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Investigating the anticancer potential of boric acid against human gastric adenocarcinoma cells

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ABSTRACT

Gastric cancer is a major contributor to cancer-related mortality worldwide, and the search for effective, targeted, and biocompatible therapeutic agents remains ongoing. Boric acid, a biologically active and low toxicity boron compound, has recently gained attention for its potential anticancer properties. This study aimed to investigate cytotoxic and pro-apoptotic effects of boric acid on human gastric adenocarcinoma cells (AGS) in vitro. The cell viability was assessed using a cytotoxicity assay following 24 h boric acid treatment, the IC_{50} value for AGS cells can be confidently stated as <78.12 µM, while HEK293T non-malignant cells showed minimal sensitivity, indicating selective cytotoxicity toward cancer cells. To further evaluate the mechanism of cell death, Annexin V-FITC and 7-AAD staining was performed via fluorescence microscopy and flow cytometry. Microscopy analysis revealed a substantial rise in Annexin V-FITC and 7-AAD-positive cells in the treated group compared to controls ($p<0.0001$). Flow cytometry corroborated these results, showing 7.7% early apoptosis and 16.3% late apoptosis in boric acid-treated cells, versus minimal apoptotic activity in controls, with statistically significant increases in early and late apoptotic populations ($p<0.01$ and $p<0.001$, respectively). These results suggest that boric acid induces programmed cell death in AGS gastric adenocarcinoma cells, highlighting its promise as a potential therapeutic option in gastric cancer therapy. The data presented here provides preliminary evidence for the anticancer potential of boric acid and supports ongoing investigations into the therapeutic potential of boron compounds.

1. Introduction

Gastric cancer, also known as stomach cancer, is a malignancy that arises from epithelial cells of the inner part of the stomach [1]. It is a condition attributed to multiple factors, involving both environmental and genetic influences. Major risk factors include *Helicobacter pylori* infection, diet, smoking, chronic gastritis, and a family history of malignancy [2, 3]. Gastric cancer is a major cause of death globally, placing it as the fourth leading cause of cancer -related mortality annually [4, 5]. In Türkiye, gastric cancer ranks as the fifth most commonly diagnosed cancer and the fourth leading cause of cancer-related mortality [6].

The low survival rate of gastric cancer is largely due to the diagnostic challenges of early stages, which are frequently mistaken for other conditions and thus often identified at a later stage [7, 8]. Common symptoms of gastric cancer include indigestion, decreased appetite, early satiety, unintended weight loss, and fatigue. These vague and gradually developing symptoms often hinder early diagnosis, making timely detection difficult [1, 4]. Management of gastric cancer is guided by the physician's evaluation and tumor stage and typically consists of resection followed by reconstruction, with surgery remaining the principal treatment strategy. In addition to surgery, several alternative treatment modalities, such as endoscopic mucosal resection, endoscopic laser therapy, radiotherapy, chemotherapy, targeted therapy, and immunotherapy, are also employed and continue to be refined for the management of gastric cancer [2, 7, 8]. Since the 1970s, therapeutic strategies have progressively advanced, particularly with the introduction of combination chemotherapy regimens. Various drug combinations have been explored based on their efficacy, toxicity profiles, and patient tolerability, including agents such as fluorouracil, methotrexate, and doxorubicin [9-11].

The search for effective cancer treatment has been ongoing for years, with growing attention directed toward bioactive compounds derived from natural sources, including both botanical and mineral-based substances [12]. In this context, boron and its chemical derivatives have gathered significant interest

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as a result of their unique biological properties and therapeutic potential [13, 14]. Boron is a naturally occurring element with semiconductive properties, positioned in Group 3A of the periodic table, and plays a serious role in various applications [15, 16]. Boron compounds are widely used across industries such as glass, ceramics, agriculture, and energy, with recent studies highlighting their potential health benefits. Boron was recognized by the World Health Organization as an essential element for human health, and since then, accumulating evidence has highlighted its significant physiological roles [17-21]. It has been shown to contribute to the regulation of the immune and endocrine systems, influence lipid metabolism, and play a protective role in the prevention of osteoporosis and arthritis [22, 23]. Due to these beneficial effects, boron and its derivatives have been incorporated into various pharmaceutical and medical products, including ocular cleansing solutions, ear drop formulations, and antibacterial agents for the treatment of urinary tract infections [18, 20].

Given its broad spectrum of biological activity, boron has attracted increasing attention as a potential therapeutic agent in oncology. Recent studies have reported that boric acid and other boron-containing compounds, such as boronophenylalanine (BPA) and calcium fructoborate, exhibit anticancer activity in different tumor models, including prostate, breast, glioblastoma, and gastric cancers. These findings support ongoing research into the therapeutic potential of boron-based agents across diverse cancer types [13, 24]. Boron is a critical element in the structure of bortezomib, a boronic acid-containing proteasome inhibitor that has demonstrated substantial clinical efficacy in the treatment of hematologic malignancies [25]. In addition to its role in chemotherapy, boron is used in boron neutron capture therapy, a targeted radiotherapy technique that utilizes the preferential uptake of boron-10 isotopes by tumor cells, followed by neutron irradiation to produce localized cytotoxic damage [26, 27]. These advances underscore boron's growing relevance in the field of oncological research and its potential for further investigation as a novel anticancer strategy.

Boric acid, a naturally occurring and environmentally friendly derivative of boron, is widely recognized for its low toxicity to humans and has recently gained attention for its potential medical applications. The biological activity of boron has been well documented, with several boron-containing compounds demonstrating diverse therapeutic properties. For example, the FDA-approved drug Bortezomib confirmed boron's anticancer potential as a proteasome inhibitor, while tavaborole and crisaborole were approved for the treatment of onychomycosis and atopic dermatitis, respectively. Similarly, ixazomib was approved for multiple myeloma, and vaborbactam, a boronic acid β-lactamase inhibitor, is used in combination with meropenem for urinary tract infections. Beyond approved agents, BPA and Mercapto-undecahydro-dodecaborane (BSH) are under clinical investigation for prostate cancer, while other boron analogues, including 2-S-Amino-6-boronohexanoic acid (ABH), 2-Boronoethyl-L-cysteine (BEC), and steroidal sulfatase inhibitors, are being explored for hormone-related cancers such as breast, prostate, and ovarian cancer. Boric acid is used clinically in vaginal suppositories for yeast and bacterial infections and as an antiseptic and buffering agent in eyewash solutions. Moreover, boron supplementation has been shown to reduce inflammatory markers in humans and to enhance bone formation in animals when incorporated into bioactive glasses [13, 14, 20, 24, 27, 28]. Given these diverse biological effects and their relevance to pharmaceutical development, boron and its derivatives have become subjects of increasing interest in the field of oncology. Recent findings indicate that boron-containing compounds, such as boric acid, BPA, boronic acids, tartrolons, borophycin, calcium fructoborate and boranes, exert anticancer effects through distinct mechanisms, including the induction of apoptosis via caspase activation and mitochondrial pathways, suppression of oxidative stress responses, and direct inhibition of enzymatic processes such as proteasome activity and steroidal sulfatase function, both of which are closely associated with tumor progression. Studies on boric acid have specifically highlighted its cytotoxic and apoptotic effects across a broad range of cancer cell types, including prostate, lung, cervical, melanoma, breast, multiple myeloma, mantle cell lymphoma, ovarian, pancreatic, and head and neck cancers [24, 29, 30]. However, despite this growing body of evidence, its potential therapeutic role in gastric cancer remains largely underexplored. The current research provides only limited insight into the effects of boric acid on gastric malignancies, highlighting the need for further investigation into its potential therapeutic role.

The present study aims to address the lack of data on the cytotoxic and apoptotic effects of boric acid in gastric cancer by investigating its in vitro activity on the AGS human gastric adenocarcinoma cell line. Specifically, the study seeks to evaluate the cytotoxic and apoptosis-inducing potential of boric acid, thereby contributing to the understanding of its therapeutic relevance in gastric cancer. By identifying the cellular responses triggered by boric acid exposure, the present investigation may provide a foundation for future studies and support the development of novel boron-based compounds with oncological applications.

2. Materials and Methods

2.1. Cytotoxicity Assay

In vitro experiments were conducted using HEK293T human embryonic kidney cell line as a control and AGS cell line (ATCC, USA), which is derived from the gastric adenocarcinoma. Both cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich, USA) with 10% fetal bovine serum (FBS, PAN-Biotech, Germany) and 1% penicillin/streptomycin

(Sigma-Aldrich, USA) and incubated in a humidified atmosphere containing 5% CO_2 at 37°C. Passaging was performed using trypsin-EDTA (Gibco, USA) once the cells reached confluency, covering the entire culture surface and limiting further proliferation [31].

For the cytotoxicity assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, USA) was used. This colorimetric assay measures cell viability based on mitochondrial activity in metabolically active cells [32]. A total of 1×10^4 HEK293T and AGS cells were seeded into 96-well plates in complete growth medium. After overnight attachment, the medium was discarded, cells were rinsed with 1× phosphate-buffered saline (PBS, PAN-Biotech, Germany), and treated with decreasing concentrations of boric acid (Merck, USA). 5 mM stock solution was prepared by dissolving boric acid in the growth medium. This stock was then serially diluted to obtain final concentrations of 5000 - 2500 - 1250 - 625 - 312.5 - 156.25 and 78.12 µM. For the untreated control group, cells were resuspended in growth medium. Following 24 h of incubation, filter sterilized MTT solution (5 mg/mL in 1 × PBS) was applied to each well and incubated for an additional 3 h. The medium was then discarded, 100 µL of dimethyl sulfoxide (DMSO, Isolab, Germany) was administered to each well, and the absorbance was recorded at 540 nm using a microplate reader (BioTek Synergy H1, USA) [31, 33]. The cell viability percentage was calculated using Equation 1 from absorbance values using the following equation, where the absorbance of control cells was considered 100% [34].

$$\%\text{Viability}=100\text{x}\ \frac{(\text{Mean } A_{540} \text{ of treated cells - Mean } A_{540} \text{ of blank})}{(\text{Mean } A_{540} \text{ of control cells - Mean } A_{540} \text{ of blank})} \quad (1)$$

The half-maximal inhibitory concentration (IC_{50}) values were calculated using nonlinear regression analysis in GraphPad Prism v.10 (GraphPad Software Inc., USA). The data of cytotoxicity were fitted into a four-parameter logistic model, with the log-transformed concentrations of boric acid plotted on the X-axis and the corresponding percentage of cell viability on the Y-axis [35].

2.2. Flow Cytometric Analysis

Apoptosis was assessed using the Annexin V-FITC/7-AAD assay and analyzed by flow cytometry. The apoptosis assay was based on the externalization of phosphatidylserine during early apoptosis, which bound to FITC-conjugated Annexin V in the presence of calcium, allowing apoptotic cells to be detected by fluorescence imaging methods. After boric acid treatment, AGS cells were harvested by trypsinization, centrifuged at 300×g for 5 min, and washed with cold PBS. 1×10^6 cells/mL were suspended in 100 µL of binding buffer firstly, then 5 µL of Annexin V-FITC (BioLegend, USA) and 5 µL of 7-AAD (BioLegend, USA) were added to each tube. After gentle mixing and incubation in the dark for 10 min, 400 µL of binding buffer (BioLegend, USA) was distributed and all samples were analyzed using flow cytometer (BD Accuri C6, USA). For flow cytometric analysis, 3.5×10^4 cells were counted per sample, and all events were analyzed using standard gating strategies to differentiate between live, early apoptotic, late apoptotic, and necrotic populations based on Annexin V-FITC and 7-AAD staining. Cells positive for Annexin V-FITC and negative for 7-AAD were classified as early apoptotic, while those positive for both markers were considered late apoptotic or necrotic [31, 36].

2.3. Florescence Microscopy Analysis

The apoptotic cell death percentage was determined using Annexin V-FITC & 7-AAD staining, visualized under a fluorescence microscope. AGS cells were seeded into a 12-well plate at a density of 2.5×10^5 cells in 1 mL of growth medium/well. The experimental group was treated with boric acid, while the control group received only growth medium. Both groups were incubated at 37°C with 5% CO_2 for 24 h. After incubation, 5 µL of Annexin V-FITC, 5 µL of 7-AAD and 10 µL of DAPI (Genetex, USA) in cell binding buffer were added to each well, followed by gentle mixing. The plate were incubated in dark for 15 min. Following the incubation, cells were counted under a fluorescence microscope (Zeiss Axio Observer A1, Germany) using appropriate filters to visualize Annexin V-FITC (green fluorescence) and 7-AAD (red fluorescence). Cells positive for Annexin V-FITC and negative for 7-AAD were considered early apoptotic, while those positive for both stains were classified as late apoptotic/necrotic. Untreated control cells used to establish baseline fluorescence levels. Image acquisition and analysis were performed using imaging software (Zeiss ZEN lite, Germany) [31, 36, 37].

2.4. Statistical Analysis

Statistical analyses were carried out to assess differences between groups using either Student's t-test or one-way ANOVA, as appropriate. 95% confidence interval was applied, and p-values less than 0.05 were considered statistically significant. All statistical analyses were performed using GraphPad Prism v10. (GraphPad Software Inc., USA).

3. Results

3.1. Cytotoxic Effects of Boric Acid on AGS Cells

To evaluate the cytotoxic effects of boric acid on HEK293T human embryonic kidney cells and AGS gastric cancer cells after 24 h incubation, a range of concentrations was tested using the MTT assay. Considering gastric physiology, orally administered compounds typically remain in the stomach for only 3-6 h before passing into the small intestine, where

systemic absorption begins [38, 39]. To mimic this physiological time frame while allowing sufficient interaction with the cells in vitro, a 24 h incubation period was selected for cytotoxicity assessment and the results were plotted against the logarithmic values of each dose (Figure 1A). This approach also enables the analysis of early response parameters while minimizing potential secondary, indirect, or extracellular effects that may arise during extended incubation periods. The boric acid concentration range used in the current study was selected based on previously reported effective concentrations in gastrointestinal cancer cell models. Prior studies in colon cells demonstrated minimal cytotoxicity at ≤10 mM, with significant viability reduction only at ≥50 mM after 24 h exposure [40]. In pancreatic cancer cells, 48 h IC_{50}values were reported as ~15 mM, respectively, with induction of apoptosis and endoplasmic reticulum stress [41]. Similarly, in HepG2 liver cancer cells, substantial cytotoxic and pro-apoptotic effects were observed within the 5-25 mM range [42]. Considering these findings, the concentration range used in the methodology was designed to encompass sub-cytotoxic doses as well as higher doses approaching or exceeding reported IC_{50} values. This enabled comparative assessment of boric acid's effects across a physiologically relevant spectrum and facilitated alignment with existing literature on GI tract cancers.

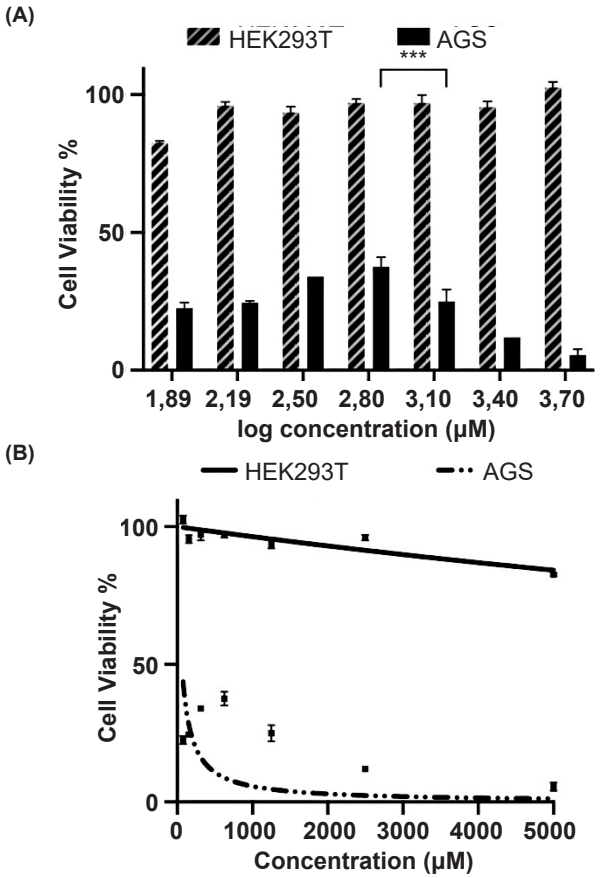

Figure 1. Boric acid induced cytotoxicity in HEK293T human embryonic kidney cells and AGS gastric cancer cells. a) The percentage of cell viability was determined by MTT assay after 24 h treatment with increasing log-transformed concentrations of boric acid. b) Dose-response curve showing the effect of boric acid. Data are expressed as mean ± standard deviation (SD) of three replicates: *p≤0.001.

The cell viability was determined using Equation 1. The data for the AGS cell line demonstrated a clear dose-dependent response, with increased boric acid concentrations correlating with reduced cell viability. At lower concentrations (156.25 and 78.12 µM), the mean cell viability remained relatively moderate, ranging from approximately 22.5% to 34%, suggesting limited cytotoxicity. Interestingly, at 625 µM, a slight but statistically significant increase in cell viability was observed compared to 312.5 µM (p≤0.001), although overall viability remained below 50%. This minor increase may reflect biological variation rather than a protective effect, as higher concentrations again reduced viability significantly. However, as the boric acid concentration increased beyond 1.25 mM a significant decline in cell viability was observed. At 2.5 mM, viability dropped sharply to an average of 12%, and at the highest concentration tested (5 mM), it fell to approximately 5.5%. In contrast, HEK293T human embryonic kidney cells exhibited minimal cytotoxicity across the same concentration range. At the highest dose, HEK293T viability remained above 82-83%, while other doses of boric acid yielded viabilities between 92% and 104%, indicating negligible loss of metabolic activity in non-malignant cells. These results indicate that boric acid exerts a strong cytotoxic effect on AGS cells at higher concentrations, with a steep loss of viability between 1.25 to 5 mM. Based on the MTT assay data, in AGS cells tested concentrations reduced viability below 50%, indicating strong cytotoxicity even at the lowest tested dose. The IC_{50}value for AGS cells can be stated as <78.12 µM, in contrast, HEK293T cells maintained >80% viability even at the highest concentration tested, suggesting that the IC_{50} for the non-malignant cell line is >5 mM. These findings indicate a clear difference in sensitivity between AGS and HEK293T cells, supporting selective cytotoxicity of boric acid toward gastric cancer cells.

The cytotoxicity findings highlight the selective activity of boric acid, with AGS cells displaying high sensitivity, while non-malignant HEK293T cells showed resistance across the tested range. The precision of IC_{50} determination in the current investigation was limited by the concentration range tested, which restricted the ability to generate fully defined dose-response curves for both AGS and HEK293T cells. Nevertheless, the selective response is consistent with the biological context: cancer cells exhibit elevated metabolic demands, impaired antioxidant defenses, and greater susceptibility to oxidative stress, which may enhance boric acid's apoptotic activity. In contrast, HEK293T cells likely benefit from intact redox balance, stable mitochondrial function, and potentially limited uptake of boric acid, which together could explain their resistance. This selectivity underlines boric acid's potential as an anticancer agent while sparing normal

cells, although further mechanistic studies, particularly at the molecular and protein levels, are warranted. The difference between AGS and HEK293T cells suggests that boric acid preferentially targets malignant gastric cancer cells over healthy kidney cells. Comparable findings have been observed in prostate and breast cancer models, where boric acid selectively reduced tumor cell viability while preserving normal epithelial cells, supporting its potential therapeutic window. In the study, boric acid exhibited a dose-dependent cytotoxic effect on AGS cells. This is notably lower than the IC_{50} value of 31.25 µg/mL (~505 µM) reported by Kar et al. in U251 glioblastoma cells [43]. The difference in IC_{50} values indicates that AGS cells are significantly more sensitive to boric acid compared to glioblastoma cells under similar treatment conditions. A similar trend was observed in the findings reported by Cebeci et al. who investigated various boron derivatives in lung cancer cell lines. In their study, boric acid and its derivatives significantly reduced cell viability, with IC_{50} values ranging between 80-100 µg/mL [44]. Also, Güneş et al. reported that pancreatic cancer cell lines (PANC-1 and MIA PaCa-2) showed IC_{50} values of ~16 mM following 24 h treatment [41]. This variation between studies may reflect differences in cellular uptake mechanisms, antioxidant capacities, or apoptotic signaling pathways between gastric and other tumor cells. The lower effective concentration in this investigation underscores boric acid's potential as a more potent agent in gastric cancer therapy compared to other cancers. Also, as a healthy cell line, HEK293T cells exhibited substantially higher viability, with no significant cytotoxicity observed at doses near the IC_{50} of AGS. This differential response suggests a degree of selectivity for malignant cells, as boric acid impaired AGS viability more effectively than that of HEK293T cells. These findings reinforce the importance of tailoring boron-based anticancer strategies to the biological context of each cancer type and further highlight the promise of boric acid as a selective and effective compound in gastric malignancies.

In a related study, Wang et al. designed a novel boron delivery agent named Lap-BPA, in which L-p-boronophenylalanine is covalently coupled to the tyrosine kinase inhibitor lapatinib via an amide bond, with additional hydrophilic groups to improve solubility and selectivity for tumor cells and evaluated its cytotoxicity across several cancer cell lines, including AGS cells [45]. Their findings demonstrated that their delivery agent induced minimal toxicity to AGS cells at concentrations up to 10 µM, with cytotoxic effects becoming apparent only at higher doses (>20 µM). In contrast, this study using unmodified boric acid revealed substantial cytotoxicity in AGS cells after 24 h. This suggests that simple boric acid may be more effective at lower concentrations than the more complex agents. These differences could reflect distinct cellular uptake mechanisms, compound stability or intracellular targeting efficiency.

The findings of this study support the hypothesis that boric acid itself can be a potent cytotoxic agent against gastric cancer cells, potentially offering a more cost-effective and simpler alternative to specialized boron delivery molecules. These findings further reinforce the dose-dependent cytotoxic potential of boric acid, consistent with previous studies that have reported similar growth-inhibitory effects of boron compounds in various cancer cell lines. In HEK293T cells, boric acid treatment did not induce a notable reduction in cell viability across the tested concentrations, and the calculated IC_{50} value was markedly high. These results indicate that boric acid exerts negligible cytotoxicity toward healthy cells under the experimental conditions. Given the absence of significant toxicity in HEK293T cells, further apoptosis-related analyses were not pursued for this cell line, as such experiments would be unlikely to yield meaningful apoptotic responses.

3.2. Apoptotic Effects of Boric Acid Assessed by Annexin V-FITC/7-AAD staining

Following the cytotoxicity assay, apoptotic cell death was assessed using Annexin V-FITC and 7-AAD staining, analyzed with flow cytometry, and visualized using a fluorescence microscope. The experimental group was treated with 61 µM boric acid, while the control group received only growth medium. The selected boric acid dose reduced AGS cell viability to approximately 50%, representing a biologically relevant and effective concentration that induced measurable cell death while preserving sufficient viable cells for apoptotic analysis.

Firstly, to evaluate the pro-apoptotic effect of boric acid on AGS cells, Annexin V-FITC & 7-AAD double staining was done and determined by flow cytometry. The dot plot analysis revealed clear differences between the control and boric acid treated groups (Figure 2a). In the control sample, the majority of cells (94%) were located in the Annexin V-$FITC^-$/7-AAD^- quadrant (Q2-LL), indicating viable cells. Only 2.5% of cells were in early apoptosis (Annexin V-$FITC^+$/7-AAD^-, Q2-LR) and 3.2% in late apoptosis (Annexin V-$FITC^+$/7-AAD^+, Q2-UR), while necrotic cells were negligible (0.1%). In contrast, treatment with boric acid caused a significant shift in cell populations. The percentage of viable cells decreased to 76%, while early apoptotic cells increased to 7.7%, and late apoptotic cells rose markedly to 16.3%. This shift indicates that boric acid effectively induced programmed cell death in AGS cells. Quantitative analysis confirmed these observations (Figure 2b). The percentage of early and late apoptotic cells was significantly higher in the boric acid-treated group compared to the control ($p<0.01$ and $p<0.001$, respectively). The reduction in live cell population and concurrent increase in apoptotic fractions support the hypothesis that boric acid triggers apoptotic mechanisms rather than nonspecific necrosis.

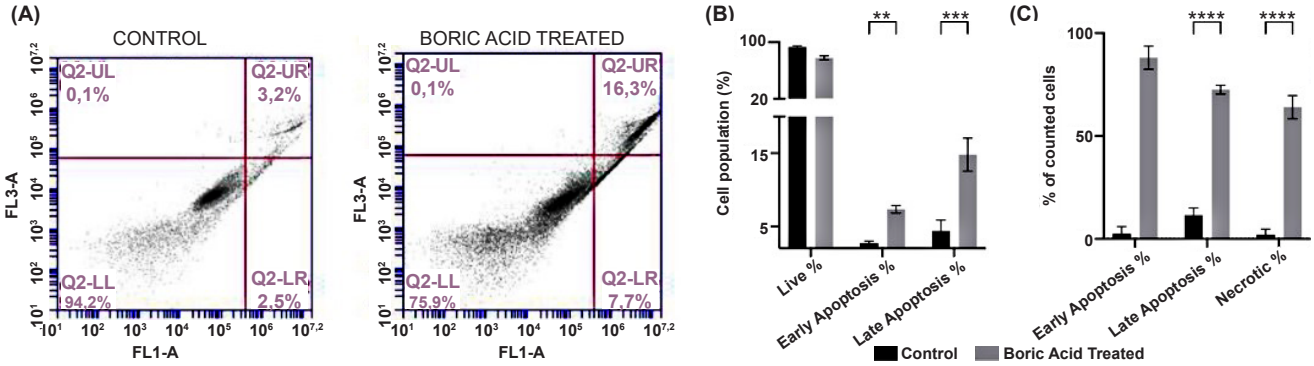

Figure 2. Analysis of boric acid induced apoptosis in AGS cells using flow cytometry and fluorescence microscopy. a) Flow cytometry dot plots showing Annexin V-FITC/7-AAD double staining of control (left) and boric acid-treated (right) AGS cells. Cells in the lower left quadrant (Q2-LL) represent live cells, lower right (Q2-LR) early apoptotic, upper right (Q2-UR) late apoptotic and upper left (Q2-UL) necrotic populations. b) Quantification and statistical analysis of cell populations from flow cytometry analysis. c) Quantitative and statistical analysis of fluorescence microscopy images based on Annexin V-FITC/7-AAD staining. Data are presented as mean ± SD. Statistical significance: **p<0.01 , ***p<0.001 , ****p<0.0001.

Apoptotic cell death was also visualized under a fluorescence microscope (Figure 3). In the control group (top panel), most cells stained only with DAPI (blue), indicating viable nuclei, with negligible green (Annexin V-FITC) or red (7-AAD) fluorescence, suggesting minimal apoptotic or necrotic activity, as expected. In contrast, the boric acid-treated group (bottom panel) exhibited a marked increase in green fluorescence (Annexin V-FITC$^+$ cells), indicating early apoptosis, as well as red fluorescence (7-AAD$^+$), representing late apoptotic or necrotic cells. The merged image confirms the co-localization of Annexin V-FITC and 7-AAD signals in AGS cells, demonstrating that boric acid treatment significantly induced apoptotic cell death in AGS cells compared to the untreated control.

Quantitative analysis of florescence microscopy revealed significant differences in cell death profiles between control and boric acid-treated AGS cells (Figure 2c). In the control group, nearly all cells were viable, with minimal detection of apoptotic or necrotic cells. In contrast, treatment with boric acid resulted in a substantial shift in cell viability. The proportion of early apoptotic cells increased significantly, as indicated by Annexin V-FITC positivity, along with a marked rise in both late apoptotic and necrotic cell populations ($p<0.0001$ for all comparisons). These findings confirm that boric acid induces programmed cell death in AGS cells and support the qualitative observations made via fluorescence microscopy. The combined results strengthen the evidence that boric acid has a significant pro-apoptotic effect on human

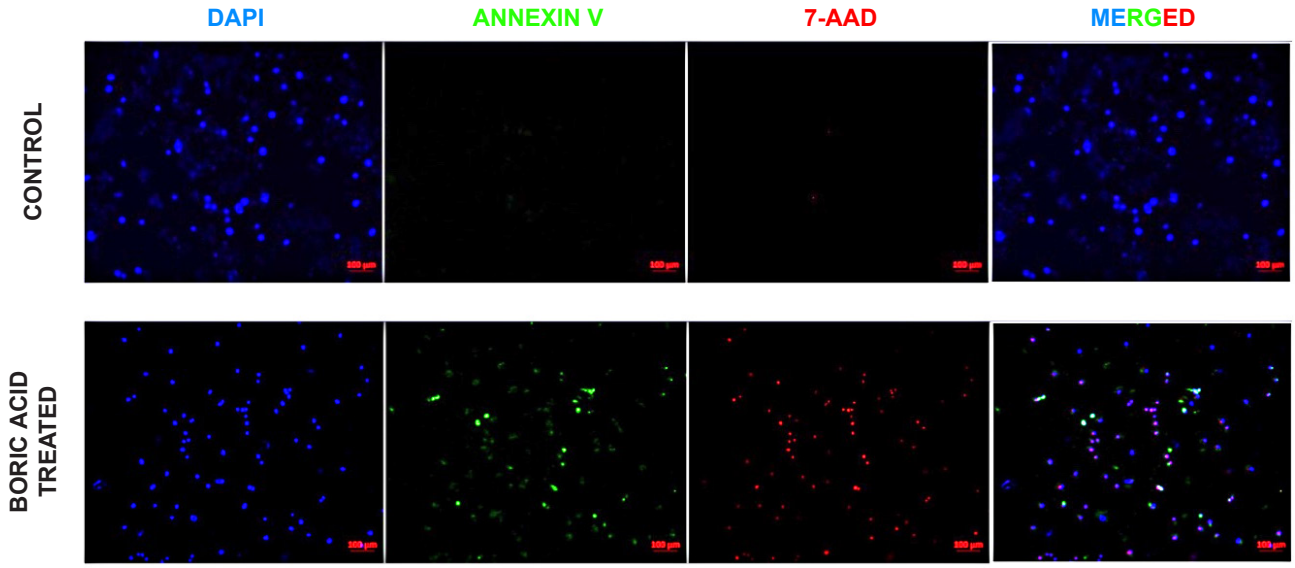

Figure 3. Fluorescence microscopy images showing apoptotic and necrotic AGS cells stained with Annexin V-FITC, 7-AAD, and DAPI. Cells were treated with 61 µM boric acid (bottom row) or growth medium only (control, top row) for 24 h. After incubation, cells were stained using Annexin V-FITC (green), 7-AAD (red) and DAPI (blue). Merged images confirm the co-localization of apoptotic and necrotic markers. Magnification: x 100, Scale bar: 100 µm.

gastric cancer cells and may serve as a promising candidate for further anticancer research.

These findings are consistent with the proposed mechanism of boron-based compounds promoting apoptosis through oxidative stress and mitochondrial disruption, as supported by previous studies. Hacıoğlu et al. reported that boric acid triggered oxidative stress, growth inhibition, and apoptosis in the prostate cancer cell line (DU-145) in a dose-dependent manner, accompanied by increased Bax expression and morphological alterations [46]. In medullary thyroid cancer cells, a recent study observed that boric acid (IC_{50} = ~35 μM at 48h) significantly activated caspase-3 and caspase-9, upregulated pro-apoptotic genes, and increased TUNEL positivity, indicating intrinsic apoptosis via mitochondrial pathways [47]. Although the treatment duration differed, the IC_{50} value is closely comparable to the findings of this research's results, reinforcing boric acid's potent pro-apoptotic effect at similar concentrations across cancer types. It was also found that boric acid consistently reduces proliferation and induces apoptosis in breast cancer cell lines across both hormone receptor-positive and triple-negative subtypes. Comparative studies indicate that boric acid's anti-invasive and apoptotic effects are dose-dependent in breast cancer and contribute to reduced cell proliferation, induction of programmed cell death, suppression of metastatic potential, and modulation of key apoptotic genes, highlighting its potential as a supportive therapeutic agent in breast cancer treatment strategies [24, 48-51].

Collectively, these findings indicate a safe, dose-dependent apoptotic response to boric acid across diverse cancer types. The presented study provides additional evidence that AGS gastric cancer cells are particularly sensitive to boric acid, responding with apoptosis at lower concentrations than those effective in prostate or breast cancers. Future research should focus on delineating the specific molecular signaling pathways involved and validating efficacy in in vivo models to assess therapeutic potential.

4. Conclusions

The presented study demonstrates that boric acid exerts a concentration-dependent cytotoxic effect on AGS human gastric adenocarcinoma cells compared to the healthy HEK293T cell line and significantly induces apoptosis, as evidenced by both MTT-based viability assays and Annexin V-FITC/7-AAD staining analyzed via fluorescence microscopy and flow cytometry. The consistent results across multiple assays support the hypothesis that boric acid may interfere with cellular survival pathways and activate apoptotic mechanisms in gastric cancer cells. While the outcomes of this work demonstrated effects of boric acid on AGS cells, several limitations should be acknowledged. The investigation focused specifically on morphologic and flow cytometric detection of apoptosis, without exploring the underlying molecular mechanisms at the gene or protein level. Similarly, no evaluation was conducted on signaling pathways potentially involved after boric acid treatment, such as oxidative stress or cell cycle regulation. The study was limited to in vitro assays and therefore the therapeutic potential of boric acid remains to be confirmed in in vivo models. Boric acid holds potential for further investigation as a complementary or standalone agent in gastric cancer therapy, and addressing these limitations in future research would provide a more comprehensive understanding of boric acid's anticancer potential.

In conclusion, the current study provides valuable preliminary evidence supporting the anticancer activity of boric acid and offers a foundation for future research into boron-based strategies for gastric cancer treatment and pharmaceutical development.

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6. Author Contribution Statement

Aslıhan Kartal: Conceptualization, methodology, data curation, writing-original draft preparation, visualization, investigation.

Sezen Atasoy: Methodology, data curation, writing-original draft preparation, visualization, investigation, validation, writing-review, and editing.

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