

# Boric Acid Inhibits Cell Growth in SH-SY5Y Neuroblastoma Cell Lines

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**Abstract:** Boric acid (BA) form of boron is the most common in humans. BA is a weakly unionized acid at the physiological pH. In the current study, we investigated to cytotoxic effect of BA on SH-SY5Y cell line. BA used in this study is commercially purchased. It was dissolved in water by probe sonicator under certain conditions and characterization analyses were carried out. The synthesized BA was applied to the neuroblastoma SH-SY5Y cell line and the cytotoxic effect of BA were determined by using MTT method. SH-SY5Y cells were treated with different concentrations of BA (1-200µg/ml) for 24, 48 and 72 hours. The effects of BA-water on the neuroblastoma SH-SY5Y cell line were compared to the control group and IC<sub>50</sub> values were found for 24, 48 and 72 hours. In this study, it was shown that the effect of BA -water system on neuroblastoma SH-SY5Y cell line was inhibitory to growth in cancer cells compared with control group. However, there is no study on BA-water so far. This study will provide new information in that regard.

**Keywords:** Neuroblastoma, SH-SY5Y, Boric acid, MTT

## 1. Introduction

Neuroblastoma treatment continues to be a difficult pediatric cancer, with progression of disease with high-risk tumors. Approximately 15% of such patients have a 5-year survival probability [1]. While many of the high-risk tumors respond to initial chemotherapy, most of these patients eventually develop chemotherapy-resistant diseases [2,3]. Progressive neuroblastomas after chemotherapy may result from altered expression of genes associated with drug resistance [4]. To overcome drug resistance, the underlying mechanisms for successful response to chemotherapy in NB cells as well as the failures of treatment should be understood. The molecular response of tumor cells to cytotoxic agents has become the focus of these efforts because it is clear that these pathways can lead to tumor cell death, and that their absence or failure may lead to resistant disease [5]. The presence of novel compounds with low toxicity and high sensitivity has an important place in cancer research [6].

Recent studies have investigated the effects of pharmacological concentrations of BA on cell morphology, proliferation and metastasis [7]. Boron has been shown to be useful for many species, but it remains unclear in the cellular part of the animals [8]. The addition of certain compounds to the boron compound gives antitumor properties in different cancer cell lines [9]. The mechanism of action of these compounds is thought not to be affected by apoptosis but by inhibition of histone deacetylase [10-12]. Proteasomes are protease complexes found in the cytoplasm of eukaryotes with several different catalytic regions. It plays an important role in the breakdown and processing of proteins, peptides and amino acids in cellular pathways [13]. In this study, we investigated BA for a new cancer treatment strategy and anticancer activity was assessed on SH-SY5Y neuroblastoma cells.

## 2. Materials and Methods

### 2.1 Materials

BA obtained commercially from Sigma Aldrich and was used as purchased. No dispersant/surfactant was used in BA-water suspension.

### 2.2 Dispersion of boric acid

The commercially available BA was prepared at 25 °C for 60 minutes in 750W power probe sonicator (Sonics & materials INC, USA) to ensure homogeneous distribution of deionized water in different mass fractions. It was observed that BA was completely dissolved in water even at ambient temperature. The prepared suspensions retained their stability even at ambient temperature for days or months.

### 2.3 Characterization

The morphology of BA was measured by a scanning electron microscope (TESCAN MIRA3 XMU). X-ray diffraction (XRD) data were obtained by a diffractometer (Rigaku DMAX IIIC). In order to homogeneously disperse BA in water, a probe sonicator (Sonics & amp; materials INC, USA) at 750W power was used.

### 2.4 Cell Culture

SH-SY5Y neuroblastoma cells were maintained in DMEM medium, containing 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (10 mg/L). Cells were grown in at 37 °C, 5% CO<sub>2</sub> and 95 % air in a humidified incubator. For each cell line, 70-80% confluent cell culture flask was trypsinized and cells were seeded in 96 well plates.

## 2.5 Cytotoxic effect of BA on SH-SY5Y cells

Cytotoxicity effect of the BA against SH-SY5Y cell lines was performed with the MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay according to the Skehan's method [11]. Briefly, cells were trypsinized and plated into 96-well plates (Corning, USA) in 0.1 mL of complete culture medium at a density of  $1 \times 10^5$  cells per well and allowed to attach for 24 h. 1  $\mu$ L of test substance at concentrations ranging between 1-200  $\mu$ g/ml were added into each well containing the cells. The plates were incubated at 37°C with an internal atmosphere of 5% CO<sub>2</sub>. After 24, 48 and 72 h incubation, with different concentrations of compounds, MTT (5 mg/ml dissolved in PBS) 10  $\mu$ l/well was added directly to all the wells and incubated for 2 hours at 37°C. After mixing with a mechanical plate mixer for 15min, the absorbance of plates was recorded at 570 nm on a microplate reader (Bio-Tec, USA).

## 3. Results and Discussion

### 3.1. Boric acid

According to the scanning electron microscopy data (Figure 1), boric acid powder is polydisperse systems. As can be observed from the above-mentioned micrograph, the particles are spherical and have quasi-agglomerated structures.

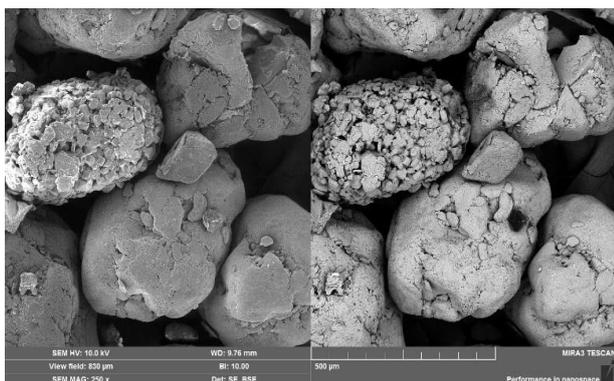


Figure 1: SEM micrograph of boric acid.

XRD analyses were performed at 35kV and 20 mA by using Cu-K $\alpha$  radiation. In Fig. 2, the maximum intensity percentages (I%) of boric acid are observed at the 2 $\theta$  values of 27.68°, 14.82°, and 14.28°. In accordance with the XRD pattern of boric acid, boric acid mineral is determined as Sassolite with the powder diffraction file of "01-073-2158" and the XRD score of 62.

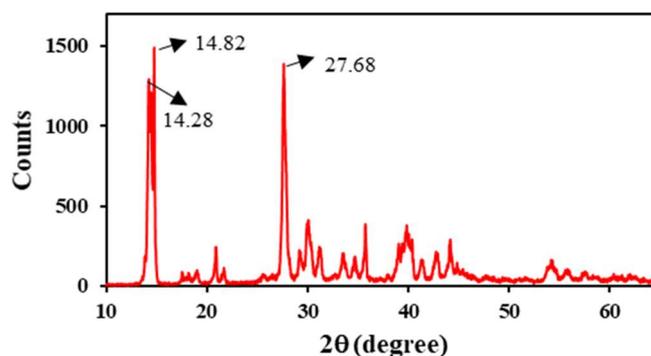


Figure 2: XRD pattern of boric acid

### 3.2. Cytotoxicity activities of BA on SH-SY5Y cells

The boron has a high affinity to oxygen and, depending on the pH, is present in the aqueous solution either as BA (B(OH)<sub>3</sub>) or borate (B(OH)<sub>4</sub><sup>-</sup>). Since the pK<sub>a</sub> of the equilibrium between B(OH)<sub>3</sub> and borate B(OH)<sub>4</sub><sup>-</sup> is 9.2, at intracellular pH (7.4) free boron exists as the weak Lewis acid, BA. BA, a small molecule with a mass of 61.83, is rapidly absorbed from the human intestine and excreted in the urine with a half-life of 21 hours [14,15]. The most common inorganic form of Boron is BA. Several studies have shown that BA has anti-tumor properties [16-18]. However, there is not enough in vivo study to verify the toxicity of boron / BA or to demonstrate its usefulness as an anti-cancer agent [19]. A study conducted in vitro found that BA inhibited the growth of human prostate cancer cell lines [16]. In our study, SH-SY5Y cells were exposed to a range of concentrations of BA rate was examined by MTT (Figure 3). Compared to the control group, BA, SH-SY5Y neuroblastoma cells showed significantly decreased tumor survival rate after 24h, 48h and 72h of incubation. Cell survival rates in BA after 24h, 48h and 72 h of incubation were significantly decreased than those in the control group. Effect of BA on SH-SY5Y cells was the most active for 72 h of incubation. In addition, the most active BA IC<sub>50</sub> values for 24, 48 and 72 hours were 73,11  $\mu$ g/ml, 51,55  $\mu$ g/ml and 21,40  $\mu$ g/ml respectively (Table 1).

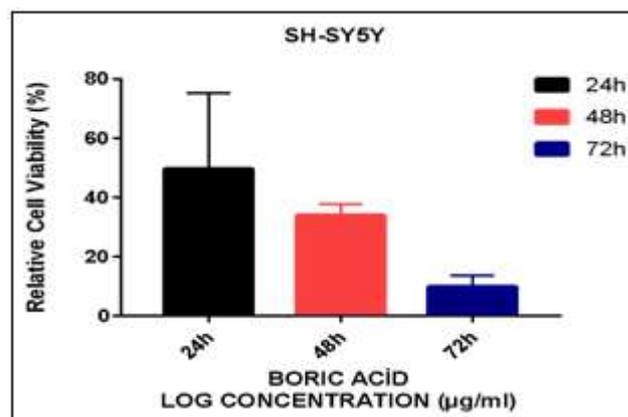


Figure 3: Cytotoxicity activities of of BA on SH-SY5Y cell line

Table 1: Comparison of IC<sub>50</sub> values between BA on SH-SY5Y after 24 h, 48 h and 72 h of incubation.

Time	Boric Acid IC <sub>50</sub> (µg/mL)
24h	73,11
48h	51,55
72h	21,40

#### 4. Conclusions

BA was prepared homogeneously in the water with the help of probe sonicator in ambient temperature and observed that the solution was stable. In summary, this work showed that BA has a cytotoxic effect on SH-SY5Y cells compared with control group. We indicate that high concentrations of BA affect SH-SY5Y cells in 24, 48 and 72h times. There is not enough data is available about BA toxicity, so BA use as cancer treatment can be possible if new toxicity studies are performed. In our study, BA application for the treatment of neuroblastoma was demonstrated. Apart from this study, more studies are needed to determine the effects of boric acid completely and precisely.

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