ANTIMICROBIAL EFFECT OF A MEANS FOR ENZYME EXCAVATION (BRIX 3000) AND PHOTODYNAMIC THERAPY IN CARIOUS LESIONS OF PRIMARY TEETH – IN VITRO EXPERIMENT

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ABSTRACT

The selective removal of the caries dentin via enzyme methods for excavation appears to be an alternative to the conventional treatment of carious lesions in childhood. Photodynamic therapy is an effective alternative for the reduction of cariesogenic microorganisms. Brix 3000 is an enzyme-based material for excavation of carious dentin.

Aim: To study the antimicrobial effect of means for enzyme excavation (Brix 3000) and photodynamic therapy with FotoSan 630 Intro Kit to the two main cariesogenic microorganisms – S. mutans and L. acidophilus, in vitro experiment;

Materials and Methods: Eighty plates were prepared: group 1- 20 plates only with Brix 3000; group 2 – 20 plates only with FotoSan; group 3 – 20 plates with a combination of Brix 3000 and FotoSan; group 4 – 20 plates without an active agent. 

In the agar of each plate, three 7 mm wells in diameter were made, where the Brix 3000 gel was placed, as well as discs soaked with the dye and irradiated with FotoSan and a combination of them. After 24 hours, the zone of inhibition was measured.

Results: Compared to the control group, Brix 3000 and FotoSan have a defined antimicrobial activity against S. mutans and L. acidophilus, in vitro experiment;

Conclusion: A combination of enzyme-based excavation and photodynamic therapy could be used successfully in the treatment of carious lesions in primary teeth.

Keywords: S. mutans, Lactobacillus spp., photodynamic therapy, Brix 3000,
**MATERIAL**

For the experimental study of the antimicrobial efficacy of Brix 3000 and PDT with FotoSan 630 Intro Kit, 80 plates were prepared, grouped as follows:

- Group 1 – 10 plates each inoculated with *S. mutans* and *Lactobacillus spp.* processed with Brix 3000;
- Group 2 – 10 plates each inoculated with *S. mutans* and *Lactobacillus spp.* processed with FotoSan 630 Intro Kit;
- Group 3 – 10 plates each inoculated with *S. mutans* and *Lactobacillus spp.* treated with the combination Brix 3000 + FotoSan 630 Intro Kit;
- Group 4 – 10 plates each inoculated with *S. mutans* and *Lactobacillus spp.* without an active agent-control group.

**MICROBIOLOGICAL METHOD:**

Lyophilized strains of *S. mutans* and *Lactobacillus spp.* come to life after culturing in broth and repeated subculturing of blood agar and CO₂ atmosphere. After obtaining a pure 24-hour culture, it was standardized according to McFarlan 0.5 Mueller-Hinton agar plates (EUCAST 2020) were inoculated with 5% horse blood and 20 mg/I NAD using a sterile tampon.

In the agar of each plate were made three wells with a diameter of 7mm., where the Brix 3000 gel was applied, as well as discs soaked with the dye and irradiated with FotoSan (photodynamic therapy) and the combination of them.

The formulation thus prepared was incubated for 24-48 hours in a thermostat at 36°C in a CO₂ atmosphere. Inhibition zones were reported, including a well with a diameter of 7mm. and the zone of actual inhibition. The average of the zones of inhibited growth was calculated in each group.

**Image 1. Zone of inhibition obtained by the combined action of Brix 3000 and FotoSan**

The SPSS (version 19, SPSS Inc., USA) was used for the statistical processing of the results. A 95% confidence interval (p<0.05) was chosen as a level of significance at which the null hypothesis is rejected.

**RESULTS**

1. Antimicrobial activity of Brix 3000, FotoSan and their combination to *S. mutans*

   The next table represents the values of reported zones of *S. mutans* inhibition growth in millimeters.

   **Table 1. Antimicrobial activity of Brix 3000, PDT with FotoSan and their combination to *S. mutans***

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean± SD</th>
<th>Ind T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 – Brix 3000</td>
<td>10</td>
<td>9.60 ± 0.699</td>
<td>t₁,₂ = 4.837   p₁,₂&lt; 0.05</td>
</tr>
<tr>
<td>Group 2 – FotoSan</td>
<td>10</td>
<td>8.30 ± 0.483</td>
<td>t₁,₃ = -20.813 p₁,₃ &lt; 0.05</td>
</tr>
<tr>
<td>Group 3 – Brix 3000+FotoSan</td>
<td>10</td>
<td>17.20 ± 0.919</td>
<td>t₁,₄ = 11.759 p₁,₄&lt; 0.05</td>
</tr>
<tr>
<td>Group 4 – control group</td>
<td>10</td>
<td>7.00 ± 0.00</td>
<td>t₂,₃ = -27.110 p₂,₃ &lt; 0.05</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>t₂,₄ = 8.510  p₂,₄&lt; 0.05</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>t₃,₄ = 35.101  p₃,₄&lt; 0.05</td>
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</tbody>
</table>

The average value of the antimicrobial activity of Brix 3000 only against *S. mutans* is 9.60 mm. The antimicrobial activity of Brix 3000 is double when compared to that of FotoSan, in separate usage. The best antimicrobial effect could be observed in ingroup 3, which includes a combination between Brix 3000 and FotoSan (p<0.05).

2. Researching the antimicrobial activity of Brix 3000, FotoSan and their combination to *Lactobacillus spp.*

Table 2 presents the antimicrobial activity of Brix 3000, FotoSan and their combination to *Lactobacillus spp.*
Used separately, Brix 3000 demonstrates a higher antimicrobial activity compared to FotoSan (p<0.05). The two materials combined have higher antimicrobial activity in comparison to their individual use.

Table 2. Antimicrobial activity of Brix 3000, FotoSan and their combination to Lactobacillus spp.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean ± SD</th>
<th>Ind T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 – Brix 3000</td>
<td>10</td>
<td>11.20 ± 0.789</td>
<td>t_{1,2} = 8.547</td>
</tr>
<tr>
<td>Group 2 – FotoSan</td>
<td>10</td>
<td>8.70 ± 0.483</td>
<td>t_{1,3} = -20.889</td>
</tr>
<tr>
<td>Group 3 – Brix 3000+FotoSan</td>
<td>10</td>
<td>19.20 ± 0.919</td>
<td>t_{1,4} = 16.837</td>
</tr>
<tr>
<td>Group 4 – control group</td>
<td>10</td>
<td>7.00 ± 0.00</td>
<td>t_{2,3} = -31.983 t_{2,4} = 11.129</td>
</tr>
</tbody>
</table>

It could be observed from the table that the antimicrobial activity of Brix 3000, used separately, is higher than Lactobacillus spp, when compared to S. mutans. FotoSan demonstrates a relatively similar antimicrobial activity to both microorganisms. The stronger antimicrobial effect of the combination of both materials used simultaneously is to Lactobacillus spp.

**DISCUSSION**

Our results revealed that both Brix 3000 and FotoSan possess an expressed activity with regards to the two main studied microorganisms, S. mutans and Lactobacillus spp. The papain, which is the main component of Brix 3000, is an end protein with bacteriostatic, bactericidal and anti-inflammatory activity. [9,10]. The papain could inhibit the growth of the bacteria since it could degrade the peptide bonds in microorganisms to dipeptides and amino acids. The enzyme papain is included in the sulfhydryl proteases group. This shows that it has a sulfhydrylene residue in its active place, which affects the bacterial cell wall and the cytoplasmic membrane [11].

The essence of photodynamic therapy is the creation of free radicals or of the active oxygen with a short life span. These radicals cause the immediate death of the cell, in which the photosensitizer lies. They have a very short life span and could be deactivated very rapidly, and thus they do not endanger the neighboring cells [11].

Brix 3000 has a higher antimicrobial activity to S. mutans and Lactobacillus spp in comparison to the photodynamic therapy as an individual activity. When the two materials are combined, the activity is increased. This proves a potentiated antimicrobial effect of the combination between Brix 3000 and photodynamic therapy with FotoSan to both microorganisms - S. mutans and Lactobacillus spp.

A number of studies prove the antimicrobial activity of the papain, which is the main component of Brix 3000. Goyal et al. compare the conventional method of excavation with the enzyme one by studying 25 children aged between 5 and 9 years. The microbiological samples are taken before and after excavation of the carious dentine. The results obtained reveal a significant reduction in the number and amount of S. mutans and Lactobacillus spp. Similar results are also achieved by Singh et al. by discovering a reduction in the amounts of S. Mutans and Lactobacillus spp.

Various authors conduct in vitro studies of the antimicrobial effect of the photodynamic therapy against S. mutans and Lactobacillus spp by researching different sources of light and different photosensitizers. Araujo et al. demonstrate a high antimicrobial effect of the photodynamic therapy to S. Mutans and Lactobacillus spp by using curcumin as a photosensitizer and blue light with wavelength 450 nm [14]. In their research, Melo et al. use a diode laser and toluidine blue and prove the high antimicrobial effect of the photodynamic therapy [15]. Their results concur with ours. The same results are achieved by Ricatto et al. in their work, although they use methylene blue as
photosensitizer [16].

The chemio-mechanical technique of excavation of the carious dentine with Brix 3000 in primary teeth demonstrates excellent results in the context of the minimally invasive method. Through the technique, a controlled excavation of the completely damaged dentine only, and only the dentine, which could stimulate a tertiary dentinogenesis and internal remineralization, is reserved.

The potentiated antimicrobial effect, proven by us, of the means of enzyme excavation and follow-up procedure of photodynamic therapy with FotoSan, represents a good reason to recommend the studied means in selective excavation to soft, partially infected dentine in the course of treatment of deep dentine caries or asymptomatic closed pulps. The method is sparing and achieves dentine serving for internal remineralization and stimulation of the tertiary dentinogenesis as well as a healing process on the part of the dental pulp.

CONCLUSIONS:
Brix 3000 for enzyme excavation has a two times higher antimicrobial activity when compared to FotoSan, used separately, to S. mutans and Lactobacillus spp.

By combining Brix 3000 with photodynamic therapy with FotoSan, their antimicrobial activity is significantly increased;

S. mutans is more resilient to Brix 3000 and the photodynamic therapy with FotoSan in comparison to Lactobacillus spp.

This contribution is published in accordance with project Grant-2019 with Contract No.86/23.04.2019 themed “Micro-invasive and Antimicrobial Effect of Chemo-mechanical Excavation Means in Primary Teeth Caries.”

Abbreviations:
PDT - photodynamic therapy

REFERENCES:

Please cite this article as: Lazarova Z, Rashkova M, Gergova G, Stavros Tsitou V-M. Antimicrobial Effect of a Means for Enzyme Excavation (Brix 3000) and Photodynamic Therapy in Carious Lesions of Primary Teeth – in vitro Experiment. J of IMAB. 2021 Oct-Dec;27(4):4048-4051. DOI: https://doi.org/10.5272/jimab.2021274.4048

Received: 19/04/2021; Published online: 18/10/2021

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