RESEARCH ARTICLE

Antifungal efficacy of pure boron on yeast and mold isolates causing superficial mycosis

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Abstract

Objective: To examine the in vitro antifungal effects of water-soluble pure elemental boron with an alkaline solution against *Candida species*, *Trichophyton species*, and *Aspergillus fumigatus* that cause superficial mycosis.

Method: The study was conducted at the microbiology laboratory of Kahramanmaras Sutcu Imam University Hospital, Kahramanmaras, Turkey, from June to December 2018, and comprised fungal strains isolated from patients with superficial mycosis who visited the dermatology clinic. The in vitro antifungal effects of the boron solution at various concentrations were determined using the microbroth dilution method. *Candida albicans* ATTC 90028 and *Candida albicans* MYA 274 served as the quality control strains, while fluconazole and amphotericin B were used as comparator antifungal agents. Data was analysed using SPSS 22.

Results: Of the 58 strains, 28(48.3%) were *Candida albicans*, 9(15.5%) non-*Candida albicans*, 12(20.7%) *Trichophyton rubrum*, 4(6.9%) *Trichophyton mentagrophytes*, 2(3.4%) *Trichophyton species* and 3(5.2%) were *Aspergillus fumigates*. Boron at a concentration of 78.125 µg/mL inhibited the growth of *Candida albicans*. The 50% and 90% minimum inhibitory concentrations of the solution in non-*Candida albicans* were 78.125 and 312.5 µg/mL, respectively, whereas those in *Trichophyton rubrum* were 312.5 and 625 µg/mL, respectively. The 50% minimum inhibitory concentration of the solution in *Aspergillus fumigatus* was 625 µg/mL, whereas the 90% minimum inhibitory concentration could not be determined.

Conclusion: Boron is an inexpensive, non-antibiotic element with potential uses as an antifungal agent.

Keywords: Antifungal agents, Antimicrobial susceptibility, Boron, Superficial mycoses, Minimum inhibitory concentration. (JPMA 72: .1330 2022) **DOI: https://doi.org/10.47391/JPMA.2219**

Introduction

Boron is a ubiquitous element in nature even at low concentrations.[1] This naturally-occurring element has two stable isotopes: 10B (19.8%) and 11B (80.2%).[2] Boron is consistently found covalently bound to oxygen as boric acid or tetraborate. These covalent bonds cannot be broken without extreme laboratory manipulation because of the ionic form of boron, which is controlled by both the acid dissociation constant (pKa) of the molecule and potential hydrogen (pH) of the aqueous medium.[1] Boric acid, which is also referred to as boracic or orthoboric acid, is produced by exposing a concentrated solution of borax to hydrochloric or sulfuric acid. Boric acid is highly preferred for treating burns and surface-level wounds as a mild antiseptic, and it is also one of the main ingredients in eye creams.[3]

As boric acid is a weak acid, it is essentially present as an undissociated acid (H_3BO_3) at physiological pH in an aqueous medium.[1] Boron compounds are expected to have similar toxicity. Boron oxide has similar effects and reacts with water in the body to form boric acid.[2] Boric acid may form complexes with carbohydrates and proteins in the body.[4] It is the sole boron compound detected in urine and has been found at levels >90% of the dose of ingested boron.[2] There is no evidence in literature that boric acid is degraded in the body. Moreover, research has indicated that boric acid and borate compounds are present in the body in the undissociated form, which accumulates in the bone and spreads to soft tissues.[5]

Boric acid, as a fungistatic agent, has been preferred for treating recurrent vulvovaginal candidiasis, particularly for non-*Candida albicans* and *Saccharomyces cerevisiae* infections.[6] Several studies have investigated the efficacy of boric acid and organoboron compounds against yeasts and molds.[4,5,6] However, boric acid is potentially toxic, particularly if it is ingested or systematically absorbed.[7-13]

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In addition to being non-soluble in water, elemental boron has limited industrial applications, and no study has cited its potential antifungal activity. By contrast, several studies have described the antimicrobial and antifungal properties of boric acid and boron compounds.[8-12]

The current study was planned to investigate the in vitro antifungal effects of pure boron with pH 7.89, diluted and dissolved in distilled water against yeasts and moulds isolated from patients with superficial mycosis.

Materials and Methods

The study was conducted at the microbiology laboratory of Kahramanmaras Sutcu Imam University Hospital, Kahramanmaras, Turkey, from June to December 2018. After approval from the institutional ethics review committee, via distillation at 2,000°C, boron solution of a minimum purity of 99% was obtained by reducing boron oxide. The solution was diluted in distilled water to the desired concentration of 2% boron (pH 7.89 at 24.6°C). The boron solution has been submitted for a patent.[14] Because the application has not been approved yet, no details are provided to ensure the confidentiality of the procedures used and allow the patent administration system to run the process for the first-time submission.

The concentration of the boron-containing solution is expressed in percentages and can be converted to mg/L as follows: 2% boron is equal to 20,000 ppm and 20,000 mg/L. The 50% and 90% minimum inhibitory concentrations (MIC_{50} and MIC_{90}, respectively) are the concentrations at which microbial growth is inhibited by 50% and 90%.

Strains of *Candida (C.) albicans*, non-*C. albicans*, *Trichophyton (T.) rubrum*, *T. mentagrophytes*, *Trichophyton species*, and *Aspergillus (A.) fumigatus* were isolated from patients with superficial mycosis who visited the dermatology clinic. In the microbiology laboratory, the isolates were cultured on Sabouraud's dextrose agar (SDA) (BD, USA), which was supplemented with gentamicin and cycloheximide, and incubated at 35°C. All isolates were identified by conventional methods, which included the macroscopic and microscopic examination of the culture strains. *Candida* isolates were simultaneously tested in the BD Phoenix 100 automated system (BD, USA) using the Yeast ID Panel (Phoenix). The isolates were stored at 20°C in skim milk until used.

For the determination of MICs, Roswell Park Memorial Institute 1640 (RPMI 1640) was used, containing L-glutamine and a pH indicator but no bicarbonate (Sigma, Milan, Italy), supplemented with glucose at a final concentration of 2% and buffered with 3-(N-morpholino) propanesulfonic acid (Sigma) at a final concentration of 0.165 mol/L with pH 7.0.

Each inoculum was prepared by suspending approximately five colonies from an 18–24-h culture on SDA in sterile distilled water. The final inocula displayed a range between 0.5×10^5 and 2.5×10^5 mL, with adjustment to a cell density of 0.5 McFarland standard.

The isolates were subcultured on potato dextrose agar at 35°C for 2-5 days to produce conidia. The fungal colonies were covered with 5 mL of 0.1% Tween 20, and the suspensions were made by slightly probing the surface of the colony and transferred with a pipette to a sterile tube. The suspension was vortexed for 15s using a rotating vortex mixer at approximately 2,000rpm. Inoculum was quantified by counting microconidia via microscopic examination. The suspension was then diluted 1:10 using sterile distilled water to obtain a final working inoculum of 2×10^5 to 5×10^5 cfu/mL.

Antifungal susceptibility testing was performed in vitro for yeasts using the EUCAST E.DEF 7.3.1 broth microdilution method, and for moulds using the EUCAST E.DEF 9.3.1 broth microdilution method. The test was performed in flat-bottom microdilution plates. Each isolate was tested in 1 row of 12 wells. In total, 50 µL of boron solution containing 2% (20,000m g/L) pure elemental boron in distilled water was dispensed by two-fold serial dilution at the concentrations of 9.7, 19.5, 39, 78.125, 156.25, 312.5, 625, 1,250, 2,500 and 5,000 mg/L. RPMI 1640 (100 µL) was added into all the wells, and 50µL of the fungal suspension was added to the first 11 wells of each row. In the 12th well of each row, 50 µL of sterile deionised water was added. The 12th well of each row was used as a sterile control, and the 11th well without boron was used as a growth control, labelled as the positive control well. The plates were incubated at 35°C for 24-48 h and absorbance was measured at 450 nm (Figure 1). The minimum fungicidal concentrations (MFCs) were examined by transferring approximately 10 µL from two wells of the microdilution plate without visible growth and diluting the solutions in 10 mL saline. Further, 100 µL of the suspensions were spread on to SDA and incubated at 35°C for 24-48 h. MFCs were determined as the lowest concentrations that resulted in no growth on the subculture after 2 days for *Candida species* and 5 days for moulds.

C. albicans ATTC 90028 and *C. albicans* MYA 274 were used as quality control strains in each microplate experiment, and fluconazole and amphotericin B were used as comparator antifungal agents to test the antifungal susceptibility of *C. albicans* ATTC 90028 and *C. albicans* MYA274. Fluconazole and amphotericin B were dissolved in dimethyl sulfoxide (Sigma Chemical Co.). MIC for

amphotericin B was interpreted as the lowest concentration that inhibited growth by ≥90% relative to the drug-free control, whereas that for fluconazole was interpreted as the lowest concentration that inhibited growth by ≥50%.

Data was analysed using SPSS 22. The distribution of variables was examined using Shapiro-Wilk test. Kruskal-Wallis H test was used to compare the variables that did not show normal distribution, and Dunn's test was used for pairwise comparisons. Statistical parameters were expressed as medians. P<0.05 denoted statistical significance.

Results

Of the 58 strains, 28(48.3%) were *C. albicans*, 9(15.5%) non-*C. albicans*, 12(20.7%) *T. rubrum*, 4(6.9%) *T. mentagrophytes*, 2(3.4%) *Trichophyton species* and 3(5.2%) were A. fumigates. The most sensitive strain was C. albicans, whereas the most resistant strain was A. fumigatus, with an MIC ranging between 312.50 µg/mL and >5,000 µg/mL. MIC_{50} and MIC_{90} were both 78.125 µg/mL for *C. albicans* ATCC 90028,

Table: In vitro activities of boron against yeasts and moulds.

Species (no. of strains tested)	MIC (µg/mL)	MIC (GM) (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Candida albicans (28)	39.06–625.00	78.125	78.125	78.125
Non-*Candida albicans* (9)	39.06–312.50	156.25	156.25	156.25
Trichophyton rubrum (12)	19.53–>5,000	352.12	312.50	1,250.00
Trichophyton mentagrophytes (4)	78.125–>5,000	473.66	312.50	1,250.00
Aspergillus fumigatus (3)	312.50–>5,000	625.00	625.00	625.00
Candida albicans (ATCC 90028) (Amphotericin B- and fluconazole-susceptible strain)	-	-	39	78.125
Candida albicans (MYA 274) (Amphotericin B-resistant and fluconazole-susceptible strain)	-	-	78.125	78.125

GM: Geometric mean; MIC: Minimum inhibitory concentration

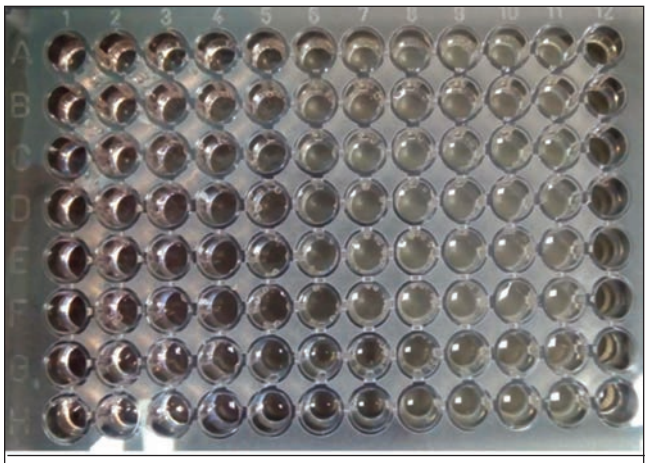

Figure-1: In vitro antifungal susceptibility testing in yeasts.

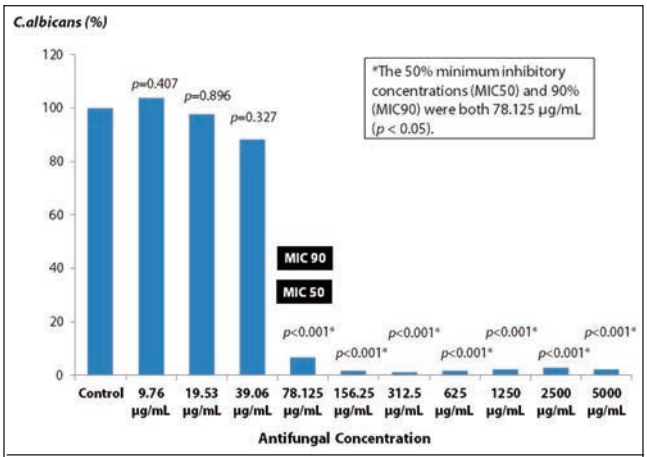

Figure-2: *Candida albicans* growth rates in microplates in the presence of various boron concentrations.

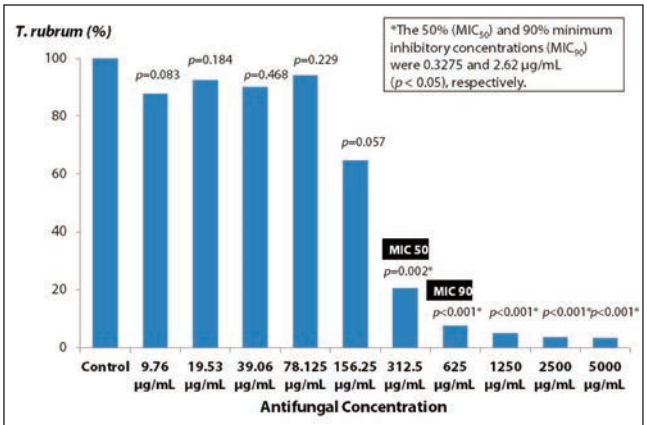

Figure-3: *Trichophyton rubrum* growth rates in microplates in the presence of various boron concentrations.

whereas the values for *C. albicans* MYA274 were 39 µg/mL and 78.125 µg/mL, respectively. The MICs for fluconazole and amphotericin B ranged 1-2 µg/mL for *C. albicans* ATTC 90028 and 0.5-4 µg/mL for *C. albicans* MYA 274. *C. albicans* MYA274 was considered resistant to amphotericin B (Table).

The antifungal effects of boron on the different strains were significantly different compared to the positive control ($p<0.001$). Boron inhibited the growth of both *C. albicans* and non-*C. albicans* at low concentrations (Figure 2). MIC_{50} and MIC_{90} for non-*C. albicans* were 78.125 µg/mL and 312.5 µg/mL ($p<0.05$). The lowest MIC was observed for *T. rubrum*; MIC_{50} 312.5 µg/mL ($p=0.002$) and MIC_{90} 625 µg/mL ($p<0.001$ (Figure 3). Regarding the 3(5.2%) A. fumigatus strains, MIC_{50} was 625µg/mL, whereas MIC_{90} could not be determined ($p>0.05$).

Discussion

The current study aimed at determining the antifungal activity of an alkaline boron solution against yeasts and

moulds. To the best of our knowledge, this is the first in vitro antifungal study of a pure elemental boron solution at an alkaline pH. We propose that boron is nontoxic to humans at an alkaline pH, and it could have several advantageous pharmacological properties, including the reduction of inflammation, erythema, or irritation, on surfaces, such as the vaginal mucosa, skin, and eyes, when used as topical antifungal agent. By contrast, the long-term use of azoles and their derivatives can lead to side-effects and drug resistance.

The effects of boric acid and other boron compounds have been investigated for many years. Although boric acid has been used as a topical antifungal medication for vaginal and skin infections in literature,[15,16] the current study aimed at scrutinising the effects of pure boron in an alkaline composition. In some clinical studies, boric acid was also used for treating chronic suppurative otitis media.[8,9] The ototoxic potential of boric acid was investigated in some animal studies, and results revealed a tympanic membrane toxicity of 4% boric acid in 70% alcohol.[8-10] Microbiological studies showed that applying acid to an infected surface lowers pH and negatively affects bacterial growth. An acidic pH also affects the release of oxygen, which is necessary for cell survival, and several studies have proposed that boric acid inhibits yeast growth because it is a weak acid.[16] However, other organic acids with significantly lower pKa values than boric acid did not exert inhibitory effects on *Candida* growth.[17] In the present study, a boron solution with a minimum purity of 99% at an alkaline pH restricted the growth of *Candida species* and moulds. Boron derivatives used in studies so far have been related to the applications of acidic and basic boron salts in liquid form. The boron solution used in the present study was purified and prepared at a near-neutral pH of 7.89.

These findings support another mechanism of the antifungal efficacy of boron and its compounds. A study noted that low concentrations of boric acid reduce the corresponding ergosterol content of yeast membranes. Moreover, sodium borate at an alkaline pH intrinsically has stronger growth inhibitory effects than boric acid.[11]

Inhibition studies have also been performed using organoboron compounds, which are particularly used as oral nutritional supplements.[8,12,13] The growth of drug-resistant *C. albicans* and *C. glabrata* has previously been found to be inhibited by boric acid and organoboron compounds.[11] From this viewpoint, the advantageous characteristic of boron as an antifungal treatment lies in the pharmacological properties of the agent.

De Seta et al. noted that >90% of *Candida* strains could not grow in the presence of 10,000 mg/L boric acid and that broth dilution showed that the growth >90% of strains was inhibited by 31,000 mg/L boric acid.[12] To confirm these results, the broth microdilution method was used in the present study, and 96.2% of the tested strains were inhibited by 3,125 mg/L boric acid. Moreover, prolonged culture at 50,000 mg/L was fungicidal.[12] The current study observed that boron was fungistatic, but it did not exhibit fungicidal effects, as revealed via the subculture of yeasts and moulds with boron. To provide information to physicians interested in using boron as a remedy, new studies are required to determine the fungicidal concentrations of boron. In another study, 0.5% and 4% boric acid inhibited the growth of 50% and 90% of *C. albicans* strains, respectively.[18] According to the findings of Larsen et al., 1% boric acid restricted the growth of drug-resistant *C. albicans* and inhibited hyphal growth. The MICs of boric acid ranged between 0.07% and 0.31% (0.013-0.051 mg/L).[11] In this respect, the growth inhibition results of the current study agree with the findings related to the inhibitory effects of boron and related compounds.

The current study confirmed that boron inhibited the growth of *Candida species* and moulds that cause mycosis. A concentration as low as 0.0078% (78.125 µg/mL) was sufficient to inhibit the growth of *C. albicans* isolates. For all yeasts, MICs fell within a range between 78.125 µg/mL and 156.25 µg/mL. In a prior broth microdilution study, MICs ranged between 0.025% and 3.2%, with 1% boric acid being equal to a concentration of 10 mg/mL. In particular, the MICs of boric acid ranged between 0.05% and 0.1% for the control strain *C. parapsilosis* ATCC 22019.[19] The MIC_{50} and MIC_{90} of boron for the control strain *C. albicans* (ATCC 90028) were 39 µg/mL and 78.125 µg/mL (range: 00.0039-0.0078%), respectively, whereas those for amphotericin B-resistant *C. albicans* MYA 274 were 78.125 µg/mL and 156 µg/mL (range: 0.0039-0.0156%).

Regarding the antifungal effect of boric acid on dermatophytes, the growth of *T. rubrum* was inhibited in liquid and solid media at low boric acid concentrations of 0.03-0.05%, and a fungicidal effect was observed at 0.1%.[20] The present study suggested that RPMI 1640 is an appropriate medium for testing the effects of boron on yeasts and moulds. MIC_{90} for *Trichophyton species* varied from 19.53 µg/mL to >5,000 µg/mL, whereas those for *Aspergillus species* ranged from 312.50 µg/mL to >5,000 µg/mL. As such, *T. mentagrophytes* is seen to have low boron tolerance among filamentous fungi. The mean MIC_{50} and MIC_{90} differed among the species and were higher in moulds. Boric acid can cause damage to cells owing to the generation of oxygen-free radicals, thereby inhibiting the growth of microorganisms. With the activation of signalling and secretion pathways, boric acid stimulates the synthesis

of extracellular matrix glycoproteins and proteoglycans at lower concentrations. These findings provide evidence that boric acid inhibits mycelial growth and stimulates damaged skin regeneration.[21,22] Recent systematic analyses on the antimicrobial activity of boron emphasise its value as an inexpensive non-antibiotic alternative for microbial control. Boric acid exhibits low toxicity in humans. It is not absorbed through the intact skin and is safe for oral ingestion at doses up to 18 mg/day in adults in its elemental form.[22,23] However, the use of boric acid and borate salts in the first line setting is limited by their acidic pH.

Conclusion

Boron inhibited the growth of both yeasts and moulds. Boron, diluted in distilled water at an alkaline pH, exhibited antifungal effects at considerably low concentrations. The topical application of boron alone or in combination with other anti-fungals could be an alternative treatment for superficial mycosis in patients who respond to standard anti-fungals.

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