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Research paper

Boric acid as a promising agent in the treatment of ovarian cancer: Molecular mechanisms

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ABSTRACT

Purpose: The aim of this study is to determine the therapeutic effects of boric acid cell proliferation, invasion, migration, colony formation, cell cycle and apoptosis mechanisms in ovarian cancer cell line under *in vitro* conditions.
Methods: MDAH-2774 ovarian cancer cells were employed. Real-time PCR test was used to investigate changes in genes and proteins of cell cycle and apoptosis and identified miRNAs under the addition of boric acid. The apoptosis rates were calculated by TUNEL assay. Matrigel invasion, colony formation and Wound healing tests were used to determine invasion and migration. Oxidative stress index value was calculated for oxidative stress.
Results: Boric acid inhibited cell proliferation, invasion, migration and colony formation, but induces apoptosis and oxidative stress. Also, the expression of miRNA-21, miRNA-200a, miRNA-130a and mi-RNA-224 (which are indicators of poor prognosis of ovarian cancer) decreased significantly.
Conclusion: The potential of boric acid as a natural molecule may supports its effectiveness in reducing adverse effects arising from conventional ovarian cancer treatments.

1. Introduction

Ovarian cancer (OC) is the leading cause of mortality among all gynecological malignancies (Siegel et al., 2016). Ovarian cancers are frequently detected in advanced stages, although some genetic approaches give an opportunity to provide an early diagnosis (Bookman, 2019; Staicu et al., 2020; Vafadar et al., 2020). When it is diagnosed, metastases are already present to lymph nodes and distant organs, because the disease is usually asymptomatic in the initial stages (Leffers et al., 2009; Tognon et al., 2013; Holschneider and Berek, 2000). Tumor prognosis depends on the use of cytoreductive surgery with chemotherapy or neoadjuvant chemotherapy and subsequent interval debulking (Sioulas et al., 2017; Al Rawahi et al., 2013). To date, these treatment protocols present the gold standard treatment for OC. Unfortunately, the efficiency of systemic chemotherapy is low, and patients usually develop chemoresistance, also side effect profile is high (Mihanfar et al., 2019). This drug resistance reduces the effectiveness of standard chemotherapies, and increases the morbidity and mortality rate in these patients. Considering the prognosis of ovarian cancer, it requires the identification of new effective and promising agents or new treatment strategies.

Boron (B) is the only nonmetallic one in the group 13 elements of the periodic table. In nature, B is not found in the elemental form. It is present as a component of boric acid (BA), kernite, borax, borates, ulexite and colemanite (Kot, 2009; Simmons and Ahsian, 2007). In humans, 90% of the administered dose of boron is absorbed by the respiratory tract epithelium, mucosal membranes of gastrointestinal tissues, mouth, vagina, and anus, and distributed evenly throughout the

Abbreviations: B, Boron; BA, Boric acid; Bcl-2, B-cell lymphoma 2; Bcl-xL, B-cell lymphoma-extra large; BID, BH3 interacting domain death agonist; cDNA, Complementary DNA; CCND1, Cyclin D1; CCND2, Cyclin D2; CDKs, Cyclin dependent kinases; CDK4, Cyclin-dependent kinase-4; CDK6, Cyclin-dependent kinase-6; FADD, Fas-associated protein with death domain; FBS, Fetal bovine serum; IC50, Inhibitory concentration that induces 50% of cell death; miRNA, microRNA; OSI, Oxidative stress index; OC, Ovarian cancer; RT-PCR, Real time PCR; TAS, Total antioxidant status; TOS, Total oxidant status; TRADD, Tumor necrosis factor receptor type 1-associated DEATH domain protein.

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body as boric acid $B(OH)_3$ (Hunt, 1998; Bakirdere et al., 2010). In previous studies, the antioxidant and anti-osteoporotic effects of boron have been reported (Ince et al., 2010; Cengiz, 2018; Ayhanci et al., 2020; Cengiz et al., 2019; Scorei and Rotaru, 2011; Miggiano and Gagliardi, 2005). It was also shown that boron may alter the metabolic processes of several enzymes and minerals, and immune system regulation (Khaliq et al., 2018). Furthermore, borates are becoming increasingly attractive owing to the recent publications indicating that they may possess anti-carcinogenic effects in a variety of cancer types of prostate (Cui et al., 2004), cervix (Korkmaz et al., 2007), lung (Mahabir et al., 2008) and breast tissues (Scorei et al., 2008) and malign melanoma (Acerbo and Miller, 2009).

This study was intended to determine the effects of boric acid on human ovarian cancer MDAH-2774 cell lines *in vitro* conditions. The assays regarding cell viability, colony formation, migration, proliferation, apoptosis, and changes in gene expression were conducted to explore whether there is an anticancer activity of boric acid and to elucidate the exact mechanism by which boric acid induces this activity. The most important outcome obtained from this study is that it will provide evidence-based information based on the molecular mechanism of boric acid for future research. To the best of our knowledge, we present the first work investigating the anticancer activity of boric acid on ovarian cancer.

2. Materials and methods

2.1. Cell culture

Human ovarian cancer cell line MDAH-2774 (RRID: CVCL_0420) and human lung fibroblast cell line WI-38 [WI-38 (ATCC® CCL-75™)] were used in this study. WI-38 human fibroblast cell line was used to test the selectivity of boric acid against normal cells and used for control purposes only in the XTT (2,3-bis(2–methoxy–4nitro–5–sulfophenyl)–2H-tetrazolium-5-carboxanilide) experiment. Hence, we did not study the gene expression pattern in WI38 cell line. Since we used the WI-38 cell line as a fibroblast control cell line in our study, further experiments with WI-38 cell line were not planned.

Both cell lines were cultured under suitable conditions at 37 °C in 5% CO_2 and cells were grown in Dulbecco Modified Eagle Medium (DMEM; Sigma) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Capricorn Scientific), 20 units/mL penicillin and 20 μg/mL streptomycin, 0.1 mM amino acid solution (Biological Industries) and 1 mM sodium pyruvate (Biological Industries). Different concentrations (10 μM, 20 μM, 35 μM, 50 μM, 75 μM, 100 μM, 200 μM, 500 μM) of boric acid (Etimaden) were applied to the cells in a time and dose-dependent manner.

2.2. Cell proliferation XTT assay

Effects of boric acid on cell proliferation of human ovarian cancer cell line MDAH-2774 and human lung fibroblast cell line WI-38 cells were detected by XTT assay according to manufacturer procedure (Cell Proliferation Assay with XTT Reagent-Cell Proliferation Kit; Biotium cat no: 30007). MDAH-2774 and WI-38 cells were seeded into 96-well plates at a concentration of 1 × 104 cells per well. After 24 h of incubation, the cells were treated with 10 μM, 20 μM, 35 μM, 50 μM, 75 μM, 100 μM, 200 μM, 500 μM concentrations of boric acid during 24, 48 and 72 h. Untreated cells were used as control cells. After incubation period, XTT mixture was added and then formazan formation was determined spectrophotometrically at 450 nm (reference wavelength 630 nm) by a microplate reader (Biotek). Viability (%) of cells was calculated using the background-corrected absorbance as follows:

Viability (%) = Absorbance of experiment well/Absorbance of control well × 100

2.3. RNA Isolation, cDNA Synthesis, and Real-time PCR (RT-PCR)

Total RNA from control and dose group of MDAH-2774 ovarian cancer cells were isolated by Trizol Reagent (Invitrogen, USA) according to manufacturer's instructions. Complementary DNA (cDNA) was synthesized using the high capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA), according to the manufacturer's protocol.

CCND1 (cyclin D1), CCND2 (cyclin D2), cyclin-dependent kinase-48 (CDK4), CDK6, caspase-3, caspase-9, caspase-8, caspase-10, B-cell lymphoma 2 (Bcl-2), B-cell lymphoma-extra large (Bcl-xL), BH3 interacting domain death agonist (BID), Fas-associated protein with death domain (FADD), tumor necrosis factor receptor type 1-associated DEATH domain protein (TRADD) and *Bax* genes were used for cell cycle, and cell apoptosis pathway expression analysis in this study and expression profiles were calculated using the beta-actin (house-keeping gene) as the reference. Real-Time PCR tests were performed by Real-Time Reverse Transcription PCR (RT-PCR) according to the SYBR Green qPCR Master Mix (Applied Biosystems, USA) protocol. The sequences of primers were given in supplementary materials (Supplementary Table 1).

2.4. Micro-RNAs (miRNAs) selection

miRNA selection was guided by the literature analysis with the requirement that these miRNAs had to be involved in the ovarian carcinogenesis, and they were shown to exhibit differential expressions in between ovarian cancer tissue and normal tissue. miRNAs expressed in advanced stage, chemotherapy-resistant ovarian carcinomas or different subtypes (serous, endometrioid, clear cell etc.) of ovarian carcinomas were preferred. In addition, miRNAs that may have a potential role in determining the diagnosis, prognosis and recurrence of ovarian carcinomas were selected. Four miRNAs matching these criteria (miR-21-5p, miR-200a-3p, miR-224-5p, miR-130a-3p) were selected for the present study.

Micro-RNAs (miRNAs) expression changes were determined by using RT-PCR. miRNA cDNA synthesis kit (ABM) was used for cDNA synthesis and subsequently relative quantification of hsa-miR-21-5p, hsa-miR-224-5p, hsa-miR-200a-3p, hsa-miR-130a-3p was analyzed by RT-PCR according to EvaGreen (ABM) mastermix (ABM) protocol. miRNAs expressions were normalized to U6 as human endogenous control.

2.5. TUNEL assay

The apoptotic effects of boric acid on MDAH-2774 ovarian cancer cells were evaluated by TUNEL assay according to manufacturer's instructions. The control and dose group of MDAH-2774 ovarian cancer cells were fixed with 4% (w/v) paraformaldehyde, and the cell apoptosis was analyzed using a commercial kit (Tunel In Situ Cell Detection Kit, AAT Bioquest) according to manufacturer's protocol. MDAH-2774 ovarian cancer cells were stained with Hoechst dye and then observed under a fluorescence microscope (Olympus Inc., Tokyo, Japan). The number of total cells and TUNEL-positive cells were counted in 10 randomly selected fields in the fluorescence microscope. The results were expressed as a percentage of TUNEL-positive cells defining the ratio of apoptotic cells to the total cells.

2.6. Matrigel-invasion assay

Number of invasive control and dose group MDAH-2774 ovarian cancer cells were performed using Matrigel invasion chambers (Thermo scientific LOT: 159467) following the manufacturer's instructions. 2×10^5 cells were plated onto the upper chambers of Matrigel-coated filter inserts. The assays were performed in triplicate. Percentage of invasion was computed using the following equation:

Invasion (%) = the number of cells in matrigel matrix basement

membrane/the number of cells in control membrane X 100

2.7. Colony formation assay

Colony formation assay was performed to examine the colony formation of MDAH-2774 cells treated with boric acid. The seeding of the cells into a six-well plate was done at a density of 10^3 cells/well. The medium was changed every three days for 2 to 3 weeks. When macroscopically visible colonies appeared in the culture dish, colonies were fixed in methanol for 10 min and stained with crystal violet. The morphology of colonies was observed under a microscope, and the numbers of colonies were counted.

2.8. Wound-healing migration assay

Anti-migratory effects of boric acid on MDAH-2774 ovarian cancer cells were determined by wound-healing assay. The control and dose group MDAH-2774 cells were seeded in 60 × 15 mm style cell culture dishes at 10^6 cells per well and allowed to adhere overnight at 37 °C with 5% CO_2. After the growing cell layers had reached about 90% confluence, a scratch was made via sterile 200-μL plastic pipette tip. Cells were further incubated for 24 h with IC50 dose of boric acid. Then, the closure is evaluated at 24 h using a light microscope. Images of the MDAH-2774 cell proliferation were taken. The assays were performed in triplicate.

2.9. Total antioxidant status (TAS) – Total oxidant status (TOS) and oxidative stress index (OSI) measurements

Total antioxidant status (TAS) and the total oxidant status (TOS) in control cells and boric acid treated cells were determined Rel Assay commercial kits (Rel Assay Kit Diagnostics, Gaziantep, Turkey) according to manufacturer's test protocol. TAS and TOS values were measured by using a microplate reader (BioTek). The oxidative stress index value (OSI) is a marker for the degree of oxidative stress as a combined ratio between pro-oxidants (TOS) and antioxidants (TAS). To determine OSI, the mmol unit of TAS and the μmol unit of the TOS were cross-converted. "OSI (arbitrary unit) = TOS (μmol H_2O_2 Eq/l)/TAS (mmol Trolox Eq/l) × 100" formula was used for calculation of oxidative stress index value, as previously reported (Alp et al., 2010; Karkucak et al., 2010).

2.10. Statistical analysis

The ΔΔCt method was used for the statistical analysis of the findings and quantitated with a computer program. The comparison of the groups has been performed with the 'Volcano Plot' analysis, from 'RT2 Profiles PCR Array Data Analysis', which is assessed statistically using the 'student *t*-test.' IBM SPSS Version 23 (SPSS Inc., Chicago, IL, USA) analysis program was used to perform the parametric and nonparametric analysis of dose and control groups. $p < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Boric acid decreases cell proliferation of MDAH-2774 ovarian cancer cells

Anti-proliferative activity of boric acid in MDAH-2774 ovarian cancer cells was determined by XTT assay. Furthermore, effects of cell viability on WI-38 cell line were evaluated by using XTT assay. WI-38 cell line was used as a control cell line. In this way, the effect of boric acid on the MDAH-2774 cell line was investigated depending on time and dose and IC50 (inhibitory concentration that induces 50% of cell death) dose of boric acid was found to be 51.63 μM at the 24th hour (Figs. 1 and 2).

3.2. Boric acid changes mRNA expressions of genes

cDNAs, which are synthesised from total RNA from control and dose group cells were performed, and Real Time PCR was used for detecting the expression of genes and miRNAs which are missioned in apoptosis pathways and cell cycle. It was observed that boric acid in MDAH-2774 ovarian cancer cell line caused a significant increase in the apoptosis related genes expressions of *Bax, Bid, caspase-3* and *caspase-9* which are inducing apoptosis and a significant decrease in the apoptosis related proteins expressions of *Bcl-2* and *BcL-xL* which are negative regulators of apoptosis (Table 1). *Caspase-8, Caspase-10, Cyclin D1, Cyclin D2, CDK6, CDK4, FADD, TRADD, FAS, DR4* and *DR5* gene mRNA expression changes were found insignificant in boric acid treated group compared with control. mRNA expression changes to these genes were given in supplementary materials (Supplementary Table 2).

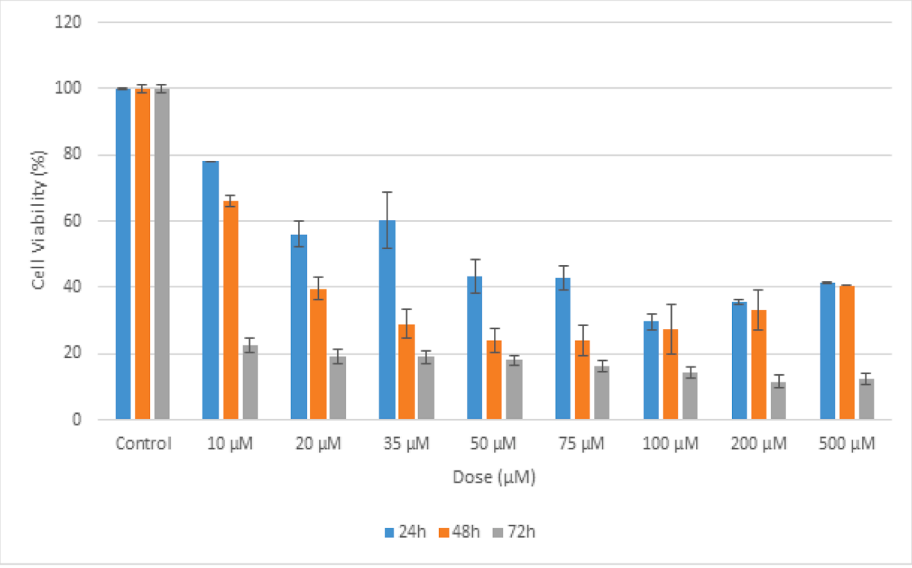

Fig. 1. Effect of Boric acid on the viability of MDAH-2774 ovarian cancer cell line. The cells were treated with boric acid at different concentrations and time intervals and their proliferation was assessed by XTT assay.

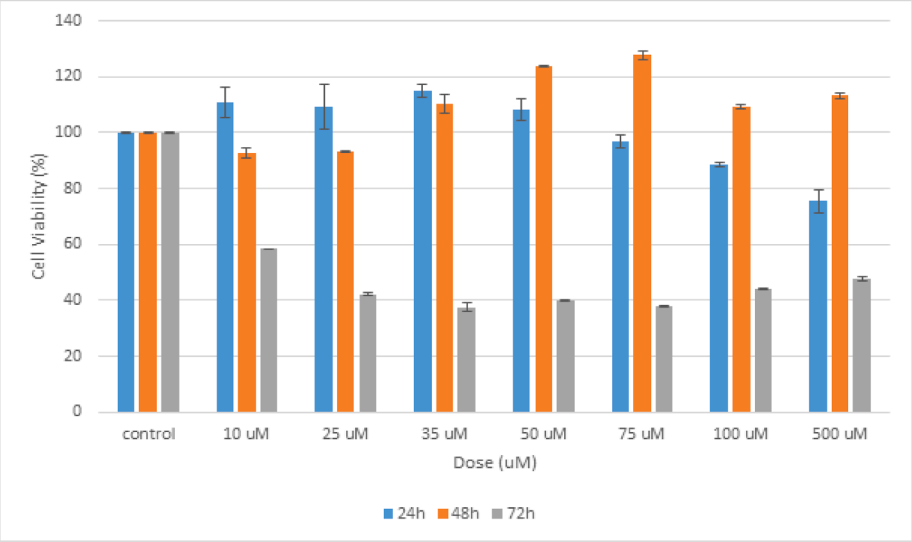

Fig. 2. Effect of Boric acid on the viability of WI-38 cell line. The cells were treated with boric acid at different concentrations and time intervals and their proliferation was assessed by XTT assay.

Table 1
The mRNA expression changes of cell cycle and apoptosis genes in MDAH-2774 ovarian cancer cells treated with boric acid, compared with the control group cells. Data were obtained by RT-PCR assay via ΔΔCt method in RT2 Profiles PCR Array Data Analysis online program.

Gene Names	Fold Regulation	p value
Bcl-XL	−3.08055	0.012[a]
Blc-2	−2.509633	0.03[a]
Bax	1.629	0.036[a]
Bid	2.3663	0.007[a]
Caspase-3	1.5945	0.0117[a]
Caspase-9	2.008	0.0426[a]

[a] $p < 0.05$ statistically significant.

3.3. Boric acid affects miRNA expression

We have compared the effects of boric acid on miRNA expression patterns in control and boric acid treated groups. The expression of *miR-21, miR-200a, miR-130a* and *miR-224* were significantly decreased in dose group compared to control group (-10.4 times ($p = 0.0025$), −6.61 times ($p = 0.0373$), −3.99 times ($p = 0.026$), −2.96 times ($p = 0.0422$), respectively) (Table 2).

3.4. Boric acid induces apoptosis in MDAH-2774 cells

TUNEL test was used to evaluate the data about apoptosis index between groups. In the light of the data obtained, the percentage of apoptotic cells was compared between the dose group and the control group. In the control group, the rate of apoptotic cells was determined as 3.0 ± 1.0%, and in the dose group in which IC_{50} dose of boric acid was added, the rate of apoptotic cells was determined as 15.0 ± 2.44% (Fig. 3A and 3B).

Table 2
The miRNA expression changes between control and boric acid treated groups; data were obtained by RT-PCR assay via ΔΔCt method in RT2 Profiles PCR Array Data Analysis online program.

miRNA Names	Fold Regulation	p value
hsa-miR-21-5p	−10.4035	0.0025[a]
hsa-miR-200a-3p	−6.6058	0.0373[a]
hsa-miR-130a-3p	−3.9968	0.026[a]
hsa-miR-224-5p	−2.9564	0.0422[a]

[a] $p < 0.05$ statistically significant.

3.5. Boric acid inhibits invasion of MDAH-2774 cells

Matrigel invasion chamber assay was used to detect the effect of boric acid on invasion in MDAH-2774 cell line. It was observed that boric acid inhibited the invasion in dose treatment groups, compared with the control groups (Fig. 4A). According to the results, the invasion capacities of the control cells were 79.0 ± 1.63%, while the percentage of invasion was 36.66 ± 1.24% in the dose group administered boric acid (Fig. 4B).

3.6. Boric acid reduces colony formation capacity of MDAH-2774 cells

Colonies larger than 0.1 cm were evaluated to determine the effect of boric acid on colony formation. While the average number of colonies in the control group cells was 96.0 ± 3.77, the number of colonies was determined as 72.0 ± 1.4 in the dose group cells treated with boric acid. When the colony images of the cells were evaluated, it was remarkable that the number of colonies between the control group colony number and the boric acid administered dose group was significantly different, and there was significant decrease in the number of colonies of the MDAH-2774 cell line after boric acid (Fig. 5).

3.7. Boric acid inhibits migration of MDAH-2774 cells

Wound healing migration assay was performed to detect of effect of boric acid on migration in MDAH-2774 cell line. According to our results, boric acid reduced migration of MDAH-2774 ovarian cancer cells, compared with the control group. 0th and 24th hour images were given in Fig. 6.

3.8. Boric acid increases oxidative stress in MDAH-2774 cells

To evaluate the effects of boric acid on oxidative stress in MDAH-2774 ovarian cancer cells, TAS, TOS, and OSI were measured. Calibration and relative readings are based on the Trolox equivalent standard solution for TAS assay. Calibration and relative readings are based on micromolar H_2O_2 equivalent per liter for TOS assay. While TOS levels were decreased, TAS levels were increased in MDAH cells treated with boric acid. Oxidative stress index increased in the group treated with boric acid in MDAH-2774 ovarian cancer cells (Fig. 7).

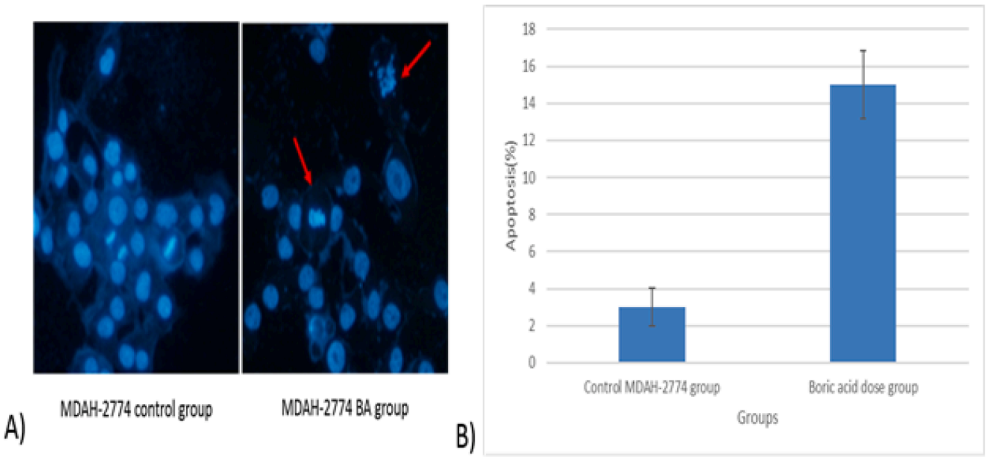

Fig. 3. (A) The cells were stained with hoechst dye and imaged at 20x magnification in a fluorescent microscope. The red arrows show the apoptotic cells, (B) The number of apoptotic cells was significantly increased in both MDAH-2774 cells treated by boric acid compared with the control cells (Data were expressed as mean ± SD, n = 10). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

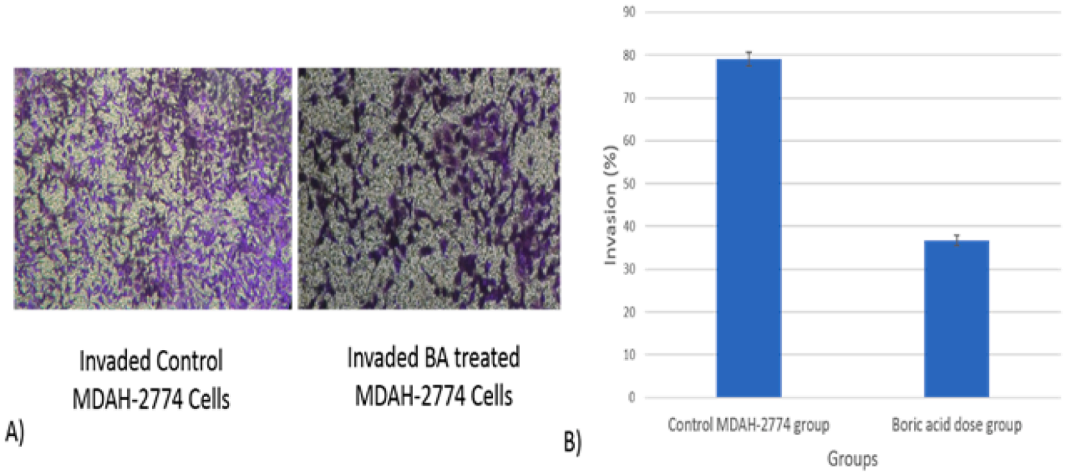

Fig. 4. (A) Crystal viole stained images of invaded cells in control and boric acid treated cells (Magnification: 40x), (B) % Invasion Rate in MDAH-2774 ovarian cells with boric acid compared to control group (Data was presented as mean ± SD. n = 3).

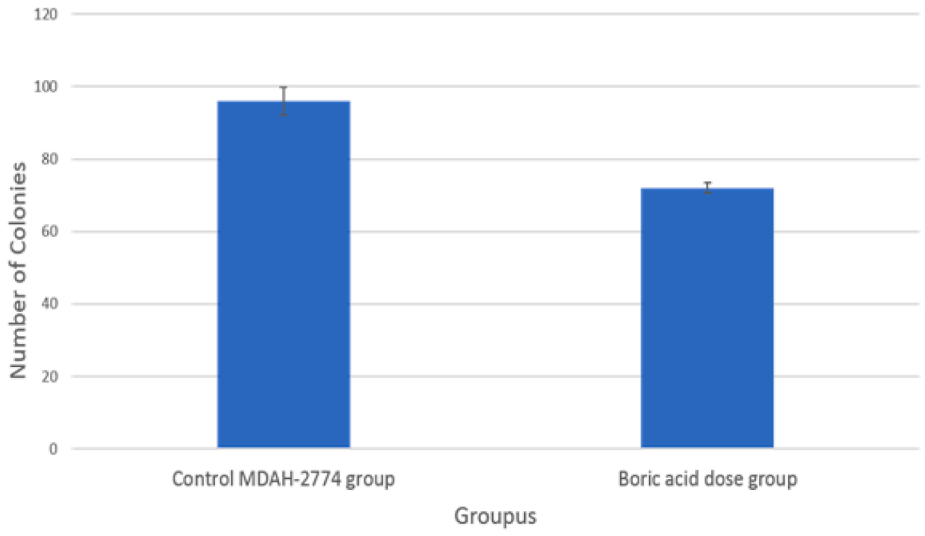

Fig. 5. Colony numbers were counted in the control and dose groups (Data are expressed as mean ± SD, n = 3).

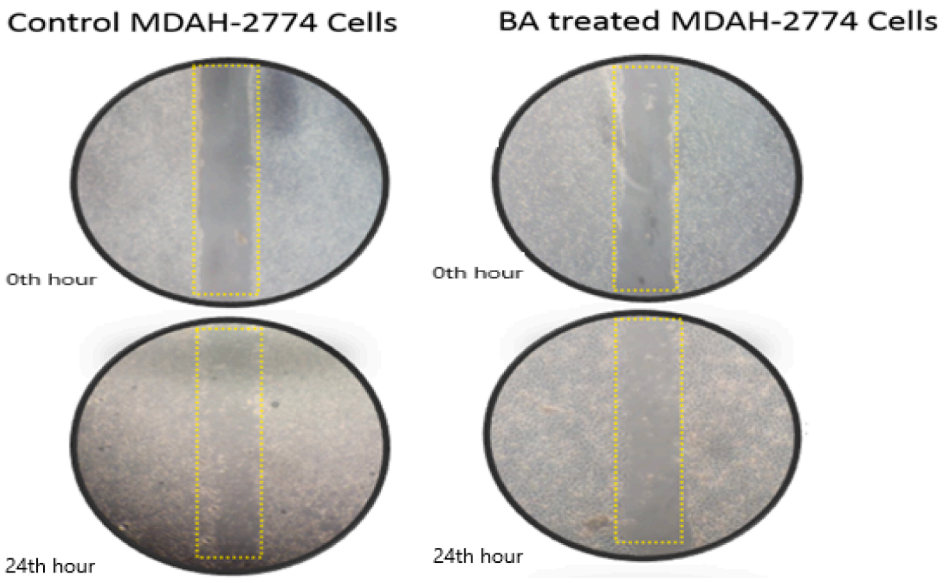

Fig. 6. Wound healing petri images in control and boric acid added dose groups. The results showed that boric acid decreased cell migration. Images of control land dose group cells at 0 h and 24 h were given.

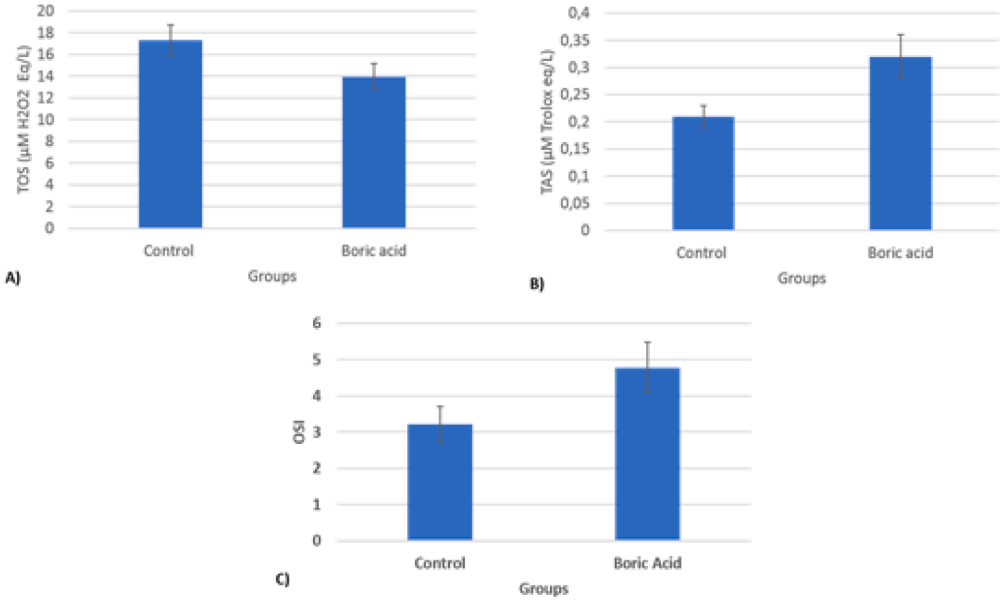

Fig. 7. TAS, TOS and OSI values in study groups.

4. Discussion

Many novel agents are being researched in terms of anticancer properties to support the standard chemotherapy used in cancer treatment nowadays. Boron element and Boron origin compounds are among these agents. In current study, the *in vitro* cytotoxic effects and possible anti-cancer properties of boric acid were investigated by examining colony formation, migration, invasion, cell proliferation, cell cycle, apoptosis, miRNAs, and oxidative stress in human ovarian cancer cell line MDAH-2774.

The caspase-cascade system has a pivotal role in the induction of apoptotic signals (Creagh et al., 2003; Harwood et al., 2005). Caspase-9, and especially Caspase-3 activate the apoptotic pathway (Salvesen, 2002; Boatright and Salvesen, 2003). The activation and function of caspases are regulated by a variety of molecules. *Bcl-xL*; *Bcl-2*, and *Bax Bcl-2* gene family members; *Bcl-xL* and *Bcl-2* act as anti-apoptotic proteins, preventing the secretion of pro-apoptotic factors like cytochrome *c*. On the other hand, *Bax* plays an active role in the release of cytochrome-c and pro-apoptotic factors from mitochondria (Korsmeyer, 1995). In our study, we observed that boric acid caused significant increases in the apoptosis related genes expressions of *Bax, Bid, caspase-3* and *caspase-9* which are inducing apoptosis; conversely, there were considerable decreases in the expression of apoptosis related proteins *Bcl-2* and *BcL-xL* which are negative regulator of apoptosis. The rate of apoptotic cells of the control group and boric acid dose group were determined as $3.0 \pm 1.0\%$ and $15.0 \pm 2.44\%$, respectively, so we can say that boric acid induced apoptosis was supported by our TUNEL assay outcomes.

Cell division is the mechanism by which genes are passed from one generation of cells to the next. There is a cell cycle control system including a set of molecules that control the cell cycle through positive and negative regulation. Cyclin dependent kinases (CDKs) and cyclins

are located at the heart of cell control points. The conversion of CDKs into active kinases by forming complexes with cyclins is necessary for the continuation of the cell cycle (Satyanarayana and Kaldis, 2009). We found insignificant changes in mRNA expression of *Caspase-8, Caspase-10, Cyclin D1, Cyclin D2, CDK6, CDK4, FADD, TRADD, FAS, DR4* and *DR5* gene in boric acid treated group compared with control. These results elucidate a boric acid-induced apoptotic pathway in MDAH-2774 cells that are involved in the up-regulation of apoptotic genes and the down-regulation of anti-apoptotic genes. Nevertheless, boric acid did not affect cell cycle arrest related genes in ovarian cancer cells. These results suggested that boric acid induced apoptosis by using the mitochondrial pathway rather than through the cell cycle. These results were partially in line with the observations of some previous reports (Psurski et al., 2019), but not with others (Scorei et al., 2008; Barranco et al., 2007).

miRNAs which are endogenous, small non-coding RNAs are involved in the regulation of post-transcriptional processes of gene expression in cytoplasm (Deb et al., 2018; Mirzaei et al., 2016). miRNAs are involved in multiple biological activities, but dysregulation of miRNAs has been broadly observed in different stages of human cancers including ovarian cancer (Razavi et al., 2021; Shabaninejad et al., 2019). miRNAs could act as either tumor suppressors or oncogenes under certain conditions (Di Leva et al., 2014). Recently some studies have indicated that miRNAs have a potential to be used as biomarkers in the diagnosis and prognosis of ovarian cancer (Pal et al., 2015; Yoshida et al., 2020). For instance, *miR-21, miR-200a, miR-130a* and *miR-224* are indicators of a poor prognosis of ovarian cancer (Mahmoud et al., 2018; Zhao et al., 2014; Shi et al., 2019; Chao et al., 2014). Echevarría-Vargas et al. showed that microarrays and additional methods were used to evaluate miRNA expression in ovarian cancer tissue, and found that 12 miRNAs were upregulated, including miR-21 (Echevarría-Vargas et al., 2014). Additionally, it has been shown that miR-21 was overexpressed in ovarian cancer tissues, ovarian cell lines, and serum of patients with ovarian carcinoma (Robelin et al., 2020; Song et al., 2020). Among the dysregulated miRNAs, miR-21 (upregulation) was identified to have the highest change between normal and ovarian cancer (Nam et al., 2008). miR-21 promotes the cell proliferation, invasion and migration abilities in ovarian epithelial carcinomas (Lou et al., 2010). Also it has been demonstrated that the advanced stage (FIGO III/IV) ovarian cancer had comparatively high expression of miR-21 as compared to early stage of ovarian cancer (Paliwal et al., 2020). Jiang et al. demonstrated that miR-200a expression was significantly elevated in ovarian cancer tissues and cell lines. Their results indicated that overexpressed miR-200a promoted the proliferation and invasion of ovarian cancer cells (Jiang et al., 2018). Also, miR-200a is highly up-regulated in different epithelial ovarian cancer subtypes such as serous, endometrioid and clear cell (Iorio et al., 2007). miR-200a is associated with high-grade and late-stage tumors (Coppola et al., 2008). miR-200a is also possibly involved in the pathogenesis of ovarian cancer and is a promising biomarker for the prognosis of ovarian cancer (Shi et al., 2019). miR-130a promotes proliferation/invasion and induces epithelial-mesenchymal transition (EMT) of ovarian cancer cells *in vitro* (Wang et al., 2017). Upregulation of miR130a might be associated with MDR1/P-glycoprotein-mediated drug resistance in SKOV3/CIS cells, while downregulating miR-130a expression might overcome cisplatin resistance (Yang et al., 2012). Over-expression of miR-130a contributes to the development and regulation of cisplatin resistance in ovarian cancer cells. Inhibiting the expression of miR-130a is a novel approach for overcoming drug resistance in ovarian cancer (Li et al., 2015). miR-224 inhibits the expression of KLLN, subsequently suppress cyclin A expression and then promotes epithelial ovarian cancer cell proliferation (Hu and Liang, 2017). miR-224 functions as an oncogene and induces platinum resistance in ovarian papillary serous carcinoma (Zhao et al., 2014). In our study, we found that miR-21, miR-200a, miR-130a and miR-224 expressions were significantly decreased in dose group when compared with the control group. These results indicate that boric acid induced anti-tumor effect may be associated with alterations in miRNAs.

Cell motility and migration are main components of inflammation and wound healing and vital to normal development. However, dysfunctions in the signaling pathways that control its regulation can lead to pathological processes of cancer cell invasion and metastasis (Tahtamouni et al., 2019). Understanding the mechanisms underlying metastasis is very crucial for the development of effective cancer therapies (Jiang et al., 2015). Boric acid has already been shown to reduce invasion capacity of cancer cells (Barranco and Eckhert, 2006). In our study, matrigel invasion chamber assay outcomes revealed that boric acid reduced invasion capacity (*79.0 ± 1.63% in the control group cells, and 36.66 ± 1.24% in the dose group cells*) of MDAH-2774 ovarian cancer cells. Also, wound healing migration assay demonstrated that boric acid reduced cell migration of ovarian cancer cell under *in vitro* conditions. Moreover, results of colony formation test indicated that, numbers of colonies in the dose group cells treated with boric acid were suppressed (*96.0 ± 3.77 in the control group cells, and 72.0 ± 1.4 in the dose group cells*). Our outcomes suggested that boric acid decreased cell invasion, migration and colony formation capacity in MDAH-2774 ovarian cancer cells.

Oxidative stress emerges when there is an imbalance between the productions of free radicals and antioxidant system. It is considered to be a major cause of several pathological conditions such as cancer, particularly in terms of molecular damage to cellular structures (Gupta et al., 2014). The relationship between increased oxidative stress and ovarian cancer is widely known (Zhang et al., 2019). Although there were publications showing that boric acid stimulated the antioxidant system (Çelikezen et al., 2014; Cengiz et al., 2020), our findings showed the opposite. Interestingly, in our study, oxidative stress index increased in the dose group cells treated with boric acid. It will be logical to assume that boric acid can induce cell death by increasing oxidative stress process in MDAH-2774 ovarian cancer cells.

5. Conclusions

Inhibition of carcinogenesis including dysfunction of cell cycle control, invasion, and migration is very important point for anti-cancer agent development. To reveal its therapeutic potential, the effects of boric acid on ovarian cancer cells under *in vitro* circumstances were investigated in current study. The presence of boric acid as a natural molecule may potentiate its effectiveness in reducing side effects attributed to conventional ovarian cancer treatments. Based on our findings, we would like to put emphasis on the need for future advanced animal studies.

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CRediT authorship contribution statement

Umit Cabus: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration. **Mucahit Secme:** Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft. **Cihan Kabukcu:** Conceptualization, Formal analysis, Visualization. **Nazli Cil:** Validation, Investigation, Resources, Visualization. **Yavuz Dodurga:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing - review & editing. **Gulcin Mete:** Methodology, Validation, Resources, Writing - review & editing. **Ibrahim Veysel Fenkci:** Conceptualization, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2021.145799.

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