Original Article

Comparative evaluation of antibacterial efficacy of active oxygen containing versus other dentifrices against *Streptococcus mutans* count in children with early childhood caries: A clinico-microbiological study

Tanvi Jindal, Nikhil Srivastava, Vivek Rana, Noopur Kaushik, Preeti Sharma[1], Tushar Pruthi

Departments of Pediatric and Preventive Dentistry and [1]Oral Pathology and Microbiology, Subharti Dental College and Hospital, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India

ABSTRACT

Background: Fluoridated dentifrices have been used for the prevention of dental caries since ages. However, to avoid the risk of fluorosis, the use of newer nonfluoridated options in dentifrices has shown a great interest in reducing *Streptococcus mutans* (SM) in early childhood caries (ECC). **Aim:** The study aimed to evaluate the antimicrobial efficacy of active oxygen (AO)-based dentifrice with amine fluoride (AF)-, sodium monofluorophosphate (SMP)-, herbal (HB)-, and tricalcium phosphate (TCP)-based dentifrices on SM count in children with ECC. **Materials and Methods:** Two hundred and fifty children aged 3–6 years with def ≥4 were selected and randomly divided into five groups of 50 each, based on the type of dentifrices used; Group I: AO-based, Group II: TCP, Group III: SMP, Group IV: AF, and Group V: HB dentifrice, and were asked to brush twice daily for 15 days. The saliva samples were collected at baseline, and after 15 days, cultured for SM colonies count. **Results:** Difference in colony-forming units (CFU)/ml between baseline and 15 days was highly significant in all the five groups ($P < 0.001$). Significant difference was observed in SM count after 15 days between Groups I and IV ($P = 0.017$), while nonsignificant differences were seen when compared with Groups II, III, and V ($P = 0.975$, 0.137, and 0.992). **Conclusions:** All the dentifrices were efficacious in reducing SM count in children with ECC. Although AO dentifrice showed superior results when compared to SMP, TCP, and HB, it did not prove to be superior to AF.

KEYWORDS: Active oxygen, dentifrices, early childhood caries, fluoridated dentifrice, *Streptococcus mutans*

Address for correspondence:
Dr. Tanvi Jindal,
Department of Pediatric and Preventive Dentistry,
Subharti Dental College and Hospital, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India.
E-mail: drtanvijindal94@gmail.com

Access this article online

Quick response code

Website: https://journals.lww.com/jped

DOI: 10.4103/jisppd.jisppd_135_23

Introduction

Early childhood caries (ECC) is one of the most prevalent diseases in India and in the world. Its global prevalence as reported by Uribe *et al*. was 48%, and in

How to cite this article: Jindal T, Srivastava N, Rana V, Kaushik N, Sharma P, Pruthi T. Comparative evaluation of antibacterial efficacy of active oxygen containing versus other dentifrices against *Streptococcus mutans* count in children with early childhood caries: A clinico-microbiological study. J Indian Soc Pedod Prev Dent 2023;41:57-62.

Submitted: 14-Mar-2023 **Revised:** 23-Mar-2023
Accepted: 24-Mar-2023 **Published:** 01-Jun-2023

India, it was found to be 49.6%.[1,2] The consequences of ECC often include a higher risk of new carious lesions in both the primary and permanent dentitions, hospitalizations and emergency room visits, high treatment costs, loss of school days, diminished ability to learn, and diminished oral health-related quality of life.[3] The predominant microorganism of the oral microflora is *Streptococcus mutans* (SM), and its increased levels in saliva have shown to be the most substantial cause of ECC. SM is a nonmotile, catalase-negative, and gram-positive streptococcus. This facultative anaerobe acts by breaking down the sugars on the tooth surface, further producing an acidic environment that ultimately demineralizes calcium in the tooth.[4,5]

The most feasible method of prevention of decay is the use of mechanical oral hygiene aids, i.e., the use of a toothbrush and toothpaste with a few chemical compounds. For decades, fluoride-containing tablets, dentifrices, and mouthwashes have been used to prevent the initiation of dental decay. Different types of fluoride formulations are available in dentifrices, such as sodium monofluorophosphate (SMP), sodium fluoride, and amine fluoride (AF) either alone or in combination.[6] AF, which was developed years ago, works by disrupting the membrane stability after adhering to the cell surface of bacteria.[7]

SMP is the widely used active constituent in dental formulations due to its effective anticaries action. Nonfluoridated toothpastes containing tricalcium phosphate (TCP) for children are also being popularized by various manufacturers. It works by releasing calcium, which can inhibit demineralization, and hydroxide and phosphate ion release may aid in acid buffering. Toothpastes containing TCP have shown a significant reduction in SM levels in the saliva of children.[8] However, there is a shift in the mindset of public owing to a herbal (HB) resurgence all over the globe. Neem (Azadirachta indica), an age-old herb, has been used by Asians and South Indians since time immemorial is known for its strong antibacterial properties and is an active ingredient of many HB dentifrices.[9]

Recently, a novel dentifrice containing honey ensuring active oxygen (AO), lactoferrin, and xylitol as its main ingredients has been introduced. AO formula of honey and lactoferrin contributes to the antibacterial action of the dentifrice,[10] while xylitol present in this toothpaste inhibits the growth and adhesion of SM.[11]

Since ECC is the most prevalent disease in children among all the oral diseases, the upsurge of new products for its prevention is always a novel approach. There have been very limited research studies to compare the efficacy of various medicated dentifrices against SM in children with ECC. Thus, the present study was planned to determine the efficacy of AO-based dentifrice with TCP-, SMP-, AF-, and HB-based dentifrices on the SM count in children with ECC.

Materials and Methodology

The present clinico-microbiological study was conducted in the Department of Pediatric and Preventive Dentistry in association with Department of Oral Pathology and Microbiology, Subharti Dental College and Hospital, Meerut, Uttar Pradesh. Sample size for the present study was done by applying the following formula:

$$N = \frac{(r+1)\left(z_{\alpha|_2} + z_{1-B}\right)^2 \sigma^2}{rd^2}$$

The permission of the Institutional Ethical Committee was obtained before the study. Two hundred and fifty healthy children between the age group of 3–6 years with ECC and def ≥4 and cooperative child patient with Frankl's Rating Scale 3 and 4 were included. Children with any systemic illness/recent systemic antibiotic administration, children with special health-care needs, recent history of professionally applied topical fluoride, or marked intraoral soft tissue pathology were excluded from the study. Children were checked for caries experience, and then, the saliva sample of each child was collected for baseline value for SM. The children were randomly divided using table of numbers into five groups, namely, Group I AO toothpaste (Bluem® AO toothpaste fluoride free), Group II TCP, (Mee Mee Fluoride-Free Strawberry Flavor Toothpaste), Group III SMP toothpaste having 458 ppm fluoride (Cheerio Fluoride Medicated Oral Gel), Group IV AF with 500 ppm (manufactured by Group Pharmaceuticals Ltd.), and Group V HB toothpaste (Patanjali Dant Kanti Junior Dental Cream), and were asked to brush using pea-sized dentifrice (according to the American Academy of Pediatric Dentistry [AAPD] 2020 guidelines) twice daily by horizontal scrub brushing method for 15 days. Patients were asked to sit in an upright position, and the saliva samples were obtained 2 h after breakfast to minimize diurnal variation. Then, patients were asked to spit the saliva into a sterile container. Thus, 2–3 ml of unstimulated saliva was collected and termed as baseline sample. The saliva sample was sent immediately to the department of oral pathology and microbiology for the microbiological analysis. The samples were inoculated onto freshly prepared mitis-salivarius-bacitracin agar (MSB agar) and were incubated for 48 h under aerobic conditions. Colonies were expressed as the number of colony-forming units per ml of saliva using colony counter (Yorco Company, UP). Unstimulated saliva was again collected after 15 days of intervention and was inoculated/incubated, and colonies were counted.

Results

The data were compiled on a MS Office Excel Sheet (v 2019© Microsoft, USA) and were subjected to statistical analysis using the Statistical Package for

the Social Sciences (IBM SPSS Inc., Chicago II, USA, version 23.0). Data were tested for normality using the Chi-square test, Wilcoxon signed-rank test, Kruskal–Wallis test, and Mann–Whitney test. The comparison of colony-forming units (CFU)/ml at baseline and after 15 days in each group was done using the Wilcoxon signed-rank test.

The results of the study found that the difference between baseline and 15 days count in all the five groups was very highly significant ($P < 0.001$). The most significant reduction in CFU/ml was observed in Group IV (AF), then Group I (AO), followed by Group III (SMP), Group V (HB), and Group II (TCP) at 15-day follow-up [Table 1 and Figure 1].

When the pair-wise comparison of all the groups was done, the difference in CFU/ml count between Group I (AO) and II (TCP) (mean difference = 7.80), Group I (AO) and IV (AF), and Group I (AO) and V (HB) was found to be statistically significant ($P = 0.043$, 0.017, and 0.018, respectively) [Table 2].

Discussion

ECC negatively affects a child's lives in several ways; its short-term consequences include difficulty in drinking hot or cold liquids, in chewing and biting, reduction in appetite, and having trouble in sleeping.[12,13] The main microorganism responsible for human dental caries is the bacteria SM, which also has a significant impact on the etiology of ECC. The colonization of SM in human dentition occurs in early childhood before the onset of dental caries.[14] In the present study, saliva was used for the estimation of bacterial count in children with ECC as there is a strong association between ECC and Mutans Streptococci, mainly in saliva and biofilm samples.[15] Unstimulated saliva is preferred for collection of saliva as it minimizes the dilution of analytes, and passive drooling is considered gold standard for young children.[16] The AAPD supports the use of fluoridated dentifrices in no more than a pea-sized amount for children aged 3–6 years.[17] Different types of fluoride and nonfluoride formulations available in dentifrices are SMP, sodium fluoride, AF, calcium phosphate, and HB formulations either alone or in combination, which have been added to children's dentifrices nowadays.[18]

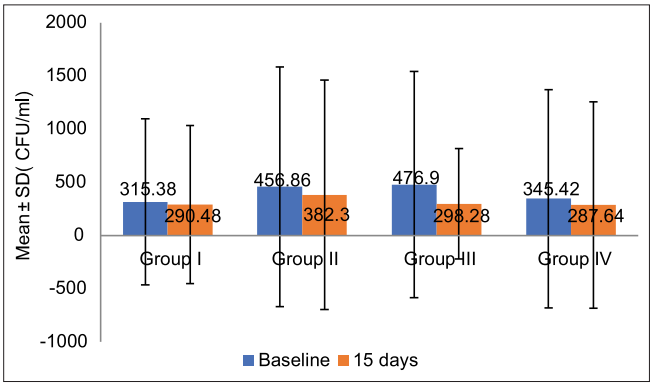

Figure 1: Comparison of CFU/ml at baseline and after 15 days in each group. CFU: Colony-forming units

In the present clinico-microbiological study, a total of 250 children with ECC in the age group of 3–6 years were randomly selected. The reason for selection of this age group was probably the presence of the window of infectivity at this age interval, increased acquisition, and colonization of SM occurs, making the oral cavity, particularly molars, more prone to caries.[19,20] Horizontal scrub was advised in the present study for children, as it was proven to be the best technique for children up to 6 years of age.[21] MSB agar was used to count colonies of SM in aerobic conditions as it is considered to recover the maximum number of colonies of SM.[22,23] Further, for quantification of bacteria, CFU determination on agar plating was done as it is considered a standard method to estimate the viability of bacteria.[24] The reason for counting colonies after 15 days was based on previous studies conducted, which evaluated the colony count after 7–14 days.[25,26] However, one study that evaluated CFU/ml after 15 and 30 days did not find any statistically significant difference at these two intervals.[27] Another reason for recalling the patient after 15 days was that one tube of dentifrice lasted for 15 days.

In the present study, marked reduction of bacterial count was observed after 15 days of intervention was

Table 1: Comparison of CFU/mL at baseline and after 15 days in each group

Group	Interval	Mean	SD	Difference	*P*
I	Baseline	386.80×10³	983.92×10³	143.38	<0.001* (VHS)
	At 15 days	243.42×10³	605.98×10³		
II	Baseline	315.38×10³	779.49×10³	24.90	0.001* (VHS)
	At 15 days	290.48×10³	740.42×10³		
III	Baseline	456.86×10³	1126.21×10³	74.56	<0.001* (VHS)
	At 15 days	382.30×10³	1078.08×10³		
IV	Baseline	476.90×10³	1063.05×10³	178.62	<0.001* (VHS)
	At 15 days	298.28×10³	51w7.61×10³		
V	Baseline	345.42×10³	1024.97×10³	57.78	<0.001* (VHS)
	At 15 days	287.64×10³	969.08×10³		

SD=Standard deviation; VHS=Very highly significant

Table 2: Pair-wise comparison of CFU/mL after 15 days between all the groups

Group	Mean difference	*P*
I versus II	47.06	0.975 (NS)
I versus III	138.88	0.137 (NS)
I versus IV	54.86	0.017*
I versus V	44.22	0.992 (NS)
II versus III	91.82	0.218 (NS)
II versus IV	7.80	0.043*
II versus V	2.84	0.956 (NS)
III versus IV	84.02	0.243 (NS)
III versus V	94.66	0.154 (NS)
IV versus V	10.64	0.018*

*Significant difference at $P \leq 0.05$. Mann–Whitney test. NS=Nonsignificance

seen in all the five groups. When the mean comparison of posttreatment in Group I was compared with Groups II, III, and V, it was seen that AO dentifrice, TCP, SMP, and HB dentifrices were effective in reducing the bacterial load, and the difference among them was nonsignificant. The exact mechanism of action of toothpaste containing AO and lactoferrin against SM in children with ECC has not been reported in the literature till date. However, the various components present in this include active enzymes such as hydrogen peroxide, sodium perborate, honey, xylitol, and lactoferrin. This new over-the-counter toothpaste's producer claims that its underlying mechanism of action is based on the regulated delivery of reactive oxygen species to damage areas and the presence of AO and lactoferrin in its formulation.[10] In addition, xylitol is assumed to inhibit the development of pathogens and possesses antibacterial-like action specifically against Mutans streptococci.[28] Thus, it could be recommended that it is safer and can be used in children with ECC. When the comparison of AO containing toothpaste with Group IV (AF) was made, superior results were shown by AF containing dentifrice. As there is no literature as of now comparing both the dentifrices, it could be hypothesized that the superiority of AF may be due to its organic component, amine, which improves the bioavailability of fluoride.

On comparison of TCP (Group II) with SMP (Group III), AF (Group IV), and HB (Group V) dentifrices after 15 days, the results were found to be comparable, and no significant difference was seen between these groups in reducing the CFU/ml against SM. The reduction was due to the high alkaline pH of calcium ions in dentifrice containing TCP; the release of these calcium ions prevent further demineralization, while the release of phosphate and hydroxide ions might potentially help with acid buffering, contributing to its anti-plaque properties.[8,29] Similar results were found in various *in vitro* studies showing a comparable antimicrobial activity against the SM strain with other nonfluoridated and fluoridated dentifrices.[18,30] However, when comparison was done with Group II with Group IV (AF dentifrice), it was inferred that AF-containing dentifrice was more effective than TCP dentifrice (Group II).

When Group III (SMP-containing dentifrice with 458 ppm fluoride) was compared with Group IV (AF dentifrice with 500 ppm fluoride) and Group V (HB dentifrice) after 15 days, the results were found to be comparable, and no significant difference was seen between these groups in reducing the CFU/ml against SM. The antibacterial action of sodium monofluorophosphate is attributed to its inhibition of bacterial glycogen. It basically decreases the internal polysaccharide content of S. mutans.[31] The presence of fluoride in an acidic environment reduces the dissolution of calcium hydroxyapatite.[32,33] The results obtained in the present study were in accordance with an *in vitro* study conducted by Suetenkov *et al.*,[32] in which they concluded that toothpaste containing AF, sodium fluoride, and SMP showed a significant antibacterial activity than toothpaste containing xylitol. However, contrasting results were found in the study conducted by Randall *et al.*,[34] in which fluoridated dentifrices showed a greater antimicrobial activity against SM compared to HB dentifrices.

When the posttreatment comparison was made between Group IV (AF) and Group V (HB) dentifrice, superior results were shown by AF-containing dentifrice. This may be attributed to the hypothesis that HB toothpaste used by the children in the study had an unacceptable taste and they might have skipped using it regularly. The greatest antimicrobial activity of AF-containing dentifrice in this present study might be due to its mechanism of action. In AF, the amine portion is positively charged, and it readily binds to the enamel surface.[35] Various studies have shown similar results when AF toothpaste with 500 ppm of fluoride was compared with other toothpastes.[18,29,36]

The reduction in HB dentifrice used is due to the presence of active ingredients like neem which has got antiseptic, antibacterial, and antifungal activity.[37] The secondary metabolites, including alkaloids, flavonoids, polyphenols, and lectins present in neem, attribute to its antibacterial properties.[38,39] Similar results showing the efficacy of HB toothpaste against SM were also obtained in a study conducted by Patil *et al.*,[9] where HB toothpaste containing neem and fluoridated toothpastes was equally efficacious in the prevention of caries in children.

Thus, when the efficacy of all the dentifrices used in the study was compared, all proved to be efficacious against SM. However, AF containing dentifrice showed maximum reduction in CFU/ml, followed by AO, SMP, and HB dentifrice and least reduction was found in dentifrice with TCP at 15 days. However, a short follow-up period of 15 days and a limited sample size may be considered major limitations of this study,

and further studies are needed to be conducted on a larger sample size and with a long-term follow-up.

Conclusions

All the dentifrices were found to be efficacious in reducing the CFU/ml in children with ECC. The maximum reduction in CFU/ml was shown by dentifrice containing AF (Group I). Dentifrice containing TCP (Group II) showed the least reduction in the SM count compared to other groups. Furthermore, AO dentifrice showed superior results compared to other dentifrices except AF dentifrice. Thus, newer dentifrices containing AO can be considered safer to be used in children with ECC.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Uribe SE, Innes N, Maldupa I. The global prevalence of early childhood caries: A systematic review with meta-analysis using the WHO diagnostic criteria. Int J Paediatr Dent 2021;31:817-30.
2. Ganesh A, Muthu MS, Mohan A, Kirubakaran R. Prevalence of early childhood caries in India – A systematic review. Indian J Pediatr 2019;86:276-86.
3. Policy on early childhood caries (ECC): Classifications, consequences, and preventive strategies. Pediatr Dent 2016;38:52-4.
4. Pintone D. *Streptococcus mutans* and the Oral Microbiome. Honors Program Contracts; 01 April, 2021. Available from: https://scholarworks.merrimack.edu/honors_component/23. [Last accessed on 2023 14 Feb].
5. Jauhari D, Srivastava N, Rana V, Chandna P. Comparative evaluation of the effects of fluoride mouthrinse, herbal mouthrinse and oil pulling on the caries activity and *Streptococcus mutans* count using oratest and dentocult SM strip mutans kit. Int J Clin Pediatr Dent 2015;8:114-8.
6. Malhotra R, Singla S, Shashikiran ND. Comparison of antimicrobial activity of child formula dentifrices at different concentrations: An *in vitro* study. Int J Clin Pediatr Dent 2017;10:131-5.
7. Naumova EA, Weber L, Pankratz V, Czenskowski V, Arnold WH. Bacterial viability in oral biofilm after tooth brushing with amine fluoride or sodium fluoride. Arch Oral Biol 2019;97:91-6.
8. Thomas JM, Bhat SS, George SS, Prabha EA, Mohan AP, Somson HM. Effect of tricalcium phosphate and calcium sucrose phosphate on the inhibition of *Streptococcus mutans*: An *in vivo* study. J Contemp Dent Pract 2021;22:1422-5.
9. Patil S, Venkataraghavan K, Anantharaj A, Patil S. Comparison of two commercially available toothpastes on the salivary *Streptococcus mutans* count in urban preschool children – An *in vivo* study. Int Dent SA 2010;12:72-82.
10. Cunha EJ, Auersvald CM, Deliberador TM, Gonzaga CC, Esteban Florez FL, Correr GM, Storrer CLM. Effects of Active Oxygen Toothpaste in Supragingival Biofilm Reduction: A Randomized Controlled Clinical Trial. Int J Dent 2019;2019:3938214
11. Salli KM, Gürsoy UK, Söderling EM, Ouwehand AC. Effects of xylitol and sucrose mint products on *Streptococcus mutans* colonization in a dental simulator model. Curr Microbiol 2017;74:1153-9.
12. Singh N, Dubey N, Rathore M, Pandey P. Impact of early childhood caries on quality of life: Child and parent perspectives. J Oral Biol Craniofac Res 2020;10:83-6.
13. Lam PP, Chua H, Ekambaram M, Lo EC, Yiu CK. Does early childhood caries increase caries development among school children and adolescents? A systematic review and meta-analysis. Int J Environ Res Public Health 2022;19:13459.
14. Taghdisi-Kashani A, Gholamshahi A, Fathizadeh H, Azadchehr M, Rahimi M, Forouzandeh H, *et al*. Antimicrobial activity of children's toothpaste on the bacteria causing dental caries. Iran J Med Microbiol 2022;16:399-404.
15. Parisotto TM, Steiner-Oliveira C, Silva CM, Rodrigues LK, Nobre-dos-Santos M. Early childhood caries and mutans streptococci: A systematic review. Oral Health Prev Dent 2010;8:59-70.
16. Pidamale R, Sowmya B, Thomas A, Jose T, Madhusudan KK, Prasad G. Association between early childhood caries, *Streptococcus mutans* level and genetic sensitivity levels to the bitter taste of, 6-N propylthiouracil among the children below 71 months of age. Dent Res J (Isfahan) 2012;9:730-4.
17. American Academy of Pediatric Dentistry. Fluoride therapy. In: The Reference Manual of Pediatric Dentistry. Chicago, Ill.: American Academy of Pediatric Dentistry; 2021. p. 302-5.
18. Lodaya R, Venkataraman S, Lakade L, Bhat C, Kunte S, Jajoo S. Comparative evaluation of antimicrobial efficiency of marheted children's fluoridated toothpastes at diluted concentrations against *Streptococcus mutans* – An *in vitro* study. Int Dent Med J Adv Res 2018;4:1-5.
19. Sampaio-Maia B, Monteiro-Silva F. Acquisition and maturation of oral microbiome throughout childhood: An update. Dent Res J (Isfahan) 2014;11:291-301.
20. Jindal T, Srivastava N, Rana V, Kaushik N. Recent and conventional concepts of mechanical plaque control aids in children – A literature review. Int J Enhanc Res Med Dent Care 2023;10: 34- 40.
21. Muller-Bolla M, Courson F. Toothbrushing methods to use in children: A systematic review. Oral Health Prev Dent 2013;11:341-7.
22. Gutiérrez de Annan S, Ruíz de Valladares RE, Benito de Cárdenas IL. Mitis salivarius-bacitracin 10% sacarose agar for oral streptococci and *Streptococcus mutans* counts. Acta Odontol Latinoam 1997;10:47-53.
23. Zeng Y, Youssef M, Wang L, Alkhars N, Thomas M, Cacciato R, *et al.* Identification of non- *Streptococcus mutans* bacteria from predente infant saliva grown on mitis-salivarius-bacitracin agar. J Clin Pediatr Dent 2020;44:28-34.
24. Thieme L, Hartung A, Tramm K, Graf J, Spott R, Makarewicz O, *et al.* Adaptation of the start-growth-time method for high-throughput biofilm quantification. Front Microbiol 2021;12:631248.
25. Sharma M, Pandit IK, Srivastava N, Gugnani N, Gupta M. A comparative evaluation of efficacy of *Streptococcus mutans*

counts in saliva: An *in vivo* study. Int J Clin Pediatr Dent 2018;11:94-9.
26. Gudipaneni RK, Kumar RV, Jesudass G, Peddengatagari S, Duddu Y. Short term comparative evaluation of antimicrobial efficacy of tooth paste containing lactoferrin, lysozyme, lactoperoxidase in children with severe early childhood caries: A clinical study. J Clin Diagn Res 2014;8:ZC18-20.
27. Bhati N, Jaidka S, Somani R. Evaluation of antimicrobial efficacy of *Aloe vera* and Meswak containing dentifrices with fluoridated dentifrice: An *in vivo* study. J Int Soc Prev Community Dent 2015;5:394-9.
28. Milgrom P, Ly KA, Tut OK, Mancl L, Roberts MC, Briand K, *et al.* Xylitol pediatric topical oral syrup to prevent dental caries: A double-blind randomized clinical trial of efficacy. Arch Pediatr Adolesc Med 2009;163:601-7.
29. Samuel V, Ramakrishnan M, Halawany HS, Abraham NB, Jacob V, Anil S. Comparative evaluation of the efficacy of tricalcium phosphate, calcium sodium phosphosilicate, and casein phosphopeptide – Amorphous calcium phosphate in reducing *Streptococcus mutans* levels in saliva. Niger J Clin Pract 2017;20:1404-10.
30. Reddy D, Selvan A, Paul ST, Azher U. Antimicrobial efficacy of commercially available low-fluoride and fluoride-free dentifrices for children. Int J Clin Pediatr Dent 2021;14:183-6.
31. Demonte AM, Asencion Diez MD, Naleway C, Iglesias AA, Ballicora MA. Monofluorophosphate blocks internal polysaccharide synthesis in *Streptococcus mutans*. PLoS One 2017;12:e0170483.
32. Suetenkov DE, Sukhorukova MD, Petrova AP, Sayutina LV. Antimicrobial activity of toothpastes against early childhood caries. Saratov Med J 2022;3:e0104.
33. Buzalaf MA, Pessan JP, Honório HM, Ten Cate JM. Mechanisms of action of fluoride for caries control. Monogr Oral Sci 2011;22:97-114.
34. Randall JP, Seow WK, Walsh LJ. Antibacterial activity of fluoride compounds and herbal toothpastes on *Streptococcus mutans*: An *in vitro* study. Aust Dent J 2015;60:368-74.
35. Sh P, Raghu R, Shetty A, Gautham P, Reddy S, Srinivasan R. Effect of organic versus inorganic fluoride on enamel microhardness: An *in vitro* study. J Conserv Dent 2013;16:203-7.
36. Amaechi BT, AbdulAzees PA, Alshareif DO, Shehata MA, Lima PP, Abdollahi A, *et al.* Comparative efficacy of a hydroxyapatite and a fluoride toothpaste for prevention and remineralization of dental caries in children. BDJ Open 2019;5:18.
37. Chandhru TP, Anusha VR, Peedikayil FC, Gufran Ahmed MB, Kottayi S, Narasimhan D. Evaluation of antifungal activity of six children's toothpaste on *Candida albicans* isolated from early childhood caries patients. J Indian Soc Pedod Prev Dent 2020;38:152-7.
38. Prabhuswamy B, Mallikarjun N, Nagaraj K, Simpi B. Comparative evaluation of anticariogenic activity of commercially available herbal dentifrices. SRM J Res Dent Sci 2018;9:58-62.
39. Selvaraj K, Bharath N, Natarajan R, Dinesh S, Murugesan S, Selvaraj S. Comparative evaluation of antimicrobial efficacy of toothpastes containing probiotic and neem as primary ingredient on salivary *Streptococcus mutans* in Melmaruvathur population: An *in vivo* study. J Pharm Bioallied Sci 2020;12:S595-600.