Comparative Evaluation of Mouth Rinsing with Probiotic, Herbal, and Chlorhexidine Mouthwash on Salivary pH of Children: A Randomized Control Trial

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KEYWORDS

ABSTRACT

BACKGROUND: Maintaining an optimal salivary pH is essential for pediatric oral health to prevent dental caries and other oral diseases. Mouthwashes like Probiotic and Grape Seed Extract (GSE) are emerging as safer alternatives to traditional Chlorhexidine mouthwash.

OBJECTIVE: To compare the efficacy of probiotics, grape seed extract, and chlorhexidine mouthwashes on salivary pH in children aged 6 to 11 years.

DESIGN: A randomized controlled trial (RCT) was conducted.

SETTING: Private Dental Institute

PATIENTS AND METHODS: Thirty-six children aged 6 to 11 years were randomized into three groups: Group A: Probiotic mouthwash Group B: Grape Seed Extract mouthwash Group C: Chlorhexidine mouthwash Salivary pH was measured using a digital pH meter at baseline (day 1) and after one week (day 7) of daily use. Data were analyzed using one way ANOVA and Tukey's post hoc test.

MAIN OUTCOME MEASURES: Change in salivary pH from baseline to day 7. SAMPLE SIZE: Thirty-six children aged 6 to 11 years RESULTS: The mean salivary pH was comparable at baseline. After one-week, probiotic mouthwash significantly increased salivary pH compared to GSE and chlorhexidine. Overall, differences among groups were statistically significant.

CONCLUSION: Probiotic mouthwash was most effective in increasing salivary pH, highlighting its potential as a safe alternative for pediatric oral health..

1. Introduction

Dental caries is among the most widespread chronic conditions impacting children globally.[1,2] It is a multifactorial disease caused by the interaction of dietary sugars, oral microbiota, and host factors, ultimately leading to the demineralization of the tooth surface.[3,4] Maintaining an optimal salivary pH plays a critical role in oral health as it influences the remineralization and demineralization balance in dental tissues.[5] A pH below 5.5 is considered critical for enamel demineralization, making salivary pH a key factor in preventing dental caries.[6]

Mouthwashes are commonly used adjunct to mechanical plaque control measures, such as toothbrushing. They help reduce the microbial load, alter oral microenvironment conditions, and enhance salivary pH.[7,8] Among the available options, **Chlorhexidine mouthwash** is considered the gold standard because of its potent antimicrobial properties.[9] However, its prolonged use is associated with side effects, such as staining of teeth, altered taste sensation, and mucosal irritation, necessitating the exploration of alternative mouthwash formulations.[10] In recent years, interest in natural and probiotic-based mouthwashes as alternatives to conventional chemical formulations has grown. **Probiotic mouthwashes** are designed to restore the microbial balance in the oral cavity by introducing beneficial bacteria that suppress pathogenic strains, such as *Streptococcus mutans*.[11] Studies have demonstrated the potential of probiotics to reduce plaque levels, gingival inflammation, and caries risk.[12] Moreover, probiotic mouthwashes are generally well-tolerated and do not produce adverse effects associated with chemical mouthwashes.

Similarly, herbal mouthwashes have gained attention owing to their natural composition and bioactive properties. **Grape seed extract (GSE)** is rich in polyphenols and flavonoids and exhibits strong antioxidant, anti-inflammatory, and antimicrobial properties.

It has been shown to inhibit cariogenic bacteria and promote enamel remineralization, making it a promising natural alternative for maintaining oral health.[13] Additionally, grape seed extract can neutralize acidic oral environments, thereby maintaining a salivary pH conducive to remineralization.[14]

Given the potential benefits of probiotics and grape seed extract, this study was designed to assess their effectiveness with that of the chlorhexidine mouthwash in altering salivary pH among children. The rationale for focusing on children lies in the heightened vulnerability of this age group to dental caries and the need for preventive oral care strategies. By evaluating the ability of these mouthwashes to elevate and stabilize salivary pH over a one-week period, this study aimed to provide evidence-based recommendations for the selection of effective, safe, and patient-friendly mouthwashes in pediatric populations.

The aim of this study was to assess the effectiveness of Probiotic, Herbal, and Chlorhexidine mouthwashes on salivary pH in children aged 6-11 years. The specific objectives included evaluating the efficacy of probiotic mouthwash on salivary pH in children within this age group, assessing the efficacy of herbal mouthwash on salivary pH, and determining the efficacy of chlorhexidine mouthwash on salivary pH.

Materials and Methods

This study adhered to the principles of the Declaration of Helsinki and was approved by the Institutional Ethics Committee. Furthermore, the trial was registered with the Clinical Trials Registry of India (CTRI) under the registration number **CTRI/2024/07/069853.** Written informed consent was obtained from the parents or guardians of all the participants prior to their inclusion in the study.

Study Design

This study was conducted as a randomized controlled trial to evaluate and compare the efficacy of Probiotic, GSE (herbal) and Chlorhexidine mouthwashes in altering the salivary pH in children.

Inclusion and Exclusion Criteria

The inclusion criteria for participation were children aged **6–11 years**, free from any systemic illnesses, and whose parents or guardians provided **written informed consent**. Children diagnosed with **periodontal diseases**, children with **congenital anomalies**, physical or mental disabilities, and those with **severe medical illnesses** or known allergies to the mouthwash components were excluded from the study.

Study Setting

The study was conducted in the Department of Pedodontics and Preventive Dentistry. Participants were selected among children who were seeking dental care. Then they were screened to ensure that they met the inclusion and exclusion criteria. This process ensured that only eligible children participated, thus minimizing confounding factors that could influence the results.

Materials Used

The study utilized the following materials for intervention and data collection:

1. **Probiotic Mouthwash:** Perfora Probiotic Rinse, India.
2. **Grape Seed Extract Mouthwash:** HERBAL ENGINE, 100% Natural Black/Blue Grape Seed Powder, Punjab, India.
3. **Chlorhexidine Mouthwash:** Rexidine, Warren India.
4. **Digital pH Meter:** Metravi Digital pH Meter (PH-600)
5. **Distilled water**

Preparation of Grape Seed Extract Mouthwash

A standardized procedure was followed to prepare the herbal grape seed extract mouthwash in a sterile setting.

1. **Mixing**: In a clean beaker, 100 ml of distilled water were combined with 1.563grams of powdered grape seed extract.

2. **Dissolution**: Using a magnetic stirrer, the mixture was heated to 60°C until it completely dissolved.

3. **Dilution:** To reach a final volume of 1L, the dissolved solution was diluted with 900mL of distilled water.

4. **Storage**: To ensure stability, the prepared mouthwash was placed in sterile, amber-colored bottles and kept in a refrigerator at 4°C.

Study Procedure

A structured protocol was followed in this study. Before the study started, the parents or guardians received comprehensive information about its goals and methods. We obtained written informed consent from each participant.

Three groups were randomly selected among the participants. Depending upon the group assigned, participants were provided with the samples of mouthwashes.

Group A: Probiotic mouthwash diluted 1:1 with water. The participants were rinsed with **10 mL** of diluted solution for **2 minutes daily**.

Group B: GSE mouthwash, prepared as described above, was used without dilution. The participants were rinsed with **10 mL** of the solution for **2 minutes daily**.

Group C: Chlorhexidine mouthwash was diluted 1:1 with water. The participants were rinsed with **10 mL** of diluted solution for **2 minutes daily.**For all groups, saliva samples were collected at two time points:

1. **Baseline (Day 1):** Before the first mouth rinse.
2. **Post-intervention (Day 7):** After one week of daily mouth rinsing.

A **Digital pH** meter was used to measure the pH of saliva samples that had been collected in sterile containers. (**Figure 1**)

Figure 1: A Digital pH meter was used to measure the pH of saliva samples that had been collected in sterile containers.

Sample Size Determination

The sample size was derived for an experimental study utilizing a two-sided test with a significance level of 5% and a power of 80%, calculated based on the outcome variable on a ratio scale and null hypothesis testing.

The following formula was used to calculate the sample size:

$$n = 2\frac{S^2(Z1 + Z2)^2}{(M1 - M2)^2}$$

A priori power analysis was done by using G*Power version 3.0.1 (Franz Faul, University of Kiel, Germany) to determine the necessary sample size for a one-way ANOVA with fixed effects and omnibus testing. The input parameters for the three groups included an effect size (f) of 0.55, a significance level (α) of 0.05, and a power (1-β) of 0.80 for three groups. The

analysis yielded a non-centrality parameter (λ) of 10.89, a critical F-value of 3.24, with numerator degrees of freedom (df) = 2 and denominator df = 33. The total calculated sample size was 36 participants (12 per group), providing an actual power of 0.8125 to detect statistically significant differences between the groups

Randomization

A **simple random sampling technique** was utilised to choose participants, ensuring an unbiased distribution into three groups. Participants were randomly divided three groups:

Group A: Probiotic mouthwash

Group B: Grape seed extract powder (GSE) (herbal) mouthwash

Group C: Chlorhexidine mouthwash

Randomization was conducted using the lottery method, ensuring an equal allocation ratio of 1:1:1. The process was managed by an intern who was not involved in the data collection or statistical analysis.

Blinding

To eliminate bias, the participants were blinded to the mouthwash allocation. Mouthwashes were distributed in small containers which were labelled with codes (A, B and C) to conceal the identity of the mouthwash**.** (**Figure 2**)

Figure 2: Mouthwashes were distributed in small containers which were labelled with codes (A, B and C) to conceal the identity of the mouthwash.

Statistical Analysis

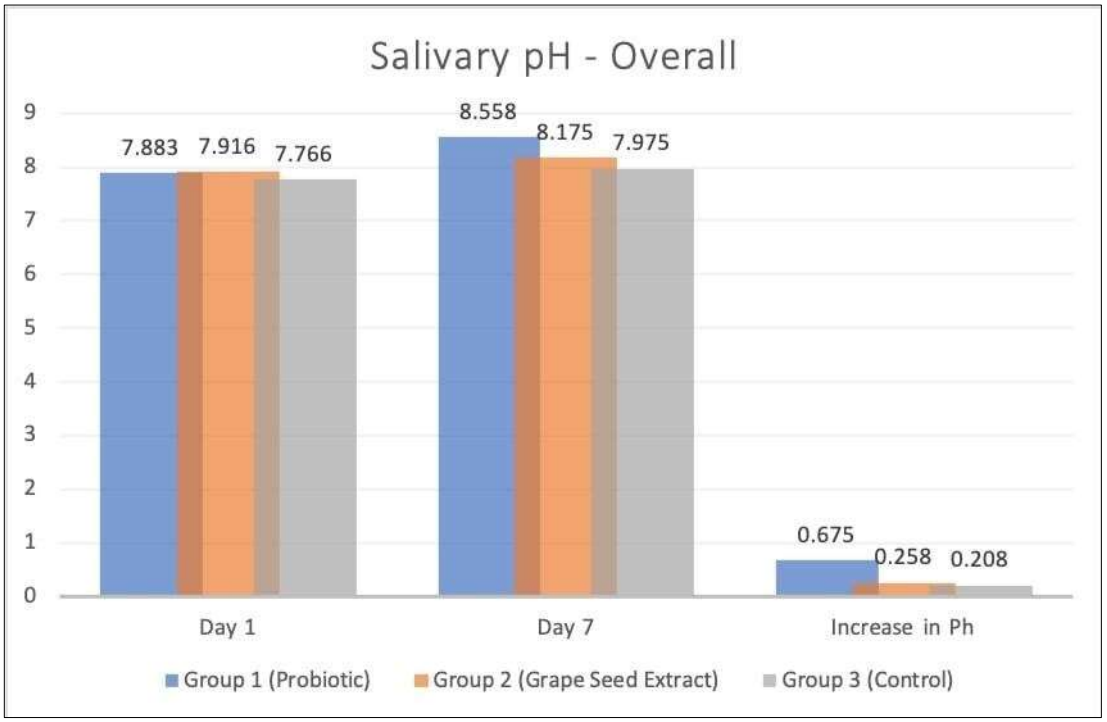
Statistical analysis was performed using **SPSS software (version 21)**. Descriptive statistics were expressed as **mean ± standard deviation (SD)**. Data normality was assessed with the help of the **Shapiro-Wilk test**. Intergroup comparisons were performed using **one-way ANOVA** to evaluate the overall differences between the three groups. For pairwise comparisons of the groups, **Tukey's post hoc test** was used. A **confidence interval of 95%** was maintained with the **level of significance (α)** set at **0.05**. The power of the study was ensured to be **80%**, aligned with the calculated sample size.

Results

On **Day 1,** the mean salivary pH across all groups was comparable, with no statistically significant difference being observed (Probiotic: 7.883 [0.79]; GSE: 7.916 [0.71]; Control: 7.766 [0.45]; F = 0.167, *P = .847*). This indicated that the initial salivary pH of the participants in all groups was similar.

By **Day 7,** there was a notable rise in the mean salivary pH of all groups, with the probiotic group (SD 8.558 [0.49]) showing the highest pH compared to the GSE (8.175 ± 0.65) and the control group (SD 7.975 [0.53]). The difference in pH across groups was statistically significant (F = 3.310, *P = .049).*

The mean increase in salivary pH from day 1 to 7 further highlighted the differences between the groups. The Probiotic group demonstrated the greatest increase (SD 0.675[0.43]), followed by the GSE group (SD 0.258 [0.32]), and the control group (SD 0.208[0.246]). Intergroup differences in pH increase were statistically significant (F = 6.664, *P =.004*). These results suggest that the probiotic mouthwash was the most effective at elevating salivary pH over the study period **(Table 1 and Figure 3).**



Graph 1: Overall intergroup comparison of efficacy of Probiotics, Herbal and Chlorhexidine mouthwash on salivary pH of children.

	Day 1 Mean (SD)	Day 7 Mean (SD)	Increase in pH Mean (SD)
Group 1 (Probiotic)	7.883 (0.79)	8.558 (0.49)	0.675 (0.43)
Group 2 (GSE)	7.916 (0.71)	8.175 (0.65)	0.258 (0.32)
Group 3(Control)	7.766 (0.45)	7.975 (0.53)	0.208 (0.246)
One-way Anova F-test value	F = 0.167	F = 3.310	F = 6.664
P value, Significance	p= .847	p= .049*	p= .004*

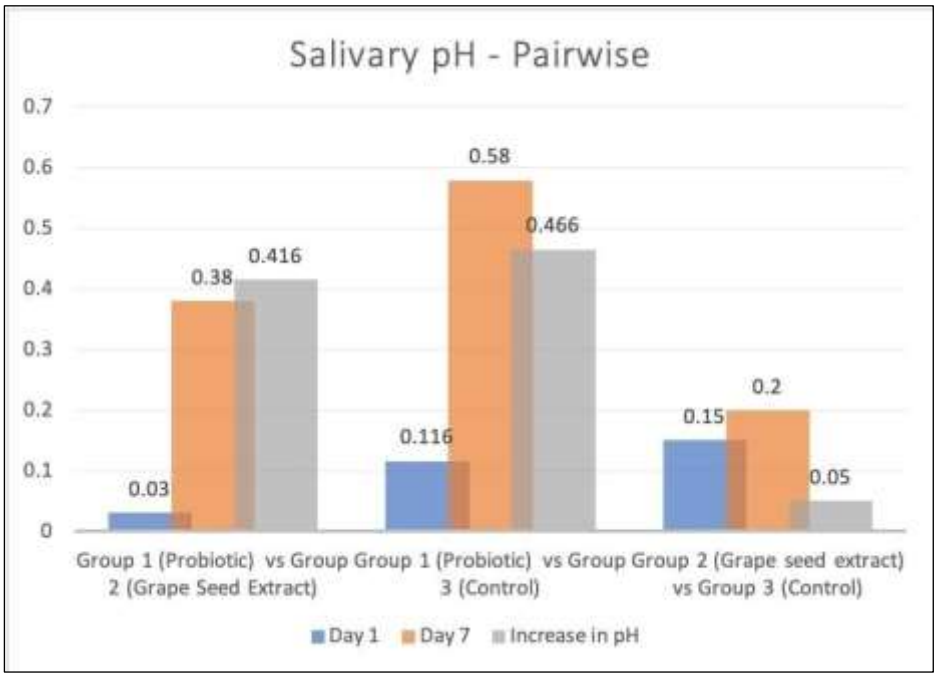
p>.05 (no significant difference) *p<.05 (significant difference)

Table 1: Overall intergroup comparison of efficacy of Probiotics, Herbal and Chlorhexidine mouthwash on salivary pH of children respectively using One-way Anova F test.

The **pairwise comparisons on Day 1** revealed no significant differences in salivary pH between the groups. The Probiotic group showed similar pH levels as the GSE group (mean difference= 0.03, *P = .992)* and the control group (mean difference = 0.116, *P = .904*). Similarly, no significant difference was observed between the GSE mouthwash and Control groups (mean difference = 0.15, *P = .848*). These findings confirmed that baseline pH levels were consistent across all groups.

On **Day 7**, significant differences in salivary pH were observed. The Probiotic group exhibited higher pH than the GSE group (mean difference = 0.416, *P = .015)* and the control group (mean difference = 0.466, *P = .006).* However, the GSE and Control groups showed no significant differences in salivary pH (mean difference = 0.05, *P =.933).*

Salivary pH increased from day 1 to day 7, with significant pairwise differences between the Probiotic and Control groups (mean difference = 0.58, *P = .042*). However, the differences between the Probiotic and GSE groups (mean difference = 0.38, *P = .234*), and between the GSE and Control groups (mean difference = 0.2, *P = .664)* were not statistically significant. This further reinforces the superior efficacy of probiotic mouthwashes in improving salivary function **(Table 2 and Figure 4).**

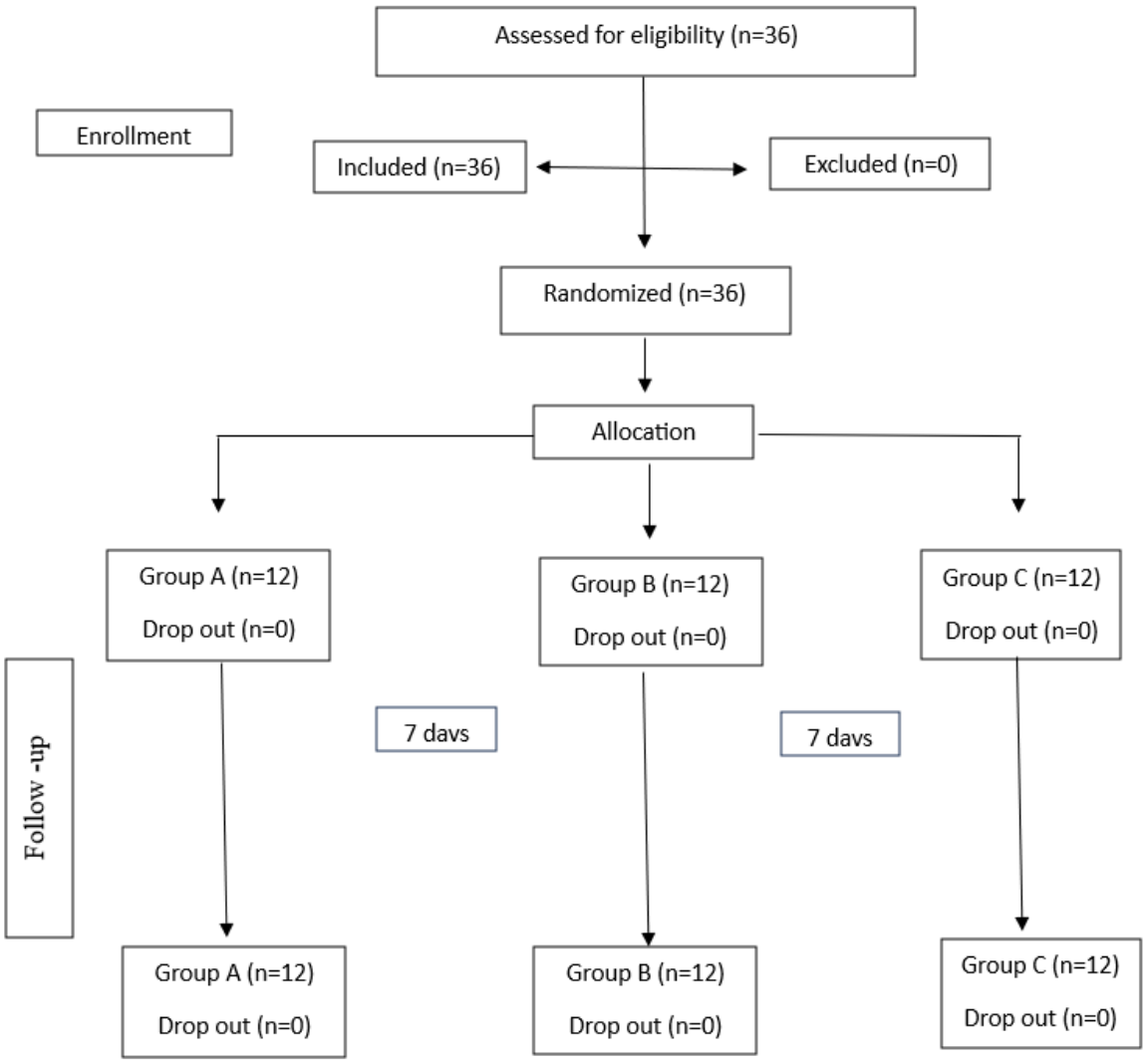


Graph 2: Pairwise comparison of efficacy of Probiotics, Herbal and Chlorhexidine mouthwash on salivary pH of children.

	Day 1	Day 7	Increase in pH
Group 1 (Probiotic) Vs Group 2 (GSE)	Mean difference = 0.03, p = .992	Mean difference =0.38, p = .234	Mean difference = 0.416, p = .015*
Group 1 (Probiotic) vs Group 3 (Control)	Mean difference = 0.116, p = .904	Mean difference = 0.58, p = .042*	Mean difference = 0.466, p = .006*
Group 2 (GSE) vs Group 3 (Control)	Mean difference = 0.15, p = .848	Mean difference = 0.2, p = .664	Mean difference = 0.05, p = .933

p>.05 (no significant difference) *p<.05 (significant difference)

Table 2: Pairwise comparison of efficacy of Probiotics, Herbal and Chlorhexidine mouthwash on salivary pH of children respectively using Tukey's post hoc test.



Discussion

The study shown that all three mouthwashes increased salivary pH after one week of use, with the probiotic mouthwash showing the most pronounced effect. Salivary pH influences the dynamic between enamel demineralization and remineralization and is essential for preserving oral balance. A pH below 5.5 facilitates enamel demineralization, leading to dental caries, whereas a neutral or slightly alkaline pH promotes remineralization.[15] Consequently, the ability of mouthwashes to raise and stabilize salivary pH is essential for caries prevention. These results are consistent with earlier studies, which have also shown that therapeutic mouthwashes can significantly impact salivary pH over time.[16,17]

Probiotic Mouthwash: Efficacy and Mechanism

The probiotic mouthwash demonstrated the highest efficacy in increasing salivary pH, with a mean increase of 0.675 units over one week. This finding is supported by data in the literature highlighting the ability of probiotics to modulate oral microbiota and suppress acidogenic bacteria, such as *Streptococcus mutans*.[18] Probiotic strains, including *Lactobacillus* and *Bifidobacterium*, release antimicrobial peptides and create a competitive environment that inhibits pathogenic bacteria, leading to reduced acid production and an increase in pH.[19] Moreover, probiotics are known to enhance salivary flow and buffer capacity, further contributing to a neutral oral environment.[20] The findings of this investigation correlate with earlier research that demonstrated probiotics improved salivary parameters and significantly lowered plaque pH.[21,22] Unlike chlorhexidine, probiotics do not pose the risks of staining or taste alteration, making them a safer and more acceptable option for long-term use, especially in pediatric populations.

Grape Seed Extract: A Natural Alternative

The GSE (Herbal) mouthwash also showed a modest increase in salivary pH, with a mean change of 0.258 units. It is rich in polyphenols, particularly proanthocyanidins, which exhibit antimicrobial, antioxidant, and anti-inflammatory properties. These substances have been demonstrated to decrease the formation of biofilms and stop the growth of cariogenic bacteria.[13] Additionally, GSE enhances the remineralization of enamel by interacting with hydroxyapatite and stabilizing calcium and phosphate ions in saliva.[14]

Even though the pH rise using grape seed extract was less than that with probiotics, it was still much greater than that of the control group. This implies, grape seed extract is a safe and natural substitute for dental cavities.

Chlorhexidine Mouthwash: The Gold Standard

Chlorhexidine, often regarded as the benchmark in mouthwashes, caused a modest increase in salivary pH (mean increase of 0.208 units). Its primary mechanism of action is its broad- spectrum antimicrobial activity, which disrupts bacterial cell membranes and inhibits biofilm formation. However, chlorhexidine primarily targets bacterial reduction rather than direct modulation of salivary pH.[9]

The limited increase in pH observed in this study may reflect the indirect effect of bacterial reduction on acid production. This finding aligns with that of Badr et al., who reported that while chlorhexidine significantly reduced bacterial counts, its effect on salivary pH was less pronounced. Furthermore, the use of chlorhexidine in pediatric populations is restricted due to its long-term side effects, which include staining and taste alteration.[9]

Comparative Efficacy

Comparative analysis revealed that the probiotic mouthwash was significantly more effective than both GSE and Chlorhexidine mouthwashes in elevating salivary pH. This superiority can be attributed to the dual action of probiotics in reducing acidogenic bacteria and enhancing salivary buffering capacity.[23,24] In contrast, the herbal mouthwash provided a natural but less potent effect, whereas the pH modulation by chlorhexidine was secondary to its antimicrobial action.

Limitations and Future Directions

The one-week duration of this study limits the ability to evaluate the long-term effects of the mouthwashes. Future research with extended follow-up periods is necessary to assess long-term variations in salivary pH and their impact on dental caries incidence. Additionally, this study focused solely on salivary pH as an outcome measure. Incorporating parameters such as microbial load, plaque indices, and enamel remineralization markers would allow for a more comprehensive assessment of efficacy.

Although the sample size was statistically adequate, it was relatively small. Larger studies involving diverse populations are required to validate these findings. Furthermore, participant compliance with the rinsing protocol was not assessed, which may have influenced the results. Future studies should include objective measures of adherence, such as periodic saliva sampling.

Clinical Implications

The findings of this study have significant effects on clinical practice. The significant pH- modulating effect of probiotic mouthwash suggests its potential as a primary preventive measure in children at a high risk for dental caries. It offers a safe, natural, and effective alternative to chlorhexidine, which is better suited for short-term use under acute conditions GSE mouthwash, with its natural composition and antimicrobial properties, represents another viable alternative, particularly for patients seeking natural remedies.

Moreover, the findings highlight the importance of tailoring oral care interventions according to individual needs. For instance, children with poor oral hygiene and high caries risk may benefit more from probiotics,

whereas those with mild risk may benefit more from herbal formulations.

Conclusion

This study demonstrated that Probiotic, GSE, and Chlorhexidine mouthwashes effectively increased salivary pH in children, with the probiotic mouthwash showing the most significant improvement. These findings support the potential of probiotics and natural formulations as safe and effective alternatives to chlorhexidine, particularly in the pediatric population. These alternatives may play a vital role in the prevention of dental caries by modulating salivary pH and promoting oral health. Further research is warranted to explore its long-term benefits and broader applications in preventive dentistry.

Ethics Approval: The study was approved by the Institutional Ethics Committee of the Chhattisgarh Dental College and Research Institute.

Additionally, under registration number **CTRI/2024/07/069853**, the trail was registered with the **Clinical**

Trials Registry of India (CTRI).

All participants completed an informed consent form, and all of them had the option to leave the study at any time without stating a reason, and the privacy and confidentiality of the obtained data were guaranteed.

Availability of data and material

The datasets used during the current study are available.

Competing Interests

Not applicable.

Funding Statement

Not applicable

Authors' Contributions

Dr. Rasika Talmale: Conception and design, data acquisition, data analysis, and interpretation
Dr. Nagarathna PJ: Reviewed and approved the final version of the manuscript.
Dr. Rashmi Sunil Lokhande: Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved.
Dr. Shilpa Kochar: Performed the background literature review.
Dr. Sanchita Dhote: Revised the manuscript.
Dr. Ayushi Verma: Drafting the article and critically revising it for important intellectual content

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