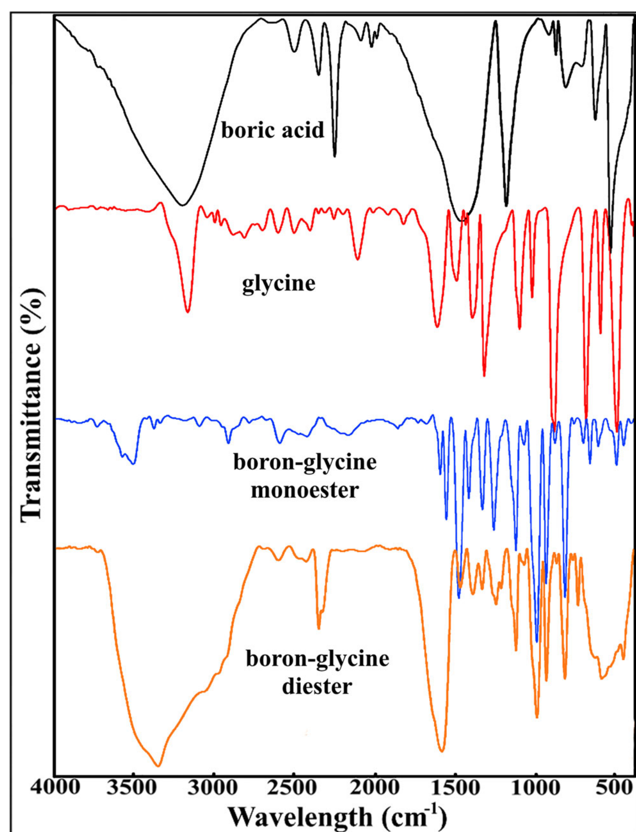


Fig. 1 FT-IR spectra of boron-glycine ester compounds

Because of its rapid metabolism and impaired cellular signaling mechanism, cancer cells have high ROS production. High ROS levels are generally detrimental to cells, and the redox status of cancer cells is different from that of normal cells. Cancer cells, therefore, maintain ROS levels above a low cytostatic level, but below levels that would be cytotoxic, at a moderately high tumorigenic level [6]. In the increase of ROS levels in cancer, the type of the produced radical, the region where the radical is located, and the local concentration are important. ROS-sensitive signaling pathways involved in cell growth/proliferation, differentiation, protein synthesis, glucose metabolism, cell survival, and inflammation in many types of cancer are also constantly increasing.

In cancer cells, higher than normal ROS production is balanced by an equal degree of antioxidant activity to maintain redox balance [7]. Catalase, one of the powerful antioxidants in the cell, is the primary enzymatic defense system. Hydrogen peroxide (H_2O_2) is metabolized by catalase into the water and molecular oxygen [6]. Catalase is a primary enzyme of the antioxidant system in defense against oxidative stress that occurs in different cancer types. Rajneesh et al. [69]

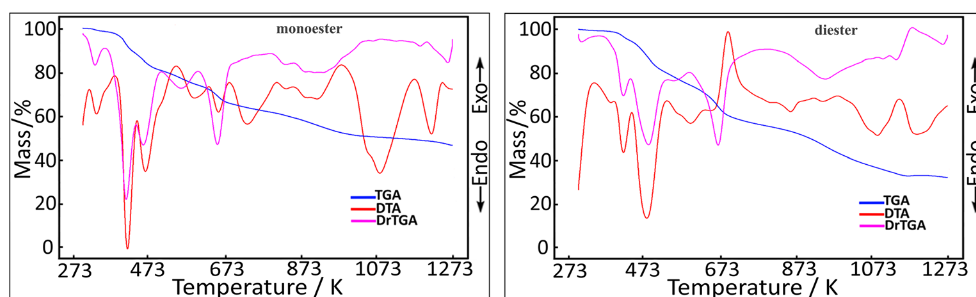


Fig. 2 TGA/DTA/DrTGA curves of boron-glycine ester compounds

found that catalase levels increase in cancer patients. Hacıoğlu et al. [68] reported that the catalase enzyme activity decreased in a dose-dependent manner in DU-145 human prostate cancer cells treated with boric acid. In another study, boric acid has been shown to reduce CAT activity in mouse TM3 Leydig cells [70]. In our study, it was found that catalase enzyme level decreased as a result of the treatment of hepatocellular carcinoma cells with boron-derived compounds. These findings show that the complexes have properties to increase intracellular and intercellular oxidative damage. This has shown us that boron compounds can be effective against cancer by inhibiting the antioxidant defense system of cancer cells.

Acid and alkaline phosphatase belong to the phosphomonoesterase enzyme family. Acid phosphatases are enzymes that transfer oxygen from water to inorganic phosphate in an acidic environment and catalyze the nonspecific hydrolysis of phosphate monoesters. Alkaline phosphatase (ALP) is an enzyme that catalyzes the hydrolysis of phosphate esters in an alkaline environment [71]. ALP is an enzyme responsible for the supply of phosphate groups required for the kinases involved in the cell cycle. These enzymes are used as a biological marker in studies on cancer cells with the impaired cell cycle. However, in this study, boron-glycine compounds did not change the ALP enzyme activity in hepatocellular carcinoma cells; ACP enzyme activity was reduced.

Kaynar et al. [72] examined antioxidant enzyme activities in cancer patients and found erythrocyte malondialdehyde, nitric oxide, total glutathione levels, and erythrocyte superoxide dismutase, catalase, and xanthine oxidase activities to be significantly higher than the control group. These studies have shown that there are significant changes in the antioxidant defense system caused by increased oxygen radicals in patients with cancer. The cancer cell has detoxified itself from ROS by increasing the level of antioxidant protein expression and ensures its safety. Thus, a delicate balance of intracellular ROS levels is considered necessary for the function of the cancer cell. For example, cancer cells can adapt to increased ROS levels by increasing intracellular antioxidants such as glutathione (GSH) and heme oxygenase-1 (HO-1) [73]. In another study, the GSH level did not change at low doses in Leydig cells, where boric acid was applied at increasing doses, but decreased at high doses [70]. In this study, no significant change was observed in the GSH level in hepatocellular carcinoma cells treated with BGM and BGD. GSH depletion or downregulation of GSH-related metabolic enzymes supports the restoration of active substance sensitivity in resistant cells. Therefore, the GSH antioxidant system plays an important role in the treatment of cancer. Given its potential as a target for the GSH antioxidant system, antitumor therapy, and reversal of drug resistance, it has recently become an attractive focus in cancer research [74, 75].

Lipid peroxidation disrupts the function of the membrane and cell. The liver is exposed to oxidative stress more than other tissues due to its high antioxidant enzyme activity [76]. This situation supports the claim that unsaturated fatty acids in the membrane structure are most affected by lipid

Table 3 The thermal decomposition data of boron-glycine ester compounds

Ester molecules		Temp. range (°C)	DTA_{max} (°C)	Removed group	Mass loss (%)		Remained product (%)		Decomp. product	Color
					Exp.	Calc.	Exp.	Calc.		
$Na[B(OH)_2(C_2H_3NO_2)]$	1	53–89	67	$H_2O_{(moisture)}$	1.18	-				White
BGM	2	95–174	149	H_2O	11.85	12.78				
$C_2H_2BNNaO_4$	3	176–275	195	NH	10.11	10.65				
	4	426–880	450; 641; 861	C_2H_2O	28.94	29.85	47.92	46.71	$NaBO_2$	Black
$Na[B(C_2H_3NO_2)_2]$	1	75–123	112	$H_2O_{(moisture)}$	0.80	-				White
BGD	2	125–236	145;207	2NH	15.97	16.68				
$C_4H_6BN_2NaO_4$	3	238–890	323; 587; 814	$2C_2H_2O$	45.97	46.74	37.26	36.58	$NaBO_2$	Black

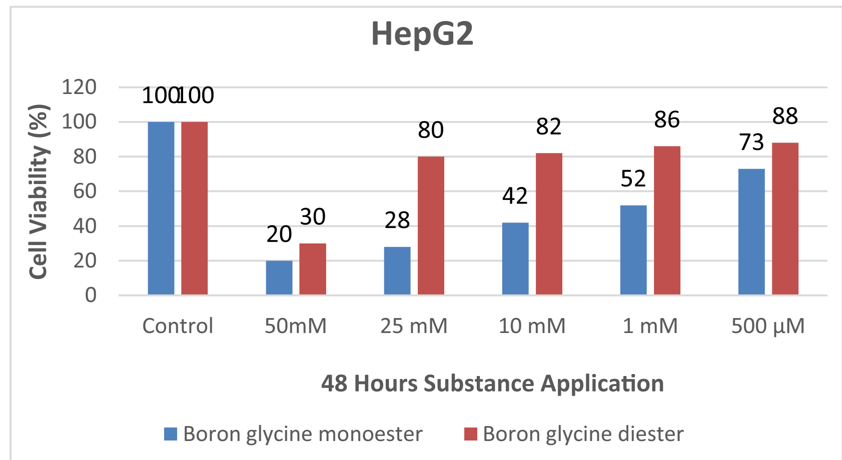


Fig. 3 Cytotoxic effect of different concentrations of boron compounds in HepG2 cells. After the treatment of HepG2 cells with 50 mM, 25 mM, 10 mM, 1 mM, and 500 µM boron-glycine monoester and boron-glycine diester for 48 h, the MTT test was performed

peroxidation. The measurement of MDA is most commonly done by the thiobarbituric acid (TBA) method [77]. Since all measured MDA is a secondary product of lipid peroxidation, this method is thought to provide a reliable measure of lipid peroxidation in terms of MDA equivalents [78]. It has been observed that catechin and resveratrol increase lipid peroxidation in rat C6 glioma cells [79]. In another study, it was reported that the level of MDA increased in prostate cancer cells treated with boric acid [68]. In this study, it was observed that the level of MDA in hepatocellular carcinoma cells treated with boron compounds was increased.

It is known that boron, boric acid, their salts, and compounds have a significant antimicrobial effect on fungal and bacterial infections. Various studies have shown that boron has an antimicrobial effect against *C. albicans* [80], *S. aureus*, *P. aeruginosa*, *E. coli*, *Acinetobacter septicus*, *Aeromonas hydrophila*, *Brucella melitensis*, *B. abortus*, *Vibrio anguillarum*, and *Lactococcus garvieae* [81–84].

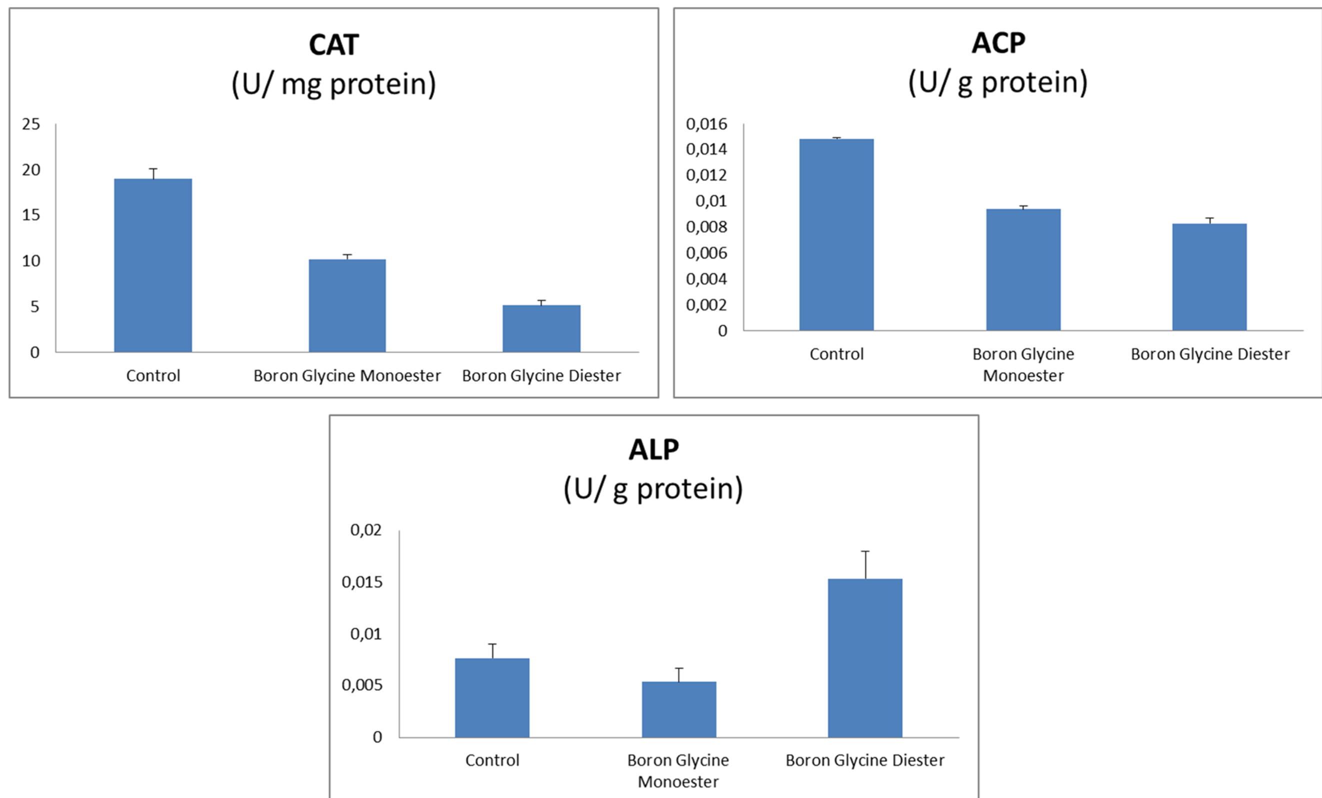


Fig. 4 Results of 48 h BGM (9.9 mM) and BGD (24 mM) in HepG2 cells with IC50 values applied. **a** Catalase (CAT) enzyme activity. **b** Acid phosphatase (ACP) enzyme activity. **c** Alkaline phosphatase (ALP) enzyme activity

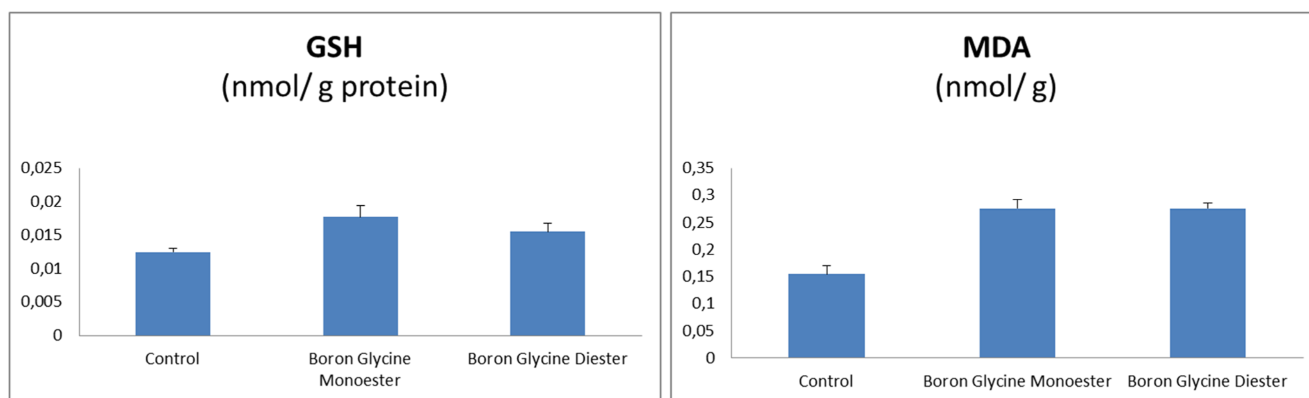


Fig. 5 Results of 48 h BGM (9.9 mM) and BGD (24 mM) in HepG2 cells with IC50 values applied. **a** Total glutathione (GSH) level. **b** Malondialdehyde (MDA) level

Table 4 Inhibition zone diameters of BGM and BGD solutions against test microorganisms (mm)

	Staphylococcus aureus ATCC 29213	*Enterococcus faecalis* ATCC 29212	*Pseudomonas aeruginosa* ATCC 277853	*Escherichia coli* ATCC 25922	*Candida parapsilopsis* ATCC 22019
	Inhibition zone (mm)				
BGM (9.9 mM)	13	0	0	16	0
BGD (24 mM)	7	0	0	11	0
Vancomycine/amphotericine-B	19	17	0	0	0

Also, boron compounds come to the fore with their antimicrobial effects in new drug development studies due to the increase in antibiotic-resistant strains. Since this situation is thought to affect other properties, the antimicrobial effects of boron-containing compounds have been tested in addition to the anticancer and antioxidant effects.

The disk diffusion method was used to evaluate in vitro antimicrobial activities. In the study, it was determined that BGM showed a higher antibacterial effect against test strains than BGD. BGM showed the highest antimicrobial activity against *E. coli* ATCC 25922, a gram-negative bacteria. The IC50 dose of both compounds had no antifungal effect on *C. parapsilopsis* ATCC 22019. Yılmaz [82] investigated the antimicrobial effects of boric acid and sodium tetraborate, which are boron compounds, and revealed that these compounds have antibacterial effects on *S. aureus* and *E. coli*, similar to our study. The BGM and BGD that we used could not show activity against *P. aeruginosa*, but boric acid and sodium tetraborate were shown to have antibacterial effects on *P. aeruginosa* [82].

Conclusions

Increased lipid peroxidation and changes in enzyme activities in HepG2 cells treated with growth inhibitory BGM and BGD concentration for 48 h clearly show that these substances are affected by the cancer cells undergoing oxidative stress. It was concluded that BGM and BGD against *S. aureus* and *E. coli*, which are important human pathogens, have high potential in terms of antibacterial activity; therefore, it would be beneficial to conduct more comprehensive studies involving different bacterial species. Further molecular studies can help to understand BGM and BGD effects and mechanisms.

Code Availability Not applicable

Author Contribution All authors contributed equally

Funding The authors received support from the National Boron Institute (BOREN) for the research project (2020-30-06-30-002).

Data Availability Not applicable

Declarations

Ethics Approval Not applicable

Conflict of Interest The authors declare no competing interests.

References

1. Hashem B, El-Serag (2020) Epidemiology of hepatocellular carcinoma. In: Arias IM, Alter HJ, Boyer JL, Cohen DE, Shafritz DA, Thorgeirsson SS, Wolkoff AW (eds) The liver: biology and pathobiology, 6th edn. John Wiley & Sons Ltd. Published, pp 758–772
2. McGlynn KA, Petrick JL, London WT (2015) Global epidemiology of hepatocellular carcinoma: an emphasis on demographic and regional variability. Clin Liver Dis 19(2):223–238. https://doi.org/10.1016/j.cld.2015.01.001
3. Singal AG, El-Serag HB (2015) Hepatocellular carcinoma from epidemiology to prevention: translating knowledge into practice. Clin Gastroenterol Hepatol 13(12):2140–2151. https://doi.org/10.1016/j.cgh.2015.08.014
4. Zender L, Spector MS, Xue W, Flemming P, Cordon-Cardo C, Silke J, Fan ST, Luk JM, Wigler M, Hannon GJ, Mu D, Lucito R, Powers S, Lowe SW (2006) Identification and validation of oncogenes in liver cancer using an integrative oncogenomic approach. Cell 125:1253–1267. https://doi.org/10.1016/j.cell.2006.05.030
5. Bruix J and Sherman M; American Association for the Study of Liver Diseases (2011) Management of hepatocellular carcinoma: an update. Hepatology 53:1020–1022. https://doi.org/10.1002/hep.24199
6. Halliwell B (2006) Oxidative stress and neurodegeneration: where are we now? J Neurochem 97(6):1634–1658. https://doi.org/10.1111/j.1471-4159.2006.03907.x
7. Çiftçi N (2017) Oksidatif Stresin Kanserdeki Rolü: Antioksidanlar Kanser Progresyonunun Yakıtı Olabilir mi? Ahi Evran Tıp Dergisi 1:8–13
8. Schumacker PT (2015) Reactive oxygen species in cancer: a dance with the devil. Cancer Cell 27(2):156–157. https://doi.org/10.1016/j.ccell.2015.01.007
9. Scorei R (2012) Is boron a prebiotic element? A mini-review of the essentiality of boron for the appearance of life on earth. Orig Life Evol Biosph 42:3–17. https://doi.org/10.1007/s11084-012-9269-2
10. Murray FJ (1998) A comparative review of the pharmacokinetics of boric acid in rodents and humans. Biol Trace Elem Res 66:331–341. https://doi.org/10.1007/BF02783146
11. Nielsen FH (2008) Is boron nutritionally relevant? Nutr Rev 66:183–191. https://doi.org/10.1111/j.1753-4887.2008.00023.x
12. Singh NP, Danner DB, Tice RR, Brant L, Schneider EL (1990) DNA damage and repair with age in individual human lymphocytes. Mutat. Res 237:123–130. https://doi.org/10.1016/0921-8734(90)90018-m
13. Tombuloglu A, Copoglu H, Aydin-Son Y, Guray NT (2020) *In vitro* effects of boric acid on human liver hepatoma cell line (HepG2) at the half-maximal inhibitory concentration. J Trace Elem Med Bio 62:126573. https://doi.org/10.1016/j.jtemb.2020.126573
14. Wei Y, Yuan FJ, Zhou WB, Wu L, Chen L, Wang JJ, Zhang YS (2016) Borax-induced apoptosis in HepG2 cells involves p53, Bcl-2, and Bax. Genet Mol Res 21:15(2). https://doi.org/10.4238/gmr.15028300
15. Eijssen LMT, Jaillard M, Adriaens ME, Gaj S, de Groot PJ, Müller M, Evelo CT (2013) User-friendly solutions for microarray quality control and pre-processing on ArrayAnalysis.oRg. Nucleic Acids Res:W71-6. https://doi.org/10.1093/nar/gkt293
16. Dai M, Wang P, Boyd AD, Kostov G, Athey B, Jones EG, Bunney WE, Myers RM, Speed TP, Akil H, Watson SJ, Meng F (2005) Evolving gene/transcript definitions significantly alter the interpretation of GeneChip data. Nucleic Acids Res 10;33(20):e175. https://doi.org/10.1093/nar/gni179
17. Axtell JC, Saleh LM, Qian EA, Wixtrom AI, Spokoyny AM (2018) Synthesis and applications of perfunctionalized boron clusters. Inorg Chem 57:2333–2350. https://doi.org/10.1021/acs.inorgchem.7b02912
18. Hawthorne MF, Maderna A (1999) Applications of radiolabeled boron clusters to the diagnosis and treatment of cancer. Chem Rev 99:3421–3434. https://doi.org/10.1021/cr980442h
19. Qian EA, Wixtrom AI, Axtell JC, Saebi A, Jung D, Rehak P, Han Y, Moully EH, Mosallaei D, Chow S (2017) Atomically precise organomimetic cluster nanomolecules assembled via perfluoroaryl-thiol S N Ar chemistry. Nat Chem 9:333–340. https://doi.org/10.1038/nchem.2686
20. Hawthorne MF (1993) The role of chemistry in the development of boron neutron capture therapy of cancer. Angew Chem Int Ed Engl 32:950–984
21. Aydin HE, Koldemir Gündüz M, Kizmazoğlu C, Kandemir T, Arslantaş A (2020) Cytotoxic effect of boron application on glioblastoma cells. Turk Neurosurg:1–5. https://doi.org/10.5137/1019-5149.JTN.30316-20.1
22. da Silva FJ, Williams RJ (1991) The biological chemistry of the elements: the ınorganic chemistry of life. Oxford University Press Oxford, UK, pp 58–63
23. Murray FJ (1995) A human health risk of boron (boric acid and borax) in drinking water. Regulatory Toxicology and Pharmacology 22:221–230. https://doi.org/10.1006/rtph.1995.0004
24. Hunt CD (1998) Regulation of enzymatic activity: one possible role of dietary boron in higher animals and humans. Biology Trace Elem Res 66:205–225. https://doi.org/10.1007/BF02783139
25. Köse DA, Zumreoglu-Karan B, Hökelek T (2011) A comparative examination of mono- and bis-chelate salicylatoborate complexes and the crystal structure of layered magnesium bis-salicylatoborate. Inorganica Chimica Acta 375:236–241. https://doi.org/10.1016/j.ica.2011.05.012
26. Köse DA, Zümreoglu-Karan B (2012) Mixed ligand complexes of boric acid with organic biomolecules. Chemical Papers 66(1):54–60. https://doi.org/10.2478/s11696-011-0108-0
27. Köse DA (2008) Preparation and structure investigation of biopotent boron compounds with hydroxy-functionalized organic molecules. PhD Thesis, Hacettepe University, Science Institute, Ankara
28. Zumreoglu-Karan B, Kose DA (2015) Boric acid: a simple molecule of physiologic, therapeutic and prebiotic significance. Pure and Applied Chemistry 87(2):155–162. https://doi.org/10.1515/pac-2014-0909
29. Köse DA, Karan-Zümreoğlu B (2009) Complexation of boric acid with vitamin C. N J Chem 33:1874–1881. https://doi.org/10.1039/B902812A
30. Miggiano GA, Gagliardi L (2005) Diet, nutrition and bone health. Clin Ter 156(1-2):47–56. https://doi.org/10.4103/jmsr.jmsr4118
31. Frommer WB, von Wirén N (2002) Plant biology: ping-pong with boron. Nature. 420:282–283. https://doi.org/10.1038/420282a
32. Miwa K, Fujiwara T (2010) Boron transport in plants: co-ordinated regulation of transporters. Ann. Bot. 105:1103–1108. https://doi.org/10.1093/aob/mcq044
33. Park M, Li Q, Shcheynikov N, Zeng W, Muallem S (2004) NaBC1 is a ubiquitous electrogenic Na+-coupled borate transporter essential for cellular boron homeostasis and cell growth and proliferation. Mol. Cell. 16:331–341. https://doi.org/10.1016/j.molcel.2004.09.030
34. Park M, Li Q, Shcheynikov N, Muallem S, Zeng W (2005) Borate transport and cell growth and proliferation: not only in plants. Cell Cycle. 4:24–26. https://doi.org/10.4161/cc.4.1.1394
35. Zhang W, Ogando DG, Bonanno JA, Obukhov AG (2015) Human SLC4A11 is a novel NH3:H+ co-transporter. J. Biol. Chem. 290:16894–16905. https://doi.org/10.1074/jbc.M114.627455

36. Zangi R, Filella M (2012) Transport routes of metalloids into and out of the cell: a review of the current knowledge. Chem. Biol. Interact. 197:47–57. https://doi.org/10.1016/j.cbi.2012.02.001
37. Kao L, Azimov R, Shao XM, Frausto RF, Abuladze N, Newman D, Aldave AJ, Kurtz I (2016) Multifunctional ion transport properties of human SLC4A11: comparison of the SLC4A11-B and SLC4A11-C variants. Am. J. Physiol. - Cell Physiol. 311:C820–C830. https://doi.org/10.1152/ajpcell.00233.2016
38. Zhang W, Ogando DG, Kim ET, Choi MJ, Li H, Tenessen JM, Bonanno JA (2017) Conditionally immortal Slc4a11-/- mouse corneal endothelial cell line recapitulates disrupted glutaminolysis seen in Slc4a11-/- mouse model. Investig. Opthalmology Vis. Sci. 58: 3723–3731. https://doi.org/10.1167/iovs.17-21781
39. Mukhopadhyay R, Bhattacharjee H, Rosen BP (2014) Aquaglyceroporins: generalized metalloid channels. Biochim Biophys Acta - Gen Subj 1840:1583–1591. https://doi.org/10.1016/j.bbagen.2013.11.021
40. Scorei IR (2013) Popa R (2013) Sugar-borate esters – potential chemical agents in prostate cancer chemoprevention. Anticancer Agents Med Chem 13:901–909
41. Donoiu I, Militaru C, Obleagă O, Hunter JM, Neamțu J, Biță A, Scorei IR, Rogoveanu OC (2018) Effects of boron-containing compounds on cardiovascular disease risk factors - A review. J Trace Elem Med Biol 50:47–56. https://doi.org/10.1016/j.jtemb.2018.06.003
42. Hunter JM, Nemzer BV, Rangavajla N, Biță A, Rogoveanu OC, Neamțu J, Scorei IR, Bejenaru LE, Rău G, Bejenaru C, Mogoșanu GD (2019) The fructoborates: part of a family of naturally occurring sugar-borate complexes-biochemistry, physiology, and impact on human health: a review. Biol Trace Elem Res 188(1):11–25. https://doi.org/10.1007/s12011-018-1550-4
43. Kose DA, Necefoglu H, Sahin O, Buyukgungor O (2011) Synthesis, spectral, thermal and structural study of monoaquabis (acetylsalicylato-κO)bis(nicotinamide κN) copper(II). Journal of Chemical Crystallography 41(2011):297–305. https://doi.org/10.1007/s10870-010-9876-6
44. Westerink WMA, Willem GEJ (2007) Schoonen Phase II enzyme levels in HepG2 cells and cryopreserved primary human hepatocytes and their induction in HepG2 cells. Toxicol In Vitro 21(8): 1592–1602. https://doi.org/10.1016/j.tiv.2007.06.017
45. Köse DA, Karan Zümreoğlu B, Hökelek T, Şahin E (2010) Boric acid complexes with organic biomolecules: Mono-chelate complexes with salicylic and glucuronic acids. Inorganica Chimica Acta 363:4031–4037
46. Yerlikaya A, Okur E, Şeker S, Erin N (2010) Combined effects of the proteasome inhibitor bortezomib and Hsp70 inhibitors on the B16F10 melanoma cell line. Mol Med Rep 3:333–339. https://doi.org/10.3892/mmr00000262
47. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72:248–254. https://doi.org/10.1006/abio.1976.9999
48. Ledwozyw A, Michalak D, Stepien A, Kadziolka A (1986) The relationship between plasma triglycerides, cholesterol, total lipids and lipid peroxidation products during human atherosclerosis. Clin Chim Acta 155(3):275–283
49. Beutler E (1975) Glutathione in red cell metabolism: a manual of biochemical methods, 2nd edn. Grune and Stratton, NY, pp 112–114
50. Aebi H (1974) Catalase invitro. In: Bergmeyer HU (ed) 2 nd ed, FLMethods of enzymatic analysis, pp 121–126
51. Walter K, Schült C (1974) Acid and alkaline phosphatase in serum (two point method). In: Bergmeyer HU (ed) 2nd ed, FLMethods of Enzymatic Analysis, pp 856–886
52. CLSI-Clinical and Laboratory Standards Institute (2012) Performance standards for antimicrobial disk susceptibility tests; approved standard—Eleventh Edition. In: CLSI document M02-A11. PA. USA, Wayne
53. Boesekem J (1949) The use of boric acid for the determination of the configuration of carbohydrates. Adv Carbohydr Chem 4:189–210. https://doi.org/10.1016/S0096-5332(08)60049-1
54. Zittle C (1951) Reaction of borate with substances of biological interest. Adv Enzymology 12:493–527. https://doi.org/10.1002/9780470122570.ch9
55. Van Duin M, Peters JA, Kieboom APG, Van Bekkum H (1984) Studies on borate esters ı: the pH dependence of the stability of esters of boric acid and borate in aqueous medium as studied by 11B NMR. Tetrahedron 40:2901–2911. https://doi.org/10.1016/S0040-4020(01)91300-6
56. Chapelle S, Verchere JF (1988) A 11B-and 13C-NMR determination of the structures of borate complexes of pentoses and related sugars. Tetrahedron 44(14):4469–4482. https://doi.org/10.1016/S0040-4020(01)86149-4
57. Kliegel W (ed) (1980) Bor in biologie medizin und pharmazie, Springer- Verlag. USA, New York
58. Shao C, Matsuoka S, Miyazaki Y, Yoshimura K (2001) Studies on the complexation of boric acid with polyhydroxyl compounds. Analytical Sciences 17:i1475–i1478. https://doi.org/10.14891/analscisp.17icas.0.i1475.0
59. Tepedelen EB, Korkmaz M (2017) A study on the anticarcinogenic effects of calcium fructoborate. Biol Trace Elem Res 178:210–217. https://doi.org/10.1007/s12011-016-0918-6
60. Militaru C, Donoiu I, Crăciun A, Scorei ID, Bulearcă AM, Scorei IR (2013) Oral resveratrol and calcium fructoborate supplementation in subjects with stable angina pectoris: effects on lipid profiles, inflammation markers, and quality of life. Nutrition 29:178–183. https://doi.org/10.1016/j.nut.2012.07.006
61. Köse DA, Zumreoglu-Karan B, Sahin O, Büyükgüngör O (2014) Boric acid complexes with thiamine (vitamin B1) and pyridoxine (vitamin B6). Inorganica Chimica Acta 413:77–83. https://doi.org/10.1016/j.ica.2013.12.045
62. Alvarez-Ros MC, Sanchez-Cortes S, Garcia-Ramos JV (2000) Vibrational study of the salicylate interaction with metallic ions and surfaces. Spectrochim Acta A 56:2471–2477. https://doi.org/10.1016/S1386-1425(00)00328-0
63. Ross SD (1972) The infrared spectra of minerals', ed. V.C. Farmer, The Minerological Society, London, p.205.
64. Davis HB, Mott CJB (1980) Interaction of boric acid and borates with carbohydrates and related substances. J.C.S. Faraday I 76: 1991–2002
65. Altinoz MA, Topcu G, Elmaci İ (2019) Boron's neurophysiological effects and tumoricidal activity on glioblastoma cells with implications for clinical treatment. International Journal of Neuroscience 129(10):963–977. https://doi.org/10.1080/00207454.2019.1595618
66. Meiyanto E, Susidarti RA, Jenie RI, Utomo RY, Novitasari D, Wulandari F, Kirihata M (2020) Synthesis of new boron containing compound (CCB-2) based on curcumin structure and its cytotoxic effect against cancer cells. Journal of Applied Pharmaceutical Science 10(02):060–066. https://doi.org/10.7324/JAPS.2020.102010
67. Canturk Z, Tunali Y, Korkmaz S, Gulbas Z (2016) Cytotoxic and apoptotic effects of boron compounds on leukemia cell line. Cytotechnology 68:87–93. https://doi.org/10.1007/s10616-014-9755-7
68. Hacioglu C, Kar F, Kacar S, Sahinturk V, Kanbak G (2020) High concentrations of boric acid trigger concentration-dependent oxidative stress, apoptotic pathways and morphological alterations in DU-145 human prostate cancer cell line. Biol Trace Elem Res 93(2):400–409. https://doi.org/10.1007/s12011-019-01739-x

69. Rajneesh CP, Manimaran A, Sasikala KR, Adaikappan P (2008) Lipid peroxidation and antioxidant status in patients with breast cancer. Singapore Med J 49(8):640–643
70. Yalcin CO, Abudayyak M (2020) Effects of boric acid on cell death and oxidative stress of mouse TM3 Leydig cells *in vitro*. J Trace Elem Med Biol 3(61):126506. https://doi.org/10.1016/j.jtemb.2020.126506
71. Çelik SY, Demir N, Demir Y (2017) The *in vitro* effect of lisonopril on serum alkaline phosphatase and acid phosphatase enzymes activity. Celal Bayar Üniversitesi Fen Bilimleri Dergisi 13(1):233–237. https://doi.org/10.18466/cbayarfbe.302653
72. Kaynar H, Meral M, Turhan H, Keles M, Celik G, Akcay F (2005) Glutathione peroxidase, glutathione-S-transferase, catalase, xanthine oxidase, Cu-Zn superoxide dismutase activities, total glutathione, nitric oxide, and malondialdehyde levels in erythrocytes of patients with small cell and non-small cell lung cancer. Cancer Lett 227(2):133–139. https://doi.org/10.1016/j.canlet.2004.12.005
73. Kachadourian R, Brechbuhl HM, Ruiz-Azuara L, Gracia-Mora I, Day BJ (2010) Casiopeína IIgly-induced oxidative stress and mitochondrial dysfunction in human lung cancer A549 and H157 cells. Toxicology 268(3):176–183. https://doi.org/10.1016/j.tox.2009.12.010
74. Backos DS, Franklin CC, Reigan P (2012) The role of glutathione in brain tumor drug resistance. Biochemical pharmacology 83(8):1005–1012. https://doi.org/10.1016/j.bcp.2011.11.016
75. Fojo T, Bates S (2003) Strategies for reversing drug resistance. Oncogene 22(47):7512–7523. https://doi.org/10.1038/sj.onc.1206951
76. Uysal N, Gönenç S, Topçu A, Kayatekin BM, Açıkgöz O (2005) Adölesan SIçan Beyinde Antioksidan Enzim Aktiviteleri ve Lipidperoksidasyon Düzeyleri. Ege Tıp Dergisi 44:75–79
77. Zabłocka-Słowińska K, Płaczkowska S, Skórska K, Prescha A, Pawełczyk K, Porębska I, Kosacka M, Grajeta H (2019) Oxidative stress in lung cancer patients is associated with altered serum markers of lipid metabolism. PLoS One 14(4):e0215246. https://doi.org/10.1371/journal.pone.0215246
78. Ray G, Batra S, Shukla NK, Deo S, Raina V, Ashok S, Husain SA (2000) Lipid peroxidation, free radical production and antioxidant status in breast cancer. Breast Cancer Res Treat. 59(2):163–170
79. Sarı P (2011) Kültüre edilmiş C6 glioma hücrelerinde epigallokateşin gallat ve resveratrolün biyokimyasal etkilerinin incelenmesi (Master's thesis, İstanbul Bilim Üniversitesi, Sağlık Bilimleri Enstitüsü.).
80. Pointer BR, Boyer MP, Schmidt M (2015) Boric acid destabilizes the hyphal cytoskeleton and inhibits invasive growth of Candida albicans. Yeast 32(4):389–398. https://doi.org/10.1002/yea.3066
81. Ahmad S, Haque MM, Ashraf SM, Ahmad S (2004) Urethane modified boron filled polyesteramide: a novel anti-microbial polymer from a sustainable resource. Eur Polym J 40:2097–2104. https://doi.org/10.1016/j.eurpolymj.2004.05.013
82. Yılmaz MT (2012) Minimum inhibitory and minimum bactericidal concentrations of boron compounds against several bacterial strains. Turk J Med Sci 42(2):1423–1429. https://doi.org/10.3906/sag-1205-83
83. Sarac N, Ugur A, Boran R, Elgin ES (2015) The use of boron compounds for stabilization of lipase from Pseudomonas aeruginosa ES3 for the detergent industry. Journal of Surfactants and Detergents 18(2):275–285. https://doi.org/10.1007/s11743-014-1653-7
84. Sayin Z, Ucan US, Sakmanoglu A (2016) Antibacterial and antibiofilm effects of boron on different bacteria. Biol Trace Elem Res 173:241–246. https://doi.org/10.1007/s12011-016-0637-z

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.