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# *Investigation of Antifungal Effects of Different Remineralization Agents on Salivary Candida amount in children with Early Childhood Caries*

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**Research Article**

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**ABSTRACT**

**Objectives:** In recent years, *Candida* has been linked to dental caries in children, with *Candida albicans* being particularly associated with the development of early childhood caries (ECC). In children with ECC, *Candida* species, along with *mutans streptococci* and *lactobacilli*, are commonly present in the oral cavity. This study aimed to evaluate the effects of different remineralization agents on *Candida* counts in children diagnosed with Severe Early Childhood Caries (S-ECC).

**Material and Methods:** Fifty-four healthy children, aged 3 to 5 years, diagnosed with S-ECC, were assessed, and 21 *Candida*-positive children were selected for inclusion in the study. The children were randomly divided into three groups: a control group using 500 ppm NaF toothpaste, a 10% Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) group, and a Calsium Glycerophosphate (CaGP) + 12% Xylitol group. Oral hygiene instructions were provided, and participants were advised to apply the remineralization agents three times daily for 3-5 minutes over a 2-week period. Unstimulated saliva samples were collected and cultured to quantify *Candida* counts. The number of *Candida* colonies was measured at baseline, the second week, and at the first and fourth months. Data analysis was performed using SPSS Statistics software (version 23), with statistical significance set at a p-value of less than 0.05.

**Results:** A significant difference was observed in Candida counts between baseline and the first and fourth months in the CaGP + 12% Xylitol group ($p < 0.05$). The CaGP group exhibited a notable decrease in salivary Candida levels ($P = 0.028$) after 4 months of treatment. However, while reductions in Candida counts were seen in the NaF toothpaste and CPP-ACP groups, these changes did not reach statistical significance ($p>0.05$).

**Conclusion:** These findings indicate that remineralization agents may reduce Candida counts, suggesting their potential effectiveness in caries management.

**Keywords:** Early Childhood Caries, CPP-ACP, CaGP, Xylitol, Candida Albicans.

# Erken Çocukluk Çağı Çürüğü Olan Çocuklarda Farklı Remineralizasyon Ajanlarının Tükürükteki Candida Miktarı Üzerine Antifungal Etkilerinin İncelenmesi

**Research Article**

*Süreç*

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**ÖZ**

**Amaç:** Son yıllarda Candida türlerinin çocuklarda diş çürüğü ile ilişkili olduğu bildirilmiştir. *Candida albicans*' ın Erken Çocukluk Çağı Çürüğü (EÇÇ) başlangıcındaki rolü iyi bilinmektedir. *Mutans streptokokları* ve *laktobasillerin* yanı sıra, Candida türleri de EÇÇ' li çocukların ağız boşluğunda sıklıkla bulunmaktadır. Bu çalışmanın amacı, Şiddetli Erken Çocukluk Çağı Çürüğü (Ş-EÇÇ) olan çocuklarda farklı remineralizasyon ajanlarının tükürükteki Candida miktarına etkisini karşılaştırmaktır.

**Gereç ve Yöntemler:** Bu araştırmada, yaşları 3-5 arasında değişen, Ş-EÇÇ tanısı konmuş 54 sağlıklı çocuk değerlendirildi ve Candida-pozitif olan 21 çocuk çalışmaya dahil edildi. Çocuklar rastgele 3 gruba ayrıldı: 500 ppm sodyum fluorid (NaF) diş macunu grubu (kontrol), %10 kazein fosfopeptid–amorf kalsiyum fosfat (CPP-ACP) grubu ve kalsiyum gliserofosfat (CaGP )+ %12 ksilitol grubu. Tüm çocuklara ağız hijyeni eğitimi verildi ve remineralizasyon ajanlarını iki hafta boyunca günde üç kez, 3-5 dakika süreyle kullanmaları istendi. Candida kolonilerinin kültürü için uyarılmamış tükürük örnekleri toplandı. Tükürükteki Kandida değerlendirilmesi, başlangıç, 2. hafta, 1. ay ve 4. ayda toplanan tükürüklerde gerçekleştirildi. Tüm analizler SPSS Statistics Version 23 ile yapıldı ve anlamlılık düzeyi $p < 0,05$ olarak kabul edildi.

**Bulgular:** CaGP + %12 Ksilitol grubunda, başlangıç ile 1. ay ve 4. ay arasındaki Candida miktarı açısından istatistiksel olarak anlamlı bir azalma bulundu ($p < 0,05$). Ayrıca, CaGP grubu, 4 aylık uygulama sonrasında tükürükteki Candida miktarında anlamlı bir azalma gösterdi ($p = 0,028$). Ancak, NaF diş macunu ve CPP-ACP gruplarındaki Candida miktarındaki azalmalar, istatistiksel olarak anlamlı düzeyde değildi ($p > 0,05$).

**Sonuç:** Sonuçlar, remineralizasyon ajanlarının Candida miktarını azaltabileceğini ve bu nedenle diş çürüğü yönetiminde etkili olabileceğini göstermektedir.

**Anahtar Kelimeler:** Erken Çocukluk Çağı Çürüğü, CPP-ACP, CaGP, Ksilitol, Candida Albicans.



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## Introduction

Early childhood caries (ECC) is defined by the occurrence of at least one decayed lesion (either cavitated or non-cavitated), a tooth lost due to caries, or a restored tooth surface in any primary tooth of a child aged 71 months or younger. For children under the age of 3, the detection of caries on smooth surfaces signifies severe early childhood caries (S-ECC).[1] ECC remains the most prevalent chronic disease in children, with nearly 1.8 billion new cases reported globally each year.[2] S-ECC develops at an earlier age than ECC, often leading to more severe health complications and typically necessitating extensive dental treatment under general anesthesia, which may include several tooth extractions, restorations, and crowns.[3] Although not life-threatening, the disease significantly affects individuals and communities by diminishing quality of life due to pain and functional impairment, and it negatively impacts a child's growth, body weight, and overall development.[4] Therefore, S-ECC represents a significant challenge to public health, necessitating a deeper understanding of its etiopathogenesis and the development of more effective treatment strategies to reduce recurrence and associated costs.[5]

In germ-free animal studies, it was observed that caries did not occur despite a high carbonhydrate diet, and the primary effect of oral microorganism in caries etiology has been proven.[6] The microbial origin of S-ECC is often linked to a polybacterial infection of the teeth. However, microbiological studies also indicate that fungal organisms play a role in this pediatric oral condition. *Candida* species were found in significantly higher levels in the oral cavities of children with S-ECC compared to caries-free children, with their presence correlating positively with both the severity of caries and the colonization of *Streptococcus mutans*. In the studies, researchers stated that *C. albicans* is highly acidogenic and has the ability to dissolve hydroxyapatite crystals of enamel more than *S.mutans.*[7,8] These factors show that *C.albicans* contributes to the severity and aggresive nature of the S-ECC. Research on chemotherapeutic approaches to reduce or prevent *Candida* levels in ECC is limited, emphasizing the need to assess the effectiveness of various agents in decreasing caries incidence in children.[9]

A common approach to minimizing the risk of ECC involves brushing teeth twice daily using fluoride toothpaste. In addition to fluoride, chlorhexidine, silver, povidone iodine, xylitol, probiotics and caseine containing preparations (Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP/ACP) and Casein Phosphopeptide-Amorphous Calcium Fluoride Phosphate (CPP/ACFP)) are also used. In a meta-analysis study, it has been reported that, these antibacterial and remineralization agents effect is low in the prevention of ECC, and there is not enough evidence about the effectiveness of the agents.[10]

There is limited research evaluating the antimicrobial effects of CPP-ACP on primary teeth.[11-14] However, no research has compared the antifungal effect of CPP- ACP, xylitol containing CaGP paste and low-fluoride containing tooth paste in children with ECC. Therefore the aim of this study was to compare three approaches: (1) brushing twice daily with low-fluoride toothpaste; (2) brushing twice daily combined with the application of 10% CPP-ACP paste three times daily; and (3) brushing twice daily combined with the application of a paste containing CaGP, MgCl2, and 12% xylitol three times daily, to evaluate their effects on *Candida* counts in children with S-ECC.

## Materials and Methods

### *Ethical considerations*

The study protocol received approval from two authorities: (1) the Clinical Research Ethics Committee of Marmara University, School of Dentistry (approval number 2019-283) and (2) the Turkish Medicines and Medical Devices Agency, Republic of Turkey Ministry of Health (approval number 20-AKD-167). The research adhered to the principles outlined in the Declaration of Helsinki (1964). Written informed consent was obtained from the parents or guardians of all participants.

### *Sample size calculation*

The sample size was determined using G*Power 3.1 software, with the significance level set at 0.05 and a statistical power of 0.8. The calculation indicated a total of 18 participants (at least 6 per group) was required. To account for an anticipated dropout rate of 15%, the recruitment target was set at a minimum of 21 participants, with 7 individuals in each group.

### *Study design*

This randomized clinical trial adhered to the Consolidated Standards of Reporting Trials (CONSORT) guidelines.[15] The study was carried out with systemically healthy, cooperative children aged 3–5 years diagnosed with S-ECC, who were patients at the Department of Pediatric Dentistry, Marmara University, Istanbul, Turkey. All participants met the American Academy of Pediatric Dentistry's criteria for the definition of S-ECC. Exclusion criteria included the presence of a systemic disease, recent antibiotic or anti-inflammatory use within the previous month, or receiving specialized dental care such as orthodontic treatment, the use of space maintainers, or other dental appliances. After being informed about the study, the parents of 54 children provided consent, and these children were included in the trial. The CONSORT flow diagram outlining the study process is presented in Figure 1.

Sociodemographic and oral hygiene behavior characteristics were recorded. All children were examined by a single examiner (CG). Before the study commenced, the examiner underwent training and calibration, and the initial patients enrolled were assessed through a peer-reviewed consensus process. Caries experience in children was evaluated using visible light, a standard dental mirror, and a probe designed for the Community Periodontal Index.

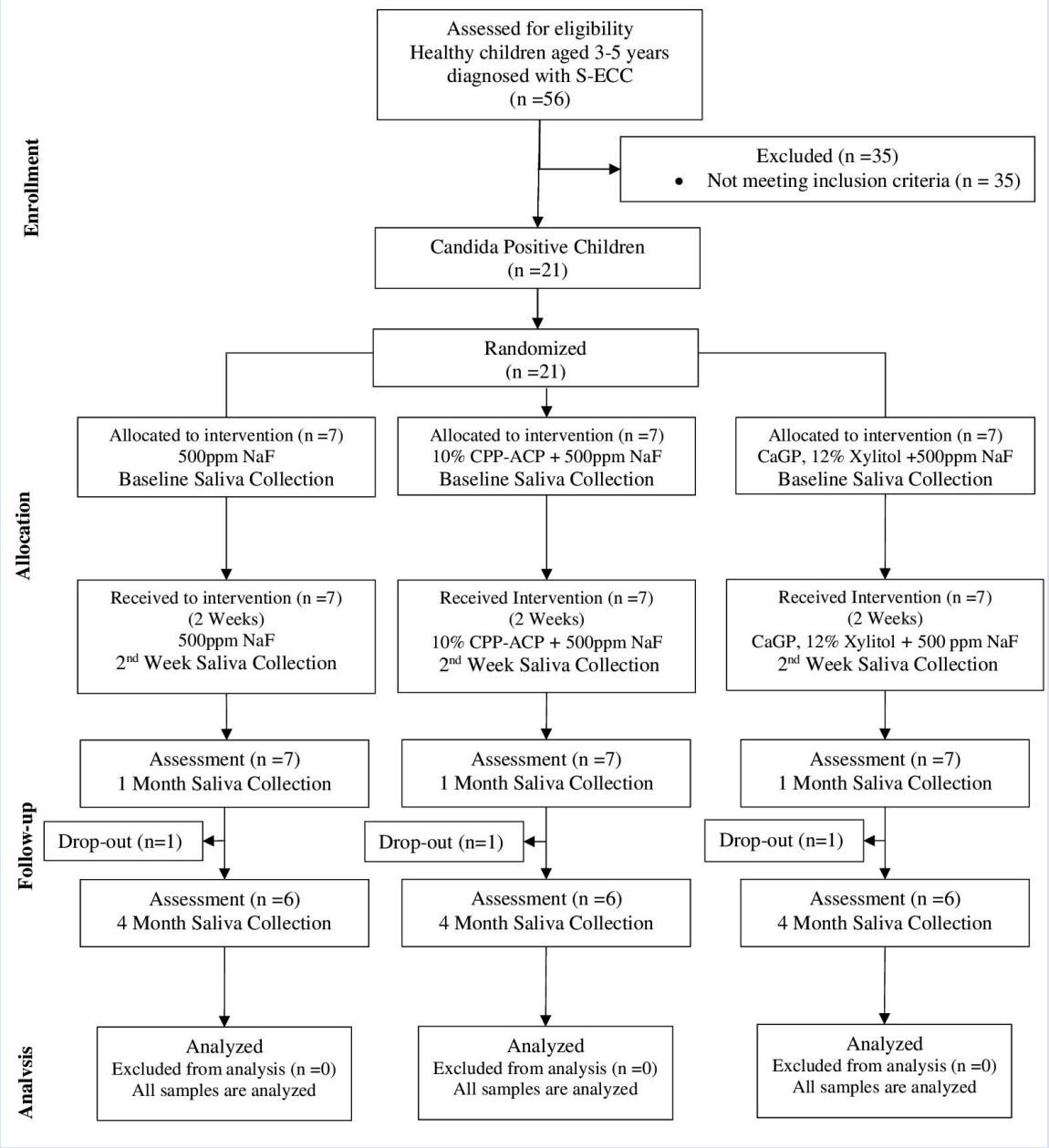


*Figure 1:* Consolidated Standards of Reporting Trials flow diagram.

### *Randomization and blinding*

Randomization and blinding procedures were conducted using sealed, opaque envelopes, each individually numbered and limited to single selections. These envelopes contained details about group allocation and identification numbers. Participants were asked to draw a sealed envelope at random from the collection of concealed envelopes. The maintenance of allocation concealment was independently overseen by FE. The trial intervention remained blinded to patients, the microbiologist, and the data analyst. The microbiologist was not involved in the investigative team conducting the trial intervention, did not participate in salivary sample collection, and had no access to randomization sheets. Salivary samples were uniformly collected by a single investigator (CG), who was unaware of the patients' group allocations.

### *Saliva collection*

Saliva samples were collected from 54 children at 9:00 to 11:00 am to reduce potential circadian variations. Prior to collection, participants were asked to swallow to eliminate any saliva that had accumulated in the mouth. Unstimulated saliva samples were collected at baseline from 54 children using a dental saliva ejector. The collected samples were forwarded to the laboratory at the Department of Basic Medical Sciences, Faculty of Dentistry, Istanbul University, for *Candida* culture testing. The study population was constituted by children with a positive Candida value (≥100 cfu/ml) as determined by the results of the Candida culture.

### *Intervention*

Baseline saliva samples were obtained from all participants, and the 21 children were randomly divided

into three groups, each comprising 7 participants. Participants received (Group 1) tooth paste including 500 ppm NaF, (Colgate Oral Pharmaceuticals, New York, NY) (Control Group); (Group 2) 500 ppm NaF + 10% CPP-ACP containing paste (GC Tooth Mouse, Europe, Tokyo, Japan) and (Group 3) 500 ppm NaF + CaGP and 12% Xylitol containing paste (R.O.C.S Medical Minerals gel, DRC Group, Moscow, Russia). Application procedure for remineralization agents were 2 weeks, 3 times per day; 1) after brushing the teeth in the morning after breakfast, 2) at any time of the day and 3) after brushing the teeth before going to bed at night as chickpea size and advised to applied to all tooth surfaces under parent's supervision. Parents were advised to wait 60 minutes between tooth brushing and gel application and to avoid eating or drinking any solid or liquid items for 40-50 minutes after applying the gel. All participants received the same toothpaste as the control group and oral hygiene education. The participants were continuously monitored to ensure adherence to the prescribed regimen. Throughout the intervention phase (2 weeks) and follow-up periods, the subjects were advised to continue their regular oral hygiene routine during each check-up session. Saliva samples were obtained at baseline, followed by collections after 2 weeks, 1 month, and 4 months, and the salivary *Candida* counts were measured. Each sample tube was marked with a code that included the participant's identification number and the corresponding control session of the study.

### *Salivary Candida counts*

Two milliliters of unstimulated saliva were utilized for microbiological analysis. Sabouraud Dextrose agar (Merck KGaA, Darmstadt, Germany) medium was utilized for *Candida* isolation in the study. A volume of 0.01 ml from collected saliva samples was inoculated by spreading onto the agar surface. Subsequently, incubation was carried out in an aerobically controlled environment at 37°C for 48. At the end of the appropriate time, the typical colonies seen in the medium were counted and the colony count per milliliter (cfu/ml) was calculated. For *candida*, counts less than $<10^3$ cfu/ml were considered as low level, between $>10^3$ - $<10^4$ cfu/ml as moderate level, and $>10^4$ cfu/ml as high level. The count below the detection limit of 10 cfu/ml with no detectable growth was accepted as '0'.[16]

### *Statistical Analysis*

Data analysis was conducted using SPSS software, version 23 (SPSS Inc., Chicago, IL, USA). The results were presented as frequencies (%), as well as mean ± standard deviation (SD) with descriptive statistics presented as mean ± SD. The normality of the data distribution was assessed using the Kolmogorov-Smirnov test. Values that did not have normal distribution and were followed at 4 different times were analyzed with the Friedman's two-way ANOVA test. To identify which specific pairs of groups differed significantly, All Pairwise tests were used. The level of statistical significance (*p*-value) was set at <0.05.

## Results

This study included 54 children diagnosed with S-ECC who consented to participate in the research. In forming the research group, initial Candida counts were considered, and to assess the significance of changes in Candida counts, 21 children with counts greater than 100 cfu/ml were included. The mean age of these 21 children was 4.08 ± 0.68 years, with 14 boys (66.7%) and 7 girls (33%).

Table 1 shows the the changes in low, moderate and high level of *Candida* at baseline, after 2 weeks, 1 month and 4 month of intervention. When comparing baseline and 4th-month results, a reduction in the moderate level of *Candida* count was observed across all groups.

The changes in the median *Candida* levels of the children in the study group according to the follow-up sessions were evaluated for each group. No statistically significant difference was observed between baseline and follow-up values in the 500 ppm NaF and 10% CPP-ACP groups ($p > 0.05$). However, a statistically significant difference was found between baseline and follow-up values in the CaGP + 12% Xylitol group ($p = 0.012$) (Table 2).

The difference between the baseline mean values and the first-month mean values in the CaGP + 12% Xylitol group was statistically significant ($p = 0.018$), as was the difference between the baseline mean values and the fourth-month mean values ($p = 0.028$) (Table 3).

*Table 1. The evaluation of changes in Candida count between the groups.*

| *Candida* | 500ppm NaF (Control) n=7 | | | 10% CPP-ACP n=7 | | | CaGP + 12% Xylitol n=7 | | |
|---|---|---|---|---|---|---|---|---|---|
| Baseline | Low $<10^3$ cfu/ml n(%) | Moderate $>10^3$ - $<10^4$ cfu/ml n(%) | High $>10^4$ cfu/ml n(%) | Low $<10^3$ cfu/ml n(%) | Moderate $>10^3$ - $<10^4$ cfu/ml n(%) | High $>10^4$ cfu/ml n(%) | Low $<10^3$ cfu/ml n(%) | Moderate $>10^3$ - $<10^4$ cfu/ml n(%) | High $>10^4$ cfu/ml n(%) |
| | 3(42.9%) | 3(42.9%) | 1(14.3%) | 2(28.6%) | 5(71.4%) | 0(0) | 4(57.1%) | 2(28.6%) | 1(14.3%) |
| 2nd-week | 4(57.1%) | 3(42.9%) | 0(0%) | 4(57.1%) | 3(42.9%) | 0(%0) | 6(85.7%) | 1(14.3%) | 0(0%) |
| 1st-month | 5(71.4%) | 1(14.3%) | 1(14.3%) | 4(57.1%) | 3(42.9%) | 0(0%) | 5(71.4%) | 2(28.6%) | 0(0%) |
| 4th-month | 4(66.7%) | 0(0%) | 2(33.3%) | 5(83.3%) | 1(16.7%) | 0(%0) | 5(83.3%) | 1(16.7%) | 0(0%) |

*Table 2. Distribution of changes in Candida counts (median [%25-%75]) at the baseline, 2nd week, 1st and 4th month according to the groups.*

| *Candida* counts (logCFU/ml) | | Median [%25-%75] | P |
|---|---|---|---|
| 500ppm NaF (Control) | Baseline | 1800 [700 -9200] | 0.168 |
| | 2nd-week | 100 [0 -3800] | |
| | 1st-month | 200 [100 -2000] | |
| | 4th-month | 800 [0 -19000] | |
| 10% CPP-ACP | Baseline | 3300 [300 -4800] | 0.093 |
| | 2nd-week | 700 [200 -1200] | |
| | 1st-month | 100 [0 -1200] | |
| | 4th-month | 600 [100 -1100] | |
| CaGP+12% Xylitol | Baseline | 800 [500 -9000] | **0.012*** |
| | 2nd-week | 200 [0 -800] | |
| | 1st-month | 200 [0 -1900] | |
| | 4th-month | 400 [100 -700] | |

Friedman's two-way ANOVA test, *p<0,05 statistical significance.

*Table 3. Statistical change in Candida count in the CaGP + 12% Xylitol group according to control sessions.*

| *Candida* count (CFU/ml) | | CaGP+12% Xylitol *p* |
|---|---|---|
| Baseline | 2nd-week | 0.128 |
| | 1st-month | **0.018*** |
| | 4th-month | **0.028*** |
| 2nd-week | 1st-month | 0.833 |
| | 4th-month | 0.528 |
| 1st-month | 4th-month | 0.686 |

All pairwise test, *p<0.05 statistical significance.

## Discussion

S-ECC is a widespread public health issue that necessitates intricate treatment procedures and results in significant costs. It has been suggested that the prevention of S-ECC is positively correlated with the understanding of etiopathogenesis involving cariogenic bacteria.[7] However, it has long been recognized that the microbiota of caries-associated biofilms consists of a diverse range of microorganisms, including Fusobacterium, Bifidobacterium, Actinomyces, Prevotella, Scardovia, Veillonella, Atopobium, and Candida species.[17]

Raja et al., in their study of healthy children in the mixed dentition period, showed that dental caries was associated with *Candida* carriage.[18] Given their ability to colonize tooth surfaces, infiltrate dentinal tubules, contribute to microbial biofilm formation, and generate substantial amounts of acid that lead to enamel demineralization and hydroxyapatite crystal dissolution, Candida species are hypothesized to play a significant role in the advancement of caries lesions.[19,20] In their study, de Carvalho et al. demonstrated a significant association between *Candida albicans* and the occurrence of ECC.[21] Akdeniz et al., investigated *Candida* carriage in children with and without caries in their study and stated that environmental factors such as oral hygiene status, high concentration of dietary sugar and the presence of widespread caries may be an important factor for the high prevalence of *Candida*.[22] On the other hand, certain clinical studies have found no notable differences in oral Candida prevalence between caries-free and caries-active populations, nor a clear positive correlation between the presence of *Candida albicans* and caries risk in children.[7] Due to the controversial results of studies on *Candida* and ECC, this study evaluated the antifungal effect of two remineralization agents and low-fluoride toothpaste on *Candida* known to be causative in S-ECC.

Two systematic reviews have demonstrated that numerous studies have investigated either a single or combination of various agents (e.g., chlorhexidine, iodine, fluoride, silver compounds, xylitol, CPP-ACP, probiotics and triclosan) combined with different application methods (e.g., m outh rinses, gels, varnishes, cleaning wipes, restorative materials) for the prevention of ECC.[23,24] In our study, remineralizing gels containing CaGP+12% Xylitol or 10% CPP-ACP, and a toothpaste containing 500 ppm NaF were used. A literature review revealed studies investigating the remineralization effects of the agent containing 10% CPP-ACP[25] but it was found that research on its antimicrobial effects was limited.[14] However, our study is the first to investigate the antifungal effect of the 10% CPP-ACP and 12% Xylitol + CaGP-containing remineralizing agents on ECC.

The effectiveness of fluoride-containing toothpaste in preventing dental caries among children and adolescents has been consistently confirmed by numerous systematic reviews.[26,27] The anticariogenic effects of low fluoride levels in toothpaste are unclear; however, most studies have shown that toothpastes containing less than 500 ppm fluoride are less effective compared to those containing 1000 ppm fluoride.[13] Zaze *et al.* investigated the effects of varying concentrations of calcium glycerophosphate (CaGP) in low-fluoride toothpastes on enamel demineralization. Their findings indicated that a

low-fluoride toothpaste (500 µg F/g) supplemented with 0.25% CaGP could achieve a level of efficacy comparable to that of a higher-fluoride toothpaste (1,100 µg F/g). This suggests that adding CaGP to low-fluoride toothpastes may effectively enhance their ability to prevent enamel demineralization, potentially offering a valuable alternative in oral care formulations.[28] Cavazana et al. examined the effect of CaGP, with or without fluoride, on the pH of dual-species biofilms consisting of *Streptococcus mutans* and *Candida albicans*. They concluded that CaGP had an impact on the dual-species biofilm formed by *S. mutans* and *C. albicans*.[29] In this study, the changes in *Candida* count during follow-up sessions after regular use of toothpaste containing 500 ppm fluoride were also evaluated. A decrease in *Candida* count was observed between sessions, but this reduction was not statistically significant. In both developed and developing countries, regular use of fluoride toothpaste has been identified as a key factor in reducing dental caries.[30] However, discussions on fluoride use have been increasing in recent years, and the emerging need has led to an increase in studies focused on developing alternative agents that are as effective as fluoride.[31]

Research suggests that the anticariogenic properties of CPP-ACP result from its combined effects on remineralization and bacterial displacement.[32] The literature is conflicting on CPP-ACP's antibacterial properties. In their in vitro study, Erdem et al. showed that CPP-ACP reduced S. mutans biofilm formation, while Rahiotis *et al.* found that the presence of CPP-ACP delayed bacterial biofilm formation.[11,12] In an in vivo study, Schüpbach et al. demonstrated that CPP reduced the ability of mutans streptococci (MS) to adhere to treated enamel surfaces.[33] In a study by Plonka et al., it was shown that the daily application of a CPP-ACP-containing remineralizing agent from the eruption of the first tooth until the child reached approximately 24 months was more effective than chlorhexidine in reducing the presence of MS.[13] In contrast, Grychtol et al. reported that a CPP-ACP-containing preparation had no significant effect on the initial bacterial colonization of enamel and dentin, and thus could not be recommended for biofilm prevention and management.[34] While the antibiofilm potential of *S. mutans* is established, the antibacterial properties of casein and calcium phosphate have not yet been confirmed.

Al-Batayneh et al. evaluated the combined effects of fluoride, CPP-ACP, and both agents on *S. mutans* in children at high risk of caries at the 3rd and 6th months. They found a significant reduction in the number of *S. mutans* positive children from baseline to the 3rd month in all groups except the CPP-ACP group, but no significant difference between the groups throughout the study period. However, at the 6-month follow-up, the CPP-ACP group exhibited the most significant reduction in *S. mutans* levels. These findings were associated with the better performance of CPP-ACP over time, with a significant difference reported in the fluoride group at the 3-month mark and a similar trend observed in the combination group. It was reported that the similarity in the combination group was due to fluoride being applied before CPP-ACP.[14] In this study, the effect of a remineralising agent containing 10% CPP-ACP on *Candida* was examined after being used three times a day for two weeks. A decrease in *Candida* count between sessions was observed, with a slight increase at the 4-month follow-up session, which did not reach baseline levels; however, these changes between sessions were not statistically significant. A literature review revealed no studies evaluating the antimicrobial efficacy of CPP-ACP against *Candida*, making it impossible to compare the results. Further studies are required to assess the antibacterial effects of CPP-ACP.

Antibacterial activity in materials used for the remineralization of dental hard tissues is a valuable property for preventing and reversing dental caries. To achieve this, various antibacterial agents can be incorporated into remineralization agents.[35] In our study, two different remineralization agents containing CaGP+12% xylitol and 10% CPP-ACP were used for this purpose.

Since the 1960s, xylitol has been approved by the US. Food and Drug Administration for use in foods, and research has demonstrated its effectiveness as an anticaries agent.[36] Studies on xylitol have included various applications such as tablets, gum, syrup, and cleaning wipes in children ranging from 6 months to 5 years old.[37-39] Li and Tanner, in their meta-analysis, demonstrated that xylitol-containing agents generally led to a significant reduction in mutans streptococci (MS) colonization in young children.[23] Milgrom et al found that applying xylitol syrup (8g/day) twice a day from the appearance of the first primary tooth could prevent up to 70% of dental caries.[37] In our study, the effects of a remineralization agent containing 12% xylitol were evaluated after its use three times a day for two weeks. A statistically significant change in *Candida* count was observed between sessions. Regular use of the 12% xylitol-containing agent led to a significant reduction in *Candida* count compared to baseline. The changes between baseline and the 1-month follow-up session, as well as between baseline and the 4-month follow-up session, were statistically significant. In light of these findings, it was found that the effect of the mentioned remineralization agent on *Candida* continued for up to 4 months following two weeks of use. The use of this remineralization agent every 4 months for two weeks in preschool children at high caries risk appears to be a reasonable approach.

To summarize, the results of this study, which investigated the antifungal effects of different remineralization agents on children with S-ECC, indicate that the effects of CaGP + 12% Xylitol containing gel on *Candida* are significantly acceptable throughout all sessions. This antimicrobial efficacy is thought to be due to the xylitol content in the used agent. Additionally, no studies have been found in the literature investigating the effect of this remineralization agent on *Candida* in

vivo. Therefore, our study is significant as it is the first to address this issue. We believe that further research involving larger sample sizes, different application and examination methods, and longer follow-up periods is needed to obtain more definitive data on the effects of the agent containing CaGP + 12% Xylitol on other bacterias. Considering regular oral hygiene, the use of CaGP + 12% Xylitol, which is easy to use, effectively acceptable, and economically advantageous compared to other remineralization agents, appears appropriate to be included in preventive application protocols for preschool children at high risk of caries.

## Conclusions

These results suggest that remineralization agents can reduce the role of Candida in the pathogenesis of caries by reducing the count of Candida, thus it can be effective in caries management.

## Acknowledgments

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## Conflict of Interest

The authors have no conflicts of interest to declare.

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